Single-use vs. Multi-use Needles for PIT Tagging of Summer Steelhead (*Oncorhynchus mykiss*) at the Hagerman National Fish Hatchery

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Abstract

The Hagerman National Fish Hatchery Hatchery Evaluation Team compared on-station survival and wound recovery of steelhead smolts PIT tagged with pre-loaded (single-use) PIT tag needles from Biomark (www.biomark.com) to standard multi-use PIT tag needles. On-station survival was 96.9% for smolts tagged with single-use needles and 96.4% for smolts tagged with multi-use needles. Mean incision length was 2.50 mm for both groups. Mean incision width was significantly ($p<0.05$) wider for multi-use needles (0.86mm vs 0.52mm). Multi-use needles also produced more qualitative ragged incisions (44% vs 3%). At 7 days post tagging, only 18% of ragged incisions had healed compared to 44% of clean incisions. Despite wider wound width and higher percentage of ragged incisions, we did not observe a difference in healing time between the single-use and multi-use methods. Approximately 30% of wounds were healed 7-days post-tagging and 97% were healed by 14 days post-tagging for both groups. Single-use needles produced narrower and cleaner incisions, but both methods produced satisfactory results in this study.

Introduction

The Hagerman National Fish Hatchery (Hatchery) annually rears 1.46 million summer steelhead smolts under the Lower Snake River Compensation Plan (LSRCP) Program. The Hatchery rears A-run steelhead destined for release at the Sawtooth Weir (790K), Sawtooth stock steelhead destined for the Yankee Fork (440K), Sawtooth stock destined for Salmon River Section 18 Tunnel Rock release (60K), and East Fork Naturals for release at the East Fork Trap (170K). As part of Monitoring and Evaluation for the LSRCP program, the Idaho Department of Fish and Game (IDFG) or Pacific States Marine Fisheries Commission (PSMFC) routinely mark Hatchery steelhead smolts with Passive Integrated Transponder (PIT) tags. Prior to Brood Year (BY) 2007, the number of PIT tagged smolts was 300 per release group or 1200-1500 tags for the entire Hatchery. In BY2007 to 2009, the number of PIT tagged smolts increased to 30,000 (Table 1). The PIT tag increase was associated with the Comparative Smolt Survival Study.

After PIT tagging operations in BY2007, the Hatchery experienced an increase in daily mortality rates that peaked 7 to 8 weeks post-PIT tagging (Figure 1). The Hatchery did not experience a mortality increase after PIT tagging in BY2006. As a result, the Hagerman Hatchery Evaluation Team (HET) and IDFG shifted PIT tagging from February to early January during BY2008. However, mortality rates again increased after BY2008 PIT tagging (Figure 1).

The Idaho Fish Health Center identified Coldwater and Furunculosis epizootics during the mortality events in BY2007 and 2008. Coldwater disease and Furunculosis had last been identified at the Hatchery in BY2002. BY2007 smolts were too close to release for medicated feed treatment. BY2008 mortality was reduced with a successful treatment with Aquaflor® medicated feed.

As a result of the disease episodes with BY2007 and 2008 steelhead, the Hagerman HET recommended PIT tagging in November when loading rates were lower and the average smolt size (125 mm) was closer to the 80-150 mm size recommended by PIT Tag Steering Committee (1999). The HET also recommended an examination of pre-loaded (single-use) PIT tag needles from Biomark (www.biomark.com) to reduce the risk of disease transmission between smolts, allow for quicker healing time, reduce shed tags, and reduce mortality.

Materials and Methods

BY2009 Sawtooth stock steelhead smolts destined for release at the Sawtooth weir were used for a comparison of on-station survival, downstream survival, and wound recovery of smolts PIT tagged with single-use needles as compared to conventional multi-use PIT tag needles. Two treatments of 6600 PIT tags each were distributed between 11 raceways. Each treatment raceway contained 600 single-use and 600 multi-use needle PIT tagged smolts except for raceways 85, 92, and 94 which each had an additional 100 multi-use needle PIT tagged smolts to meet overall tagging goals. Raceways were chosen to represent first, second, and third-use rearing and multiple egg-take lots (Appendix I). A PSMFC crew installed tags via the multi-use needle methods and a Biomark crew installed single-use needle PIT tags. We realize the discrepancy in marking crews, but assumed that each crew was considered "experts" for the needle type they consistently install. Hence, we compared the best installers available for each method.

PIT Tagging

PSMFC and Biomark crews randomly PIT tagged 1200 smolts from each study raceway (600 for each method and 700 multi-use needle PIT tagged in raceways 85, 92, and 94) on November 18, 2009 following standard PSMFC and Biomark protocols. In addition to standard protocols, fish handling, oxygen, water temperatures, and crowding was carefully monitored to minimize fish stress. Dissolved oxygen in holding tanks, vats, etc, was maintained as close to 8 ppm as possible. Water temperature remained as close to 15^0C as possible and never above 16^0C or below 13^0C. Steelhead smolts were held under anesthesia for less than 5 minutes.

Hatchery Standard Operating Procedures were followed for PIT tagging operations: Steelhead were held off feed for 3 days prior to PIT tagging. No feeding occurred during PIT tagging and feeding resumed 3 days after PIT tagging. Raceways were crowded to approximately 20 feet (1/5 of total raceway length). Smolts were transferred to the PIT tagging trailers via water-filled six gallon buckets at a loading rate of no more than 100 fish per bucket (7 pounds) and a transfer time of less than 3 minutes. Two-ten foot adjacent sections were maintained with crowd racks as recovery areas for multi-use needle PIT tagged smolts and single-use needle PIT tagged smolts. Smolts were held in recovery for approximately 30 minutes. PSMFC and Biomark PIT tagging personnel monitored fish behavior and number of incoming smolts and advised Hatchery personnel as needed.

Wound Healing

150 smolts were quarantined from each treatment (300 total) for a period of 4 weeks for wound healing comparison. The smolts were randomly selected from the recovery areas of raceways 38, 47, 59, 65, 82, and 92 immediately following PIT tagging. All wound healing smolts were held off feed for three days and were returned to feeding rates matching the raceway population. Wound healing smolts were held in 6 experimental tanks (2'x 2'x 2'). Each tank contained 25 smolts from each treatment (50 total). Wound observations were conducted blindly on randomly selected smolts. The same observer was used for all measurements and qualitative observations.

Wound healing observations occurred at 1d, 7d, 14d, 21d, and 28d post-PIT tagging. All wound observation smolts (300 total) were anesthetized with MS-222 and scanned for PIT tag codes with a Destron-Fearing ™ FS2001F-ISO PIT reader. The puncture wound was digitally photographed and qualitatively classified as healed or not healed. The observer also recorded bruising and ragged incisions. These additional observation criteria were chosen from the PIT Tag Steering Committee (1999) report that specified the wound should be a straight incision about 1-2mm of length rather than a ragged hole. Digital photographs were measured using Abode Acrobat 7.0 Professional software to determine the lengths and widths of the incisions (1d post-tagging) and resulting wounds (7-28d post-tagging). Length and width measurements ceased when a wound was considered "healed". Wounds were considered healed when red inflammation subsided and/or a membrane over the wound was observed. Bruises were defined as the bruised or damaged area around the incision that was discernibly darker than the surrounding tissue. Off centered wounds not visible in photographs were recorded as "n/a".

Several statistical analyses were completed. Mean wound length and width were compared with a single factor ANOVA. The percent healed for each sampling day was compared using a single factor ANOVA after arcsine transformation of the data. A Tukey Test was than performed for multiple comparisons. This same statistical process was performed for bruising and incision quality between the two methods. After examination, the smolts were returned to the recovery tanks. Length and weight measurements were taken at 1d and 28d checks and compared with a single factor ANOVA.

Shed Tags

PIT tagged raceways were swept with magnetic sweepers for shed tags two weeks and four weeks post-PIT tagging. An additional sweep for shed tags occurred just prior to release. Shed rates in wound healing tanks were determined by number of smolts with missing tags.

On Station Survival

Hatchery personnel scanned smolt mortalities from PIT tagged raceways and wound recovery tanks daily with a Destron-Fearing ™ FS2001F-ISO PIT reader. Hatchery personnel also recorded total number of PIT tags recovered from each raceway daily. PIT tag codes were collected on an internal file and downloaded from the Destron-Fearing ™ FS2001F-ISO PIT reader bi-weekly

Downstream Survival

The Hatchery notified IDFG Nampa Research upon release of smolts from PIT tag raceways. Estimated survival and travel time to Lower Granite Dam will be calculated using PIT tag interrogations at the four Lower Snake river dams (Lower Granite, Little Goose, Lower Monumental, and McNary Dams).

Fish Health

During disease episodes (mortality >0.1% per day), moribund samples were examined to determine fish health through basic necropsy. Samples of skin mucus and gill filaments were examined for external parasites or the presence of other abnormalities. The fish were opened and examined for visual signs of parasites or other indications of bacterial or viral disorders. Kidney imprints were taken from fish that exhibit signs that vary from 'normal' i.e. swollen and inflamed hindgut, pink fat, anemia, swollen kidney, etc. Imprints, when gram stained, show the presence of *Nucleospora salmonis* spores and/or bacteria. Kidney samples were collected and then combined into two-fish pools in tissue lysis buffer for Polymerase Chain Reaction (PCR) assays. Individual kidneys were streaked onto Tryptic Soy Agar and Tryptone Yeast Extract plus Salts Agar to isolate bacteria, primarily *Flavobacterium psychrophilum* (causes Coldwater Disease) and *Aeromonas salmonicida* (causes Furunculosis).

PIT Tag Array

Several treatment raceways were scanned with a PIT tag array during smolt distribution activities in April. The array consisted of four independent antennas that were connected to four Destron-Fearing ™ FS2001F-ISO PIT readers. The four antennas were mounted on the 6” suction hose of Nielson style fish pump. The antennas were tuned daily to account for antenna interference from metal objects and other antenna fields. Expected PIT tag detections were calculated as Total Tags installed minus Shed Tags and Mortalities. Efficiency was calculated as:

$$E = 1-(((T-X_1)/T)* ((T-X_2)/T)* ((T-X_3)/T)* ((T-X_4)/T))$$

Where:

E = Efficiency
T = Total Expected Tags in the Raceway
X_1 = Detected Tags by Antenna #1
X_2 = Detected Tags by Antenna #2
X_3 = Detected Tags by Antenna #3
X_4 = Detected Tags by Antenna #4

Results

Wound Healing

Table 2 summarizes observations from the wound healing study. 23% of the single-use needle group and 1% of the multi-use needle group were excluded because tag placement was not visible in photographs (Appendix 2). Overall healing rates between the two groups were not significantly different ($p>0.05$) during the time intervals measured. Seven days post-tagging, 32% of the wounds in both groups were considered "healed". Fourteen days post-tagging, 97% of the single-use needle group and 98% of the multi-use needle group were considered "healed". One smolt from the multi-use needle group never healed during the study period because the PIT tag had herniated from the abdominal cavity.

Mean incision length between the two groups was not significantly different ($p>0.05$). Initial mean incision length was 2.45mm and 2.55mm for the single-use and multi-use needle groups respectively.

Mean incision width was significantly ($p<0.05$) wider for multi-use needles (0.86mm vs 0.52mm). Mean wound width seven days post-tagging was also significantly ($p<0.05$) wider for multi-use needles (1.00mm vs 0.73mm).

The incisions of the multi-use needle group were significantly ($p<0.05$) more ragged than the single-use needle group. Of the incisions, 44% of the multi-use needle group was considered ragged compared to 3% with the single-use needle group. Most of the ragged incisions were caused by "X" shaped tearing at the insertion point (Appendix 2). Smolts with torn incisions took longer to heal than those without. One week post PIT tagging, only 18% of the smolts with ragged incisions had healed as compared to 44% of smolts with clean incisions. By 14 days, both clean and ragged incisions had almost completely healed (Table 2).

Bruising rates were the same for both methods ($p>0.05$). We observed 34% bruising in the single-use needle group and 33% bruising in the multi-use needle group 1 day post tagging.

There was no significant difference ($p > 0.05$) in weight or length gained by fish tagged with the two different types of needles. The fish tagged with single-use needles gained 11.2g and grew 7.65mm. The fish tagged with multi-use needles gained 11.0g and grew 7.37mm.

Shed Tags

We recovered four shed tags (1.3%) and one broken tag (0.3%) found during the wound healing tank study. Two of these tags were found one week post PIT tagging and the other three were found two weeks post PIT tagging. The single-use needle insertion method accounted for four of the five shed/broken tags in the study.

We recovered two shed tags in the raceways during this study. These two tags account for a shed rate of 0.03% and were both inserted with the multi-use needle method.

On Station Survival

Survival was 100% during the wound healing study. Survival rates for fish tagged with the single-use, multi-use needle, and non-tagged fish were all high as illustrated in Table 3. Of the 6,542 fish tagged in single-use needle group, 203 mortalities were recovered for an overall survival rate of 96.9%. There were 6,749 fish tagged in the multi-use needle group with 246 mortalities recovered for an overall survival rate of 96.4%. Survival rate for non-tagged smolts in PIT tagged raceways was 95.8%. There was no significant difference ($p > 0.05$) between survival rates of fish tagged with the single-use, multi-use needle, or non-tagged fish in the same raceway. During data collection of mortality of PIT tagged raceways, some data was lost. The backup battery in the PIT tag reader failed and approximately 61 mortality codes (0.2%) were lost from March 16-27, 2010. Mortality from this time period was removed from the analysis for treatment and control groups.

Raceway survival was not significantly different between all Sawtooth stock raceways containing PIT tags (94.9%) compared to those that were not PIT tagged (94.6%).

Downstream Survival

This section will be updated as downstream survival information becomes available.

Fish Health

The Hatchery experienced several disease epizootics and sporadic elevated mortality levels throughout the raceways in brood year 2009. Fish Health observations are reported in Table 6. Furunculosis outbreaks were treated with Aquaflor on the top deck on 10/5/09, the middle and bottom deck on 10/26/09, and spot treated in several raceways on

the top and middle deck on 2/5/10. Additional furunculosisis outbreaks were treated with Romet in raceways 57 and 58 on 11/26/09 followed by a facility wide treatment with Romet at the end of December 09. Raceway 47 was treated with potassium permanganate on April 14, 15, and 16 at 0.5, 1.0, and 2.0 ppm for bacterial gill disease and columnaris.

PIT Tag Array

Detection rates by the PIT Tag Array were not significantly different ($p > 0.05$). The PIT tag array detected 91.6% of expected tags in the multi-use needle group and 90.9% of expected tags in the single-use needle group (Table 5). The calculated efficiency for the PIT tag array was 99.4% assuming random probability of detection between antennas.

Discussion

PIT tagging operations for BY2009 went well. Both tagging procedures yielded overall survival rates post-PIT tagging over 95%. However, BY2009 smolts still exhibited comparable mortality rates during the smolting period (February to April) as previous brood years (Figure 1). This suggests that smolting stress is significant during this time period regardless of tagging timing. PIT tagged raceways had similar survival rates to non-PIT tagged raceways which was different than previous Brood Years that were tagged closer to smolting periods (HNFH 2008).

Mean incision width was significantly wider for the multi-use needle group. The large percentage (44%) of ragged incisions in the multi-use needle group likely impacted this measurement. Despite wider wound width and higher percentage of ragged incisions, we did not observe a difference in healing time between the single-use and multi-use methods. However, in the multi-use needle group, only 18% of the smolts with ragged incisions were healed at 7 days post tagging as compared to 44% of the smolts with clean incisions. A more frequent sampling protocol between seven and fourteen days would have provided additional data resolution to determine a difference in healing rates. Ragged incisions were likely higher in the multi-use needle group because multiple uses dull the needles causing the skin to be torn rather than sliced at the incision point. The handling of multi-use needles intuitively leads to some dulling of the needles. After use, the needles are dropped into collection trays. These trays allow contact between different needles. The needles are then dumped from these initial collection trays through the sterilization process in bunches. Intuitively, this jumbling of needles likely causes additional wear on the sharpened edge of the PIT needles. Conversely, the single-use disposable needles were consistently sharp resulting in cleaner incisions.

Mean incision length was not significantly different between the two groups. However, mean incision length for both groups (2.47 mm) was longer than the recommended 1 to 2 mm length (PIT Tag Steering Committee 1999).

Detection rates by the PIT tag array were not significantly different. The PIT tag array

detected 91.2% of the expected tags with a calculated efficiency of 99.4%. Errors in shed and mortality estimates could have affected the expected number of tags. We lost 61 tag codes (0.2%) due to a back-up battery failure. Mortality codes may also have been missed by operator error; scanning two tags simultaneously causes antenna interference and neither tag is recorded. We were aware of this limitation, so each deceased smolt was scanned individually. We believe the error in mortality tracking was small (<1%) because the mortality rates of untagged to tagged fish in the same raceway were not significantly different (Table 3). Shed tags recovered from the raceways were very low (0.03%). We suspect that shedding rates could have been much higher since we recovered 1.3% shed/broken tags in the more controlled wound recovery experiment.

We tested our shed recovery efficiency in December 2009 by placing twenty-five tags into 4 raceways (41, 53, 65, and 72) via a standpipe (so they were not ingested while falling into the raceways). Four days later, a blind routine magnetic sweeping of the raceways yielded zero tags. After the blind sweeping, we returned to the four experimental raceways and re-swept them with magnets and inspected likely areas for shed tags. These additional efforts recovered zero PIT tags. However, 46% of the PIT tags were detected from raceways 41 and 65 with the PIT tag array. We hypothesized that the loose PIT tags were ingested and lodged in the intestinal tract. Some of the ingested tags may have occurred in already tagged smolts or smolts that ingested multiple tags effectively cancelling both PIT tags. Ingestion could explain the lack of shed tag recoveries and some of the lower detection rate.

Costs of installing PIT tags with single-use and multi-use needles were compared superficially. Costs of single-use-injectors included $.31 per injector, plus per diem, and operating costs. Personnel and equipment costs for multi-use needles appeared similar. Determining exact costs for PIT tag insertion using multi-use needles is difficult because PIT tagging at Hagerman is only a small part of the IDFG fish marking (CWT, PIT, Ad-clipping) program. Adding costs of each part of the program across multiple hatcheries may not sum to the total marking program cost without additional effort.

Both PIT tagging methods worked well and produced low shed rates and high survival rates (96%). The single-use needle method did produce cleaner, narrower, and more consistent incisions. Despite the cleaner incisions, we did not find significant differences in shedding rates or overall survival. Wound healing time was not significantly different in our analysis, but additional observations during days 7 to 14 post tagging would have likely discerned a quicker healing time for single-use needles. We base this assumption on the greater percentage of healed wounds from clean incisions by 7 days post tagging. The clean incisions of the single-use method should be a motivation to continually evaluate the sharpness of multi-use needles when deciding to replace and or re-sharpen. Sharp needles produce cleaner incisions which should result in faster healing rates.

Acknowledgments

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Tables and Figures

Table 1. PIT tagging of steelhead, Hagerman National Fish Hatchery, BY2006-2009

Brood Year	Marking Dates	PIT Tagged Smolts	Fish Per Pound	Length (mm)	Total Mortality Scanned	PIT Tags Recovered	PIT Rwys (66 ttl)
2006	February 2007	1500	5.5	200	n/a	7	5
2007	February 24-26, 2008	31,070	5.9	196	12,743	1,532	15
2008	January 6-8, 2009	26,832	9.3	168	34,446	1,883	25
2009	November 17-19, 2009	28,982	30.8	113	22,787	1,164	21

Table 2. Percent of PIT tag wounds healed by needle type and mean wound length and width of unhealed steelhead smolts, PIT Tag study, Hagerman National Fish Hatchery BY2009.

	Single-Use Needles			Multi-Use Needles		
Days Post PIT Tagging	Mean wound length (mm)	Mean wound width (mm)	Healed (%)	Mean wound length (mm)	Mean wound width (mm)	Healed (%)
1	2.45[z]	0.52[m]	0.0[a]	2.55[z]	0.86[o]	0.0[a]
7	2.41[z]	0.73[n]	32.7[b]	2.45[z]	1.00[o]	32.7[b]
14	*	*	96.5[c]	*	*	98.0[c]
21	*	*	99.1[c]	*	*	99.3[c]
28	*	*	100.0[c]	*	*	99.3[c]

* Means were not included in because sample size was insufficient

**35 smolts in the single-use group and 2 smolts in the multi-use group were excluded because the tag incision was not completely visible in the photo for measurement

Table 3. Survival rates of single-use needle, multi-use needle, and non-PIT tagged steelhead smolts at Hagerman National Fish Hatchery, BY2009.*

	Single-Use Needles				Multiple-Use Needles				Non-PIT Tagged
Race way	Mort ality	Sheds Tags	Total Tags	Surviv al	Morta lity	Sheds Tags	Total Tags	Surviv al	Survival
38	14	0	592	97.6%	21	1	594	96.5%	95.2%
41	7	0	595	98.8%	13	0	592	97.8%	97.4%
44	3	0	596	99.5%	3	0	595	99.5%	99.3%
47	10	0	594	98.3%	15	0	598	97.5%	97.4%
59	14	0	593	97.6%	15	1	596	97.5%	96.2%
65	17	0	597	97.2%	13	0	597	97.8%	97.0%
67	15	0	595	97.5%	13	0	595	97.8%	95.9%
82	36	0	594	93.9%	30	0	598	95.0%	93.8%
85	29	0	591	95.1%	34	0	695	95.1%	93.2%
92	30	0	599	95.0%	42	0	693	93.9%	93.4%
94	28	0	596	95.3%	47	0	596	92.1%	94.5%
AVG	18	0	595	96.9%[a]	22	0	614	96.4%[a]	95.8%[a]
TTL	203	0	6542		246	2	6749		

[a]No significant difference ($p > 0.05$) in mortality rate between two tagging methods.
*Mortality Tag codes from March 16 - 27, 2010 were lost from the Destron-Fearing ™ FS2001F-ISO PIT reader due to back-up battery failure.

Table 5. PIT tag array detections of steelhead PIT tagged by single-use or multi-use needles, Hagerman National Fish Hatchery, BY2009.*

	Single-Use			Multi-Use		
Raceway	Found	Expected**	% Detected	Found	Expected**	% Detected
41	581	588	98.81	572	579	98.79
44	557	593	93.93	548	592	92.57
47	529	584	90.58	534	583	91.60
65	525	580	90.52	523	567	92.24
82	501	558	89.78	530	568	93.31
85	483	562	85.94	562	661	85.02
92	492	569	86.47	570	651	87.56
AVG			90.86[a]			91.58[a]

[a]Not significantly different ($p > 0.05$)

*Tag codes from March 16 - 27, 2010 were lost due to back-up battery failure.

**Expected tags = Total Tagged Smolts - (Shed Tags + Mortality)

Table 6. Results Fish Health Exams of BY09 summer steelhead from raceways PIT tagged with both single-use and multi-use needle methods, Hagerman NFH.

	17-Dec-09	17-Dec-09	17-Dec-09	17-Feb-10	17-Feb-10	10-Mar-10
Obs.	Raceway 37,39,41,47,48,52	Raceway 61,62,67,71,79	Raceway 81,86,89,91,93	Raceway 41	Raceway 81,82	Raceway 37,38,39
External	Saddle back, fungus eyes	Saddle back	Saddle back	Severe lesions, fungus, no skin left on fish, no bacteria or fungus in lesion	Discoloration of skin, lesions, caudal fins severly eroded	Lesions on skin, bad eyes, frayed tails, gyrodactylus
Gills	Pale and anemic	Bad	Normal	Gas bubbles, swollen, clubbed, and irritated	Gas bubbles	Necrotic gill, small amount of fungus and bacteria
Internal	Swollen kidneys, enlarged spleen, hemoraged intestine and visceral fat	Swollen kidneys, enlarged spleen, hemoraged intestine and visceral fat	Swollen kidneys, enlarged spleen, hemoraged intestine and visceral fat	Swollen kidneys, enlarged spleen, hemoraged intestine	Swollen kidneys, enlarged spleen, hemoraged intestine and visceral fat, spleen and fat soft and mushy	Swollen kidneys, enlarged spleen, hemoraged intestine and visceral fat
Bacterial	Aeromonas salmonicida	Aeromonas salmonicida	Aeromonas salmonicida	Aeromonas salmonicida, Flavobacterium columnare, Aeromonas hydrophila	Flavobacterium columnare, Aeromonas hydrophila	Flavobacterium columnare, Cold Water Disease
PCR	neg	Nucleospora salmonis	Nucleospora salmonis	Nucleospora salmonis	Nucleospora salmonis	Nucleospora salmonis

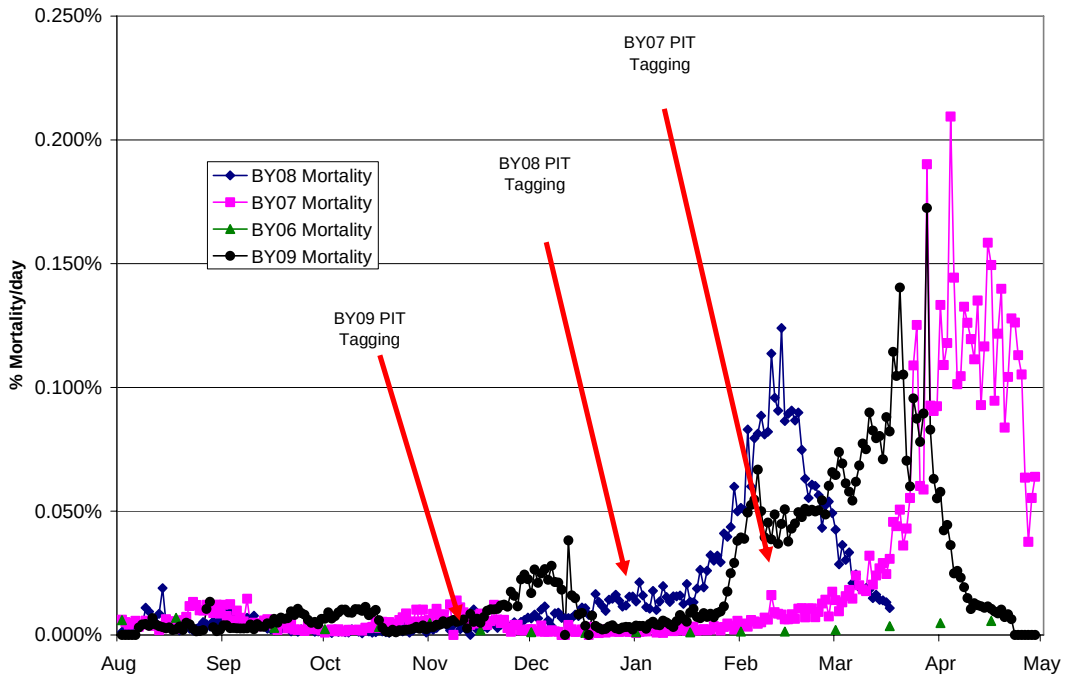


Figure 1. Daily steelhead mortality, Hagerman National Fish Hatchery, BY06-09.

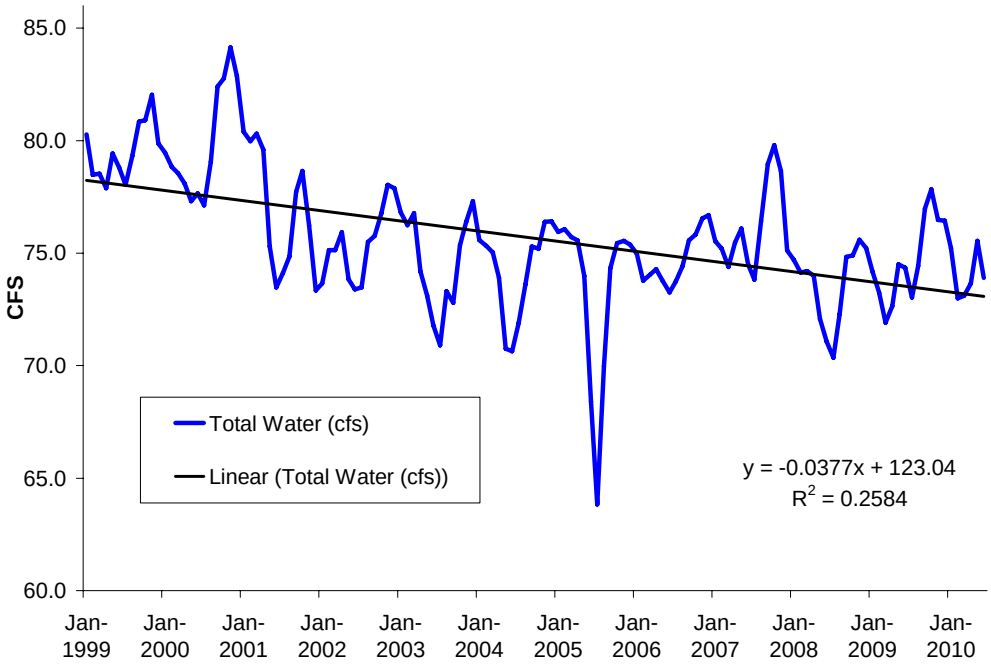


Figure 2. Total spring water discharge tributary to Riley Creek, Hagerman National Fish Hatchery, 1999-2010.

Appendices

Appendix 1. PIT Tag Placement for BY2009 Summer Steelhead, Hagerman National Fish Hatchery.

37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58
S	S	S	S	S	S	S	S	S	S	S	S	YF	YF	YF	YF	YF	YF	YF	YF	YF	YF
L1	L1	L1	L1	L3	L3	L5	L5	L5	L6	L7	L7	L1	L2	L2/3	L3	L3	L3/4	L4	L5	L5	L6
25k	25k	25K	22K	25k	25k	25K	25K	25K	25K	20K	20K	23k	23k	23k	23k	23k	23k	23k	24K	24k	23k
Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad	NoAd	NoAd	NoAd	NoAd	NoAd	NoAd	NoAd	NoAd	NoAd	NoAd
		CWT							CWT												
LD	LD	LD	LD	LD	LD	ND	ND	ND	ND	ND	ND										
	PIT			PIT			PIT			PIT			PIT			PIT			PIT		
	1200			1200			1200			1200			1200			1300			1300		
	Pre			Pre			Pre			Pre											
	Wd									Wd											
24692	24987	24997	22206	24693	24694	25002	24692	24688	24689	20278	20315	22906	22956	22966	23675	23871	22891	22867	24669	24489	23868

59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
S	S	S	S	S	S	S	S	S	EF	EF	EF	YF	YF	YF	YF	YF	YF	YF	YF	YF	YF
L3	L3	L3	L3	L3	L3	L4	L4	L4				L2	L2/3	L3	L3	L3/4	L4	L4	L5	L5	L6/7
24K	21K	24K	24K	24K	21K	24K	24K	24k	19K	19K	17K	23K	23k	23k	23k	23k	23K	22K	22K	22K	17K
Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad	NoAd	NoAd	NoAd	Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad
									CWT	CWT	CWT	CWT			CWT		CWT			CWT	
LD	LD	LD	LD	LD	LD	ND	ND	ND				ND	ND	ND	ND						
PIT						PIT		PIT		PIT			PIT			PIT			PIT		
1200						1200		1200		2400			1200			1300			1300		
Pre						Pre		Pre													
Wd						Wd															
23992	20631	23993	24000	23998	20882	24127	23993	23998	19487	19238	16818	22999	23060	23015	22978	22982	22907	21771	20994	21186	17252

81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102
S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	TR	TR	TR	EF	EF	EF	EF
L2	L2	L2/3	L3	L3	L3	L3	L4	L4	L4	L5	L5	L5	L6	L7	L1/2	L4	L7				
			CWT							CWT						CWT		CWT	CWT	CWT	CWT
23k	23k	23k	23K	23k	23k	23k	23k	23k	23k	23K	23k	23k	23k	23k	23k	23K	25K	19K	19K	16K	16K
Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad	Ad	NoAd	NoAd	NoAd	NoAd
	PIT			PIT							PIT		PIT		PIT			PIT		PIT	
	1200			1300							1300		1300		1000			2300		2400	
	Pre			Pre							Pre		Pre								
	Wd										Wd										
23003	22987	23034	22993	22989	22983	23002	22997	22994	23008	22989	22995	23005	23008	22989	22993	22991	24690	19133	19234	18084	16578

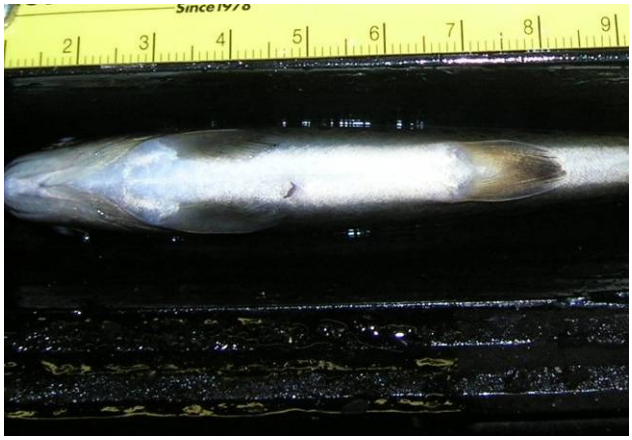
S	Sawtooth (4/15-30/2010)	LD	Low Early Rearing Density		
EF	East Fork Naturals (5/3-4/2010)	ND	Normal Early Rearing Density (Control)		
YF	Yankee Fork (5/4-15/2010)	Pre	Pre-loaded PIT study		
TR	Tunnel Rock Release	Wd	Wound retention smolts (50 each)		
CWT	Coded Wire Tagged	PIT	PIT Tagged		
Ad	Ad Clip				

Release Site	Inventory	PIT
East Fk. Salmon R.	126,572	7100
Tunnel Rock	70,534	1000
Sawtooth Weir	842,295	13500
Yankee Fk.	454,312	7600
Total	1,493,713	29200

Appendix 2. Photographs post PIT tagging BY2009 Summer Steelhead, Hagerman NFH

Day One Single-Use PIT Needle

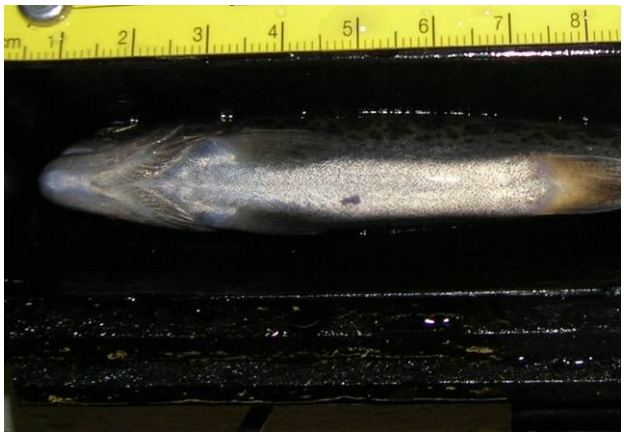
Day One Multiple-Use PIT Needle



Day Seven Single-Use PIT Needle

Day Seven Multiple-Use PIT Needle

Day Fourteen Single-Use PIT Needle

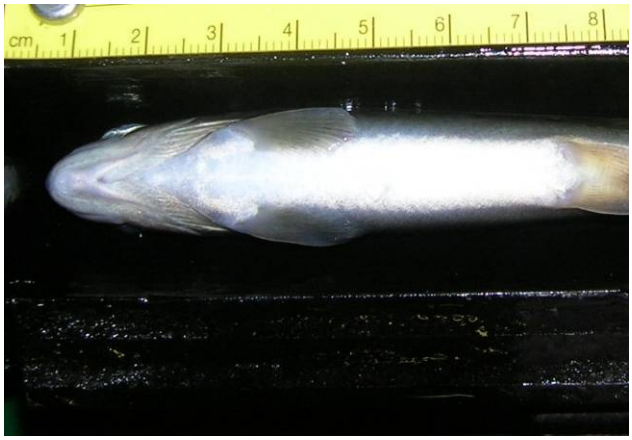


Day Fourteen Multiple-Use PIT Needle

Appendix 2 cont.. Photographs post PIT tagging BY2009 Summer Steelhead, Hagerman NFH

Day Twenty-One Single-Use PIT Needle

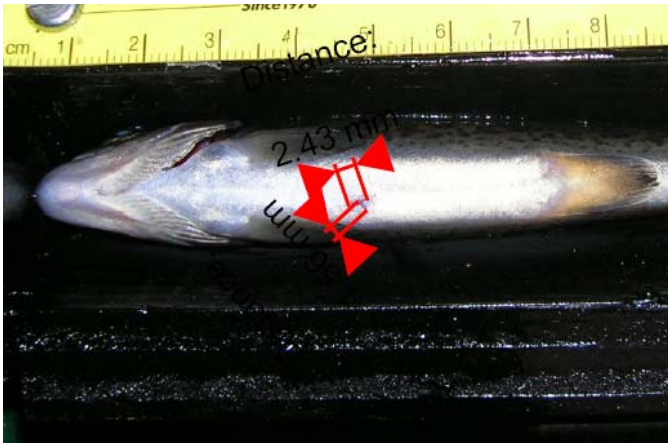
Day Twenty-One Multiple-Use PIT Needle



Day Twenty-Eight Single-Use PIT Needle

Day Twenty-Eight Multiple-Use PIT Needle

Example Single-Use PIT Needle Measurement



Example Multiple-Use PIT Needle Measurement

Appendix 2 cont.. Photographs post PIT tagging BY2009 Summer Steelhead, Hagerman NFH

Example of Clean Incision 1 Day Post PIT Tagging with Single-Use Needle Method

Example of Clean Incision 1 Day Post PIT Tagging with Multiple-Use Needle Method

Example of Ragged Incision 1 Day Post PIT Tagging with Single-Use Needle Method

Example of Ragged Incision 1 Day Post PIT Tagging with Multiple-Use Needle Method

Example of Bruised Incision 1 Day Post PIT Single-Use Needle Method

Example of Bruised Incision 1 Day Post PIT Tagging Multiple-Use Needle Method

Appendix 2 cont.. Photographs post PIT tagging BY2009 Summer Steelhead, Hagerman NFH

Example Protruding Tag 28 Days Post Tagging Multiple-Use Needle Method

Example of Photographed Fish Excluded from Study Because Wound is not visible

Example of Incision Wound that Led to Tag Shedding of Single-Use Needle Method

Example of Incision Wound that Led to Tag Shedding of Multiple-Use Needle Method

Example of Incision 1 Day Post Tagging, Not Healed, Single-Use Needle Method

Example of Incision 7 Days Post Tagging, Healed, Single-Use Needle Method

Appendix 2 cont.. Photographs post PIT tagging BY2009 Summer Steelhead, Hagerman NFH

Example of Incision 1 Day Post Tagging, Not Healed, Multiple-Use Needle Method

Example of Incision 7 Days Post Tagging, Healed, Multiple-Use Needle Method