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FLUORESCENT PIGMENT MARKING OF SEVEN MINNESOTA FISH SPECIES

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FLUORESCENT PIGMENT MARKING OF SEVEN MINNESOTA FISH SPECIES[1]

by

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ABSTRACT

The use of sprayed fluorescent pigment for marking small fish was tested on chinook salmon (Oncorhynchus tshawytscha), rainbow trout (Salmo gairdneri), lake trout (Salvelinus namaycush), muskellunge (Esox masquinongy), channel catfish (Ictalurus punctatus), largemouth bass (Micropterus salmoides), and walleye (Stizostedion vitreum vitreum). More than 96% of rainbow trout marked as emergent fry (mean total length 25 mm) retained marks throughout a 267 d assessment period. Emergent fry were adequately marked only when the spraying force was high enough to cause 25% mortality. Initial pigment marks were readily visible and short-term retention was greater than 95% for fingerlings of all species and for yearling lake trout. Walleye was the only species that had unacceptable (>10%) post-marking mortality and that mortality was associated with harvest stress of the lake-reared fingerlings. Pigment marking did not influence growth of rainbow trout or lake trout fingerlings. Pigment marked lake trout yearlings held in raceways for 95 d grew slightly less than unmarked controls.

Particles from bulk pigment were separated into four size ranges and tested for mark quality in an experiment with rainbow trout fingerlings. Particles less than 250 μm in diameter comprised approximately 88% of the bulk pigment but did not provide adequate marks.

A few fish of several species were recaptured more than 1 yr after pigment marking. Long-term mark retention remains problematical and should be monitored.

INTRODUCTION

Fisheries managers and researchers frequently need to mark large numbers of juvenile fish with lasting and easily identifiable marks. Removal of external body parts, such as fins, is a common marking technique. Fin removal is labor intensive, prohibitively so for smaller fish, and few suitable alternatives are available. The feasibility of marking by fin removal for collection of unbiased biological information is questionable.

Investigations into the impact of fin removal on fish survival have yielded highly variable results (Nicola and Cordone 1973; Mears and Hatch 1976). The inconsistent conclusions imply that fin excision bears high potential for causing increased mortality among treated fish.

Although retarded growth resulting from fin removal appears to be less of a problem, it has been reported for some salmonids (Shetter 1951; Saunders and Allen 1967; Cleaver 1969; Phinney and Mathews 1969). Bonham (1968) similarly concluded that maxillary bone removal retarded growth of chinook salmon (Oncorhynchus tshawytscha). Much of the reported excess mortality of marked fish, however, is possibly an indirect result of retarded growth and increased vulnerability to predation (Ricker 1949).

Regeneration of clipped fins may present problems in recognition of marks during long-term studies. Fin regeneration has been noted for centrarchids (Crawford 1958; Ricker 1975), esocids (Koshinsky 1972; McNeil and Crossman 1979) and salmonids (Armstrong 1949; Slater 1949; Shetter 1951; Hallock et al. 1952; Hale 1954; Mears 1976; Mears and Hatch 1976). Maxillary regeneration on sockeye salmon (Oncorhynchus nerka) was reported by Weber and Wahle (1969).

Jackson (1959) pioneered a mass marking technique for embedding fluorescent pigment granules in the epidermis of fish by spraying with compressed air. Phinney et al. (1967) demonstrated that two to four people could pigment mark several thousand juvenile salmonids per hour. Phinney and Mathews (1969) compared unmarked controls with pigment marked and finclipped coho salmon (Oncorhynchus kisutch) and concluded that pigment marking had no apparent detrimental effect on survival or growth while finclipping did.

Marking with sprayed pigments has been used primarily with salmonids, but successful applications of the technique have been reported for channel catfish (Ictalurus punctatus) and centrarchids (Ware 1969), cyprinids (Andrews 1972; Rinne and Deacon 1973), and largemouth bass (Micropterus salmoides) (Engelhardt 1977). Although reports of pigment retention by advanced juveniles are encouraging, there remains uncertainty about the potential for effectively marking scaleless juveniles. Mattson and Bailey (1969) found nearly all their caged pink salmon (Oncorhynchus gorbuscha) and chum salmon (O. keta) fry retained some pigment after 14 d, but they did not recapture any released fish less than 43 mm fork length. Hennick and Tyler (1970) reported 76% of marked scaleless pink salmon retained marks after 14 d and after 31 d. Phinney et al. (1967) and Bax (1983) reported mark retention between 55 and 100% for various juvenile salmonids.

This study examined potential of the pigment spraying technique for marking game fish in Minnesota. Guidelines for pigment spraying were established for chinook salmon, rainbow trout (Salmo gairdneri), lake trout (Salvelinus namaycush), muskellunge (Esox masquinongy), channel catfish, largemouth bass and walleye (Stizostedion vitreum vitreum).

METHODS

A granular fluorescent pigment marketed by Scientific Marking Materials[2] of Seattle, WA was used in all experiments. Pigment from a 0.95 L cannister was delivered by a sandblast gun with a 2.4 mm diameter siphon stem orifice and a 4.8 mm diameter delivery tube orifice. An air pressure regulator was fitted at the storage tank end of the 15 m air hose to assure constant pressure during pigment delivery, and an inline pressure gage was attached to the air gun handle.

With one exception, unanesthetized fingerling and yearling fish were held for marking in a 343 mm square by 137 mm deep crib with a perforated aluminum bottom. In the exception, a 724 mm square crib of similar construction was used. The nozzle of the air gun was held vertically 30-38 cm from the fish during mark application. The cannister was filled with pigment to approximately two-thirds capacity and refilled when pigment had diminished to approximately one-third capacity. A single pigment application consisted of spraying the area of the crib twice at opposing angles using side to side motions. This took about 4 s with the smaller crib. Most treatments were two pigment applications separated by an intervening rinse.

Pigment delivery force was measured by directing the air discharge from the spray nozzle, with the cannister removed, onto a platform scale. McAfee (1982) emphasized the unreliability of gage pressure recordings and suggested the alternative. Gage pressures were also recorded on occasion. The relationship between delivery force and

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[2] Use of company name does not imply product endorsement.

gage pressure was linear at the onset of spray marking in 1983 (Fig. 1), however, this relationship did not hold throughout the investigation so all treatments were described as delivery force in grams.

Fish lengths were measured to the nearest mm total length (TL) and all weights were measured to the nearest gram. A portable 365 nm wavelength ultraviolet lamp powered by two 6 V batteries was used for pigment detection in all mark retention assessments.

RESULTS

Emergent Rainbow Trout Fry

Fry sizes were assessed the day before pigment application. The mean rate of three 20-26 g lots of fry was 6,822/kg (SD=257/kg). Total lengths of 92 fry were measured after storage in 10% formalin for 7 d. Lengths ranged from 23-27 mm (mean=25 mm; SD=1 mm).

The fry were anesthetized and contained for marking in a Mattson and Bailey (1969) marking apparatus. A smaller mesh size (3.2 mm) on the containment frame to confine the small fish was the only modification. Marking procedure consisted of two passes of the spray gun on each side of the frame.

A preliminary test was conducted with seven small lots of fry to assess survival at various mark application forces. Six lots were marked and one was held for a control. Mark application forces were 70, 100, 135, 200, 265 and 330 g. Survival was evaluated the following day.

The next marking experiment was conducted with 16 lots of approximately 500 fry/lot at delivery forces of 100, 155, 210 and 265 g. The highest and lowest forces tested in the preliminary

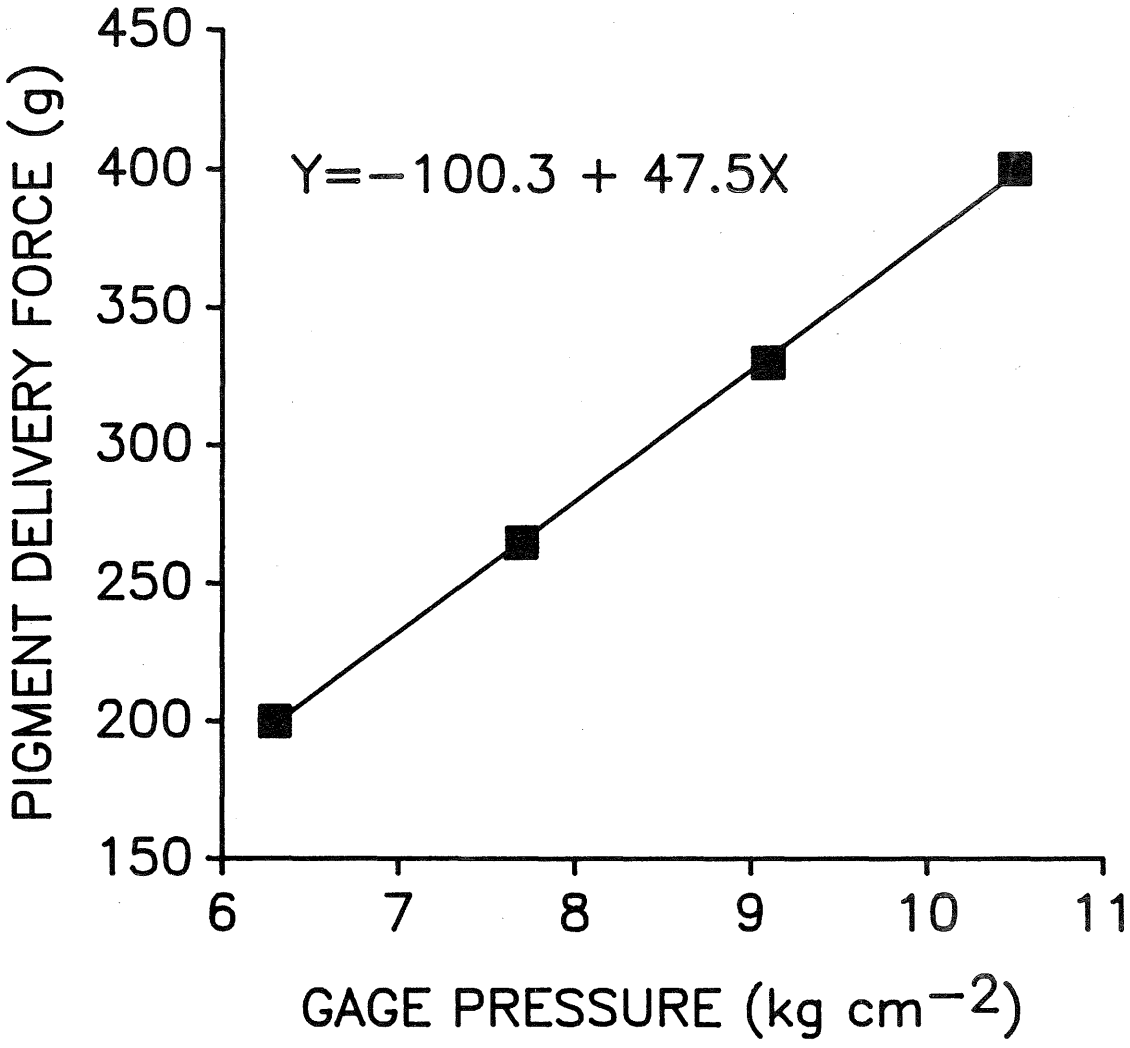

Figure 1. Relationship between pigment delivery force and gage pressure at the onset of fluorescent pigment marking.

experiment were abandoned because they produced high mortality and low quality marks, respectively. The four marking treatments were duplicated and eight lots of fry were held for controls. Fry were rate counted and approximately 250 were marked at a time. Following treatment, all lots were confined in raceways.

Fry in all marked lots were hand counted the following day. Mark retention was examined after 7, 28, 77, 104 and 267 d. Following the 104 d assessment, the fish marked with 155, 210 and 265 g forces were combined according to treatment and retained in three deep tanks through day 267. Fish given the 100 g treatment were discarded when tank space became limited because their marks were least visible.

Survival. - Rainbow trout fry survival in the preliminary assessment varied from 100% for the control and the 70 g application to 3% for the 330 g treatment (Table 1). Correlation between survival and pigment delivery force was high (Fig. 2).

Most of the mortality from the experimental treatments occurred during the first day following mark application (Table 2). Suffocation appeared to be a major cause of death, and there was much compacted pigment around the gill openings of the dead fish.

Mortality between days 0 and 1 of the two groups of fry marked with a delivery force of 265 g is unknown because a problem with raceway retaining screens resulted in substantial fry losses. Since the number of fry were weight estimated on day 0 and the marked fish not counted until day 1, the number of missing fish could only be estimated. Ranges of possible survival during the interval day 0-1 for fry marked at 265 g were estimated by treating all missing fish as mortalities for the lower end of a range and all as survivors for the upper end.

Table 1. Results of a 1 d preliminary survival assessment of pigment marked emergent rainbow trout fry.

Pigment delivery force (g)	Number of fish	Survival rate
0	73	1.00
70	57	1.00
100	73	0.95
135	46	0.74
200	42	0.45
265	48	0.21
330	90	0.03

Delayed mortality was indicated by significantly lower survival among marked fry than among unmarked controls during the interval day 1-6. Delayed mortality was particularly evident among fry marked with the higher delivery forces. It appeared, however, that most, if not all, mortality caused by the marking procedures occurred during the first 6 d. Although the marked fry exhibited slightly lower mean survival rates than controls between days 6 and 12, the differences were not significant.

The two treatments at 265 g exhibited considerable variation in fry survival as indicated by large standard deviations. A delivery force of 265 g during the preliminary treatments 2 d earlier yielded a 1 d survival rate of 0.21 (Table 1, Fig. 2). Perhaps as delivery forces are increased, fry become increasingly sensitive to subtle and unavoidable differences in procedure from treatment to treatment.

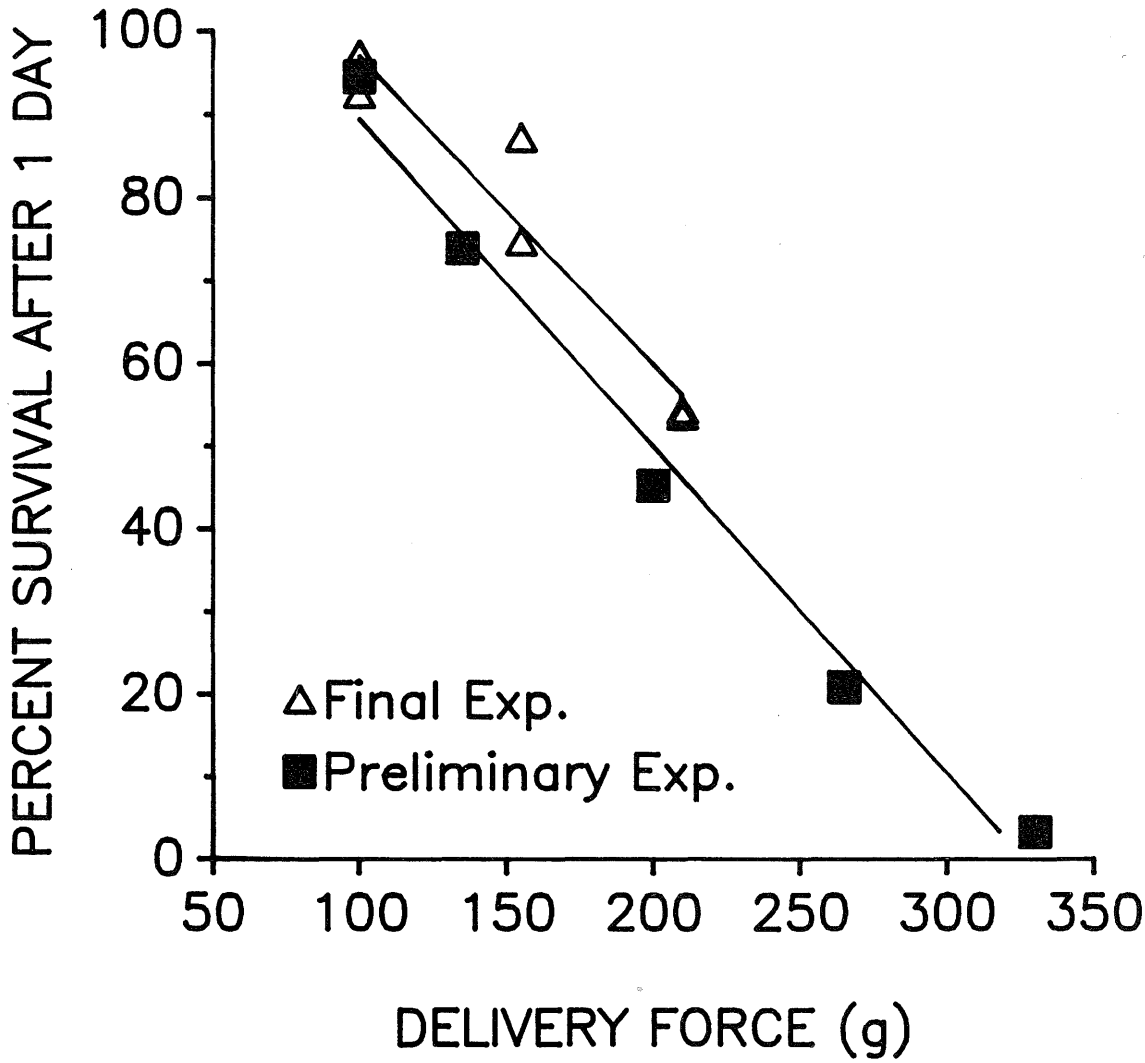

Figure 2. Regressions of first day rainbow trout fry survival on pigment delivery force. In preliminary experiments $y = 129 - 0.395\ x$ with $r^2 = 0.98$. In final experiments $y = 134 - 0.371\ x$ with $r^2 = 0.92$.

Table 2. Mean survival rates of pigment marked and unmarked emergent rainbow trout fry and results of one-way analyses of variance of arc sine of square root transformations. Estimated ranges in parentheses.

	Pigment delivery force (g)					ANOVA	
	0	100	155	210	265	F	P
Number of replicates	0	2	2	2	2		
Day 0-1							
Mean	0.998	0.947	0.809	0.540	(0.377-0.541)		
SD	0.003	0.034	0.088	0.005	(0.198-0.230)		
Day 1-6							
Mean	0.991	0.966	0.953	0.832	0.806	10.44	0.0010
SD	0.006	0.018	0.027	0.044	0.185		
Day 6-12							
Mean	0.973	0.931	0.934	0.949	0.971	1.64	0.2322
SD	0.022	0.051	0.026	0.023	0.024		

Two days difference in age between fish marked in the preliminary and final experiments appeared to make a substantial difference in their sensitivity to pigment application (Fig. 2). The older fish had higher survival at all application forces (ANCOVA, test of equality of intercepts, F=6.71, P=0.032). Slopes and residual variances did not differ (F=0.16; P=0.699 and F=1.28; P=0.437, respectively).

Mark Retention. - Initial mark retention was 100% among fry subjected to the 210 and 265 g treatments (Table 3). Pigment was found on body and fins and poor quality marks were rare. No difference in overall mark quality between the two treatments was perceived. Although most fish marked at 155 g had good marks, quality

Table 3. Results of pigment retention assessments on rainbow trout marked as emergent fry.

	Pigment delivery force (g)							
	100	100	155	155	210	210	265	265
Day 7								
Number present	450	460	342	419	209	234	239	79
Number examined	200	200	200	200	200	200	200	79
Percentage marked	98.5	98.0	100	99.5	100	100	100	100
Day 28								
Number present	346	395	256	321	191	196	204	74
Number examined	200	200	200	200	191	196	204	74
Percentage marked	96.0	94.5	100	99.5	100	100	100	100
Day 77								
Number present	334	388	250	315	175	182	198	46
Number examined	200	200	200	200	175	182	198	46
Percentage marked	86.0	81.5	99.5	98.5	99.4	98.9	99.5	100
Day 104								
Number present	334	388	250	314	175	182	198	46
Number examined	200	200	200	200	175	182	198	46
Percentage marked	91.0	91.5	99.0	99.0	98.9	98.4	99.0	100
Mean TL (mm)	60	52	63	53	77	72	69	80
n	50	50	50	50	50	50	50	45
SD	9	8	11	10	9	10	7	7
Day 267								
Number present	---[a]		437[b]		351[b]		232[b]	
Number examined	---		232		232		232	
Percentage marked	---		96.1		97.4		95.7	
95% conf. limit	---		2.5		2.0		2.6	
Mean TL (mm) (n = 100)	---		140		156		157	
SD	---		27		25		23	

[a] Fish marked with an application force of 100 g were discarded after day 104.

[b] Lots having same treatment were combined after day 104.

progressively declined at delivery forces less than 210 g. Pigment detection on several fry marked at 100 g required considerable effort.

Between days 7 and 28, quality of marks declined in all groups but the same relationships between groups seemed to hold. Mark quality declined further by day 77, but marks were still easily recognized on most fish marked at 155, 210, and 265 g. At this time, mark quality among fish marked at 155 g appeared equal to that of fish marked with the higher delivery forces. Quality of marks on fish marked at 100 g was substantially lower.

No further decline in mark quality was perceived among any of the groups on day 104. Larger percentages of fish in the 100 g treatment lots were identified as having marks on day 104 than on day 77. Reasons for this might be sampling error, the poor quality marks from the 100 g treatment, or that larger fish were easier to examine on day 104.

Mark retention was similar, about 96%, among the combined groups marked at 155, 210 and 265 g on day 267 when the final assessment was made. Two investigators agreed that they could not perceive differences in mark quality between the three treatments in general. Most fish had fewer than four marks and many had only one. Most marks were readily visible although some were faint and took extra effort to detect.

Most marks on day 267 were on or near the head of the fish--on opercula, around the eyes and mouth, and at the base of pectoral fins. Marks were also at the base of other fins and on the ventral side of the abdomen. None were seen on pigmented areas of the abdomen. The smaller size of the fish marked with the 155 g delivery force reflects crowding during the raceway phase of the experiment.

Preliminary 5 d Fingerling Survival and Pigment Retention Assessments

Preliminary assessments of survival and mark retention at various delivery forces were made with fingerlings of all species except rainbow trout. Fingerlings were confined in a 343 mm square crib and subjected to a double pigment application with an intervening rinse. Pigment delivery forces were 200, 265, 330 and 400 g. The assessment period was 5 d.

All species took the mark well and only one fingerling, a chinook salmon, failed to retain any pigment for 5 d (Table 4). Scaled fish usually had numerous marks on the body, but channel catfish retained fewer pigment granules and these were usually confined to the fins, particularly the pectorals. Ware (1969) reported similar results with channel catfish.

Only walleye exhibited excessive mortality. The first walleye trial was aborted prematurely when 81% (400 g lot) to 95% (unmarked control lot) of the fish were dead the morning after marking. The fingerlings exhibited severe stress symptoms, presumably resulting from pond harvest. A second group of walleye fingerlings was marked 1-2 d after harvest. Although this trial went to completion, mortality was still excessive. The walleye were infected with columnaris disease and heavy mortality began on the third day.

With the possible exception of walleye, pigment marking procedures did not cause excessive mortality among the various species at the highest delivery force. More force probably could have been applied to most species successfully. The 400 g application force (9.8 kg/cm^2) was near maximum capacity of the air compressor (11.2 kg/cm^2) and was the highest force that could be sustained during spraying.

Table 4. Results of 5-day survival and pigment retention assessments on fingerlings. All marked individuals retained pigment except one chinook salmon.

	No./kg	Pigment delivery force (g) 0	200	265	330	400
Chinook salmon	600					
No. of fish		100	0	100	100	100
Percentage survival		100	---	97	96	99
Lake trout	91					
No. of fish		50	50	50	53	51
Percentage survival		98	98	100	92	96
Muskellunge	10					
No. of fish		27	23	23	23	23
Percentage survival		100	100	100	100	100
Channel catfish	430					
No. of fish		100	100	99	100	100
Percentage survival		96	92	99	98	95
Largemouth bass	22					
No. of fish		50	50	50	50	50
Percentage survival		100	100	100	100	100
Walleye[a]	23					
No. of fish		100	100	100	97	125
Percentage survival		41	34	33	21	34

[a] 84% of surviving walleye exhibited gross symptoms of columnaris infection.

Chinook Salmon Fingerlings

On 9 May 1984, 51,000 chinook salmon numbering 595/kg were marked with red pigment at a delivery force of 365 g. They were subjected to a double application without an intervening rinse. After 33 d, the right pelvic fin was removed from 10,101 of the pigment marked fish and the left pelvic fin from 10,092 unmarked control fish of the same stock. All pigment marked and fin clipped control fish were then

released in the French River, a tributary of Lake Superior. The pigment marked fish comprised approximately one-half the total French River spring 1984 chinook salmon release.

Most mortality caused by pigment application occurred during the first 2 d after marking (Table 5). Mortality attributable to pigment marking was only 0.5%.

Examination of 1,200 fish 21 d after the pigment application yielded 11 unmarked fish (0.9%). Three chinook salmon with right pelvic fin clips and one with a left pelvic clip have been recaptured to date. Pigment was not seen on a 522 mm fish 1.4 yr after pigment marking. Two salmon were recaptured 3.1 yr after pigment marking. One of those fish (714 mm total length) had two particles of pigment on a cheek; the other fish (865 mm total length) had no pigment.

Rainbow Trout Fingerlings

Four lots of 200 rainbow trout fingerlings (119/kg) were confined in separate raceways. Mean total length of 400 was 94 mm (SD=8 mm). Two lots of fish were each given a double pigment application with an intervening rinse. Delivery force was 380 g. The other two lots of trout were held as controls for assessments of survival, growth and condition.

No mortalities occurred until more than 3 mo after treatment. Total mortalities through the conclusion of the assessment period (232 d after marking) were two marked fish and three control fish.

All 400 treated fingerlings had good quality pigment marks after 9 d. Of the 398 marked fish surviving to day 232, 395 (99.2%) had visible pigment. Multiple marks were common and most were recognizable at a glance. Extra effort was needed to identify pigment on a few fish (<10%).

Table 5. Mean number of chinook salmon mortalities per lot in 6 pigment marked lots and 31 unmarked lots. Approximately 8,500 fingerlings per lot.

Days after marking	Marked lots	Unmarked lots
1	37.8	2.9
2	9.5	2.1
3	2.3	1.2
4	2.7	0.9
6	3.3	3.2
7	0.8	2.5
8-20	13.0	18.1

Influence of pigment marking on growth of rainbow trout was not indicated (Table 6). Total lengths of marked fish and control fish did not differ significantly at the time of treatment nor 232 d later. An analysis of the influence of marking on condition factors was unclear because significant differences occurred within treatments.

Lake Trout Fingerlings and Yearlings

Lake Superior. - Some of the hatchery-raised lake trout for the 1984 Lake Superior stocking were marked as fingerlings in fall with red pigment and the remainder were marked as yearlings in spring with yellow pigment. Fingerlings numbered 91/kg and mean total length was 113 mm (SD=10 mm). Yearlings numbered 22/kg and mean total length was 176 mm (SD=20 mm). Both treatments were double applications with an intervening rinse and a 365 g delivery force. The fish were held for marking in a 343 mm square crib.

Both groups were finclipped after the spring pigment marking. Lake trout marked as fingerlings with red pigment on 7-8 November 1983 were

Table 6. Mean total lengths and mean condition factors of pigment marked and unmarked rainbow trout and results of analyses of variance.

	Treatment/lot number				ANOVA	
	Marked		Unmarked			
	1	2	3	4	F	P
Total length (mm)						
Day 0 (n=100)	94	94	95	95	1.05	0.369
SD	7	8	7	8		
Day 232 (n=95)	243	244	247	243	0.65	0.585
SD	21	18	18	19		
Condition factor (K) (n=95)						
Day 232	1.17	1.23	1.21	1.18		
SD	0.09	0.11	0.12	0.09		
Between treatments					0.03	0.881
Within treatments					8.54	0.000

given right pectoral and dorsal fin clips. Lake trout marked as yearlings with yellow pigment on 16-17 May 1984 were given left pectoral and right pelvic fin clips. Final rate counts of marked lake trout released in Lake Superior on 14 June 1984 were 21,300 red pigment marked and 26,700 yellow pigment marked fish.

Two lots each of fingerling-marked, yearling-marked and unmarked lake trout were isolated and confined for an additional 95 d in separate raceway compartments for mark retention and growth assessments. None of the 100 fish per lot were finclipped.

Lake trout mortality attributable to pigment marking was low. Through the first 13 d after fingerling marking, 21 mortalities occurred among the estimated 22,900 marked fish compared to four

mortalities among the estimated 28,000 unmarked fish. Discounting known mink predation, mortalities during the next 131 d were 73 and 79 among the two groups, respectively. Only 22 mortalities occurred among lake trout pigment marked as yearlings during the 28 d period (which included finclipping) between pigment marking and shipping to Lake Superior.

All captive lake trout marked as fingerlings and as yearlings initially retained pigment (Table 7). The marks were of good quality and easily recognized. A 2.4% mark loss occurred among the fingerling-marked fish during the 286 d assessment period. Mark quality declined considerably during the period and intensive examination was required to detect pigment on 8-9% of the fish. All yearling-marked fish retained marks throughout the 95 d confinement period. Mark quality declined but pigment detection was easy on most fish. The final mark retention assessments were conducted on fish that had been frozen for several months. A flow failure killed all fingerling-marked and yearling-marked fish after 95 d of raceway confinement.

A 206 mm fingerling-marked and a 208 mm yearling-marked lake trout were recaptured in Lake Superior 247 and 78 d after pigment marking, respectively. Both had visible pigment. A 259 mm lake trout examined 2.7 yr after being marked as a fingerling had a single particle of visible pigment.

Pigment marking did not influence growth of the fingerling-marked lake trout (Table 8). A sample of marked fish averaged slightly smaller than a sample of unmarked fish at the time of marking and the reverse was true after 198 d. Conversely, the yearling-marked lake

Table 7. Results of pigment retention assessments of captive Lake Superior lake trout.

Age at marking	Days after marking	Mean total length (mm)	Number of fish examined	Percentage of fish marked
Fingerling	0	113	---	---
	14	---	299	100.0
	198	178	500	98.6[a]
	286	201	198	97.6[b]
Yearling	0	176	---	---
	7	---	500	100.0
	95	195	200	100.0

[a] Percentage of marked fish adjusted 1% upward because 292 unmarked fish escaped into the enclosure with the approximately 22,900 marked fish.

[b] There was a 1% mark loss between 198 and 286 d.

trout grew somewhat less than unmarked fish during the 95 d raceway confinement period. Significant differences in mean length occurred within treatment lots at the beginning and end of the assessment period, so data from lots with similar treatment were combined for analysis of variance. Yearling-marked fish grew 7 mm less, on average, than unmarked fish and the difference was significant. Individually, the marked lots grew 4 and 9 mm less, on average, than the control lots combined.

Inland Lakes. - Forty-seven thousand lake trout yearlings numbering 20/kg were marked with green pigment on 5-6 June 1984. The fish were confined for marking in a 724 mm square crib and were subjected to a single application and a pigment delivery force of 365 g.

Table 8. Mean total lengths of pigment marked and unmarked Lake Superior lake trout and results of one-way analyses of variance.

Lot number	Marked 1	Marked 2	Unmarked 1	Unmarked 2	ANOVA F	ANOVA P
Fingerling						
Day 0 (n=100)						
TL (mm)	113[a]		116[a]		3.03	0.079
SD	10		10			
Day 198						
TL (mm)	178	178	176	175	0.54	0.659
n	100	99	100	100		
SD	17	21	20	22		
Yearling						
Day 0 (n=200)						
TL (mm)	176[b]		175[b]		0.21	0.648
SD	20		21			
Day 95						
TL (mm)	195[b]		201[b]		6.59	0.010
n	200		198			
SD	20		21			

[a] Single lots only.
[b] Lots combined.

Six (0.6%) unmarked lake trout were found among 1,000 examined 5 d after marking was completed. Mark quality was poorer than with the Lake Superior lake trout applications which employed a smaller holding crib and in which the fish were double marked. Pigment detection was difficult on several fish.

Shortly thereafter the adipose fin was removed from 4,000 of the fish and all marked lake trout were released in three Cook County lakes. None was withheld for further pigment retention assessment. Final disposition was: Daniels Lake - 3,860 lake trout with green

pigment and adipose fin clips; Gunflint Lake - 21,400 lake trout with green pigment only; Seagull Lake - 19,200 lake trout with green pigment only. Daniels Lake is scheduled for assessment in summer 1987.

Muskellunge Fingerlings

A total of 555 muskellunge fingerlings numbering 9.1/kg were marked with red pigment between 3 and 10 October 1983. The fish were given double pigment applications with an intervening rinse. Delivery force was 400 g. Mean total length of 219 fingerlings was 283 mm (SD=23 mm). Right maxillary bone clips were given to 354 of the fish and they were released in French Lake, Rice County. Two hundred perished in a failed attempt to overwinter them in captivity.

Only one (0.2%) mortality occurred during a 3 d holding period following pigment application. All 200 fingerlings examined 3 d after treatment had good quality pigment marks. Five of the muskellunge were recaptured in French Lake the following April, more than 6 mo after marking. All had easily recognized pigment marks. The clipped maxillary bones were red and slightly shredded at the severance point. In August 1984 (10 mo after marking), a 357 mm marked muskellunge was recaptured. Both the pigment mark and maxillary clip were readily detected. The severed maxillary bone had healed and no regrowth was evident. A 465 mm muskellunge was recaptured on 22 April 1986 (2.5 yr after marking). Nineteen distinct pigment marks were counted on the fish and a few more faint marks were later identified indoors. Maxillary bones on each side of the fish appeared anatomically identical.

On 20 September 1984, 1,000 muskellunge numbering 7.7/kg were similarly marked with red pigment at a delivery force of 380 g. They

were given left maxillary bone clips and released in French Lake the following day. No initial pigment retention assessment was conducted.

Twenty-five (2.5%) mortalities occurred between marking and release in French Lake. Difficulty in containing the large fingerlings in the 137 mm deep crib may have contributed to mortality, which seems excessive in light of the 1983 muskellunge marking results. The crib was carried 5-10 m to the marking site and fingerlings repeatedly jumped out. None has been recaptured to date.

Channel Catfish Fingerlings

A pigment marking experiment with channel catfish fingerlings involved four lots of 700 fish each. Two lots were double pigment marked (with an intervening rinse) at a delivery force of 400 g. Two lots of unmarked fish were held as controls. Mean total length of 200 catfish was 73 mm (SD=9 mm) and they numbered 372/kg.

Channel catfish mortality caused by pigment marking was not indicated. During the first 6 d after marking, survival was 95.7% among the marked fish and 94.3% among the unmarked fish. Subsequent mortality was high among both groups and the experiment was aborted prematurely. Survival through the 109 d assessment period was 19% of the marked fish and 16% of the unmarked fish, and morbidity was high.

All 200 catfish examined 8 d after marking had retained pigment. A total of 688 dead fish from the marked lots was examined during the period 54-109 d after marking and all had readily detected marks. The pigment was usually on fins, particularly the pectorals. Ware (1969) reported 100% retention of good quality marks on channel catfish for 8 to 10 mo.

Largemouth Bass Fingerlings

On 26 October 1983, 5,000 largemouth bass fingerlings numbering 22/kg were double pigment marked (with an intervening rinse) at a delivery force of 400 g. Mean total length of 125 bass was 152 mm (SD=19 mm). They were released the same day in a drainable pond for overwintering.

The pond was drained 188 d later and 3,700 were recovered. All 500 that were examined had numerous and readily detected pigment marks. The fish, however, had exhibited no growth since they were marked. Mean total length of 100 bass was 145 mm (SD=17 mm). The right maxillary bone was clipped on all surviving largemouth bass, and they were released in Lily Lake, Waseca County on 1 May 1984.

Six of the marked bass were recaptured from Lily Lake 398 d later (586 d after pigment marking). They ranged in total length from 183 to 209 mm. Five had a few readily visible pigment marks but the sixth required additional effort to find the faint pigment mark.

Three of the recaptured fish exhibited no regrowth of the maxillary bone. Three had nearly complete regrowth, but the bone was rounded at the posterior end and lacked the characteristic club shape of an unaltered bone. Otherwise regrowth appeared normal. Lily Lake has since winterkilled and it is unlikely that any largemouth bass survived.

On 2 May 1985, 3,000 largemouth bass yearlings numbering 19/kg were pigment marked. The delivery force was 370 g and the fish were double marked with an intervening rinse. Immediately after pigment marking they were given right maxillary bone clips and released in Loon Lake, Waseca County. None of those fish has yet been recaptured.

Walleye Fingerlings

From 13 October to 1 November 1983, 3,714 walleye fingerlings were marked with red pigment and a delivery force of 365 g. They were given a double application with an intervening rinse. The fingerlings were harvested from Tustin Lake, a nursery lake in Le Sueur County, 1-3 d before marking. The mean rate of six separate lots was 28 fingerlings/kg. After pigment marking, the right maxillary bone was removed from all fish and they were released in Clear Lake, Waseca County. No mortalities occurred before the walleye were hauled from the marking site, but the fish showed signs of stress. One fish was recaptured more than 10 mo later. Pigment and the maxillary bone clip on the 231 mm walleye were easily recognized.

On 18 October 1983, 276 Tustin Lake walleye fingerlings were similarly pigment marked and given right maxillary bone clips after a 1 d holding period. Mean total length of 72 walleye was 168 mm (SD=15 mm). Two morbid fingerlings were removed from the group the next day and the remaining 274 were released in a drainable pond for overwintering. Minnow forage was provided.

Six days later, 111 walleye fingerlings from Ida Lake, a nursery lake in Blue Earth County, were pigment marked. They were given left maxillary bone clips after a 3 d holding period. Mean total length of 30 walleye was 174 mm (SD=16 mm). All were released in the pond with the Tustin Lake fish the day after marking. Ida Lake fingerlings were selected because they were noticeably more vigorous than Tustin Lake fish when harvested. This was also true when they were released in the pond. The mean condition factor (K) of 72 Tustin Lake walleye was 0.665 (SD=0.053) and mean K of 30 Ida Lake fish was 0.734 (SD=0.038).

The means differed significantly ($t=7.35$; $P<0.0001$).

Seven mortalities of Tustin Lake fingerlings were observed in the 7 d following their release in the pond. No pond mortality of Ida Lake walleye was observed. Initial pigment retention was 100% among all fish.

Overwinter mortality was high among both groups of fish. Only two Tustin Lake fish (99.3% mortality) and 17 Ida Lake fish (84.7% mortality) were recovered when the pond was drained 1 May 1984. Pigment and maxillary bone clips were easily recognized on all fish. Mean total length of the 17 Ida Lake fish was 177 mm (SD=15 mm) and mean K was 0.800 (SD=0.058).

On 11 October 1984, 200 walleye fingerlings harvested from Sprague Lake, Rice County, were pigment marked and given upper caudal fin clips. Pigment marking procedures were the same as in 1983. Lower caudal fin clips only were given to another 200 fingerlings from the same source. After marking, the fish were given a salt bath and all 400 were released in a drainable pond. The walleye fingerlings numbered 46/kg and the mean total length of 100 was 138 mm (SD=11 mm). Mean K was 0.798 (SD=0.041). Five days later, 200 more walleye fingerlings were hauled directly from Sprague Lake and released in the same pond without receiving any marks or additional handling to represent typical harvest procedure.

Mortality was highest for fish that were doubly marked and lowest for unmarked fish. The pond was drained 23 d after release of the unmarked fish and only 15 walleye were identified as having upper caudal fin clips (92.5% mortality of double marked fish). Lower caudal fin clips were identified on 80 fingerlings (60% mortality of fish with

caudal fin clips only). There were 159 unmarked fish recovered (20.5% mortality). Besides the handling for marking, 25% of the fish in each marked group were measured and weighed.

Marking Effort and Pigment Consumption

Marking rates (number of fish/h) for double pigment applications by 2-3 workers ranged from 1,800 largemouth bass numbering 22/kg to nearly 9,500 chinook salmon numbering 595/kg (Table 9). Two workers pigment marked nearly 23,000 lake trout numbering 21/kg with a double application at a rate of more than 2,500/hr, while eight workers administered double fin clips to the same fish at a rate of about 2,000/hr. Three people did a single application on lake trout numbering 20/kg at a rate of 8,500/hr.

Nine marking operations with a 343 mm square holding crib utilized an average 29 g of pigment per double application. A single pigment application in a 724 mm square crib used 38 g per treatment. Pigment costs for double applications ranged from about $2.00 to $34.00/1,000 fish for chinook salmon (595/kg) and muskellunge (7.7/kg), respectively. The single application with lake trout (20/kg) cost $5.80/1,000 fish.

Pigment Formulation and Quality Assessment

An experiment was conducted to determine the quality of marks provided by various sizes of pigment particles contained in bulk pigment. It consisted of two replications of four treatments of rainbow trout fingerlings in eight lots of approximately 130 fish each. Treatments were applications of four different sizes of pigment particles. Bulk pigment was separated into size ranges of <125 µm, 125-250 µm, 250-355 µm and >355 µm with U.S.A. standard testing sieves.

Table 9. Summary of effort and pigment consumption for marking operations with at least 3,000 fish.

Marking procedure	Marking crib size (m^2)	No. of fish per kg	Mean no. of fish per treatment	Mean pigment consumption per 1,000 fish (g)	No. of people	Mean no. of fish marked per hr.
L.M. bass	0.12	19	31	944	3	2,440
Lake trout	0.52	20	116	329	3	8,510
Lake trout	0.12	21	35	773	2	2,550
L.M. bass	0.12	22	36	845	2	1,820
Lake trout	0.12	91	61	477	2	3,640
Chinook salmon	0.12	595	186	118	2	9,450

The fingerlings numbered 85/kg and mean total length of 200 trout was 101 mm (SD=10 mm).

Half the fingerlings in each lot were marked at a time with three passes of the spray nozzle and a delivery force of 370 g. Each replication began with 300 g of pigment in the cannister. Fish lots were confined in separate raceways and mark retention assessments were conducted at 13, 83 and 251 d after marking.

Bulk pigment was sampled at the surface, middle, and bottom of the pail and analyzed for percentage weight composition of the four particle sizes used in the experiment. Samples were 100 g each and weight was measured to the nearest 0.1 g on a triple-beam balance.

Most fish in all lots had visible pigment after 13 d (Table 10), but mark quality declined with particle size. Nearly all fingerlings among the four lots marked with particles >250 μm had readily detectable marks and mark quality from lot to lot was similar. Fish marked with 125-250 μm particles had poorer quality marks but most were

Table 10. Results of pigment retention assessment on rainbow trout 13 d following application of different particle sizes.

	Particle size (μm)							
	<125		125-250		250-355		>355	
Lot number	1	2	3	4	5	6	7	8
No. of fish	129	130	130	130	130	131	131	125
Percent marked	100	98.5	98.5	99.2	100	100	100	100

easily detected. Visible marks on fish with <125 μm particles were fewer and frequently difficult to detect.

The number of visible pigment marks on fish 83 and 251 d after marking was directly related to particle size (Tables 11 and 12). No pigment was found on 17% of the trout marked with <125 μm particles after 83 d and an additional 60% of this lot had only 1-3 visible marks. The tiny particles were usually easily seen on fins but difficult to detect on the body. Mark quality among fish marked with 125-250 μm particles was notably better, but overall quality was still poor. Pigment was found on all fish marked with particles >250 μm and over 90% had more than 10 visible marks. Visibility of marks was good, especially among the fish with >355 μm particles.

When the final mark assessment was made 251 d after marking, 99% of the trout marked with particles >250 μm had visible pigment and marks on the body were easily seen. Pigment was seen on 92% of the 125-250 μm group and on only 75% of the <125 μm group. Mark quality was poor for both groups, and in the 125 μm group, nearly all pigment was seen on fins while body marks were faint and few were readily visible.

Table 11. Percentages of rainbow trout with various numbers of visible pigment marks 83 d following application of different particle sizes.

No. of marks	Particle size (μm)			
	<125	125-250	250-355	>355
0	16.9	5.8	0.0	0.0
1	20.9	3.9	0.4	0.0
2	22.8	5.8	0.8	0.4
3	16.1	7.4	0.8	0.8
4	9.1	4.7	1.9	0.0
5	6.7	5.0	0.8	0.4
6	2.0	3.1	0.8	0.4
7	3.5	4.7	0.0	0.4
8	0.8	5.0	1.2	1.2
9	0.0	5.0	0.8	0.4
10	0.4	3.1	0.4	0.8
10+	0.8	46.5	93.1	95.3
No. of fish	254	258	259	256

Table 12. Percentages of rainbow trout with various numbers of visible pigment marks 251 d following application of different particle sizes.

No. of marks	Particle size (μm)			
	<125	125-250	250-355	>355
0	25.5	7.9	1.1	1.1
1	12.4	8.6	4.3	0.0
2	19.3	8.6	1.1	2.1
3	11.0	10.8	3.2	4.3
4	8.3	5.0	6.4	1.1
5	9.7	5.8	1.1	2.1
6	4.8	3.6	4.3	3.2
7	2.8	6.5	3.2	1.1
8	2.1	6.5	4.3	1.1
9	2.8	5.0	4.3	2.1
10	0.7	1.4	5.3	2.1
10+	0.7	30.2	61.7	79.8
No. of fish	145	139	94	94

Particles >250 μm provided mostly high quality marks but comprised only about 12% of the bulk pigment (Table 13). Smaller particles did not provide adequate marks for short-term studies. Removal of the fine particles also substantially reduced the amount of airborne particles during mark application.

Table 13. Percentage composition by particle size of three 100 g samples of fluorescent pigment used in the marking experiment. The 13.6 kg pail was sampled at the surface, middle and bottom.

Particle size (μm)	Percentage composition				
	Surface	Middle	Bottom	Mean	$t_{.05}SE$
<125	64.4	66.9	65.1	65.5	3.2
125-250	22.8	21.1	22.4	22.1	2.2
250-355	7.9	7.8	7.9	7.9	0.2
>355	4.6	4.1	4.2	4.3	0.7
Total	99.7	99.9	99.6	99.8	

DISCUSSION

Pigment marking of emergent rainbow trout fry can be used at least for investigations of limited duration. More than 96% of the scaleless fry marked with delivery forces of 155, 210 and 265 g retained visible pigment throughout the 267 d assessment period. Survival was highest among fish marked with the 155 g force and the mark quality of these fish after 38 wk was comparable to that of fish marked with the higher delivery forces. The indication is that a marking mortality of at least 25% should be expected. This might be acceptable for special investigations but may rule out routine evaluations.

Clogging of gills appeared to be a major cause of death of the young rainbow trout, so removal of fine pigment particles before spraying might reduce mortality. Delayed mortality of marked fry was indicated for about 6 d, so a mortality assessment of at least a week duration should be considered.

Poor results with pigment retention by some small ($\leq$50 mm) salmonids have been reported (Phinney et al. 1967; Strange and Kennedy 1982; Bax 1983). Hennick and Tyler (1970) reported good short-term (31 d) retention by pigment marked fry, but only about 75% initially acquired the mark. These workers sprayed fry in open nets or troughs. Mattson and Bailey (1969) reported good initial pigment retention by scaleless salmonids and described the apparatus that was used in this study to immobilize and hold fry between meshed frames for marking. White (1976), however, used the apparatus in two experiments with pink salmon and reported fair initial pigment retention but excessive losses of marks over periods of approximately 200 d.

Pigment marking mortality was not a problem with fingerlings of chinook salmon, rainbow trout, lake trout, muskellunge, channel catfish or largemouth bass. Walleye fingerlings, on the other hand, exhibited excessive mortality after marking. Columnaris disease appeared to be the major cause of walleye mortality in the preliminary treatments as mortality in the unmarked control lot was only 15% lower than in the combined marked lots.

Pond mortality of pigment marked walleye was high. Results of pond experiments with Tustin Lake fingerlings (K = 0.665; overwinter survival = 0.7%) and Ida Lake fingerlings (K = 0.734; overwinter survival = 15.3%) indicated that condition factors might influence

survival. Mean K of Sprague Lake fingerlings used in the second pond experiment was 0.798, however, less than 10% of the pigment marked fish survived 28 d in the pond. Those fish were noticeably sluggish before marking, as were the Tustin Lake walleye a year earlier. It appears that walleye pond harvest takes a heavy toll on vitality of walleye fingerlings. Handling causes physiological responses in fish that may last for weeks. Marking operations with any species should include controlled mortality assessments lasting at least 7 d.

All species initially retained pigment very well and short-term (<1 yr) assessments indicated satisfactory mark retention. The double applications provided much better marks than the single application. Few pigment marked fish have been examined more than 1 yr after marking, but results, other than with chinook salmon, are encouraging. Pigment was found on one lake trout after 2.7 yr, one muskellunge after after 2.5 yr and six largemouth bass after 1.6 yr. No unmarked fish among those species were seen. Two of three chinook salmon captured 1.4 to 3.1 yr later had lost the pigment mark.

Few longevity studies have exceeded 1 yr. Andrews (1972) reported that all fathead minnows (Pimephales promelas) surviving to the end of a 600 d study retained pigment. Phinney and Mathews (1973) found no significant change over a 2 yr period in the proportion of pigment bearing fish in experimental lots of coho salmon. Strange and Kennedy (1982) reported 100% pigment retention by brown trout (Salmo trutta) after 20 mo. Nielson and Johnson (1981) found pigment bearing cutthroat trout (Salmo clarki) 5 yr after marking. Evenson and Ewing (1985), on the other hand, noted reductions in pigment marked chinook salmon and steelhead over periods exceeding 4 yr and associated it with

fish size, gonadal maturation and sexual dimorphism which resulted in poorer retention by males.

Reliability of maxillary clips on some species is questionable. Regrowth on three of six largemouth bass was nearly complete after 1.1 yr. Although the rounded tips of the previously severed bones distinguished them from unaltered bones, uncertainty would have arisen without the confirmation of visible pigment. Six muskellunge retained recognizable clips for 6-10 mo, but a maxillary clip could not be distinguished on a muskellunge after 2.5 yr. A walleye had a recognizable maxillary clip after 10 mo.

Growth assessments with rainbow trout and lake trout fingerlings did not indicate that pigment marking influenced either species. An assessment with yearling lake trout was confounded with significant variation within treatment groups, but the two marked lots combined grew an average 7 mm less than the two control lots combined. Although the difference was small, it was significant.

Only about 12% of the bulk material provided adequate marks in the pigment quality assessment. Removal of smaller particles from the bulk pigment before use enhanced mark quality on a short term basis (<1 yr), but whether it will increase mark longevity is unknown. Mark quality on rainbow trout marked with pigment particles >250 μm progressively declined over the 251 d assessment period, but somewhat better quality appeared to be maintained than with conventional treatments that employed bulk pigment. Removal of fine pigment particles also had the advantage of a large reduction in airborne particles during spraying, resulting in more favorable working conditions and a cleaner operation.

Removal of small particles substantially increases material cost. Selection of particles larger than 125 and 250 μm increased pigment

costs by factors of about three and eight, respectively. Smaller orifices in the siphon stem or delivery tube when marking with pigment particles larger than 125 μm might reduce pigment consumption and still provide adequate marks. Phinney et al. (1967) reported clogging of a siphon stem orifice less than 2.4 mm in diameter, however, that likely was caused by compaction of fine particles and should not occur in their absence. Granules >250 μm were easily separated with sieves, but it took considerable time and effort to work the smallest particles through the 125 μm sieve.

Treatments should be described in terms of delivery force rather than gage pressures. Delivery technique and equipment influence the force with which pigment strikes the fish (McAfee 1982). Readings of delivery force on a platform scale are sensitive to differences in technique and equipment whereas pressure gage readings are not. Thus, gage readings can give rise to inconsistent results between seemingly similar operations. Scales are also less subject to malfunction than cheap pressure gages and scale accuracy is easily evaluated. Gage pressure readings relative to delivery forces repeatedly changed during this study and replacement of old gages with new added to the uncertainty.

Fingerlings of all species except walleye withstood pigment application forces to the limit of equipment capacity without excessive mortality and probably could have endured more. A larger compressor than that employed would probably enhance mark quality and longevity. It had a capacity of 11.25 kg/cm^2 but the maximum working pressure that could be sustained for an adequate length of time was less than 10.55 kg/cm^2. Prior to a pigment marking operation, a short-term

survival assessment to determine a maximum acceptable delivery force should be considered.

Pigment is visible in normal light for only a short period until excess, unembedded particles are shed. Ultraviolet light under darkened conditions is then needed to detect the mark. Pribble (1976) and McAfee (1980) have provided construction details for portable field detection boxes.

Study results indicate that pigment marking can be a suitable and economical alternative to mutilation marking for at least short-term studies with most species. A larger air delivery system than that used and the availability of better quality marking material could make fluorescent pigment marking a desirable and reliable technique for long-term studies.

MANAGEMENT IMPLICATIONS

Pigment marking of emergent rainbow trout fry can be used at least for investigations of limited duration. Removal of fine pigment particles before spraying might reduce mortality below 25%.

Marking mortality was low for fingerlings of all species except walleye. Disease and stress from harvest contribute to walleye mortality.

Short-term (<1 yr) assessments showed satisfactory pigment mark retention for all examined species. Long-term monitoring of mark retention should continue.

Pigment particles >250 μm constituted only 12% of the bulk material but yielded marks with the best quality and best short-term retention. Removal of small particles substantially increases material costs. Treatments should be described in terms of delivery force rather than gage pressures.

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