# APPENDIX IX.9

# AQUATIC ORGANISMS: COLLECTION METHODS AND TECHNICAL PROCEDURES

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# 1.0 METHODS

## 1.1 Introduction

This appendix details the technical methods followed during the collection of baseline data for the Aquatic Organisms and Habitat section (Section 9.5) of the Snap Lake environmental assessment (EA). The methods listed below fully describe those summarized in the body of the EA and provide more technical details of the specific sampling programs carried out for phytoplankton, zooplankton, benthic invertebrates, fisheries inventories and fish habitat mapping.

## 1.2 Phytoplankton Sampling

Samples for phytoplankton community and chlorophyll a (chl *a*) analysis were collected from Snap Lake and the reference lake during July, August, and September 1999 (Figure 9.5-1). Phytoplankton water samples were sub-sampled for analysis of nutrients.

In July, chl *a* and phytoplankton community structure samples were collected using a 500-millilitre (mL) integrated sampler through the top 6 metres (m) of the water column. In August and September, a Kemmerer bottle was used to collect a composite sample every 0.5 m to 1 m, depending on water column depth. Duplicate 1-litre (L) to 2-L water samples were filtered through a Whatman™ GF/C glass fibre filter using a Nalgene hand pump. The filters were then individually stored in Petri dishes, labelled, and frozen prior to chl *a* analysis. Community structure samples were stored in 125-mL Nalgene bottles and preserved with Lugol's solution. The University of Alberta Biological Science Laboratory conducted chl *a* analysis. Bio-Limno Research and Consulting of Edmonton, Alberta compiled phytoplankton community data.

## 1.3 Zooplankton Biomass and Community Structure Sampling

In July, August, and September, zooplankton samples were collected using a 76-micrometre (µm) plankton net through the top 6 m of the water column in Snap Lake and the reference lake (shown in Figure 9.5-1 in Aquatic Organisms and Habitat, Section 9.5). Biomass analysis and community structure samples from two 30-second vertical tows were preserved with 5% buffered formalin. Shallow water samples, in areas potential used by small fish, were also collected from two 30-second horizontal tows in July.

Methods used for the collection of phytoplankton for the analysis of chl *a*, phytoplankton and zooplankton community structure are outlined in Golder Associates' Technical Procedure 8.7-1 (Appendix A). Hydroqual Laboratories Ltd. (Calgary, Alberta)

calculated ash-free dry weight and Bio-Limno Research and Consulting (Edmonton, Alberta) analyzed zooplankton community structure.

### 1.4 Benthic Invertebrate Community Sampling

The benthic invertebrate community sampling program was carried out between September 8 and 13, 1999. In Snap Lake, benthic invertebrates were sampled at three sites around the northwest peninsula (shallow habitat [SH]1, SH2, and SH3) and one site in the eastern portion of the lake (water quality [WQ]3) (shown in Figure 9.5-2 in Aquatic Organisms and Habitat, Section 9.5). Four sites were sampled in the reference lake (water quality reference [WQR]1, WQR3, WQR7, and shallow habitat reference [SHR]2). All sites were close to the shore (*i.e.*, within 500 m) and were taken at a depth of 6 to 7 m.

Six replicate benthic invertebrate samples were collected at each site, using an Ekman grab of 0.023-square metres ($m^2$) bottom area (6-inch grab). Individual samples were sieved through a 250-µm mesh sieve bucket. The material retained by the sieve was preserved in 10% buffered formalin. A subsample from one additional Ekman grab sample was retained for analysis of sediment particle size, total organic carbon (TOC) and total inorganic carbon (TIC). A composite sediment sample was also collected at each site for sediment chemistry analysis. Benthic invertebrate and sediment collection methods are described in detail in Golder Associates' Technical Procedures 8.6-1 and 8.2-2 (Appendices B and C).

Qualitative kicknet samples were also collected along the shoreline in the vicinity of each benthos sampling site if shoreline vegetation was present, to ensure that all common invertebrates were captured. These samples were collected along shoreline vegetation using a 500-µm mesh D-frame kicknet. The sampler moved along the shoreline and disturbed the substrate and vegetation by kicking, while sweeping the net over the disturbed area to collect dislodged material and invertebrates.

Additional supporting measurements made at each site included water depth, Secchi depth, water temperature, dissolved oxygen, pH and conductivity. The field water quality measurements were made using a Hydrolab Multiprobe $H_20$ water quality meter and depth was determined using a graduated, weighted sounding line.

#### 1.4.1 Laboratory Methods

Benthic invertebrate samples were processed by AquaTax Consulting in Saskatoon, Saskatchewan. Each sample was washed through a 250-µm sieve and then preserved in 75 percent (%) ethanol prior to sorting. Five samples with large amounts of debris (site

SHR2, replicates 1 to 5) were subsampled using the method of Wrona *et al.* (1982), and 25% of these samples were sorted. Benthic invertebrates were removed from the detritus using a dissecting microscope at 6X to 16X magnification. All animals were removed from the sorted fractions of Ekman grab samples. Several representatives of each taxon were removed from kicknet samples for identification, and qualitative notes were made on the relative numbers of each taxon. Following identification, invertebrates were preserved in 75% ethanol. All remaining material was preserved for random checks of removal efficiency.

Benthic macroinvertebrates were identified to the lowest practical taxonomic level, usually genus. Damaged or very small specimens were typically identified to family. Chironomid midge larvae and oligochaete worms were mounted on microscope slides to facilitate identifications. Identifications were made using published keys (Davies 1971, Sawyer 1972, Edmunds *et al.* 1976, Wiggins 1977, Simpson and Bode 1980, Clarke 1981, Oliver and Roussel 1983, Wiederholm 1983, Pennak 1989, Oliver *et al*. 1990, Merritt and Cummins 1996), and comparisons with reference material. A series of representative specimens of each generic identification were prepared and have been archived for reference.

As a quality control measure, four samples were re-examined to ensure 95% of the total animals present in a sample were removed. If the number of specimens of the target groups missed by a sorter accounted for greater than 5% of the total number, all the samples in the corresponding block of samples were re-sorted. Quality control data are provided in Table IX.9-1.

**Table IX.9-1**
**Quality Control Data for Re-sorted Benthic Invertebrate Samples**

| Sample | Number Missed | Total Count | % Missed |
|---|---|---|---|
| WQR7-1 | 2 | 43 | 4.4 |
| WQR3-5 | 3 | 881 | 0.3 |
| SH2-2 | 1 | 210 | 0.5 |
| SH3-6 | 1 | 103 | 1.0 |

### 1.4.2 Data Analysis/Summarization

To prepare the benthic invertebrate data for the data summary, the data were checked for any potential data entry errors, non-benthic organisms were deleted and invertebrate abundance was expressed as numbers/$m^2$ (*i.e.*, the raw numbers were multiplied by 43).

The benthic invertebrate data were summarized as the site mean plus or minus 1 standard error for total abundance and taxonomic richness and as mean percentages of the total abundance contributed by each major invertebrate group at each site. Richness was also expressed as the total number of taxa present at each site (*i.e.*, combining the six replicates, rather than calculating a mean) and as the number of taxa collected in the qualitative shoreline kicknet samples. The supporting data directly relevant to benthic samples (water depth, sediment particle size, TOC, TIC) were also tabulated for each sampling site.

### 1.5 Fisheries Sampling

The objective of the fisheries baseline study was to characterize baseline environmental conditions of the Snap Lake area. It was initiated in 1998 by Hallam Knight Piesold and was expanded and continued in 1999 by Golder Associates. In 2001, additional baseline information was collected in relation to a small number of specific project components. The study documented existing aquatic information on pre-development conditions of waterbodies that may be affected by the Snap Lake Diamond Project or are in the vicinity of the Snap Lake Diamond Project. These waterbodies include Snap Lake as well as small lakes and streams near the Snap Lake Diamond Project (shown in Figure 9.5-3 in Aquatic Organisms and Habitat, Section 9.5). Information was collected from a reference lake that is comparable to Snap Lake but will not be affected by the Snap Lake Diamond Project. In addition, information was collected from MacKay Lake to further document regional fish health conditions (shown in Figure 9.5-4 in Aquatic Organisms and Habitat, Section 9.5). This information will be used as a baseline for comparison with monitoring data collected should the proposed project reach the operation phase.

Fish were sampled in Snap Lake in 1998 and again in 1999 when the fisheries study was expanded to include streams connecting to and from Snap Lakes and inland lakes in the vicinity of the Snap Lake Diamond Project (shown in Figure 9.5-3 in Aquatic Organisms and Habitat, Section 9.5). Waterbodies sampled in 1999 include:

- Snap Lake;
- a local reference lake;
- five inland lakes (inland lake [IL]2 to IL6) potentially near project elements;
- four northern inland lakes (northern inland lake [NL]1 to NL4) adjacent to Snap Lake; and,
- inlet and outlet streams connecting to and from Snap Lake.

In 2001 the study was expanded further to include three additional inland lakes (IL7 to IL9) that may be affected by the project footprint (Figure 9.5-3). The components of the survey on each waterbody are detailed in Table IX.9-2. General methods of the study are

listed below. Standard technical methods for each component of the survey (habitat mapping, bathymetry, fish inventory, fish tissue analysis) are detailed in the standard Golder Associates Technical Procedures 8.13-3, 8.16-0 and 8.19-0 (provided in Appendix D, E, and F).

**Table IX.9-2**
**Components of the 1999 and 2001 Fisheries Survey**

| Waterbody | Survey Details |
|---|---|
| **Snap Lake** | • shoreline lake habitat mapping<br>• bathymetry survey<br>• collection of muscle and liver tissue for metals analysis from two species of fish (round whitefish *Prosopium cylindraceum* and lake trout *Salvelinus namaycush*)<br>• general non-lethal fish inventory, building on 1998 inventory data<br>• sampling for fish in areas of potential rearing habitat<br>• fall lake trout spawning survey and habitat assessment |
| **Reference lake** | • collection of fish muscle and liver tissue for metals analysis from two species of fish (round whitefish and lake trout)<br>• sampling for fish in areas of potential rearing habitat |
| **MacKay Lake** | • collection of fish muscle and liver tissue for metals analysis from two species of fish (round whitefish and lake trout) |
| **Inland lakes** (2 to 9)<br>**Northern lakes** (1 to 4) | • lake bathymetry and shoreline habitat mapping<br>• general non-lethal fish inventory |
| **Select Snap Lake streams**: inlet and outlet tributaries to and from Snap Lake | • fish habitat survey at peak and low flows<br>• fish inventory or observations of fish in (or near) streams<br>• kick sampling for eggs and stream habitat use |

Another inland lake near snap Lake, IL1 was surveyed in the summer of 1999 IL1 has since been approved as the location of the water management pond (WMP) and was de-watered in March 2000. Results of fish habitat and bathymetry were presented in an earlier report to the Northwest Territories Water Board and the Department of Fisheries and Oceans and will not be repeated in this report.

### 1.5.1 Fish Inventory

A fish inventory was conducted on Snap Lake, reference lake, IL2 to IL6, and NL1 to NL4 from June 25 to July 19 July. In July 2001, IL6 was re-inventoried, and IL7 to IL9 were inventoried. Fish were captured by gillnets, angling (spin-cast and fly-fishing), minnow traps, seine nets, and by electrofishing. Seining was only attempted on one occasion and electrofishing on two occasions in Snap Lake; slippery conditions and

boulder substrate in the sampling areas prevented further use of these methods. Fishing was conducted according to Golder Associates' Technical Procedure 8.1-3 (Appendix D).

Gill nets and minnow traps were set in a variety of different habitat types in each lake. Nets were checked frequently to prevent fish mortality. Captured fish were released after recording the following parameters:

- length of time spent gill netting for the calculation of catch-per-unit-effort (CPUE);
- habitats sampled for fish;
- number of fish caught and mesh size they were caught in;
- species;
- state of maturity and sex (if possible);
- weight (grams);
- fork length (millimetre);
- aging structure; and,
- external health (evidence of parasites, lesions, body condition).

### 1.5.2 Fish Tissue Collection on Snap Lake, the Reference Lakes, and MacKay Lake

Adult fish from two levels of the aquatic food chain (lake trout, a predator; round whitefish, a secondary consumer) were collected from Snap Lake, the reference lake, and MacKay Lake. Snap Lake and the reference lake were sampled in July 1999, and Mackay Lake was sampled in July 2001. Ten lake trout and 14 round whitefish from Snap Lake, 10 lake trout and 10 round whitefish from the reference lake, and 10 lake trout and 15 round whitefish from MacKay Lake were retained for tissue analysis. Muscle and liver tissue were collected from each fish for determination of metal concentrations.

Samples of muscle and liver were removed from each fish according to Golder Associates' Technical Procedure 8.16-0 (Appendix E). Fish tissue from Snap Lake and the reference lake was submitted frozen to Taiga Environmental Labs in Yellowknife, NWT for determination of concentration of 23 metals. MacKay Lake samples were submitted frozen to EnviroTest Laboratories in Edmonton, Alberta for determination of metals concentration. Tissue not used in the analysis was archived for potential future analysis.

Due to the small size of the round whitefish livers, individual livers were combined and homogenized by the laboratory to form a "pooled sample". From Snap Lake, livers were pooled into four groups and analyzed. One additional liver was analyzed individually Therefore, the total sample size for Snap Lake was equal to five. Livers were pooled into

six groups from the reference site. From the MacKay Lake samples, livers were pooled into four groups (three groups containing two livers each and one group containing three livers). Six livers were analyzed individually from MacKay Lake. Pooling was based on combining fish of the same size and sex.

### 1.5.3 Lake Trout Spawning Survey of Snap Lake

Fall fish spawning surveys were conducted to identify the location of spawning habitat for lake trout. Lake trout were collected from September 1 to 13, 1999 to establish the location and confirm usage of habitat by spawning fish. Potential lake trout spawning habitats in Snap Lake were examined. After arriving at each suspected spawning site, the area was visually checked for congregations of adult lake trout. The field crew travelled the length of the shoal or shoreline while counting fish observed. If lake trout were present, the density of spawning fish was assessed primarily by angling. Results were compared based on ease of fish capture and measured in terms of the CPUE. CPUE was measured as the number of fish caught per angler per hour of effort. Gill netting was conducted, though it was minimized to avoid unnecessary mortalities. The majority of lake trout captured were returned to Snap Lake after determining fish weight, length, sex (if possible), assessment of spawning condition, and collection of a non-lethal ageing structure.

## 1.6 Bathymetry

The bathymetry of Snap Lake, IL2 to IL6, and NL1 to NL4 was surveyed during the period of June 26 to July 18, 1999. IL7 to IL9 were surveyed in July 2001. Lake bathymetry was recorded for each lake along transects with a chart recording echo-sounder. Data from the echo-sounder was used to create a bathymetric map of each lake.

## 1.7 Fish Habitat Mapping

Habitat mapping of Snap Lake, IL2 to IL6 and NL1 to NL4 occurred between June 27 and July 18, 1999. IL7, IL8, and IL9 were surveyed in July 2001. Habitat mapping was conducted according to standard Golder Associates' Technical Procedure 8.19-0 (Appendix F). Habitat mapping was done by visual estimation. During habitat mapping, shoreline characteristics were recorded on an enlarged 1:50,000-scale map of each lake. Photographs were also taken of each lake. The following characteristics were recorded for the shoreline immediately above the waterline:

- shoreline slope (flat, repose, steep, cliff, overhanging);
- shoreline soil types (silt, sand, gravel, cobble, boulder, bedrock);

- shoreline vegetation (bare, grasses, emergent macrophytes, open tundra, short willow, tall willow);
- evidence of erosion;
- location of stream inlets and outlets. Also checked the amount of flow coming in or leaving the lake and a visual inspection for fish; and,
- location and height of high watermark.

The following characteristics were recorded for the shoreline (terrestrial) and nearshore (water's edge to the 1-m contour) areas:

- terrestrial shoreline slope or gradient (flat, repose, steep, sharp drop-off) and vegetation and terrain characteristics;
- nearshore substrate types (detritus, silt, sand, gravel, cobble, boulder, bedrock);
- nearshore emergent, floating or submergent macrophytes; and,
- evidence of erosion from the shoreline or silt plumes from inlet streams.

### 1.7.1 Stream Surveys

The intent of the stream habitat surveys was to collect information on habitat capability, accessibility of the streams, as well as fish species presence and use of the streams. Streams both within the Snap Lake Diamond Project area and in the surrounding area were surveyed in order to gauge the relative stream habitat availability for the Snap Lake fish community.

A total of 30 inlet streams and two outlet streams were delineated around Snap Lake from 1:50,000 National Topographical Series maps and aerial surveys (shown in Figure 9.5-3 in Aquatic Organisms and Habitat, Section 9.5). These streams were selected to provide a representative sample of stream habitat available in the Snap Lake basin. One additional stream (stream [S]31), connecting three inland lakes (IL 3, IL4, and IL5) near the Snap Lake Diamond Project footprint was also documented.

Habitat surveys were completed on fourteen streams around Snap Lake in early June, 1999; twelve inlet streams to Snap Lake (S1[WQ], S2[WQ], S4, S7[WQ], S10[WQ], S12, S20[WQ], S22, S24, S25[WQ], S27[WQ], and S30[WQ]), and two outlet streams from Snap Lake (H1 and H2). The initial surveys conducted during the freshet (June 3 to June 7, 1999) included habitat mapping for channel characteristics, substrate composition, barrier to fish migration, water quality measurements, spawning habitat suitability and nursery habitat suitability. Aerial surveys were conducted on six additional inlet streams with poor or undefined channels during the same period (S3, S8, S15, S16, S17, S18, and S29).

A helicopter survey of the streams was conducted approximately one week later (June 17, 1999) to document fish observations in the streams and to record stream temperatures. Following this, kick sampling for fish eggs and observations of stream habitat use were conducted on June 25 and 26, 1999. A habitat survey for S31 was conducted on June 27, 1999, when it was determined that the proposed airstrip would cross this stream (culvert crossing). A mid-summer survey (July 20 and 21, 1999) of S31 was conducted again to record observations of stream conditions and fish use, particularly in relation to rearing habitat availability.

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## 3.0 UNITS AND ACRONYMS

### UNITS

| | |
|---|---|
| L | Litre |
| m | Metre |
| $m^2$ | square metre |
| mL | Millilitre |
| numbers/$m^2$ | number per square metre |
| µm | Micrometer |
| % | Percent |

### ACRONYMS

| | |
|---|---|
| CPUE | catch-per-unit-effort |
| chl *a* | chlorophyll a |
| EA | environmental assessment |
| IL | inland lakes |
| NL | northern inland lakes |
| NWT | Northwest Territories |
| S | Stream |
| SH | shallow habitat |
| SHR | shallow habitat reference |
| TIC | total inorganic carbon |
| TOC | total organic carbon |
| WMP | water management pond |
| WQ | water quality |
| WQR | water quality reference |

# APPENDIX A

# GOLDER ASSOCIATES' TECHNICAL PROCEDURE 8.7-1
# PHYTOPLANKTON AND ZOOPLANKTON SAMPLING

# 1 PURPOSE

This technical procedure describes methods for sampling phytoplankton and zooplankton for taxonomic composition (counts of organisms) or biomass analysis.

# 2 APPLICABILITY

This technical procedure is applicable to the collection of plankton samples from freshwater. The methods described below are intended primarily for sampling lakes (standing water).

# 3 DEFINITIONS

## 3.1 Analytical Request Form

Standard form provided by analytical laboratories. This form is filled out by the person collecting samples and is used to indicate how each sample is to be analyzed. This form is often combined with the Chain-of-Custody Form in a single document.

## 3.2 Chain-of-Custody Form

Standard form used to track the movement of samples from the time they are collected until they arrive at the specified analytical laboratory. The Chain-of-Custody form provides a clear record of sample transport and handling, thereby reducing the risk of sample loss during transport. This form may be combined with the Analytical Request Form in a single document.

## 3.3 Chlorophyll *a*

Plant organic molecule that is the primary photosynthetic pigment in plants. It is present in all algae and aquatic and terrestrial plants and can be used to estimate plant biomass. Approximately 1.5% of the dry weight of organic matter (ash-free dry weight) of algae is constituted by chlorophyll *a*.

## 3.4 Composite Sample

Sample containing a mixture of water collected from multiple locations or from different times at the same location.

## 3.5 Grab Sample

Sample collected during a single sampling event (i.e., water taken from a given place at a given time).

### 3.6 Plankton Net

Conical net suspended on a graduated rope, used to sample zooplankton. As the net is pulled through the water column it filters plankton from the water. It is constructed of nylon netting of known mesh size, which should be appropriate to the desired size range of plankton to be sampled.

### 3.7 Phytoplankton

Single-celled aquatic plants, referred to as algae, which live suspended in the water column.

### 3.8 Quality Assurance/Quality Control (QA/QC)

Quality assurance and quality control practices in biological studies determine data integrity and are relevant to all aspects of a study, from sample collection to data analysis and reporting. Quality assurance encompasses all management and technical practices designed to ensure that the data are of consistent high quality. Quality control is a specific aspect of quality assurance, and includes techniques used to assess data quality and remedial measures to be taken when data quality objectives are not met.

### 3.9 Replicate Samples

Replicate samples are used to evaluate within-site variation. Replicate samples are collected by filling multiple containers at a single site. They are labelled and preserved individually and are submitted separately to the analytical laboratory.

### 3.10 Specific Work Instructions (SWI)

Detailed instructions in a standardized format provided to project personnel. The SWI describe all aspects of the work to be conducted, including personnel allocation, procedures to be used, time allocation, deliverables and any additional information deemed necessary by the project or task manager.

### 3.11 Zooplankton

Small animals, raging from microscopic to a few millimetres in size, living suspended in the water column. Freshwater zooplankton is usually dominated by small crustaceans such as cladocerans (water fleas) and copepods, and rotifers.

## 4 REFERENCES AND SUGGESTED READING

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## 5 METHODS AND DISCUSSION

### 5.1 General Safety

Refer to Golder Associates Ltd. Safety Manual.

### 5.2 Methods

### 5.3 Site Location

Sampling site locations should be accurately described in the SWI. Sites should be located as near as possible to water quality sampling sites and away from aquatic vegetation unless specified otherwise. Once the exact sampling location has been determined in the field, it must be accurately described relative to permanent landmarks such as groundwater wells, effluent outfalls or distinctive landscape features. Measurements with electronic distance measuring devices and compass headings are recommended as a minimum to accurately determine position. To obtain more accurate site position data, a global positioning system (GPS) unit should be used.

### 5.4 Phytoplankton Sampling Methods

Phytoplankton samples should be collected from an anchored boat, using a bottle-type sampler such as the Van Dorn sampler, which is commonly used for collecting water samples. Sample amount should be adjusted according to the productivity of the lake sampled: up to 6 L may be required in unproductive (oligotrophic) waters; 0.5 to 1 L is usually adequate in richer waters. Water samples for phytoplankton analysis should be composites from several depths, and several replicate samples should be collected at each site to reliably estimate phytoplankton density or biomass.

The following procedure should be used to sample phytoplankton for taxonomic analysis:

1. Label sample bottle as described below.
2. Rinse sampling equipment in ambient water to remove any clinging material.
3. Set triggering device on sampler.
4. Lower sampler to desired depth and drop messenger to trigger sampler.
5. Raise sampler to the surface.

6. If only a single grab sample is to be collected, drain water from the sampler into a plastic sample bottle of appropriate size and proceed to Step 9; if a composite sample is to be collected, drain water from the sampler into a large bottle used to prepare composite sample and proceed to Step 7.
7. Collect additional samples from other depths (specified in SWI) and add them to the composite bottle.
8. Mix composite sample and fill sample bottle of appropriate size.
9. Preserve sample using Lugol's solution.
10. If required, collect additional replicate samples according to the above steps.

Samples collected for analysis after a few days of storage should be preserved by adding 0.3 mL of Lugol's solution to 100 mL sample, which imparts a tea colour to the sample. For long-term storage, samples should be preserved with 0.7 mL Lugol's solution per 100 mL sample and buffered formaldehyde to a minimum of 2.5% final concentration after 1 hour. Lugol's solution consists of 20 g potassium iodide (KI) and 10 g iodine crystals in 200 mL distilled water containing 20 mL glacial acetic acid. Samples should be stored in the dark, either refrigerated, or at ambient temperature.

Phytoplankton samples for biomass estimates (as chlorophyll *a*) should be collected in the same manner as water quality samples (refer to TP 8.3, Surface Water Sampling Methods). Briefly, the sample should be collected into a pre-cleaned water sample bottle provided by the analytical laboratory, and preserved by adding $MgCO_3$ solution also supplied by the analytical laboratory. Water samples for chlorophyll *a* analysis should be stored in the dark on wet ice, and delivered to the analytical laboratory as soon as possible.

### 5.5 Zooplankton Sampling Methods

Samples should be collected from an anchored boat. The preferred method to collect zooplankton samples is the vertical tow using a plankton net, because it provides a depth-integrated sample. However, in shallow water, oblique or horizontal tows may be necessary. Replicate samples should be collected at each site to reliably estimate zooplankton density or biomass.

Consider the following points when selecting sampling equipment and during sampling:

- A nylon, monofilament plankton net of 158 µm (Silk No. 10) mesh size collects all microcrustaceans and most rotifers and is sufficient for sampling zooplankton. However, smaller mesh sizes (e.g. 76 µm [Silk No. 20]) may be more common and are also acceptable.

- Length of the tow may have to be adjusted to prevent clogging of the net, especially when using a fine mesh net (e.g., 76 µm) in productive lakes. If the net has a pronounced green or brown colour after it is pulled up, clogging has probably occurred and the length of the tow should be reduced.

- Plankton nets with holes or rips should never be used, since such defects may cause considerable sample loss.

- If the sample bucket on the bottom of the plankton net is made of plastic, attaching a weight of 1 to 3 kilograms will cause the net to sink faster to the desired depth and is also useful to maintain vertical direction when lowering and pulling up.

- Depending on the design of the sample bucket, the sample may contain some water, which may necessitate the use of concentrated preservatives to achieve the desired concentrations.

The following procedure should be used to sample zooplankton for taxonomic analysis or biomass determination:

1. Label sample bottle as described below.
2. Rinse sampling equipment in ambient water to remove any clinging material.
3. Lower plankton net to the desired depth.
4. Pull net up vertically at an even rate of approximately 0.5 m/s.
5. Remove sample bucket from the bottom of the net.
6. Empty sample bucket (usually by removing stopper on bottom) and empty sample into sample bottle (100 to 200 mL, plastic or glass). Use a squeeze bottle containing preservative to rinse all animals from the sample bucket.
7. Preserve sample in 5% buffered formaldehyde or 70% ethanol. Ethanol is preferred if samples are to be stored.
8. If required, collect additional replicate samples according to the above steps.

Oblique tows may be carried out by casting the net some distance from the boat and, once it sank to the desired depth, pulling it back through the water. Horizontal tows may be made using the same approach, but not allowing the net to sink before pulling it back. Alternatively, if longer tows are required, the boat may be driven forward at an even, slow speed during oblique and horizontal tows. It may be necessary to modify the way in which the plankton net and the weight are suspended to collect samples using horizontal or oblique tows (i.e., attach weight on the end of a 1 to 2 m length of rope tied to the point of attachment between the plankton net and the rope on which it is suspended).

### 5.6 Sample Labelling

Sample labels must contain the following information:

- project number
- sample identifier (name of site or sample code)
- date (written as day/month/year; month abbreviated as three letters) and time of collection (24 hour notation)
- initials of collector
- type of sample (e.g., zooplankton, phytoplankton).

Fill out labels using waterproof ink and affix a label to each sample container. Plastic bottles may be labelled by writing directly on the bottle using a waterproof marker. Writing on the bottle is not recommended if samples are transported over long distances (friction may rub label off) or if bags of ice are used to keep the samples cool (water may damage label information). Double labelling of each bottle (e.g., a waterproof adhesive label affixed to the bottle and writing the same information directly on the bottle with an indelible marker) is recommended to prevent loss of label information during transport.

Transportation of Dangerous Goods (TDG) and Workplace Hazardous Materials Information System (WHMIS) regulations must be followed when handling, transporting and storing samples.

## 5.7 Field Notebooks

Field notebooks must be kept, describing all field activities and sample-related details. Format of field notes and information to be recorded should follow Golder Associates' specific guidelines. During the field survey, field notes must be maintained in a permanent, safe location at the field site or office. If possible, new entries in the field note book should be photocopied at the end of each day and copies should be stored in a safe place.

In addition to standard field notes, the information below should be recorded when sampling phytoplankton or zooplankton:

**Phytoplankton**

- sample type [grab/composite]
- number of samples used to prepare composite sample
- depth of grab sample or of individual samples used to prepare composite sample
- final sample amount
- preservative used
- if possible, basic limnological variables (e.g., water depth, temperature, dissolved oxygen concentration, pH, conductivity, depth profiles of some of these variables, turbidity, Secchi depth, presence and density of vegetation)

**Zooplankton**

- plankton net mesh size
- net design (e.g., simple conical, Wisconsin, etc.)
- type of tow (vertical, oblique, horizontal)
- preservative used
- sufficient information to allow calculation of the volume of water filtered through the net, including:
  - diameter of mouth of plankton net
  - sample depth (vertical and oblique tows)
  - horizontal distance sampled (horizontal or oblique tows)
  - angle of oblique tow
- if possible, basic limnological variables (e.g., water depth, temperature, dissolved oxygen concentration, pH, conductivity, depth profiles of some of these variables, turbidity, Secchi depth, presence and density of vegetation)

## 5.8 Chain-of-Custody Forms and Analytical Request Forms

Chain-of-Custody and Analytical Request forms must accompany all samples submitted for analysis. These forms are usually combined as a single document. The combined form must be filled out completely and the white and yellow copies should be sent along with the samples being submitted. Field personnel should retain the pink copy after it is signed by the shipper. Depending on the shipping container, these forms can either be enclosed inside the sealed container or attached firmly to the outside of the container. In either case, it is advisable to enclose the forms within a waterproof plastic bag to guard against damage.

It is important that each person having custody or control of the samples identify themselves on the Chain-of-Custody form. This means that the person collecting the sample, any intermediate persons involved in packaging, storing or transporting the sample and the person accepting the sample on behalf of the analytical lab must all be identified.

## 6 EQUIPMENT

The following are lists of recommended equipment for locating sampling sites, sampling, sample documentation and shipping, and field safety. These are only general lists and specific objectives and design of each study should be considered when selecting equipment. Boat and associated equipment are not included in the lists below.

### Sampling Site Location

- maps of study area and air photos
- long measuring tape
- electronic distance measuring device
- compass
- GPS unit
- survey flagging tape
- camera and film

### Phytoplankton Sampling Equipment

- pre-printed labels or waterproof label tape
- waterproof pens and pencils
- Van Dorn or similar sampler on graduated rope
- large, clean bottle to prepare composite sample
- sample bottles
- preservative (Lugol's solution [20 g potassium iodide (KI) and 10 g iodine crystals in 200 mL distilled water containing 20 mL glacial acetic acid]; $MgCO_3$ solution for chlorophyll *a* samples [usually provided by analytical laboratory])
- cooler and ice packs

### Zooplankton Sampling Equipment

- pre-printed labels or waterproof label tape
- waterproof pens and pencils
- plankton net with sample bucket, graduated line and weight
- sample bottles
- preservative (10% buffered formaldehyde or 95% ethanol)
- squeeze bottle containing preservative
- cooler or plastic bin to store samples

**General Water Quality Measurements**

- dissolved oxygen meter and calibration equipment
- pH meter and calibration buffers
- conductivity meter and calibration solution
- turbidity meter and calibration equipment
- Secchi disk
- thermometer
- graduated depth sounding line

**Sample Documentation and Shipping**

- waterproof pens and pencils
- bound, water-proof field logbooks
- photo log
- combined Analytical Request and Chain-of-Custody forms
- waterproof bag for forms
- packing tape

**Health and Safety Equipment**

- waders and waterproof gloves
- heavy socks, warm pants, rain gear and other articles of clothing suitable for prolonged water work
- dry bag
- cellular telephone or two-way radio
- extra set of clothes
- first aid kit
- approved personal floatation device for deep water or boat work

# APPENDIX B

## GOLDER ASSOCIATES' TECHNICAL PROCEDURE 8.6-1
## BENTHIC INVERTEBRATE SAMPLING

# 1 PURPOSE

This technical procedure describes the methods to be used for sampling benthic invertebrates for community structure analysis and tissue analysis. Detailed sampling procedures are provided for the use of the Neill cylinder, Hess sampler, Surber sampler, the Ekman and Ponar grabs, kicknet for community sampling and the hand-held net for tissue sampling.

# 2 APPLICABILITY

This technical procedure is applicable to any persons involved in the collection of benthic invertebrates from streams, rivers and lakes. Since it contains a variety of sampling techniques that are appropriate for a range of benthic habitats, it is not restricted to a given geographic area.

# 3 DEFINITIONS

## 3.1 Benthic Invertebrates (benthic macroinvertebrates, benthos, zoobenthos)

Non-vertebrate animals, such as insects, crustaceans, worms and mollusks, that inhabit the bottoms of waterbodies. Macroinvertebrates are visible to the unaided eye and are frequently defined as those animals that are larger than 0.5 mm. Benthic invertebrates may live on the surface of the substratum, between particles, or burrowed into the substratum to various depths, or on aquatic plants.

## 3.2 Benthic Habitat

The physical and biological environment which provides a place for benthic (bottom-dwelling) animals to live. Invertebrate habitat may be broadly characterized as run, riffle, backwater, pool, erosional and depositional (see below). More detailed habitat characterization is required during invertebrate surveys, as outlined in Section 5.4.

## 3.3 Chain-of-Custody Form

Standardized form used as a means of keeping close track of samples that are taken in the field and are subsequently transported to laboratories for chemical or taxonomic analysis. Whenever the samples are transported from one location to the next, the custody is relinquished from the delivery person to the receiver by signing the forms and indicating date and time. These forms substantially decrease the risk of losing samples because they provide a clear record of the chain of transport of the samples.

## 3.4 Depositional Habitat

Standing water or slow moving areas in streams and rivers where bottom sediments are soft, consisting of sand and smaller particles.

### 3.5 Erosional Habitat

Wave-washed areas of lakes and areas of streams and rivers with moderate to fast currents and hard bottoms consisting of a variety of particle sizes, but usually dominated by gravel and larger particles.

### 3.6 Exposure Area

Part of the study area that is exposed to the effluent or disturbance being monitored. Data collected from the reference area (see below) are compared with data from the exposure area to evaluate the presence and severity of environmental effects.

### 3.7 Littoral Zone

The near-shore area of lakes, where light penetration is sufficient to allow the growth of rooted aquatic plants (macrophytes) or plant-like (macrophytic) algae. The littoral zone is usually the most productive area of lakes and forms a belt of varying width around the periphery of lakes. The size and maximum depth of the littoral zone largely depends on water clarity, bottom sediment characteristics, wave exposure and the extent of water level fluctuation.

### 3.8 Profundal Zone

The deep area of lakes, where light penetration is low, characterized by exposed fine sediments free of vegetation.

### 3.9 Reference (Control) Area

Part of the study area that is not exposed to the effluent or disturbance being monitored, representing the baseline condition in the river or lake monitored. Data collected from the reference area are compared with data from the exposure area to evaluate the presence and severity of environmental effects.

### 3.10 Replicate Sample

Replicate samples are additional samples collected from a sampling site. The number of replicate samples is specific to the project and should be included in the Specific Work Instructions (SWI).

### 3.11 Specific Work Instructions (SWI)

Detailed instructions in a standardized format provided to field personnel. The SWI describe all aspects of the work to be conducted, including personnel allocation, procedures to be used, time allocation and any additional information deemed necessary by the project manager.

### 3.12 Substratum

The bottom of waterbodies, usually consisting of varying proportions of organic detritus, clay, silt, sand, gravel, cobble and bedrock.

### 3.13 Tracer

A chemical or variable such as conductivity that can be used as an indicator of the presence and approximate dilution of a discharge from a point source. Field measurements of a tracer can aid in the selection of sampling sites.

## 4 REFERENCES AND SUGGESTED READING

Alberta Environment. 1990. Selected methods for the monitoring of benthic invertebrates in Alberta rivers. Environmental Quality Monitoring Branch, Environmental Assessment Division, Edmonton, AB. 41 pp.

Environment Canada. 1993. Guidelines for monitoring benthos in freshwater environments. Prepared by EVS Consultants for Environment Canada, North Vancouver, BC. 81 pp.

Klemm, D.J., P.A. Lewis, F. Fulk and J.M. Lazorchak. 1990. Macroinvertebrate field and laboratory methods for evaluating the biological integrity of surface waters. Environmental Monitoring Systems Laboratory, Cincinnati, U.S. Environmental Protection Agency, EPA/600/4-90/030, 256 pp.

Rosenberg, D.M. and V.H. Resh (Eds.). 1993. Freshwater biomonitoring and benthic macroinvertebrates. Chapman & Hall, New York, 488 pp.

## 5 DISCUSSION

### 5.1 General Safety

Refer to Golder Associates Ltd. Safety Manual. Transportation of Dangerous Goods (TDG) and Workplace Hazardous Materials Information System (WHMIS) regulations must be followed when handling, transporting and storing samples.

### 5.2 Site Selection

Approximate site locations should be identified prior to the field survey and should be selected according to the SWI. Exact sampling sites should be selected in the field to ensure that sites within a habitat type (i.e., erosional or depositional) are as similar in terms of physical characteristics (especially current velocity, depth and substratum composition) as possible.

When sampling lakes, one's ability to assess the composition of the substratum is limited. Therefore, test grabs should be collected to ascertain that bottom sediments are suitable for grab sampling and comparable to those of other sampling locations. Special care should be taken to minimize the variation in depth among sampling sites (unless the objectives of the study indicate otherwise), since depth is one of the most important factors affecting benthic invertebrate community structure in lakes. It may also be useful to estimate the depth of the littoral zone prior to sampling, since benthic communities within the littoral zone (shallow water) are usually considerably different from those in the profundal zone (deep water).

When sampling erosional sites in rivers or streams, site selection should focus on minimizing variation in terms of current velocity and substratum composition, since most sampling devices useful in such areas can only be operated within a limited depth range. In depositional areas, minimizing variation in depth and substratum composition should be the major consideration. An initial visual survey of the study reach is highly recommended to select the habitat types that are available in all sampling areas. This is especially important during studies of effects of wastewater discharges, because benthic habitat in the exposure area may be limited to a few types, and reference sites must be as closely matched to sites sampled in the exposure area as possible.

One additional consideration when selecting sampling sites during monitoring studies is exposure to the effluent or disturbance being monitored. When monitoring the effect of a specific discharge, it is advisable to select a simple tracer of the effluent that can be measured in the field, which will allow the evaluation of the relative exposure of each site during sampling. A frequently used tracer is conductivity, since the majority of effluents have typically high conductivity compared with ambient values. Measurement of conductivity along a river transect at 1 m intervals will usually be adequate to locate the area of greatest exposure and provide an idea of the width of the plume.

Sampling sites must be accurately located relative to permanent landmarks, such as man-made structures or distinctive landscape features. If possible, measurements with long tape measure and electronic distance measuring devices should be used, in addition to coordinates obtained using a Global Positioning System (GPS) unit. Regardless of the method used for this purpose, detailed notes regarding site locations should be made in the field logbook or on the field data sheets, site locations should be marked on a topographic map and a photograph of the sampling site and relevant landmarks should be taken.

## 5.3 Sampling Methods

### 5.3.1 Neill Cylinder or Hess Sampler (erosional habitat)

The following steps should be followed to collect samples using these devices:

1. Select sampling site (Section 5.2). The area to be sampled should be undisturbed, at most 60 cm deep, in run or riffle habitat with moderate to high current velocity and gravel/cobble substratum.

2. Label sample bottle (1-L, wide mouth, plastic bottle) and attach it to the sampler net. An additional label, written with pencil on waterproof paper, should also be placed inside the sample bottle. (Shoulder-length gloves should be worn following this step to protect hands.)

3. Starting near the downstream limit of the sampling site, drive the bottom of the cylinder into the substratum and hold it there for the duration of sampling, with the sample net and attached bottle pointing downstream. Ensure that the seal at the bottom of the cylinder is adequate to prevent animals from escaping during sampling. Water should be flowing through the cylinder, entering through the circular hole at the front and exiting through the sampling net.

4. Reach into the cylinder and agitate the substratum manually to dislodge invertebrates, which will be transported into the downstream net. Gently rub the surfaces of all large rocks within the water enclosed by the cylinder and remove them until only smaller-sized particles (gravel and smaller) are left inside the cylinder. Using your hands, a small shovel, or a heavy-duty garden trowel, stir up the bottom to 5-10 cm depth. This entire step should take approximately 1 minute.

5. Allow suspended material to be transported into the net or to settle. Lift the cylinder with the net pointing down and dip it into the water a few times to transport all invertebrates clinging to the inside of the sampling net into the sample bottle.

6. Place the sampler on the shore or on a convenient surface and fold the net sampler over the mouth of the sample bottle. Pour out as much of the water as possible. When done, spray a small amount of water on the folded-over net to back-wash clinging organisms into the bottle.

7. Remove the bottle and add preservative. The 1-L sample bottle should be at most 1/2 full prior to adding preservative. Add 95% ethanol to obtain approximately 70-80% dilution, or buffered formalin to obtain approximately 10% dilution. Cap bottle, gently agitate it to distribute preservative evenly, double-check label and place it in a container for transport.

8. Rinse the cylinder and net in river water thoroughly to remove any clinging invertebrates and plant material.

Additional replicate samples should be collected using the same methods, from an undisturbed area upstream or adjacent the location of the previous replicate sample. Number of replicate samples should be specified in the SWI. Because differences in sample composition may occur due to slight differences in sampling technique among individuals, it is recommended that all samples for a study should be collected by the same person.

### 5.3.2 Surber Sampler (erosional habitat)

The operation of the Surber sampler is very similar to that of the Neill cylinder. It delineates the same area of the river bottom (0.1 $m^2$), but does not fully enclose it, which makes it prone to loss of some of the sample around the net. If given the choice of either sampler, a cylinder-type sampler (Neill cylinder or Hess sampler) should be used because it is a more quantitative sampling device. However, equipment availability, and logistic considerations (the Neill cylinder is heavy and unwieldy to carry) may

necessitate using the Surber sampler. Since Golder Associates owns a number of Surber samplers with different mesh sizes, it is important to select the right one. Mesh sizes >500 µm should not be used for benthic invertebrate sampling. Preferably, mesh size should be between 200 to 250 µm for benthic invertebrate sampling, but 500 µm mesh is sometimes acceptable. If in doubt, check SWI or verify the required mesh size with the project manager or a benthic invertebrate biologist.

The following steps should be followed to collect samples using this device:

1. Select sampling site (Section 5.2). The area to be sampled should be undisturbed, shallow enough for reaching the bottom with one's hands, in run or riffle habitat with moderate to high current velocity and gravel/cobble substratum.

2. Unfold the sampler, label a sample bottle and attach it to the sampler net. An additional label, written with pencil on waterproof paper, should also be placed inside the sample bottle. (Shoulder-length gloves should be worn following this step to protect hands.)

3. Starting near the downstream limit of the sampling site, place the bottom of the sampler on the substratum and hold it there for the duration of sampling, with the sample net and attached bottle pointing downstream. Ensure that the sampler is securely held on the bottom and that there is no space under its downstream side, which would allow invertebrates to bypass the net.

4. Reach into the enclosed area and agitate the substratum manually to dislodge invertebrates, which will be transported into the net. Gently rub the surfaces of all large rocks and remove them until only smaller-sized particles (gravel and smaller) are left in the sample area. Using your hand, a small shovel, or a heavy-duty garden trowel, stir up the bottom to a 5-10 cm depth. This entire step should take approximately 1 minute.

5. Allow suspended material to be transported into the net or to settle. Lift the sampler with the net pointing downstream and if necessary, spray the net with stream water a few times to transport all invertebrates into the sample bottle.

6. Fold the net over the mouth of the sample bottle. Pour out as much of the water as possible. When done, spray a small amount of water on the folded-over net to back-wash clinging organisms into the bottle.

7. Remove the bottle and add preservative. The 1 L sample bottle should be at most 1/2 full prior to adding preservative. Add 95% ethanol to obtain approximately 70-80% dilution, or buffered formalin to obtain approximately 10% dilution. Cap bottle, gently agitate it to distribute preservative evenly, double-check label and place it in a container for transport.

8. Rinse the sampler and net in river water thoroughly to remove any clinging invertebrates and plant material.

Additional replicate samples should be collected using the same methods, from an undisturbed area upstream or adjacent the location of the previous replicate sample. Because differences in sample composition may occur due to slight differences in sampling technique among individuals, it is recommended that all samples for a study should be collected by the same person.

### 5.3.3 Ekman and Ponar Grabs (standing water and depositional habitat)

Note that these samplers, especially the Ekman grab, require periodic maintenance even during sampling. Bolts frequently become loose during sampling and parts such as the springs and the messenger assembly (Ekman), or the hinge pin and the spring-loaded release pin (Ponar) may fall off, rendering the grab useless. For this reason, it is advisable to have a set of spare parts on the boat whenever these devices are used. The ropes attached to the grabs should also be checked periodically for wear.

The following steps should be followed to collect samples using these devices:

1. Select sampling site (Section 5.2). The area to be sampled should be undisturbed, with slow moving or standing water and soft sediments.

2. Label sample bottle. (Work gloves should be worn from this step to protect hands.)

3. Open grab and set triggering mechanism.

4. Slowly lower sampler to the bottom, at the approximate rate of 0.5 m/s, until it stops. Allowing the sampler to free-fall will generate a shock wave which invertebrates can sense and mobile animals will evacuate the area quickly. In addition, the Ponar grab is susceptible to closing before it reaches the bottom if lowered too quickly. It is advisable to determine water depth using a sonar device or a graduated sounding line before lowering the grab.

5. Send the messenger down (Ekman), or press button on top of pole (pole-mounted Ekman), or give the rope one quick, but gentle pull (Ponar) to close jaws. Pull sampler to the surface. As it comes out of the water check to see if the jaws were completely closed. If any leakage occurs, hold a sieve or sieve bucket of appropriate mesh size (200 to 500 µm, to be determined prior to sampling) below the grab as it is lifted from the water. If plant material or rocks caught in the jaws prevent complete closing, discard sample. Otherwise, continue with the next step.

6. Pour water out of the sampler through its top opening, into the sieve or sieve bucket (the sample material collected in the sieve or sieve bucket should be retained, because it is part of the sample). Set sampler down into a metal or plastic tray. Open jaws and lift sampler to remove the enclosed sediment. Examine the sample. If the grab was >60% full, with an undisturbed top layer, retain it for analysis; otherwise discard it and repeat procedure.

7. Use a spoon to scoop sample into the sieve or sieve bucket (which already contains the material that was poured from the grab after it was lifted from the water). Lower the sieve bucket into ambient water several times using “washing machine”-like circular motion or pour water into the sieve from

the top to wash out silt and clay. If there is a large amount of material, it may be necessary to sieve small amounts at a time. Adding a drop of dish-washing detergent and mixing may help if surface tension is preventing draining of the sieve. It may be more practical to do this step near the shore, after all replicates have been collected from a site, in which case the entire sample can be temporarily stored in a large, labelled Ziploc® bag prior to sieving. If this step proves to be very time-consuming or impractical, it may be skipped, but the amount of preservative and the number of sample jars may have to be increased to accommodate the larger sample amount.

8. Pour or spoon the sample into a pre-labelled sample jar and preserve. An additional label, written with pencil on waterproof paper, should also be placed inside the sample bottle. The 1 L sample bottle should be at most half full. Add 95% ethanol to fill the jar, or buffered formalin to obtain approximately 10% dilution. It may be necessary to use more than one jar per sample; if this is the case label jars as "1 of 2", "2 of 2" etc. If there is a large amount of organic material in the sample, increase the amount of preservative. Cap bottle, gently agitate it to distribute preservative evenly, double-check label and place in container for transport.

9. Rinse the sampler and tray in ambient water thoroughly to remove any sediment or clinging invertebrates.

Additional replicate samples should be collected using the same methods, from an undisturbed area.

### 5.3.4 Kicknet (erosional habitat)

Kicknet sampling may be used to collect quantitative samples that can be used to calculate densities of invertebrates, or qualitative samples that represent all species inhabiting an area but are not useful to determine densities. Use of this sampling device is different for each of these objectives. There are a variety of methods to collect samples using a kicknet and differences in sample composition due to differences between the techniques of different individuals have been commonly reported. For this reason, the quantitative procedure below is only a guideline and may be adjusted to suit individuals, but it is recommended that all samples for a study should be collected by the same person. If this is not possible, a number of sites (minimum of three) should be sampled by each individual and results should be compared to allow adjustments for potential biases.

**Procedure for Quantitative Kicknet Sampling**

Prior to collecting samples to be retained for analysis, it is necessary to determine the length of area to be sampled (usually between 3 and 5 metres) and the amount of time allocated per sample (usually between 15 seconds and 1 minute). In a productive river, both of these will have to be lower than in unproductive rivers to arrive at a sample size that is reasonable. As a general guideline, if a sample collected using the initially-chosen distance and time contains mostly organic material (detritus, algae), aim for an amount of sample material that is no more than a third of a 1-L sample jar. If it consists mostly of sand and gravel up to half of a jar may be appropriate. Once the length of area and amount of time are determined, all samples will have to be collected according to those numbers.

1. Select sampling site (Section 5.2). The area to be sampled should be undisturbed, shallow enough for safe foot-hold, in run or riffle habitat with moderate to high current velocity and gravel/cobble substratum.

2. Label a sample bottle and leave in on the shore. An additional label, written with pencil on waterproof paper, should also be placed inside the sample bottle.

3. Starting near the upstream limit of the sampling site (facing downstream), place the kicknet in your path (pointing downstream) and slowly move downstream, while kicking the substratum vigorously. Adjust distance and speed to the pre-determined values. Hold the net at the bottom to minimize escape of animals under the net.

4. Lift the net and quickly run it through river water to concentrate the sample material in its tip. Turn the net inside out and transfer sample into the sample jar.

5. Add preservative. The 1 L sample bottle should be at most 1/2 full prior to adding preservative. Add 95% ethanol to obtain approximately 70-80% dilution or buffered formalin to obtain approximately 10% dilution. Cap bottle, gently agitate it to distribute preservative evenly, double-check label and place it in a container for transport.

6. Rinse the net in river water thoroughly to remove any clinging invertebrates and plant material.

7. Collect additional replicate samples as required.

**Procedure for Qualitative Kicknet Sampling**

Since the aim of this type of sampling is to collect all species present in an area, site selection should be aimed at locating an area with a wide variety of habitats (pools, riffles, backwaters, vegetation, snags, etc.) or to spread out sampling effort in a relatively large area to ensure adequate coverage. The individual sampling should visit all potential habitats, disturb the bottom or vegetation, and sweep the net in the water to collect dislodged material. Depending on the amount of material being collected, it is simplest to restrict the sampling effort per site according to the amount of time spent sampling. Replicate samples are usually not collected when using this method. Sample preservation and labelling should follow methods provided for other devices.

### 5.3.5 Hand-held Net for Tissue Sampling (erosional habitat)

The purpose of sampling for tissues is to collect as much invertebrate material (i.e. as many animals) as possible for chemical analysis. The required sample amount usually varies between 5 and 10 g, wet weight, though certain analyses may require more or less sample amount. Always verify the amount of sample needed prior to sampling (refer to SWI). Also find out whether there is a need for extra sample material in the form of replicate samples, or for spiking (a laboratory quality control technique), which usually increases the required sample amount considerably.

To collect sufficient sample material, it is necessary to select areas of potentially high abundance of large invertebrates such as larvae of net-spinning caddisflies and nymphs of stoneflies and dragonflies. Shallow, fast riffles with low to moderate growths of benthic algae on cobble/gravel substratum are usually the most promising areas for sampling. Note that in some cases, especially in areas with gross metal contamination, even riffles may be devoid of invertebrates, preventing tissue collection altogether.

Sampling equipment and decontamination methods must be matched to the analytes. For organic chemical analysis, all equipment (sampling net, tweezers) and anything that may come into contact with the sample must be made of metal and must be pre-rinsed with appropriate solvents to remove contaminants. Insect repellents contain organic chemicals and should be avoided. For metals analysis, only plastic materials should be used and the sample container should be appropriately cleaned. Always verify sampling equipment and decontamination requirements prior to sampling (refer to SWI).

Use the following procedure to collect samples:

1. Select sampling site as above. The area to be sampled should be undisturbed and shallow enough for safe foot-hold.

2. Label a sample bottle on the outside only, pre-weigh it on a field balance to the nearest gram and leave in on the shore with the cap on.

3. Starting near the downstream limit of the sampling site, one person should hold a large (e.g. 50 x 100 cm) net in the water facing upstream. The net should be resting on the bottom to minimize the number of animals escaping under it. One or two additional persons should vigorously kick the substratum just upstream from the net for approximately a minute.

4. Remove the net and place it on the shore on a convenient surface, being careful not to allow the invertebrates on the net to come into contact with other materials. Using a net mounted on a rigid frame works well for this step. Using tweezers, remove large invertebrates and place them in the sample bottle. Weigh the sample jar periodically to keep track of sample amount. The sample bottle should be stored on dry ice if sampling is interrupted for more than 15 minutes and should be capped immediately after adding invertebrates.

5. Once all large invertebrates have been picked off, rinse the net in ambient water.

6. Repeat procedure until the desired sample amount is obtained.

Samples should be stored and shipped frozen, on dry ice. To allow taxonomic identification of the animals constituting the samples, collect representative specimens and record their approximate relative abundances in the tissue samples. Preserve these animals in 70% ethanol or 10% buffered formalin for subsequent taxonomic identification.

### 5.4 Field Measurements and Observations

Benthic invertebrate samples should be accompanied by appropriate physical measurements and field observations to allow detailed data analysis. At minimum, habitat type, current velocity, substratum composition, depth and the presence and amount of algae and plant material should be recorded at each site. However, if time and equipment are available, it is preferable to collect or record the following information:

- habitat (run/riffle/etc.) at the site;
- stream width (bankfull and wetted widths);
- riparian vegetation, degree of shading;
- current velocity and depth at each replicate sample location;
- sampler fullness (if grab sampler used);
- substratum composition in the general area of the site as percent cover by each major particle type, in standard size categories and embeddedness (see field data sheet);
- a sediment sample for analysis of texture and organic content (depositional habitat) or weights of at least three size fractions of bottom material (erosional habitat);
- relative amount of benthic algae on the substratum and a composite samples of benthic algae for analysis of chlorophyll *a* content;
- species and percent cover of aquatic macrophytes at the site;
- general water quality measurements: conductivity, pH, dissolved oxygen, water temperature, turbidity, odour;
- any pertinent observations, such as the presence of visible pollution, disturbance by animals or humans, weather conditions, etc.;
- exact site and sample location as UTM coordinates (50-position waypoint and rover file collected using GPS unit), distance from landmarks, distance from shore;
- weather conditions; and
- photograph of the sampling site, showing nearby landmarks.

### 5.5 Sample Labelling and Handling

Chain-of-Custody forms should be used to track samples. Sample labels should include:

- project number;
- sampling date;
- site location or site code;
- replicate number (separated by a hyphen from the site code); and,
- jar number (if applicable).

Preserved benthic invertebrate samples do not require special handling and holding time is indefinite at room temperature. However, if ethanol is used as the preservative and there is a large amount of organic material in the samples, the preservative should be replaced within one day of sampling with fresh 70% ethanol to prevent sample degradation. Transportation of Dangerous Goods (TDG) and Workplace Hazardous Materials Information System (WHMIS) regulations must be followed when handling, transporting and storing samples.

### 5.6 Field Records and Logbook

All pertinent information on field activities, sampling efforts and results must be recorded on the appropriate field data sheets and waterproof, bound notebook. The field crew leader is responsible for ensuring that sufficient detail is recorded in the field book. The field notes must be complete enough to enable someone unfamiliar with the project to completely reconstruct field activities without relying on the memory of the field crew. The field crew must be informed by the project manager of the objectives and requirements of the project so that they will be certain of recording the pertinent information.

The project number and title should be recorded on the front cover of the field book with an indelible felt marker. If more than one book is completed for the project, they should be numbered as 1 of 2, 2 of 2, etc. Golder identification and the company address should be recorded on the inside of the front cover. Entries in the field book and on the data sheets can be made in pencil unless the project requires the use of indelible ink.

The following procedures are required for all field notes:

- begin a new page each day
- number each page
- date each page
- a line should be drawn through the remainder of any partly used page
- all entries should be signed by the author
- all corrections made by a single-line cross-out of the error, initialled and dated

The above guidelines are designed to make the field notes for each day concise, neat and organized. When signing your entries, each section should be signed if different authors are recording notes. If only one author is recording for the day, signing the end of the day's notes is sufficient. When recording the date in the field book, always spell out the month rather than using d/m/y format.

Entries in the field book must include the following, when appropriate:

- names of field supervisor and field crew
- purpose of proposed sampling effort
- clear identification of sampling site name/number
- location of each sampling site or area (including map reference or position data such as UTM coordinates)
- description of each sampling site or area
- time of sampling
- details of sampling effort (method, area covered, etc.)
- deviations from technical procedures, if any
- sample identification codes
- field measurements (e.g., temperature, flow, D.O., etc.)
- field observations
- reference to field data sheets or any other methods used to record data
- information for photographs taken (roll no./photo no.)

- video tape reference (tape no./timer reference)
- sample shipping/tracking information

If any of the above information is recorded on the field data sheet, it does not have to be repeated in the field book. An example of the field data sheet benthic invertebrate sampling is provided in Appendix I of this technical procedure. Field forms to be used during sampling should be photocopied to waterproof paper.

Identification of the personnel who were present is very important. This should be recorded on the first day of the field job and is sufficient if the personnel does not change. On any day on which field personnel change, it should be recorded in the field book. This includes adding a field member, reducing the field crew or working with guests such as field auditors or clients. For non-Golder personnel, record the name, company and title of all personnel.

The location of the sampling site/area should be clearly identified and a reference supplied to indicate the map on which the location is recorded. Any position information that is available should also be recorded such as GPS rover file names and GPS waypoints as UTM coordinates.

In the field notes, record a reference to field data sheets used to record data from the site (e.g., "benthic sample-specific data are recorded on field data form"). Also reference any other documents that were used to record data (e.g., habitat maps, sonar tracings, video) so that there is one document that lists all of the data records available for the sampling event. All field data recorded in some form other than the field book (such as video tape, sonar tracings, etc.) should be clearly labelled as to its origin and purpose, including the following: project number, date, personnel, sampling location, and any other information that would identify it in case it was separated from the rest of the project data.

It is important to make the field data sheets as complete as possible. Not all fields on a data sheet will be applicable to every project, but it should be completed to the extent that it can be. Check for missing data before leaving the sampling site.

At the end of each day, all data records (field data sheets, etc.) that are separate from the field book should be properly organized and stored. They should not be taken into the field the next day unless they are not yet completed. Certain projects may require some level of duplication of the field data. The project manager should inform the field supervisor prior to job initiation of these requirements. This may involve recording data in duplicate while in the field or photocopying field notes and/or field forms at the end of each day, or at the end of the field program. For projects involving multiple field visits, where the field book is taken into the field on one or more different occasions, the field book should be copied between visits and the copies left at the office.

## 6 EQUIPMENT

The following is a list of the equipment recommended for benthic invertebrate sampling. It should only be used as a guideline, since the specifics of a study should dictate exact equipment requirements.

**Sampling for Community Composition**

- container for sample jars (plastic tub or cooler)
- extra sampler net and other parts that are failure-prone
- fine mesh net piece (for pouring water out of sample jar)
- garden trowel or small shovel (for Neill cylinder and Hess sampler)
- indelible ink felt tip markers
- metal or plastic tray
- preservative
- rope for grab samplers
- sample containers (1-L plastic jars recommended)
- sample jar labels (or waterproof tape)
- sampling device
- scoops or spoons
- sieve or sieve bucket of appropriate mesh size

**Sampling for Tissues**

- cooler with dry ice
- decontamination equipment (tarp, soap, brushes, containers, trays, pipettes and bulbs, distilled water, solvents, waste bottles, aluminum foil, etc.)
- field balance
- indelible ink felt tip markers
- large sample net mounted on a frame (metal or fiberglass window-screening may be used)
- sample jars and labels
- tweezers (metal or plastic depending on analytes of interest)

**Record-keeping and Site Locating/Marking**

- camera and film
- Chain-of-Custody forms
- field data sheets on water-proof paper and clipboard
- indelible ink pens and pencils
- long tape measure, electronic distance measuring device, GPS unit
- maps of area for site locations
- survey flagging tape
- GPS unit
- water-proof field logbook

**Physical Measurements**

- calibration solutions and buffers
- conductivity meter
- current velocity meter and wading rod
- dissolved oxygen meter
- pH meter

- turbidity meter
- Winkler kit (dissolved oxygen calibration)

**Health and Safety Equipment**

- approved personal floatation device for working in deep, fast water
- cellular telephone
- first aid kit

**Personal Gear and Miscellaneous Equipment**

- appropriate clothing (plus one extra set)
- drinking water
- knife
- rain gear
- sun protection
- waders (chest or hip)
- waterproof gloves (shoulder length for Neill cylinder and Hess sampler)
- work gloves

**Boat and Associated Equipment (if required)**

- air pump (if inflatable boat used)
- anchor
- approved personal floatation devices
- fire extinguisher
- fuel
- paddles
- rope
- spare keys
- spare parts
- tool box
- two-stroke oil
- water (bilge) pump

**APPENDIX I**

**FIELD DATA SHEET FOR**

**BENTHIC INVERTEBRATE SAMPLING**

***GOLDER ASSOCIATES LTD.***

*10th Floor, 940 Sixth Ave. S.W. Calgary, Alberta, T2P 3T1, Phone: (403) 299-5600, Fax: (403) 299-5606*

# *BENTHIC INVERTEBRATE SAMPLE COLLECTION*

## *FIELD DATA SHEET*

**Project:** **Station:** **Date:**

**River:**

**Crew Leader Signature:** **Start Time:** **Finish Time:**

**Crew (initials):** **Field Notes Recorded by (initials):**

***WEATHER*** Wind (dir.+vel. in km/h): Air Temp. ($^{o}$C): Precip.: Cloud Cover (%):

***SITE DESCRIPTION OR SKETCH MAP:*** **Photo #:**

**GPS Waypoint (UTM): E** **N** **Rover Filename:**

| ***FIELD WQ / HABITAT*** | | | **Substratum Particle** | **% Areal Cov.** (visual est.) |
|---|---|---|---|---|
| Diss. Oxygen (mg/L): | Conductivity (µS/cm): | Benthic Algae (N/L/M/H): | Sand/Silt/Clay (<2 mm) | |
| | | | Small gravel (2-16 mm) | |
| pH: | Water Temp. ($^{o}$C): | Bankfull Channel Width (m): | Large gravel (16-64 mm) | |
| | | Wetted Channel Width (m): | Small Cobble (64-128 mm) | |
| Odour: | | Macrophytes (species, % cover): | Large Cobble (128-256 mm) | |
| Habitat Type: | | | Boulder (>256 mm) | |
| | | Substratum Embeddedness (%) | Bedrock | |

***BENTHIC SAMPLES*** Sampling Device: Person Sampling:

Mesh Size: Preservative:

| **Sample Label** | **Distance from _____ Bank (m)** | **Depth (m)** | **Current Velocity (m/s)** | **Sampler Fullness (%) (if Ekman/Ponar)** | **Bottom Material Weights (kg) in Standard Size Categories (mm)** | | | | | **Comment** |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | **0.5-2** | **2-4** | **4-16** | **16-64** | **>64** | |
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***OTHER SAMPLES / MEASUREMENTS / OBSERVATIONS***

**Benthic algae** (circle one): **Y** **N** If Yes, record following:

Sample label(s):

No. cobbles/sample: No. replicates:

Area scraped/cobble:

Samples collected for: Chl-a _____ AFDW _____

**Water** (circle one): **Y** **N** If Yes, record following:

Sample label(s):

**Sediment** (circle one): **Y** **N** If Yes, record following:

Exact sample location relative to benthic samples:

Sample label(s):

Sampling Device: No. grabs/sample:

Colour: Texture: Current:

Depth: Odour: Top _____ cm



**CODES:** N = none; L = low; M = moderate; H = high

# APPENDIX C

## GOLDER ASSOCIATES' TECHNICAL PROCEDURE 8.2-2
## SEDIMENT SAMPLING

# 1 PURPOSE

This technical procedure describes the methods to be used for sampling bottom sediment (referred to below as sediment) for analysis of physical, chemical or toxicological characteristics. It does not apply to collection of sediment for benthic community analysis, which is covered in TP 8.6 (Benthic Invertebrate Sampling).

# 2 APPLICABILITY

This technical procedure is applicable to any persons involved in the collection of sediment and is not restricted to any geographic area.

# 3 DEFINITIONS

## 3.1 Analytical Request Form

Standard form provided by analytical laboratories. This form is filled out by the person collecting samples and is used to indicate how each sample is to be analyzed. This form is often combined with the Chain-of-Custody Form in a single document.

## 3.2 Chain-of-Custody Form

Standard form used to track the movement of sample containers from the time they leave the field until they arrive at the specified laboratory. The Chain-of-Custody form provides a clear record of sample transport and handling, thereby reducing the risk of sample loss during transport. This form may be combined with the Analytical Request Form in a single document. Golder Associates' combined form is attached as Appendix 1.

## 3.3 Chemical Analysis

Analytical procedure used to measure the *amount* of a certain compound, or group of compounds, present in a sample.

## 3.4 Quality Assurance/Quality Control (QA/QC)

Quality Assurance refers to a detailed protocol used to produce high quality products, while Quality Control refers to the process by which this protocol is tested to ensure that final products are of the specified quality. With reference to sediment sampling, QA protocol includes the use trained personnel, proper sampling methods, clean containers and equipment, proper sample preservation and transportation and detailed documentation of the entire process; field, travel and other test blanks are used for Quality Control testing.

### 3.5 Sample Types

#### 3.5.1 Grab Samples

Sample containing sediment collected during a single sampling event (i.e., sediment taken from a given place at a given time).

#### 3.5.2 Composite Samples

Sample containing a mixture of sediment collected from multiple locations or from different times at the same location.

#### 3.5.3 Replicate Samples

Replicate samples are used to evaluate within-site variation. Replicate samples are collected by filling multiple containers at a single site. They are labelled and preserved individually and are submitted separately to the analytical laboratory. Check the SWI for the number of replicate samples required per sampling site.

#### 3.5.4 Split Samples

Split samples are used to check analytical variation. A single sample (e.g. grab) is collected and is split into two sample containers. These are labelled and preserved individually and are submitted separately to the analytical laboratory.

### 3.6 Sediment

Loose material on the bottom of waterbodies, including organic material (live plants or decaying plant material) and inorganic material of varying particle size.

### 3.7 Specific Work Instructions (SWI)

Detailed instructions in a standardized format provided to field personnel. The SWI describe all aspects of the work to be conducted, including personnel allocation, procedures to be used, time allocation and any additional information deemed necessary by the project or task manager.

### 3.8 Toxicity Analysis

Analytical procedure specifically designed to examine how the health of living organisms may be affected by exposure to a given substance or sample. Toxicity tests can be based on either: acute exposures (short-term exposures lasting only a small portion of the animals life cycle, e.g. 96 hours for rainbow trout); or, chronic exposures (longer-term exposures meant to represent a significant portion of

the animal's life cycle, or a particularly sensitive portion of the animal's life cycle, e.g. 28 days for *Daphnia magna*). Responses measured in toxicity tests can be lethal (e.g. mortality), or sublethal (e.g., reduced growth or reproduction). Unlike other procedures, toxicity testing evaluates the sample as a whole, rather than describing its chemical make-up.

## 4 REFERENCES AND SUGGESTED READING

Clesceri, L.S., A.E. Greenberg and R.R. Trussell. 1989. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, D.C., U.S.A.

Environment Canada. 1993. Quality Assurance in Water Quality Monitoring. Ecosystem Sciences and Evaluation Directorate Conservation and Protection. Ottawa, Ontario.

## 5 DISCUSSION

### 5.1 General Safety

Refer to Golder Associates Ltd. Health and Safety Manual.

### 5.2 Methods

#### 5.2.1 Sampling Site Selection and Identification

General sampling locations are described in SWI. However, field crews will have a certain degree of freedom in choosing the exact locations from which to take the samples. When selecting these sites, personnel should consider the layout of the local environment, project objectives and personal safety. They should then choose areas that are both easily accessible and representative of the target waterbody or waterbodies.

Once sampling sites have been identified, they must be accurately described relative to permanent landmarks, such as groundwater wells, outfalls or distinctive landscape features; measuring the distance from permanent landmarks to each site with an appropriate compass heading is recommended. Ideally, one should try to use the Global Positioning System (GPS), but locations can also be recorded as the perpendicular distance from the shoreline and the distance upstream or downstream of a permanent landmark.

### 5.2.2 Sampling Methods

To ensure the contaminant-free collection of representative sediment samples, consider the following points:

- collect as representative a sample as possible based on the local sediment conditions and safety;
- avoid obvious sources of contamination when collecting samples, unless those sources represent the impact being investigated;
- use an appropriate sampling device, cleaned consistently with the specific requirements of the sampling program (consult SWI);
- sampling equipment should be cleaned between sites as specified in the SWI; and
- only pre-cleaned sample containers provided by the analytical laboratory or those approved by the laboratory should be used.

**Grab Samples (Ekman, Ponar, Peterson)**

1. Label sample container with indelible ink marker.

2. Grab sampler should be rinsed twice with ambient water prior to sampling to ensure no sediment or other material are attached. This should be done with the jaws open. Be sure to check that sediments have not dried on to the sampler. If so, remove dry material to prevent contamination and rinse sampler again. Additional cleaning may be required, as specified in the SWI.

3. Using a graduated line attached to the top of the sampler, lower it **slowly** until it touches the bottom. If using the Ekman grab, be sure to retain the messenger (small weight used to trigger sampler) at the surface. Be careful not to touch the bottom too abruptly as surface sediments could be disturbed by the mouth of the sampler which would result in an inaccurate sample.

4. Making sure the graduated line is as vertical as possible, release the messenger. Maintain some tension of the line to ensure that the messenger falls freely (Note: when using the Ponar or Peterson grabs, which do not have a messenger, use the appropriate method to trigger the sampler).

5. Once you feel the messenger trigger the sampler, begin to slowly raise it off the bottom. It is important to raise the grab slowly otherwise fine sediments may be lost.

6. Once the grab reaches the surface, the spring loaded jaws should be pried open and the sample put into a flat bottomed pan or similar container. The entire sample, or the top layer of the sample can then be scooped into containers. Sample containers (bottles or bags) should be stored appropriately, as instructed by the analytical laboratory.

**Core Samples**

Sediment cores are used more frequently for metals analyses than the grab samplers. Any part of core samplers that comes into contact with the sample material must be made of plastic to avoid metal contamination of samples from the sampler itself. For metals analysis, clean the sampler using

laboratory soap and rinse it with ambient water prior to sampling and between samples. Cleaning requirements may vary depending on the analyses and should be determined prior to sampling (consult SWI).

1. Label sample container with indelible ink marker.

2. For the 5-cm mouth metal core sampler, insert the plastic sleeve and an 'eggshell' stopper into the mouth of the sampler and screw on the plastic nose cone until tight.

3. If sampling from a boat, slowly lower the sampler using a graduated line until it gently touches but does not penetrate the sediment. If sampling by hand, place and hold the core sampler at the desired location on the bottom.

4. For lake sampling, raise the sampler 1-1.5 metres above the sediment and drop it vertically to collect a sample. Maintain some tension on the line to ensure the sampler falls vertically.

5. Slowly raise the sampler until it reaches the boat. Before lifting the sampler from the water, plug the bottom opening with a rubber stopper to prevent loss of fine sediments.

6. Unscrew the bottom cone and remove the plastic tube containing the sample, while holding the corer in a vertical position. Decant the entire sample, or its desired portion, into an appropriate, pre-labelled container. Sample containers (bottles or bags) should be stored appropriately, as instructed by the analytical laboratory.

### 5.2.3 Sample Documentation

The importance of proper sample documentation cannot be overemphasized. Lack of careful documentation can lead to misunderstandings and questionable test results. Components of proper documentation of field activities are described below.

**Field Notebooks**

Field notebooks must be kept, describing all field activities. Format of field notes and information to be recorded should follow Golder Associates' specific guidelines. During the field survey, field notes must be maintained in a permanent, safe location at the field site where samples are collected. If possible, new entries in the field note book should be photocopied at the end of each field day and copies should be stored in a safe place.

**Sample Labels**

Sample labels must contain the following information:

- Sample identifier (name of site or sample code);
- Date (written as day/month/year; month abbreviated as three letters) and time (24 hour clock) of collection;
- Initials of collector; and
- Analysis requested (this is usually done by the analytical laboratory in the form of a code on the sample bottle).

Fill out labels at the time of collection using waterproof ink and affix a label to each sample container. Plastic bottles may be labelled by writing directly on the bottle using a waterproof marker; however, this approach is not recommended if samples are transported over long distances (friction may rub label off) or if bags of ice are used to keep the samples cool (water may damage label information).

**Custody Seals**

If required for a project, numbered seals should be used to detect unauthorized tampering with samples in transit. Attach the seal in a way that it is necessary to break it to open the cooler containing the samples. The number on the custody seal should be recorded in the field note book and on the Chain-of-Custody and Analytical Request forms

**Chain-of-Custody Forms and Analytical Request Forms**

Chain-of-Custody and Analytical Request forms must accompany all samples submitted for analysis. These forms are usually combined as a single document. An example of Golder Associates' combined Chain-of-Custody and Analytical Request Form is provided in Appendix 1.

The combined form must be filled out completely and the white and yellow copies should be sent along with the samples being submitted. Field personnel should retain the pink copy after it is signed by the shipper. Depending on the shipping container, these forms can either be enclosed inside the sealed container or attached firmly to the outside of the container. In either case, it is advisable to enclose the forms within a waterproof plastic bag to guard against damage. It is important that each person having custody or control of the samples identify themselves on this form. This means that the person collecting the sample, any intermediate persons involved in packaging, storing or transporting the sample and the person accepting the sample on behalf of the analytical lab must all be identified.

### 5.2.4 Sample Handling

Samples need to be treated or preserved according to their specific handling protocols as prescribed by the laboratory. Storage and shipping times are very important and must be considered, as many analytical parameters require that the sample needs to be in the laboratory for analysis within a specific time frame to ensure sample integrity. Refer to SWIs for specific project requirements or check with the analytical laboratory. Contact the laboratory in advance to secure recommended sample storage and

transportation times specific to the analytical parameters. Crew leader is to confirm shipment arrival at the laboratory and to explain analysis requests if needed.

## 6 EQUIPMENT

### 6.1 Sampling Equipment

The following is a list of the equipment recommended for sediment sampling:

- precleaned sample containers from analytical laboratory
- sampling equipment
- metal tray
- coolers and ice

### 6.2 Field Location Equipment and Logs

The following is recommended for the complete documentation of sediment samples:

- field record sheets
- maps of area for site locations
- indelible ink pens and felt tip markers and pencils
- 50 metre long tape measure
- survey flagging tape
- GPS unit
- survey lathe
- Analytical Request forms
- Chain-of-Custody forms

### 6.3 Health and Safety Equipment

- waders and waterproof gloves
- suitable clothing for prolonged water work: heavy socks, warm pants, rain gear, etc.
- first aid kit
- approved personal floatation device

**APPENDIX I**

**SAMPLE CHAIN OF CUSTODY AND ANALYSIS REQUEST FORMS**

**GOLDER ASSOCIATES LTD. CHAIN-OF-CUSTODY RECORD AND ANALYTICAL REQUEST FORM**

Page ____ of ____

Field Sampler: (Signature) _____

Phone No.: _____

Shipment Date: _____

Carrier: _____

Waybill No.: _____

Ship To:

Send Results To:

Project Name: _____

Project No: _____

P.O. No.: _____

| | | | |
|---|---|---|---|
| Relinquished by: (Signature) | Received by: (Signature) | Date: | Time: |
| Relinquished by: (Signature) | Received at lab by: (Signature) | Date: | Time: |
| Relinquished by: (Signature) | Received at lab by: (Signature) | Date: | Time: |
| Relinquished from lab by: (Signature) | Received by: (Signature) | Date: | Time: |

ANALYSIS REQUEST

| Sample ID No. | Sample Description | Date/Time Sampled | Analysis Requested | Sample Condition Upon Receipt |
|---|---|---|---|---|
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Special Instructions/Comments:

Rush (surcharge): _____ Standard Turnaround Time: _____

WHITE COPY — RETURN TO GOLDER ASSOCIATES LTD.
YELLOW COPY — LABORATORY COPY
PINK COPY — RETAINED BY FIELD CREW LEADER

**GOLDER ASSOCIATES LTD. CHAIN-OF-CUSTODY RECORD AND ANALYTICAL REQUEST FORM**

Page ____ of ____

Field Sampler: (Signature) _____

Phone No.: _____

Shipment Date: _____

Carrier: _____

Waybill No.: _____

| Sample ID No. | Sample Description | Date/Time Sampled | Analysis Requested | Sample Condition Upon Receipt |
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Special Instructions/Comments:

Rush (surcharge): _____ Standard Turnaround Time: _____

WHITE COPY — RETURN TO GOLDER ASSOCIATES LTD.
YELLOW COPY — LABORATORY COPY
PINK COPY — RETAINED BY FIELD CREW LEADER

# APPENDIX D

# GOLDER ASSOCIATES' TECHNICAL PROCEDURE 8.1.3
# FISH INVENTORY METHODS

**TABLE OF CONTENTS**

**TABLE OF CONTENTS (continued)**



**LIST OF APPENDICES**

## 1. PURPOSE

This technical procedure presents the techniques and methodologies used for standard fisheries sampling during fish inventory studies for the purposes of determining species presence, distribution, relative abundance, basic population characteristics and for conducting population estimates. Decisions regarding the type of sampling gear to use, the specific techniques to be employed and the timing of sampling will be determined prior to the commencement of the field study by the project team or project manager. However, due to the nature of fisheries work, some decisions regarding sampling specifics will depend upon conditions in the field. The methods for general fisheries inventory work are covered in this technical procedure. Other technical procedures are required in addition to this one in order to conduct fish sampling for specific tasks such as biomarking/fish health studies. This technical procedure does not detail the Quality Assurance/Quality Control requirements for components of field programs, such as note taking/data recording, as they are included in other documents.

## 2. APPLICABILITY

This technical procedure is applicable to all personnel involved in fisheries surveys for lakes and streams, including all sizes and orders of streams. It covers sampling equipment and techniques currently owned/used by Golder. Additional techniques are available which may be the most suitable method for specific circumstances or project requirements. If this is the case, the project manager must authorize the use of any new technique or the purchase of additional equipment.

## 3. DEFINITIONS AND METHODS

### 3.1 Abundance, Relative

The proportional representation of a species in a sample or a community. In fisheries inventories, relative abundance is typically used to describe the relative number of fish captured for each different species at a sampling site. Relative abundance can also be determined for the same species at different sites or in different seasons. It can also be determined for different life stages of the same species.

In some limited cases, the number of fish captured can be used to describe relative abundance. This is suitable for a single effort in a single sampling area where relative abundance is simply the relative number of fish captured. For example, if 20 fish of one species and 10 fish of another species were captured in 100 seconds of electrofishing at a site, species one is determined to have a relative abundance twice that of species two.

For any sampling situation which is more complicated, Catch-Per-Unit-Effort (CPUE) values must be calculated to determine relative abundance. CPUE values take into account the sampling effort required to catch the fish as well as the number of fish captured. For example, if 20 fish of one species were captured in 100 seconds of electrofishing at one site, and 20 fish of the same species were captured in 200 seconds of electrofishing at a second site, CPUE data shows that this species has a relative abundance at the first site which is twice that of the second site. In this example, twice the effort was required to capture the same number of fish at site two. This example also shows why it would be unsuitable to derive conclusions about relative abundance based solely on the numbers of fish captured.

In order to be able to determine relative abundance, you must record all sampling efforts in a manner suitable for calculating CPUE data.

### 3.2 Ageing Structures

Ageing structures are bony parts of the fish which are taken for ageing analyses. In fish from temperate zones, these structures contain annual bands (annuli) which delineate seasonal variation in growth which can be counted to determine the fishes' age. Primary examples of these structures are scales, fin rays, saggital otoliths, cleithra and opercula. The appropriate ageing structures to collect vary according to fish species and life stage and include lethal and non-lethal sampling measures. Consult the table of "**Recommended Fish Ageing Structures**" (available in the aquatics reference file) for the appropriate structure and collection method for each species. With respect to fish ageing, all procedures used by Golder (i.e., the ageing structures which are collected and the methods used to determine age) conform to the manual of Fish Ageing Methods for Alberta (Mackay et al. 1990).

Following removal from the fish, ageing structures should then be placed in a "scale envelope", which consists of a small envelope which has been stamped with fields for recording the following information:

- date
- fish number
- species
- fork length
- weight
- life history stage
- sex
- state-of-maturity
- sampling gear
- sampling location
- ageing structure collected
- project number

Blank envelopes are ordered in batches of 1000 and must be stamped prior to use. If your project includes the collection of ageing structures, it may be necessary to order the required envelopes and stamp them before heading out into the field.

The scale envelopes should be allowed to dry overnight before being stored. Upon returning from the field, the envelopes should be stored frozen in a one of Golder's freezers.

### 3.3 Anaesthetic

An anaesthetic is used in situations requiring live fish to be removed from the water and handled for extended periods, such as during surgery to implant radio transmitters, or to quiet fish for measurements. The anaesthetic commonly used by Golder is MS-222, known as tricaine methanesulfonate. The concentration of anaesthetic to be used depends on the required level of sedation. For surgery, which requires the fish to remain sedated for a period of 5-10 minutes, a concentration of 100 mg/L is used (i.e. 4 g of MS-222 in 40 L of water). The fish is placed in the anaesthetic bath for 2-4 minutes until the desired level of sedation is reached. Care must be taken as overdoses lead to direct mortality. When monitoring the fish in the anaesthetic solution, watch for loss of coordination (when the fish no longer keeps itself upright) and respiration rate. Towards the end of the anaesthetization period, the fish will begin to "Cough".

Use of anaesthetic for quieting fish for measurements is not typically recommended unless the fish is difficult to handle or may injure itself. Fish anaesthetized with MS-222 are not recommended for consumption by anglers for a period of 2-4 weeks following exposure to the anaesthetic. Therefore, use only on fish which will not be captured and consumed or with permission of Alberta Fisheries Management Division.

### 3.4 Biomass

Biomass is the total mass (weight) of fish, or of fish of a given species, within a study area. It is a component of population estimates, as an estimate of the total number of fish in the study area is required to calculate biomass. Using either total removal data or a mark/recapture population estimate for the study site, the total biomass is calculated by multiplying the total population of fish by the average weight of the fish captured. Results can be expressed as units of weight over study area dimensions (e.g. kg/m of stream, $kg/m^2$ of lake).

### 3.5 Capture/Sampling Techniques

The following sampling techniques are used to capture fish. Some techniques are very specific to one life stage while others are more general. All sampling techniques have some degree of sampling bias associated with them with respect to fish size selectivity and sampling efficiencies based on environmental parameters such as water depth, conductivity, stream size etc. It is important to understand these biases when designing or implementing a study plan and when interpreting the data and drawing conclusions from the results.

#### 3.5.1 Airlifting

Airlift sampling is used to collect fish eggs from the substrate for species which are broadcast spawners (i.e. do not bury their eggs). It can be used simply to determine if incubating eggs are present or to determine the relative density of eggs at each spawning site. The airlift sampler consists of a gas powered generator and compressor unit, a length of hose, an airlift head and couplers to connect the hose to the compressor and airlift head. The airlift head is attached to a long pole and consists of a 4" or 6" diameter hollow tube with a 90° bend at the upper end. The lower end of the airlift head has an internal tube which runs around the internal circumference and which is perforated. With the lower end of the airlift head held against the substrate, air is pumped from the compressor through a hose and into the perforated tube. Air rising inside the airlift head creates a vacuum effect which lifts loose particles up from the substrate. A removable collection bag placed over the upper end of the airlift head collects the particles. The sample is dumped into a sampling tray and examined for the presence of eggs.

This technique is employed when sampling water too deep to kick sample or when a quantitative sample is required. Since the area ($cm^2$) of the airlift head is known, simply count the number of times the head is touched to the substrate for each sample in order to determine the number of eggs/$cm^2$ in the sample. Quantitative sampling can be used to determine the relative use of the spawning areas sampled, as determined by egg density. Remember to record the size of the airlift head used.

### 3.5.2 Angling

Angling refers to the use of angling gear, such as rod and reel, to sample for fish. Angling is an active technique using lures, bait or flies. Leaving a static, baited line in one place is referred to as a Set Line and is not an angling technique. On the other hand, jigging with a baited line would be an angling technique.

Sampling effort should be recorded as both the number of hours angled and the number of angling tools used. It would be recorded as angler-hours, or as rod-hours or some equivalent if more than one piece of angling gear is used per angler. The types of hooks, size of hooks, and number of hooks should also be recorded. In addition, notes on the types of habitats fished and the length of shore line covered if trolling is conducted should be recorded.

### 3.5.3 Drift Net

Drift net is a passive sampling technique for use in flowing water for the capture of life stages which are moving or drifting downstream. A drift net consists of a long, tapering net with an open mouth at the upstream end and a detachable sample bottle at the downstream end. Drift nets are anchored in place in the stream and filter the water passing through them, collecting materials from the water column. They can be placed to sample the bottom, middle or top of the water column or can be stacked to sample the entire water column. At regular intervals, the nets are removed and cleaned by dumping the collection jars into a sampling tray and examining the sample for the presence of fish. Typically the drift nets are checked and cleaned twice per day, once first thing in the morning and once again in the evening. Record the catch separately for each period in order to be able to determine diurnal patterns.

Sampling effort is usually recorded as the number of hours between net cleanings to determine catch/hour. If more detail is required, it is also possible to estimate the volume of water sampled by the net during the period between net cleanings to determine the catch/$m^3$. To do this, measure the velocity of the water at the sampling site before setting the drift net and again after lifting the net for cleaning to determine the average water velocity through the net. Multiply the average velocity (m/s) by the area of the net mouth ($m^2$) to get the volume sampled per unit time ($m^3$/s) (remember to record the size of the drift net mouth). Multiply this value by the time the net was in place to calculate the total volume sampled. For this calculation, the drift net mouth must be completed submerged.

### 3.5.4 Electrofishing

Electrofishing refers to the use of electricity to stun and capture fish. An electrical current is passed between electrodes placed in the water and the resulting electrical field attracts passing fish (galvanotaxis) toward the positive electrode (anode). As fish pass close to the anode they encounter an increasingly stronger current gradient which acts as a narcotic and stuns the fish (galvanonarcosis), allowing them to be easily dip-netted from the water. Once captured, the fish may be identified, weighed, measured, tagged and then returned to the water. Fish taken by electrofishing revive quickly when returned to the water. Effort is automatically recorded by the electrofishing unit as the number of seconds of active electrofishing (i.e. the time current is applied to the water). **Record the effort (seconds) immediately after completion of sampling and reset the timer to zero.** Electrofishing techniques require experienced operators in order to reduce injury to the fish and to eliminate potential

injury to the personnel involved. Safety training or working with experienced personnel is required for operating electrofishing equipment.

**Backpack Electrofishing**

Backpack electrofishing is a sampling technique for small, wadable streams. A backpack electrofisher consists of a portable electrofishing unit and a power source (12v battery or mini generator) attached to a pack frame. It is equipped with a hand held, button-operated anode pole and a cathode plate which is left trailing in the water. The operator wears the pack unit and uses the button switch to activate the anode in order to stun fish while wading instream. One or more assistants wading next to the operator use dip nets to capture the stunned fish. The assistant also adjusts the electrofisher settings for the operator and monitors the electrical output. Sampling is normally conducted while moving upstream so that fish are not disturbed, prior to being sampled, by disturbances to the stream bed and material moving downstream with the flow.

**Boat Electrofishing**

Boat electrofishing is an extremely effective sampling technique for moderately shallow water and is used for intermediate streams, large rivers and shallow littoral areas in lakes. Two types of boat electrofisher are available, both of which consist of an electrofishing control box which is powered by a 5,000 watt generator. The ***portable boat electrofisher*** has a free control box and generator which can be loaded into an inflatable boat (Zodiac) and is ideal for small or intermediate sized rivers which cannot be waded and which cannot be effectively sampled by the low current outputs provided by a backpack electrofisher. Two anode configurations are possible, depending on stream size, and include either a hand-held, button operated anode pole or a foot-switch operated portable boom system. In both cases, a floating cathode plate is employed. The boat can be drifted downstream or an outboard jet can be used to provide increased mobility. In comparison, an ***electrofishing boat*** consist of an 18' aluminum river boat with an integral electrofisher control box and generator. It is also equipped with a work platform and flow-through live well for holding fish. It has a foot-switch operated anode boom system and uses the boat hull as the cathode. Boat electrofishers are designed for any intermediate or large river which is deep enough to allow a boat of this size to float and which has a site with a suitable boat launch. This unit has the largest anode/cathode surface area and is capable of generating the largest electrical field and the highest current outputs. Boat electrofishing sampling for both types of units is usually conducted while floating downstream, as this makes fish easier to dipnet and puts less stress on the dipnets and anodes.

### 3.5.5 Emergent Trap

An emergent trap is a passive sampling technique specifically designed to capture fry as they emerge from the substrate following hatching. A typical emergent trap consists of a square metal frame (0.3m x 0.3m) covered with a small mesh net and collection bottle. The mouth of the trap is placed on top of the substrate at a known or suspected spawning area where incubating eggs are known or thought to be present. It is left in place through the incubation period. Once the fry have hatched and absorbed their yolk sacs they emerge from the substrate. The fry from the eggs which were located under the trap mouth will be captured by the trap.

Emergent traps can be used to verify a suspected spawning area or to check for hatching success at a know spawning site.

### 3.5.6 Fry Traps

A fry trap is a passive sampling technique used to capture fry which are drifting downstream in flowing water. It is suitable for capturing fry which are larger than post-emergent size but which are not yet strong swimmers. The fry trap is anchored to the stream bed using 2 rebar posts and consists of a large metal frame open at the upstream end and otherwise covered with small mesh metal screening. "Wings" lead from the trap mouth into a low velocity area at the downstream end of the trap where the fry accumulate. The trap is designed so that it will pivot at the anchor point on the stream bed. To check the trap, simply tilt it forward and hold a collection bucket in front of the "top" of the low velocity holding cell. Water and fry from the holding cell will pour into the bucket as the trap is tilted. Typically the traps are checked and cleaned twice per day, once first thing in the morning and once again in the evening. Record the catch separately for each period in order to be able to determine diurnal patterns.

Sampling effort is usually recorded as the number of hours between trap cleanings to determine catch/hour. If more detail is required, it is also possible to estimate the volume of water sampled by the trap during the period between trap cleanings to determine the catch/$m^3$. To do this, measure the depth and velocity of the water at the sampling site before setting the trap and again after checking the trap to determine the average water depth and velocity through the trap during the sampling period. Multiply the average depth (m) by the average velocity (m/s), then by the width of the trap mouth (m) to get the volume sampled per unit time ($m^3$/s) (remember to record the width of the trap mouth). Multiply this value by the time the trap was in place to calculate the total volume sampled.

### 3.5.7 Gill Netting

A method of capturing fish that involves the setting of nets of various mesh sizes anchored in place in a river or lake. A gill net consists of netting suspended between a weighted "lead" line and buoyant "float" line which, when set, forms a vertical wall of netting. The lead line is attached at both ends to heavy weights to hold it in place and keep the net taught. The float line is attached at either end to floats. In Alberta, the floats must each consist of a pole which stands upright at the water surface and extends above the water surface for a minimum of 1.0 m. The top of the poles must have a blaze red or orange flag measuring at least 20 cm x 20 cm and marked with the Fish Collection Licence Number in 20 mm high letters. Typically, we use sandbags filled with rocks or sand from the gill net site for lead line weights. This way, all we have to carry with us to the site is a few empty sandbags. New gill nets need to have a length of sideline attached to either end which extends from the float line to the lead line to take the tension when the net is lifted to ensure that the mesh does not rip.

Gill nets are designed to function by catching on the gill covers of fish as they attempt to swim through. Fish of a size for which the gill net mesh size is designed swim into the net but can only pass partway through the mesh. When the fish struggles the twine slips behind the gill covers (opercula) and the fish becomes "gilled". Therefore, the mesh size of the gill net is important when selecting a net or nets for your sampling activity as gill netting can be a very size selective technique.

Gill net mesh size can be measured as either the stretch measure or square measure of the openings in the mesh. At Golder, we always use the stretch measure to identify our gill nets and when reporting results. The stretch measure is the distance between two opposite corners of the square mesh opening, when the square is stretched flat. Gill net mesh sizes typically range from 1.9 to 14.0 cm (3/4"-5.5"). As most gill nets are sold using imperial units of measure, the following table will help you convert mesh sizes to metric units.

Stretch Mesh Sizes:

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Imperial (inches) | 3/4 | - | 1.0 | - | 1.5 | - | 2.0 | - | 2.5 | - | 3.0 | - | 3.5 | - | 4.0 | - | 4.5 | - | 5.0 | - | 5.5 |
| Metric (cm) | 1.9 | - | 2.5 | - | 3.8 | - | 5.1 | - | 6.3 | - | 7.6 | - | 8.9 | - | 10.2 | - | 11.4 | - | 12.7 | - | 14.0 |

Gill net meshes are constructed either of monofilament or nylon. Monofilament is sturdier and longer lasting but gill nets made from this material do not compress and take up a much larger volume than a nylon net of the same dimensions. For longer nets, the volume of a monofilament net becomes significant.

Gill nets can be simple or multi-mesh. Simple nets consist of one mesh size only, although different nets may have different lengths and depths. Multi-mesh nets are also called "gang" nets and consist of more than one mesh size. Each mesh size occurs in a discreet section of the net which is called a panel. Gang nets typically have from two to five different mesh sizes or panels. Usually, each panel has the same length, although this is not always the case. An important component of recording sampling effort is to record the dimensions of all gill nets that are set. Record the depth of each net as well as the total length. Also record the number of panels, the mesh size of each panel and the length of each panel. Effort should also be recorded as the number of hours the net is set and CPUE is expressed as either duration (hrs), panel-hours, or meter-hours, depending on the type and variety of nets set.

Since the size of the mesh will have a major role in determining the size of fish (i.e. species or life stages) that will be captured, it is extremely important to record the mesh sizes of any gill net used. It is also important to record the catch for each individual panel or mesh size. The field form used to record the catch has a space for recording the mesh size for each fish captured. When removing fish from the gill net, the fish must be separated by mesh size.

Selecting a gill net or nets to be used for a project will vary depending on your sampling goals. Long gang nets with several different mesh sizes, from small to large mesh, are best for general inventory sampling and have the smallest level of sampling bias. For single mesh nets or nets with few panels, it is generally true that the larger the mesh size used the larger the fish that will be captured. The small 1.9 cm mesh nets will capture fish as small as the larger minnow species and juvenile life stages of larger fish. Mesh sizes in the range of 5.1-7.6 cm are typically used for salmonid species while larger mesh sizes will be employed to capture adult northern pike and burbot. Most gill nets will capture a larger size range of fish than mesh size would dictate as some species will be captured without necessarily being "gilled". For example, suckers may be entangled by their large lips and northern pike often bite and roll in the mesh, becoming entangled in mesh sizes too small to capture them by gilling. Bullheads on the other hand are often captured in mesh sizes too large to gill them when their pectoral and dorsal spines become entangled in the mesh.

Nets selected for sampling in rivers are generally different from those used in lakes. River gill nets typically have large floats attached to the float line for added buoyancy. Shorter nets are used as they must be set in low velocity pockets such as backwaters or pools and heavy weights are used to anchor the net so that it will remain in position in flowing water. Caution should be taken when setting nets in a river at high stage if floating debris is moving downstream which could damage or move the net. In lakes, much longer nets can be used if required and, since lakes typically have greater depths than rivers, nets can be set at a variety of depths. Lake nets can be set so that they float near the surface, are set along the lake bed or are positioned in mid column. For floating sets, nets with large floats attached to the float line can be used and long leads are tied to the weights to allow the net to remain at the surface. For sinking sets, nets without additional floats or with small floats are used. For bottom sets, the weights are tied tight to the lead line and long leads are tied to the floats so that the net will sit on the bottom and the floats will remain at the surface. For mid column sets, leads are attached to both the weights and floats so the net will be positioned between the bottom and the surface.

Gill netting is a sampling technique that can be used in the winter as nets can be set under the ice. In lakes where there is no current a jigger is used to run a length of sideline under the ice. A large hole is opened in the ice and the jigger is placed under the ice. The sideline is tied to the jigger and the lever arm is manipulated to send the jigger moving away from the hole. Once the jigger has moved far enough it must be relocated, either by sight if the ice is clear or by sound as the jigger is equipped with a "clicker" device. A hole is drilled at the location of the jigger and a hook is used to pull the sideline up the hole. In rivers or in the case of thick lake ice a Murphy stick is used to set the net. A Murphy stick consists of two sections of aluminum pipe hinged together which extends as an under-ice probe. The far end of the probe has an eye-hook at the end and a float a short distance back. A length of sideline a little longer than the gill net is tied to the eye-hook and the far end of the probe is pushed down through one hole in the ice and maneuvered towards a second hole where the attached sideline is hooked and pulled up through the hole. The process is repeated several times to extend the rope as far as desired. Once the sideline has been placed under the ice it is then attached to one end of the gill net and used to pull the net under the ice.

As a sampling technique, gill nets can have a high mortality rate if the fish are left in the net for a prolonged period or if water temperatures are high. If fish mortality is a concern, the nets should be cleaned of fish on a regular basis (e.g. every two hours). If mortality is desirable (i.e. fish are to be sacrificed) or not a concern, nets should be set overnight in order to sample day and night periods of fish movements and to allow capture of fish which may avoid the net if it is visible during daylight hours in low turbidity water.

### 3.5.8 Hoop Net (Fyke Net)

A hoop net is cylindrical net distended by a series of hoops or frames with one or more internal funnel-shaped throats whose tapered ends are directed inward from the mouth to prevent fish from escaping once they enter the net. A fyke net is a hoop net with two wings or leads of webbing attached to the mouth to guide fish into the enclosure. Our hoop nets have large square hoops at the front of the net and taper to a smaller diameter with smaller ring hoops at the back end. Webbing extends inwards and backwards between the sides of the first square hoop to form a "V" slot at the net mouth and a funnel is attached to the back of the second square hoop. The chamber between the funnel and the rear of the net is termed the "pot". The net is tapered at the rear end and held closed with a draw string which can be opened to permit removal of the trapped fish from the pot, although trapped fish may also be present

between the "V" slot and the funnel. The funnel also has a draw string which allows removal of fish from this chamber. If it is desirable to have a fyke net, use two lengths of webbing tied to the sides of the hoop net mouth to convert the hoop net to a fyke net.

Fyke nets are typically set at a time and location where fish will be moving through the area in a direction that will lead them into the net mouth. They are very effective when set in small tributaries to lakes or larger rivers during a spawning run but can also be used in shallow areas of lakes and larger rivers. The net and wings are anchored in place by tying them to rebar posts embedded in the substrate. The wings of the net should be set at a 45° to the axis of the hoop net.

As the holding chambers in the fyke nets are small, they should be checked and cleaned of fish on a regular basis, particularly during an active spawning run. Try to set the net so that fish in the holding chamber will not be subjected to high water velocities. Sampling effort is usually recorded as the number of hours between net cleanings. Record fyke net dimensions such as mesh size, mouth size, wing lengths and, when used in streams, whether full or partial channel blockage was achieved and whether the net mouth was oriented upstream or downstream.

### 3.5.9 Kick Sampling

Kick sampling is used to collect fish eggs from the substrate in spawning areas, both for species which are broadcast spawners and for those which bury their eggs (i.e. from trout redds). It can be used to determine if incubating eggs are present but it is generally considered a qualitative (i.e. non-quantitative) sampling technique and, unlike airlifting, is not suitable for determining the relative density of eggs. The kick sampler is attached to a pole and consists of a tapered net attached to a metal frame which forms the mouth of the net. It is generally used in flowing water. To use, grasp the pole and place the kick net against the substrate. Stand upstream of the net mouth and use your feet to disturb the substrate, letting the disturbed materials float into the net. Remove the net from the water and examine the contents of the net for eggs.

Kick sampling can only be conducted in water shallow enough or which is flowing slow enough to allow instream wading. This technique is simpler to use than the airlift sampler and requires considerably less equipment. It is a very efficient and fast technique for identifying spawning areas in wadable streams, particularly over long lengths of stream.

### 3.5.10 Minnow Trap

Minnow trapping is a passive sampling technique used to sample for the presence of minnow species and small life stages (i.e. fry) of larger species which can be difficult to capture using other techniques such as electrofishing or gill netting. The traps we use are Gee Minnow Traps which consist of two pieces which are clipped together to form a small cylinder slightly tapered at either end. Each end has a funnel which leads into the centre of the trap which allows fish to enter but prevents them from escaping. The traps are generally placed on the substrate in the shallow shoreline areas of lakes and streams with the long axis of the trap parallel to the shoreline. A length of sideline is used to tie the trap to a stake or anchor on shore to keep it in place. The anchor site is usually flagged so that the site can be easily found when returning to check the trap. The traps can be baited or unbaited, depending on if the intent is to trap fish moving through the area or attract fish to the trap.

Sampling effort is recorded as the number of hours that the trap is set.

### 3.5.11 Observation

Underwater observation involves the use of either snorkeling or SCUBA techniques to observe, count or record the activities of fish. Scuba diving is generally restricted to lake habitats but may also be employed in deeper rivers. It is a fairly intrusive technique and is considered to be more disruptive than snorkeling and requires that the observer have a valid scuba certificate. Snorkeling is commonly employed by Golder to conduct fish observations in stream habitats which have low turbidities. It is less disruptive than SCUBA and logistically simpler. Equipment used for snorkeling includes a diving mask, snorkel, dry suit, diving gloves and an underwater writing slate. A wet suit can be used in place of a dry suit in warm water but a dry suit is preferable as it increases observation time. To date, snorkeling has been used by Golder to study the habitat preferences of some fish species but the technique can also be used to determine fish abundance and distribution.

### 3.5.12 Post-Emergent Trap

Post-emergent traps are a passive sampling technique for use in flowing water to sample for the presence of post-emergent fry. Unlike emergent traps which capture the fry as they emerge from the substrate, post-emergent traps capture the fry as they drift downstream following emergence. Unlike emergent traps, it is not required that they be set at a spawning site overtop of incubating eggs, there only needs to be a spawning area somewhere upstream of the set location. Post-emergent traps are essentially extremely large drift nets. Each trap consist of a tapered, small-mesh net attached to a metal frame which forms the trap mouth. The trap mouths are 0.9 x 0.9 m in size. Each net is equipped with a removable sample bottle attached at the downstream end of the net. A post-emergent trap is set by anchoring two rebar poles into the substrate and looping the four hoops attached to the trap over the poles and sliding the trap down until the bottom of the trap sits on top of the substrate with the mouth facing upstream.

Post-emergent traps should be checked at a minimum of twice per day, once in the morning and once in the evening. Definite diurnal/nocturnal patterns have been observed using these traps, so be sure to record the catch separately for each sampling period. To check the catch, remove the trap from the stream and wash all materials from the netting into the sample bottle. Dump the contents of the bottle into a sampling tray to look for the fry. Post-emergent fry are extremely small and almost transparent. They are best seen by looking for the large, dark eyes which will be their most obvious feature. They may also be seen to be swimming around in the sampling tray. It is also prudent to check the mesh of the trap for additional fry as they are so small that some become “gilled” on the mesh and do not wash down into the collection bottle. If more than one species may be hatching at the time and location of your study and you are not sure of the identification of fry in the sample, the sample should be preserved in 5% buffered formalin for laboratory identification.

Sampling effort is recorded as either catch/hr or catch/$m^3$, as described for fry traps (Section 3.5.6). Post-emergent traps are used to check for the presence of post-emergent fry in the study area, either as proof of spawning activity in upstream areas or simply to tell if this life stage or a certain species is present. They are also used in entrainment studies, which are conducted to determine if fish are entering man-made structures such as diversion canals or water intakes. In addition, they may be used to

determine the timing of hatching periods and the relationship between hatching and environmental parameters such as discharge or water temperature.

### 3.5.13 Seine Netting

Seine netting refers to the use of a specifically designed net to catch fish by dragging it through the water. Seine nets consist of netting suspended between a float line and a lead line. The netting is constructed of thicker net material than gill nets so that fish do not become gilled in the mesh. Mesh sizes vary but most nets are constructed of minnow netting which has a small mesh size and is suitable for catching forage fish and small life stages of larger fish species. Larger mesh seine nets are also available for sampling for large fish and are much easier to drag through the water. Two types of seining operations are possible, beach seining and boat seining.

Beach seining is accomplished by two people dragging the net through the water while wading and is used in shallow water areas in lakes and streams. To beach seine, each person grabs one end of the net by placing one foot in the loop at the end of the lead line and holding the loop at the end of the float line in their hands. One person walks out from shore to a suitable depth. Both people then walk parallel to shore dragging the net between them. The lead line is kept in contact with the substrate to prevent fish from escaping under the net by dragging the foot looped to the lead line along the bottom. As they walk through the water, fish are herded in front of the net. The person near shore moves slower than the person further out. When the further person has passed the near shore person they curve back to shore, meeting the near shore person at the waters edge and bringing the two ends of the net together forming a pen holding the captured fish. Both people then drop the float lines and pick up the lead lines and standing side-by-side pull the net up on shore, ensuring that the lead line remains in contact with the substrate at all times. The trapped fish will congregate in the end of the looped net and will be dragged up onto shore.

Boat seining is a specialized technique used in water too deep to wade. It usually involves the use of long, large mesh seine nets for the capture of large fish. It is particularly useful in areas where fish congregate such as spawning areas of lakes or snye areas in rivers. The principle is similar to beach seining except that a boat is used to move the offshore end of the net through the water. A pole is attached to both the lead and float lines, at the boat end of the net, and is used to keep the lead line on the bottom.

Seine netting is a suitable technique only where the bottom is fairly smooth. If large substrate particles, debris, or aquatic vegetation is present which will cause the lead line to lift off the bottom as it passes, the technique will not be very efficient and most or all fish will escape. Seine netting is typically used to sample for the presence and abundance of small fish and life stages which are not effectively sampled for using other inventory techniques.

Sampling effort is recorded as the number of seine hauls made and either the distance (m) or the area ($m^2$) seined for each haul. Record the dimensions of the seine net used (length/depth/mesh size) and the shoreline distance of each seine haul. If area is required, multiply the length of the seine haul by the length of the seine net used.

### 3.5.14 Set (Trot) Line

A set line is a series of leaders and baited hooks strung from one central line which is anchored to shore. Set lines are used to catch predatory fish and are usually set out overnight. Golder set lines are 30 m in length, which includes a 10 m lead with no hooks and 20 m of line with a total of 10 leaders/hooks set at 2 m intervals. A large lead weight is attached to the end of the line to keep it in place once it is set. The 10 m lead is used to set the baited hooks well out from shore or can be tied short to keep the hooks near shore, as desired.

Sampling effort is recorded as the number of hours the line is set or the number of hook-hours if set lines of different lengths and number of hooks are used. Record the size of the hooks that are used (e.g. #8 hooks).

### 3.5.15 Trap/Counting Fence

Fish traps or counting fences are a passive sampling technique used to capture fish as they move past a specific location. They consist of one or more trap boxes with fences (wings) which stretch out in front of the entrances of the boxes to lead fish into the trap. The trap boxes are large holding pens enclosed on four sides as well as on the bottom with metal or plastic mesh. The front of each box has an opening equipped with a funnel which leads into the interior of the trap box. The boxes are also equipped with locking plywood lids to protect the fish as they congregate in the traps. The fences consist of angular aluminum frames with a series of holes into which are fitted round aluminum rods to form a barrier to fish passage. The counting fence is installed by attaching the components to rebar posts driven into the stream bed and by placing sandbags on cradles included in the fence design. The fences or wings are set as close as possible on a 45° angle to the trap box entrance.

Two types of counting fence set-up are possible, the **one-way fence** and the **two-way fence**. The one-way fence has only one trap box and one set of wings and is used to capture fish moving in one direction. The two way fence has two trap boxes facing in opposite directions, each with its own set of wings, to capture fish moving in both directions. Counting fences can be used to sample portions of the shoreline in lakes or large rivers but are typically used in small or medium sized streams to close off the entire channel and capture all fish moving past the trap location. In this case, the box which captures fish moving upstream is called the upstream trap and the box catching fish moving downstream is called the downstream trap. In streams, the trap boxes should be set in a location where the water velocity is not too high so that the fish caught in the trap can rest. If no such site is available, a piece of plywood placed upstream of the trap will provide a velocity shelter

The counting fence should be checked a minimum of twice a day, once first thing in the morning and once again in the evening and the fish removed from the traps using a dipnet. The fence should also be cleaned of debris to keep the water flowing freely through it and to reduce the build up of pressure on the fence. Record the day, time and catch each time the fence is checked. During an active spawning run, the fence may need to be checked more frequently so that the number of fish holding in the trap boxes does not become too large. Record the catch separately for each sampling period. After removing the fish from the trap boxes they should be released in the direction that they were traveling so that they can continue in that direction (i.e. fish from the upstream trap should be released upstream of the counting fence while fish from the downstream trap should be released downstream of the fence).

Counting fences are used to determine the species, relative abundances and timing of movements of fish past the sampling site. They are typically used to capture fish during their spawning runs in the spring or fall or to quantify the movements of fish into and/or out of tributary streams.

### 3.6 Catch-Per-Unit-Effort (CPUE)

Catch-Per-Unit-Effort is a measure which relates the catch of fish, with a particular type of gear, to the sampling effort expended; it is expressed as: ***number of fish captured/unit of effort***. Results can be given for a particular species or the entire catch. CPUE is used to define species relative abundance and to compare abundances between sites and/or seasons. Effort can be expressed a number of ways depending on the sampling equipment. If CPUE data is required, sampling effort must be recorded. Following are common CPUE calculations for traditional sampling gear:

- electrofishing (boat and backpack) — No. of fish/100 seconds (of active electrofishing)
- gill net — No. of fish/net-hour, or /panel-hour, or/100m of net-hour
- set line (trot line) — No. of fish/hour, or /hook-hour
- angling — No. of fish/hour, or /angler-hour, or /rod-hour
- minnow trap — No. of fish/hour, or /trap-hour
- seining — No. of fish/area seined ($m^2$), or /length of shoreline seined (m)
- counting fence (fish trap) — No. of fish/hour
- drift net/post-emergent trap — No. of fish/hour, or /volume of water ($m^3$)

It is important to recognize the components of the effort inherent in the sampling technique being employed so that effort will be recorded properly. Most field forms will have fields specifically designed to record the pertinent information. Record all aspects of your sampling effort (e.g., number of set lines used and number of hooks per line) so that CPUE can be calculated. CPUE values will be used in our own studies to establish relative abundance. Our data may also be used in a more historical context to compare the abundances we record with past or future research, using both similar and different sampling gear, and CPUE values may need to be recalculated to conform to other studies. The more detailed used when recording sampling effort, the easier it will be to accommodate these needs.

### 3.7 Coldwater Fish

When dealing with the general suitabilities of freshwater habitats for game fish species, temperature regime is often used to describe the habitat potential and the species assemblage which could possibly be present. Although the terms are not definitive or precise, the designations of habitats as “coldwater” or “coolwater” habitats and the associated fish fauna as “coldwater” or “coolwater” species are often used.

Coldwater fish are those which have a preference for summer water temperatures ranging from about 10-18 °C. In Alberta, this encompasses all of the salmonid species including the trouts, whitefishes and Arctic Grayling. Within this group the species will have differing temperature preferences and tolerances (see section 3.50 - Temperature Criteria).

### 3.8 Condition Factor (Ponderal Index)

Condition factors are used to describe the plumpness and, by inference, the well-being of individual fish. Formulas are used to calculate condition factors using the fish's length and weight and are based on the principle that the weight of a fish will vary with the cube of its length. Any variation in the shape or plumpness will be measured using the formula. Golder primarily uses the coefficient of condition *K*, also called the Fulton condition factor. The formula (**using metric length and weight data**) is as follows:

$$K = [\text{weight (g)} \times 10^5] / \text{fork length}^3 \text{ (mm)}$$

Condition factor is believed to reflect the nutritional state or well-being of an individual fish. The *K* value will be 1.0 for fish whose weight is equal to the cube of its length. Fish which have a *K* value >1.0 are more plump and are thought to have a higher degree of well-being or better nutritional state-of-health, whereas fish with a value <1.0 are considered to be less robust.

Condition factors vary with season, sex, sexual maturity, age and various other factors. Therefore, if sufficient data is available, average condition factors for a species should be calculated separately for each sex and should exclude young-of-the-year fish. Condition factors also vary by species, particularly if they have different shapes, and should not be used to compare well-being between fish species. They can, however, be used to determine differences in the condition of fish of the same species in different years or at different sites. Fulton's condition factor is also limited for comparisons between fish populations in different lakes because of differences in growth parameters. Other formulas for condition factor calculations are available and would be designated by the project manager if they are required.

### 3.9 Coolwater Fish

Coolwater fish are those which generally prefer summer water temperatures ranging from about 18-26°C. Alberta species generally considered to belong to this group include northern pike, walleye, sauger, yellow perch, goldeye, mooneye and lake sturgeon (see also Section 3.7 - Coldwater Fish).

### 3.10 Creel Census

The term "creel" refers to the basket a fisherman uses to hold the fish which have been angled and a creel census refers to a survey in which recreational fisherman are censused in order to determine aspects of the recreational fishery. Important survey goals typically include determining angler effort and success (i.e. fishing pressure and harvest) and may include examining the fisherman's catch for tagged fish or to collect ageing structures.

### 3.11 Dissolved Oxygen Criteria

The dissolved oxygen concentration in the water is an important habitat component. Different fish species have different dissolved oxygen requirements and have different tolerances to low dissolved oxygen levels. Dissolved oxygen criteria provide minimum dissolved oxygen levels that are necessary to

protect various life stages and have been developed for selected game fish species. Golder has prepared a document which list the criteria for selected Alberta species (Taylor and Barton 1992).

### 3.12 Fecundity

The most common measure of reproductive potential in fish. Female reproductive potential is the total number of eggs (ova) in both ovaries of a gravid female fish. Fecundity normally increases with the size of the female within a given species. For most studies conducted by Golder, fecundity is determined for female fish only. Fecundity is determined by recording the total weight (g) of both ovaries and removing a small sub-sample of known weight from the middle of the ovaries (usually a 1.0 g sample). Count the number of eggs in the sub-sample to determine the number of eggs/g of ovary. Multiply this value by the total ovary weight to calculate the total number of eggs.

### 3.13 Field Forms

Golder uses a number of specially designed field forms to aid in recording field data. They are not meant to replace the use of a field book or the recording of detailed field notes. They are intended to provide a template showing the type of supporting data that must be recorded for each sampling technique and provide an organized method of recording the sampling results. For each specific or general type of sampling technique there is a ***Catch Record Form*** (e.g. Gill Net Catch Record Form) for recording sampling information such as location, technique, effort and is used to summarize the results. The main form for recording the catch results is the ***Fish Sample Record Form*** which has fields for recording length and weight data and other particulars for each individual fish. On the back of this form you will find a list of all abbreviations to be used when recording data.

A copy of each field form is kept in the aquatics reference file located at Carole Collins desk (Aquatic Ecology Group Secretary). Copy the forms you will require onto waterproof paper and return the originals to the file.

### 3.14 Fish Collection Licence

Fish collection licences or permits are granted by provincial governments or by DFO and are required for all fisheries sampling activities. Obtaining a license varies from province to province. In Alberta, a Fish Collection Licence is granted to Golder by Alberta Environmental Protection, Fisheries Management Division. Each Licence is specific to the waterbody(s) being sampled and is valid for a specified time period. To obtain a Licence you must forward a letter of request to the F & W District office for the region in which you wish to sample. Include in the letter the reason for sampling, the location(s) to be sampled, the period the permit should be valid for, the capture techniques to be employed, the fate of the fish captured (i.e. will any be sacrificed), and the personnel to conduct the sampling. They will then send a Licence granting permission to carried out the proposed activities. They may impose specific restrictions on the licence (i.e., restricted number of fish allowed to be sacrificed, designation of a certain landfill for fish disposal, or specific reporting requirements) and the permits should be read carefully to ensure all restrictions will be followed. The original permit or licence should be immediately placed in the project file and a copy of the document given to the field personnel. You must be prepared to produce a copy of the permit while conducting any field sampling.

The Fish Collection Licence will also specify a date by which a permit return is to be submitted to the issuer. In Alberta, the permit return is a form which accompanies the Licence. The form requests information regarding the sampling conducted under authority of the Licence, such as sampling locations and results. Fill out the form and send it to the office which issued the Collection Permit following completion of sampling activities and prior to the date specified on the Licence.

### 3.15 Forage Fish

A general term applied to smaller species of fish that "forage" on small invertebrate animals or plant materials. This includes minnow species and other small fish such as sculpins, stickleback, trout-perch and darters.

### 3.16 Game (Sport) Fish

Fish used by anglers for recreational fishing or sought after by the commercial fishing industry, e.g., northern pike, walleye, trout, etc.

### 3.17 Geographical Position

All sampling sites, whether they are point locations (such as a minnow trap site) or sections (such as a section of river that was electrofished), should be recorded on a map of the study area. The standard is to use a 1:50,000 NTS topographical map but other maps or airphotos can be used if they provide greater detail. The geographical position of sampling sites can also be recorded using Universal Transverse Mercator (UTM) grid coordinates or by degrees of latitude/longitude. UTM coordinates are particularly useful in case the map is lost as they can be used to pinpoint the sampling site on a new map.

UTM and latitude/longitude are two different systems of grid coordinates used to establish geographical location. Both systems appear in the margins of 1:50,000 scale National Topographical Service maps. A calibrated ruler is used to calculate coordinates of any point on the mapsheet. Golder always uses UTM coordinates rather than lat/long, unless otherwise specified by the client.

The most accurate way to record the position of the sampling site is to use Geographical Position System (GPS) technology. If possible, use a GPS rover unit to record a position file at the sampling site that can be stored for differential correction. You should also use the GPS unit to record a "real-time" waypoint in the event that the stored file is lost or accidentally deleted. If you do not have a GPS unit capable of differential correction, a simpler unit will allow you to record a waypoint, which will be less accurate.

### 3.18 Gradient

Gradient refers to the vertical drop in elevation along a watercourse over a horizontal distance. It is recorded as the percent gradient. To determine the gradient over a length of stream, measurements are taken off of a 1:50,000 scale NTS map of the watercourse. Locate a point upstream and downstream of the study area on the map where contour lines cross the stream and determine the difference in elevation

(m) between these two points. Measure the distance (m), following the channel, between the same two points using a map wheel. The gradient is calculated as follows:

gradient (%) = [difference in elevation (m)/distance (m)] x 100

In very flat terrain determining gradient from a map may not be possible. In these situations, gradient may also be measured in the field using a clinometer. With this method one person with a clinometer stands at the upstream end of the section to be measured, a second person moves as far downstream as possible while still visible to the upstream person. Both individuals stand at the very edge of the stream with their feet at the water surface. The upstream person uses the clinometer to measure the angle from his or her eyes to the eyes of the other person. If your clinometer measures in % then this value should be recorded. If the clinometer measures in degrees, then percent can be calculated by taking the tangent of that number and multiplying by 100. This technique may need to be repeated several times and averaged to determine the gradient of a large section of stream.

### 3.19 Growth

Fish show indeterminate growth in that they continue to grow throughout their lives rather than stop growing once they reach an "adult size". However, growth rate is asymptotic, meaning the growth rate decreases with increasing age approaching some maximum value for the individual or population. As growth rate is a function of time, true growth rates can only be determined when fish length and age is known. Two parameters related to growth rate are: 1) the maximum size which is possible for fish in a given population, and; 2) the rate at which maximum size is achieved. The maximum size value indicates whether the population is "stunted" (i.e. does not have the potential to reach the normal maximum size for the species) and differentiates between populations that are stunted and those which do not achieve their potential maximum due to a short life span. If the maximum size for the population is at the lower end of the normal range for the species, than the population is slow growing rather than stunted. See Mackay et al. (1990) for methods of calculating maximum size and rate.

### 3.20 Gonads

Organs which are responsible for producing haploid reproductive cells in multicellular animals. In the male, these are the testes and in the female, the ovaries. In fish they are located in the peritoneal cavity, extending between the diaphragm and the cloaca, and running along the dorsal side of the cavity along both sides of the spine. When the fish is gravid, the gonads will fill much of the peritoneal cavity.

### 3.21 GSI (Gonadal:Somatic Index)

Gonad-Somatic Index is the proportion of reproductive tissue in the body of a fish to total body weight. It is calculated by dividing the total weight (g) of the gonads by the total body weight (before gonad removal) and multiplying the result by 100. It is used as an index of the proportion of growth allocated to reproductive tissues in relation to somatic growth. It is believed to be an indicator of fish health in that a fish with a comparatively low GSI for its species is considered to not have sufficient energy available for proper gonad growth. Fish are seasonal spawners and the size of the gonads changes dramatically as

they pass through the various stages of gamete maturation. It is preferable to conduct GSI measurements for fish just prior to the spawning season when the gonads are fully developed (i.e. gravid).

### 3.22 Habitat

Fish habitat refers to aspects of the physical environment which provide the requirements of a fish community, species or life stage. Habitat evaluations conducted for fisheries studies generally involve measurements or evaluations of macro- and/or micro-habitat conditions in order to determine the types of fish or life stages an area might support, the quality of available habitats or habitat limitations.

#### Macro-habitat

Macro-habitat refers to habitat components which are attributable to a general region or section of the study area. They are general conditions related to geographical location, climate, stream order, lake type, etc. For macro-habitat evaluations, we typically measure general water quality parameters (dissolved oxygen, temperature regime, pH, conductivity, turbidity, visibility (secchi depth), stream gradient), as they relate to describing coldwater and coolwater habitats and the types of fish species which may be present. Different fish species have different tolerances for macro-habitat conditions which affect their abundances and distribution.

#### Micro-habitat

Micro-habitat conditions are the physical conditions at a specific location. For micro-habitat assessments we measure or evaluate water depth, velocity, substrate particle size and condition, and the availability of cover for fish. Cover includes instream cover (i.e. any objects which provide velocity shelters) and overhead cover (i.e. anything which provides visual isolation). Each fish species has a range of micro-habitat conditions which are suitable, ranging from barely useable to optimal. In addition, each species has a series of life stages which may also have different habitat requirements. These life stages include spawning, incubation/embryo, nursery, rearing, feeding (adult summer) and overwintering.

Knowledge of the suitable and preferred habitat conditions for different species and life stages is very useful when conducting fisheries inventories, habitat evaluations and impact assessments. Information concerning these habitat requirements is available in the form of Habitat Suitability Index (HSI) models and Habitat Preference Criteria (HPC). HSI models were developed by the U.S. Fish and Wildlife Service and are species-specific models, with each model containing information for all life stages of one fish species. The models include all the habitat variables (macro- and micro-habitat) that accumulated research has determined to be significant to each species with respect to population abundance. Each habitat variable is provided along with the range of suitable and optimal conditions. HPC are species-specific curves showing suitable and preferred conditions for micro-habitat variables (depth, velocity, substrate and cover). HPC curves are available for a limited number of game fish species and were developed from snorkeling observations of the different species and life stages (developed for the most part by Golder from streams in Alberta).

Measurements of macro- and micro-habitat conditions in lakes and streams are useful in combination with inventory data and existing information to establish habitat potential for a study area. Habitat based

assessments are being used more frequently to provide a complete picture of habitat potential, with respect to use by different fish species and life stages, rather than relying on fish inventory data from a specific point in time.

### 3.23 Length

Refers to the whole body length of a fish. There are three types of length measurements: standard length, fork length, and total length. The measurement most commonly used in Canada and ***required for use by Golder*** is the ***Fork Length*** and is ***always recorded in millimetres (mm)***. Fork length is the distance from the most anterior point on the head to the tip of the median caudal fin rays. The fork length of captured fish is measured on a fork length board, which is a trough or flat board with a ruler attached to the surface and a vertical block at the anterior (zero mm) end. Place the fish on the board with its head flush with the block and spread the caudal fin to show the mm mark under the anterior point of the fork.

Some fish species such as burbot, sculpins and darters do not have a fork in their caudal fins. For these species, the standard measurement is Total Length, which is the distance from the most anterior part of the head to the distal tip of the longest caudal fin ray.

The fish which must be measured for length and weight may vary between projects. You will always be measuring game species but will not necessarily have to measure rough or forage fish. The project manager will be able to tell you what is required. For instances where large numbers of individuals are being captured and the time required to measure length and weight is excessive, it may be possible to measure length only for some fish. A large number of lengths are required to produce a complete length-frequency distribution (see section 3.25) while a lesser number of weight measurements are required to provide an accurate length-weight analysis (see section 3.26). If fish are being preserved, always measure length and weight before preserving.

### 3.24 Length-at-Age

Length-at-age analysis is used to determine the average length of fish in each age class in the population. This analysis can only be conducted for individuals for which age is known. For each age class (i.e. 1 year old fish, 2 year old fish, etc.) calculate the range of lengths, mean length and the standard deviation of the mean. Plot this data graphically showing the range, mean and standard error (error bars) (see section 3.47 standard error and standard deviation) with age as the X-axis.

### 3.25 Length-Frequency Analysis

Length and weight data provide the statistics that are the cornerstone of fisheries research and management. Rate of change of length in individuals and length-frequency distributions are key attributes of fish populations. Length-frequency analyses provide an important description of population structure and are used to provide information for the interpretation of age and growth, especially for young fish. Length-frequency distributions reflect the interaction of rates of reproduction, growth and mortality of the population. However, when interpreting length-frequency data it is important to evaluate sampling biases for the capture technique that was used, particularly with respect to size selectivity. The length-frequency distribution of a population is shown graphically by plotting the number of fish in each

size class using a histogram chart. Typically, size classes include every 50 mm fork length interval (i.e. 0-50 mm, 51-100 mm, 101-150 mm..... etc.) but may be more frequent if you have a large sample size. When plotting the length-frequency distribution using Microsoft Excel, label the size classes on the X-axis of the graph using the complete label (i.e. 0-50 mm, not 50 mm).

Using the length-frequency analysis to determine fish age and growth rates is called the Peterson method. The plot of the length-frequency analysis is examined for peaks which are believed to represent each of the year classes in the population. The peak closest to the Y-axis would represent zero aged fish (young-of-the-year) and each peak after that should represent another year class. Great care must be exercised when conducting age analysis with this technique. Typically, distinct peaks are only evident for the first few year-classes. Individual fish exhibit different growth rates and as they get older, the overlap in size ranges for each age class becomes too great and the peaks in the length-frequency distribution are lost. In addition, this method requires measurement of a large number of fish which represent an unbiased sample of the population. The size intervals (fork length classes) chosen for plotting these data are particularly important, as size intervals which are too large or too small will obscure the peaks. Other problems with this method include dominant year-classes which may obscure the peaks of weaker year-classes and divergent growth rates of male and female fish complicates the analysis as does the small incremental changes in length which occur in older fish. However, the Peterson method is quite suitable for some forage fish populations where the life-span is short. It is the recommended ageing method for some minnow species which may have life-spans as short as three years.

### 3.26 Length-Weight Relationships

Length-weight relationships can be used in order to assess the state of well-being of a fish population. These relationships can be used to compare the condition or “fatness” of fish in a population to other populations, or to that in previous years. As a fish population size increases and/or food resources decline, individual fish become thinner and the ratio of weight to length decreases.

The relationship between fish length and body weight is curvilinear, and can normally be represented by the following function:

$$W = aL^b$$

where W = weight, L = length, and ‘a’ and ‘b’ are constants which are characteristic of the population being examined. The constant ‘b’ reflects the rotundness of the fish or the rate at which weight increases for a given increase in length. In general, a value of ‘b’ less than 3.0 represents fish becoming less rotund as length increases, and ‘b’ greater than 3.0 indicates a population where fish become more rotund as length increases. If ‘b’ is equal to 3.0, growth is isometric, meaning shape does not change as fish grow.

The length-weight relationship that we typically use is called length-weight regression analysis. The length-weight relationship can be changed from curvilinear to linear (straight line) using a $\log_{10}$ transformation of both length and weight. The relationship between length and weight becomes:

$$\log W = \log a + b \log L$$

where log a is the 'Y' intercept of the regression line and b is the slope of the line. A regression analysis can be conducted from length and weight measurements of a sub-sample of the fish population. Be sure to measure fish which are representative of the size range in the population, that is an even number of fish should be measured from all size groups in the population, from the smallest to the largest fish. A general rule is that at least 30 fish should be measured to provide a large enough sample size to calculate an accurate regression. The regression analysis plots the log weight versus log length for all the fish measured and then produces the "best fit" straight line that approximates the mathematical relationship between length and weight. The regression analysis can be conducted by entering the length-weight data on a computer spread sheet (Microsoft Excel) and having the program conduct the log transformation of the data. The computer program will provide the regression equation, including the values for 'a' and 'b'. When conducting a regression analysis, you should also record the 'R' value (coefficient of determination) that the computer calculates as this value represents properties of the linear relationship. The higher the 'R' value, the more closely the data conforms to a straight line and the better the regression equations represents the data.

Differences often exist in the body weight to length relationship for males and females in the same population. If possible, length-weight regressions should be calculated separately for the two sexes. The relationship also changes throughout the annual growing season, particularly for females, as gonad size and weight increases, so care should be taken when comparing various sets of data. Prior to conducting a length-weight regression analysis, the length-weight data should be plotted on a scatter diagram in order to spot 'outlying' data points. Points which are well outside the range represented by the other data points should be checked for accuracy to make sure both length and weight were recorded properly.

### 3.27 Lesion

Lesions are the result of a pathological change in body tissue. External hemorrhagic lesions (bloody sores) may be observed on the body surface of the fish and should be recorded on the Fish Sample Record form. Reddened areas and lesions on the body surface are evidence of systemic (widespread, internal) infections of bacteria or superficial bacterial infections. Skin lesions in wild fish are seen most often in the early spring when rising water temperatures encourage bacterial growth at a time when fish are least resistant to it. An increased prevalence of skin lesions also has been associated with fish from water with a high organic load and bacterial community, such as below a sewage outfall.

### 3.28 LSI (Liver:Somatic Index)

Liver-Somatic Index is also known as hepato:somatic index. It is the ratio of liver weight (g) versus total body weight, expressed as a percentage of total body weight. The LSI is used as an indicator of fish health. Energy is stored in the liver in the form of glycogen and the relative size of the liver is believed to correlate with nutritional state.

### 3.29 Marking/Tagging

Identification of individual fish or simply identification of fish which have been captured is required for some projects. Different marking techniques are available, depending on the goals of the study.

### 3.29.1 Anchor (Floy) Tagging

A practical and inexpensive method of permanently marking individual fish. The tag, shaped like an inverted "T", is most commonly inserted through the fishes' back at the base of the rear portion of the dorsal fin and anchored between the epipleural bones of the dorsal fin using a special tag-gun. The tip of the gun is a hollow needle which is inserted through the skin and muscle. As the handle of the tag-gun is depressed, an injector rod pushes the anchor portion of the tag out the end of the gun through the needle. The tag-gun needle will not pass through fish scales. In order to insert the needle, use the tip of the needle to lift the posterior edge of a scale and slip it in under the scale. Fully insert the needle through the skin by inserting it to the base of the needle and depress the handle. Once the tag-gun handle has been fully depressed, hold it in the depressed position while giving the gun a quarter turn to free the tag from the needle. Still with the handle depressed, remove the tag-gun needle from the fish and the tag will remain anchored in place.

The posterior portion of the Floy tag remains outside the fishes' body and is usually brightly coloured and carries a numeric identification code. This tagging method is used when conducting mark-recapture population estimates and basic fish movement studies. It is also the preferred marking technique when seeking angler return data to aid in establishing fish movements. Tags marked with the researchers address and the phrase "$2 reward" are often used to ensure angler response.

When sampling, always record the recapture of marked fish, even if the tag is not one that was inserted during your present study. It is common to catch fish carrying old Floy tags inserted by other agencies who will provide the date and location the fish was tagged; information which will provide movement data for all of the researchers involved. Older tags will usually have a build up of algae and will need to be scraped clean with a knife in order to read the tag number and other information.

Floy tags will usually carry the name and address of the client/agency that Golder is working for and, therefore, the tags are usually provided by the client. If this is not the case, Floy tags will need to be ordered and discussion with the client may be necessary to decide what writing the tags will carry.

### 3.29.2 Visual Implant (VI) Tagging

A "micro-tag" method using tags which are inserted under the skin. VI tags are suitable for use when a tagging method is required which has minimal effects on the swimming and feeding efficiency of the fish. Good for tagging smaller fish than is possible with the anchor tag method, such as small fish species or juvenile fish. Each tag consists of a small metal strip with an individual alpha-numeric code (typically three digits) which is inserted using an injector into a clear tissue somewhere on the fishes body (e.g., post-ocular tissue for salmonids). If working with non-salmonids, it will be necessary to determine a suitable implant location for the fish species you are working with. The implant location should have a sufficiently thick layer of clear tissue so that there will be room to insert the flat injector needle and the tag can be read through the tissue. Record in the field notes the location (including left or right side) of tag insertion for each fish species that you are tagging. To tag a fish, insert the injector needle into the selected tissue, depress the injector and hold it down while removing the needle from the fish.

### 3.29.3 Batch Marking

A marking method which does not distinguish between individual fish. Common methods are fin clipping or dye marking. Batch marking can be used to distinguish fish from specific sites by varying the location on the fishes' body which is dye marked, the colour of the dye or varying which fin is clipped by sampling site. This method is suitable for simple movement studies and for simple mark-recapture population estimates. This method is also used when extremely large numbers of fish need to be marked, as it is simple and more economical than anchor or VI tagging.

Dye marking is accomplished by injecting a small amount of a coloured dye or liquid plastic sub-cutaneously. It can be used for marking very small fish, such as minnows and other forage fish, since a very small hypodermic needle can be used as the injector. One disadvantage of dye marking forage fish is that it is difficult to avoid using a colour which is readily visible to the researcher without increasing the probability of predation of the marked individuals.

Fin clipping includes removing or distinctively altering a fin in a recognizable manor. Fin removal is usually only conducted for non-essential fins such as the adipose fin on salmonids. For other fins such as the pectoral or pelvic fins, the first two fin rays may be removed. For larger fish, a hole punch can be used to make a distinctive mark on a fin. When clipping a fin, it is important to make straight, regular cuts to distinguish the mark from naturally frayed or eroded fins. Record the fin which is marked for each sampling site.

### 3.29.4 Radio Tagging

Attachment of a battery powered radio transmitter to a fish in order to follow its movements using a radio telemetry receiver. The transmitter is affixed externally or surgically implanted in the body cavity. To avoid adverse effects on swimming ability, the transmitter should be <2% of the fishes' body weight. Ground, boat or aerial surveys are conducted with the telemetry receiver in order to follow the fishes movements.

## 3.30 Maturity (State-of-Maturity)

Maturity refers to the state of gonad maturation of an individual fish at the time it is examined. It does not refer to whether or not the fish is "mature" (i.e. adult); classification of a fish as juvenile or adult is referred to as life-history stage (see Section 3.46).

For adult fish, the gonads will typically progress through a series of conditions or phases of maturation each year during the seasonal development cycle. Although juvenile fish have only one possible state-of-maturity, adult fish can be one of several maturities. The state-of-maturity is used to determine the current reproductive status of the individual. For fish populations, state-of-maturity data can be used to determine the size or age at first spawning, the proportion of the stock that is reproductively active, or to illustrate the nature of the reproductive cycle.

Golder uses a system that includes **9 maturity categories**. The 9 categories, their definitions and abbreviation codes are presented on the back of the Fish Sample Record forms used to record the data.

More detailed definitions and descriptions of each maturity category, for both males and females, are provided in Appendix I. Maturity is best determined by conducting an internal examination of the gonads, which requires sacrificing the fish. Maturity can sometimes be determined by external examination of the fish based on fish size and by knowing the typical spawning period for the fish in relation to the capture date or, for some species, by external secondary sexual characteristics which become pronounced during the spawning season (see Section 3.41). The classification system includes an "unknown" category for fish which are examined externally and for which maturity cannot be determined.

For many studies, most or all fish will be released live and only external examinations will be conducted. For other studies, a sub-sample of fish captured will be sacrificed for definitive state-of-maturity data. The following are some hints for establishing state-of maturity from external examination. *Pre-spawning* fish will be found immediately prior to the species spawning season. Fish of a size large enough to be adult or displaying secondary sexual characteristics at this time and with a strongly distended body cavity may be *Pre-spawning*. During the spawning season, gametes (milt or roe) can be extruded from the fish with gentle pressure on the abdomen and it will be obvious that the fish is *Ripe*. *Spent* female fish can be identified by a flaccid, concave abdomen resulting from shedding of the large egg mass and abdominal abrasions obtained during spawning activity. They may extrude a small number of residual eggs in response to pressure on the abdomen. *Spent* males may also have abdominal abrasions and will probably still extrude milt with abdominal pressure, but the milt may appear "watery". Other maturity classifications are very difficult to determine from external examination.

### 3.31 Milt

Milt is a milky white fluid extruded by male fish during spawning activity and contains the sperm. During spawning season, ripe male fish will extrude milt in response to pressure on the abdomen.

### 3.32 Necrosis

The death of a tissue due to injury or disease.

### 3.33 Parasites

Fish are subject to several types of internal and external parasites. A complete parasitological examination requires sacrificing of the subject and microscopic examination of some tissues. For general fisheries inventories, the occurrence of macro-parasites which can be readily observed by the an-aided eye should be recorded on the Fish Sample Record Form. A basic external examination is conducted while measurements of length and weight are conducted. An internal examination is conducted for fish which have been sacrificed. Common external parasites include body lice, gill lice, leeches and lamprey. Common internal parasites include tapeworms, nematodes and flukes associated with the gastro-intestinal tract and other internal organs.

### 3.34 Pathology

For fisheries inventory studies, pathology refers to the field examination of captured fish for indications of parasites, disease and abnormalities, without the use of special procedures (e.g. tissue collection) or tools (e.g. microscope). This can include either external pathology or external and internal pathology.

#### External Pathology

Examination of the body surface, fins, eyes, gills and gill chamber for signs of parasites, disease or abnormalities (deformations). Components of the external examination include body form, body surface, lips and jaws, snout, barbels, opercles, isthmus, eyes, fins, gills, pseudobranch, branchial cavity, anus, and the urogenital opening. A basic external examination can be conducted for most fish while measurements of length and weight are being conducted and the results recorded on the Fish Sample Record Form.

#### Internal Pathology

Examination of the body cavity and internal organs for signs of parasites, disease and abnormalities. Components of the internal examination include body cavity, mesenteric fat, liver, gall bladder, hind gut, stomach, pyloric caeca, intestines, spleen, gas bladder, kidney, gonads, and muscle. A basic internal examination can be conducted for fish which have been sacrificed.

### 3.35 Population Estimates

Population estimates are used to determine or approximate the total number of fish, for one species or a number of species, within a study area. Population estimates may be calculated for a portion of a waterbody (e.g. a section of stream - #fish/km) or an entire waterbody (e.g. a lake - #fish/ha). Two basic types of population estimates are used; Removal and Mark-Recapture.

**Removal** (Reference - Armour et al. 1983)

Removal population estimates involve the isolation of the study area using a physical barrier to block fish movements followed by the removal of fish from the area to provide a population estimate. This technique is restricted to study areas which can be isolated and is typically used in small streams. Small-mesh blocking nets are placed at the upstream and downstream boundaries of the study area to prevent immigration or emigration of fish from the study area. Long minnow seine nets are used as blocking nets and are held in place using rebar posts embedded in the substrate. Care must be taken to ensure the bottom of the net remains in contact with the stream substrate to form an effective barrier.

Electrofishing is used as the capture technique, typically backpack or portable boat electrofishing, depending on stream size and water depth. It is vital that the capture technique be very efficient. If the stream is too deep or wide for effective sampling by backpack electrofishing, the portable boat electrofisher should be used or use two backpack units working simultaneously. Multiple electrofishing passes are conducted within the study area and the catch (species and length) and sampling effort are recorded for each pass. Captured fish are retained in a holding pen or are released outside the study area.

The catch will decline with each pass as the number of fish in the study area is reduced. Ideally, the catch on the final pass will be zero as total removal is achieved, however, total removal is not required. What is required is that the capture efficiency must be high enough that the probability of capture for each individual is high. When this requirement is met, most of the fish in the study area will be captured on the first pass. After two electrofishing passes, the capture probability is calculated (Armour et al. 1983). If the capture probability is 0.8 or greater, the capture efficiency is high enough to provide an accurate population estimate and a sufficient number of passes has been conducted. In practice, capture probabilities as high as 0.8 are uncommon and additional passes must be conducted. Typically, 3 or 4 passes must be conducted to get a good estimate of capture efficiency and to get enough data to calculate a population estimate. If after 4 passes the number of fish being captured has not declined to near zero, the sampling technique is not sufficiently effective and the population estimate will have poor accuracy. A population estimate can be calculated from such data, but the confidence intervals will be very large.

It is very important that the diminishing catch on subsequent passes be due to the reduced number of fish in the study area and not to a reduced amount of sampling effort. It is vital that a similar effort be expended on all passes. The number of seconds of electrofishing and the search pattern in the study area should be similar for all passes. Monitor the electrofishing seconds throughout each pass in order to ensure this requirement is met.

If total removal is achieved, the population estimate for each species is equal to the total number of individuals captured. If total removal is not achieved, formulas are used to calculate the population estimate. Two formulas are available; the first is a simple formula for computations for two removal passes and the second is more complex for computations for more than two removal passes (Armour et al. 1983). Both of these formulas are presented on a Microsoft Excel spreadsheet in the G:\Aquatics directory. Simply type in your data for each species (i.e. number of fish captured on each pass) and the spreadsheet will calculate capture probability, population estimate, standard error and the 95% confidence interval. The lower limit for the 95% confidence interval is sometime lower than the number of fish that was captured. If this is the case, the lower limit should be changed to equal the number of fish captured as this number represents the minimum population size.

**Mark-Recapture**

Mark-recapture population estimates are used in situations where isolation of the study area is not possible or for situations where removal of a significant portion of the population is not practical. Using this technique, a sub-population of fish is captured, marked and released. These fish are then allowed to mix with the larger unmarked population. A sub-sample of fish is then captured and the number of marked and unmarked fish is used to determine the proportion of the total population represented by the marked sub-population. As the size of the marked sub-population is known, the size of the total population can be calculated. This technique is useful in large and intermediate sized streams and in lakes. Any sampling technique with good sampling efficiency can be used but is typically limited to electrofishing, particularly in flowing waters. The mark-recapture technique assumes a closed population (no immigration/emigration) which is not usually true in many situations. Study design should include aspects to reduce the effects of immigration/emigration of fish. For size selective sampling techniques such as electrofishing, population estimates should be conducted separately for different size classes.

For most mark-recapture population estimates, it is recommended that multiple sampling passes be conducted to capture and mark fish. This is followed by a few days without sampling to allow mixing of

marked fish in the general population. A sampling pass (census) is then conducted to determine the portion of marked to unmarked fish in the census sample. Batch marking (see section 3.29) can be used for this technique. The population estimate is calculated using the Chapman modification of the Peterson method (Ricker 1975) as follows:

$$N = (M+1)\ (C+1) / R+1$$

where N = population estimate, M = number of marked fish, C = sample taken for census, and R = number of marked fish in the census sample.

At Golder we generally use the ***CAPTURE*** program (Otis et al. 1978) for mark-recapture population estimates. For this method, the fish marking technique must be Floy or VI tagging (see section 3.29) as each individual fish must be identifiable. Multiple sampling events are conducted in order to tag fish and to keep daily counts of the number of tagged and untagged fish that are captured. The results are then arranged in a matrix which has one line for each individual fish that was captured, along with the day or days it was captured/tagged and recaptured. This matrix is used by the CAPTURE software to provide the population estimate. The CAPTURE program is located in the G:\Aquatics directory. The CAPTURE software tracks the capture/recapture history for each individual fish over each pass and calculates the population estimate based on these results. This technique is believed to provide a more accurate result than the single census-pass estimate presented above. This technique does not require a rest period between the marking passes and a census pass and is more suitable for use in open populations where fish movements in or out of the study area may occur.

### 3.36 Riparian

With respect to fisheries habitat evaluations, riparian areas are terrestrial habitats bordering water bodies (lakes and streams). Riparian areas are not included within the boundaries of the waterbody but are significant in providing habitat features such as overhanging vegetation, inputs of large-woody-debris, sediment stabilization, shading, moderation of surface water run-off, nutrient inputs, etc. Riparian conditions, including species of bank vegetation and floodplain vegetation when possible, are an important part of habitat evaluations.

### 3.37 Roe

Fully developed, unfertilized eggs produced in the ovaries of adult female fish. During spawning season, ripe female fish will extrude roe in response to pressure on the abdomen.

### 3.38 Rough Fish

Large fish species (i.e. non-forage fish) which are not included as game fish. Primarily sucker species.

### 3.39 Sacrifice

Fish which are killed in order to allow internal examination or collection of ageing structures are referred to as sacrificed. For each fish captured, information on whether or not the fish was sacrificed is recorded on the Fish Sample Record Form (i.e. capture code), which helps to identify fish which have been examined internally versus those which were only examined externally. Fish which are sampling mortalities (accidentally killed as a result of capture) are also recorded as sacrificed. Even if intentionally sacrificing fish is not a part of the study design, dead fish should be examined internally for definitive sex and state-of-maturity data, as well as stomach contents and internal pathology when time allows.

### 3.40 Sampling Bias

Sample inaccuracy caused by bias or imprecision in sampling; e.g., bias towards large fish because of the type of sampling gear. In statistics, a sampling bias may be represented as skewedness or as variance.

### 3.41 Sex

Sex refers to the sex of the individual fish, usually recorded as either male or female. However, since determination of sex may be difficult from external examination or from internal examination of juvenile fish, sex may also be recorded as unknown.

#### Sex Determination (Lethal)

To determine the sex of a fish, an incision should be made on the ventral surface of the body from a point immediately anterior of the anus toward the head to a point immediately posterior to the pelvic fins exposing the gonads. If necessary, a second incision may be made on the left side of the fish from the initial point of the first incision toward the dorsal fin. To observe the gonads, fold back the tissue. Ovaries appear whitish to greenish to orange and have a granular texture. The eggs will be readily apparent in developed ovaries. Testes appear creamy white and have a smooth texture.

#### Sex Determination (Non-Lethal)

Determination of sex from external examination of the fish is generally more difficult. For some species, sex may be determined from external secondary sexual characteristics, observable either during the spawning season or, for some species, at any time of year. For most fish species, sex of adult fish can be determined during the spawning season by forcing extrusion of the sexual product (milt/roe).

Secondary sexual characteristics are external physical characteristics displayed by fish which distinguish sex. Some species do not display secondary sexual characteristics. Other species show secondary sexual characteristics during the spawning season and these characteristics are only useful for distinguishing sex for adult fish during the spawning season. Still other species have morphological differences which allow determination of sex from external examination at any time.

Mountain whitefish develop small tubercles (raised bumps) on the lateral scales prior to spawning. These tubercles are generally more pronounced in males than in females but, alone, tubercles may not be a reliable indicator of sex. Trout may show differences in jaw morphology with females having a rounded jaw and male developing a kype (extended, upwardly hooked lower jaw). This characteristic is not reliable in that the male may not develop a kype, particularly in smaller adults. Males for most sucker species develop obvious tubercles which show as hard nodules in the pelvic, lower caudal and, particularly, the anal fin during the spawning season and which are very reliable for determining sex in adult fish. Many species, such as minnows, suckers and some trout develop distinct body coloration or markings during the spawning season which may aid in separating the sexes. Two species, goldeye and mooneye, show a difference in anal fin structure between mature male and female fish which is a reliable external indication to distinguish sex at any time. In the female, the longest rays of the anal fin are the first four and all of the anal fin rays are slender. The overall shape of the fin is "smoothly concave". The first half of the anal fin of the male has long rays followed by much shorter rays at the back, giving the fin a "lobed" appearance. In the male, the anterior rays are thick near the base. This characteristic is not reliable for juvenile fish.

### 3.42 Spawning Surveys

Spawning surveys refer to the visual observation of spawning activity or sampling for the presence of incubating eggs and are used to determine if a site has been used as a spawning area, to determine the distribution of spawning sites within a study area, or to collect micro-habitat data (Habitat Preference Criteria) at known spawning areas. Spawning occurs when eggs (roe) and milt (sperm) are extruded by the fish so as to mix and produce fertilized ovum. This is accomplished in a number of ways by different species. Most game fish species for which spawning surveys are typically conducted are either spring or fall spawning species. There are two basic types of spawning surveys (***egg surveys or redd surveys***) depending on the spawning strategy of the species involved.

#### Egg Surveys

Some species, such as mountain whitefish, lake whitefish, lake trout, walleye and sauger are ***broadcast spawners*** which distribute their eggs over the substrate in areas of suitable depth, velocity and substrate type. The eggs fall into the interstitial spaces (crevices) in the substrate to incubate, although some species will spawn over hard sand if rocky substrates are not available. Spawning surveys for broadcast spawners are conducted using kick sampling and/or airlift sampling techniques (see sections 3.5.1 and 3.5.9). If the study area is small, systematic sampling can be used to examine the entire area for eggs. In large study areas where this type of sampling is impractical, sampling is conducted by examining areas of suitable spawning habitat for the target species. Habitat preference information (see section 3.22) is used to determine the habitat types that should be examined. The section of the stream or portion of lake that is examined during the survey and the location of all spawning sites where incubating eggs are recovered should be identified on maps of the study area. The standard is to use 1:50,000 scale topographical maps but other maps or air photos may be used if they provide greater accuracy. The number of eggs recovered is also recorded for each spawning site and, depending on the sampling technique, sampling effort may also be recorded at each site.

If incubating eggs are found in a study area where more than one species may be spawning, measure egg diameter for the recovered eggs and use egg size, colour and features such as the presence or absence of oil globules to identify the eggs. Egg diameter can be measured using an egg measuring trough. Place 10

eggs in the trough and measure the total amount of the ruler covered, divide this distance by 10 to get an average egg diameter. Scott and Crossman (1973) provide egg descriptions for most species. If egg identification is still doubtful, collect a sample of eggs, measure the egg diameter, and preserve the sample in 5% buffered formalin.

Some fish species use spawning strategies which are part-way between broadcast spawners and species which construct spawning nests. These species include Arctic grayling and several sucker species such as longnose and white sucker. No actual nest or redd is prepared but spawning occurs close over the substrate while the fish are vigorously vibrating and the fertilized eggs become somewhat covered by the substrate material stirred up during this vibration. In some cases, such as spawning areas used by a large number of suckers, disturbances of the substrate can be visually observed but it is not possible to enumerate the number of spawning acts or the number of fish involved. For species such as Arctic grayling, these disturbances are indistinct. Spawning surveys for these species are conducted using egg surveys, as for broadcast spawners.

Still other species, such as northern pike and yellow perch, attach their incubating eggs to submerged vegetation (aquatic macrophytes or flooded terrestrial vegetation). Spawning surveys for these species are conducted by searching for eggs in areas of submerged vegetation. A kick sampling net or other small mesh net is swept through the vegetation and the net contents are examined for eggs.

**Redd Surveys**

Most trout species (including brook, brown, bull, cutthroat and rainbow trout) construct excavations in the substrate into which the fertilized eggs are deposited. A similar excavation immediately upstream of the depression is dug and the materials from this excavation are used to cover the incubating eggs. These excavations or spawning "nests" are termed ***redds*** and are typically constructed in flowing water, although areas of ground-water upwellings in lakes may also be used. As the algae and silt covered rocks are turned over during redd construction, the redds can usually be readily observed due to their "clean" nature and distinctive shape (i.e. distinct depression upstream of a mound). Redd surveys are conducted by one or more observers walking or floating through a study area, enumerating the redds observed, and recording the locations of the redds on a 1:50,00 map of the study area. The study area (section of stream or portion of lake) examined should also be recorded on the map. Not all excavations are redds which contain incubating eggs and it may sometimes be difficult to determine if a disturbance of the streambed is truly a redd. Therefore, redds should be enumerated and classified into the following categories: 1) Class A redd - large or distinct, well formed or spawning fish present; 2) Class B redd - less distinct, most likely an active redd; 3) Class C redd - small or indistinct, possible redd but not definite.

If more than one trout species may be spawning in the study area, enumeration of the redds by species may be difficult. If this is the case, species identification for each redd is best facilitated by conducting the redd survey during the active spawning period so that it is likely that the fish will be present at the redds to aid in identification. Knowing the species and size of the fish in the study area will also help, as some species build larger redds than others. If only one species is expected to be spawning in the study area, the redd survey is usually conducted towards the end of the spawning season when the maximum number of redds will be present.

Repeated redd surveys in the same study area can be used to define the spawning season if required. Surveys are conducted at regular intervals from the start of the spawning season and the number and location of redds on each successive survey is used to determine the length and peak of spawning activity.

### 3.43 Species Code

Standard abbreviation of fish species names is based on the following rules (MacKay et al. 1990):

a) use a four letter abbreviation
b) for a one word name - use the first four letters
   e.g., GOLD for goldeye
c) two word names - use the first letter in each word plus the next consonant in each word
   e.g., ARGR for Arctic grayling,
   LKWH for lake whitefish, and,
   WHSC for white sucker
   (exception - due to duplication, use BRTR for brook trout and BNTR for brown trout)
d) three word names - use the first letter in the first two words and the first letter and next consonant in the last word
   e.g., NRDC for northern redbelly dace

The species codes for all Alberta species are presented on the back of the Fish Sample Record Form.

### 3.44 Species Composition

A term that refers to the species found in the sampling area.

### 3.45 Species Distribution

Where the various species in an ecosystem are found at any given time. Species distribution varies with season and life history stage.

### 3.46 Stage (Life History Stage)

Stage refers to the life history stage (or life stage) of the individual fish. Three stage categories are used to describe free swimming fish: ***fry, juvenile or adult***. The incubating egg is also a life stage and is referred to as the embryo stage.

Fry are also called young-of-the-year (YOY) and are fish from their hatching date until the first anniversary of their hatching date. Juvenile fish are fish from one year old until reaching sexual maturity. Adult fish are fish which are sexually mature.

Definitive life history stage is determined for an individual by internal examination of the gonads. Fry and juvenile fish would have undeveloped gonads and would be classified as immature with respect to state-of-maturity. Fry can usually be separated from juvenile fish by their small size (i.e. smallest fish in

the population) and, for some species, by secondary characteristics such as parr marks. Adult fish are sexually mature fish which have spawned in the past or will spawn in the upcoming spawning season. Their state-of-maturity can be one of several categories, from maturing to spent.

Determination of stage from external examination is not always possible. Identification of fry is based on their small size. However, it is not always possible to tell large juvenile fish from small adult fish, in which case an ***unknown*** category is provided in addition to the three main categories. Evidence of sexual maturity, such as secondary sexual characteristics or extrusion of milt or roe during the spawning season can be used to identify adult fish.

### 3.47 Standard Error and Standard Deviation

Standard error (SE) and standard deviation (SD) both express the variability of results around the mean. However, standard error takes the sample size into consideration when calculated. By including sample size, SE gives an indication of how well we've measured the entire population. This is particularly true if you have very different sample sizes for the groups you are comparing; the larger the sample size, the more confidence you have that the data represents the population.

Standard error is calculated as: $SE=SD \div \sqrt{n}$; where n=sample size. Microsoft Excel will calculate SD automatically. In order to calculate SE the formula in Excel would be " =StDev(cells with data)/(sample size)^0.5". The "^.05" denotes square root (by asking excel to calculate to the power of 0.5).

Standard error is now considered to be the appropriate measure to use in any technical presentation of data and should be used in any figures or tables of fish population statistics.

### 3.48 Stomach Content/Gut Analysis

Stomach content analysis is used to determine the diet and food preferences of fish. The stomach is removed from the sacrificed individual and opened to allow examination of its contents. Record stomach fullness as the percentage of fullness, from 0 to 100%. Record the contents of the stomach as percentage of the material in the stomach, not as percentage of the total stomach volume (e.g. a stomach that was half full, with all the contents being mayflies would be recorded as follows: 50% full, 100% mayfly).

For invertebrates in the stomach contents, record the contents to the lowest taxonomic level possible. Family level is usually required, but Genus should be recorded if known. Unidentifiable, overdigested invertebrates should be recorded as IR (invertebrate remains) and unidentifiable fish remains should be recorded as FR (fish remains).

### 3.49 Study Site/Sampling Location

A study site or sampling location is the portion of a study area at which sampling is conducted. The site may be a ***point location*** (such as a gill net or set line location) a ***transect*** (cross section of a stream channel or lake) or a ***section*** (such as a section of stream electrofished or an area of a lake which is seined). In any event, the location of the sampling site must be recorded in the field notes. For large

studies or studies with multiple sampling locations on the same waterbody, you may wish to number each sampling site. For a single waterbody, sample site may be numbered sequentially (i.e. #1, #2, etc.). For multiple waterbodies, you may wish to combine the number with an abbreviation for the waterbody (e.g. BR1 = Bow River Site #1). You may also wish to identify the type of sampling conducted (e.g. GN1 = gill net set #1). All study site abbreviations must be clearly identified in the field notes. At a minimum, all study sites should be recorded on a 1:50,000 scale topographical map. Other maps or air photos may also be used if they provide greater detail than the 1:50,000 map. See section 3.17 for additional methods of recording location.

Study areas on flowing watercourses are often divided into homogeneous sections called reaches. A ***reach*** is a relatively homogenous section of stream having a uniform set of characteristics and habitat types. A reach is relatively uniform with respect to channel morphology, flow volume, gradient and habitat types and is separated from other reaches by changes in these characteristics. Conventionally, reach numbers are assigned in an upstream ascending order starting from the mouth of the stream. Typically, reach lengths are too long to sample in their entirety, in which case representative study sections will be selected in each reach for determining species distribution and abundances.

### 3.50 Temperature Criteria

Water temperature is a very important habitat component. Different fish species have different temperature requirements and have different tolerances to high water temperatures. Temperature regime in lakes and rivers can affect the presence, distribution and abundance of fish species (see Sections 3.7 and 3.9). Temperature criteria provide maximum temperature levels that are tolerable by various life stages and have been developed for selected game fish species. Golder has prepared a document which list the criteria for selected Alberta species (Taylor and Barton 1992).

### 3.51 Underwater Video

Underwater video equipment includes a remote control underwater camera, light and above surface monitor and video recorder. Underwater video is used to determine fish presence, general abundance and activity. It is not generally useful for recording fish numbers. It is a sampling technique that is effective in both the open water season and for winter sampling under the ice.

### 3.52 Water Quality

Water quality is a basic aspect of fisheries habitat and can influence fish survival, distribution, abundance and reproductive success. Basic water quality parameters that are measured for fisheries studies include; temperature, dissolved oxygen, pH, conductivity, visibility (secchi depth), turbidity, total suspended solids (TSS) and total dissolved solids (TDS).

### 3.53 Weight

Weight refers to the total body weight (wet weight) of fish. It is measured for live fish before they are released or for sacrificed fish immediately after they have been killed. Along with length, weight is one of the most basic parameters measured evaluate the key attributes of fish populations.

Weight should be measured **in grams (g)** using a properly calibrated dial scale or electronic scale, depending on fish size. Golder uses dial scales fitted with fork length troughs for measurements of intermediate and large fish. Two types of dial scale are used; small scales which are rated for 0-4 kg in weight are used for most fish species, large scales rated for 0-25 kg are used for large fish species. For forage fish species and fry life stages of large fish species, more sensitive digital electronic scales are used.

### 3.54 Weight-at-Age

Weight-at-age analysis is used to determine the average weight of fish in each age class in the population. This analysis can only be conducted for individuals for which age is known. For each age class (i.e. 1 year old fish, 2 year old fish, etc.) calculate the range of weights, mean weight and the standard deviation of the mean. Plot this data graphically with age as the X-axis, showing the range, mean and standard deviation (error bars). Weight -at-age is usually plotted on the same graph as length-at-age data.

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## 5. DISCUSSION

All basic aspects of each fisheries sampling program should be clear before commencement of field work. The field supervisor and field crew should be appraised by the project manager of all study design details. This will include study objectives, delineation of the study area, sampling techniques, data requirements and budgeting. Conditions at the field site may require alteration of the study design. The field crew should act in coordination with the project manager regarding changes to sampling protocols.

# APPENDIX I

## MATURITY CODES AND DEFINITIONS

**UNKNOWN (UN):** This category is used when state-of-maturity cannot be determined. This will most often occur for fish which have only been examined externally, where no examination of the gonads has been conducted. It may also be used following internal examination of the gonads when the observer cannot definitely determine the maturity of the fish. The gonads have been examined but the observer is unsure which maturity category to use, or the conditions of the gonads do not appear to match any of the maturity categories. If this is the case, record a complete description of the gonads and, if possible, collect a sample for microscopic examination.

**IMMATURE (IM):** This category is for immature fish (fry or juvenile life stages); defined as fish which have never spawned before and will not spawn in the coming spawning season. The gonads will be undeveloped and will be small and largely transparent. They will be string-like organs situated on the dorsal surface of the body cavity (dorsal to other internal organs) and will lie close under the vertebral column. In very young or small fish, determination of sex from examination of the immature gonads may be difficult or impossible.

*Male:* The testes will typically be smooth in texture and yellow, pink or white in colour. In suckers and percids, immature male testes can be identified by the position of the testicular artery. The artery is usually totally or partially imbedded in the organ.

*Female*: The ovaries will typically have a granular texture and will be yellow or pink in colour. In suckers and percids, immature female ovaries can be identified by the position of the ovarian artery. The artery is usually completely outside the organ, resting on top of the surface tissue and attached with connective tissue.

**MATURING (MA):** A maturing fish is a fish which has not spawned before but will spawn in the coming spawning season. This category refers to a fish whose gonads are developing for the first time. Fish in the maturing category are, for the first time, considered adult fish as they are hormonally similar to sexually mature individuals. Since the gonads are developing for the first time, development may not be complete at the time the fish is examined. The gonads may be developed (enlarged and showing sperm or egg development) primarily at the anterior end. The posterior end of the gonad may still be undeveloped and appear thinner (similar to an immature gonad). This category can be difficult to interpret in the field, being difficult to tell from the *Green* category, and examination of the gonads by microscope may be required. In general, the gonads of a maturing fish will be smaller than those for a *Green* fish.

*Male*: In the field, maturing testes will be smaller and paler than those of fully developed males but considerably larger than immature testes. If unsure, take a sample for histological analysis and designate the fish as *Green* (GN).

*Female*: In the field, maturing ovaries will be smaller and paler than those of fully developed females but considerably larger than immature ovaries. If unsure, take a sample for histological analysis and designate the fish as *Green* (GN).

**SEASONAL DEVELOPMENT (SD):** Fish in this category are sexually mature adults which have spawned in one or more previous spawning seasons and will spawn in the coming spawning season. The gonads are undergoing their seasonal development following the last spawning season. This is the longest of the sexually mature stages as it extends from just after the post-spawning period until the next pre-spawning period, as the fish utilizes its resources to produce new gametes. For spring spawning fish (e.g. walleye, northern pike, longnose sucker, rainbow trout, etc.), this category would last from late May to early April of the next year. For fall spawning fish (e.g. lake whitefish, mountain whitefish, bull trout, brook trout, etc.) this category would last from the end of the fall spawning season one year (September to November) through to the fall of the next year. However, for most fish, gonadal development occurs primarily during the growing season with only limited gonadal development during the winter months.

*Male*: The testes will vary greatly in size and colour within this category depending on the time of year the fish is examined. Early in development (i.e. after the post-spawning period), the testes will be small and yellow to light orange in colour. By early fall (i.e. after the primary gonad development period in the summer), they will have grown to nearly mature size and be white in colour. At this point, the testes will be large and distinct. Note: Suckers have a black coloured testicular membrane which may mask the white colour of the testes.

*Female*: The ovaries will vary greatly in size and colour within this category depending on the time of year they are sampled. Early in development (i.e. after the post-spawning period), the ovaries will be small and yellow to light orange in colour. Developing oocytes will be small and dark orange in colour and will give the ovary a granular appearance. By early fall (i.e. after the primary gonad development period in the summer), the ovaries will have grown considerably to nearly mature size and be bright yellow to orange in colour. The individual eggs will be readily apparent.

**PRE-SPAWNING (PR):** Fish in this category are sexually mature adults which have spawned in one or more previous spawning seasons and will spawn in the coming spawning season. The *Pre-spawning* category follows right after the *Seasonal Development* category, with respect to both time and stage of gonadal development, and occurs when the gonads have completed their seasonal development prior to the spawning season. This is a short term condition which extends from time the gonads are fully developed until the start of spawning activity.

*Male*: Externally the abdomen will be slightly distended. Semen can sometimes be extruded with pressure to the abdomen. If this is the case, small amounts of loose semen will be extruded followed by more viscous semen if pressure is re-applied. Internally, the testes will be large and white and will fill much of the body cavity. Pre-spawning condition can also be inferred by the capture location of the male. Males will usually only enter spawning condition once they are on the spawning grounds and around mature females. Thus a male caught away from the spawning grounds as the spawning season approaches is most likely still in pre-spawning condition, even if some sexual products can be extruded. Note: Semen can be extruded from sexually mature males as early as February in spring spawning species.

*Female*: Externally the abdomen will be noticeably distended. Sometimes a few eggs can be extruded with strong pressure to the abdomen. Care must be taken when applying pressure as the eggs are difficult to extrude and injury to the female can occur. The abdomen will feel tight and hard. Internally, the ovaries will be large and bright yellow to bright orange in colour. The size can be up to 25% of the total body weight and the gonads will fill much of the body cavity. Individual eggs will be large, round and obvious, some eggs will be translucent. Pre-spawning condition can also be inferred by capture location. Females will usually only enter spawning condition once they are on the spawning grounds and around mature males. Thus a female caught away from the spawning grounds as the spawning season approaches is most likely still in pre-spawning condition, even if some sexual products can be extruded.

**RIPE (RP):** Fish in this category are sexually mature adults. *Ripe* is the term for the spawning condition. The *Ripe* category follows right after the *Pre-spawning* category, with respect to both time and stage of gonadal development, and occurs when the gametes (semen and eggs) have become loose in the gonads. This is a short term condition which extends from start to the end of spawning activity. Externally the fish will appear as they do during the *Pre-spawning* stage but extrusion of the gametes will occur in response to slight pressure on the abdomen.

*Male*: Externally the abdomen will be slightly distended. Semen can be extruded with light pressure to the abdomen. Large amounts of loose semen will be produced if pressure is applied. Internally, the testes will be large and white.

*Female*: Externally the abdomen will be greatly distended. Eggs immersed in ovarian fluid can be extruded with light pressure to the abdomen. Large amounts of loose eggs will be produced if pressure is applied. Internally, the ovaries will be large and yellow or orange. The eggs will be large and translucent and some will appear to be loose as the ovarian tissue is weak (i.e. the ovarian sac will be transparent and thin). Eggs will be loose inside the sac and they will be immersed in clear ovarian fluid.

**SPENT (SP):** Fish in this category are sexually mature adults. *Spent* is the term for the post-spawning condition. The *Spent* category follows right after the *Ripe* category, with respect to both time and stage of gonadal development, and occurs following spawning activity when the gametes (semen and eggs) have been largely extruded during spawning. This length of time a fish will spend in this category depends on how long it takes for the fish to begin the next cycle of seasonal gonadal development, at which time the fish will again be classified as *Green*.

*Male*: Externally, the abdomen will be slightly flaccid, especially ventrally. Some semen can still be extruded with pressure to the abdomen but it will most likely be watery (i.e. not as intense a white colour as in spawning males). Internally, the testes will be reduced in size and gray to creamy-white in colour. Hemorrhaging and distended blood vessels on the surface of the organ are common. Post-spawning males are known to stay on the spawning grounds for some time (up to 2 weeks) so capture location is not always a reliable indication of whether the fish has finished spawning.

*Female*: Externally, the abdomen will be noticeably flaccid, especially ventrally. The surface of the abdomen may be red or roughened with abrasions and the urogenital opening may be extended or swollen. Some eggs can still be extruded with pressure but will be few in number and they will be associated with watery ovarian fluid. Internally, the ovaries will be greatly reduced in size and dark orange to brown in colour. Hemorrhaging and distended blood vessels on the surface of the organ as

well as within it are very common and normal. Some residual eggs (from a few up to 25% of the ovary volume) are common. It is not common for post-spawning females to stay on the spawning grounds, most spawn and leave the area immediately. However, capture location is not always reliable indicator.

**REABSORBING (RB):** Fish in this category are sexually mature fish which have developed to some extent for the coming spawning season but, instead of completing gonadal development or instead of spawning after completing gonadal development, these fish are reabsorbing materials from the gonads back into the body. This category represents arrested gonadal development or interrupted spawning activity. There are several reasons why a fish may terminate gonadal development or decide not to spawn after completing gonadal development. These include the condition of the fish with respect to nutrition and/or health, aspects of population dynamics or environmental cues such as improper water temperatures, poor water quality conditions or adverse water level conditions. Interrupted gonadal development can occur at any stage of development and prior to entering the reabsorbing category the fish may have been *Maturing*, undergoing *Seasonal Development* or in *Pre-spawning* condition.

*Male*: This condition is *extremely rare in males* and difficult to observe as reabsorption of the semen by the testes is usually a rapid process. Very rarely will a case be observed of a male actually retaining the entire contents of the testes for re-absorption. Should you suspect this condition the testes should be preserved and stage verified by a qualified biologist.

*Female*: This condition is primarily observed in females. Reabsorption of the eggs by the ovary is usually a lengthy process which can take up to a full year. Some females may retaining the entire contents of the ovaries for re-absorption. Identification of this stage is not always easy. Externally, the female will still have a distended abdomen if caught within a few months of the spawning season. The abdomen will feel unusually hard as compared to normally developing females. Later in the season, it will be impossible to distinguish a normally developing female from a reabsorbing one without an internal examination. Internally, reabsorbing ovaries go through a series of distinct stages. Early in the reabsorption process, the ovary is dark orange to brown in colour. The eggs are dark and flaccid. Heavy amounts of watery ovarian fluid collect at the posterior of the ovary. This fluid most often is ejected readily if the fish is handled. Later, the ovary becomes smaller and hard. The colour becomes darker and the eggs become atritic. Atritic eggs are easily identified as they are small, hard and white. Ovaries in the later stages of eggs reabsorption have few new oocytes. The remnants of the old eggs collect in the middle of the organ. New oocytes production is restricted to the periphery of the ovary. Should you suspect this condition the ovaries should be preserved and stage verified by a qualified biologist. Occasionally, females have been observed which aborted spawning activity after they had became *Ripe*. Functionally speaking, eggs at this stage are no longer connected to the ovaries and cannot be reabsorbed. Instead they remain in the body cavity. Internal examination of a fish in this condition will show the newly developed gonad as well as residual (brown, desiccated) eggs which could not be reabsorbed in the posterior portion of the body cavity.

**RESTING (RS):** Fish in this category are sexually mature adults which have spawned in one or more previous spawning seasons but will not spawn in the coming spawning season. These fish are different from *Reabsorbing* fish in that their gonads are either not developing or are developing too slowly to be ready for the upcoming spawning season. This is a common condition for fish which do not spawn every year (alternate year spawners).

*Male*: This condition is *extremely rare in males*. It can only be used as an alternative to the Green category. A few cases of males in the resting condition have been observed. They are most common in northern latitudes where the growing season is short or in ultra-oligotrophic lakes. Testes will appear flaccid and dirty-white to yellow in colour. They will be larger in size than the testes of immature fish. A good indication is the size of the testicular artery in relation to the organ. In immature fish this artery is very thin whereas in resting males the testicular artery is much larger because of prior testicular development. Should you suspect this condition the testes should be preserved and stage verified by a qualified biologist.

*Female*: This condition is primarily observed in females but is still relatively infrequent, affecting usually only 0.5 to 1% of the population. This stage can only be used as an alternative to the *Green* category. It is most common in northern latitudes where the growing season is short or in ultra-oligotrophic lakes. The ovaries will appear to have some oocytes but they will be few in number and arrested in their development. The colour of resting ovaries varies greatly with fish species but most often they are a light orange. They will be larger in size than the ovaries of immature fish. A good indication is the size of the ovarian artery in relation to the organ. In immature fish this artery is very thin whereas in resting females the ovarian artery is much larger because of prior egg development. Should you suspect this condition the ovaries should be preserved and stage verified by a qualified biologist.

# APPENDIX E

## GOLDER ASSOCIATES' TECHNICAL PROCEDURE 8.16
## HEALTH ASSESSMENTS - METALS

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## 1 PURPOSE

The purpose of this technical procedures document is to:

- describe sampling methods for fish health assessment;
- provide standardization of these methods amongst personnel, sites, sample times, and studies; and,
- document the standard fish health sampling methods employed by Golder Associates Ltd.

The following methods are covered in this technical procedure document:

- special precautions for samples taken for metal contaminant analysis;
- general sampling procedures for fish health assessment;
- sample packaging, preservation, labelling, and shipping procedures; and,
- field documentation.

## 2 APPLICABILITY

These technical procedures are applicable to all personnel involved in fish health surveys. **These procedures are to be used only at sites where there is potential exposure of fish to metals.** At other sites, where potential fish exposure to organic compounds, or mixtures of organics and metals are an issue, refer to TP 8.15 and 8.17, respectively. These technical procedures assume that fish have been captured according to methods outlined in TP 8.1-3 Fish Inventory Methods.

## 3 DEFINITIONS

### 3.1 Ageing Structures

Parts of the fish that are taken for ageing analyses. These structures contain bands (annuli) that delineate seasonal variation in growth; these bands can be counted to estimate age. Primary examples of these structures are scales, fin rays, otoliths, cleithra and opercula. The appropriate ageing structures to collect vary according to fish species and lifestage and include lethal and non-lethal sampling measures (Table 1).

### 3.2 Archive Samples

Extra samples which are taken and kept in storage for possible later analysis.

### 3.3 Bile

An alkaline secretion of the vertebrate liver, which is temporarily stored in the gall bladder. It is composed of organic salts, excretion products and bile pigment. It is responsible primarily for emulsifying fats in the small intestine.

### 3.4 Biomarker

Biomarker refers to a chemical, physiological or pathological measurement of exposure or effect in an individual organism from the laboratory or the field. Examples include levels of: liver detoxification enzymes (e.g. metallothionein); metabolized contaminants in bile; sex steroids in serum.

### 3.5 Chain-of-Custody Forms

Standardized forms used as a means of keeping close track of samples that are taken from the field and transported to laboratories for analysis. Whenever the samples are transported from the field, the custody is relinquished from the delivery person to the receiver by signatures on the forms. These forms substantially decrease the risk of losing samples because they provide a clear record of the chain of transport and handling of the samples.

### 3.6 Contaminants

A general term referring to any chemical compound added to a receiving environment in excess of natural concentrations. The term may include chemicals not generally regarded as “toxic”, such as nutrients, colour and salts.

### 3.7 Electroshocking

The use of electricity to stun and capture fish. An electrical current is passed between electrodes placed in the water; this current causes passing fish (galvanotaxis) to be attracted toward the positive electrode (anode). Once fish pass close to the anode the current acts as a narcotic and stuns the fish (galvanonarcosis), allowing them to be easily netted. Once captured, the fish may be identified, weighed, measured, tagged and then returned to the water. Fish taken by electrofishing revive quickly when returned to the water. Effort is automatically recorded by the electrofishing unit as the number of seconds of active electrofishing (i.e. time current is applied to the water).

### 3.8 Fecundity

The most common measure of reproductive potential in fish. It is the total number of eggs in the ovary of a gravid female fish. Fecundity normally increases with the size of the female within a given species.

### 3.9 Gonads

Organs that are responsible for producing haploid reproductive cells in multicellular animals. In the male, these are the testes and in the female, the ovaries.

### 3.10 GSI

Gonad-Somatic Index. The proportion of reproductive tissue in the body of a fish. It is calculated by dividing the total weight of the gonad by the total body weight and multiplying the result by 100. It is

used as an index of the proportion of growth allocated to reproductive tissues in relation to somatic growth.

### 3.11 LSI

Liver-Somatic Index. Ratio of liver versus total body weight. Expressed as a percentage of total body weight.

### 3.12 Lesions

Pathological change in body tissue.

### 3.13 Metallothionein

Metallothioneins (MT) are a group of proteins present in fish that are responsible for binding and maintenance of metals, including heavy metals. MTs are inducible, that is, exposure to metals causes increase MT production. Therefore, MT levels in fish tissue are a useful indicator of exposure to heavy metals.

### 3.14 Necrosis

The death of a tissue due to injury or disease.

### 3.15 Reference Site

A site used for comparison with a site exposed to the discharge being studied. Ideally, reference sites should be as similar as possible to the exposed site, but without the discharge.

### 3.16 Sampling Error

Sample inaccuracy caused by bias or imprecision in sampling; e.g., bias towards large fish because of the type of sampling gear. In statistics, sample error is expressed by the standard deviation, which expresses the variability of results around the mean.

### 3.17 Secondary Sex Characteristics

External sexual characteristics displayed by fish, particularly during spawning season. Examples are tubercles on fins or body coloration.

### 3.18 Sex Determination (Lethal)

Sex can be determined by examining the gonads during the internal examination. Ovaries appear whitish to greenish to orange and have a granular texture. Testes appear creamy white and have a smooth texture (Texas Water Commission, 1990).

### 3.19 Sex Determination (Non-Lethal)

For some species, sex may be determined from external secondary sexual characteristics, observable either during the spawning season (e.g. suckers - tubercles in males) or at any time of year (e.g. goldeye - anal fin morphology). For most fish species, sex can be determined during the spawning season by forcing extrusion of the sexual product (milt/roe)

### 3.20 Standard Deviation

A measure of the variability or spread of the measurements about the mean. It is calculated as the positive square root of the variance.

### 3.21 SWI

Specific Work Instructions

### 3.22 TDG

Transport of Dangerous Goods

### 3.23 WHMIS

Workplace Hazardous Materials Information System

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# 5 DISCUSSION

## 5.1 General Safety

Refer to Golder Associates Ltd. Safety Manual for general safety procedures.

All solvents and preservatives required for field work must be packaged, labelled, shipped, and used according to WHMIS and TDG regulations.

All used “sharp” dissecting/sampling equipment (needles, scalpel blades, etc.) must be placed in a designated “sharps” disposal container.

## 5.2 Sampling Procedures for Fish Health Assessment

### 5.2.1 General Considerations

The procedures outlined here assume that fish have been captured according to methods outlined in TP 8.1-3, and that associated supporting receiving environment measurements have been recorded. These measurements may include: water temperature, pH, dissolved oxygen, conductivity, secchi reading and current weather conditions (e.g., cloud cover, air temperature, approximate wind speed, precipitation, etc.). Also, relevant data for fish population status assessment may be recorded according to TP 8.1-3. These data may include: species identification, weight (g), length (mm), life history staging (fry, juvenile, adult), sex (refer to Section 3.27) and sexual maturity (if possible), and presence of abnormal external pathology (e.g., fin erosion, ulcers, skeletal anomalies, neoplasms). A fish sample number may be assigned according to TP 8.1-3; and aging structures may be collected. Ageing materials to be collected for each fish species are summarized in Table 1 (MacKay et al. 1990).

Concerns regarding the effect of capture and holding stress on fish, particularly on sensitive physiological biomarkers, may require that fish are captured using specific techniques, such as electroshocking. Refer to specific work instructions (SWI) for instructions.

Prior to sampling for fish health, fish can be held temporarily in a live holding facility, such as a live well, holding pen, or tub. If necessary, fish can be marked at the time of capture using temporary tags (floy tags or fin clipping) for later identification during sampling.

For fish selected for biomarker processing, record time (24-hour clock) of capture. Large fish that are moribund or dead should be fully processed for biological data (sex maturity, internal pathology) unless time limits between capture and processing for specific parameters (e.g. histopathology) have been established.

The full set of procedures outlined in this document may not be required for a particular project. Always refer to Specific Work Instructions (SWI) for specific instructions.

**Samples must not be allowed to thaw once frozen. Protect sample integrity by ensuring adequate dry ice levels in cooler and then take measures to expedite shipping to the analytical laboratory.**

**Ensure that histology samples are properly preserved in 10% neutral buffered formalin. In order for total preservation to take place, 10% neutral buffered formalin should be added so that there is a formalin to tissue ratio of 10 to 1. This is best accomplished by having one nalgene container dedicated to all the histology samples from one fish. Place all tissue cassettes for an individual fish into a single nalgene container, and fill the container with 10% neutral buffered formalin.**

### 5.2.2 General Preparations for Sampling

All new personnel must read the technical procedures for Fish Inventory (TP 8.1-3), and Fish Health Assessment (TP 8.15-0, 8.16-0, or 8.17-0 - whichever is applicable). Personnel must understand the protocol for fish health assessment, have it demonstrated and then practice the procedures on at least 2 practice fish.

Battery operated balances are to be checked daily. Level balance at work area and check calibration using standard weights. A vial, weigh paper; anything that has been weighed on a calibrated lab balance may be used. Shield from wind if necessary.

All biomarker data are to be recorded in waterproof field notebooks, Biomarker forms (Exhibit A), External and Internal Examination forms (Exhibit B and C).

### 5.2.3 Special Precautions to Prevent Contamination of Samples Taken for Metals Analysis

**Special care is required to minimize the chance of contaminating samples for metals analysis.**

If you are collecting contaminant samples, use a fresh filleting knife and dissecting equipment for each fish. Fresh syringes must also be used for each bile and blood sample.

During the dissection process, take care that the tissues designated as metal samples are dissected on a washable plastic surface covered with a plastic sheet which is changed after each dissection. The fish is to be placed on the plastic dissection surface and kept there while the fillet for metals are taken. The fillet for metals must be placed in a labelled plastic bag. If other organs are also being taken for contaminants analysis (e.g. liver, kidney) special care will have to be taken to isolate the section of the organ to be used for metals and take it while still on the plastic and then carefully remove the other section of the organ without contacting the plastic.

**All samples to be analyzed for metals must be placed in plastic packaging, not foil.**

Stainless steel dissecting instruments are made predominantly of chromium and nickel. If these metals are not of concern (refer to SWI), the use of high-quality, corrosion-resistant stainless steel sample processing equipment is acceptable (USEPA 1993). Knives with titanium blades and PTFE handles are recommended for performing tissue resections (Lowenstien and Young 1986, USEPA 1993) but clean plastic handles can also be used. Following use, dirt and tissues should be removed from the instruments with distilled water before washing. For washing, utensils and containers should be cleaned thoroughly with a detergent solution, rinsed with tap water, soaked in acid, and then rinsed with metal-free water. Quartz, PTFE, glass, or plastic containers should be soaked in 50% $HNO_3$ for 12 to 24 hours at room

temperature (USEPA 1993). Stainless steel parts may be cleaned as stated for glass or plastic, omitting the acid soaking step (Stober 1991, in USEPA 1993).

All dissecting equipment is to be wrapped in heavy plastic wrap during storage; and all dissecting equipment, sample containers, sample wrapping and wash equipment must be shipped and stored in clean waterproof containers with leak-proof lids.

### 5.2.4 Specific Steps Involved in Sampling for Fish Health Assessment

This section provides detailed step-by-step instructions for sampling for fish health assessment; these steps are summarized in the flow chart entitled "Protocol for fish health sampling at metals sites" (Figure 1). **Throughout these procedures, refer to specific work instructions (SWI) for project applicable procedures.**

1. Insure the dissection surface (i.e. cutting board) is covered in clean, heavy plastic wrap and that all instruments are clean and readily available to you. Check with the data recorder that all is in order before dissection begins (e.g. sample numbers, labels, dry ice supply, etc.).

2. Assign a biomarker number to fish based on instructions in Section 5.3

3. Put on a clean pair of non-chlorinated, non-powdered latex examination gloves.

4. The next step taken is governed by whether fish is alive or dead, and if dead, for how long. If fish is alive, fish will be processed for the full series of biomarkers and tissue sampling called for in the SWI; in this case proceed to step 5. If fish has been dead for less than 4 hours, proceed to step 8. If fish has been dead for more than 4 hours, then the fish must be rejected for histological sampling, but other measurements may be taken; in this case, proceed to step 8, but disregard instructions for collecting histological samples.

5. Select live fish from holding facility. Excessive handling of fish and stress is to be avoided. Ensure that the skin on the specimen has not been lacerated during sampling. If there has been laceration and loss of fluids, reject the specimen.

6. **Collect blood**: Specific blood sample collection and treatment methods will depend on the objectives of the work; refer to SWI for specific project applicable methods. Special requirements for serum steroid or other types of analyses may require that the fish be sampled within 1 hour of capture; refer to SWI for project specific instructions. Generally, blood collection must be done as quickly as possible, while minimizing stress to the fish. Blood collection proceeds as follows: place fish on dorsal surface into a foam block; withdraw blood from caudal vein using a clean, disposable syringe (size of syringe depends on size of fish - 1 to 5 ml may be suitable); label vial; and, place blood on wet ice for later preparation according to SWI.

7. Sacrifice the fish with a blow to the head. Note sacrifice time (24 hour clock) on log sheet.

8. Rinse the fish in ambient water to remove any foreign material from the external surface.

9. **Weigh and measure the fish** (to nearest gram and mm) and record results. Use fork length measurement except for species with no anal fin indentation (e.g. burbot) which should be measured for total length.

10. Place the fish on the cutting board.

11. **Collect gill samples for histopathology**: The gill tissues begin to degrade almost immediately after death, and must be removed as quickly as possible. Open the opercula and find the second gill arches on both sides of the fish. Cut out the second gill arch with a pair of scissors taking care to cut as close as possible to the dorsal and ventral insertions. Place the gill arches in a labelled histology cassette and immerse in a nalgene container filled with 10% neutral buffered formalin.

12. Examine the fish for external abnormalities and note on External Examination Form (Exhibit B).

13. **Collect fillet samples for metals analysis:** Remove two approximately 100 g fillets with a filleting knife that has been cleaned according to instructions outlined above. Remove skin from fillets and ensure that no part of the fillet touches a surface that has not been covered in plastic. Place each fillet on a piece of plastic and record weight. Wrap each fillet in heavy plastic wrap, insert a label in the plastic (between wrapping) taking care not to touch the fillet. Securely tape a second label onto the outside of the plastic. Label one fillet per fish as an archive sample for possible later analysis. Place one fillet sample from each fish in a cooler with dry ice and store there until shipment to a lab. Place the archive fillet sample per fish in a cooler with dry ice clearly marked "archive samples or QC samples". Note: an alternative packaging for contaminant samples is a plastic bag.

14. **Collect skeletal muscle sample for histology**: After removing a fillet, locate the spinal cord just posterior to the dorsal fin. Remove a piece of skeletal muscle approximately 3 cm $\times$ 1 cm $\times$ 0.5 cm. Place the skeletal muscle in a labelled histology cassette and immerse in a nalgene container filled with 10% neutral buffered formalin.

15. Open body cavity by making an incision on the ventral surface of the body from a point immediately anterior to the anus toward the head to a point immediately posterior to the pelvic fins; cut intestinal tract at both ends; remove intestinal tract and set aside; remove gonads and set aside. Throughout this procedure and the procedures to follow, examine and record the internal condition of the fish, including the presence of parasites, on the Internal Examination Form. Preserve any abnormal tissues and parasites in 10% neutral, buffered formalin for later analysis. Ensure that histology samples contain both internal labels (waterproof paper and pencil) and external labels. Record tissues taken on the Internal Examination Form (Exhibit C) and in field notebook.

16. **Collect gall bladder:** Remove liver from body cavity, taking care not to puncture gall bladder. Identify the gall bladder, clamp bile duct using hemostat forceps, and remove gall bladder. Collect bile from gall bladder using a clean syringe, place in a cryovial, and freeze on dry ice. Alternately (depending of size of the organ), you may place the entire gall bladder intact into a cryovial and freeze on dry ice.

17. **Measure and record weight of entire liver.** Place liver onto a tared piece of plastic on scale, record weight.

18. **Collect sample for liver histology:** Make a longitudinal section through the middle of the liver and remove a strip of tissue approximately 3 cm X 1 cm X 0.5 cm. Place the sample into a tissue cassette; and preserve in 10% neutral buffered formalin in a nalgene container. For histology samples, the liver tissue should originate from a portion of the liver away from the bile duct.

19. **Collect sample for liver metallothionein analysis:** Collect an approximately 1 gm. sample of liver for metallothionein analysis. Record the exact weight, wrap the sample in plastic wrap with internal (placed between wrappings, not touching the sample), and external labels, and freeze on dry ice.

20. **Collect sample for liver metal contaminant analysis:** Take the remainder of the liver, weigh it, and wrap in heavy plastic wrap for contaminants analysis. Insert label in the plastic (between wrapping) taking care not to touch the tissue; and securely tape a label onto the outside of the plastic. Freeze the sample on dry ice.

21. **Collect sample for spleen histology:** The spleen is a dark purple or wine coloured organ at the distal end of the intestines. It is usually housed in a thin, clear mesentery which is sometimes surrounded in adipose tissue. Remove the spleen in its entirety and place it in a tissue cassette and preserve in 10% neutral buffered formalin inside a nalgene container.

22. **Remove and record weight of entire kidney.** The kidney is difficult to remove without disrupting the integrity of the organ; usually, the organ has to be scraped from the fish. **However, histological analysis must be performed on intact undisturbed tissue.** To sample the kidney, perform the following steps: place a piece of clean plastic on the scale, and place a tissue cassette onto the plastic; tare the scale; using a scalpel, obtain two 1 cm thick portions of kidney (one near anterior end, one near posterior end) for histological analysis, place these portions into the cassette on the scale; remove the remainder of the kidney from the fish by scraping with a scalpel, placing the pieces of tissue next to the histological samples on the scale; record total kidney weight.

23. **Collect sample for kidney histology:** Preserve the histological samples previously placed in the tissue cassette in 10% neutral buffered formalin in a nalgene container.

24. **Collect sample for kidney metal contaminant analysis:** Wrap the remaining kidney tissue in heavy plastic wrap for contaminants analysis. Insert label in the plastic (between wrapping) taking care not to touch the tissue; and securely tape a label onto the outside of the plastic. Freeze the sample on dry ice.

25. **Collect sample for gonad histology:** Examine gonads, remove and weigh to nearest 0.1 g. Note sex and assign maturity rating (refer to Table 2 for maturity codes). Make a cross-sectional cut through the middle of the organ after weighing and determining state of maturity. Take the cross-section of testes or ovary and place it in a tissue cassette and preserve in 10% neutral buffered formalin inside a nalgene container.

26. **Collect samples for fecundity and egg diameter determination (perform this step only if fish is a pre-spawning female):** Remove approximately 1 gram of eggs from the midregion of the ovary and place them in round histology cassettes that are lined with foil. Tare the balance to the weight of any empty cassette and then weigh the samples. Label the cassette. A minimum of 50% of the total sample size per species per site must have fresh measurements of egg diameter. Measure 30 individual fresh eggs/female using a micrometer for egg diameter. Record, label and store each egg individually in a histology cassette with 10% neutral, buffered formalin. After the minimum sample size for fresh measurements has been reached, store 30 eggs/female together in a histology cassette with 10% neutral, buffered formalin for analysis at the lab. At the lab, the individual eggs measured fresh in the field will be remeasured and calculated for % shrinkage. The % shrinkage will be used to correct measurement of the samples that were not measured fresh in the field. Volumetric determinations of egg size are made by counting 100 eggs and placing in a graduated cylinder with a pre-measured volume of water. Measure the new volume after the eggs have been added and record. Eg. Pre-volume = 5 ml. Volume with eggs = 5.5 ml. Volume of eggs = 0.5 ml. Precision of volumetric measurements will be dependent upon the graduated cylinder used. NOTE: The precision required for volumetric measurements of egg size in Environmental Effects Monitoring studies is $\pm$ 1%. (E.g. if a 10-ml cylinder is used, measurements are expected to be precise to 0.1 ml. This may

not be achievable with 100 very small eggs. If not achievable, make note in the field notes and record the actual precision - e.g. 0.5 ml).

27. **Collect sample for heart histology:** Cut away the pectoral girdle that protects the heart. Grasp the anterior portion of the heart, pull caudally and very gently separate the connection to the ventral aorta. The heart can be pulled far enough out of the body to expose and sever the sinus using a scalpel. For histology samples, make a sagittal cut through the heart, place in a tissue cassette and preserve in 10% neutral buffered formalin inside a nalgene container.

28. **Collect sample for thyroid histology:** Cut away the ventral insertions of the gill arches, anterior to the heart. The isthmus of tissue in which the gill arches insert contains the thyroid gland although there is no obvious structure. Cut out this triangle of and place it in a tissue cassette and preserve in 10% neutral buffered formalin inside a nalgene container.

29. **Open and examine intestinal tract:** Observe and record qualitative stomach contents on internal examination form. Qualitative measurement of stomach contents is to be done by estimating the % of total volume of contents taken up by each food item. Be as specific as possible. For example, mayflies, stoneflies, caddisflies, water boatmen, water striders, beetles, not just “insects”. Include % sediment or detritus and % plant material. Identify fish to species if possible, e.g., longnose sucker. Identify amphibian, bird or mammal prey as accurately as possible. If stomach contents are to be retained (refer to SWI), then place the stomach contents into a prelabelled whirlpak bag, and preserve by adding 10% neutral buffered formalin.

30. **Collect ageing structures:** Collect two different ageing structure materials (i.e., scales, pectoral fin ray, otoliths) as per species requirements in Table 1, unless otherwise specified in the Specific Work Instructions. Place ageing materials in an “ageing materials” envelope and label.

31. Discard the remains of the specimen into a sealed bag for later disposal in an appropriate manner (e.g. temporary storage in a freezer, or disposal at a landfill). Discard latex gloves.

32. Rinse off cutting board with ambient water. Put on a clean pair of latex gloves and place a fresh piece of washed foil on the board. Proceed to the next fish.

Once all samples have been taken from one site, ensure adequate dry ice levels in the cooler, attach a Chain-of-Custody (Exhibit D) to the inside lid of the cooler and then seal the cooler using duct tape. Do not mix samples from different sites in one cooler.

### 5.3 Assigning Fish Sample Numbers

All fish that are selected for fish health surveys are to be given an individual biomarker number. This is in addition to the fish number assigned at the time of capture. The biomarker number is to be recorded on all individual sample labels. The biomarker number is a unique number which identifies the fish by project, species type, site, season and year.

The following format is to be used for biomarker numbering:

e.g.,

| WLD | 95 | P | 2A | LNSC | 013 |
|---|---|---|---|---|---|
| Project | Year | Season | Site | Species | Fish No. |

**Project** - a unique 3 character code relating to the project.
**Year** - use the last two numbers of the year e.g., 1986 = 86.
**Season** - a one character code relating to season.
P - Spring
U - Summer
F - Fall
W - Winter

**Site** - a one or two alphanumeric code relating to the site the sample was caught.

**Species** - a four character abbreviation for species, based on the following rules (MacKay et al. 1990):

a) use a four letter base abbreviation
b) for a one word name - use the first four letters
e.g., GOLD for goldeye
c) two word names - use the first letter in each word plus the next consonant in each word
e.g., ARGR for Arctic grayling,
LKWH for lake whitefish, and,
WHSC for white sucker
d) three word names - use the first letter in the first two words and the first letter and next consonant in the last word
e.g., NRDC for northern redbelly dace

**Fish No.** - a three digit consecutive number. Individual numbering scheme for each species are to be used.

**This labelling scheme may be superseded by labelling requirements specific to a project. However, a special labelling scheme may only be used at the authorization of the project manager and QA officer.**

### 5.4 Instructions for Preparing a Composite Tissue Sample

All fish fillet samples for each composite will be placed in a labelled bag. The bag containing the samples to be composited will be labelled in indelible ink with the following information:

- name of the composite
- type of sample (i.e. bile, fillet etc.)
- project number
- name of the collecting company (i.e. Golder)
- analysis requested

Upon arrival at the laboratory all samples should be kept in the labelled bag and returned to it upon completion of preparation of the composite.

For fish fillets the following procedure should be used to prepare the composite:

- unwrap the individual samples that are to form one composite
- take a portion of fillet from each individual sample to use in the composite
- make sure to retain a portion of each individual sample and rewrap it
- the remaining portion of each individual sample should be returned to the labelled composite bag and archived (frozen to -25° C)

For bile samples the following procedure should be used to prepare the composite:

- take a small amount of bile from each cryovial that is to form part of the composite
- if possible, leave some of the bile in each cryovial to be archived for future analysis. In some circumstances when the volume of bile is small (< 0.1 ml), the whole sample may have to be used
- the remaining portion of each individual sample should be returned to the labelled composite bag and archived (frozen to -25° C)

For serum samples the following procedure should be used to prepare the composite:

- take a small amount of serum from each cryovial that is to form part of the composite
- leave some of the serum in each cryovial to be archived for future analysis
- each remaining portion of the individual samples should be returned to the labelled composite bag and archived (frozen to -25° C)

### 5.5 Sample Identification Label

**The use of pre-printed labels is strongly encouraged.**

#### 5.5.1 Labelling for Individual Samples for Contaminant Analysis

Each label must be completed in indelible ink for each sample. For contaminant samples, the following information must be included on the label:
Project number
Collecting Agency or Firm-Golder
Biomarker number
Sampling date/time (24 hour clock)
Sample type: F = fillet, W = whole, ungutted, L = liver, B = bile, G = gonad, S = stomach, K = kidney.
Time-frame for analysis - immediate or archive

A completed sample identification label must be taped securely onto each foil-wrapped or bagged sample.

### 5.5.2 Labelling Composite Samples for Contaminant Analysis

Each label must be completed in indelible ink for each sample. For contaminant samples, the following information must be included on the label:

Project number
Collecting agency or firm
Sampling date/time (24 hour clock)
Sample Site
Sampler (name and signature)
Composite number
Species abbreviation
Sample type: F = fillet, W = whole, ungutted, L = liver, B = bile, G = gonad, S = stomach, K = kidney.
Chemical analysis requested - or refer to an accompanying Chain-of-Custody Form or Analysis Request Form
Time-frame for analysis - immediate or archive

A completed sample identification label must be taped securely onto each wrapped or bagged sample. An additional label identifying the composite sample must be placed on each plastic bag containing the wrapped or bagged samples. The same type of label may also be used for archive samples; simply indicate on the label that the samples are to be archived.

### 5.5.3 Labelling for Liver Metallothionein, Blood and Bile Samples

Each label must be completed in indelible ink for each sample; and, the following information must be included on the label:

Biomarker number
Sampling date/time (24 hour clock)

Then place a label on outside of the dewar, bag or cooler containing several bile, blood or liver metallothionein samples and including the following information on the label:

Project number
Collecting agency or firm
Sampler (name)
Time frame for analysis - immediate or archive
General sample type (e.g., bile, liver, etc.)

### 5.5.4 Labelling for Histology and Egg Samples

Each label must be completed in indelible ink for each sample. For histological and egg samples, the following information must be included on the label:

Project number
Sampling date/time (24 hour clock)
Biomarker number
Tissue type:

| | |
|---|---|
| O = ovary | T = testes |
| L = liver | S = spleen |
| H = heart | K = kidney |
| G = gill | I = intestine |
| ST = stomach | SK = skin |
| F = fin | AB = air bladder |

An additional label must be placed on the jar or plastic bag identifying the several cassettes or jars of preserved specimens contained within. The label must include the following:

Project number
Collecting agency or firm
Time frame for analysis - immediate or archive
General sample type (e.g., eggs for fecundity, histology samples)

## 5.6 Documentation

### 5.6.1 Field Record Keeping

For proper interpretation of field survey results, thorough documentation of all field sample collection and processing activities is required. All logbooks should be perfect-bound and waterproof, forms should be preprinted on waterproof paper, and only indelible ink and pencil (if form or paper is wet) should be used.

To document field activities, sample identification labels, field logbooks, Biomarker Forms (Exhibit A), External (Exhibit B) and Internal (Exhibit C) Examination Forms, and Chain-of-Custody forms (Exhibit D) should be used, in addition to forms described for fish capture records (see TP 8.1-3). This will serve as an overall "Chain-of-Custody", documenting all field samples and field events beginning with sample collection through biomarker processing and preservation and shipment to the laboratory.

### 5.6.2 Chain-of-Custody Form

Sample possession and proper handling of samples must be traceable from the time of sample collection, through laboratory and data analysis. A Golder Chain-of-Custody form must be completed and signed in indelible ink for each shipping container (e.g. ice cooler) used. Two copies of the Chain-of-Custody form must be sealed in a plastic bag and taped to the outside cover of the cooler. Ensure that the carrier responsible for delivering the samples also signs and dates all Chain-of-Custody forms.

### 5.6.3 Field Records and Logbook

All pertinent information on field activities and sampling efforts must be recorded in an appropriate (i.e., waterproof) bound logbook. The field crew leader is responsible for ensuring that sufficient detail is

recorded in the logbook. The logbook must be complete enough to enable someone unfamiliar with the project to completely reconstruct field activity without relying on the memory of the field crew. All entries must be made in indelible ink, with each page numbered, signed and dated by the author, and a line drawn through the remainder of any partly used page. All corrections are made by a single-line cross-out of the error, entering the correct information, dating and initialing the change. Upon return to the office, all field notes must be photocopied and placed in the appropriate project files.

Entries in the field logbook must include:

- Purpose of proposed sampling effort.
- Date and time (24 hour clock) of sampling.
- Names of field crew leader and team members.
- Description of each sampling site, including information on any photographs that may be taken.
- Location of each sampling site, name and number, applicable navigational coordinates, waterbody name/segment number.
- Details of sampling method and effort, particularly deviations from Specific Work Instructions.
- Clear identification of site names and sample numbers.
- Field observations.
- Field measurements taken (e.g., pH, temperature, flow, dissolved oxygen, secchi, weather conditions).
- Sample shipping information.

The field logbook should also be used to document any additional information on sample collection activities, hydrologic conditions, boat or equipment operations, or any unusual activities observed or problems encountered that would be useful to the project manager when evaluating the quality of the monitoring data.

A biomarker logbook should also be kept. All pertinent information on fish biomarkers must be recorded in an appropriate (i.e., waterproof) bound logbook. The field crew leader is responsible for ensuring that sufficient detail is recorded in the logbook. The logbook must be complete enough to enable someone unfamiliar with the project to completely reconstruct fish biomarker field activity without relying on the memory of the field crew. All entries must be made in indelible ink, with each page numbered, signed and dated by the author, and a line drawn through the remainder of any partly used page. All corrections are made by a single-line cross-out of the error, entering the correct information, dating and initialing the change. Upon return to the office, all field logbooks and notes must be photocopied and placed in the appropriate project files.

Entries in the fish biomarker field logbook must include:

- Date and time (24 hour clock) of sampling.
- Names of field crew leader and team members.
- Site name and number.
- Secchi, water temperature, conductivity,
- Fish number and Biomarker number.
- Capture time/sacrifice time (24 hour clock)
- Length/ Total Weight.
- Sex and stage
- Liver: total weight, sample weights, sample time (24 hour clock), canister storage number, colour

- Fillet: sample weights
- Bile: colour, volume, canister storage number
- Gonads: total weight (testes or ovaries), egg weights, total fecundity count
- Abnormal tissues collected and preserved
- Ageing structures collected

Biomarker Forms, Catch Records, Fish Sample Records, External/Internal Examination Forms and Photo-Log Sheets are to be filled out, dated and signed. All forms should be cross-referenced to the appropriate field record via the fish number and/or composite number.

### 5.7 Change of Procedures

Variations from the established procedure requirements may be necessary due to unique circumstances in the field. All variations from established procedures shall be documented on Procedure Alteration Checklists (Exhibit G) and reviewed by the Project Manager and the QA Manager.

The Project Manager may authorize the individual Field Crew Members to initiate variations as necessary. If practical, the request for variations shall be reviewed by the Project Manager and the QA Manager prior to implementation. If prior review is not possible, the variation may be implemented at the direction of the Field Biologist, provided that the Project Manager is notified of the variation within 24 hours of implementation and the Procedure Alteration Checklist is forwarded to the Project Manager and the QA Manager for review within 2 working days of implementation. If the variation is unacceptable to either reviewer, the activity shall be repeated or action shall be taken as indicated in the Comments section of the checklist.

All completed Procedure Alterations Checklists shall be maintained in project records.

### 5.8 Shipping of Samples

Samples are to be shipped by the fastest possible means to the analytical laboratory. The primary QA consideration in shipping samples is protecting sample integrity. Preserve sample integrity by ensuring adequate ice levels in coolers before shipment to laboratory. Coolers are to remain sealed throughout shipment. Weigh-bill numbers are to be noted on the copies of the Chain-of-Custody form retained after sealing the coolers. Each transfer of custody is to be noted and signed for. The coolers should be labelled as Perishable/Keep Cold/Time-Sensitive. Clearly indicate the analytical laboratory address as well as a Golder contact person and phone number. The crew leader is to telephone the processing laboratory and inform them of the upcoming delivery. The crew leader is also required to phone the processing laboratory to confirm arrival of the shipment and that analysis instructions are clear.

## 6 RESPONSIBILITY

All aquatic field crew members engaged in conducting fish inventories or fish biomarking studies are responsible for compliance with this procedure.

# 7 EQUIPMENT AND MATERIALS

## 7.1 General Supplies

First aid kit (including emergency phone numbers of local hospitals, family contacts for each crew member)
Topographical maps of sampling sites
Flagging material
Tool box
Fish tubs

## 7.2 Record Keeping

Field logbook (perfect-bound, waterproof)
Labels
Chain-of-Custody forms
Fish Sample Records
Unique Catch Records (boat, backpack, gillnet, seine net, etc.)
Indelible pens
Pencils
Applicable MSDS sheets and TGD placards

## 7.3 Biomarking Equipment (to be stored in waterproof, sealable equipment containers)

Specific Work Instructions
20 Litre pails for transfer and holding of fish
Fish measuring board (metric)
Balance (metric), calibration weights, balance levels and 9 volt batteries
Stainless steel forceps
Stainless steel filleting knives
Stainless steel dissecting scissors
Stainless steel scalpels
Stainless steel scalpel blades
Centrifuge (if taking blood samples)
Small whirlpacks
Nalgene bottles for histology samples
Histology cassettes
Hemostats for clamping off gall bladder
5 ml Cryovials
Blood tubes
Tube rack
Paper towels
0.15 M KCl
Non-chlorinated, non-powdered latex surgical gloves
Plastic wrap
10 ml syringe
18 g needle
5 ml syringe

27 g needle
Pipettes (if taking blood smears or serum samples)
Pipette Bulbs
Goggles
Gloves
“Sharps” disposal containers
Wash-tubs for field-washing of dissecting equipment
Detergent solution
Metal-free water
$HNO_3$
Distilled water
Used washing solution containers
Plastic cutting boards (washable)
Fish bonker
Folding tables (for biomarking stations)
Biomarker tent
Teflon wash bottle with distilled water
Medical tape
String
Several sizes of plastic bags including garbage bags
Cage material for holding fish in situ, if live-wells or fish tubs not available or too small

### 7.4 Sample Preservation and Shipping Supplies

Ice (wet ice and/or dry ice)
10% neutral buffered formalin
0.15% KCl
Scale envelopes
Ice chests
Duct tape
Clear shipping tape for Chain-of-Custody forms
Pre-printed labels

**TABLE 1:**

**RECOMMENDED FISH AGEING STRUCTURES (FROM MACKAY ET AL. 1990).**

| SPECIES | AGEING STRUCTURE (most preferred structure in bold) | | | |
|---|---|---|---|---|
| | LETHAL | | NON-LETHAL | |
| | Preferred | Secondary | Preferred | Secondary |
| lake sturgeon | otoliths | none | first pectoral fin ray[A] | none |
| Arctic grayling | sagittal otoliths | none | scales[B] | pectoral fin rays |
| cisco | sagittal otoliths | none | scales[B] (if fast growing) | none |
| lake whitefish | sagittal otoliths | undetermined | scales[B] (if fast growing) | pelvic fin rays |
| mountain whitefish | sagittal otoliths | none | scales[C] | undetermined |
| lake trout | sagittal otoliths | none | first three pelvic fin rays[A] | scales (for immature fish) |
| bull trout | sagittal otoliths | none | none (scales not suitable) | none |
| brook trout | sagittal otoliths | none | scales[C] (if < 3 yrs. old) | none |
| brown trout | sagittal otoliths | none | scales[D] (if < 3 yrs. old) | none |
| rainbow trout | sagittal otoliths | none | scales[E] (if fast growing) | none |
| cutthroat trout | sagittal otoliths | none | scales (unreliable) | none |
| northern pike | cleithrum (freeze) | opercular bones and vertebrae | first three pelvic fin rays[A] | scales[D] (fish up to 3 yrs. old) |
| goldeye | operculum | none | first three pelvic fin rays[A] | scales[C] (fish up to 5 yrs.) |
| mooneye | operculum | none | first three pelvic fin rays[A] | scales[C] (fish up to 5 yrs.) |
| yellow perch | opercular bone | none | pelvic spine and first two fin rays[A] | two anal spines[A] |
| walleye | opercular bones | otoliths | pelvic spine and first two fin rays[A] | dorsal spine |
| sauger | opercular bones | otoliths | pelvic spine and first two fin rays[A] | dorsal spine |
| burbot | sagittal otoliths | none | none | none |
| suckers spp. | none | none | pectoral fin rays[A] | scales (if <5 yrs.) |
| trout-perch | otoliths | none | none | none |
| sculpin spp. | otoliths | none | length-freq. analysis | none |
| cyprinids | otoliths | none | scales | length-freq. analysis |
| flathead chub | otoliths | none | pectoral fin ray[A] | scales |
| sticklebacks | otoliths | none | length-freq. analysis | none |

[A] proximal end
[B] collect 10-15 scales from the left side between the front edge of the dorsal fin and the lateral line
[C] collected between the dorsal fin and the lateral line
[D] collected from above the lateral line just posterior to the dorsal fin
[E] collected from immediately dorsal to the lateral line, between the posterior edge of the dorsal fin and origin of the anal fin

**TABLE 2:**

**MATURITY CODES AND DEFINITIONS**

**UNKNOWN (UN):** This category is used when state-of-maturity cannot be determined. This will most often occur for fish which have only been examined externally, where no examination of the gonads has been conducted. It may also be used following internal examination of the gonads when the observer cannot definitely determine the maturity of the fish. The gonads have been examined but the observer is unsure which maturity category to use, or the conditions of the gonads do not appear to match any of the maturity categories. If this is the case, record a complete description of the gonads and, if possible, collect a sample for microscopic examination.

**IMMATURE (IM):** This category is for immature fish (fry or juvenile life stages); defined as fish which have never spawned before and will not spawn in the coming spawning season. The gonads will be undeveloped and will be small and largely transparent. They will be string-like organs situated on the dorsal surface of the body cavity (dorsal to other internal organs) and will lie close under the vertebral column. In very young or small fish, determination of sex from examination of the immature gonads may be difficult or impossible.

*Male*: The testes will typically be smooth in texture and yellow, pink or white in colour. In suckers and percids, immature male testes can be identified by the position of the testicular artery. The artery is usually totally or partially imbedded in the organ.

*Female*: The ovaries will typically have a granular texture and will be yellow or pink in colour. In suckers and percids, immature female ovaries can be identified by the position of the ovarian artery. The artery is usually completely outside the organ, resting on top of the surface tissue and attached with connective tissue.

**MATURING (MA):** A maturing fish is a fish which has not spawned before but will spawn in the coming spawning season. This category refers to a fish whose gonads are developing for the first time. Fish in the maturing category are, for the first time, considered adult fish as they are hormonally similar to sexually mature individuals. Since the gonads are developing for the first time, development may not be complete at the time the fish is examined. The gonads may be developed (enlarged and showing sperm or egg development) primarily at the anterior end. The posterior end of the gonad may still be undeveloped and appear thinner (similar to an immature gonad). This category can be difficult to interpret in the field, being difficult to tell from the *Green* category, and examination of the gonads by microscope may be required. In general, the gonads of a maturing fish will be smaller than those for a *Green* fish.

*Male*: In the field, maturing testes will be smaller and paler than those of fully developed males but considerably larger than immature testes. If unsure, take a sample for histological analysis and designate the fish as *Green* (GN).

*Female*: In the field, maturing ovaries will be smaller and paler than those of fully developed females but considerably larger than immature ovaries. If unsure, take a sample for histological analysis and designate the fish as *Green* (GN).

**SEASONAL DEVELOPMENT (SD):** Fish in this category are sexually mature adults which have spawned in one or more previous spawning seasons and will spawn in the coming spawning season. The gonads are undergoing their seasonal development following the last spawning season. This is the longest of the sexually mature stages as it extends from just after the post-spawning period until the next pre-spawning period, as the

fish utilizes its resources to produce new gametes. For spring spawning fish (e.g. walleye, northern pike, longnose sucker, rainbow trout, etc.), this category would last from late May to early April of the next year. For fall spawning fish (e.g. lake whitefish, mountain whitefish, bull trout, brook trout, etc.) this category would last from the end of the fall spawning season one year (September to November) through to the fall of the next year. However, for most fish, gonadal development occurs primarily during the growing season with only limited gonadal development during the winter months.

*Male*: The testes will vary greatly in size and colour within this category depending on the time of year the fish is examined. Early in development (i.e. after the post-spawning period), the testes will be small and yellow to light orange in colour. By early fall (i.e. after the primary gonad development period in the summer), they will have grown to nearly mature size and be white in colour. At this point, the testes will be large and distinct. Note: Suckers have a black coloured testicular membrane which may mask the white colour of the testes.

*Female*: The ovaries will vary greatly in size and colour within this category depending on the time of year they are sampled. Early in development (i.e. after the post-spawning period), the ovaries will be small and yellow to light orange in colour. Developing oocytes will be small and dark orange in colour and will give the ovary a granular appearance. By early fall (i.e. after the primary gonad development period in the summer), the ovaries will have grown considerably to nearly mature size and be bright yellow to orange in colour. The individual eggs will be readily apparent.

**PRE-SPAWNING (PR):** Fish in this category are sexually mature adults which have spawned in one or more previous spawning seasons and will spawn in the coming spawning season. The *Pre-spawning* category follows right after the *Seasonal Development* category, with respect to both time and stage of gonadal development, and occurs when the gonads have completed their seasonal development prior to the spawning season. This is a short term condition which extends from time the gonads are fully developed until the start of spawning activity.

*Male*: Externally the abdomen will be slightly distended. Semen can sometimes be extruded with pressure to the abdomen. If this is the case, small amounts of loose semen will be extruded followed by more viscous semen if pressure is re-applied. Internally, the testes will be large and white and will fill much of the body cavity. Pre-spawning condition can also be inferred by the capture location of the male. Males will usually only enter spawning condition once they are on the spawning grounds and around mature females. Thus a male caught away from the spawning grounds as the spawning season approaches is most likely still in pre-spawning condition, even if some sexual products can be extruded. Note: Semen can be extruded from sexually mature males as early as February in spring spawning species.

*Female*: Externally the abdomen will be noticeably distended. Sometimes a few eggs can be extruded with strong pressure to the abdomen. Care must be taken when applying pressure as the eggs are difficult to extrude and injury to the female can occur. The abdomen will feel tight and hard. Internally, the ovaries will be large and bright yellow to bright orange in colour. The size can be up to 25% of the total body weight and the gonads will fill much of the body cavity. Individual eggs will be large, round and obvious, some eggs will be translucent. Pre-spawning condition can also be inferred by capture location. Females will usually only enter spawning condition once they are on the spawning grounds and around mature males. Thus a female caught away from the spawning grounds as the spawning season approaches is most likely still in pre-spawning condition, even if some sexual products can be extruded.

**RIPE (RP):** Fish in this category are sexually mature adults. *Ripe* is the term for the spawning condition. The *Ripe* category follows right after the *Pre-spawning* category, with respect to both time and stage of gonadal development, and occurs when the gametes (semen and eggs) have become loose in the gonads. This is a short term condition which extends from start to the end of spawning activity. Externally the fish will

appear as they do during the *Pre-spawning* stage but extrusion of the gametes will occur in response to slight pressure on the abdomen.

*Male*: Externally the abdomen will be slightly distended. Semen can be extruded with light pressure to the abdomen. Large amounts of loose semen will be produced if pressure is applied. Internally, the testes will be large and white.

*Female*: Externally the abdomen will be greatly distended. Eggs immersed in ovarian fluid can be extruded with light pressure to the abdomen. Large amounts of loose eggs will be produced if pressure is applied. Internally, the ovaries will be large and yellow or orange. The eggs will be large and translucent and some will appear to be loose as the ovarian tissue is weak (i.e. the ovarian sac will be transparent and thin). Eggs will be loose inside the sac and they will be immersed in clear ovarian fluid.

**SPENT (SP):** Fish in this category are sexually mature adults. *Spent* is the term for the post-spawning condition. The *Spent* category follows right after the *Ripe* category, with respect to both time and stage of gonadal development, and occurs following spawning activity when the gametes (semen and eggs) have been largely extruded during spawning. This length of time a fish will spend in this category depends on how long it takes for the fish to begin the next cycle of seasonal gonadal development, at which time the fish will again be classified as *Green*.

*Male*: Externally, the abdomen will be slightly flaccid, especially ventrally. Some semen can still be extruded with pressure to the abdomen but it will most likely be watery (i.e. not as intense a white colour as in spawning males). Internally, the testes will be reduced in size and gray to creamy-white in colour. Hemorrhaging and distended blood vessels on the surface of the organ are common. Post-spawning males are known to stay on the spawning grounds for some time (up to 2 weeks) so capture location is not always a reliable indication of whether the fish has finished spawning.

*Female*: Externally, the abdomen will be noticeably flaccid, especially ventrally. The surface of the abdomen may be red or roughened with abrasions and the urogenital opening may be extended or swollen. Some eggs can still be extruded with pressure but will be few in number and they will be associated with watery ovarian fluid. Internally, the ovaries will be greatly reduced in size and dark orange to brown in colour. Hemorrhaging and distended blood vessels on the surface of the organ as well as within it are very common and normal. Some residual eggs (from a few up to 25% of the ovary volume) are common. It is not common for post-spawning females to stay on the spawning grounds, most spawn and leave the area immediately. However, capture location is not always reliable indicator.

**REABSORBING (RB):** Fish in this category are sexually mature fish which have developed to some extent for the coming spawning season but, instead of completing gonadal development or instead of spawning after completing gonadal development, these fish are reabsorbing materials from the gonads back into the body. This category represents arrested gonadal development or interrupted spawning activity. There are several reasons why a fish may terminate gonadal development or decide not to spawn after completing gonadal development. These include the condition of the fish with respect to nutrition and/or health, aspects of population dynamics or environmental cues such as improper water temperatures, poor water quality conditions or adverse water level conditions. Interrupted gonadal development can occur at any stage of development and prior to entering the reabsorbing category the fish may have been *Maturing*, undergoing *Seasonal Development* or in *Pre-spawning* condition.

*Male*: This condition is ***extremely rare in males*** and difficult to observe as reabsorption of the semen by the testes is usually a rapid process. Very rarely will a case be observed of a male actually retaining the entire contents of the testes for re-absorption. Should you suspect this condition the testes should be preserved and stage verified by a qualified biologist.

*Female*: This condition is primarily observed in females. Reabsorption of the eggs by the ovary is usually a lengthy process which can take up to a full year. Some females may retaining the entire contents of the ovaries for re-absorption. Identification of this stage is not always easy. Externally, the female will still have a distended abdomen if caught within a few months of the spawning season. The abdomen will feel unusually hard as compared to normally developing females. Later in the season, it will be impossible to distinguish a normally developing female from a reabsorbing one without an internal examination. Internally, reabsorbing ovaries go through a series of distinct stages. Early in the reabsorption process, the ovary is dark orange to brown in colour. The eggs are dark and flaccid. Heavy amounts of watery ovarian fluid collect at the posterior of the ovary. This fluid most often is ejected readily if the fish is handled. Later, the ovary becomes smaller and hard. The colour becomes darker and the eggs become atritic. Atritic eggs are easily identified as they are small, hard and white. Ovaries in the later stages of eggs reabsorption have few new oocytes. The remnants of the old eggs collect in the middle of the organ. New oocytes production is restricted to the periphery of the ovary. Should you suspect this condition the ovaries should be preserved and stage verified by a qualified biologist. Occasionally, females have been observed which aborted spawning activity after they had became *Ripe*. Functionally speaking, eggs at this stage are no longer connected to the ovaries and cannot be reabsorbed. Instead they remain in the body cavity. Internal examination of a fish in this condition will show the newly developed gonad as well as residual (brown, desiccated) eggs which could not be reabsorbed in the posterior portion of the body cavity.

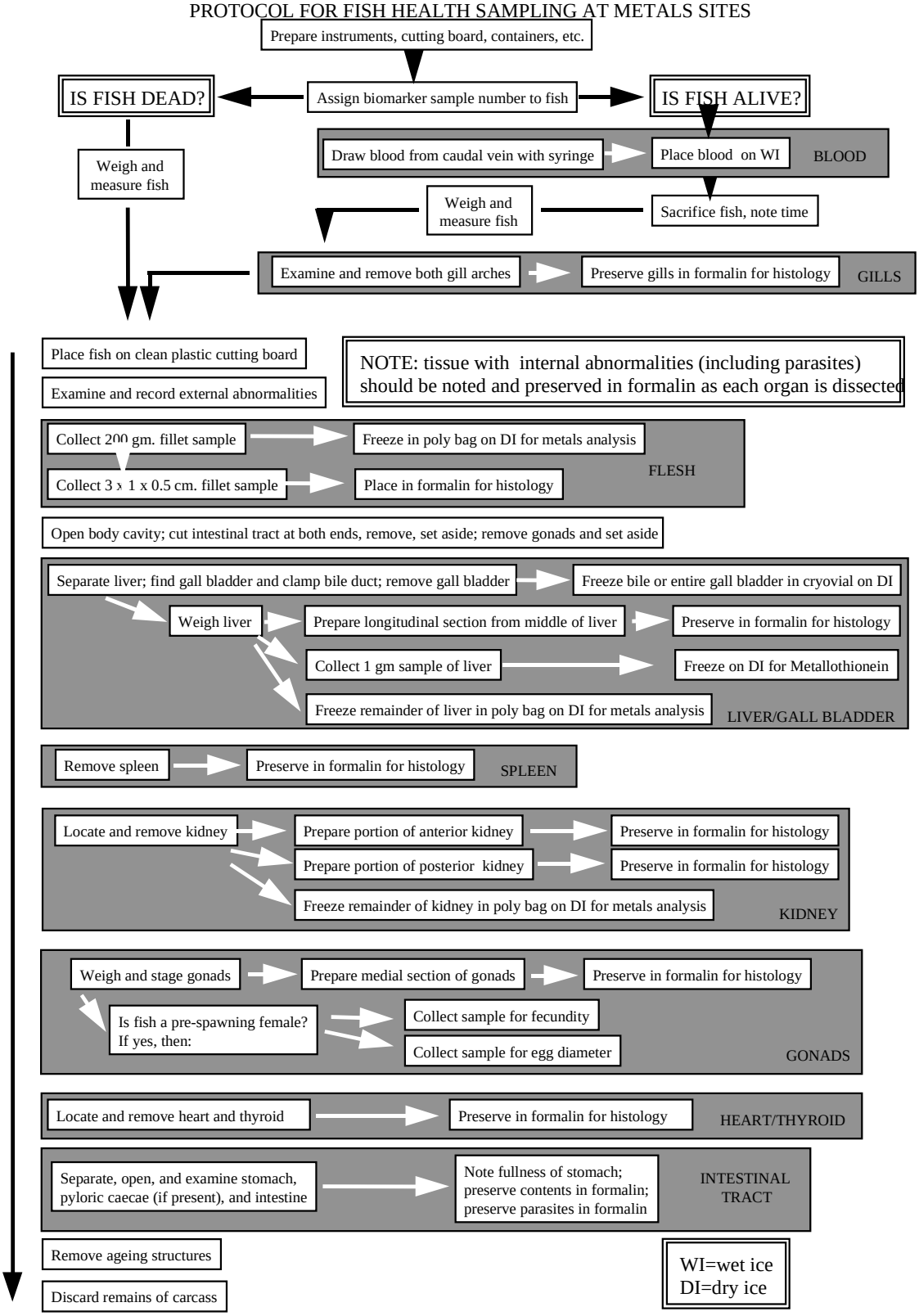
**RESTING (RS):** Fish in this category are sexually mature adults which have spawned in one or more previous spawning seasons but **will not** spawn in the coming spawning season. These fish are different from *Reabsorbing* fish in that their gonads are either not developing or are developing too slowly to be ready for the upcoming spawning season. This is a common condition for fish which do not spawn every year (alternate year spawners).

*Male*: This condition is ***extremely rare in males***. It can **only** be used as an alternative to the Green category. A few cases of males in the resting condition have been observed. They are most common in northern latitudes where the growing season is short or in ultra-oligotrophic lakes. Testes will appear flaccid and dirty-white to yellow in colour. They will be larger in size than the testes of immature fish. A good indication is the size of the testicular artery in relation to the organ. In immature fish this artery is very thin whereas in resting males the testicular artery is much larger because of prior testicular development. Should you suspect this condition the testes should be preserved and stage verified by a qualified biologist.

*Female*: This condition is primarily observed in females but is still relatively infrequent, affecting usually only 0.5 to 1% of the population. This stage can **only** be used as an alternative to the *Green* category. It is most common in northern latitudes where the growing season is short or in ultra-oligotrophic lakes. The ovaries will appear to have some oocytes but they will be few in number and arrested in their development. The colour of resting ovaries varies greatly with fish species but most often they are a light orange. They will be larger in size than the ovaries of immature fish. A good indication is the size of the ovarian artery in relation to the organ. In immature fish this artery is very thin whereas in resting females the ovarian artery is much larger because of prior egg development. Should you suspect this condition the ovaries should be preserved and stage verified by a qualified biologist.

FIGURE 1:

PROTOCOL FOR FISH HEALTH SAMPLING AT METALS SITES



**FISH HEALTH ASSESSMENT**

Waterbody: _____ Site: _____ Date: _____

Fish Species:_____ Biomarker Fish # _____ Fisheries Inventory # _____

Fork length (mm): _____ Total Length (mm) _____ Total Weight (g): _____

Carcass Weight (g): _____ Capture Method: _____ Aging Structure Taken: FR Sc Ot Op Cl

**Internal and External Examination**

Eyes:
N B1 B2 E1 E2 H1 H2 M1 M2 OT(other):_____

Gills:
N F C M P OT (other):_____

Pseudobranchs:
N S L I OT (other):_____

Thymus:
0 1 2 3 comments:_____

Skin:
0 1 2 3 comments:_____

Bodyform Deformaties:
Description:_____

Fins
0 1 2 3 comments:_____

Opercles:
0 1 2 comments:_____

Hindgut:
0 1 2 3 comments:_____

Sex:
M F U Maturation stage: IM MA SD PR RP SP RS RB UN

Mesentaric Fat
0 1 2 3 4 comments:_____

Liver:
A C D E F OT (other):_____

Spleen
B G D E OT (other):_____

Gall Bladder:
0 1 2 comments:_____

Kidney:
N S M G U OT (other):_____

Parasites:
0 1 2 3 comments:_____

General Comments: _____
_____
_____
_____
_____

**BIOMARKER DATA** Project No._____ CUTTER:_____ (Full name)

Waterbody: _____ Site: _____ Species: _____

Biomarker Fish #: _____ Fisheries Inventory #:_____

Fork length (mm): _____ Total length (mm):_____ Total Weight (g): _____

Carcass Weight (g) _____ Sex:_____ Maturity:_____

Capture Time: Date _____ Hr._____ Capture Method: _____

Sacrifice Time: Date_____ Hr._____ Sacrifice Method: _____

**COLLECTION DETAILS:**

STOMACH:
Observed: _____
Collected: _____
% Fullness: _____
Description/%of Contents: _____
Other Observations: _____

HISTOLOGY EXAMINATION:

| | Abnormality | Preserved (g) |
|---|---|---|
| Liver | _____ | _____ |
| Spleen | _____ | _____ |
| Heart | _____ | _____ |
| Gill | _____ | _____ |
| Kidney | _____ | _____ |
| Other | _____ | _____ |

BLOOD:
Time Taken _____
Total Volume: _____ mL
Time of Centrifugation: _____
Plasma Volume: _____ mL
Plasma Colour: _____
Hematocrit: _____ %
_____ %
Leucocrit: _____ %
_____ %
Plasma Protein: _____ g/dL

LIVER:
MFO / MT: _____ g
Contaminants: _____ g
Histology: _____ g
Archive: _____ g
Total Liver Weight: _____ g
Time MFO Sample Taken: _____

KIDNEY:
MT: _____ g
Contaminants: _____ g
Histology: _____ g
Total Kidney Weight: _____ g

FILLETS:
Organics: _____ g
Metals: _____ g
Other: _____ g

BILE:
Gall Bladder Fullness (%): _____
Colour: _____
Volume Collected: _____ mL

GONADS:
Contaminants: _____ g
Histology: _____ g
Region Collected From: _____

Fecundity Subsample Weight: _____ g
Region Collected From: _____
No. of Eggs in Subsample: _____

Ave. Egg Diameter: ____ mm
No. of Eggs Measured: _____

Total Gonad Weight _____ g

Recorder (Full Name)_____ _____

| VARIABLE | VARIABLE CONDITION | ORIGINAL FIELD DESIGNATION |
|---|---|---|
| Eyes | No aberrations; good “clear” eye | N |
| | Blind; an opaque eye (one or both) | B |
| | Swollen, protruding eye (one or both) | E |
| | Hemorrhaging or bleeding in the eye (one or both) | H |
| | Missing one or both eyes | M |
| | Other; any manifestation not fitting the above | OT |
| Gills | Normal; no apparent aberrations | N |
| | Frayed; erosion of tips of gill lamellae resulting in “ragged” gills | F |
| | Clubbed; swelling of the tips of the gill lamellae | C |
| | Marginate; gills with light, discolored margin along tips of the lamellae | M |
| | Pale; very light in color | P |
| | Other; any observations not fitting above | OT |
| Pseudobranchs | Normal; flat, containing no aberrations | N |
| | Swollen; convex in aspect | S |
| | Lithic; mineral deposits, white, somewhat amorphous spots | L |
| | Inflamed; redness, hemorrhage, or other | I |
| | Other; any condition not covered above | OT |
| Thymus | No hemorrhage | 0 |
| | Mild hemorrhage | 1 |
| | Moderate hemorrhage | 2 |
| | Severe hemorrhage | 3 |
| Skin | Normal; no aberrations | 0 |
| | Mild skin aberrations | 1 |
| | Moderate skin aberrations | 2 |
| | Severe skin aberrations | 3 |
| Fins | No active erosion | 0 |
| | Light active erosion | 1 |
| | Moderate active erosion with some hemorrhaging | 2 |
| | Severe active erosion with hemorrhaging | 3 |
| Opercle | No shortening | 0 |
| | Mild shortening | 1 |
| | Severe shortening | 2 |
| Hindgut | Normal; no inflammation or reddening | 0 |
| | Slight inflammation or reddening | 1 |
| | Moderate inflammation or reddening | 2 |
| | Severe inflammation or reddening | 3 |
| Mesentaric Fat | None | 0 |
| | < 50 % | 1 |
| | 50 % | 2 |
| | > 50 % | 3 |
| | 100 % | 4 |
| Liver | Normal; solid red or light red color | A |
| | “Fatty” liver; “coffee with cream” color | C |
| | Nodules in the liver; cysts or nodules | D |
| | Focal discoloration; distinct localized color changes | E |
| | General discoloration; color change in whole liver | F |
| | Other; deviation in liver not fitting other categories | OT |
| Spleen | Normal; black, very dark red, or red | B |
| | Granular; rough appearance of spleen | G |
| | Nodular; containing fistulas or nodules of varying sizes | D |
| | Enlarged; noticeable enlarged | E |
| | Other; gross aberrations not fitting above categories | OT |
| Gall Bladder | Normal | 0 |
| | Enlarged | 1 |
| | Parasites | 2 |
| Kidney | Normal; firm dark red color, lying relatively flat along the length of the vertebral column | N |
| | Swollen; enlarged or swollen wholly or in part | S |
| | Mottled; gray discoloration | M |
| | Granular; granular appearance and texture | G |
| | Urolithiasis or nephrocalcinosis; white or cream-colored mineral material in kidney tubules | U |
| | Other; any aberrations not fitting previous categories | OT |
| Parasites | No observed parasites | 0 |
| | Few observed parasites | 1 |
| | Moderate parasite infestation | 2 |
| | Numerous parasites | 3 |

| Maturity Codes: | IM = Immature MA = Maturing SD = Seasonal Development PR = Pre spawning<br>RP = Ripe SP = Spent RS = Resting RB = Reabsorping UN = Unknown |
|---|---|

**GOLDER ASSOCIATES LTD.**
**CHAIN-OF-CUSTODY RECORD**
**AND ANALYTICAL REQUEST FORM**

Page ____ of ____

Field Sampler: (Signature) _____

Phone No.: _____

Shipment Date: _____

Carrier: _____

Waybill No.: _____

Ship To:

Send Results To:

Project Name: _____

Project No: _____

P.O. No.: _____

| | | | |
|---|---|---|---|
| Relinquished by: (Signature) | Received by: (Signature) | Date: | Time: |
| Relinquished by: (Signature) | Received at lab by: (Signature) | Date: | Time: |
| Relinquished by: (Signature) | Received at lab by: (Signature) | Date: | Time: |
| Relinquished from lab by: (Signature) | Received by: (Signature) | Date: | Time: |

ANALYSIS REQUEST

| Sample ID No. | Sample Description | Date/Time Sampled | Analysis Requested | Sample Condition Upon Receipt |
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Special Instructions/Comments:

Rush (surcharge): _____ Standard Turnaround Time: _____

WHITE COPY — RETURN TO GOLDER ASSOCIATES LTD.
YELLOW COPY — LABORATORY COPY
PINK COPY — RETAINED BY FIELD CREW LEADER

**GOLDER ASSOCIATES LTD.**
**CHAIN-OF-CUSTODY RECORD**
**AND ANALYTICAL REQUEST FORM**

Page ____ of ____

Field Sampler: (Signature) _____

Phone No.: _____

Shipment Date: _____

Carrier: _____

Waybill No.: _____

| Sample ID No. | Sample Description | Date/Time Sampled | Analysis Requested | Sample Condition Upon Receipt |
|---|---|---|---|---|
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Special Instructions/Comments:

Rush (surcharge): _____ Standard Turnaround Time: _____

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# APPENDIX F

# GOLDER ASSOCIATES' TECHNICAL PROCEDURE 8.19-0
# LAKE HABITAT MAPPING

# 1 PURPOSE

This technical procedure presents the standard Golder Associates method for describing and mapping features of lakes for the purpose of identifying and classifying habitat for fish, for determining the ability of the lake to support fish, or for determining the suitability of the lake for one or more specific fish species.

# 2 APPLICABILITY

This technical procedure applies to all personnel involved with habitat mapping waterbodies. Methods described within are relevant for the basin-wide assessment of small or shallow lakes/ponds and the assessment of shoreline and shoal habitats in large/deep lakes. These techniques are valid for lakes throughout North America, including all geographical regions, and are applicable regardless of the size of the waterbody.

# 3 DEFINITIONS

## 3.1 Cover

Refers to cover for fish species. Cover in lakes refers to physical features which provide visual isolation or shading for fish. Examples of cover features include large substrate particles such as boulders, submerged or floating logs and debris piles. (Aquatic macrophytes also provide cover but are evaluated separately as aquatic vegetation.)

## 3.2 Drop-off

The place at which the lakebed exhibits a dramatic change in bottom slope, observable as a rapid increase in depth; occurs moving away from shore or shoal areas. For clear-water lakes, the drop-off will typically mark the boundary between where the bottom is visible (i.e. where substrate and vegetation features can be mapped) and water that is too deep for the bottom to be seen.

## 3.3 Embeddedness

Refers to the degree to which rocky substrates (gravel/cobble/boulder) on the lakebed are covered with fines. Fines are fine sediments <2 mm diameter and include clay, silt and sand particles. Covering of the rocky substrates and filling of the interstitial spaces between the substrate particles can potentially affect the suitability of the area as spawning habitat for some fish species.

## 3.4 Habitat Transect

A method for conducting detailed investigations of lake habitats designed to provide quantitative data in support of the more general habitat mapping process. The method effectively describes the depth profile (slope), percentage composition of substrate materials, availability of cover, and vegetation

characteristics along a line perpendicular to the shoreline. In shallow lakes, the habitat transect may bisect the entire lake, while in deep lakes the transect may end at the drop-off. The transect may be conducted with particular emphasis on the habitat needs of particular fish species.

### 3.5 Inlet/Outlet

Respectively, a location or locations where water enters or leaves a lake. Typically these will be inflow or outflow channels (creeks or rivers) which may have significant habitat features or be of significant importance to fish populations in the lake.

### 3.6 Lake

Any body of water with definable boundaries that does not exhibit measurable current, so as to be differentiated from pools or flat areas in streams and rivers. Deepest areas tend be centrally located, however some lakes have very uneven bottoms and may not follow this trend. In this document, the term 'lake' is interchangeable with the term 'waterbody' and is not dependent on the size of the waterbody.

### 3.7 Lake Habitat Transect Form

A field sheet specifically designed for recording information about lake habitat that is used during all habitat transect evaluations.

### 3.8 Limnetic Zone

The open-water region of a lake that is too deep to support rooted aquatic vegetation.

### 3.9 Littoral Zone

The shallow area of a lake that extends from the waterline to the lakeward limit of rooted aquatic vegetation.

### 3.10 Macrophytes

Macrophytes are aquatic plants which are large enough to be seen with the unaided eye, as opposed to phytoplanktonic organisms. Macrophytes are specifically adapted to aquatic habitats, growing either in the water or along the margins of waterbodies. Flooded terrestrial vegetation does not qualify. There are two basic types of macrophytes which are distinguished when habitat mapping lakes; emergent and submergent macrophytes.

*Emergent Macrophytes*

Macrophytes which grow in wet or boggy areas or which are rooted in water but extend above the water surface. Typical examples include Cattails, Bulrushes and Sedges.

*Submergent Macrophytes*

Macrophytes which are rooted and which grow under the water. This includes floating-leaved aquatic plants and plants which may have flower stalks that extend above the surface. Typical examples include pondweeds, Coontail and water lily.

### 3.11 Pinnacle

A small, isolated point of lake bottom which is exposed above the water line and surrounded by a shoal. Can be thought of as a very small island or a shoal which has a small portion above water.

### 3.12 Shelf

Frequently observed in deep lakes; it is the area of the bottom located near shore, between the waterline and the drop-off. The distance between these points can be highly variable. In areas of shallow bottom slope, the shelf may be extensive, whereas, in areas of steep bottom slope the shelf may not exist. In deep, clear lakes, the shelf is typically the primary area that is habitat mapped since the bottom is visible and characteristics of substrate, cover and vegetation can be recorded.

### 3.13 Shoal

A shallow but submerged area isolated from the shorelines of a lake. Can be composed of a variety of materials depending upon regional geology. May be "clean" (free of fines) if shallow enough to be affected by wave action and may have a significant potential to provide spawning habitat. Therefore, shoal areas in large, deep lakes receive particular attention during habitat mapping.

### 3.14 Slope

The change in vertical height relative to the change in horizontal distance. For lakes, this measurement describes the steepness of the shoreline above and below the water surface.

*Bottom Slope*

The slope of the lakebed on a line perpendicular to shore, from the waterline to the lakeward side.

*Shore Slope*

The slope of the lake shore immediately above the waterline.

### 3.15 Substrate

The material which comprises the bottom of a water body. Can be highly variable depending on regional geology, weathering, location within the lake, etc. Described by substrate particle size. Particle sizes

include fines (clay, silt and sand), gravel, cobble, boulder and bedrock. Golder employs specific criteria for particle size classes which are defined in this document.

### 3.16 Transect

A line of travel (usually a straight line) across or within a lake that is either boated or waded that allows particular parameters to be measured at various points along the distance travelled. Parameters generally include depth, substrate type, cover availability and/or aquatic vegetation.

## 4 REFERENCES AND SUGGESTED READING

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## 5 DISCUSSION

### 5.1 General Safety

Refer to Golder Associates Ltd. Health and Safety Manual.

## 5.2 Methods

### 5.2.1 Study Area/Site Selection

The study area should be firmly defined before beginning field work. For smaller lakes, this will include the number and location of waterbodies to be examined. For large lakes, it may be necessary to subsample a portion of the waterbody; determine the amount of shoreline (length) that will be examined and have an idea of the number and size of shoals that will be visited. Shoals may also need to be subsampled, depending the number present. The amount of time necessary to complete the habitat survey will dictate the area to be covered. The study area should be specified by the project manager in the Specific Work Instructions for the project.

### 5.2.2 Creation of Habitat Base Maps (lake outline maps)

Maps of the lake or lakes in the study area should be produced and copied to waterproof paper, preferably at the 1:10,000 or 1:15,000 scale. The map should include an outline of the lake shoreline and any islands or pinnacles in the lake. This can be accomplished by enlarging topographical maps or air photos using a photocopier and then tracing the enlarged waterbody onto waterproof paper. Be sure to enlarge the map scale along with the map as **the exact scale of the habitat map must be known in order for area or shoreline length calculations to be conducted.** Ideally, maps should show only a single line representing the shoreline of the lake. This will allow markings to be made on field maps that represent habitat features both above and below the waterline. If available, a digital mapsheet of the area can be produced showing the required area using Autocad software. For large areas, several smaller maps can be created at a desired scale from the larger digital map (usually 1:250,000 scale). **Note:** The tracing method is usually more accurate than using digital maps for features such as small lakes (<2 km across).

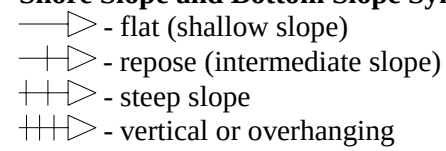
On the habitat base map, include the locations of all inlets and outlets and any man-made structures which would affect lake habitat or potential fish use of any area in the lake.

Shallow areas (**shoals**) located away from shorelines of lakes provide excellent spawning habitat for many species of fish. Maps depicting shoals are easily produced with the aid of a bathymetric map showing depth contours. With this information, shallow areas can be identified for crews to visit and perform necessary shoal habitat assessments. If bathymetric maps are not available, areas that are markedly shallower than the rest of the lake can be located using a depth sounder. However, this method is only efficient for small lakes or portions of larger ones. Airphoto analysis may also be a useful method for detecting shoal areas in lakes. Shallow areas are sometimes identifiable (depending upon water clarity when the photo was taken) and locations can be noted for visitation later by determining accurate UTM coordinates.

### 5.2.3 Required Habitat Mapping Symbols/Abbreviations

The following symbols/abbreviations should be used to indicate the particulars of lake habitats during the habitat mapping process:

**Substrate Particle Size Classes**
Si - silt/clay (<0.06 mm in diameter)
Sa - sand (0.06-2.0 mm)
Gr - gravel (2-64 mm)
Co - cobble (64-256 mm)
Bo - boulder (>256 mm)
Bd - bedrock

**Shore Slope and Bottom Slope Symbols**

- flat (shallow slope)
- repose (intermediate slope)
- steep slope
- vertical or overhanging

**Shore Vegetation Types**
BA - bare
OT - open tundra
MU - muskeg/bog
GF - grass/forbes
SH - shrub
DF - deciduous forest
CF - coniferous forest
MW - mixed wood forest

**Aquatic Macrophyte Abbreviation**
Sm - submergents (provide *Genera*)
Em - emergents (provide *Genera*)

**Shore Instability Ratings**
Aggrading
Eroding
Slumping
Gullying

### 5.2.4 Habitat Assessment and Mapping Methods

#### 5.2.4.1 General Considerations

**NOTE:** It is critical that habitat maps prepared in the field are legible. Use of good waterproof paper and a sharp pencil will help this cause. The large majority of field maps are given to the drafting department for digitization using AutoCad or GIS software. The resulting maps are eventually included in reports for presentation to our clients. **Therefore, legibility is of utmost priority.**

**Label the habitat map** with the following:

- Project Number
- Date
- Personnel
- Waterbody Name or Identifier
- Location (if mapping a subsample area of a large lake)
- North Arrow
- Map Scale (must be accurate)
- Lake Water Level (if known)

**During the habitat mapping process, the following information is to be recorded on the habitat map**.

- Shore vegetation type, soil type, and stability
- Shore slope
- Bottom slope

- Bottom substrate characteristics
- Distribution of macrophytes
- General cover features
- Depth at drop-off (deep lakes)

The process for conducting the habitat mapping is somewhat different for small, shallow waterbodies versus large, deep lakes. Shallow lakes are waterbodies in which the entire lake bottom, or a significant portion of it, is visible, allowing substrate, cover and vegetation to be mapped throughout much of the basin. For deep lakes, these characteristics can only be mapped for shoreline, shelf and shoal areas.

General habitat mapping procedures include recording the height of high water marks above the current waterline, and taking some representative pictures of all habitat types encountered.

Whenever fisheries inventory sampling is conducted in conjunction with the habitat mapping procedure, it is best to complete the lake habitat map prior to sampling, if possible. During the fisheries inventory, sampling effort and catch results should be recorded by habitat type, as per the habitat map. Specific effort should be made to sample each habitat type present in the lake to determine how the habitats present effect fish distribution and abundance and to determine habitat use by life stage (i.e. spawning, nursery, rearing and adult feeding areas).

#### 5.2.4.2 Shore Vegetation Type, Soil Type and Stability

Record the shore vegetation present above the waterline using the categories presented in Section 5.2.3. This should include the dominant vegetation at the margin of the waterbody which would potentially affect cover, shading, surface run-off and allochthonous inputs. For small waterbodies the entire circumference of the lake should be mapped, while large lakes may require the mapping of representative sections only. For each shore vegetation type, the boundaries should be delineated using lines drawn from the waterline into the uplands, perpendicular to shore. Within these boundaries, record the *shore vegetation type symbol*.

Also record on the map any additional information for the area above the waterline which could affect land stability and the nature of runoff from the area. This will include soil type (use *substrate particle size symbols*) and any evidence of instability (use *shore instability ratings*). All shore areas are assumed to be stable unless an instability rating is applied. **IMPORTANT: for all inlet and outlet streams, record whether they are flowing or non-flowing.**

#### 5.2.4.3 Shore Slope

Record the slope of the shore immediately above the waterline. Record shore slope by drawing the appropriate *slope symbol* on the upland side of the waterline, perpendicular to shore. Slope symbols should be draw around the entire circumference of the lake or for the subsampled sections, depending on lake size.

#### 5.2.4.4 Bottom Slope

Record the general slope of the lake bottom on a line perpendicular to shore and extending from the waterline out into the lake. For deep lakes the bottom slope is usually recorded for the area extending from waterline to the drop-off (i.e. the slope of the shelf area). If the bottom is not visible due to turbid water, a sounding line will help to describe bottom slope. Record shore slope by drawing the appropriate *slope symbol* on the lake side of the waterline, perpendicular to shore. Slope symbols should be draw around the entire circumference of the lake or for the subsampled sections, depending on lake size

#### 5.2.4.5 Bottom Substrate Characteristics

Draw in the boundaries between sections of the lake bottom with different substrate types and, for each section, record the *dominant and co-dominant* substrate particle sizes using the *substrate particle size symbols*. Substrate size assessment is conducted by visual estimation of the percent occurrence of each particle size class, when possible. If the water is too turbid record what habitat characteristics are visible at the waterline. Record substrate sizes in order of dominance, most abundant size first, and use a slash to separate them (e.g. an area that is 70% boulder and 30% silt is recorded as Bo/Si). For turbid water where the bottom cannot be seen, a sounding line or pole should be used to get a general idea of substrate type (i.e. is it silt, small rock or boulder). In small shallow lakes, substrate symbols should be recorded for the entire lake basin. For large deep lakes, bottom substrate should be evaluated for the shelf area only.

#### 5.2.4.6 Distribution of Macrophytes

Draw in the boundaries of all areas containing aquatic macrophytes, using separate boundaries for submergents and emergents and label each area with the appropriate *aquatic macrophyte abbreviation*. Within the boundaries, identify the dominant and subdominant *Genera*, either by scientific or common name, using a plant key if required. For some projects designed to map macrophytes, species names may be required. In small shallow lakes, macrophytes should be mapped for the entire shoreline and lake basin. For large deep lakes, macrophytes should be mapped in the shoreline and littoral areas where they occur.

#### 5.2.4.7 General Cover Features

The location and extent of any feature which provides cover for fish should be included on the habitat map. Draw in the feature and provide an identification label (e.g. submerged logs, stumps, overhangs, etc.). If cover is provided by a feature already included on the habitat map such as substrate (boulders) or vegetation, include a general rating of the quality of the cover provided (poor, moderate, good, excellent).

#### 5.2.4.8 Depth at Drop Off

For deep lakes, draw a line on the habitat map which approximates the location of the drop-off. This should produce a line which runs around the lake, inside the waterline and roughly parallel to the shoreline. This line defines the extent of the shelf and will be close to shore in areas of steep bottom slope and further from shore in areas of shallow bottom slope. Use a sounding line to measure the depth at the drop-off, which represents the maximum depth to which bottom features will have been mapped.

### 5.2.5 Habitat Assessment Methods – Shoals

Once shoals have been located using bathymetry mapping or airphoto analysis, shoals should be visited and a habitat assessment procedure be conducted. To assess the fisheries habitat provided by shoals, the following steps should be taken:

1. Begin by slowly cruising around the area to determine the extent of the shoal. Quality of fish habitat can vary significantly depending upon the side of the shoal you are on.

2. Locate the edge of the shoal and determine the depth using a sounding line.

3. Move to the middle of the shoal (the shallowest point) and again measure the depth.

4. Make an estimate of the slope of the shoal (0 - 90°).

5. Record information about the substrate including the following:
   - substrate particle size - report actual estimated % for each size class rather than simply by dominant/subdominant classes
   - number of interstitial spaces (none, few, moderate, many)
   - embeddedness (cleanliness) - amount of fines (clay, silt or sand) lying on rock material
   - aspect relative to prevailing wind/wave action
   - cover by macrophytes

The location and estimated size of each shoal should be recorded on the habitat map and all information regarding shoals should be written in an appropriate field book and should include shoal number and GPS location. Shoal sites should be marked on a topographical map to aid in finding the site if spawning surveys are planned for a later date.

### 5.2.6 Habitat Transect Methods

In general, habitat transects are conducted to provide quantitative data from representative habitat types for lakes that have been habitat mapped. They provide measurements or more detailed estimates of the variables recorded during the habitat mapping process and serve to better describe each habitat type. Transects are not required for all projects; for some jobs habitat mapping alone is sufficient. The project manager will determine if transects should be conducted in addition to habitat mapping.

Habitat transects should be conducted in each of the habitat types available in the lake, as identified on the habitat map, in order to describe conditions in each habitat type. If numerous transects are to be conducted, the proportion of transects conducted in each habitat type should be equal to the proportion of occurrence of that habitat in the lake as a whole. Further, transects should be conducted in the middle of a habitat type to avoid any edge effects from adjacent types. ‘Habitat types’ are not specifically defined and may vary for each lake. In general, transects are conducted in different types of habitats such as; shallow versus steeply sloped bottoms, rocky versus silty areas, macrophyte beds versus bare areas, etc.

The results of the transect surveys should be recorded in the field note book and on **Lake Habitat Transect Data Forms** (see Exhibit A). Each transect should be numbered for identification and the locations should be marked on the habitat map. It may be appropriate to record the endpoints of the transect using GPS technology.

**Habitat transects should be performed by following these steps:**

- Establish a straight line transect perpendicular to the shoreline using a tape measure and record the transect total length (m), using the shoreline as the zero point. In deep lakes, transects should extend from shoreline to the drop-off. In shallow lakes, transects should be long enough to provide a good description of the habitat.

- Along the transect for a width of 2.0 m (1 m on either side of the line), make a detailed account of the substrates present. Divide the transect length into sections of uniform substrate composition and **for each section** record the start and end point on the transect length (m), provide a visual estimate of the % occurrence of each substrate particle size class, and record the % of macrophyte cover. Also record the degree of embeddedness, which is the degree to which fine sediments (i.e. clay, silt or sand particles < 2 mm) cover rocky substrates and fill interstitial spaces. Record as *clean (i.e. no fines)*, *low*, *moderate*, or *high*.

- Produce a depth profile of the lake bottom by measuring water depth at stations along the length of the transect. Provide the bottom slope category, as used on the habitat map, and calculate the actual bottom gradient as follows:

$$\% \text{ Gradient} = ([\text{Depth}_{(\text{transect end})} - \text{Depth}_{(\text{transect start})}] / \text{Transect Length}) \times 100$$

- Record the percentage, by length, of the transect with emergent and submergent macrophyte cover and the plant species present.

- Record the degree of algae growth on the substrates.

- Provide a rating of the average cover availability for the transect, from poor to excellent.

- Provide the shore slope category, as used on the habitat map, and measure the actual shore gradient using as clinometer.

- Record the average substrate characteristics for the shore area by visual estimation of the percent composition of the substrate particle sizes.

- Provide the shoreline vegetation type, as used on the habitat map, and provide details of the vegetation composition. Provide a visual estimate of the percent occurrence of each category on the transect data form and record the dominant species present in the shrub and tree canopies.

- Provide the bank stability rating, as recorded on the habitat map.

- Record specifics of any inlet or outlet areas present, as per the transect data sheet.

### 5.2.7 Data Analysis and Post-Field Activities

Data should be reviewed at the end of every day. Insure that all data has been properly recorded and that all the required data is present (GPS readings are the most commonly forgotten item). Fill out note books according to the protocols established by the QA officer (if your not sure, have you field notes verified by a crew leader). Once maps have been filled out, they must not be taken back into the field. If a large map is only partially complete at the end of a day, every effort should be made to make a photocopy of the map before taking it back out the next day to complete the area.

Calculations that can be made from the habitat map relate to determining the proportions of available habitat types. This is done as percentage of shoreline length for large/deep waterbodies for which only shoreline areas could be mapped or as percentage of lake area for small/shallow waterbodies where the entire lake basin was mapped. Again, 'habitat types' are not defined units. Rather, each lake will typically have a few identifiable habitat types (combinations of shore and bottom types) which are repeated throughout the lake and which can be used as the basis for the analysis of habitat composition. Alternatively, percentage of occurrence can be calculated for different parameters; such as shore areas by vegetation type, bottom areas by slope or substrate type, etc.

## 6 EQUIPMENT AND MATERIALS

### 6.1 Sampling Equipment

Necessary equipment may include the following:

- habitat base map(s) photocopied onto waterproof paper (clear plastic waterproof bag - optional)
- mechanical pencils/eraser
- 50 or 100 metre measuring tape or tagline
- graduated pole (2.0 metres or longer)
- sounding line
- clinometer
- chart recording sonar (optional)
- camera/film (disposable box cameras may be appropriate)
- lake habitat mapping technical procedure
- Shoreline Habitat Transect Forms (if required)

### 6.2 Health and Safety Equipment

Health and safety equipment when conducting habitat mapping by boat should include:

- lifejackets (one for each person in the crew)
- chest waders with wader belt
- two-way radio
- GPS/topographical maps and compass
- first-aid kit
- survival kit
- appropriate clothing and gear
- sunglasses, sunscreen, bug spray

### 6.3 Boat and Associated Equipment

The following equipment should be included with each boat working in the field:

- boat and motor
- oars/paddles in good condition
- extra fuel (ensure fuel/oil mixture is correct)
- engine tools (spark plug wrench and spare spark plug (keep dry!), spare propeller and pull cord, appropriate screwdrivers)
- zodiac rubber patch kit (if relevant)
- anchor and line (line length should be appropriate for water depths in the region)
- mechanical bailer, horn

## EXHIBIT A

## Lake Habitat Transect Data Form

**Project #:**_____ **Date (d/m/y):**_____ **Personnel:**_____

**Lake:**_____ **Sampling Area:**_____ **Transect Number:**_____

**Length of Transect:**____**m** **GPS Coordinates: Start**_____ **End**_____

| **Substrates** | | | | | | | | | | **Depth Profile** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Distance (m)** | | **% Composition by Size Class (mm)** | | | | | | | | | |
| **Start** | **End** | Si/Cl | Sand | Gravel | Cobble | Boulder | Bedrock | Deg. of Emb. * | Macr. Cover (%) | Distance (m) | Depth (m) |
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*Embeddedness Categories: Clean, Low, Moderate, High

**Shore Characteristics (above waterline)**

Shore Slope: Flat___ Repose___ Steep___ Vertical___ Overhanging___ Shore Gradient (%): _____

Shoreline Particle Size (%): Organic Soil___ Silt/clay___ Sand ___ Gravel___ Cobble___ Boulder___ Bedrock___

Shoreline Vegetation Type: _____

Shoreline Vegetation (%): Bare___ Open Tundra___ Muskeg___ Grass/forbes___ Shrub (species)_____

Tree: Deciduous (species)_____ Conifer (species)_____

Bank Stability: Stable___ Aggrading___ Eroding___ Slumping___ Gullying___

Inlet Present: Yes___ No___ Size of Stream (width m): ____ Flowing: Yes___ No___

Height of High Watermark above waterline:_____ m

**Lakebed Characteristics (below waterline)**

Bottom Slope: Flat___ Repose___ Steep___ Vertical___ Overhanging___ Bottom Gradient (%): _____

% of Transect length with Emergent Macrophyte Cover:____ Species (% Occurrence):_____

% of Transect length with Submergent Macrophyte Cover:____ Species (% Occurrence):_____

% of Substrates Covered by Algae:_____% Mean Thickness of Algae Cover: Thin ___ Moderate ___ Thick ___

Number of Interstitial Spaces: None ___ Few ___ Moderate ___ Many ___

Cover Availability Rating: Poor ___ Moderate ___ Good ___ Excellent ___

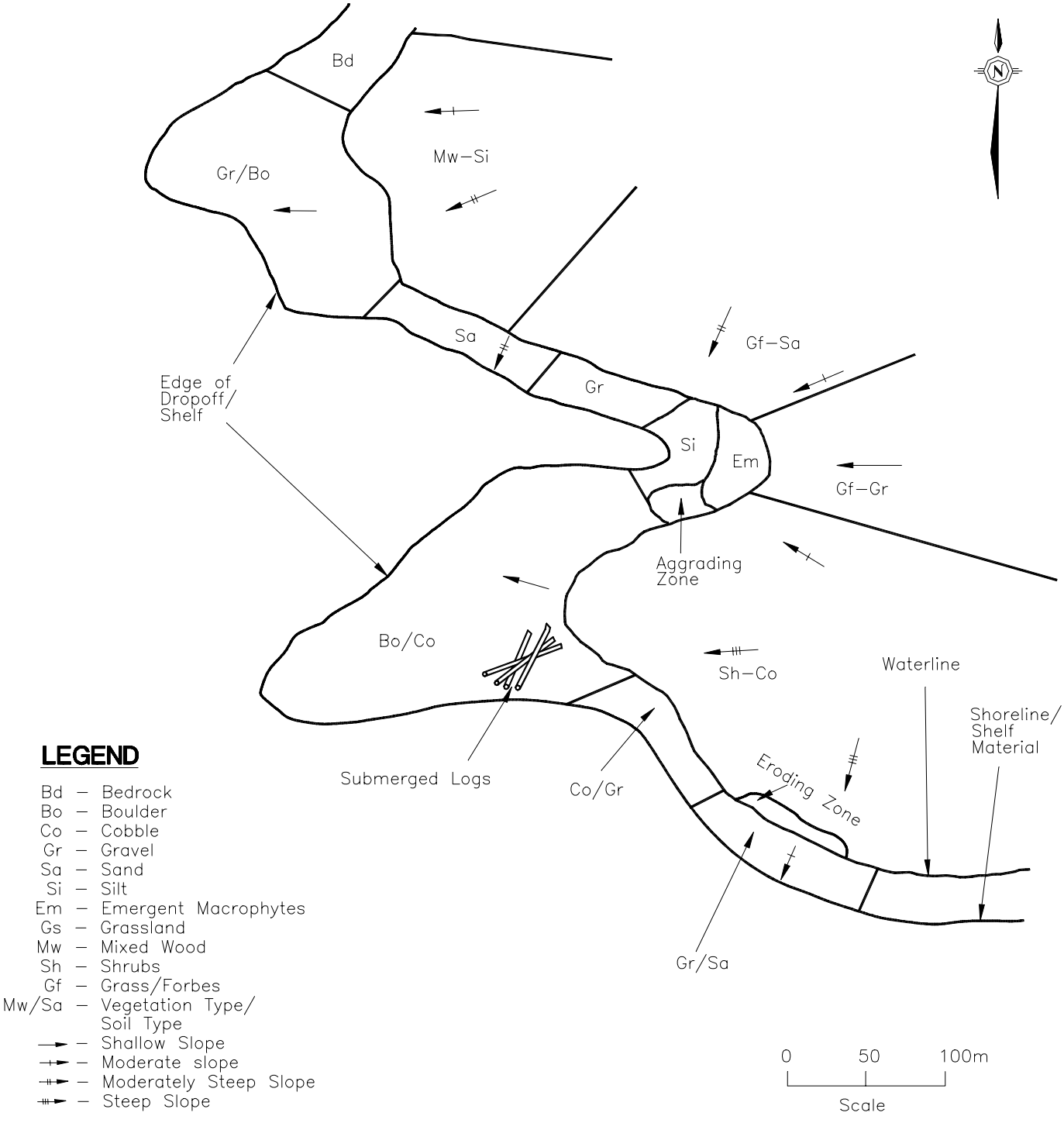
Other Habitat Observations:_____

_____

_____

**EXHIBIT B**

**Sample Shoreline Habitat Map**




**LEGEND**

Bd – Bedrock
Bo – Boulder
Co – Cobble
Gr – Gravel
Sa – Sand
Si – Silt
Em – Emergent Macrophytes
Gs – Grassland
Mw – Mixed Wood
Sh – Shrubs
Gf – Grass/Forbes
Mw/Sa – Vegetation Type/ Soil Type
→ – Shallow Slope
→ – Moderate slope
→ – Moderately Steep Slope
→ – Steep Slope