Marking Salmonids with Strontium Chloride at Various Life History Stages

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A long-standing challenge faced by fishery researchers and managers has been how to tag or mark small salmonids without injuring them. Numerous methods have been developed. Some like fin clipping and half-length CWTs (Thrower and Smoker 1984) require that each fish be individually handled. Others, for example, fluorescent spray marking, can be applied simultaneously to many fish but will leave some unmarked, are inherently stressful, and become more difficult to detect over time. Thermal marking of otoliths, on the other hand, appears to be a benign and universal way to mark embryonic salmonids. It does, however, require that fish be cultured or held for several days or longer. In some instances, it is desirable to quickly mark wild fish or those produced from spawning channels or other large incubation settings where thermal marking is not practical.

About forty years ago, Trefethen and Novotny (1963) recommended that stable isotopes be introduced into fishes to create recognizable marks. Since then investigators have introduced bone-seeking cations into fishes by feeding, injections, and immersion baths. For example, Behrens Yamada and Mulligan (1982, 1987) exposed salmonids to strontium chloride by holding them in dilute baths (1 ppm) or using strontium enriched diets. Their methods produced recognizable marks but took weeks to complete. We modified their approach by exposing salmonid fry to strontium baths containing up to 9000 ppm for 24 hrs (Schroder et al. 1995).

Calcified tissues from these fish were analyzed with inductively coupled mass spectrometry (ICPMS), and clear marks were discerned. ICPMS is a bulk analytical tool that requires examined specimens to be dissolved in ultra pure nitric acid before analyses can take place. Because entire structures, e.g., otoliths, centra, and so on, are analyzed, the relative concentration of introduced Sr becomes diluted as a fish grows. One way to circumvent such dilution is to use micro-probe analytical procedures. These techniques do not destroy a sample; instead they examine the elemental composition of a specimen in discrete and relatively small locations. Wave-length dispersive spectrometry (WDS) is one such method. In this case, a specimen is bombarded with primary electrons from an artificial source. These electrons interact with the specimen to produce backscattered electrons, secondary electrons, and x-rays. X-rays are detected and used to identify elemental composition, and backscattered electrons can be used to create backscattered electron images (BEIs). BEIs taken from otoliths collected from fish we marked clearly showed where strontium had been deposited at the time of marking. Such deposits will last throughout a fish's lifetime.

The value of any marking technique will be increased if multiple marks can be produced. Experiments showed that varying marking bath concentrations and exposure times creates distinct strontium marks. In addition, if the fish being marked can be held for several days it is possible to use multiple immersion events to create bar codes much like those employed in thermal marking.

Some work by Kalish (1990) suggested that it might be possible to create trans-generational marks in fishes by using strontium. He showed that female salmonids maturing in marine waters passively absorb strontium and incorporate it into their eggs. During early ontogeny, their offspring deposit this strontium into their otoliths. We speculated that artificial trans-generational marks could be induced in fishes by injecting high levels of dissolved strontium into gravid females (Buckley et al., personal communication). This marking approach was tried on several different species of marine fishes, where gravid females were injected with 9000, 30000 ppm Sr or a control saline solution. Otoliths collected from the offspring of these fish showed that those coming from mothers injected with Sr had elevated levels of this element in their otoliths.

A review of ion regulation in teleost eggs by Alderdice (1988) also suggested that salmonids could be marked with strontium at fertilization. He recounted that in salmon eggs, negatively charged proteins associated with the plasma membrane are inverted into the space between the chorion and plasma membrane immediately after activation. The proteins absorb water and also attract cations that are then bound to the proteins in the perivitelline space or moved across the plasma membrane into the yolk material. Absorbed cations mainly Ca, Na, and K may be essential for further embryonic development, particularly in waters that have low hydro-mineral content. The

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capacity to absorb cations in this fashion continues for about three hours post fertilization, and after that the chorion becomes relatively impervious to ion exchange (Alderdice 1988). Given the proclivity to absorb cations, we felt that recently activated eggs would readily absorb strontium. To test this idea, we exposed newly fertilized chum salmon eggs to 5000, 2500, 500, and 50 ppm strontium chloride solutions for three hours. ICPMS analyses showed that an unambiguous increase in Sr occurred in otoliths collected from fry treated with the 5000 ppm Sr bath at fertilization. Moreover, recent WDS scans made on otoliths obtained from fry exposed to our marking baths disclosed that these fish possessed elevated levels of Sr in the inner portions of their otoliths. Hence, clear Sr marks can be induced on salmonids at fertilization by simply activating their gametes in solutions containing this element.

Salmonid alevins also appear to be an ideal life-history stage to mark with strontium because they are able to absorb ions through their gills, intestinal epithelia, and yolk sac (Behrens Yamada and Mulligan 1987). We exposed brown trout alevins to 1000 and 100 ppm marking solutions for 24 hrs or 4 hrs. All the alevins were subject to four separate marking episodes that were conducted at either two or five day intervals. BEI images of otoliths collected from these fish illustrated that all the treatments produced visible marks.

In summary, salmonid fishes can be marked in mass by using strontium solutions at diverse life history stages. Trans-generational marks also appear to be possible. The widespread use of this method will depend on its acceptance by federal regulatory agencies. Canadian researchers using our methods have marked large numbers of sockeye salmon. One of us (Pete Hagen) is working closely with the U.S. Food and Drug Administration (FDA) and has gained approval to mark 26 million sockeye fry. With continued FDA support we anticipate that strontium marking will be used in diverse research and management settings.

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