GREAT LAKES FISHERY COMMISSION
RESEARCH COMPLETION REPORT [1]

STUDIES OF HOMING AND REPRODUCTIVE BIOLOGY OF LAKE TROUT (PART 3--REARING AND STOCKING OF EARLY LIFE STAGES) [2]

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[2] Includes: (1) a manuscript which was presented at the International Symposium and Workshop on Fish-Marking Techniques held in Seattle, Washington during June, 1988. The manuscript will be published in the symposium proceedings. (2) an appendix to the manuscript, detailing marking activities during 1988. (3) video tape of the 1988 fry stockingavailable from the secretariat.

Abstract

The otoliths of 676,000 sac fry of lake trout Salvelinus namaycush in 1986 and 1,100,000 in 1987 were marked by daily manipulation of water temperature and stocked into Lake Huron in the spring. Otolith marks consisted of groups of daily growth rings accentuated into recognizable patterns by steadily raising and lowering the temperature about 10°C (from a base of 1-4°C) over 14 h. In 1987, groups of marked and control fish were held for 6 months. The otoliths were removed from samples of the fish, embedded in epoxy, thin-sectioned by grinding in the sagittal plane, etched, and viewed by using a combination of a compound microscope (400X-1000X) and a video enhancement system. One or more readable otolith sections were obtained from 39 of a sample of 40 fish. Three independent readers examined 41 otoliths for marks and correctly classified the otoliths, with accuracies of 85, 98, and 100%, as being from marked or unmarked fish. The exact number of rings in a recognizable pattern sometimes differed from the number of temperature cycles to which the fish were exposed. Counts of daily rings within groups of six rings varied less than within groups of three rings.

Introduction

During the 1940s and 1950s, lake trout Salvelinus namaycush became severely depleted in Lake Superior and probably reached or neared extinction in the other Great Lakes. The sequence of events leading to the declines was reviewed in detail for Lake Huron by Berst and Spangler (1973), for Lake Ontario by Christie (1973), for Lake Erie by Hartman (1973), for Lake Superior by Lawrie and Rahrer (1973), and for Lake Michigan by Wells and McLain (1973). The declines were generally attributed to a combination of fishery exploitation and lethal attacks by the sea lamprey Petromyzon marinus. Control of the sea lamprey with a selective lamprey larvicide (Pearce et al. 1980; Smith and Tibbles 1980) and the later stocking of more than 100 million yearling lake trout (Great Lakes Fishery Commission 1983) did not result in appreciable reproduction, except in Lake Superior where remnant populations contributed to the recovery (Eshenroder et al. 1984).

Inadequate use of appropriate spawning sites has been hypothesized as being a factor contributing to the failure of the restoration of natural lake trout reproduction (Eshenroder et al. 1984). Lake trout were routinely stocked as yearlings at historic spawning sites, but the fish apparently did not imprint, and returned to spawn at those sites in lower

proportions than native fish (Krueger et al. 1986). Binkowski (1984) and Foster (1984) suggested that the stocking of earlier life stages might be more effective in imprinting lake trout to potential spawning sites. However, an evaluation of this hypothesis requires a marking method that differentiates between fish stocked as fry and fish stocked as yearlings, as well as between stocked and naturally produced young.

Because the methods currently used for marking lake trout in the Great Lakes (fin clipping and coded-wire tags) are most applicable to fingerlings or larger fish, Brothers (1988, this volume) tested in the laboratory a technique for placing thermal marks on the otoliths of very young lake trout. We used this technique to mark large numbers of lake trout sac fry stocked on an offshore reef in Lake Huron during 1986-87. We here describe the large-scale implementation of an inexpensive and effective method (Brothers 1988, this volume) for thermally marking the otoliths of large numbers of young fish by subjecting them to diel temperature cycles, describe and evaluate preparation of the otoliths for decoding, and evaluate the readability of the mark.

Methods

Marking

We subjected three groups of lake trout sac fry to diel temperature cycles, as suggested by Brothers (1988, this volume), to produce identifiable marks on their otoliths. Otoliths of lake trout of the Lake Superior strain were marked during spring in 1986 and 1987, and otoliths of lake trout of the Seneca Lake strain were marked only in spring 1987. In both years, control groups of lake trout of the Lake Superior strain were held under identical conditions. All lake trout were obtained from the Iron River, Wisconsin National Fish Hatchery as eyed eggs and incubated at the Hammond Bay Biological Station in eight 16-tray Heath[1] incubators. Each incubator was supplied with filtered Lake Huron water at ambient temperatures at the rate of 7 L/min. Fry density was about 10,000 per tray, as judged by estimates of total numbers of eggs and number of trays used.

During marking, each of four incubators was supplied with 4.0 L/min of water from a 1,900-L mixing tank in place of the normal supply. The mixing tank was supplied with a constant flow (16 L/min) of ambient temperature water and two potential flows (each 2.1 L/min) of heated water (58°C) controlled by solenoid valves. Water was heated in a propane-fired water

heater. The solenoid valves were opened and closed by timers at the same times each day to increase and decrease the temperature over the ensuing 14 h. During each temperature cycle, one solenoid valve was on for the first 3 h, both for the next 4 h, and one for the next 3 h. The water temperature in the mixing tank then returned to ambient in about 4 h, and remained there until the start of the next cycle. The large volume of water in the mixing tank resulted in relatively even changes in temperature (Figure 1). For all marks applied, the mean ambient temperature at the start of the cycle and the mean rise in temperature (minimum and maximum values in parentheses) were 2.4°C (1.1-4.4°C) and 10.6°C (9.4-12.8°C), respectively. Control fish were held at ambient temperatures.

Marks consisted of one or two groups of accentuated daily rings that formed recognizable patterns. Sac fry were exposed to the described temperature cycle for a number of consecutive days to produce a corresponding number of accentuated daily rings (i.e., a group of rings) on the otoliths. If more than one group of rings was desired, a space between them was produced by holding the sac fry at ambient temperatures for 9-13 d.

Lake trout of the Lake Superior strain stocked in 1986 were marked with a group of five rings followed by a group of two (mark = 5,2). On 23 April 1986, 570,000 sac fry marked

5,2 were released on Six Fathom Bank in Lake Huron (Figure 2). Lake Superior strain lake trout stocked in 1987 were marked with a group of three accentuated daily rings followed by a group of six (mark = 3,6). However, because heated water could be supplied to only four incubators at a time, the sac fry were marked in two lots. On 14 April 1987, 1,100,000 sac fry marked 3,6 were released on Six Fathom Bank (Figure 2). Seneca Lake strain lake trout stocked in 1987 were marked with a single group of four accentuated daily rings (mark = 4). On 13 March 1987, 106,000 sac fry marked 4 were released near Arnold Island in Lake Huron (Figure 2).

Reference collections from each marked group were taken 2-4 d after marking was completed. In addition, control fish and fish marked 3,6 were held at the Hammond Bay Biological Station for 6 months after they were marked. All lake trout sacrificed for the reference collections were preserved and stored in 95% ethanol until the otoliths were removed.

During spring 1988, we conducted an experiment to measure the effect of the marking procedure on survival. Four groups of 500 sac fry each were exposed to nine consecutive diel temperature cycles as described above. Four groups of 500 sac fry each were also held as controls. The mean numbers dead in control and marked groups after 90 d were compared by analysis of variance.

Sectioning of Otoliths

The otoliths (sagittae) were located and removed from the preserved lake trout with the aid of a dissecting microscope (at 12-50x) and air dried. Beyond this point, the method of preparation differed, depending on the size of the otolith.

Each of the small otoliths taken from sac fry were individually placed sulcus side up in a drop of thin-section epoxy (Hillquest, Seattle, Washington, USA) on a glass slide. Slides were then placed in an oven at 55°C for 5 to 10 min to remove air bubbles. Upon removal from the oven, the otoliths were reexamined for proper orientation and repositioned if necessary. Any remaining air bubbles were removed with a needle. Setting of the epoxy required overnight storage at room temperature.

Small otoliths were ground to the mid-sagittal plane with 600-grit, silicon carbide sand paper, followed by 1,000-grit, silicon carbide grinding abrasive (Bruce Bar, Bruce Products, Howell, Michigan, USA) on a glass plate. It was not difficult to locate the mid-sagittal plane. The first area of continuous growth surrounding the otolith core structure (Geffen 1983; Brothers 1988, this volume) was relatively featureless and the hatching mark formed a well-defined reference mark (Figure 3). We periodically checked each

otolith microscopically (40-400X) during the grinding process and continued to remove material until the hatching mark was visible. If it was visible, any thermal marks laid down outside it would also be visible.

After the otoliths were ground, they were polished with aluminum oxide polishing compound on a wet felt lap and etched with 1% (0.03 N) HCl for 15 to 30 s to enhance microstructural features. The weak acid accented the surface contours by removing material from the proteinaceous or dark portion of the growth increment (Pannella 1980; Mugiya et al. 1981). A small camel's-hair brush was used to expose the reactive surface to the acid and also to remove any gas bubbles that blocked the field of view. Etching time was best regulated by observing the process under a dissecting microscope; continuous observation was particularly important for small otoliths because it helped prevent over-etching and loss of microstructural features.

Larger otoliths (from fingerling lake trout held for 6 months after marking) were more opaque and required grinding on both sides. Caps from 1.5-mL microcentrifuge test tubes (Bio-Rad Laboratories) were used as molds for embedding the otoliths. The caps were tapered and slightly wider at the open end, which helped secure the preparation while the first side of the otolith was ground. The caps were filled with

thin-section epoxy and an otolith was positioned sulcus side down in each. The epoxy was then heated as previously described.

After setting overnight, the epoxy plugs were pried from the caps, placed on a hard flat surface with the otolith end down, and the caps were forced partly over them so that the embedded otolith remained beyond the cap lip. The cap was then used to grip the epoxy plug during grinding. Otoliths were ground to the mid-sagittal plane on the first side with 600-grit, silicon-carbide sand paper, followed by 1000-grit, silicon-carbide grinding abrasive on a glass plate. The epoxy plugs were periodically checked microscopically (40-400X) to ensure that they were ground precisely to the mid-sagittal plane.

After the first side of the otolith was ground, the flat surface was washed with 95% ethanol, polished, air dried, and bonded to a glass slide with a drop of cyanoacrylate glue. Epoxy resin overlying the otolith was removed with 400-grit sand paper before the second side was ground, polished, and etched as described for small otoliths.

To evaluate the success rate of our sectioning technique, we attempted to remove and section the otoliths from a sample of 40 lake trout of the Lake Superior strain (20 control fish

and 20 marked 3,6) sacrificed 6 months after marking. Sections were permanently mounted and later evaluated for mark recognition.

Mark Recognition

The otolith sections were wetted with mineral or immersion oil and viewed without a coverslip by using a compound microscope and transmitted light. The magnification required to detect a thermal mark on an otolith varied from 400X for the best sections to 1,000X for most other sections. Counting the number of accentuated daily rings within a group of rings always required 1000X. The camera port on the microscope was fitted with a high resolution video camera that enhanced both resolution and contrast. The camera was helpful in the counting of individual rings and resolving uncertainties about some of the marks.

In an evaluation of the readability of the marks, three readers independently examined 41 permanently mounted otolith sections. Two of the readers had previous experience in aging fish from otoliths; the third, an experienced histologist, did not. The test sample consisted of 19 otolith sections from unmarked control fish and 22 from fish marked 3,6 (1 each from 18 fish and 2 each from 2 fish). The slides were viewed in a random sequence with knowledge that the marked fish had been

marked 3,6. The reader was asked to (1) judge whether each fish was marked or unmarked and (2) count the number of rings evident in each group of rings. Because the reading of each otolith had two possible outcomes (correct or incorrect), the proportion of fish correctly identified as marked or unmarked by the three readers and the 95% confidence intervals were calculated by using the binomial distribution.

Results and Discussion

Marking

The sac fry showed neither unusual behavior nor increased mortality during the marking process. Although the rise in temperature was as much as 12.8°C during a temperature cycle, the sac fry never appeared distressed. Daily estimates of mortality were not attempted, but the numbers of sac fry lost in 1986 and 1987 were low from the start of marking through stocking, and no increases in mortality associated with marking were noted. In 1988, the mean number dead after 90 d in four lots of 500 control fry was 10.5 (SD = 4.65) and in four lots of 500 marked fry was 13.8 (SD = 6.34). The number dead in the marked groups was not significantly different (ANOVA, $P=0.44$) than in the control groups. Examination of a

more delayed mortality caused by the temperature changes was not within the scope of this study but should be investigated.

The marking process was inexpensive relative to the number of fish that could be marked. The flow of heated water was controlled by washing-machine solenoid valves that were, in turn, controlled by ordinary appliance timers. Because no adjustments were required after a series of temperature cycles began (Figure 1), personnel costs were low because only periodic checks of the equipment were required. The largest cost was that for propane used to heat the water. Assuming a 100% efficiency, each cycle (Figure 1) required 8.2 kg of propane to supply heated water to four 16-tray incubators, or about 74 kg for the 3,6 mark. Considering that our apparatus was used to mark about 650,000 fry at a time, these costs were low.

Sectioning of Otoliths

We successfully prepared sections of one or both otoliths from 39 of 40 fingerling lake trout. We believe that, with additional practice, readable sections could be prepared from nearly all fish attempted.

Because thermal marks were not equally clear and well defined in all sectors of an otolith, it was critical that the

section be kept in the sagittal plane. On most otoliths, daily growth increments, including thermal marks, were better defined on the posterior portion. In some, only a limited sector toward the posterior end of the otolith contained enough detail to allow interpretation. If that end of the otolith was tilted downward during grinding, the area where the thermal marks were best defined could be lost before the hatching mark was revealed.

The sectioning of otoliths marked in an early life stage and later recovered from fish at a larger size is a difficult but manageable task. Although we sectioned only otoliths taken from fingerlings, we are confident that the technique will also yield reliable results with those from larger fish. Because of the small size of the otoliths at marking, the greatest challenge was to obtain a section through the small area that contained the marks. If the absence of marks is also of interest, it is absolutely critical that the section be made in an appropriate plane. Otherwise, marks will not be seen, and the fish would be misclassified as unmarked. Use of the hatching mark to establish the proper plane consistently resulted in sections that were suitable for determining the presence or absence of marks. Volk et al. (1984) used the otolith core as a guide for establishing sections through the centers of otoliths. Either the core or hatching mark should serve well as a reference mark. However, if marking was done

before hatching, the otolith core would of course have to be used as the guide.

The process of grinding, polishing, and mounting otoliths by hand was very exacting and severely limited the number of sections that could be prepared each day. One person could process no more than 25 otoliths in a day if both sides were to be ground. With experience, greater productivity should be possible and elimination of acid etching might further accelerate the sectioning process without reducing readibility (E. Brothers, EFS Consultants, Ithaca, New York 14850 USA, personal communication). The number of otoliths that can be sectioned per day might also be increased by grinding multiple otoliths of similar size at the same time (E. C. Volk, Washington Department of Fisheries, Olympia, Washington 98504 USA, personal communication).

Mark Recognition

Daily marks on the otoliths consisted of a broad translucent zone, followed by a narrow opaque zone. Differences in the transparency of these zones gave the appearance of hills (translucent zones) and valleys (opaque zones). The temperature cycles increased both the width and the contrast of the daily increments. The most visible feature of daily marks was the narrow opaque zones (Figure 4)

that Brothers (1988, this volume) associated with the end of each temperature cycle (thermal night). He also believed that the fluctuations in temperature accentuated the contrast between these elements. A series of temperature cycles produced a regular pattern that was difficult to mistake for one produced by random events (Figure 4). The broader translucent zones were formed during the periods of elevated temperature and were wider than normal daily increments at ambient temperatures.

In counting rings, we believed it was best to concentrate on the opaque zones because the inner edge of the first translucent zone was often poorly defined (see the inner edge of the 6-ring group in Figure 4). However, it was also important to realize that a distinct opaque zone preceding the first translucent zone may not have been due to the marking process. If a distinct random mark (as in Figure 3) preceded the marking process by a day, the illusion of an additional mark could be created (as on the inner edge of the 3-ring group in Figure 4).

Otoliths from control fish were not featureless. Some of the daily rings on otoliths from control fish were as distinct as those accentuated by the temperature cycles (Figure 3). These usually appeared in random patterns, but in some cases a series of two or three strong rings (Figure 3) could be

mistaken for thermal marks. The distinctness of natural daily rings tended to increase with distance from the otolith core. Confusion between strong natural daily rings and thermally accentuated rings on lake trout otoliths would be minimized if marking occurred before or soon after hatching.

Otolith sections from reference collections of sac fry taken 2-4 d after marking failed to show all the rings from the most recent mark. The outer rings appeared to be crowded near the edge and were difficult to count (Figure 5). However, unsectioned otoliths examined fresh at the time the reference collections of sac fry were taken all clearly showed the presence of the most recent marks. We are not sure of the reason for this discrepancy. Because the marks were clearly present in fresh specimens 2-4 d after marking and in specimens sacrificed 6 months later, the marks must have been present when the reference collections were taken. The outer marks were either obscured by diffraction at the edge of the otolith, or etching and sectioning affected the thin outer margin. Rings should be visible shortly after marking is completed, but to minimize the possibility of losing information near the edge, we advise holding fish for at least a week after marking before any reference collections are taken. Because of this problem, our evaluations were confined to collections taken in 1987, 6 months after marking.

Independent reading of a blind sample of 41 otolith sections from fish held 6 months after marking indicated that thermally marked fish could be separated from control fish with reasonable certainty. Of 41 sections examined all were properly classified by the first reader, 40 (98%) by the second, and 35 (85%) by the third. Overall, two marked fish were misclassified as controls and five control fish as marked. The 95% confidence intervals for the proportions of otolith sections that would be classified correctly by the three readers in similar samples of the same size were 93-100%, 92-100%, and 73-97%.

Our success in classifying marked and control fish suggested that, if otoliths are properly sectioned and if the information is retained on the otoliths of adults, we could identify thermally marked adult lake trout. The three readers combined correctly identified 94% of the otoliths examined, and the two readers who were experienced at aging fish from otoliths together correctly identified 99%. This observation further suggests that a reader with experience should be able to identify marked fish with almost no errors.

Counts of individual rings within groups of rings frequently differed from the number of temperature cycles to which the fish had been exposed. None of the counts for the six-ring groups differed from the expected value by more than

one ring (Table 1). For the three readers combined, 89% of the six-ring groups were judged as containing six rings. The variability in counts was greater for the three-ring groups (Table 1), where counts ranged from zero (-3 rings) to four (+1 ring). For the three readers combined, only 58% of the three-ring groups were judged as containing three rings. The readers were aware of the expected counts, and variation in a blind test would probably have been greater.

The variability in our counts of individual rings within the 3-ring and 6-ring groups suggests two conclusions: (1) with the marking procedure we used, counts often vary by at least one from the count expected and (2) it was less difficult to count the rings in the 6-ring group than in the 3-ring group. Because the thermally induced marks were usually strong, we believe that the reader errors were largely caused by uncertainty about whether naturally occurring rings were also part of the mark. Because the regular pattern established in a group of six consecutive rings made it easier to judge whether a ring was part of the pattern, we believe that five or more rings should be grouped together. Control of lighting and ambient temperatures (not done in our study) might also reduce the number of strong, naturally occurring rings and thereby reduce variability of the counts.

Thermal marking of otoliths, as we approached it, would have its greatest utility in situations where a large number of marks are not required. Otherwise, the variability in ring counts would result in confusion between marks. Because we had few groups of fish to separate, it was possible to use marks that were very different and not easily confused. Many important issues concerning contributions of hatchery and wild fish could also be settled using a small number of distinct marks. With more precise temperature control, more elaborate marks are possible (Brothers 1988, this volume).

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[1] Reference to trade names does not imply endorsement by the U.S. Fish and Wildlife Service.

Table 1.--Distribution of the number of rings counted (percentages in parentheses) by three independent readers on 22 otoliths marked with groups of three and six daily rings accentuated by temperature manipulations. Because readers 1 and 3 misclassified a marked fish as unmarked and readers 2 and 3 also did not assign a count to a three-mark group, row totals are less than 22.

	Number of rings counted in three-mark group					Number of rings counted in six-mark group		
Reader	0	1	2	3	4	5	6	7
1		1 (5)	9 (43)	11 (52)			19 (90)	2 (10)
2	2 (10)	1 (5)	7 (33)	9 (43)	2 (10)	2 (9)	19 (86)	1 (5)
3			4 (20)	15 (75)	1 (5)		19 (90)	2 (10)
Total	2 (3)	2 (3)	20 (32)	35 (56)	3 (5)	2 (3)	57 (89)	5 (8)

Figure Captions

Figure 1.--Water temperatures during two temperature cycles used to mark otoliths of lake trout sac fry, 30-31 March 1987. Heating cycles began at about 0800 hours each day.

Figure 2.--Stocking sites for lake trout sac fry with thermally marked otoliths stocked during 1986-1987.

Figure 3.--Section of an otolith taken from an unmarked fingerling lake trout (control) sacrificed 6 months after the marking period. H = hatching mark. Bar = 200 µm.

Figure 4.--Section of an otolith marked 3,6 taken from a fingerling lake trout sacrificed 6 months after the marking period. OZ = opaque zone. TZ = translucent zone. Bar = 20 µm.

Figure 5.--Section of an otolith marked 3,6 taken from a lake trout sac fry sacrificed 2 d after the marking period. E = otolith edge. Bar = 20 µm.

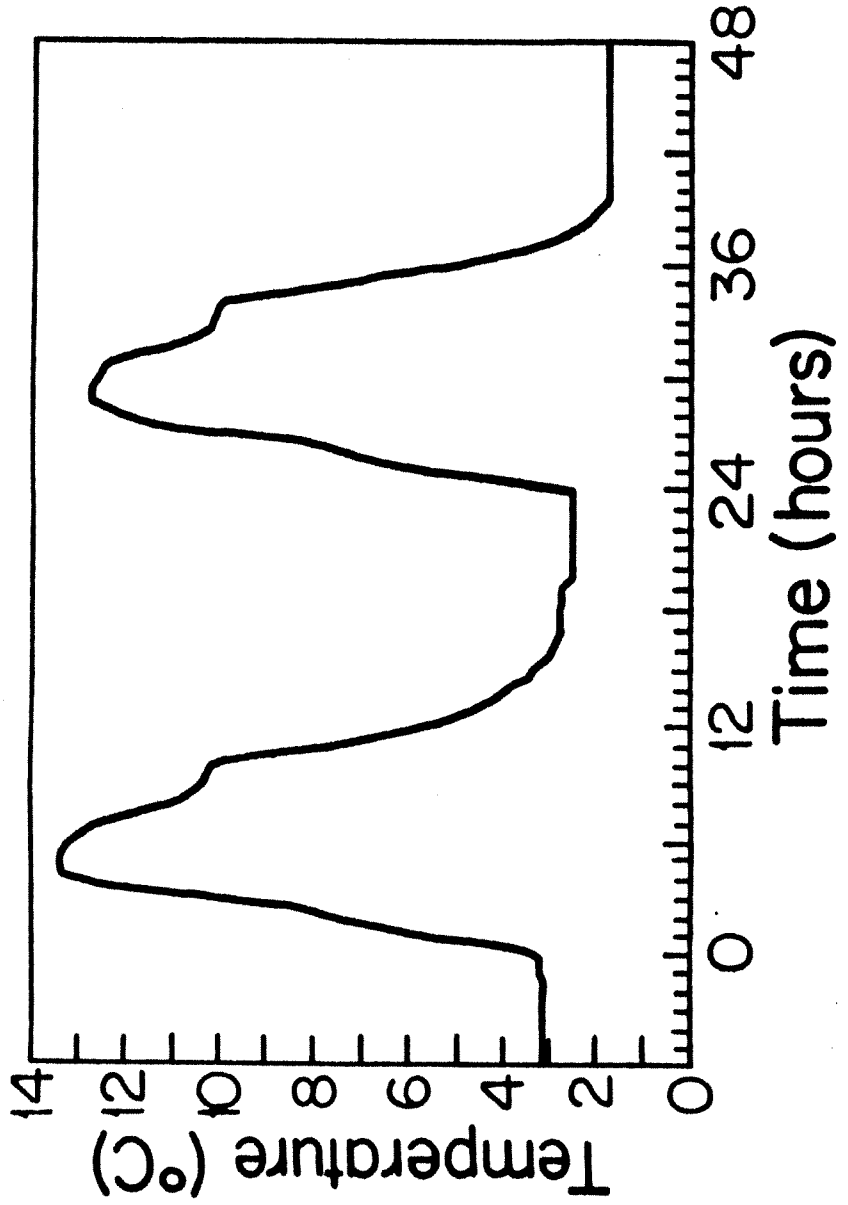

Fig. 1

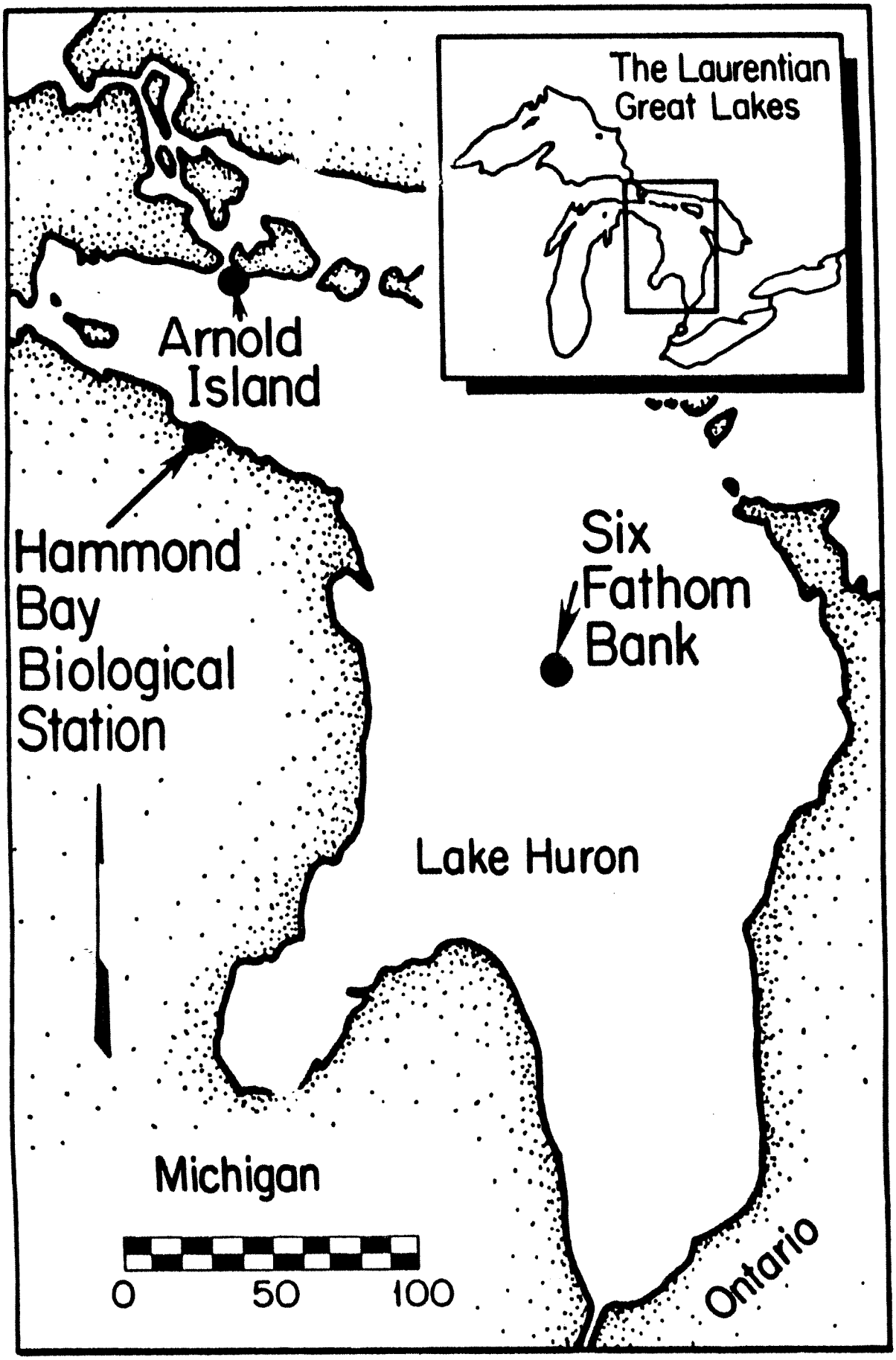
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Fig. 2

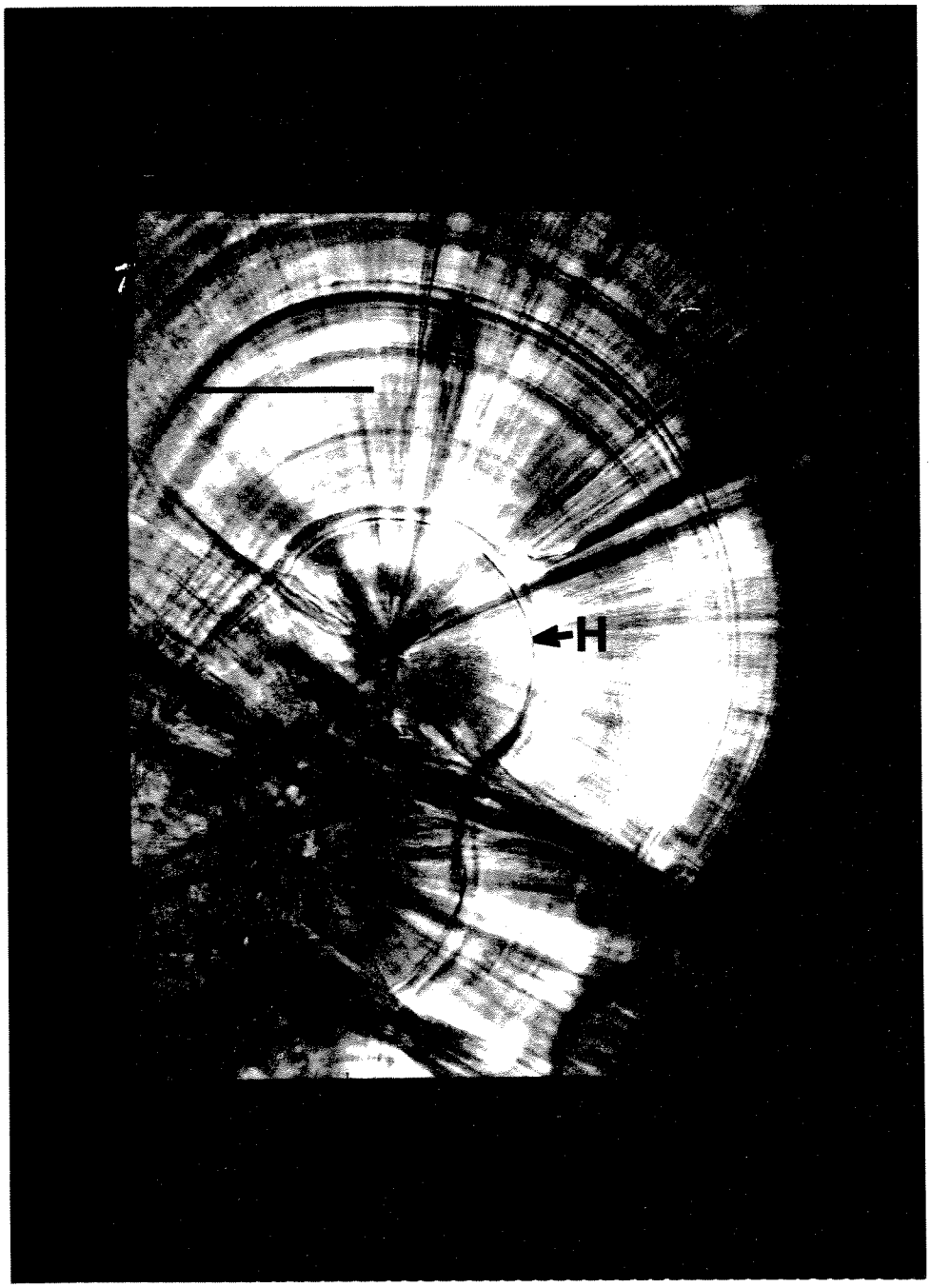


Fig. 3

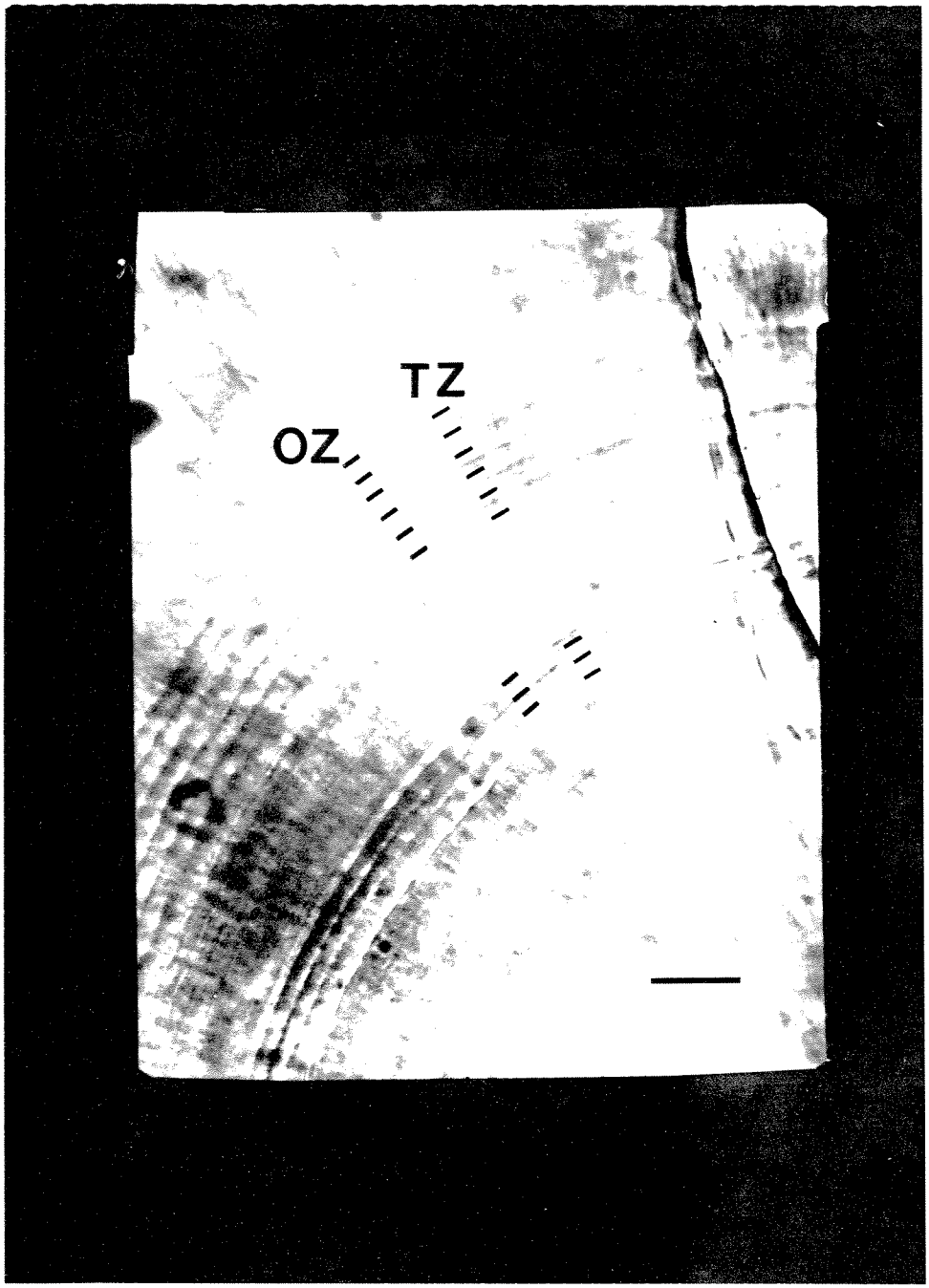


Fig. 4

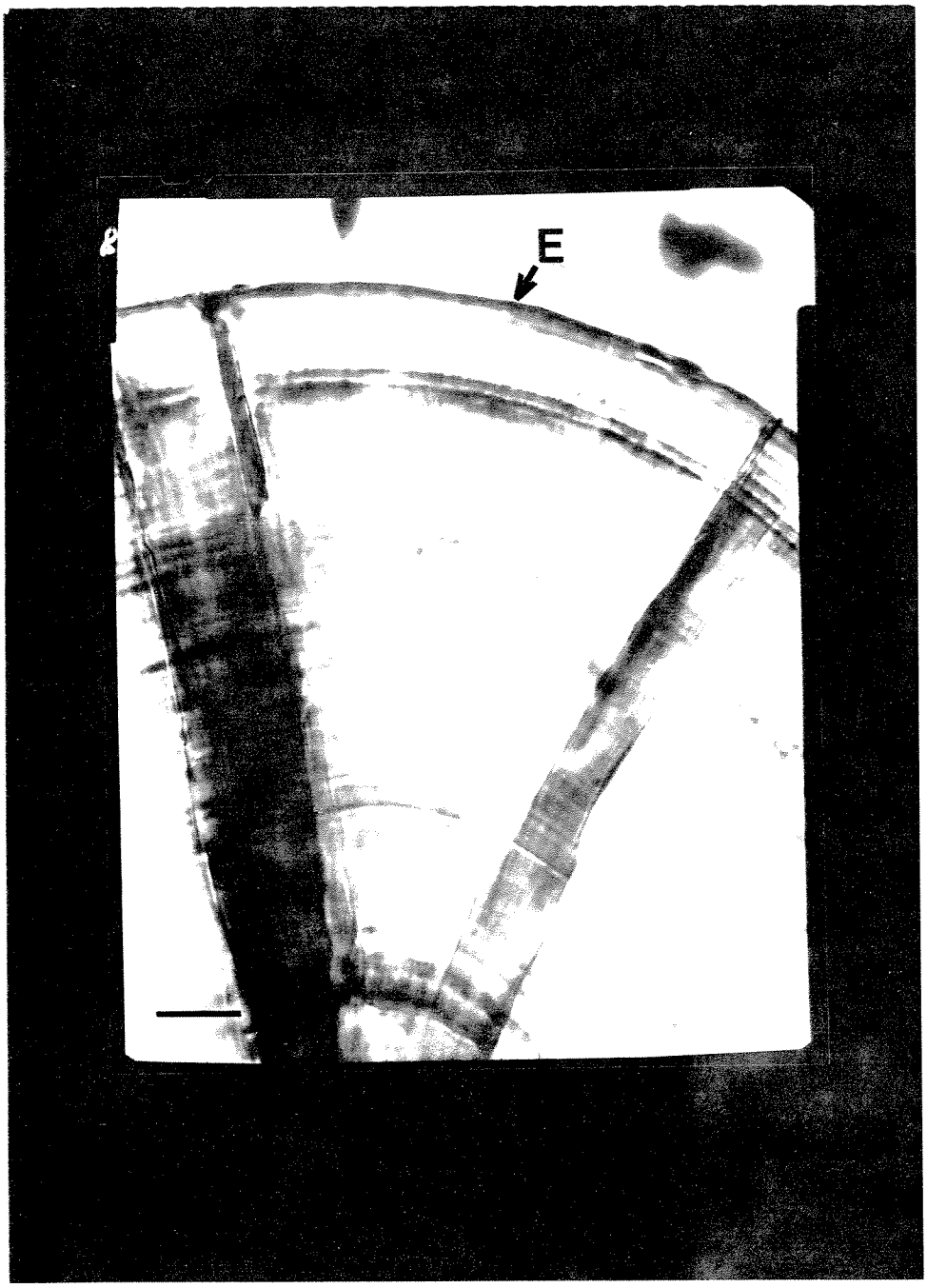


Fig. 5

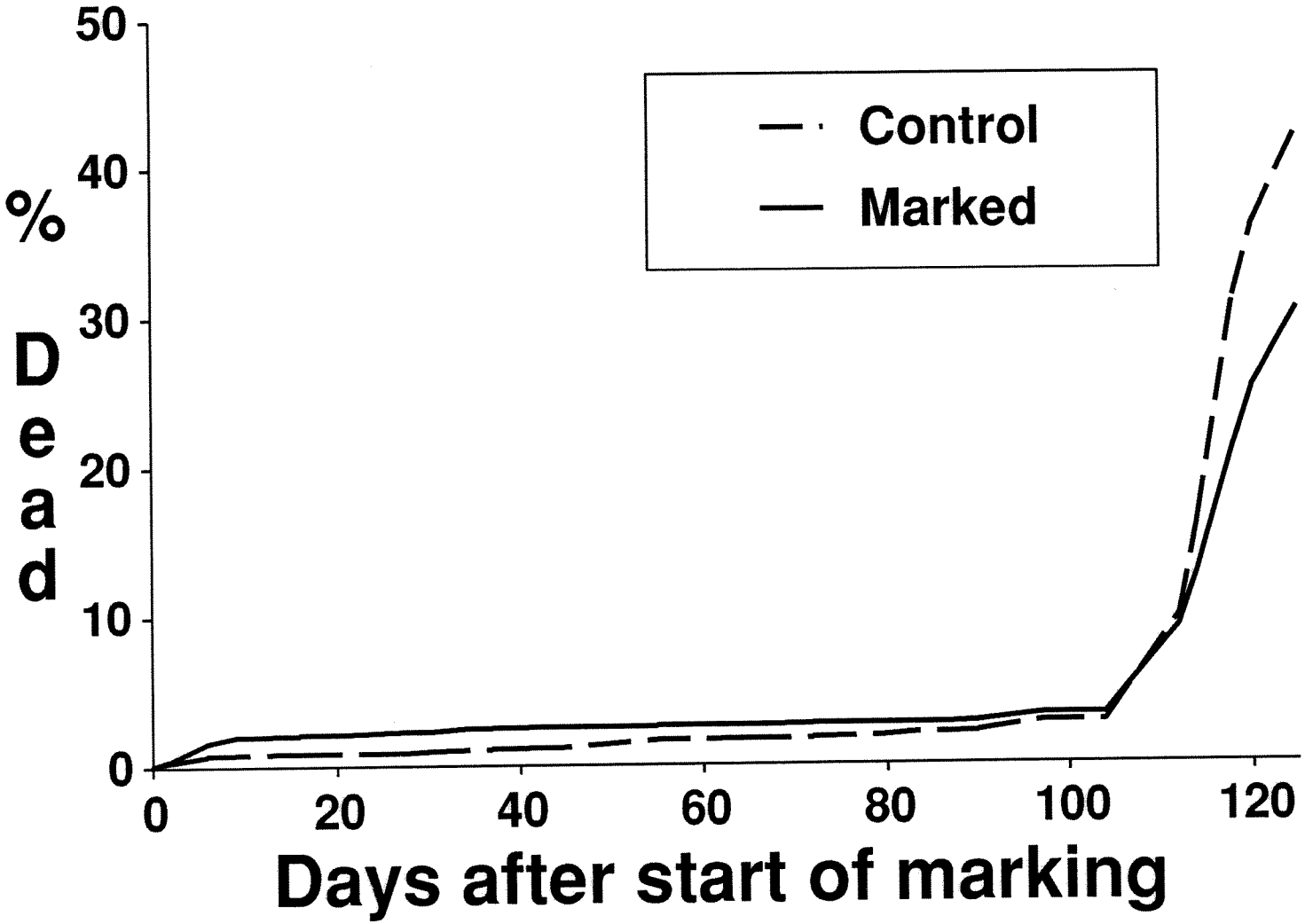
Appendix A: Marking in 1988

We received 960,000 eggs and recently-hatched fry from the Jordan River Hatchery on 15 January 1988. Approximately 90% of the eggs had hatched at that time. We experienced some initial losses to fungus, but the problem was quickly brought under control. Those initial losses were estimated at 100,000, leaving 860,000 for marking.

The fry were marked with nine consecutive temperature cycles administered between 9 and 17 February 1988. The marking procedure was identical in all other respects to that described in the manuscript. Losses between the end of the marking period and stocking were 10,000, leaving approximately 850,000 to be stocked.

Stocking took place on 31 March 1988. The fry were transported to Alpena, Michigan between 0650 and 0740, where they were placed aboard the R/V Grayling. The Grayling arrived on Six Fathom Bank at 1245. The fry were discharged by gravity low to within about a meter of the bottom while drifting between 44° 48' 26.5"N, 82° 29' 00.6"W and 44° 48' 27.3"N, 82° 28' 57.3"W. Water depth was 70 to 75 feet. Bottom type and condition of the fry were monitored with both an underwater video camera attached to the end of the discharge hose and in a remotely operated vehicle. The substrate in the area of the release appeared excellent and the fry also appeared to be in good health. Use of the remotely operated vehicle allowed some short-term observations of the fate of the fry. This was also encouraging, because soon after settling to the bottom, most fry were seen moving into the spaces between the rocks.

A mortality study was also conducted in 1988. Four lots of 500 fry each were placed in randomly selected trays in the incubator stacks being marked. Four lots of 500 fry each were also held as controls. The raw data and a summary figure are attached. Mortality through 100 days after marking was not significantly different between marked and control groups. Between 100 and 120 days mortality rose sharply with mortality in the control groups higher. Sharp increases in mortality are often seen at about this stage with lake trout in the Great Lakes, and we do not feel that it is likely that there is any connection between the marking process and our handling of the fry and that mortality. The lower mortality of the marked fish also suggests that the marking process did not adversely affect the marked fry.


50
40
30
20
10
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Days after start of marking