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A Study to Determine the Biological Feasibility of a New Fish Tagging System

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Studies Division
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A STUDY TO DETERMINE THE BIOLOGICAL
FEASIBILITY OF A NEW FISH TAGGING SYSTEM, 1985-1986

by

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ABSTRACT

An ongoing cooperative project between the Bonneville Power Administration and the National Marine Fisheries Service was initiated in 1983 to evaluate the technical and biological feasibility of adapting a new identification system to salmonids. The system is based upon the passive integrated transponder (PIT) tag. This report discusses the work completed in 1985 and is divided into laboratory and field studies. All studies were conducted with the tag implanted into the body cavity of the test fish via a 12-gauge hypodermic needle.

Laboratory studies with juvenile chinook salmon and steelhead showed no adverse effect of the tag on growth or survival. Once the tag was established in the body cavity, its location was found to be consistent over time. Behavioral tests showed no significant effect of the tag on opercular rate, tail beat frequency, stamina, or post fatigue survival on juvenile steelhead. Active swimming did not affect tag retention in steelhead. Tests revealed a minimum size threshold for tag retention in juvenile steelhead at 8.5 g before acceptable tag retention levels were achieved. No effect on growth or survival was observed for juvenile chinook salmon or steelhead.

The polypropylene encapsulated tags had an unacceptable failure rate due to moisture contacting the tag's electronic circuitry. The use of polypropylene encapsulated PIT tags was not recommended. The tag manufacturer now produces the tag encapsulated in glass--which should provide significant improvements in tag longevity and tag retention.

No evidence of infection due to tagging procedures was observed in tagged fish. Nevertheless, it was demonstrated that the PIT tag and tagging apparatus could be disinfected against Aeromonas salmonicida by exposure to a 50% or stronger solution of ethanol for a minimum of 1 minute.

Maturing Atlantic salmon were PIT tagged. In males, tag retention was 100% prior to and after spawning. Females had 100% tag retention prior to spawning and 83% retention after multiple hand strippings. Lost tags accompanied the egg mass during strippings and were easily detected in the spawning bucket.

All field tests using juvenile salmonids were conducted at McNary Dam, whereas tests using adult fish were conducted at Bonneville Dam. The PIT tag monitoring equipment is described and discussed. The tag monitoring equipment showed a high degree of reliability, efficiency, and accuracy. During the 6-month testing period, tag reading efficiency exceeded 90%, and tag reading accuracy for juvenile chinook salmon was 100%. Only two minor equipment failures occurred during the testing period.

Field studies used migrant spring and fall chinook salmon; no significant effects of the tag on survival could be determined when compared to traditional tagging and marking methods. No significant difference was observed in the recovery rate between branded and PIT tagged juvenile fall chinook salmon released into McNary reservoir and recovered at the dam. The PIT tag data were acquired with 90% fewer PIT tagged fish being released than branded fish and a 33-fold reduction in the number of tagged fish being physically handled to recover the data. Adult steelhead were successfully PIT tagged and automatically interrogated as they passed through a PIT tag monitor installed on a Denil fish ladder. It was concluded that a PIT tag monitor for adults can be installed at any location that can accommodate a coded wire tag monitor.

Future work related to PIT tag systems development is described and discussed.

CONTENTS

	PAGE
INTRODUCTION	1
PART I: LABORATORY STUDIES.....	3
Study 1: Comparison Between Functional and Sham PIT Tags.....	3
Introduction.....	3
Methods and Materials.....	3
Results and Discussion.....	5
Study 2: PIT Tag Longevity.....	11
Introduction.....	11
Methods and Materials.....	11
Results and Discussion.....	12
Study 3: PIT Tag Effect on Locomotive Ability.....	16
Introduction.....	16
Methods and Materials.....	17
Results and Discussion.....	21
Swimming Stamina.....	21
Stride Efficiency.....	22
Opercular Beat Rate.....	27
Post-Test Survival and Tag Retention.....	31
Study 4: Serial Tagging to Determine Minimum Fish Size for Tagging...33	
Introduction.....	33
Methods and Materials.....	34
Results and Discussion.....	34
Study 5: Tag Placement in Adult Salmon.....	37
Introduction.....	37

	PAGE
Methods and Materials.....	38
Results and Discussion.....	38
Study 6: Sterilization Technique for Tagging Equipment.....	41
Conclusions and Recommendations.....	41
PART II: FIELD STUDIES.....	43
Study 1: Evaluate Juvenile PIT Tag Monitor Reliability.....	43
Introduction.....	43
Methods and Materials.....	43
Results and Discussion.....	45
Study 2: Evaluate Tag Reading Efficiency of the Juvenile PIT Tag Monitor	48
Introduction.....	48
Methods and Materials.....	49
Test 1.....	49
Test 2.....	50
Test 3.....	50
Results and Discussion.....	51
Test 1.....	51
Test 2.....	51
Test 3.....	53
Study 3: Comparison of the PIT Tag to Traditional Tagging and Marking Methods.....	55
Introduction.....	55
Methods and Materials.....	55
Results and Discussion.....	56

	PAGE
Study 4: McNary Reservoir Release.....	58
Introduction.....	58
Methods and Materials.....	58
Results and Discussion.....	60
Study 5: Monitoring PIT Tags in Adult Fish.....	62
Introduction.....	62
Methods and Materials.....	63
Results and Discussion.....	65
Conclusions and Recommendations.....	67
PART III: SYSTEMS DEVELOPMENT.....	69
Study 1: PIT Tag Injection Devices.....	69
Study 2: Quality Control Monitor for Tagging.....	69
Study 3: Hatchery Release Monitor.....	71
Study 4: Design and Placement of Future Monitoring Systems	72
Conclusions and Recommendations.....	76
ACKNOWLEDGMENTS.....	77
LITERATURE CITED.....	78
APPENDIX A--Preliminary Investigation of the Inactivation of <u>Aeromonas salmonicida</u> , a Fish Pathogen.....	80
APPENDIX B--Budget Information.....	88

INTRODUCTION

A cooperative program between the National Marine Fisheries Service (NMFS) and the Bonneville Power Administration (BPA) to evaluate the technical and biological feasibility of the passive integrated transponder (PIT) tag for salmonid research has been under way since 1983. The PIT tag is being developed as a research and management tool for monitoring the movements of juvenile and adult salmonids in the Columbia River Basin. Preliminary results show that fish injected with this tag can be automatically recognized by detecting/recording devices strategically located within the collection facilities at hydroelectric dams. The PIT tag is an electronic tag 10 mm long by 2.1 mm in diameter that can be coded with one of 35 billion unique codes. The tag can be automatically detected and decoded in situ--eliminating the need to sacrifice, anesthetize, handle, or restrain fish during data retrieval.

In 1983 and 1984, juvenile and adult salmon were injected with sham (non-functional) PIT tags to determine suitable anatomical areas for tag placement, develop tag injection techniques, and determine the effect of the tag on growth and survival. The body cavity was selected as the best area for tag placement for most applications from a biological and social standpoint.

From 1984 to 1985, work continued to evaluate the effect of the tag on growth and survival of juvenile fish and to further refine the tagging technique. Functional PIT tags were used in studies for the first time. Prototype juvenile and adult PIT tag monitoring systems were evaluated in field tests. Tag decoding efficiency averaged 90.5% for four different tests using juvenile fish and 94.4% in tests using adult fish. Tag reading accuracy was 100% for all tests.

This report covers the work conducted under the 1985 to 1986 work plan and is divided into three parts. Each of these studies concentrate on different developmental aspects for the PIT tag. The species of fish used in these studies varies, and was governed both by availability and applicability. The Laboratory Studies (Part I) focus on tag retention, reliability, and effects on behavior. This study establishes minimum fish size criteria for tagging with the polypropylene encapsulated tag. The Field Studies (Part II) evaluate the PIT tag monitors and compare the PIT tag to the traditional tagging and marking methods. Systems Development (Part III) focuses on design and quality control measures needed to develop the PIT tag for use in large scale studies.

PART I: LABORATORY STUDIES

Study 1: Comparison Between Functional and Sham PIT Tags

Introduction

All laboratory tests through 1984 used sham, non-functional, tags. The sham tags were the same size and shape as functional tags and had the same external coating. These tests defined an acceptable anatomical area for tag placement (intraperitoneally near the mid-ventral line and posterior of the pectoral fins) and resulted in techniques for implanting the tag. The objective of the 1985 study was to compare results obtained from fish injected with sham tags to those injected with functional tags.

Methods and Materials

The study was conducted at the University of Washington's Big Beef Creek Research Station. Juvenile fall chinook salmon, Oncorhynchus tshawytscha, were initially maintained in 2.4-m diameter tanks with running fresh water (surface water). Standard husbandry practices were followed in maintaining the fish. Fish were randomly selected from the main population on 15 April 1985 to establish five groups: functional tag, functional tag sacrifice, sham tag, sham tag sacrifice, and control. At the time the groups were established and at the termination of the study (20 August), a sub-sample of 10 fish from each group was weighed (± 0.5 g) and measured (± 3.0 mm). The number of replicates and number of fish per replicate are shown in Table 1.

The PIT tags and sham tags were injected into the body cavity of the fish using a 12-gauge hypodermic needle. The control fish were handled, but not injected with the hypodermic needle. During tag insertion, the needle was angled in a posterior direction, 2 to 3 mm to either side of the mid-ventral

Table 1.--Test plan for Part I, Studies 1 and 2 using fall chinook salmon.

Study	Treatment	Rearing area	Number of replicates	Number of fish per replicate
1	Control	Fresh water	6	100
1	Functional tag	Fresh water	6	100
1	Functional tag sacrifice	Fresh water	2	100
1	Sham tag	Fresh water	6	100
1	Sham tag sacrifice	Fresh water	2	100
2	Control	Seawater	1	300
2	Functional tag	Seawater	1	300

line at the posterior end of the pectoral fins. Tagging methods are still being developed and automated, however, they generally followed methods described by Prentice et al. 1985. At tagging, a single tag was loaded into the barrel of a needle and, upon needle insertion into the fish, the tag was released via a push-rod attached to the plunger of the hypodermic syringe. Tag location within the body cavity as well as tissue response to the tag were determined by examining fish that died or were sacrificed. The first two sacrifice groups were terminated and examined on 25 and 29 May, the third on 19 July, and the fourth group on 20 August. All fish that died during the study were examined for tag retention and cause of death. At the termination of testing, all tagged fish were sacrificed and examined for tag location and tissue response to the tag.

Results and Discussion

No significant difference ($P < 0.05$) in length or weight was seen between replicates within a treatment or between treatments at the start of the study. Similarly ($P < 0.05$), growth rates were not different at the end of the study (127 elapsed days). These results are similar to that previously reported (Prentice et al. 1984 and 1985), suggesting the PIT tag does not suppress growth.

Tag retention (sham and functional) was poor ranging from 58 to 93% at 127 days (Table 2). No explanation can be given for the one sham tag replicate with only a 58% tag retention, whereas the next lowest tag retention value was 74%. The overall percentages (combined replicates) of tag retention for sham and functionally tagged fish were 80 and 86%, respectively.

Tag retention among the sacrificial groups was also poor. The first sham group was sacrificed on Day 40 of the test with a 5% tag loss. The second sham group was sacrificed on Day 97 showing a 25% tag loss. Similar high and

Table 2.--Summary of data comparing functional and sham tagged fish to control fish.

Treatment	Replicate	Test period (days)	Starting number	Percent survival	Starting length (mm)		Ending length (mm)		Starting weight (g)		Ending weight (g)		Percent tag retention	Percent functional tags
					Ave.	SD	Ave.	SD	Ave.	SD	Ave.	SD		
Control	1	127	100	98	78	5.29	130	5.98	4.7	1.01	21.3	5.85	-	-
	2	127	100	97	76	4.37	118	6.61	4.2	0.70	16.8	3.15	-	-
	3	127	100	97	76	4.99	103	6.56	4.3	0.86	9.8	2.20	-	-
	4	127	100	100	81	5.04	127	7.32	5.4	0.97	21.3	4.17	-	-
	5	127	100	99	79	5.66	120	4.28	4.5	1.13	16.9	1.76	-	-
	6	127	100	100	79	5.23	140	11.80	4.6	0.91	27.4	6.55	-	-
PIT tagged	1	127	100	99	79	5.03	139	8.59	5.1	0.93	27.5	5.23	76	96
	2	127	100	99	79	4.44	125	10.40	4.8	0.76	20.3	3.50	90	84
	3	127	100	96	80	2.58	131	6.00	5.1	0.67	25.6	3.76	92	95
	4	127	100	98	78	6.03	130	5.69	4.6	1.08	24.3	5.65	89	98
	5	127	100	95	77	4.65	114	4.83	4.4	0.18	15.4	2.49	82	99
	6	127	100	98	80	3.78	126	6.89	4.7	0.65	22.5	5.33	89	99
PIT sacrifice	1	45	100	99	78	5.70	94	5.04	4.3	0.86	9.3	1.48	85	90
	2	127 ^{1/}	103	99	81	3.43	114	7.09	4.8	0.87	17.1	3.41	93	95
Sham tagged	1	127	100	99	80	4.40	128	6.96	4.6	1.02	22.7	3.94	83	-
	2	127	100	100	79	2.95	114	5.79	4.5	0.62	15.6	2.63	74	-
	3	127	100	97	77	2.74	114	5.59	4.4	0.65	15.3	2.50	93	-
	4	127	100	89	77	2.91	121	5.85	4.6	0.72	19.9	3.18	85	-
	5	127	100	99	77	5.06	130	7.01	4.5	0.85	24.5	4.47	88	-
	6	127	100	95	78	4.69	129	6.89	4.6	0.90	24.5	4.61	58	-
Sham sacrifice	1	40	100	96	78	5.51	95	5.08	4.6	0.99	-	-	95	-
	2	97	69 ^{2/}	99	78	6.46	116	6.22	3.9	0.69	18.5	3.35	75	-

^{1/} The experiment was extended 30 days after the sham sacrifice.

^{2/} Sample size varies due to availability of fish.

variable tag loss occurred with the functional PIT tag sacrificial groups. At the end of 45 days, 15% of the tags were lost from the first sacrificial group, whereas by the end of 127 days, only 7% were lost from the second sacrificial group. No good explanation can be offered for the variability in tag retention between replicates, but the small size of the fish (about 80 mm) at tagging was thought to be a factor controlling tag retention.

During the experiments, tags were observed protruding through the body wall (Table 3). Some fish that had shed tags showed two scars in the abdominal region, one from the tagging needle and a second scar presumed to be where the tag exited the body cavity.

The exact mechanism by which the tag is rejected is unknown. It is probable, however, that the peritoneal cavity of small fish is very limited in its capacity to accommodate a proportionally large foreign body such as the PIT tag. If at the time the tag is injected into the body cavity of small fish, it does not lie nearly parallel to the body axis, but lies at some angle, the body organs (intestine, gut, etc.) may exert pressure against the tag. Since there would be limited room in the peritoneal cavity under these conditions for the tag to reorient itself, the force against the tag could cause the tag to be pushed through the body wall. This condition could be further aggravated if there was a slight tissue reaction to the encapsulating material or to the rough edges that occasionally occur on the tag. We have seen very few incidences of tissue reaction to the tag, however, such reactions may be short term and/or difficult to detect. The above condition may not occur in large fish with peritoneal cavities of greater volume.

A large number of functional tags failed to operate after 127 days of testing. The percent of functional tags ranged from 84 to 99% (Table 2). Within the sacrificial group, 10% of the tags failed within 45 days. The tag manufacturer was made aware of the failure problem and is in the process of modifying production techniques to increase the tag's longevity.

Table 3.--Summary of wound condition after tagging and tag location within the body cavity of juvenile fall chinook salmon over time with a description of wound condition and tag location codes.

Code	Days post tagging		
	40-45	97	127
Wound code ^{a/}	Percent fish within a classification code		
A	7.3 ^{b/}	0	0.6
B	8.3 ^{b/}	0	0.2
C	84.4 ^{b/}	100.0	99.2
Tag location code ^{c/}			
A	2.1 ^{d/}	0	3.9
B	86.5 ^{d/}	69.1	83.3
C	0.0 ^{d/}	4.4	1.0
D	5.2 ^{d/}	25.0	6.9
E	6.3 ^{d/}	1.5	4.9

^{a/} A = An open wound.

B = A wound that is closed by a thin membrane and is healing; at times a slight red or pinkish coloration is noticeable in the area of the wound.

C = A wound completely healed and may or may not be noticeable by the presence of a scar. There is no red or pink coloration in the area of the wound.

^{b/} Percentage based on data from the combined sham and functional PIT tagged groups examined from Days 40-45.

^{c/} A = Tag located between the pyloric caeca and mid-gut.

B = Tag located near the abdominal musculature and often embedded in the posterior area of pyloric caeca near the spleen or in the adipose tissue at the posterior area of the pyloric caeca.

C = Tag found in an area other than those noted; generally between the mid-gut and air bladder or between the liver and pyloric caeca.

D = No tag present.

E = Tag partially protruding through abdominal wall.

^{d/} Percentages based only on the sham sacrificial group examined on Day 40.

Survival was high among all groups, ranging from 89 to 100% (Table 2). Control fish showed a slightly (but not significantly) higher survival (97 to 100%) than sham tagged (89 to 100%) or functionally tagged fish (95 to 99%). The difference in survival between the control group and the other two treatment groups was attributed to initial tagging mortality. Initial tagging mortality was from perforation of the intestine or laceration of the kidney with the tagging needle at the time of tagging. Fish suffering such injuries died within the first 4 days after tagging. All other mortalities among test and control fish were attributed to bacterial kidney disease or bacterial gill disease.

No correlation was seen between tag retention and survival ($r=0.030$, $P<0.05$) among any test group (Table 2). The passing of the tag through the body wall did not cause an increase in mortality. No infection or other disease problems were visually observed among fish that were rejecting or had rejected their tag.

Tag wound condition and tag placement were documented for fish in four sacrificial groups (two sham and two functionally tagged groups) (Table 3). Nearly 85% of the fish examined ($n=195$), regardless of treatment, showed the tagging wound to be completely healed with only a scar indicating the area of needle insertion by Days 40 and 45. During this same period, 7.3% of the fish showed an open wound and 8.3% showed a wound that was closed but slightly discolored. All fish ($n=99$) sacrificed after 90 days showed the wound to be completely healed. At the termination of the study (127 days), 102 fish from a functional sacrifice group were examined, and 99.2% of the fish had completely healed wounds, 0.6% showed open wounds, and 0.2% had wounds that were closed but slightly discolored.

In a previous study, data for juvenile steelhead Salmo gairdneri, showed that after 30 days, all tagging wounds were completely healed (Prentice et al. 1985). The fish used in that study were larger than the fall chinook salmon used in the present study. A second difference between the studies was that the number of fish used per observation was limited (n=6) in the earlier work, thus the precision of the estimate is not comparable to the present study. In spite of the slight difference in results between the two studies, it is our opinion that no problem exists from the tagging. To date there has been no evidence of infection or excessive mortality resulting from PIT tagging fish.

Tag location within the body cavity was consistent regardless of the treatment (sham or functional PIT tag) or time observed (Table 3). The majority of the tags were observed near the abdominal musculature either embedded in the posterior area of the pyloric caeca near the spleen or in the adipose tissue at the posterior area of the pyloric caeca. These results are consistent with those obtained in a previous study, where 96% of the tags were found in similar locations (Prentice et al. 1985).

Tag retention was a problem among both the test replicates and sacrificial groups regardless of treatment. Tag loss occurred throughout the study and showed signs of continuing by the presence of tags protruding from the body wall. Close examination of these fish did not reveal where the tags may have been within the body cavity prior to their migration through the abdominal wall.

Study 2: PIT Tag Longevity

Introduction

The only information pertaining to the longevity of the functional PIT tag is from the tag manufacturer who thoroughly tests the tags under laboratory conditions. Field testing is necessary, however, to provide valuable information, unobtainable in the laboratory, that is needed to design studies and interpret their results. The objective of the study was to determine, under field conditions, the longevity of functional tags placed in juvenile salmon.

Methods and Materials

Juvenile fall chinook salmon were obtained from the same populations utilized in Study 1. On 2 April 1985, two 300-fish test groups were established at Big Beef Creek: one control and one functional tag group (Table 1). Tags were injected into the body cavity of fish as previously described. All fish in each test group were weighed (± 0.5 g) and measured (± 3.0 mm) at the time the test groups were established. The identification number of each fish was recorded. The two test groups were maintained in separate tanks in fresh water until smolted.

At the time of smoltification, as determined by visual observations, all fish were transported to the NMFS Manchester Marine Experimental Station near Manchester, WA, (5 May); vaccinated against Vibrio sp.; and acclimated to seawater over a 5-day period. All fish in each test group were counted and the presence of the functional tag verified prior to placement in seawater.

The PIT tag and control groups were maintained in separate seawater net-pens. Standard husbandry practices were followed for the duration of the study. All dead fish were examined for cause of death, and the presence of

the tag was verified if applicable. Additional observations as to tag presence and functionality took place on 6 March, 21 August, and 5 November, 1986. At termination of the study on 6 March 1986, all fish were measured, and a subsample of 25 fish from each treatment was weighed.

Results and Discussion

A total of 35 days elapsed from the time the fish were tagged to the time they were transferred to seawater (Table 4). During that period, two tagged fish died of kidney damage that occurred during tagging. Four control fish died during freshwater rearing; one from jumping from a rearing tank and the other three from unknown causes. During seawater culture (306 days), a total of 9 tagged fish and 16 control fish died. The cause of death was bacterial kidney disease.

No significant difference ($P < 0.05$) in growth between control fish and tagged fish was observed during 341 days of rearing (Table 4 and Figs. 1 and 2). The mean starting fork lengths of control and tagged fish were $70.0 \text{ mm} \pm 3.8$ (SD) and $69.8 \text{ mm} \pm 3.8$ (SD), respectively. After 341 days, the mean lengths were $254 \text{ mm} \pm 26.0$ (SD) for control fish and $256 \text{ mm} \pm 24.8$ (SD) for tagged fish.

Tag longevity was poor. A total of 40 tags out of the initial 300 failed (13.3%) after 341 days in fish (Table 4). The nonfunctional tags were returned to the manufacturer for inspection. They concluded that body fluids entered the tag through the ends of improperly sealed tags. At the time the tags are manufactured, they are pressure tested to several atmospheres using a leak indicator. It was discovered however, that micro-openings occur occasionally in the end seal of the tag. These openings closed under pressure testing, and the defective tags were not detected. However, under normal

Table 4.--Summary of growth, survival, and tag retention and longevity information for PIT tagged and control fall chinook salmon cultured for 341 days.

Treatment	Period (days)	Starting number of fish ^{a/}	Ending number of fish	Percent survival	Starting weight		Ending weight		Starting length		Ending length		Percent tag retention	Percent functional tags
					Mean (g)	SD (g)	Mean (g)	SD (g)	Mean (mm)	SD (mm)	Mean (mm)	SD (mm)		
PIT tagged	0-35	300	297	99.0	7.5	0.64	5.6	1.62	70.0	3.77	79.0	7.00	91.7	98.7
	36-106	268	264	98.5	5.6	1.62	-	-	79.0	7.00	139.6	9.61	93.7	96.3
	107-217	237	233	98.3	-	-	-	-	139.0	9.61	202.0	8.27	100.0	92.4
	218-341	215	209	97.2	-	-	225.1	64.49	202.0	18.27	256.3	24.78	100.0	98.3 ^{c/}
Control	0-35	300	296	98.7	3.6	0.62	6.2	1.11	70.0	3.80	82.4	4.83	-	-
	36-106	296	287	97.0	6.2	1.11	-	-	82.0	4.83	139.0	9.96	-	-
	107-217	287	280 ^{b/}	97.6	-	-	-	-	139.0	9.96	198.0	17.72	-	-
	218-341	280	261 ^{b/}	97.9	-	-	247.5	68.45	198.0	17.72	254.0	25.95	-	-

a/ The number of fish at the start of the period has been adjusted for mortalities, missing fish, fish with no tag, and fish with non-functional tags.

b/ Thirteen fish were missing from the group due to predators. The percent mortality is calculated on the basis of accounted for mortality.

c/ Over an elapsed period of 341 days, 40 tags out 300 (13.3%) failed.

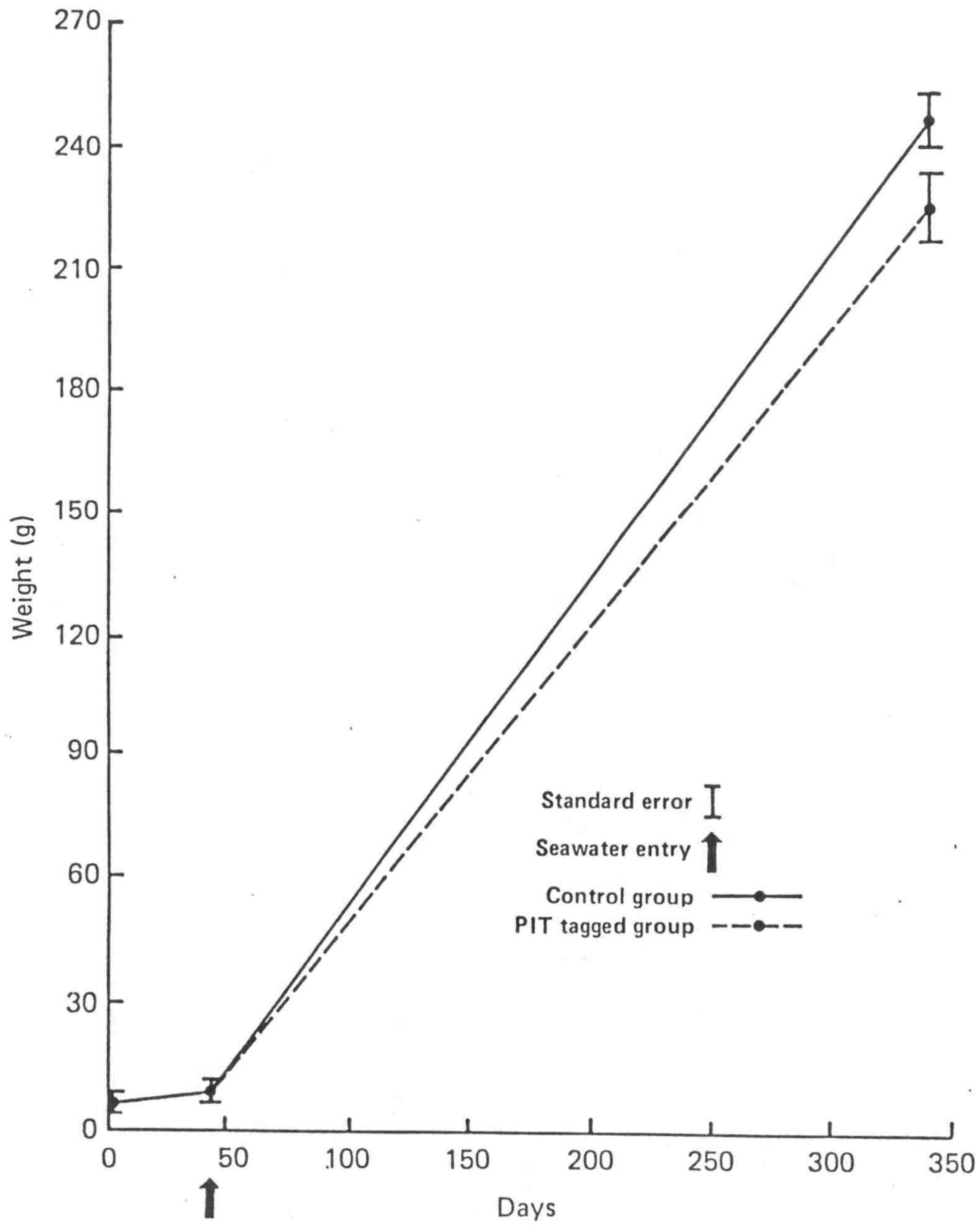


Figure 1.--Comparison of weight change of PIT tagged and control fish over time.

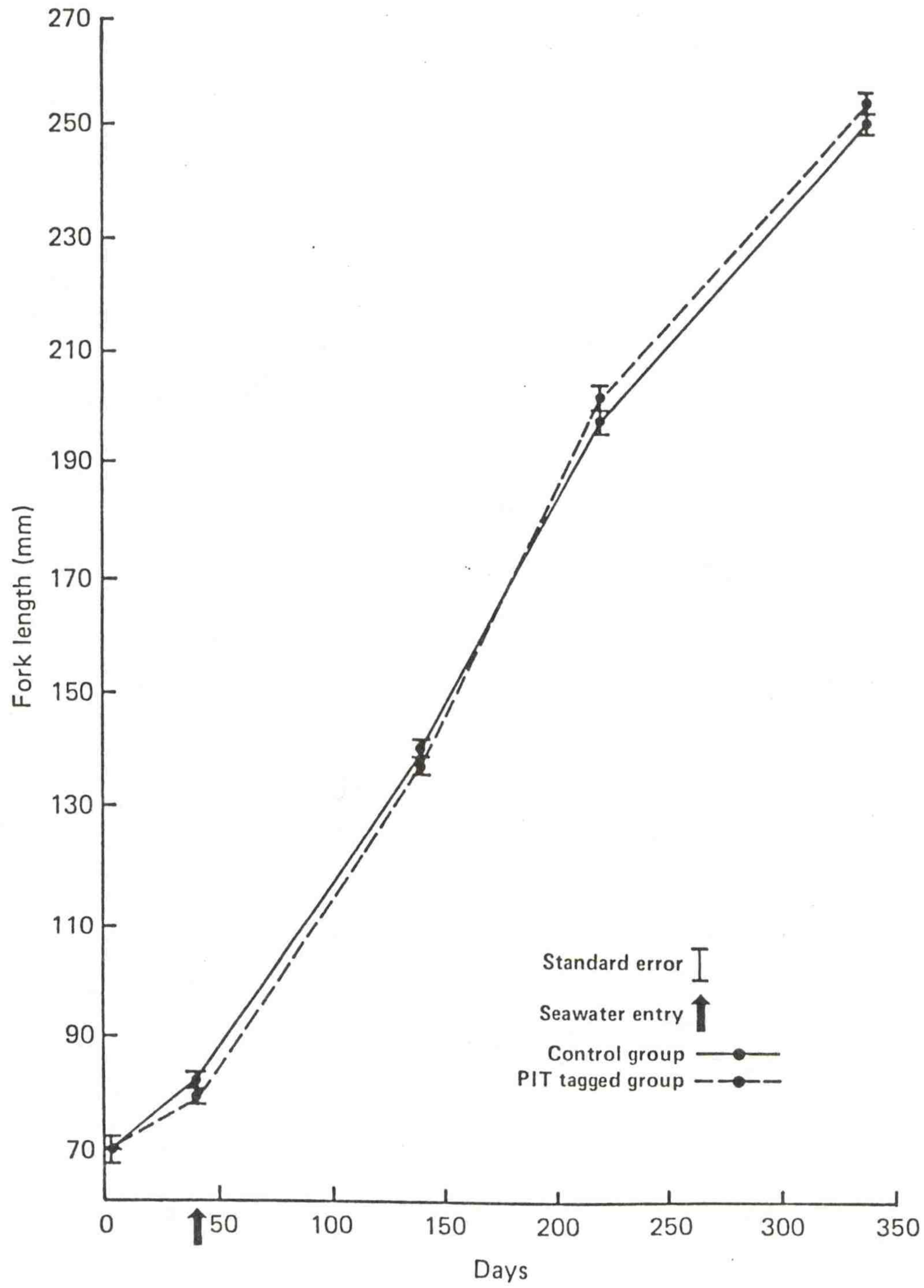


Figure 2.--Comparison of length change of PIT tagged and control fish over time.

conditions, capillary action drew fluids into the tag and caused shorting of the electronic circuitry. The manufacturer of the tag will be providing tags with a glass enclosure in 1986. This change in manufacturing should eliminate leakage problems and substantially increase tag longevity.

Tag retention initially was poor. In the first 35 days of culture, 8.3% of the tags (25 tags) were not retained within the body cavity. During the next 107 days of rearing, an additional 6.3% of the tags were rejected. Tag rejection, however, was zero during the following 234 days. The increase in fish size during the last 234 days of the study may have accounted for the improved tag retention.

The tag rejection process did not jeopardize the survival of the fish. During the 341 days of culture, 17 tagged fish died (vs 26 control fish), while 42 tags were rejected. The exact mechanism of tag rejection remains unknown.

Study 3: PIT Tag Effect on Locomotive Ability

Introduction

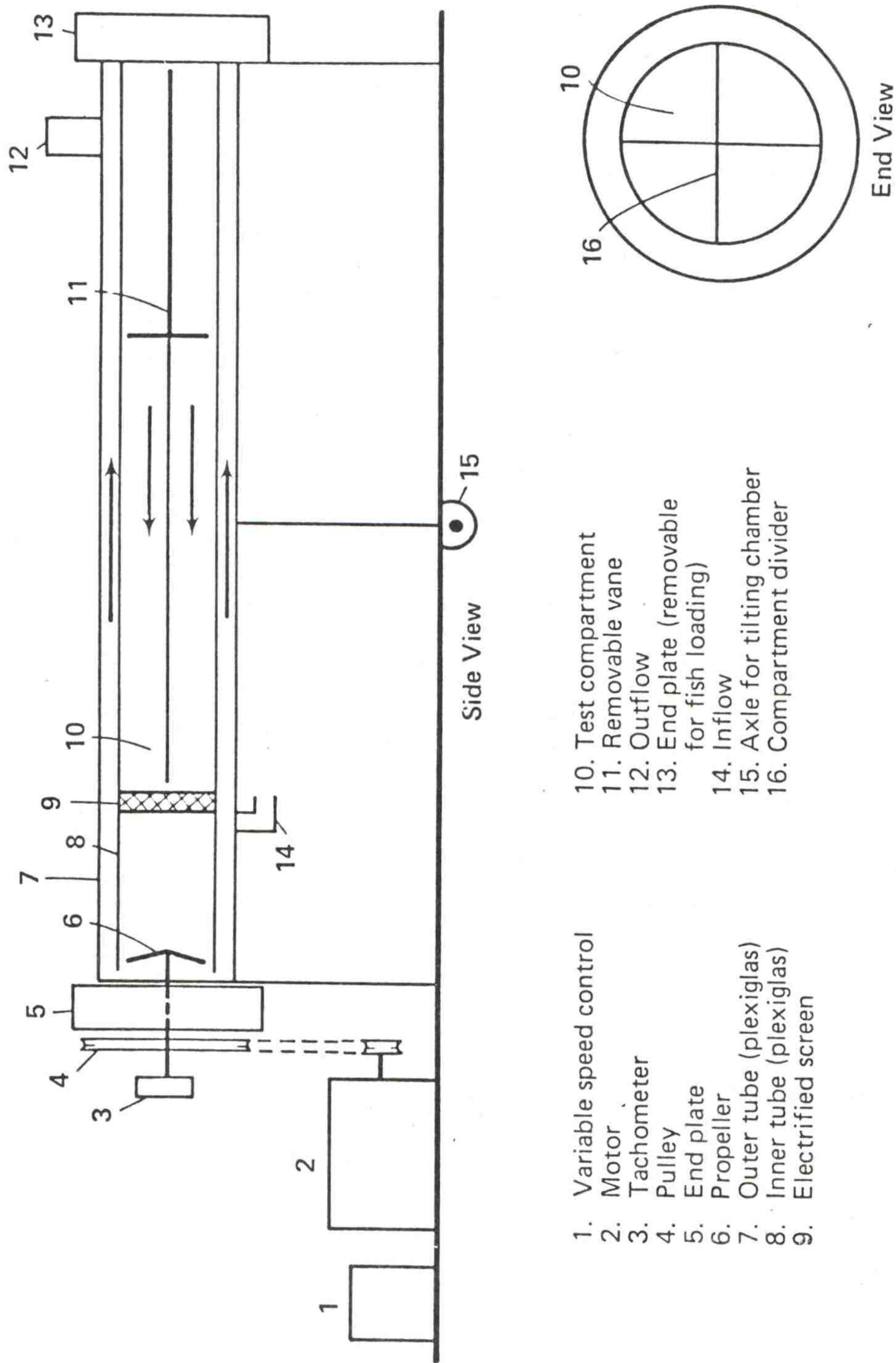
Both internal and external ultrasonic telemetry tags have been shown to adversely effect the fishes swimming ability and respiratory rate (McCleave and Stred 1975; Lewis and Muntz 1984) and, therefore, could potentially alter migratory ability. Though the PIT tag is only about 3% of the volume of commonly used juvenile radio telemetry tags (Monan 1985), there is concern that swimming performance could be affected. The present study evaluated the physiological/behavioral effects of the PIT tag on locomotive ability for two size ranges of steelhead, these tests are ongoing and will eventually include other size ranges of steelhead and chinook salmon. Locomotive performance was

evaluated by assessing swimming stamina, tail beat (swimming) proficiency, and respiratory rates.

Methods and Materials

Two size ranges of steelhead were evaluated in the present study: fingerling fish tested in July 1985 averaged $83 \text{ mm} \pm 8$ (SD) in length and $6.5 \text{ g} \pm 1.8$ (SD) in weight. In October 1985, juvenile steelhead were tested, these fish averaged $112 \text{ mm} \pm 9$ (SD) in length and $17.2 \text{ g} \pm 4.4$ (SD). At testing, random samples ($n=200$) were removed from the main population and intraperitoneally tagged with the PIT tag using procedures described by Prentice et al. (1984). A control, non-tagged, group ($n=200$) was also established from the main population at this time. Swimming performance tests were conducted on Days 0 (same day as tagging) 1, 2, 3, 4, 7, 9, 11, 14, 17, 21, and 25, following tagging, with 12 tagged and 4 control fish tested each day.

Swimming tests were conducted in a modified version of the Blaska respirometer-stamina chamber described by Smith and Newcomb (1970) (Fig. 3). These chambers were divided into multiple compartments to allow the simultaneous testing of four fish. Each test chamber was equipped with an electrified screen at the downstream end, assuring maximum fish performance. In these tests, fish were individually anesthetized [tricaine methane-sulfonate (MS-222)], weighed ($\pm 0.1 \text{ g}$) and fork length measured ($\pm 1 \text{ mm}$), and then placed into a test compartment. After a 1-h recovery period, the initial water velocity was set at 1.5 body lengths per second (l/s) and increased 0.5 l/s every 15 minutes until all the fish reached fatigue (i.e., could no longer hold position in the current and remained impinged against the electrified screen).



- | | |
|---------------------------|--|
| 1. Variable speed control | 10. Test compartment |
| 2. Motor | 11. Removable vane |
| 3. Tachometer | 12. Outflow |
| 4. Pulley | 13. End plate (removable for fish loading) |
| 5. End plate | 14. Inflow |
| 6. Propeller | 15. Axle for tilting chamber |
| 7. Outer tube (plexiglas) | 16. Compartment divider |
| 8. Inner tube (plexiglas) | |
| 9. Electrified screen | |

Figure 3.--Diagram of modified Blaska respirometer-stamina chamber, showing side and end views. For loading, the chamber is tilted, partially filled with water, and end plate and vane are removed. Fish are placed in the test compartments, vane and end plate are replaced, and chamber is filled with water and leveled. Water flow is produced with motor driven propeller and varied via motor speed controller. Direction of water flow is toward propeller in inner tube, water is turned at the end plate, and returned through the space between the inner and outer tubes (see arrows).

In these studies, the step-wise 1/s value was based on the mean length of the four fish in the chamber. The swimming speed of each fish was calculated from the relationship of the mean length of the fish in the chamber, and length of each individual fish, to the water flow within the chamber by the formula:

$$S_p = (l_i/l_{ii}) \times V$$

where: S_p = swimming speed of individual fish in body lengths
per second (1/s)

l_i = mean length of the four fish (mm)

l_{ii} = length of the individual fish (mm)

V = water velocity in the chamber (1/s)

Individual swimming speed was corrected for the effects of solid blocking (for any fish whose size was greater than 10% of the cross-sectional area of its swimming compartment) using the formula of Bell and Terhune (1970):

$$V_f = V_t \left[\frac{1 + \frac{A_o/A_t}{1 - A_o/A_t}}{1 - A_o/A_t} \right]$$

where: V_f = effective velocity (1/s)

V_t = average velocity through the empty test section (1/s)

A_o = maximum cross-sectional area of the object in the test
section (mm²)

A_t = test section area (mm²)

A swimming stamina profile (U-critical) was established for each group, using the swimming speed at fatigue and the time of fatigue as an integrated time/velocity measure of impingement, by the methods described by Beamish (1978):

$$U\text{-critical} = U_i + (t_i/t_{ii} \times U_{ii})$$

where: $U\text{-critical}$ = critical swimming speed (l/s)

U_i = highest velocity maintained for the prescribed period (l/s)

U_{ii} = velocity increment (l/s)

t_i = time (in minutes) fish swims at fatigue (impingment)
velocity

t_{ii} = prescribed period of swimming (in minutes)

Swimming (tail beat) proficiency was determined for all tested fish by documenting the number of tail beats per minute over the range of swimming speeds using a video camera with a superimposed stop watch function. Respiratory rate was determined by documenting the number of opercular beats per minute.

Tail-beat frequency (TBF) and opercular beat rate (OBR) per minute were monitored using a video camera. Data were recorded with fish maintaining position in the central portion of the swimming tunnel and not moving relative to the video recording equipment. The TBF and OBR were documented two to three times throughout each 15-minute velocity increment. Stride length (distance traveled per tail beat) was calculated by the formula:

$$S_L = Sp/TBF$$

where: SL = stride length

Sp = swimming speed of individual fish in body lengths per
second (l/s)

TBF = tail beat frequency, complete cycles per minute

Stride efficiency (number of tail beats per minute required to maintain a unit swimming speed of one body length per second) was calculated for each water velocity increment from the tail beat frequency data by the formula:

$$S_E = \text{TBF}/S_p$$

where: S_E = stride efficiency

TBF = tail beat frequency, complete cycles per minute

S_p = swimming speed of individual fish in body lengths per second (l/s)

All tested fish (tagged and control) were held for 14 days post-test to establish stress survival profiles. These fish were fed daily, and the populations were inspected regularly to document mortality. At the end of the 14-day holding period, all fish were examined to determine tag retention.

The swimming stamina data, stride efficiency data, and respiratory rate data were compared between tagged and control fish, and between post-tag testing dates, using the non-parametric Mann-Whitney test. Swimming proficiency profiles for tagged and control fish were calculated using standard regression techniques. All data analyses followed the methods of Sokal and Rohlf (1981).

Results and Discussion

Swimming Stamina.--Changes in swimming stamina levels have proven to be a reliable indicator of significant stressors in fish (Beamish 1978; Flagg 1981). Depressions in swimming stamina levels have been noted in teleost fish upon exposure to many stressors, including both external and internal telemetry tags (McCleave and Stred 1975; Lewis and Muntz 1984). The present study indicates that neither the act of tagging nor the presence of the PIT tag is a significant stress to steelhead, as measured by swimming stamina tests.

The Mann-Whitney statistical tests indicate that the PIT tag does not compromise the swimming stamina (U-critical) of steelhead. Fish were tested during Days 0-25 post-tag and there were no statistical differences ($P < 0.01$) between tagged and control fish at any test day (post-tag) for either fingerling (Table 5) or juvenile (Table 6) steelhead. The swimming stamina of PIT tagged and control fish varied slightly between test days (Figs. 4 and 5), however, no trend is evident, and the data suggest that a swimming stamina level (U-critical) of 4.6-5.2 body lengths per second is representative of the fish used in this study (Tables 5 and 6). This swimming stamina level is within limits documented by other authors (Beamish 1978) and indicates that the PIT tagged steelhead in these studies had good locomotive ability.

Stride Efficiency.--Measures of tail beat frequency have been used by researchers to document changes in physiological condition of fish (Beamish 1978; Stevens 1979; Flagg and Smith 1982). Recently, Lewis and Muntz (1984) showed that external ultrasonic tagging adversely affects the tail beat frequency of rainbow trout, Salmo gairdneri. However in our tests, the PIT tag did not affect the tail beat efficiency of steelhead. These data suggest that tagging and the presence of the PIT tag are not significant physiological impairments to steelhead.

Stride efficiency (number of tail beats per minute required to maintain a unit swimming speed of one body length per second) was documented as a comparative measure of propulsive efficiency. The Mann-Whitney statistical tests showed there were no statistical differences ($P < 0.01$) between test (PIT tagged) and control (non-tagged) fish at any post-test day (0-25) for either size range of steelhead tested (Tables 5 and 6).

Table 5.--Stride efficiency, opercular beat rate, and swimming stamina of PIT tagged and control fingerling steelhead (6.5 g average).

Test day post tag	Group ^{a/}	Stride efficiency ^{b/}		Opercular beat rate ^{b/}		Swimming stamina ^{b/}	
		\bar{x}	SE	\bar{x}	SE	\bar{x}	SE
0	T	86.7	6.1	133.3	5.4	4.4	0.19
	C	74.3	10.0	131.0	8.3	4.8	0.43
1	T	94.1	4.3	145.1	2.9	4.2	0.07
	C	93.8	6.8	145.1	5.2	4.1	0.09
2	T	88.0	3.3	150.5	3.8	4.6	0.19
	C	83.4	4.4	146.3	5.6	5.2	0.25
3	T	95.3	4.0	154.0	2.7	5.7	0.18
	C	89.1	7.6	155.4	6.2	5.3	0.35
4	T	101.6	4.2	144.3	3.5	4.9	0.33
	C	102.0	8.6	147.3	6.7	5.9	0.55
7	T	89.9	3.5	150.8	3.0	5.4	0.16
	C	84.2	6.4	144.6	4.9	5.4	0.31
9	T	95.8	3.1	139.9	3.5	5.2	0.20
	C	99.4	6.1	126.6	4.9	4.9	0.00
11	T	95.5	3.4	143.5	2.6	5.3	0.15
	C	105.3	8.6	144.5	5.5	5.2	0.40
14	T	100.3	3.7	141.9	3.4	5.3	0.20
	C	97.7	5.3	141.8	7.1	5.4	0.35
17	T	102.9	4.2	143.0	3.3	5.1	0.16
	C	108.6	8.0	143.4	3.9	4.9	0.90
21	T	93.6	3.0	136.0	3.5	5.8	0.09
	C	95.3	4.7	136.9	5.6	5.6	0.00
25	T	103.5	4.2	143.0	3.4	5.3	0.30
	C	102.3	5.4	150.0	6.4	<u>c/</u>	
\bar{x} tagged		95.6		143.8		5.1	
\bar{x} control		94.6		142.7		5.2	

a/ T = PIT tagged, n = 12 tagged fish tested each day

C = control, n = 4 control fish tested each day

b/ \bar{x} = mean

SE = standard error

* = significantly different, P<0.01; (note: there were no statistical differences noted in these data)

c/ No data due to equipment malfunction.

Table 6.--Stride efficiency, opercular beat rate, and swimming stamina of PIT tagged and control juvenile steelhead (17.2 g average).

Test day post tag	Group ^{a/}	Stride efficiency ^{b/}		Opercular beat rate ^{b/}		Swimming stamina ^{b/}	
		\bar{x}	SE	\bar{x}	SE	\bar{x}	SE
0	T	105.8	2.8	153.8*	1.6	4.0	0.09
	C	92.4	6.9	139.3*	4.3	3.9	0.20
1	T	105.5	2.9	150.4	2.6	4.1	0.14
	C	98.8	4.5	153.0	3.0	4.5	0.22
2	T	100.2	3.1	149.5	3.7	4.2	0.26
	C	103.9	7.8	151.4	4.3	4.9	0.09
3	T	97.5	3.0	145.2	2.7	4.7	0.19
	C	100.6	4.5	148.6	2.4	4.8	0.03
4	T	105.5	3.7	145.5	1.9	4.5	0.15
	C	92.6	4.7	147.0	5.4	4.3	0.62
7	T	90.7	3.1	144.7	3.2	4.9	0.14
	C	93.4	5.2	147.0	6.6	4.4	0.54
9	T	100.7	3.2	145.1	2.3	4.8	0.01
	C	96.3	4.1	154.1	6.1	4.9	0.13
11	T	102.4	3.1	152.1	3.0	4.7	0.21
	C	93.9	4.2	146.3	6.2	5.2	0.15
14	T	94.4	2.4	145.2	3.1	4.9	0.03
	C	89.6	4.2	152.3	5.1	5.1	0.28
17	T	104.1	3.9	142.2	3.3	4.7	0.18
	C	89.2	4.6	141.0	6.8	4.9	0.10
21	T	98.2	2.8	149.5	2.6	4.8	0.08
	C	97.1	4.9	152.7	4.0	4.9	0.07
25	T	97.2	2.5	151.6	2.7	4.6	0.13
	C	98.7	5.5	148.5	6.1	4.6	0.19
\bar{x} tagged		100.2		147.9		4.6	
\bar{x} control		95.5		148.4		4.7	

a/ T = PIT tagged, n = 12 tagged fish tested each day

C = control, n = 4 control fish tested each day

b/ \bar{x} = mean

SE = standard error

* = significantly different, P<0.01

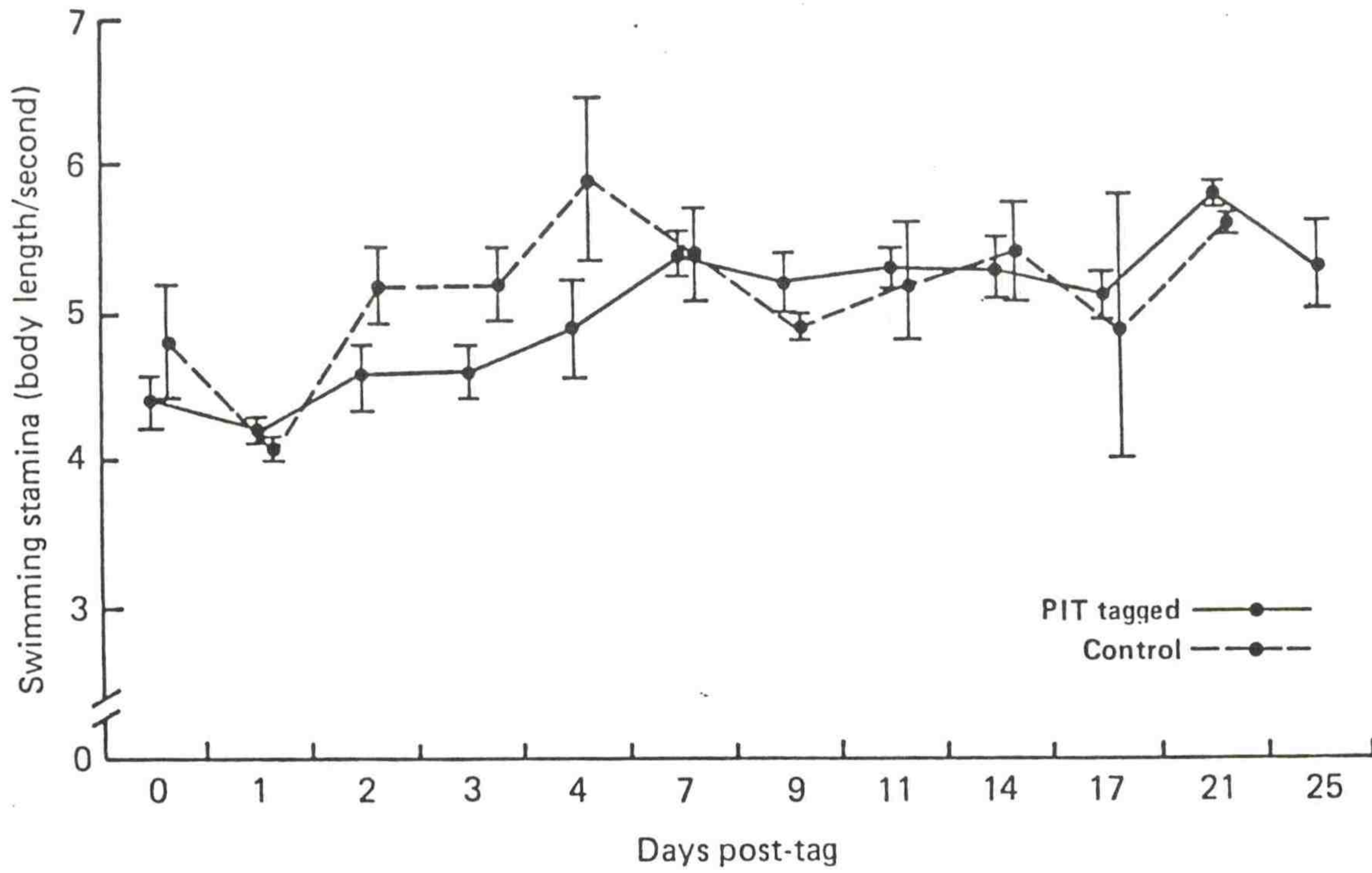


Figure 4.--Mean swimming stamina (U-critical) of PIT tagged and control fingerling steelhead (6.5 g average) trout during Days 0-25 post-tag. Brackets indicate \pm one standard error.

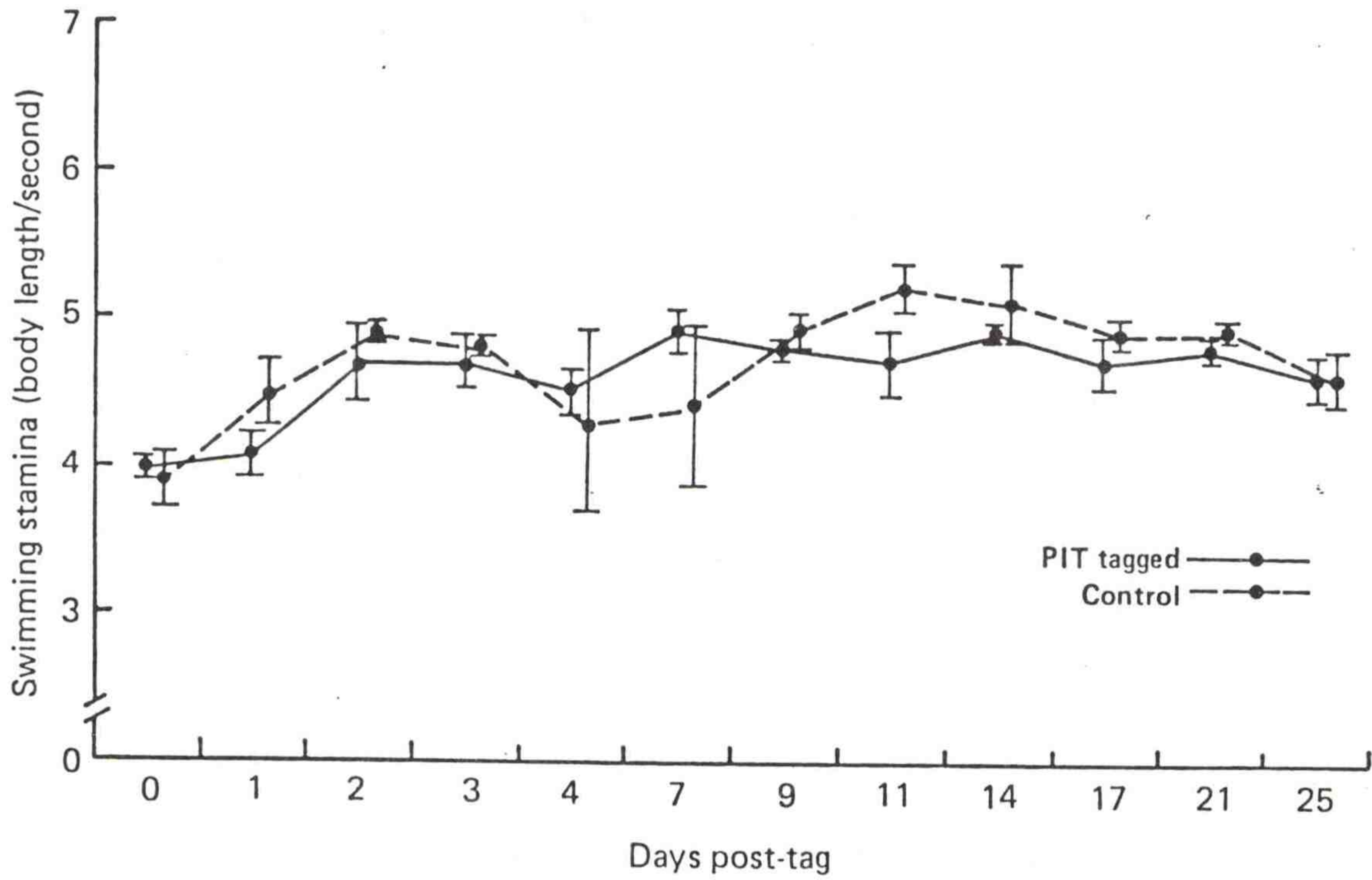


Figure 5.--Mean swimming stamina (U-critical) of PIT tagged and control juvenile steelhead (17.2 g average) trout during Days 0-25 post-tag. Brackets indicate \pm one standard error.

Stride efficiency varied slightly between test days, and on Day 0 was reduced, although not significantly ($P < 0.01$), from control levels for both fingerling and juvenile fish. In addition, the control fish were slightly, but not significantly ($P < 0.01$), more stride efficient throughout the tests (Tables 5 and 6; Figs. 6 and 7). However, this advantage varied between test days, and no clear trend was evident--suggesting that a stride efficiency of 94.6-100.2 tb/l/s is representative of fish used in this study. The results of this test suggest that interperitoneally tagging with the PIT tag does not affect the stride efficiency of steelhead.

Opercular Beat Rate.--Changes in respiratory metabolism have also been used by researchers to document changes in the physiological condition of fish. Lewis and Muntz (1984) showed that external ultrasonic tags raise the respiratory (opercular beat) rate, and the authors suggested that these type tags cause physiological compromises in rainbow trout. In the present study, OBR was documented as a comparative measure of respiratory efficiency. The data suggest that the PIT type tags do not physiologically compromise steelhead.

In the tests on fingerling steelhead, OBR exhibited an unexplained progressive increase during the first 4 days (for both test and control fish), and subsequently, peaked and stabilized (Table 5 and Fig. 8). However, the Mann-Whitney statistical tests indicated there were no statistical differences ($P < 0.01$) between the PIT-tagged and control fish (fingerling steelhead) at any test day in this series of tests (Table 5). Therefore, it seems probable that some external environmental influence caused the variations in OBR level noted in tests on fingerling steelhead.

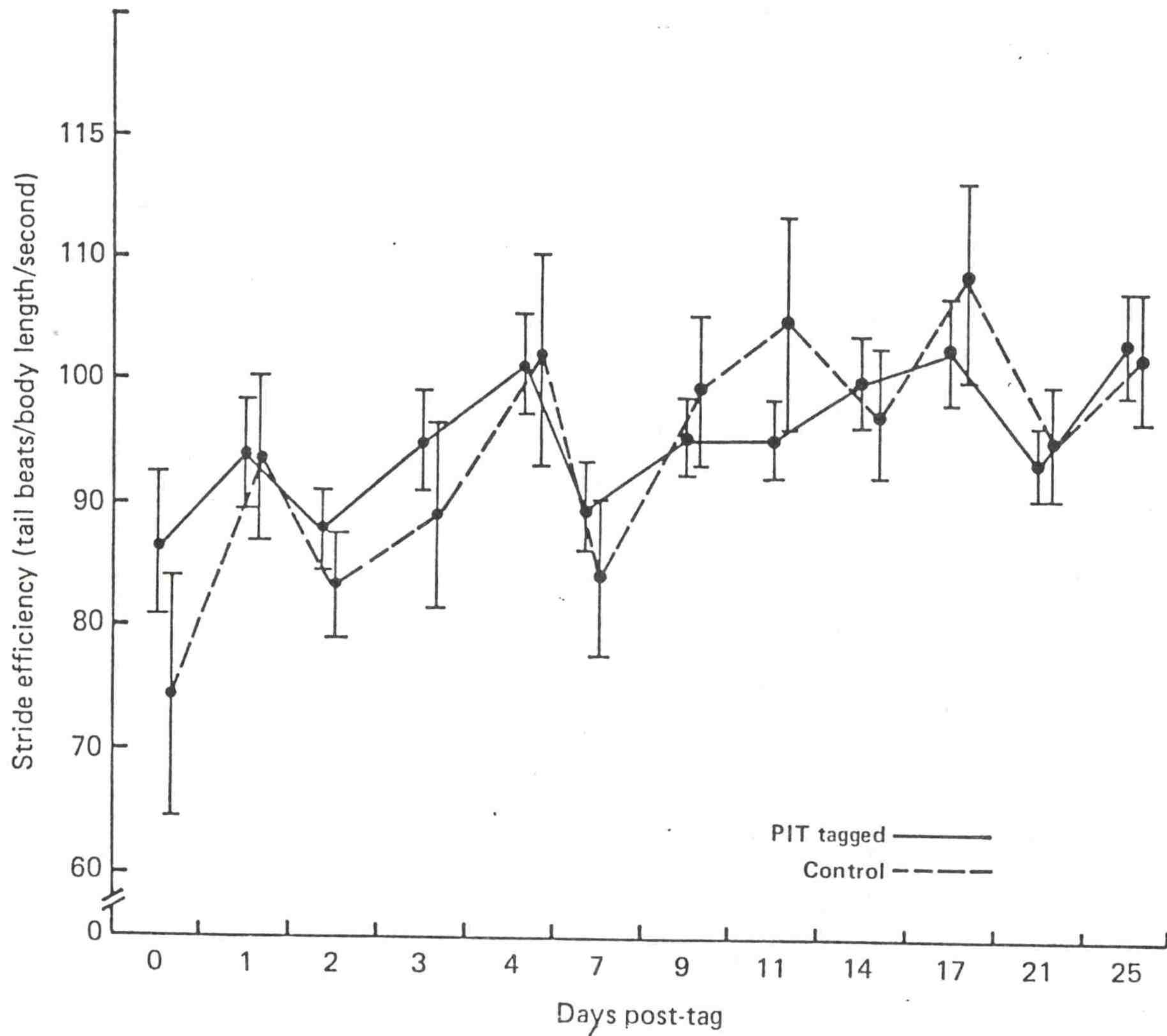


Figure 6.--Mean stride efficiency of PIT tagged and control fingerling steelhead (6.5 g average) trout during Days 0-25 post-tag. Brackets indicate \pm one standard error.

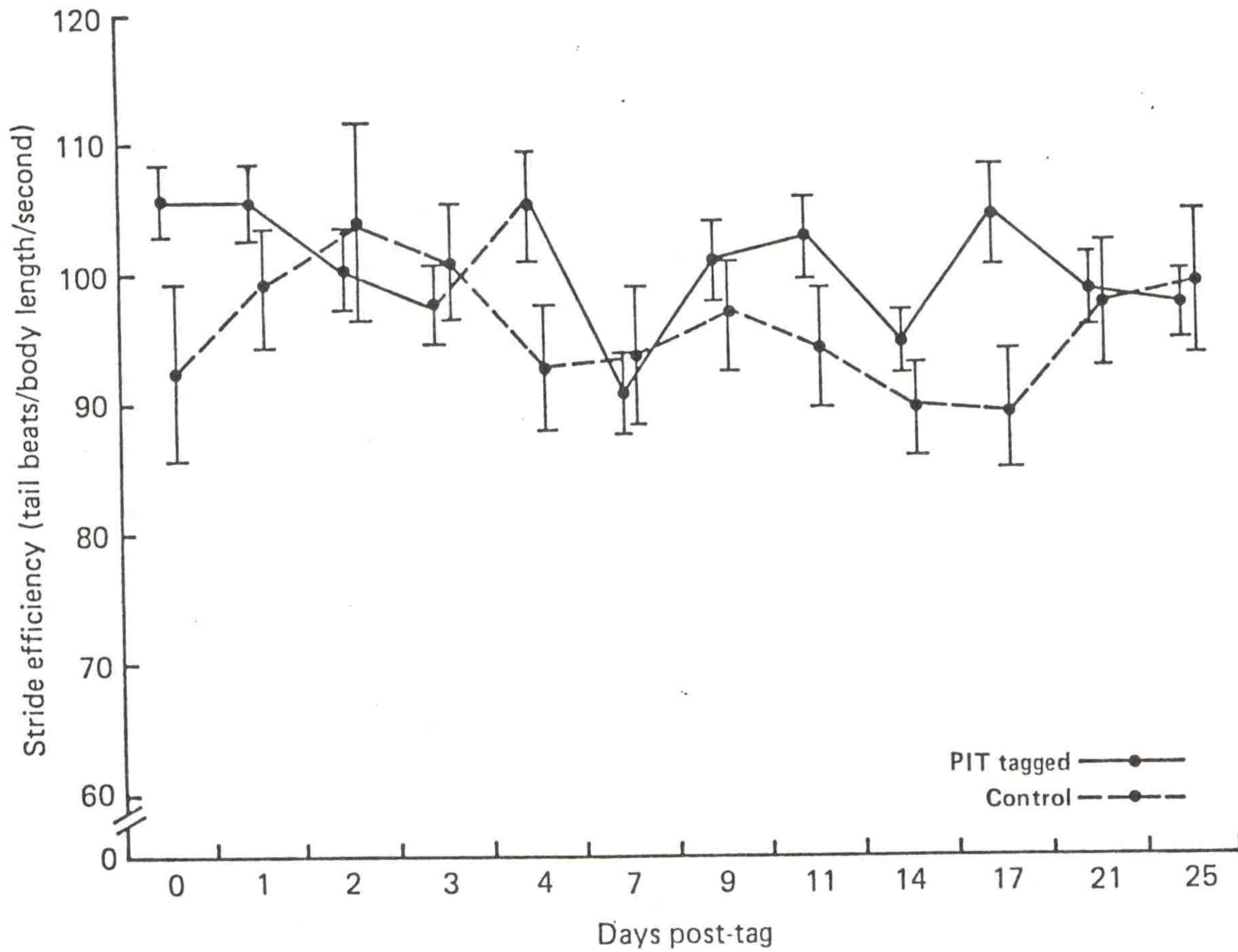


Figure 7.--Mean stride efficiency of PIT tagged and control juvenile steelhead (17.2 g average) trout during Days 0-25 post-tag. Brackets indicate \pm one standard error.

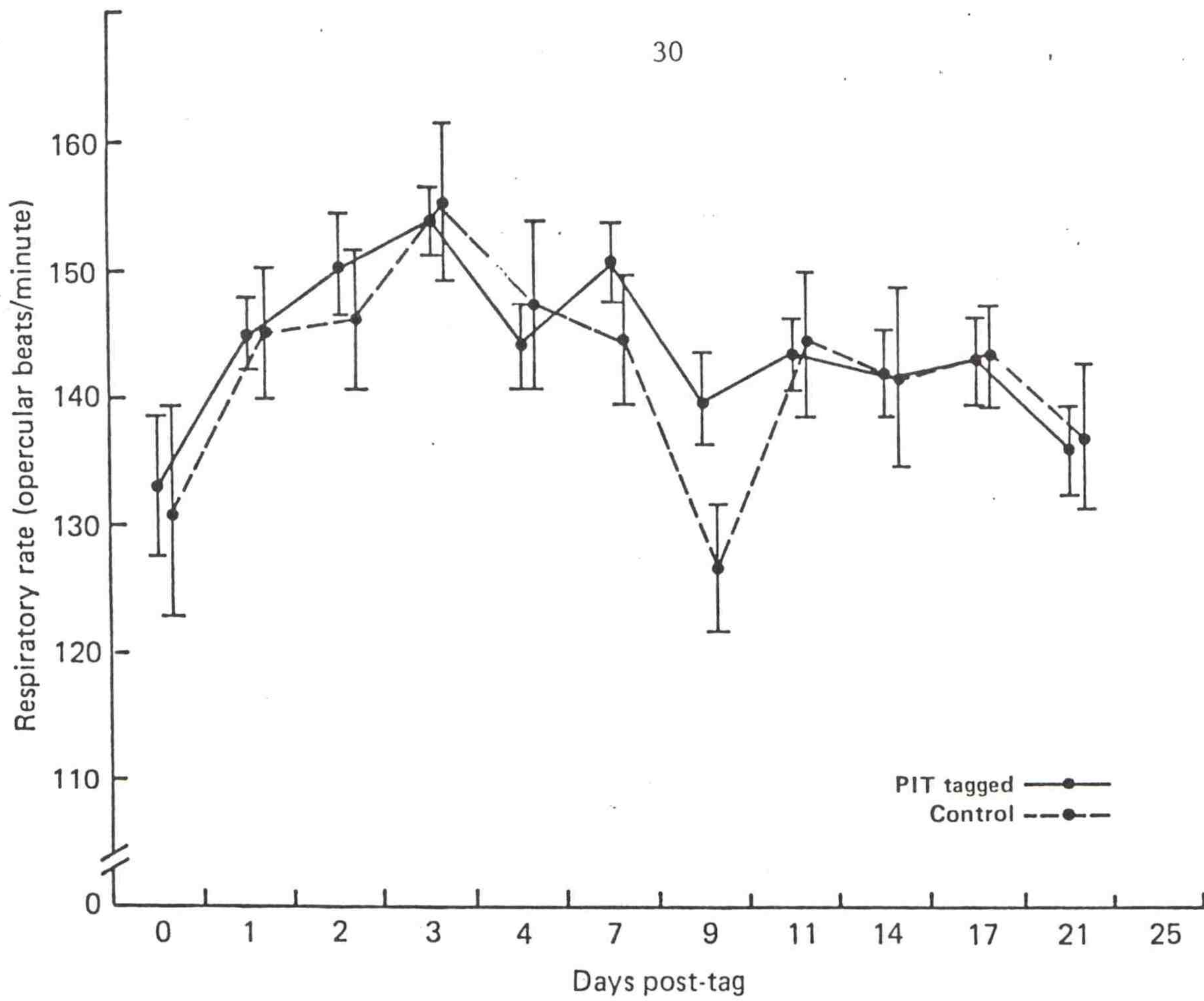


Figure 8.—Mean opercular beat rate of PIT tagged and control fingerling steelhead (6.5 g average) trout during Days 0-25 post-tag. Brackets indicate + one standard error.

In the tests on juvenile steelhead, control OBR was significantly ($P=0.01$) reduced from that of PIT-tagged fish at Day 0 post-tag. However, by Day 1 post-tagging, control OBR had increased to that of the PIT-tagged fish, and there were no further significant differences ($P<0.01$) between test and control fish for the remainder of the test series (Table 6 and Fig 9). Since control OBR increased to equal the PIT-tagged fish by Day 1 post-tagging, the significance of the lower OBR for control fish at Day 0 is unclear.

However, since only one of the test days (out of 24 observations) showed a statistical difference from controls, it is apparent that neither tagging nor the presence of the PIT tag normally compromise the respiratory efficiency of steelhead. The data suggest that an OBR of 140-150 is most commonly representative of (swimming) steelhead (Tables 5 and 6).

Post-Test Survival and Tag Retention.---The effects of tagging on fish can vary due to tag type, size, and placement. Recent tagging/survival studies using juvenile salmonids indicated that the PIT tag has excellent (up to 99%) retention and does not adversely affect survival (Prentice et al. 1985). However, the potential interactions of tagging and stress have not been fully documented. Severe exercise, such as swimming to fatigue, is a stress that has the potential to induce trauma (possibly causing tag rejection) or even death (Black 1958; Beamish 1978; Flagg et al. 1983).

In the present study, all fish were held 14 days after their stamina test, and survival and tag retention were documented to assess whether the act of tagging and/or the presence of the PIT tag were detrimental to fish encountering a severe secondary stress (e.g., swimming to fatigue).

Neither the act of tagging nor the presence of the PIT tag had any effect on the fishes post-stress (fatigue test) survival. Of the 414 steelhead

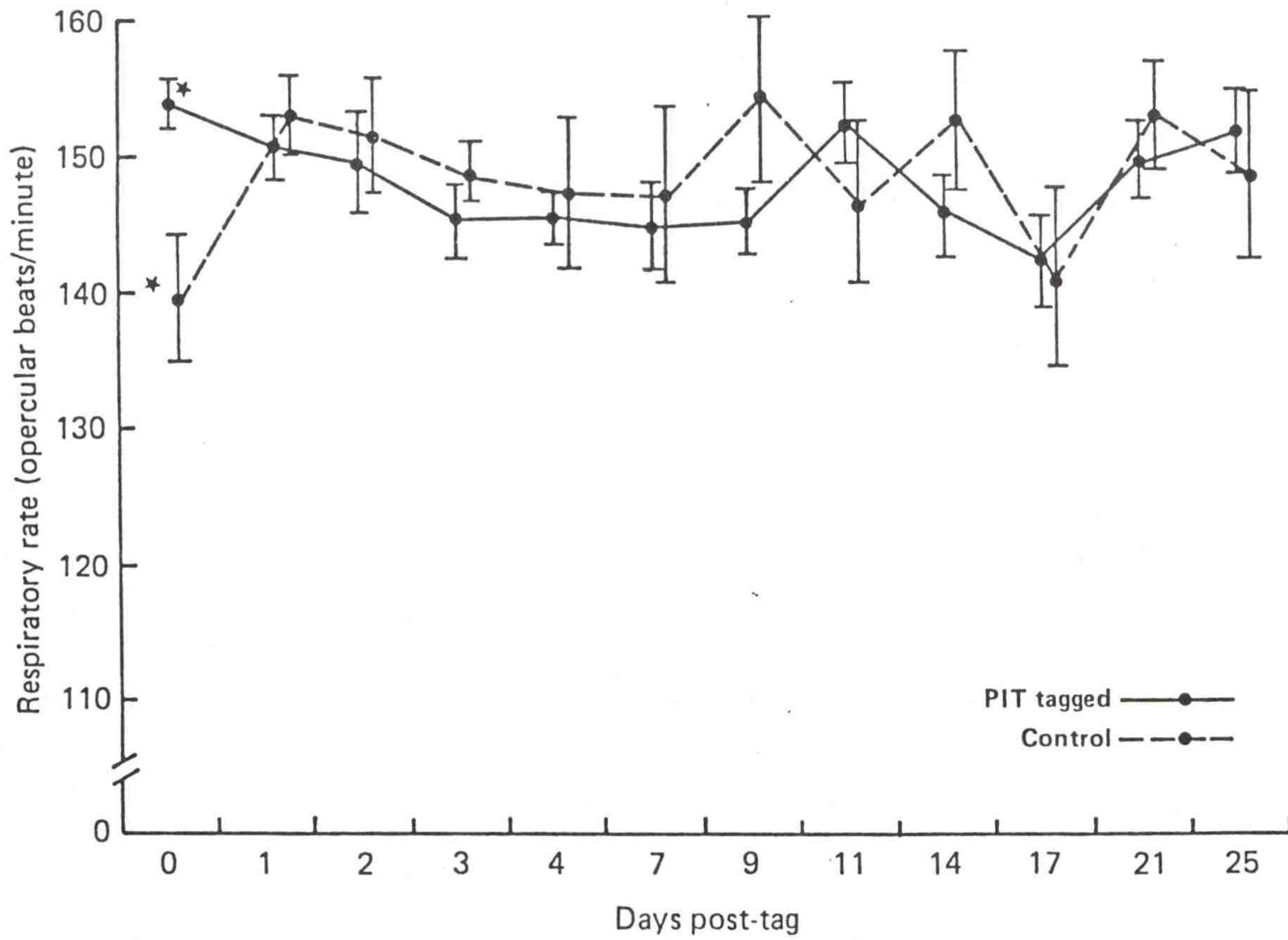


Figure 9.--Mean opercular beat rate of PIT tagged and control juvenile steelhead (17.2 g average) trout during Days 0-25 post-tag. Brackets indicate \pm one standard error. Asterisk (*) indicates significant (tagged vs. control) difference ($P < 0.01$).

surveyed during these series of tests, none of 312 PIT-tagged nor 102 control fish died (100% survival). In addition, PIT tag retention was 100%. At the termination of the 14-day holding, all PIT-tagged fish were sacrificed and necropsies performed to determine tissue reaction to the tags. No adverse tissue reaction and no tag migration were noted.

Thus, it appears that the PIT tag does not impact the fish's ability to survive severe secondary stress (e.g., swimming to fatigue). It also appears that this type of severe stress (even during the first few days post-tag) does not compromise tag retention.

This study indicates that the PIT tag does not compromise the swimming efficiency, swimming stamina, or respiratory rate of either fingerling or juvenile steelhead. In addition, this study supports previous work showing excellent PIT tag retention and survivability. However, the full physiological/behavioral effect of the PIT tag on smolting or migrating fish is still not known. During the 1986 season, these type tests will continue in the hatchery using smolting steelhead and three size ranges of chinook salmon. In addition, locomotion tests will be conducted on migrating spring chinook salmon, fall chinook salmon, and steelhead at McNary Dam.

Study 4: Serial Tagging to Determine Minimum Fish Size for Tagging

Introduction

PIT tag retention in juvenile fish has been variable (Prentice et al. 1984 and 1985). In 1985, we conducted a study to compare the functional PIT tag to sham tags (see Part I, Study 1 of this report), and tag loss varied between 7 and 42%. Similarly, a study to determine tag longevity (Part 1, Study 2) showed a high tag rejection rate (8.3% within the first 35 days).

The objective of this study was to evaluate the relationship between fish size at tagging and tag retention. The criteria for successful tagging was 96% or greater tag retention over a 45-day period. The study was not outlined in our 1984-85 BPA Work Plan, but was conducted after the results of Studies 1 and 2 of this report were available.

Methods and Materials

Juvenile steelhead were used for the study. The population was maintained in a 2.4-m diameter tank with running fresh water; standard husbandry practices were followed. The study was conducted at the Big Beef Creek Research Station. Fish were randomly selected from the main population to establish eight test groups, each consisting of two replicates of 150 fish each (Table 7). Each replicate was maintained in a 1.2-m diameter tank with running fresh water. One test group was established about every 14 days between 1 August and 23 October 1985. Thirty-two days elapsed between the establishment of the seventh and eighth test groups.

All fish were injected with functional PIT tags in a manner similar to that described previously. Fifty fish in each replicate were weighed to the nearest 0.5 g, and all fish were measured to the nearest 3.0 mm (fork length) at the start and end of the study (45 elapsed days). Each test tank was examined for rejected tags at 1- to 3-day intervals. All fish were sacrificed at the end of the study and examined for tag presence.

Results and Discussion

All data for the study are summarized in Table 7. The data presented are for combined replicates since there were no apparent differences between replicates for weight, length, number of tags rejected, or survival.

Table 7.--Summary of serial tagging study to determine the minimum fish size for optimal tag retention and survival.

Group	Number ^{a/} of fish	Starting weight		Starting length		Ending weight		Ending length		Percent survival	Percent tag retention
		Mean (g)	SD (g)	Mean (mm)	SD (mm)	Mean (g)	SD (g)	Mean (mm)	SD (mm)		
1	301	3.3	0.7	63.9	4.3	8.5	2.5	84.1	10.1	96.3	86.4
2	300	4.3	1.2	69.4	4.7	7.6	2.0	83.6	7.9	98.3	95.0
3 ^{b/}	300	4.3	1.0	71.8	4.9	8.8	2.6	89.5	9.1	98.0	94.0
4	301	6.1	1.2	75.9	4.7	10.5	2.5	92.7	6.7	99.7	91.7
5	302	8.5	2.1	85.0	6.8	13.4	3.6	100.6	5.3	98.3	98.0
6	301	10.8	2.8	92.9	7.6	23.5	5.3	123.0	8.5	100.0	100.0
7	300	15.8	4.4	103.7	13.6	22.6	4.4	121.5	7.9	99.0	98.0
8	301	28.0	6.9	128.6	10.6	32.8	8.0	138.0	11.9	99.3	99.7

a/ Summary data are for combined replicates since no significant difference was seen between replicates for growth, survival, or tag retention.

b/ Group 3 fish were from a separate population than all other test groups.

Our criteria for successful tag retention was 96% over a 45-day period. This criteria was achieved only with fish within the fifth through the eighth test groups. The mean weight and length of fish within the fifth group was $8.5 \text{ g} \pm 2.1 \text{ (SD)}$ and $85 \text{ mm} \pm 6.8 \text{ (SD)}$. The poorest tag retention was observed in the first test group (13.6% tag rejection). Tag rejection occurred on the first day after tagging and continued throughout the 45 days of testing in Groups 1 through 4. The majority of the tags were rejected between Days 13 and 30. The few tags that were rejected from Groups 5 through 8 occurred after Day 26, with the exception being two tags rejected from Group 7 on Day 8. The exact number of tags rejected during a specific period was difficult to ascertain. Once tags were rejected, fish had a tendency to ingest them and were capable of passing them through the intestinal tract at a later date. All fish within each test group were sacrificed at the end of 45 days, and the presence or absence of the tag within the body cavity was confirmed. Upon examination of the fish, we found up to four tags in the stomach of one fish and several other fish that had ingested one or two tags. How many fish had ingested tags and passed them prior to the termination of the study is unknown.

Survival was high between test groups, ranging from 96.3 to 100% (Table 7). The lowest survival was in the first test group, which had the smallest fish. Damage to the intestinal tract from the tagging needle accounted for a number of the initial (first 4 days) mortalities among the fish. This was especially true with the smaller fish.

No clear relationship was seen between survival and tag loss if the percent survival for each group of fish is compared to the percent tag rejection (Table 7). Thus, there appears to be no severe adverse effect to

the fish during the tag rejection process. The exact mechanism or reason for tag rejection is unknown at this time. We have observed fish with either a scar or a partially protruding tag through the abdominal wall; no infection or other adverse tissue reaction to the tag could be observed in such fish. We have further observed fish with protruding tags. If these fish are left in the population, they will continue to grow and survive.

Presently the minimum size fish that meets our criteria for successful tag retention weighs $8.5 \text{ g} \pm 2.1$ (SD) and measures $85 \text{ mm} \pm 2.1$ (SD). High survival (greater than 96%), however, can be achieved with fish much smaller than the above size restriction. The process of rejecting the tag does not appear to compromise the health or survival of the fish. The mechanism or reason for tag rejection between various size groups of fish is unknown. Modification to the tag's encapsulation material from polypropylene to glass and altering tagging procedures slightly may improve tag retention.

Study 5: Tag Placement in Adult Salmon

Introduction

Numerous morphological and physiological changes take place as a salmon matures. These changes may alter the response of a fish to foreign material such as a PIT tag within its body cavity. For instance, since wound healing ability may be impaired in maturing fish, tag implantation may subject the fish to infection and thus increase the chance for tag loss through an open wound or cause premature death. Furthermore, the questions of whether a tag placed in the body cavity would cause internal damage to eggs and whether a tag would be retained during spawning need to be answered. The objective of this study, therefore, was to obtain information on wound healing, tag retention, and tag effect during spawning in maturing adult salmon.

Methods and Materials

The study was conducted at the Manchester Marine Experimental Station and the Northwest and Alaska Fisheries Center (NWAFC) in Seattle, Washington. A total of 84 maturing female and male Atlantic salmon, Salmo salar, were used in the study. The fish were reared to near maturity in seawater net-pens at Manchester.

All fish were PIT tagged intraperitoneally on 15 October 1985. Initially the tag was injected through the abdominal musculature about 3 to 5 cm anterior to the pelvic girdle along the mid-ventral line. This procedure was subsequently modified by moving the injection point about 1 to 2 cm to either side of the mid-ventral line. Tag insertion was made with a 12-gauge needle and a modified hypodermic syringe.

The fish were divided into two groups. One group consisted of 10 males and 33 females retained in seawater until spawning. The second group consisted of 11 males and 30 females transported to fresh water at the NWAFC. All fish were weighed to the nearest 100 g and measured (fork length) to the nearest 1 cm. Fish weight ranged from 2,500 to 10,000 g, and lengths ranged from 61 to 80 cm. All fish were examined for wound healing, readiness to spawn, and general condition on 18, 21, 22, 23, 25, and 29 October and 4 November. The study was terminated on 5 November. When fish were determined to be ripe, eggs were collected by squeezing the peritoneal cavity by hand (stripping). All fish were lightly anesthetized (MS-222) for spawning and scanned for tag code using a hand-held scanning unit. Individuals that spawned were subjected to 3 to 4 strippings.

Results and Discussion

During the study, no adverse reaction by the tissue to the tag was noted. All tagging wounds were closed and healing on the first day of

observation (3 days post tagging). No infection or discoloration was noted in the area of the tagging wound.

Three fish in the seawater group were removed from the study immediately after tagging when severe external bleeding was noted in the area of the tag wound. The bleeding problem was eliminated in subsequent tagging by moving the tag injection site about 1 to 2 cm to either side of the mid-ventral line thereby avoiding the ventral artery. We recommend this change in tagging procedure for all size ranges of fish. The distance from the mid-ventral line should vary, however, with the size of fish being tagged.

All 21 males matured, and milt was collected from each fish. A total of 48 females were spawned from the population of 60 fish in the study (Table 8). At the termination of the study (5 November), 12 fish had not yet ripened.

Overall, there was 100% tag retention among male fish and 83% among females. Four tags were passed during the first stripping and four during the second to fourth strippings (Table 8). There was no clear relationship between tag retention among freshwater or seawater test groups. No adverse effects could be noted to the eggs from the tag's presence. When a tag was passed, it was easily observed among the eggs.

All fish were easily identified with one or two scans of a portable tag detector using lightly anesthetized fish. During the observation periods, the fish were placed in a 1.2-m diameter tank and guided to the tank's side where tag detection was accomplished from the exterior of the tank without removing the fish from the water.

Table 8.--Spawning dates and PIT tag rejection for Atlantic salmon females.

Date spawned	No. females spawned per date	Cumulative no. spawned	No. tags not retained
21 Oct	21	21	1 ^{a/}
22 Oct	4	25	0
23 Oct	7	32	0
25 Oct	7	39	2 ^{b/}
29 Oct	3	42	3 ^{c/}
4 Nov	6	48	2 ^{d/}

a/ One tag not retained during first stripping.

b/ One tag not retained during third and fourth stripping.

c/ One tag not retained during first, second, and fourth stripping.

d/ Two tags not retained during first stripping.

Study 6: Sterilization Technique for Tagging Equipment

Presently, the PIT tag is injected into the fish's body cavity using a 12-gauge hypodermic needle attached to a modified syringe. The same unit (needle and syringe) is used for consecutive fish. This procedure has not resulted in any documented disease transfer from fish to fish; however, the fish used in the tests were healthy. To reduce the potential of transferring diseases from fish to fish via the tagging apparatus, a practical means of disinfection is needed. Battelle Northwest, Sequim Marine Laboratory, was contracted to evaluate the problem and to provide recommendations. Their report is presented in Appendix A.

Conclusions and Recommendations

1. The presence of the PIT tag within the body cavity of juvenile fall chinook salmon and steelhead will not significantly ($P < 0.05$) affect growth or survival.
2. Tag retention can be expected to be high (tag retention of 96% or greater over 45 days) for juvenile steelhead weighing more than 8.5 g and measuring greater than 85 mm.
3. The exact mechanism for tag rejection in juvenile fall chinook salmon and steelhead is unknown but may be primarily mechanical and, in part, related to fish size.
4. There is no correlation between survival and tag rejection for juvenile fall chinook salmon and steelhead.
5. Tag location in juvenile fall chinook salmon is consistent (greater than 90%) within the body cavity over time, suggesting that once the tag is established within the cavity it remains stationary.

6. Even though no infection in the area of the tagging wound was noted, and survival of tagged fish was not significantly different from control fish, we recommend that both the tags and tagging apparatus be disinfected (when practical) to reduce the chance for disease transmission from fish to fish.

7. Tag longevity was poor with up to 8.3% of the tags failing to function after 35 days due primarily to liquids entering the tag through faulty end seals on the polypropylene capsule. We do not recommend the use of the polypropylene encapsulated PIT tags at this time. We believe however, that this problem will be overcome by the introduction of glass encapsulated tags in 1986.^{1/}

8. The PIT tag does not have a significant effect on the opercular rate, tail beat frequency, stamina, and post fatigue survival of fingerling or juvenile steelhead.

9. Active swimming does not affect tag retention in fingerling or juvenile steelhead (100% retention over 14 days in all tests). The PIT tag will not significantly affect locomotive ability of juvenile steelhead in the size range tested.

10. The PIT tag can be injected safely into maturing adult salmon without jeopardizing their health, survival, and egg or sperm viability.

^{1/} Preliminary 1986 data show that by encapsulating the tags in glass, tag longevity and retention are greatly improved.

11. The PIT tag is retained within the body cavity of adult female salmon at a high rate even after multiple hand-strippings.

12. We recommend that until additional laboratory and field tests are conducted and the data analyzed, that a cautious approach be taken in the use of the PIT tag, even though all the information to date is encouraging. Premature use of the tag may give biased results stemming from a lack of understanding of the technical limitations of the tag and monitoring system and an incomplete understanding of the biological ramifications of injecting the tag into fish. We believe that if test results continue to be as encouraging as they are, the tag should be ready for use in the field by 1987.

PART II: FIELD STUDIES

Study 1: Evaluate Juvenile PIT Tag Monitor Reliability

Introduction

The objective of the study was to determine the reliability of juvenile PIT tag monitoring equipment installed at McNary Dam during the 1985 field season. The continuous operation of the equipment is essential not only to ensure the accuracy and reliability of the collected data but also to determine areas for design improvement.

Methods and Materials

The study was conducted at McNary Dam on the Columbia River near Umatilla, Oregon. Two juvenile PIT tag monitors were installed directly on the fish discharge ports of the juvenile wet separator (Fig. 10). Water velocity through the monitors was up to 0.3 m/sec. Monitor A was 147.3 cm long by 20.3 cm high by 30.5 cm wide and had three monitoring loops. Monitor B was 122.0 cm long by 20.3 cm high by 30.5 cm wide and had two monitoring loops. Both monitors were made of clear PVC and had a plastic

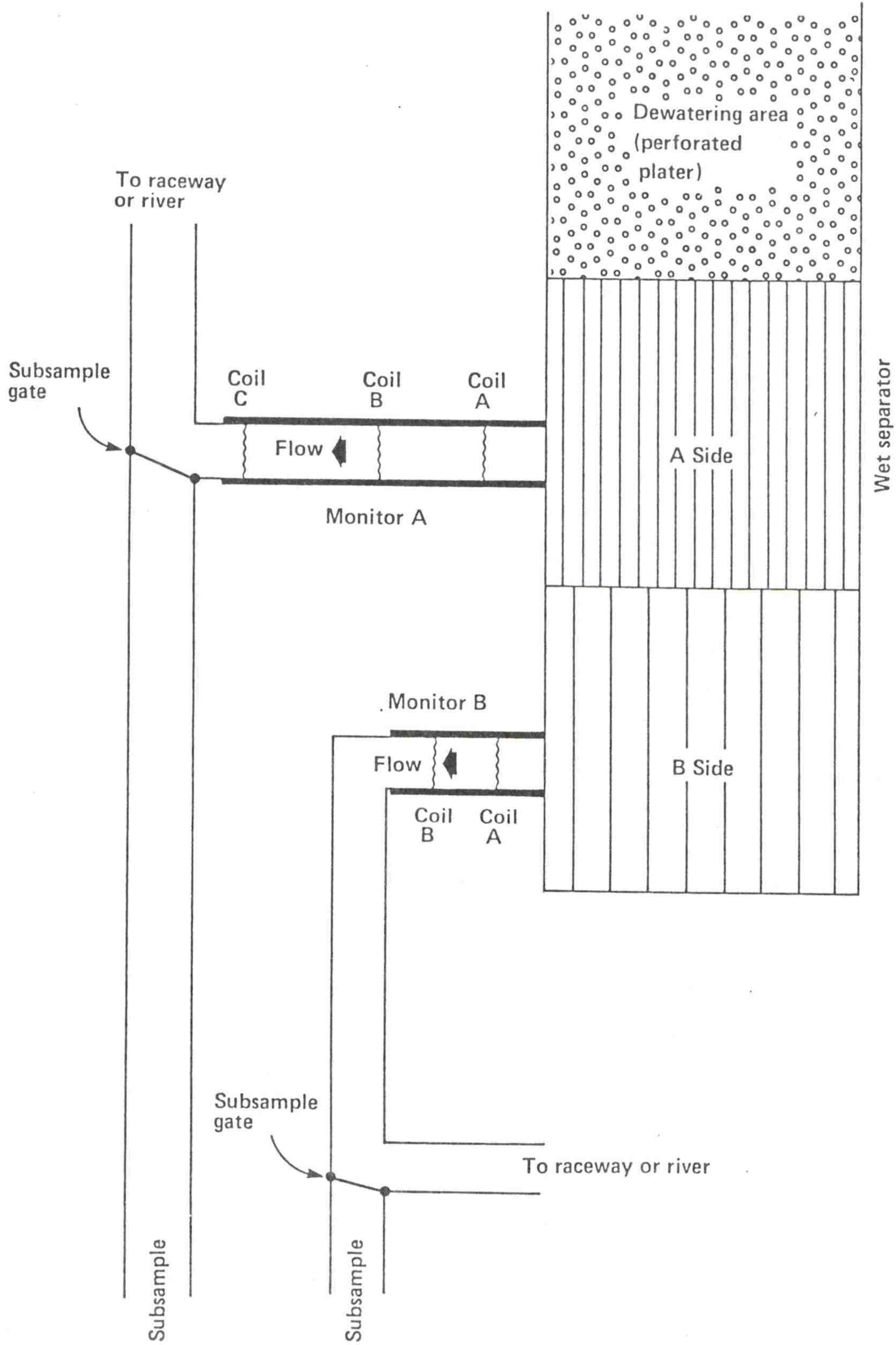


Figure 10.--Location of juvenile salmon PIT tag monitors at McNary Dam during 1985.

shield to protect the loops from weather. Monitor A was operated by a triple excitor and power supply mounted in a single housing and wired directly to the loops. Monitor B was operated by a dual excitor and power supply mounted and wired as Monitor A. The exciters of Monitors A and B were connected to individual controller units, printers, and computers (Fig. 11).

To evaluate the reliability of the electronic components of the PIT tag monitoring system, all equipment except the printer and computer were left continuously in an operational mode from 27 April to 20 July 1985. The equipment was again activated from 4 August to 28 September 1985. During the active period, a total of 16 tests (8 tests per monitor) were conducted to determine monitor tag reading reliability.

The tests were conducted on a monthly basis from April to September 1985 (Table 9). Each test consisted of releasing neutrally buoyant plastic fishing bobbers (5.8 cm long by 2.5 cm diameter) containing a functional PIT tag. The number of bobbers release per test ranged from 8 to 204 (Table 9). The bobbers were released into the entrance of each monitor and recovered upon their exit for reuse.

Results and Discussion

The prototype juvenile PIT tag monitoring equipment performed well during the 1985 field season with only two electronic equipment problems. The monitoring equipment was turned off on 20 July while a leak in a section of the flume was repaired. Monitor A malfunctioned during power-up on 4 August. Two controller cards within the controller malfunctioned, and two capacitors failed within the power supply. The failure of the capacitors probably caused the controller cards to malfunction. All repairs were made in the field within 1 hour.

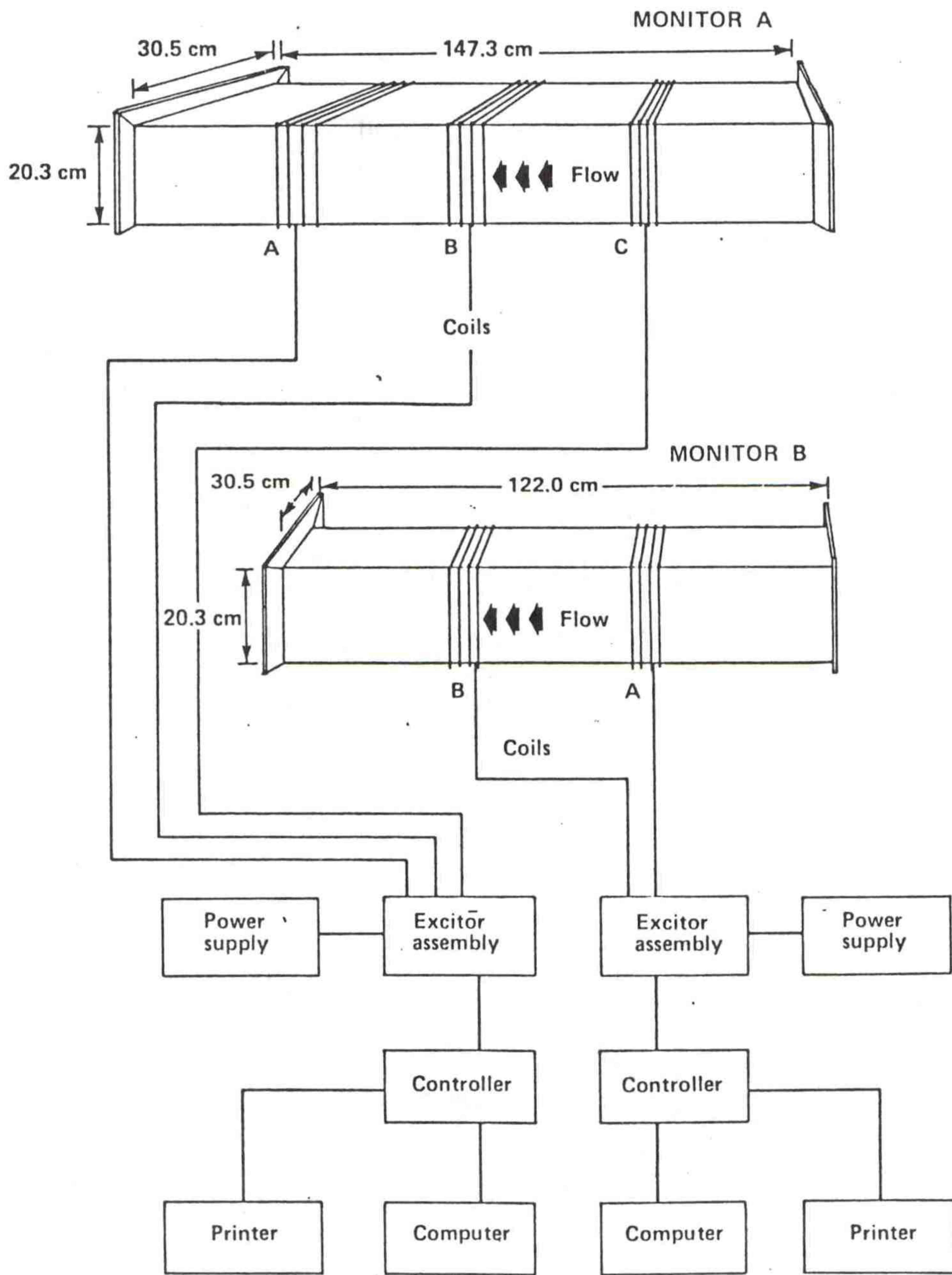


Figure 11.--Diagram of the juvenile salmon PIT tag monitoring system at McNary Dam during 1985.

Table 9.--Summary of reliability tests conducted at McNary Dam in 1985.

Test date	Monitor	Number of trials	Number of bobbers per trial	Total number of bobbers	Number of bobbers not read	Percent bobbers read	Number reading errors
4/29	A	51	4	204	4	98	2
	B	50	4	200	4	98	0
5/10	A	52	3	156	0	100	0
	B	49	4	196	4	98	0
5/22	A	51	3 to 4	184	7	96	0
	B	51	4	204	5	98	0
5/28	A	26	4	104	3	97	0
	B	23	4	92	3	97	0
6/5	A	26	4	104	0	100	0
	B	27	4	108	1	99	0
7/17	A	2	4	8	0	100	0
	B	2	4	8	0	100	0
8/2	A	10	4	40	1	98	0
	B	12	3 to 4	42	0	100	0
9/25	A	28	4	112	10	91	0
	B	32	4	128	0	100	0
Total	A	246	3 to 4	912	25	97	2
Total	B	246	3 to 4	978	17	98	0

Monitor A again malfunctioned sometime after 5 August. One of the three monitoring loops failed which caused the detuning of the remaining two loops. We estimated that a 6 to 7% decrease in tag reading ability resulted from this failure and detuning (Table 9).

Results of the monthly tag reading tests are shown in Table 9. A total of 912 tags were passed through Monitor A, and 978 tags through Monitor B during 8 tests per monitor. Out of 1,890 tags, two tags were misread. No explanation can be given for the two reading errors. No other misreadings were experienced in any other study conducted during 1985 using PIT tags. Overall reading efficiency for all tests was 97 and 98% for Monitors A and B, respectively. The slight difference in overall tag reading efficiency between Monitors A and B was due to the detuning of the detector loops on Monitor A as previously discussed.

The overall results of the reliability tests suggest that the PIT tag monitoring equipment can withstand the rigors of field operation over an extended time. The results of the tag reading tests with the bobbers showed a high degree of reliability in reading efficiency, and the results were similar to those obtained with fish. This suggests that the bobbers used in these tests are a dependable substitute for fish in determining monitor reliability.

Study 2: Evaluate Tag Reading Efficiency of the Juvenile PIT Tag Monitor

Introduction

Juvenile PIT tag monitors were evaluated for tag reading efficiency under simulated field conditions in 1984 (Prentice et al. 1985). Results showed a mean reading efficiency of 90.5%. However, a question remained whether this level of reading efficiency could be obtained under actual test conditions in the field. This study was designed to answer that question.

Methods and Materials

Two juvenile PIT tag monitors installed on the wet separator at McNary Dam on the Columbia River were evaluated. The monitors are described in Part II, Study 1 of this report (Fig. 10). Three tests were conducted, two using juvenile migrant spring chinook salmon and one with migrant fall chinook salmon.

Test 1.---Juvenile migrant spring chinook salmon used in the study were randomly collected from the juvenile salmon collection and inspection facility at McNary Dam on 8 May 1985. At the facility, a subsample of fish passing through the juvenile collection system was diverted into an inspection room where they were dipnetted; anesthetized; and inspected for fin clips, descaling, injuries, species composition, and brands. Only fish showing limited scale loss and no previous marks, tags, or injuries were used in the study. The fish were PIT tagged in the same manner as previously described. Twenty-five groups of fish, 20 fish per group, were tagged, measured to the nearest 3 mm (fork length), and recorded on a computer file and printer. The fish ranged in length from 95 to 215 mm and averaged 147 mm. Each group of fish was held in a 132-liter holding container receiving a continuous supply of aerated ambient river water.

The fish were held between 20 and 25 h prior to their release directly into the wet separator (Fig. 10). Prior to release, each group was examined for tag loss and mortality. All mortalities were replaced with fish from the 25th group of fish. The individual code and length of the replacement fish were substituted for the removed mortalities, thus all release groups had 20 fish. Two groups of fish were released into the wet separator at 30-min intervals, one in the A side, the other in the B side (Fig. 10).

All fish were allowed to pass through the wet separator on their own volition. All PIT tagged fish were interrogated, and PIT tag codes were recorded automatically using the systems previously described. The code of each PIT tagged fish, monitor, detection loop, date of passage, and time of passage (hour, minute, and second) were recorded into a computer and printer file.

Test 2.--At the termination of the study, comparing the PIT tag to traditional tagging and marking methods (Part II, Study 3), all surviving PIT tagged fish within each of four test groups were retained. On 3 June 1985, additional fish obtained from the inspection facility were tagged and added to each of the four groups as needed to adjust the total number of fish per group to 26. A fifth group of 20 fish was tagged as replacement fish for any subsequent mortalities. All fish handling, holding, releasing (two releases of two groups per release), and tag monitoring were conducted in a manner similar to Test 1.

Test 3.--Juvenile migrant fall chinook salmon ranging in length from 85 to 160 mm were used in the test. The fish were obtained from two sources. Groups 1 through 13 were obtained from the subsample as were the fish in Test 1. These fish had up to 24 h of rest prior to being handled. Low numbers of fish in the subsample made it necessary to obtain the needed fish for Groups 14 through 24 from a raceway system. Many fish from the raceway did not have an opportunity to recover from stress resulting from their passage through the dam's collection facility before being handled for tagging. After tagging, all test groups were held for 24 h. The rest of the methods and materials, number, and size of the test groups were all similar to Test 1; the main differences between Tests 1 and 3 were the species used, time of year, and prevailing environmental conditions.

Results and Discussion

Test 1.--A total of 480 PIT tagged spring chinook salmon were released into the wet separator; 9 fish were not detected for an overall tag reading efficiency of 98.1%. All of the tags that were detected were read correctly (100% reading accuracy).

The elapsed time for spring chinook salmon to exit the wet separator in Test 1 ranged from 16 seconds to 36 h 27 min (Fig. 12). Eighty-one percent of the fish were detected within the first 30 min after release, 9% in the next 30 min, and 5% within the following 60 min. Two fish resided in the wet separator for extended periods: 20 h 13 min and 36 h 27 min. No explanation can be given for the long residence time for these two fish; however, this phenomenon has been observed previously (Park et al. 1984).

Based upon our 1984 work, our criteria for acceptable tag reading efficiency was 90% with 99% reading accuracy (Prentice et al. 1985). The results of this test far exceeded that criteria.

Test 2.--The results obtained in Test 2 were similar to that of Test 1. Overall tag reading efficiency for Test 2 was 97.1% (3 fish were not detected out of 104 fish released). All tags that were detected were correctly decoded (100% tag reading accuracy).

Passage time of PIT tagged fish out of the wet separator was similar to that for Test 1 (Fig. 12). Within the first 30 min, 74% of the fish exited the system, an additional 11% passed through the system in the next 30 min, and 12% within the following 60 min. No fish remained in the separator longer than 3 h and 44 min.

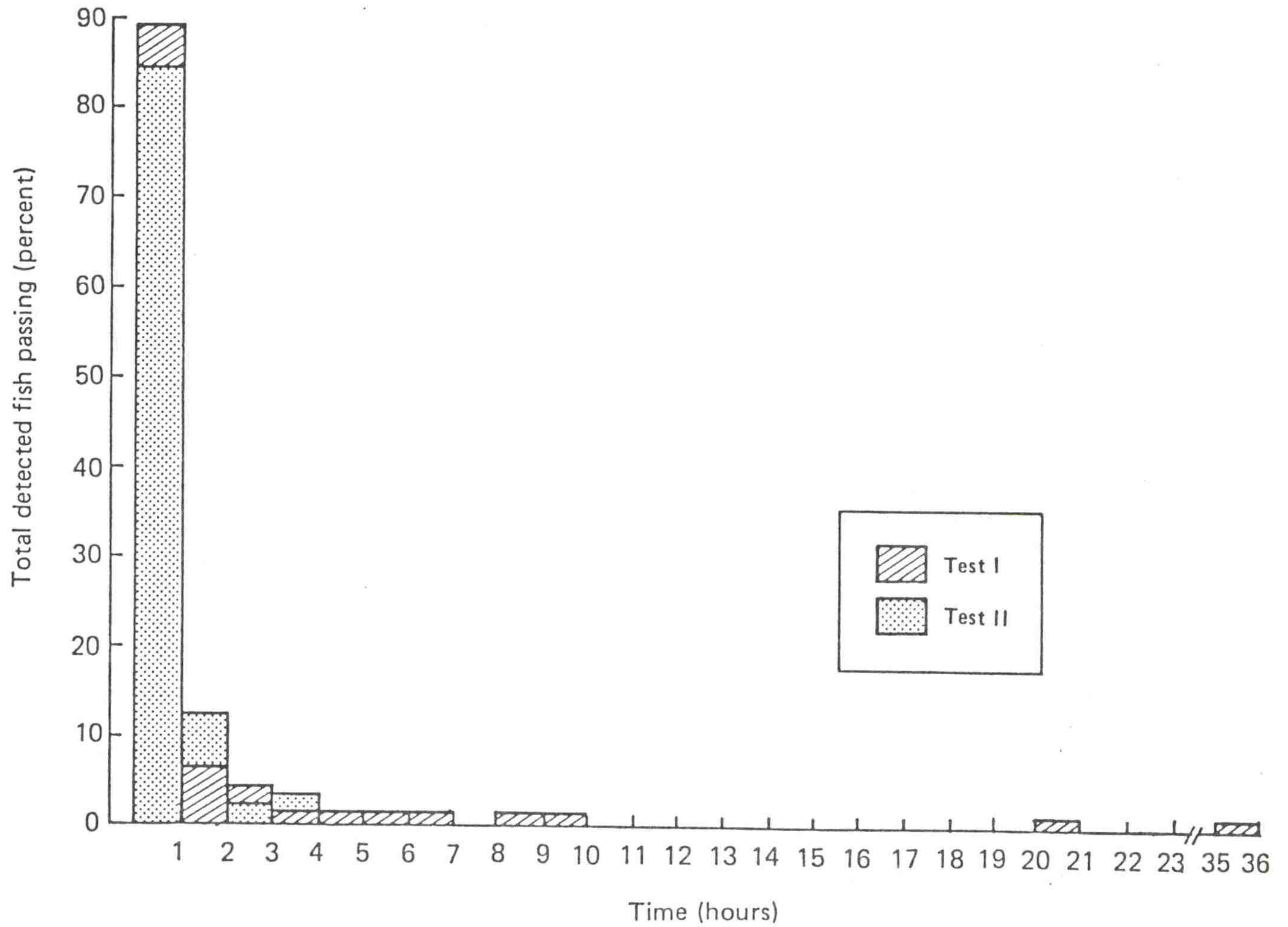


Figure 12.--Percentage of PIT tagged spring chinook salmon detected while exiting the McNary Dam wet separator.

Test 3.--Post tagging mortality was different between the two sources of fish. Fish from the subsample (Groups 1 through 13) showed a 0.38% mortality (1 fish died), whereas fish from the raceway showed a 4.1% mortality (11 fish died). Overall 24-h post tagging mortality was 2.2%. Raceway mortality (non-tagged fish) during the same period was 1.7%. The difference in mortality between the two sources of fish likely indicates the effect of stressing a fish twice within a short period without sufficient recovery time.

Overall tag reading efficiency was 92.5%, with all tags being read correctly (100% tag reading accuracy). We believe, however, that the tag reading efficiency was affected by fish dying within the wet separator. Tag reading efficiency was different between the two sources of fish: Group 1 through 13 (n=260), 95.4% and Groups 14 through 24 (n=220), 89.1%.

We believe that the difference in mortality between the two sources of fish continued after release into the wet separator. Since the residence time for the fall chinook salmon in the wet separator was long (Fig. 13), there was a high probability for mortality to occur. After death, a fish would have decayed rapidly and lost its tag in the 20° to 21°C water present during the test. Tags lost in this manner would not be available for detection but would drop through the wet separator's perforated floor.

The time for fall chinook salmon to exit the wet separator was much different than for spring chinook salmon in Tests 1 and 2 (Figs. 12 and 13). Within the first 30 min, 16.1% of the fish in Test 3 exited the separator compared to 81 and 74% for fish in Tests 1 and 2, respectively. Similar differences were seen in exit times during the next 30 min, with only 0.2% of the fish in Tests 3 exiting in this test compared to 8.9 and 10.9% in Tests 1 and 2, respectively. Within the first 24 h in Test 1 and 2, 99.8 and 100%,

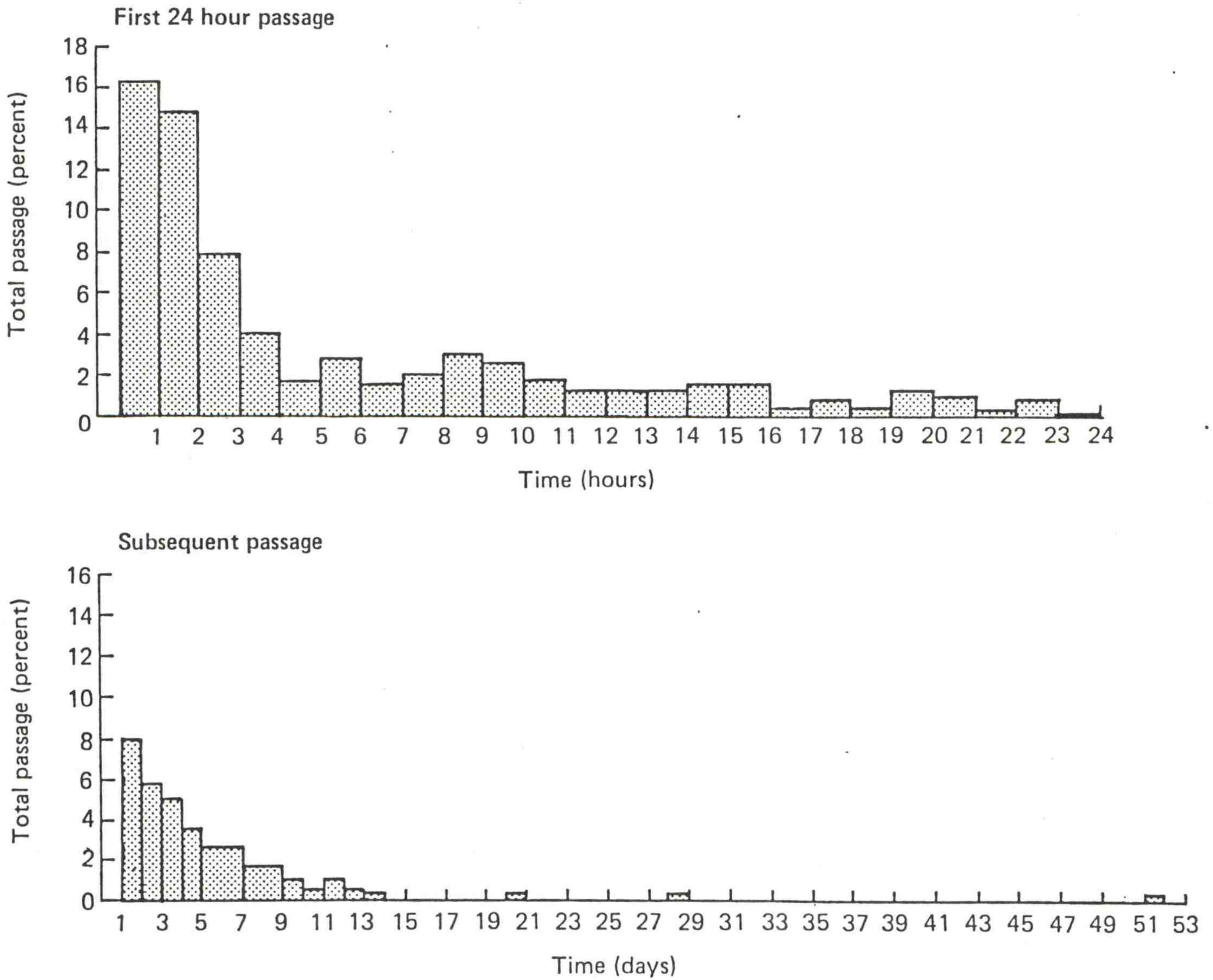


Figure 13.--Percentage of PIT tagged fall chinook salmon detected while exiting the McNary Dam wet separator in the first 24 h and subsequent days.

respectively, exited the wet separator, whereas in Test 3 only 67.3% exited in the same period. No definitive explanation can be given for the long residence time in the wet separator.

Study 3: Comparison of the PIT Tag to Traditional Tagging and Marking Methods

Introduction

Branding and coded wire tags (CWT) have traditionally been used as means of identifying groups of fish on the Columbia River. Often fish must be randomly collected at dams during periods of elevated water temperatures and then branded and/or tagged. Although marking fish during these conditions is stressful to salmonids and normally should be avoided, situations often necessitate such an approach. The objective of Study 3 was to compare the survival of fish injected with PIT tags to survival of fish tagged and marked by traditional methods. If no adverse effects to marking or tagging were seen under these harsh field conditions, it is unlikely that severe problems would result under more favorable conditions.

Methods and Materials

The comparative study between traditional methods of marking and tagging and marking with the PIT tag was conducted at McNary Dam. Outmigrating fall chinook salmon collected from the juvenile collection and inspection facility were used in the study. The fish ranged in fork length from 104 to 181 mm. The study was conducted from 21 May to 9 June 1985.

The survival of PIT tagged fish was compared to that of control fish (handled, but not tagged or marked), CWT, CWT and branded, and branded fish. Traditional tagging and branding techniques were used in the study. All

treatments were combined and held as four replicate groups since each treatment could be recognized by its identifying tag or mark (Table 10). Twenty-five fish per treatment for a total of 125 fish per group were used in the study. The fish were held for 14 days in four knotless nylon nets suspended within a raceway receiving a continuous supply of untreated ambient river water. The fish were examined daily for mortality. The data were analyzed for differences using the G^2 statistic at the $P=0.05$ level (Sokal and Rohlf 1981).

Results and Discussion

No statistical difference ($G^2=6.14$ $df=4$, probability 0.19) between the survival of fish injected with the PIT tag and other treatment groups was shown at the end of 14 days of holding (Table 10). During the first 7 days of holding, only one control and one PIT tag fish died out of the 500 fish in the study. A total of 4 control, 13 PIT tagged, 6 branded, 8 CWT, and 7 CWT plus branded fish died during the 14 days of holding. At the termination of the study, two control and two CWT fish were heavily infected with a fungus and would probably not have survived an additional 1 to 2 days. The condition of all fish in the test groups was rapidly deteriorating at the end of the 14 days of holding.

All dead fish were usually examined for cause of death. The fish examined showed descaling and fungus infection in the caudal area. No signs of disease or fungus were seen on live or dead fish in the vicinity of the wound made by the injection needle. All PIT tagged fish showed complete closure of the injection wound.

The holding of migrant fall chinook salmon captured at a collection facility during the late part of the run and during a period of elevated water

Table 10.--Summary of survival data comparing PIT tagged fish and traditionally marked and or tagged fish after 14 days of holding.

Replicate	Treatment	Starting (n)	Dead (n)	Ending (n)
I	Control	25	0	25
	PIT tag	25	5	20
	Brand	25	2	23
	CWT	25	2	23
	CWT + brand	25	1	24
II	Control	25	2	23
	PIT tag	25	2	23
	Brand	25	0	25
	CWT	25	3	22
	CWT + brand	25	3	22
III	Control	25	0	25
	PIT tag	25	5	20
	Brand	25	2	23
	CWT	25	0	25
	CWT + brand	25	0	25
IV	Control	25	2	23
	PIT tag	25	1	24
	Brand	25	2	23
	CWT	25	3	22
	CWT + brand	25	3	22

temperature is a stressful situation. It is believed, however, since no adverse effect of the PIT tag to survival was seen under these conditions, that under more favorable conditions of capture, tagging, and holding, the PIT tag would not create any severe problems to migrant fall chinook salmon.

Study 4: McNary Reservoir Release

Introduction

The 1985 workplan did not include a reservoir release study, however, based on the encouraging results of our planned 1985 field tests, we felt that a reservoir release would provide valuable information for future planning purposes. A test plan was prepared and approved by BPA and the Columbia River Fish Passage Committee. The objective of the study was to compare the collection ratio of freeze branded fish to PIT tagged fish at the McNary Dam juvenile fish collection facility.

Methods and Materials

Testing was conducted from 7 August to 26 September 1985 at McNary Dam. A total of 4,400 juvenile outmigrant fall chinook salmon ranging in fork length from 90 to 172 mm were marked and tagged over a 5-day period. Each day a replicate consisting of 880 fall chinook salmon was randomly sampled from the juvenile collection facility. No weak, highly descaled, or previously marked fish or species other than fall chinook salmon were used in the study. Of the 880 fish, 80 fish were randomly subsampled, injected with PIT tags, and measured. The remaining 800 fish were marked with a freeze brand (Park and Ebel 1974), and the upper caudal fin was clipped.^{2/} All fish were transferred via flowing water to a 1,800-liter transport tank located on a

^{2/} Freeze brands are difficult to read until about 4 days after marking, thus a upper caudal clip is generally used by researchers as a flag whenever brands are expected to be read prior to 4 days.

truck. Brands were changed for each replicate (daily), and each PIT tagged fish had an individual code. Both PIT tagged and branded fish were held together in the truck transport tank for 24 h with flow through water prior to being transported to McNary Yacht Harbor at Hat Rock, Oregon, 11 km upstream from McNary Dam. The fish were transferred from the truck via gravity flow through a hose to a barge containing a transport tank receiving a continuous supply of river water. The fish were then barged to the main river channel and released. Prior to release, all dead fish were collected for tag and mark observation comparisons.

PIT tag detection was performed by two automatic monitoring systems located on the wet separator at McNary Dam (see Part II, Study 1 for a description). The tag monitor systems required no handling of fish and automatically stored tag codes and time of tagged-fish passage through the detectors on computer files and a printer. The monitor systems were positioned to interrogate 100% of the fish passing through the juvenile collection facility (Fig. 10).

Branded fish were monitored by NMFS personnel at the juvenile salmon collection and inspection facility at McNary Dam. A subsample of the fish exiting the wet separator was diverted to an inspection room; the subsample diversion gates were located downstream from the PIT tag monitors (Fig. 10). The gates were operated by a timer system which allowed sampling for 1.4 min, 3 times per hour or 7% of the time fish passed out of the separator. The subsampled fish were dipnetted; anesthetized; and inspected for fin clips, descaling, injuries, and brands. The subsampled fish were then diverted to a raceway for transport downstream. The study was terminated when the collection system shut down for the season on 26 September 1985.

Results and Discussion

Results of the reservoir release comparative study are summarized in Table 11. No statistical difference was observed ($P < 0.001$) between the recovery of branded and PIT tagged fish. The total number of PIT tagged fall chinook salmon detected exiting from the collection facility was 64 (16%). This represented 100% of the PIT tagged fish that were guided and passed through the collection facility at McNary Dam. The 758 branded fish (19%) is an estimate. The estimate is based upon expanding the actual number of fish observed in the subsample (53) by a factor of 14.3 to adjust for the subsampling rate.

In all, 13,239 fish were handled for branding and brand sampling to obtain the 53 fish in the subsample. To obtain statistically equal data, only 400 fish were handled during PIT tag marking, and an estimated 138,926 fish were passively monitored. Therefore, 97% more fish were handled to obtain brand information in comparison to PIT tag data. This handling difference equates to a ratio of 33:1. In addition, 99% of the fish sampled for the brand evaluation during this testing period were non-branded and were unnecessarily stressed.

Post branding mortality (24-h) was slightly higher among branded fish than the PIT tagged fish, --2.3 vs 1.5%. The water temperature at the time of tagging ranged between 20° and 21°C. The branded fish, as noted, received a small caudal clip as a marker. The combination of clipping the caudal fin and high water temperature may explain the mortalities that occurred prior to release of the fish. Upon recapture, several of the branded fish showed deterioration of the caudal fin in the clipped area. We do not believe this factor biased the data, however in future studies, we will avoid using any fin clip under adverse environmental conditions.

Table 11.--Recovery of branded and PIT tagged fall chinook salmon at McNary Dam.

Treatment ^{a/}	Total number of fish	Pre-release mortality (%)	Total fish handled	Actual number fish observed	Expanded ^{b/} number fish observed	Percent observed	Standard deviation (%)
Brand	4,000	2.3	13,239	53	758	19	±9
PIT tagged	400	1.5	400	64	64 ^{c/}	16	±4

a/ All data are for combined replicates.

b/ The expanded value is based upon adjusting the actual observed number of fish in the subsample by 14.3 to adjust for the subsampling rate.

c/ No expansion factor is required since the number of fish observed represents 100% of the PIT tagged fish passing through the collection facility.

The initial comparison between the PIT tag and brand showed very encouraging results, with the PIT tag being considered a more statistically reliable marking method than marking with brands. Also, significantly fewer fish were stressed during the marking and sampling procedures with the PIT tag.

We recommend that further testing be conducted, as outlined in our 1986 workplan, using: (1) releases of steelhead, spring chinook salmon, and fall chinook salmon; (2) releases made at both inriver sites as well as from hatcheries; and (3) monitoring conducted at both Lower Granite and McNary Dams. If results for 1986 are as conclusive as those we have seen in 1985, we could recommend the use of the PIT tag as a tool for obtaining data to address some of the problems on the Columbia River system in 1987.

Study 5: Monitoring PIT Tags in Adult Fish

Introduction

The PIT tag has significant potential as a tool to identify adult fish returning to a river system. The tag can either be: (1) placed in smolts resulting in data being recovered during their outmigration at dams equipped with automatic tag monitors and again, when as adults, they pass monitors on their upstream spawning migration or (2) placed in adults at some point on their spawning migration, with data subsequently recovered as in (1) above. The former use may replace current CWT or freeze branding techniques. The latter use would complement radio-tracking and CWT/freeze branding studies where research is needed on adult losses, migration delays, stock identifications, and fall-back problems at dams or other migratory obstacles.

If the PIT tag is to have broad application for research, detection and automatic data recording must be assured under a variety of field

conditions. Therefore, our objectives were to: (1) evaluate the feasibility of monitoring PIT-tagged adult salmonids in a variety of situations applicable to Columbia River dams and (2) assess the accuracy and reliability of the PIT tag detector system when used with adult salmonids.

The 1985 PIT tag studies expanded the 1984 research by: (1) conducting the research at an existing CWT trapping station instead of a simulated site, (2) modifying the detection system to provide more power and thus increasing tag reading efficiency, (3) improving the PIT tag quality, (4) increasing detection by using a tandem detection system (multiple loops), and (5) adding additional testing on the use of a PIT jaw tag.

Methods and Materials

Since this phase of testing was to be under actual field conditions, an existing adult trap was necessary for a testing site. The interim fish trap located at the north shore fish ladder at Bonneville Dam was chosen due to its proximity to the newly completed fish-collecting facility and because this existing trap could be used without interfering with normal fish passage (Fig. 14).^{3/} Two modifications to the interim trap were necessary: (1) a screen was installed in the approach channel from the fish ladder, providing a closed system and (2) a 2.7-m long section of the flume was removed immediately below a magnetic CWT detector located at the exit of a Denil fish ladder. This flume section was replaced with two PIT tag detectors joined end to end (Fig. 14). Each detector consisted of a 1.2-m long section of 30-cm

^{3/} The interim fish trap was constructed upon the completion of the Bonneville Second Powerhouse in 1981 to provide a north shore adult fish trap during the interim time before the completion of the north shore fish collection facility.

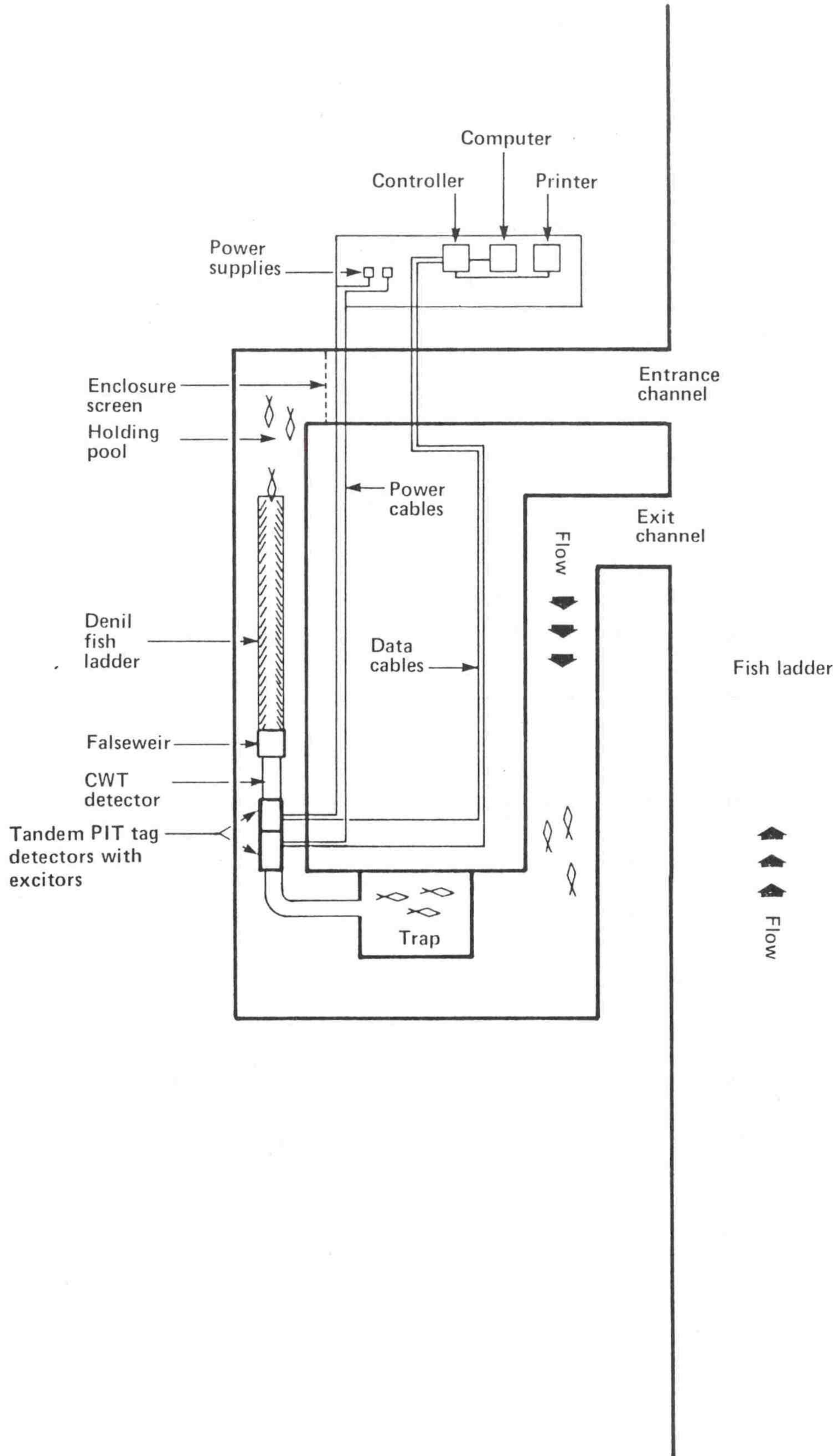


Figure 14.--Bonneville Dam interim fish trap and testing facility, 1985.

diameter PVC pipe containing two detector loops shielded with 4.8-mm thick aluminum. A dual excitor was located inside the shielded box, and the power supply, controller, computer, and printer were located in a mobile office stationed 100 feet away. The PIT tags used for this test were improved by the manufacturer to provide more range than those used in the 1984 study.

Testing was conducted from 11 to 19 July 1985, using adult steelhead ranging in fork length from 51 to 82 cm. Steelhead entering the new trapping facility were diverted directly into an anesthetic tank containing 40 ppm MS-222. The anesthetized fish from all 10 replicates (10 fish per replicate) were then internally tagged with PIT tags (Prentice et al. 1985). For Replicates 1 and 3, the fish were also tagged with PIT jaw tags (Prentice et al. 1985). All fish were measured and placed into a 568-liter transport container. After recovering from the anesthetic, the fish were transported to the interim trap and released (water-to-water) into the holding area. The time of release, length of fish, and PIT tag number were entered on the computer to create a release file. The holding pool had only one exit, the 6.7-m long Denil fish ladder used as an approach to the magnetic CWT detector, the PIT tag detector, and the holding trap. Codes from the PIT tags were read automatically as the fish passed through the tunnel at flow velocities up to 0.3 m/second. These data along with the passage time were simultaneously placed on hard copy and floppy disk for storage.

Results and Discussion

Results of tests conducted under actual field conditions with the automatic detection system for PIT-tagged adult salmonids are summarized in Table 12. Detection efficiency ranged from 90 to 100%, with an average detection of 98%. These results should be representative of fish tagged internally as juveniles and detected upon returning as adults.

Table 12.--Detection of PIT tags placed in adult steelhead at working coded wire trapping facility on Bonneville Dam, July 1985.

Replicate	Release date/time	No. fish released	No. fish detected	Mean length (mm)	Mean passage time (h)	Detection (%)
1 ^{a/}	11 Jul - 1100	10	10	625	9.75	100
2	12 Jul - 0900	10	10	645	18.32	100
3 ^{a/}	13 Jul - 0925	10	10 ^{b/}	698	8.56	90
4	13 Jul - 1358	10	9	636	2.39	90
5	14 Jul - 1009	10	10	656	12.28	100
6	14 Jul - 1104	10	10	672	4.96	100
7	15 Jul - 0840	10	10	627	3.12	100
8	16 Jul - 0924	10	10	627	8.16	100
9	16 Jul - 1434	10	10	637	16.60	100
10	17 Jul - 0842	<u>10</u>	<u>10</u>	<u>613</u>	<u>4.70</u>	<u>100</u>
	Totals	100	98			
	Ave.			644	8.88	98

^{a/} Replicates 1 and 3 were double tagged with PIT internal tag and PIT jaw tag. In both cases, jaw tag data are identical to that shown for internal.

^{b/} All internal PIT tags were detected, however, for replicate three, one PIT jaw tag was not detected.

In some instances, the PIT tag may be used to obtain adult information only. In this case, the fish could be externally tagged. In the two replicates where the fish were double tagged with both an internal PIT tag and a PIT jaw tag, both methods of tagging performed equally, with a mean detection of 95% and each non-detections occurring on separate fish.

One of the primary goals of any research or management activity, where living organisms will be returned to the environment, is to reduce handling stress. After testing the PIT tag on adult salmonids, we believe this objective was met. In fact, the primary advantage of this system is the ability to recover data (read tags) from moving fish, thus totally eliminating additional handling stress to that fish and other fish which would be trapped in the sampling process. Furthermore, the 98% detection rate achieved during the test of the adult PIT tag system exceeded the design criteria of 95% detection. For these reasons, we feel that this system could be used at existing CWT trapping facilities to increase data collection as well as enhance the quality of the data and fish collected.

The performance of the PIT jaw tag was equal to the internal PIT tag, suggesting that the PIT jaw tag could be a viable method of tagging adult salmonids when returns from non-automated sources are necessary (i.e., commercial or sport fisheries).

Conclusions and Recommendations

1. The PIT tag monitors can be installed at dams and give consistent and reliable results. We recommend that a minimum of two independent double loop assemblies be used wherever PIT tags are to be remotely detected, and one controller, exiter, and power supply be maintained in a convenient location to serve as an emergency replacement unit in case of a component failure.

2. The PIT tag can be read efficiently and accurately in juvenile fall and spring chinook salmon that are moving up to 0.3m/sec as they pass volitionally through a PIT tag detection system.

3. The PIT tag does not significantly impair survival of juvenile migrant fall chinook salmon compared to the survival of traditionally tagged and marked fish.

4. Based on branded and PIT tagged juvenile fall chinook salmon released in McNary reservoir being collected at the McNary juvenile fish collection facility in the same ratio, PIT tagged fish behave and survive in a manner similar to fish traditionally marked.

5. The use of the PIT tag, in many types of juvenile salmon studies could reduce the number of test fish required by up to 90% and reduce stress to the fish by only requiring the fish to be handled at the time of tagging. All data collection can be automatic without handling the fish or restraining their passage.

6. Adult steelhead migrants can be successfully PIT tagged and automatically interrogated as they volitionally pass through a PIT tag detection system installed on a Denil fish ladder.

7. With properly installed tag detection equipment, PIT tag reading efficiency for adult migrant steelhead can be expected to be greater than 95% with 100% accuracy.

8. The PIT tag detection system for adult salmonids can be used at existing coded wire tag trapping facilities with minimal revision.

9. The use of the PIT tag with adult migrant fish can increase data collection and enhance the quality of the data collected.

PART III: SYSTEMS DEVELOPMENT

Study 1: PIT Tag Injection Devices

PIT tags are presently injected into fish with a modified hypodermic syringe and needle. Each injector is loaded by hand, requiring a tag to be manually inserted into the needle. This procedure has been satisfactory for test purposes requiring small numbers of fish, however, as greater numbers of fish are tagged, a more efficient means of placing the tag in the needle is required. Complicating the design of a tagging system is a self-imposed requirement that both the needle and tag be disinfected prior to use. Presently, several designs for a tagging system that meet our requirements are under evaluation. The final design and implementation of an automatic tag injection system must wait until the tag manufacturer decides upon a packaging system (tags in a strip, cartridge, etc).

Study 2: Quality Control Monitor For Tagging

At the time a fish is PIT tagged, every assurance must be made that the tag injected into the fish is functional and can be interrogated and the data recorded. Furthermore, since each fish can be identified by a unique identification number, individual information such as length and/or weight can be recorded and associated with the identification number at the time of tagging. Figure 15 shows a quality controlled tagging system to be evaluated in 1986. The system will consist of two similar tagging stations. Each station will have a 150-cm diameter tag detection loop, a tag monitor, an electronic measuring board and balance, and a controlled fish release area. The components of the two stations are connected to a multiplexer, computer, and printer. The tagging procedure at a station would require a number of steps. A fish would be removed from an anesthetic tank and injected with a

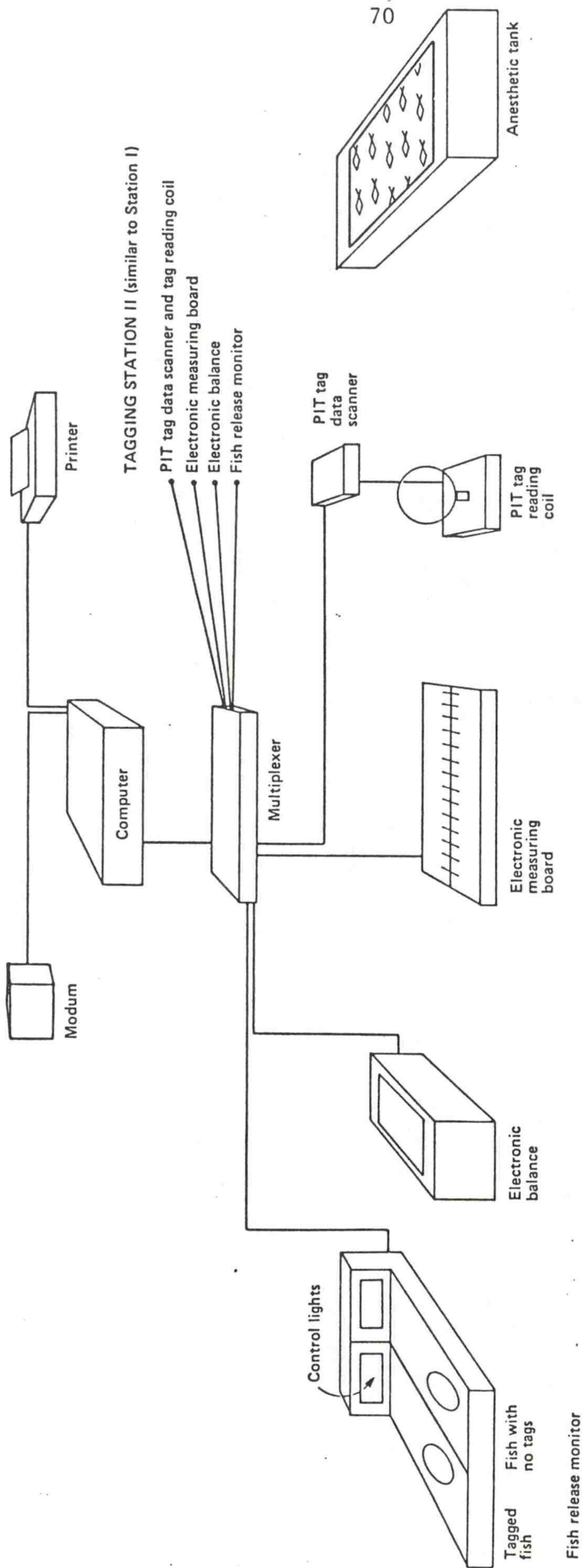


Figure 15.---Conceptual drawing of a quality control system for tagging.

PIT tag. While holding the fish in hand, the fish would be placed through the tag detection loop. A message would appear on the tag monitor's screen if the tag was successfully read and entered into the computer. The operator would then place the fish on the electronic measuring board and touch a stylus to the fork of the tail to obtain fork length. The fish would then be weighed on an electronic balance. The data from the measuring board and balance would be entered into the computer automatically. If all data were entered successfully, a green light would show and a rubber gate would open allowing the operator to release the fish. All data would be automatically entered on computer files and a hard copy made. If for some reason not all the data entered the computer, a red light would show on the tagging console and a rubber gate over a repeat exit would open. The two stations could be operated simultaneously since the multiplexer acts as a controller and a buffer for the system.

To date, not all the components have been linked together and fully tested. However, we have individually tested the tag detection loop, tag monitor, electronic balance and measuring board, multiplexer, computer, and printer. Actual field testing of the system awaits the 1986 field season. The design of this system has been reviewed by U.S. Fish and Wildlife Service personnel associated with fish tagging.

Study 3: Hatchery Release Monitor

Mortality and tag loss may occur between the time fish are tagged and released at a hatchery. Therefore, it is essential to know the actual identification of each fish at the time of release so that tags that are no longer a part of the study can be eliminated from the data base.

Monitoring tagged fish at time of release from a hatchery is challenging, since the highest concentration of tagged to non-tagged fish will occur within a hatchery rearing system when all the fish will be released within a short time. Under these conditions, precautions are needed to reduce the likelihood of two tagged fish entering a monitoring loop simultaneously to prevent reading error. Furthermore, the monitoring system must be designed to rapidly monitor fish without stress.

Design work was completed on a hatchery monitor under the 1985-86 workplan (Fig. 16). The monitor consists of four pipes measuring 10.2 cm in diameter by 61.0 cm long. Each pipe is equipped with two PIT tag monitoring loops connected to tag monitoring equipment. All of the monitors are connected to a computer and printer. As each PIT-tagged fish passes through a monitor, its number will be recorded automatically on a computer file and be printed. After the release, the release file will be compared to the file created at the time of tagging and missing fish will be noted. The release monitor will be tested at Dworshak National Fish Hatchery in March 1986.

Study 4: Design and Placement of Future Monitoring Systems

The results obtained during the 1985 field season at McNary Dam provided valuable insight into the future design and placement of juvenile monitoring equipment at collector dams. Initial monitor design and placement made it difficult to clean the orifices on the wet separator and, thus, could potentially increase debris problems within the fish collection system. Suggested modifications include narrowing the monitor entrance and adding a dewatering section. In addition, it was determined that a series of two monitors (with two detector coils each) per flume should provide optimal PIT tag reading efficiency. Based upon this experience, an improved new PIT tag

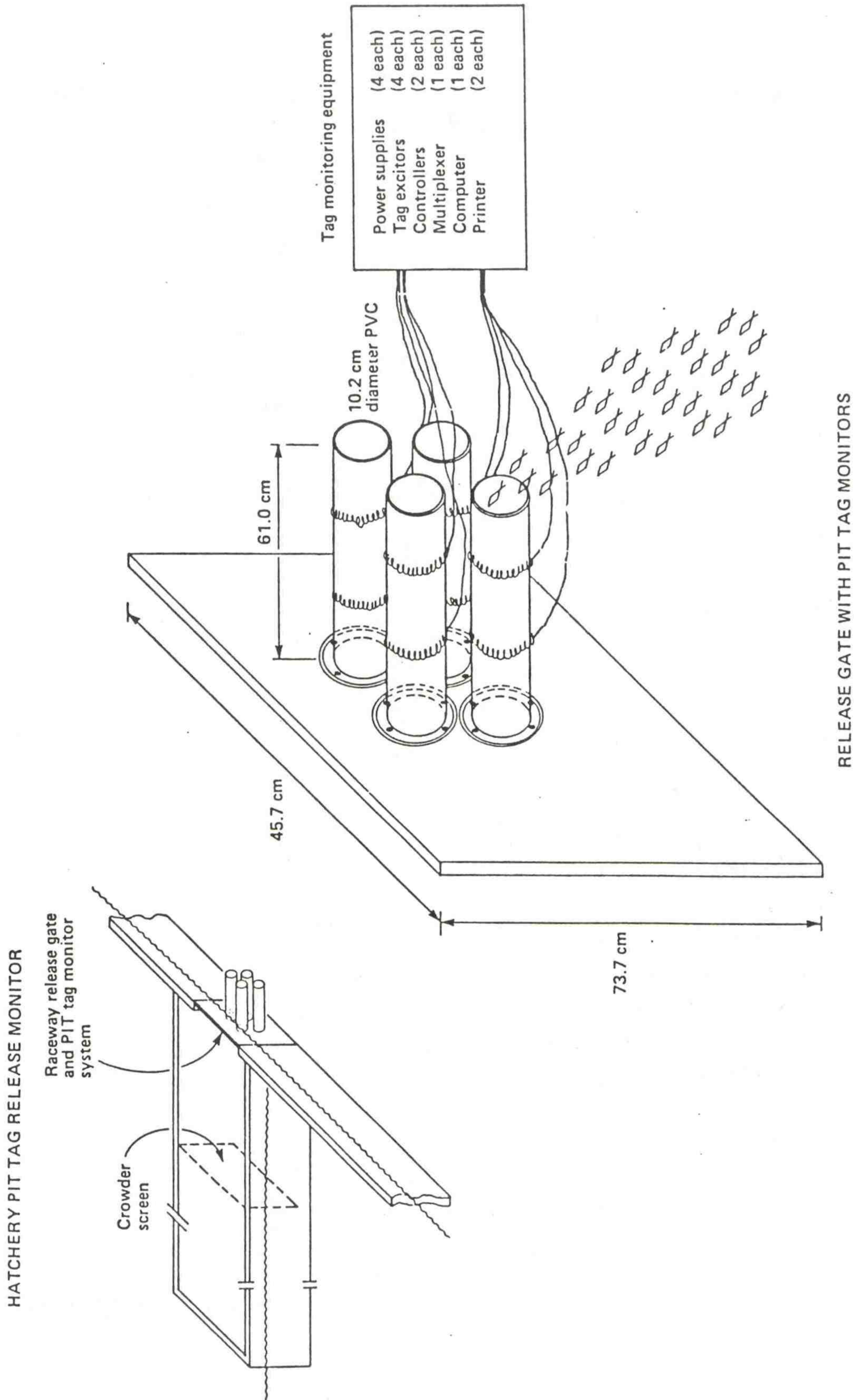


Figure 16.--Hatchery PIT tag release monitor system.

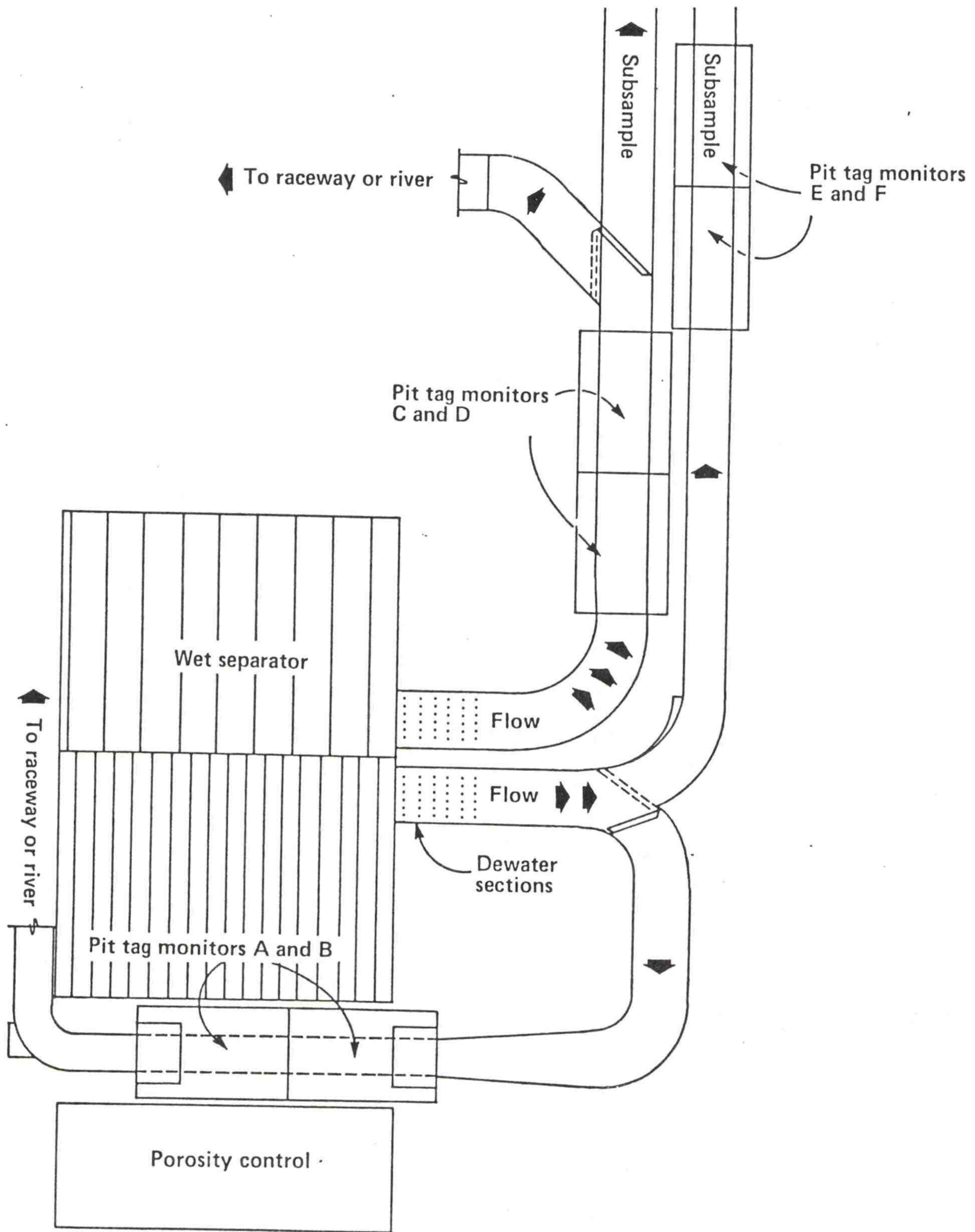


Figure 17.--Proposed location of juvenile PIT tag monitors at McNary Dam.

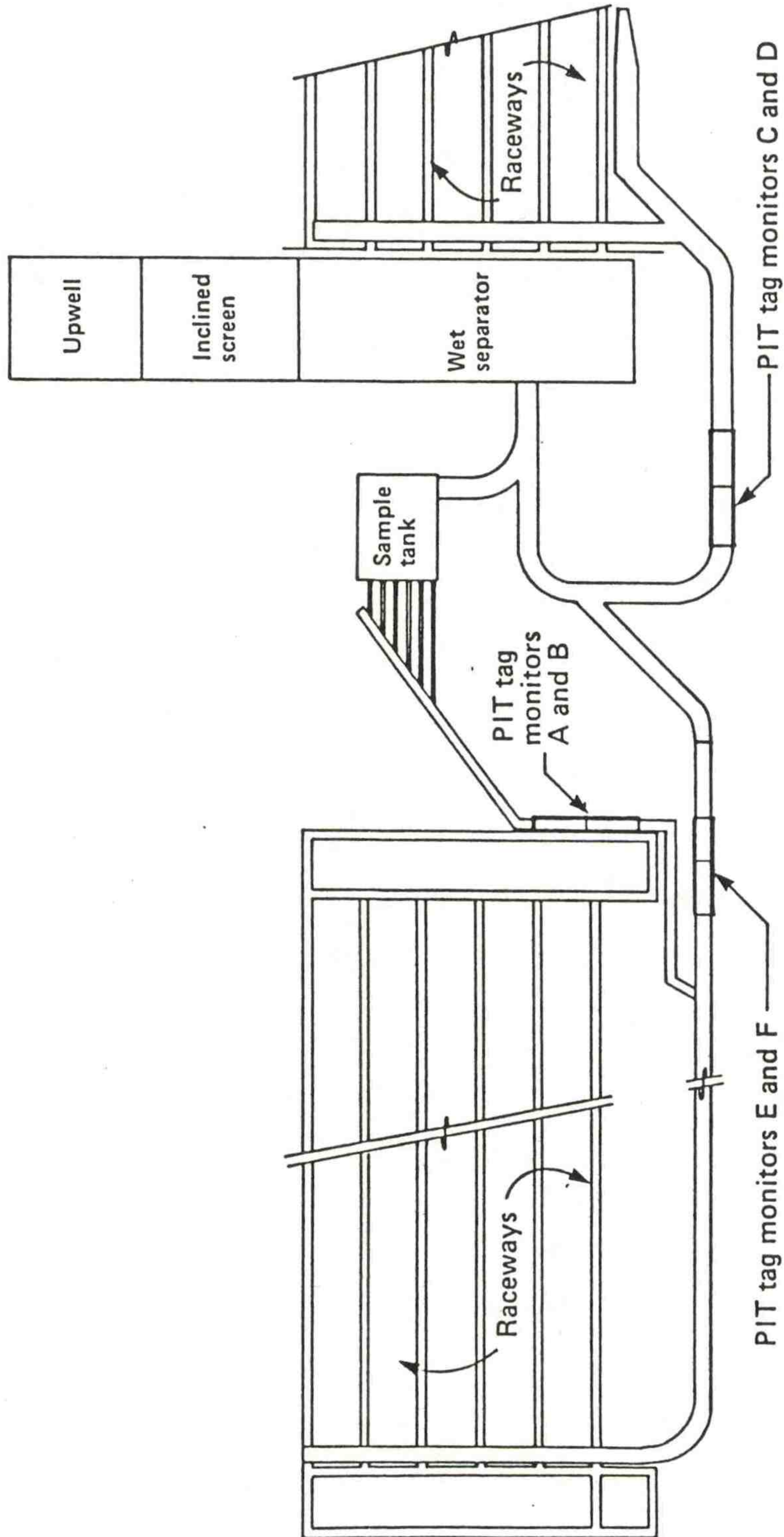


Figure 18.--Location of juvenile PIT tag monitors at Lower Granite Dam.

monitoring system will be installed at McNary Dam for the 1986 field season (Fig. 17). This unit should improve operational efficiency by lowering debris problems in the system. A similar system will be installed at Lower Granite Dam (Fig. 18). The U.S. Army Corps of Engineers and the Fish Passage Committee have approved the design and installation of the new monitors at both dams.

A tag monitor system has also been designed for Little Goose Dam. A series of controlled tests incorporating both the PIT tag monitors and fish counters working in close proximity to one another must be completed before the design is available for review.

Conclusions and Recommendations

1. As soon as the PIT-tag manufacturer decides on final tag design and packaging, we recommend a semi-automatic or automatic tag injection system be developed to reduce the time required to tag a population of fish.
2. We recommend that a PIT tag tagging station and quality control system be designed and fully tested in 1986. Such a system should be designed on the same principle as that used for CWT.
3. We recommend that the system to monitor PIT-tagged fish leaving hatchery raceways be evaluated in 1986.
4. We recommend that an improved PIT tag detection system be installed at McNary Dam to overcome the potential debris problem that existed at the wet separator in 1985.
5. PIT tag detection systems can be installed at Lower Granite Dam without major modifications to the existing system; we recommend that such a system be installed in 1986.

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Special thanks is given to David Cross for his technical support. We also thank Lee Ferguson, Carol Ranck, and their crews for the support given during the project. Thanks is also given to Brad Ebby (COE) and his crew, for without their assistance the field studies could not have proceeded.

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APPENDIX A

Final Report

PRELIMINARY INVESTIGATION OF THE
INACTIVATION OF AEROMONAS SALMONICIDA,
A FISH PATHOGEN

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INTRODUCTION

In order to provide some preliminary indication of the ability of a sterilizing agent to inactivate a common fish pathogen, initial studies described here were conducted. These studies were in support of a tagging program in which electronic fish tags (PIT tags) were used to mark salmonid fishes from a variety of Columbia River Basin stocks. The studies resulted from a concern that the repeated use of fish tagging injectors could serve as a vector for fish pathogens. It was realized that an exhaustive investigation of sterilizing agents on various pathogens of differing degrees of sensitivity to the sterilants was beyond the scope of the effort here. Thus the results provided here utilizing a relatively sensitive indicator and easy to detect bacterium provide a guideline for the minimal conditions which should be used in the maintenance of sterilizing solutions. Further extensive work with a variety of pathogens such as Renibacterium salmoninarum and infectious pancreatic necrosis virus would be required to definitively establish the efficacy of the concentrations of ethanol used here or other sterilants for their inactivation.

METHODS

Injectors used for intraperitoneal injection of fish were obtained from the National Marine Fisheries Service (NMFS) as well as the tagging devices (PIT tags). Injector tips were dipped in sterile petroleum jelly prior to the test in order to simulate conditions of actual use in the field. An isolate of Aeromonas salmonicida was also obtained from the NMFS.

Bacterial suspensions of A. salmonicida were prepared by inoculating tryptic soy broth with a loopful of the isolate. Density of 18 to 24 hour cultures and an approximation of cell concentration was made by measuring optical density at a wavelength of 620 nm. Sterilized tag injectors were dipped into the bacteriological broth (to a depth of about 1 cm) containing between 1×10^6 and 1×10^7 organisms per ml. The tags were expelled after the devices were withdrawn from the broth and placed in the sterilizing solutions for the appropriate test time. Untreated controls were given a similar immersion in the bacterial broth but were not subjected to the dip in the test sterilizing solutions.

Following the sterilizing treatments, the injectors were swabbed with sterile cotton tip applicators which were then used to qualitatively inoculate tryptic soy agar plates. Plates were incubated for up to 3 days and examined for the presence or absence of bacteriological growth. A series of four experiments was conducted to determine the minimal concentration of ethanol which would completely sterilize all test injectors.

RESULTS

A summary of the test results is given in Table 1. Preliminary experiments suggested that a concentration of as low as 30% ethanol would inactivate the bacterium. Further experimentation (Experiments 3 and 4, Table 1) with 30% and 50% ethanol indicated that the lower concentration (30%) was not effective in inactivation (only 2/10 test samples were inactivated) but that 50% ethanol was effective in inactivating 10/10 test samples. The inactivation occurred after one minute of exposure to the ethanol solution. The first experiments with small sample sizes had suggested that exposure of the contaminated injectors to the sterilant resulted in sterilization within one minute although several injectors were tested with a five minute treatment in the sterilant. The results thus indicate that for Aeromonas salmonicida or for microorganisms of similar sensitivity to ethanol that a one minute exposure of the PIT tag injecting devices in 50% ethanol is sufficient to kill the bacteria.

TABLE 1. Inactivation of *Aeromonas salmonicida* with an ethyl alcohol rinse.

PROPORTION OF POSITIVE BACTERIOLOGICAL PLATES

Experiment Number	Concentration of Alcohol in Sterilizing Solution	Duration of Treatment*	
		1 Minute	5 Minutes
1	90%	0/2	0/2
	50%	0/2	0/2
	Untreated Control	2/2	2/2
2	50%	0/2	0/2
	30%	0/2	0/2
	10%	2/2	2/2
	Untreated Control	2/2	2/2
3	30%	8/10	-
	Untreated Control	10/10	-
4	50%	0/10	-
	Untreated Control	10/10	-

*Proportion of total plates with bacterial growth for each indicated treatment.

DISCUSSION

It must be noted that the results presented here cannot be applied to other microorganisms which may not have the same sensitivity to ethanol. For example, the cell wall of the gram positive fish pathogen, Renibacterium salmoninarum, could render it more resistant to the treatments which were effective for Aeromonas salmonicida. This possibility can only be verified by further testing.

Wedemeyer et al. 1979, found that A. salmonicida was more resistant to both chlorine and ozone treatment for inactivation than was the etiologic agent of enteric redmouth disease (ERM), Yersinia ruckeri. A concentration of 0.05 mg/L inactivated Y. ruckeri 30s while a concentration of 0.1 mg/L for 30s was required to inactivate A. salmonicida. The inactivation of infectious hematopoietic necrosis virus (IHNV) in hard lake water required chlorine at 0.5 mg/L for 10 minutes or 1.0 mg/L for 30s. Under similar conditions, 0.7 mg/L chlorine destroyed infectious pancreatic necrosis virus (IPNV) within 2 minutes. These values may provide some indication of the relative resistance of the microorganisms to inactivating agents but can not be assumed to be directly proportional to the sensitivity of the same microorganisms to ethanol since the mechanism of inactivation may be different.

One important component of the approach to the control of diseases through the use of tagging equipment is to determine which diseases are known or considered to be probable to exist in a given watershed. Obviously, if infectious agents which are potentially more resistant to a given method

of inactivation are not present in a particular drainage, then these agents would not be considered in the inactivation of fish handling equipment.

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APPENDIX B

Budget Information

BUDGET INFORMATION

A. Summary of expenditures

Personnel Services and Benefits	87.9K
Travel & Transportation of Persons	9.3K
Transportation of Things	5.5K
Rent, Communications & Utilities	0
Printing & Reproduction	0.1K
Contract & Other Services	7.5K
Supplies & Materials	280.2K
Equipment	276.3K
Grants	0
Support Cost (Including DOC ovhd.)	<u>33.6K</u>
TOTAL	693.7K

B. Major items purchased

1. PIT tags (50,000)--Contract 85-ABC-00182
2. PIT tag monitoring systems for juvenile migrants at Lower Granite and McNary Dams--Contract 50-ABNF-6-0048.

FIGURES

- Figure 1.--Comparison of weight change of PIT tagged and control fish over time.
- Figure 2.--Comparison of length change of PIT tagged and control fish over time.
- Figure 3.--Diagram of modified Blaska respirometer-stamina chamber, showing side and end views. For loading, the chamber is tilted, partially filled with water, and end plate and vane are removed. Fish are placed in the test compartments, vane and end plate are replaced, and chamber is filled with water and leveled. Water flow is produced with motor driven propeller and varied via motor speed controller. Direction of water flow is toward propeller in inner tube, water is turned at the end plate, and returned through the space between the inner and outer tubes (see arrows).
- Figure 4.--Mean swimming stamina (U-critical) of PIT tagged and control fingerling steelhead (6.5 g average) trout during Days 0-25 post-tag. Brackets indicate \pm one standard error.
- Figure 5.--Mean swimming stamina (U-critical) of PIT tagged and control juvenile steelhead (17.2 g average) trout during Days 0-25 post-tag. Brackets indicate \pm one standard error.
- Figure 6.--Mean stride efficiency of PIT tagged and control fingerling steelhead (6.5 g average) trout during Days 0-25 post-tag. Brackets indicate \pm one standard error.
- Figure 7.--Mean stride efficiency of PIT tagged and control juvenile steelhead (17.2 g average) trout during Days 0-25 post-tag. Brackets indicate \pm one standard error.
- Figure 8.--Mean opercular beat rate of PIT tagged and control fingerling steelhead (6.5 g average) trout during Days 0-25 post-tag. Brackets indicate \pm one standard error.
- Figure 9.--Mean opercular beat rate of PIT tagged and control juvenile steelhead (17.2 g average) trout during Days 0-25 post-tag. Brackets indicate \pm one standard error. Asterisk (*) indicates significant (tagged vs. control) difference ($P < 0.01$).
- Figure 10.--Location of juvenile salmon PIT tag monitors at McNary Dam during 1985.
- Figure 11.--Diagram of the juvenile salmon PIT tag monitoring system at McNary Dam during 1985.

Figure 12.--Percentage of PIT tagged spring chinook salmon detected while exiting the McNary Dam wet separator.

Figure 13.--Percentage of PIT tagged fall chinook salmon detected while exiting the McNary Dam wet separator in the first 24 h and subsequent days.

Figure 14.--Bonneville Dam interim fish trap and testing facility, 1985.

Figure 15.--Conceptual drawing of a quality control system for tagging.

Figure 16.--Hatchery PIT tag release monitor system.

Figure 17.--Proposed location of juvenile PIT tag monitors at McNary Dam.

Figure 18.--Location of juvenile PIT tag monitors at Lower Granite Dam.