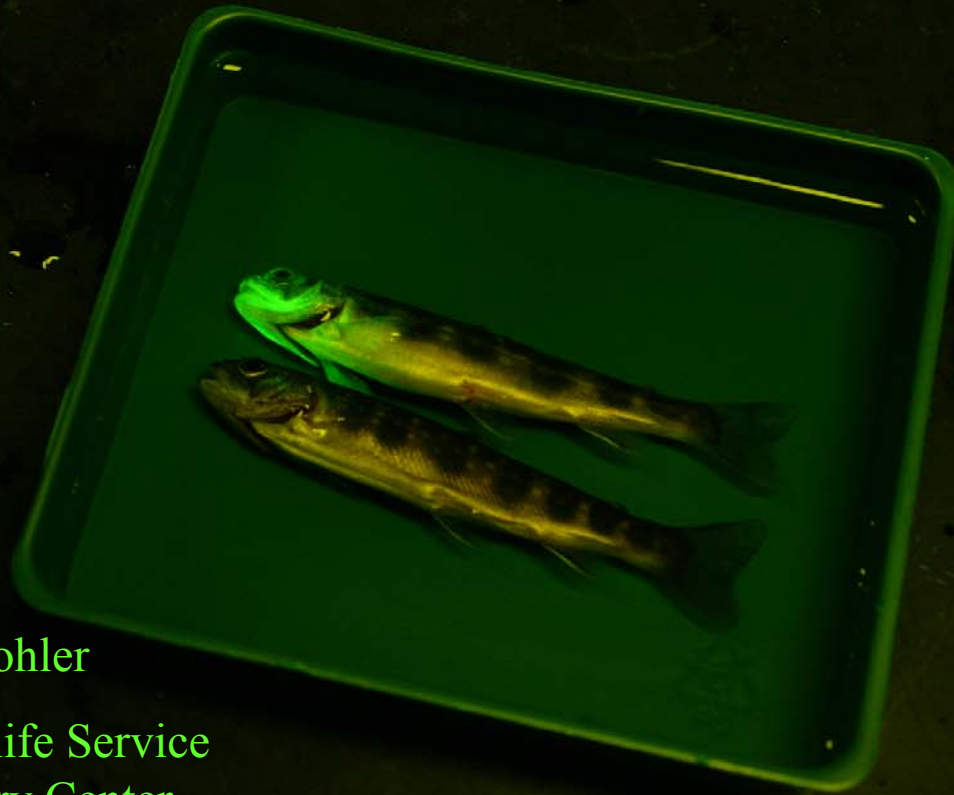




Calcein Marking, a Promising New Tool for Hatchery Product Evaluation



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What is calcein?

- Fluorochrome dye; yellow-orange powder, water soluble, green color in solution
- Chemical formula: $C^{30}H^{26}N^2O^{13}$
- Chemically binds with calcified tissues (fin rays, bones, scales, otoliths)
- Once bound, it remains invisible to the naked eye ... but fluoresces bright green under specific optical conditions
- Not yet approved by FDA for use on food fish, but INAD permit exemption application is in progress by USFWS-Bozeman, MT

What is calcein used for?

- First synthesized in 1956 as an indicator for calcium content of water and limestone.
- Bone label studies in mice (1966) and lab rats (2000)
- Used in 1972 to assess fit for soft contact lenses due to its non-irritating properties
- Used experimentally as a fluorescent dye to trace blood flow through the eye (angiography) in 1990
- In fish, first reported as a fluorescent marker for otoliths by Wilson, Beckman, and Dean. 1987
- In 1995, NEFC-Lamar began calcein evaluations with Atlantic salmon to develop a fry marking technique

History of calcein mark development and use at NEFC

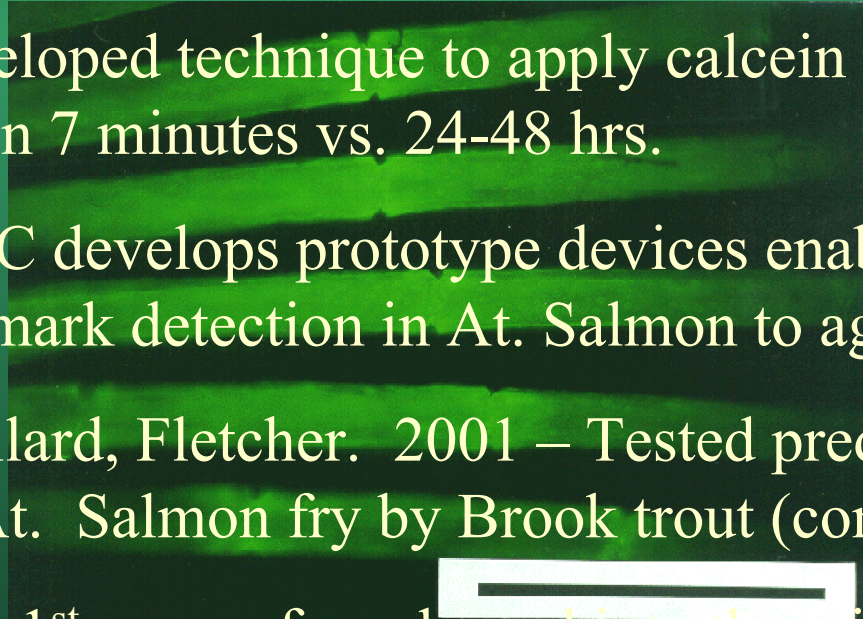
- Mohler 1997. Reported that calcein could be used as a fluorescent label in fins rays of Atlantic salmon fry (48-h passive immersion @ 125-250 mg/L) with microscopic non-lethal detection.

- 1998 – Developed technique to apply calcein mark to Atlantic salmon fry in 7 minutes vs. 24-48 hrs.

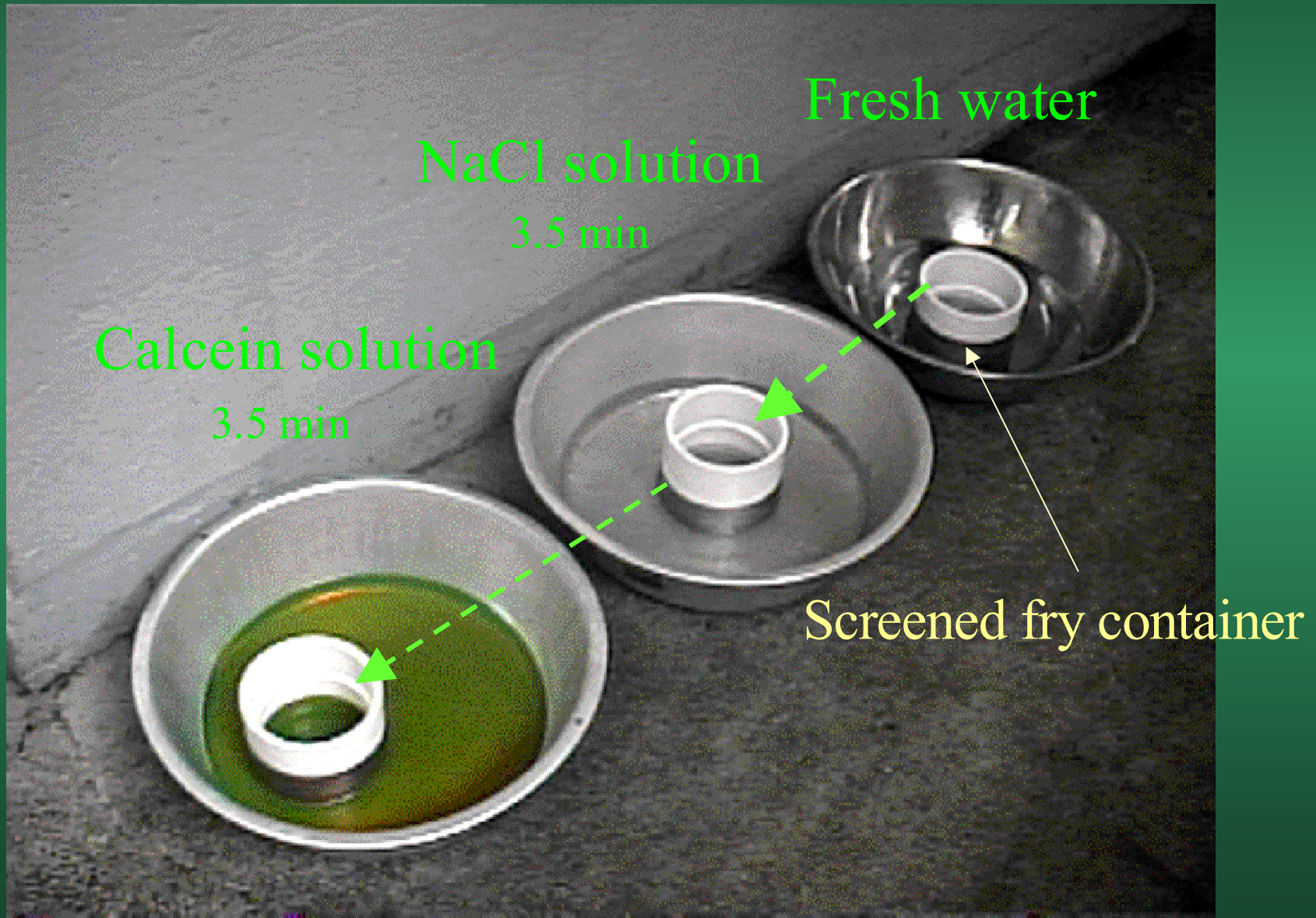
- 1999 - NEFC develops prototype devices enabling macroscopic non-lethal mark detection in At. Salmon to age 3

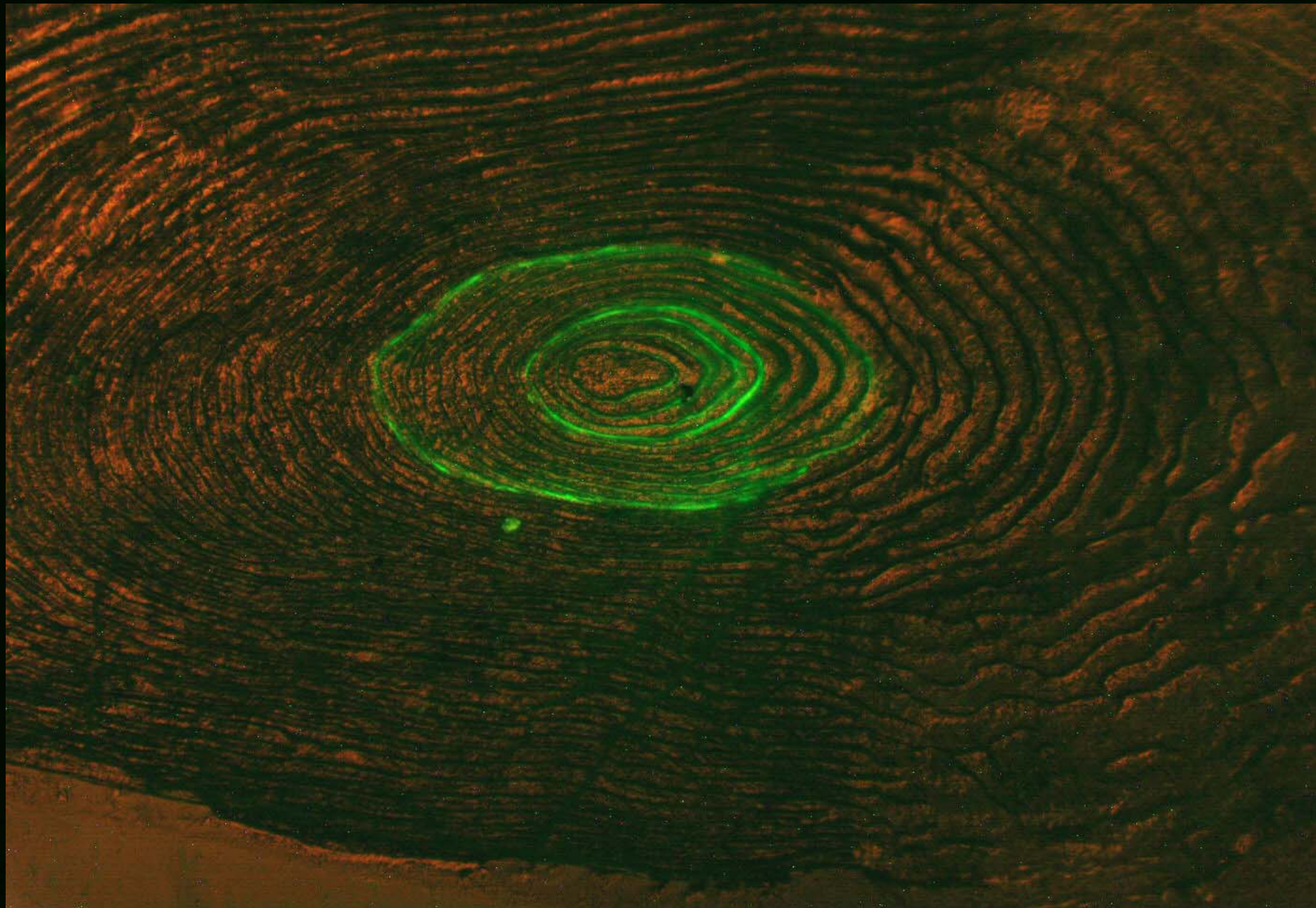
- Mohler, Millard, Fletcher. 2001 – Tested predation of marked vs unmarked At. Salmon fry by Brook trout (controlled study)

- 2001/2002 - 1st use on fry released into the wild – endangered stocks of Atlantic salmon in the Sheepscot River, Maine



Applying the calcein mark via immersion (osmotic induction method; 7-min mark)





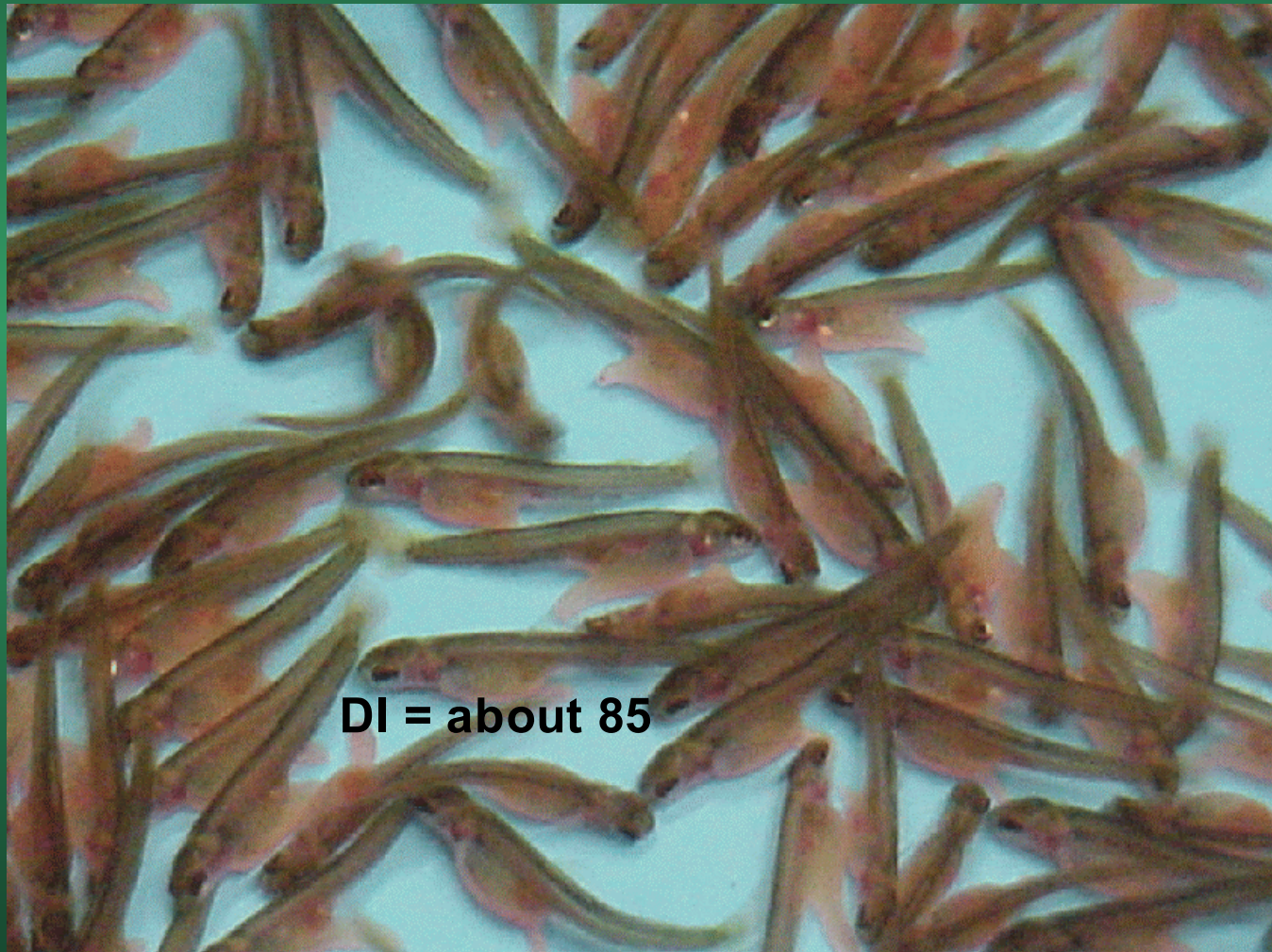
Case Study with endangered Atlantic salmon stocks

Using about 60K fry produced at Craig Brook NFH from Sheepscot River stocks in 2001/02, we:

- Determined whether fish which were mass-marked with calcein in the hatchery as sac-fry and released into the W. Br. Sheepscot could be differentiated from their stocked, unmarked counterparts after 4 months in the wild.
- Tested the hypothesis that natural mortality of calcein-marked salmon fry is equal to that of unmarked fry
- Evaluated calcein mark detection techniques under field conditions

Fry marking

- April, 2001/02 at Craig Brook
National Fish Hatchery



30K fry marked / \$300 = \$0.01 ea

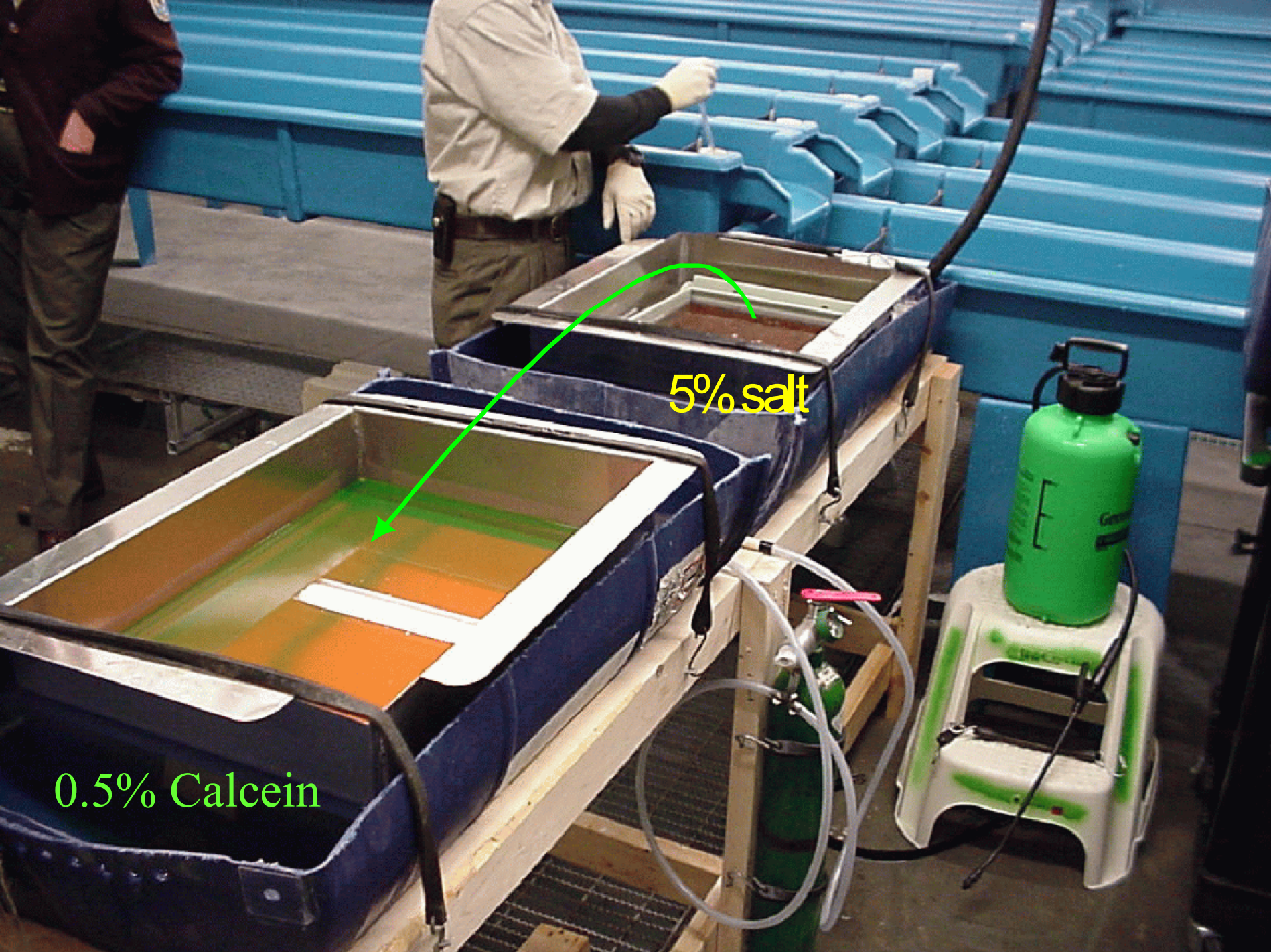


Calcein (5 g/L)

**\$300 to mark 7
trays**



Ambient temp. water bath



5% salt

0.5% Calcein

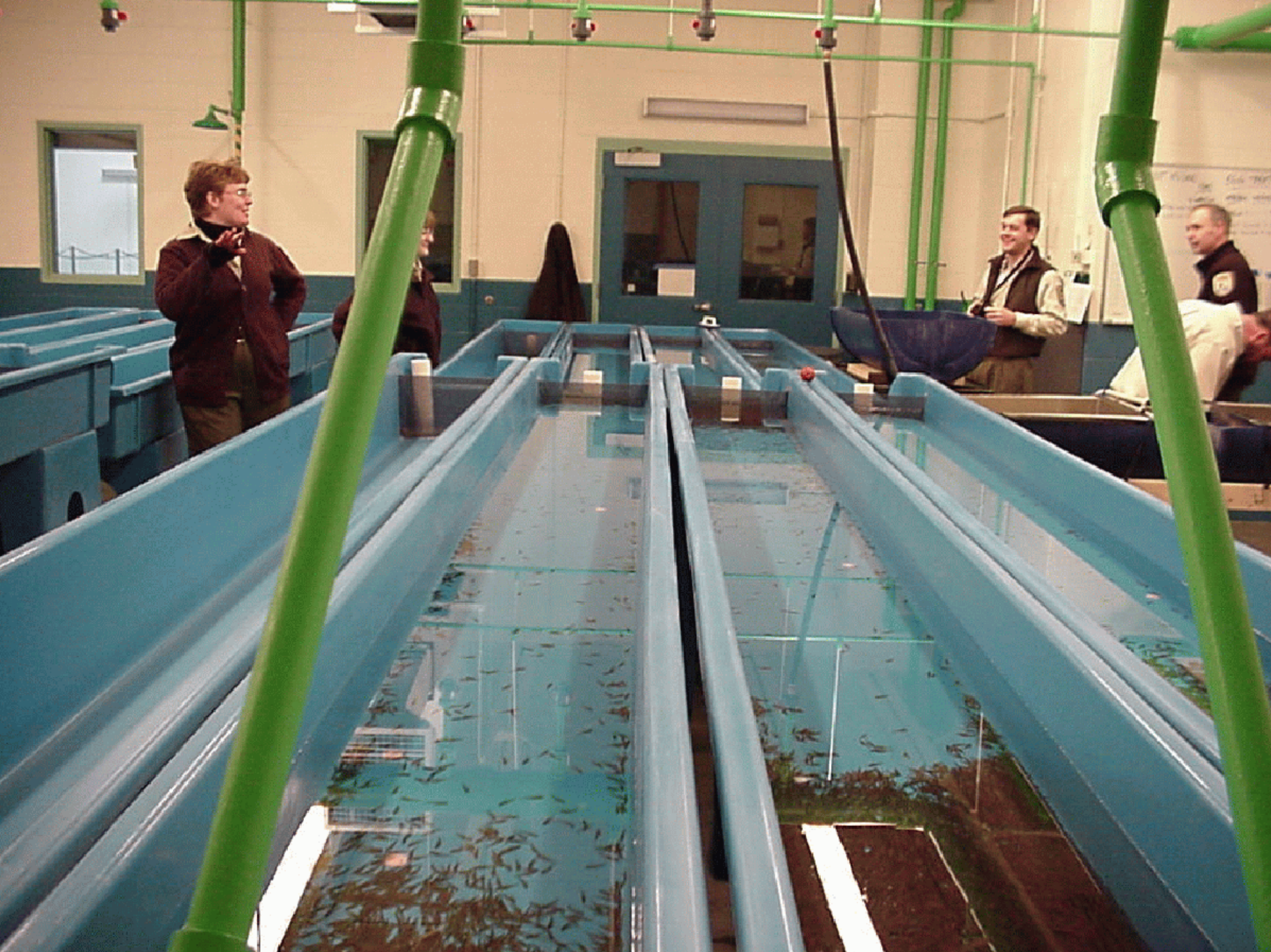


**30K fry were marked (7 trays)
30K were not marked**

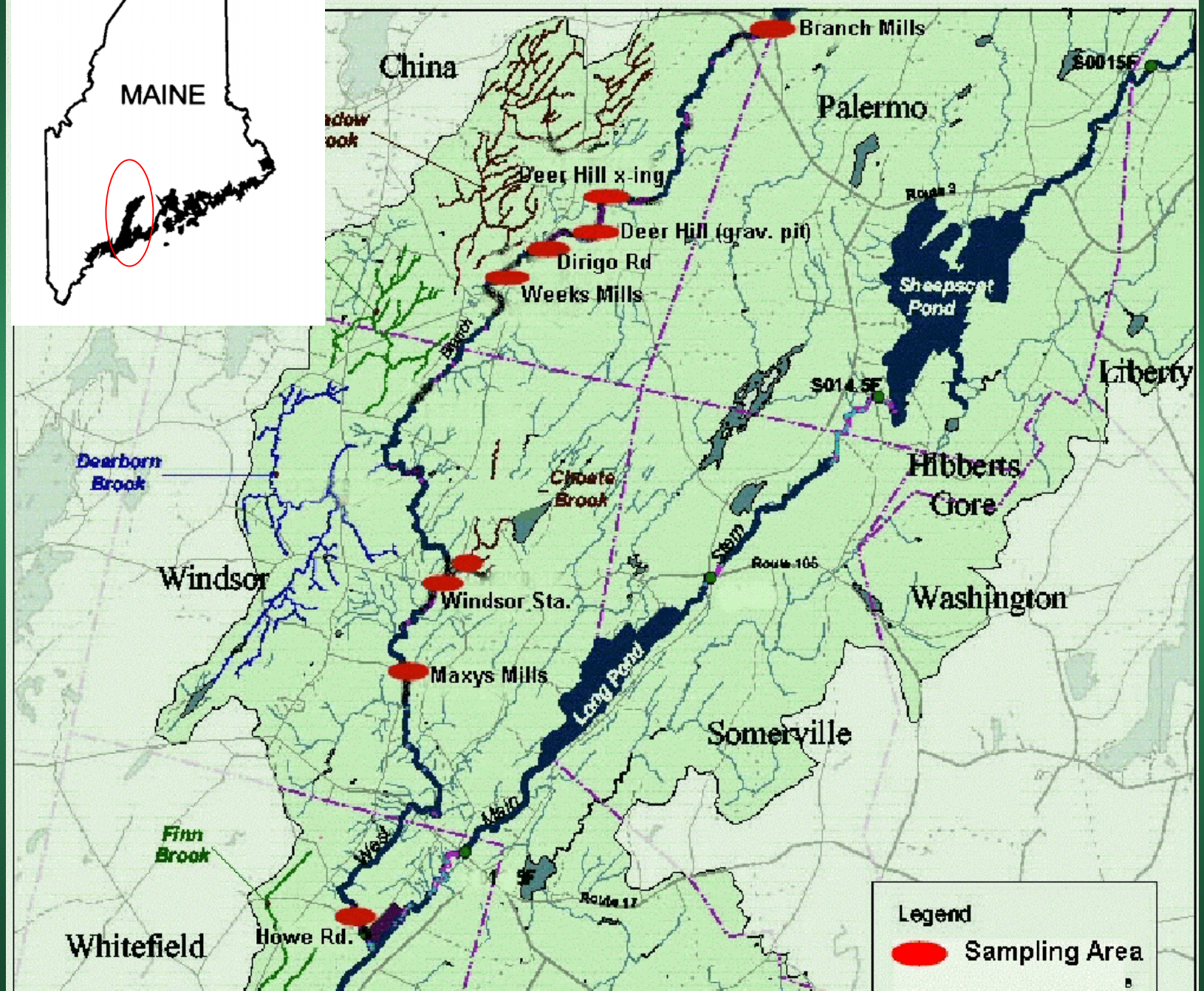
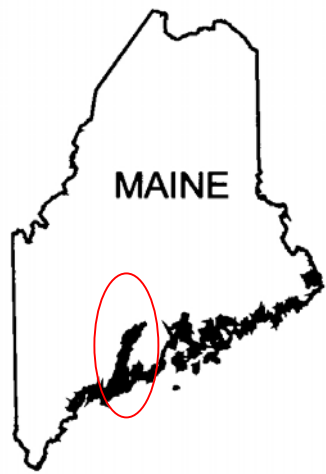
**Heath tray insert
(4 - 5K fry)**

Gently rinsing off excess calcein solution
into a waste container





Sheepscot River Watershed



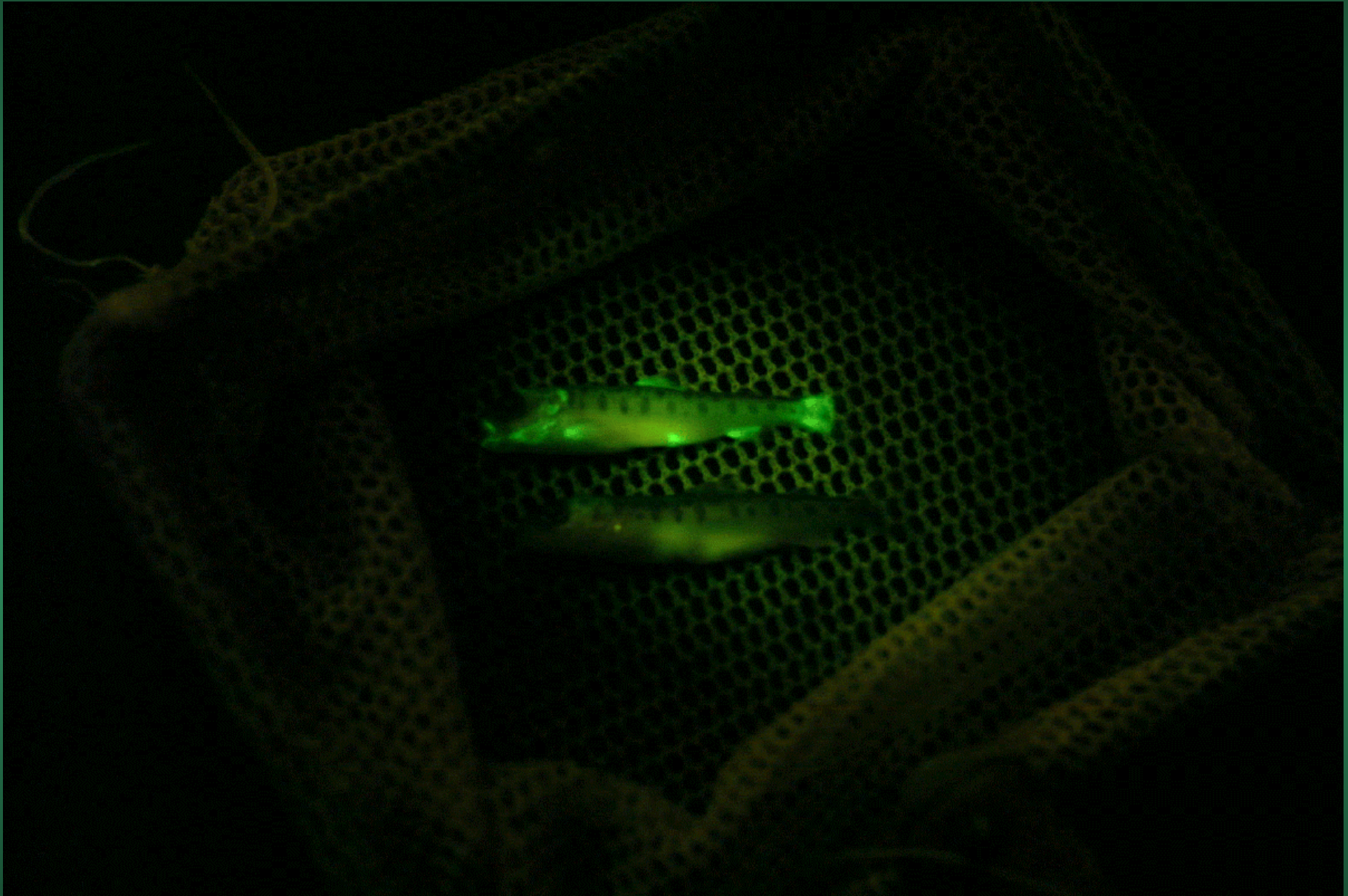


- Fry were anesthetized,
measured, and classified as
marked or un-marked



Portable darkroom and battery-powered calcein detector





- ' *Hypothesis:* Natural mortality of calcein-marked Atlantic salmon fry is equal to that of unmarked fry (1:1 ratio)
- ' *Analysis:* Data were tested using Replicated Goodness-of-Fit (G-Statistic) at alpha level of 0.05

Individual G values for sampling sites (2001)

Site	Unmark	Marked	G_i	G_c	df	Significant?
Howe Rd3	18	7	5.009	3.841	1	Yes
Deer HI Pit	24	28	0.308			No
Branch Mills	5	4	0.111			No
Up. Choate	33	21	2.689			No
Low Choate	13	13	0.000			No
Trout Brook	10	15	1.006			No

Goodness-of-fit analysis

	Unmark	Marked		G_c	df	results
Pooled data	103 (54%)	88 (46%)	G_H 7.945	12.592	6	homogeneous
			G_P 1.179	3.841	1	fits expected ratio
			G_T 9.124	14.067	6	fits expected ratio

Individual G values for sampling sites (2002)

Site	Unmark	Marked	G_i	G_c	df	Significant?
Weeks Mls	65	5	61.016	3.841	1	Yes
Dirigo Rd	9	2	4.818			Yes
Deer Hill	113	34	44.780			Yes
Windsor Sta.	10	3	4.818			Yes
Choate Br.	31	22	1.536			No
Smokey Cp.	2	4	0.680			No
Trout Brook	15	11	.931			No

Goodness-of-fit analysis

	Unmark	Marked		G_c	df	results
Pooled data	236 (75%)	78 (25%)	G_H			
			30.529	11.070	5	Ratios not homog.
			G_P			
			87.208	3.841	1	Does not fit ratio
			G_T			
			117.737	12.592	6	Does not fit ratio

Total length comparison: marked vs. unmarked

2-way ANOVA (allowed for sample site effect)

Least square means:

Calcein-marked $67.7 \pm 0.9\text{mm}$

Unmarked $66.8 \pm 0.8\text{mm}$

$P=0.45$ (no significant difference)

Additional observations from 2002 field work:

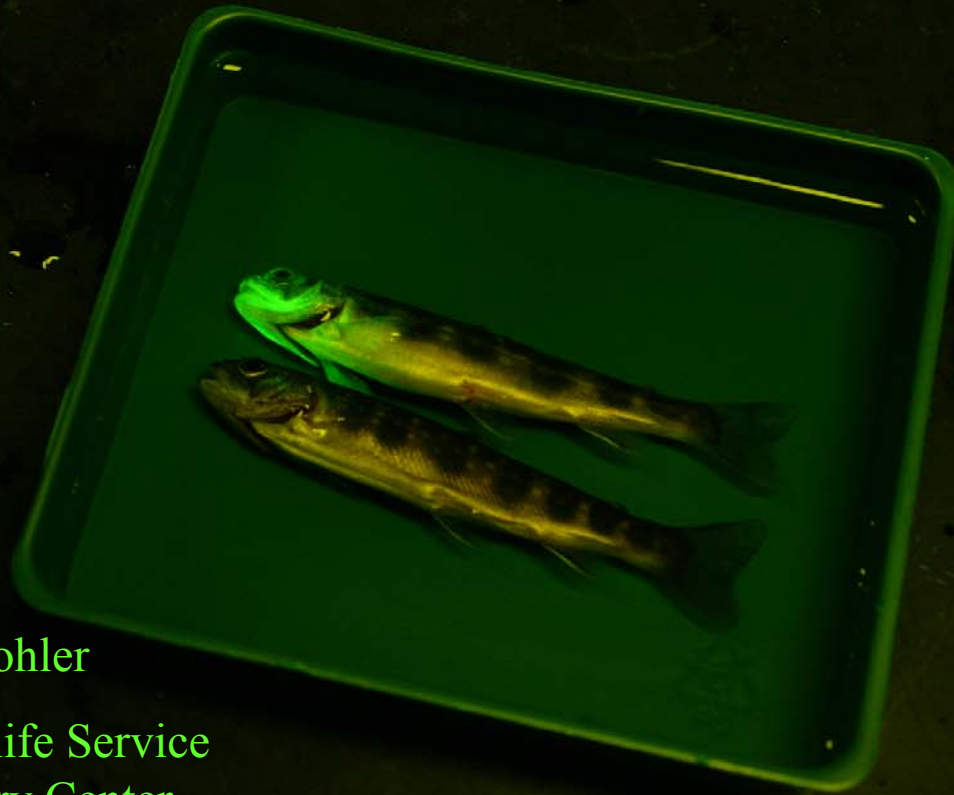
- Out of 52 parr captured (age 1 or greater), 13 had calcein marks which had been applied in April 2001
- Age 1 calcein marks were best viewed on the ventral side of the mandible
- 5 natural redds were identified in the study area

Conclusions:

- Results of Goodness-of-fit may not be accurate given that numbers of wild YOY captured are unknown
- The calcein marking technique shows promise as an inexpensive and practical way to batch-mark ATS fry for hatchery product evaluations
- Field detection equipment worked reliably and resulted in instantaneous mark classification
- More tightly-controlled studies needed
- Calcein marks persisted in the wild for at least one year in At. salmon marked as sac-fry



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