

Evaluation of Calcein Immersion and Medicated Feed to Mark Snake River Cutthroat Trout and Shovelnose Sturgeon



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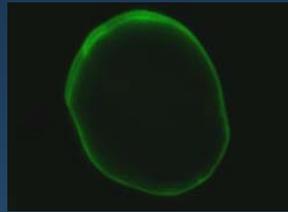
U.S. Fish & Wildlife Service

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BACKGROUND



Calcein

- fluorescent chromophore that binds to calcium
- used in ophthalmology in humans
- used in bone growth and formation studies
- more recently been used in fish as a marking compound

INVOLVEMENT

1987 First published paper of Calcein use in fisheries
“... Marking Otoliths of Larval and Juvenile Fish” (Wilson et al.)

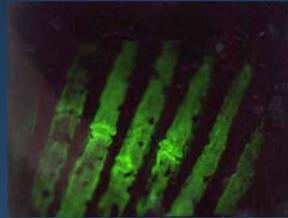
1993 Biologists at Northeast Fishery Center started researching a low-cost tagging/mass-marking technology which would allow for non-lethal detection

Jerre Mohler (NEFC)

- extensive work with Atlantic sturgeon and salmon
- developed the SE-MARK™ detector
- peaked interest of others to expand research



BACKGROUND



Calcein

- chemically binds with calcium phosphate in tissues such as otoliths, fin rays, scutes, and scales
- results in non-lethally detectable fluorescent marks
- able to achieve double-marks

INVOLVEMENT

CONTINUED

2003 INAD #10-987 Calcein (SE-MARK™) Immersion

USFWS – AADAP Program

Sponsor: Western Chemical Inc., Ron Secor

Product: SE-MARK™



IMMERSION

Immersion Mass-Marking

- 🐟 Folks are experiencing some difficulties
 - Handling stress (some mortality)
 - Time consuming
 - Disposal of the SE-MARK™ waste
 - Species sensitivity to salt bath and/or SE-MARK™
 - SE-MARK™ gets everywhere (“my waders are glowing”)
- 🐟 Does Feeding Calcein to Fish Produce a Mark?



PROJECT

Sound Science Project Proposal (submitted Summer 2005)

USGS Northern Appalachian Research Laboratory
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USFWS Lamar Fish Technology Center / Northeast Fishery Ctr
John Fletcher
Thomas Kehler
Jerre Mohler

Bozeman Fish Technology Center
Greg Kindschi

AADAP Program

Tom Bell
Jim Bowker
Molly Bowman



GOAL / OBJECTIVE



Goal

To develop adequate information regarding Calcein administration via feed to justify pursuit of an INAD for oral administration of Calcein as a non-lethal marking agent.

Objectives

- (1) To evaluate the effectiveness of Calcein fed to fish
Does It Work?
- (2) Mark retention – 3 year study (lab conditions vs sunlight)

Note: Currently under INAD 10-987 Calcein can only be used as an immersion agent

CONCERNS

Palatability

Preliminary trials with rainbow trout were done to confirm an effective dose range

Binding

Does Calcein bind to free-calcium in feed products

Dissolving

Will Calcein dissolve immediately in water

Potential Resolution ?



Encapsulation

ENCAPSULATION



Process to encapsulate Calcein and then incorporate it into the feed

- “The Coating Place” in Verona, WI
- 10 min retention time before coating dissolves in 75°F water
- as small as possible to be incorporated into starter diets
- once ingested, coating has to dissolve or otherwise make the Calcein available to the fish

STUDY DESIGN

WRITTEN DESIGN - Bozeman

Test Species

- ▶ Snake River Cutthroat Trout
- ▶ Shovelnose Sturgeon
- ▶ 200 fish / tank & 2 g fish

Treatment Conditions

- ▶ negative control (no treatment)
- ▶ positive control (immersion – marked fish)
- ▶ encapsulated feed – 40 mg Calcein / kg fish / day
(5, 10, or 15 d treated)
- ▶ top-coated feed - 40 mg Calcein / kg fish / day
(5, 10, or 15 d treated)

Replication

- ▶ triplicate (24 tanks of fish)



STUDY DESIGN

WRITTEN DESIGN - Bozeman

Sampling

Histology

- tissue samples taken from each tank 1 d post treatment

Mark-Evaluation

- fish evaluated 1 d post-treatment to evaluate mark with SE-MARK™ Detector (score of 0 – 3)

Luminosity

- fish sampled 30 d post-treatment and sent frozen to NEFC for evaluation



PROBLEMS

Deviations from the Written Design

Palatability of Calcein in feed

- ▶ SR cutthroat did not eat top-coated feed
- ▶ 3 treatment conditions (eliminated top-coated feed)

Size of fish

- ▶ CTT – about 3 g n = 200 fish/tank
- ▶ SNS – about 10 g n = 80 fish/tank

Feed Amount

- ▶ did not have enough feed
 - treated SR CTT for 15 days only
 - 9 tanks of SR CTT



STUDY DESIGN

ACTUAL DESIGN

Shovelnose Sturgeon

Negative Control

Positive Control

Encapsulated

5 d

10 d

15 d

Top-Coated

5 d

10 d

15 d

Snake River Cutthroat Trout

Negative Control

Positive Control

Encapsulated

15 d



IMMERSION

Positive Control

- 🐟 **Shovelnose Sturgeon**
 - 3% salt solution for 3.5 min
 - 0.5% SE-MARK™ solution for 5 min
 - 50 fish / 2 L of solution
- 🐟 **Snake River Cutthroat Trout**
 - 4% salt solution for 3.5 min
 - 0.5% SE-MARK™ solution for 5 min
 - 25 fish / 2 L of solution



No Fish Died During the Immersion Marking Process

IMMERSION



FEEDING TRIAL



BioOregon Feed
40 mg Calcein / kg of fish / day
Fed at 2% Body Weight



SE-MARK™ DETECTOR



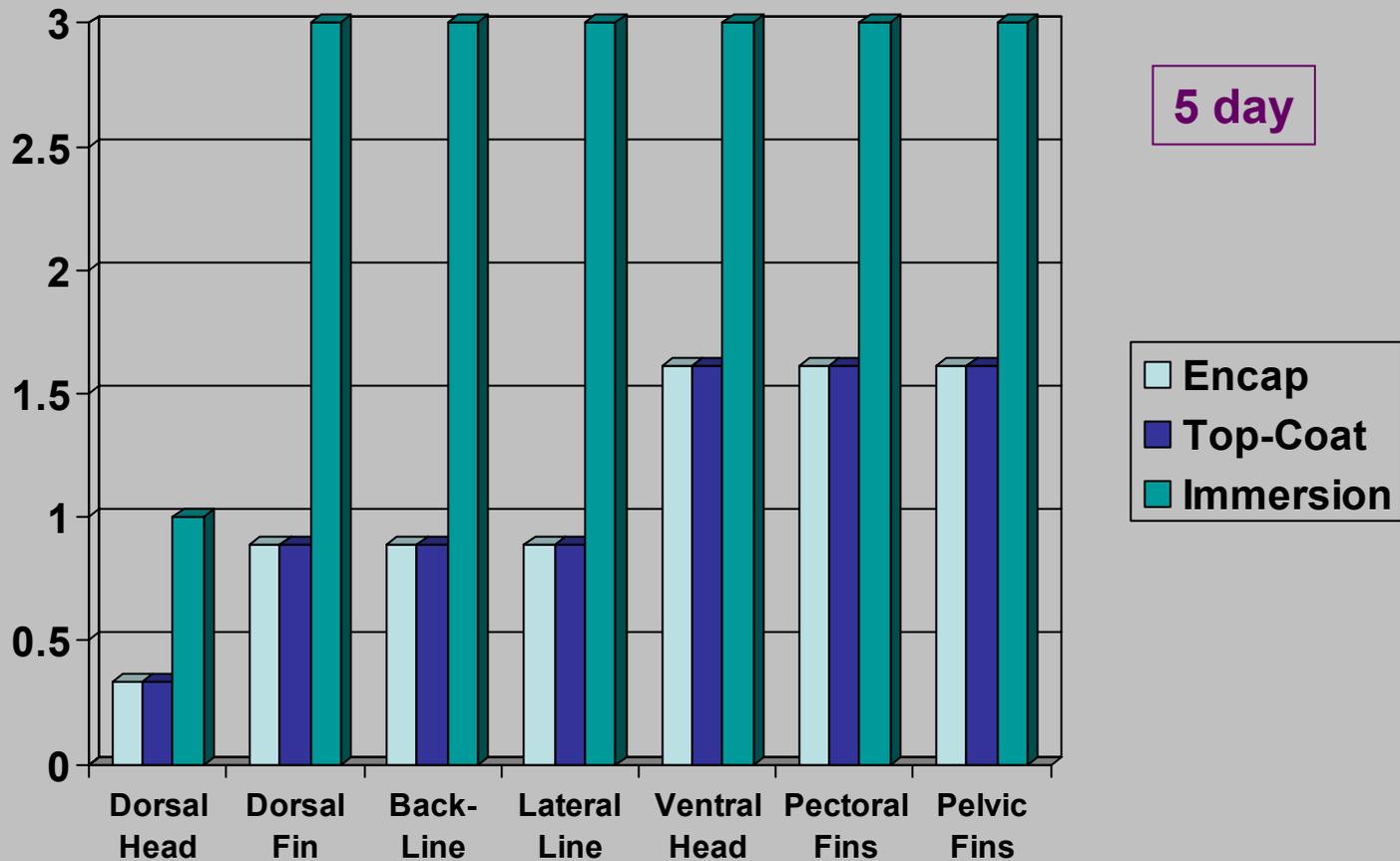
- ◆ Wave length = 495 nm
- ◆ Barrier filter = 520 nm

P R E L I M I N A R Y

R E S U L T S

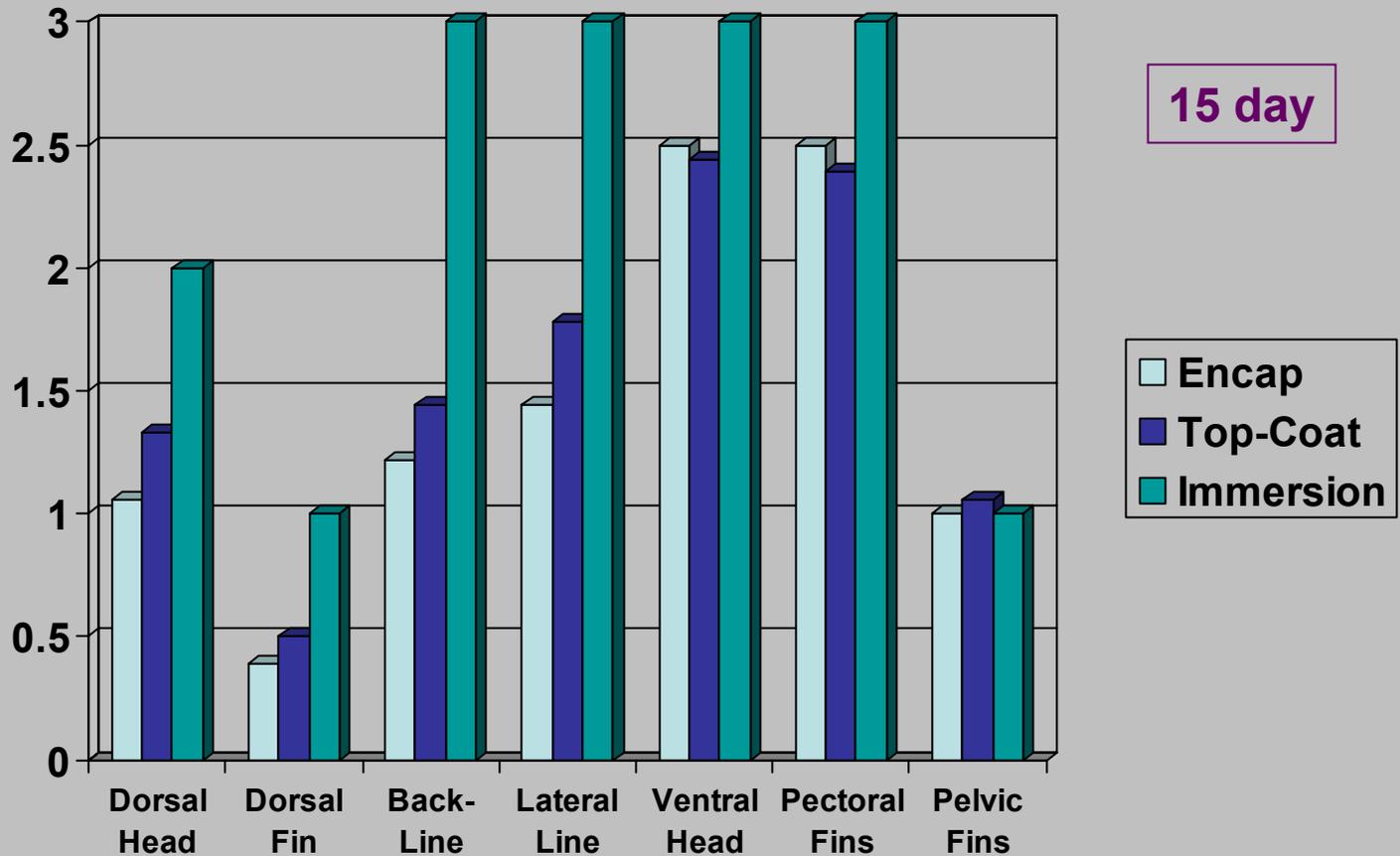
MARK INTENSITY

Shovelnose Sturgeon



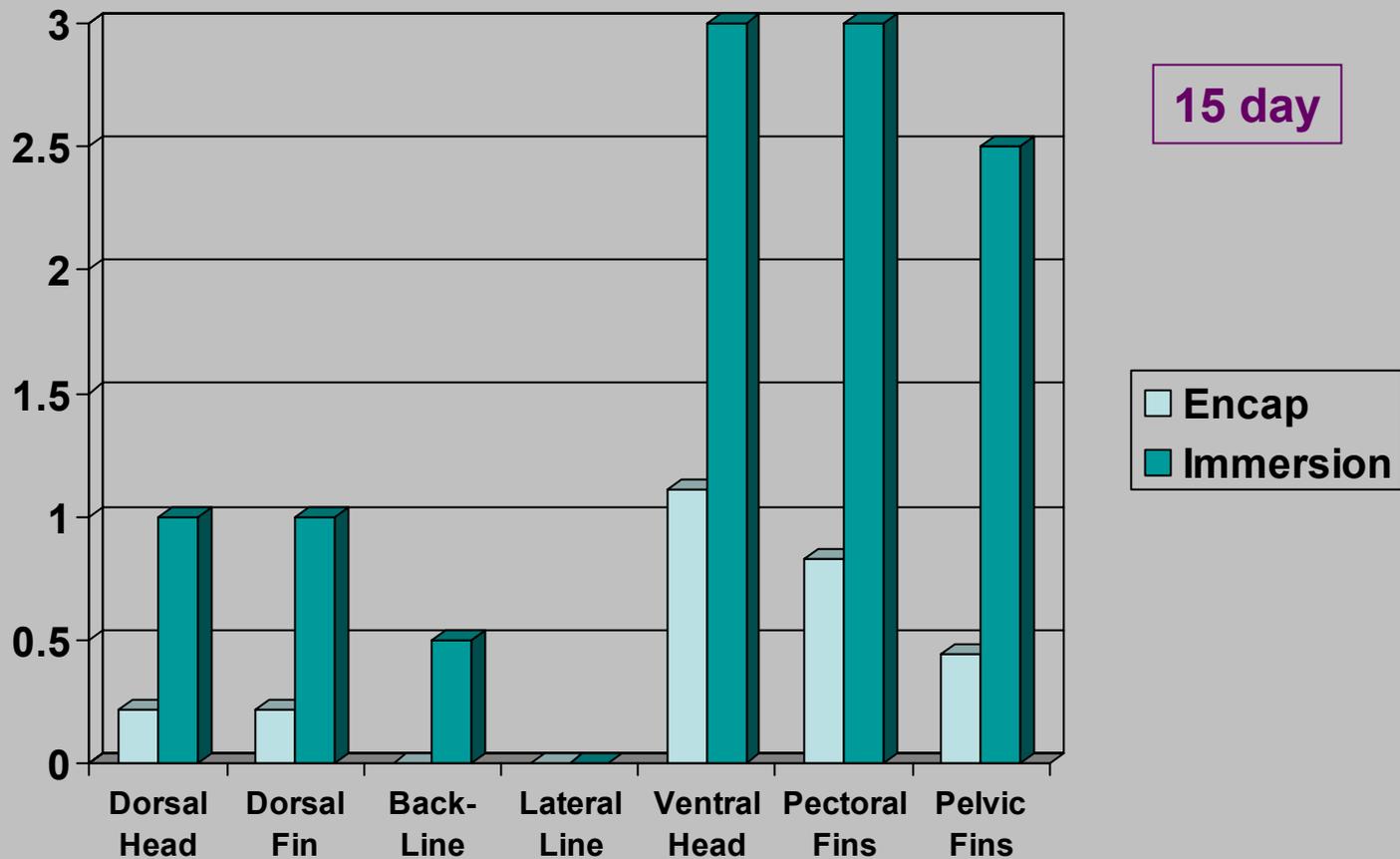
MARK INTENSITY

Shovelnose Sturgeon



MARK INTENSITY

Snake River Cutthroat Trout



IMMERSION

Shovelnose Sturgeon

5 d



10 d



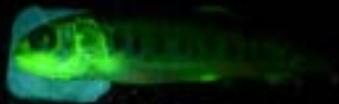
15 d



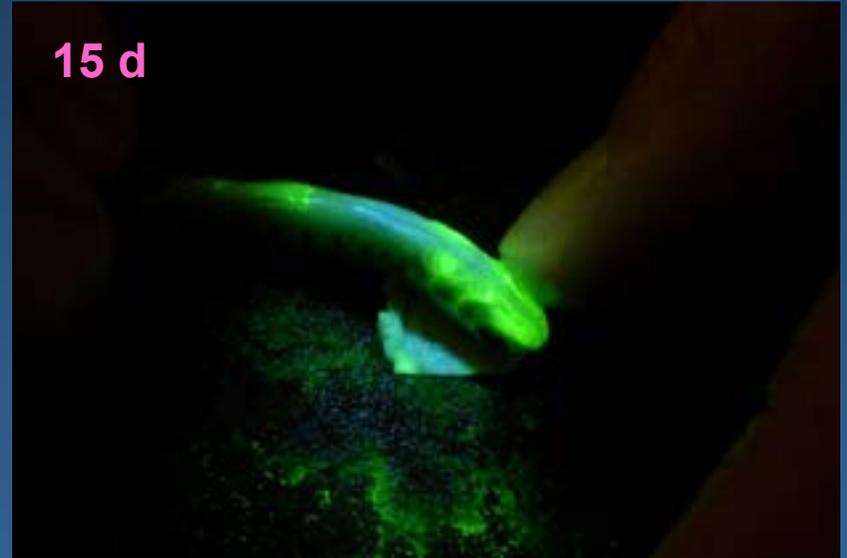
IMMERSION

Snake River Cutthroat Trout

5 d



15 d



TOP-COATED

Shovelnose Sturgeon

5 d

10 d

15 d



ENCAPSULATED

Shovelnose Sturgeon

5 d



10 d



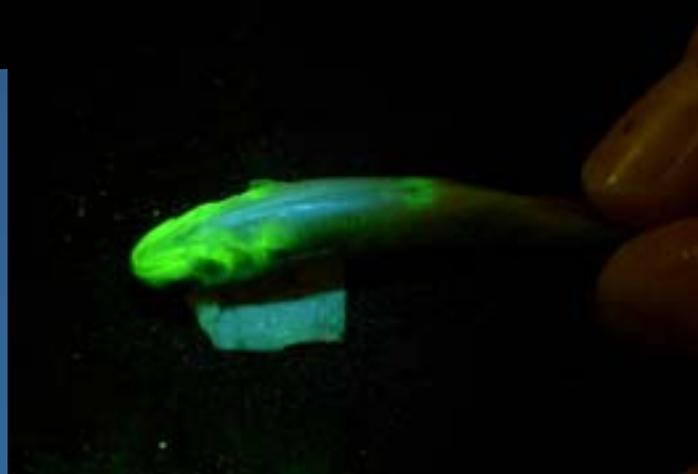
15 d



ENCAPSULATED

Snake River Cutthroat Trout

15 d



SUMMARY

PRELIMINARY RESULTS

Shovelnose Sturgeon

- 🐟 15 days of feed = “Best Mark”
- 🐟 5 and 10 days of feed = “Good Mark”
- 🐟 No apparent difference between top-coated and encapsulated diets



SUMMARY

PRELIMINARY RESULTS

Snake River Cutthroat Trout

- 🐟 Fish consumed feed (observed daily)
- 🐟 15 days = “Poor Mark”

We're Not Sure Why

- Physiological differences
 - Different fish size (SNS = 10 g and CTT = 3 g)
 - Digestive abilities
 - Different species
 - Different temperatures (SNS = 18°C and CTT = 12°C)
- Encapsulation coating more easily dissolved?

Possibly try lower concentration of top-coated feed to test palatability
(10 - 20 mg Calcein / kg fish / d)



SAMPLING

CONTINUED

- **Sample at 30 days post-exposure for all medicated-feed treatment groups**
- **Send frozen samples to the Northeast Fishery Center**
- **Marks will be evaluated by fluorescent microscopy**
 - **scale and scute samples to be evaluated**
 - **luminosity will be quantified with Adobe Photoshop 7.0**
- **“Best-Mark” treatment group will be kept and evaluated periodically for 3 years (best does not necessarily mean brightest)**
 - **will evaluate effects of UV / sunlight on marked fish**

? QUESTIONS ?



AADAP Program Website:

www.fws.gov/fisheries/aadap