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Field Test of a Bioassay Procedure for Assessing Habitat Quality on Fish Spawning Grounds

BRUCE A. MANNY

U.S. Fish and Wildlife Service, National Fisheries Research Center–Great Lakes
1451 Green Road, Ann Arbor, Michigan 48105, USA

DAVID J. JUDE

Center for Great Lakes and Aquatic Sciences, University of Michigan
Ann Arbor, Michigan 48109, USA

RANDY L. ESHENRODER

Great Lakes Fishery Commission, 1451 Green Road
Ann Arbor, Michigan 48105, USA

Abstract.—A bioassay procedure to assess habitat quality was tested on Port Austin reef in southern Lake Huron, a spawning area of lake trout *Salvelinus namaycush*. In 1986, Plexiglas incubators filled with fertilized lake trout eggs were buried by scuba divers in rock rubble at two sites. The incubators then were attached to chains between large trap-net anchors on the bottom and left over winter. At one site, egg hatch rate was significantly higher in incubators that remained buried in substrate (24%) than in incubators that were dislodged out onto the substrate (13%). At the other, more exposed site, no significant difference was found in percent hatch between eggs that incubated in (10%) and on (8%) the substrate. Percent hatch at both sites was significantly lower than that (40%) of eggs from the same source that were incubated in controlled laboratory conditions. In autumn, concentrations of dissolved ammonia, hydrogen sulfide, and nitrate near bottom and in the substrate posed no threat to lake trout embryos and were not correlated with hatch rate; concentrations differed significantly between the two sites. During winter, 15 cm of sediment settled from the water onto the reef but did not accumulate or smother the eggs. The bioassay procedure is easy to implement, is recommended for use in the Great Lakes, and could be adapted easily for use elsewhere.

Most Great Lakes fishes have demersal eggs (Scott and Crossman 1973; Crowder 1980; Auer 1982; Goodyear et al. 1982). These include species that have long supported valuable fisheries (Baldwin et al. 1979; Talhelm 1988). The consequences of cultural eutrophication (Beeton 1969) on the productivity of fish spawning grounds in the Great Lakes therefore need to be addressed. In 1980, biologists recognized that the loss or degradation of spawning habitat by eutrophication had led to depletion of native fish stocks (Great Lakes Fishery Commission 1980). In 1985, an ad hoc committee[1] of the Great Lakes Fishery Commission formulated a field bioassay procedure for assessing whether reproduction by lake trout *Salvelinus namaycush* had been reduced substantially by cultural eutrophication (Eshenroder 1988). Our goal was to test in the field the procedure developed by this committee.

Eutrophication may physically impede lake trout reproduction by fouling spawning grounds with decaying plant matter (Eshenroder 1988; Sly 1988). Low dissolved oxygen concentrations and waste products that result from plant decomposition (ammonia and hydrogen sulfide) could interfere with embryogenesis, as may have occurred in Seneca Lake, New York (Sly and Widmer 1984). Plastic incubators can be used to study the effects of water degradation on the survival of lake trout embryos on Great Lakes spawning shoals (Sly 1984). It is assumed that eggs in the incubators experience the same environmental stresses as naturally spawned eggs, except for predation and clumping. Two previous attempts to incubate fertilized lake trout eggs on nearshore spawning shoals in Lake Huron met with failure because poorly designed incubators trapped silt and allowed eggs to clump together, and because equipment losses resulted from a combination of inadequate anchoring devices, strong water currents, and ice scour (B. A. Manny, unpublished data). To test the bioassay procedure in the Great Lakes, we

[1] Members of the committee: T. A. Edsall, R. L. Eshenroder (Chair), D. J. Jude, J. R. M. Kelso, J. A. MacLean, and J. W. Peck.

examined performance of the anchoring system and incubators (placement, retrieval, durability, and egg hatching rate), interstitial water chemistry, and rates of sedimentation. The latter two aspects affect development of fish embryos and correlate with embryo survival (Sly 1988) and thereby affect procedure results. Here we summarize results of the first field test.

Methods

Factors not directly associated with water quality or sedimentation, such as predation and fungus infection, affect survival of lake trout eggs under natural conditions (Sly 1984). Therefore, mortality from these sources was avoided by enclosing individual eggs in plastic incubators to relate differences in survival to habitat conditions, which was the focus of the bioassay. We used a Plexiglas incubator similar to that developed by Kennedy (1980) and modified by Gunn and Keller (1984) for research on the effects of acidity on lake trout eggs in small lakes. This incubator held 50 eggs singly in 50 individual compartments, was easy for divers to handle and anchor, and trapped little silt. To make our incubators more durable than those of Gunn and Keller (1984), we used stainless steel fasteners and a thicker (9-mm) center piece with a large hole at one end for attachment to leader chains (Figure 1). The eggs were enclosed in the incubators on either side by 2-mm-mesh Nitex. These incubators exposed embryos to ambient (interstitial) conditions and removed the need for plankton sampling to estimate embryo survival.

Study Site

We conducted our test of the bioassay procedure at two sites in an area where lake trout once reproduced: Port Austin reef in southern Lake Huron (Figure 2; Goodyear et al. 1982). This reef is a submersed limestone outcropping in outer Saginaw Bay (44°03'N, 83°00'W). We expected high sedimentation rates in this area because prevailing currents carry nutrient-enriched water from Saginaw Bay (Ayers et al. 1956). Sites of incubator placement on the reef represented the best spawning substrate as determined both from maps of the bottom compiled from side-scan sonar data (C. L. Brown, National Fisheries Research Center–Great Lakes, unpublished data) and by examination of the reef with underwater television in 1986. Substrate at both sites consisted of a shallow (marginal) layer of angular and rounded limestone rocks and rounded metamorphic rocks underlaid by gravel, sand, and limestone bedrock.

On November 4, 1986, eggs from four male × four female pairings of lake trout (1974 Marquette domestic strain) were fertilized at the Jordan River National Fish Hatchery in Elmira, Michigan. The eggs were water-hardened, given one 10-min treatment with diluted (1:600 by volume) formalin to counteract infection from handling, and placed in incubators. Then the incubators were buried by divers among the rocks on the reef within 48 h. Each incubator was attached to the end of a 3-m leader chain with self-closing nylon ties passed through the large hole at one end of the center piece of the incubator. Ten leader chains were fastened to a 16-m center chain attached at

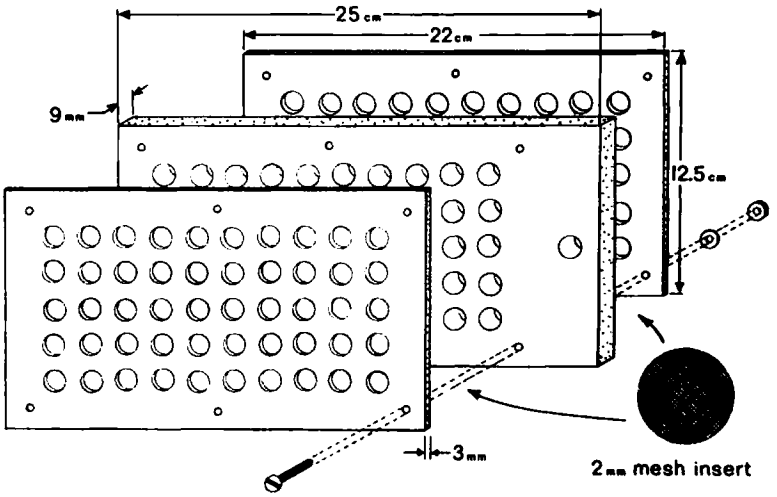

FIGURE 1.—Exploded view of a Plexiglas incubator (including fastener) for fish eggs.

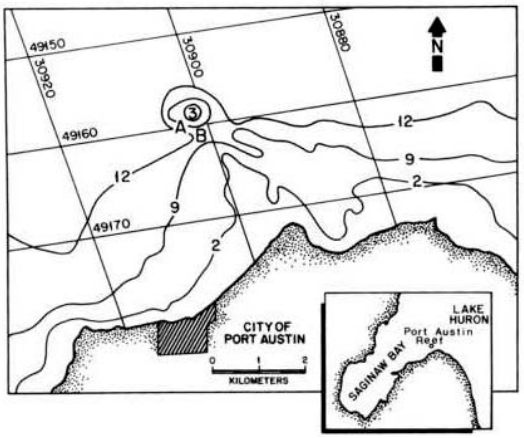

FIGURE 2.—Map showing location of Port Austin reef in Saginaw Bay (insert) and locations of sites A and B, which are superimposed on Loran coordinates. Depth contours are in meters.

each end to large trap-net anchors (Figure 3). Two center chains (20 incubators) were placed at each of the two sites. Site A was about 1.2 km offshore north of Port Austin harbor (Loran coordinates 30903.3 and 49159.6), and site B was about 0.4 km northwest of the Port Austin lighthouse (coordinates 30902.1 and 49161.2). Water depth at both sites was 11–12 m. To test incubator design and performance, divers placed the incubators on edge (lengthwise) on the reef. Sixteen incubators were buried about 10 cm deep in the rock rubble, sometimes beside a boulder. However, 24 incubators were placed on bedrock and covered with a mound of small (3–15 cm) rocks because rock rubble deep enough to bury incubators was not available.

To ensure that eggs were viable and would hatch in the incubators, we filled four incubators with eggs, handled them as we did the others, and then held them with about 3,200 loose eggs (i.e., not in incubators) from the same fertilization in flowing well water at constant temperature (7.5 ± 0.5°C) at the National Fisheries Research Center–Great Lakes, Ann Arbor, Michigan. We removed dead eggs from the loose eggs three times per week but removed none from the Plexiglas incubators. In April 1987, we retrieved the incubators from site B, 9 d after they were retrieved from site A. Retrieval required 2 d and was geared to the divers' schedules. Water temperatures were relatively constant in nearshore Lake Huron, and there were no strong winds at Port Austin during April 14–23, 1987. Therefore, we do not think that hatch or sedimentation changed substantially during the additional 9 d that incubators remained at site B. The chains were easily located by a diver towed on an underwater sled. Because some incubators were dislodged from the substrate by water currents, incubators were classified as being "in" (undisturbed) or "on" the substrate (dislodged by currents) by examination of photographs taken by the diver before retrieval and from scratches and abrasion on the incubators after retrieval.

We assessed water chemistry on the reef on November 6, 1986, by collecting six water samples from interstitial spaces among the rocks and six water samples 1 m above bottom at each site with 50-mL plastic syringes and 2-L "jug" samplers (D. J. Jude, unpublished data). These 24 samples were analyzed for 5-d biochemical oxygen demand (at 20°C), chlorophyll, total ammonia, nitrate, soluble reactive phosphorus, silica, and chloride (Da-

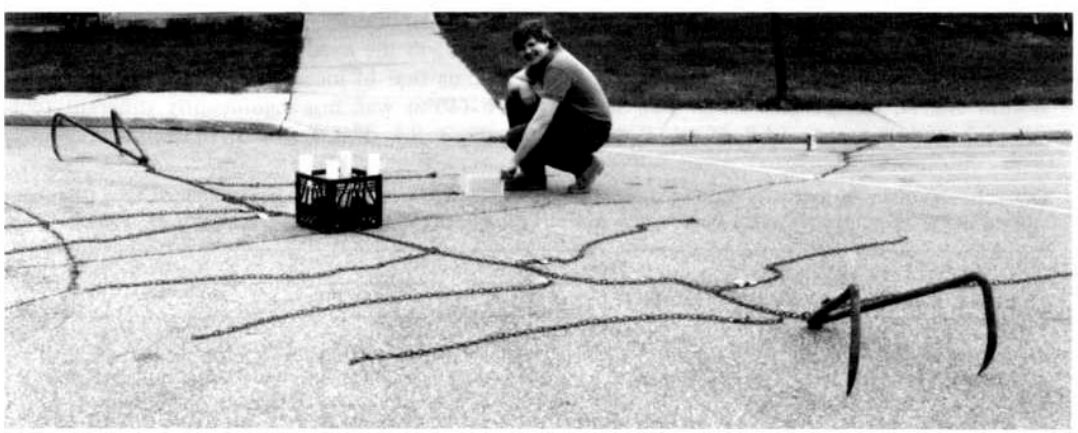

FIGURE 3.—Photograph of the chain, sediment trap, and anchors to which the buried Plexiglas incubators were attached.

vis and Simmons 1979). Water temperatures were measured in situ with a mercury (stem) thermometer. Ice conditions prevented winter measurements of interstitial water chemistry.

Sediment traps consisted of four polyvinyl chloride tubes (52 mm internal diameter, 360 mm high) wired upright in the corners of a cubical plastic frame (40 × 40 × 40 cm); the frames were filled with rocks and anchored by divers to the bottom near one of the center chains at each site in November 1986. The ratio of tube height to tube diameter exceeded 5, as recommended for areas subject to high current velocity (Hargrave and Burns 1979; Bloesch and Burns 1980). In April 1987, divers retrieved the sediment traps and incubators. In the laboratory, we examined the sediments for the presence of decaying plant material (Sly and Widmer 1984; Sly 1988), measured their height in the tube, determined their water, organic matter, and ash content, and measured their particle size composition after ignition (Buchanan 1971). We used chi-square and Student's t-tests to evaluate hatching results and analysis of variance to evaluate water chemistry results.

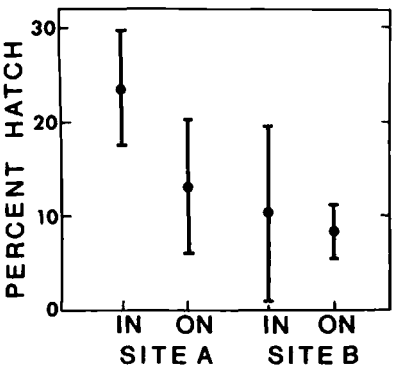

FIGURE 4.—Mean hatch of fertilized lake trout eggs in Plexiglas incubators at two sites on Port Austin reef in southwestern Lake Huron. Vertical lines represent 95% confidence intervals. IN signifies incubators remained buried in the substrate; ON signifies incubators were dislodged out onto the subtrate. Number of incubators used: 11 for IN and 7 for ON at site A, 4 for IN and 16 for ON at site B.

Results

Stainless steel fasteners used to hold our incubators together loosened and allowed eggs to escape from 2 of the 40 incubators; this left 38 intact incubators (18 at site A and 20 at site B; Table 1). Of these 38 incubators, 7 at site A and 16 at site B were dislodged, presumably by water currents, and 15 remained buried.

Scouring by water currents was more severe at site B than at site A. At site B, the sediment trap was overturned; coarse sand was found in the incubators; many incubators were dislodged from the substrate; and incubators that were dislodged were extensively scratched and rounded by abrasion.

Within site A, the percent hatch was significantly higher (chi-square = 14.8, df = 1, $P \leq 0.01$) for eggs in incubators that remained buried in the substrate than for eggs in incubators that were dislodged. Within site B, there was no significant difference between percent hatch in and on the substrate (chi-square = 1.0, df = 1, $P \leq 0.33$). Between sites A and B there was no significant difference in percent hatch for eggs in incubators that remained buried in the substrate or for eggs in incubators that were dislodged (Figure 4). The percent hatch for eggs in control incubators in the laboratory (40 ± 16%, N = 4, 95% confidence interval; Table 2) was not significantly different from that for eggs in the substrate at site A ($P \leq 0.05$) but was significantly higher than that for eggs on the substrate at site A and that for eggs either in or on the substrate at site B (Figure 4).

In the laboratory, incubator effects were minor; survival to the eyed stage in incubators was the same as that of loose eggs (62%), and the hatch rate (40%) was not significantly different (chi-square = 8.1, df = 4, $P \leq 0.10$) from that of loose

TABLE 1.—Number of fertilized eggs used, number of sac fry retrieved, and percent hatch of lake trout eggs incubated in Plexiglas incubators on Port Austin reef in Lake Huron, November 6, 1986–April 23, 1987.[a]

Site and position	Number of incubators	Eggs		Fry		Mean % hatch (SE)
		Mean (SE)	N	Mean (SE)	N	
Site A						
In substrate	11	49 (0.5)	540	12 (1.4)	129	24 (2.7)
On substrate	7	49 (0.4)	345	7 (1.5)	46	13 (2.9)
Site B						
In substrate	4	48 (0.6)	191	5 (1.2)	20	10 (2.5)
On substrate	16	47 (0.7)	752	4 (0.6)	62	8 (1.4)

[a] Incubators were retrieved on April 14, 1987, from site A and on April 23, 1987, from site B. Sac fry were 15–18 mm long.

TABLE 2.—Percent eye-up and hatch of fertilized lake trout eggs held loosely and in Plexiglas incubators at the National Fisheries Research Center–Great Lakes in Ann Arbor, Michigan, November 6, 1986–January 30, 1987.

Egg treatment	Number of eggs	Eyed eggs		Number of fry	% hatch
		N	%		
Loose	3,191	1,979	62	1,438	45
In incubator					
1	50	30	60	19	38
2	50	34	68	27	54
3	45[a]	25	56	13	29
4	47[a]	30	64	18	38
Average	48	30	62	19	40

[a] Corrected for dead eggs present in incubators at start of incubation.

eggs (45%; Table 2). Fungus infecting dead eggs in the incubators did not spread to live eggs in adjacent compartments.

Water chemistry values were lower than those that are lethal to lake trout eggs or fry (Thurston et al. 1979). Ammonia, nitrate, and silica concentrations in November were significantly higher, and chloride concentrations were significantly lower at site A than at site B (Tables 3, 4). Nitrate concentrations were significantly higher in near-bottom waters than in interstitial waters at site B. No other differences in water chemistry were significant. Biochemical oxygen demand of interstitial waters was relatively low (4.1–5.8 mg/L). Lake water temperature was 8.5°C during incubator placement and 5.8°C at the time of retrieval. Chlorophyll concentrations in the water column over Port Austin reef (3.9–4.8 μg/L) were in the range normally associated with oligotrophic to mesotrophic waters (Vollenweider et al. 1974; Wetzel 1975), which would produce small to moderate amounts of organic matter.

The trap at site A collected 15 cm of sediment (Table 5), but at site B, the trap was overturned by strong currents so no measurement of sediment accumulation was made. Sediments in each tube were a dark-brown mixture of sand and mud plus a small amount of fibrous organic matter. They contained no decaying plant material and little organic matter, and they were predominantly (70%) medium and fine sands, 125–500 μm in diameter.

TABLE 4.—Analysis of variance of water chemistry results from six replicate measurements of each variable made once at each of two locations (L) and depths (D); 24 observations are included. Asterisks indicate significant values ($P \leq 0.05$*).

Variable	Source of variation	Sum of squares	*F*-value[a]
Ammonia	L	156.264	7.41*
	D	0.009	0.00
	L × D	13.620	0.65
Nitrate	L	0.023	2,163.46*
	D	0.000	18.85*
	L × D	0.000	3.46
Silica	L	0.378	2,188.43*
	D	0.001	4.08
	L × D	0.000	0.02
Chloride	L	23.443	7.83*
	D	2.369	0.79
	L × D	1.664	0.56
Phosphorus	L	26.230	1.89
	D	27.843	2.01
	L × D	14.183	1.02

[a] All with 1 df.

Discussion

Coarse substrates on shoals in nearshore waters of the Great Lakes are often rearranged by storms (Sly and Schneider 1984). Because our anchoring system remained in place and none of our equipment was lost, we believe the design and deployment of the bioassay gear described here were adequate to withstand storm forces likely to be encountered in nearshore areas of large waters. Evidence of ice scour on the bottom to depths of 10 m has been observed on spawning shoals used by lake trout in eastern Lake Ontario (P. G. Sly, Rawson Academy of Aquatic Science, personal communication). To avoid ice scour, the anchoring system and bioassay gear should be deployed in waters greater than 10 m deep. However, lake trout often spawn in the Great Lakes on shoals in

TABLE 3.—Mean values (SE) of chemical variables in near-bottom and interstitial waters at two sites on Port Austin reef in Lake Huron, November 6, 1986; $N = 6$.

Site	Stratum	Ammonia (μg/L)	Nitrate (mg/L)	Silica (mg/L)	Chloride (mg/L)	Soluble reactive phosphorus (μg/L)
A	Near-bottom	11.2 (2.5)	0.200 (0.000)	0.54 (0.00)	8.1 (0.1)	2.1 (0.4)
	Interstitial	12.7 (1.8)	0.197 (0.002)	0.55 (0.01)	6.9 (1.4)	2.8 (0.4)
B	Near-bottom	7.6 (2.1)	0.140 (0.000)	0.29 (0.00)	9.5 (0.1)	1.6 (0.2)
	Interstitial	6.0 (0.3)	0.132 (0.002)	0.30 (0.00)	9.4 (0.0)	2.2 (0.5)

TABLE 5.—Physical and chemical characteristics of sediments collected in traps on Port Austin reef in Lake Huron, November 6, 1986–April 14, 1987; $N = 4$.

Characteristic	Mean (SE)
Height in trap (cm)	15 (0.4)
Wet weight (g)	550 (8)
Dry weight (g)	262 (23)
Ash weight (g)	255 (24)
Water content (%)	51 (4)
Organic matter content (%)	3 (1)
Ash content (%)	46 (4)
Substrate composition (particle size in μm)[a]	
Coarse sand (>500)	11 (2)
Medium sand (500–250)	55 (2)
Fine sand (250–125)	15 (1)
Very fine sand (125–63)	11 (1)
Silt and clay (<63)	8 (2)

[a] Means for substrate components are given as percent of total ash weight.

3–6 m of water (Goodyear et al. 1982; Wagner 1982) where deployment of such gear becomes a calculated risk.

The stainless steel fasteners held two of the Plexiglas incubators together inadequately. We used steel fasteners in preference to nylon (Gunn and Keller 1984) because steel is more durable. We have since learned that nylon is superior to steel because nylon swells slightly under water and thus prevents fastener failure. None of the 96 Plexiglas incubators fastened with nylon nuts and bolts and deployed over winter in the Great Lakes since this test was conducted have come apart (Manny, unpublished data).

Other aspects of incubator performance were satisfactory. Fungus infecting dead eggs in the incubators did not affect live embryos in adjoining compartments under either field or laboratory conditions. Also, silt was not trapped in the incubators, and periphyton growth did not reduce mesh porosity. In these respects, performance of the Plexiglas incubators was superior to that of stainless steel incubators perforated with 3-mm openings that were deployed in 1985 at four nearshore locations in western Lake Huron, including Grindstone City, 8 km south of Saginaw Bay (Manny, unpublished data). Despite the larger porosity of the steel incubators and greater distance of Grindstone City from sources of cultural eutrophication in Saginaw Bay, much silt and periphyton accumulated in the steel incubators and all eggs in them died. Therefore, we believe our 1986 study on Port Austin reef was an adequate test of whether the Plexiglas incubators trap silt or impede water flow when exposed to cultural eutrophication. However, we did not test whether conditions in the incubator chambers deviated from natural physiochemical conditions outside the incubator.

Interpretation of our results was complicated because a large percentage of the incubators (39% and 80% at sites A and B, respectively) did not remain buried in the shallow (marginal) substrates as we intended. We did not know exactly where lake trout had spawned previously on Port Austin reef (Goodyear et al. 1982; Nester and Poe 1987), so we placed the incubators on substrate found by divers that most resembled "ideal" spawning habitat described by Wagner (1982) (i.e., rock rubble with deep interstices). Using a video camera mounted on a remotely operated vehicle, we subsequently examined the lake bottom at 17 other areas on and near Port Austin reef in 1987 and found four areas of rock rubble with deeper interstitial spaces than were present at sites A and B (National Fisheries Research Center–Great Lakes, unpublished data). Forty-eight incubators were buried in these four areas in 1987 as described previously; they all remained buried throughout the winter (Manny, unpublished data). Therefore, the many dislodged incubators found during our 1986 test were strictly a result of the shallow substrates at sites A and B and not the result of any shortcoming in this procedure. High mortality of embryos in dislodged incubators that were extensively scratched and rounded by abrasion may have been caused by mechanical shock to eggs before the eyed stage, when trout embryos are sensitive to jarring (Piper et al. 1982). We deployed too few incubators at each site (20) to detect significant differences in percent hatch between sites. Low hatch rates of eggs both in and on lake substrates (8–24%) relative to laboratory controls (40–45%) indicated that some mortality was induced by an unknown factor in the lake. However, some eggs hatched successfully in incubators on Port Austin reef, and this suggests that spawning substrate capable of producing viable fry exists in this area.

In November on lake trout spawning reefs in Lake Ontario, total ammonia concentrations in interstitial waters (0.5–1.7 mg/L; Sly 1988) exceeded those we measured on Port Austin reef, but the portion present as nonionized ammonia nitrogen was still less than the amount that is toxic to salmonid fry (Thurston et al. 1979). The lower (sometimes equal) ammonia values in interstitial waters compared with overlying waters (Table 3) suggest that no decomposition products accumulated on Port Austin reef in November. However, winter measurements would be needed to deter-

mine whether any subsequent organic matter decomposition affected embryogenesis.

We believe that the sedimentation rates we measured did not accurately portray sediment accumulation in and on the spawning substrate because underwater photographs made in April 1987 showed that little sediment actually accumulated on the reef during winter. However, we think that sedimentation rates measured in tubes are useful because they integrate environmental conditions over the period of incubation and permit comparisons among sites of the amount and kind of sediments falling onto spawning grounds. Such measurements may partly explain differences in embryo survival among sites. Sediments falling on Port Austin reef were probably resuspended from areas inside Saginaw Bay and carried to the reef by prevailing currents (Ayers et al. 1956). Their physical and chemical composition resembled that of sediments near shore in Saginaw Bay (Robbins 1986).

In conclusion, the Plexiglas incubators performed well by exposing individual embryos to lake bottom conditions, preventing the spread of fungus, and allowing sediments to pass through. To ensure that incubators remain buried in substrate, they should be buried 5–10 cm below the substrate surface among rock rubble with deep (>30 cm) interstices. Interstitial water chemistry measurements could be made at intervals during the incubation period by small dialysis chambers in the spawning substrates (Sly 1988). In the Great Lakes, such measurements may be impractical for fall-spawning fishes because winter access is restricted. The simple, inexpensive sediment trap we used is adequate if it is anchored independently of the incubators and buried until the tube tops are at the spawning substrate surface to avoid snagging by debris.

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