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Assessing Juvenile Salmonid Passage Through Culverts: Field Research in Support of Protocol Development

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Sequim, Washington

September 2001

Prepared for the
Washington State Department of Transportation
Research Office; Olympia, Washington
under a Related Services Agreement
with the U.S. Department of Energy
under Contract DE-AC06-76RLO 1830

Pacific Northwest National Laboratory
Richland, Washington 99352

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Protocol Development for Field Assessment of Juvenile Salmonid Passage through Culverts

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Executive Summary

Under guidance from the Washington State Department of Transportation (WSDOT), the Battelle Marine Sciences Laboratory is conducting research developing a standardized field protocol to quantitatively assess juvenile fish passage through road culverts in the field. Ultimately, validated field estimates of juvenile passage will provide an inventory of culverts with specific passage deficiencies, and will enable WSDOT to correct "problem" culverts. This report summarizes findings from field studies conducted April through July, 2001 designed to refine techniques and examine field situations under which a standardized protocol could be applied to assess juvenile coho salmon (*Oncorhynchus kisutch*) passage through road culverts.

Research was guided by preliminary reviews of the fish passage literature (Annotated Bibliography SOW Task 1), likely field locations (Test Site Selection SOW Task 2), and appropriate sampling methods (Comparison of Measurement Techniques SOW Task 3). Pilot studies of field techniques were conducted in Snow and Andrews Creeks during April and May 2001, and actual protocol trials began in May 2001 at two sites (Parish and Dogfish Creeks) with culvert retrofits and large populations of resident juvenile coho salmon. Field evaluations focused on capture-mark-recapture experiments that allowed complex analysis of fish movement patterns and identification of cues inducing these movements.

The availability and abundance of juvenile coho in potential testing locations was one of the primary factors driving study site selection. Although a number of factors were necessary for experimental manipulations to be of net benefit, many of the potential study sites did not appear to have adequate numbers of juvenile coho to justify testing protocol designs. Unfortunately, the unavailability of current information on coho presence/absence and abundance for any particular stream led to front-loaded delays in planning time and effort. Furthermore, potential study locations were characterized by a wide variety of stream sizes, habitats, and culvert configurations.

A number of capture methods, including dip nets and seines, resulted in high catch rates of juvenile coho salmon for marking. These collection methods were rapid (averaging 115 fish/hr combined) and could be accomplished in most field situations; trials provided estimates of relative capture efficiency with each method. Other collection methods were deemed inappropriate due to very low capture efficiency and CPUE rates (minnow traps) or possible evidence of behavioral modification and injury (electrofishing).

Long-term marking techniques provided the best approach to tracking fish movement within a stream. Small (<35 mm FL), recently emerged age 0+ coho were marked successfully using subcutaneous injection of photonic dyes, whereas larger fish (>75 mm FL) were marked even more rapidly using devices that employed a burst of compressed air to lodge dye under the skin. Over a 5-week monitoring span, these subcutaneous dyes were retained extremely well and provided unquestionable evidence of mark presence or absence.

A combination of direct collection and observation techniques provided the most reliable detection of marked fish in a variety of stream habitats. A total of 729 marked 0+ coho were collected over 42 recapture attempts, encompassing both study sites and marking techniques. Dip nets and seines yielded large catches of juvenile coho salmon in most stream habitats, with recapture rates of up to 64% within release reaches. Snorkel surveys supplemented information derived from recapture attempts, and provided valuable information on the abundance and distribution of coho and other species within, and in proximity to, the test culverts. Another observational technique, underwater videography, proved to be an extremely useful tool for evaluating behavioral effects after marking and in the vicinity of traps or culverts.

Recapture rates of marked fish were highest in reaches where they had been previously released, regardless of mark type or recapture technique. However, only 10 fish (<2%) were recaptured outside of their initial marking reach, resulting in few instances where movement metrics could be calculated. Monitoring from May 21 to July 19 resulted in one confirmed incident of upstream movement at Dogfish Creek, and two of downstream movement at Parish Creek. In part, study timing (two months in early summer) and frequency may have been inadequate to encompass high movement periods in these coastal streams.

In general, increasingly complex study designs can yield greater information on fish movement, with related increases in time, effort, and cost. Further consultation from WSDOT is necessary to balance these limitations with the desired level of knowledge to determine optimal protocol study designs.

The following recommendations are offered to facilitate refinement and application of the final protocol design:

1. Before testing, acquire an understanding of site conditions and fish presence/absence from a comprehensive source.
2. A standard, yet flexible sampling design (e.g., a suite of methods) must be used that is transferable to a variety of locations and situations.
3. Use rapid collection methods that involve minimal handling and stress.
4. Use subcutaneous injection to mark fish permanently, assigning various colors and fins to code for release locations and mark dates.
5. For recapturing marked fish, use a combination of dip net and seining methods with estimated capture efficiencies.
6. Direct observation techniques using snorkel surveys should be integrated with primary recapture methods into any final protocol design.
7. Additional work should be pursued to identify or design video systems and image recognition technology that can be used to quantitatively evaluate fish passage at culverts.
8. Continue monitoring of permanently marked fish periodically throughout the year to encompass periods of high movement.
9. Base inferences of movement upon the percentage of marked fish recaptured, not the percentage of recaptures in home reaches.
10. When planning the layout of a proposed study site, habitat characteristics (e.g., riparian cover, stream habitat distribution) should be fairly similar between study reaches. Sampling methods and effort should be consistent between sampling reaches.
11. Use standardized data forms to facilitate complete data collection and comparability of data between sites.

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INTRODUCTION

Problem Description

The passage of juvenile salmon (*Oncorhynchus* spp.) through culverted road crossings is a significant Endangered Species Act (ESA) issue throughout the Pacific Northwest, with related implications for other aquatic species throughout the country. Much of recent research and engineering has focused on increasing passage of returning adult salmon (see Bates et al. 1999). However, stream-dwelling salmonid juveniles are highly mobile, and in-stream movement and dispersal is critical to growth and survival (Kahler and Quinn 1998). Because a large percentage of the existing culverts beneath roads in the Pacific Northwest are judged as blocking juvenile salmon from thousands of miles of potential rearing habitat, determining the barrier status of culverts has both substantial environmental implications and costs.

Existing assessment protocols use a combination of hydraulic and physical habitat measurements to assess culverts as possible fish passage barriers (Ralph 1990, Washington Department of Fish and Wildlife 2000). At best, these protocols rely upon physical models (e.g., Love 2000), laboratory-measured swimming capabilities of adult and subadult fish, and/or best professional judgment. However, no standardized field protocol has been developed for biologically evaluating the passage success of juvenile salmonids through road culverts.

Goals and Objectives

The Washington State Department of Transportation (WSDOT) has developed a strategic plan to identify regional needs for juvenile fish passage and culvert-related research (http://www.wsdot.wa.gov/ppsc/research/salmon.htm). A principle objective of this plan is the development of a standardized field protocol to quantitatively assess juvenile fish passage through road culverts in the field. Field estimates of juvenile passage will provide an inventory of culverts with specific passage deficiencies, and will enable retrofit programs to correct "problem" culverts (Johnson et al. 2001). Fish passage under various culvert designs, slopes, and flow regimes in the field may also be validated in the future by reproducible experiments in culvert test bed facility, currently under development.

The primary goal of our research in spring/summer 2001 was to refine techniques and examine field situations under which a standardized protocol could be applied to assess juvenile coho salmon (*Oncorhynchus kisutch*) passage through road culverts. Field evaluations focused on capture-mark-recapture methods that allowed analysis of fish movement patterns, estimates of culvert passability, and potential identification of cues inducing these movements. At this stage, 0+ age coho salmon fry 30 mm to -65 mm long (fork length) were the species and age class of interest. Ultimately, the protocol will provide rapid, statistically rigorous methods for trained personnel to perform standardized biological assessments of culvert passability for a number of juvenile salmon species. Questions to be addressed by the research include the following:

- Do hydraulic structures such as culverts restrict habitat for juvenile salmonids?
- How do existing culverts and retrofits perform relative to juvenile salmonid passage?
- Do some culvert characteristics and hydraulic conditions provide better passage than others?
- Does the culvert represent a barrier to certain size classes of fish?

The WSDOT-sponsored effort parallels concurrent research programs by federal agencies, as well as Alaska and Oregon Departments of Transportation (ADOT and ODOT, respectively) to assess fish passage at road crossings in the field. In some cases, these programs are focusing on different species or age classes of fish, under unique environmental conditions. For example, Oregon State University, under contract with ODOT, has been studying movement of resident cutthroat trout greater than 90 mm predominantly, because of limited availability of other species (Gregory and Klingeman 2000). University of Alaska Fairbanks has also been studying culvert passage issues for several years under contract with ADOT, and has recently completed studies of juvenile coho salmon movement through culverts (Kane et al. 2000). The Northwest Indian Fisheries Commission has also developed standardized culvert monitoring and research program guidelines (Cupp et al. 1999), although data are not currently available from these field efforts.

STUDY SITE SELECTION

Culverts for testing protocol procedures were selected as described within the test-site selection report (Statement of Work [SOW] Task 2). This site evaluation was based on a variety of criteria, which included some measure of coho salmon relative abundance, a brief assessment of stream and culvert conditions, and proximity to the Battelle Marine Sciences Laboratory (MSL). Juvenile coho presence and abundance was obtained from a variety of sources, including the Washington Department of Fish and Wildlife (WDFW) stream catalogs, StreamNet, WSDOT Fish Passage Barrier Removal Program progress reports (Johnson et al., 2001), and personal communications with WSDOT/WDFW biologists (e.g., Larry Cowan, Alex Uber, Randy Cooper). Culvert retrofit sites in the Olympic Region were input to a selection matrix that lists various descriptive site and culvert parameters (Appendix A). In addition, data from several status "unknown" sites and some planned retrofit sites known to have coho present were also compiled. Distance from MSL was also considered to maximize field-study efforts, and was calculated from Johnson Creek, which crosses West Sequim Bay Road and intersects Highway 101 at mile point 267.2. Individual site visits were conducted to verify the accuracy of collected data and to visually assess the site for appropriateness of intensive testing of the protocol.

Based on the criteria outlined above, two sites in Kitsap County were selected for protocol development: Dogfish Creek near Poulsbo and Parish Creek near Gorst. In addition, Snow and Andrews Creeks near Discovery Bay in Jefferson County were used for pilot studies comparing capture methods and marking techniques, as well as early development of the protocol concepts.

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Dogfish Creek

Dogfish Creek drains a 5.08-square-mile watershed into Liberty Bay, Kitsap County, Washington (Figure 1). The creek is used by Chinook (*Oncorhynchus tshawytscha*), chum (*O. keta*), and coho salmon, as well as cutthroat trout (*O. clarki*) (Washington Department of Fish and Wildlife 1981). The Dogfish Creek/Highway 305 road crossing (mile point 12.8) was retrofitted with a second parallel culvert during widening of Highway 305 in 1997-1998. The

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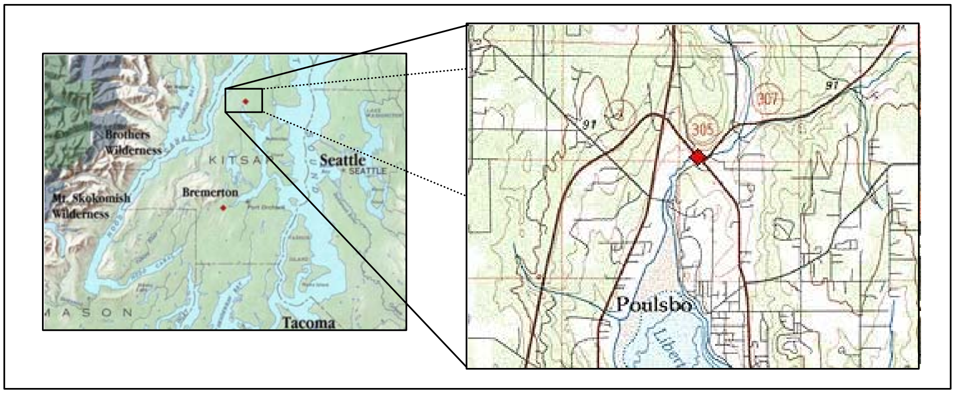

Figure 1. Location of Dogfish Creek Highway 305 road crossing in Kitsap County, Washington.

original culvert was a 1.52-m diameter x 36.5-m smooth concrete pipe. The retrofit is a 1.49-m diameter x 36.5-m smooth steel pipe (Appendix B). The new culvert is also 0.42 m lower in elevation, and is partially embedded with natural material (Figure 2). During the course of protocol development, the lower culvert was the only pathway used for downstream water flow, although the old culvert still functions during higher flows (Figures 2 and 3). Prior to retrofit installation, this culvert was considered to be a fish-passage barrier, estimated at 75% passability by WDFW field crews (Johnson et al., 2001). Just over 100 meters upstream of the Highway 305 crossing, Dogfish Creek also crosses Highway 307. The upstream culvert is considered a barrier with 67% passability (Johnson et al., 2001), and juvenile coho appear to be abundant beyond it (visual assessment).

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Figure 2. Dogfish Creek culvert inlet (left photo) and outlet (right photo), June 2001.

Figure 3. Dogfish Creek culvert inlet (left photo) and outlet (right photo), March 1999. Courtesy of Washington State Department of Transportation.

Parish Creek

Parish Creek drains a 1.67-square-mile watershed into Gorst Creek, a tributary to Sinclair Inlet, in Kitsap County, Washington (Figure 4). Parish Creek is used by coho salmon and cutthroat trout (StreamNet Website 2001). Spawning and rearing habitat is available until river mile 1.86, above which the creek regularly runs dry. The Parish Creek/Highway 3 road crossing (mile point 33.7), located at river mile 0.53, was retrofitted with a fishway and baffles in 1992 in order to provide fish passage. The culvert consists of a 1.83-m-high x 2.13-m-wide x 61.59-m-long concrete box, with elliptical (2.74-m-high x 2.44-m-wide) corrugated steel pipe on either end; the total length of the culvert is 96.95 m (Figure 5, Appendix C). Within the box culvert is a series of 33 angled, steel baffles, each covering approximately 79% of the culvert width (Figure 6). Water flows freely through the remaining channel on the left bank, while low-velocity pools are created behind each baffle. The outlet pipe contains 8 vee-notch baffles that form plunge pools

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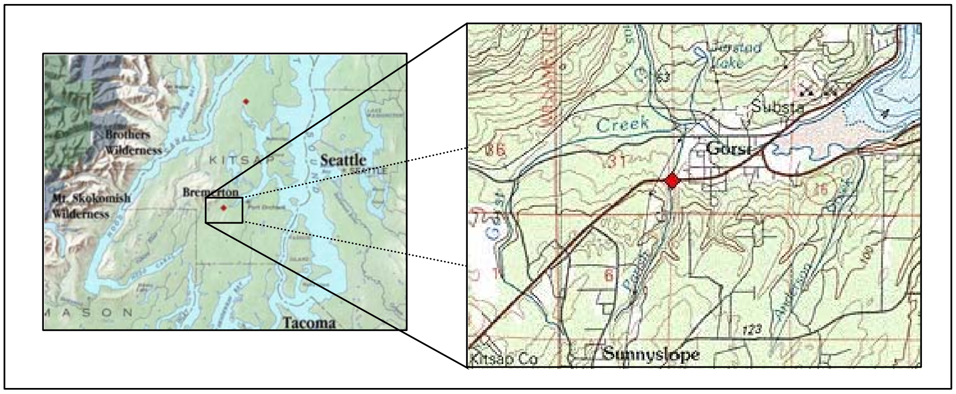

Figure 4. Location of Parish Creek Highway 3 road crossing in Kitsap County, Washington.

Figure 5. Parish Creek culvert inlet (left photo) and outlet (right photo), June 2001.

with increased water depth, creating a fishway at the downstream end (Figure 6). The outlet of the culvert is partially submerged in the downstream pool, with very slight backwater influence. It appears that a small outfall drop may be created at very low flows. Juvenile coho are very abundant downstream, and are present to a lesser extent upstream of the culvert. Prior to retrofit installation, the culvert was rated at 0% passability (Johnson et al., 2001).

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Snow/Andrews Creeks

Snow Creek drains an 11.2-square-mile watershed into Discovery Bay in Jefferson County, Washington (Figure 7). Andrews Creek, a tributary to Snow Creek, drains 10.2 square miles. Both creeks are used by coho and chum salmon, and cutthroat and steelhead trout (*O. mykiss*) (Washington Department of Fish and Wildlife 1981, StreamNet Website 2001). In these two streams, WDFW operates remote site incubators (RSIs) for wild coho salmon, which are captured and strip-spawned at a weir downstream. These sites were also the subject of a concurrent trapping study to determine juvenile coho upstream movement characteristics (Southard 2000, Southard et al., 2001). Due to the abundance of juvenile fish, Snow and

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Andrews Creeks provided an opportune location for pilot testing of methods. In addition, a small non-barrier road crossing (two parallel, round, embedded culverts, each 1.3-m diameter x 20-m length) is located at the convergence of Andrews and Snow Creeks under Highway 101, at mile point 285.7 (Figure 8).

Figure 6. Photo of baffle and wier configurations inside Parish Creek culvert.

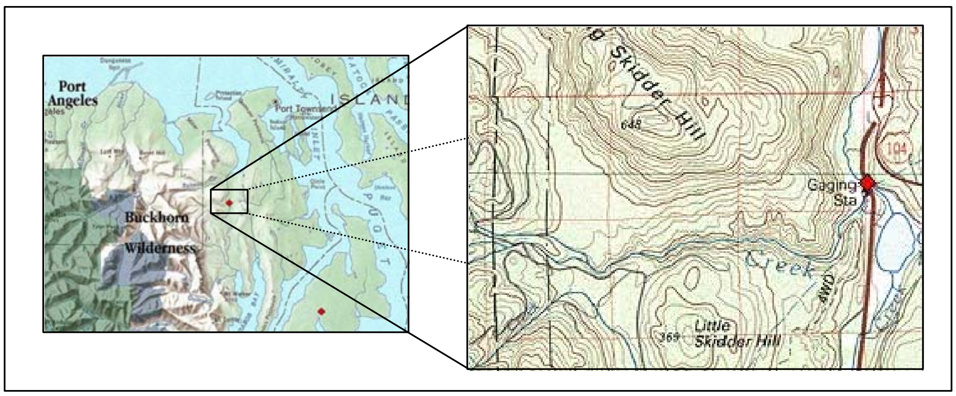

Figure 7. Location of Snow/Andrews Creek Highway 101 road crossing in Jefferson County, Washington.

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The field activities undertaken for methods evaluation and protocol design development are summarized below (Table x.). Brief descriptions are given in order to illustrate the evolving nature of the field work. ¶
Table x. Summary of activities during protocol development.¶
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Figure 8. Snow/Andrews Creek convergence and culvert inlet (Andrews Creek, left photo) and outlet/convergence (right photo), August 2001.

APPROACH

Initial Capture

In our review of animal movement studies (SOW Task 3), capture-mark-recapture methods allowed complex analysis of fish movement patterns and identification of cues inducing these movements (e.g., Warren and Pardew 1998, Kane et al. 2000, Riley et al. 1992). As a result, our field evaluation focused on associated capture techniques. Criteria that guided the selection of initial capture methods included ability to collect adequate quantities of fish, time of deployment, cost, fish handling time, and effects on fish behavior.

Based on these criteria, initial fish capture techniques relied upon minnow trapping, dipnetting, and seining. Electrofishing posed risks of injury and behavioral alteration that were considered too high to use this technique during initial capture, although a small electrofishing and marking pilot study was incorporated into a capture efficiency calibration experiment.

During all fish capture activities, fish were moved immediately from the collecting device into an aerated bucket, which was kept in the flowing stream to maintain water temperatures.

Minnow Traps

Based upon behavioral observations noted by Kane et al. (2000), the initial stages of protocol development used standard cylindrical Gee minnow traps baited with salmon roe as a primary capture method. Traps were allowed to fish for one-half hour to an hour in pools or low-velocity areas. Trap openings were restricted to 1 cm to reduce entry by larger fish, and traps were at least two-thirds submerged in capture locations.

Pilot studies at Snow Creek examined catch rates of juvenile coho salmon using minnow traps based on the following factors: bait type (bread, salmon roe, or none), frequency of trap check (0.5 and 3.0 hour), and time of day (time period A: 1010-1240; B: 1457-1720; C: 1900-2030; D: 2230-0100). Analysis of variance tests showed that none of these factors significantly affected catch rates ($p<0.05$) (Figures 9 and 10).

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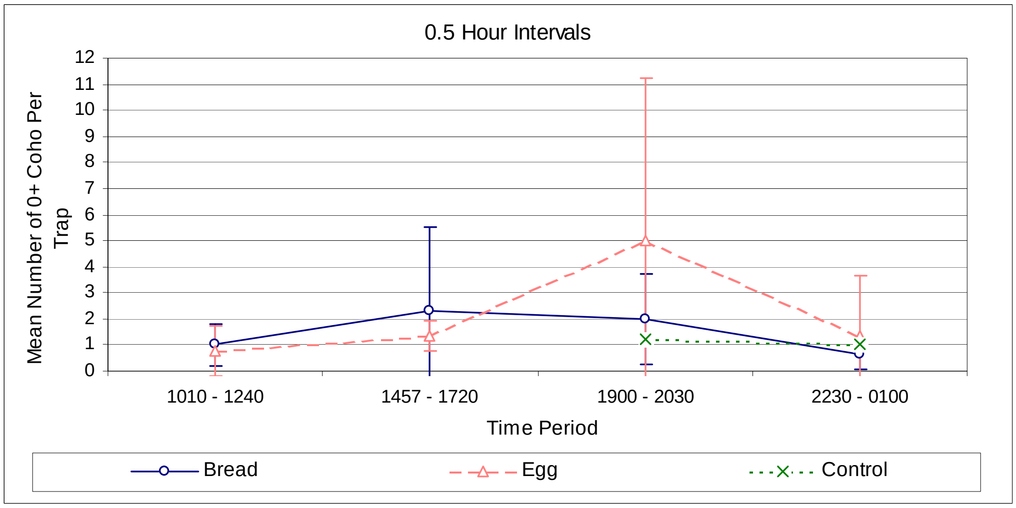


Figure 9. 0+ Coho captures in minnow traps at Snow Creek checked at 0.5 hr intervals; error bars represent $\pm$1 standard error (SE).

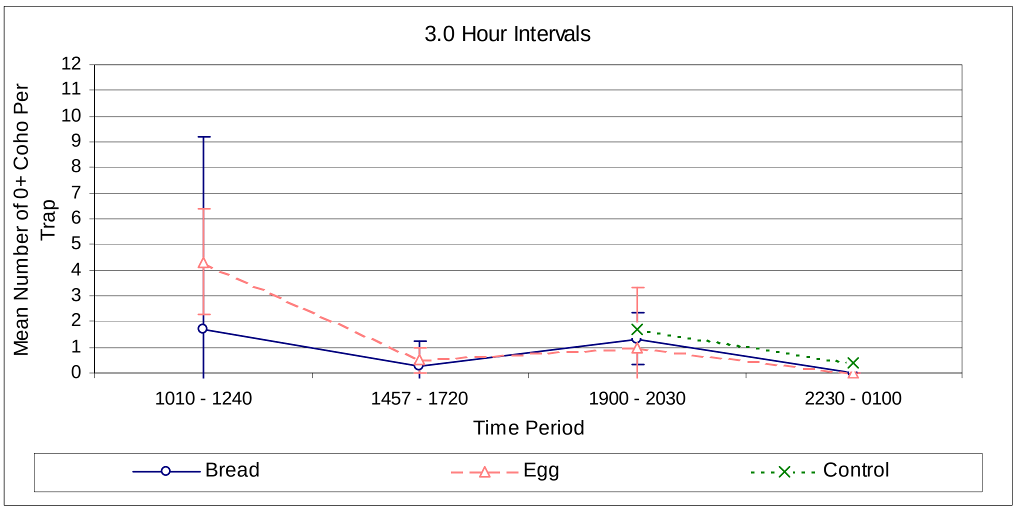


Figure 10. 0+ Coho captures in minnow traps at Snow Creek checked at 3.0 hr intervals; error bars represent $\pm$1 SE.

Dip net

A 30- x 45-cm rectangular rim dip net (90-cm bag depth) was used for most fish capture during protocol development, especially in small streams with numerous obstructions. An operator (using polarized glasses when appropriate) would fish the net slowly through pools and along banks, targeting 0+ coho visible in these areas. Dip netting was repeated at each location until catch rates declined.

Seine

Seining was done using a small bag seine (1 m deep x 4 m wide x 2.7 m bag depth). Bag mesh was 3-mm square delta, with a wing mesh of 6-mm. The seine was spread across the stream section to be sampled, and swept through the water by two operators manning poles on either end. Small sweeps of the seine were done to reduce the possibility of catching more fish than could be safely handled in one capture event. When a specified portion of the reach had been covered, the net was pulled to the bank and fish removed. This process was repeated at each location until catch rates declined substantially.

Electrofishing

The relative capture efficiency of minnow traps and dipnetting was compared with that of electrofishing during a pilot study in Parish Creek on June 27, 2001. A 51.5-m reach of stream above the Parish Creek culvert was block-netted, divided into sections by habitat type (pool/riffle, approximately 10 m each), and sampled with each method. Snorkel surveys were done first to identify the effectiveness of snorkeling as an observational technique, and to establish habitat relationships within the reach. Following the snorkel survey, minnow traps (eight total) were placed in each habitat type and allowed to fish for one hour. Captured fish were measured and released back into respective capture locations. Dipnetting was then done in each section, with captured fish also measured and released back into respective capture locations. Finally, two-pass electrofishing was done to remove all possible fish from the reach. Capture numbers for each method were then compared with the total number of fish removed through electrofishing.

Fish collected by electrofishing on 6/27/01 were held briefly, given a distinct mark, and released downstream in a location that contained other previously marked fish. The goal was to complement the population of marked fish in this stream while evaluating differences in recapture rate, movement, and behavior that might be associated with electrofishing.

This trial study was completed during ongoing mark-recapture studies, and was positioned above the culvert to minimize behavioral alteration of marked fish, and to maximize the possibility of recapturing fish that had moved into this reach through the culvert.

Marking

Several fish marking methods were evaluated, including tissue stains and subcutaneous dyes. During protocol development tests, only age 0+ coho were marked. Fork length (FL) of all marked fish was recorded to the nearest millimeter in the field, using a small measuring device.

Bismarck Brown Y

Bismarck Brown Y dye was used to stain the tissues of fish an orange-brown tint. Juvenile coho were placed in an aerated dye solution (38 mg dye/L stream water) for between 45 and 60 minutes to allow uptake of the stain. During each staining event, several individuals were retained in sealed minnow traps held in-stream to serve as reference fish for mark retention identification; all others were released directly into the nearest downstream pool. At Snow/Andrews, Parish, and Dogfish Creeks, a total of 1008 coho were stained with Bismarck Brown Y dye and released for protocol testing on 12 separate dates (Table 1). A total of 33 individuals were held as reference fish and released after completion of the tests.

Table 1. Bismarck Brown Marking Activities at Snow/Andrews, Dogfish, and Parish Creeks During Protocol Testing

Site	Reach	Date	Number of Coho Released[a]	Mean Length[b] (fork length, mm)
Snow/Andrews	B	5/8/01	80	n/d
Snow/Andrews	B	5/16/01	15	n/d
Parish	B	5/29/01	40	46
Parish	B	5/30/01	28	43
Parish	B	5/31/01	50	44
Parish	B	6/5/01	101	44
Parish	B	6/7/01	53	46
Dogfish	B	5/21/01	287	34
Dogfish	B	5/29/01	132	38
Dogfish	B	5/30/01	56	40
Dogfish	B	5/31/01	44	42
Dogfish	B	6/5/01	122	42

[a]Number of 0+ coho released with stain, minus mortalities and reference fish held for the duration of the test.
[b]n/d - no data.

Subcutaneous Injection

Subcutaneous dye was also explored as a marking option on small 0+ coho fry using both compressed air and hypodermic needle injection. Photonic dye solutions (latex microspheres suspended in deionized water) were used, which fluoresce when exposed to ultraviolet light. In all cases, fish were first placed in a bath of Tricaine (MS-222) solution (75 mg/L) for approximately one minute to induce sedation, quickly marked on a damp surface, revived in fresh aerated water, and released in an appropriate location.

Dye injection was first attempted in the field with a handheld, spring-loaded device (Micro-Ject BMX 1000) that uses a burst of compressed air to lodge dye under the skin. Based on our trials, the compressed air system resulted in excessive fin damage on fish smaller than 55 mm to 60 mm FL; on larger fish (90-mm to 120-mm FL), the technique provided an efficient and easily recognizable mark. Based on these results, we discarded this technique as a viable marking option.

Fluorescent latex dye was also injected into the base of fins with a hypodermic needle placed just under the skin surface (Figure 11). Coho as small as 31 mm (fork length) were successfully marked using the hypodermic method. During subsequent protocol trials, mark color (orange or green) and anatomical location (anal, dorsal, or caudal fin) were specified as unique identifiers for each sample reach and mark date. At Parish and Dogfish Creeks a total of 364 coho were marked using subcutaneous injection during nine separate marking events on five dates (Table 2).

Figure 11. Subcutaneous injection of photonic dye at the anal fin base of a juvenile coho salmon.

Table 2. Subcutaneous Injection Activities at Parish and Dogfish Creeks During Protocol Testing

Site	Reach	Date	Mark Color and Location	Number of Coho Released[a]	Mean Length (fork length, mm)
Parish	A	6/15/01	Green Anal Fin	48	47
Parish	A	6/28/01	Green Caudal Fin	31	49
Parish	B	6/15/01	Orange Anal Fin	79	49
Parish	B	6/27/01	Orange Anal Fin/Green Dorsal Fin	71	49
Parish	B	6/28/01	Orange Caudal Fin	35	52
Dogfish	B	6/13/01	Orange Anal Fin	27	42
Dogfish	B	6/29/01	Orange Caudal Fin	14	43
Dogfish	C	6/13/01	Green Anal Fin	33	45
Dogfish	C	6/29/01	Green Caudal Fin	26	48

[a]Number of 0+ coho released with mark indicated, minus mortalities.

Recapture/Observation

Recapture of 0+ coho was attempted on multiple dates after the initial marking effort by using three of the methods outlined above: minnow traps, dipnetting, and seining. Each method was deployed in the same manner as that used for initial capture. However, minnow traps were generally allowed to fish overnight during recapture, with checks taking place upon arrival at the study site each day. In most cases, the number, fork length, and sample location of marked and unmarked 0+ coho was recorded.

In addition to these capture methods, observational techniques (snorkel surveys and underwater videography) were used to identify marked fish and view fish behavior without physical handling.

Snorkel Survey

Snorkel surveys were done periodically to provide information on fish abundance, movement, and relative capture efficiency. Observers would swim upstream with a dry suit and snorkel gear, using underwater flashlights for illumination as conditions required. Surveys were done with one snorkeler and one recorder, using two teams operating in different stream sections. Snorkelers would identify and count fish, noting location (meters from reference point) and habitat type (pool, riffle, bank, debris presence, etc.), and relay this information to a recorder on the streambank.

Underwater Videography

Underwater videography was regularly used to provide data on fish behavior after release and on the effects of marking on interactions and movement. A high-resolution color video camera, sealed in a Plexiglass/PVC housing, was deployed for various time periods with a VCR on the streambank recording activity. The camera was typically located in pools above or below each culvert, with the inlet or outlet in view to note fish entering and exiting. Car batteries were used to operate the equipment for up to 6 hours.

Estimating Movement

Two basic study designs were tested during protocol development, and fish-movement data collected from each design were used to calculate relevant movement metrics. The initial design tested (Option 1) consisted of two sample reaches, one above (Reach C) and one below (Reach B) the culvert (Reach Z) (Figure 12, based on Kane et al. 2000). Fish were captured throughout both reaches, marked, and released downstream of the culvert. On successive dates, the upstream reach was sampled to determine whether marked fish had moved through the culvert. Bismarck Brown Y dye was used for all marking during the early, short-term tests of this design.

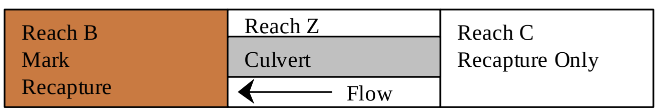


Figure 12. Basic mark-recapture study design (design option 1).

Data on marked 0+ coho that were recaptured outside of their release reach were subjected to the following measures for estimation of movement:

- Percentage passed (# PASSED) - the number of marked fish recaptured above the culvert is expressed as a percentage of total fish marked and released in the reach below the culvert.
- Comparison of means (COM) - mean length of marked fish recaptured above the culvert is compared with that of fish marked and released below the culvert to identify potential size-class barriers imposed by culverts. Fish lengths are subjected to a standard two-sample t-test, and analyzed for statistical differences in the mean.
- Minimum length of passing fish (Min L)- the smallest marked fish recaptured above the culvert is compared with the size range of fish marked and released below the culvert to identify potential size-class barriers.

A second study design (Option 2) was later implemented resembling that recommended by Cupp et al. (1999), and used previously by Bolton and Moss (2001) and Warren and Pardew (1998). This design consists of three sampling reaches (A, B, C), separated by a treatment reach (Z; the culvert) and a natural control reach (N) of equal length (Figure 13). Fish are

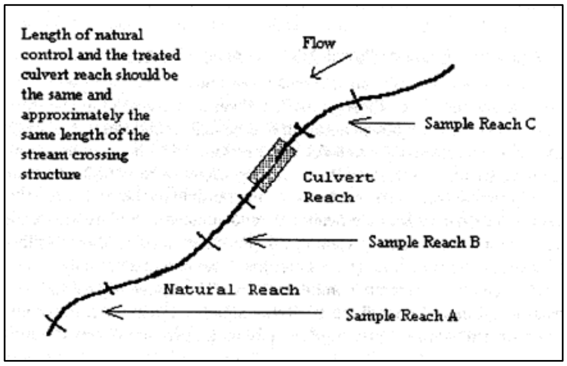


Figure 13. Study design proposed by Cupp et al. (1999).

initially collected, marked, and released in sampling Reaches A and B. All sampling reaches (A, B, C) are then resampled on subsequent dates to recapture marked fish. The control reach (N) provides a reference for determining natural upstream movement of 0+ coho fry through a passable reach of the stream being studied. This information can then provide some index of natural movement that may be compared to movement through the culverted reach (Z).

Due to differences of downstream (reach A) habitat and coho abundance at Dogfish Creek, Design Option 2 differed slightly for each site. As such, the study design for each creek was designated under the Option Subtype 2a or 2b. The Parish Creek design (Option 2a) followed the basic conceptual design presented in Cupp et al. (1999) in terms of study design layout. Standard sampling reach lengths are not indicated in Cupp et al., so we established reaches that were half of the culvert length each, based on available habitat and reasonable sampling effort. The culvert (Z) and natural reach (N) lengths were both 96 meters, and the sampling reaches were approximately 46 meters each (±-1 m to 2 m to correspond to naturally occurring habitat divisions) (Figure 14). At Dogfish Creek (Option 2b), reach A was eliminated, the natural reach (N) was moved above reach C, and an additional sampling reach (D) was located further upstream (Figure 15). The Dogfish Creek culvert is much shorter than Parish, so the treatment, control, and sample reaches all remained the same length, 37 meters.

Reach A - 46 m Mark Recapture	Reach N- 96 m Natural Control	Reach B - 46 m Mark Recapture	Reach Z - 96 m Culvert ← Flow	Reach C - 46 m Recapture Only

Figure 14. Study design used for Parish Creek site (design option 2a).

Reach B - 37 m Mark Recapture	Reach Z - 37 m Culvert ← Flow	Reach C - 37 m Mark Recapture	Reach N_2 - 37 m Natural Control	Reach D - 37 m Recapture Only

Figure 15. Study design used for Dogfish Creek site (design option 2b).

Movement indices calculated for Design Option 2 include the metrics listed above for Option 1, as well as additional ones as described below:

- Comparison of means (COM) - mean length of fish moving through the culvert is compared with that of fish moving through the natural reach, in addition to the mean analysis described previously.
- Fish passage efficiency (FPE) - FPE calculates the efficiency of a structure for passing fish, relative to a control reach within the same system. FPE is calculated as $T * N^{-1}$, where T is the proportion of marked fish that moved upstream through the treatment reach, and N is the proportion of marked fish that moved upstream through the natural reach.
- Directional daily movement (DDM) - DDM relates the number of marked fish that move between reaches to the total number of marked fish recaptured in the original marking reach. DDM is calculated as $M * R^{-1} * D^{-1}$, where M is the total number of marked fish that moved through a reach, R is the total number of similarly marked fish recaptured in the reach of origin, and D is the number of days since initial marking. Direction of movement (upstream or downstream) is expressed via assignment of a positive or negative value to the calculated result. DDM values between the treatment and control reach can be compared qualitatively for a given date. Positive DDM values can also be used to calculate FPE for a culvert in relation to time.

Comment [NRE1]: table

The anatomical location and color of subcutaneous marks on fish used for Design Option 2 varied according to reach and marking date, as outlined in Table 3. Reach coloration on each figure (14 and 15) denotes the color of latex dye used.

Table 3. Summary of Marking Activities According to Study Design and Mark

Date	Location	Study Design Option	Reach	Mark Type	Number of Coho Released[a]
5/8/01	Snow/Andrews	1	B	Bismarck Brown	80
5/16/01	Snow/Andrews	1	B	Bismarck Brown	15
5/29/01	Parish	1	B	Bismarck Brown	40
5/30/01	Parish	1	B	Bismarck Brown	28
5/31/01	Parish	1	B	Bismarck Brown	50
6/5/01	Parish	1	B	Bismarck Brown	101
6/7/01	Parish	1	B	Bismarck Brown	53
5/21/01	Dogfish	1	B	Bismarck Brown	287
5/29/01	Dogfish	1	B	Bismarck Brown	132
5/30/01	Dogfish	1	B	Bismarck Brown	56
5/31/01	Dogfish	1	B	Bismarck Brown	44
6/5/01	Dogfish	1	B	Bismarck Brown	122
6/15/01	Parish	2a	A	Green Anal Fin	48
6/28/01	Parish	2a	A	Green Caudal Fin	31
6/15/01	Parish	2a	B	Orange Anal Fin	79
6/27/01	Parish	2a	B	Orange Anal Fin/Green Dorsal Fin	71
6/28/01	Parish	2a	B	Orange Caudal Fin	35
6/13/01	Dogfish	2b	B	Orange Anal Fin	27
6/29/01	Dogfish	2b	B	Orange Caudal Fin	14
6/13/01	Dogfish	2b	C	Green Anal Fin	33
6/29/01	Dogfish	2b	C	Green Caudal Fin	26

[a]Number of 0+ coho released with mark indicated, minus mortalities or reference fish (when applicable).

FINDINGS

Summary of Field Activities

As noted previously, the methods used during development of the protocol were subject to ongoing evaluation and revision. Many of our initial assumptions were based on information obtained during the literature reviews and have been modified by encounters with actual field situations.

The field activities undertaken for methods evaluation and protocol design development are summarized below (Table 4). Brief descriptions are given to illustrate the evolving nature of the field work.

Table 4. Summary of Activities During Protocol Development

Date	Location	Activities
4/06/01 - 5/05/01	Snow Creek	Pilot studies evaluating minnow traps and marking techniques.
5/08/01 - 5/11/01	Snow/ Andrews Creeks	Initial deployment of protocol concepts with Bismarck Brown and minnow traps at Snow/Andrews Creek convergence. Fish for marking were obtained with the seine at Snow Creek. Baited minnow traps were placed upstream and checked daily. Reference and control fish held for duration of test.
5/13/01 - 5/15/01	Various	Numerous site visits for study site selection.
5/16/01 - 5/21/01	Snow/ Andrews Creeks	Protocol concepts deployed, similar to first time, with seining also done daily above culvert.
5/21/01 - 5/25/01	Dogfish Creek	Protocol test using Bismarck Brown. Fish obtained by seining large culvert outfall pool, baited minnow traps placed upstream. Seining daily above culvert; dipnetting implemented at end of week. Reference and control fish held for duration of test.
5/29/01 - 6/01/01	Dogfish Creek	Protocol test using Bismarck Brown. Fish obtained by dipnetting upstream and downstream of culvert. Baited minnow traps placed upstream of culvert; dipnetting done daily above culvert. All new unmarked captures marked and released downstream of culvert. Reference and control fish from initial marking held for duration of test.
5/29/01 - 6/01/01	Parish Creek	Protocol test using Bismarck Brown. Fish obtained by dipnetting upstream of culvert. Baited minnow traps placed upstream of culvert; dipnetting done daily above culvert. All new unmarked captures marked and released downstream. Supplemental catch for daily additional marking done downstream of culvert. Reference and control fish from initial marking held for duration of test.
6/05/01 - 6/08/01	Dogfish Creek	Protocol test using Bismarck Brown. Fish obtained by dipnetting upstream and downstream of culvert. Baited minnow traps placed upstream of culvert, dipnetting done daily upstream of culvert. Dipnetting also done daily below culvert for recapture. Snorkel survey done in place of dipnetting on the 8th. Reference and control fish held for duration of test.
6/05/01 - 6/08/01	Parish Creek	Protocol test using Bismarck Brown. Fish obtained by dipnetting upstream and downstream of culvert. Baited minnow traps placed upstream of culvert, dipnetting done daily upstream of culvert. Dipnetting also done daily below culvert for recapture. Additional unmarked captures marked on the 7th. Snorkel survey done in place of dipnetting on the 8th. Reference and control fish from both marking events held for duration of test.
6/13/01 - 7/19/01	Dogfish Creek	Advanced protocol testing using subcutaneous injection of photonic dye. Fish marked on two separate dates, recapture attempted weekly in study reaches. Minnow trapping discontinued. Dip net used as primary mode of capture, replaced by seine in final 2 weeks.
6/15/01 - 7/19/01	Parish Creek	Advanced protocol testing using subcutaneous injection of photonic dye. Fish marked on two separate dates, recapture attempted weekly in study reaches. Methods calibration experiment using electrofishing performed on 6/27, with electroshocked fish marked uniquely. Minnow trapping discontinued. Dip net used as primary mode of capture.

Initial Capture

Minnow Traps

Baited minnow traps were used extensively throughout both Dogfish and Parish Creeks to collect 0+ coho. Cumulative fishing time for all traps exceeded 960 hours during 20 capture events, with 242 coho collected (Table 5). In general, minnow trap capture rates were low and ranged from 0.06-2.00 coho per trap/hr, with a mean of 0.25 coho per trap/hr during protocol development.

Table 5. Summary of Recorded Catch-per-unit-effort Rates of Capture Methods Evaluated During Protocol Development

Location	Method	Number of Capture Events	Total Time Fishing (hrs)	Number of Coho Captured	Catch Per Unit Effort (# coho/hr)
Dogfish	Minnow Trap	11	617.25	195	0.32
Parish	Minnow Trap	9	346.00	47	0.14
Both	Minnow Trap	20	963.25	242	0.25
Dogfish	Dip net	20	8.00	756	94.50
Parish	Dip net	28	15.70	1360	86.62
Both	Dip net	48	23.70	2116	89.28
Dogfish	Seine	9	2.58	885	343.02
Parish	Seine	1	0.08	26	325.00
Both	Seine	10	2.66	911	342.48
Parish	Electrofishing	1	0.69	75	108.70

During the catch efficiency calibration trial conducted in Parish Creek, eight minnow traps set for 1 hour captured an average of 8.0% of fish present, at a rate of 0.75 coho per trap/hr (Table 6).

Table 6. Summary of Captures During Electrofishing Calibration Experiment at Parish Creek

Method	Efficiency[a]	Total Time Fishing (hrs)	Number of Coho Captured	Catch Per Unit Effort (# coho/hr)
Snorkel	.8267	0.50	62	124.00
Minnow Trap	.0800	8.00	6	0.75
Dip net	.2400	0.49	18	36.96
Electrofishing	1.0	0.69	75	108.70

[a]Relative to electrofishing.

Underwater video documentation of baited minnow traps showed little behavioral orientation of coho to these traps, besides the occasional use of the trap as a flow shelter (Figure 16).

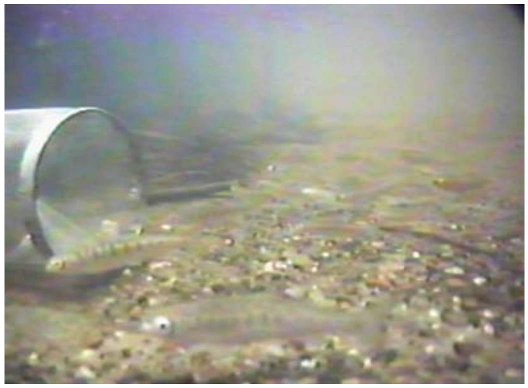


Figure 16. Underwater video frame of 1+ age coho near baited minnow trap.

Dip net

Dipnetting was used to collect 0+ coho during 48 capture events at both Dogfish and Parish Creeks (Table 5). Over 23 cumulative hours of active capture effort yielded 2116 coho (range: 13.33- to 344.00 coho/hr), for a mean catch per unit effort (CPUE) of 89.28 coho/hr. During the catch efficiency calibration trial, dipnetting captured 24.00% of fish present within the reach, at a rate of 36.96 coho/hr (Table 6). Fish collected by dip net did not display any adverse physical or behavioral effects immediately after release, as demonstrated by underwater videography, and control fish survival (fish held for duration of each test to evaluate mortality associated with capture and mark techniques).

During each capture event, CPUE tended to decline as fishing time exceeded 0.5 hrs, likely due to diminishing returns as fish were depleted within the selected habitats (Figure 17). In addition, the overall capture efficiency of dipnetting appeared to decline over the course of the season at some locations. At Dogfish Creek (the larger stream of the two primary study sites) coho fry became more difficult to capture as mean size increased and they moved to deeper pool habitats (Figure 18). Fish also became noticeably better at avoiding the dip net as time progressed, responding earlier and more quickly to the presence of the net in the water. At Parish Creek, coho fry growth was much lower and trends in corresponding CPUEs were not readily apparent (Figure 19).

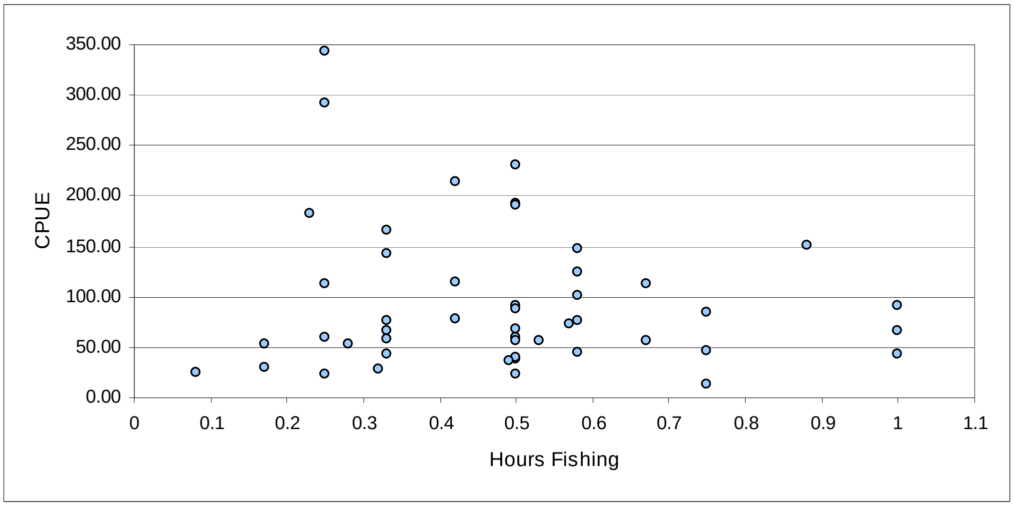


Figure 17. Dip net CPUE vs. time continuously fishing.

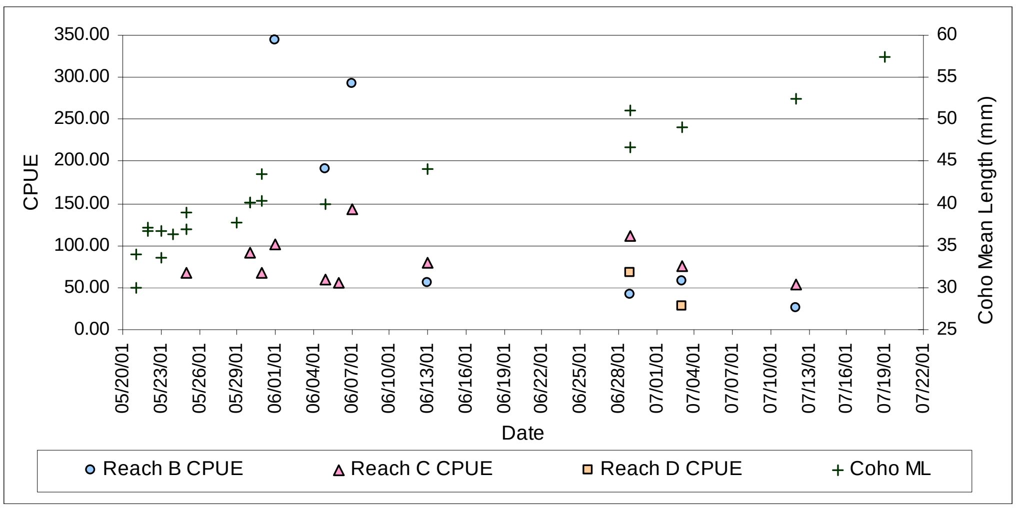


Figure 18. Dip net CPUE vs. coho mean length over time, Dogfish Creek.

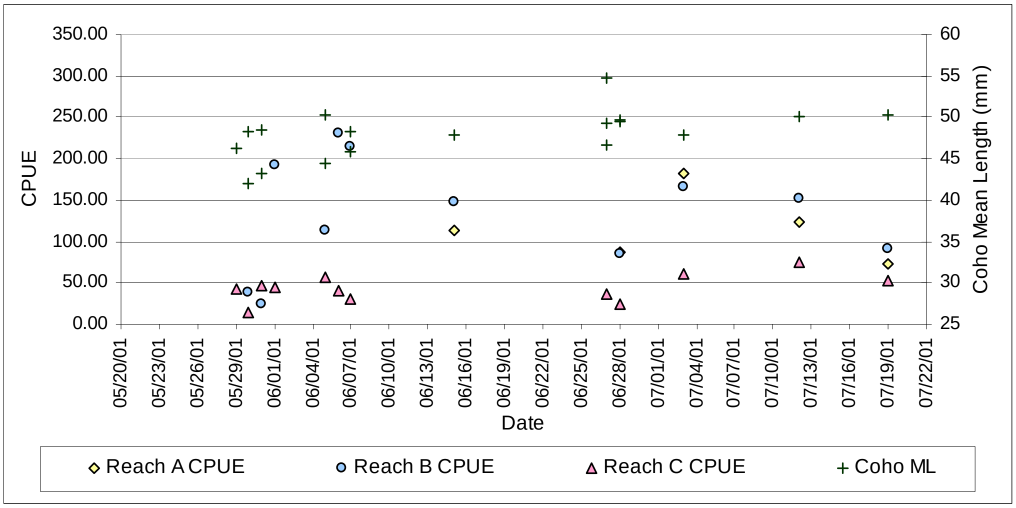


Figure 19. Dip net CPUE vs. coho mean length over time, Parish Creek.

Seine

Initial testing of the seine at Snow Creek resulted in large catches in the broad and deep pool and open riffle habitats. However, at Dogfish and Parish Creeks, which are more shallow, narrow, and complex, the seine provided mixed utility for initial collection of 0+ coho. At these primary study locations the seine was used to collect 0+ coho during 10 initial capture events over 2.7 cumulative hours of active capture effort (Table 5). Seining at these locations yielded 911 coho, for a mean CPUE of over 342 coho/hr. Despite high apparent catch rates, limitations in the availability of deepwater habitat at Parish Creek reduced its overall effectiveness at this location. At Dogfish Creek, which is faster and deeper than Parish, the seine was particularly effective in the large culvert outfall pools and large glide areas between pools. Catches with the seine also appeared to increase in efficiency during the latter part of the season as coho mean sizes increased and they moved into deeper pool habitats.

Seine-associated mortality was observed on one occasion, likely due to large amounts of debris in the net that reduced efficiency of fish removal. Small sweeps with the seine eliminated this problem by limiting the amount of fish and debris caught in any single capture event.

Electrofishing

Although electrofishing posed risks of injury and behavioral alteration that were considered too high for use during initial capture efforts, it was used on one occasion in order to evaluate the effectiveness of other methods. Electrofishing is assumed to capture nearly 100% of fish present in small streams (1996), and this method was chosen to establish a benchmark for coho densities in a 51.5 m reach of Parish Creek (Table 6). During one, two-pass collection effort spanning 0.69 hours of total fishing time, 75 0+ coho were collected, for a CPUE of 108.7 coho/hr (Tables 5 and 6).

Spinal deformities not previously detected in the population were observed at this time, and are considered to be associated with electrofishing methods. One mortality was observed during collection, 4 mortalities (including one fish that exhibited spinal damage) occurred during holding following marking, and one marked 0+ coho was released alive with spinal deformity. On a later date, one coho with a spine deformity was recaptured and died in holding, and possibly one other coho with spinal deformity was captured 2 weeks prior. In addition, at least two trout had visible burn marks on the skin during initial capture.

Marking

Bismarck Brown Y

Bismarck Brown Y dye resulted in an orange-brown stain to the skin and fins, which remained for up to 1 week (Figure 20). Stained fish were visibly identifiable underwater soon after marking (Figure 20). In general, the dye faded from the skin fairly rapidly (1 to 2 days), although the snout tended to retain a brown coloration for up to 1 week. This mark varied considerably between fish, and longevity was observed to be different between test sites and batches. In most cases, marked reference fish held in-stream were essential for differentiating marked from unmarked fish.

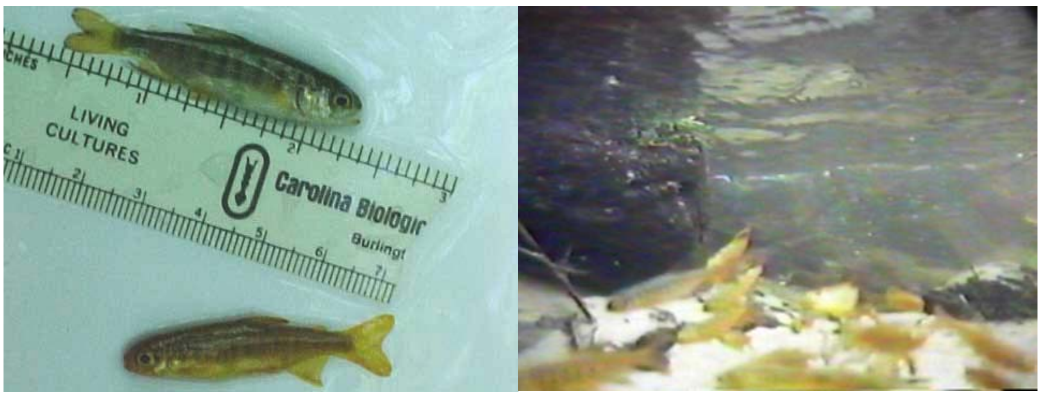


Figure 20. Comparisons of stained (bottom) and unstained (top) juvenile coho out of the water (left photo), and using underwater videography (right photo).

Only 12 mortalities (1.19%) were observed during marking, although this could not be attributed to either capture or marking technique. Video observations showed no behavioral abnormalities after release, with marked fish moving and feeding in the same manner as other individuals in the stream. Stained coho often appear brighter and more visible from the stream bank than unmarked coho for 1-2 days after marking, which may make them more susceptible to predators. Video observations documented a small trout (approximately 120 mm length) attempting to feed on a stained coho fry (Figure 21), and kingfishers were also observed preying upon coho fry (no marking observed) at Dogfish Creek.

Figure 21. Underwater video frames of predation attempt on juvenile coho by juvenile trout (right photo). Left photo is coho school just prior to attack.

Subcutaneous Injection

Subcutaneous injection of photonic dye with hypodermic needles was a fairly rapid (50 to 100 fish/hour) and efficient technique for creating longer-term marks on small fish. The mark typically formed a small streak or globule (1- to 4-mm^2 surface area), and recognition of the mark was very apparent on light skin such as the anal or caudal fin base (Figures 22 and 23). The photonic marks were also readily recognizable after several weeks, with little apparent fading or dissolution. In addition, the dye fluoresced well under UV light, making the mark identifiable on video under low-light conditions (Figure 24).

Figure 22. Photonic orange anal (left photo) and caudal (right photo) fin marks on age 0+ coho out of water.

Figure 23. Photonic orange anal and caudal fin marks, underwater (fish are in a small aquarium).

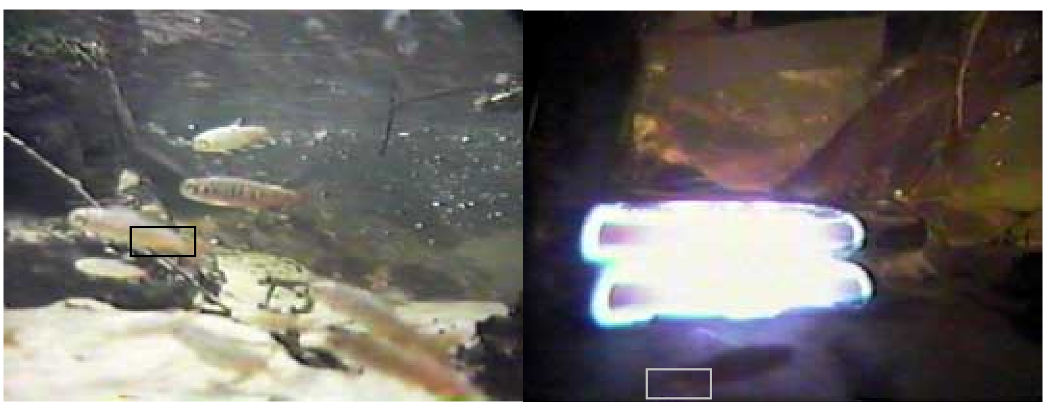


Figure 24. Underwater video frame of orange anal fin mark. Left photo is under ambient light in the early afternoon. Right photo demonstrates fluorescence under handheld UV light (mark highlighted in small box at bottom of photo).

Incidental mortality of 20 individuals (5.49%) was recorded during marking, although this could not be attributed to either capture or marking technique. Underwater video of fish during the hours following marking does not reveal any apparent behavioral modification.

Recapture/Observation

Recapture rates of marked fish were highest in reaches where they had been previously released, regardless of mark type or recapture technique. These observations suggest that most marked fish remained in the vicinity of the release point from 1 to 5 weeks after release. In general, mean recapture rates were higher at Parish Creek than at Dogfish Creek (Figure 25). Bismarck stained fish were recaptured at slightly higher mean rates than photonically marked fish at these respective locations. However, this might be related to sampling frequency in proximity to release date, as Bismarck-stained fish were resampled intensively immediately following release.

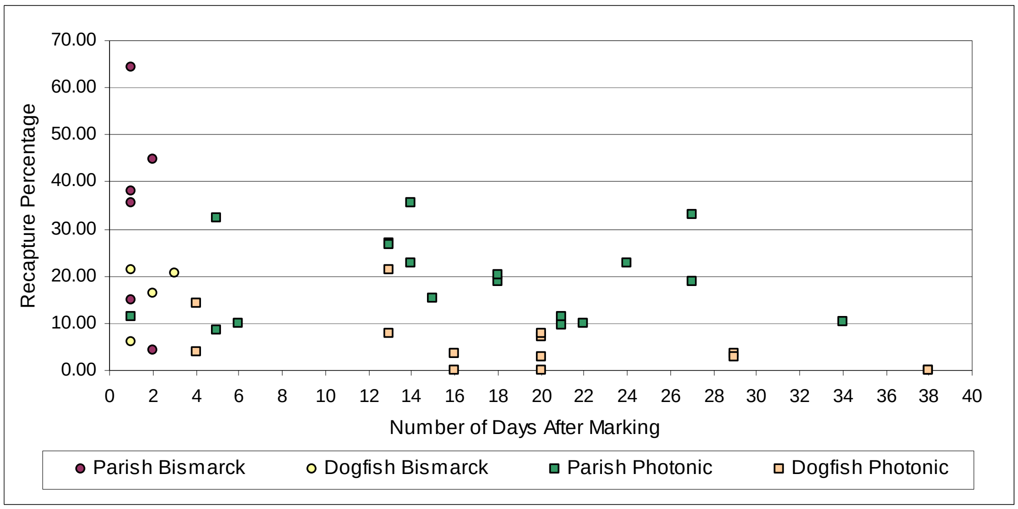


Figure 25. Recapture rates of marked coho over time at Parish and Dogfish Creeks.

We also examined the relationship between number of fish initially marked and recapture rate. Using photonic tags, our activities consisted of nine marking events, ranging in sample size from 14 to 79 fish (Figure 26). Fewer fish were marked at Dogfish Creek (14 to 33 fish over 4 marking events) than Parish Creek (31 to 79 fish over 5 marking events). No distinct relationship was seen between number initially marked and recapture rate; rather, location (e.g., habitat differences) may have been a stronger factor in determining recapture efficiency.

Results of our mark-recapture with electroshocked fish show declines in recaptures as compared with batches of fish initially collected using other methods. On subsequent days, recapture (via dip net) of these fish was lower than that of other previously marked fish at Parish Creek (9.9% to 15.4% of initially electroshocked, versus 20.3% to 32.9% of initially dipnetted coho in release reach) (Figure 25 and 26). Additional dip-net captured fish marked after the electrofishing event also had higher recapture rates (8.60% to 22.90%).

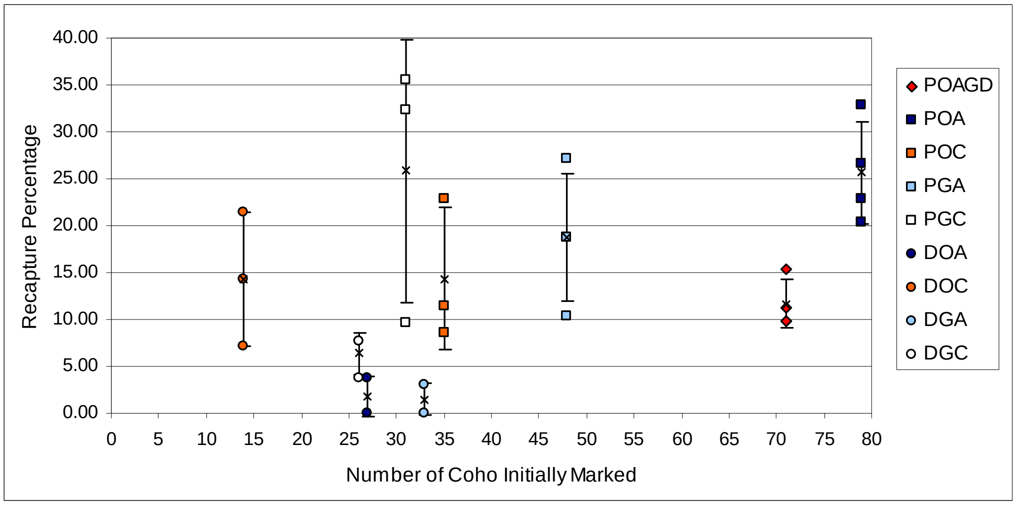


Figure 26. Recapture rates of photonically marked coho versus number initially marked; coding letters represent location (P - Parish Creek, D - Dogfish Creek), mark color (O - orange, G - green), and mark location (A - Anal fin, C - Caudal fin); additional GD (Green Dorsal) coding signifies mark of fish initially collected by electrofishing.

Recapture Methods

We compared recapture methods (minnow traps, dip net, seine) using recapture rates from the release reaches. Data on fish recaptured outside of release reaches are evaluated in the Estimating Movement section (page 32).

During pilot studies using minnow traps at Snow Creek in early May, photonically marked 1+ coho were recaptured on subsequent dates at a single Snow Creek release point, with recapture rates of 5.3, 7.9, 7.9, and 2.6% over 14 days. No photonically marked 1+ coho were caught in minnow traps outside the immediate vicinity of the release point.

During protocol development at the Dogfish and Parish Creek sites, minnow traps did not capture any 0+ coho marked with either the Bismarck Brown stain or photonic dye. As a result of these low recapture rates, and low initial capture efficiencies, minnow traps were not used for recapture during advanced protocol testing.

Coho fry were primarily recaptured using the dip net, although the seine was used more intensively at Dogfish Creek as the season progressed. Recaptures of Bismarck Brown stained fish occurred within 1 week following release and ranged from 4.40% to 64.4% of initially marked fish (Table 7, Figure 25). The mean recapture rate of Bismarck-stained fish at both sites combined exceeded 26% over 8 recapture events (total of 626 marked 0+ coho). Coho recapture rates were considerably different between streams, averaging 33.70% at Parish Creek and 14.58% at Dogfish Creek.

Later in the season, recapture attempts of 0+ coho marked with photonic dyes occurred at weekly intervals. Recapture rates ranged from 0.00% to 35.5% of initially marked fish, averaging 15.39% over 24 recapture attempts (364 coho marked) (Table 7, Figure 25). Again, these rates were generally higher at Parish (19.14%) than Dogfish creek (4.13%).

Table 7. Summary of Recapture Attempts in Reaches Where Coho Had Been Released (No Movement Observed)

Recapture Date	Location	Reach	Mark Type	# Coho	Mean Length[a]	Recapture Percent	Recapture Method
5/30/01	Parish	B	Bismarck Brown Stain	6	n/d	15.00	Dip net
5/31/01	Parish	B	Bismarck Brown Stain	3	n/d	4.40	Dip net
6/1/01	Parish	B	Bismarck Brown Stain	45	n/d	38.10	Dip net
6/6/01	Parish	B	Bismarck Brown Stain	65	n/d	64.40	Dip net
6/7/01	Parish	B	Bismarck Brown Stain	45	n/d	44.60	Dip net
6/8/01	Parish	B	Bismarck Brown Stain	55	n/d	35.70	Snorkel
6/28/01	Parish	A	Green Anal Fin	13	49	27.10	Dip net
7/3/01	Parish	A	Green Anal Fin	9	44	18.80	Dip net
7/12/01	Parish	A	Green Anal Fin	9	48	18.80	Dip net
7/19/01	Parish	A	Green Anal Fin	5	47	10.40	Dip net
7/3/01	Parish	A	Green Caudal Fin	10	46	32.30	Dip net
7/12/01	Parish	A	Green Caudal Fin	11	48	35.50	Dip net
7/19/01	Parish	A	Green Caudal Fin	3	44	9.70	Dip net
6/28/01	Parish	B	Orange Anal Fin	21	50	26.60	Dip net
7/3/01	Parish	B	Orange Anal Fin	16	50	20.30	Dip net
7/12/01	Parish	B	Orange Anal Fin	26	52	32.90	Dip net
7/19/01	Parish	B	Orange Anal Fin	18	54	22.80	Dip net
7/3/01	Parish	B	Orange Caudal Fin	3	49	8.60	Dip net
7/12/01	Parish	B	Orange Caudal Fin	8	53	22.90	Dip net
7/19/01	Parish	B	Orange Caudal Fin	4	53	11.40	Dip net
6/28/01	Parish	B	Orange Anal Fin/ Green Dorsal Fin	8	50	11.30	Dip net
7/3/01	Parish	B	Orange Anal Fin/ Green Dorsal Fin	7	51	9.90	Dip net
7/12/01	Parish	B	Orange Anal Fin/ Green Dorsal Fin	11	52	15.40	Dip net
7/19/01	Parish	B	Orange Anal Fin/ Green Dorsal Fin	7	49	9.90	Dip net
6/1/01	Dogfish	B	Bismarck Brown Stain	14	n/d	6.03	Dip net
6/6/01	Dogfish	B	Bismarck Brown Stain	26	n/d	21.30	Dip net
6/7/01	Dogfish	B	Bismarck Brown Stain	20	n/d	16.40	Dip net
6/8/01	Dogfish	B	Bismarck Brown Stain	25	n/d	20.50	Snorkel
6/29/01	Dogfish	B	Orange Anal Fin	1	45	3.70	Dip net
7/3/01	Dogfish	B	Orange Anal Fin	0	n/d	0.00	Dip net
7/12/01	Dogfish	B	Orange Anal Fin	1	52	3.70	Seine
7/19/01	Dogfish	B	Orange Anal Fin	0	n/d	0.00	Seine
7/3/01	Dogfish	B	Orange Caudal Fin	2	50	14.30	Dip net
7/12/01	Dogfish	B	Orange Caudal Fin	3	42	21.40	Seine
7/19/01	Dogfish	B	Orange Caudal Fin	1	47	7.10	Seine
6/29/01	Dogfish	C	Green Anal Fin	0	n/d	0.00	Dip net
7/3/01	Dogfish	C	Green Anal Fin	1	60	3.00	Dip net
7/12/01	Dogfish	C	Green Anal Fin	1	56	3.00	Seine
7/19/01	Dogfish	C	Green Anal Fin	0	n/d	0.00	Seine
7/3/01	Dogfish	C	Green Caudal Fin	1	53	3.80	Dip net
7/12/01	Dogfish	C	Green Caudal Fin	2	65	7.70	Seine
7/19/01	Dogfish	C	Green Caudal Fin	2	62	7.70	Seine

[a]Fork length, nearest mm. n/d - no data.

Observation Methods

Marked fish were easily distinguished from unmarked fish underwater using snorkel survey and video methods. Snorkel surveys provided data on marked and unmarked fish abundance and distribution at the study sites on June 8, 2001 (Figures 27 and 28). Both surveys revealed a lack of marked fish within or above the culverts, with most of the marked fish remaining in the release pool, confirming the findings of our other capture methods. At Dogfish Creek, 20.50% of the marked fish were observed during the snorkel survey; in general, unmarked coho were much more abundant upstream of the culvert than downstream. At Parish Creek, 35.70% of the marked fish were observed and most were observed downstream of the culvert.

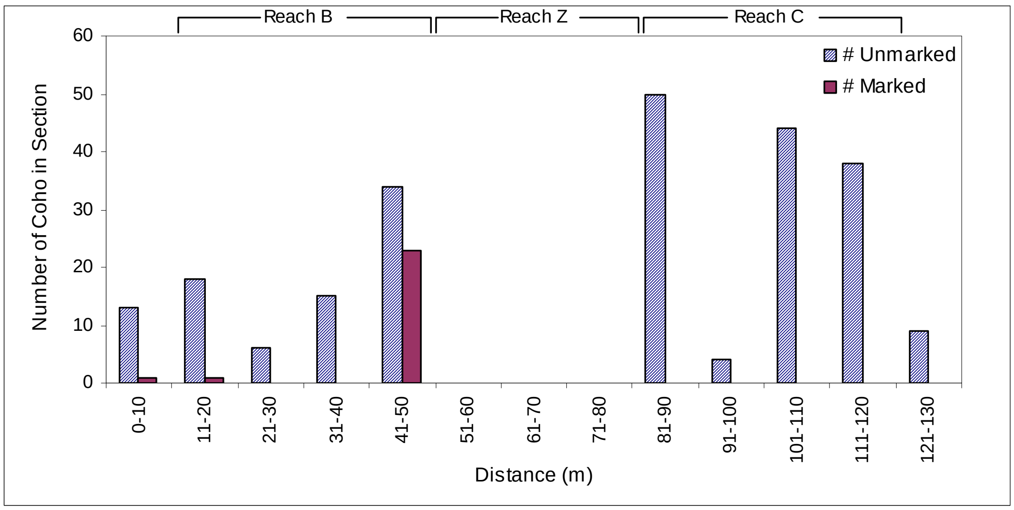


Figure 27. Number of marked and unmarked 0+ coho sighted in 10 meter sections at Dogfish Creek during snorkel survey. Survey started 45 meters below the culvert ("0"); culvert is present at 45-83 meters.

Snorkel survey methods documented 82.67% of fish present during the electrofishing calibration trial in exactly 0.50 hours of observation time (124 coho/hr). Duplicate visual counts likely occurred on two occasions when more fish were counted by snorkel survey than were captured with electrofishing.

Videography was used on 7 occasions over 22.75 hours to document movement and behavior of marked 0+ coho in all of the study locations. Marked fish were readily identifiable at various distances from the lens using underwater videography. Marked fish exhibited no apparent behavioral effects, and were observed feeding and holding with unmarked fish soon after release. Furthermore, we were able to document possible predation events and intraspecific interactions (schooling behavior) for extended periods in a natural environment. No fish movement into or through culverts was documented on video.

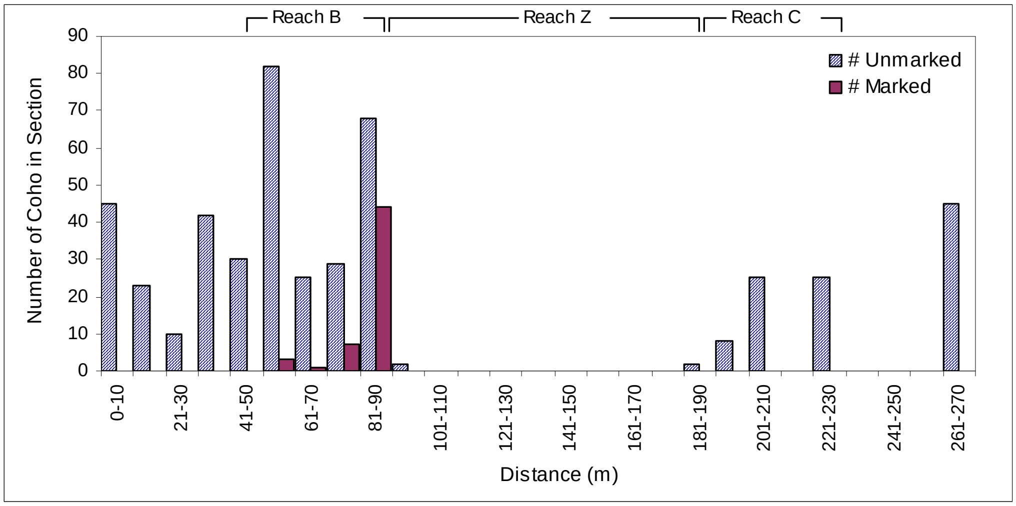


Figure 28. Number of marked and unmarked 0+ coho sighted in 10 meter sections at Parish Creek during snorkel survey. Survey started 91 meters below the culvert ("0"); culvert is present at 91-187 meters.

Estimating Movement

Snow/Andrews Creeks

No marked fish were recovered at the Snow/Andrews Creek convergence and culvert, nor was movement observed during pilot studies.

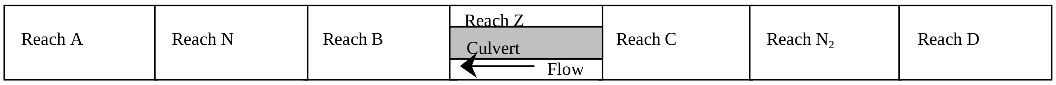
Dogfish Creek

During May testing of Design Option 1, one incident of upstream movement was documented. Three marked coho (5.36% of released) were recaptured upstream of the culvert on May 31, 2001 (Table 8). These fish had clearly been stained the day before. Mean length of fish passing the culvert (54 mm) was greater than the mean length of marked fish released below the culvert (40 mm), although this was not statistically different ($p = 0.11$, 2 df, $t = -2.73$) due to low sample sizes and high variance. The smallest fish passing this culvert had a fork length of 44 mm.

No coho movement was documented between reaches at Dogfish Creek while testing study Design Option 2, which took place during June and July.

Table 8. Summary of Movement Recaptures, with Selected Movement Metrics

Location	Mark type	Release Reach	Recapture Reach	# Coho	# Days After Marking	Recapture Percent	%PASSED	COM[a]	FPE	DDM
Dogfish	Bismarck Brown Y dye	B	C	3	1	5.36	5.36	0.11	N/A	N/A
Parish	Orange caudal dye injection	B	A	1	15	2.90	N/A	N/A	N/A	-0.0083
Parish	Orange anal/ green dorsal dye injection	B	A	3	16	4.20	N/A	0.34	N/A	-0.0170
Parish	Orange anal/ green dorsal dye injection	B	A	3	23	4.20	N/A	0.51	N/A	-0.0186



[a]p value from t-test (∀ = .05)

Parish Creek

Using study Design Option 1, no marked coho were recaptured above the culvert at this site.

During testing with Design Option 2, no coho moved upstream through the control or treatment reaches, although seven coho were recaptured that had moved downstream through the control reach. On July 12, 2001, 15 days after release, one orange caudal-marked coho (40 mm) was captured in sample reach A below the natural reach (2.86% of released). Also on July 12, 2001, 16 days after release, three orange-anal/green-dorsal marked fish were recaptured in sample Reach A (4.23% of released). The mean length of fish captured downstream of the release point was not significantly different than the mean length of fish released initially ($p = 0.34$, 2 df, $t = 1.23$). The following week, on July 19, three more orange-anal/green-dorsal marked coho were caught in sample Reach A (4.23% of released). Again, the mean length of fish captured downstream was not significantly different than the mean length of fish released initially ($p = 0.51$, 2 df, $t = -0.80$). The total of six orange-anal/green-dorsal marked coho found in Reach A represent 8.45% of total marked, and they moved 142 to 188 meters downstream from the release point. No fish were marked above the culvert, so comparison of negative DDM values between control and treatment reaches was not possible.

DISCUSSION AND CONCLUSIONS

During the spring/summer 2001 field program, considerable progress was made in refining techniques and examining scenarios under which a standardized protocol could be applied to assessing juvenile coho salmon passage through road culverts. Field evaluations focused on capture-mark-recapture methods that allowed analysis of fish movement patterns and potential identification of cues inducing these movements. Where possible, we also critically examined the approaches of other researchers (e.g., Cupp et al. 1999, Kane et al. 2000, Gregory and Klingeman 2000) to guide the process. Our findings have led to several refinements in sampling techniques and study design. Below, we outline our primary conclusions, organized by sampling method or procedure, and follow with additional discussion of relevant issues. As the protocol continues to develop and moves into advanced stages of testing, these findings will be implemented into a final standardized testing procedure.

Study Site Selection

- The availability and abundance of juvenile coho in potential testing locations was one of the primary factors driving study site selection.

Culvert study locations were selected after conducting visits to a number of existing retrofit sites outlined under the WSDOT Fish Barrier Removal Program (Johnson et al. 2001). Although a number of factors were necessary for experimental manipulations to be of net benefit, many of the potential study sites did not appear to have adequate numbers of juvenile coho to justify testing protocol designs.

Ideally, any culverted stream reach should be subject to biological evaluation with the protocol. However, determining a reasonable estimate of passability requires a minimum number of study subjects. Early estimates of fish abundance were based on rapid visual surveys; preliminary recapture data may allow us to develop preliminary guidelines on the “adequate” number of fish available for marking in the stream before passage can be assessed (see Estimating Movement Section).

Unfortunately, the unavailability of current information on coho presence/absence and abundance for any particular stream led to front-loaded delays in planning time and effort. Current information systems that strive to inventory the status of salmon stocks and habitat (e.g., StreamNet, WDFW Stream Catalog, SASSI) were either out of date, inaccurate, incomplete, not standardized by stream and watershed, or did not provide the level of information needed (i.e., juvenile abundance) for preparation in advance of field visits.

- Potential study locations were characterized by a wide variety of stream sizes, habitats, and culvert configurations.

In general, the physical attributes of any particular study site (i.e., stream size, habitat types, culvert configurations) were characterized by more differences than similarities. In other words, no single site conformed to any single, preconceived categorization of these physical attributes. Acquisition of engineering design plans for road culverts, both pre- and post-retrofit, may be extremely helpful for advance preparation before field tests are initiated.

Initial Capture

- A number of capture methods, including dip nets and seines, resulted in high catch rates of juvenile coho salmon for marking. These collection methods were rapid (averaging 115 fish/hr combined) and could be accomplished in most field situations.

The ultimate success of a mark-recapture study often depends upon the collection and marking of a large sample size of animals at the outset. A combination of fish collection methods resulted in high 0+ coho catch rates and was best suited to the wide variety of habitat types encountered in these stream settings. Targeted dipnetting appeared to be the most useful capture method across all study locations. While more labor-intensive than some other methods, the dip net was deployable in the widest variety of habitat types encountered. A small seine provided another efficient method that supplemented fish catches in open, low-complexity habitats and deep pools. In general, catch rates using these methods declined after 0.5 hours of collection within any stream reach.

Minnow trapping was used extensively at the outset of the study based on the successes described by Kane et al. (2000) in Alaska. Despite high ambient densities of coho, these techniques were generally not effective in our stream situations and eventually were discontinued because of very low capture efficiency and CPUE rates. Traps might be useful as a supplemental capture method prior to marking, although dependence on the method for all fish collection is not likely to result in large sample sizes.

- Initial collection of fish for marking must be accomplished while minimizing stress and subsequent behavioral modification.

Minimization of stress and injury is a requirement in any study that ultimately measures animal behavior in a natural setting. However, any collection method can be expected to result in at least some rate of injury as a result of capture and handling related stresses. When conducted with care, fish collected by minnow trap, dip net, or seine exhibited few behavioral alterations after release, as confirmed by video camera observations.

Electrofishing was used as an initial collection method on one occasion and is not highly recommended based on this trial. Groups of fish released after collection using this method exhibited lower subsequent recapture rates within their release reach (11.6% vs. 21.3% by other methods), and high rates of spinal deformities. Furthermore, this group of fish exhibited much higher downstream movement rates (six of seven total fish that were recaptured downstream – see Estimating Movement section) than fish collected by other methods. While additional study is needed to determine whether the observed movement of these fish is attributable to electroshocking effects, relocation, or natural cues, the substantial differences in observed movement offer conservative evidence that behavior of these fish had been altered in some way.

Marking

- Long-term marking techniques provided the best approach to tracking fish movement within a stream.

Juvenile salmonid movements can occur throughout the period of residency (up to 1+ years for juvenile coho salmon). Because the primary cues inducing fish movement have not been clarified, associated monitoring must encompass this entire residence period. Subcutaneous injection of photonic dyes to the base of fins was a very rapid (>60 fish/hr) and reliable approach to "permanently" mark small coho salmon with very low incidental mortality. Over a 5-week monitoring span, these subcutaneous dyes were retained extremely well and fluoresced when exposed to UV light. The presence or absence of a mark was unquestionable, although mortality rates were slightly higher (5%) than those obtained with immersion dyes. It is anticipated that mark retention will extend throughout the period of stream residency (12-15 months) and marking mortality rates will decline as techniques are further refined and operators gain additional experience.

Small (<35 mm FL), recently emerged age 0+ coho were marked successfully using subcutaneous injection, whereas larger fish (>75 mm FL) may be marked even more rapidly using devices that employ a burst of compressed air to lodge dye under the skin. The small mark did not appear to significantly alter the fish's natural camouflage, and behavioral effects after marking were not observed. Multiple dye colors and fin marking locations can provide coding that designates individual fish (e.g., Kahler 1999), particular stream release points, or dates of release (Cupp et al. 1999). Other researchers are currently using subcutaneous marking (Alcian blue dye) with compressed air on fish >80 mm FL with fairly high success (Gregory and Klingeman 2000). Both Gregory and Klingeman (2000) and Bolton and Moss (2001) do not recommend this technique on smaller size classes due to tissue damage.

Mark retention of pigmented immersion dyes such as Bismarck Brown Y varied by stream, was short-lived, and was only useful for very short-term applications. Although positive results were achieved with the technique, many possible recaptures were discounted because of uncertainty of mark recognition after about 48 hours. Other researchers have reported suitable identification of these dyes from 48 hours up to 4-5 days (Mueller et al. 1995, Carlson et al. 1998, Kane et al. 2000, Bolton and Moss 2001). The dye technique does not appear to have associated behavioral effects, but might affect camouflaging attributes of the fish's natural coloration and increase mortality via predation.

Several other viable marking techniques were evaluated and not used in our studies for a variety of reasons (see SOW Deliverable 3). Fin clipping, although quick and simple, could alter the behavioral/movement patterns of the fish, or it may affect fish passage abilities under various hydraulic conditions. Coded wire tags offer an excellent means of quickly marking a large number of individual fish, but high initial cost for equipment ($15,000 for implant and detection equipment) and the need to sacrifice fish for tag interpretation negated its use at this stage. Finally, PIT (Passive Integrated Transponder) tags can relay precise information about the location of fish without requiring the fish to be captured or handled, but tag size (12 mm long) at this stage of technological development limits its use to larger size classes of fish.

<u>Recapture/Observation</u>

- A combination of direct collection and observation techniques provided the most reliable detection of marked fish in a variety of stream habitats.

A variety of field conditions and stream habitats were encountered while testing recapture techniques during protocol development. Although some approaches (e.g., minnow traps) were eventually discarded because of poor recapture rates in field tests, we found that using a combination of other techniques was suitable to diverse situations. Flexibility in methods selection enabled higher recapture rates in appropriate habitat types through the course of the season as fish behavior and habitat preferences changed over time.

As observed during initial capture efforts, dip nets and seines yielded large catches of juvenile coho salmon in most stream habitats, with recapture rates of up to 64%. The dip net was generally the most effective collection method in the widest variety of habitat types, although dip net efficiency was reduced when fish could not be visibly tracked, such as in larger streams, deep, shaded pools, or during adverse weather conditions. Later in the season as fish grew larger, began to inhabit deeper habitats, and were better able to avoid the dip net, the small seine provided superior performance and catch efficiency. Bolton and Moss (2001) successfully used dip nets to capture juvenile coho salmon in several Washington streams, whereas Dill and Fraser (1984), and Kahler (1999) used seining techniques.

Snorkel surveys supplemented information derived from recapture attempts, and provided good indications of fish movement with lower associated risks. Snorkel surveys provided valuable information on the abundance and distribution of coho and other species within, and in proximity to, the test culverts. Accordingly, we recommend inclusion of these observational methods within any final assessment procedure. In pilot studies, snorkel surveys documented over 80% of the fish (unmarked) present in a stream reach (assuming 100% efficiency estimates by electrofishing), as well as the distribution patterns of Bismarck Brown-marked fish relative to culverts. It should be noted that positive mark identification can be affected by stream conditions (e.g., turbidity) and light levels. Snorkel surveys have been used extensively in streams by other researchers to assess salmon movement (Kahler 1999) and distribution patterns relative to culverts (Gregory and Klingeman 2000).

Another observational technique, underwater videography, proved to be an extremely useful tool for evaluating behavioral effects after marking and in the vicinity of traps or culverts (see also Kane et al. 2000). Videography also documented predation events and was useful for assessing mark viability.

Minnow trap recapture rates and capture efficiencies were unacceptably low (<8% with age 1+ coho; 0% age 0+ coho), even with baited traps placed directly at release points. Associated disadvantages of minnow traps included the possibility of coho fry escaping as trap set time increased (Kane et al. 2000) and predation by larger fishes (e.g., trout, sculpins) that might have entered traps. However, other investigators have reported clear responses of fish to introduction of baited traps in Alaskan streams, with fairly high recapture rates (6 to 23%) over the course of hours to days (Kane et al. 2000).

Electrofishing was not extensively used in our field efforts because of concerns related to fish behavioral alteration and injury (Schroeder 1996, Resources Inventory Committee 1997, Reynolds 1996). In pilot studies, electrofishing provided superior collection efficiency over other recapture techniques. Electrofishing techniques have been widely used for fish population and movement studies (Cederholm and Scarlett 1981, Peterson 1982, Rimmer et al. 1983, Belford 1986, Belford and Gould 1989, Elliott 1986, Nielsen 1992, Riley et al. 1992, Foy 1995, Gowan and Fausch 1996, Warren and Pardew 1998, Rosenfeld et al. 2000, Bolton and Moss 2001), and this approach is currently being used, or is recommended, by a number of other researchers pursuing similar studies (Gregory and Klingeman 2000, Cupp et al. 1999). Electrofishing still remains a viable option under a protocol design that limits recapture efforts to stream reaches above culverts; however, it is currently not recommended under protocol designs that include control reaches and multiple resample locations below culverts. Had other recapture or observation techniques not proven effective, we might have moved toward this approach.

Weirs and stream-crossing traps were other potential recapture methods not employed in our field program at this stage of development because they did not meet our criteria for rapid deployment, minimal alteration of the stream environment, and permanence (see SOW Deliverable 3). Weirs and stream-crossing traps are commonly employed in field studies to assess movement of marked fish (Bjornn 1971, Cederholm and Scarlett 1981, Rimmer et al. 1983, Elliott 1986, Hartman and Brown 1987, Riley et al. 1992, Foy 1995, Gowan and Fausch 1996, Carlson et al. 1998, Kahler 1999) and are described in available protocols (Cupp et al. 1999). While the capture efficiency of weirs and stream-crossing traps may be very high (up to 100%)(Cupp et al. 1999), the high cost and time necessary for weir construction limits their use to longer-term studies (Schroeder 1996).

- A total of 729 marked 0+ coho were collected over 42 recapture attempts, encompassing both study sites and marking techniques.

Distinct differences in recapture rates (number recaptured / number initially marked) were observed between locations, with consistently higher mark recaptures observed at Parish Creek (mean 22.78%) than at Dogfish Creek (mean 7.76%). Other studies have reported a broad range of recapture rates, attributed to season, methods, or species of interest. For example, Warren and Pardew (1998) investigated the effect of road crossings on movement of fish in southwestern Arkansas streams using subcutaneous latex paint tags and electrofishing. They documented recapture rates of 18% in spring and 21% in summer, with the mean percentage of tagged fish recaptured in the same stream segment in which they were tagged varying by location relative to the culvert (upstream 27%, downstream 15%). In comparison, Bolton and Moss (2001) recaptured only 5% to 6% of marked juvenile coho in Olympic Peninsula streams using fin-clips, Bismarck Brown dye, and electrofishing.

<u>Estimating Movement</u>

- Recapture rates of marked fish were highest in reaches where they had been previously released, regardless of mark type or recapture technique.

Recapture rates of marked fish averaged 16.3% (range: 0% to 64%) within their release reach. Recapture rates in these reaches averaged 26.6% in trials that extended only 1 to 4 days past initial marking with Bismarck Brown, the maximum period of recognition with this method. In trials that extended up to 5+ weeks after initial marking with subcutaneous dyes, recapture rates averaged 13.1% across both locations, with recapture rates as high as 33% at Parish Creek almost 4 weeks post-marking.

- Only 10 fish (<2%) were recaptured outside of their initial marking reach, resulting in few instances where movement metrics could be calculated.

Monitoring from May 21 to July 19 resulted in one confirmed incident of upstream movement and two of downstream movement. Upstream movement was documented in May 2001, when three fish, representing 5.4% of the initially marked population, moved through the Dogfish Creek culvert and were recaptured in the upstream sampling reach. Downstream movement was documented on two separate dates in mid-July when a total of 7 fish (4 on July 12, 3 on July 19) moved through the Parish Creek control reach into the downstream sampling reach.

Movement metrics (i.e., COM and FPE) associated with Design Option 2 are comparative in nature. Without documentation of fish movement through both the control and culvert reach, no index can be calculated (see Table 8). While directional daily movement (DDM) can be calculated if fish move through either one of these reaches, this metric is more meaningful when placed within a comparative framework.

Although 1372 juvenile coho salmon were marked (364 permanently with subcutaneous dyes) in our study reaches, we documented very little movement in over 7 weeks of monitoring. It is likely that the low movement rates reflected actual conditions in the streams, as all of the protocol testing coincided with summer low-flow periods. Previous studies of juvenile salmonids in coastal streams have suggested that few individuals move during summer low-flow periods. Rather, there are two major movement events prior to seaward migration: dispersal of fry following emergence in the spring and early summer, and the redistribution of parr to low velocity or off-channel rearing area in the autumn and winter (Kahler and Quinn 1998).

- The methods used in this study were one way to measure fish movement, and could be subject to bias. Possible characteristics of the sampling design that could have affected results included timing of monitoring, scale of movement, sampling methods, and number of marked fish released from each location.

As previously suggested, study timing and frequency may have been inadequate to encompass high movement periods in these coastal streams. Definitive cues that elicit juvenile salmonid movements (e.g., environmental stimuli and internal motivations) have not yet been clarified in the literature, and may vary by species, age class, season, and stream system (Kahler and Quinn 1998). Fish might move in response to changing water temperature, stream discharge, and behavioral interactions related to population density (Kahler and Quinn 1998). Although water temperatures and stream discharge were monitored during the course of our study, the monitoring period encompassed a very limited seasonal range of conditions. No obvious relationship was discerned between these factors and the few cases of documented movement (Figures 29 and 30).

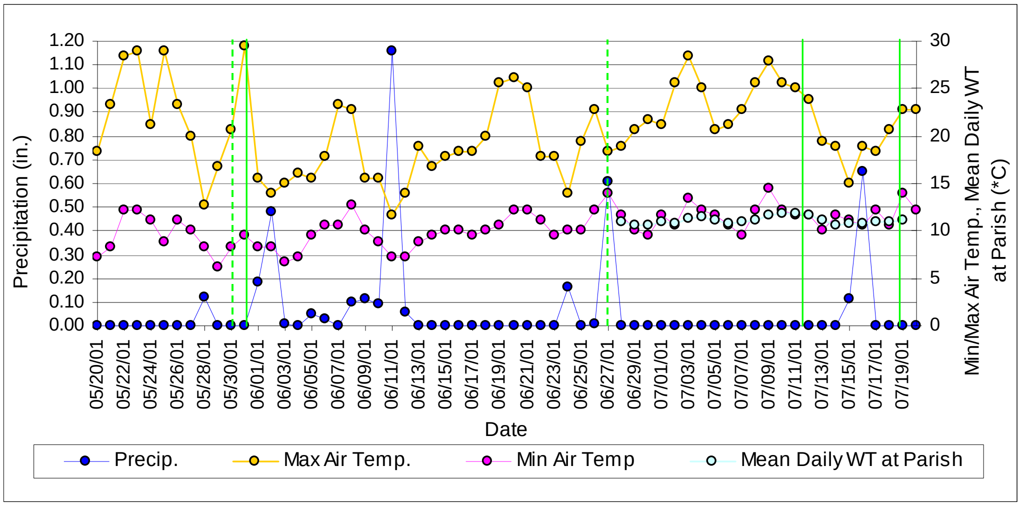


Figure 29. Precipitation (in., SeaTac), air temperature (°C, SeaTac), and water temperature (WT, °C, Parish Creek) during protocol testing. Solid green lines denote dates when fish were recaptured outside release reaches. Dashed green lines denote the date of initial marking.

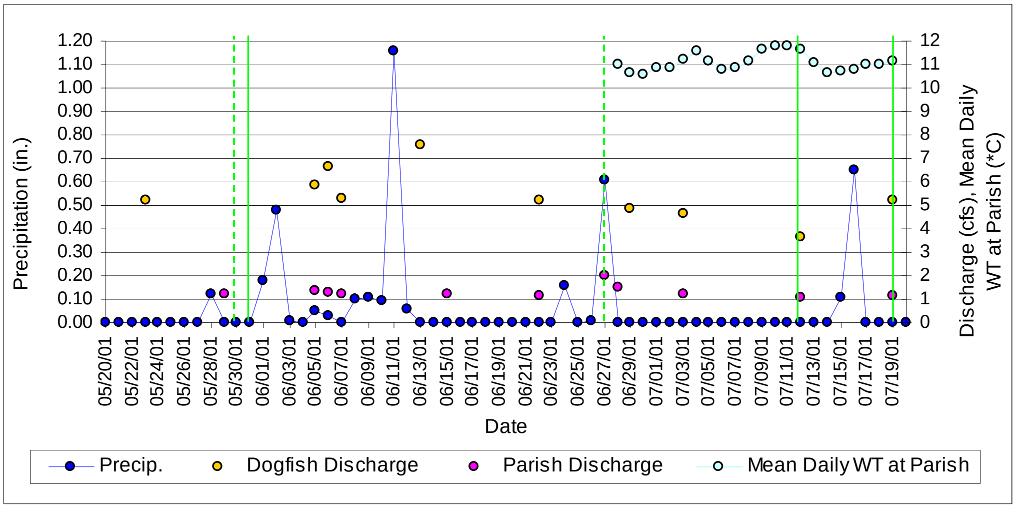


Figure 30. Precipitation (in., SeaTac), stream discharge (cfs, Parish and Dogfish Creeks), and water temperature (°C, Parish Creek) during protocol testing. Solid green lines denote dates when fish were recaptured outside of release reaches. Dashed green lines denote dates of initial marking.

Defining movement is subjective, scale-specific, and might yield different conclusions depending on sampling design and methods (Gowan and Fausch 1996). According to our study design, movement was quantified only when a marked fish was recaptured outside of the stream segment from which it had been released. In all cases, sampling reaches were separated by stream segments equal in length to the study culvert (96 m at Parish Creek, 37 m at Dogfish Creek), and sole reliance on recaptures in sample reaches limited the ability to discern fine-scale (habitat-unit based) movements therein. Recent research has called into question whether summer movement of juvenile salmonids is actually reduced. Kahler (1999) found that summer movement estimates for tagged juvenile coho fry in several Western Washington coastal streams ranged from 28% to 60%, with upstream movement exceeding downstream movement in 3 of 4 cases. In his study, Kahler (1999) used periodic snorkel surveys to identify the precise distance (individual habitat units) that subcutaneously marked coho had moved, and used weirs to detect movement beyond study sections. In most cases mean range of movement spanned 2- to 6 habitat units, which averaged approximately 4 m to 16-m in length, in either direction.

Our sampling may not have been sufficiently rigorous or frequent enough to detect movement. Over 98% of recaptured fish were caught within the same study reach in which they were originally released. Most movement studies show similar results, although more than 50% of fish originally released are rarely ever recaptured (Gowan and Fausch 1996). Fish moving at high rates may quickly go beyond the boundaries of the study reach and become unavailable for recapture, whereas those moving slowly tend to remain within the designated study reach. As a result, researchers may mistakenly conclude that fish are sedentary. Accordingly, it is recommended that inferences about movement should be based upon the percentage of marked fish recaptured, not the percentage of recaptures in home reaches (Gowan and Fausch 1996).

Finally, the number of fish marked and released from each location may have influenced results. During the second phase of protocol testing, sample sizes of fishes marked and released ranged between 14 and 79 fish depending upon collection date, with a total of 264 permanently marked fish in Parish Creek and 100 in Dogfish Creek. Recapture rates (%) were much higher in Parish Creek, where a large sample size of fish had been marked initially. However, it is possible that movement would have been detected more readily had more fish been marked in either study location (i.e., testing did not encompass the upper range of sample sizes needed to detect minimum movement). In either case, movement must be expressed in terms of percentages to account for discrepancies in initial sample sizes.

Determining sampling frequency and minimum sample size needed to detect fish movement in a stream is dependent on movement probability and capture efficiency. As previously noted, movement probability is likely affected by a combination of seasonal (i.e., temperature, flow), habitat, and species parameters, as well as on the scale of defined movement (distance, habitat units). Movement probability may be estimated by periodic snorkel surveys, which can be used to determine marked fish movement, gain insight into potential barrier locations at culverts, and validate the accuracy of capture methods. In addition, there are no associated fish handling stresses that may alter volitional movement. This information can be used to establish sampling intervals that maximize the probability of recapturing fish that have moved between reaches, while minimizing the potential for undetected fish migration completely through a reach

Few collection methods offer the probability of 100% fish recapture efficiency, although many methods with the highest efficiencies (e.g., electrofishing, weirs) offer their own disadvantages (see approach section). Recapture efficiencies of selected methods may be estimated through a series of collection trials in blocked stream reaches; snorkel surveys should also be used to validate the accuracy of capture methods.

Assuming movement probability and recapture efficiency are known, minimum sample sizes needed to detect movement and differences in fish length can be estimated by using tables of statistical probability (Appendix D). Because movement was rarely detected throughout the course of our field study, we have little basis for validating these estimates at this time.

Other Considerations

Study Design:

- In general, increasingly complex study designs yield greater information on fish movement, with related increases in time, effort, and cost. Further consultation from WSDOT is necessary to balance these limitations with the desired level of knowledge to determine optimal protocol study designs.

It is clear that study designs may need to be adaptable to the specific site. The initial design (Design Option 1) with short-term marking provides little information beyond the physical passability of the culvert during a short time period, and false negatives ('impassable culvert') are very likely. Timing of fish movement is critical, and short term (one week) evaluation periods may not occur at the time fish are motivated by natural cues to move upstream. Attempts to motivate fish through false means (i.e., bait) did not appear to affect results. As a result, the initial assumption of designing a very quick protocol is unlikely to be met.

A more likely approach for obtaining conclusive data is to set up the test for long-term monitoring, such as was begun in Design Option 2. One critical aspect of this protocol option is the inclusion of control reaches considered passable to juveniles migrating upstream that can be used to assess natural movement rates and compare to culverted reaches.

RECOMMENDATIONS

Based on results and conclusions of our protocol testing, the following points are recommended during further testing, protocol development, and protocol application.

Study Site Selection

- Before testing, acquire an understanding of site conditions and fish presence/absence from a comprehensive source that includes both historic and current levels of fish species presence, abundance, and distribution (including data on juvenile fish). This comprehensive database should also integrate existing barrier culvert information (Johnson et al. 2001) for listed streams. Much of this information should be available pending completion of the Salmon and Steelhead Habitat Inventory and Assessment Project (SSHIAP) (http://www.wa.gov/wdfw/hab/sshiap/).
- Site variability highlights the need for a standard, yet flexible sampling design (e.g., using a suite of methods) that must be transferable to a variety of locations and situations. For advance preparation, acquire engineering design plans for road culverts, both pre- and post-retrofit, and existing habitat and fish passage evaluations from agency records.
- When implemented in the field, prioritization of culvert testing should be based on retrofit status. Use of the protocol to evaluate road crossings before a planned retrofit occurs (pre-retrofit) can yield valuable information on the effectiveness of the retrofit design when compared with post-retrofit results.
- Reliable, site-specific evaluation of historic and current fish populations within a particular watershed could provide some indication of the impact an impassable barrier has had on these populations, while clarifying the potential benefits of removing this barrier. In cases in which historic populations have been extirpated or compromised, the protocol could be implemented via the introduction of fry from adjacent systems. However, this is a future decision point that must be made in consultation with managers from WDFW and potentially, NMFS.

Initial Capture

- Use rapid collection methods that involve minimal handling and stress. Hold fish for a short time in aerated containers maintained at ambient stream temperatures. Electrofishing is not recommended, although the long-term ramifications of this technique are still under evaluation.
- A variety of capture techniques may present the best range of options for collecting fish in a particular stream system. In our streams, both dip nets and small seines were successfully used for capturing a large number of age 0+ coho for marking. These methods might not be appropriate at all locations under a range of conditions; it should be left within the judgement of local biologists to determine which methods are appropriate during each sampling event, provided that they meet the general criteria laid forth in the protocol. To increase collection efficiency, use a number of sampling teams throughout a range of habitats.

Marking

- Use subcutaneous injection to mark fish, assigning various colors and fins to code for release locations and mark dates. A large collection and marking effort on one date early in the field season (immediately following coho emergence times in the spring) is preferred over multiple marking dates in order to minimize complexities related to data collection, compilation and analysis. While subcutaneous injection using hypodermic needles was the best marking procedure for small fish (<60 mm FL), a spring-loaded dye injection system would be preferable in the field due to safety concerns. This technical option must be further pursued.

Recapture/Observation

- For recapturing marked fish, use a combination of dip net and seining methods with estimated capture efficiencies. Electrofishing may become a viable option pending final approval of protocol design, but must be approved in further discussion with agencies (WSDOT, WDFW, NMFS, Tribes).
- Direct observation techniques using snorkel surveys should be integrated with primary recapture methods into any final protocol design. Additional work should be pursued to identify or design video systems and image recognition technology that can be used to quantitatively evaluate fish passage at culverts. As with snorkel surveys, confidence in mark identification by observation is subject to prevailing stream conditions to a greater degree than by physical recapture.

Estimating Movement

- Continue monitoring of permanently marked fish periodically throughout the year to encompass periods of high movement. These observations will expand our understanding of local movement and culvert passability, and allow refinement of the proposed field protocol. Include observational methods using snorkel surveys to detect timing and scale of movement.
- Base inferences of movement upon the percentage of marked fish recaptured, not the percentage of recaptures in home reaches.
- When planning the layout of a proposed study site, habitat characteristics (e.g., riparian cover, stream habitat distribution) should be fairly similar between study reaches. Sampling methods and effort should be consistent between sampling reaches.
- Use standardized data forms to facilitate complete data collection and comparability of data between sites.

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SUMMARY OF FIELD ACTIVITIES

The field activities undertaken for methods evaluation and protocol design development are summarized below (Table x.). Brief descriptions are given in order to illustrate the evolving nature of the field work.

Table x. Summary of activities during protocol development.

Date	Location	Activities
4/06/01 - 5/05/01	Snow Creek	Pilot studies evaluating minnow traps and marking techniques.
5/08/01 - 5/11/01	Snow/ Andrews Creeks	Initial deployment of protocol concepts with Bismarck Brown and minnow traps at Snow/Andrews Creek convergence. Fish for marking were obtained with the seine at Snow Creek. Baited minnow traps were placed upstream and checked daily. Reference and control fish held for duration of test
5/13/01 - 5/15/01?	Various	Numerous site visits for study site selection.
5/16/01 - 5/21/01	Snow/ Andrews Creeks	Protocol concepts deployed, similar to first time, with seining also done daily above culvert.
5/21/01 - 5/25/01	Dogfish Creek	Protocol test using Bismarck Brown. Fish obtained by seining large culvert outfall pool, baited minnow traps placed upstream. Seining daily above culvert, dip-netting implemented at end of week. Reference and control fish held for duration of test.
5/29/01 - 6/01/01	Dogfish Creek	Protocol test using Bismarck Brown. Fish obtained by dip-netting upstream and downstream of culvert. Baited minnow traps placed upstream of culvert, dip-netting done daily above culvert. All new unmarked captures marked and released downstream of culvert. Reference and control fish from initial marking held for duration of test.
5/29/01 - 6/01/01	Parish Creek	Protocol test using Bismarck Brown. Fish obtained by dip-netting upstream of culvert. Baited minnow traps placed upstream of culvert, dip-netting done daily above culvert. All new unmarked captures marked and released downstream. Supplemental catch for daily additional marking done downstream of culvert. Reference and control fish from intial marking held for duration of test.
6/05/01 - 6/08/01	Dogfish Creek	Protocol test using Bismarck Brown. Fish obtained by dip-netting upstream and downstream of culvert. Baited minnow traps placed upstream of culvert, dip-netting done daily upstream of culvert. Dip-netting also done daily below culvert for recapture. Snorkel survey done in place of dip-netting on the 8^{th}. Reference and control fish held for duration of test.
6/05/01 - 6/08/01	Parish Creek	Protocol test using Bismarck Brown. Fish obtained by dip-netting upstream and downstream of culvert. Baited minnow traps placed upstream of culvert, dip-netting done daily upstream of culvert. Dip-netting also done daily below culvert for recapture. Additional unmarked captures marked on the 7^{th}. Snorkel survey done in place of dip-netting on the 8^{th}. Reference and control fish from both marking events held for duration of test.
6/13/01 - 7/19/01	Dogfish Creek	Advanced protocol testing using subcutaneous injection. Fish marked on 2 separate dates, recapture attempted weekly in study reaches. Minnow trapping discontinued. Dip-net used as primary mode of capture, replaced by seine in final 2 weeks.
6/15/01 - 7/19/01	Parish Creek	Advanced protocol testing using subcutaneous injection. Fish marked on 2 separate dates, recapture attempted weekly in study reaches. In addition, a methods calibration experiment using electrofishing was performed on 6/27, with electroshocked fish marked uniquely. Minnow trapping discontinued. Dip-net used as primary mode of capture.