A PROPOSAL FOR A BIOASSAY PROCEDURE TO ASSESS IMPACT OF HABITAT CONDITIONS ON LAKE TROUT REPRODUCTION IN THE GREAT LAKES

(Report of the Ad Hoc Committee to Assess the Feasibility of Conducting Lake Trout Habitat Degradation Research in the Great Lakes)

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Great Lakes Fishery Commission

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INTRODUCTION

In 1985 an ad hoc expert committee was formed by the Secretariat of the Great Lakes Fishery Commission to develop a method to assess whether chemical and physical degradation of the Great Lakes is a major impediment to reproduction of lake trout Salvelinus namaycush. It was hoped that a promising method could be identified so fishery agencies, working cooperatively, could make quantitative comparisons among the Great Lakes, especially between areas where lake trout are reproducing and areas where successful reproduction is wanting. After considering several methodologies and conducting field tests, the committee is prepared to recommend a bioassay technique as a basis for comparative studies. misreportof the committee identifies the heed for conducting field bioassays that measure impacts of differing chemical-physical conditions on lake trout reproduction, it outlines a tested, experimental design, and it recommends a coordinated implementation by concerned agencies.

The uncertainties associated with a deteriorated environment were recognized as one of three critical, first-order research areas at the 1983 Conference on Lake Trout Research (Eshenroder 1984). A quantification of water quality effects on lake trout reproduction would be valuable for linking water quality and fishery goals in the Great Lakes. Lake trout are being stocked over almost all of their former range in the Great Lakes with the expectation that they will reproduce and establish self-sustaining populations. However, much of this habitat has been enriched, contaminated, or altered since the loss of native populations, and there is no asmmnce that such areas remain suitable for lake trout reproduction (Sly 1984; Willford 1984). Areas of major concern are southern Lake Michigan (where lake trout became extinct in the 1950s), outer Saginaw Bay (where lake trout disappeared in the 1940s), eastern Lake Erie (where lake trout virtually disappeared in the 1930s), and Lake Ontario (where reproduction ceased in the 1950S). Each of these waterbodies has been affected, because of increased cultural eutrophication during the period when lake trout were absent (Beeton 1969), and each lacks significant recruitment from planted stocks (Eshenroder et al. 1984).

It is generally thought that enriched or contaminated water would interfere with the life cycle of lake trout at the embryo or fry stages (Willford 1984; Sly 1984). Enrichment could physically impact lake trout reproduction by fouling the spawning shoals with decaying plant matter resulting in the suffocation of embryos as is thought to have occurred in Seneca Lake, New York (Sly and Widmer 1984). Organic contaminants could interfere with the early ontogeny of lake trout, particularly the onset of exogenous feeding (emergence) which is a critical event in the early life history (Balon 1984), and in fact, Mac et al. (1985) suspected that such contaminantswere responsible for observed mortalities of lake trout fry fmnmmmstemIake Michigan. It is also possible that enriched or contaminated water could impact lake trout by altering the fish community, but this hypothesis is beyond the scope of the bioassay discussed here.

. . GENERAL APPROACH

We emphasize a field rather than a laboratory bioassay in our approach to enhance our ability to assess differences in performance of lake trout that have

been documented in the Great Lakes. This choice does, however, complicate the issue of controls, which are more manageable in the laboratory. Because we will be using differences in survival of embryos and fry among experimental sites as measures of reproductive and, hence, environmental impairment, in the strictest sense we lack controls. Our general approach uses a shoal in Lake Superior where lake trout are reproducing as a "control" site and "treatment" sites (shoals) in the other Great Lakes where lake trout are not reproducing.

It could be argued that the present chemical-physical conditions in Lake Superior my hot mirror earlier conditions in the other Great Lakes when lake trout were reproducing, and that Lake Superior cannot serve as a "control" for the pre-degradation situation in the other lakes, which were always more productive than Superior. However, an examination of the record of lake trout landings from Lakes Michigan, Huron, and Ontario suggests that in the past the chemical-physical environment in these waterbodies must have been adeguate for lake trout reproduction or the productivity and resiliency of the native stocks (Berst and Spangler 1972; Christie 1972; and Wells and McLain 1972) would not have been possible. Even Lake Erie at the turn of the century was a fair producer of lake trout, if catch is compared against the relatively mall area of the eastern basin where they were fished (Smith 1972). The caveat in our approach is that to establish linkage with the past the treatment must be done on sites where former stocks reproduced (Goodyear et al. 1982).

The rather long embryonic developmental period of lake trout requires conducting the proposed bioassay in two phases. Since adverse conditions associated with enrichment (law dissolved oxygen and suffocating plant debris) would most likely affect unhatched embryos and since embryoes and alevins may be capable of avoidance, the bioassay is partitioned by these life stages. Mac et al. (1985) reported that mortalities associated with contaminant burdens were highest after hatch. Thus, the bioassay spans about 8-9 months with the first stage encompassing the period from fertilization to hatch (approximately late October to April), and the second stage lasting from hatch to the early alevin stages (Balon 1980), which depending on rearing conditions, could extend until July (approximately 1100 TU total).

Factors not directly associated with water quality such as predation and dislodgement (Sly 1984) can affect survival of unhatched embryos in natural systems. Therefore, mortality from the resources must be avoided or accounted for in this field bioassay. Also, it must be possible to relate survival differences to habitat conditions, the focus of the bioassay. To work within these constraints some artificial conditions are imposed in the design. Thus, incubating cages are to be used to secure and protect unhatched enbqmsihthefirstphaseofthe bioassay. In contrast to naturally spawned or seeded eggs, cages offer more control over potentially confounding effects such as differences in predatory losses among sites. Properly designed cages fully expose the test specimens to ambient lake conditions. Furthermore, cages negate the use of a capture process to estimate the fraction surviving.

In the second phase of the bioassay, the incubating cages will be retrieved near the time of hatch, and surviving embryos will be reared in laboratory aquaria. Embryo retrieval is necessary because we know of no practical way to hold and feed hatched embryos in a reef environment. By design, the first phase of the bioassay provides la&-conditioned embryos for the second phase. Although the most

faithful simulation of exposure to ambient water chemistries would involve rearing the hatched embryos in lake water, Mac et al. (1985) did not find differences in mortality after hatching caused by rearing in lake versus well water. Consequently, lake rearing, with its substantially increased costs, does not appear to be warranted for the second phase of the bioassay.

Two sources of embryos will be compared in the bioassay. A wild source collected in the vicinity of each treatment site is needed to provide green eggs maternally contaminated with toxics at ambient levels. A second source of Lake Superior wild stock will also be deployed in a reciprocal arrangement at the Lake Superior site (the control) and at each treatment site in the other Great Lakes. This second source of embryos will help to isolate site effects as a key variable in the bioassay. Thus, in the first phase of the bioassay each treatment site will have embryos from two wild sources (local and Lake Superior), and the control site in Lake Superior will have local plus sources from each of the treatment sites in the other Great Lakes.

Although Lake Superior is the "control" for the other lakes, laboratory-reared embryos will also be needed to account for differences in fertilization rates among the various batches of embryos. Therefore, the interlake comparisons in phase one of the bioassay will be based on differences in survival after fertilization rather than on absolute differences in survival among the lakes.

The committee had considered using a hatchery source of eggs in addition to eggs collected from wild stocks at each experimental site, but this idea evolved to using Lake Superior wild stock as a common, reciprocal test organism at all sites. Mac et al. (1985) noted that eggs from a domestic supply did not survive as well as wild eggs from Lake Michigan when reared in ambient Lake Michigan water. The existence of this phenomenon weighs against the use of domestic eggs in this bioassay. Also, the performance of hatchery eggs in natural systems is not consistent with the concept of assessing differences in performance of wild lake trout in natural systems, the theme of the bioassay.

RESULTS FROM FEASIBILITY STUDIES

Selection and evaluation of an incubating cage for use in the first phase of the bioassay was a major task. Various incubators used heretofore on Great Lakes spawning reefs had drawbacks. Specific problems with other incubators were vulnerability to displacement or loss in storms, a tendency to trap silt, problems with excessive handling (scuba diving) time, and a tendency to cause egg clumping which resulted in transmission of fungus. The committee selected an incubating cage used by Kennedy (1980) and Gunn and Keller (1984) in studies of acidity in inland lakes for testing in Lake Huron during winter, 1986-87. This plexiglass cage (Figure 1), which holds 50 eggs in individual compartments was small, which made anchoring and handling easy, it prevented eggs from clumping, and it minimized the trapping of silt.

Port Austin Reef, a limestone outcropping in outer Saginaw Bay (Figure 2), was the site for a test of the plexiglass incubator. This reef is fully exposed to north, northeast, and easterly storms. Also, high sedimentation rates, which would encourage the trapping of silt by incubators, were suspected in this area because of proximity to enriched waters of Saginaw Bay. In preparation for

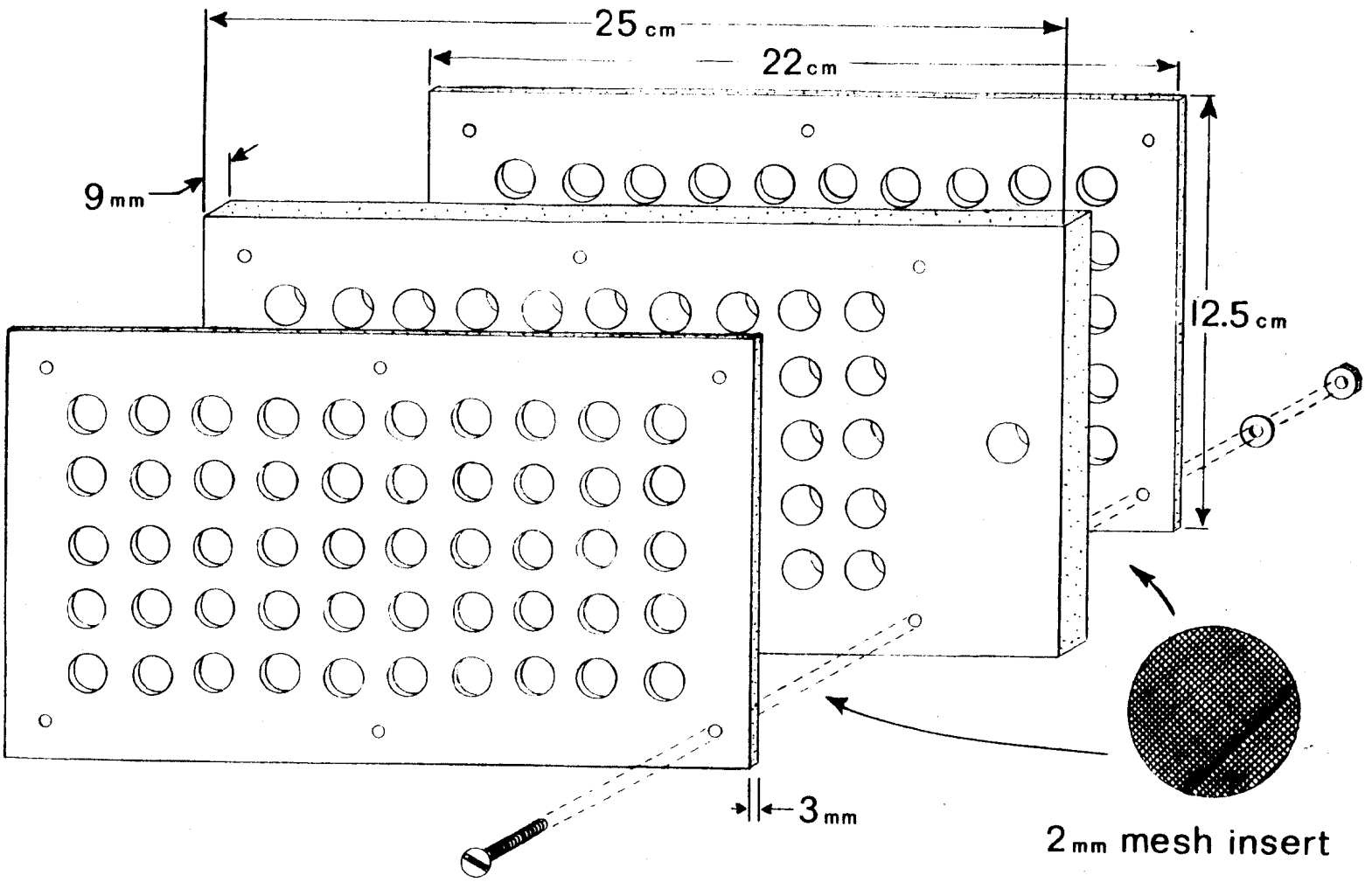

Figure 1. Detail of plexiglass incubator with nylon fasteners.

testing the incubators, the best substrates on the reef were identified with side-scan-sonar and underwater TV. Forty-three incubators containing green eggs from a domestic brood stock were buried in cobble-gravel substrates at the 12 m depth contour in late October 1986 and retrieved in April 1987. In addition, four incubators were held as controls at the National Fisheries Center-Great Lakes in Ann Arbor, Michigan. The incubators in Lake Huron were fastened with chain leaders to a longer chain secured with trap net anchors.

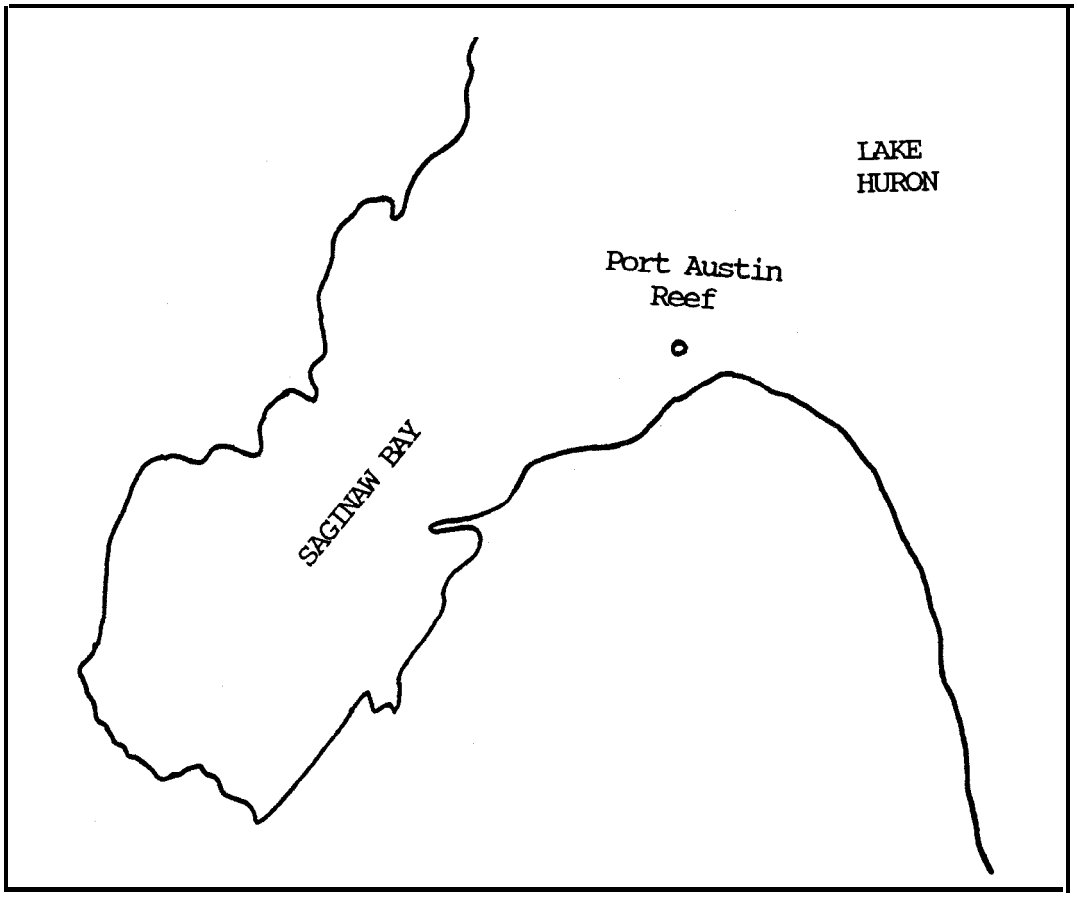

Figure 2. Location of Port Austin Reef in central Lake Huron.

The plexiglass incubator appears suitable (personal communication, B. Manny, National Fisheries Center-Great Lakes) for the bioassay proposed here. Although 24 incubators out of 43 deployed were dislodged from their original locations, all remainedsecured to anchor lines. Those that were dislodged did so because they were partially exposed above marginal (thin) substrates, which would be avoided in future bioassays. The incubators did not appear to accumulate sufficient silt to interfere with embryo development. Survival in the stationary incubators was 21%, in the dislodged incubators 11%, and in the controls 38%. Performance of the incubators was judged by the committee to be highly satisfactory. They were relatively inexpensive to make, easy to load, easy to deploy and retrieve by scuba

divers, and they prevented escapement of embryos and predation, inhibited fungal transmissionbetween embryos, and allowed about as much internal water circulation as is possible with any cage-like device.

BIOASSAY PROCEDURES

Siting Superficial substrates on "candidate" bioassay sites were mapped in each of the Great lakes. Other sites may be desirable and can be used in the bioassay. However, because site mapping is time consuming and requires specialized equipment and because a high degree of coordination is needed with the reciprocal transfer of embryos, the committee undertook mapping of candidate reefs in each Great Lake so that this task would not impede agencyparticipation in the bioassay. Maps indicating depth contours and particle sizes referenced by Loran coordinates are available for each site from the National Fisheries Center-Great lakes. Mapped sites are as follows:

Control	
Lake Superior	Partridge Island Reef
Treatments	
Lake Michigan	Wilmette Reef
Lake Huron	Fort Austin Reef
Lake Erie	Brockton Shoal
Lake Ontario	Charity Shoal

The sites selected for the bioassay met the following criteria: 1) once used for spawning by native lake trout, 2) submerged and structurally discrete from the shorebank, and 3) spawning gravels exist at depths readily worked by scuba divers. In addition, sites in Lake Huron and Michigan were favored because of their location in areas most impacted by cultural eutrophication.

Lacking information on the specific locations where native lake trout spawned on the sites selected for the bioassay, researchers should deploy the incubating cages in cobble-rubble habitats that appear most promising for lake trout reproduction (Nester and Poe, 1987). Reef maps plus additional observations with underwater TV will help in the selection process. It may be necessary to deploy incubators at more than one location on each site, depending upon habitat complexity. The selected substrates should have sufficient thickness to prevent dislodgement of naturally spawned eggs had they been deposited. Selection of incubator locations should be accomplished well before the spawning season. Anchoringchains and chain leaders to which the incubators will be attached can be rigged and set in the summer months, when field work is easier. Anchoringchains at least 30 m in length should be secured with heavy trapnet anchors.

Egg Sources/Fertilization A target number of 8-12 males and females should be used to produce each source of embryos. This number of parents will result in an excess supply. If 48 incubators (4 strings of 12 each) are deployed at a "treatment" site, 4,800 embryos will be needed for a reciprocal transfer. Requirements for "control" embryos (Lake Superior source) are 2,400 for the Lake Superior site plus a multiple of 2,400 for each "treatment" site in the bioassay. Rnbryos for assessment of eye-up and contaminant burdens will also be needed as discussed later. Sex, length, weight, finclip (if marked), and a scale sample

should be taken from each lake trout parent. Spawnersshculdbenettedinthe vicinity (within 25 km) of each site. Eggs from 8-12 live, anesthetized (100 ppm MS-222) females should be mixed and 2.5 L of eggs retained for dry fertilization with a mixture of milt from 8-12 live males. The anesthetic solution must be rinsed from the parents prior to stripping as it could be toxic to fish sperm and possibly eggs. After water hardening, the~~os~~dbedisinfectedwith100 ppm iodine solution for 10 minutes. All transportation, deployment, or placement of embryos should be accomplished within 2 days of fertilization.

Incubator Deployment Incubators loadedwith embryos can be stored overnight and transported to the site in coolers filled with lake water. At the site the incubators will be attached with nylon self-locking ties to each leader on the anchor line, which should have been previously located by Loran and buoyed. Twelve incubators of each egg source (treatment and control) will be deployed in each habitat type selected for bioassay at each site. Because of differences in spawning times between the lakes, it is unlikely that "controls" and "treatments" will be deployed on the same day. The "control" site in Lake Superior will harbor incubators containing Lake Superior eggs and additional incubators for the other lakes in the bioassay.

Eachim=ubatoristobe~iedverticallyonitslongedgeasdeepaspossible in the spawning gravels. Experience indicates that this depth will normally be 0.5 m or less. Scubadivers shouldbury individual incubators along the anchoring chain at points where dislodgement is unlikely. The maximum area in which the 24 incubators at each site can be buried is approximately 120 m^2, i.e. along a 2-m swath (leader length) on either side of the 30 m anchoring chain. Individual incubators must be numbered to facilitate data recording at the time of retrieval. Also, the upper edge should be marked so that it will be possible to determine which embryos were highest in the sub&rates. After incubators are buried, the marking buoys should be removed from the anchoring line to prevent uprooting of the gear by ice fields later in the winter.

In addition to the caged embryos deployed in the Great Lakes, 3,000 loose embryos of each source will be held under laboratory conditions to determine mortality to the eye-up stage. To keep handling differences between caged and loose embryos to a minimum, the loose embryos should be transported with the caged embryos to the sites and then be returned ashore and placed in a laboratory for rearing. At the laboratory the loose embryos shouldbeheld at simulated ambient temperatures until eye-up as described by Mac et al. (1985).

Each source of embryos should be tested for heavy metals, PCBs, and chlorinated pesticides and scanned by GS/MS for organic contaminants at the green egg stage. In the past most of the analytical focus was on chlorinated organic contaminants, but these toxics have been declining (Devault et al. 1985). Therefore, 400 eggs in lots of 50 will be prepared and tested as described by Mac et al. (1985). However, to increase comparability between years and laboratories, testing should be deferred to a single investigator after the biossay is complete. Parent lake trout and subsequent developmental stages of embryos will not be analyzed for contaminants. It will not be possible to relate survival in individual incubators with parent fish, because of the pooled egg samples. Thus, the burdens in embryos are of most interest.

Incubator Retrieval After ice-out in early spring (probably April) the anchoring lines must be located for incubator retrieval. A combination of visual bearings, compass bearings, depths, and Loran coordinates can be used to approximate the location for search by underwater television/scuba divers. Once located, the individual incubators can be removed from the leaders by severing the nylon ties. However, before removal from the gravels, the interstitial depth, position of the incubator (dislodged or lodged), presence of organics, and incubator number must be recorded. Recording will be greatly simplified by two-way communications between divers and personnel in the tender vessel. Photographs or video of each incubator after excavation, but before removal, may be helpful in accounting for differences in embryo survival among incubators.

The retrieved incubators should be returned intact in well oxygenated water to a laboratory. The embryos at this time will probably be hatching or recently hatched. At the laboratory the cages should be opened and the number of live and dead embryos recorded by life stage and position in the cage.

Laboratory Rearing After enumeration, live embryos from each incubator will be reared separately in aquaria for approximately 140 days to assess delayed mortality associated with contaminants. Relatively small aquaria (39 L) with individual compartments can be used to rear the hatched embryos. The experience in Lake Huron indicated that 10 incubators would produce roughly 150 embryos for laboratory rearing. Rearing procedures and data recording should follow Mac et al. (1985).

Analysis Results from each phase of the bioassay can be analyzed by analysis ofvariance. Differences between lakes andembryo sources are the main effects of interest. Embryos from each incubator, habitat type, source, and site should retain their identity throughout the bioassay. If an experimental effect is evident, it my show as a survival difference between sources at the same site or between sites for the same source. Effects in phase one of the bioassay (fertilization to hatch) would probably show as between site differences, whereas in phase two (hatch plus 140 days), differences between sources are anticipated. To enhance the analysis, equal numbers of incubators of each embryo source should bedeployed at each habitat type.

LIMNOLOGY

It is very desirable to have basic measurements of water chemistry at each experimental site for correlation with embryo survival. Unfortunately, such measurements have little value unless they can be taken at frequent intervals during the incubation period. Manual collection is usually impractical because of restricted winter access, and alternatively, continuous monitoring system are expensive and not readily available. Therefore, water chemistry is not a requirement for the bioassay. However, if the bioassays indicate major survival problems, the employment of continuous monitors may be warranted.

Although water chemistries are not required for the bioassay, the committee does recommend the placement of sediment traps in each habitat type assessed at each lake site. Such traps can provide a measure of organic sedimentation which may correlate with embryo survival. A simple trap designed by B. Manny of the National Fisheries Center - Great Lakes is recommended (Figure 3). However, improve-

merits in securing this trap are needed. Anchoring the sediment traps to an incubator anchoring line may jeopardize the incubators if the traps, which protrude above the gravels, are snagged by ice or woody debris. Therefore, the sediment traps should be anchored independent of the incubators. Analysis of organic matter and particle size of sediments from the traps should be as described by Buckhanan (1971) and Manny et al. (1978). In addition to the sediment data, water temperatures should be recorded when the incubators are deployed and retrieved.

Figure 3. Sedimnttrapconstruchd with 2 inch PVC pipe plugged with No. 11 stopper.

RESULTS/PUBLICATION

A minimum of 2 and more likely 3 years of assessment would be needed to account for year to year variations in the results from the bioassay. Individual investigators/agencies are encouraged to publish their results in major journals either independently or on a collaborative basis. The GLFC'S Technical Report Series is also available for publication. Naturally, results from the control site in lake Superior are crucial for interpretation of any effects observed at the treatmentsites.

Results from phase two of the bioassay would be stronger if all the surviving fry were reared in the same facility so that rearing variables are minimized. The research in phase two of the bioassay is, thus, highly collaborative in that one investigator my rear embryos supplied by another. Therefore, authorship arrangements should be made by the principals before any incubators are deployed.

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