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Methods Manual IV: Sediment toxicity testing, field and laboratory methods and data management

Prepared by:

Trefor B. Reynoldson, Craig Logan, Danielle Milani, Timothy Pascoe and Sherri P. Thompson

Aquatic Ecosystem Restoration Branch

National Water Research Institute

Environment Canada

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Introduction

Over the past ten years the National Water Research Institute has conducted major programmes in both the Great Lakes and Fraser River as part of a programme to establish a national reference database on benthic invertebrates for Canada. A critical part of this programme is the establishment of a standard set of protocols and methods for all phases of data collection and processing. This document attempts to provide that written record of the methods being used at the institute.

There are three components to the database. Data sets describing the invertebrate fauna, data sets describing the toxicity of sediments based on four invertebrate tests and data sets describing the environmental attributes of sites. In this protocols document we have described the methods being used in a sequential manner. The first section deals with field procedures, collection of both biological and environmental data, in both lentic and lotic habitats. The second section addresses the laboratory procedures regarding sample handling and sample processing for biological samples. The third addresses the issue of data management. In addition we have provided a set of tables and forms used by the programme.

We hope that this will provide a useful resource for others involved in this type of work.

Field Procedures

Estimation of functional response of benthic invertebrates to contaminants associated with fine grained sediment requires acquiring both samples of the material for testing in the laboratory and associated information on the local environment.

Sampling

In studying benthic communities from lentic environments measurements of both water and sediment are important. In many studies initial data can often be overlooked or forgotten but proves to be critical at a later date. When several procedures are required in the field and several different samples are being taken, things can accidentally be overlooked. Once critical parameters are determined, creation of a field data sheet will help to make sure these parameters are measured and properly recorded.

A copy of the NWRI lentic field sheet is included to illustrate these points (Appendix A).

Water column sampling

Using a Van dorn sampler a water sample is obtained ½ meter from the bottom and the following samples are taken:

i. 125 mls for nutrients: measured are nitrates/nitrites and total Kjeldahl nitrogen

ii. 125 mls for total unfiltered phosphorus preserved with 1 ml of 30 % sulfuric acid. This can be added to the bottle before going into the field.

iii. 125 mls for alkalinity

iv. a sample for pH and temperature measured immediately in the field

v. a sample for Dissolved oxygen measured immediately in the field

The samples are obtained and stored according the NLET procedures. This involves keeping the samples cool until submitted for analysis. When taking a sample this close to the bottom it is very important that the depth is known. Water samples that are extremely silty should be discarded and retaken, as the Van dorn was probably dropped onto the surface, disturbing the sediment. Proper, legible labels, with the appropriate Site Code marked using a water- and solvent-proof marker make sample identification easier later.

Bio-Assay Sediment Collection

Often bioassays are to be conducted in conjunction with benthic community assessments. Five field replicates are obtained using a miniponar. Two liters of sediment is required per bioassay. Each full miniponar contains approximately 2 liters of sediment and is placed in separate bags and sealed. If a full miniponar is not obtained then a second one should be taken and added to the first.

Sample Handling

The five bags are placed in a labeled 10 liter bucket and sealed. Each container must be marked with proper, legible labels, with the appropriate Site Code and replicate number marked using a water- and solvent-proof marker. Samples should be kept cool until returned to the lab.

Sediment Sampling

In assessing sediment toxicity or sampling for the benthic community, sediment parameters play a key role and must be looked at.

If samples are taken for invertebrate community structure using the methods described in Reynoldson et al 1988, then the methods described there can be used to obtain samples for sediment analyses. Otherwise and additnal mini-ponar sample is suggested and the following steps are taken:

i. the sample of sediment is placed in a glass dish. Approximately 1 liter of sediment should be removed.

ii. the 1L of sediment is homogenized.

> **Note:** The type of equipment used to stir the sediment should be appropriate for the analysis, i.e. metals analysis requires a non-metal stirring apparatus.

iii. a 125 ml sample is removed for organic analysis. This sample is placed in a hexane rinsed glass bottle and covered with a hexane rinsed piece of tin foil before the lid is placed on.

Note: Remember to use tweezers to handle the tin foil.

iv. a 100 ml sample is removed for particle size

v. a 500 ml sample is removed for chemical analysis.

Sample Handling

Samples should be kept cool until returned to the lab for analysis. The sample taken for chemical analysis are tested for the following parameters:

major ions	metals
loss on ignition	total organic carbon
total Kjeldahl nitrogen	total phosphorus

added in 1:2 ratio water to formalin.

Laboratory Procedures for Sample Management and Analysis

Just as the sampling process for any study is critical to the success of the research, so is the management and analysis of those samples once they arrive at the laboratory from the field. A carefully structured framework for managing both the samples themselves, as well as data generated from their analysis is required. Without such a framework, samples can be lost or unanalyzed and results can become misplaced or misconstrued. By identifying specific responsibilities for each individual involved in a project, significant reductions in wasted time and resources can be achieved.

NWRI has established guidelines for sample and data management. These guidelines are broken down into sections, each identifying the proper procedure(s) and personnel responsible at each stage.

Project Initialization

Upon the initialization of a new project, a hard-copy Project Folder is started by the Senior Community Structure Lab (SCSL) technician (Craig Logan). The Project Folder acts as a data warehouse for raw field sheets and any other hard-copy data generated during a project, until its entry into the electronic data base (see Laboratory Protocols for Data Management). Individuals responsible for specific results must ensure that their data sheets are placed in the Project Folder once analysis has been completed. Project Folders are stored in the Benthic Ecology laboratory (L572).

Arrival of Samples

Delivery

When project samples collected in the field arrive at CCIW, they are delivered to the Benthic Ecology laboratory (L572). The SCSL technician is responsible for the initial inspection and subsequent re-distribution of samples to their appropriate laboratories for analysis. The inspection of samples includes:

i. entering each sample into the Project Folder log.

ii. checking against the project field sheets to ensure samples from all sampling locations are accounted for.

iii. identifying any samples which have been damaged/lost during transport. Damaged/lost samples are noted, and a comment must be placed in the Comments section of the benthic data information system (see Laboratory Protocols for Data Management).

iv. separating samples into their specific components for delivery to appropriate laboratories for analysis.

Distribution

Sample components are sent to the following laboratories for analysis:

i. ***Bio-assay Sediment:*** sent to the Bio-Assay lab (W234) for storage and processing by Senior Toxicology technician (Danielle Milani).

ii. ***Sediment and Water Chemistry Samples:*** sent to the Senior Project Technologist(Sherri Thompson) for storage and subsequent delivery to appropriate laboratories for processing.

Laboratory Sample Protocols

Bio-Assay Sediment Samples

Samples arriving at the Bio-Assay lab (W234) are the responsibility of the Senior Toxicology technician (Danielle Milani). Samples must be checked against the Project Folder log to ensure that no samples have been lost during delivery to the laboratory. Once all of the samples have been verified, the Senior Toxicology technician must initiate a project journal, recording the date of a project's sample(s) arrival to the lab, and the subsequent dates of sediment sieving, bio-assay test initiation, and bio-assay test completion.

Sample Storage

After verification, samples must be placed in storage at 4° C until processing and sediment preparation can be done.

Sample Processing

Depending on the requirements of the particular study, up to four different organisms are used to perform bio-assays: *Chironomus riparius*, *Hyalella azteca*, *Hexagenia spp.*, and *Tubifex tubifex*. All test results are recorded on lab data summary sheets and parameter sheets (Appendix D), specific for each of the 4 species. Endpoint calculations are also done on these sheets. On completion of each test, these sheets are filed in the appropriate project folder (see section 1), to await entry to the data base.

Chironomus riparius Culturing Protocol

Setup of Tank(s)

a) Add 1.5 - 2.0 cm silica sand (thoroughly rinsed with distilled water prior to use) and approximately 10 cm of culture water to a 20 L aquarium.

b) Place a plexiglass addition (40 cm x 19.5 cm x 20.5 cm) on top of the aquarium (fitted into the lip of the tank), preventing the escape of adults, and allowing emerged adults further room to mate. A circular screened hole is cut in the addition to access the tank without removal of the upper addition. (Note: Any kind of addition will do that contain the organisms and allow them further room mate).

c) Add three hatched egg masses onto the substrate (more may result in crowding, and subsequently varied growth rates). Note: While the animals are considered hatched, they are still at this point attached to the egg mass.

d) Label the aquarium with the date of egg deposition, and label with the date of emergence when it occurs (emergence typically occurs in ~ 3 weeks at 23°C).

e) Aerate cultures gently, and incubate at 23 °C ± 1.0°C.

f) Light/Photoperiod: 500 - 100 Lux/16L:8D

g) Replace water lost by evaporation if necessary.

h) Disassemble tank after egg deposition is complete.

Removal of Egg Masses

a) Remove egg masses from culture tanks daily with a net and wash into a petri dish containing culture water (use tweezers to remove the egg masses from the net if they are stuck).

b) Examine daily under a dissecting microscope; separate egg masses that have begun to hatch from those that have not (this ensures animals of the same age for testing purposes). Place egg masses in containing 100-150 mL culture water. Discard egg masses which contain fungus.

c) Use hatched masses (at least 3) to initiate another tank, or keep a minimum of three egg masses aside for testing purposes.

d) Remove dead adults and exuviae from tank daily with a net, and discarded.

Cleaning

a) Disassemble tank and thoroughly rinse the silica sand with distilled water. Heat the sand at 150°C for two hours. Rinse again and follow procedures for setup as outlined above. Generally the silica sand is reused once, then discarded.

Feeding

Food: Commercial fishfood flakes (Nutrafin®/Tetramin™) finely ground with a mortar and pestle.

a) Feed chironomids ad libitum, when they look to be actively searching for food (i.e. they are outside of their cases or swimming in the water column). Adjust feeding rate if necessary (i.e. reduce if accumulation of food or fungus occurs).

b) Moisten food with distilled water before adding to tank.

The hatched egg masses set aside for testing purposes do not need to be fed.

Chironomus riparius Test Protocol - 10 day Survival and Growth Test

Test Structure

The life stage used in the 10 day growth test is the first instar of *C. riparius* that have been cultured in the lab. Tests are performed in a temperature controlled facility. Temperature is maintained throughout the test at 23 ± 1 °C. Tests are conducted in 250

mL beakers fitted with plastic lids (petri dishes). A hole is drilled in the lid for airline passage. Photoperiod for the test is 16 hours of light, and 8 hours of darkness.

Sediment Preparation

Homogenize each sediment sample, then add as much sediment as needed to a 1L glass jar. Fill to the top of the jar with culture water. Mix the sediment/culture water mixture, then pour through a 250 µm mesh sieve. Continue to add water and pour through the sieve until most of sediment has passed through. Discard the residue. Use a 4:1 ratio of sediment to culture water mixture when sieving. Allow the sieved sediment to settle a minimum 24 hours, then decant the overlying water into a separate plastic bag. This water is to be used as the overlying water in the test.

Addition of Sediments to Beakers

Add 50 mL of sediment and 200 mL of overlying water to each beaker. Allow the sediment to settle overnight in the test facility. Once settled, aerate the beakers for 24 hours prior to the introduction of the organisms.

Addition of Organisms (Day 0)

Gently pour hatched 1st instar chironomids into a petri dish. Using a dissecting microscope, pipette (7 mm) organisms as groups of five, and add randomly to assigned treatments until 15 per beaker is attained. Add the organisms below the water line and be sure to rinse pipette between each transfer.

Feeding

Feed each replicate jar 8 mg of crushed Nutrafin® on day 0 and twice per week thereafter for a total of three times throughout the test.

Monitoring

Check water levels and daily, and airlines twice daily to ensure proper levels. Replace water lost to evaporation with distilled water. Measure water chemistry variables (dissolved oxygen (mg/L), pH, conductivity (µS), and temperature (°C)) on day 0, day 5, and day 10 and record values. Measure total ammonia concentration (mg/L) on day 0 and day 10 for each sample by taking a composite overlying water sample from each replicate.

Takedown of Test (Day 10)

Sieve the contents of each replicate beaker through a 250 µm mesh sieve. Wash the contents from each sieve back into the beakers. Record the number of surviving organisms and measure wet weights. Dry organisms for a minimum 24 hours at 60 °C, then measure and record dry weights.

Endpoint(s)

Results are expressed as percent survival of organisms and mean dry weight (mg) per treatment. Lethal and sublethal response in the sample is compared to those of control and reference sediments by multivariate analysis.

Note: The *Chironomus riparius* toxicity test is a standardized Environment Canada

protocol. This protocol (referenced below) contains modifications from the test outlined above (i.e. number of organisms per beaker, feeding regime).

Hexagenia spp. Culturing Protocol

Culture Initiation/Maintenance

Mayfly nymphs are reared at various stages of growth to ensure a weekly turnover of animals of the proper size for testing purposes. Generally, two tanks are initiated per week if there is a high demand.

a) Add 2.5 cm culture sediment and 10 cm culture water to a 20 L aquarium.
b) Mix food (see diet and Table 1 below) into the culture sediment. Allow sediment to settle, then aerate the tank 2 - 5 days prior the introduction of the young (this allows a bacterial base to build). Table 1 depicts the amount of food to add initially and per feeding (based on animal density/tank size).
c) Add young (up to 15 at a time) close to the surface of the sediment (see Table 1 for density recommendations) until 300 per aquarium have been added. Remove the airstone for approximately 10-15 minutes to allow the young to settle into the sediment.
d) Aerate cultures gently, and incubate at 23°C± 1.0°C.
e) Feed cultures once per week (you may need to adjust this depending on the organic content of your sediment).

Animals are ready for testing purposes after 6 weeks of incubation.

Removal of Eggs from Storage

a) Store eggs immediately upon arrival from supplier (J. Ciborowski, U of Windsor) at 4°C.
b) Remove approximately 1-2 mL eggs from storage and add to a petri dish containing 50 ml culture water weekly.
c) Incubate at 23°C ± 1.0°C until hatched, and then transfer to aquaria as described above.

Feeding

Diet: *4 g crushed Nutrafin®*
3 g Cerophyl
3g brewers yeast
100 mL distilled water

a) Mix ingredients, producing a thick concentrate.
b) Store prepared food at 4°C.

Table 1. Recommended density and food requirements for *Hexagenia spp.*

Tank Size (L)	Number of Organisms per Tank	mL Food Added Initially	mL Food/ Feeding
10	150	10	2.0
20	300	20	4.0
40	600	40	8.0

Hexagenia spp. Test Protocol - 21 day Survival and Growth Test

Test Structure

Hexagenia spp. nymphs that have been reared in the lab and are between 5 - 8 mg wet weight are to be used in tests. Tests are to be performed in a temperature controlled facility. Temperature is maintained throughout the test at 23 ± 1 °C. Tests are conducted in 1 L widemouth jars fitted with plastic lids. A hole is drilled in the lid for airline passage. Photoperiod for the test is 16 hours of light, and 8 hours of darkness.

Sediment Preparation

Homogenize each sediment sample, then add as much sediment as needed to a 1L glass jar. Fill to the top of the jar with culture water. Mix the sediment/culture water mixture, then pour through a 250 µm mesh sieve. Continue to add water and pour through the sieve until most of sediment has passed through. Discard the residue. Use a 4:1 ratio of sediment to culture water mixture when sieving. Allow the sieved sediment to settle a minimum 24 hours, then decant the overlying water into a separate plastic bag. This water is to be used as the overlying water in the test.

Addition of Sediments to Beakers

Add 150 mL of sediment and 650 mL of overlying water to each jar. Allow the sediment to settle overnight in the test facility. Once settled, aerate the beakers for 24 hours prior to the introduction of the organisms.

Addition of Organisms (Day 0)

Scoop sediment from culture tank onto a 500 µm mesh sieve and immerse in culture water, separating nymphs from sediment. Wash contents of sieve into a 5 cm deep sorting tray containing culture water. Sort nymphs of uniform size with a pipette (7 mm) and randomly add to large plastic dishes until 10 per dish is attained. Pre-weigh the mayfly nymphs as groups of ten by pouring contents of each dish onto a 250 µm mesh sieve. Blot the sieve with Kimwipes® to absorb excess water, and transfer to a tared plastic weighing dish with the aid of a soft paintbrush. Record wet weights, then randomly allocate

containers to treatment beakers immediately.

Note: Initial wet weights are converted to dry weights (mg) by the following conversion:

$$\text{Initial dry weight} = (\text{Initial wet weight} + 1.15)/7.35$$

Feeding

Feed each replicate jar 50 mg of prepared food slurry (see diet above) day 0, and once per week thereafter for a total of three times throughout the test.

Monitoring

Check water levels and daily, and airlines twice daily to ensure proper levels. Replace water lost to evaporation with distilled water. Measure water chemistry variables (dissolved oxygen (mg/L), pH, conductivity (µS), and temperature (°C)) on day 0, day 10, and day 21 and record values. Measure total ammonia concentration (mg/L) on day 0 and day 21 for each sample by taking a composite overlying water sample from each replicate.

Takedown of Test (Day 21)

Sieve the contents of each replicate jar through a 500 µm mesh sieve. Wash the contents from each sieve back into the beakers. Record the number of surviving organisms and record wet weights. Dry organisms for a minimum 24 hours at 60 °C, then measure and record dry weights.

Endpoint(s)

Results are expressed as percent survival of organisms and mean growth (mg) per treatment (growth is determined by subtracting the initial dry weights from the final dry weights). Lethal and sublethal response in the sample is compared to those of control and reference sediments by multivariate analysis.

Hyalella azteca Culturing Protocol

A complete *H. azteca* culture should contain 1 or more main tanks and 20 - 25 brood jars that are used to harvest the young on a weekly basis. This number of brood jars will produce 400-1200 young per week. The main culture tank(s) act as adult backup and weekly young can be added to tank(s) when not used in tests.

Set up of Main Tank(s)

a) Add 2.5 X 2.5 cm strips of gauze (pre-soaked for a minimum of 24 hours in culture water prior to use) and 8 L culture water to a 10 L aquarium (add 1gauze strip per 25-30 animals) (recommended holding capacity is 20 - 30 animals/L, or 200-300 animals per tank).

b) Aerate cultures gently and keep at 23°C± 1.0°C.

c) Light/photoperiod: 500 - 1000 Lux/16L:8D.

d) Replace evaporated water if necessary.

e) Replace gauze strips when they get fouled.

Set up of Brood Jars

a) Fill 20 - 25 2 L jars or equivalent **each** with 1 L culture water, one 2.5 X 2.5 cm strip of pre-soaked gauze (pre-soak for 24 hours in culture water), and add approximately 25 - 30 adults (preferable mating pairs) to each jar.

b) Do not aerate jars, and incubate at 23°C± 1.0°C.

c) Light/photoperiod: 500 - 1000 Lux/16L:8D.

Counting Young

A complete water change is performed on each brood jar once weekly, and the young are counted.

a) Pour contents of brood jar onto a small (6 cm diameter) 500 µm (top) and 250 µm (bottom) screen sequentially (the top screen will collect the adults and the bottom screen will collect the young). Gently rinse the amphipods from the screens into petri dishes.

b) Shake gently the gauze strip and rinse it off into a petri dish to dislodge remaining young and adults.

c) Clean the brood jar, rinse the gauze strip, and add fresh culture water (1 L) to the jar.

d) Count the number of young and add via a pipet (below the water line) to a separate jar until needed for testing purposes (or added to a main culture tank).

e) Count the number of adults (note mating pairs) and add back to the brood jar.

f) Replace dead adults using amphipods from your main tank(s).

g) Keep a permanent record on the number of young produced per week., and the number of adults and mating pairs/jar.

Feeding Cultures

Food: Commercial fishfood flakes (Nutrafin®/Tetramin™) finely ground with a mortar and pestle.

a) Feed main tank(s) and brood jars three times per week on non consecutive days (feed brood jars after the water change is performed).

Brood jars: Add 5 mg of food to each, swirl into water.

Main tank(s): Adjust above feeding rate accordingly to the number of animals in your tank(s).

b) Adjust feeding rate if needed (i.e. reduce if accumulation of food/fungus occurs).

Note: Feed the young that were counted and kept aside for testing purposes.

Hyalella azteca Test Protocol - 28 Day Survival and Growth Test

Test Structure

H. azteca that have been cultured in the lab and are 2 - 9 days old are to be used in tests. Tests are to be performed in a temperature controlled facility. Temperature is maintained throughout the test at 23 ± 1 °C. Tests are conducted in 250 mL beakers fitted with plastic lids (petri dishes). A hole is drilled in the lid for airline passage. Photoperiod for the test is 16 hours of light, and 8 hours of darkness.

Sediment Preparation

Homogenize each sediment sample, then add as much sediment as needed to a 1L glass jar. Fill to the top of the jar with culture water. Mix the sediment/culture water mixture, then pour through a 250 µm mesh sieve. Continue to add water and pour through the sieve until most of sediment has passed through. Discard the residue. Use a 4:1 ratio of sediment to culture water mixture when sieving. Allow the sieved sediment to settle a minimum 24 hours, then decant the overlying water into a separate plastic bag. This water is to be used as the overlying water in the test.

Addition of Sediments to Beakers

Add 50 mL of sediment and 200 mL of overlying water to each beaker. Allow the sediment to settle overnight in the test facility. Once settled, aerate the beakers for 24 hours prior to the introduction of the organisms.

Addition of Organisms (Day 0)

Pour gently the young amphipods (2 - 9 days old) onto a small (6 cm diameter) 250 µm mesh sieve and rinse into a petri dish. Pipet amphipods randomly to plastic dishes containing culture water until 15 is attained. Add randomly the dishes to the replicate beakers. Monitor for floaters. Gently add a drop of water to floaters to push below water line. Replace the organism if floating persists.

Feeding

Feed each replicate jar 8 mg of crushed Nutrafin® on day 0 and twice per week thereafter for a total of eight times throughout the test.

Monitoring

Check water levels and daily, and airlines twice daily to ensure proper levels. Replace water lost to evaporation with distilled water. Measure water chemistry variables (dissolved oxygen (mg/L), pH, conductivity (µS), and temperature (°C)) on day 0, day 14, and day 28 and record values. Measure total ammonia concentration (mg/L) on day 0 and day 28 for each sample by taking a composite overlying water sample from each replicate.

Takedown of Test (Day 28)

Sieve the contents of each replicate beaker through a 250 µm mesh sieve. Wash the contents from each sieve back into the beakers. Record the number of surviving amphipods and measure wet weights. Dry organisms for a minimum 24 hours at 60 °C, then measure and record dry weights.

Endpoint(s)

Results are expressed as percent survival of organisms and mean dry weight (mg) per treatment. Lethal and sublethal response in the sample is compared to those of control and reference sediments by multivariate analysis.

Note: The *Hyalella azteca* toxicity test is a standardized Environment Canada protocol (see references). This protocol contains modifications from the test outlined above (i.e. number of organisms per beaker, feeding regime).

Tubifex tubifex Culturing Protocol

Culture Initiation/Maintenance

a) Maintain the worm cultures under no photoperiod in 20cm X 20cm X 20cm Plexiglas aquaria with fitted lids (any similar apparatus will do).

b) Week 1, add approximately 6 cm of control sediment (pre-sieved through 250 µm sieve) and 10 cm of culture water to an aquarium. Allow the sediment to settle.

c) Add approximately 200 **full** *Tubifex* cocoons (can be at various stages of development) to the aquarium. Continue this process weekly until 7-8 tanks are achieved.

d) Aerate cultures and incubate at 23°C± 1.0°C. Animals are ready for testing purposes after 7-8 weeks of incubation (at this point they should be sexually mature if your culture sediment is organically rich enough).

e) On week 7-8, sieve the tank that was initiated week 1 through a 500 µm sieve, removing sexually mature worms and cocoons. Full cocoons are used to initiate another culture repeating the cycle. This ensures that the animals being used for testing purposes are all of the same relative age (7-8 weeks ± 1 week since cocoons take approximately this long to hatch) and that there is a weekly turnover of worms 7-8 weeks from the first culture initiation.

Note: You may not want to initiate a new tank every week if you don't have many tests to perform. This culturing technique is based on performing tests on a weekly basis.

Feeding

Your control sediment may be organically rich enough to sustain cultures for 7-8 weeks without the need to supplement with food (our control sediment (Long Point, Lake Erie, has a TOC of ~ 8%). However, if not then sediment can be supplemented with crushed Nutrafin®/Tetramin™ flakes.

Tubifex tubifex Test Protocol - 28 Day Adult Survival and Reproduction Test

Test Structure

T. tubifex that have been cultured in the lab and are sexually mature are to be used in tests. Sexually mature adults are identifiable by the presence of gonads. Tests are to be

performed in a temperature controlled facility. Temperature is maintained throughout the test at 23 ± 1 °C. Tests are conducted in 250 mL beakers fitted with plastic lids (petri dishes). A hole is drilled in the lid for airline passage. The worms are tested under no photoperiod.

Sediment Preparation

Homogenize each sediment sample, then add as much sediment as needed to a 1L glass jar. Fill to the top of the jar with culture water. Mix the sediment/culture water mixture, then pour through a 250 µm mesh sieve. Continue to add water and pour through the sieve until most of sediment has passed through. Discard the residue. Use a 4:1 ratio of sediment to culture water mixture when sieving. Allow the sieved sediment to settle a minimum 24 hours, then decant the overlying water into a separate plastic bag. This water is to be used as the overlying water in the test.

Addition of Sediments to Beakers

Add 100 mL of sediment and 100 mL of overlying water to each beaker. Add 80 mg crushed Nutrafin® to each beaker, and stir until well mixed. Allow the sediment to settle overnight in the dark in the test facility. Once settled, aerate the beakers for 24 hours prior to the introduction of the organisms.

Addition of Organisms (Day 0)

Remove sexually mature worms from a 7 -8 week culture tank by adding the sediment to a 500 µm sieve and gently immersing in culture water. Wash the contents of the sieve into petri dishes containing culture water. Sort the sexually mature adults with a probe and randomly add to plastic dishes until each dish contains four worms. Randomly allocate the worms in the dish to the replicate beakers.

Monitoring

Check water levels and daily, and airlines twice daily to ensure proper levels. Replace water lost to evaporation with distilled water. Measure water chemistry variables (dissolved oxygen (mg/L), pH, conductivity (µS), and temperature (°C)) on day 0, day 14, and day 28 and record values. Measure total ammonia concentration (mg/L) on day 0 and day 28 for each sample by taking a composite overlying water sample from each replicate.

Takedown of Test (Day 28)

Sieve the contents of each replicate beaker through a 500 µm mesh sieve (top) and a 250 µm sieve (bottom) sequentially. Wash the contents from each sieve separately into gridded petri dishes for enumeration with a dissecting microscope. The number of original adults, full cocoons, empty cocoons and worms >500 µm are counted from the top 500 µm mesh sieve. The number of worms <500 µm are counted from the bottom 250µm mesh sieve (some cocoons and adults may also pass through to bottom sieve). Note any abnormalities in the condition of the worms or cocoons (i.e. deformities, lack of maturity in original adults, etc.). The contents of the two sieves may be preserved in 4% Formalin containing a stain (Rose Bengal) for future enumeration if time constraints exist, but preservation is not recommended.

Endpoint(s)

Results are expressed as percent survival of adults, number of cocoons per adult, percentage cocoons hatched, and number of young per adult. Lethal and sublethal response in the sample is compared to those of control and reference sediments by multivariate analysis.

Quality Assurance/ Quality Control (QA/QC)

Control Sediment

A control sediment is included in each test to determine the health of the test organisms and the consistency and precision of test results. The sediment used is a natural marsh sediment from Long Point, Lake Erie. This sediment has been used for approximately six years and is shown to have a high organic carbon content and an appropriate particle size for all species. The measured endpoints for all four species in this control sediment are plotted in warning charts. The number that equals two times the standard deviation above and below the mean for each endpoint measured (the upper and lower 95% confidence limits) are plotted. These warning limits indicate the normal variability in the test responses. If test data fall inside the limits, they are included in the data set. These charts are updated continually to compensate for different batches of this control sediment collected over time.

Reference Toxicant Tests

Reference toxicant tests are also performed regularly using $CuSO_4$, to again monitor the health of the test organisms. Results are expressed in warning charts as described above.

Monitoring of Water Quality

Water used for culturing and testing purposes is City of Burlington, Lake Ontario tap water. This water is aerated and carbon filtered prior to use. This water is analyzed monthly for nutrients, major ions and water hardness/alkalinity to ensure it is within acceptable standards.

Sediment and Water Chemistry Samples

Sediment and Water Chemistry Samples are delivered to the Senior Project Technologist for delivery to the appropriate laboratory for analysis. Each standard sample is comprised of 3 sediment chemistry containers: 1-500ml tub, 1-125ml contaminant jar, 1-100ml pill jar. The sediment containers are labeled top and bottom when the sediment is collected. Each standard sample is also comprised of 3 water chemistry containers: 1 for alkalinity, 1 for total phosphorus and 1 for nutrients). Water bottles are also labeled at time of collection.

i. Water samples

A sample submission form for NLET with the sample ID's, the analysis to be done (SCHEMA) and sample date is filled out (Appendix E). Samples are then sorted such that

the 3 bottles for each site are together, and delivered to the NLET submission office (W236, Don Marsh).

ii. Sediment Chemistry

Sediment chemistry samples are prepared for freezing and freeze drying in the bio-assay lab (W234). Preparation consists of removing each sample container lid and replacing it with a Kimwipe which is held on by elastics. Samples are then either: 1) placed in the freezer in W234 until a sufficient number of samples have accumulated for submission for freeze drying, or 2) taken directly to the Sediment Laboratory (R125) for freeze drying.

Samples taken to the Sediment Lab are placed in the freezer there. The sheet on top of the freezer must be filled out (# of samples, type of container, who submitted samples). A submission form must then be filled out and given to the Sediment Laboratory (John Dalton, R124), who is responsible for the actual freeze drying process.

Once samples have been freeze dried, they are picked up from the Sediment Laboratory by the Senior Project Technologist, taken to L572, and the Kimwipes are replaced with proper lids for each sample. Three types of analysis are then carried out on the freeze dried samples:

a) Particle Size Analysis - samples are returned to the Sediment Lab (R125), and a submission form is filled out for particle size analysis (Appendix E).

b) Contaminant Analysis - samples are stored in L572 until required. If contaminant analysis is requested, samples are delivered to the appropriate lab (George Garbai).

c) Major Elements and Total Metals - the large tubs of sediment which have been freeze dried are packaged in L572 by the SCSL technician and shipped to Seprotek.

Data sheets generated by sediment analysis are returned from two sources. First, the particle size analysis sheets from the Sediment Lab are delivered to the Senior Project Technician. Seprotek data sheets are returned to the SCSL technician. All data sheets are placed in the appropriate Project Folder to await entry to the electronic data base.

Laboratory Procedures for Data Management

The management of data resulting from field collection and laboratory analysis is the final step in establishing a complete project data base. The success or failure of a project can be determined as much by the management of project data, as by the success or failure at any other stage of a project's duration. Like the collection and analysis of samples, resulting data must be handled within a well-established framework.

During the field sampling and laboratory analysis portion of a project, raw data sheets are warehoused in the Project Folder initiated by the SCSL technician, which represents the first stage in data management. However, the Project Folder is not the final storage media for data collected during a project. Standard data storage methods enable scientists to manipulate and analyze data with much more accuracy and fewer problems. NWRI has established a computer-based information storage and retrieval system for use during projects involving Benthic Community Structure and Environmental Attributes in Aquatic Ecosystems.

ELECTRONIC BENTHIC DATA INFORMATION SYSTEM

Electronic Project Initialization

The Project Folder represents the hard-copy equivalent of the final electronic data base. Unlike the Project Folder, however, the Benthic Data Information System (BDIS) is designed to store data from multiple projects. For this reason, the first stage in transferring data from hard-copy to electronic format is establishing a unique Study Name for identification in BDIS (Appendix F). The initialization of a new study within BDIS can be undertaken at any time during a project. However, the most efficient routine is to establish a new electronic project at the same time that field-work is begun. New projects are initiated by the SCSL technician.

Study Name Protocol

Any alphanumeric name which uniquely identifies a project and associated data within the data base.

Site Code Entry

Individual sample sites for a specific project are identified by unique Site Codes within BDIS. These are established before field work is undertaken, and are used to identify samples as they are collected in the field. Each Site Code must be entered into the information system (Appendix F), and this is the responsibility of the SCSL technician. The entry of site codes is done only once during a project. Once these codes have been established, they are automatically maintained by BDIS as new data for each site is entered into the system from the Project Folder. This eliminates data entry errors, and the possibility of data being lost due to conflicting or contradicting site codes. No data entry can take place until the SCSL technician has entered Site Codes for a particular project.

Site Code Protocol

i. **Lake Projects** - projects involving lentic environments are assigned a ten digit numeric code consisting of a 4 digit number identifying the station, and 6 digits for the date a sample was taken, in the format dd/mm/yy.

ii. **River Projects** - projects involving lotic environments are assigned a nine digit alpha-numeric consisting of: three letters identifying the major river on which samples were taken, 2 digits for the sample number for that river, 2 digits for the replicate number at the site, and 2 digits for the year of the sample.

Tracking Project and Sample Status

BDIS is capable of tracking the status of six different sample types typically taken during field collection (Appendix F). These are:

i. **Community Structure**

a) Sample Location - where are the samples are currently stored.

b) Picked - are the samples picked yet.

c) Identified - are the samples identified yet.

d) Entered to BDIS - have the hard-copy data sheets been entered to BDIS.

ii. **Periphyton Structure**

a) Sample Location - where are the samples are currently stored.

b) Entered to BDIS - have the hard-copy data sheets been entered to BDIS.

iii. **Particle Size Analysis**

a) Sample Location - where are the samples are currently stored.

b) Entered to BDIS - have the hard-copy data sheets been entered to BDIS.

iv. **Sediment Chemistry**

a) Sample Location - where are the samples are currently stored.

b) Entered to BDIS - have the hard-copy data sheets been entered to BDIS.

v. **Water Chemistry**

a) Sample Location - where are the samples are currently stored.

b) Entered to BDIS - have the hard-copy data sheets been entered to BDIS.

vi. **Bio-Assay**

a) Sample Location - where are the samples are currently stored.

b) Test Undertaken - which of the 4 species are being tested.

c) Entered to BDIS - have the hard-copy data sheets been entered to BDIS.

Once a study has been initiated in the system, project members may use the tracking system to determine a project's status. Project members must also update the tracking system if changes to the status of samples or data which they are responsible for occur.

Data Entry

i. Hard-Copy Study Binder

As data for each stage is entered into the benthic data information system, the raw data sheets are removed from the Project Folder, and placed into a permanent binder for the project. These binders are stored in the Community Structure Lab (L572).

ii. Field Sheets (Primary and Physical Data)

Field Sheets for benthic assessment include the site number, date sampled, location, and other physical parameters collected while in the field (Appendix F). Field sheets are placed in the Project Folder immediately after a return from the sampling trip, and are the responsibility of the SCSL technician. The entry of data from the Field sheets is also undertaken by the SCSL technician, and must be completed before any other data can be entered to BDIS. Once field data has been entered, field sheets are moved from the Project Folder to the Hard-Copy Study Binder.

Note: Since Primary data is the first stage of data entry completed from raw data sheets, it is the SCSL technician's responsibility to create a new project Hard-Copy Study Binder at this point, to store all raw data sheets from subsequent data entry stages.

iii. Chemistry Data

Chemistry data is entered from the data sheets returned by the analysis labs listed in appropriate sections above. The entry of chemistry data is the responsibility of the Senior Data Base Manager (Timothy Pascoe). Once data for a particular analysis (e.g. Water Chemistry) for a project has been entered, data sheets are moved from the Project Folder to the Hard-Copy Study Binder.

iv. Particle Size Analysis

Particle size data is entered from the data sheets returned from the Sediment Lab (John Dalton, R125). The entry of particle size data is the responsibility of the Senior Data Base Manager. Data sheets are moved from the project folder to the project binder once all data has been entered into the data base.

v. Bio-Assay

The entry of bio-assay data is the responsibility of the Senior Toxicology Technician (Appendix F). Data sheets are moved from the project folder to the project binder once all data has been entered into the data base.

vi. Special Cases or Procedures

a) Automated Computations

Several forms in the data entry system automate the computation of values:

Decimal Degrees

Location data in the field is typically recorded using a Global Positioning System (GPS), and is measured in Degrees, Minutes, and Seconds of Latitude and Longitude. For statistical analysis, however, Latitude and Longitude must be converted to Decimal Degrees. BDIS automatically computes Decimal Degrees for the user, as Degrees, Minutes and Seconds are entered.

Mortality Compensation in Bio-Assay Results

During bio-assay tests (especially *Tubifex tubifex*) it is sometimes difficult to determine how many individuals are responsible for reproduction; for example, the computation of Cocoons/Individual in the case of a *Tubifex tubifex* test where only 3 of the 4 individuals survived. In these cases, the protocol is to use the mean value of adults as the denominator in the equation. Thus, if the test began with 4 adults, and only 3 survived, the Cocoons/Individual would be based on a value of 3.5 individuals.

Appendix

Appendix A: Lentic Sampling Field Sheet

*** General Section:**

Site No.:_____ Lake:_____ Ecodistrict:_____

Latitude:_____ Longitude:_____

Sample Date (DD/MM/YR):____/____/____ QA/QC: Yes No

*** Biological Section:**

1. Water depth:_____

2. Benthic community samples:
   Operator:_____ No. of Replicates:_____

   Sampling device:_____ Sediment depth in box core:_____

3. Bioassay samples:
   Operator:_____ No. of Replicates:_____

   Sampling device:_____

*** Physical/Chemical Section:**

1. Field measurements:
Water surface temperature:_____ Water bottom temperature:_____ DO:_____ pH:_____

2. Water samples:
Nutrients:_____ Total phosphorus:_____ Alkalinity:_____

3. Sediment samples:
Chemistry (450mls):_____ Organics (125 mls):_____ Particle size (50 mls):_____

*Comments.
Sediment type (eg: sand, clay, mud, etc.)

sediment colour:

No. of attempts with sampling device:

Appendix B: Bio-Assay Laboratory Recording Sheets

Chironomus Bioassay Data Sheet

	DAY 0						
Treatment Number/Rep:							Notes
Temperature							
D.O.							
pH							
Conductivity							

Notes:

	DAY						
Treatment Number/Rep:							Notes
Temperature							
D.O.							
pH							
Conductivity							

Notes:

	DAY 10						
Treatment Number/Rep:							Notes
Temperature							
D.O.							
pH							
Conductivity							

Notes:

Hexagenia Bioassay Data Sheet

	DAY 0						
Treatment Number/Rep:							Notes
Temperature							
D.O.							
pH							
Conductivity							

Notes:

	DAY						
Treatment Number/Rep:							Notes
Temperature							
D.O.							
pH							
Conductivity							

Notes:

	DAY 21						
Treatment Number/Rep:							Notes
Temperature							
D.O.							
pH							
Conductivity							

Notes:

Hyalella Bioassay Data Sheet

	DAY 0						
Treatment Number/Rep:							Notes
Temperature							
D.O.							
pH							
Conductivity							

Notes:

	DAY 14						
Treatment Number/Rep:							Notes
Temperature							
D.O.							
pH							
Conductivity							

Notes:

	DAY 28						
Treatment Number/Rep:							Notes
Temperature							
D.O.							
pH							
Conductivity							

Notes:

Tubifex Bioassay Data Sheet

	DAY 0						
Treatment Number/Rep:							Notes
Temperature							
D.O.							
pH							
Conductivity							

Notes:

	DAY 14						
Treatment Number/Rep:							Notes
Temperature							
D.O.							
pH							
Conductivity							

Notes:

	DAY 28						
Treatment Number/Rep:							Notes
Temperature							
D.O.							
pH							
Conductivity							

Notes:

6. APPENDIX I

SUMMARY SHEET

Chironomus riparius 10 day survival and growth test

SEDIMENT SOURCE_____
SAMPLING DATE_____

STORAGE CONDITIONS_____
HANDLING_____

TEMPERATURE_____
TEST VOLUME_____

START DATE_____
END DATE_____

REPLICATE	1	2	3	4	5
No. Live Animals					
Survival (%)					
Mean Wet Wt (mg)					
Mean Dry Wt (mg)					
Endemic Species					

COMMENTS:

SUMMARY SHEET

Hexagenia spp. 21 day survival and growth test

SEDIMENT SOURCE_____
SAMPLING DATE_____
STORAGE CONDITIONS_____
HANDLING_____

TEMPERATURE _____ TEST VOLUME _____
START DATE _____ END DATE _____

REPLICATE	1	2	3	4	5
No. Live Animals					
Survival (%)					
Mean Initial Wet Wt (mg)					
Mean Initial Dry Wt (mg) (wet+1.15/7.35)					
Mean Wet Weight (mg)					
Mean Dry Wt (mg)					
Growth (mg)					
Endemic Species					

COMMENTS:

SUMMARY SHEET

Hyalella azteca 28 day survival and growth test

SEDIMENT SOURCE_____
SAMPLING DATE_____

STORAGE CONDITIONS_____
HANDLING_____

TEMPERATURE_____
TEST VOLUME_____

START DATE_____
END DATE_____

REPLICATE	1	2	3	4	5
No. Live Animals					
Survival (%)					
Mean Wet Wt (mg)					
Mean Dry Wt (mg)					
Endemic Species					

COMMENTS:

SUMMARY SHEET

Tubifex tubifex 28 day survival and reproduction test

SEDIMENT SOURCE_____
SAMPLING DATE_____
STORAGE CONDITIONS_____
HANDLING_____

TEMPERATURE _____ START DATE
TEST VOLUME _____ END DATE

REPLICATE	1	2	3	4	5	1	2	3	4	5
ADULTS										
COCOONS EMPTY										
COCOONS FULL										
COCOONS TOTAL										
HATCHED (<500u)										
YOUNG (>500u)										
TOTAL OFFSPRING										

COMMENTS:

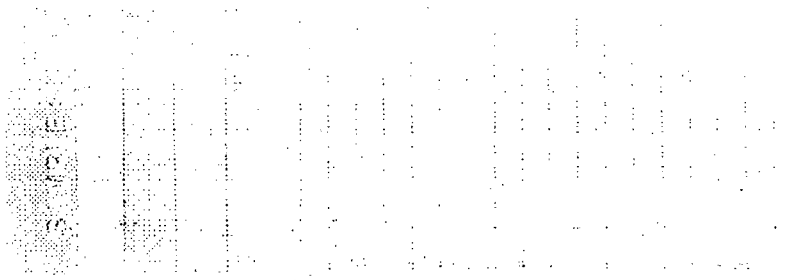
SEDIMENT SOURCE_____
SAMPLING DATE_____
STORAGE CONDITIONS_____
HANDLING_____

TEMPERATURE _____ START DATE
TEST VOLUME _____ END DATE

REPLICATE	1	2	3	4	5	1	2	3	4	5
ADULTS										
COCOONS EMPTY										
COCOONS FULL										
COCOONS TOTAL										
HATCHED (<500u)										
YOUNG (>500u)										
TOTAL OFFSPRING										

COMMENTS:

Appendix E: Analytical Laboratory Submission Forms



Environment Canada | Environnement Canada

SAMPLE SUBMISSION FORM - NAQUADAT/MISCELLANEOUS
EXPÉDITION D'ÉCHANTILLONS PRÉLEVÉS DANS L'ENVIRONNEMENT

NATIONAL LABORATORY FOR ENVIRONMENTAL TESTING
ECB - HEALTH BRANCH
LABORATOIRE NATIONAL DES ESSAIS ENVIRONNEMENTAUX
DIRECTION DE LA SANTÉ DES ÉCOSYSTÈMES

NLET Project No. / Projet du LNEE | Project Leader / Chef de projet | Survey Coordinator / Responsable | Sp. No. / N° de sp.

Date Shipped / Expédié le | Via / Par | Date Rec'd / Reçu le | NAQUADAT Submitter ID / ID NAQUADAT du client

No. of Bottles / Nbre de bouteilles	Client Sample No. / N° de l'échant. (max 10 chars.)	NLET Scheme Name or Water Scheme Number / Nom du régime du LNEE ou numéro du régime	NAQUADAT Station ID / ID de la station NAQUADAT (10 chars.)	Collected / Prélèvement: Date (YYMMDD)	Time / Heure (HHMM)	Zone / Fuseau	Remarks / Remarques

SRP Analytical: Sample Submission Form
For
MAJOR AND TRACE METAL
AND
TRACE ORGANIC ANALYSIS

Submitted by:

Branch/Study #:

# of Samples:

Sample Type:

Analysis Type / Organics		
☐ PAHs	☐ n-ALKs	☐ Congener PCBs
☐ PCB total	☐ OCs	☐ Chlorobenzenes
☐ Pesticides	☐ Herbicides	☐ Custom Target Compounds
☐ Qualitative	☐Method Development	☐ Confirmation
☐ Others		

Analysis Type/ Metals		
☐ Aluminum	☐ Copper	☐ Silicon
☐ Barium	☐ Iron	☐ Sodium
☐ Calcium	☐ Lead	☐ Thallium
☐ Cadmium	☐ Magnesium	☐ Vanadium
☐ Cobalt	☐ Manganeese	☐ Zinc
☐ Chromium	☐ Nickel	☐

Comments

Date:

Signiture:

SAMPLE SUBMISSION FORM
For
PARTICLE SIZE ANALYSIS
AND
OTHER RELATED ANALYSIS

Submitted by:

Branch/Study #/Leader

# of Samples:

Sample Container Type:

ANALYSIS TYPE/	PROCEDURE TYPE	
☐ SEDIGRAPH/SIEVE	☐ORGANIC CONT:(LOI)	☐ FRACTIONATION
☐ SIEVE	☐ DENSITY	☐ CORE SECTIONING
☐ CARBON/ORGANIC	☐ MOISTURE CONTENT	☐ PHOTOGRAPH CORES
☐ CARBON/INORGANIC	☐FREEZE DRYING	☐ ATTERBERG LIMITS
☐ CARBON/TOTAL		

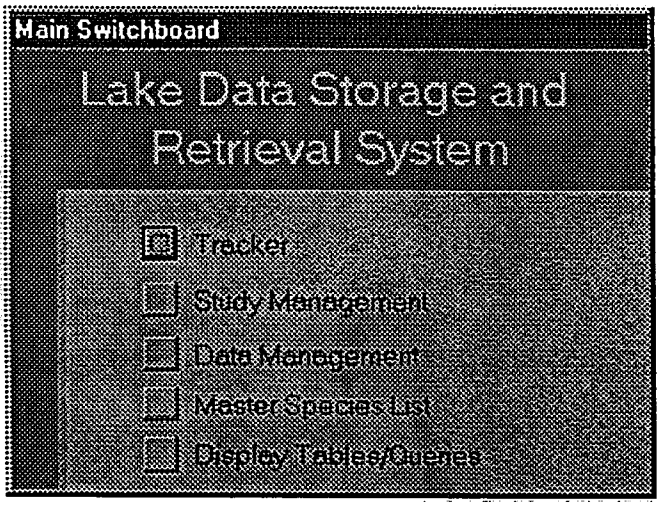
Analysis Type/ other/and Equipment usage		
☐ Fractionation for XRD	☐ Equipment usage	☐
☐ Fractionate for carbonate	☐ General Lab usage	☐

Comments

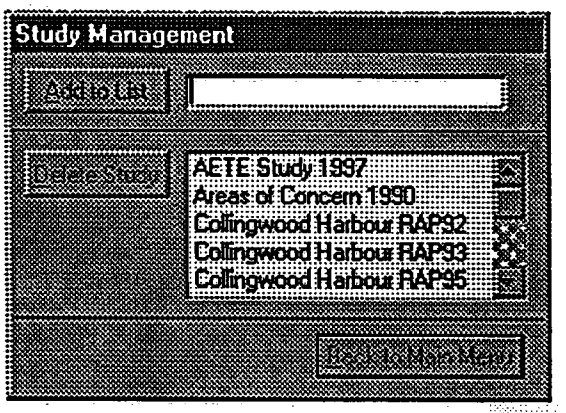
Phone # for Contact person:

Appendix F: Benthic Data Information System Forms

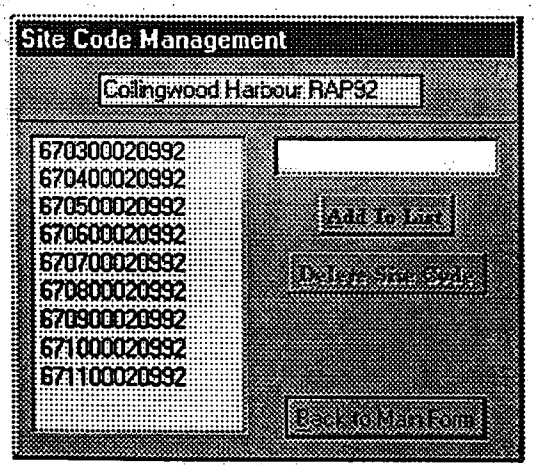
Main Menu:



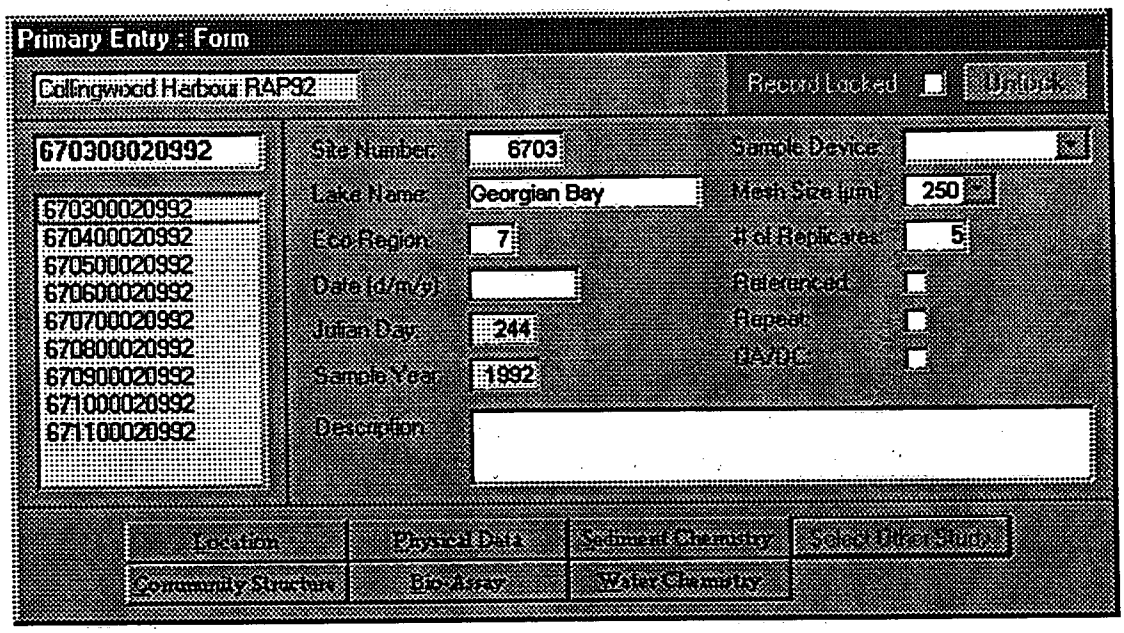
New Project Name:



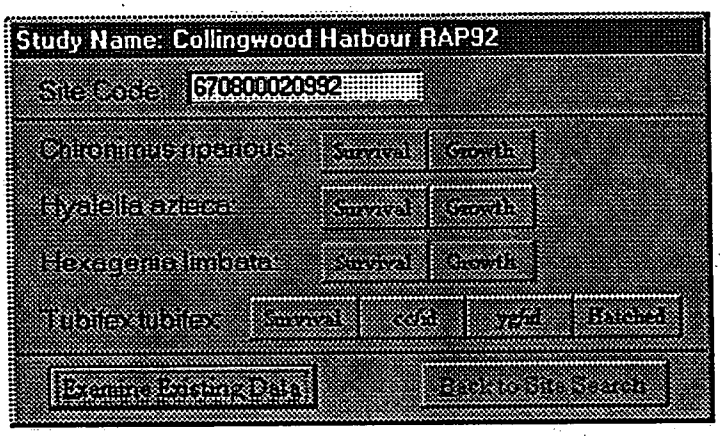
Site Code Entry:



Primary Site Information Entry:



Bio-Assay Data Entry:



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