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# Great Lakes Fish Monitoring Program

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## Quality Assurance Project Plan for Sample Collection Activities



*Prepared by:*

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**DRAFT**



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Field Sampling Teams (identified annually)





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Appendix A	Fish Collection Standard Operating Procedure
Appendix B	Fish Homogenization Standard Operating Procedure
Appendix C	Field Recording Form and Chain-of-Custody Record
Appendix D	Packing and Shipping Instructions for the Great Lakes National Program Office's Fish Monitoring Program
Appendix E	List of Sample Collection Port Codes and Grid Numbers



## 1.0 PROJECT ORGANIZATION

This Quality Assurance Project Plan (QAPP) describes the quality assurance (QA) and quality control (QC) activities and procedures associated with collecting samples of fish tissue for the Great Lakes Fish Monitoring Program (GLFMP). The purpose of this document is to present the methods and procedures that are used for the collection of whole and fillet fish from the five Great Lakes as part of cooperative efforts to monitor the concentrations of contaminants in fish tissues. This document addresses *only* the sample collection effort of the GLFMP. This QAPP is accompanied by three documents:

- ▶ *Trends in Great Lakes Fish Contaminants Quality Assurance Project Plan*, submitted to Great Lakes National Program Office (GLNPO) by the Principal Investigator Deb Swackhamer, which outlines the quality assurance activities associated with the analytical component of this project,
- ▶ *The Great Lakes Fish Monitoring Program Quality Management Plan*, developed by GLNPO, which outlines overall project objectives and associated project-level quality control activities, and
- ▶ *The Great Lakes Fish Monitoring Program Historical Document*, which provides a history of the project design and implementation prior to 2003.

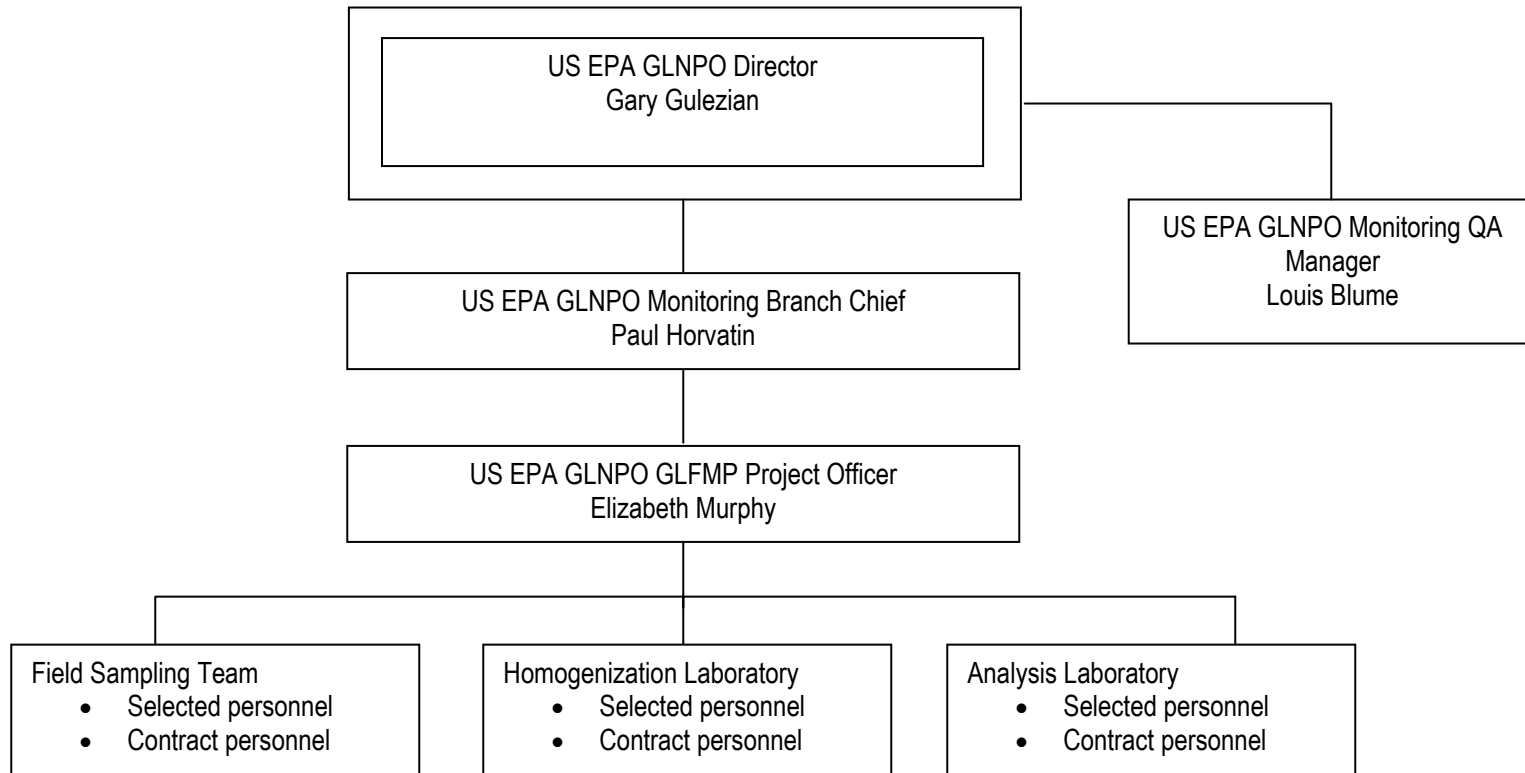
### 1.1 Project Management

The project is implemented by staff from the United States Environmental Protection Agency's (US EPA) Great Lakes National Program Office, the Great Lakes states, and selected state resource agencies or commercial resources, and Native American Tribes. GLNPO oversees and administers the project. The Great Lakes States that include Illinois, Ohio, Michigan, Minnesota, Pennsylvania, Indiana, Wisconsin, and New York, provide sampling and technical support. In some cases, Tribes or commercial fisherman also provide sampling support. A Principal Investigator (PI), Deb Swackhamer of the University of Minnesota, provides analytical and technical support. Finally, a sample preparation laboratory is responsible for preparing homogenized composites of the fish samples and distributing them to the PI.

GLNPO is organized into five functional teams that specialize in specific areas of interest and expertise for data gathering activities including the Environmental Monitoring and Indicators Team, Health and Safety Team, Information Management and Data Integration Team, Quality Management (QM) Team, and Management Team. The Environmental Monitoring and Indicators Team and QM Team provide the majority of the GLFMP project management support.

This section describes the overall management and lines of authority within GLNPO and the participants supporting GLFMP. It includes an organization chart illustrating the relationships between groups participating in the major study activities (Figure 1).

**Figure 1. Participants in the Great Lakes Fish Monitoring Program**



## 1.2 Project Implementation

### 1.2.1 Director of the Great Lakes National Program Office

The **GLNPO Director**, Gary Gulezian, is responsible for providing financial and staff resources necessary to meet project objectives and implement the requirements described in this QAPP. The Director is responsible for establishing GLNPO quality policy and resolving related issues, which are identified through the Quality Management Team and study participants.

### 1.2.2 GLNPO Quality Assurance Manager

The **GLNPO Quality Assurance Manager**, Louis Blume, is responsible for reviewing and approving all QAPPs and reports directly to the GLNPO Director. Additional GLNPO QA Manager responsibilities include the following:

- ▶ reviewing and evaluating field procedures,
- ▶ conducting external performance and system audits of the procedures, and
- ▶ participating in Agency QA reviews of the study.

### 1.2.3 Monitoring Indicators and Reporting Branch Chief

The **Chief** of GLNPO's Monitoring Indicators and Reporting Branch, Paul Horvatin, reports directly to the GLNPO Director and is responsible for providing overall direction concerning all aspects of the GLFMP.

### 1.2.4 GLNPO Project Officer

The **GLNPO Project Officer**, Elizabeth Murphy, reports directly to the Monitoring Indicators and Reporting Branch Chief and is responsible for supervising the assigned project personnel. Additional GLNPO Project Manager responsibilities include the following:

- ▶ providing oversight for development of study design,
- ▶ ensuring adherence to study design and accomplishment of project objectives,
- ▶ reviewing and approving the project work plan, QAPP, and other materials developed to support the project, and
- ▶ coordinating with contractors, grantees, and US EPA Regions/States/Tribes to ensure technical quality and contract adherence.

### 1.2.5 Field Sampling Teams

**Field sampling teams** are selected by GLNPO and can include the following:

- ▶ State personnel such as field biologists or fisheries biologists,
- ▶ Native American Tribes,

- ▶ commercial fisherman, and
- ▶ contracted field staff (including subcontracted organizations).

Field sampling teams are selected by GLNPO each year prior to the sampling event. A field sampling team leader is identified as the primary contact for study implementation. Sample collection personnel are responsible for performing fieldwork, including collection, preparation, and shipment of fish tissue samples and completion of field sampling records. The field sampling teams must adhere to the established sample collection protocols (see Appendix A, Fish Collection SOPs). They must perform all work in adherence with the project work plan and the QAPP. Labeling of individual fish is required to ensure the integrity of the samples and to maintain proper sample identification during handling. In this role, field sampling teams are responsible for:

- ▶ reviewing SOPs and this QAPP prior to sample collection,
- ▶ determining the appropriate sampling techniques,
- ▶ choosing and preparing appropriate sampling gear,
- ▶ inspecting sampling gear prior to use,
- ▶ collecting fish samples,
- ▶ receiving and inspecting the sample containers,
- ▶ completing, reviewing, and signing appropriate field records (these are provided in Attachment 1),
- ▶ preparing and adhering labels to each individual fish sample,
- ▶ maintaining custody of the samples by controlling and monitoring access to samples while in their custody,
- ▶ shipping samples to appropriate destinations, and
- ▶ ensuring all sampling and sample handling activities are in compliance with GLNPO procedures as described in this QAPP and the attached SOPs.

#### *1.2.6 Homogenization Laboratory*

Individual samples of salmon/rainbow trout and lake trout will be used to prepare composite samples for the analyses of contaminants. The compositing and homogenization of these samples is conducted in the homogenization laboratory. Currently, homogenization is being conducted by Axys Analytical in Sydney, British Columbia, Canada.

The laboratory must adhere to the compositing methods, sampling handling and custody requirements, and quality control requirements outlined in the *Fish Homogenization Standard Operating Procedure* (Appendix A). Any deviations from the SOP should be approved prior to implementation by the GLNPO Project Officer or if the deviation was unintentional, reported immediately to the GLNPO Project Officer.



### 1.2.7 Analysis Laboratory

The analyses of the fish tissue samples are *not* covered by this QAPP. Details regarding this component of the project can be found in *Trends in Great Lakes Fish Contaminants Quality Assurance Project Plan*, submitted to GLNPO by the principal investigator Deb Swackhamer.

## 2.0 PROBLEM DEFINITION

The US Geological Survey Biological Research Division (formerly known as the US Fish and Wildlife Service) began fish monitoring in the mid-1960s to measure the contaminant levels of various organic substances in lake trout in the Great Lakes ecosystem. In 1977, the USGS-BRD and GLNPO collaborated and modified the original study design to generate more data by including additional species, sampling locations, and contaminants. The study was further modified in 1980, when the US EPA, US Food and Drug Administration (FDA), USGS-BRD, and the eight Great Lakes states began a cooperative effort to monitor and better define the fish contaminant problem in the Great Lakes. The project is implemented by GLNPO with cooperation from the Great Lakes States, selected state agencies, and Native American Tribes.

Game fish and predatory fish are collected from the five Great Lakes. Game fish are collected by the eight states surrounding the Great Lakes (Ohio, Illinois, Michigan, New York, Pennsylvania, Wisconsin, Minnesota, and Indiana). Game fish include Coho and Chinook salmon in Lakes Michigan, Superior, Ontario, and Huron and rainbow trout in Lake Erie. Each state collects game fish using their own methods and techniques (see Section 8.3) and may use the information gained from their collections in various ways. However, due to funding constraints, the individual states may not provide game fish for the GLFMP every year. Predatory fish are collected from all five Great Lakes on an annual basis. Predatory fish include lake trout in Lakes Michigan, Superior, Ontario, and Huron, and walleye in Lake Erie. The GLFMP organizes collections through cooperative agreements with other agencies or by purchasing predatory fish. Therefore, fish sampling teams for each year are identified by GLNPO prior to the annual collection.

Over the life of the GLFMP, a wide variety of metals and organic chemicals have been analyzed in fish samples collected in the Great Lakes Basin. The list of analytes has changed in response to both budgetary constraints and information about new and emerging contaminants. The current list of analytes of interest is shown in Table 1, consisting of a wide variety of organic contaminants and mercury, a metal contaminant of specific concern in the Great Lakes. The actual list of analytes for a given year of the study may be modified to match the funding appropriated for the program.

**Table 1. Analytes of Interest**

Analyte	
Polychlorinated biphenyl (PCB) congeners	<i>cis</i> -Nonachlor
co-planar PCBs	<i>trans</i> -Nonachlor
Hexachlorobenzene	p,p', o,p-DDD
Pentachlorobenzene	p,p', o,p-DDE
Octachlorostyrene	p,p', o,p'-DDT
$\Delta$ -HCH (Lindane)	Endrin
A-HCH	Mirex
Aldrin	Toxaphene & homologs
Dieldrin	Polychlorinated dibenzo- <i>p</i> -dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)
Heptachlor epoxide a	
Heptachlor epoxide b	Polybrominated diphenyl ethers (PBDEs)
<i>cis</i> -Chlordane	Polychlorinated naphthalenes (PCNs)
<i>trans</i> -Chlordane	Mercury
Oxychlordane	Lipid fraction
Perfluorooctyl sulfonate	

### 3.0 PROJECT DESCRIPTION

The overall goals of the GLFMP include:

- ▶ to monitor temporal trends in bioaccumulative organic chemicals in the Great Lakes using top predator fish as biomonitors,
- ▶ to assess potential human exposure to organic contaminants found in these fish, and
- ▶ to provide information on new compounds of concern entering the lakes ecosystem.

The GLFMP goals are broken down further into two elements:

- ▶ Element 1: Open Lake Trend Monitoring
- ▶ Element 2: Game Fish Fillet Monitoring

Element 1 (Open Lake Trend Monitoring) is directed at monitoring contaminant trends in the open water of the Great Lakes, and assisting in evaluating the impacts of contaminants on the fishery. The program provides for collection and analysis of whole-fish composites of lake trout (*Salvelinus namaycush*) in the size range from 600 mm to 700 mm from Lakes Michigan, Huron, Ontario, and Superior, and of walleye (*Stizostedion vitreum*) in the size range of 450 mm to 550 mm in Lake Erie. Composites of each species, consisting of five individual fish, are analyzed for contaminants to assess temporal trends in organic contaminants in the open waters of the Great Lakes, using fish as biomonitors. These data can also be used to assess the risks of such contaminants on the health of this important fishery, and on wildlife that consume them.

Element 2 (Game Fish Fillet Monitoring) is directed at monitoring potential human exposure to contaminants through consumption of popular sport species, as well as providing temporal trend data for top predator species, which have shorter exposures than the lake trout collected in Element 1. Coho salmon (*Oncorhynchus kisutch*) and Chinook salmon (*Oncorhynchus*

*tshawytscha*) are collected from Lakes Michigan, Huron, Ontario, and Superior, and rainbow trout (*Salmo gairdneri*) are collected in Lake Erie during the fall spawning run. Composites of each species, consisting of five individual fish fillets, are analyzed for organic contaminants to assess potential human exposure. These data complement those from Element 1. The Coho salmon spawn at 3 years of age while the lake trout may integrate exposures over 4 to 10 years, depending on the lake. Therefore, body burdens of the salmon reflect a more focused and consistent exposure time, compared to the lake trout. Chinook salmon spawn after 4 to 5 years, and have higher (and thus more detectable) contaminant concentrations than the Coho salmon and also represent a consistent exposure time. Ideally, the composite should focus on the larger individuals commonly harvested by the local population. The size ranges specified by this study (see Table 4) are designed to meet this goal. For salmon, however, the fish are being provided on a cooperative and voluntary basis from the Great Lakes States that have their own fish monitoring goals. States collect the salmon as needed as a way to verify consumption advisories in each state. Advisories are based upon water body and size of fish. Therefore, salmon are collected in the size ranges of small, medium, and large in order to follow the protocol for issuing State consumption advisories. Sample composites, consisting of five fish each in the same category, are prepared and analyzed for the target analytes for each fish species. Trends are not meant to be concluded from Element 2, as the voluntary nature of the program does not allow for consistent collection of salmon from year to year. For trend analysis, GLNPO is currently using only the fish tissue contaminant data for Coho salmon from Lake Michigan that are larger than 500 mm.

Each year, project planners conduct an annual review to determine the number and type of samples to be collected, the analyses to be conducted, and the field sampling teams to be assembled by the States or GLNPO. A generalized work schedule is shown in Table 2. Planning is particularly important because the type of fish and locations from which they are collected alternate according to an even or odd year specification (see Section 7.2).

**Table 2. Work Schedule**

<b>Date</b>	<b>Action</b>	<b>Person Responsible</b>
January prior to fall collection	Organize and coordinate collection efforts	Project Officer
August prior to fall collection	Coordination of collection SOPs and sampling materials	Project Officer
October - November of collection year	Samples to be collected	Identified collection personnel
November - December of collection year	Coordination of sample shipment to homogenization laboratory	Project Officer
December - February following a collection year	Homogenization of samples	Identified homogenization personnel
February following a collection year	Coordination of shipment of homogenized samples to analysis laboratory	Project Officer
March - May following a collection year	Analysis of homogenized samples and report preparation	Identified analysis personnel
May – July following a collection year	Review of sample analysis report	Project Officer, QA Manager
July - September following a collection year	Final preparation and release of finalized report	Project Officer, QA Manager, Branch Chief

#### **4.0 QUALITY OBJECTIVES AND CRITERIA FOR MEASUREMENT DATA**

##### **4.1 Project Quality Objectives**

Data of known and documented quality are essential to the success of any monitoring program. Data quality objectives (DQOs) are qualitative and quantitative statements that clarify the intended use of the data, define the type of data needed to support the decision, identify the conditions under which the data should be collected, and specify tolerable limits on the probability of making a decision error due to uncertainty in the data. DQOs are developed by the data users. Sources of error or uncertainty include the following:

- ▶ Sampling error: The difference between sample values and *in situ* true values from unknown biases due to collection methods and sampling design.
- ▶ Measurement error: The difference between sample values and *in situ* true values associated with the measurement process. This includes error sources or biases associated with compositing, sample handling, storage, and preservation.
- ▶ Natural variation: Natural spatial heterogeneity and temporal variability in population abundance and distribution.

This QAPP addresses the sample collection activities for this project. Therefore, the relevant quality objectives are primarily related to sample collection and handling issues. The DQOs for the overall monitoring project are described in *The Great Lakes Fish Monitoring Program*

*Quality Management Plan.* The DQOs for the analytical activities for this project are described in *Trends in Great Lakes Fish Contaminants Quality Assurance Project Plan.*

## **4.2 Sampling Quality Objectives**

The goal of the sample collection effort is to collect 15 to 50 fish of each of five species in specific size ranges at specific sampling locations throughout the Great Lakes (50 fish for Element 1 and 15 fish for Element 2). While it is understood that both size and age affect contaminant concentrations, resource limitations had precluded routine aging of fish, and the analysis of individual fish. The program has therefore focused on collection of fish in a narrow size range that are approximately the same age and analysis of composite samples (Devault, 1996). Ideally, the target species composite should focus on the larger individuals commonly harvested by the local population. The size ranges specified by this study (see Table 4) are designed to meet this goal. For salmon, the fish are provided on a cooperative basis from the Great Lakes States that have their own fish monitoring goals often involving setting fish advisories. States collect fish in size ranges categorized as small, medium, and large fish. Sample composites, consisting of five fish each in the same category, are prepared and analyzed for the target analytes for each fish species. Accurate taxonomic identification is essential in assuring and defining the organisms that have been composited and submitted for analysis. Under no circumstances should individuals from different species be used in a single composite sample.

Fish lengths are recorded to the nearest mm (or sixteenth of an inch) by the field personnel. Field personnel use hand-held rulers to measure fish and are instructed not to use cooler lids or other less accurate means. The fish length also is measured in the homogenization laboratory as a QC check for the field length. In the field, fish are weighed to the nearest gram.

The field personnel identify the species of each fish during collection. The accuracy of the identifications is assured through use of experienced field personnel. Personnel that are collecting fish meet at least one of the following requirements: 1) they are responsible for field collection for state-sponsored fish monitoring studies; or 2) they are established commercial fisherman conducting business in the Great Lakes. In the second case, they have participated in fish monitoring studies for other state or federal agencies.

Field sampling personnel are required to submit to GLNPO a field recording form and chain of custody record at the time that fish are shipped (see Appendix C). This recording form is used to document information regarding each fish and the sample collection effort and to document shipment and handling from the field personnel to the sample homogenization laboratory. For example, the port code or grid number where each fish was collected (port codes are listed in Section 7.4 and Appendix E) is documented by field personnel. Additionally, the latitude and longitude where each fish was collected also is documented as a means of verifying the port or grid where the fish was collected.

The GLNPO PO reviews the records submitted by the field personnel. If any discrepancies or questions arise, the GLNPO PO will contact the field personnel and document the resolution. All field data are submitted to the homogenization and analytical laboratories to ensure proper identification of each fish in the study. These laboratories are instructed to inform GLNPO of any discrepancies noted among sample labels (discussed in Section 6) and documentation.

The field personnel also are required to document other data as described in Section 6. To ensure accurate documentation, GLNPO will discuss the SOP and specifics regarding the field data with field personnel prior to sampling.

## **5.0 SPECIAL TRAINING REQUIREMENTS**

Each field sampling team is required to have the necessary knowledge and experience to perform all field activities. This includes both knowledge and experience in the collection and identification of fishes, in the use of fisheries sampling gear needed to successfully implement the study, and in the operation of small boats. The GLNPO PO annually reviews the study requirements, sample collection procedures, and documentation with all field personnel and verifies that they do not have any questions.

The field sampling crews are primarily composed of state, tribal, and regional fisheries biologists or contracted biologists with a strong technical background in fisheries sampling activities. However, GLNPO sometimes selects a commercial fishing operation to supplement the field sampling personnel. When possible, GLNPO identifies commercial fisherman through recommendations from state or federal agencies that have used them for their fish monitoring studies. The GLNPO PO provides expanded oversight, if necessary, to commercial fisherman involved in the study to ensure proper implementation. Commercial fishermen are properly informed of the collection procedures and are expected to follow the protocols. They also are informed of the documentation and records they are required to maintain.

## **6.0 DOCUMENTATION AND RECORDS**

Thorough documentation of all field sample collection and handling activities is necessary for proper identification in the laboratory and, ultimately, for the interpretation of study results. Field sample collection and handling is documented in writing on specific forms that have been created by GLNPO for this project. This documentation includes a field recording form and chain-of-custody record, and a sample label.

Field sampling personnel are required to submit to GLNPO a field recording form and chain of custody record (Appendix C) at the time that fish are shipped to the homogenization laboratory. The field recording form is used to document the sample collection effort and includes specific information regarding each fish specimen such as length, weight, and species. The form also is used to document shipment and handling of all fish from the field personnel to the sample homogenization laboratory. Field sampling teams are provided with *Packing and Shipping*

*Instructions for the Great Lakes National Program Office's Fish Monitoring Program* (located in Appendix D) that provides information on filling out the required forms.

The field recording form is designed to capture a unique tracking number for each fish composite. This tracking number is used by GLNPO and the homogenization and analytical laboratories to identify each composite and report results. The tracking number or composite ID includes the following:

- ▶ A two-character code for each lake (e.g., LO for Lake Ontario, LM for Lake Michigan, etc.),
- ▶ The fish species code,
- ▶ The four digit year of collection,
- ▶ The grid number or port code, and
- ▶ A sequential number indicating the number of each composite from a specific location and year (e.g., 001, 002, etc.).

Data specific to each fish specimen is recorded on sample specific labels that are prepared by the field personnel and adhered to each fish. The data recorded on the label adhered to each fish are listed in Table 3.

Coded wire tags and fin clips found in any fish specimens are removed by the homogenization laboratory and shipped with the homogenized samples to the PI. Fin clips and wires removed from any of the fish samples will be reviewed against the field documentation. The PI will maintain the clips for five years. The GLNPO PO will resolve any discrepancies between the tags and the field documentation with the field sampling team.

**Table 3. Types of Field Data to be Collected & Recorded in Association with Fish Tissue Sample Collection**

<b>Data Type</b>	<b>Measurement Units or Allowed Entries</b>
Lake Name	Erie, Michigan, Huron, Ontario, Superior
Grid Number or Port Code of Sample Collection	See Section 7.4 and Appendix E for a list of grid numbers and port codes
Latitude and Longitude of Site	Degrees, minutes, seconds
Fish Species	Taxonomic identification
Fish Length	Millimeters (mm), total length
Fish Sex (if available)	Not applicable
Water Depth at Collection Site	Meters
Fish Weight	Grams
Method of Collection	Dip Net, Cage Trap, Boat Shocker, Nets, Seines, Gill Net, Trawl, Electrofishing Unit
Date of Collection	Month/Day/4-digit year
Collector Identification	Vessel and collector's name
Fin Clip(s)	If none, record none

All records and reports pertaining to sample collection are sent to the GLFMP Project Officer, Elizabeth Murphy, as soon as collection has been completed. All field records and electronic correspondence are retained by the GLNPO PO for at least five years. The records and other study reports and documentation are retained by the GLNPO PO or GLNPO's document control coordinator as appropriate.

## **7.0 SAMPLING PROCESS DESIGN**

The objectives of the GLFMP are to monitor time trends in bioaccumulative organic chemicals in the Great Lakes using top predator fish as biomonitors, and to assess potential human exposure to organic contaminants found in these fish.

In so doing, the study will provide the following types of information:

- ▶ information on the trends of fish from the open lakes,
- ▶ information on emerging problems associated with contaminants in fish from harbors and tributary mouths, and
- ▶ information on potential human exposure to toxic substances found in the most commonly consumed species.



The goal of the sample collection effort is to collect 15 to 50 fish of each of five species in specific size ranges at specific sampling locations throughout the Great Lakes. While it is understood that both size and age affect contaminant concentrations, resource limitations had precluded routine aging of fish, and the analysis of individual fish. The program has therefore focused on collection of fish in a narrow size range and analysis of composite samples (Devault, 1996). Beginning in 2003, collected fish will be aged, when possible, to investigate these relationships and if necessary, refine the sampling design.

## 7.1 Sample Type

To meet the study objectives, the GLFMP includes composite sampling of specific fish species from each Great Lake. For Element 1, Open Lake Trend Monitoring, lake trout and walleye are collected and for Element 2, Game Fish Filet Monitoring, salmon and rainbow trout are collected. Sample composites, consisting of five fish each, are prepared and analyzed for the target analytes for each fish species. The species, size ranges, and numbers of fish collected and analyzed from each lake are listed in Table 4 for elements 1 and 2 of the study.

**Table 4. Number and Type of Samples Collected**

Study Component	Lake	Species	Size Range (mm)	Number of Fish	Number of Composites	Sample Type
Element 1	Michigan, Huron, Superior, Ontario	<i>Salvelinus namaycush</i> (Lake trout)	600 to 700	50	10	Whole fish
	Erie	<i>Stizostedion vitreum</i> (Walleye)	450 to 550	50	10	Whole fish
Element 2	Michigan, Huron, Superior, Ontario	<i>Oncorhynchus kisutch</i> (Coho salmon) and <i>Oncorhynchus tshawytscha</i> (Chinook salmon)	Small, medium, and large fish	15	3	Filet (skin-on)
	Erie	<i>Salmo gairdneri</i> (Rainbow trout)	600 to 700	15	3	Filet (skin-on)

Fish retained for a composite sample must meet the following criteria:

- ▶ All be of the same species,
- ▶ Satisfy any legal requirements of harvestable size (or weight), or at least be of consumable size if no legal harvest requirements are in effect,
- ▶ Be of similar size so that the smallest individual in a composite is no less than 75% of the total length of the largest individual, and
- ▶ Be collected as close to the same time and location as possible, but no more than one week apart.

For salmon, where possible, filets should be composites that represent large, medium and small fish. If sufficient numbers of fish within the designated size range cannot be obtained by a reasonable sampling effort, it is acceptable to expand the size range by approximately 5%. However, if possible, an attempt should be made to include similar numbers of fish above and

below the designated size range so that the mean size of fish remains near the middle of the range.

Fish identified for a composite are homogenized together in the homogenization laboratory to prepare a single homogeneous fish paste suitable for chemical analysis. Accurate taxonomic identification is essential in assuring and defining the organisms that have been composited and submitted for analysis. Under no circumstances should individuals from different species be used in a single composite sample. This is assured through four standard procedures:

1. use of experienced field personnel,
2. use of standard documentation for field sampling (Appendix C),
3. review of field documentation by the PO, and
4. notification of the PO by the homogenization laboratory of field record and specimen discrepancies and occurrences where fish from a single composite do not appear to be the same species.

## 7.2 Sampling Period

The field sampling activities take place in the fall months (defined here as August – November) as the fish begin their spawning runs, and are repeated every year. Coho salmon are collected in even-numbered years and Chinook in odd-numbered years. Rainbow trout, lake trout and walleye are collected and analyzed every year; however, sampling locations change depending if sample collection is occurring in an even-numbered or odd-numbered year. The sampling period for each species is detailed in Table 5.

**Table 5. Species Collected by Lake and Even or Odd Year**

Lake	Lake Trout	Walleye	Chinook	Coho	Rainbow Trout
Lake Michigan	E,O	—	O	E	—
Lake Superior	E,O	—	O	E	—
Lake Erie	—	E,O	—	—	E,O
Lake Huron	E,O	—	O	E	—
Lake Ontario	E,O	—	O	E	—

E = even year collection  
O = odd year collection

### **7.3 Selection of Sites within Lakes for Sampling**

Selection of sampling sites is based on availability of fish populations and other factors depending on the study element. For Element 1, lake trout and walleye sites represent both industrial and non-industrial areas in each of the lakes. In addition, sites were chosen to represent offshore fishing grounds (i.e., sampling sites should represent open water populations of fish) and are well removed from tributaries or other potential sources of contaminants. For element 2, Coho and Chinook salmon and rainbow trout collection sites are based on historical collection by states for fish advisories. These sites are representative of human consumption.

Sampling locations for each lake are identified in Table 6. Each sampling site has been identified with a grid number or port code and associated longitude and latitude. It is appropriate to collect fish from grids immediately adjacent to the designated grid if the specified grid does not contain good fishing grounds, if collection from that grid will cause conflicts with management practices (e.g., excessive impact on native fish versus hatchery produced fish), or if it is impractical. The guiding rule should be that the site sampled represents offshore fishing grounds (i.e., open-water populations of fish) and is relatively remote from tributaries or other potential sources of contaminants.

**Table 6. Sampling Locations for the Great Lakes Fish Monitoring Program**

Year	Lake/State	Port Name	Species	Port Code	Grid Number	Latitude/Longitude
Odd-numbered	Lake Superior	Keweenaw Point	Lake Trout	NA	1028	87°35'W/47°25'N
	Lake Michigan	Sturgeon Bay	Lake Trout	NA	0906	87°05'W/44°45'N
	Lake Michigan	Charlevoix (as back-up only)	Lake Trout	NA	0516	85°25'W/45°25'N
	Lake Huron	Port Austin	Lake Trout	NA	1413	82°45'W/44°05'N
	Lake Erie	Dunkirk	Walleye	NA	0424	79°35'W/42°25'N
	Lake Ontario	North Hamlin	Lake Trout	NA	0713	77°55'W/43°25'N
	New York	Salmon River	Chinook	0601	NA	43°32'N/75°59'W
	Pennsylvania	Trout Run	Steelhead Trout	0527	NA	42°3.54'N/80°16.38'W
	Michigan	St. Joseph's River	Chinook	0201	NA	41°56.63'N/86°19.7'W
		Grand River	Chinook	0225	NA	42°56'N/84°54'W
		Platte River	Chinook	0213	NA	44°43.18'N/86°8.21'W
		Thompson Creek	Chinook	0251	NA	41°65'N/86°36'W
		Swan River	Chinook	0300	NA	45°23'N/83°44'W
		Ausable River	Chinook	311A	NA	44°24.93'N/83°19.84'W
	Indiana	Trail Creek	Chinook	0227	NA	41°43'N/86°53'W
	Wisconsin	Root River	Chinook	0237	NA	42°43.955'N/87°46.72'W
		Kewaunee River	Chinook	0247	NA	44°27.533'N/87°33.383'W
		Lake Superior, Pike Creek	Chinook	0139	NA	46°47.154'N/90°51.953'W
	Minnesota	French River	Chinook	0143	NA	46°52'N/91°54'W
		Knife River	Coho	0145	NA	46°45'N/91°45'W
Ohio	Grand River	Steelhead Trout	0523	NA	41°45'N/81°16'W	
Even-numbered	Lake Superior	Apostle Islands	Lake Trout	NA	1311	90°25'W/46°55'N
	Lake Michigan	Saugatuck	Lake Trout	NA	2210	86°25'W/42°35'N
	Lake Huron	Rockport	Lake Trout	NA	0710	83°1'W/45°15'N
	Lake Erie	Middle Bass Island	Walleye	NA	0904	82°55'W/41°35'N
	Lake Ontario	Oswego	Lake Trout	NA	0623	76°15'W/43°35'N
	New York	Salmon River	Coho	0601	NA	43°32'N/75°59'W
	Pennsylvania	Trout Run	Coho	0527	NA	42°3.54'N/80°16.38'W
	Michigan	St. Joseph's River	Coho	0201	NA	41°56.63'N/86°19.7'W
		Grand River	Coho	0225	NA	42°56'N/84°54'W
Platte River		Coho	0213	NA	44°43.18'N/86°8.21'W	

Year	Lake/State	Port Name	Species	Port Code	Grid Number	Latitude/Longitude
Even-numbered	Michigan	Thompson Creek	Coho	0251	NA	41°65'N/86°36'W
		Swan River	Coho	0300	NA	45°23'N/83°44'W
		Ausable River	Coho	311A	NA	44°24.93'N/83°19.84'W
	Indiana	Trail Creek	Coho	0227	NA	41°43'N/86°53'W
	Wisconsin	Root River	Coho	0237	NA	42°43.955'N/87°46.72'W
		Kewaunee River	Coho	0247	NA	44°27.533'N/87°33.383'W
		Lake Superior, Pikes Creek	Coho	0139	NA	46°47.154'N/90°51.953'W
	Minnesota	French River	Coho	0143	NA	46°52'N/91°54'W
	Ohio	Grand River	Steelhead Trout	0523	NA	41°45'N/81°16'W

## **8.0 SAMPLING METHODS**

### **8.1 Target Species**

Five distinct ecological groups of fish in the Great Lakes will be included as target fishes for this study: Coho salmon, Chinook salmon, and lake trout (in Lakes Michigan, Huron, Ontario, and Superior), and walleye and rainbow trout (in Lake Erie). These groups permit monitoring of a wide variety of habitats, feeding strategies, and physiological factors that might result in differences in bioaccumulation of contaminants. Every effort will be made to collect the desired species and number of fish specified in Section 7.1. However, the outcome of field sampling efforts will ultimately depend on the natural diversity and abundance of fish in the study lakes. Documentation of collection site physical data for each fish is recorded on the *Field Recording Form and Chain-of-custody Record* (Appendix C).

### **8.2 Sample Collection**

The objective of the GLFMP is to obtain representative composite samples of each target species from each lake. Each composite must consist of all the same species and size ranges as discussed in Sections 7 and 8.3. The composites are intended to estimate the mean fish tissue contaminant concentration for the lake for each target parameter. Composite sampling is discussed in Section 8.3.

Fish collection methods can be divided into two major categories, active and passive. Each method has advantages and disadvantages. Active collection methods involve a wide variety of sampling devices including electro fishing units, seines, trawls, and boat shocker. The active collection methods generally require more field personnel and more expensive equipment than passive collection methods. Passive collection methods employ a wide array of sampling devices, including gill nets, dip nets, trap net and cage trap. Passive collection devices (e.g., gill nets) must be checked frequently (e.g., at least once every 24-hours) to ensure a limited time lag between fish entrapment and sample preparation. Passive collection methods, while time-consuming, generally require less fishing effort than active methods, but normally yield a much greater catch than would be required for a contaminant-monitoring program. Although active collection requires greater fishing effort, it is usually more efficient than passive collection for covering a large number of sites and catching the relatively small number of individuals needed from each site for tissue analysis. Table 7 provides a list of collection techniques that may be used in this study.

Field sampling personnel choose the collection method and choose appropriate sampling gear to meet the study objectives pertinent for their fish collection effort. Each sampling team may determine the sampling technique that best fits the situation. Selection of the most appropriate gear for a particular target lake will be at the discretion of the experienced on-site fisheries biologists or collection personnel.

As soon as fish are obtained via active collection methods, or removed from passive collection devices, they are identified to species. Species identification should be conducted only by experienced personnel knowledgeable of the taxonomy of species in the water bodies included in the fish contaminant-monitoring program. Nontarget species, collected by the field team should be returned to the water. Field sampling personnel are instructed to wear clean nitrile gloves (provided by GLNPO if not already available to personnel) to handle fish and sample handling equipment. Individuals of the selected target species are rinsed in ambient water to remove any foreign material from the external surface) and placed in clean holding containers (live well, buckets, etc.) to prevent contamination. The buckets are cleaned according to the procedures outlined in the SOP and described in Section 9.2. Each fish of the selected target species is measured to determine total body length (mm). Maximum body length should be measured and is defined as the length from the anterior-most part of the fish to the tip of the longest caudal fin ray (when the lobes of the caudal fin are depressed dorsoventrally). Once the fish have reached shore, fish should then be wrapped in acetone-washed foil, placed in polyethylene bags, labeled according to the SOP, and immediately placed in freezer at -20°C for temporary storage.

**Table 7. Sampling Location and Method of Collection, by Lake**

Lake	Sampling Location	Collection Method
Michigan	St. Joseph River, Grand River	Dip Net/Cage Trap
	Platte River, Thompson Creek, Swan River	Dip Net/Cage Trap
	Ausable, Black Rivers	Boat Shocker
	Sturgeon Bay	Gill Net
	Saugatuck	Gill Net
	Trail Creek	Electro Fishing Unit
	Root River Weir	Nets/Seines
	Kewaunee	Nets/Seines
Superior	French River	Seine
	Apostle Islands	Gill Net
	Keweenaw Point	Gill Net
	Pikes Creek	Nets/Seines
Ontario	North Hamlin	Gill Net/Trawls
	Oswego	Gill Net
Erie	Middle Bass Island	Gill Net/Trap Net
	Dunkirk	Gill Net/Trap Net
	Trout Run (West of City of Erie)	Dip Net/Seine
Huron	Rockport	Not Available
	Port Austin	Not Available

### 8.3 Composite Sampling

Composite samples are a cost-effective means for estimating average tissue concentrations of target analytes in target species populations, and compositing ensures adequate sample mass for analysis of all target analytes. For each target species, composite samples, consisting of five fish each, will be prepared and analyzed. The composites are intended to estimate the mean fish tissue contaminant concentration for the lake for each target parameter. For Element 1 of the study, lake trout and walleye will be homogenized as whole fish with guts intact. For Element 2, skin-on fillets for Chinook and Coho salmon and rainbow trout will be homogenized. As described in Section 7, fish retained for a composite sample must meet the following criteria:



- ▶ All be of the same species,
- ▶ Satisfy any legal requirements of harvestable size (or weight), or at least be of consumable size if no legal harvest requirements are in effect,
- ▶ Be of similar size so that the smallest individual in a composite is no less than 75% of the total length of the largest individual, and
- ▶ Be collected as close to the same time and location as possible, but no more than one week apart.

Fish identified for a composite are homogenized together in the homogenization laboratory to prepare a single homogeneous fish paste suitable for chemical analysis.

## **9.0 SAMPLE HANDLING AND CUSTODY REQUIREMENTS**

### **9.1 Sample Handling**

Instructions for sample handling are provided in the *Fish Collection Standard Operating Procedure* (located in Appendix B) and in the *Packing and Shipping Instructions for the Great Lakes National Program Office's Fish Monitoring Program* located in Appendix D. Sample handling is described for Element 1 in Section 9.1.1 and Element 2 in Section 9.1.2.

#### *9.1.1 Element 1: Lake Trout and Walleye*

After fish collection, the fish are brought ashore and prepared for storage and shipment to the homogenization laboratory. All fish collected for this project are kept in their own fish box separate from other collected fish. The importance of placing the fish in a separate box in order to avoid any contamination from other fish, fuels, or other sources is emphasized to the field sampling teams. Once fish are brought ashore, they are wrapped in acetone-washed foil and immediately placed in polyethylene plastic bags cut to size (# 27-4-16, Associated Bag, Milwaukee, WI). Members of the field sampling team wear clean nitrile gloves while handling fish. Lake trout and walleye are bagged singly. All fish must be whole and without incisions. Each sample is labeled with the information listed in Table 3, and described in Section 6.0 and also in the fish collection SOP. The field sampling teams prepare two labels for each sample. One label is placed inside the bag with the fish and the second label is secured to the outside of the bag. After five fish are collected that meet the specifications for a composite as described in Section 8.3, the individually bagged fish can be combined into one liner bag (# 47-3-25C, Associated Bag, Milwaukee, WI).

Once packaged, samples should be immediately frozen for shipment or placed on ice for transport to a processing facility where fish will be immediately frozen. All fish must be kept at  $\leq -20^{\circ}\text{C}$ , and maintained frozen until they reach the designated homogenization laboratory. Collection facilities must be able to retain frozen samples for at least 4 weeks, or until the US EPA-GLNPO Project Officer has specified a shipment date. All records and reports pertaining

to sample collection should be sent to the GLFMP Project Officer, Elizabeth Murphy, as soon as collection has been completed.

### *9.1.2 Element 2: Coho and Chinook Salmon and Rainbow Trout*

Fillets are prepared for Coho and Chinook salmon and rainbow trout. In most cases, the state personnel or other samplers will prepare the fillets and in other cases the homogenization laboratory will prepare the fillets.

After collection, the fish are prepared for storage and shipment to the homogenization laboratory. All fish collected for this project are kept separate from any fish collected for other monitoring studies. Members of the field sampling team wear clean nitrile gloves while handling fish. Once fish are collected, they are wrapped in acetone-washed foil, immediately placed in polyethylene plastic bags, labeled, and either transported on ice to a processing facility, or frozen for later delivery to the processing facility. To minimize contamination, samples are not placed directly on dry ice, but are wrapped in acetone-washed foil and a plastic bag prior to setting on ice. At the processing facility, if the fish arrive on ice, they are either processed the day after collection or frozen for later processing. Fish are filleted with the skin on and filets are wrapped individually in acetone-washed foil. The fish filets are wrapped in acetone-washed foil and immediately placed singly in plastic bags. If the homogenization laboratory is doing the filleting of the fish, samples are wrapped in acetone-washed foil as whole fish and placed singly in plastic bags.

Each sample is labeled with the information listed in Table 3, and described in Section 6.0 and also in the fish collection SOP. The field sampling teams prepare two labels for each sample. One label is placed inside the bag with the fish and the second label is secured to the outside of the bag. After five fish filets are prepared that meet the specifications for a composite as described in Section 8.3, the individually wrapped fish can be combined into one liner bag (# 47-3-25C, Associated Bag, Milwaukee, WI).

Once packaged, samples should be immediately frozen for shipment. All fish must be kept at  $\leq -20^{\circ}\text{C}$ , and maintained frozen until they reach the designated homogenization laboratory. Collection facilities must be able to retain frozen samples for at least 4 weeks, or until the GLNPO Project Officer has specified a shipment date. All records and reports pertaining to sample collection should be sent to the GLFMP Project Officer, Elizabeth Murphy as soon as collection has been completed.

## 9.2 Sample Integrity

A critical requirement of the GLFMP is the maintenance of sample integrity from the time of collection to the arrival at the analytical laboratory. Sample integrity involves preventing loss of target analytes that might be present in the sample and taking precautions to avoid possible introduction of contaminants during handling. The loss of target analytes can be prevented in the field by minimizing the laceration of fish skin. Proper storage of the fish also will prevent loss of target analytes.

Special precautions must be taken by field sampling personnel to prevent contamination of the fish with any foreign materials. Sources of contamination include the sampling gear, oils and greases on boats, spilled fuel, skin contact, contact with soil or sand, boat motor exhaust, and other foreign materials. All potential sources should be identified prior to and during sample collection, and appropriate measures should be taken to minimize or eliminate them. Examples of preventative measures include the following:

- ▶ collection nets should be free of any potential contaminants,
- ▶ the use of tarred collection nets is prohibited,
- ▶ boats should be positioned so that engine exhaust does not fall on the deck area where samples are being handled,
- ▶ ice chests and other sample storage containers should be scrubbed clean with detergent and rinsed with distilled water prior to use (containers originating from the sample control center will be prewashed and rinsed), and
- ▶ samples should not be placed directly on dry ice, but should be stored inside acetone-washed foil, and plastic bags first.

## 9.3 Custody Requirements

As soon as possible following collection, the field sampling teams begin the process of identifying, labeling, packaging, and storing the samples. Each sample will be identified and tracked with labeling information listed in Table 3 and described in Section 6.0 and in the Field Collection SOP. Fish are then shipped on dry ice to the homogenization laboratory.

Field sampling personnel are required to submit to GLNPO a field recording form and chain of custody record (located in Appendix C) at the time that fish are shipped to the homogenization laboratory. This form is used to document shipment and handling of all fish from the field personnel to the sample homogenization laboratory. The form requires signatures of field personnel shipping the fish and signatures for the personnel at the homogenization laboratory receiving the fish. Field sampling teams are provided with *Packing and Shipping Instructions for the Great Lakes National Program Office's Fish Monitoring Program* (located in Appendix D) that provides information on filling out the required forms.

When fish are shipped to the homogenization laboratory, GLNPO or GLNPO contractors notify the lab of the shipment, and any tracking numbers associated with the shipment are provided.

The laboratory is instructed to confirm receipt of the shipment in writing. The laboratory also notes on this confirmation if the fish arrived frozen and in good condition.

Custody requirements for shipment of fish samples from the homogenization to the analytical laboratory are discussed in *The Great Lakes Fish Monitoring Program Quality Management Plan*.

## **10.0 ANALYTICAL METHODS REQUIREMENTS**

After processing at the homogenization laboratory, fish homogenate samples are shipped to the Principal Investigator (PI), Deb Swackhamer of the University of Minnesota, who provides analytical support. Sample processing and analytical testing are discussed in *The Great Lakes Fish Monitoring Program Quality Management Plan* and in *Trends in Great Lakes Fish Contaminants Quality Assurance Project Plan*.

## **11.0 QUALITY CONTROL REQUIREMENTS**

Data quality is addressed by use of knowledgeable field sampling teams as described in Sections 1.15 and 4.2, use of SOPs, and consistent performance of the procedures documented in the SOPs. The SOPs include instructions for preventing loss of target analytes from the fish and preventing contamination of the fish from sampling equipment or other sources. The GLNPO PO annually reviews the study requirements, sample collection procedures, and documentation with all field personnel and verifies that they do not have any questions.

The field sampling team leader is responsible for ensuring all sampling equipment is in good working condition and is used properly by the field sampling personnel. The field sampling team leader is responsible for reviewing all required documentation discussed in Section 6. After review, the leader signs the field recording form (located in Appendix C) indicating their review and approval of the documentation.

Fish lengths are recorded to the nearest mm (or sixteenth of an inch) by the field personnel. The fish length also is measured in the homogenization laboratory as a QC check for the field length. In the field, fish are weighed to the nearest gram. Latitude and longitude coordinates are recorded for each sampling location using Global-positioning systems (GPS).

The field personnel identify the species of each fish during collection. The accuracy of the identifications is assured through use of experienced field personnel. The homogenization laboratory is instructed to notify the GLNPO PO if any fish targeted for a single composite not appear to be the same species.

Coded wire tags and fin clips found in any fish specimens are removed by the homogenization laboratory and shipped with the homogenized samples to the PI. Fin clips and wires removed from any of the fish samples will be reviewed against the field documentation. The GLNPO PO will resolve any discrepancies with the field sampling team.

The GLNPO PM also reviews all field documentation for discrepancies or anomalies and contacts the field sampling team leader to resolve any issues.

## **12.0 INSTRUMENT/EQUIPMENT TESTING, INSPECTION, AND MAINTENANCE REQUIREMENTS**

All field equipment is inspected by the field sampling team leader prior to each sampling event to ensure that all equipment is in good working condition. For example, the team leader must verify that boats or electro fishers are operating correctly and that the sample nets do not have any defects. Additional maintenance includes ensuring that:

- ▶ collection nets are free of any potential contaminants,
- ▶ collection nets are not tarred, and
- ▶ ice chests and other sample storage containers should be scrubbed clean with detergent and rinsed with distilled water prior to use (containers originating from the sample control center will be prewashed and rinsed).

## **13.0 INSTRUMENT CALIBRATION AND FREQUENCY**

No instruments need to be calibrated for this project.

## **14.0 INSPECTION/ACCEPTANCE REQUIREMENTS FOR SUPPLIES AND CONSUMABLES**

Careful and thorough planning is necessary to ensure the efficient and effective completion of the field sample collection task. It is the responsibility of each field sampling team to gather and inspect the necessary sampling gear prior to the sampling event and to inspect the sample packaging and shipping supplies received from the laboratory. The field record form includes verification that sampling equipment and supplies were inspected and found to be suitable for use. Additional inspections include verification that:

- ▶ collection nets are free of any potential contaminants,
- ▶ collection nets are not tarred, and
- ▶ ice chests and other sample storage containers should be scrubbed clean with detergent and rinsed with distilled water prior to use (containers originating from the sample control center will be prewashed and rinsed).

## **15.0 DATA ACQUISITION REQUIREMENTS (NONDIRECT MEASUREMENTS)**

Nondirect measurements are not used in the sample collection component of this study.

## **16.0 DATA MANAGEMENT**

Samples will be labeled and tracked via Sample Identification Labels detailed in Table 3 and Section 6.0. Because the sampling effort is a cooperative one involving many different partner agencies and groups, the diligence of the field sampling teams in completing the proper records is essential. Field team leaders will be responsible for reviewing all completed documentation as described in Section 6. Any corrections should be noted, initialed, and dated by the reviewer. Shipment of samples to the sample preparation laboratory (Section 9.1) must be conducted by a delivery service that provides constant tracking of shipments (e.g., Federal Express). The data collected in the sampling component of this project are documented on field recording forms and generated by the homogenization laboratory. All records and reports pertaining to sample collection are sent to the GLFMP Project Officer, Elizabeth Murphy as soon as collection has been completed. All field records and electronic correspondence are retained by the GLNPO PO for at least five years. The records and other study reports and documentation are retained by the GLNPO PO or GLNPO's document control coordinator as appropriate. Laboratory sample receipt and data management procedures are discussed in *The Great Lakes Fish Monitoring Program Quality Management Plan* and in *Trends in Great Lakes Fish Contaminants Quality Assurance Project Plan*.

## **17.0 ASSESSMENT AND RESPONSE ACTIONS**

Due to the voluntary participation of the states in fish collection, the assessment and response actions for this study are limited. The GLNPO PO will serve as the primary contact for any questions or issues that arise from the field sampling teams. The GLNPO PO will contact GLNPO scientists or the PI if needed to provide recommendations to field personnel.

If during review of the field documentation or through communication with the samplers the GLNPO PO identifies a discrepancy, the sampler will be contacted to resolve the issue. If an error in the sample collection process is identified that will significantly impact the results of the study, the field sampling team will, if possible, obtain additional samples.

Coded wire tags and fin clips found in any fish specimens are removed by the homogenization laboratory and shipped with the homogenized samples to the PI. Fin clips and wires removed from any of the fish samples will be reviewed against the field documentation. The GLNPO PO will resolve any discrepancies with the field sampling team.

The PI will be notified of any issues that arose with the sampling teams. The PI also will be notified of any errors in the field documentation or discrepancies identified during review of the fin clips against the documentation. Ideally, sample collection anomalies will be discussed in reports developed by the PI.

## **18.0 REPORTS TO MANAGEMENT**

The GLNPO PO provides project status to management at regularly scheduled meetings or at least once per month.

## **19.0 DATA REVIEW, VALIDATION, AND VERIFICATION REQUIREMENTS**

Field team leaders will be responsible for reviewing all completed documentation as described in Section 6. Any corrections should be noted, initialed, and dated by the reviewer.

The GLNPO PO reviews all field documentation for discrepancies or anomalies and contacts the field sampling team leader to resolve any issues. This includes all data recorded on the field recording form and chain-of-custody record that can be verified by the PO including sample locations, fish lengths and weights, and fish species. Coded wire tags and fin clips found in any fish specimens are removed by the homogenization laboratory and shipped with the homogenized samples to the PI. Fin clips and wires removed from any of the fish samples will be reviewed against the field documentation. The GLNPO PO will resolve any discrepancies with the field sampling team.

Data review regarding the sample homogenization and shipment to the analytical laboratory is discussed in *The Great Lakes Fish Monitoring Program Quality Management Plan*.

## **20.0 VALIDATION AND VERIFICATION METHODS**

The GLNPO PO will employ data verification and validation methods to ensure the data documented for the field effort is complete and correct to the best of her knowledge. She will perform point-by-point comparisons for all field documentation against documentation submitted by the homogenization laboratory and against her expertise regarding fish populations in the Great Lakes.

Coded wire tags and fin clips removed from any of the fish samples will be reviewed point-by-point against the field documentation. The GLNPO PO will resolve any discrepancies with the field sampling team.

The GLNPO PO also will perform a 100% review of all port codes and grid numbers and verify latitudes and longitudes reported in the field documentation against the location information provided in Table 6.

## **21.0 RECONCILIATION WITH DATA QUALITY OBJECTIVES**

Project data quality objectives are discussed in *The Great Lakes Fish Monitoring Program Quality Management Plan*.

## 22.0 LITERATURE CITED

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## **Appendix A**

# **Standard Operating Procedure for Fish Collection for the Great Lakes Fish Monitoring Program**



# **Standard Operating Procedure for Fish Collection For the Great Lakes Fish Monitoring Program**

## **1.0 Scope and Application**

These standard operating procedures are used to collect fish in support of the Great Lakes Fish Monitoring Program administered by the US EPA Great Lakes National Program Office. This SOP is accompanied by the *Great Lakes Fish Monitoring Program Quality Assurance Project Plan (QAPP) for Sample Collection Activities*. For each field sampling team a team leader is identified as the primary contact for study implementation and communication with GLNPO. The number and type of fish targeted for collection will be communicated to the field sampling team leader each year. Each field sampling team must have the required permit to collect the required amount of fish.

Adherence to this SOP will ensure that field sampling activities will be performed the same way every time and that they are standardized for all sampling participants.

## **2.0 Summary of Procedures**

Lake trout in Lakes Michigan, Superior, Ontario, and Huron and walleye in Lake Erie are collected at pre-specified sampling sites. Similarly, Coho salmon and Chinook salmon in Lakes Michigan, Superior, Ontario, and Huron and rainbow trout in Lake Erie are collected at pre-specified sampling sites. Field personnel are responsible for determining the appropriate sampling techniques for each particular sampling site that may include use of gill nets, cage traps, or seines. Fish are collected in specific size ranges for preparation of sample composites. For each target species, composite samples, consisting of five fish each, are prepared and sent to the homogenization laboratory for processing. For lake trout and walleye, composites are prepared from whole fish and for Coho salmon, Chinook salmon, and rainbow trout, composites are prepared from skin-on filets. Special precautions are taken to prevent contamination of the fish with any foreign materials and ensure the integrity of the sample. Fish are immediately wrapped in foil and placed in plastic bags and labeled. Fish are frozen as soon as possible after collection and maintained at -20°C. Fish are packaged in groups of five fish intended for a single composite according to specific criteria. Fish are shipped frozen to the sample homogenization laboratory. Data regarding each individual fish and associated sample location are recorded on standard forms and sent with the fish to the laboratory. If any questions or concerns arise regarding the collection, storage, or shipping procedures contact Beth Murphy at (312) 353-4227.

### **3.0 Sampling Equipment**

Fish collection methods can be divided into two major categories, active and passive. Each has advantages and disadvantages. Active collection methods involve a wide variety of sampling devices including electro fishing units, seines, trawls, and boat shocker. Passive collection methods employ a wide array of sampling devices, including gill nets, dip nets, trap net and cage trap. Passive collection devices (e.g., gill nets) must be checked frequently (e.g., at least once every 24-hours) to ensure a limited time lag between fish entrapment and sample preparation.

Careful and thorough planning is necessary to ensure the efficient and effective completion of the field sample collection task. It is the responsibility of each field sampling team to gather and inspect the necessary sampling gear prior to the sampling event and to inspect the sample packaging and shipping supplies received from the laboratory. The field recording form (attached to this SOP) includes verification that sampling equipment and supplies were inspected and found to be suitable for use. Additional inspections include verification that:

- ▶ collection nets are free of any potential contaminants,
- ▶ collection nets are not tarred, and
- ▶ ice chests and other sample storage containers should be scrubbed clean with detergent and rinsed with distilled water prior to use (containers provided by GLNPO will be pre-washed and rinsed).

### **4.0 Fish Collection**

Field sampling personnel choose the collection method and choose appropriate sampling gear to meet the study objectives pertinent for their fish collection effort. Each sampling team may determine the sampling technique that best fits the situation. Selection of the most appropriate gear for a particular target lake will be at the discretion of the experienced on-site fisheries biologists or collection personnel.

As soon as fish are obtained via active collection methods, or removed from passive collection devices, they are identified to species. Species identification should be conducted only by experienced personnel knowledgeable of the taxonomy of species in the water bodies included in the fish contaminant-monitoring program. Nontarget species, collected by the field team should be returned to the water. Field sampling personnel should wear clean nitrile gloves (provided by GLNPO if not already available to personnel) to handle fish and sample handling equipment. Individuals of the selected target species are rinsed in ambient water to remove any foreign material from the external surface) and placed in clean holding containers (live well, buckets, etc.) to prevent contamination. The buckets are cleaned according to the procedures outlined in Section 3 above and described in Section 9.2 of the QAPP. Each fish of the selected target species is measured to determine total body length (mm). Maximum body length should be measured and is defined as the length from the anterior-most part of the fish to the tip of the longest caudal fin ray (when the lobes of the caudal fin are depressed dorsoventrally).

## 5.0 Species, Size, and Number of Fish

Collect 50 lake trout and walleye in the size range specified in Table 1 at each location cited in Section 6.0, Table 2 (unless otherwise specified by GLNPO). Collect 15 Coho salmon, Chinook salmon, and rainbow trout in the size range specified in Table 1 at each location cited in Section 6.0, Table 2 (unless otherwise specified by GLNPO). If sufficient numbers of fish within the designated size range cannot be obtained by a reasonable sampling effort, it is acceptable to go outside the size range, approximately 5% above or below. However, try to include similar numbers of fish above and below the designated size range so that the mean size of fish remains near the middle of the range.

**Table 1. Species Collected by Lake and Even or Odd Year**

Lake	Lake Trout	Walleye	Chinook	Coho	Rainbow Trout
Lake Michigan	E,O	—	O	E	—
Lake Superior	E,O	—	O	E	—
Lake Erie	—	E,O	—	—	E,O
Lake Huron	E,O	—	O	E	—
Lake Ontario	E,O	—	O	E	—
Size Range (mm)	600 to 700	450 to 550	small, medium, and large	small, medium, and large	600 to 700

E = even year collection

O = odd year collection

Sampling teams group samples into composites of five fish each. Fish are placed singly in bags and the five fish grouped for a composite are placed in a larger bag. The five fish that make up a specific composite must meet the following criteria:

- ▶ All be of the same species,
- ▶ Satisfy any legal requirements of harvestable size (or weight), or at least be of consumable size if no legal harvest requirements are in effect,
- ▶ Be of similar size so that the smallest individual in a composite is no less than 75% of the total length of the largest individual, and
- ▶ Be collected as close to the same time and location as possible, but no more than one week apart.

Fish identified for a composite are homogenized together in the homogenization laboratory to prepare a single homogeneous fish paste suitable for chemical analysis.

## 6.0 Sampling Sites

Grid locations for each station are listed in Table 2. If the specified grid does not contain good fishing grounds or if collection from that grid will cause conflicts with management practices (e.g., excessive impact on native fish versus hatchery produced fish) it is appropriate to collect from grids immediately adjacent to the designated grid. The guiding rule should be that the site sampled represents offshore fishing grounds (i.e., open water populations of fish) and is relatively remote from tributaries or other potential sources of contaminants.

**Table 2. Sampling Locations for the Great Lakes Fish Monitoring Program**

Year	Lake/State	Port Name	Species	Port Code	Grid Number	Latitude/Longitude
Odd-numbered	Lake Superior	Keewanaw Point	Lake Trout	NA	1028	87°35'W/47°25'N
	Lake Michigan	Sturgeon Bay	Lake Trout	NA	0906	87°05'W/44°45'N
	Lake Michigan	Charlevoix (as back-up only)	Lake Trout	NA	0516	85°25'W/45°25'N
	Lake Huron	Port Austin	Lake Trout	NA	1413	82°45'W/44°05'N
	Lake Erie	Dunkirk	Walleye	NA	0424	79°35'W/42°25'N
	Lake Ontario	North Hamlin	Lake Trout	NA	0713	77°55'W/43°25'N
	New York	Salmon River	Chinook	0601	NA	43°32'N/75°59'W
	Pennsylvania	Trout Run	Steelhead Trout	0527	NA	42°3.54'N/80°16.38'W
	Michigan	St. Joseph's River	Chinook	0201	NA	41°56.63'N/86°19.7'W
		Grand River	Chinook	0225	NA	42°56'N/84°54'W
		Platte River	Chinook	0213	NA	44°43.18'N/86°8.21'W
		Thompson Creek	Chinook	0251	NA	41°65'N/86°36'W
		Swan River	Chinook	0300	NA	45°23'N/83°44'W
		Ausable River	Chinook	311A	NA	44°24.93'N/83°19.84'W
	Indiana	Trail Creek	Chinook	0227	NA	41°43'N/86°53'W
	Wisconsin	Root River	Chinook	0237	NA	42°43.955'N/87°46.72'W
		Kewaunee River	Chinook	0247	NA	44°27.533'N/87°33.383'W
		Lake Superior, Pikes Creek	Chinook	0139	NA	46°47.154'N/90°51.953'W
	Minnesota	French River	Chinook	0143	NA	46°52'N/91°54'W
		Knife River	Coho	0145	NA	46°45'N/91°45'W
Ohio	Grand River	Steelhead Trout	0523	NA	41°45'N/81°16'W	
Even-numbered	Lake Superior	Apostle Islands	Lake Trout	NA	1311	90°25'W/46°55'N
	Lake Michigan	Saugatuck	Lake Trout	NA	2210	86°25'W/42°35'N
	Lake Huron	Rockport	Lake Trout	NA	0710	83°1'W/45°15'N



Year	Lake/State	Port Name	Species	Port Code	Grid Number	Latitude/Longitude
Even-numbered	Lake Erie	Middle Bass Island	Walleye	NA	0904	82°55'W/41°35'N
	Lake Ontario	Oswego	Lake Trout	NA	0623	76°15'W/43°35'N
	New York	Salmon River	Coho	0601	NA	43°32'N/75°59'W
	Pennsylvania	Trout Run	Coho	0527	NA	42°3.54'N/80°16.38'W
	Michigan	St. Joseph's River	Coho	0201	NA	41°56.63'N/86°19.7'W
		Grand River	Coho	0225	NA	42°56'N/84°54'W
		Platte River	Coho	0213	NA	44°43.18'N/86°8.21'W
		Thompson Creek	Coho	0251	NA	41°65'N/86°36'W
		Swan River	Coho	0300	NA	45°23'N/83°44'W
		Ausable River	Coho	311A	NA	44°24.93'N/83°19.84'W
	Indiana	Trail Creek	Coho	0227	NA	41°43'N/86°53'W
	Wisconsin	Root River	Coho	0237	NA	42°43.955'N/87°46.72'W
		Kewaunee River	Coho	0247	NA	44°27.533'N/87°33.383'W
		Lake Superior, Pike Creek	Coho	0139	NA	46°47.154'N/90°51.953'W
	Minnesota	French River	Coho	0143	NA	46°52'N/91°54'W
Ohio	Grand River	Steelhead Trout	0523	NA	41°45'N/81°16'W	

## 7.0 Sample Integrity and Quality Control

Sample integrity involves preventing loss of target analytes that might be present in the sample and taking precautions to avoid possible introduction of contaminants during handling. The loss of target analytes can be prevented in the field by minimizing the laceration of fish skin. Proper storage of the fish also will prevent loss of target analytes. All fish collected for this project are kept in their own fish box separate from other collected fish. The importance of placing the fish in a separate box in order to avoid any contamination from other fish, fuels, or other sources cannot be over-emphasized. Special precautions must be taken by field sampling personnel to prevent contamination of the fish with any foreign materials.

Sources of contamination include the sampling gear, oils and greases on boats, spilled fuel, skin contact, contact with soil or sand, boat motor exhaust, and other foreign materials. All potential sources should be identified prior to and during sample collection, and appropriate measures should be taken to minimize or eliminate them. Examples of preventative measures include the following:

- ▶ collection nets should be free of any potential contaminants,
- ▶ the use of tarred collection nets is prohibited,
- ▶ boats should be positioned so that engine exhaust does not fall on the deck area where samples are being handled,
- ▶ ice chests and other sample storage containers should be scrubbed clean with detergent and rinsed with distilled water prior to use (containers originating from the sample control center will be pre-washed and rinsed), and
- ▶ samples should not be placed directly on dry ice, but should be stored inside acetone-washed foil and plastic bags first.

The field sampling team leader is responsible for ensuring all sampling equipment is in good working condition and is used properly by field personnel.

## 8.0 Documentation and Records

Field sampling personnel are required to complete and submit a field recording form and chain of custody record (Appendix C) at the time that fish are shipped to the homogenization laboratory. Because the sampling effort is a cooperative one involving many different partner agencies and groups, the diligence of the field sampling team in completing the proper records is essential. The field recording form is used to document the sample collection effort and includes specific information regarding each fish specimen such as length, weight, and species. The form also is used to document shipment and handling of all fish from the field personnel to the sample homogenization laboratory. Field sampling teams are provided with *Packing and Shipping Instructions for the Great Lakes National Program Office's Fish Monitoring Program* (located in Appendix D) that provides information on filling out the required forms.

The field record form is designed to capture a unique tracking number for each fish composite. Specific instructions for assigning these Composite IDs are included on the form. This tracking number is used by GLNPO and the homogenization and analytical laboratories to identify each composite and report results. The tracking number or composite ID includes the following:

- ▶ A two-character code for each lake (e.g., LO for Lake Ontario, LM for Lake Michigan, etc.),
- ▶ The fish species code,
- ▶ The four digit year of collection,
- ▶ The grid number of port code, and
- ▶ A sequential number indicating the number of each composite from a specific location and year (e.g., 001, 002, etc.).

Field team leaders are responsible for reviewing all completed documentation and signing the *Field Recording Form and Chain-of-custody Record*.

## 9.0 Sample Handling, Storage, and Shipping

All fish collected for the GLNPO study should be kept separate from other collected fish in their own fish box. It is very important that all fish collected be placed into the box as soon as possible after they are harvested so as to avoid any contamination from other fish, any oils or fuel, or other sources. Field sampling personnel should wear clean nitrile gloves (provided by GLNPO if not already available to personnel) to handle fish and sample handling equipment. Specific steps for fish collection are provided below followed by sample handling and shipping instructions specific to whole fish (lake trout and walleye) and fillets (Coho and Chinook salmon and rainbow trout).

- 9.1 As soon as fish have been obtained via active or passive collection methods, rinse fish in ambient water to remove any foreign material from the external surface and place fish in holding containers (e.g., buckets, livewells) cleaned according to specific procedures in Section 3. Nontarget fishes or small specimens are returned to water.
- 9.2 Accurate taxonomic identification is essential in assuring and defining the organisms that have been composited and submitted for analysis. Under no circumstances should individual fish of different species be used in a single composite.
- 9.3 The fish should then be brought ashore and the steps described below should be taken to prepare the fish for storage and shipping to the homogenization lab for processing. GLNPO will arrange for shipping of fish to a lab for analysis. Follow instructions described in *Packing and Shipping Instructions for the Great Lake's National Program Office's Fish Monitoring Program* for shipping fish to the location specified by GLNPO. As described in these instructions, the field team must include all field and sample handling documentation including the *Field Recording Form and Chain-of-custody Record* (provided by GLNPO) with the fish shipment. Collection bags and labels will be provided to collectors by GLNPO.
- 9.4 Measure each fish to determine total body length. Measure total length of each specimen in millimeters or inches (to the nearest millimeter or sixteenth of an inch) from the anterior-most part of the fish to the tip of the longest caudal fin ray (when the lobes of the caudal fin are depressed dorsoventrally).
- 9.5 Weigh each fish to the nearest gram.
- 9.6 Prepare a sample label (provided by GLNPO) for each fish. If not using labels provided by GLNPO, prepare a sample label for each fish that includes all information listed in Table 3. Fill out *Field Recording Form and Chain-of-custody Record* or fill out forms at completion of sample handling. Provide hard copy and electronic copy to Project Officer.

- 9.7 Whole Fish (lake trout and walleye): To minimize contamination, samples are not placed directly on dry ice, but are wrapped in acetone-washed foil and placed in a plastic bag prior to setting on ice. Once fish are brought ashore, or as soon as they are collected, wrap each fish separately in acetone-washed foil and immediately place in polyethylene plastic tubes (# 27-4-16, Associated Bag, Milwaukee, WI) or polyethylene plastic bags. If using the plastic tubing, cut the plastic tube to the length of the fish and fasten ends of tube with provided cable ties. All fish must be whole and without incisions. Attach the sample label prepared in section 9.6 to one of the cable ties fastened to the end of the tubing or to the polyethylene bag. After five fish are collected that meet the specifications for a composite as described in Section 5, combine the individually bagged fish into one liner bag (# 47-3-25C, Associated Bag, Milwaukee, WI) or large polyethylene bag. Once packaged, samples should be immediately frozen for shipment or placed on ice for transport to a processing facility where fish will be immediately frozen. All fish must be kept at  $\leq -20^{\circ}\text{C}$ , and maintained frozen until they reach the designated homogenization laboratory. Collection facilities must be able to retain frozen samples for at least 4 weeks, or until the GLNPO Project Officer has specified a shipment date.
- 9.8 Fish filets (Coho salmon, Chinook salmon, and rainbow trout): To minimize contamination, samples are not placed directly on dry ice, but are wrapped in acetone-washed foil and placed in a plastic bag prior to setting on ice. Once fish are brought ashore or as soon as they are collected, wrap each fish separately in acetone-washed foil and immediately place in polyethylene plastic tubes (# 27-4-16, Associated Bag, Milwaukee, WI) or polyethylene plastic bags. If using the plastic tubing, cut the plastic tube to the length of the fish and fasten ends of tube with provided cable ties. Attach sample label prepared in section 9.6 to one of the cable ties fastened to the end of the tubing or to the polyethylene bag. After five fish are collected that meet the specifications for a composite as described in Section 5, the individually bagged fish can be combined into one liner bag (# 47-3-25C, Associated Bag, Milwaukee, WI) or large polyethylene bag. For salmon and rainbow trout, once the fish have reached shore or as soon as they are collected, they are wrapped in acetone-washed foil, immediately placed in polyethylene plastic bags, labeled, and either transported on ice to a processing facility, or frozen for later delivery to the processing facility. At the processing facility, if the fish arrive on ice, they are either processed the day after collection or frozen for later processing. Fish are filleted with the skin on. Wrap each fish filet in acetone-washed foil and immediately place singly in plastic bags. Attach the sample label prepared in section 9.6 to the polyethylene bag. If the homogenization laboratory is doing the filleting of the fish, samples are wrapped in acetone-washed foil as whole fish and placed singly in plastic tubing with cable ties fastened to each end or in polyethylene plastic bags. Attach the sample label prepared in section 9.6 to one of the cable ties fastened to the end of the tubing or to the polyethylene bag. After five fish are collected that meet the specifications for a composite as described in Section 5, the individually bagged fish can be combined into one liner bag (# 47-3-25C, Associated Bag, Milwaukee, WI) or large polyethylene bag.

9.9 Each bag must be accurately labeled with the information in Table 3.

**Table 3. Types of Field Data to be Collected & Recorded on Sample Labels**

Data Type	Measurement Units or Allowed Entries
Lake Name	Erie, Michigan, Huron, Ontario, Superior
Grid Number or Port Code of Sample Collection	See Section 7.4 and Appendix E for a list of grid numbers and port codes
Latitude and Longitude of Site	Degrees, minutes, seconds
Fish Species	Taxonomic identification
Fish Length	Millimeters (mm), total length
Fish Sex (if available)	Not applicable
Water Depth at Collection Site	Meters
Fish Weight	Grams
Method of Collection	Dip Net, Cage Trap, Boat Shocker, Nets, Seines, Gill Net, Trawl, Electro Fishing Unit
Date of Collection	Month/Day/4-digit year
Collector Identification	Vessel and collector's name
Fin Clip(s)	If none, record none

9.10 Follow instructions described in *Packing and Shipping Instructions for the Great Lake's National Program Office's Fish Monitoring Program* for shipping fish to the location specified by GLNPO. The field team must include all field and sample handling documentation including the *Field Recording Form and Chain-of-custody Record* with the fish shipment. The field sampling team leader signs and dates the forms.



## **Appendix B**

# **Standard Operating Procedure for Fish Homogenization for The Great Lakes Fish Monitoring Program**





# **Standard Operating Procedures for Homogenization of Fish Samples for Contaminant Analysis for the Great Lakes Fish Monitoring Program**

## **1.0 Scope and Application**

These standard operating procedures are used to prepare fish tissue homogenates in support of the Great Lakes Fish Monitoring Program administered by the US EPA Great Lakes National Program Office. This SOP is accompanied by the *Great Lakes Fish Monitoring Program Quality Assurance Project Plan (QAPP) for Sample Collection Activities*. When modifications of this procedure are necessary for specific studies, they will be explained in detail in the associated study plans.

## **2.0 Summary of Method**

Fish arrive frozen at the homogenization laboratory. Fish are slowly thawed. Fin clips are removed and maintained and all information recorded. Fish tissue is homogenized together to create sample composites consisting of five fish each. Fish to be included in a given composite arrive at the facility packaged together and labeled with the composite ID. Each individual fish comprising a composite sample is measured, weighed, sexed, and aged. A homogenizer appropriate for the total mass of the composite is chosen. All homogenization equipment and storage jars are pre-cleaned according to specific procedures to prevent contamination of the sample. Fish are ground until a smooth, creamy paste is obtained and placed into sample jars. Jars are placed in a freezer (approximately -20°C) until shipped frozen to the analytical laboratory.

## **3.0 Sample Receipt**

Upon receipt of samples, the following steps must be taken to ensure accuracy of samples:

- ▶ Inspect shipping container for damage and verify that samples have arrived frozen and in good condition.
- ▶ Verify that all paperwork is in tack and legible.
- ▶ Ensure that all samples were properly labeled and packaged.
- ▶ Sign and distribute chain of custody document.
- ▶ Enter data from label into lab notebook.
- ▶ Unwrap and inspect fish for damage.

Fish to be included in a given composite arrive at the facility packaged together and labeled with the composite ID.

## 4.0 Interferences

Glass jars (4-oz and 16-oz) are first washed in a dishwasher and rinsed with Reverse Osmosis water. They are rinsed (in sequence) with 1 N HNO<sub>3</sub>, Reverse Osmosis water, acetone, and again with Reverse Osmosis water. The jars are allowed to dry completely. Both the 4-oz and the 16-oz lids to be used for organics analysis should be lined with aluminum acetone-washed foil (acetone rinsed) before lids are applied.

Cross contamination of samples is a potential problem during fish grinding and processing. All equipment used for processing a sample group is cleaned thoroughly before it is used to process the next sample group. Measuring boards, balances, scalpels, spatulas, and all other pieces of equipment coming into contact with the sample are washed with detergent and then rinsed with acetone (methanol for plastic pieces), nitric acid, and Reverse Osmosis water. The same rigorous cleaning procedure also applies to the larger pieces of equipment such as food processors and the Hobart choppers and mixers.

## 5.0 Safety

Procedures outlined in this method involve the use of powerful high-speed equipment and high strength acids. Details of safe operational practices are too elaborate to be covered by this document. No person(s) are allowed to work unsupervised in the fish grinding and processing operation until they are approved to do so by the regular staff working in the fish processing facility. Each chemical compound used in this procedure should be treated as a potential health hazard. A reference file of material safety data sheets and regulations regarding the safe handling of all chemicals must be available to all personnel involved in the fish processing operation.

## 6.0 Apparatus

- ▶ Virtis Super 30 homogenizer, 1/5 horsepower, variable speed. Virtis Inc.
- ▶ Stephan Machinery Vertical Choppers, models VCM-12 and VCM-40. Stephan Machinery Inc, Columbus, Ohio.
- ▶ Robot Coupe Homogenizer, Robot Coupe Inc.
- ▶ Aluminum acetone-washed foil
- ▶ 4-oz glass jars. Porter Bottle Co.
- ▶ 16-oz glass jars. Porter Bottle Co.
- ▶ Acetone
- ▶ Nitric Acid
- ▶ Reverse Osmosis Water
- ▶ Balances (at least 0.1 g accuracy)

## **7.0 Sample Storage**

Whole frozen fish are stored in freezers (intact, with all body fluids and no incision) in tagged (external and internal) polyethylene bags at -20°C. The tags include at least: species, date of collection, and location of collection.

### **7.1 Preparations**

Prior to homogenization, while the fish are still frozen, coded wire tags are removed from any fish found to have an 'AD' fin clip.

At the end of the day prior to homogenization, the fish are removed from the freezer and allowed to thaw slowly over approximately a 12-hour period in their sealed bags. Smaller species are allowed to thaw in a refrigerated area while larger species are placed on a metal table in the homogenization room and allowed to thaw at room temperature.

Samples should not be rinsed with distilled water prior to homogenization. Samples are cleaned in field.

### **7.2 The Process**

All hardware is washed and solvent rinsed as described in Section 3.

Composites are made up of five whole fish for lake trout and walleye and five skin-on fillets for salmon (Chinook and Coho) and rainbow trout (one fillets per fish for a total of 5 fillets per composite). Groups of five fish should have been prepared at the collection site and bagged together, Section 9 of Field QAPP. In the situation where 50 fish are not collected or received for a given lake, composites will continue to be made as stated above. Left over fish can be disposed of.

Each individual fish comprising the composite sample is measure, weighed (including any fluids contained in the bag) and is sexed by opening the body cavity to examine the reproductive organs. Fillets should also be weighed and measured. When appropriate, fin clips are also recorded. All values are recorded in a laboratory data book.

Scales are collected for age analysis by carefully removing scales from the area between the dorsal fin and the lateral line.

A homogenizer appropriate for the total mass of tissue is chosen (generally a 40-quart homogenizer) and the fish or fillets are placed in the homogenizer. All parts of the fish including any fluids that have collected in the polyethylene bag are placed in the homogenizer.

The sample group is then homogenized as whole fish for lake trout and walleye and as skin-on fillets for salmon (Chinook and Coho) and rainbow trout. The fish are ground until a smooth,

creamy consistency is obtained. Precleaned spatulas (Section 3) are used to stir the homogenate, if needed.

## **8.0 Subsampling and Storage of Ground Fish**

For the Great Lakes Monitoring Study, 80 grams of the homogenate are weighed into each of three labeled 4-oz jars, providing there is sufficient sample. One of the 4-oz jars should be capped with a non-PTFE lid for future PFOS analysis. From each sample or composite at a sampling site, 30 grams are put into each of two 16-oz jars labeled as the working and the historical archive samples. These archives are subsamples of the sampling site and are usually used for investigative monitoring for new contaminants.

The jars are then placed in the freezer (approximately -20°C) to be kept for future use.

## **9.0 Shipping and Archiving**

After homogenization is complete, two of the 4-oz jars (one jar with Teflon lid and one jar without Teflon lid) should be shipped to the EPA specified analysis laboratory. The remaining sample jars are to be archived at -20°C until further notice.

## **Appendix C**

# **Field Recording Form and Chain-of-custody Record**

# Great Lakes Fish Monitoring Program

Page \_\_\_\_\_ of \_\_\_\_\_

Fish Collector Name:
Affiliation:
Address/Phone:
Contact Name/Phone:

## FIELD RECORDING FORM & CHAIN-OF-CUSTODY RECORD

**Site/Sample Location Information** (Enter the appropriate information for each site/sample location visited.)

Site/Sample Location 1	
Lake sampled or lake associated with waterbody sampled:	
Sample Location (Grid Number or Port Code):	
Latitude & Longitude (decimal degrees):	
Estimated Water Depth (m):	
Fish Species:	

Site/Sample Location 2	
Lake sampled or lake associated with waterbody sampled:	
Sample Location (Grid Number or Port Code):	
Latitude & Longitude (decimal degrees):	
Estimated Water Depth (m):	
Fish Species:	

Site/Sample Location 3	
Lake sampled or lake associated with waterbody sampled:	
Sample Location (Grid Number or Port Code):	
Latitude & Longitude (decimal degrees):	
Estimated Water Depth (m):	
Fish Species:	

Site/Sample Location 4	
Lake sampled or lake associated with waterbody sampled:	
Sample Location (Grid Number or Port Code):	
Latitude & Longitude (decimal degrees):	
Estimated Water Depth (m):	
Fish Species:	

Site/Sample Location 5	
Lake sampled or lake associated with waterbody sampled:	
Sample Location (Grid Number or Port Code):	
Latitude & Longitude (decimal degrees):	
Estimated Water Depth (m):	
Fish Species:	

Site/Sample Location 6	
Lake sampled or lake associated with waterbody sampled:	
Sample Location (Grid Number or Port Code):	
Latitude & Longitude (decimal degrees):	
Estimated Water Depth (m):	
Fish Species:	

Sampled by: <i>(signature)</i>	Date/Time:	Shipped by: <i>(signature)</i>	Date/Time:
Received by: <i>(signature)</i>	Date/Time:		

Please see *Packing and Shipping Instructions for the Great Lakes Fish Monitoring Program* for further information on filling out and distributing this form.

# Great Lakes Fish Monitoring Program

## Fish Sample Collection and Description Information

Page \_\_\_\_\_ of \_\_\_\_\_

Composite ID* (see below for an example)	Fish Number	Date of Collection (Month/Day/Year)	Method of Collection	Estimated Collection Depth (m)	Sample ID	Field Length (mm)	Field Weight (g)	Sex (M=male, F=female)	Fin Clips If none, record none.
	1								
	2								
	3								
	4								
	5								
	1								
	2								
	3								
	4								
	5								
	1								
	2								
	3								
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	1								
	2								
	3								
	4								
	5								

\*For a given composite, minimum individual size should be no less than 75% maximum individual size.

Composite ID Sample Example: LM Chinook 2003 0225 001  
 (LMChinook20030225001) (Lake) (Fish Species) (Year Fish Collected) (Grid # or Port Code) (Composite #)

Please see *Packing and Shipping Instructions for the Great Lakes Fish Monitoring Program* for further information on filling out and distributing this form.

# Great Lakes Fish Monitoring Program

Page \_\_\_\_\_ of \_\_\_\_\_

Composite ID	Fish Number	Date of Collection (Month/Day/Year)	Method of Collection	Estimated Collection Depth (m)	Sample ID	Field Length (mm)	Field Weight (g)	Sex (M=male, F=female)	Fin Clips (If none, record none.)
	1								
	2								
	3								
	4								
	5								
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	5								



# Great Lakes Fish Monitoring Program

Page \_\_\_\_\_ of \_\_\_\_\_

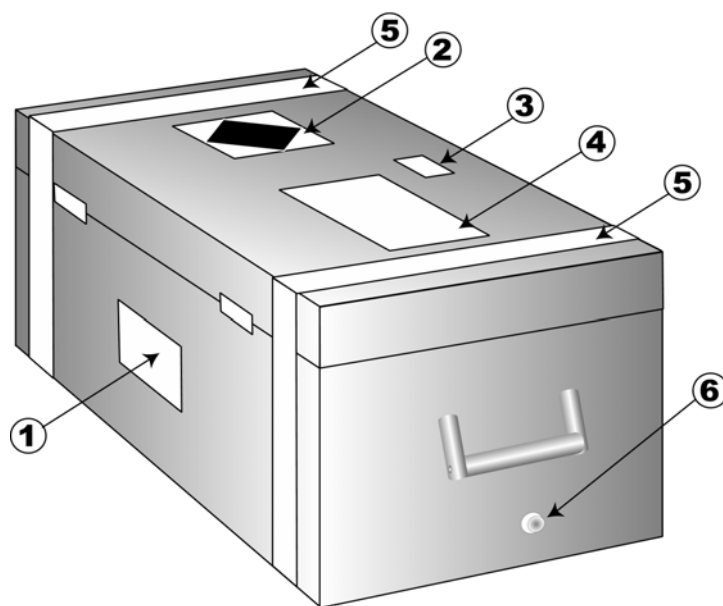
Composite ID	Fish Number	Date of Collection (Month/Day/Year)	Method of Collection	Estimated Collection Depth (m)	Sample ID	Field Length (mm)	Field Weight (g)	Sex (M=male, F=female)	Fin Clips (If none, record none.)
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**Appendix D**  
**Packing and Shipping Instructions for the Great Lakes**  
**National Program Office's Fish Monitoring Program**

## **Packing and Shipping Instructions for the Great Lakes National Program Office's Fish Monitoring Program**

These instructions apply to fish collected for US EPA's Great Lakes fish monitoring program (GLFMP). These fish are being sent to Axys Analytical (British Columbia, Canada) for sample preparation prior to analysis. These instructions supplement the Fish Collection SOPs provided by US EPA GLNPO. We are requesting that you fill out a Field Recording Form & Chain-of-custody Record, provided with these instructions, and send it to Axys with the fish. This will allow tracking of the shipments, identify all fish being shipped to Axys, and confirm that fish remained frozen during transport. This is in addition to any data sheets you may be providing. If you receive dry ice with the ice chests, please ship samples within 24 hours of receipt. If you did not receive a sampling kit from EPA's contractor, DynCorp (CSC), composite bags are provided with these instructions. Please follow the instructions detailed below.

1. Complete a Field Recording Form & Chain-of-custody Record (Attachment 1), provided in the FedEx package, for all fish being shipped. All entries must be in black ink and coincide with fish information on the Sample Identification Labels adhered to the samples (as described in EPA's Fish Collection SOPs). An example Field Recording Form & Chain-of-custody Record is included as Attachment 2. In order to simplify entering data onto the form, feel free to use ditto marks as shown in the example form. This form also is available electronically, although it will need to be printed and signed prior to sending. If you would like to receive this form electronically, or if you have any questions regarding fill out the form, please contact Judy Schofield at (703) 461-2027.
  - a. For identifying sample location (Grid number or Port code), please use Attachment 3, *Sample Collection Port Codes, Grid Numbers, & Latitude/Longitude Coordinates for the Great Lakes Fish Monitoring Program*.
  - b. For composite number, please assign numbers to each set of five fish being used for a composite. Assign 001 to the five fish being used for one composite, 002 to the next five fish being used for a composite, etc. This is a component of the sample identification numbering scheme that is being requested by the analytical laboratory.
2. Please fax the Field Recording Form & Chain-of-custody Record to Judy Schofield at 703-461-8056 before or immediately after shipping. This is critical for monitoring shipments to ensure they reach Axys Analytical before the samples thaw. If faxing is not possible, please call Judy Schofield at (703) 461-2027 and provide the **shipping date** and **airbill numbers** for all ice chests being shipped.
3. Make a copy of the Field Recording Form & Chain-of-custody Record. Place the original Field Recording Form & Chain-of-custody Record in a waterproof bag. If you have additional forms containing sampling or specimen data, place them in the waterproof bag as well and seal. Place the sealed forms in the ice chest.
4. Please double-bag all fish to protect sample integrity in the event of a tear in the plastic bag due to packing the fish in dry ice. If you used food grade tubing provided by EPA's contractor DynCorp (CSC), place each set of five fish being used for a composite inside the composite bag provided in your sampling kit and seal with a cable tie. If food grade tubing was not used, please use the composite bag as your second bag for packaging the fish.
5. As soon as each sample is packaged, place it immediately on dry ice for shipment in the ice chest. Try to surround all fish with dry ice. If possible, fill the ice chest with dry ice.
6. If possible, keep all (five) fish designated for a particular composite in the same shipping container (ice chest) for transport.
7. Secure each ice chest with packaging tape. Prepare the ice chests with the labels and other information as described on pages 2-3. Ship each ice chest to the laboratory via priority overnight express delivery service. If you are not using FedEx, please follow the appropriate courier procedures for shipping "dangerous goods" to Canada.



1. **Laboratory Address Label:** Ensure the Axy's Analytical label is secure and completely taped over with clear tape.
2. **Class 9 Dangerous Goods Label:** List the amount of dry ice in kg (2 lbs = 1 kg). Place the label on top of the cooler and completely tape over the label with clear tape.
3. **Perishable Goods Label:** Be sure to completely tape over this label with clear tape.
4. **FedEx Airbill:** To fully complete the FedEx airbill, please enter the information in the following sections:
  - ▶ Section 1 (sender's name, date)
  - ▶ Section 4 (number of total packages, total weight, commodity description, value for customs)
  - ▶ **Commodity Description: Please enter the following "Environmental Samples for RESEARCH PURPOSES ONLY."**
  - ▶ Section 7 (dry ice weight in kg)
  - ▶ Section 10 (signature, date)

**Commercial Invoice:** When filling out form, please press hard enough to ensure the information can be read on the last carbon copy. Complete the commercial invoice by entering the following information:

- A. International air waybill number - Enter the FedEx Tracking Number (12 digits in lower right hand corner of FedEx Airbill)
- B. Date of exportation - Date the cooler is shipped
- C. Your name under the shipper/exporter information
- D. Number of packages
- E. Quantity - Number of samples
- F. Unit of measure
- G. Weight - Weight of samples
- H. **Total value - \$100 per cooler. REGARDLESS OF THE NUMBER OF SAMPLES BEING SHIPPED, EACH COOLER WILL BE VALUED AT \$100.**
- I. Total weight - Combined weight of samples, dry ice, and cooler
- J. Total invoice value - Should be the same as the total value
- K. Signature of shipper/exporter
- L. Date

**NOTE:** *Include the commercial invoice with the FedEx airbill.*

5. **Packaging Tape:** Each end of the cooler needs to be wrapped with strapping tape at least three (3) times.
6. **Cooler Drain Hole:** Please make sure the cooler drain hole has been taped so that the drain hole is OPEN. If the tape has been removed, please tape the plug so that the drain hole remains OPEN. This is critical to ensure proper venting of the dry ice.

NOTE: If you have any questions regarding the packing or shipping of these samples or if you need assistance filling out the paperwork, please contact Judy Schofield at (703) 461-2027.

### **Special Instructions:**

- Not all FedEx locations will accept shipments containing dry ice. Please be sure to call in advance (800-Go-FedEx) to ensure that your FedEx drop-off location accepts dry ice. In the event that you cannot locate a station in your area that accepts dry ice, simply call for a pickup (800-Go-FedEx) and explain that you have a shipment containing dry ice. FedEx will gladly pick-up these shipments.
- FedEx Dangerous Goods personnel have given approval for shipment of these packages using the instructions listed above. Failure to follow these instructions could result in the package being “bumped” and therefore, not reaching its destination. If you have any problems with FedEx personnel accepting your package, please contact Judy Schofield at (703) 461-2027 immediately.
- Immediately after sample shipment, please call Judy Schofield at (703) 461-2027 and provide the **shipping date** and **airbill numbers**. This is critical for monitoring shipments to ensure they reach Axys Analytical before the samples thaw. If possible, please fax the Field Recording Form & Chain-of-custody Record to Judy Schofield at 703-461-8056 immediately after shipping.

## **Appendix E**

### **List of Sample Collection Port Codes and Grid Numbers**

Table E-1. Sample Collection Port Codes, Grid Numbers, Latitude/Longitude Coordinates for the Great Lakes Fish Monitoring Program

Year	Lake/State	Port Name	Species	Port Code	Grid Number	Latitude/Longitude
Odd-numbered	Lake Superior	Keewanaw Point	Lake Trout	NA	1028	87°35'W/47°25'N
	Lake Michigan	Stugeron Bay	Lake Trout	NA	0906	87°05'W/44°45'N
	Lake Michigan	Charlevoix (as back-up only)	Lake Trout	NA	0516	85°25'W/45°25'N
	Lake Huron	Port Austin	Lake Trout	NA	1413	82°45'W/44°05'N
	Lake Erie	Dunkirk	Walleye	NA	0424	79°35'W/42°25'N
	Lake Ontario	North Hamlin	Lake Trout	NA	0713	77°55'W/43°25'N
	New York	Salmon River	Chinook	0601	NA	43°32'N/75°59'W
	Pennsylvania	Trout Run	Steelhead Trout	0527	NA	42°3.54'N/80°16.38'W
	Michigan	St. Joseph's River	Chinook	0201	NA	41°56.63'N/86°19.7'W
		Grand River	Chinook	0225	NA	42°56'N/84°54'W
		Platt River	Chinook	0213	NA	44°43.18'N/86°8.21'W
		Thompson Creek	Chinook	0251	NA	41°65'N/86°36'W
		Swan River	Chinook	0300	NA	45°23'N/83°44'W
		Ausable River	Chinook	311A	NA	44°24.93'N/83°19.84'W
	Indiana	Trail Creek	Chinook	0227	NA	41°43'N/86°53'W
	Wisconsin	Root River	Chinook	0237	NA	42°43.955'N/87°46.72'W
		Kewanee River	Chinook	0247	NA	44°27.533'N/87°33.383'W
		Lake Superior, Pike Creek	Chinook	0139	NA	46°47.154'N/90°51.953'W
	Minnesota	French River	Chinook	0143	NA	46°52'N/91°54'W
		Knife River	Coho	0145	NA	46°45'N/91°45'W
Ohio	Grand River	Steelhead Trout	0523	NA	41°45'N/81°16'W	
Even-numbered	Lake Superior	Apostle Islands	Lake Trout	NA	1311	90°25'W/46°55'N
	Lake Michigan	Saugatuck	Lake Trout	NA	2210	86°25'W/42°35'N
	Lake Huron	Rockport	Lake Trout	NA	0710	83°1'W/45°15'N
	Lake Erie	Middle Bass Island	Walleye	NA	0904	82°55'W/41°35'N
	Lake Ontario	Oswego	Lake Trout	NA	0623	76°15'W/43°35'N
	New York	Salmon River	Coho	0601	NA	43°32'N/75°59'W
	Pennsylvania	Trout Run	Coho	0527	NA	42°3.54'N/80°16.38'W
	Michigan	St. Joseph's River	Coho	0201	NA	41°56.63'N/86°19.7'W
		Grand River	Coho	0225	NA	42°56'N/84°54'W
		Platt River	Coho	0213	NA	44°43.18'N/86°8.21'W
		Thompson Creek	Coho	0251	NA	41°65'N/86°36'W
		Swan River	Coho	0300	NA	45°23'N/83°44'W
		Ausable River	Coho	311A	NA	44°24.93'N/83°19.84'W
	Indiana	Trail Creek	Coho	0227	NA	41°43'N/86°53'W
	Wisconsin	Root River	Coho	0237	NA	42°43.955'N/87°46.72'W
		Kewanee River	Coho	0247	NA	44°27.533'N/87°33.383'W
		Lake Superior, Pike Creek	Coho	0139	NA	46°47.154'N/90°51.953'W
	Minnesota	French River	Coho	0143	NA	46°52'N/91°54'W
	Ohio	Grand River	Steelhead Trout	0523	NA	41°45'N/81°16'W

**Table E-2. Corner Coordinates for Sample Collection Grids for the Great Lakes Fish Monitoring Program**

Lake	Grid	Name	Corner	Degree (W)	Minute (W)	Degree (N)	Minute (N)
Michigan	2210	Saugatuck	NW	86	30	42	40
			NE	86	20	42	40
			SW	86	30	42	30
			SE	86	20	42	30
Michigan	906	Sturgeon Bay	NW	87	10	44	50
			NE	87	0	44	50
			SW	87	10	44	40
			SE	87	0	44	40
Michigan	516	Charlevoix	NW	85	30	45	30
			NE	85	20	45	30
			SW	85	30	45	20
			SE	85	20	45	20
Huron	1413	Port Austin	NW	82	50	44	10
			NE	82	40	44	10
			SW	82	50	44	0
			SE	82	40	44	0
Huron	710	Rockport	NW	83	20	45	20
			NE	83	10	45	20
			SW	83	20	45	10
			SE	83	10	45	10
Erie	904	Middle Bass Island	NW	83	0	41	40
			NE	82	50	41	40
			SW	83	0	41	30
			SE	82	50	41	30
Erie	424	Dunkirk	NW	79	40	42	30
			NE	79	30	42	30
			SW	79	40	42	20
			SE	79	30	42	20
Ontario	713	North Hamlin	NW	78	0	43	30
			NE	77	50	43	30
			SW	78	0	43	20
			SE	77	50	43	20
Ontario	623	Oswego	NW	76	20	43	40
			NE	76	10	43	40
			SW	76	20	43	30
			SE	76	10	43	30
Superior	1028	Keewenaw Pt.	NW	87	40	47	30
			NE	87	30	47	30
			SW	87	40	47	20
			SE	87	30	47	20
Superior	1311	Apostle Islands	NW	90	30	47	0
			NE	90	20	47	0
			SW	90	30	46	50
			SE	90	20	46	50

Indicate a portion of the grid that overlaps with land.