MN DNR Fisheries
Investigational Report #402

LONG-TERM RETENTION OF FLUORESCENT PIGMENT MARKING OF CHINOOK SALMON[1]

by

Mary T. Negus
Fisheries Biologist

and

David A. Belford
Fisheries Biologist

and

Steven E. Colvin
Fisheries Biologist

Minnesota Department of Natural Resources
Section of Fisheries

---

[1] This project was funded in part by the Federal Aid in Sport Fish Restoration (Dingell-Johnson) Program. Completion Report, Study 309, D-J Project F-26-R Minnesota.

ABSTRACT

Chinook salmon (Oncorhynchus tshawytscha) of approximately 50 mm total length were spray-marked in spring 1984 with red fluorescent pigment. Spray-marked fingerlings, along with an equal number of control fingerlings, were fin-clipped and released into the French River, a tributary to Lake Superior. Marked chinook salmon were recaptured as age 2+ to 5+ spawning adult migrants. Primary locations for pigment retention were the body side and the transparent tissue near the eye. Eighty-eight percent of the adult females and 63% of the males retained at least one pigment granule. Spray-marking did not appear to influence growth, but some increased mortality was indicated, possibly due to the cumulative stress of spray-marking and fin-clipping.

INTRODUCTION

Chinook salmon (Oncorhynchus tshawytscha) comprise approximately 15% of the angler catch in Minnesota waters of Lake Superior (D. Schreiner, MN Dept. Nat. Res., personal communication 1990), and Minnesota supplements that population by annual stocking of 350,000 to 500,000 fingerlings. Assessment of stocked chinook salmon is an important component of Lake Superior management. Giving each cohort a unique and readily-identifiable mark would assist in assessing relative contributions of Minnesota stocked chinook salmon to the sport fishery, straying and migration patterns, and relative survival. Identifying marks must be readily recognizable and retained for the lifetime of the fish; must be economical; must not adversely affect survival, growth, or behavior; and must not increase vulnerability to predators or fishing gear (Arnold 1966).

Fin-clipping has been the traditional marking method for chinook salmon, and excision of the posterior portion of the maxillary bone is becoming more popular. These techniques require no specialized marking or identification equipment, but they are labor-intensive when marking large numbers of small fish. Differential survival and growth of clipped fish may confound data interpretation (Weber and Wahle 1969; Nicola and Cordone 1973; Phinney 1974). Fin regeneration, particularly in fish clipped at a small size, has also been noted (Shetter 1950; Hale 1954; Mears 1976)

and has been observed in Lake Superior chinook salmon (D. Schliep, MN Dept. Nat. Res., personal communication 1990).

Fluorescent spray-marking is an alternative to fin-clipping for mass marking chinook salmon fingerlings. Marking and identification procedures are economical, efficient, easy to learn, require relatively simple equipment, do not require excessive handling or anesthetization, and have low mark-related mortality (Phinney et al. 1967; Pribble 1976, Chart and Bergersen 1988). Spray-marking fish with fluorescent pigments was first described by Jackson (1959). Pigment granules are sprayed with sufficient force to penetrate the epidermis and dermis (Phinney et al. 1967). Results on a variety of species have indicated excellent short-term mark retention and low mortality in fish marked after scale formation (Phinney et al. 1967; Engelhardt 1977; Bandow 1987). Experiments with scaleless fish and fry have been less successful (Hennick and Tyler 1970; White 1976; Moodie and Salfert 1982). Experiments with chinook and coho salmon (Oncorhynchus kisutch) fingerlings have yielded satisfactory short-term (<1 year) mark retention and survival (Phinney and Mathews 1969, Bandow 1987).

Long-term pigment retention (>1 year) has not been extensively studied and results have been variable. Andrews (1972) reported 100% pigment retention in fathead minnows after nearly 2 years. Similar results were

obtained with fingerling coho salmon after 2 years in a hatchery pond (Phinney and Matthews 1973). Pigment retention in coho smolts after 2 years in the wild, however, was only about 68% (Gray et al. 1978). Pigment retention of adult chinook salmon marked as fingerlings has not been fully investigated, although Evenson and Ewing (1985) report 50-60% mark retention after 4.5 years in chinook salmon marked as yearlings. Long-term effects on growth and survival have not been evaluated for spray-marked fingerlings.

This study evaluated fluorescent pigment marking as a tool to identify stocks of chinook salmon as they returned to French River in spawning runs. We determined whether chinook salmon marked as fingerlings still retained identifiable fluorescent pigment when they returned as age 2+ to 5+ adults; we quantified pigment concentration in adult chinook; we identified body locations most likely to retain pigment granules; and we determined the effect of fluorescent pigment spraying on survival and growth in Lake Superior.

METHODS

On 9 May 1984, over 51,000 chinook salmon fingerlings (approximately 50 mm total length) were spray-marked with red fluorescent pigment. Details of pigment application are described by Bandow (1987). After 33 d, the right pelvic fin was removed from 10,101 of the spray-marked fish and the left pelvic fin from 10,092 unmarked control fish

of the same stock. All pelvic-clipped spray-marked and control fish were stocked into the French River, a tributary of Lake Superior.

Marked chinook salmon were captured in the French River trap, located approximately 100 m upstream from Lake Superior, during the 1986 through 1989 spawning migrations. Fin-clipped fish were weighed, measured, and sexed, and scale samples were taken. Fish with right pelvic clips were examined under ultraviolet light; the presence and locations of fluorescent dye granules were noted.

Survival of spray-marked versus control fish was compared using a Heterogeneity Chi-square test (Snedecor and Cochran 1973). Growth of spray-marked and control fish was compared by back-calculating lengths in each year of life (Missouri Department of Conservation 1989). A body-scale constant of 38 mm was used in back-calculations. A two-way analysis of variance (ANOVA) of total body length in each year of life was done using mark (spray-marked versus control) and sex as the independent variables (Snedecor and Cochran 1973). The Kolmogorov-Smirnov/Lilliefors test was used to check for normality within each cell of the ANOVAS (Lilliefors 1967). Levene's test was used to check for homogeneity of variance within each cell (Levene 1960). Hypotheses were tested at the 0.05 level. Length-weight relationships at capture of spray-marked versus control fish were compared using analysis of covariance (ANCOVA) on log transformed data.

All statistical analyses except the Chi-square test were computed with SYSTAT (Wilkinson 1988).

RESULTS

A total of 54 pelvic-clipped chinook salmon returned in spawning migrations to French River from 1986 to 1989 (Table 1). Proportions of spray-marked versus control fish were not significantly different between years ($\chi^2 = 6.73$, df = 3, P >0.50), but significantly fewer marked fish returned than control fish ($\chi^2 = 5.35$, df = 1, P <0.025).

Pigment granules retained their bright color throughout the experiment, but often only one to four granules remained per fish. Determining the location of granules was often confounded by fluorescing unembedded extraneous material, so identification of granules sometimes took over a minute. Pigment was retained by a higher percentage of females (87.5%) than males (62.5%) (Table 2), and more granules were found per female than male.

The length-weight relationships (Table 4) at the time of capture for spray-marked versus control chinook salmon were not significantly different (ANCOVA, test of adjusted intercepts, P = 0.182). There was no significant mark or sex effect on length at ages 1, 2, or 3 (P >0.05). At age 4 the mark*age interaction was marginally significant (P = 0.045). The back-calculated lengths at age (Table 3) for each mark (spray-marked and control) and sex group were

Table 1. Numbers of spray-marked and control chinook salmon in spawning migrations from 1986 through 1989. F = female, M = male, ? = sex unknown.

Year	Spray-marked	Control
1986 (age 2)	1 F	1 F, 1 ?
1987 (age 3)	0 F, 6 M	4 F, 5 M
1988 (age 4)	8 F, 3 M	17 F, 6 M
1989 (age 5)	0	2 F

Table 2. Location of fluorescent pigment granules on the bodies of chinook salmon captured as adults during spawning migrations. No notation of pigment presence or absence was made on one female (age 2+) and one male (age 4+).

		Location of pigment granules			
Year	Sex	Eye	Preopercle	Body side	Caudal peduncle
1987 (age 3)	M		X		
1987 (age 3)	M	X			
1987 (age 3)	M	no pigment present			
1987 (age 3)	M	no pigment present			
1987 (age 3+)	M	pigment present, location unknown			
1987 (age 3+)	M	no pigment present			
1988 (age 4+)	M			X	
1988 (age 4+)	M			X	
1988 (age 4+)	F			X	
1988 (age 4+)	F			X	X
1988 (age 4+)	F	X		X	X
1988 (age 4+)	F	X		X	
1988 (age 4+)	F	X	X	X	X
1988 (age 4+)	F	X			
1988 (age 4+)	F	X			
1988 (age 4+)	F	no pigment present			

Table 3. Mean back-calculated lengths (mm) ± SD of spray-marked and control chinook salmon. Sample size is shown in parentheses.

Age	Spray-marked fish (right pelvic clipped) Female	Spray-marked fish (right pelvic clipped) Male	Control fish (left pelvic clipped) Female	Control fish (left pelvic clipped) Male
1	285±24 (9)	279±26 (9)	274±34 (23)	300±34 (10)
2	483±28 (8)	484±40 (9)	478±38 (23)	522±61 (9)
3	659±28 (7)	673±36 (9)	665±57 (23)	719±71 (9)
4	867±53 (7)	800±65 (3)	837±61 (19)	878±71 (5)

Table 4. Mean lengths ± SD, and mean weights ± SD at capture of chinook salmon.

Mark	N	Length (mm)	Weight (g)
Spray-marked females (right pelvic clipped)	9	870 ± 78	6861 ± 1739
Spray-marked males (right pelvic clipped)	9	794 ± 66	5178 ± 1015
Control females (left pelvic clipped)	24	870 ± 75	7283 ± 1989
Control males (left pelvic clipped)	11	880 ± 59	7664 ± 1942

normally distributed (P >0.05), except for spray-marked females at age 1 (P = 0.049). When lengths were log-transformed, this distribution was normalized (P = 0.083). The variances between cells (mark vs. sex) at ages 2, 3, and 4 were homogeneous, but variances were not homogeneous at age 1 (P = 0.041). Variances within cells at age one were relatively low, thus making between-cell variances seem relatively high (see Table 3).

DISCUSSION

The transparent tissue surrounding (but not in) the eye has been reported as one of the most common and visible areas of pigment retention in chinook salmon (Evenson and Ewing 1985) and cyprinids (Andrews 1972). The caudal peduncle is another common area, reported for chinook salmon and steelhead (Oncorhynchus mykiss) (Evenson and Ewing 1985) and largemouth bass (Micropterus salmoides) (Englehardt 1977). On those fish retaining marks in this study, the body side and the tissue surrounding the eye were the most common locations for pigment retention, followed by the caudal peduncle and the preopercle (Table 2).

Overall, 25% of the fish originally spray-marked retained no pigment; others retained only 1 - 4 granules. Bandow (1987) reported that 0.9% of the spray-marked fish were actually unmarked after 21 d. Additional granule loss could have resulted from sloughing off with skin tissue or from further embedding of pigment granules to a depth where

they were no longer visible (Evenson and Ewing 1985). Ware's (1968) work with largemouth bass suggested increased mark loss in fast growing fish; the growth rate of chinook salmon is faster still, which may contribute to pigment loss. The greater retention of pigment granules by female chinook salmon than males corresponds to the findings of Evenson and Ewing (1985), who attributed this phenomenon to sexually dimorphic skin characteristics associated with sexual maturation.

Mortality attributable to initial pigment application was only 0.5% for the fish used in this study (Bandow 1985), which corresponds to the low initial mortality seen in other studies when spray pressures were carefully adjusted (Jackson 1959; Phinney et al. 1967; Hennick and Tyler 1970). Long term survival (from stocking to sexual maturity) of our fish was somewhat diminished, however, in contrast to other studies which report no long-term mortality effects (Phinney et al. 1969; Phinney 1974; Hennick and Tyler 1970). The spray-marked fish exhibited this increased mortality regardless of age at maturity. Fin-clipping is widely reported to adversely affect survival of fish (Weber and Wahle 1969; Nicola and Cordone 1973), and it is possible that the cumulative effects of spray-marking and fin-clipping resulted in some increase in mortality among those fish. Migratory straying or misidentification due to fin regeneration may have caused underestimation of the total number of fish surviving to

adulthood, but relative numbers of spray-marked and control fish were assumed constant.

Spray-marking had no obvious effect on growth or condition. Marginally significant length differences seen in spray-marked males at age 4 could be related to the small sample size.

Selective predation upon spray-marked fish was considered as a possible source for increased mortality. Immediately following spray-marking the color is very obvious on the fish in visible light, but most of this unembedded pigment is sloughed off within a few hours (Phinney et al. 1967; Bandow 1987), while the fish are still being maintained in holding tanks. Thereafter, pigment granules are visible only under ultraviolet light. While such lighting conditions may exist underwater, enabling predators to see the pigment, the scarcity of embedded granules and the presence of other fluorescent particles in the environment diminish the chance of selective predation. Phinney (1974) found no selective predation on spray-marked coho salmon.

MANAGEMENT IMPLICATIONS

Fluorescent pigment marking is an efficient, economical means of mass-marking fish, and has definite potential for short-term studies. Long-term (2-4 yr) retention of fluorescent pigment by chinook salmon was achieved in this study, but stock assessments using this technique should consider the following qualifications:

1) Pigment retention and initial survival is highly dependent upon spray pressure (Phinney et al. 1967; Hennick and Tyler 1970; Bandow 1987).

2) Using pigment particles >250 μm could increase penetration and reduce mortalities resulting from clogged gills (Phinney et al. 1967; Strange and Kennedy 1982; Bandow 1987), but this also increases the cost of materials.

3) Resistance to the treatment and retention of pigment varies with fish age (both at the time of marking and the time of monitoring) and species (Hennick and Tyler 1970; Moring and Fay 1984; Bandow 1987).

4) Disease and stress could contribute to mortality, particularly if spray-marking is used in combination with fin-clipping. Some researchers recommended treatment in malachite green following marking (Phinney et al. 1967; Pribble 1976), however that drug can no longer be used.

5) In this study, the chinook were dead when examined for pigment granules. The tiny granules were not always obvious, and use of this technique in the field to identify

stocks of vigorous adult chinook salmon (mean weight 6.5 kg) in an ultraviolet light box would be difficult.

6) Differential pigment retention due to sex and stage of maturity should be considered (Evenson and Ewing 1985).

7) Retention of pigment granules $\geq$5 yr is untested, though continuous loss of granules beyond what was seen at age 4 in chinook salmon would render mark detection unlikely.

8) This technique lacks permutations for individual fish identification; the red pigment is reported to be most readily detectable (Ware 1968). Also, pigment is visible only under ultraviolet light, so it is undetectable to anglers.

LITERATURE CITED

Andrews, A.K. 1972. Survival and mark retention of a small cyprinid marked with fluorescent pigments. Transactions of the American Fisheries Society 101:128-133.

Arnold, D.E. 1966. Marking fish with dyes and other chemicals. Technical Paper 10: Bureau of Sport Fisheries and Wildlife. U.S. Government Printing Office. 44 pp.

Bandow, F. 1987. Fluorescent pigment marking of seven Minnesota fish species. Minnesota Department of Natural Resources, Division of Fish and Wildlife, Investigational Report No. 393.

Chart, T.E., and E.P. Bergersen. 1988. Methods for long-term identification of salmonids: a review. Biological Report 88(37), U.S. Fish and Wildlife Service, U.S. Department of the Interior, Washington DC. 18 pp.

Engelhardt, W.H. 1977. Retention of fluorescent pigment marks by two strains of largemouth bass. Transactions of the American Fisheries Society 106:64-66.

Evenson, M.D., and R.D. Ewing. 1985. Long-term retention of fluorescent pigment marks by spring chinook salmon and summer steelhead. North American Journal of Fisheries Management 5:26-32.

Gray, P.L., K.R. Florey, J.F. Koerner, and R.A. Marriott. 1978. Coho salmon (Oncorhynchus kisutch) fluorescent pigment mark-recovery program for the Tako, Berners, and Chilkat rivers in southeastern Alaska (1972-1974). Alaska Department of Fish and Game Information Leaflet No. 176:75 pp.

Hale, G. 1954. Regeneration of pelvic fins on fingerling brook trout following fin clipping. Minnesota Department of Conservation, Division of Game and Fish, Bureau of Fisheries Investigational Report No. 149:3 pp.

Hennick, D.P., and R.W. Tyler. 1970. Experimental marking of emergent pink salmon (Oncorhynchus gorbuscha) fry with sprayed fluorescent pigment. Transactions of the American Fisheries Society 99:397-400.

Jackson, C.F. 1959. A technique for mass-marking fish by means of compressed air. New Hampshire Fish Game Department, Technical Circular No. 17. 8 pp.

Levene, H. 1960. Robust tests for equality of variance. In I. Olkin (Ed.), Contributions to probability and statistics. Palo Alto, California: Standard University Press, pp. 278-292.

Lilliefors, H.W. 1967. On the Kolmogorov-Smirnov test for normality with mean and variance unknown. Journal of the American Statistical Association 64:399-402.

Mears, H.C. 1976. Overwinter regeneration of clipped fins in fingerling brook trout. Progressive Fish-Culturist 38:73.

Missouri Department of Conservation. 1989. Fisheries analysis tools (F.A.T.) manual, reference and users' guide to FishCalc89 and DisBCal89 microcomputer software packages. Conservation Commission of the State of Missouri, Jefferson City, MO. 396 pp.

Moodie, G.E.E., and I.G. Salfert. 1982. Evaluation of fluorescent pigment for marking a scaleless fish, the brook stickleback. Progressive Fish-Culturist 44:192-195.

Moring, J.R., and C.W. Fay. 1984. Identification of U.S. stocks in non-U.S. waters: non-tag marking techniques. U.S. Department of Commerce. NOAA, National Marine Fisheries Service, Woods Hole, MA. 84 pp.

Nicola, S.J., and A.J. Cordone. 1973. Effects of fin removal on survival and growth of rainbow trout (Salmo gairdneri) in a natural environment. Transactions of the American Fisheries Society 102:753-758.

Phinney, D.E. 1974. Growth and survival of fluorescent-pigment-marked and finclipped salmon. Journal of Wildlife Management 38:132-137.

Phinney, D.E., and S.B. Matthews. 1969. Field test of fluorescent pigment marking and fin-clipping of coho salmon. Journal of the Fisheries Research Board of Canada 26:1619-1624.

Phinney, D.E., and S.B. Matthews. 1973. Retention of fluorescent pigment by coho salmon after two years. Progressive Fish-Culturist 35:161-163.

Phinney, D.E., D.M. Miller, and M.L. Dahlberg. 1967. Mass-marking young salmonids with fluorescent pigment. Transactions of the American Fisheries Society 96:157-162.

Pribble, H.J. 1976. Pressure spray marking fish with granular dyes. Oregon Department of Fish and Wildlife, Information Report Series, Fisheries No. 76-1, 12 pp.

Shetter, D.S. 1950. The effect of fin removal on fingerling lake trout (Cristivomer namaycush). Transactions of the American Fisheries Society 80:260-277.

Snedecor, G.W., and W.G. Cochran. 1973. Statistical Methods, Sixth Edition. Iowa State University Press, Ames, Iowa.

Strange, C.D., and G.J.A. Kennedy. 1982. Evaluation of fluorescent pigment marking of brown trout (Salmo trutta L.) and Atlantic salmon (Salmo salar L.). Fisheries Management 13(3):89-95.

Ware, F.J. 1968. Mass-marking warm water fish by compressed air and fluorescent pigment. Proceedings of the Annual Conference of the Southeastern Association of Game and Fish Commissions 22:339-342.

Weber, D., and R.J. Wahle. 1969. Effect of finclipping on survival of sockeye salmon (Oncorhynchus nerka). Journal of the Fisheries Research Board of Canada 26:1263-1271.

White, L.E. 1976. Fluorescent pigment retention by pink salmon marked as scaleless fry. Progressive Fish-Culturist 38:184-186.

Wilkinson, L. 1988. SYSTAT: The System for Statistics. Evanston, Illinois: SYSTAT, Inc.

ACKNOWLEDGMENTS

We thank C. Anderson and T. Close for guidance in statistical analyses of the data and review of this manuscript. T. Close also assisted in data collection.

Special thanks are extended to D. Schliep, and the Duluth Fisheries Management Crew for recapture of the adult chinook salmon in this study.

Edited by:

C.S. Anderson, Coldwater Fisheries Research Supervisor