Protocol for detection of SE-MARK® calcein marks in fish.
9/10/04

Introduction

Perhaps the 3 most important principles concerning actual detection of calcein marks in fish are: **(1)** the lower the level of ambient light present during detection, the easier it will be to detect the fluorescent-green calcein mark, **(2)** the level of calcein-mark fluorescence is directly proportional to the intensity of blue light emitted from the detector which strikes the fish. and **(3)** Calcein labels all bony and calcified tissues present in the fish during the marking procedure.

Protocol

The only commercially available calcein detection device is the SE-MARK® detector (Plate 1) Western Chemical, Co., Ferndale, Washington (360) 384-5898.

To detect the calcein mark, examine all bony structures available: fin rays of all fins (especially pectoral and pelvic), operculum areas, jaw bones (dorsal, ventral, and lateral), and scales (Plate 2) using the following considerations:

Plate 1.- SE-MARK®
calcein detector

(1) ambient light levels.- During field-recovery and assessment of calcein marked vs. non-marked fish, investigators are obviously at a disadvantage due to presence of sunlight. This must be overcome through use of some technique for decreasing the amount of light in the immediate area of detection. At stream-side or in a boat this can be accomplished with something so simple as a sheet of dark-colored, opaque plastic or a poncho draped over the body of the investigator while fish are examined with the battery-powered SE-MARK® detector. If permanent sampling stations are established, then more elaborate dark room environments may be constructed and equipped with electrical-powered SE-MARK® detection units.

2) intensity of blue light striking the fish.- To obtain the greatest intensity of blue light from the battery-powered detector, make sure that the batteries are not weak. Using fresh batteries, observe the intensity of the blue light when the activated detector is shone on your hand, held from a distance of about 1 inch. This will provide a frame of reference for you to judge whether batteries are becoming weak as field work progresses. The other consideration for intensity of blue light is the distance the detector is held from the specimen. Obviously, the closer the light

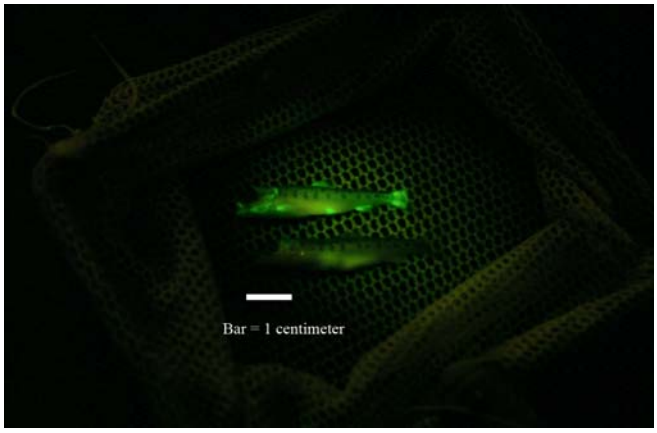

Plate 2.- Marked vs. non-marked Atlantic salmon fry with fin rays and other calcified tissues showing the characteristic green fluorescence of SE-MARK® calcein.

is held to the specimen, the greater is the intensity of light striking the specimen. Normally, the detector need not be held any closer than 1/2 inch from the specimen for adequate mark detection but the detector can be pressed against the specimen if desired.

Other factors which affect the ability to detect calcein marks are: (a) quality of the original mark as a function of marking technique and quality of the calcein compound itself, (b) size and life stage of the fish when it was marked, (c) elapsed time since fish were marked (d) the ability of the investigator to see the color green, which is the color of the fluorescence emitted from calcein-marked structures on the fish, (e) condition of the fish, and (f) exposure of marked fish to direct natural light.

(a) quality of the original mark .- To obtain highest quality calcein marks always follow established protocols for marking the particular species and life stage of fish being marked and use SE-MARK® calcein solution. Mohler (in progress) found that marks induced using SE-MARK® were more brilliant and longer-lasting those induced using calcein purchased from Sigma-Aldrich, Inc. (St. Louis, Missouri).

(b) size and life stage of the fish.- The amount of calcein which becomes bound to fish tissues is directly proportional to the amount of calcified tissues present. Therefore, fingerling and larger sized fish will obviously have more skeletal and supportive tissue development than larval fish. It follows that mark detection will be maximized in fish which are marked in advanced life stages as opposed to larvae. However, this concept is sometimes offset by the fact that it may be more efficient and less stressful on the fish to apply the mark at larval or pre-feeding life stages.

(c) elasped time since fish were marked.- Calcein marks are retained at their point of origin in calcified fish tissues. Since growth of fin rays in fish is terminal, the calcein mark will be most intense at the base of fins especially if much growth has occurred since fish were first marked. Growth of scales is also terminal and similar to growth rings of a tree, therefore calcein marks will be found on scales at the same location at which they were first induced (Plate 3). The presence of calcein-marked scales on a fish can be observed with the detection device, but verification of multiple marks as a banding

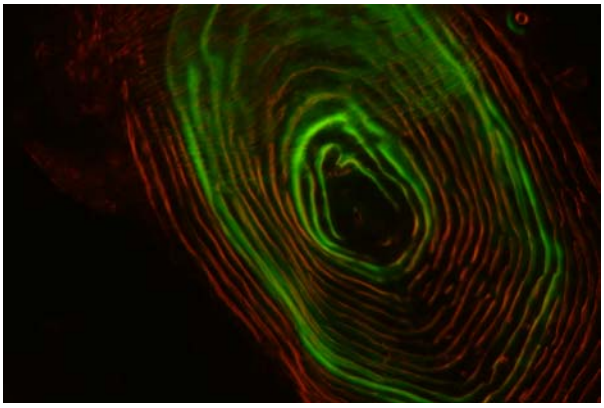

Plate 3. - Scale from an Atlantic salmon which underwent 2 separate calcein-marking episodes. The earliest mark is in the center of the scale.

pattern may require use of fluorescence microscopy techniques. For inexperienced investigators, it is helpful to have an unmarked fish of the same age/size available for comparison with marked fish. **Note: Scales removed from a fish which will not be microscopically examined immediately for calcein marks must be maintained in 190-proof grain alcohol, 200-proof absolute alcohol, or frozen in a block of ice to preserve the readability of the calcein mark. Use of denatured alcohols, untested solutions, or otherwise drying of calcein-marked tissues dramatically increases non-specific fluorescence making it more difficult if not impossible to see calcein marks.**

(d) color recognition of investigator.- Since the calcein mark is manifested as a green fluorescence, the ability of the investigator to recognize this color is obviously essential to mark recognition.

(e) condition of the fish.- Fish are often held in the investigators hand or laid on some other surface during calcein mark detection. Therefore it is recommended that fish be anesthetized prior to examination to minimize trauma to the fish as well as to facilitate close examination of fin rays and other calcified structures. In fish which have been marked up to 6 months, a quick glance through the activated detection unit should immediately reveal the fluorescent mark if one exists and anesthesia may not be needed, but in fish which have been calcein-marked from 6 months to 1 year, anesthesia is highly recommended so that fish are not mis-classified as unmarked.

(f) exposure of marked fish to direct natural light.- Calcein marks have been documented to last 14 months in fish liberated into the wild in Maine (Mohler 2004), but also were undetectable after only 5 months in the wild in Pennsylvania (Mohler et al. in progress a). Experimentation has shown that direct exposure to natural light has a deleterious effect on mark quality (Mohler and Farrell in progress b), therefore the calcein-marking technique may not be suitable for applications which involve allowing marked fish to remain at-large in aquatic environments exposed to high levels of direct natural light.

Non-lethal mark detection in Atlantic salmon and rainbow trout maintained indoors has been documented as long as 3 years post-marking (Mohler 2003; Negus and Tureson 2004). Therefore, if marked fish are to be released into the wild, it is desirable to maintain them indoors or in covered, outdoor tanks until they are released.

(3) Calcein labels all bony tissues present.- All bony or calcified tissues present in fish during the marking process will become calcein-labeled, such as: fin rays, skeletal bones, scales, and otoliths. Depending upon the species being marked and conditions in the environment, some structures will likely retain or reveal the calcein mark better than others, so researchers should examine the entire fish. For some fishery evaluations, it may be desirable to examine internal bony tissues such as otoliths (Plate 4) or spines. When sectioned and polished, these structures clearly reveal marking episodes.

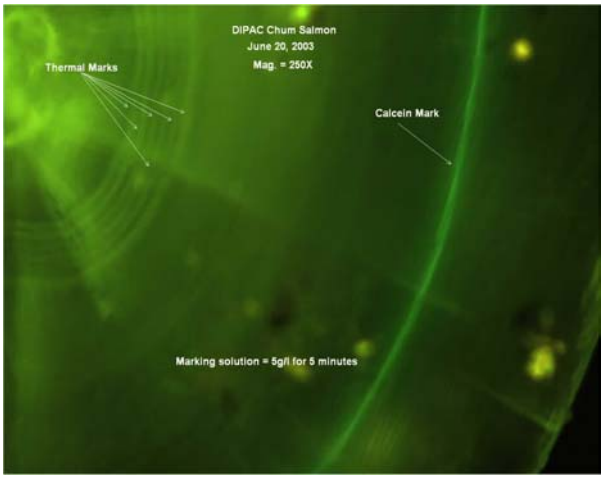

Plate 4.- Polished otolith from a chum salmon showing a calcein mark (outer band) as well as numerous thermal marks (inner bands). Photo courtesy of Don Mortensen, National Marine Fisheries Service - Auke Bay Lab, Alaska.

References

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