Strontium Chloride (SrCl – 6H2O) as a Mass-Marker for Salmonid Otoliths in Alaska

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As the number of thermally marked salmonids released from hatcheries increase, fewer mark patterns become available, and the risk of repetitive mark releases increases. Consequently, there is a need to develop alternative, reliable, and cost-effective methods of mass-marking hatchery-raised salmon. We evaluated the usefulness of strontium chloride hexahydrate (SrCl - 6H2O) to mass-mark salmonid otoliths in Alaska. In 2000, emergent sockeye fry raised at the Gulkana Hatchery, Prince William Sound, Alaska, were exposed to dilute concentrations of SrCl (500, 1,000 and 3,000 ppm) for a variety of durations (2, 4, 8, 12, and 24 hours) to determine which protocol resulted in a clear, unambiguous mark. Voucher specimens were collected spring 2001 from each mark group and held for two weeks in collection boxes to allow additional growth to occur on the otolith beyond the strontium mark to eliminate the potential for edge effects that may occur while viewing the mark.

Approximately 20 fry were sampled from each mark group, preserved in 90% ethanol, and sent to Alaska Department of Fish and Game’s Mark, Tag, and Age Laboratory in Juneau, Alaska for dissection and otolith preparation. Approximately 1 million fry exposed to strontium chloride for 24 hours at 3,000 ppm were released into Paxson, Crosswind and Summit Lakes. All remaining fry were destroyed. Adults are expected to be recovered as part of the Copper River salmon fishery beginning in the summer of 2003. Otoliths were removed from fry and mounted directly onto petrographic glass slides with clear thermoplastic cement. Only left sagittal otoliths were mounted. Right sagittae were stored dry to determine if the mark could be detected without any kind of sample preparation (e.g. grinding and polishing). All mounted otoliths were ground down to the primordia using 9-micron aluminum oxide lapping film, then finished with three and one micron polishing film to eliminate scratches. During this preparation process, the smoothness of the otolith’s surface was evaluated using a Leica reflected light microscope. Once polished, specimens were examined for mark presence using an FEI Quanta 600 environmental scanning electron microscope (ESEM) capable of operating at high pressure (low vacuum) that employed backscatter electron detectors (BSE) as the primary imaging source.

Fry exposed to the highest concentration (3,000 ppm) for the longest duration (24 hours) experienced minimal stress and average survival rates. Mortality did not exceed 0.8%, which was considered to be an average rate of mortality at the Gulkana Hatchery. During growth, strontium displaced calcium in structures made of calcium carbonate (CaCO3) to form strontium carbonate (SrCO3). Sequential exposure produced a series of strontium carbonate bands in otoliths that were similar to those patterns produced using thermal marking procedures. The higher molecular weight strontium layer scattered a greater number of electrons than the surrounding matrix and was consequently displayed as a bright band against the darker lower molecular weight calcium of the otolith (Fig. 1). Strontium rings were only visible in polished specimens. The surface topography of whole, unprepared otoliths scattered the electron beam to such a great degree that the strontium mark was not detectable. Mark recovery procedures indicated exposure duration was more important than concentration and that modern, low vacuum ESEMs with BSE had high enough sample processing rates to support mark recovery efforts while a fishery was in progress. From a purely subjective standpoint, otoliths exposed to at least 1,000 ppm of SrCl for 24 hours consistently exhibited the clearest, most unambiguous marks (Fig. 1). Advantages of strontium for mass-marking include 100% effectiveness, relatively short exposure times, generation of marks that can be detected in the otolith any time after hatch, and creation of marks at a reasonable cost, especially under circumstances where thermal marking is impractical. Because strontium marks cannot be detected with a light microscope, the primary disadvantage is the high cost of the ESEM needed for mark recovery. Traditional SEMs operate only at high.
vacuum, which cannot be used to examine non-conductive materials without preparation (e.g. conductive coating), both of which increase sample handling times. ESEMs operate at low vacuum and require no sample preparation, which significantly increases sample processing rates. Because strontium occurs naturally in the environment in dilute concentrations, there should be little environmental concern over its handling or disposal.

**Fig. 1.** Otoliths from sockeye salmon fry exposed to dilute concentrations of strontium chloride for a variety of durations. The bright ring occurring towards the edge of each otolith is the strontium mark.