DEVELOPMENT OF LASER MARKING AS A MASS MARKING TECHNIQUE

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Prepared by
H. Lee Blankenship and Dan Thompson

Washington Department of Fisheries

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U.S. Department of Energy
Bonneville Power Administration
Division of Fish and Wildlife
Post Office Box 3621
Portland, Oregon 97208-3621

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ABSTRACT

The first year of work with development of lasers as a mass marking technique provided both disappointing and encouraging results. A Coumarin Dye 480 laser was used to mark coho salmon in a variety of body locations and with varying energy levels. A “bleached” white mark was made void of any pigment. Areas marked included the nape area behind the head and in front of the dorsal fin, slightly above the anal fin, the upper lobe of the caudal fin, the dorsal fin and on the operculum. The mark appeared immediately after being lasered but started to gradually fade after one month and was fairly completely re-pigmented after three months. Complete removal and notching of the adipose fin was also attempted with a Carbon Dioxide laser. This surgical method of fin removal appears to have advantages over scissor excision (no bleeding or regeneration), and has possible application as part of a device or system which could be employed for mass marking.

INTRODUCTION

The need for relatively inexpensive, benign, and easily identifiable marks which can be applied to large numbers of individuals is nothing new to fisheries management. The Endangered Species Act and declining fisheries, however, have accentuated the need for mass marking in two major areas. The first relates to broodstock management for the purpose of maintaining genetic integrity within the area of artificial propagation and supplementation. The second involves harvest management needs where weak, threatened, or endangered stocks need protection in mixed-stock fisheries where it is desirable to harvest healthier stocks (selective fisheries). To address this pressing need, PSMFC's Regional Mark Committee appointed a Subcommittee on Mass Marking in September 1991 to evaluate mass marks. Their report on "Mass Marking Anadromous Salmonids: Techniques and Options" did not provide fishery managers with any new viable options to solve their mass marking needs. The report, however, did recommend the need to investigate the use of lasers as a potential new technique.

Marking animals with lasers in not a new idea. Dr. R. K. Farrell from Washington State University, patented the process in 1975 (U.S. Patent 3,916,143; October 28, 1975) and marked catfish with a ruby laser. The ruby laser was the first laser invented and this work was done with what is now considered an ancient laser. The marks were visible for at least one year (Brock and Farrell, 1977). Hawkes (1973, 1976) reported on the effects of ruby laser light on fish skin. Unfortunately, Dr. Farrell died shortly after his initial work and acquiring the patent. His work and Hawkes' work left many unanswered questions in regards to mark clarity and longevity.

Pigmentation of salmon skin comes from three sources: melanophores, xanthophores, and iridophores. Melanophores and xanthophores present color through optical absorption of the unpresented wavelengths. Melanophores, which are optically black, absorb light in the ultra-violet, visible and near infrared regions of the spectrum (with their absorbtivity increasing linearly with decreasing wavelength), and thus are susceptible to disruption by a broad range of laser wavelengths - given that the laser has sufficient peak power. Xanthophores are visually yellow-orange when illuminated by natural light, apparently indicating they absorb blue and green colors making them sensitive to those wavelengths. It should be noted that Hawkes' studies indicated that xanthophores were not damaged by the deep red light of the ruby laser she used. Iridophores most often operate by optical interference, being layered structures with dimensions comparable to a wavelength of visible light and were damaged along with the irradiated melanophores in Hawkes' studies.

METHODS AND MATERIALS

The laser chosen to mark the fish by “bleaching” the skin or destroying the pigment cells with minimal collateral damage to adjacent cells was a linear flashlamp pumped dye laser model LFO8-8 from Cynosure in Bedford, Maine. The Coumarin 480 Dye (CD) Laser System included a 3KW switchmode power supply with remote control, CFDCC 40 dye and coolant circular with dye lifetime extender for C-480 dye, 600 M fiber attachment, laser head with 24-inch gain length head, thyration switched modulator and boardband optics for C-480. The technical specifications include an initial pulse energy of 500 millijoules (MJ), pulse energy of 500 MJ, and pulse energy exceeding 450 MJ after 10,000 pulses without dye change.

The laser was mounted on an optional breadboard and installed in a Dodge Maxivan. Retrofitting the maxivan and initial testing of the laser was done at Oregon Graduate Institute, Hillsboro, Oregon. The maxivan was driven to Washington Department of Fisheries (WDF) George Adams Hatchery near Shelton, Washington for marking March 20, 1993. The maxivan was parked adjacent to a WDF Mobile Coded Wire Tagging Trailer (Schurman and Thompson 1990). Power was supplied to the laser via the tagging trailer and fiber optics led from the maxivan to a marking station inside the tagging trailer.

In addition to the CD laser, a Carbon Dioxide (CO_2) laser was tested to see if fin removal with lasers was feasible. The CO, laser energy levels are much greater than the CD laser and cuts and burns rather than “bleach marks”.

Nine groups of yearling coho were marked with the CD laser and four with the CO, laser on March 20 (Table 1). The coho averaged 110 mm fork length.

The CD laser was used to apply marks to five different body locations: nape (on dorsal side behind head) above the anal fin, caudal fin, dorsal fin, and the operculum. Increasing energy levels within these body areas were also attempted depending upon the amount of energy that could be absorbed without causing excessive collateral tissue damage. Increased energy levels were applied by using repeated pulses of energy with each pulse equating to about 700 MJ/CM^2. Initial results from this experiment led to two additional groups which were marked on June 13, 1993. Both groups were marked in the nape area but with much lower energy levels of 130 and 30 MJ/CM^2 (Table 1).

The CO, laser was used to burn across the fin rays on the dorsal and caudal fins and to cut a-notch in the adipose fin as well as its complete removal (Table 1).

The fish were held in separate tanks for three months for observations, photographs and histological samples. The marks were observed and photographed weekly for the first four weeks and every two weeks thereafter.

Table 1. List of Coho marked with Carbon Dioxide Laser and Coumarin Dye Laser along with the associated body location and energy level.

LASER	AREA OF MARK	ENERGY LEVEL (Millijoules/cm^2)
Coumarin Dye	On back behind head	1 Pulse - 700
Coumarin Dye	On back behind head	3 Pulses - 2100
Coumarin Dye	On back behind head	5 Pulses - 3500
Coumarin Dye	Above anal fin	1 Pulse - 700
Coumarin Dye	Above anal fin	3 Pulses - 2100
Coumarin Dye	Operculum	1 Pulse - 700
Coumarin Dye	Upper lobe caudal fin	1 Pulse - 700
Coumarin Dye	Upper lobe caudal fin	2 Pulses - 1400
Coumarin Dye	Dorsal fin	1 Pulse - 700
Carbon Dioxide	Notch in adipose fin	Not measurable
Carbon Dioxide	Remove adipose fin	Not measurable
Carbon Dioxide	Mark across caudal fin	Not measurable
Carbon Dioxide	Mark across dorsal fin	Not measurable
Coumarin Dye	On back behind head	30
Coumarin Dye	On back behind head	130

RESULTS AND DISCUSSION

Immediately after being lasered with the CD laser, a good visible “bleach mark” was obtained at all the different body areas attempted and at all energy levels utilized (Figure 1). If the target area had scales, the initial blast avulsed the scales and epidermis down to the surface of the stratum compactum. The damage appears to be due to acoustic shock, in that the margins of the hole are torn off and not burned. Usually all pigment cells above the stratum compacturn are likewise removed. The stratum compactum looks initially unaffected and only in the most intensely radiated locations are collagen strata swollen, presumably by heat denaturation. Pigment cells under the stratum compacturn are much more resistant to ablation. Only with 3-5 laser hits are the deep melanophores extirpated, at which time enormous blisters are formed under the stratum compacturn. The adjacent muscle looks slightly edematous but nearly normal. The blister cavity is usually filled with fibrin clots and some blood. Connective tissue strips between fin rays of the tail often will curl out of the plane of the skin by having been severed from the adjacent rays.

The epidermis recovers from the laser blast quickly. It closed within a week over all but the very largest injuries, which are completely re-epithelialized a few days later. The upper layers of the epidermis remain open textured (spinous) for some time as a function of the underlying connective tissue injury, but return to normal morphology after about five weeks. Goblet cell return is variable, sometimes sparse, in other instances profuse, but within the limits of normal. The aggressive growth of the epithelium along the convenient substratum of the stratum compacturn precludes any discernible bacterial or fungal invasion. Loose pieces of scales or connective tissue are often surrounded by the advancing epithelial front and subsequently purged from the tissue. Macrophages quickly come into the area where cell damage has occurred and quickly clean up the damaged area. Pigment cells return with the macrophages to such an extent that the area actually appears hyper-pigmented. Figures 2-4 show the marks one week after marking and again after 8 weeks.

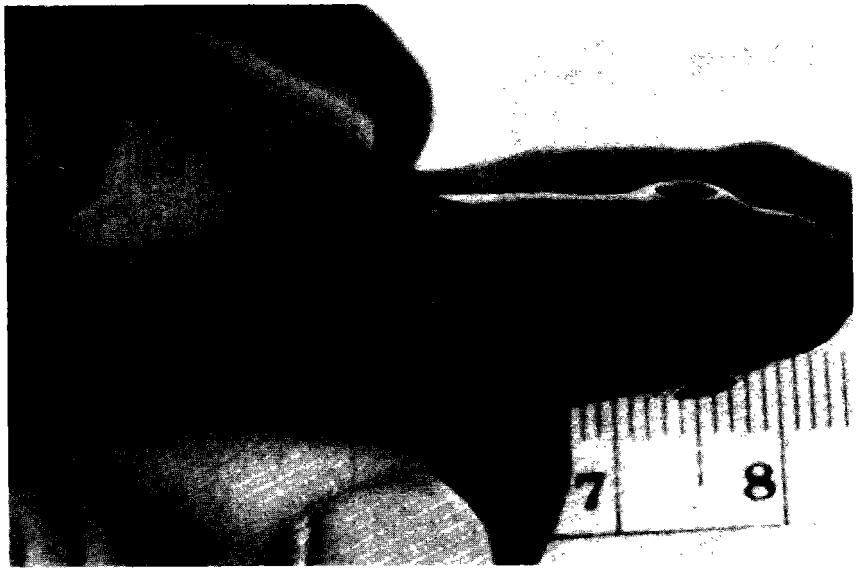
Subepidermal pigment cells are reestablished as a layer of small, but perfectly ordered and mutually properly organized pigment cells of all three types after two months. An additional month of growth returns the epidermis and its pigment cell complement to the normal state. This re-introduction of pigment cells occurred at all body locations and at all energy levels. The second group of marking at the lower energy level was done because it was thought with minimal collateral tissue damage the ensuing migration of macrophages and re-introduction of pigment cells could be minimized. This occurred to some extent, but the lower energy levels didn’t destroy the pigment cells below the stratum compacturn. Further work based on histological research should be carried out to determine if a feasible way of destroying the chromatophores can be accomplished without the ensuing migration of macrophages (which results in hyper-pigmentation). At this time however the feasibility of obtaining long-lasting marks by bleaching the chromatophores with lasers is looking less promising than originally envisioned.

FIGURE 1

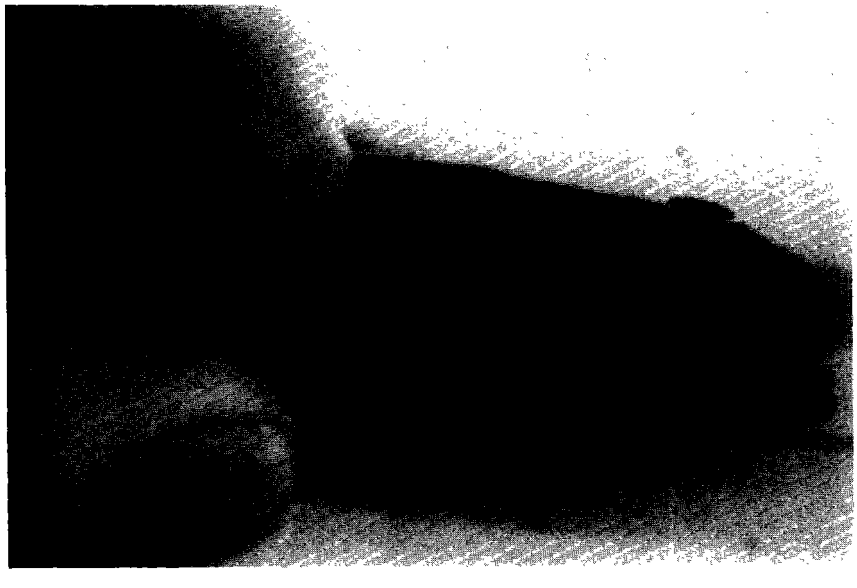
A photograph of a lasered “bleach mark” on a coho salmon smolt immediately after marking.

FIGURE 2

Photographs of a lasered mark on the nape area of a coho salmon smolt 1 week and 8 weeks after marking. Background scale in millimeters.



Week #I

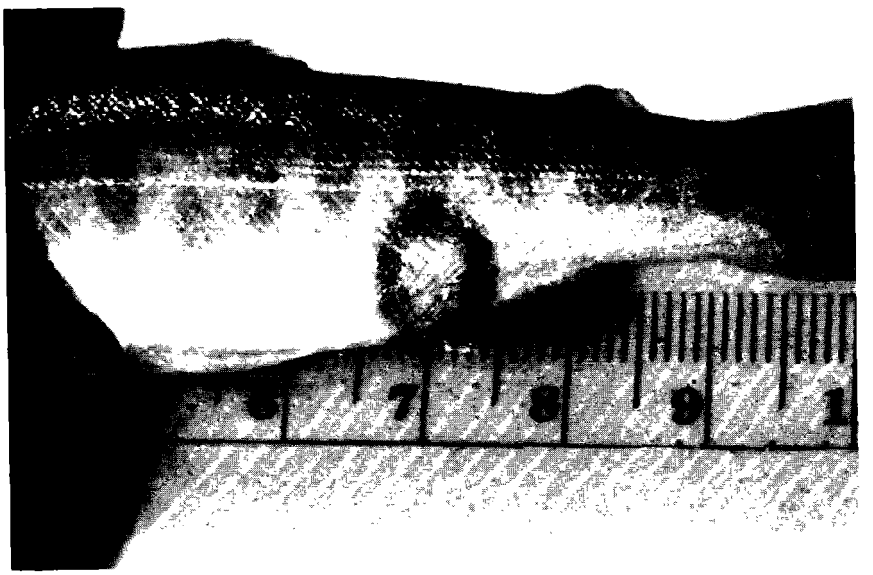


Week #8

FIGURE 3

Photographs of a lasered mark above the anal fin of a coho salmon smolt 1 week and 8 weeks after marking background scale in millimeters.

Week #I



Week #2

FIGURE 4

Photographs of a lasered mark on the upper lobe of the caudal fin of a coho salmon smolt 1 week and 8 weeks after marking. Background scale in millimeters.

Week #I

Week #8

The dorsal and caudal fins which were burned with the CO, laser showed similar results to tissue damage as described above but often with more substantial surface disruption. Here, however, nerves and blood vessels traveling between fin rays are evidently sufficiently damaged that a progressive degenerative process from the injury site distad sets in. As a result, all the tissue distal to the burn or injury involuted and was lost to be replaced by normal longitudinal growth of the more proximal, healthy tissue. Even though the fin repaired itself, it remained moderately visible by a distortion in the fin rays (Figure 5).

Laser hits on the adipose fin using the CO_2 laser would immediately ablate or notch the fin (Figure 6). Heat generated by the CO_2 laser immediately cauterized the incised area prohibiting fin regeneration. Adipose fins entirely and even partially incised did not regenerate over the study period. It appears that patterned notching of the adipose might be feasible. From personal experience a similar notch or partial cut on the adipose made with scissors would probably have regenerated beyond recognition.

Additional work using surgical lasers for notching or complete removal of the adipose fin looks promising. The advantages of no bleeding and no regeneration over conventional scissor removal could be very significant when coupled with the need for mass marking of all hatchery fish.

FIGURE 5

Photograph of a coho salmon smolt with a Carbon Dioxide lasered mark across caudal fin after 8 weeks. Background scale in millimeters.

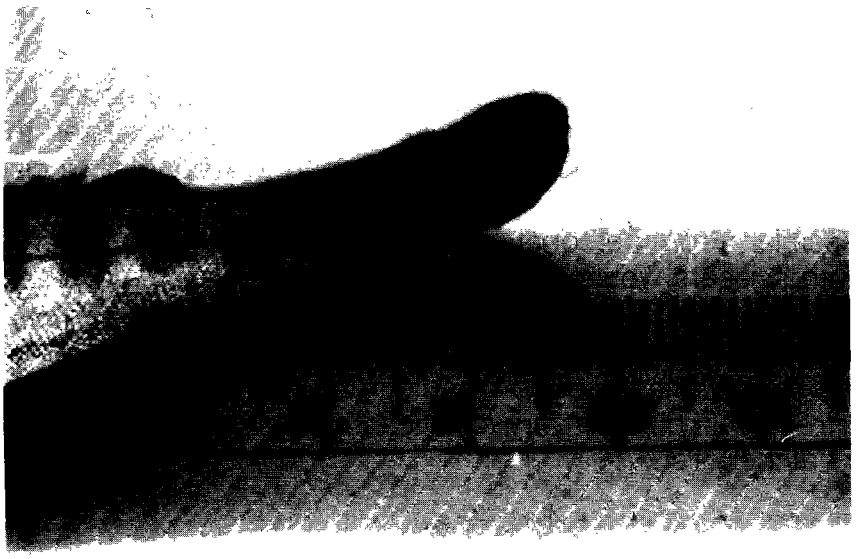
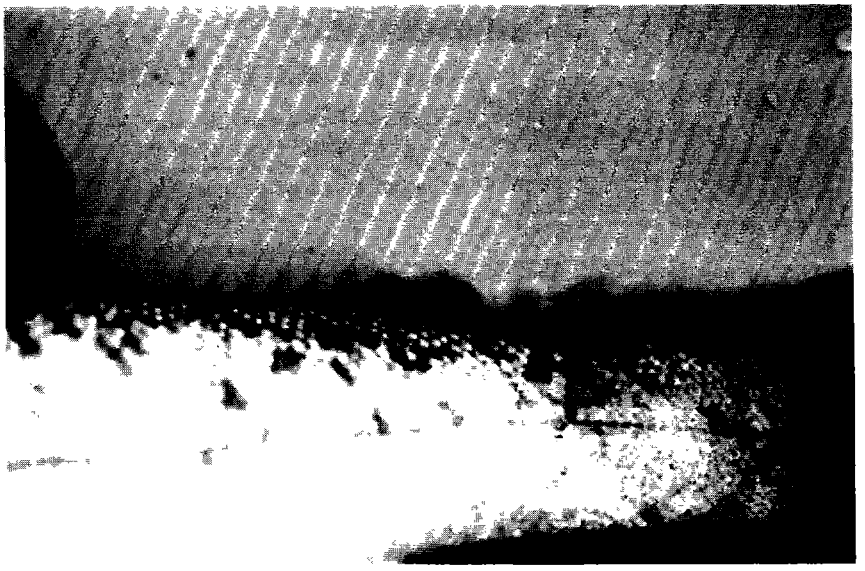


FIGURE 6

Photograph of a coho salmon with a notched adipose fin after 8 weeks that was marked with a Carbon Dioxide laser.



REFERENCES

Brock, J. A. and R. K. Farrell. 1977. Freeze and laser marking of channel catfish. Prog. Fish-Cult. 39: 138.

Hawkes, J. W. 1973. The effect of laser branding on fish chromatophores. Anat. Record 175(2): 339.

Hawkes, J. W. 1976. The effects of laser irradiation on fish chromatophores. IN: Pigment Cell (Karger, Basel, 1976), Vol. 3: 345-356.

Schurman, G. C. and D. A. Thompson. Washington Department of Fisheries' Mobile Tagging Units: construction and operation. American Fisheries Society Symposium 7: 232-236.