Great Lakes Fish Monitoring and Surveillance Program

Quality Assurance Project Plan for Sample Collection Activities

Version 2.0, November 2012

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Great Lakes Fish Monitoring and Surveillance Program

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Revision History

Version/Date	Description	Affected QMP Sections
Ver. 1.0, 2008	Original QAPP prepared/signed	All
Ver. 2.0, 2012	1. Updated program name to Great Lakes Fish Monitoring *and Surveillance* Program 2. Updated program history, recent activities, and program changes since 2008 3. As the program has matured, more detail has been documented regarding program elements including quality objectives and criteria, quality control, reports to management, data review, validation and verification requirements, etc.	1. Throughout 2. Throughout 3. Throughout

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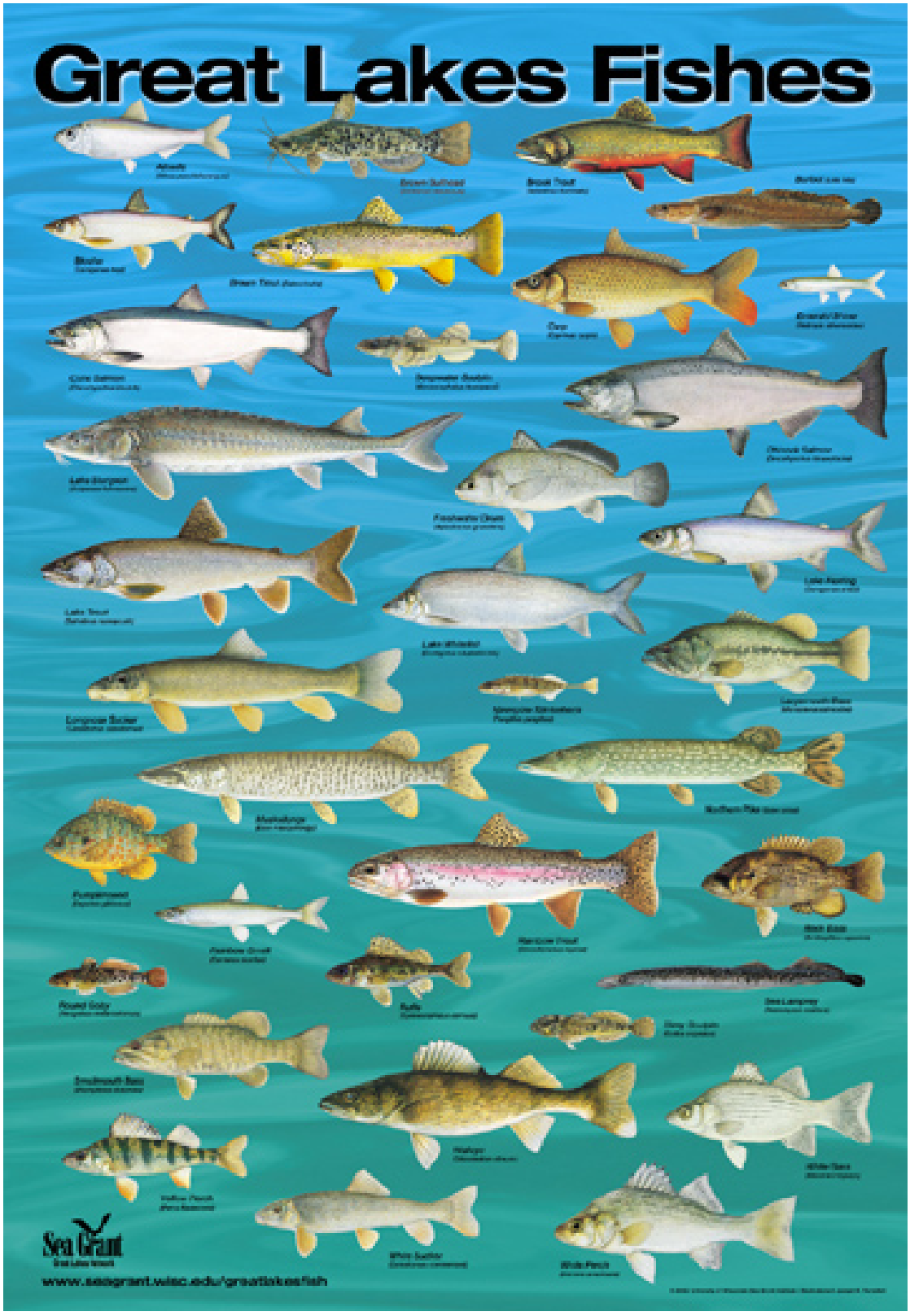
Great Lakes Fishes
Sea Grant
www.seagrant.wisc.edu/greatlakesfish

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Acronyms and Abbreviations

BEC	Binational Executive Committee
BTS	Binational Toxics Strategy
CEC	Contaminants of Emerging Concern
CSC	Computer Sciences Corporation
CSMI	Cooperative Science and Monitoring Initiative
CWTs	Coded Wire Tags
DNR	Department of Natural Resources
DQOs	Data Quality Objectives
EMIT	Environmental Monitoring and Indicators Team
EPA	U.S. Environmental Protection Agency
FAA	Fatty Acid Analysis
GLAS	Great Lakes Accountability System
GLENDA	Great Lakes Environmental Database
GLFC	Great Lakes Fisheries Commission
GLFMP	Great Lakes Fish Monitoring Program
GLFMSP	Great Lakes Fish Monitoring and Surveillance Program
GLNPO	Great Lakes National Program Office
GLRI	Great Lakes Restoration Initiative
GLWQA	Great Lakes Water Quality Agreement
GPS	Global-Positioning System
IAG	Interagency Agreement (outdated term)
LaMP	Lakewide Management Plans
LOY	Lake of the Year
MIRB	Monitoring, Indicators and Reporting Branch
MQOs	Measurement Quality Objectives
PI	Principal Investigator
QA/QC	Quality Assurance/Quality Control
QM	Quality Management
QMP	Quality Management Plan
QAPP	Quality Assurance Project Plan
SCA	Stomach Contents Analysis
SIA	Stable Isotope Analysis
SOLEC	State of the Great Lakes Ecosystem Reporting and Conference
SOP	Standard Operating Procedure
US EPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration
USFWS	United States Fish and Wildlife Service
USGS	United States Geological Survey
USGS-BRD	United States Geological Survey – Biological Resource Division
USGS-GLSC	United States Geological Survey – Great Lakes Science Center

A.3 Distribution List

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Principal Investigator(s) of the Great Lakes Fish Monitoring and Surveillance Program (identified every 5 years)

Field Sampling Teams (identified annually)

QA Contractor

A.4 Project/Task 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 and Surveillance Program (GLFMSP). The purpose of this document is to present the methods and procedures that are used for the collection of 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 fish sample collection efforts of the GLFMSP.

A4.1 Project Management

The GLFMSP is implemented by staff from the United States Environmental Protection Agency's (US EPA) Great Lakes National Program Office (GLNPO), the Great Lakes states, 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. Field samplers typically ship fish samples to a homogenization laboratory; in some instances, samples are shipped directly to an analytical laboratory. The homogenization laboratory is responsible for taking physical measurements (e.g., weight, length, gender and maturation stage assessments, etc.), identifying abnormalities, collecting samples for aging purposes (e.g., scales, otoliths, and coded wire tags) and compositing and homogenizing the samples. The homogenization laboratory is determined on an annual basis. The homogenization laboratory prepares aliquots of each sample composite and ships them to an archival facility and the analytical laboratory. The analytical laboratory is responsible for analyzing the samples received from the homogenization laboratory and any samples received directly from the field samplers.

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

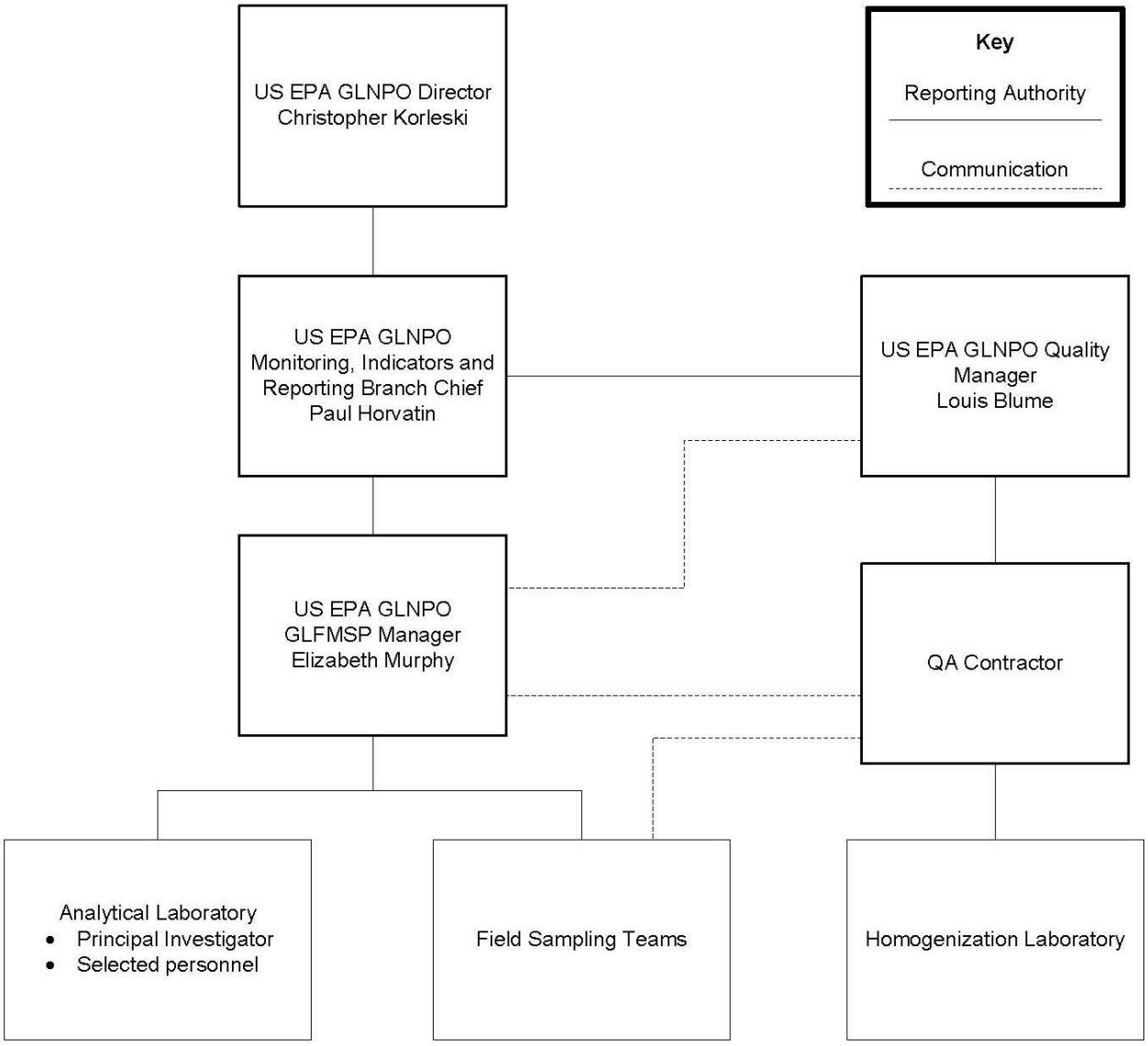

Figure 1. Participants in the Great Lakes Fish Monitoring and Surveillance Program

A4.2 Project Implementation

Director of the Great Lakes National Program Office

The **GLNPO Director**, Chris Korleski, 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 Manager and study participants.

Monitoring Indicators and Reporting Branch Chief

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

GLNPO Quality Manager

The **GLNPO Quality Manager**, Louis Blume, is responsible for reviewing and approving all QAPPs and reports directly to the MIRB Chief. Additional GLNPO Quality 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.

GLFMSP Manager

The **GLFMSP Manager**, Elizabeth Murphy, reports directly to the MIRB Chief and is responsible for supervising the assigned project personnel. Additional GLFMSP 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,
- coordinating with contractors, grantees, and US EPA Regions/States/Tribes to ensure technical quality and contract adherence, and
- maintaining all official copies of GLFMSP documents and materials.

Field Sampling Teams

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

- state personnel such as field biologists or fisheries biologists,
- federal agencies,
- 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, 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). They must perform all work in adherence with the project work plan and the QAPP, to the best of their abilities. 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 standard operating procedures (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 recording form and chain-of-custody record (Appendix B),
- 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.

Field sampling teams ship samples to the homogenization laboratory or analytical laboratory.

QA Contractor

The QA contractor is responsible for coordinating with field samplers to create a schedule for shipping sampling supplies. The QA contractor creates sampling kits and shipping kits and ships them along with coolers to the field sampling teams. The QA contractor also updates and provides the field sampling teams with hardcopy versions of the field recording form and chain-of-custody record, SOPs, and fish sample identification labels on an annual basis. QA contractor staff arrange for the shipment of samples between the field sampling teams, homogenization laboratory, and analytical laboratory. The QA contractor annually reviews and determines the homogenization laboratory and coordinates homogenization services support to the GLFMSP through a purchase order (PO) with the homogenization laboratory.

The QA contractor processes and formats the field data submitted by field sampling teams to the Great Lakes Environmental Database (GLENDA) reporting standard, conducts checks to ensure that all necessary information has been provided, and seeks to resolve any discrepancies. The QA contractor also enters data provided by the homogenization laboratory into the GLENDA files for the applicable sampling year and conducts a check on the data to ensure accuracy of data already provided in the GLENDA files. The QA contractor seeks to resolve any discrepancies in the data. Data assessments also are performed on laboratory-submitted data, and focus on data completeness, and data consistency. Data completeness checks are performed by comparing the field and laboratory data to identify any missing or non-unique sample analyses, while data consistency checks verify that the data correctly follow the GLENDA standard. The QA contractor has been Computer Sciences Corporation (CSC) since 2003. All support provided to the GLFMSP by CSC is done according to procedures described in the *Quality Assurance Project Plan for CSC Support to the Great Lake Fish Monitoring and Surveillance Program* (Appendix A.2 of GLFMSP Quality Management Plan [QMP]).

Homogenization Laboratory

Field sampling teams send fish samples to the homogenization laboratory. The homogenization laboratory records physical measurements, collects coded wire tags, scales, and otoliths when applicable, records any abnormalities (e.g., tumors, fins missing, wounds, etc.), prepares composites of the samples, homogenizes the samples, and prepares mega-composites of the samples. Each mega-composite includes tissue from all "regular" composites from a single site. The homogenization laboratory also prepares aliquots from composites, individual samples, and mega-composites and sends them to an archival facility and the analytical laboratory. Homogenization services were provided by AXYS Analytical in Sydney, British Columbia, Canada between 2003 and 2010. In 2011, Aquatec Biological Sciences, Inc. in Williston, Vermont, began providing homogenization services.

The laboratory must adhere to the sample receipt requirements, sample preparation and physical data collection requirements, homogenization requirements, aliquot creation requirements, sampling handling and custody requirements, and QC requirements outlined in their Statement of Work (SOW) as determined through the PO with the QA contractor. The SOW for the homogenization laboratory is updated and reviewed annually by the QA contractor. The homogenization lab must have approved SOPs in place prior to beginning work. Any deviations from the SOPs should be approved prior to implementation by the GLFMSP Manager or if the deviation was unintentional, reported immediately to the GLFMSP Manager. Aquatec Biological Sciences, Inc.'s GLFMSP SOP can be found in Appendix A.1 of the GLFMSP QMP.

Analytical Laboratory

Field sampling teams send some samples directly to the analytical laboratory. The majority of samples are sent to the analytical laboratory by the homogenization laboratory. The analyses of the fish tissue samples are *not* covered by this QAPP. Clarkson University was awarded chemical analysis of the GLFMSP tissue samples in 2004 following submissions and approval of quality documentation. Thomas Holsen serves as the Principal Investigator (PI) providing analytical and technical support and will continue to do so through the 2015 research year. Details regarding this component of the project can be found in the QAPP submitted to GLNPO by Thomas Holsen: *The Great Lakes Fish Monitoring and Surveillance Program: Pushing the Science (GLFMSP)*(Holsen *et al.*, 2012) (Appendix A.4 of GLFMSP QMP).

The QAPP for analysis of tissue samples prior to 2004 can be found in *Trends in Great Lakes Fish Contaminants Quality Assurance Project Plan* (Swackhamer, 2004) (Appendix B.9 of GLFMSP QMP), submitted to GLNPO by Deb Swackhamer of the University of Minnesota who served as PI from 1999-2003.

A.5 Problem Definition/Background

The GLFMSP is designed to examine the health of fish and fish-consuming wildlife through trend analysis, improve understanding of contaminant cycling throughout food webs in the Great Lakes, and screen for emerging chemicals in fish tissue to help identify new chemicals for future trend analysis.

The overall goals of the GLFMSP include:

- Monitor temporal trends in bioaccumulative organic chemicals in the Great Lakes using top predator fish as biomonitors,
- Gather information regarding the contaminant cycling throughout food webs in the Great Lakes, and
- Provide information on new compounds of concern entering the lakes ecosystem.

The GLFMSP has evolved over time, with the number of lakes, sampling locations, species, and contaminants changing as resources allowed and scientific knowledge demanded. The program is currently implemented by GLNPO with cooperation from selected federal or local agencies, Great Lake states, and Native American Tribes. The present design of the GLFMSP includes three programs:

1. the Open Lakes Trend Base Monitoring Program (hereafter referred to as the "Base Monitoring Program"),
2. the Emerging Chemical Surveillance Program, and
3. the Cooperative Science and Monitoring Initiative (CSMI) / Special Studies Program

More information about these programs is provided in Section A6. Table 1 provides a brief history of the GLFMSP.

Table 1. GLFMSP History

Date	Event
Mid 1960s	U.S. Geological Survey Great Lakes Science Center (USGS-GLSC) (formerly known as U.S. Fish and Wildlife Service Great Lakes Fishery Laboratory) begins monitoring fish in Lake Michigan to measure the contaminant levels of various organic substances in lake trout in the Great Lakes ecosystem.
1977	USGS-GLSC collaborates with US EPA/GLNPO to form the Great Lakes Fish Monitoring Program (GLFMP) to monitor top predator fish in the Great Lakes. The original study design is modified to generate more data by including additional species, sampling locations, and contaminants. Through this cooperative agreement, a partnership is formed, with USGS collecting and processing the fish, and US EPA funding the analyses.
Early 1980s	GLFMP is expanded to include sport fish (coho and chinook salmon) to directly link the condition of the Great Lakes to the health of it users. Each Great Lakes State collects 15 fillets from Coho of Chinook salmon at designated sites. The Great Lakes States and the U.S. Food and Drug Administration (USFDA) become additional partners, with the States voluntarily collecting sport fish and the USFDA processing and analyzing the samples for toxic chemicals.
1998	USFDA withdraws from the cooperative agreement to analyze contaminants in sport fish.
2003	USGS-GLSC discontinues cooperative agreement to analyze contaminants in whole fish, leaving GLNPO as the sole supporter of the program, both financially and through staff support.
2005	A program review sponsored by the US EPA occurred. The program review included an overview of the history of the GLFMP, current sampling plan, historical record of target analytes and data management, current program, Quality Management Program data storage, stakeholder use of GLFMP data, and technical charge. The GLFMP review panel made recommendations for consideration by GLNPO to help revise and enhance the GLMFP to better fit with current environmental conditions and better serve stakeholders.
2007	A peer review is conducted on the GLFMP to enhance the quality and validity of the program and ensure that the data generated under the program are statistically sound and representative of the current environment.

Date	Event
2009	In response to suggestions provided in peer review and careful assessment by GLNPO Management, Sport Fish Monitoring is eliminated from the GLFMP. The Emerging Chemical Surveillance Program is added to the GLFMP. The program name changes from GLFMP to Great Lakes Fish Monitoring and Surveillance Program (GLFMSP).
2010	Great Lakes Restoration Initiative (GLRI) establishes a task force of 11 federal agencies to devise and implement an action plan to proactively rehabilitate the Great Lakes. This task force identifies goals, objectives, and specific actions addressing each of five focus areas including the identification of toxic substances with an emphasis on their impact on ecosystems and the entire food web.
2011	In response to a Request for Proposals, Lake of the Year monitoring is proposed by Clarkson University's Principal Investigator and accepted. The CSMI/Special Studies Program is added to the GLFMSP.

Over the life of the GLFMSP, 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.

Table 2 provides the current list of analytes of interest that are monitored for and a list of emerging contaminants that are screened for on an annual basis. The actual list of analytes for a given year of study may be modified to match the funding appropriated for the program. For a complete list of analytes, refer to the Clarkson University QAPP, *The Great Lakes Fish Monitoring and Surveillance Program: Pushing the Science (GLFMSP)* (Holsen *et al.*, 2012) (Appendix A.4 of GLFMSP QMP).

Table 2. Analytes of Interest

Base Contaminant Analytes	
Polychlorinated biphenyl (PCB) congeners	*cis*-Nonachlor
co-planar PCBs	*trans*-Nonachlor
Hexachlorobenzene	p,p', o,p-DDD
PPCPs	p,p', o,p-DDE
Octachlorostyrene	p,p', o,p'-DDT
Δ-HCH (Lindane)	Endrin
Alpha BHC	Mirex
APEs	Toxaphene & homologs
Dieldrin	Polychlorinated dibenzo-*p*-dioxins (PCDDs)
BFRs	Polychlorinated dibenzofurans (PCDFs)
Heptachlor epoxide b	Polybrominated diphenyl ethers (PBDEs)
cis-Chlordane	Polychlorinated naphthalenes (PCNs)
t*rans*-Chlordane	Mercury
Oxychlordane	Lipid fraction
Emerging Contaminant Analytes	
PentaBromoChlorocycloHexane	Dibutyl chlorendate solution
1,2-Dibromo-4-(1,2-dibromoethyl)-cyclohexane	SAYTEX® BT-93W
Hexachlorocyclopentadiene	Hexabromocyclododecane
Cyanuric chloride	Chlorocyclopentane
Pentachlorothiophenol	Perbromophthalate/benzoate
3,5-Dichloro-2,4,6-trifluoropyridine	Decabromodiphenyl Ethane
Pentachloropyridine	Tetradecabromo-1,4-diphenoxybenzene
2,4,6-Tribromophenol solution	Tetrabromophthalic anhydride
Decabromodiphenyl ethane	

A.6 Project/Task Description

The GLFMSP consists of the Base Monitoring Program, the Emerging Chemical Surveillance Program, and the CSMI/Special Studies Program. These programs assist the GLFMSP in achieving its overall goals of (1) monitoring temporal trends in bioaccumulative organic chemicals in the Great Lakes using top predator fish as biomonitors, (2) gathering information regarding the contaminant cycling throughout food webs in the Great Lakes, (3) providing information on new compounds of concern entering the lakes ecosystem.

A6.1 Open Lakes Trend Base Monitoring Program

The Open Lakes Trend Base Monitoring Program (referred to as the Base Monitoring Program throughout this QAPP) is directed at monitoring the health of the Great Lakes ecosystem, using whole top predator fish (lake trout and walleye) as biomonitors, for select contaminants to determine general trends and to provide support to the research community and the public through collection of high quality data using identified and approved

methodology. These data also can be used to assess the risks of such contaminants on the health of this important fishery, and on wildlife that consume them. The Base Monitoring Program involves collection and analysis of predatory fish from all five Great Lakes on an annual basis, with slight differences in design during even and odd years. Top predator fish like lake trout and walleye have been extensively used as bioindicators of the overall condition of the Great Lakes system and serve as excellent contaminant biomonitors by encompassing both water and sediment exposures through pelagic and benthic routes. During odd years, 50 lake trout (*Salvelinus namaycush*) in the size range of 600 mm to 700 mm will be collected in each of the five Great Lakes. During even years, 50 lake trout in the size range of 600 mm to 700 mm will be collected from four of the five Great Lakes (Huron, Michigan, Ontario, and Superior) and 50 walleye (*Stizostedion vitreum*) in the size range of 400 mm to 500 mm will be collected from Lake Erie. The GLFMSP organizes collections through cooperative agreements with other agencies or by purchasing predatory fish. Composites of each species, consisting of five individual fish, are analyzed for contaminants to assess temporal trends in organic contaminants and mercury in the open water of the Great Lakes. Because this part of the program was designed to assess the overall effects of toxic chemicals on fish, whole fish are used for analysis, including parts not routinely eaten by humans such as liver and bones.

Each lake contains two sampling sites, one representing an industrial area and one representing a non-industrial area. Chosen sites 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. These locations are sampled alternately each year at approximately the same time of year. Detailed information on GLFMSP sampling sites is provided in Section B.

The goals of the Base Monitoring Program are to:

- Provide an indication of environmental quality,
- Identify contaminant levels in fish and their trends,
- Assess the impact of regulatory controls on whole lake conditions,
- Provide an early warning for new contaminants,
- Identifying potential harm to fish stocks, and
- Identifying transboundary contamination.

A6.2 Emerging Chemical Surveillance Program

The Emerging Chemical Surveillance Program is directed at screening for emerging chemicals in fish tissue according to their persistent, bioaccumulative, and/or toxic chemical properties. This program utilizes samples collected for the Base Monitoring Program and the CSMI/Special Studies Program to determine the presence of Contaminants of Emerging Concern (CEC), identify and guide State and Federal monitoring programs in the development of their analyte lists and priority setting, and to incorporate emerging CECs into the routine analyte list for the Base Monitoring Program and the CSMI/Special Studies Program. Identification of CECs is accomplished through two methods. The first method involves performing a detailed "Full Scan" analysis of Great Lakes fish by screening for a set of previously identified contaminants in composite samples and analyzing extracts for previously unidentified peaks (non-legacy contaminants). The second method involves working from the US EPA sponsored *Potential Emerging Contaminant List* developed by Muir and Howard (*Howard & Muir, 2010)*. This list is based on high and medium production volume chemicals in the United States and Canada. Retrospective analysis may be conducted upon archived samples if a CEC is identified.

The goals of the Emerging Chemical Surveillance Program are to:

- Screen for a set of previously identified CECs,
- Determine the presence of CECs,
- Identify and guide State and Federal monitoring programs in the development of their analyte lists and priority setting, and
- Incorporate CECs into the routine analyte list for the Base Monitoring Program and the CSMI/Special Studies Program.

A6.3 CSMI / Special Studies Program

The Cooperative Science and Monitoring Initiative (CSMI) was established by the Binational Executive committee (BEC) to address greater coordination of science and monitoring activities in the Great Lakes Basin pursuant to the obligations under the Great Lakes Water Quality Agreement (GLWQA).

The CSMI is a forum and a process to foster and coordinate collaboration for binational monitoring and research to meet key Lakewide Management Plan (LaMP) information needs, as well as support other science needs under the GLWQA (such as science needs of the Great Lakes Binational Toxics Strategy [BTS], Great Lakes Fishery Commission [GLFC], and the State of the Lakes Ecosystem Conference [SOLEC]. CSMI recognizes a five year rotational cycle of research and monitoring on the Great Lakes, in which science activities address one of the Great Lakes each year, but accommodate multiple lake activities simultaneously when necessary and practical. Within the five year rotational cycle, years one and two involve identification of priorities for collaboration and planning, year three involves intensive field activities, year four involves analysis and data work-up, and year five involves synthesis and communicating out to partners such as the Binational Executive Committee (BEC), the LaMP, and the public. In any given year, each lake is at a different stage in the cycle.

The GLFMSP participates in the CSMI through additional sample collection efforts and analyses as identified by the Principal Investigator (PI) when funding is available.

The Great Lakes Restoration Initiative (GLRI) allowed for the inclusion of special studies in the five year award for the GLFMSP beginning in 2010. The current PI proposed to incorporate the CSMI into the GLFMSP through these special studies by conducting Lake of the Year (LOY) monitoring to improve our understanding of contaminant cycling throughout food webs in the Great Lakes by expanding research efforts in one lake each year. The LOY is chosen based on the schedule proposed by the CSMI.

The PI's proposal was accepted and in 2011 the CSMI/Special Studies Program was added to the GLFMSP to incorporate LOY monitoring. Unless otherwise designated, CSMI/Special Studies Program collections occur at the same locations in each lake as Base Monitoring Program collections.

The CSMI/Special Studies Program is directed at addressing issues raised by the other components of the GLFMSP and supports additional research that will improve our understanding of pollutant impacts on the fishery and help properly gauge the efforts of remediation and pollutant reduction efforts in the Great Lakes. The CSMI/Special Studies Program was not designed to meet a specific DQO. Rather, it was designed to provide data needed to supplement the Base Monitoring Program and improve our understanding of pollutant cycling in the Great Lakes. The biological structure and composition of food webs are important in determining the flow of energy, nutrients, and ultimately contaminants through ecosystems

CSMI/Special Studies Program collection efforts include the collection of ten individual lake trout or walleye (depending on the sampling location) per site at both sampling locations in the LOY (20 samples total). Top predator fish like lake trout and walleye have been extensively used as bioindicators of the overall condition of

the Great Lakes system and serve as excellent contaminant biomonitors by encompassing both water and sediment exposures through pelagic and benthic routes. Lake trout in the size range of 600 mm to 700 mm and walleye in the size range of 400 mm to 500 mm are collected.

Stomach contents and eggs are collected from the individual fish. The purpose of collection of stomach contents is to assist in the evaluation of the movement of contaminants in complex Great Lakes food webs. This requires data on pollutant concentrations and fluxes (diet) for the top predator and the prey species at the supporting lower trophic levels. The eventual body burden of contaminants in the individual fish depends on the feeding preferences and food availability at lower trophic levels and the contaminant burden of each prey species. The purposes of the collection and analysis of contaminant levels in parent individual fish and associated eggs are to evaluate the relationship of parent-egg contaminant levels, potentially identify new emerging contaminants, assess critical contaminant trends, and support LaMPs. Eggs are separated when the stomachs are being removed for fish gut analysis, and analyzed. This long-term data is used to assess if "batch egg samples" can act as a surrogate for long-term trends of fish contaminant loads. The number of egg samples collected is determined by the number of gravid females collected (maximum 10).

Benthic invertebrates, phytoplankton, zooplankton, and water samples are also collected from two locations within the LOY to improve our understanding of contaminant cycling throughout food webs in the Great Lakes.

CSMI/Special Studies Program collection efforts also include the collection of forage fish species (e.g., bloaters, siscowets, sculpins, ciscos, smelts, etc.). At both sampling locations in the LOY, forage fish of varying species are collected. Ideally at each site, 30 fish of each of the three most abundant forage fish species and 10 fish of the fourth and fifth most abundant forage fish species (110 fish total) are collected. The purpose of the collection of forage fish is to improve our understanding of the food availability at lower trophic levels and the contaminant burden of each prey species.

Because CSMI/Special Studies Program collection efforts and analyses are temporary and issued on a periodic basis, limited quality information is available for them. When possible, SOPs and QAPPs for additional sample collection and analysis are provided.

The goal of CMSI/Special Studies Program is to gather information regarding the contaminant cycling throughout food webs in the Great Lakes.

A6.4 Project Design

Field sampling teams collect samples for the Base Monitoring Program and the CSMI/Special Studies Program. Collection methods are determined by the field sampling teams. Samples include lake trout and walleye (which are grouped into composites), individual lake trout and walleye, fish eggs and stomach contents, forage fish, benthic invertebrates, phytoplankton, zooplankton, and water samples. Field sampling teams must adhere to the established sample collection protocols for collection, labeling, and shipping samples (Appendix A), to the best of their abilities. This QAPP focuses *only* on fish sample collection efforts.

When sample collection efforts are complete, field sampling teams ship their samples to the homogenization laboratory or analytical laboratory. Samples that are sent to the homogenization laboratory include: Base Monitoring Program fish, CSMI/Special Studies Program forage fish, and CSMI/Special Studies Program individual fish and their associated stomach contents.

The homogenization laboratory performs the following steps:

- Collect physical data on the samples,
- Identify external abnormalities (e.g., tumors, fins missing, wounds, etc.),
- Collect samples for aging purposes (e.g., scales, otoliths, and coded wire tags),
- Prepare composites of samples,
- Homogenize composites or individual fish,
- Prepare mega-composites of samples from each site (representing all samples from a given lake or site),
- Prepare aliquots, and
- Ship the aliquots to the analytical laboratory and an archival facility.

Samples that are shipped directly to the analytical laboratory include CSMI/Special Studies Program individual fish eggs.

The analytical laboratory analyzes the fish tissue samples. Details regarding this component of the project can be found in Clarkson University's QAPP: *The Great Lakes Fish Monitoring and Surveillance Program: Pushing the Science (GLFMSP)* (Holsen *et al.*, 2012) (Appendix A.4 of GLFMSP QMP).

Sample analysis provides information including:

- Contaminant trends in Great Lakes fish,
- New chemicals of interest in fish tissues, and
- Contaminant cycling throughout food webs in the Great Lakes.

This data can be used for site-specific statements, not lake-wide statements.

Figure 2 is a simplified illustration of the processing of fish samples collected in support of the Base Monitoring Program.

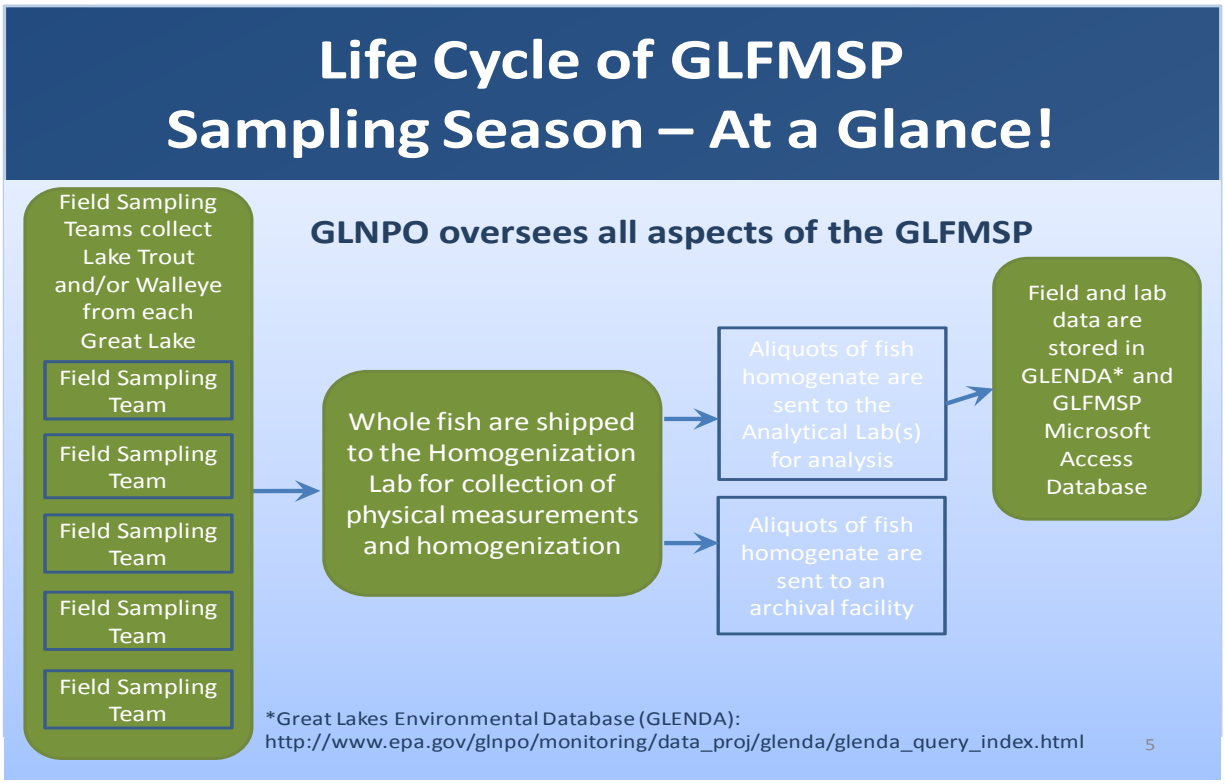

Figure 2. Life Cycle of the Base Monitoring Program Sampling Season

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 3.

Table 3. Work Schedule

Date*	Action	Person Responsible
January prior to fall collection	Organize and coordinate collection efforts	GLFMSP Manager
April – May prior to fall collection	Coordination of collection SOPs and sampling materials	GLFMSP Manager and QA Contractor
June – October of collection year	Samples to be collected	Identified collection personnel
October – December of collection year	Coordination of sample shipment to homogenization laboratory	GLFMSP Manager
January – April following a collection year	Homogenization of samples	Identified homogenization personnel
April following a collection year	Coordination of shipment of homogenized samples to analytical laboratory and archival facility	QA Contractor
May following a collection year	Coordination of shipment of coded wire tags, otoliths, fish scales, and fin clips to QA contractor	QA Contractor
May following a collection year	Analysis of homogenized samples and report preparation	Identified analysis personnel
June following a collection year	Submission of final homogenization deliverable package to QA contractor	Identified homogenization personnel
July following a collection year	Review of final homogenization deliverable package and submission to GLFMSP Manager	QA Contractor
May – July following a collection year	Review of sample analysis report	GLFMSP Manager, Quality Manager

** These are estimated timeframes.*

The PI provides progress reports quarterly and final reports yearly. Data is released according to the *Great Lakes Fish Monitoring and Surveillance Program Data Release Guidelines* (Appendix C.10 of GLFMSP QMP). Journal publications serve as the method for final data reporting. The GLFMSP Manager will make a public announcement annually when data that have been checked for completeness and consistency are available in GLENDA and the GLFMSP Microsoft Access Database. Data will be available through a request to the GLFMSP Manager. Section C2 of this QAPP provides detailed information on GLFMSP data reporting.

A.7 Quality Objectives and Criteria

A7.1 Representativeness

Several factors played a role in the selection of lake trout for the GLFMSP. Lake trout are representative of the offshore zone in the three upper lakes, but not in Lakes Erie or Ontario due to the fact that the populations sampled are relatively local to their spawning areas. Lake trout are top predators and long lived and were considered to be excellent concentrators of contaminants. Walleye were originally chosen to be collected in Lake Erie because they have similar characteristics to lake trout and were available in greater abundance. Lake trout and walleye serve as biomonitors for select contaminants to determine general trends and to provide

support to the research community and the public through collection of high quality data using identified and approved methodology. These data also can be used to assess the risks of such contaminants on the health of this important fishery, and on wildlife that consume them.

A limitation of the GLFMSP is the fact that fish are difficult to use as indicators of environmental quality without adequate past history information. Scientists do not know exactly where each fish has been and without that information, it is difficult to describe what the sample represents. GLFMSP data is used for site-specific statements, not lake-wide statements. Because each lake is a unique ecosystem, a discussion of each individual lake's representativeness is included.

Lake Michigan

There are two GLFMSP sampling sites located in Lake Michigan:

- Saugatuck in the southeastern part of the lake, and
- Sturgeon Bay in the northwestern part of the lake.

Patrick Schmalz and others studied a population of lake trout in northwestern Lake Michigan to determine the distances that they would travel (Schmalz et al., 2002). Based on other lake trout movement studies that have been completed in Lake Michigan and Lake Superior, they hypothesized that lake trout would occupy an area within 80 km of the tagging location. Their results concurred with the prior studies, showing that lake trout recaptured during 1983-1997 in northwestern Lake Michigan did not travel far, but rather remained within a fairly well-defined area with a radius of approximately 68 km. The lake trout tagged in the fall did return to the same spawning reefs in successive years, but it appeared to the researchers that the lake trout occupied the same general area during the whole year, rather than demonstrate distinct movement patterns in the fall. The researchers also noticed that lake trout movement tended to be greater along the western shore than across the open waters of Lake Michigan. The lake trout in the study would only have had to travel 80 km directly east to reach the Michigan shore; however, only nine recaptures were made on the Michigan side, compared to 182 along the western shore at distances more than 80 km. This suggests that areas of open water may separate lake trout stocks in northern Lake Michigan.

The two GLFMSP sites in Lake Michigan represent distinct populations of lake trout. Based upon their locations, it can be assumed that there is very little transfer of fish between the sites. However, there may always be exceptions to the rule. Some factors that could cause lake trout to move further than expected could be increased adult population density, which can lead to increased dispersal radius, spawning, food and environmental conditions. Other movement is simply random. Fish collected at each GLFMSP site in Lake Michigan have most likely integrated and thus are representative of an area of approximately 68 km surrounding the collection site.

Lake Superior

There are two GLFMSP sites in Lake Superior:

- The Apostle Islands in the western half of Lake Superior on the Wisconsin side, and
- Keweenaw Point in the eastern half of Lake Superior on the Michigan side.

Steve Schram, from the Wisconsin Department of Natural Resources (DNR) Bayfield Office, has participated in GLFMSP lake trout collections from the Apostle Islands site since the early 1990s. The Wisconsin DNR has collected the fish in 25-65 feet of water off of the Gull Island Shoal during spawning in mid-October. Recaptures and tag returns from anglers have helped biologists determine that many lake trout stay within the

Gull Island Refuge after spawning, while some travel east to the Keweenaw Peninsula in Michigan. Some lake trout may travel around the Peninsula into Keweenaw Bay. Generally, the fish collected at the Apostle Islands represent the western end of Lake Superior and are not influenced by Duluth Harbor (Schram, 2005). Sean Sitar, from the Michigan DNR in Marquette has assisted in collection of lake trout from the Keweenaw Point site in the past. Michigan DNR has suggested that lake trout in Lake Superior travel about 50 km from their home spawning area. In addition to the physical separation between the two Lake Superior sites, GLNPO may also need to take into consideration the differences between the types of fish in Lake Superior (e.g., siscowet lake trout versus lean lake trout). The Keweenaw Point site has a large siscowet population and these fish tend to live in deeper waters while lean lake trout (collected for GLFMSP) tend to inhabit more shallow waters. A concern exists that siscowets are frequently found in more shallow water and can be mistaken for lean lake trout when collected by inexperienced staff. In order to avoid this situation, Michigan DNR has suggested to GLNPO to take lateral head photographs and whole body shots to create a photographic archive to decrease variability in future collections. At a minimum, fish collectors should be trained to distinguish between the two morphologies before annual collections (Sitar, 2005).

Lake Superior lake trout are mostly wild fish and have homing instincts to return to their spawning reefs every fall. For this reason, fish are collected from the same population every year at each site and those populations are distinct from one another. Historically, before the wild lake trout populations stabilized, some hatchery fish were collected along with wild fish. Although the hatchery fish do not have the homing instincts of the wild fish, they tend to stay in the same general area where they were released so distinct populations were still most likely being collected. Because the diets of the hatchery fish and wild fish are similar (e.g., smelt, whitefish, chubs and herring), the mix of hatchery and wild lake trout most likely did not significantly affect measured contaminant concentrations. A publication studying the movement of lake trout in Lake Superior from 1973 to 2001 (Kapuscinski et al., 2005) suggests that a fair proportion of the fish do not travel long distances, but rather stay within about 42 km of the spawning reef. Some lake trout do travel further distances and may integrate more of the lake. However, based on the observations of scientists in the field and the Kapuscinski manuscript, it can be assumed that each site represents an area of about 50 km.

Lake Huron

There are two GLFMSP sites located in Lake Huron:

- Rockport in the northwest part of the lake, and
- Port Austin in the southwest part of the lake.

Both sites are located on the Michigan side of Lake Huron in U.S. Territory. The Michigan side of Lake Huron is essentially divided into three separate management units and populations of lake trout (MH1 – MH3) (Johnson et al., 2004). MH1 extends from the Straights of Mackinaw south to Rogers City (Northwest of Rockport) and is the coldest part of the lake. Lake trout are slower growing in MH1 due to the limited nutrients associated with this area and seem to have the most lamprey wounds compared to the other two regions. MH2 is located in the Rockport area and extends from Rogers City south to the Black River Harbor (south of Thunder Bay). The GLFMSP site of Rockport is included in this management area. MH3 is located in the Port Austin area and extends from River Harbor to the southernmost point in the lake. The GLFMSP site of Port Austin is included in this management area. There is a gradient across the management units with increasing growth rates and decreasing lamprey wounding rates from north to south. The MH1 fish do not migrate into any other management units (McClain et al, 1998), while the MH2 fish do sometimes migrate to the MH1 area. MH3 fish tend to migrate in a southeasterly direction (McClain et al., 1998), although some do migrate into Saginaw Bay, which is not included in MH3 (Johnson, 2005). There is some mixing between Canadian and U.S. lake trout in the Lake's main basin, but there is no mixing between the Georgian Bay and the North Channel and the main

basin. Lake Huron has a lot of structural diversity which helps explain why the fish are separate populations and do not travel far from their management units.

Lake Erie

There are two GLFMSP sites located in Lake Erie:

- Dunkirk in the eastern part of the lake along the New York coastline, and
- Middle Bass Island in the western part of the state off the coast of Ohio.

Lake Erie can be separated into three distinct basins, which are linked together along an east-west axis and separated by shoals and reefs. The western basin extends from Toledo in the U.S. at the western tip of the lake to Point Pelee in Ontario and is the shallowest basin with an average depth of around seven meters. The central basin extends from Point Pele to Long Point in Ontario and averages about 20 meters in depth. The eastern basin extends from Long Point to Buffalo, NY in the U.S. and has an average depth of about 40 meters. At the time the program was designed, walleye were selected for the GLFMSP due to the limited availability of lake trout. Currently, the lake trout population is thought to have become self-sustaining in the eastern basin of Lake Erie; therefore, lake trout are now being collected in the eastern basin during odd years. Collection of lake trout in Lake Erie allows for data comparison with lake trout collected in the other Great Lakes. (***Note:*** GLNPO continues to evaluate if this change will be permanent to the program.) Lake Erie's sample collection site in the western basin is still not capable of supporting a self-sustaining lake trout population; therefore, walleye are still collected there during even years.

Several tag-recapture studies have been completed over the years examining walleye movement and distribution in Lake Erie. These studies include two New York State Department of Environmental Conservation reports, "Distribution of Marked Walleye in New York Waters of Lake Erie and A Preliminary Examination of Walleye Distribution" (Einhouse and Shepard, 1988) and "Exploitation in the Eastern Basin of Lake Erie Using Tag-Recapture Data" (Einhouse and Haas, 1995). Both of these studies suggest that the eastern walleye occupying New York waters are essentially local and do not seem to stray much from their original spawning sites. The 1995 study, however, also demonstrated that unlike the eastern walleye, the western walleye do tend to migrate large distances and thus contribute to lake-wide fisheries. Large female walleye were typically the segment of the western basin tagged walleye population that has a range extending into eastern Lake Erie. These studies also demonstrated the homing behavior of walleyes to their spawning site each spring. Although the GLFMSP fish sampling teams collect lake trout during their fall spawning season, the walleyes are still migrating during the fall collection and do not return home until the spring to spawn. Because of this migration, it can be assumed that some western basin walleye are collected each fall in the Dunkirk site. The Middle Bass Island collection is most likely composed of western basin walleye, some which have integrated the entire lake. These fish also may have traveled north after spawning into the Detroit River, Lake St. Clair, and into Lake Huron.

Lake Ontario

There are two GLFMSP sites located in Lake Ontario along the New York coastline:

- North Hamlin in the central part of the lake, and
- Oswego in eastern part of the lake.

North Hamlin is located approximately equidistant from the western and eastern coasts near Rochester, N.Y. Oswego is located well east of North Hamlin, near the mouth of the Oswego River. There have been two main studies published regarding lake trout dispersal in Lake Ontario (Elrod, 1987, Elrod, et al., 1996), and both have concluded that most of the lake trout remain in the same general region where they were initially stocked. According to these studies, fish stocked east of the Niagara River rarely crossed the river mouth into Canadian waters west of the river. Also, few lake trout moved across Lake Ontario between the north and south shores, or

between the eastern outlet basin and the main lake basin. North Hamlin is one of the stocking sites in Lake Ontario, and both studies found that fish stocked at North Hamlin tend to disperse both east and west. Joseph Elrod (Elrod, 1987) found that the dispersal was not caused by random swimming movements, but was greatly affected by currents. North Hamlin is one of four south-shore stocking sites, and 84% of the fish stocked at those sites were found within 30 km of where they were stocked (Elrod, 1987). Although Oswego is not one of Lake Ontario's four stocking sites, it does lay between two of the south-shore sites, Sodus and Mexico Bay. Based on the 1996 study, fish stocked at North Hamlin tend to move along most of the southern shore and do not typically travel west of the Niagara River or into the eastern basin of Lake Ontario. Fish stocked near Oswego, also tend to integrate much of the southern shore of Lake Ontario. However, fish stocked at North Hamlin appear to be spending more time integrating the western side of the southern shore, while the Oswego fish appear to spend more time integrating the eastern side of the southern shore (Elrod et al., 1996). It appears that mature lake trout do have some tendencies to return to their stocking sites in preparation for fall spawning. However, this tendency appears to be weak (Elrod et al., 1996).

A7.2 Comparability

The Base Monitoring Program focuses on collection of fish between the ages of six and eight. Size is used as a surrogate to age; therefore fish in a narrow size range (Table 5) are targeted each year. These fish are collected from the same sampling location every other year at approximately the same time of year, allowing for the assessment of trends across years.

A7.3 Project Quality Objectives

The Data Quality Objective (DQO) process can be used for systematic planning and is described in US EPA's document *Guidance for the Data Quality Objective Process* (US EPA, 2006). Generally, 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. Essentially, the DQO design is intended to answer the primary question of the program. It is the responsibility of the GLFMSP Manager to define this allowable uncertainty and develop DQOs with the PIs and cooperators.

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.

Original Project Quality Objectives

GLNPO entered into a cooperative agreement with the USGS-GLSC in 1977 (Appendix C.8 of GLFMSP QMP). This cooperative program built upon an existing USGS-GLSC Lake Michigan lake trout monitoring program that originated in the 1960s. This existing USGS-GLSC program was used to estimate the appropriate

sample size and any resulting uncertainties from collection. The original sampling design stated that a 10% change in contaminant residue levels was statistically significant in a sample size of 40-60 individuals or 12 composites of 10 in the 240-280 mm size range (*Contaminants Surveillance Program for the Great Lakes, Rationale and Design* [Appendix C.6 of GLFMSP QMP)]. The original design of the GLFMSP attempted to balance the USGS-GLSC's sampling design with a limited budget. The resulting goal of the GLFMSP Base Monitoring Program became the ability to detect a 20% change from current contaminant levels by analysis of variance where alpha = .05 and beta = .20 with a minimum collection of 20 fish within a specified size range. The GLFMSP's collection scheme called for the collection of 60 lake trout (or walleye) per site each year in 3 size categories, small (300-450 mm), medium (451-650 mm), and large (>650 mm), with 20 fish in each category. To reduce analytical costs, the fish were grouped into four composite samples consisting of five fish each within each size category. According to Dave DeVault, the original GLNPO coordinator of the GLFMSP, the program was initially designed to compare contaminant levels in fish, both temporally and spatially, in the three size categories collected from each site through analysis of covariance (DeVault *et al*., 1986).

The original sampling design of the GLFMSP was adhered to following the creation of the cooperative agreement. However, the design was not sufficient to meet the program's goal of a 20% detectable change in contaminant concentration between consecutive sampling periods at each site within the 95% confidence interval. In 1979, the analytical and collection designs of the program were revised to use mean statistics for specific size ranges of fish to compare contaminant concentrations between sites and within sites over time.

Deviations from GLFMSP Standard Operating Procedures

Sample Size

The number of fish collected for the GLFMSP may vary from year to year due to unforeseen circumstances when collecting live fish. When fewer than the target of 50 fish (ten composites of five fish) are collected at a site for the Base Monitoring Program, then fewer than the target number of composites are analyzed, while keeping the number of fish per composite constant at five in order to maintain consistency in data at the individual composite level. For example, if only 47 fish are caught at a site in a given year, then nine composites of five fish would be prepared, rather than creating seven composites of five fish and three composites of four fish (or a different alternate scheme). An alternative approach may be considered if the number of fish collected is significantly lower than the target. Variability in the data should be taken into account when making final decisions.

The original sampling design for the GLFMSP Base Monitoring Program called for the collection of walleye from Lake Erie in the 400 – 500 mm size range (DeVault *et al*., 1996). However, according to the USGS QAPP for sample collection, *Monitoring Trends of Selected PCB Congeners and Pesticides in Fish from the Great Lakes, 1991, 1992, and 1993* (Appendix B.4 of GLFMSP QMP), the range for walleye collection was between 450 and 550 mm. USGS was responsible for fish collections for the GLFMSP from 1977 through 2003 and performed chemical analysis for the program in the early and mid 1990s. Historical data shows that the mean walleye length falls below the 450-550 mm size range in the years 1977, 1978, 1979, 1980, 1981 and 1982 and that the mean length values have never been above 500 mm. Thus the data supports a walleye size range of 400-500 mm as documented by DeVault in his 1996 manuscript. However, mean walleye length does range between 450 – 550 mm for 2003 and 2004 fish and returns to the 400 – 500 mm mean length in the following years. This deviation occurred following the introduction of a new program manager to the GLFMSP in 2003, the dissolution of the USGS – GLSC cooperative agreement with US EPA, and the incorrect quality documentation in the USGS QAPP.

Analytical Methods

Prior to the creation of the GLFMSP, USGS-GLSC was collecting and analyzing Lake Michigan lake trout for their own program (1960 – 1976). These data (1972 – 1976) are included in the GLFMSP long-term trend for Lake Michigan. It is important to note, however, that the methods were different for these five years of analysis and that individual whole fish between 500-700 mm total length were analyzed instead of composites (Willford *et al*., 1976).

Current Project Quality Objectives

The GLFMSP is a long-term trends program of exceptional value, providing documentation of changes in contaminant levels in the Great Lakes ecosystem. A DQO was developed for the GLFMSP Base Monitoring Program to help guide the program's sampling and analytical efforts and maintain consistent monitoring, and was reflective of the high and changing concentrations of contaminants in fish. The original DQO for the GLFMSP stated that the program should be able to detect a 20% change between consecutive sampling periods at each site within the 95% confidence interval.

Current concentrations of total PCBs in lake trout appear to be reaching a steady state, with tissue concentrations at or below 2mg/kg. As a steady state is approached, year to year changes in tissue concentration become more difficult to detect because these changes are small. For example, in 1999, the mean concentration in Lake Superior lake trout from the Keweenaw Peninsula was .27 mg/kg. In order to meet the original DQO, the GLFMSP would have to be able to detect a .05 mg/kg difference between two sampling periods. This would be difficult even with the best methodology and a large sample size.

To address this issue, a GLFMSP review (Appendix C.9 of GLFMSP QMP) was conducted in February of 2005. Great Lakes stake holders, the current PI for the GLFMSP, and previous and present GLFMSP managers were in attendance. A recommendation to conduct statistical power analysis on the GLFMSP in order to revise and/or develop DQOs was presented to GLNPO management. In order to address the recommendation of the program review panel, GLNPO has revised its DQO for the GLFMSP Base Monitoring Program. The revised DQO for the GLFMSP is described in the *Great Lakes Fish Monitoring and Surveillance Program Data Quality Objective Revision Report* and is to have an **80% probability of detecting a 10% change in contaminant concentration per year over a three to four year sampling period at the 95% confidence level using log transformed data** (Appendix B.1 of GLFMSP QMP).

The CSMI/Special Studies Program is directed at addressing issues raised by the other components of the GLFMSP and supports additional research that will improve our understanding of pollutant impacts on the fishery and help properly gauge the efforts of remediation and pollutant reduction efforts in the Great Lakes. The CSMI/Special Studies Program was not designed to meet a specific DQO. Rather, it was designed to provide data needed to supplement the Base Monitoring Program and improve our understanding of pollutant cycling in the Great Lakes. The biological structure and composition of food webs are important in determining the flow of energy, nutrients, and ultimately contaminants through ecosystems.

MQOs for the data generated in support of the GLFMSP are summarized in Clarkson University's QAPP, *The Great Lakes Fish Monitoring and Surveillance Program: Pushing the Science (GLFMSP)* (Holsen *et al*., 2012)(Appendix A.4 of GLFMSP QMP). No single measurement parameter is critical to success of this project. 90% completeness is the goal of this project.

A7.4 Sampling Quality Objectives

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. 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.

Fish lengths are recorded to the nearest mm (or sixteenth of an inch) by the field personnel (when applicable) using hand-held rulers. Field sampling teams are instructed not to use cooler lids or other less accurate means. All fish collected for the Base Monitoring Program and lake trout and walleye collected for the CSMI/Special Studies Program are weighed to the nearest gram (if applicable). Forage fish collected for the CSMI/Special Studies Program are not measured. An aggregate weight (to the nearest gram) is collected for each species of forage fish.

Base Monitoring Program

The goal of this sample collection effort is to collect 50 fish (lake trout or walleye) in specific size ranges at each designated sampling location in all five lakes. Each lake contains two sampling sites, one representing an industrial area and one representing a non-industrial area. Chosen sites 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. These locations are sampled alternately each year at approximately the same time of year.

While it is understood that both size and age affect contaminant concentrations, resource limitations have 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 5) are designed to meet this goal.

Composites of each species, consisting of five individual fish, are analyzed for contaminants to assess temporal trends in organic contaminants and mercury in the open water of the Great Lakes. 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 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.

CSMI/ Special Studies Program

CSMI/Special Studies Program collection efforts include the collection of ten individual lake trout or walleye (depending on the sampling location) in specific size ranges per site at both sampling locations in the LOY (20 samples total).

Stomach contents and eggs are collected from the individual fish. The purpose of collection of stomach contents is to assist in the evaluation of the movement of contaminants in complex Great Lakes food webs. This requires data on pollutant concentrations and fluxes (diet) for the top predator (lake trout) and the prey species at the supporting lower trophic levels. The eventual body burden of contaminants in the individual fish depends on the feeding preferences and food availability at lower trophic levels and the contaminant burden of each prey species. The goal of the stomach gut analysis is to physically assess diets of lake trout (species composition by weight) and characterize the availability of macro invertebrates, zooplankton, and phytoplankton. Results from this analysis will provide critical information to quantify the movement of contaminants from their source through the food web to the final concentrations in lake trout. The biological, physical, and chemical information compiled by this project will provide a lake-specific and comprehensive assessment of factors effecting long-term monitoring of top predator fish contaminant bioaccumulation.

The purposes of the collection and analysis of contaminant levels in the fish eggs and associated parent fish are to evaluate the relationship of parent-egg contaminant levels, potentially identify new emerging contaminants, assess critical contaminant trends, and support LaMPs. Eggs are separated when the stomachs are being removed for fish gut analysis, and analyzed. This long-term data is used to assess if "batch egg samples" can act as a surrogate for long-term trends of fish contaminant loads. The number of egg samples collected is determined by the number of gravid females collected (maximum 10).

CSMI/Special Studies Program collection efforts also include the collection of forage fish species (e.g., bloaters, siscowets, sculpins, ciscos, smelts, etc.). The purpose of the collection of forage fish is to better interpret long-term monitoring for contaminants in lake trout and walleye, which is dependent on changes in prey types and overall food web structure.

A.8 Special Training/Certification

The GLFMSP Manager is required to take a grants management refresher training course every three years.

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 GLFMSP Manager 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 GLFMSP Manager 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.

A.9 Documents 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, chain-of-custody record, and sample labels.

Field sampling personnel are required to submit to GLNPO a field recording form and chain-of-custody record (Appendix B) at the time that the samples are shipped to the homogenization or analytical 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 or analytical laboratory. Field sampling teams are provided with *Packing and Shipping Instructions for Great Lakes Fish Monitoring and Surveillance Program* (Appendix C) that provides information on filling out the required forms. Field recording forms and chain-of-custody records for Base Monitoring Program and CSMI/Special Studies Program collections are located in Appendix B.

Base Monitoring Program

The field recording form is designed to capture a unique tracking number for each fish composite collected for the Base Monitoring Program. This tracking number is used by GLNPO and the homogenization and analytical laboratories to identify each composite and report results. The field sampling teams generate the composite IDs for the fish collected for the Base Monitoring Program. Field sampling teams record composite IDs on sample labels and field recording forms and chain-of-custody records.

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.).

Sample specific data is recorded on labels and adhered to each sample. The type of data recorded for the Base Monitoring Program fish collection efforts is detailed in the attached SOPs and their associated field recording forms (Appendix A.1). Table 4 provides a list of types of data recorded for each fish collected for the Base Monitoring Program.

Table 4. Types of Field Data Recorded for the Base Monitoring Program.

Data Type	Measurement Units or Allowed Entries
Lake Name	Erie, Huron, Michigan, Ontario, Superior
Collector Identification	Vessel and collector's name
Collection Date	MM/DD/YY
Fish Length	Millimeters (mm) or inches, total length
Fish Weight	Grams
Composite ID	First Letter of Lake Name [e.g., LS = Lake Superior], Fish Species, Year Fish Collected, Grid #, Composite # [e.g., 001, 002, etc.] Example: 1st composite of Lake Superior lake trout collected from grid # 1028 in year 2011 = LSLakeTrout20111028001

CSMI/Special Studies Program Individual Fish, Eggs, and Stomach Contents

The field recording form is designed to capture a unique tracking number for each individual lake trout or walleye and associated eggs and stomach contents collected for the CSMI/Special Studies Program. This tracking number is used by GLNPO and the homogenization and analytical laboratories to identify each individual fish and report results. The field sampling teams generate the individual fish IDs. Field sampling teams record individual fish IDs on sample labels and field recording forms and chain-of-custody records.

Note: Individual fish and their associated eggs and stomach contents collected for the CSMI/Special Studies Program are not composited and therefore, do not require a composite ID.

The tracking number or individual fish 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 name (Lake Trout or Walleye),
- The four digit year of collection,
- The grid number, and
- A sequential number indicating the number of each fish from a specific location and year (e.g., F001, F002, F003, etc.).

Sample specific data is recorded on labels and adhered to each sample. The type of data recorded for the individual fish, eggs, and stomach contents collected for the CSMI/Special Studies Program is detailed in the attached SOPs and their associated field recording forms (Appendix A.2). Table 5 provides a list of types of data recorded for each individual fish collected for the CSMI/Special Studies Program.

CSMI/Special Studies Program Forage Fish

The field recording form is designed to capture information about forage fish collected for the CSMI/Special Studies Program at a given site. Field Sampling teams fill out field recording forms for forage fish, but the homogenization laboratory generates composite IDS and individual fish IDs for these fish per the requirements

in their SOW. The IDs generated by the homogenization laboratory are used by the homogenization laboratory, GLNPO, and the analytical laboratory to identify each fish and composite an report results. Field sampling teams record composite IDs on sample labels and field recording forms and chain-of-custody records.

The tracking number or composite ID generated by the homogenization laboratory includes the following:

- A two character code for lake (LH),
- The fish species name (e.g., DeepwaterSculpin),
- The four digit year of collection (2012),
- The grid number or port code (1413 = Port Austin, 710 = Rockport), and
- A sequential number indicating the number of each composite from a specific location and year (e.g., 001, 002, etc.).

The individual fish ID consists of the composite ID followed by F1, F2, F3 etc. representing each specific fish in that composite.

Coded wire tags (CWTs), otoliths, scales, and fin clips found in any fish specimens are removed by the homogenization laboratory and shipped to the QA contractor. CWTs, otoliths, scales and fin clips removed from any of the fish samples will be reviewed against the field documentation. The QA contractor will maintain the fin clips for five years. The GLFMSP Manager will resolve any discrepancies between the tags and the field documentation with the field sampling team.

All records and reports pertaining to sample collection are sent to the GLFMSP Manager, as soon as collection has been completed. All field records and electronic correspondence are retained by the GLFMSP Manager for at least five years. All study reports and documentation are retained by the GLFMSP Manager.

B.1 Sampling Process

Field sampling teams collect samples for the Base Monitoring Program and CSMI/ Special Studies Program according to the attached SOPs (Appendix A) and ship them to the homogenization or analytical laboratory.

This section provides details on the sampling process design for Base Monitoring Program and CSMI/Special Studies Program fish collections.

B1.1 Sample Number and Type

Due to expensive contaminant analyses, the goal for sample size when the GLFMSP was created was to find the least number of samples necessary to detect statistically annual changes in contaminant concentrations. Decreasing the variability between replicate samples was one way to increase the ability to detect change. The use of whole fish was recommended as a way to decrease both biological and analytical variance. Biological variance was less for whole fish, because while there were seasonal differences in contaminant concentrations in various fish body tissues, total body burden varied little on a seasonal basis. Analytical variance can be introduced through sample preparation, including filleting, packaging, homogenization, etc., due to human error or inconsistent technique.

Shortly after the creation of the GLFMSP, it was decided that fish would begin to be collected according to size as an indication of age (see GLFMSP significant events, Significant Events of the Great Lakes Fish Monitoring and Surveillance Program [Appendix D.1 of GLFMSP QMP]).At the time that the GLFMSP was created, appropriate size data were limited. The original design of the program called for extensive sampling in the first year to establish a statistically reliable sampling protocol. The most reliable data available at the time were produced by the USFWS (Willford, 1982). These data indicated a 10% change in contaminant residue levels was statistically significant in a sample size of 40–60 individuals or 12 composites of 10 in the 240–280 mm size range (*Contaminants Surveillance Program for the Great Lakes, Rationale and Design* [Appendix C.6 of GLFMSP QMP]).

The original design of the GLFMSP attempted to balance the USGS-GLSC's sampling design with a limited budget. The GLFMSP's collection scheme called for the collection of 60 lake trout with 20 fish in the small category (300–450mm), 20 fish in the medium category (451–650 mm) and 20 in the large category (>650 mm). Both a spatial and temporal comparison of samples was to be conducted using all three size categories of fish using analysis of covariance techniques. Unfortunately, the data did not meet the requirements for the test and the original design had to be abandoned and was replaced by the use of mean statistics with specific size ranges of fish. Lake trout in the larger size range (>650 mm) were not available in Lake Ontario until 1982. Smaller lake trout were collected at the Lake Ontario sites prior to 1982, and the resulting data were probably skewed lower in contaminant concentrations than they would have been if fish of the appropriate size had been available.

In 1982, a final program design for the Base Monitoring Program was adopted. This program design called for fish between the ages of six and eight to be collected. Size is used as a surrogate to age; therefore fish in a narrow size range are targeted each year. 50 lake trout in the size range of 600 mm to 700 mm are collected from Lakes Huron, Michigan, Ontario, and Superior each year. 50 walleye in the size range of 400 mm to 500 mm are collected from Lake Erie each year. Beginning in 2011, lake trout began to be collected in the Eastern basin sampling site of Lake Erie during odd years. For each lake, these 50 fish are grouped into 5 composites of 10 whole fish.

For the CSMI/Special Studies Program, ten individual lake trout or walleye (depending on the sampling location) are collected per site at both sampling locations in the LOY (20 samples total). Lake trout in the size range of 600 mm to 700 mm and walleye in the size range of 400 mm to 500 mm are collected. Stomachs are collected from each fish. The number of egg samples collected is determined by the number of gravid females collected (maximum 10).

CSMI/Special Studies Program collection efforts also include the collection of forage fish species (e.g., bloaters, siscowets, sculpins, ciscos, smelts, etc.). At both sampling locations in the LOY, forage fish of varying species are collected. Ideally at each site, 30 fish of each of the three most abundant forage fish species and 10 fish of

the fourth and fifth most abundant forage fish species (110 fish total) are collected. The purpose of the collection of forage fish is to improve our understanding of the food availability at lower trophic levels and the contaminant burden of each prey species.

The species, size ranges of samples (if applicable), number of samples per location, and number of locations for each collection effort are listed in Table 5.

Table 5. Number and Type of Samples Collected

Collection Effort	Species	Size	Samples/Location	Number of Locations
Base Monitoring Program	Odd years: Lake trout in all lakes Even years: Lake trout in Lakes Huron, Ontario, Superior and Michigan and walleye in Lake Erie	Lake Trout: 600 to 700 mm Walleye: 400 to 500 mm	50	1 per lake (5 total)
CSMI/Special Studies Program Individual Fish, Eggs, and Stomach Contents	Lake trout	600-700 mm	10	2 in LOY
CSMI/Special Studies Program Forage Fish	Any forage fish species (Ideally at each site, the 5 most abundant forage fish species)	All sizes	30 fish of each of the three most abundant forage fish species and 10 fish of the fourth and fifth most abundant forage fish species (110 fish total)	2 in LOY

Fish collected for the Base Monitoring Program and forage fish collected for CSMI/Special Studies Program are grouped into composites prior to shipment to the homogenization laboratory (***Note:*** Individual fish collected for the CSMI/Special Studies Program are homogenized individually and therefore are not grouped into composites). Base Monitoring Program fish grouped together for a composite sample must meet the criteria listed in Section B2.3.

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 B),
3. review of field documentation by the GLFMSP Manager, and
4. notification of the GLFMSP Manager 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.

B1.2 Selection of Sites within Lakes for Sampling

Selection of sampling sites is based on the availability of fish populations. Each lake contains two sampling sites, one representing an industrial area and one representing a non-industrial area. Chosen sites 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.

Sampling locations for each lake are identified in Table 6. These locations are sampled alternately each year for the Base Monitoring Program (i.e., one site is sampled during even years and one site is sampled during odd years). Both sampling sites in the LOY are sampled for the CSMI/Special Studies Program. Each sampling site has been identified with a grid number 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. Fish Sampling Locations for the GLFMSP

Lake	Grid	Site	Year	Longitude	Latitude
Michigan	2210	Saugatuck	E	86°25'	42°35'
Michigan	906	Sturgeon Bay	O	87°15'	44°45'
Huron	1413	Port Austin	O	82°45'	44°05'
Huron	710	Rockport	E	83°15'	45°15'
Erie	904	Middle Bass Island	E	82°55'	41°35'
Erie	424	Dunkirk	O	79°35'	42°25'
Ontario	713	North Hamlin	O	77°55'	43°25'
Ontario	623	Oswego	E	76°15'	43°35'
Superior	1028	Keweenaw Pt.	O	87°35'	47°25'
Superior	1311	Apostle Islands	E	90°25'	46°55'

E = even year collection
O = odd year collection

B1.3 Sampling Period

Because there are seasonal variations in body burdens of fish, it was determined that collections needed to be made at the same time every year. The fall was suggested as a time when there would be the greatest availability of fish and also the least likelihood of shifts in body burdens of contaminants caused by spawning, except by fall spawners.

When the GLFMSP was created, the IJC recommended that at a minimum, sampling occur annually at four locations offshore in late summer and fall, until after a baseline had been established. Once enough data had been collected for a baseline, the sampling could occur every two years. In order to address locally impacted

nearshore areas or suspected impacted nearshore areas, it was recommended that these sites be monitored annually. Control sites were to be monitored annually for comparison purposes.

Currently for the Base Monitoring Program, sampling sites in each lake are sampled alternately each year at approximately the same time of year. Table 7 details species collected by lake and even or odd year for the Base Monitoring Program.

Research efforts are expanded in one lake each year for the CSMI/Special Studies Program. The LOY is chosen based on a schedule proposed by the CSMI and both sites in the LOY are sampled each year.

Sample collection activities commence in June and are completed before November. Because the field sampling teams establish their schedules individually, this time frame can change based on local conditions such as weather.

B.2 Sampling Methods

B2.1 Target Species

Base Monitoring Program

According to the *Contaminants Surveillance Program for the Great Lakes, Rationale and Design* (Appendix C.6 of GLFMSP QMP), when the GLFMSP was created, the selection of species to be sampled was dependent upon several different factors including population distribution, availability, longevity, contaminants to be monitored, representativeness, lake to lake comparison, and importance in commercial and sport fisheries. Overall, eight species were considered as strong candidates: smelt, yellow perch, walleye, carp, alewife, lake trout, whitefish and coho salmon.

The GLFMSP species collection originally included lake trout (walleye in Lake Erie and Lake St. Clair) and smelt. However, smelt collection was never fully funded or implemented. Sport fish were added to the program in the early 1980s (but discontinued in 2009). Several factors played a role in the selection of lake trout for the GLFMSP. Lake trout are representative of the offshore zone in the three upper lakes, but not in Lakes Erie or Ontario due to the fact that the populations sampled are relatively local to their spawning areas. Lake trout are top predators and long lived and were considered to be excellent concentrators of contaminants. Walleye were originally chosen to be collected in Lake Erie because they have similar characteristics to lake trout and were available in greater abundance. Beginning in 2011, lake trout began to be collected in the eastern basin sampling location in Lake Erie during odd years because the lake trout population was thought to have become self-sustaining. Walleye continued to be collected in the western basin sampling location in Lake Erie which was still not capable of supporting a self-sustaining lake trout population. Collection of lake trout in Lake Erie allows for data comparison with lake trout collected in the other Great Lakes (Note: GLNPO is still evaluating if this change will be permanent to the program).

Lake trout and walleye serve as biomonitors for select contaminants to determine general trends and to provide support to the research community and the public through collection of high quality data using identified and approved methodology. These data also can be used to assess the risks of such contaminants on the health of this important fishery, and on wildlife that consume them.

CSMI/Special Studies Program

The CSMI/Special Studies Program is designed to provide data needed to supplement the Base Monitoring Program and improve our understanding of pollutant cycling in the Great Lakes. Individual lake trout or walleye (depending on the sampling site) and their associated eggs and stomach contents are collected to assist in the evaluation of the movement of contaminants in complex Great Lakes food webs. Stomach contents provide information on pollutant concentrations and fluxes (diet) for the top predator and the prey species at the supporting lower trophic levels. The eventual body burden of contaminants in lake trout and walleye depends on the feeding preferences and food availability at lower trophic levels and the contaminant burden of each prey species. The purposes of the collection and analysis of contaminant levels in the lake trout eggs and associated parent fish are to: evaluate the relationship of parent-egg contaminant levels, potentially identify new emerging contaminants, assess critical contaminant trends, and support lake-wide management plans.

CSMI/Special Studies Program collection efforts also include the collection of forage fish species (e.g., bloaters, siscowets, sculpins, ciscos, smelts, etc.). Collected forage fish provide enough biological mass from species that comprise a typical lake trout diet to assist in the determination of legacy and emerging contaminants.

Every effort will be made to collect the desired species and number of fish specified in Section B1.1. 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 Records* (Appendix B).

B2.2 Sample Collection

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.

Field sampling personnel choose the collection method and 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.

Forage fish collected for the CSMI/Special Studies Program are collected by trawling and other methods, as specified by the field sampling team leader. Collection techniques for individual lake trout or walleye collected for the CSMI/Special Studies Program can vary due to the change in lake sampled each year.

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 waters of the Great Lakes. Nontarget species, collected by the field sampling team should be returned to the water. Field sampling team personnel are instructed to wear clean nitrile gloves (provided by GLNPO if not already available) 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 described in Section B3.2.

Each fish collected for the Base Monitoring Program and individual lake trout or walleye collected for the CSMI/Special Studies Program is measured to determine total body length (mm). (***Note:*** Forage fish are not measured in the field.) 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). Other physical measurements are recorded according to the procedures detailed in the sample collection SOPs (Appendix A).

When collection efforts are complete, fish are wrapped in acetone-washed foil, placed in polyethylene tubing, placed in large composite bags (if applicable) according to the sample collection SOPs, and immediately placed in freezer at -20°C for temporary storage. Sample handling procedures are detailed in Section B3.1.

B2.3 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. Composite samples will be prepared and analyzed for fish collected for the Base Monitoring Program and forage fish collected for the CSMI/Special Studies Program. Composites for the Base Monitoring Program will consist of five fish each. Composites for forage fish collected for the CSMI/Special Studies Program will consist of varying number of fish, as the goal of this study component is to provide enough biological mass from species that comprise a typical lake trout diet to assist in the determination of legacy and emerging contaminants. Composites are intended to estimate the mean fish tissue contaminant concentration for the lake for each target parameter. All fish will be homogenized as whole fish with guts intact. Base Monitoring Program 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 (for the Base Monitoring Program), 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.

Note: Individual fish and their associated eggs and stomach contents collected for the CSMI/Special Studies Program are homogenized individually.

B.3 Sample Handling and Custody

B3.1 Sample Handling

This section describes sample handling procedures. Instructions for sample handling also are provided in the fish collection SOPs in Appendix A and in the *Packing and Shipping Instructions for the Great Lakes Fish Monitoring and Surveillance Program* in Appendix C.

When fish are collected, they are identified to species by experienced personnel knowledgeable of the taxonomy of species in the Great Lakes. Field sampling personnel wear clean nitrile gloves to handle fish and sample handling equipment. All fish must be whole and without incisions. Individual fish are rinsed in ambient water to remove any foreign material from the external surface and placed in clean holding containers (e.g., live well, buckets, etc.) to prevent contamination. The buckets are cleaned according to the procedures described in Section B3.2. Each individual fish is placed in a separate container in order to avoid any contamination from other fish, fuels, or other sources. The importance of this procedure is emphasized to the field sampling teams. When the fish are brought ashore they are prepared for storage and shipment to the homogenization laboratory.

For all collection efforts, 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 ≤ -20°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 GLFMSP Manager has specified a shipment date. All records and reports pertaining to sample collection should be sent to the GLFMSP Manager, as soon as collection has been completed.

Base Monitoring Program

As soon as possible after collection, fish are wrapped in acetone-washed foil and immediately placed in pre-cut heavy-duty polyethylene tubing. Each sample is labeled with the information described in Section A9 and also in the GLFMSP Base Monitoring Program Sample Collection SOP in Appendix A.1. 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 B2.3, the individually bagged fish can be combined in groups of five into large composite bags.

CSMI/ Special Studies Program

<u>Individual Fish, Eggs, and Stomach Contents</u>

As soon as possible after collection, parent lake trout are placed on acetone-washed foil. All eggs and the entire stomach gut contents are harvested from each individual fish according to the procedure described in the GLFMSP CSMI/Special Studies Program Collection of Individual Fish, Eggs, and Stomach Contents SOP in Appendix A.2. Eggs and stomach contents from each fish are transferred each to separate jars. The parent fish and jars of eggs and stomach contents are each labeled according to the details provided in Appendix A. Each parent fish is then wrapped in acetone-washed foil and placed in pre-cut heavy-duty polyethylene tubing. Individually bagged fish are then combined into groups of five in large composite bags.

Forage Fish

As soon as possible after collection, forage fish are grouped by species, wrapped in acetone-washed foil and immediately placed in pre-cut heavy duty polyethylene tubing. The exact number of specimens in each group varies. Each group of fish is labeled with the information described in the GLFMSP CSMI/Special Studies Program Collection of Forage Fish SOP in Appendix A.3.

B3.2 Sample Integrity

A critical requirement of the GLFMSP 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.

B3.3 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 described in Section A9 and in the field collection SOPs (Appendix A). Base Monitoring Program fish, CSMI/Special Studies Program forage fish and CSMI/Special Studies program individual fish and stomach contents are then shipped on dry ice to the homogenization laboratory. Fish eggs are shipped on dry ice directly to the analytical laboratory.

Field sampling personnel are required to submit to GLNPO a field recording form and chain-of-custody record (Appendix B) 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 Fish Monitoring and Surveillance Program (Appendix C) that provides information on filling out the required forms.

When fish are shipped to the homogenization laboratory, the QA contractor notifies the laboratory of the shipment, and any tracking numbers associated with the shipment are provided. Chain-of-custody forms accompany all shipments. 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.

When fish are shipped to the analytical laboratory, the QA contractor notifies the GLFMSP Manager and the laboratory of the shipment, and any tracking numbers associated with the shipment are provided. Chain-of-custody forms accompany all shipments. 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.

When fish are shipped to the archival facility, the QA contractor notifies the archival facility, and any tracking numbers associated with the shipment are provided. Chain-of-custody forms accompany all shipments. The archival facility is instructed to confirm receipt of shipment in writing. The archival facility also notes on this confirmation if the fish arrived frozen and in good condition.

When CWTs, scales, otoliths, and fin clips are shipped to the QA contractor, the QA contractor reviews the chain-of-custody form to ensure all samples have been received in good condition.

B.4 Analytical Methods

After processing at the homogenization laboratory, fish homogenate samples are shipped to the analytical laboratory. Sample processing and analytical testing are discussed in the PI's approved QAPP: *The Great Lakes Fish Monitoring and Surveillance Program: Pushing the Science (GLFMSP)*(Holsen *et al*., 2012) (Appendix A.4 of GLFMSP QMP).

B.5 Quality Control

Sample Collection Quality Control

Data quality is addressed by use of knowledgeable field sampling teams, 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 GLFMSP Manager annually reviews the study requirements, sample collection procedures, and documentation with all field personnel and verifies that they do not have any questions.

The accuracy of the taxonomic identification in the field 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.

Fish lengths are recorded to the nearest mm (or sixteenth of an inch) by the field personnel (when applicable) using hand-held rulers. Field sampling teams are instructed not to use cooler lids or other less accurate means.

All lake trout and walleye are weighed to the nearest gram. An aggregate weight (to the nearest gram) is collected for each species of forage fish.

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 A9. After review, the leader signs the field recording form indicating their review and approval of the documentation.

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 (Appendix B). 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 grid number where each fish was collected (grid numbers are listed in Table 6) is documented by field personnel. Additionally, the latitude and longitude where each fish was collected also are documented as a means of verifying the grid where the fish was collected (latitude and longitude coordinates are listed in Table 6). Latitude and longitude coordinates are recorded for each sampling location using Global-Positioning Systems (GPS).

The field personnel also are required to document other data as described in Section A9. To ensure accurate documentation, GLNPO will discuss the SOP and specifics regarding the field data with field personnel prior to sampling.

Additional Quality Control Checks

The GLFMSP Manager reviews the records submitted by the field personnel. If any discrepancies or questions arise, the GLFMSP Manager 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 A9) and documentation.

Accurate taxonomic identification is essential in assuring and defining the organisms that have been composited and submitted for analysis. The homogenization laboratory is instructed to notify the QA contractor if any fish targeted for a single composite do not appear to be the same species. The QA contractor then notifies the GLFMSP Manager. The GLFMSP Manager will resolve any discrepancies with the field sampling teams.

Fish lengths, weights, and other physical data recorded in the field are measured in the homogenization laboratory as a QC check for the field data. The homogenization laboratory is instructed to notify the QA contractor if any lab data do not appear to match field data. The QA contractor then notifies the GLFMSP Manager. The GLFMSP Manager will resolve any discrepancies with the field sampling teams.

Fish that are collected outside the acceptable length range will be flagged in GLENDA using Llong and/or Lshort in the Field Remark Code field of the field sample worksheet.

CWTs, fin clips, scales, and otoliths found in fish specimens are removed by the homogenization laboratory according to their SOW and shipped to the QA contractor. The QA contractor reviews all fin clip information against the field documentation and ensures that all CWTs, fin clips, scales, and otoliths are received. The GLFMSP Manager will resolve any discrepancies with the field sampling teams.

Based on the availability of funding, fish collected for the Base Monitoring Program are supplemented with control fish collected from inland lakes as designated by the PI. Theoretically, these control fish have been

exposed primarily to contaminants deposited into the lake through atmospheric deposition. Comparing concentrations in these control fish to the Great Lakes fish helps determine sources of the compounds that are identified and the relative importance of atmospheric deposition. These control fish are treated like normal sample and analyzed for both legacy and emerging contaminants by the analytical laboratory.

B.6 Instrument/Equipment Testing, Inspection, and Maintenance

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.

B.7 Instrument/Equipment Calibration and Frequency

Field sampling teams ensure scales are calibrated according to manufacturer requirements prior to the collection of physical measurements (weight) of each fish.

B.8 Inspection/Acceptance of 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 recording 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.

B.9 Non-Direct Measurements

Non-direct measurements are not used in the sample collection component of this study.

B.10 Data Management

Samples will be labeled and tracked via sample identification labels and field recording forms and chain-of-custody records as detailed in Section A9. Because the sampling efforts are cooperative and involve 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 A9. Any corrections should be noted, initialed, and dated by the reviewer. Shipment of samples to the homogenization laboratory (Section B3) must be conducted by a delivery service that provides constant tracking of shipments (e.g., Federal Express). Sampling data are documented on field recording forms by field sampling teams and generated by the homogenization laboratory. All records and reports pertaining to sample collection are sent to the GLFMSP Manager as soon as collection has been completed. All field records and electronic correspondence are retained by the GLFMSP Manager for at least five years. All study reports and documentation are retained by the GLFMSP Manager.

Laboratory sample receipt and document control, records, and information management procedures are discussed in *The Great Lakes Fish Monitoring and Surveillance Program Quality Management Plan* (US EPA, 2012) and in Clarkson University's QAPP: *The Great Lakes Fish Monitoring and Surveillance Program: Pushing the Science (GLFMSP)* (Holsen *et al*., 2012) (Appendix A.4 of GLFMSP QMP).

C.1 Assessment and Response Actions

External and internal audits will be carried out to evaluate the project progress and the adherence to QAPP. External audits will be carried out as directed by GLNPO. Internal audits (i.e., systems audits, performance evaluations and data audits) will be carried out to monitor the program in terms of the degree of adherence to QAPP and procedures. Internal audits are conducted by the GLNPO, US EPA Quality Staff, Inspector General and others, who are independent of the area to be evaluated. QA audit assessment procedures are described in the following. Furthermore, we will seek the consultancy or suggestions of experts in this field to better carry out the project evaluation.

System Audits

Due to the voluntary participation of the field sampling teams supporting the fish collection efforts, the assessment and response actions for field work are limited. The GLFMSP Manager will serve as the primary contact for any questions or issues that arise from the field sampling teams. The GLFMSP Manager will contact GLNPO scientists or the PI if needed to provide recommendations to field personnel. The GLFMSP Manager, with support from the GLNPO Quality Manager, is responsible for corrective actions and the GLFMSP Manager is responsible for ensuring corrective actions are verified and documented.

If during review of the field documentation or through communication with the samplers, the GLFMSP Manager 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 obtain additional samples if possible.

The PI will be notified of any issues that arise 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.

The GLFMSP Manager makes the following assessments:

- Completeness of the chain-of-custody forms,
- Safe storage of the documents and backup of the computer files, and
- Any correction actions and the results.

Expert Reviews

Discussions with other researchers experienced in similar studies can be valuable for identifying overlooked problems. This will be arranged via various ways of communication. Review of the comments and suggestions obtained will be discussed by the project participants, and when applicable, actions will be taken accordingly.

C.2 Reports To Management

The GLFMSP Manager provides project status to GLNPO management (GLNPO Quality Manager, MIRB Chief, and MIRB staff members) at regularly scheduled meetings or at least once per month. The reports will document results of performance evaluations and audits, results of periodic data quality assessments, and any significant QA problems.

The analytical laboratory is required to fulfill reporting requirements as defined in the special conditions of the grant agreement including annual and semi-annual reporting and ensuring the Great Lakes Accountability System (GLAS) is up to date and accurate. The PI provides progress reports quarterly and final reports yearly. Data is released according to the *Great Lakes Fish Monitoring and Surveillance Program Data Release Guidelines* (Appendix C.10 of GLFMSP QMP). Journal publications serve as the method for final data reporting.

The QA contractor fulfills the requirements in the *Quality Assurance Project Plan for CSC Support to the Great Lakes Fish Monitoring and Surveillance Program* (CSC, 2011) (Appendix A.2 of GLFMSP QMP) which include providing the GLFMSP Manager with monthly updates on project status.

D.1 Data Review, Verification, and Validation

The QA contractor updates and provides the field sampling teams with a hardcopy version of the field recording form on an annual basis. The field sampling teams complete the recording form and typically submit the form via facsimile. There are instances where the field sampling teams do not utilize the program field recording form but provide the necessary data elements using their own reporting forms. Field sampling team leaders will be responsible for reviewing all completed documentation as described in Section A9. Any corrections should be noted, initialed, and dated by the reviewer.

Upon receipt of the completed field recording forms, the QA contractor conducts a 100% completeness check to ensure all necessary information has been provided. The following checks are performed:

- Assure all individual fish samples are included in both the Sample Collection and Individual Organism files.
- Assure all composite samples are included in both the Sample Collection and Multiple Organism files.
- Assure the link between all individual fish and the composites are defined in the Sample Group file.

- Assure all collected species of fish are identified in the Class Definition file, and all defined species were collected.
- Assure all sample collections are defined in the Station Visit file, and all defined station visits have associated samples.

Instances where the data are missing or illegible are raised to the field sampling team for resolution. All resolutions are documented.

The QA contractor processes and formats the field data submitted by field sampling teams to the GLENDA reporting standard, as documented at http://www.epa.gov/glnpo/monitoring/data_proj/glenda/rptstds/index.html. This ensures capability that data can be directly imported to GLENDA and ensures the comparability of field data collected in multiple years. The data is formatted using the GLENDA Allowable Codes (http://www.epa.gov/glnpo/monitoring/data_proj/glenda/codes/codes.html).

The GLFMSP Manager 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 GLFMSP Manager 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 to the QA contractor. Fin clips and coded wire tags removed from any of the fish samples will be reviewed against the field documentation. The GLFMSP Manager will resolve any discrepancies with the field sampling team.

The fish homogenization laboratory provides the laboratory lengths and weights for each fish in their final report. The QA contractor enters these data into the GLENDA files for the applicable sampling year. The fish homogenization laboratory's final report also provides the field lengths and weights for each fish. The QA contractor conducts a 100% check while entering the fish lab lengths and weights to ensure accuracy of the fish field lengths and weights already provided in the GLENDA files. If a discrepancy exists, the QA contractor views a copy of the fish sample identification label provided in the homogenization laboratory's final report and uses that value to serve as the final fish field length or weight. If the fish sample identification label is not legible, then QA contractor staff flags the value for resolution by another staff member. The team member reviews the hardcopy field recording forms, fish sample identification labels, and fish homogenization final report in an attempt to resolve the discrepancy. If QA staff is unable to resolve the discrepancy, they may contact the field sampling team for input. All resolutions are documented. The QA contractor conducts a 50% check on the entry of the fish lab lengths and weights using a different QA contractor staff member. Differences in lab and field lengths and weights are assessed and entered into the GLENDA files. The fish homogenization laboratory also provides the fish gender and age in a separate Microsoft Excel spreadsheet. These data are transferred to the GLENDA files and entered for each fish.

As an additional assessment, the homogenization laboratory documentation is reviewed to confirm that the homogenization laboratory ID and the GLNPO identifiers are consistent throughout all documentation.

Data review regarding sample homogenization and analysis is discussed in *The Great Lakes Fish Monitoring and Surveillance Program Quality Management Plan* (US EPA, 2012).

D.2 Verification and Validation Methods

The GLFMSP Manager 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. Fin clips removed from any of the fish samples will be reviewed point-by-point against the field documentation. The GLFMSP Manager will resolve any discrepancies with the field sampling team.

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

D.3 Reconciliation With User Requirements

Data is released according to the *Great Lakes Fish Monitoring and Surveillance Program Data Release Guidelines* (Appendix C.10 of GLFMSP QMP). Publications will be prepared for highly ranked peer-reviewed scientific journals. The results of this research will be presented at scientific conferences to disseminate the findings and to obtain feedback. GLNPO will work with the scientists and administrators in the Great Lakes region to respond to any requests regarding the program findings, and to design the most effective way to disseminate the results of this program. Issues and challenges encountered during the study will be clearly communicated through the reports and publications. The GLFMSP Manager will make a public announcement annually when data that have been checked for completeness and consistency are available in GLENDA and the GLFMSP Microsoft Access Database. Data will be available through a request to the GLFMSP Manager. GLFMSP data can be used for site-specific statements, not lake-wide statements.

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Appendix A

Fish Collection Standard Operating Procedures

Appendix A.1

GLFMSP Base Monitoring Program Sample Collection SOP

Standard Operating Procedure for the Collection of Fish for the Great Lakes Fish Monitoring and Surveillance Program Base Monitoring Program

1.0 Scope and Application

This standard operating procedure (SOP) is used to collect fish for base monitoring in support of the Great Lakes Fish Monitoring and Surveillance Program administered by the U.S. EPA Great Lakes National Program Office. 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 Method

Lake trout in Lakes Michigan, Superior, Ontario, Huron and Erie and walleye 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, seines, etc. Fish are collected in specific size ranges for preparation of sample composites. For each target species, composite samples consisting of five whole fish each, are prepared and sent to the homogenization laboratory for processing. 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 solvent rinsed and baked foil, placed in polyethylene plastic tubes, and labeled appropriately. Fish are packaged in plastic bags in groups of five fish intended for a single composite according to specific criteria. Fish are frozen as soon as possible after collection and maintained at -20°C. Fish are then 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. 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 Collection Procedures

Field sampling personnel choose the collection method and appropriate sampling gear to meet the study objectives pertinent for their fish collection effort. Each field 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. Non-target 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 (e.g., live well, buckets, etc.) to prevent contamination. The buckets are cleaned according to the procedures outlined in Section 3.0 above. 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). Detailed sample handling instructions are provided in Section 9.0.

5.0 Species, Size, and Number of Fish

The field sampling teams collect 50 lake trout or walleye 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, field sampling teams should 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
Lake Erie	O	E
Lake Huron	E,O	—
Lake Michigan	E,O	—
Lake Ontario	E,O	—
Lake Superior	E,O	—
Size Range (mm)	600 to 700	400 to 500

E = even year collection
O = odd year collection

Field sampling teams group samples into composites of five fish each. Fish are placed singly in polyethylene tubing secured with cable ties and the five fish grouped for a composite are placed in a large 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 Collection of Fish for the GLFMSP

Lake	Grid	Site	Longitude	Latitude
Michigan	2210	Saugatuck	86°25'	42°35'
Michigan	906	Sturgeon Bay	87°15'	44°45'
Huron	1413	Port Austin	82°45'	44°05'
Huron	710	Rockport	83°15'	45°15'
Erie	904	Middle Bass Island	82°55'	41°35'
Erie	424	Dunkirk	79°35'	42°25'
Ontario	713	North Hamlin	77°55'	43°25'
Ontario	623	Oswego	76°15'	43°35'
Superior	1028	Keewenaw Pt.	87°35'	47°25'
Superior	1311	Apostle Islands	90°25'	46°55'

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 will help to prevent loss of target analytes. All fish collected for this project should be 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 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 including composite ID, fish number, date of collection, method of collection, estimated collection depth, sample ID, field length, field weight, sex, and fin clip location. The form also is used as a chain-of-custody record 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* that provides information on filling out the required form.

The field recording 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
- 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* (provided by GLNPO).

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 a 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.

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, etc.) that have been cleaned according to the specified procedures in Section 3.0. Return any non-target fishes or small specimens to the 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 listed below should be taken to prepare the fish for storage and shipping to the homogenization lab for processing. 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 wait to fill it out upon completion of sample handling.

9.7 To minimize contamination, samples are not placed directly on dry ice, but are wrapped in acetone-washed foil and placed in polyethylene plastic tubes prior to setting on ice. Once fish are measured and weighed, wrap each fish separately in acetone-washed foil and immediately place in pre–cut polyethylene plastic tubes. If the fish do not fit in the pre-cut 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.

9.8 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 or large polyethylene bag.

9.9 Ensure that each individually bagged fish is accurately labeled with the information in Table 3 and that five individually bagged fish all belonging to one composite are combined in one large bag.

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

Data Type	Measurement Units or Allowed Entries
Lake Name	Erie, Huron, Michigan, Ontario, Superior
Collector Identification	Vessel and collector's name
Collection Date	MM/DD/YY
Fish Length	Millimeters (mm) or inches, total length
Fish Weight	Grams
Composite ID	First Letter of Lake Name [e.g., LS = Lake Superior], Fish Species, Year Fish Collected, Grid #, Composite # [e.g., 001, 002, etc.] Example: 1st composite of Lake Superior lake trout collected from grid # 1028 in year 2011 = LSLakeTrout20111028001
Fish Number	Fish 1 - 5 of each composite (e.g., F1, F2, etc.)

9.10 Once packaged, samples should be immediately frozen for shipment or placed on dry ice for transport to a processing facility where fish will be immediately frozen. All fish must be kept at ≤ -20°C, and maintained frozen until they reach the designated homogenization laboratory. Collection facilities must be able to retain frozen samples for at least four weeks, or until the GLFMSP Manager has specified a shipment date.

9.11 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 sampling 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. The field sampling team leader signs and dates the form. Provide a hard or electronic copy to the GLFSMP Manager, Beth Murphy (electronically to Murphy.Elizabeth@epa.gov or via fax to 312-886-6869) and Marian Landon of CSC (electronically to mlandon3@csc.com or via fax to 703-461-8056).

Appendix A.2

GLFMSP CSMI/Special Studies Program Collection of Individual Fish, Eggs, and Stomach Contents SOP

Standard Operating Procedure for the Collection of Individual Fish, Stomach Contents, and Eggs for the Great Lakes Fish Monitoring and Surveillance Program CSMI/Special Studies Program

1.0 Scope and Application

This standard operating procedure (SOP) is used to collect individual lake trout and their stomach contents and eggs in support of the Great Lakes Fish Monitoring and Surveillance Program (GLFMSP) administered by the U.S. EPA Great Lakes National Program Office (GLNPO). This collection effort was added to the routine sampling efforts for GLFMSP effective the spring of 2011. The purpose of collection of the stomach contents will be to assist in the evaluation of the movement of contaminants in complex Great Lakes food webs. This requires data on pollutant concentrations and fluxes (diet) for the top predator (lake trout) and the prey species at the supporting lower trophic levels. The eventual body burden of contaminants in lake trout depends on the feeding preferences and food availability at lower trophic levels and the contaminant burden of each prey species. The purposes of the collection and analysis of contaminant levels in the fish eggs and associated parent fish are to: evaluate the relationship of parent-egg contaminant levels, potentially identify new emerging contaminants, assess critical contaminant trends, and support lake-wide management plans.

A team leader is identified for each field sampling team 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 Method

Individual lake trout, in the lake identified by the Cooperative Science and Monitoring Initiative (CSMI) for each sampling season, are collected at both nearshore and offshore routine monitoring sites (refer to Table 2). At each site, lake trout will be collected by methods specified by the field sampler team leader. Ideally at each site, samplers will collect ten individual lake trout. Eggs and stomach contents will be extracted from the individual fish and stored separately in jars until shipped to the appropriate laboratory for processing. Each parent fish will be bagged, labeled, and stored frozen until shipped to the homogenization lab. Special precautions are taken to prevent contamination of the fish with any foreign materials and ensure the integrity of the sample. Eggs and stomach contents are immediately removed from the fish and placed into sterile jars. Fish are immediately wrapped in foil, then placed in polyethylene tubing. Fish and their associated eggs and stomach contents are frozen as soon as possible after collection and maintained at -20°C. No PTFE (Teflon tape) should come into contact with the sample contents or container. Samplers store frozen fish at a secure location until directed to ship to the appropriate 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, please contact Beth Murphy immediately 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 (at least once every 24 hours) to ensure a limited time lag between fish entrapment and sample preparation.

If fish are in nets for over 24 hours, please ensure only live fish are collected.

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 GLNPO. The *Field Recording Form and Chain-of-custody Record* (provided by GLNPO) 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).

The following materials have been provided to aid in collection of eggs and stomach contents:

- 500-mL amber glass collection jars with unlined polypropylene plastic lids
- Small plastic bags
- Labels
- Bubble wrap

4.0 Collection Procedures

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. Non-target 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 (e.g., live well, buckets, etc.) to prevent contamination. The buckets are cleaned according to the procedures outlined in Section 3.0 above.

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). Note: These measurements must be collected and recorded prior to the removal of the eggs and stomach contents.

Procedures for handling and storing individual fish and collecting eggs and stomach contents are detailed in Section 9.0.

5.0 Species, Size, and Number of Fish

At each site, samplers are directed to collect at least ten individual lake trout within the 600-700 mm length range. 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.

After collection of eggs and stomach contents, fish are placed singly in bags and then five fish are placed into a larger composite bag. Each of the ten fish should:

- 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, and
- Be collected as close to the same time and location as possible, but no more than one week apart.

Fish are homogenized individually 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 1. 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 1. Sampling Locations for the Collection of Fish for the GLFMSP

Lake	Grid	Site	Longitute	Latitude
Michigan	2210	Saugatuck	86°25'	42°35'
Michigan	906	Sturgeon Bay	87°15'	44°45'
Huron	1413	Port Austin	82°45'	44°05'

Lake	Grid	Site	Longitute	Latitude
Huron	710	Rockport	83°15'	45°15'
Erie	904	Middle Bass Island	82°55'	41°35'
Erie	424	Dunkirk	79°35'	42°25'
Ontario	713	North Hamlin	77°55'	43°25'
Ontario	623	Oswego	76°15'	43°35'
Superior	1028	Keewenaw Pt.	87°35'	47°25'
Superior	1311	Apostle Islands	90°25'	46°55'

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 and its associated eggs and stomach gut also will prevent loss of target analytes. All fish and their associated eggs and stomach contents 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 provided by GLNPO 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 the *Field Recording Form and Chain-of-custody Record* (provided by GLNPO) at the time that fish are shipped to the appropriate 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 and Chain-of-custody Record* is used to document the sample collection effort and includes specific information regarding each fish specimen such as length, weight, and species. The form is used to document shipment and handling of all fish from the field personnel to the appropriate laboratory. Field sampling teams are provided with *Packing and Shipping Instructions for the Great Lakes National Program Office's Fish Monitoring Program* that provides information on filling out the required forms.

The *Field Recording Form and Chain-of-custody Record* is designed to capture unique parent fish IDs that are used as tracking numbers for each fish. Specific instructions for assigning these parent fish IDs are included on the form. Parent fish IDs are used by GLNPO and the homogenization and analytical laboratories to identify each fish and report results. Each parent fish 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 name (Lake Trout or Walleye),
- The four digit year of collection,
- The grid number, and
- A sequential number indicating the number of each fish from a specific location and year (e.g., F001, F002, F003, 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, Egg and Stomach Contents Collection, 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.

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 3.0. Non-target fish or small specimens are returned to water.

9.2 Accurate taxonomic identification is essential in assuring and defining the organisms that have been submitted for analysis. Under no circumstances should individual fish of different species be used.

9.3 The steps described below should be taken to prepare the fish for storage and shipping to the homogenization lab for processing.

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). (Note: This measurement must be collected and recorded prior to the removal of the eggs and stomach contents.)

9.5 Weigh each fish to the nearest gram. (Note: This measurement must be collected and recorded prior to the removal of the eggs and stomach contents.)

9.6 Prepare three sample labels (provided by GLNPO) for each fish (one label for the parent fish, one label for the eggs, and one label for the stomach contents). Make sure the Parent fish ID (detailed in Section 8.0) is listed on the label.

9.7 Collection of eggs should utilize the following method:
1. Place the parent lake trout onto a piece of acetone-washed and baked aluminum foil.
2. Harvest all eggs using a stripping technique. If the fish is not ripe, remove and send the skein that holds the eggs.
3. Transfer all eggs to a single jar. Do not fill the jar completely to allow room for expansion during freezing.
4. Make sure the parent fish ID has been listed on the label prepared in Section 9.6 and place the label onto the outside of the jar. Clear tape should be placed over the labels to ensure they stay on the jars and that the markings on the labels do not become smeared.

9.8 Collection of stomach contents should utilize the following method:
1. Place the parent lake trout onto a piece of acetone-washed and baked aluminum foil.
2. Make a 3-5 inch incision with a clean knife in the belly of the fish.
3. Pull out and remove the stomach (anterior esophagus to pyloric sphincter) and all its contents. The spleen and any other organs or excess flesh that may be attached to the stomach should

be placed back inside the fish. If the stomach appears to be empty, open it to verify that it is completely void. Void stomachs need not be kept.

4. Transfer the entire stomach gut to a single jar. Do not fill the jar completely to allow room for expansion during freezing.
5. Make sure the parent fish ID has been listed on the label prepared in Section 9.6 and place the label onto the outside of the jar. Clear tape should be placed over the labels to ensure they stay on the jars and that the markings on the labels do not become smeared.

9.9 After eggs and stomach contents have been collected, each individual fish should be wrapped separately in acetone-washed foil and immediately place in polyethylene plastic tubing or in a polyethylene plastic bag. If using the plastic tubing, cut the plastic tube to the length of the fish and fasten ends of tube with provided cable ties.

9.10 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. Make sure the parent fish ID has been listed on the label.

9.11 After ten fish are collected that meet the specifications as described in Section 5.0, combine the individually bagged fish into two liner bags or large polyethylene bags (5 individually wrapped fish per 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.

9.12 Fill out *Field Recording Form and Chain-of-custody Record* or fill out form at completion of sample handling. Provide a hard or electronic copy to the GLFSMP Manager, Beth Murphy (electronically to Murphy.Elizabeth@epa.gov or via fax to 312-886-6869) and Marian Landon of CSC (electronically to mlandon3@csc.com or via fax to 703-461-8056).

9.13 All fish, eggs, and stomach contents must be kept at ≤ -20°C, and maintained frozen until they reach the designated homogenization laboratory. Collection facilities must be able to retain frozen samples for at least ten weeks, or until the GLFMSP Manager has specified a shipment date.

9.14 The following steps should be taken before shipping jars of eggs and stomach contents:

1. Secure the lid tightly and place in two small plastic bags.
2. Place jars into small cooler and surround jars with bubble wrap to minimize breakage of jars while in transport.
3. Completely surround jars with dry ice to ensure samples are frozen while in transit.

9.15 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* with the fish shipment.

Use overnight delivery services and only Monday through Thursday to ensure samples are not delivered on the weekend.

Ship parent fish, eggs, and stomach contents using the *Packing and Shipping Instructions for the GLFSMP* (provided by GLNPO with shipping supplies).

For additional questions, please contact:

Beth Murphy
GLFMSP Program Manager
(312) 353-4227
Murphy.Elizabeth@epa.gov

Appendix A.3

GLFMSP CSMI/Special Studies Program Collection of Forage Fish SOP

Standard Operating Procedure for the Collection of Forage Fish for Great Lakes Fish Monitoring and Surveillance Program CSMI/Special Studies Program

1.0 Scope and Application

This standard operating procedure (SOP) is used to collect forage fish (e.g., bloaters, sculpins, alewives, gobies, and smelts) in support of the Great Lakes Fish Monitoring and Surveillance Program (GLFMSP) administered by the U.S. EPA Great Lakes National Program Office (GLNPO). The sampling of forage fish was added to the routine sampling efforts for GLFMSP effective the spring of 2011. The purpose of collection is to provide enough biological mass from species that comprise a typical lake trout diet to assist in the determination of legacy and emerging contaminants.

A team leader is identified for each field sampling team 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

Forage fish in the lake identified by the Cooperative Science and Monitoring Initiative (CSMI) for each sampling season are collected at both nearshore and offshore routine monitoring sites (refer to Table 2). At each site, forage fish of varying species are collected by trawling or other methods, as specified by the field sampler team leader. Ideally at each site, samplers will collect 30 fish of each of the three most abundant forage fish species and 10 fish of the 4th and 5th most abundant forage fish species (110 fish total) (refer to Table 1). Site conditions may prohibit the collection of this quantity of fish and under such circumstances the goal would be to collect as many fish as possible for all species. However, if additional fish are collected than the requested amount, nearly all fish will be utilized and should be provided to the homogenization lab.

After collection by trawl (or other method), forage fish are bagged and stored frozen until shipped to the homogenization lab for processing. Special precautions are taken to prevent contamination of the fish with any foreign materials and ensure the integrity of the sample. Fish are immediately grouped and wrapped in foil, then placed in plastic bags. Fish are frozen as soon as possible after collection and maintained at -20°C. No PTFE (Teflon tape) should come into contact with the sample contents or container. Samplers store frozen fish at a secure location until directed to ship to the sample homogenization laboratory. Data regarding each forage fish collection effort (e.g., date and time of collection, sample location, etc.) are recorded on a *Field Recording Form and Chain-of-custody Record* (provided by GLNPO) which are sent with the fish to the laboratory. If any questions or concerns arise regarding the collection, storage, or shipping procedures, please contact Beth Murphy immediately 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 GLNPO. A chain-of-custody form (provided in Sampler Information Packet from GLNPO) 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 select the collection method and 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.

Field sampling personnel should wear clean nitrile gloves (provided by GLNPO if not already available to personnel) to handle fish and sample handling equipment. As soon as fish are obtained via active collection methods, or removed from passive collection devices, they should be grouped by species. Individuals of each group should be rinsed in ambient water to remove any foreign material from the external surface and each group should be placed in clean holding containers (live well, buckets, etc.) to prevent contamination. The buckets should be cleaned according to the procedures outlined in Section 3. Each group should be weighed in aggregate. Each group should then be wrapped in foil and bagged in polyethylene tubing (plastic bags that foil comes in can be used instead of tubing if fish are small). If using the polyethylene tubing, cut the tube to the length of the fish and fasten ends of tube with provided cable ties. A label should be filled out with pencil to indicate the location, date, species, number of individuals, and aggregate weight of each group (labels are provided by GLNPO). This label should then be placed in a small plastic bag and included in the tubing with each group. Bags should be placed on ice.

Collection of forage fish should include any and all available species. However, anticipated forage fish species include: bloaters (*Coregonus hoyi*), sculpins (*Cottus* sp.), alewives (*Alosa pseudoharengus*), gobies (*Apollonia melanostomus* or *Proterorhinus semilunaris*) and smelts (*Osmerus mordax*).

5.0 Species, Size, and Number of Fish

At each site, samplers are directed to collect 30 fish of each of the three most abundant forage fish species and 10 fish of the 4th and 5th most abundant forage fish species (110 fish total) (refer to Table 1). All collected forage fish should be shipped to the homogenization laboratory, regardless of whether more or

less than the requested quantities are collected. Fish should be collected as close to the same time and location as possible, but no more than one week apart.

Table 1. Requested Quantity of Forage Fish to be Collected at Each Site

Species	Number of Fish per Site
1st abundant species	30
2nd abundant species	30
3rd abundant species	30
4th abundant species	10
5th abundant species	10
Total	**110**

Fish will be identified, composited and homogenized at 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 Collection of the Forage Fish for the GLFMSP

Lake	Grid	Site	Longitute	Latitude
Michigan	2210	Saugatuck	86°25'	42°35'
Michigan	906	Sturgeon Bay	87°15'	44°45'
Huron	1413	Port Austin	82°45'	44°05'
Huron	710	Rockport	83°15'	45°15'
Erie	904	Middle Bass Island	82°55'	41°35'
Erie	424	Dunkirk	79°35'	42°25'

Lake	Grid	Site	Longitute	Latitude
Ontario	713	North Hamlin	77°55'	43°25'
Ontario	623	Oswego	76°15'	43°35'
Superior	1028	Keewenaw Pt.	87°35'	47°25'
Superior	1311	Apostle Islands	90°25'	46°55'

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* (provided by GLNPO) 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 form 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* (provided in the Sampler Information Packet from GLNPO) that provides information on filling out the required forms.

The *Field Recording Form and Chain-of-custody Record* (provided by GLNPO) is designed to capture information about the collection of forage fish at a given site and includes the following fields:

- Date
- Time
- Sampler Name
- Sampler Contact Information
- Site Name
- Lake Name
- Latitude and Longitude Coordinates for the Site
- Comments from Sampler(s) (e.g., observations on fish species [not required])
- Species and Scientific Names
- Method of Collection
- Depth of Collection
- Number of Individuals
- Aggregate Weight (g)

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

9.0 Sample Handling, Storage, and Shipping

All fish collected for the GLFMSP should be kept separate from other collected fish in their own fish box. It is very important that all fish collected be placed into a 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.

9.1 As soon as fish have been obtained via 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.

9.2 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 chain-of-custody record (provided by GLNPO) with the fish shipment. Collection bags will be provided to collectors by GLNPO.

9.3 Complete the chain-of-custody record in the field or at completion of sample handling. Provide a hard or electronic copy to the GLFSMP Manager, Beth Murphy (electronically to

Murphy.Elizabeth@epa.gov or via fax to 312-886-6869) and Marian Landon of CSC (electronically to mlandon3@csc.com or via fax to 703-461-8056).

9.4 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 a group of fish in acetone-washed foil and immediately place in polyethylene plastic tubes or polyethylene plastic bags (bags that foil comes in can be used). 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. 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 ≤ -20°C, and maintained frozen until they reach the designated homogenization laboratory. Collection facilities must be able to retain frozen samples for at least four weeks, or until Beth Murphy has specified a shipment date.

Appendix B

Field Recording Forms and Chain-of-Custody Records

Appendix B.1

GLFMSP Base Monitoring Program Collection of Fish Field Recording Form and Chain-of-Custody Record

Great Lakes Fish Monitoring and Surveillance Program
Field Recording Form and Chain-of-custody Record
Base Monitoring Program

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

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:	

Sampled by: (signature)	Date/Time:	Shipped by: (signature)	Date/Time:
Received by: (signature)	Date/Time:		

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

Great Lakes Fish Monitoring and Surveillance Program

Fish Sample Collection and Description Information

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								
	1								
	2								
	3								
	4								
	5								
	1								
	2								
	3								
	4								
	5								
	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:	LO	Lake Trout	2009	0713	001
(LOLakeTrout20090713001)	(Lake)	(Fish Species)	(Year Fish Collected)	(Grid # or Port Code)	(Composite #)

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

Great Lakes Fish Monitoring and Surveillance Program

Fish Sample Collection and Description Information

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								
	1								
	2								
	3								
	4								
	5								
	1								
	2								
	3								
	4								
	5								
	1								
	2								
	3								
	4								
	5								
	1								
	2								
	3								
	4								
	5								

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

Great Lakes Fish Monitoring and Surveillance Program

Fish Sample Collection and Description Information

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								
	1								
	2								
	3								
	4								
	5								
	1								
	2								
	3								
	4								
	5								
	1								
	2								
	3								
	4								
	5								
	1								
	2								
	3								
	4								
	5								

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

Appendix B.2

GLFMSP CSMI/Special Studies Program Collection of Individual Fish, Eggs, and Stomach Contents Field Recording Form and Chain-of-Custody Record

Great Lakes Fish Monitoring and Surveillance Program
Field Recording Form and Chain-of-custody Record
CSMI/Special Studies Program Individual Fish, Eggs, and Stomach Contents

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

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:	

Sampled by: (signature)	Date/Time:	Shipped by: (signature)	Date/Time:
Received by: (signature)	Date/Time:		

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

Great Lakes Fish Monitoring and Surveillance Program

Fish Sample Collection and Description Information

Fish ID	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.	Eggs Collected (Y = Yes, N = No)	Stomach Collected (Y = Yes, N = No [If stomach was empty, enter E])

Fish ID Sample Example: LO Lake Trout 2009 0713 F001
(LOLakeTrout20090713F001) (Lake) (Fish Species) (Year Fish Collected) (Grid # or Port Code) (Fish #)

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

Appendix B.3

GLFMSP CSMI/Special Studies Program Collection of Forage Fish Field Recording Form and Chain-of-Custody Record

Great Lakes Fish Monitoring and Surveillance Program
Field Recording Form and Chain-of-custody Record
CSMI/Special Studies Program Forage Fish

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

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 (Optional):	

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 (Optional):	

Sampled by: (signature)	Date/Time:	Shipped by: (signature)	Date/Time:
Received by: (signature)	Date/Time:		

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

Great Lakes Fish Monitoring and Surveillance Program

Fish Sample Collection and Description Information

Species	Scientific Name	Date of Collection (Month/Day/Year)	Method of Collection	Sample ID	Estimated Collection Depth (m)	Number of Individuals	Aggregate Weight (g)

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Great Lakes Fish Monitoring and Surveillance Program

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Appendix C

Packing and Shipping Instructions for the Great Lakes Fish Monitoring and Surveillance Program

Packing and Shipping Instructions for the Great Lakes Fish Monitoring and Surveillance Program

These instructions apply to fish collected for the United States Environmental Protection Agency's (U.S. EPA) Great Lakes National Program Office (GLNPO) Great Lakes Fish Monitoring and Surveillance Program (GLFMSP). These fish are being sent to Aquatec Biological Sciences, Inc. (Aquatec) for sample preparation prior to analysis. These instructions supplement the fish collection SOPs provided by the GLFMSP. We are requesting that you complete a chain-of-custody and field recording form, provided when you received your sampling kits, to send to Aquatec with your fish. This will allow tracking of the shipments, identify all fish being shipped to Aquatec, and confirm that fish remained frozen during transport. This is in addition to any data sheets you may be providing. If you received dry ice, please ship samples within 24 hours of receipt. Please follow the instructions detailed below.

1. Complete a chain-of-custody and field recording form provided with the sampling kits, 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 GLFMSP fish collection SOPs). In order to simplify entering data onto the form, feel free to use ditto marks as seen on 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 Marian Landon at (703) 461-2351.
    a. For identifying sample location (grid number), please use the List of Grid Numbers and Port Codes for the GLFMSP that you received with your sampler information packet prior to sampling.
    b. For composite numbers, 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 or scan and email the chain-of-custody and field recording forms to Marian Landon (Fax: 703-461-8056, Email: mlandon3@csc.com) before or immediately after shipping. This is critical for monitoring shipments to ensure they reach Aquatec before the samples thaw. If faxing or scanning and emailing is not possible, please call Marian Landon at (703) 461-2351 and provide the **shipping date** and **airbill numbers** for all coolers being shipped.

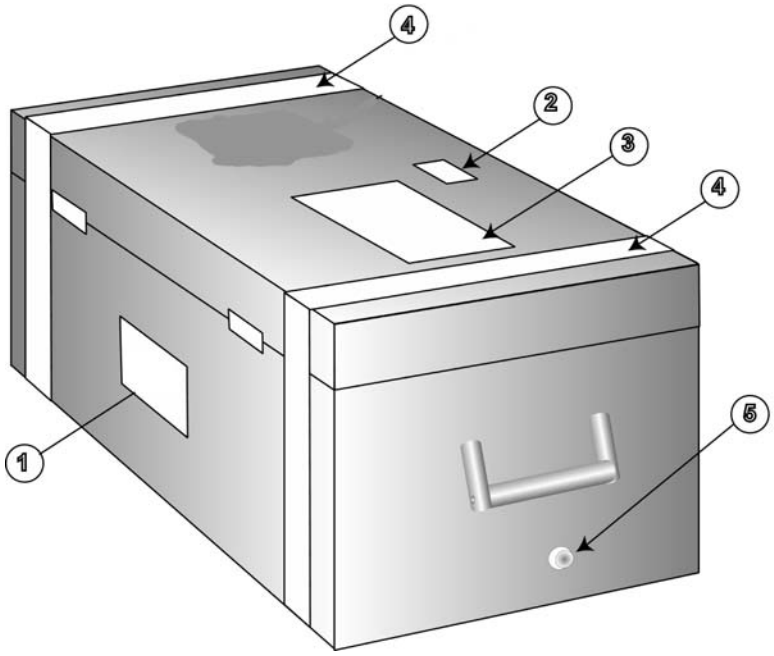
3. Make a copy of the chain-of-custody and field recording form. Place the original chain-of-custody and field recording form in a waterproof bag. If you have additional forms containing sampling or specimen data, place them in the waterproof bag as well and seal. Place the sealed forms in one of the coolers with the samples.

4. Please bag each fish in its own piece of tubing and seal the other end with a cable tie. Place each set of five fish being used for a composite inside the large composite bag provided in your sampling kit and seal with a cable tie.

5. As soon as each sample is packaged, place it immediately on dry ice for shipment in the cooler. Try to surround all fish with dry ice. If possible, fill the entire cooler with dry ice.

6. If possible, keep all five fish designated for a particular composite in the same cooler for transport.

7. Secure each cooler with packaging tape. Prepare the coolers with the labels and other information as described on page 2. Ship each cooler to Aquatec via FedEx Priority Overnight delivery service. All costs can be charged to the provided FedEx account number.



1. **Class 9 Dangerous Goods Label:** List the amount of dry ice in kg (2 lbs = 1 kg) on each label. Place one label on each long side of the cooler (number 1 on the figure above and in the same position on the opposite side of the cooler). Completely tape over the labels with clear tape.

2. **Perishable Goods Label:** Be sure to completely tape over this label with clear tape.

3. **FedEx Airbill** (please be sure to secure airbill with clear tape)**:** To fully complete the FedEx airbill, please enter the information in the following sections:
   Section 1: Date
   Section 6: Dry Ice weight in kg

4. **Packaging Tape:** Each end of the cooler needs to be wrapped with strapping tape at least three (3) times.

5. **Cooler Drain Hole:** Please make sure the cooler drain hole has been taped so that the drain hole is CLOSED. If the tape has been removed, please tape the plug so that the drain hole remains CLOSED.

6. **Custody Seal** (not pictured in figure above): Tape custody seal over the cooler to help ensure that cooler is unopened during shipping.

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 Marian Landon (703) 461-2351.***

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 Marian Landon at (703) 461-2351 immediately.

- Please make sure you have faxed or scanned and emailed the chain-of-custody and field recording forms to Marian Landon (703) 461-8056. If faxing or scanning and emailing are not possible, please

call Marian Landon at (703) 461-2351 and provide the **shipping date** and **airbill numbers** for all coolers being shipped.

Contact Information:

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