Minnesota Department of Natural Resources
Investigational Report 457

THERMAL MARKING OF LAKE TROUT OTOLITHS, AND EVALUATION OF METHODS FOR STOCKING FRY[1]

Mary T. Negus

Minnesota Department of Natural Resources
Section of Fisheries
500 Lafayette Road
St. Paul, MN 55155-4012

Abstract. The ability to distinguish hatchery produced lake trout *Salvelinus namaycush* from their wild counterparts is essential for evaluation of restoration efforts in Lake Superior. While conventional external marking procedures are inappropriate for early life history stages, thermal marking of otoliths is an accepted procedure for Pacific salmon *Oncorhynchus spp.* fry, but knowledge of the technique with lake trout fry is inadequate. This study adds to existing knowledge of thermal marking with insight into factors that affect lake trout. Several thermal marking regimes with lake trout sac fry were evaluated by varying the number, range, duration, and spacing of temperature pulses. Pulses produced individual bands on otoliths that together comprised the mark. Mark visibility varied within treatments, but in general, the highest quality marks were achieved when fry were subjected to pulses of 10°C or higher for 8 h or more on alternate days. These marks were visible on fry otoliths at 40X magnification, and individual bands were visible at 40X-100X. Higher magnification was needed to distinguish individual bands when fish were marked with consecutive-day pulses. As fish grew older, 200X magnification was required to distinguish bands. Precision in preparing otoliths for examination was also a factor governing mark visibility. Marked fish are being maintained in the hatchery until maturity, and mark recognition has been 100% for up to 5 years. Nearly 1.5 million marked lake trout sac fry were released on a rocky reef in Lake Superior during the 3 year period 1994-1996, and mark evaluation of these fish will be attempted when they mature. Several methods to deliver the fry safely and directly to the reef surface were evaluated.

[1]This project was funded in part by the Federal Aid in Sport Fish Restoration (Dingel-Johnson). Completion Report, Study 695, D-J Project F-26R Minnesota.

Introduction

The evaluation of management procedures is an economic necessity, especially in the current climate of budget reductions, and fiscal and environmental accountability. Evaluation of fisheries stocks necessitates the ability to distinguish between fish that were naturally reproduced and those that originated in a hatchery. The Minnesota Department of Natural Resources is attempting to determine the most expedient methods to restore and promote self-sustaining fish populations, since hatchery supplementation is subject to budget cuts, loss of genetic variability, and disease outbreaks. Stocking also may supply fish that behave differently from native populations, reducing their ability to survive, reproduce, and supplement the sport fishery.

The Great Lakes Fishery Commission has promoted reestablishment of lake trout *Salvelinus namaycush* in Lake Superior because they are the native top predator, they are particularly suited to the cold deepwater food chain, and they are the only salmonine (among those currently stocked) that have the potential to become self-sustaining at their current levels of abundance (Eshenroder et al. 1995b). Efforts to reestablish lake trout by stocking yearlings (marked with a fin clip) into Lake Superior and the other Great Lakes have met with varying success. While target population levels have been reached in most of Lake Superior (except Minnesota waters), recovery has been slow (Hansen et al. 1995), and spawning populations have not returned to historical spawning reefs (Eshenroder et al. 1995a; Krueger et al. 1986). The survival of stocked lake trout has been declining in U.S. waters of Lake Superior since the 1960s, despite continued stocking, probably due to predation and gill net fishing (Hansen et al. 1995; 1996). At the same time, wild stocks have increased in some areas. Restoration of lake trout may depend on management of these naturally reproducing stocks.

Lake trout stocked as yearlings tend to spawn in shallow water along shorelines where wave action, ice scour, and siltation limit recruitment (Eshenroder et al. 1995a). Wild lake trout home to their natal reefs to spawn, but when yearling lake trout were stocked directly onto reefs they apparently failed to imprint, providing evidence that imprinting occurs at an earlier life stage (Krueger et al. 1986). Stocking of eggs or sac fry directly onto historical spawning reefs (where natural reproduction is low) in the season when that life stage would normally be found in the wild, has been recommended to promote imprinting and survival of lake trout and their progeny (Horrall 1981; Krueger et al. 1986; Eshenroder et al. 1995b; Marsden et al. 1995).

Early life history stages are economical to stock, and they provide benefits in terms of imprinting and genetic diversity, but evaluation of their long-term contribution presents some difficulties. Any feature used to distinguish lake trout stocked at the egg or fry stage must endure for at least 7 years, which is the age of maturity when the fish first home to their natal (or stocking) reef. Ideally, the distinguishing feature should last the lifetime of the fish, which could be more than 20 years. Indirect evidence has been used to determine that lake trout eggs have promise for reestablishing populations (Swanson 1982). Unmarked eggs were stocked on spawning reefs in alternate years, and success was evaluated by tracking the abundance of spawners resulting from those year classes (Charles Bronte, U.S. Geological Survey - Great Lakes Science Center, Ashland, WI, personal communication).

Otoliths provide useful records of early life history in fish because they begin forming during the embryonic stage just before eyes become pigmented and visible without a microscope (Campana and Neilson 1985; Brothers 1990). They are the first calcified structure to appear in salmonine embryos, and they are the only hard structures with the potential for marking in embryos and fry. Otoliths are composed of calcium carbonate in the aragonite form, and they are never resorbed (Mugiya et al. 1981; Campana and Neilson 1985), so they provide a more complete growth record of the fish than do scales.

Thermal marking of otoliths is becoming an accepted technique for mass marking early life history stages of Pacific salmon

Oncorhynchus spp., and is being used in Washington and Alaska on a production scale for identification and management of stocks (Blankenship and Volk 1991; Munk et al. 1993; Volk et al. 1994; Hagen et al. 1995). Mark retention in these fish has been confirmed up to 5 years. This method has been tried with lake trout (Bergstedt et al. 1990; Brothers 1990; Bouchard 1994), but the marks were never confirmed in recaptured fish, and marked individuals were not held for examination beyond 6 months. Thermal marks are recognizable, distinct, non-random patterns created in the otoliths of small fish by exposing embryos or fry to temperature changes. Thermal marking can be done either by dropping or raising temperatures for various periods of time, followed by the return to ambient temperatures. Lake trout eggs incubated at ambient Lake Superior temperatures hatch at about 1-2°C, so temperature increases are required to produce marks.

To define what is meant by an otolith thermal mark, one must be familiar with the microstructural growth increments in otoliths that are composed of two zones. One zone is optically dense or opaque in transmitted light, because of a protein matrix deposited in this area (Casselman 1983; Campana and Neilson 1985; Brothers 1990). The other zone is more translucent, and is composed of calcium carbonate crystals (aragonite). The opaque zone is deposited at night or during lower or declining water temperatures, and the translucent zone is formed during daylight hours or when temperatures are elevated or rising. Temperature effects are generally more pronounced and mask the effects of photoperiod (Brothers 1990). The opaque zone, which appears dark under transmitted light, is generally referred to as the "ring" or "band", and the brighter translucent zone appears as space between bands. Temperature increases broaden these translucent zones, and declines emphasize the dark opaque zones, which is the essence of thermal marking (Figure 1). Daily growth increments, composed of one translucent zone plus one opaque zone, are seen in many species of young fish for at least 100 d (Brothers et al. 1976; Campana and Neilson 1985). Distinct otolith increments are more difficult to distinguish in fish held at constant temperature than in those exposed to diel fluctuations, because the opaque and translucent zones have less contrast (Campana and Neilson 1985; Brothers 1990). However, at high magnification, faint daily increments deposited during constant temperature can often be seen between distinct bands caused by temperature changes.

Thermal marking of otoliths is a relatively inexpensive method that reliably marks 100% of the fish. It is accomplished without handling individual fish, at a life stage in the hatchery when many fish are concentrated in a relatively small area, and it causes little or no mortality (Brothers 1990; Volk et al. 1990; Munk et al. 1993). Fin clips and coded wire tags are inappropriate for fry. Naturally occurring trace materials or applied chemical markers such as tetracycline, alizarin complexone, calcein, fluorescein, acetazolamide, strontium, and rare earth elements have all been used with some success to distinguish stocks of fish of a variety of sizes, but each has its drawbacks. Detection of some of these materials may require sophisticated equipment and techniques. Thermal marks require only a compound microscope with transmitted light (Brothers 1990). Chemical markers must be applied by immersion of fish too young to feed or too small to inject, and mortality is sometimes high (Brothers 1990). The success of tetracycline immersion may vary by species, but these differences may also be due to variations in concentration or buffering. This method created weak marks in lake trout fry (Brothers 1990), and effectively marked only 88% of juvenile black crappies (Conover and Sheehan 1996), although more success has been achieved in coastal or marine species (Hettler 1984; Secor et al. 1991; Thomas et al. 1995). Immersion can be a problem when young fish are reared in flow-through systems. Fluorescent marks may be somewhat labile when exposed to light during dissection and storage. Food and Drug Administration regulations also limit the use of most chemical treatments on fish that are potential food sources.

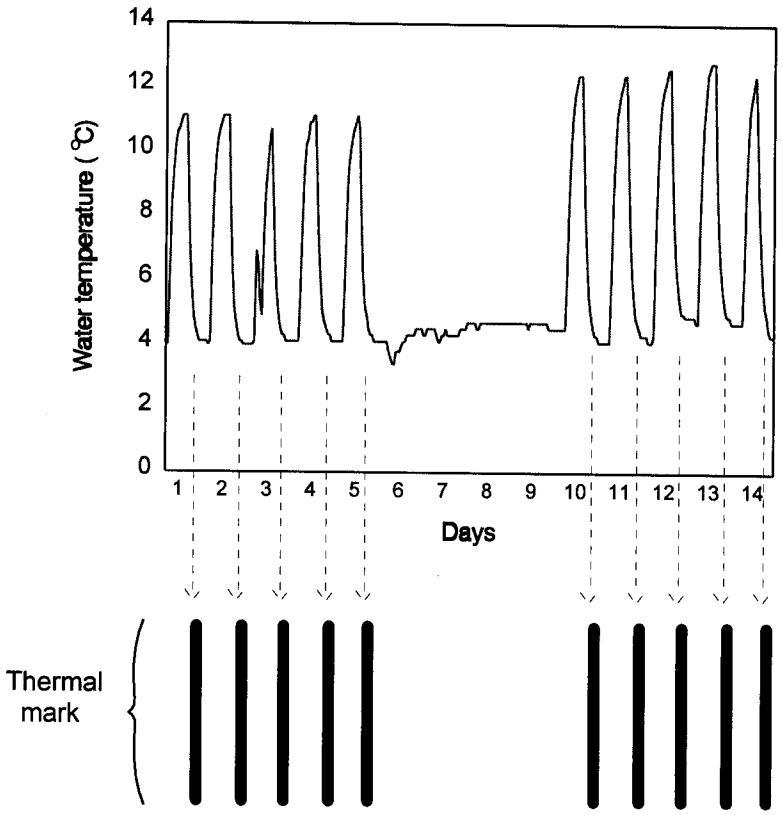

Figure 1. Demonstration of temperature pulses delivered to sac fry and the corresponding marking pattern created in the otoliths. Otolith bands correspond to the rapid declines in temperature.

The objectives of this study were to develop the techniques to mass-mark otoliths of lake trout sac fry; to develop the techniques to prepare otoliths for evaluation of temperature marks; to field test the technique with lake trout sac fry; and to devise a method to deliver marked sac fry directly to the surface of a potential spawning reef.

Methods

The study was conducted in three phases including initial small-scale marking experiments in 1992, a large-scale pilot project in 1993, and large-scale production runs in 1994, 1995, and 1996. Lake trout eggs were obtained in the fall and over-wintered in Lake Superior water at ambient temperatures to produce fry for stocking in the spring when this developmental stage would normally be found in the wild. All marking experiments were conducted on sac fry, and all marking and rearing took place at the Duluth Area Fisheries Headquarters near French River (Figure 2).

Small scale, initial experiments, 1992

Initial temperature marking experiments were conducted in spring 1992 in 38 L aquaria with flow-through systems that supplied unheated Lake Superior water. Heat for marking was supplied by heaters placed in each aquarium, and the extent of temperature increase was determined by the flow rate of the water. The aquarium heaters were connected to a programmable 24 h timer.

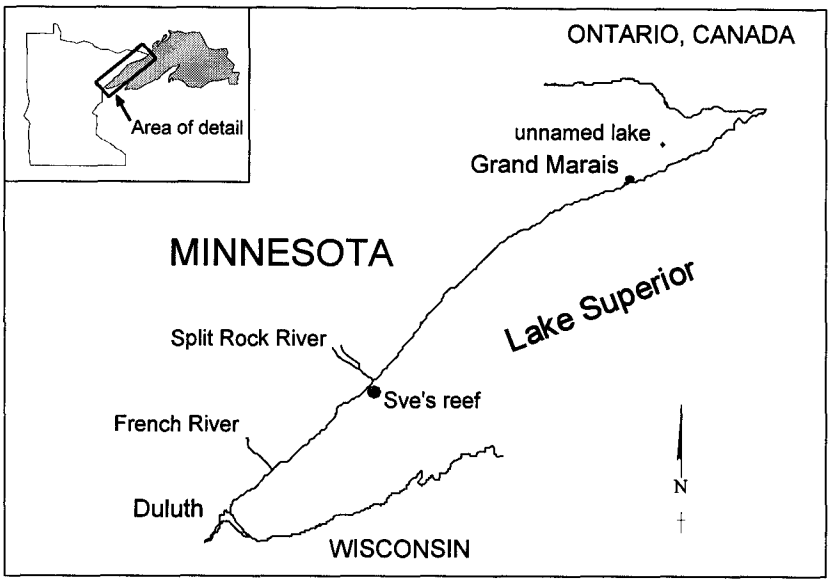

Figure 2. Minnesota's Lake Superior shoreline, showing the location of the unnamed lake where lake trout fry were stocked in 1993 and 1995, and the location of Sve's reef where fry were stocked in 1994-1996.

Three different temperature patterns (A, B, and C) were used to mark different batches of sac fry, and each pattern was tested at three different temperature amplitudes (Figure 3). Only one temperature pattern (or mark) was implemented at a time in three aquaria. Cup-shaped containers to suspend batches of lake trout sac fry in the aquaria were constructed from plastic window screen suspended from styrofoam rings. Each of these "fry boats" could hold more than 50 sac fry in one layer on the bottom.

Fish were placed in seven fry boats about one week after hatching. One fry boat was placed in each of the three aquaria with heaters, and four fry boats were placed in an unheated aquarium. The electrical timer was programmed to activate the heaters on a predetermined schedule. Fry in the three boats held in the heated aquaria thus experienced the entire warming and cooling cycle within those aquaria. Three more fry boats were subjected to each temperature pulse by transferring them abruptly from the unheated aquarium into the heated aquaria when temperatures approached their maxima, and later returning them abruptly to the cold aquarium just before the heaters shut off. During transfers, the fry boats were carried in a dish of water, so the fry remained submerged. One fry boat stayed continuously in the unheated aquarium to serve as a control. Fry were preserved in ethanol about one week after marking was completed, and otoliths were examined later.

Approximately 100 fry with Mark A (Figure 3) were reared for periodic sampling to confirm mark retention, and for practice in otolith removal and preparation as the fish grew. Some of these fish will be maintained for sampling until maturity.

Large scale, pilot study, 1993

A system was developed to supply ambient or heated water to incubator trays at varied time intervals (Negus and Dexter 1995). This

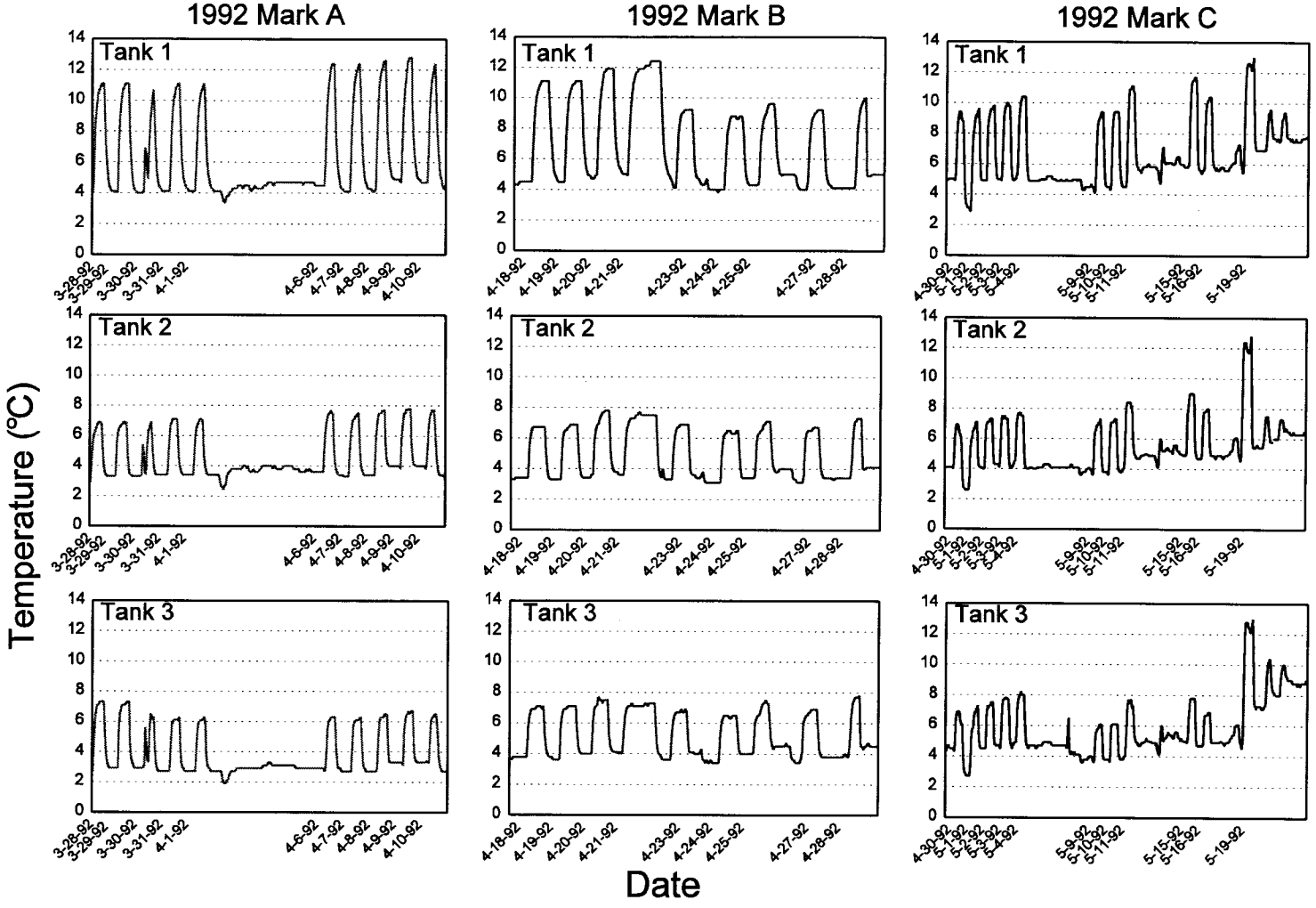

Figure 3. Temperature regimes used to mark lake trout sac fry in small-scale initial experiments, beginning 8-10 d after hatch. Temperature pulses lasted 8.5 h.

system was used to mass-mark the otoliths of lake trout sac fry on a production scale. A 7 d programmable timer controlled the water delivery schedule. This system was capable of heating water 8-10°C above ambient Lake Superior temperatures and delivering it to one 16-tray incubator stack at a time with a flow of 15 L/min. Temperatures could be varied by adjusting flow rates.

A pilot run of the rearing and marking procedure using this equipment was completed in spring 1993 with 84,600 eggs of the Isle Royale strain. Two sets of six temperature pulses were used to mark the fish beginning approximately 9 d after hatching (Figure 4). The first set of temperature pulses was delivered on alternate days, to provide greater spacing between bands. The temperature pulses in the second set were paired on adjacent days, with 2 d between pairs. Durations of the temperature pulses varied from 4 h to 12 h between incubator stacks.

Approximately 30,000 sac fry from this pilot run were stocked on 5 May 1993 into a 3.6 ha unnamed lake with a maximum depth of 11 m, located in Township 63N, Range 3E near Tom Lake northeast of Grand Marais. This lake is stocked biennially with brook trout *Salvelinus fontinalis* fingerings, and contained no other lake trout.

Large scale, production runs, 1994-96

Lake trout eggs of Isle Royale strain and Gull Island Shoal strain were obtained on several dates in fall 1993 for production-scale rearing and marking (Table 1). The eggs were placed in four 16-tray incubator stacks, and the developmental stages of the eggs within each stack were synchronized by temporarily applying heated water to the younger eggs, while keeping older eggs in ambient temperature Lake Superior water. Sac fry of both strains were marked in spring 1994 using two sets of five 8 h temperature pulses (Figure 5). The timing of the temperature pulses was varied slightly to create a distinguishable difference in the spacing of the otolith bands between the two strains. Marking was begun 2 weeks after the hatch, and completed in 17 d.

These incubation procedures were repeated with Isle Royale eggs obtained in fall 1994 and 1995, but different thermal marks were used for each year class (Table 1; Figure 5). Marking in spring 1995 and 1996 was begun at the time of hatching and repeated until just prior to stocking. Fry were stocked on Sve's reef (Figure 2) prior to swim-up in early May of 1994, 1995, and 1996.

Table 1. Lake trout reared for production-scale thermal marking and stocking, 1993-1996. Fry were stocked onto Sve's reef in Lake Superior unless otherwise noted.

Spawn dates	Broodstock strain	No. of eggs spawned	Survival from spawn to stock	No. of sac fry stocked	Date stocked
10/7/93-11/17/93	Isle Royale	785,233	35.1%	275,580	5/5/94
10/26/93	Gull Island Shoal	>312,000[a]		275,497	5/5/94
10/18/94-11/15/94	Isle Royale	1,092,478	50.9%	540,104 16,040[b]	5/5/95 5/1/95
10/10/95-11/08/95	Isle Royale	946,482	37.8%	357,982	5/1/96

[a] These were surplus eggs obtained at the eyed stage from the Iron River federal hatchery, Wisconsin.
[b] These sac fry were stocked into an unnamed lake northeast of Grand Marais, Minnesota.

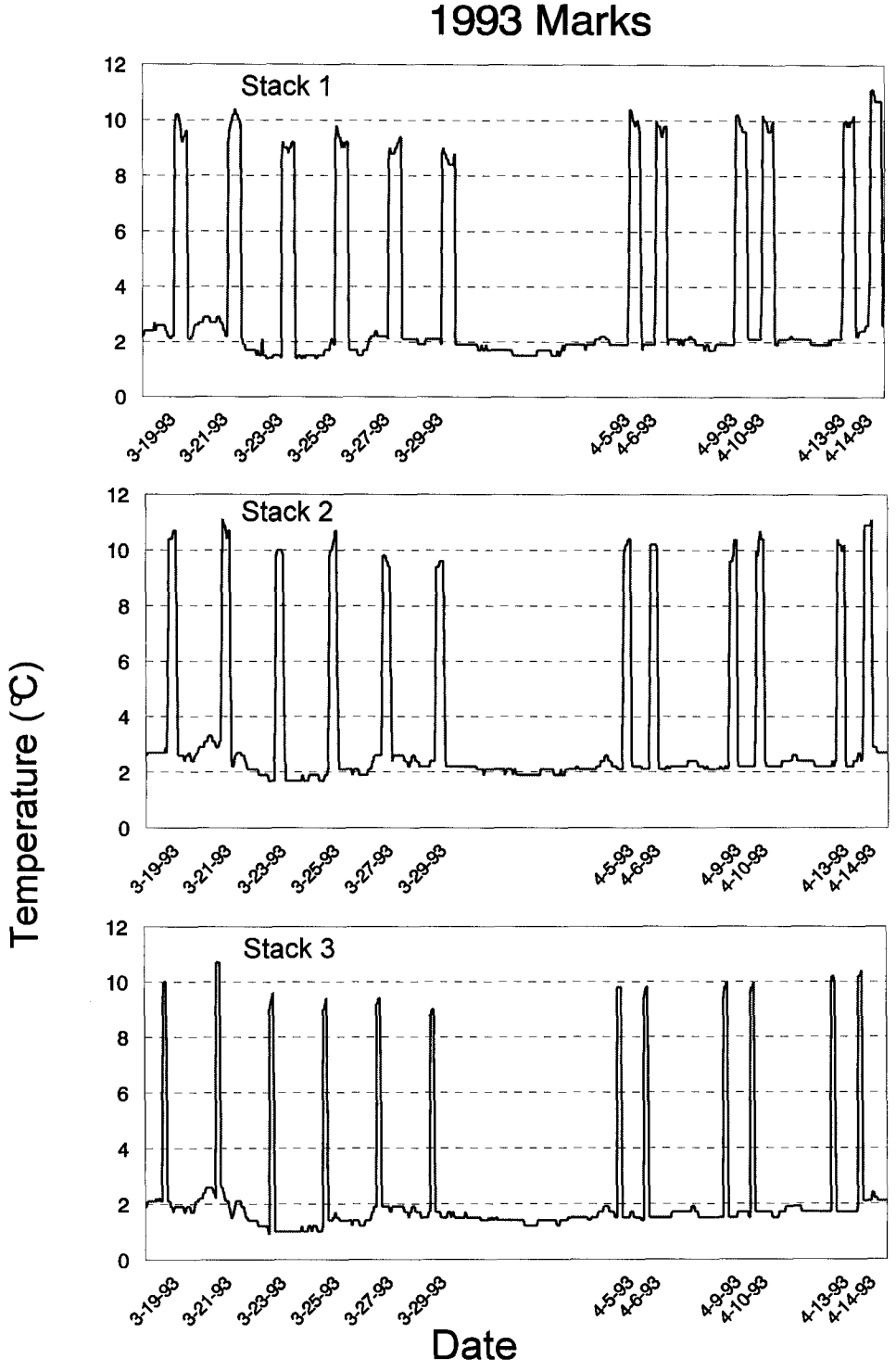

Figure 4. Temperature regimes used to mark lake trout sac fry in the pilot run of the production-scale equipment, beginning about 9 d after hatch. Fry in Stack 1 experienced 12 h temperature pulses, Stack 2 experienced 8 h pulses, and Stack 3 experienced 4 h pulses.

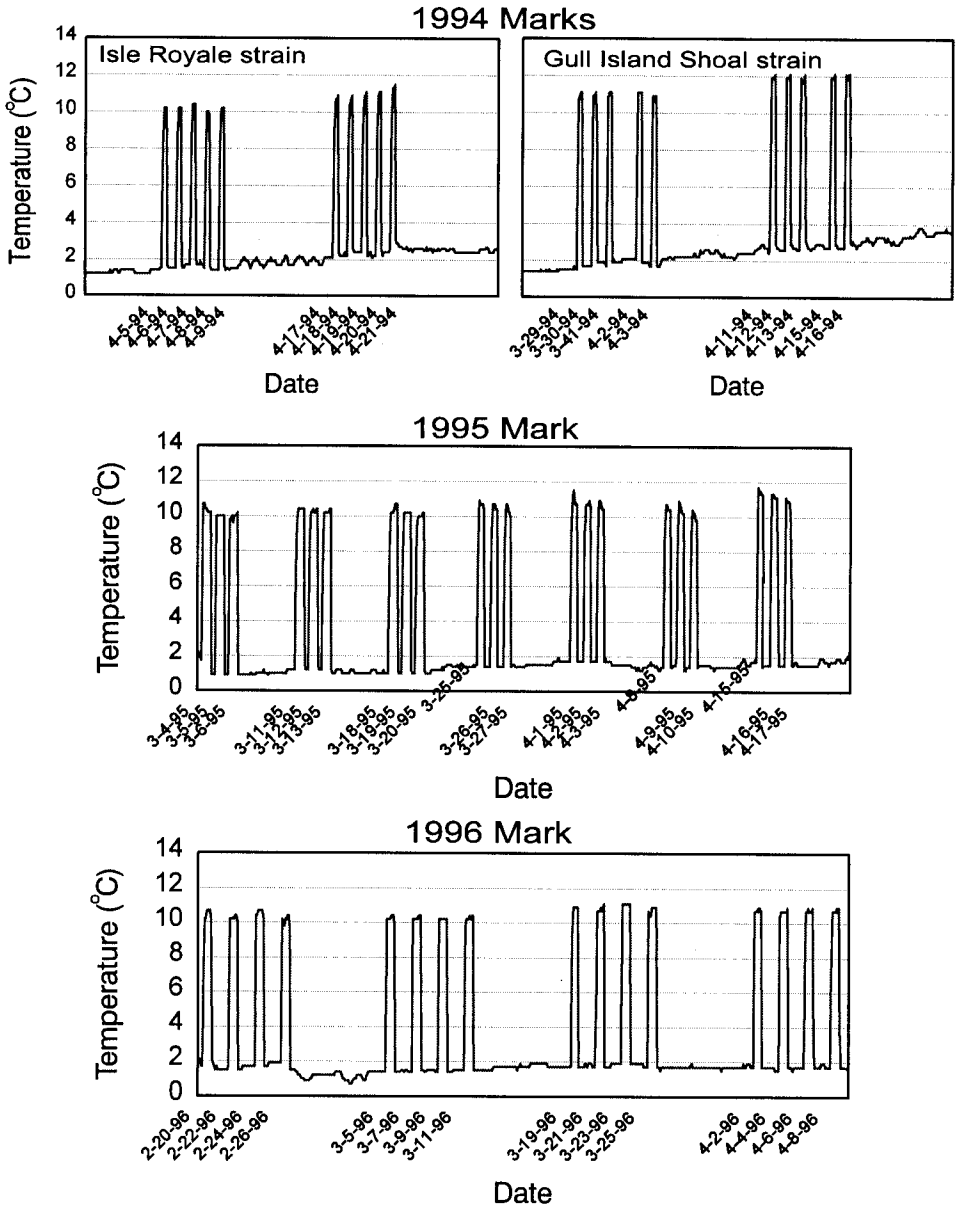

Figure 5. Temperature regimes used to mark lake trout sac fry during the production runs in 1994 - 1996. Marking in 1994 was begun about two weeks after hatching, and temperature pulses lasted 8 h. Marking in 1995 and 1996 was begun at the time of hatching and continued until just prior to stocking. Temperature pulses in 1995 lasted 8 h. Temperature pulses in 1996 lasted 16 h when only one stack was being marked per day, and 12 h when two stacks were being marked per day. Note that the graphs from 1995 and 1996 do not depict the entire duration of the marking period.

Otolith preparation

Only sagittal otoliths were used for mark identification, and their removal from preserved lake trout fry required the aid of a dissecting microscope. Fry specimens were placed in isopropyl alcohol at least one week after marking was completed, to insure some growth after the last band. Otolith embedding and preparation followed the procedures outlined by Bergstedt et al. (1990), with some minor variations. All sectioning was done on the sagittal plane. A slide warmer was used in place of an oven to cure the epoxy used in otolith mounting. Etching with 1% HCl was not done, in accordance with recommendations by Campana and Neilson (1985). Larger otoliths that required grinding on both sides were embedded sulcus side up in epoxy on plastic mounting cylinders (the type used for thin sections). After grinding one side to the mid-sagittal plane, the otolith in epoxy was pried off the cylinder with a scalpel, and the freshly ground surface was bonded to a glass slide for the second grind.

Otoliths from fry marked in the small- and large-scale trials were evaluated for mark recognition by comparing them with otoliths from the control fish and native fish captured in Lake Superior. Otoliths from fry were examined with a compound microscope and transmitted light immediately after they were embedded in epoxy with no sectioning. Some of these otoliths were ground to the mid-sagittal plane for observation and photographs. Otoliths from fish up to at least 2.5 years post-hatch were observed after grinding only one side. Marks were examined at 40X to 1000X magnification. Photographs were taken at 400X and 1000X.

Stocking methods - hatchery trials

Three stocking methods to transport fry directly and safely to an artificial reef surface were compared on 2 June 1992. These trials were all conducted in a 7 m long by 0.75 m wide by 0.5 m deep trough in the Duluth Area Fisheries Headquarters. The behavior and survival of fry stocked with each method were noted. Observations of the fry were conducted during daylight hours and after dark immediately after turning on overhead lights.

Method 1) An artificial turf "sandwich", like those used in lake trout egg stocking studies in Wisconsin (Swanson 1982) was used as a sac fry incubator. The "sandwich" consisted of five layers (two facing pieces of artificial turf equalled one layer) within a wooden frame. Sac fry were loaded at a rate of 2,000 fish/layer. Only five layers (instead of the original six layers used for stocking eggs) were used to avoid crushing the fry. The "sandwich" was anchored to the tank bottom between two rock piles consisting of rocks approximately 15 cm in diameter.

Method 2) About 5,400 sac fry were flushed through a funnel connected to tubing (2 cm inner diameter) that extended to just above a rock pile at the tank bottom. Some of the fry were also flushed over the bare tank bottom away from the rocks.

Method 3) A white plastic 19 L bucket with a lid was modified to contain sac fry. Two 10 cm by 30 cm windows were cut near the bucket rim and covered with 6 mm square mesh plastic screen. About 5,000 fry were placed in the bucket, which was lowered into the tank and anchored near a rock pile.

Stocking methods - field trials

The suitability of Sve's reef in Lake Superior (Figure 2) as potential spawning habitat was evaluated by SCUBA diving in October 1993. The emergent rock portion of the reef is located approximately 0.3 km offshore. The nearshore (west) side of the reef is about 5 m deep, composed of angular rocks approximately 0.3 m across with deep interstitial spaces. The offshore (east) side of the reef is about 15 m deep, composed of large boulders from 0.3 m to 3 m across. The south side is a gradation between the east and west characteristics, and the north side has a sandy bottom. Fry were planted on the east, south, and west sides of the emergent reef. All fry were transported from the hatchery to the reef in 19 L collapsible jugs at a rate of 5,000 fry with about 5 L water per jug. Jugs were filled

to capacity with oxygen prior to transport. Two methods of stocking were compared in 1994 in efforts to transport the fry unharmed directly to the reef surface.

About 419,000 fry were flushed through a 5 cm diameter hose to a depth within 3 m of the reef surface. Another 132,000 fry were lowered to the reef surface in weighted buckets that allowed fry to escape at will. The buckets were modified from the earlier version, by drilling holes throughout the sides, and using plastic screening only on the lids. These buckets were retrieved 2 weeks later. Two small mesh (1.25 cm bar measure) gill nets, each 15 m long and 2 m deep, were set overnight in the stocking area to sample smelt and other opportunistic predators.

To evaluate their tolerance of the stocking procedure, approximately 200 fry were lowered to the reef surface (14 m) in a bucket, left for 5 minutes, then retrieved and transported back to the hatchery for observation.

Results

Small scale, initial experiments

The desired temperature differentials of about 8°, 6°, and 4° C were not maintained accurately in the three aquaria, because flows could not be controlled precisely. However, the differentials in Tank 1 were sufficiently higher than those in the other two tanks to make a difference in thermal marks. Thermal control at later dates was also hindered by springtime warming of the lake water. Mark A in tank one with the 7-9° C temperature differential (Figure 3) produced the most visible banding patterns in 1992. Marks B and C were difficult to distinguish from random marks in the otoliths, even at the highest temperature differentials. Marks created in fry that were moved abruptly from one temperature to another were virtually indistinguishable from those created in fry that experienced the entire range of warming and cooling. No mortality was observed in marked or unmarked fry. Fish with Mark A have been sampled up to age 5, with 100% mark retention. The mark could be recognized at 200X magnification.

Large scale, pilot study

All fry marked in spring 1993 were combined in an emergency rescue attempt when a water line broke, so marks created from temperature pulses of different durations could not be compared. In general, this mark (Figure 4) was not as dark and distinct as Mark A from 1992 (Figure 3). The entire mark was fairly close to the otolith primordia, and the paired marks from temperature pulses on consecutive days were very close together. Two juvenile lake trout were recovered during a survey conducted in fall 1993 of the unnamed lake that received marked fry in spring 1993. Their otoliths were ground, and the temperature marks were located.

Large scale, production runs

Equalizing the egg development stage within each incubator stack allowed marking of all fish in a stack at the same stage following hatching. Marks created in 1994 were not quite as distinct as the 1992 Mark A, but the Isle Royale mark could be distinguished from the Gull Island Shoal mark. Bands created from temperature pulses on consecutive days were very close together. The regularity of the 1995 pattern over a wide portion of the otolith was the most visible feature at low magnification. The clusters of 3 bands appeared to be one wide band at low magnification, but individual bands could be seen at higher magnification. Marks created in 1996 were the most obvious of all the thermal marks, due to the relatively wide spaced, high density bands.

Otolith preparation.

Examinations of otoliths from fish which had undergone thermal treatments revealed that 100% contained marks, although some marks were more visible than others. Otoliths from fry were quite transparent, and did not need to be sectioned to observe the thermal mark. To obtain clear photographs of the marks, however, fry otoliths often required some grinding. Larger, less transparent otoliths required grinding to the mid-sagittal plane, using the primor-

dia or the hatching check for reference, in order to obtain the clearest view. By focusing through some depth in the otolith, one could often see marks clearly even if the thin section had not been ground to the exact plane of the mark on all sides of the core. Often parts of the mark were lost as other parts were clarified, since the otoliths were not perfectly oriented within the epoxy during grinding. Marks could often be recognized as distinct clusters of bands at lower magnification, and the bands could then be counted at higher magnification. Preparation of otoliths for photographs was more exacting, because focus was fixed, and magnification was usually higher.

The best thermal marks in sac fry otoliths (in 1992 and 1996) could be recognized without grinding at 40X magnification under transmitted light. Individual bands could be counted at 100X or 200X in the 1992 Mark A, or at 40X or 100X in the 1996 mark. Other marks (from 1993-1995) could be recognized at 100X and counted at 200X. Larger otoliths taken from older fish required grinding to a thin section on one or both sides, and mark recognition sometimes depended on the quality of the preparation. Magnification at 200X was usually necessary for positive mark recognition in the larger otoliths from the 1992 year class.

Bands created in the lake trout otoliths using daily temperature pulses that lasted 8 h (1992, 1994, and 1995 temperature regimes) were about 1.4-2.2 μm apart. Bands created by alternate day temperature pulses lasting 8 h (1993 temperature regime) were about 2.1-2.6 μm apart. Bands created by alternate day pulses lasting 12-16 h (1996 temperature regime) were about 2.5-3.2 μm apart.

Stocking: hatchery trials

The sac fry actively sought cover in the rock piles even when they escaped or were released over the bare tank bottom. They remained in the crevices until their yolks were absorbed. Only deformed individuals were found on the bare tank bottom away from the rocks. The yolk sacs of some fry were absorbed and they began swimming up on 8 June (6 d after stocking), and most fish swam up during the following week. The three methods of stocking produced the following results:

Method 1) Artificial turf "sandwiches" - During the process of loading 10,000 sac fry into the layers, 960 fish escaped into a catch basin, and a few others located near the edges of the turf washed out during deployment. Most of the fry stocked in the "sandwich" remained alive within the turf layers until their yolk sacs were absorbed, and then were able to escape into the tank. Many of the fry appeared to escape vertically through holes in the layers, rather than sideways between the grass blades to the edge. By 22 June (20 d after stocking), long after most of the fry had swum up, 786 live fry without yolk sacs remained in the turf. Approximately 10-15 dead fry were found in each layer, with more toward the center layers and fewer near the top and bottom.

Method 2) The fry flushed down the hose were in good condition. Those that landed on rock surfaces moved into crevices within minutes of stocking. Those released over the open bottom actively sought cover.

Method 3) Most of the fish deployed in the bucket remained in the bucket until their yolks were absorbed, and then swam out the screen windows. Some fish fell out through the screen when the bucket tipped during stocking. Only a few fish, primarily deformed individuals, remained after one week.

Stocking: field trials

Some fish were seen washing out of the screen-lidded buckets when they were initially lowered to the reef. Of the 132,000 fish originally placed in buckets, approximately 8,250 dead fry were recovered when the buckets were retrieved, and most had large yolk sacs. The fry that were lowered to the reef, retrieved on the same day, and transported back to the hatchery sustained no mortality after one week. The mortality of fry stocked by flushing down a hose could not be evaluated. Two rainbow smelt *Osmerus mordax* with empty stomachs were captured in gill nets set in the vicinity of the stocked lake trout.

Discussion

Otolith marking and specimen preparation

When using temperature to mark fish otoliths, every effort should be made to increase mark visibility. Bold distinct bands that are well spaced in a regular nonrandom pattern are the key to future recognition. An important factor in viewing marks from older fish is proper grinding and preparation of the otoliths, but perfection is unnecessary for mark recognition.

Ability to recognize of marks at low magnification allows rapid initial screening, and avoids extensive and precise grinding and searching. The best marks in this study were recognizable at 40X, and bands could be counted at 40X or 100X in fry otoliths. Marks could be recognized and counted in older (>1-2 year) otoliths at 200X. The magnification required to detect a thermal mark in lake trout fry otoliths from Bergstedt et al. (1990) varied from 400X for the best sections to 1,000X for most other sections, and counting the number of bands always required 1,000X. Bouchard's (1994) lake trout fry otoliths also required 1,000X magnification for good mark recognition. Thermal marks used for Pacific salmonids have typically required lower magnification for mark recognition. Volk et al. (1990; 1994) were able to count thermal bands and recognize differential spacing at 200X in otoliths from juvenile Pacific salmonids marked as embryos or fry. Munk et al. (1993) used 100X magnification to confirm marking in pink salmon *O. gorbuscha* from 3 d to 3 weeks after marking.

The spacing of the bands within each mark was proportional to the time between temperature declines, and the duration of the warmer temperature pulse, which is typical of marks created in other studies (Brothers 1990; Munk et al. 1993; Bouchard 1994; Volk et al. 1994) (Figure 1). In this study, band spacing ranged from about 1.4 μm to 3.2 μm. The 1996 temperature regime, which included the longest time between temperature pulses (2 d) and the longest pulse duration (12 - 16 h) provided wider spacing between bands - a pattern more visible at lower magnification than the other marks.

The magnitude of the temperature difference between relatively warm and relatively cool water appeared to affect the optical density of the otolith mark, and greater changes produced clearer marks. Changes of 3 to 4°C created much less visible marks than those created by 7 to 9°C differentials (Figure 3) or 9 to 10°C differentials (Figures 4 and 5). Similar results have been reported in other studies, but mark clarity appears to vary by species. Volk et al. (1990) created distinct bands in chum salmon embryos with temperature changes as small as 1.7°C when hatchery temperatures were tightly controlled, but larger temperature changes were required in hatcheries where ambient temperatures varied somewhat. Current Alaska guidelines based on mark quality require a minimum temperature differential of 3°C for marking Pacific salmonids (Munk et al. 1993), and Volk et al. (1994) selected a temperature differential of 5°C to create finely distinguished banding patterns in Pacific salmonids. Bouchard (1994) created faint marks in lake trout otoliths with changes of 2°C, but mark quality progressively improved with 4°C and 6°C changes. Bergstedt et al. (1990) used temperature changes of about 10.6°C and Brothers (1990) used 12°C changes to mark lake trout with good results.

Marks created by rapid temperature changes in 1992 were indistinguishable from those created during gradual changes, but even the "gradual" changes in 1992 occurred within the space of 0.5 h. The large-scale marking apparatus used from 1994 - 1996 reached its maximum temperature in about 0.5 h, and the temperature decline was more rapid as the heated water valve closed abruptly and the unheated valve opened. Although Bouchard (1994) reported that a slightly better quality mark was usually produced in lake trout using a gradual temperature change, the rates of change were not reported, and graphs of the "gradual" changes suggest that they occurred within 0.5 h from maximum to minimum. Volk et al. (1990; 1994) reported that a rapid decline in temperature produced high quality marks in Pacific salmonids.

The dark bands in otoliths that are created during periods of slow or no growth are apparently emphasized when the fish experience some stress. Thus, a gradual or small temperature change that causes little stress may also reduce mark clarity. Lake trout prefer temperatures around 10°C (Negus 1995; Peck 1982; Wismer and Christie 1987), so temperatures in that vicinity are likely to produce minimal stress, which may also diminish mark clarity. Brothers (1990) determined that 4 d cycles of gradually rising then falling temperatures created less distinct bands in lake trout otoliths than shorter-term cycles of 6 h to 2 d. However, the temperature change was so gradual that a low of about 4°C was reached only briefly, and the mean temperature during the decline was 10°C. Bouchard (1994) found that 16 h warm and 32 h cold cycles created somewhat less distinct bands in lake trout otoliths than 16 h warm and 8 h cold cycles, even though these temperature changes were fairly rapid. However, the minimum temperature was about 6.8°C and the range of temperatures experienced was only 4°C. The lack of definition in the dark bands in both studies may be due to the relatively warm temperatures experienced during the "cold" phase of the marking regimes. Growth rates and otolith deposition under these conditions may have been affected little by the temperature decline, creating more diffuse bands than those created when temperatures were lower and changes were larger or more abrupt. Also, daily increments deposited between these temperature bands appeared to reduce mark clarity.

In general, marks appeared slightly less obvious when the fry were marked shortly after hatch than when marking was delayed 2 weeks or more. A similar trend was seen by Bouchard (1994) when comparing fish marked one week after hatch versus 3 weeks after hatch. Marking fish repeatedly from the time of hatch to the time of stocking (as done in 1995 and 1996) maximized the chances of grinding to a marked plane within the otoliths. In 1992 Mark A, the second set of five bands was usually clearer than the first. The exceptional clarity of Mark A relative to most marks created in later years may be related to the exposure of these fish to room light or daylight in the open fry boats. Only the density of the bands created in 1996 equalled or exceeded Mark A.

Hatchery conditions, life histories, growth patterns, and otolith characteristics of different species can present advantages and challenges to thermal marking. For example, compared to lake trout, most Pacific salmonids have large otoliths as fry, and short life spans - from 2 to 5 years. The otoliths of these species thus contain fewer natural increments spaced at larger intervals than those created in lake trout. Otoliths from chinook salmon *O. tshawytscha* measure about 450 μm along the long axis at the sac fry stage, and about 11 mm in 4-5 year old adults. In contrast, lake trout otoliths measure about 300-350 μm along the long axis in fry, and reach only about 6mm after more than 20 years. Various hatchery temperature regimes also influence the types of possible marks. Warmer rearing temperatures cause faster growth and wider translucent zones in daily increments. When dense optically opaque bands are then induced by temperature declines, these bands are well spaced. This type of temperature regime was used by Volk et al. (1990; 1994) to mark chum salmon and chinook salmon, and the widely spaced banding patterns were distinguishable under low magnification. If rearing temperatures are low (as in this study), and thermal marks are created by raising and then lowering the temperature, the bands tend to be closer together.

The potential formation of otoliths composed of the vaterite polymorph of calcium carbonate instead of the aragonite polymorph should not pose a problem in the recognition thermal marks. This malformation causes otoliths to have a translucent or crystalline appearance, is more prevalent in fish of hatchery origin, and seems to originate at or before the time of stocking (Casselman 1986; David et al. 1994; Murray 1994). Because the core of many vaterite otoliths display the normal alternation of aragonite-protein matrix zones, marks deposited near the core at an early stage of development should not be affected.

While prolonged periods at warmer temperatures could have provided wider spacing between thermal bands in the lake trout otoliths, two factors made this approach undesirable. First, the small water heating system could provide heated water to only one incubator stack at a time, so marking two stacks in one day limited each to a 12 h duration. By alternating days, four stacks could be marked during the same week on 12 h cycles. Second, my objective was to stock the fry just before swim-up in the spring when they would naturally occur in the wild, but I also had to wait until ice left the shoreline of Lake Superior in order to launch a boat. Warmer temperatures accelerated fry development, and even with the temperature regimes used in 1994-1996 the fry were ready to be stocked immediately at ice-out.

Thermal marking of the otoliths of lake trout sac fry was 100% effective, relatively inexpensive, caused no mortality, and was sufficient for distinguishing year classes. This procedure is particularly well suited to species that have relatively large otoliths, and inhabit fairly constant temperature during the life stage when marking is done, so that temperature marks provide a distinct contrast. Preparation of the otoliths for mark recognition is the most time consuming aspect of mark retrieval, so a mark that is highly visible at low magnification saves time and money. While the persistence of thermal marks throughout the potential lifespan of lake trout was not demonstrated, marks were identified after five years, and persistence is assumed since otoliths are not resorbed. This technique is recommended for the evaluation of fry stocking, with the following guidelines:

1) Select a simple, nonrandom pattern that forms band clusters recognizable at low magnification so that unmarked otoliths can be rejected during time-saving precursory examinations.

2) Variations in the time between temperature declines can be used to change increment widths, to distinguish different stocks of fish, but make sure the overall pattern is simple and obvious.

3) Wait at least 5 d between repetitions of marking cycles to create well-defined separations between patterns. Munk et al. (1993) recommended that the space between clusters of bands should be at least 2.5 times the space between individual bands.

4) Begin marking at the time of hatching, and repeat the pattern as many times as possible until just before swim-up. This creates a large marked region facilitating otolith preparation. Marking could be started with embryos after eye-up, but when more heat is applied the fish mature faster and will need to be stocked sooner.

5) Large temperature differentials up to 10°C or 11° C create the best marks. Larger differentials have not been attempted.

6) Use 12 - 16 h intervals of heated water for each temperature pulse. Longer periods were not tried but may work well.

7) Deliver temperature pulses every other day (or even less frequently) to provide sufficient spacing between bands. Longer periods between pulses were not tried in this study, but may provide better band spacing.

8) Keep background temperatures as constant as possible, to avoid the creation of spurious marks.

Stocking trials and spawning habitat

The rubble-boulder substrate and sloped orientation of Sve's reef were characteristic of historic lake trout spawning reefs. Lake trout spawning habitat is defined by the presence or absence of olfactory cues for homing, reef location relative to shoreline, water depth, proximity to nursery areas, reef size, contour, substrate size and shape, depth of interstitial spaces, water temperature, water quality, and the presence of egg and fry predators (Marsden et al. 1995). Rocky substrates vary from 8 cm to 3 m in diameter, with interstitial spaces to at least 1 m deep (Fitzsimons 1995; Marsden et al. 1995; Schreiner et al. 1995). Spawning sites often have steep slopes, which may provide better water quality due to currents, and may provide access to deepwater habitat for juveniles (Marsden et al. 1995).

Survival evaluations suggested that the three methods of fry stocking could be equally effective providing that fry escape from the equipment and take refuge in rock crevices. Some fry were swept out of the bucket before reaching the reef, and some fry became trapped in the artificial turf sandwich layers. The hose method was preferred because of its relative simplicity. The bucket and sandwich methods required more effort loading and deploying them and more equipment, boat space, trips to shore, and time to complete. They also were vulnerable to storm damage or loss, and both required a second trip to retrieve them.

Fry should not be stocked in Lake Superior until just prior to swim-up. Fry that were stocked prematurely, when their yolk sacs were large, appeared to sustain higher mortality. The rapid descent to the reef surface did not appear to harm fry that were stocked at the appropriate time, as evidenced by those that were retrieved and maintained in the hatchery. Direct observations of the fry as they are delivered to the reef surface would verify whether or not they seek shelter in rock interstices as they did in the hatchery tank. The lack of opportunistic predators captured in gill nets set in the vicinity of the stocked lake trout was a favorable sign, but a more extensive effort is needed to rule out their importance.

Spawning adults from the thermally marked 1994-1996 plants should begin to appear in spawning assessment gill nets on Sve's reef beginning about the year 2001. Otoliths from these adults can then be sampled for temperature marks to determine the effectiveness of fry stocking.

References

Bergstedt, R. A., R. L. Eshenroder, C. Bowen II, J. G. Seelye, and J. C. Locke. 1990. Mass-marking of otoliths of lake trout sac fry by temperature manipulation. American Fisheries Society Symposium 7:216-223.

Blankenship, H. L., and E. Volk. 1991. Effects of coded-wire tagging on the survival of spring chinook salmon. Annual Report FY-1990-91, Project No. 88-163, Washington State Department of Fisheries, Olympia.

Bouchard, L. 1994. Expérimentation de thermo-marquage d'otolithes de touladi. Rapport du Centre Écologique du Lac St-Jean inc. à la Vice-présidence Environnement, Hydro-Québec. Saint-Félicien, Québec, Canada.

Brothers, E. B. 1990. Otolith marking. American Fisheries Society Symposium 7:183-202.

Brothers, E. B., C. P. Mathews, and R. Lasker. 1976. Daily growth increments in otoliths from larval and adult fishes. Fishery Bulletin 74:1-8.

Campana, S. E., and J. D. Neilson. 1985. Microstructure of fish otoliths. Canadian Journal of Fisheries and Aquatic Sciences 42:1014-1032.

Casselman, J. M. 1983. Age and growth assessment of fish from their calcified structures - techniques and tools. NOAA (National Oceanic and Atmospheric Administration) Technical Report NMFS (National Marine Fisheries Service)8:1-17.

Casselman, J. M. 1986. Scale, otolith, and growth characteristics of juvenile lake trout - criteria for discriminating between indigenous and hatchery fish from the natural environment. Great Lakes Fishery Commission, Research Completion Report. Contribution No. 86-00 of the Ontario Ministry of Natural Resources, Maple, Ontario, Canada.

Conover, G. A., and R. J. Sheehan. 1996. Evaluation of oxytetracycline marks in sagittal otoliths of juvenile black crappies.

North American Journal of Fisheries Management 16:686-688.

David, A. W., C. B. Grimes, and J. J. Isely. 1994. Vaterite sagittal otoliths in hatchery-reared juvenile red drums. The Progressive Fish-Culturist 56:310-303.

Eshenroder, R. L., C. R. Bronte, and J. W. Peck. 1995a. Comparison of lake trout-egg survival at inshore and offshore and shallow-water and deepwater sites in Lake Superior. Journal of Great Lakes Research 21(Supplement 1):313-322.

Eshenroder, R. L., E. J. Crossman, G. K. Meffe, C. H. Olver, and E. P. Pister. 1995b. Lake trout rehabilitation in the Great Lakes: an evolutionary, ecological, and ethical perspective. Journal of Great Lakes Research 21(Supplement 1):518-529.

Fitzsimons, J. D. 1995. Assessment of lake trout spawning habitat and egg deposition and survival in Lake Ontario. Journal of Great Lakes Research 21(Supplement 1):337-347.

Hagen, P., K. Munk, B. Van Alen, and B. White. 1995. Thermal mark technology for inseason fisheries management: a case study. Alaska Fishery Research Bulletin 2(2):143-155, Juneau.

Hansen, M. J., M. P. Ebener, R. G. Schorfhaar, S. T. Schram, D. R. Schreiner, J. H. Selgeby, and W. W. Taylor. 1996. Causes of declining survival of lake trout stocked in U.S. waters of Lake Superior in 1963-1986. Transactions of the American Fisheries Society 125:831-843.

Hansen, M. J., J. W. Peck, R. G. Schorfhaar, J. H. Selgeby, D. R. Schreiner, S. T. Schram, B. L. Swanson, W. R. MacCallum, M. K. Burnham-Curtis, G. L. Curtis, J. W. Heinrich, and R. J. Young. 1995. Lake trout (*Salvelinus namaycush*) populations in Lake Superior and their restoration in 1959-1993. Journal of Great Lakes Research 21(Supplement 1):152-175.

Hettler, W. F. 1984. Marking otoliths by immersion of marine fish larvae in tetracycline. Transactions of the American Fisheries Society 113:370-373.

Horrall, R. M. 1981. Behavioral stock-isolating mechanisms in Great Lakes fishes with special reference to homing and site imprinting. Canadian Journal of Fisheries and Aquatic Sciences 38:1481-1496.

Krueger, C. C., B. L. Swanson, and J. H. Selgeby. 1986. Evaluation of hatchery-reared lake trout for reestablishment of populations in the Apostle Islands Region of Lake Superior, 1960-84. Pages 93-107 *in* R. H. Stroud, editor. Fish culture in fisheries management. American Fisheries Society, Fish Culture and Fisheries management sections, Bethesda, Maryland.

Marsden, J. E., J. M. Casselman, T. A. Edsall, R. F. Elliot, J. D. Fitzsimons, W. H. Horns, B. A. Manny, S. C. McAughey, P. G. Sly, and B. L. Swanson. 1995. Lake trout spawning habitat in the Great Lakes - a review of current knowledge. Journal of Great Lakes Research 21(Supplement 1):487-497.

Mugiya, Y., N. Watabe, J. Yamada, J. M. Dean, D. G. Dunkelberger, and M. Shimizu. 1981. Diurnal rhythm in otolith formation in the goldfish, *Carassius auratus*. Comparative Biochemistry and Physiology 68A:659-662.

Munk, K. M., W. W. Smoker, D. R. Beard, and R. W. Mattson. 1993. A hatchery water-heating system and its application to 100% thermal marking of incubating salmon. The Progressive Fish-Culturist 55:284-288.

Murray, C. B. 1994. A method for preparing chinook salmon otoliths for age determination, and evidence of its validity. Transactions of the American Fisheries Society 123:358-367.

Negus, M. T. 1995. Bioenergetics modeling as a salmonine management tool applied to Minnesota waters of Lake Superior. North American Journal of Fisheries Management 15:60-78.

Negus, M. T., and D. J. Dexter. 1995. A compact, inexpensive, automatic system to deliver water at alternating tempera-

tures for hatchery studies. The Progressive Fish-Culturist 57:315-319.

Peck, J. W. 1982. Extended residence of young-of-the-year lake trout in shallow water. Transactions of the American Fisheries Society 111:775-778.

Schreiner, D. R., C. R. Bronte, N. R. Payne, J. D. Fitzsimons, and J. M. Casselman. 1995. Use of egg traps to investigate lake trout spawning in the Great Lakes. Journal of Great Lakes Research 21(Supplement 1):433-444.

Secor, D. H., M. G. White, and J. M. Dean. 1991. Immersion marking of larval and juvenile hatchery-produced striped bass with oxytetracycline. Transactions of the American Fisheries Society 120:261-266.

Swanson, B. L. 1982. Artificial turf as a substrate for incubating lake trout eggs on reefs in Lake Superior. The Progressive Fish-Culturist 44:109-111.

Thomas, L. M., S. A. Holt, and C. R. Arnold. 1995. Chemical marking techniques for larval and juvenile red drum (*Sciaenops ocellatus*) otoliths using different fluorescent markers. Pages 703-717 *in* D. H. Secor, J. M. Dean, and S. E. Campana, editors; A. B. Miller, associate editor. Recent developments in fish otolith research. University of South Carolina Press, Columbia.

Volk, E. C., S. L. Schroder, and K. L. Fresh. 1990. Inducement of unique otolith banding patterns as a practical means to mass-mark juvenile Pacific salmon. American Fisheries Society Symposium 7:203-215.

Volk, E. C., S. L. Schroder, J. J. Grimm, and H. S. Ackley. 1994. Use of a bar code symbology to produce multiple thermally induced otolith marks. Transactions of the American Fisheries Society 123:811-816.

Wismer, D. A., and A. E. Christie. 1987. Temperature relationships of Great Lakes fishes: a data compilation. Great Lakes Fishery Commission, Special Publication 87-3, Ann Arbor, Michigan.

Acknowledgements

I would like to thank Fred Tureson and the rest of the French River Coldwater Hatchery staff for assistance with lake trout rearing, sorting, and counting. Daniel Dexter, from the Duluth Area Fisheries Management Station, assisted with the development of the water delivery system for otolith marking, and carried out plumbing and construction operations. Mark Dexter, an independent electrician, voluntarily designed the programmable timer that controlled the water delivery system; Lyle Mattson and Kurt Jensen, electricians from the Soudan Underground Mine, built the programmable timer. Thanks are also extended to the hatchery crew and the Duluth Area Fisheries Management staff for rescuing the lake trout eggs when pumps, pipes, and electricity failed, generally between midnight and 4 a.m. The Lake Superior Area Fisheries Management staff, and Tracy Close from Duluth Fisheries Research assisted with lake trout fry stocking. Finally, I thank Walter Sve, commercial fisherman, who cleared his boat ramp immediately after ice-out so that we could stock fish. Tracy Close, Melissa Drake, and Farrell Bandow provided editorial review of this manuscript.

Edited by:

P. J. Wingate, Fisheries Research Manager

C. S. Anderson, Fisheries Research Supervisor