Quality Assurance Project Plan: Diet Analysis for Forage Fish

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1.0 Project Description

1.1 Introduction

The Great Lakes National Program Office (GLNPO) of the US EPA has initiated a Mass Balance Study for selected toxic contaminants in Lake Michigan. The mass balance effort will be part of a "Lake Michigan Enhanced Monitoring Program," which includes tributary and atmospheric load monitoring, source inventories, and fate and effects evaluations. In general, the primary goal of this enhanced monitoring program is to develop a sound, scientific base of information to guide future toxic load reduction efforts at the Federal, State, and local levels.

A modeling team will construct a mass budget and mass balance model for a limited group of contaminants which are present in Lake Michigan at concentrations which pose a risk to aquatic and terrestrial organisms (including humans) within the ecosystem. Components to the mass balance model will be designed to predict contaminant concentrations in the water column and target fish species over a two year period, relative to loadings. Predictions of contaminant levels in three species of fish will be calculated as final output of the model. The target fish species include:

Lake trout (Salvelinus namaycush)
Coho salmon (Oncorhynchus kisutch)
Bloater chub (Coregonus hoyi)

The calibration of the food web model(s) for these target species requires data on contaminant concentrations and fluxes (diet) not only in these species, but also in the supporting trophic levels. The contaminant burden of each prey species varies based on feeding patterns at lower trophic levels.

The basic design and data requirements for the fish samples have been outlined in Tables 5 and 6 and in Appendix 4 of the Lake Michigan Mass Budget/Mass Balance (LMMB) work plan of October 14, 1993. This project addresses a subset of the work objectives for the lower trophic levels (forage fish diets and zooplankton abundance). The forage fish studied in this project include:

Bloater chub
Rainbow smelt (Osmerus mordax)
Alewife (Alosa pseudoharengus)
Slimy sculpin (Cottus cognatus)
Deepwater sculpin (Myocephalus thompsoni)

The study starts in May 1994, the field season lasts through November 1994, and the data analyses lasts nine months after the last field collection.

The specific objective of this study is to

1) determine the diets of these forage fish at each site and season.
2) determine zooplankton availability at each site and season.

The diet information of forage fish and zooplankton abundance sampled by this project will enable the modelers to quantify the movement of contaminants from their source, through the food web, and ultimately the body burden in the target fish species.

1.2 Experimental Design

Three sites (Sturgeon Bay, Port Washington, and Saugatuck) and three seasons (spring, summer, and fall) will be sampled to determine spatial and temporal effects on feeding by forage fish and availability of zooplankton (Table 1.1).

Table 1.1. Summary of critical and noncritical measurements for the evaluation of diets of forage fish and zooplankton availability.

Parameter	Sampling Instrument	Sampling Method	Analytical Instrument	Analytical Method	Reporting Units	LOD
Location	GPS Loran	SOP	NA	NA	Lake Regions	Sturgeon Bay, Port Washington, Saugatuck
Sample Date	NA	NA	NA	NA	mo/day/yy xx/xx/xx	day
Fish Length	NA	NA	measuring board ruler	SOP	mm	2 mm
Fish Weight	NA	NA	spring or electronic balance	SOP	Kg	1 g
Diet Species	NA	SOP	dissecting microscope	SOP	total number	Genus or species for common taxa
Diet Item Length	NA	SOP	ocular micrometer	SOP	mm	0.1 mm
Zooplankton Species	NA	SOP	dissecting microscope	SOP	total number	Genus or species for common taxa
Zooplankton Length	NA	SOP	ocular micrometer	SOP	mm	0.1 mm

2.0 Project Organization and Responsibilities

Paul Bertram
EPA Project Officer
Biota Co-Chair

John Gannon
NBS
Biota Co-Chair

Lou Blume
EPA QA Manager

Jacqueline Savino
NBS
Project Manager

Bruce Davis
NBS
Field Manager

Two technical positions
NBS
Laboratory Analysis

2.1 GLNPO Project Officer and Biota Co-Chair

The GLNPO Project Officer is the Agency official who initiates the grant, evaluates the proposal, and is the technical representative for EPA. The Project Officer is responsible for:

Budgeting
Program planning, scheduling, and prioritization
Developing project objectives and data quality objectives
Ensuring that project meets GLNPO missions
Technical guidance
Program and data reviews including audits
Data quality
Final deliverables

2.2 GLNPO QA Manager

The GLNPO QA Manager (QAM) is responsible for ensuring that each project funded by EPA satisfies the Agency's requirements for QA programs. The QAM is responsible for:

Offering guidance on QA techniques
Evaluating QA Project Plans (QAPjPs) and approving QAPjPs for the Agency Assisting in the coordination of audits

2.3 NBS Biota Co-Chair

The Biota Co-Chair from NBS works in partnership with the GLNPO QA Project Leader to implement the Blota portion of the Lake Michigan Mass Balance Project. Duties are:

Program planning, scheduling, and prioritization
Developing project objectives and data quality objective
Ensuring that project meets GLNPO missions

2.4 NBS Project Manager

The Project Manager is the NBS official who initiated the proposal to perform the forage fish diet portion of the LMMB project and is responsible for:

Developing the sampling plan for forage fish diet and zooplankton collection.
Administration of the forage fish diet segment of the biota objectives.
Overall supervision of field and laboratory work.
Ensures OA objectives are met
Technical supervision
Final deliverables
Data quality assessment

2.5 NBS Field Manager

The Field Manager is the NBS position that will provide daily supervision of the field collection activities and laboratory analyses and the achievement of the QA objectives. This person is responsible for:

Collecting field data
Directly supervise the field crew activities
Reviews progress toward QA objectives
Develops and implements sampling and analytical procedures
Prepares reports and deliverables
Trains field crews on sampling and analytical procedures
Data quality assessment and audits for laboratory and field segments

2.6 Field Sampling, and Analysis Personnel

The positions are responsible for the majority of the field sampling and laboratory identification. They will receive training and guidance from the Project and Field Managers, who will also audit their work to ensure QA objectives are met. Minimum qualifications are B.S. in the biological sciences or two years undergraduate experience in biological sciences and work experience.

3.0 Quality Assurance Objectives

As outlined in the Lake Michigan Mass Budget/Mass Balance Work Plan, the proposed model output should be within a factor of two of the observed concentrations in the water column and target fish. It is also estimated that the required level of model accuracy can be achieved if loadings and contaminant mass in significant environmental compartments are determined to within ±20% to 30% of the actual value.

3.1 Objectives:

1) Within each season/region strata, collect as representative a sample of coho salmon as possible so as to minimize the spatial and temporal population uncertainty (Sp2) to the extent possible (given the sample size that can be collected with the financial logistic, and biological constraints of this project).

2) To collect, package, and transport each sample, and to record, summarize, and report each physical measurement with a level of precision, accuracy, detectability, and completeness that will ensure that Measurement Uncertainty (Sm2) will be nominal compared to Sp2 and therefore not affect the interpretation of the results.

Variability in the diet of Lake Michigan forage fish can be great, especially when examined from a lakewide perspective encompassing large scale spatial and temporal gradients. The desired sample size for determining diet is to a large degree constrained by the difficulty and time required to analyze the samples.

3.2 Measurement Quality Objectives

Measurement quality objectives are designed to control various phases of the measurement process and to ensure that total measurement uncertainty is within ranges prescribed by the DQOs (Table 3.1). The MQOs can be defined in terms of data quality attributes: precision, accuracy, completeness, detectability, representativeness, and comparability. The first four can be defined in quantitative terms, while the later two are qualitative.

Precision. A measure of mutual agreement among multiple measurements of the same property, usually under prescribed similar conditions. Precision will be evaluated through auditing of data collection activities to determine whether activities are performed in a consistent manner, and by established protocol.

Accuracy. The degree of agreement between a measurement (or an average of measurements of the same thing), and the amount actually present.

Completeness. For this QAPjP, completeness is the measure of the number of valid samples obtained compared to the amount that is needed to meet the DQOs. The EMP-A completeness goal is 90%.

Detectability. The determination of the low-range critical value of a characteristic that a method-specific procedure can reliably discern or is necessary to meet program objectives.
Representativeness. Express the degree to which data accurately and precisely represent characteristics of a population, parameter variations at a sampling point, a process condition, or an environmental condition.

Comparability. Express the confidence with which one data set can be compared to another.

3.3 Laboratory MQOs

The following information describes the procedures used to control and assess measurement uncertainty occurring during laboratory analyses. Laboratory parameters in this section will include fish length, fish weight, prey number, and prey length. The majority of the uncertainties occurring in the laboratory can be alleviated by the development of detailed standard operating procedures (SOPs), and adequate training program at appropriate frequency, and a laboratory audit program. SOPs have been developed and training has occurred. Laboratory audits will be implemented during the course of the program implementation.

3.4 Precision

Another term for precision is repeatability. Repeatability in the laboratory is very important to precision, as well as data comparability. Repeatability is controlled by the development of detailed SOPs and adequate training in those SOPS. Laboratory precision can also be evaluated through the implementation of laboratory technical systems audits. These audits will be used to evaluate the adherence to the SOPS. Audits are discussed in Section 8.0.

3.5 Accuracy

As stated earlier, accuracy is based on the difference between an estimate, derived from data, and the true value of the parameter being estimated. For the laboratory measurements, the true value is dependent on the calibration of the instrument (ruler or scale). Following calibration procedures and precision requirements will provide an indication of accuracy. Following SOPs as written should reduce contamination as much as possible. Accuracy is also based on training. Therefore, during audits the trainer will remeasure 5% of the samples to determine accuracy. If accuracy requirements are not met, the trainer will review the methods with the sampler until agreement is reached.

3.6 Detectability

Detectability in this study is a function of how accurate and repeatable the measuring instruments can be maintained. Rulers or micrometers, unless broken, will be considered accurate. Therefore, detectability of length is a function of following the SOPs. Similarly, scales, if calibrated properly, should reflect an accurate weight. The SOPs will discuss ways to measure samples within the detectability requirements.

3.7 Completeness

Completeness for the field is defined as the successful collection of all viable samples in the appropriate time frame. A viable sample would be defined as any single sample whose integrity has not been effected during the collection process and would therefore not be flagged with a field qualifier.

In any case the DQOs are based on the evaluation of a statistically relevant number of samples which are effected by all errors occurring in the field and laboratory. Therefore, the overall goal is a completeness of 90%. The completeness objective for the measurement phase is 100%. As with the other data quality attributes, completeness can be controlled through the adherence to the SOPs in order to minimize contamination and sampling errors.

3.8 Representativeness

Representativeness, with respect to the overall program objectives is a function of the statistical sampling design and how well this design estimates the measurement parameters to this project. Variation in forage fish diet is expected seasonally but also from year-to-year, depending on the abundance of prey and environmental factors that might affect feeding behavior. Since the sampling period for this project is only one year, the review of past forage fish data will assist in determining how representative the 1994 diet of forage fish is to the yearly variation that can be expected.

3.9 Comparability

Comparability will be maintained by the adherence of the SOPs. Adherence of these SOPs by all samplers will allow for comparability of data among sites and throughout the project. Evaluation of comparability occurs through the implementation of the training program and the field technical systems audits.

Table 3.1. Measurement Quality Objectives for Forage Fish Diets and Zooplankton

Parameters	Sample Type	Frequency	Acceptance: Other Corrective Action
Location			The accuracy required is to regions of lake.
Fish Length			
Precision	Remeasurement	5%	2 mm of original measurement- recalibrate remeasure sample to compare to closest; add appropriate flags if unable to remeasure samples.
Accuracy	Independent remeasurement	5%	2 mm of original measurement - review protocols and remeasure another sample; add appropriate flags if unable to remeasure samples.
Completeness		NA	90%
Fish Weight			
Precision	Remeasurement	5%	1 g of the original measurement - recalibrate and remeasure sample to compare to closest; add appropriate flags if unable to reweigh samples.
Accuracy	Independent remeasurement	5%	1 g of original measurement - review protocols and remeasure another sample; add appropriate flags if unable to reweigh samples.
Completeness		NA	90%

Table 3.1. Measurement Quality Objectives for Forage Fish Diets and Zooplankton

Parameters	Sample Type	Frequency	Acceptance: Other Corrective Action
Zooplankton Species			
Precision	Re-identify, inspection	5%	95% identification, precision will be maintained through training and periodic audits to verify accuracy of identification prey items; add appropriate flags if unable to re-identify samples.
Accuracy	Independent re-identify, inspection	5%	See SOPS; add appropriate flags if unable to re-identify samples.
Completeness		NA	90%
Zooplankton Length			
Precision	Remeasurement	5%	+ 0.1 mm of original measurement - recalibrate instrument remeasure sample and compare to closest; add appropriate flags if unable to remeasure samples.
Accuracy	Independent re-identify, inspection	5%	+ 0.1 mm of original measurement - review remeasurement protocols and remeasure another sample, add appropriate flags if unable to remeasure samples.
Completeness		NA	90%

4.0 Site Selection and Sampling Procedures

Forage fish and zooplankton samples will be taken at three regions (Sturgeon Bay, Saugatuck and Port Washington) in spring, summer, and fall of 1994. Table 4.1 outlines the anticipated sampling regimes.

Table 4.1. Sampling Regimes

Biotic Element	Group	Number Collected/ Sample	Number Analyzed/ Sample	Collections	Total Analysis
Bloater	0-2 yr 4+ yr	20 20	10 10	9 (=3 seasons x 3 regions) 9	90 90
Alewife	60-120 mm >120 mm	20 20	10 10	9 9	90 90
Smelt	>100 mm	20	10	9	90
Sculpin	slimy deepwater	20 20	10 10	9 9	90 90
Total fish					630
Zooplankton		3 depth strata (hypolimnion, epilimnion, metalimnion)	3	54 = 3 seasons, 3 regions, 3 sites (within a region), 2 replicates/sites	162

Ten extra fish will be collected for each sample when possible to allow for empty stomachs. The extra fish can also be used to confirm diets if anomalous results are found in an area.

Formal chain of custody procedures are not required. However, records must be kept of sample collection, labeling, handling, transport, and laboratory analysis. Field sheets will be used to track integrity of sample from field to laboratory (Appendix). The unique sample I.D. assigned at collection will be carried through to data tabulation.

5.0 Analytical Procedures and Calibration

Standard Operating Procedures for field sampling and laboratory analyses are attached. Measurements of length and weight are the basic analytical procedures conducted for this project.

6.0 Data Reduction, Validation, and Reporting

The responsibility for data reduction, validation and reporting will be shared between Jacqueline Savino and Bruce Davis. All samples will be given a unique labeling code that identifies sample type, location, time of collection, replicate, and any other necessary information. Log books will be kept that record the sample I.D. code, pertinent collection site characteristics, and taxon (fish or zooplankton sample). All information gathered from fish preparation in the laboratory will be added to information in the log book.

Standard forms will be developed for laboratory data entry. Forms will be collected at the end of each week and checked for completeness. All data will be kept as "hard copy" and in computer files entered in ASCII data sets. Data set validation will be accomplished through comparison of hard copy with output of computer files.

Raw data will be permanently archived in mainframe computer files at the National Biological Survey - Great Lakes Science Center so that it can be referenced in the case of data entry error. Copies of all files will be held separately at the NOAA Great Lakes Environmental Research Laboratory as a means of protection against fire, vandalism, and computer failure.

The raw data will be reduced so that 1) average size of each forage fish species and their diet by taxon within a given strata (age-season-region) and 2) the average zooplankton abundance by taxon within a given strata (age-season-region-depth) can be reported (Table 6.1). The primary descriptive statistics calculated and reported will include means, frequency of occurrence, and sample sizes. The range and standard error associated with each mean will indicate the variance associated with these data.

Table 6.1. Reported Statistics Associated with Each Biotic Element

Biotic Element	Strata	Measurement	Statistics
Forage fish	age, season, region	length, weight	mean, standard error, range, sample size
Forage fish diet	age, season, region, diet taxon	number	mean, frequency of occurrence, standard error, range, sample size
		length	mean, standard error, range, sample size
Zooplankton	age, season, region, depth, taxon	number	mean, frequency of occurrence, standard error, range, sample size
		length	mean, standard error, range, sample size

7.0 Internal Quality Control Checks

Quality assurance for this project will be achieved primarily through specific training both prior to sampling and during the sampling season. Bruce Davis on the NBS staff is experienced in diet sampling and will provide training sessions on procedures before the sampling begins and while in progress. Personal observation of sample under magnification is required to provide identification of zooplankton to lowest possible taxon. Differences among observers will be checked at beginning of samples taken from each new site and season and for every 20 samples (5%) after initial checks. Replicate counts, identifications, and measurements taken by different individuals for a sample must agree within acceptance criteria in Table 3.1.

Measurements of length and weight required for this project are straightforward and their variation will be a function of the ruler or weight scale used than the person taking the measurement. The rulers or measuring boards will be examined prior to the field season to ensure the error between them is less than ±2 mm. The weight scales used for this project will be standardized against standard weights at the beginning of the project and compared to each other throughout the sampling period. The readability of the scales used is 1 g for forage fish. Size of prey individuals will be determined using dissecting microscope with an ocular micrometer. Other methods will be acceptable provided that precision requirements are met.

The PIs will review and verify all raw data. The PIs will have responsibility for all statistical analyses.

8.0 Performance and Systems Audits

Specific Audits will not be conducted as part of this sampling project. Procedures required for this project are straightforward and not complicated. The duration of the project is also short enough that the yearly checks on performance of the field and laboratory staff will serve as audit checks for this project. In yearly checks, we will use acceptance criteria in SOPs. The amount of staff involved in this project will be few, therefore, the ability to control the quality of the project will not require elaborate auditing procedures. Quality control audits at each stage of the field sampling and analysis will be conducted by the Project Manager, the Field Manager, or the EPA QA Manager. Audit reports will be kept on file at the NBS-GLC and available for review at any time.

Inadequacies in sampling procedures or the quality of the data collected will be addressed immediately by the Project Manager or Field Manager when discovered. All previous and current data collected by the person when the inadequacies will be review for accuracy.

An audit form for this project will be developed.

9.0 Calculation of Data Quality Indicators

9.1 Precision

For QA reporting we will use relative standard deviation to report precision.

$$RSD = (s/\bar{y}) \; x \; 100\%$$

Where: RSD = *relative standard deviation*
s = *standard deviation*
$\bar{y}$ = *mean of replicate analyses*

$$s = \left(\sum_{i=1}^{n} (y_i - \bar{y})^2/(n-1) \right)^{½}$$

Where: s = *standard deviation*
y_i = *measured value of the ith replicate*
$\bar{y}$ = *mean of replicate measurements*
n = *number of replicates*

However, on a case by case reporting we will use absolute differences between measurements to insure that they are within criteria stated in MQOs (Table 3.1).

9.2 Accuracy

Accuracy will be based upon expert remeasurements of a percentage of samples.

Accuracy will be evaluated by determining whether the measurements are within the acceptance limits (Table 3.1). Deviations beyond the acceptance criteria could be justification for retraining technicians.

Bias can be estimated from the theoretical "true" value of the expert measurement. "System" bias for the study may be calculated from individual samples and is defined:

$$Bias = \left\{ \sum (Y_{ik} - R_i) \right\} /n$$

Where: Y_{ik} = *the average observed value for the ith audit sample and k observations*
R_i = *is the theoretical reference value*
n = *the number of reference samples used in the assessment*

9.3 Completeness

Completeness is defined as follows for all measurements:

$$\%C = 100\% \times (V/n)$$

Where: %C = percent completeness
V = number of measurements judged valid
n = total number of measurements necessary to achieve a specified level of confidence in decision making.

9.4 Representativeness

Based upon the objectives, the three seasonal collections (spring, summer, fall) represent different forage fish diet conditions. In order to determine whether a change is statistically significant, the samples must be representative of the population, and the samples must be collected and analyzed in a consistent manner. Based on our sampling design (Table 4.1), we assume that we are getting a representative sample of fish and zooplankton within a region and season. We will evaluate representative through qualitative comparisons of past samples from Lake Michigan.

9.5 Comparability

Comparability is very similar to representativeness in that comparability is ensured through the use of similar sampling and analytical techniques. Comparability will be assessed through the evaluation of precision and accuracy measurements and technical systems audits.

10.0 Corrective Action

Table 3.1, Table 10.1, internal consistency sections, SOPs, and audit section discuss the corrective action plan. Jacqueline Savino and Bruce Davis will initiate corrective actions. Audit reports will document corrective actions through data flags. Will revise QA plans if methods change.

Table 10.1 provides an initial list of flags. PIs will develop flags as conditions warrant.

Table 10.1. List of Flags.

LAC	laboratory accident	There was an accident in the laboratory that either destroyed the sample or rendered it not suitable for analysis
FAC	field accident	There was an accident in the field that either destroyed the sample or rendered it not suitable for analysis.
ISP	improper sample preservation	Due to improper preservation of the sample, it was rendered not suitable for analysis.
UNK	unknown sex	In the case of species, indicates undetermined sex.
EER	entry error	The recorded value is known to be incorrect but the correct values cannot be determined to enter a correction.
OTL	data point outlier	When a series of data are plotted and analyzed, this point is obviously not within the normal distribution of the data, and eliminated from further analysis.
RET	returned for re-analysis	The analysis result is not approved by laboratory management and re-analysis is required by the bench analyst with no change in the method.
REN	re-analyzed	The indicated analysis results were generated from a re-analysis of the same sample.
REJ	rejected	The analysis results have been rejected for an unspecified reason by the laboratory. For any results where a mean is being determined, this data was not utilized in the calculation of the mean.
BAC	background correction	Background correction has been applied to this value.

11.0 Quality Control Reports to Management

A progress report outlining the achievement of the Quality Assurance Objectives will be provided to the Program Manager at the end of the project. The Project Manager will be notified immediately, however, if substantive changes are made to the QAPjP. The Quality Control Report will include a summary of the results of audits that were conducted, data quality assessment, and the corrective actions that were taken. The report will use statistical techniques defined in Section 9.0 and will state whether quality was better or worse than expectations defined in Table 3.1.

Appendix 1.
Standard Operating Procedures

1.0 Collecting Forage and Zooplankton

This SOP is intended to provide a step by step procedure for collecting forage fish and zooplankton to use in determining forage fish diets and zooplankton abundance in the Enhanced Monitoring Program Lake Michigan Mass Balance Study.

1.1 Overview

Forage fish and zooplankton will be collected at three regions and three seasons in Lake Michigan. Specific details of the study are documented in the Lake Michigan Mass Balance workplan and in the QA project plan. Critical and non-critical associated information, as follows, will be recorded:

Critical	Non-Critical
Location	Sample depth
Date of sample	Time of sample
Sample length	Sample weight
Age	Water temperature

These samples will be collected by NBS personnel while on their vessels. Therefore, there is a good chance that both critical and noncritical measurements will be taken.

Summary of Method

The following sampling activities will take place and are discussed in detail:

1) Collection of fish samples
2) Collection of zooplankton samples

1.2 Safety

In any field operation, emphasis must be placed on safety. Samplers must be aware of the potential safety hazards to which they are subjected. Follow all safety protocols and equipment guidelines, and be prepared for emergency situations. The sampler is responsible for his/her safety from potential hazards.

1.3 Equipment Check and Calibration

1.3.1 Serviceable Equipment

Fishing vessel equipped with
- Locational instrument (GPS, Loran)
- Sampling gear (midwater, bottom trawl)
- Plankton net
- Ice chests, including appropriate amount of ice
- Measuring board (mm markings required)
- Spring scale (1-10 Kg)

Calibrating weight

1.3.2 Consumable Equipment

Fish storage bag
Formalin
Phloxine B dye
Alka-seltzer
Sugar
Borax
Bucket
Sample labels
Reporting sheets
Marking equipment (pencils and permanent marker)
Scale envelopes
Cleaning sponge and brush
Rubber gloves for
    preserving fish
    handling fish
Glass sample jars (zooplankton)

1.3.3 Calibration and Standardization

Equipment necessary for calibration and the required frequency can be found in Table 1.1. Record calibration information (date, standards, results, and corrective action) in log books.

Table 1.1. Equipment Necessary for Calibration and Required Frequency.

Instrument	Calibration Technique	Frequency	Acceptance Criteria
Thermometer	Ice bath or boiling water	Start and end of year	±2°
Locational Device	Record pier-head position	Per trip	Can be adjusted to ±0.25 Km
Measuring Board	Check against second device	Start and end of year	±2 mm

1.4 Procedures

1.4.1 Collection of fish samples

1.4.1.1 Fish distributions are determined using acoustic instrumentation aboard large vessels, and fish are captured with a midwater or bottom trawl.

1.4.1.2 For each collection of fish captured, record all site and sample identification data

specified on the Field Data Sheet and I.D. labels.

Note: Data recorded will include: Objective (forage diet), gear, lake, region, lat./long. or statistical grid, species, date, I.D. number, lake depth/capture depth, water temperature, time of capture/time of sampling, field qualifier flag, collector's name).

1.4.1.3 Subsamples of targeted fish are taken as follows:

Within the constraints of the demarcation of forage fish for diet, sampling into the age and size groups specified in the LMMB plan of October 14, 1993, special care must be taken to assure that these fish are representative by size (and hence age) of all fish caught of the various categories being sampled.

When the trawl catch is small, the entire catch is retained and sorted by species on the sorting table in the bow of the vessel. When the catch is large. however, it is first randomly subsampled in the stern of the boat after running it into plastic fish boxes that hold about 50 lb each. The randomization is accomplished by running the fish box or boxes back and forth over a 5 gallon bucket or buckets while fish are slowly "poured" from the box. The subsample in the buckets is sorted into species in the laboratory, and each species is counted.

A further sample of the catch of fish in each diet group will be obtained by first mixing and spreading all fish in a given group on the sorting table. All fish on a section of the table will then be retained for the diet sample. This procedure is intended to avoid the inevitable bias that occurs when the sorter picks fish individually from the catch.

Because the age of bloater chubs will not be known in the field, a length cut-off based on sampling in recent years will be used to obtain an approximate separation by age into the specified age categories for chubs of 0-2 years and 4+ years of age.

1.4.1.4 Captured fish are identified to species and counted.

1.4.1.5 Each sample is placed in labeled plastic bags and then deep-frozen or placed on ice.

1.4.1.6 Frozen fish are transported to NBS-Great Lakes Science Center on ice in coolers to the laboratory freezer.

1.4.2 Collection of zooplankton samples

1.4.2.1 Zooplankton samples will be taken with stratified vertical tows.

1.4.2.2 For each collection of zooplankton, record all site and sample identification data-specified on the Field Data Sheet and I.D. labels.

Note: Data recorded will include: Objective (zooplankton), gear, lake, region, site (within region), replicate, lat./long. or statistical grid, species, date, I.D. number, lake depth/capture depth, water temperature, time of capture/time of sampling, field qualifier flag, collector's name).

1.4.2.3 The outside of the net is backwashed with water after each haul to rinse all zooplankters into the bucket.

1.4.2.4 Place the cod end of the net in a bucket of water and add an alkaseltzer tablet (narcotizing and buffering agent).

1.4.2.5 Each sample is washed from the bucket, with distilled water, into a sample jar.

1.4.2.6 Add 4 g sucrose and 2 g Borax/100 mL water.

1.4.2.7 Add buffered formalin (with 8 mg Phloxine B dye/l formalin added to enhance visibility of zooplankton) such that each sample contains 5% formalin by volume.

1.4.2.8 Zooplankton samples are transported to the NBS-Great Lakes Science Center in federal vehicles.

1.4.2.9 Integrity of samples checked upon arrival to laboratory and recorded on field sampling data sheets.

2.0 Forage Fish Diets and Zooplankton Abundance

This SOP is intended to provide a step by step procedure for analyzing stomach contents of forage fish and zooplankton availability.

2.1 Overview

Stomach contents of forage fish and zooplankton availability will be analyzed in the laboratory at NBS-Great Lakes Science Center. Specific details of the study are documented in the Lake Michigan Mass Balance workplan and in the QA project plan. Critical and non-critical associated information, as follows, will be recorded:

Critical	Non-critical
taxon identification	taxon length
taxon number	

Summary of Method

The following sampling activities will take place and are discussed in detail:

1) Preparing and analyzing fish samples
2) Analyzing zooplankton samples
3) Data reporting

2.2 Safety

In any operation, emphasis must be placed on safety. Personnel must be aware of the potential safety hazards to which they are subjected. Follow all safety protocols and equipment guidelines, and be prepared for emergency situations. The laboratory personnel is responsible for his/her safety from potential hazards.

2.3 Equipment Check and Calibration

2.3.1 Serviceable Equipment

Fume hood
Rinse water supply and rinsing bath
Rinse tray
Dissecting tray and tools (scalpel, forceps, scissors)
Dissecting microscope with ocular micrometer
Electronic balance and calibration weights
Plastic ruler (mm divisions)
Glass specimen jars
Computer and printer (with hard drive, disk drive, and necessary software)

2.3.2 Consumable Equipment/Supplies

Formalin
Rubber gloves
Paper toweling
Plastic bags
Reporting sheets and marking devices

2.3.3 Calibration and Standardization

Equipment necessary for calibration and the required frequency can be found in Table 2.1.

Table 2.1. Equipment Necessary for Calibration and Required Frequency.

Instrument	**Calibration Technique**	**Frequency**	**Acceptance Criteria**
Plastic Ruler	Check against second device	Start-end/season	±0.5 mm
Electronic Balance	Use calibration weight (300 g) and slope adjust	Daily	±0.1 g
Computer	Virus scan	Every boot-up	No viruses
Ocular Micrometer	Check against second device	Start-end/season	±0.1 mm

2.4. Preparing and Analyzing Stomach Contents of Fish

Proceed with the following steps in a well ventilated (fume hood operating if necessary) area intended for work of this nature. Wear rubber gloves when handling preserved prey items, have equipment set up, calibrated and ready for use, and start with and maintain a clean work area.

2.4.1 Fish are thawed under cool water and individually weighed to the nearest gram and measured to the nearest millimeter.

2.4.2 Record lengths and weights for fish with unique I.D. labels in log books containing all associated information.

2.4.3 Stomachs are removed using surgical scissors (from esophagus to pyloric caecum). The stomach is then preserved in 10% formalin. At this time we also determine the sex of the individual fish if possible.

2.4.4 At a later date the stomachs are opened and contents removed completely. Contents are teased apart and assessed as to whether they can be completely counted or need to be subsampled (all large prey are counted completely).

2.4.5 Contents to be subsampled are diluted to a known volume (usually 100 mL), gently stirred, and a 10% subsample is removed.

2.4.6 The contents are then identified to the lowest possible taxon, enumerated, and measured with aid of a Ward counting wheel under a dissecting microscope with an ocular micrometer. Up to 10 individuals per taxon per fish are measured to the nearest micron.

2.4.7 Record data as indicated on record sheets.

2.5 Analyzing Zooplankton Samples

2.5.1 In the laboratory, each sample is strained and drained of formalin.

2.5.2 If subsampling is necessary, the sample is diluted with water of a known volume, stirred to provide a consistent density of plankton, and then subsampled (4 mL). The subsample is returned to the original sample after processing and the procedure is repeated for a total of three subsamples. Certain taxa (such as Mysis, Bythotrephes, and amphipods) are considered too large to be subsampled; all are removed by hand using the naked eye or a magnifying light, and then processed in the same manner.

2.5.3 The zooplankters are identified to lowest possible taxon, enumerated, and measured with aid of a Ward counting wheel under a dissecting microscope with an ocular micrometer. Most mature specimens can be identified to genus and species; most immatures can be identified to family or genus. Specimens smaller than rotifers (<100 microns) will not be counted. Up to 10 individuals per species per station are measured to the nearest micron.

2.5.4 The three subsample counts are averaged and the resulting mean is used to calculate number of organisms per liter (or cubic meter).

2.5.5 Record data as indicated on record sheets.