UTILIZATION OF TWO-STAGE SINGLE-PASS ELECTROFISHING TO ESTIMATE ABUNDANCE AND DEVELOP RECOVERY-MONITORING PROTOCOLS FOR THE ENDANGERED NOOKSACK DACE (*RHINICHTHYS CATARACTAE*) IN CANADA

by

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ABSTRACT

I demonstrate the efficiency and cost-effectiveness of a two-stage sampling method for estimating abundance of Nooksack dace (*Rhinichthys cataractae*), an endangered minnow in Canada. This two-stage process involves single-pass sampling, followed by calibration of single-pass sampling capture efficiency using a mark-recapture method. Based on this two-stage method, my estimated abundances in the Brunette River, Bertrand Creek, Pepin Creek, and Fishtrap Creek were 2,763 fish (95% confidence intervals (CI): 1,823-4,537), 4,359 fish (95% CI: 2,499-7,991), 30 fish (95% CI: 12-136), and 0 fish, respectively. My presence-absence model demonstrated that mean water depth, mean water velocity, and level of substrate embeddedness are important habitat characteristics affecting presence of Nooksack dace in riffle habitats. To assess the long-term recovery of this species, I recommend conducting monitoring at 5-year intervals by sampling 15 to 25 sites in each stream using my two-stage method, resulting in a total annual monitoring cost of about $23,700.

Keywords: Species at Risk Act; Nooksack dace; *Rhinichthys cataractae*; baseline abundance; presence or absence; monitoring

ACKNOWLEDGEMENTS

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GLOSSARY

BC   British Columbia

CDC   Conservation Data Centre

COSEWIC   Committee on the Status of Endangered Wildlife in Canada

CPUE   Catch-per-unit-effort

DFO   Department of Fisheries and Oceans

FL   Fork length

GLM   Generalized linear model

ME   Mean Error

RMSE   Root mean square error

ROC   Receiver Operating Characteristics

SARA   Species at Risk Act

US   United States of America

1: INTRODUCTION

Estimating fish abundance is a fundamental aspect of fisheries research and management. Obtaining reliable and precise measures of abundance while minimizing the cost and effort of sampling has been a continuing pursuit. This endeavour is even more relevant when monitoring species at risk, where accurate information on species abundance is critical and funding for species recovery is often limited (Tisdell 2006, Scott et al. 2005, Myers et al. 2000). Monitoring species at risk allows us to assess population trends, efficiently allocate limited resources, and examine the effectiveness of recovery actions (e.g., habitat restoration or population supplementation). Unfortunately, monitoring programs often fail to provide reliable information that is needed to assess the success of recovery actions due to poor sampling design, low precision or power to detect change, and inadequate resources for monitoring (Herrick 2006, Elzinga 2001, Peterman 1990). An accurate and cost-effective sampling method is therefore necessary when developing a recovery-monitoring program.

Electrofishing, snorkeling, angling, seining, and trapping are commonly used sampling methods to estimate abundance of stream fish, but none of these methods is fully effective at providing an unbiased estimate of actual fish abundance (Peterson et al. 2004). Central to the process of obtaining such an unbiased estimate is having a reliable estimate of capture efficiency (i.e., the

proportion of actual fish abundance that is captured in a sample). Capture efficiency for a given sampling method varies by fish size and species (Price and Peterson 2010, Peterson et al. 2004, Anderson 1995, Bayley and Dowling 1993, Buttiker 1992), habitat size and complexity (Wildman and Neumann 2003, Kruse et al. 1998, Reynolds 1996, Rodgers et al. 1992), level of effort (Rosenberger and Dunham 2005, Peterson et al. 2004), and fish density (Kruse et al. 1998).

Low-intensity sampling methods (i.e., low-effort methods like snorkeling and single-pass sampling) generate biased and imprecise estimates of abundance due to observer bias, fish size or species selectivity (Peterson et al. 2004, Reynolds 1996), and low and variable capture efficiency (Temple and Pearsons 2006, Rosenberger and Dunham 2005, Fièvet et al. 1999). However, because these methods are relatively low cost and low effort, they permit sampling on a larger spatial scale for a given budget (Rosenberger and Dunham 2005). High-intensity methods (e.g., multi-pass removal; mark recapture) yield less-biased abundance estimates, but often require more time, effort, and expense, which can limit the spatial scale of sampling. Such a limited sampling scale can misrepresent fish abundance and distribution on a larger scale (Hankin and Reeves 1988). In some situations, when estimates of abundance from a low-intensity and a high-intensity method are strongly correlated, researchers calibrate estimates from the low-intensity method using the high-intensity method (an "index-to-index" comparison) (Rosenberger and Dunham 2005). However, the relationship between these two indices can vary substantially based on habitat features or other confounding factors (Williams et al. 2004).

A preferable approach to achieving an unbiased abundance estimate involves calibrating catch from a low-intensity method using an unbiased estimation method (i.e., an "index-to-unbiased estimate" comparison) (Price and Peterson 2010, Rosenberger and Dunham 2005, Peterson et al. 2004). Mark-recapture methods can provide unbiased estimates of capture efficiency, which can be used to calibrate a low-intensity method like single-pass removal to obtain reliable estimates of fish abundance (Peterson et al. 2004, Anderson 1995, Bayley and Dowling 1993, Buttiker 1992). In order to obtain unbiased estimates though, mark-recapture assumptions must not be violated (Peterson et al. 2004, Rodgers et al. 1992). Assumptions for the mark-recapture method include (1) a closed population, (2) random distribution of marked and unmarked fish, and (3) equal capture probability between marked and unmarked fish (White et al. 1982). The assumption of a closed population can be satisfied by installation of barriers to minimize escapes. For stream-dwelling fish, a recovery period of approximately 24 hours between mark and recapture events has been shown to satisfy the remaining two assumptions in previous work (Rosenberger and Dunham 2005, Peterson et al. 2004, Mesa and Schrek 1989).

I estimated the abundance of the Nooksack dace (*Rhinichthys cataractae*), an endangered fish species in Canada (*Species at Risk Act* 2002) using two-stage sampling. Nooksack dace are small (<15 cm) stream-dwelling minnows, whose known range in Canada is four streams in British Columbia's (BC) rapidly urbanizing Fraser Valley. They are considered a subspecies of the longnose dace (*R. cataractae*), and are commonly found in riffle habitats in small streams.

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Loss of critical riffle habitat appears to be the most significant threat facing this species within its Canadian range (Pearson et al. 2008). The Nooksack dace are at the northern extent of their range in BC and may be naturally rare in Canada because of this (Pearson 2004). The global range of this species includes 20 additional streams in Washington State. Because of their restricted range in Canada and the continued decline in habitat quality and availability, Nooksack dace were listed as endangered in 1996 by the Committee for the Status of Endangered Wildlife in Canada (COSEWIC 2007, McPhail 1996) and under Schedule 1 of the federal *Species at Risk Act* (*SARA*) in 2003. In BC, Nooksack dace were red-listed with a provincial rank of S1 in 2010 and a global rank of G3 in 1996 (B.C. Conservation Data Center 2011). No federal or state protection is provided for this species in the United States of America. Under *SARA* (2002), a recovery strategy and a recovery action plan must be completed for all species listed as endangered, threatened, or extirpated. The recovery strategy for the Nooksack dace was completed in 2008 (Pearson et al. 2008), and development of the recovery action plan is currently under way to identify and rank recovery actions to meet the objectives set out in the recovery strategy.

The recovery strategy for the Nooksack dace in Canada (Pearson et al. 2008) identifies two key objectives relevant to this study: (1) to increase Nooksack dace abundance to target levels in all watersheds by 2015; and (2) to ensure that at least one reach in each watershed supports a high density of Nooksack dace. To achieve these recovery objectives, it is necessary to obtain accurate and reliable data on Nooksack dace abundance in each watershed,

identify site-scale habitat features important to this species, and assess recovery of this species over time.

To help assess the status of the population against objectives in the recovery strategy, I estimated Nooksack dace abundance in each watershed, using a two-stage sampling process. Stage 1 of the process involved low-intensity single-pass electrofishing in each study stream. Stage 2 involved single-pass electrofishing coupled with mark-recapture sampling at a subset of sample sites to provide an estimate of capture efficiency at each site. Using site-scale habitat co-variates, I developed a model to predict capture efficiency, which was then used to calibrate catch at each site and estimate abundance in each stream. I examined the relative importance of site-scale habitat features for Nooksack dace by developing a presence-absence model for the species. To assess long-term recovery of the species, I developed monitoring protocols for the Nooksack dace. I discuss my results in relation to my study objectives, which include (1) developing baseline abundance estimates for the Nooksack dace, (2) developing a reliable, accurate, and cost-effective method for surveying fish in freshwater streams, and (3) developing long-term monitoring protocols for recovery of the Nooksack dace.

2: METHODS

2.1 Study Area

I conducted my study from June - August 2009 in four streams in southwestern British Columbia, where the Nooksack dace was identified as being present (Pearson et al. 2008). Three of these streams, Bertrand Creek, Pepin Creek, and Fishtrap Creek (UTM Coordinates: 534859 mE, 5427820 mN; 538661 mE, 5427853 mN; and 543375 mE, 5427889 mN; respectively) are located in the Fraser Valley Regional District (FVRD), and flow south into the Nooksack River in Washington state. The fourth stream, the Brunette River (UTM Coordinates: 507865 mE, 5451879 mN), flows from Burnaby Lake in the Metro Vancouver Regional District into the Fraser River. All streams surveyed were at or near baseflow conditions during the sampling period.

2.2 Field Methods

Sample sites (Figs. 1 and 2) were located upstream and downstream of access points (bridges, roads, trails) on each creek, beyond a 15-m buffer from these structures to avoid any effect of man-made crossings or routes (e.g., runoff from roads) on fish abundance. Sites consisted of pool, riffle, and glide habitats, identified using habitat-typing methods originally described by Bisson et al. (1981). Habitats were sampled in the natural sequence of occurrence beyond the 15-m buffer at each access point. I assumed that access points were

randomly distributed with respect to the fish population. I sampled a total of 83 riffle, 24 glide, and 28 pool habitats across study streams within potential critical habitat areas identified in the recovery strategy for the Nooksack dace (Pearson et al. 2008) (Fig. 1 and 2). In Fishtrap Creek, I sampled a limited number of sites because much of the stream was too deep for electrofishing. These conditions persisted from 0th Avenue to Marshall Road (Fig. 1), and consisted of large sections of pool resulting from several large beaver dams and from dredging work performed in 1995 (Pearson et al. 2008).

More sampling effort was allocated to riffles (ratio of riffle habitat to pool or glide habitats of approximately 3:1) because Pearson (2004) showed that adult Nooksack dace (fork length >54 mm, Inglis et al. 1994) occur primarily in riffle habitats. In some cases, however, it was not possible to attain this sample-site ratio because of instream hazards, barriers, or lack of access.

The total riffle area for Bertrand Creek, Pepin Creek, and Fishtrap Creek was estimated by walking the length of each stream, and measuring the length and wetted width of riffle habitats using a calibrated laser rangefinder (Leica DISTO^{TM} DXT). An estimate of the total riffle area in Brunette River was obtained from a similar survey performed by Pearson (unpublished raw data from a habitat survey in 2008). Available pool and glide habitat areas were not measured, because only total riffle area was used to estimate Nooksack dace abundance in each stream.
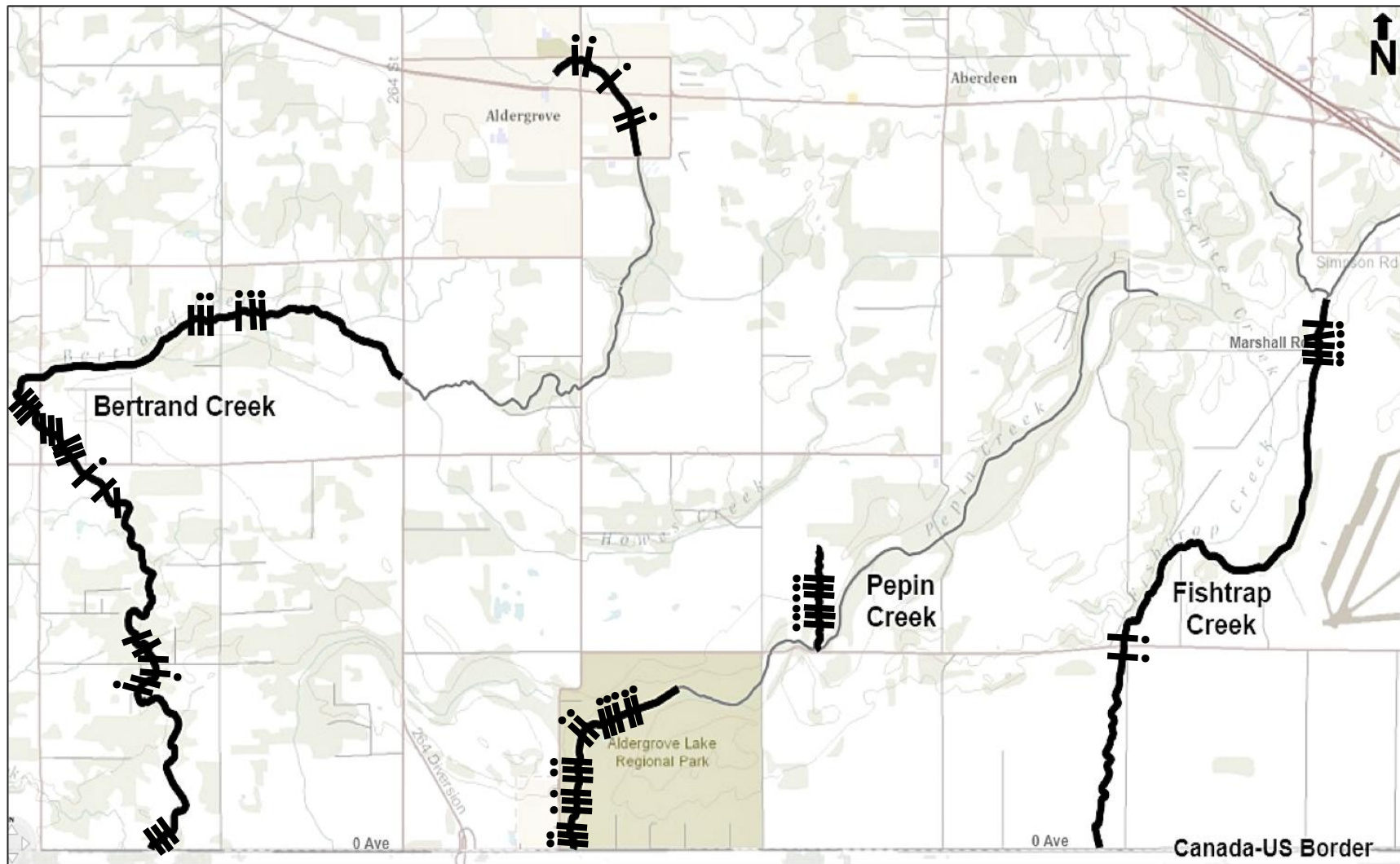
Figure 1: Single-pass riffle sites sampled in the Bertrand Creek, Pepin Creek and Fishtrap Creek in BC's Fraser Valley in 2009. The darker shaded line traced over a portion of each stream outlines potential critical habitat identified in the recovery strategy for the Nooksack dace (Pearson et al. 2008). Within this area, bars crossing the river identify the locations of riffle sites sampled, and dots next to these bars identify sites where Nooksack dace were absent.

Figure 2: Single-pass riffle sites sampled in the mainstem of the Brunette River between Brunette Avenue and the Cariboo dam, and in the small side tributary of Stoney Creek. The darker shaded line traced over a portion of the river outlines potential critical habitat identified in the recovery strategy for the Nooksack dace (Pearson et al. 2008). Within this area, bars crossing the river identify the locations of riffle sites sampled, and dots next to these bars identify sites where Nooksack dace were absent.

2.3 Stage 1: Single-Pass Sampling

At each sample site, I conducted a single electrofishing pass using a backpack electrofisher (Smith-Root, Inc.; model 12-B) with unpulsed direct current at 200–400 V and 50-70 Hz. Sampling was performed using a trained three-

person crew, with one person operating the backpack electrofisher, one person

holding a 1-meter pole seine (6.4-mm-mesh net) downstream of the electrofisher

and one person with a dip net. Sampling between the electrofisher, and pole

seine occurred in a downstream direction; however, overall sampling within a

given site was performed in an upstream direction (Fig. 3). Nooksack dace

captured at a site were weighed (g), measured for fork length (mm) and held in a

recovery bucket until active again. Other fish species caught were identified,

counted, and released.
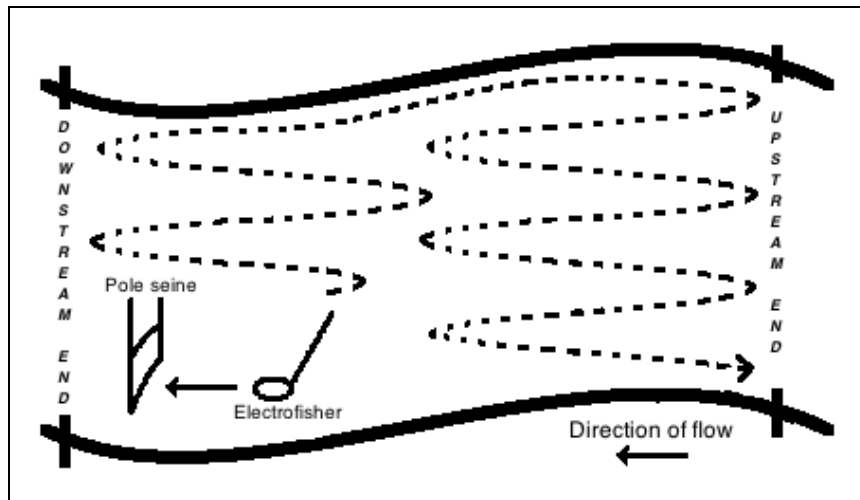
**Figure 3: Single-pass sampling technique used to sample pool, riffle and glide habitats.
Sampling between the electrofisher and the pole-seine was performed in the
downstream direction; however, the entire site itself was sampled in an
upstream direction, as indicated by the dashed line.**

Habitat characteristics were also recorded at each site (Table 1). Channel

width (m), wetted-width (m), and site-length (m) were measured using a calibrated

laser rangefinder (Leica DISTO^{TM} DXT), and site gradient (%) was measured

using a Sunnto clinometer. Mean water velocity (m/sec), mean depth (m), and

mean substrate size (cm) were estimated for each site by taking measurements at 5 evenly spaced points along a diagonal transect through the site. Water velocity and depth were measured using a Swoffer Model 2100 Series Current Velocity Meter and wading rod. Velocity was measured at 0.6 m of the total water depth at each point along the transect. Substrate size was estimated by measuring the diameter of a substrate particle immediately upstream of the wading rod.

Percent cover, proportion of instream vegetation, proportion of filamentous instream algae, and proportion of substrate types classified according to the grain-size scale developed by Kaufmann and Robinson (1993) (Table 2) were visually assessed for each site. I also measured the level of substrate embeddedness at each site, which characterizes the degree to which fine sediments surround coarse substrates on the surface of the streambed. I measured substrate embededdness by visually estimating the proportion of fine sediment (< 2 mm grain size) found between substrate pores spaces as high (>75%), medium (25-75%), and low (<25%).

Water chemistry measurements were recorded at one site on a stream on each sampling day because I assumed that water chemistry would be relatively uniform within 1 km of where the measures were recorded. These data included water temperature (^{o}C) and conductivity (μs/cm), pH, turbidity (NTU), and dissolved oxygen (mg/L). Calibrated hand-held meters were used to measure water temperature, conductivity, and dissolved oxygen (WTW LF 340/SET multi-meter), pH (Oakton Instruments waterproof pHTestr 2), and turbidity (LaMotte 2020 Turbidimeter).

Table 1: Characteristics of 83 single-pass riffle sites in Bertrand Creek, Pepin Creek, Fishtrap Creek and Brunette River in southwestern BC, sampled to estimate Nooksack dace abundance in 2009. Sampled pool and glide sites were not used in abundance estimation, and therefore are not included here.

Variable	Mean (SD) values for sampled single-pass riffle sites			
	Bertrand Creek	Pepin Creek	Fishtrap Creek	Brunette River
Site Length (m)	6.38 (2.86)	8.09 (4.50)	5.79 (2.34)	10.63 (5.09)
Mean Wetted Width (m)	3.69 (1.74)	3.57 (2.84)	3.28 (0.63)	9.08 (3.36)
Mean Depth (m)	0.16 (0.19)	0.14 (0.03)	0.15 (0.06)	0.17 (0.05)
Mean Flow Velocity (m/sec)	0.40 (0.19)	0.46 (0.23)	0.46 (0.08)	0.39 (0.13)
Mean Substrate Size (cm)	4.72 (2.77)	5.82 (4.77)	5.89 (3.69)	8.13 (6.49)
Substrate (% composition)				
Fine (sand, silt and clay)	17.36 (21.31)	21.77 (27.22)	36.43 (35.08)	9.05 (15.05)
Gravel	70.39 (30.324)	55.59 (32.80)	56.43 (41.60)	42.62 (32.81)
Cobble	11.33 (19.70)	19.68 (32.80)	5.00 (5.00)	26.76 (29.12)
Boulder	0.90 (2.67)	2.95 (12.79)	2.14 (5.67)	6.57 (17.19)
Bedrock	0 (0)	0 (0)	0 (0)	15 (28.42)
Percent Cover (%)	29.00 (23.82)	48.13 (29.19)	43.57 (25.61)	32.48 (32.80)
Instream Vegetation (%)	3.48 (5.28)	4.77 (11.4)	7.28 (10.81)	1.95 (4.27)
Instream Filamentous Algae (%)	16.81 (30.49)	10.91 (22.50)	26.28 (43.53)	53.62 (39.14)
Gradient (%)	4.14 (3.20)	3.79 (3.56)	3.14 (1.34)	3.14 (2.22)
Undercut bank (%)	1.54 (3.04)	2.86 (4.04)	0.43 (0.01)	1.14 (1.68)
Temperature (^{o}C)	17.27 (1.73)	14.72 (0.65)	17.80 (1.71)	18.3 (2.17)
Conductivity (μs/cm)	401.36 (382.4)	280.32 (6.73)	174.71 (26.84)	235.62 (20.55)
Turbidity (NTU)	8.69 (3.57)	4.66 (1.11)	9.04 (1.06)	7.52 (2.59)
Dissolved Oxygen (mg/L)	6.85 (3.43)	7.42 (2.59)	6.31 (0.48)	8.41 (3.43)

Table 2: Substrate type and size modified from Kaufmann and Robinson (1993).

Substrate Type	Diameter (mm)
Bedrock	> 4000
Boulder	> 256
Cobbles	64 – 256
Gravel	2 – 64
Fine (Sand, Silt and Clay)	< 2

2.4 Stage 2: Mark-Recapture experiments

I used mark-recapture experiments to estimate the capture efficiency of my single-pass sampling and to develop a habitat-based predictive model for capture efficiency. Sixteen riffle sites were selected in Bertrand Creek and Brunette River that spanned the available range of habitat characteristics in all study streams.

Pool and glide habitats were not included in mark-recapture experiments because negligible Nooksack dace densities were found in these habitats in Stage 1. Dace densities in Pepin Creek and Fishtrap Creek were insufficient for mark-recapture analysis and therefore no mark-recapture sites were established in these creeks. To obtain the initial marked population of fish, each mark-recapture site was sampled using the procedure described previously in the *Stage 1* section to obtain 20-30 fish. However, for 2 of the mark-recapture sites, I was unable to capture enough fish in the sampling area, and had to sample adjacent areas (within <20 m) to attain adequate numbers for marking. Captured fish were anaesthetized using clove oil (40 ppm) mixed with stream water. When fish movement was slowed, fork length (mm) and weight (g) were measured and each fish was marked with a caudal fin clip. Fin-clipped fish were visually examined for signs of stress immediately after marking. Marked fish were held in a covered perforated bucket in the stream for a 24-hour recovery period. Based on earlier work (Temple and Pearsons 2006, Rosenberger and Dunham 2005, Mesa and Shreck 1989), I assumed that this recovery period was adequate to avoid behavioural biases that could potentially result in unequal capture probabilities between marked and unmarked fish. Following the recovery period, marked fish were once again examined for signs of stress. Those fish that were perceived to be unhealthy or stressed were not included in mark-recapture experiments. Each mark-recapture site was blocked off at the upstream and downstream ends using 7-mm-mesh nets secured to the streambed by placing rocks on top of the bottom lip of each net. Marked fish were then stocked evenly through each site. Marked

fish were provided 3 hours to re-acclimate to their habitat and distribute

themselves throughout a site before I resampled the site using the single-pass

electrofishing procedure described in *Stage 1*. All fish captured had their weight

(g) and fork length (mm) measured, and were identified as 'marked' or 'unmarked'

and held in a recovery bucket. Once visibly active in the recovery bucket, fish

were released back into the site. Habitat and water chemistry characteristics

measured at sample sites in *Stage 1* were also recorded for sites in *Stage 2*.

3: DATA ANALYSIS

3.1 Modeling single-pass capture efficiency

Because fish size can affect electrofishing capture efficiency (Peterson et al. 2004, Büttiker 1992, Zalewski and Cowx 1990), I examined whether numbers of recaptured fish differed from numbers of marked fish in each size class (size range: 54 mm to >94 mm in 7 mm intervals) using a χ^2 test. Recaptures of marked fish by size class at each site were small (range: 0 - 12 recaptured fish), so I pooled data across all mark-recapture sites.

I used logistic regression to estimate capture efficiency of my single-pass sampling based on site-scale habitat and fish population characteristics at my mark-recapture sites. I developed a binomial logistic regression model because my capture efficiency data were in the form of successes (number of fish captured) and failures (number of fish not recaptured) (Price and Peterson 2010, Rosenberger and Dunham 2005, Peterson et al. 2004). Single-pass capture efficiency (q) was directly measurable at each mark-recapture site (i) by obtaining the ratio of the recaptured fish ($n_{recaptured}$) to the marked fish (n_{marked}) at each site:

(1)
$$q_i = \frac{n_{recaptured}}{n_{marked}}$$

More site-scale predictor variables (Table 1) were available than the number of mark-recapture sites. To narrow down the list of predictors for inclusion in my model, I included relevant variables from similar work (Peterson

2010, Rosenberger and Dunham 2005, Pearson 2004, Peterson et al. 2004, Alonso 2001) and also variables that seemed logically plausible, as shown below. Pearson correlations were calculated for all pairs of predictor variables to avoid inclusion of more than one of each pair of correlated independent variables (i.e., if Pearson's r > 0.3) in the global model.

Independent variables ($X_1, X_2,... X_m$) included in my global model were the proportion of instream vegetation, the proportion of substrate composed of cobble, the proportion of undercut bank, site length, percent cover, and median fork-length of captured fish:

(2) $$logit(q) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 ... + \beta_m X_m$$

where $logit(q)$ is the predicted capture efficiency expressed in log-odds (i.e., log(q/(1-q))), β_0 is the model intercept; and β_m is the parameter coefficient for the independent variable X_m.

I used Cook's distance (D_i >1) to check for data points with high leverage and found that one mark-recapture sample site could be considered an outlier. This outlier skewed the distribution of the standardized residuals away from the normal distribution, violating model assumptions. The outlier was dropped from the analysis because its removal did not influence model selection or final estimates of abundance in each creek. After testing assumptions of the global model via diagnostic plots, I used the package "MuMIn" (Bartoń 2011) in R (R Development Core Team 2011) to examine the relative support for all subsets of the global model. A subset of the most plausible models was selected using

Akaike's information criterion (Akaike 1973) corrected for small-sample bias (i.e., models with $\Delta AIC_c < 4$), and Akaike weights (AIC_c; Burnham and Anderson 2002). To avoid over-fitting the data, this plausible subset of models was further narrowed based on the relative importance of individual predictor variables to develop my final multi-model averaged model. To incorporate model selection uncertainty and increase model support, I computed model-averaged estimates of the model coefficients in equation (2) for individual predictor variables in my final averaged model (Burnham and Anderson 2002). This model was used to predict the capture efficiency of single-pass sampling at all sample sites in each stream.

I estimated the mean error (ME) of my capture-efficiency model as the mean difference between the measured and predicted capture efficiency across all mark recapture samples. I also calculated the root mean squared error (RMSE), which was simply the square root of the mean squared difference between measured and predicted capture efficiency across mark recapture samples. The mean error served as an estimate of model bias, while the root mean squared error was used to assess the combination of model precision and bias (Peterson et al. 2004). I estimated these errors as part of the bootstrapping procedure described in the following section.

3.2 Estimating total fish abundance in each stream

I examined catch from single-pass riffle sites in each stream for spatial autocorrelation using the Moran's I spatial statistic. This was done to account for the spatial location of sites in each stream and to ensure that catches between sites in each creek were independent. I used the 'ape' package (Paradis et al.

2004) in R (R Development Core Team 2011) to generate a matrix of inverse distance weights for each site using latitude and longitude data paired with the catch at each site. This matrix was then used to calculate the Moran's I statistic for each stream. Only Bertrand Creek and Brunette River were assessed for spatial autocorrelation because no Nooksack dace were detected during sampling in Fishtrap Creek, and because all sample sites that I used to estimate abundance within Pepin Creek occurred within a 570-m stretch, resulting in estimates from 9 of 12 riffle sites in that section of the creek. Thus, nearly all available riffle sites in this 570-m section of Pepin Creek were sampled, and were therefore likely spatially correlated. However, because my sample size in this section was so extensive, I obtained a better estimate of Nooksack dace abundance in Pepin Creek.

Estimates of adult Nooksack dace abundance in each stream were based on catch data from riffle sites alone because negligible densities of Nooksack dace were found in pool and glide habitats during sampling. To obtain an estimate of abundance in each stream, I first needed to calibrate the catch at each single-pass riffle site using the capture-efficiency model described previously. My first step in this process was to estimate the error in the predicted capture efficiency (q) at each single-pass riffle site (i). To do this, I conducted bootstrap sampling of the mark-recapture data with replacement (Fig. 4, Step 1), fitting the capture efficiency model at each of 10,000 sampling iterations (Fig. 4, Step 2), using the 'boot' package (Canty and Ripley 2011) in R (R Development Core Team 2011). Thus, although the model parameters were the same on all

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bootstrap iterations, the value of the averaged model coefficients changed with the resampled mark-recapture data.

Concurrently, I also conducted bootstrap sampling of single-pass habitat ($X_1, X_2,...X_m$) and catch data (C_i) 10,000 times (Fig. 4, Step 3). Thus, for each iteration at which the capture-efficiency model was fit, a bootstrap sample of single-pass riffle site habitat data was made available to the model to predict capture efficiency at each site (q_i) (Fig. 4, Step 4). The predicted capture efficiency at each single-pass riffle site (q_i) was then used to scale the catch at each site (C_i), to obtain an estimate of the abundance at each site (n_i) at that iteration (Fig. 4, Step 5):

$$(3) \qquad\qquad n_i = \frac{C_i}{q_i}.$$

Next, I estimated the mean density of Nooksack dace across single-pass riffle sites using a ratio-of-means estimator. That is, the ratio of the mean site abundance ($\bar{n}$) to the mean area ($\bar{a}$) of single-pass riffle sites was used to generate the mean density (r) (Nooksack dace/m^2) in each stream at the same iteration (Fig. 4, Step 6):

$$(4) \qquad\qquad r = \frac{\bar{n}}{\bar{a}}.$$

I then used the total estimated riffle area (A) for each stream as an expansion factor to determine the abundance of Nooksack dace in the stream at that iteration (N) (Fig. 4, Step 7):

$$(5) \qquad\qquad N = rA.$$

After 10,000 iterations, a bootstrap estimate of the total abundance based on riffle habitats (N_t) in each stream was generated along with its standard error (*SE*). Bias-corrected and accelerated 95% confidence intervals were also calculated for the total estimated abundance in each creek using the 'boot' package (Canty and Ripley 2011) in R (R Development Core Team 2011). These confidence intervals reflect adjustments for bias and skewness in the bootstrap distribution (Efron 1987).

Capture efficiency estimation

Step 1:

Re-sample
mark-recapture sites
(15 sites)

Repeat 10,000 times

Step 2:

Fit averaged
capture-efficiency model:

$logit(q) = \beta_0 + \beta_1 X_1 + ... + \beta_m X_m$

Abundance estimation

$X_1, X_2, ...X_m$

Step 3:

Sample of single-pass riffle sites
(habitat data and catch)

C_i

Step 4:

Predict capture efficiency at
each single-pass riffle site

q_i

Step 5:

Calculate riffle site fish abundance
by using capture efficiency:

$$n_i = \frac{C_i}{q_i}$$

Step 6:

Estimate mean fish density from
mean sampled riffle area

$$r = \frac{\overline{n}}{\overline{a}}$$

Bootstrap estimate of total fish
abundance in stream riffles
along with its standard error

$N_t \pm SE$

Step 7:

Estimate fish abundance in the
stream based on riffle habitats

$N = rA$

Repeat 10,000 times

Figure 4: Conceptual diagram for estimating single-pass capture efficiency (left side) and Nooksack dace abundance (right side) in riffle habitats for each study stream using bootstrapping. *Logit(q)* **represents the capture efficiency expressed as log-odds (i.e., log(q/(1 − q))),** β_0 **is the intercept, and** β_m **is the coefficient for the independent variable** X_m, q_i **is the capture-efficiency at site** *i*, C_i **is the single-pass catch at site** *i,* **and** n_i **is the calibrated single-pass catch at site** *i.* *r, A,* **and** *N* **are the stream density, total riffle area in the stream, and stream abundance, respectively, calculated in each bootstrap iteration.** N_t **is the bootstrapped estimate of total stream abundance along with its standard error (** *SE***).**

3.3 Modeling Nooksack dace presence or absence

Logistic regression is commonly used in ecology and conservation biology for predicting the presence or absence of species in habitats based on environmental data (Guisan and Thuiller 2005, Manel et al. 2001, Fielding and Bell 1997). Logistic presence or absence models have been used to predict (1) sites that might be at risk of invasion from nuisance species or diseases (Buchan and Padilla 2000, Venier et al. 1998), (2) how species may respond to changes in land use (Verheyen et al. 2003, Fitzgibbon 1997), and (3) the distribution of rare and endangered species (Engler et al. 2004, Pearson 2004, Mladenoff et al. 1999), among many other applications.

I therefore developed a logistic regression model to predict presence or absence of Nooksack dace based on data pooled from sampled riffles from Bertrand Creek, Pepin Creek, and Brunette River. I used logistic regression because my response variable (presence or absence) was binary (i.e., presence = 1, absence = 0). My goal was to predict dace presence or absence based on habitat variables at a site scale (i.e., at the scale of each individual riffle). The model also provides an estimate of the effect size of each habitat variable on the probability of presence or absence of Nooksack dace. As before, I examined Pearson pair-wise correlations between all sets of predictor variables before including them in the model (Pearson's r < 0.3). The global model was developed based on habitat variables used in a similar reach-scale analysis done by Pearson (2004) and by inclusion of logical predictor variables. After examining model assumptions, a subset of plausible models (ΔAIC_c < 4) was selected using AIC_c

and Akaike weights. This subset of plausible models included site length, mean water velocity, mean substrate size, mean depth, percent cover, site gradient, and the level of substrate embeddedness as predictor variables. My final multi-model averaged model included the full subset of plausible models. I computed model-averaged estimates of the individual predictor variables in my final model to account for model selection uncertainty (Burnham and Anderson 2002).

The performance of logistic regression models can be assessed using a Receiver Operating Characteristics (ROC) plot (Moisen et al. 2006, Manel et al. 2001, Pearce and Ferrier 2000, Fielding and Bell 1997). Unlike a 2 x 2 classification table, which is based on an arbitrary decision threshold (usually a predicted probability > 0.5) (Lindenmayer et al. 1990), a ROC plot provides a threshold-independent method of evaluating model performance (Moisen et al. 2006, Pearce and Ferrier 2000). In a ROC plot, the true positive rate (i.e., correctly predicting presence when a species is present at a site) is plotted against the false positive rate (i.e., incorrectly predicting presence when a species is absent at a site) for a range of decision thresholds from 0 to 1 (Pearce and Ferrier 2000, Fielding and Bell 1997). A model with good discrimination performance will have a high true positive rate when the false positive rate is relatively low, and will level off at the maximum true positive rate (1.0) over the range of decision thresholds. The ROC curve for a model with poor discriminatory performance will lie near the 1:1 line, where the true positive rate equals the false positive rate across all decision thresholds (i.e., the area under the curve (AUC) will equal 0.5). The AUC is therefore a good measure of overall model

performance, where AUC values close to 1.0 indicate models with good predictive performance, whereas AUC values close to 0.5 indicate poor performance (Moisen et al. 2006). To examine discrimination performance of my presence-absence model, I plotted the ROC curve and calculated the AUC using the 'ROCR' package in R (Sing et al. 2009). I calculated error bars for a range of decision thresholds in the ROC plot by bootstrapping presence/absence observations and predictions from the model 2000 times.

3.4 Variation in abundance estimates with sample size

Examining trade-offs between sample size and precision in estimates of abundance helps inform decisions about allocation of effort and funding in the development of monitoring protocols. I examined these trade-offs by calculating the coefficient of variation ($CV = \dfrac{SD}{\overline{X}}$) for bootstrap estimates of abundance generated using sample sizes from 1 to 40 single-pass riffle sites for each study stream. I used a bootstrap procedure for estimating abundance similar to that described above, except that for each sample size from 1 to 40, the population of single-pass sites from each creek was sampled with replacement to generate abundance estimates at each sample size in each study stream. In addition to propagating error from capture efficiency estimates into the estimated abundance in each creek, this method also propagates sampling error. The resulting precision in estimates of abundance can be characterized by the CV to examine the relationship between precision and sample size for each study stream.

4: RESULTS

4.1 Summary results from sampling

I sampled equal proportions of available riffle habitat in Bertrand Creek and Pepin Creek, and a larger proportion of available riffle area in Brunette River (Table 3). Nooksack dace were detected throughout identified critical habitat areas sampled in Bertrand Creek, whereas in Pepin Creek, Nooksack dace were only detected in a 570-m section of critical habitat area extending north from the US-Canada Border (Fig. 1). In the Brunette River, Nooksack dace were found throughout identified critical habitat in the mainstem of the river between Brunette Avenue and the Cariboo dam and in Stoney Creek below Government Street (Fig. 2). No Nooksack dace were detected in Fishtrap Creek.

A total of 875 Nooksack dace were captured during single-pass and mark-recapture sampling in my 4 study streams. Of this total number of fish, 95% were adults and 5% were juveniles (i.e., fork-length < 54mm). Median fork-length for adults was 75 mm, whereas median fork-length for juveniles was 32 mm. More fish were caught in Brunette River and Bertrand Creek than in Pepin Creek (Table 4). Mean catch was higher in riffle habitats than in pool and glide habitats in Bertrand Creek. A large number of Nooksack dace was found in 1 of 12 sampled pool habitats (20 fish) and 2 of 10 sampled glide habitats (5 fish and 16 fish) in Bertrand Creek. No adult Nooksack dace were detected in any other pools or glides sampled during the study (Table 4). By comparison, Nooksack dace were

found in 21 of 33 sampled riffles (158 fish) in Bertrand Creek, 15 of 21 sampled

riffles (227 fish) in Brunette River, and 4 of 9 sampled riffles (7 fish) in the lower

570 m of Pepin Creek.

Table 3: Summary of sampled riffle area and total available riffle area in identified critical habitat (Pearson et al. 2008) in Pepin Creek, Bertrand Creek, Brunette River and Fishtrap Creek.

Study Stream	Riffle Area Sampled (m^2)	Total Riffle area (m^2)	Proportion Sampled
Bertrand Creek	822	7269	0.11
Pepin Creek	214	1905	0.11
Brunette River	2069	7292	0.28
Fishtrap Creek	136	1232	0.11

Table 4: Catch of adult Nooksack dace from single-pass sampling in identified critical habitat in Bertrand Creek, Pepin Creek, Brunette River, and Fishtrap Creek. Numbers in parenthesis are the total number of single-pass sites sampled for a given habitat type in each creek. No fish were detected during sampling in Fishtrap Creek or in pool or glide habitats for Pepin Creek and Brunette River.

Study Stream	Total catch (# of fish)	Mean catch of Nooksack dace by habitat (Mean ± SE)		
		Riffles	Glides	Pools
Bertrand Creek	158	4.79 ± 1.26 (33)	2.1 ± 1.62 (10)	1.67 ± 1.19 (12)
Pepin Creek	7	0.77 ± 0.43 (22)	0 (6)	0 (4)
Brunette River	227	10.81 ± 2.18 (21)	0 (5)	0 (4)
Fishtrap Creek	0	0 (7)	0 (3)	0 (6)

During mark-recapture sampling, a total of 360 adult fish were marked and

106 of these were recaptured. Mean measured capture efficiency and predicted

capture efficiency (from the logistic model) was slightly higher in Brunette River,

but the difference was small (Table 5). Capture efficiency did not differ

significantly among size classes ($\chi^2 = 4.98$, df = 6, P = 0.55) (Fig. 5).

Table 5: Mean measured capture efficiency for mark recapture sample sites, and the mean predicted capture efficiency for Bertrand Creek, Pepin Creek, and Brunette River. Only single-pass sites in the lower 570 m of Pepin Creek in which Nooksack dace were detected were used to estimate mean predicted capture efficiency for the creek.

	Number of sites	Mean Capture Efficiency	Standard Error
Measured capture efficiency at mark-recapture sites			
Brunette River	6	0.31	0.04
Bertrand Creek	10	0.28	0.03
Overall for Brunette River and Bertrand Creek	16	0.29	0.02
Predicted capture efficiency at single-pass sites			
Brunette River	21	0.31	0.01
Bertrand Creek	33	0.26	0.01
Pepin Creek	9	0.28	0.03
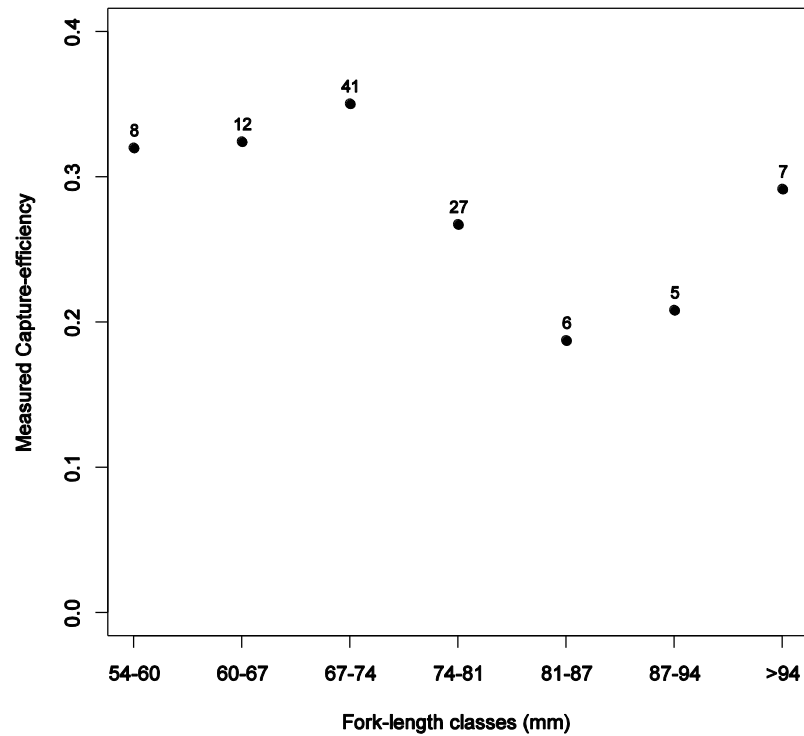
Figure 5: Measured capture efficiency by fork-length class (mm) using pooled counts from mark-recapture sites in Brunette River and Bertrand Creek. Numbers over data points represent the numbers of recaptured fish in each fork-length class.

4.2   Modeling measured capture efficiency

To calibrate catch from single-pass sampling, I used a logistic regression model to predict single-pass capture efficiency at each sample site. My most plausible subset of models ($\Delta AIC_c < 4$) included the proportion of substrate composed of cobble, the proportion of instream vegetation, the proportion of undercut bank, site length, percent cover, and median fork-length of captured fish as predictor variables (Table 6). Examination of the relative variable importance (i.e., sum of the AIC_c weights that were re-scaled relative to 1.0 for the top models that contained a given variable) of individual predictors (Table 7) showed that the proportion of substrate composed of cobble and the proportion of instream vegetation had the highest importance weights (0.76 and 0.53, respectively). To avoid over-fitting my limited mark-recapture dataset (from only 16 mark-recapture sites), I dropped all models that included predictor variables with a low (< 0.15) importance weight (Table 7). Thus, my final multi-model averaged model (i.e., averaged over Models 1-4) included only the proportion of substrate composed of cobble and the proportion of instream vegetation as predictor variables.

Table 6: The most plausible subset of candidate models ($\Delta AIC_c < 4$) for predicting capture-efficiency of single-pass sampling at riffle sites along with their AIC_c weights. β_0, $\beta_{Cobbles}$, $\beta_{Instream.Veg}$, $\beta_{Undercut}$, β_{Length}, β_{Cover}, and $\beta_{MedianFL}$ are parameters for the model intercept, the proportion of substrate composed of cobbles, the proportion of instream vegetation, the proportion of undercut bank, site length, percent cover, and median fork-length of captured fish at each site, respectively.

Model	Parameters	No. of parameters (k)	AIC_c	ΔAIC_c	Weight	Scaled Weight
1	β_0, $\beta_{Cobbles}$, $\beta_{Instream.Veg}$	3	65.99	0.00	0.16	0.21
2	β_0, $\beta_{Cobbles}$	2	66.31	0.33	0.13	0.18
3	β_0, $\beta_{Instream.Veg}$	2	67.49	1.50	0.07	0.10
4	β_0	1	67.96	1.97	0.06	0.08
5	β_0, $\beta_{Cobbles}$, $\beta_{Instream.Veg}$, β_{Length}	4	68.17	2.18	0.05	0.07
6	β_0, $\beta_{Cobbles}$, β_{Length}	3	68.72	2.74	0.04	0.05
7	β_0, $\beta_{Cobbles}$, $\beta_{Instream.Veg}$, β_{Cover}	4	68.73	2.74	0.04	0.05
8	β_0, $\beta_{Cobbles}$, $\beta_{MedianFL}$	3	68.90	2.91	0.04	0.05
9	β_0, $\beta_{Cobbles}$, β_{Cover}	3	68.93	2.94	0.04	0.05
10	β_0, $\beta_{Cobbles}$, $\beta_{Undercut}$	3	69.19	3.21	0.03	0.04
11	β_0, $\beta_{Cobbles}$, $\beta_{Instream.Veg}$, $\beta_{MedianFL}$	4	69.57	3.58	0.03	0.03
12	β_0, $\beta_{Instream.Veg}$, β_{Cover}	3	69.61	3.62	0.03	0.03
13	β_0, $\beta_{MedianFL}$	2	69.72	3.73	0.02	0.03
14	β_0, $\beta_{Cobbles}$, $\beta_{Instream.Veg}$, $\beta_{Undercut}$	4	69.78	3.80	0.02	0.03

Table 7: Relative variable importance of predictor variables present in the subset of plausible models ($\Delta AIC_c < 4$) for predicting capture-efficiency of single-pass sampling at riffle sites.

Parameter of Variable	Importance Weight
Proportion of substrate composed of cobble	0.76
Proportion of instream vegetation	0.53
Proportion of undercut bank	0.07
Site length	0.12
Percent cover	0.13
Median fork-length of captured fish at each site	0.11

The positive model-averaged parameter coefficient for the proportion of substrate composed of cobble (Table 8) suggests that the probability of capturing Nooksack dace increases with increasing proportion of cobble, whereas the large negative coefficient for the proportion of instream vegetation suggests that the probability of capturing Nooksack dace decreases strongly with increasing proportion of instream vegetation (Fig. 6). The bootstrap estimate of the mean

difference between measured and predicted capture efficiency in my model

showed that the model was almost unbiased (ME = 1% mean difference in

capture efficiency), while my estimate of the root mean squared error (RMSE =

8%) demonstrated a relatively high level of accuracy in model predictions.

Table 8: **Model-averaged parameter estimates for the logistic regression model of single-pass capture efficiency at mark-recapture sites. Sites were located in Bertrand Creek and Brunette River in southwestern British Columbia, and were sampled between June-August 2009 (CL = confidence limit).**

Parameter or variable	Parameter estimate	Lower 95% CL	Upper 95% CL
Intercept	-0.90	-1.28	-0.52
Proportion of substrate composed of cobble	0.86	-0.69	2.42
Proportion of instream vegetation	-8.96	-30.80	12.90
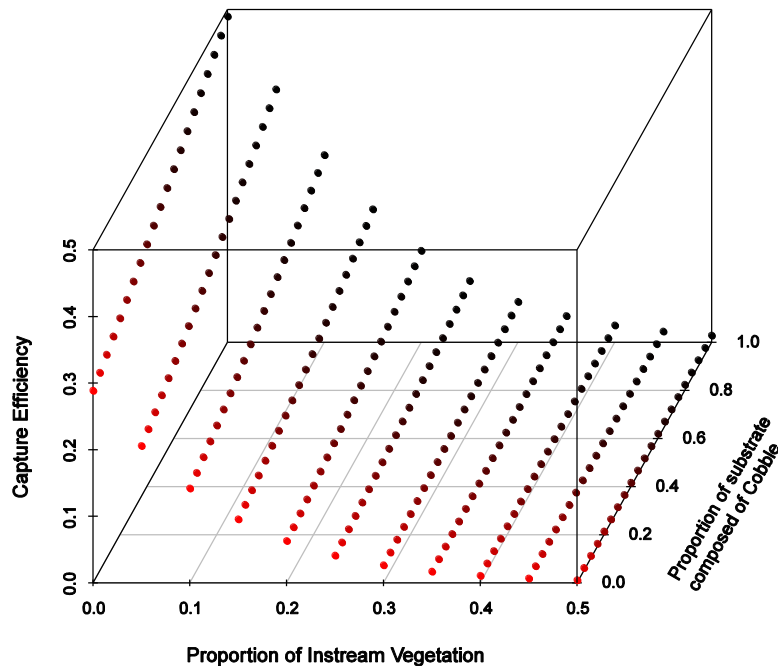
Figure 6: 3-D dot-plot showing how modeled single-pass capture efficiency varies with the proportion of instream vegetation and the proportion of substrate composed of cobble.

4.3 Estimating fish abundance in each stream

I examined single-pass sample catch from each stream for spatial autocorrelation using Moran's I. Results suggested no spatial autocorrelation for sample catch in Brunette River (Moran's I observed value = 0.02 and expected value = -0.05; P = 0.49) and Bertrand Creek (Moran's I observed value = 0.15 and expected value = -0.03; P = 0.09), indicating that the catch at a site was independent of catches at nearby sample sites in the stream.

I estimated average Nooksack dace density and total abundance in each creek using my modeled capture-efficiency and the single-pass catch at each site. Bootstrapped estimates of mean Nooksack dace abundance and density were highest in Bertrand Creek and lowest in Pepin Creek (Table 9). Estimates of total Nooksack dace abundance and density in Pepin Creek were based only on riffle sites sampled within the lower 570 m of the creek near 0th Avenue (Fig. 1) in which fish were detected.

Table 9: Bootstrapped estimates of mean Nooksack dace abundance and density (with bootstrap bias-corrected and accelerated 95% confidence limits in parenthesis (Efron and Tibshirani 1993)) based on riffle sites only for Brunette River, Bertrand Creek and Pepin Creek. No fish were detected during sampling in Fishtrap Creek. Abundance and density estimates for Pepin Creek are based only on the lower 570 m section in which Nooksack dace were detected.

Study Stream	Abundance (# of fish)	Density (Nooksack dace/m^2)
Brunette River	2,763 (1,823; 4,537)	0.37 (0.25; 0.62)
Bertrand Creek	4,359 (2,499; 7,991)	0.65 (0.37; 1.19)
Pepin Creek	30 (12; 136)	0.11 (0.03; 0.39)
Fishtrap Creek	0	0

4.4 Modeling Nooksack dace presence or absence

I used logistic regression to predict Nooksack dace presence or absence based on habitat variables measured at sampling sites. My most plausible models ($\Delta AIC_c < 4$) included site length, mean water velocity, mean substrate size, mean depth, percent cover, site gradient, and the level of substrate embeddedness as predictor variables (Table 10). Site length and level of substrate embeddedness had the highest relative variable importance followed by mean depth and mean water velocity (Table 11); the first two of these variables appeared in all plausible models. Mean substrate size had the lowest importance weight, and appeared in 6 of the 14 plausible models.   My final multi-averaged model included the full set of plausible models (i.e., Models 1-17).

Table 10: Most plausible candidate models ($\Delta AIC_c < 4$) for predicting presence or absence of Nooksack dace at riffle sites along with their AIC_c weights. β_0, β_{Length}, β_{Depth}, $\beta_{Velocity}$, $\beta_{Substrate}$, β_{Embed}, β_{Cover}, and $\beta_{Gradient}$ are parameters for the model intercept, site length, mean depth, mean water velocity, mean substrate size, level of substrate embeddedness, percent cover and site gradient, respectively. Because level of embeddedness is a categorical variable, the parameter for embeddedness (β_{Embed}) represents both low and medium site embeddedness (i.e., k = 2), whereas the intercept (β_0) represents high embeddedness.

Model	Parameters	No. of parameters (k)	AIC_c	ΔAIC_c	Weight	Scaled Weight
1	β_0, β_{Length}, β_{Depth}, $\beta_{Velocity}$, β_{Embed}, $\beta_{Gradient}$	7	76.97	0.00	0.14	0.16
2	β_0, β_{Length}, β_{Depth}, $\beta_{Velocity}$, β_{Embed}, β_{Cover}, $\beta_{Gradient}$	8	77.69	0.72	0.10	0.11
3	β_0, β_{Length}, β_{Depth}, β_{Embed}, $\beta_{Gradient}$	6	77.93	0.96	0.09	0.10
4	β_0, β_{Length}, β_{Depth}, $\beta_{Velocity}$, β_{Embed}	6	78.00	1.03	0.09	0.10
5	β_0, β_{Length}, β_{Depth}, $\beta_{Velocity}$, β_{Embed}, β_{Cover}	7	78.25	1.29	0.08	0.08
6	β_0, β_{Length}, β_{Depth}, β_{Embed}, β_{Cover}, $\beta_{Gradient}$	7	78.46	1.50	0.07	0.08
7	β_0, β_{Length}, β_{Depth}, $\beta_{Velocity}$, $\beta_{Substrate}$, β_{Embed}, $\beta_{Gradient}$	8	78.93	1.97	0.05	0.06
8	β_0, β_{Length}, β_{Depth}, $\beta_{Velocity}$, $\beta_{Substrate}$, β_{Embed}, β_{Cover}, $\beta_{Gradient}$	9	79.67	2.70	0.04	0.04
9	β_0, β_{Length}, β_{Depth}, $\beta_{Velocity}$, $\beta_{Substrate}$, β_{Embed}	7	79.84	2.88	0.03	0.04
10	β_0, β_{Length}, β_{Depth}, $\beta_{Velocity}$, $\beta_{Substrate}$, β_{Embed}, β_{Cover}	8	80.08	3.11	0.03	0.03
11	β_0, β_{Length}, β_{Depth}, β_{Embed}, $\beta_{Substrate}$, $\beta_{Gradient}$	7	80.31	3.35	0.03	0.03
12	β_0, β_{Length}, β_{Depth}, β_{Embed}	5	80.33	3.37	0.03	0.03
13	β_0, β_{Length}, β_{Depth}, β_{Embed}, β_{Cover}	6	80.35	3.39	0.03	0.03
14	β_0, β_{Length}, $\beta_{Velocity}$, β_{Embed}	5	80.42	3.45	0.03	0.03
15	β_0, β_{Length}, $\beta_{Velocity}$, β_{Embed}, $\beta_{Gradient}$	6	80.57	3.60	0.02	0.03
16	β_0, β_{Length}, $\beta_{Velocity}$, β_{Embed}, β_{Cover}	6	80.83	3.86	0.02	0.02
17	β_0, β_{Length}, β_{Depth}, $\beta_{Substrate}$, β_{Embed}, β_{Cover}, $\beta_{Gradient}$	8	80.91	3.95	0.02	0.02

Table 11: Relative importance weights of parameters in the subset of plausible models ($\Delta AIC_c < 4$) for predicting Nooksack dace presence-absence at riffle sites.

Parameter or variable	Importance Weight
Site Length	1.00
Mean Depth	0.92
Mean Water Velocity	0.71
Mean Substrate Size	0.23
Embeddedness	1.00
Percent Cover	0.43
Site Gradient	0.63

Positive parameter coefficients for some habitat variables (Table 12) indicate that the probability of finding Nooksack dace increases with site length, mean depth, mean water velocity, mean substrate size, low-to-medium substrate embeddedness, and site gradient. In contrast, the probability of detecting a Nooksack dace decreases at high levels of substrate embeddedness and with increasing percent cover.

Table 12: Parameter estimates from a logistic regression model of Nooksack dace presence-absence at individual riffle sites. Sites (pooled) were located in Bertrand Creek and Brunette River. Embeddedness – High is the model intercept because embeddedness is a categorical variable. (CL = confidence limit).

Parameter or variable	Parameter estimate	Lower 95% CL	Upper 95% CL
Intercept (Embeddedness – High)	-7.59	-11.95	-3.22
Site Length	0.29	0.10	0.47
Mean Depth	7.75	-4.18	19.68
Mean Water Velocity	2.72	-2.11	7.56
Mean Substrate Size	0.01	-0.07	0.09
Embeddedness – Low	4.18	2.05	6.32
Embeddedness – Medium	3.55	1.53	5.56
Percent Cover	-0.67	-2.82	1.48
Site Gradient	14.13	-13.74	41.99

My presence-absence model demonstrated good discrimination performance between occupied and unoccupied sample sites. The AUC score for the ROC plot (Fig. 7) describing my model was 0.92, which is close to that of a perfectly predicting model (AUC = 1.0). The ROC curve rises sharply at the origin, and levels off at a true positive rate of 1.0, indicating a higher true positive rate than false positive rate. Thus, over a range of decision thresholds, my presence-absence model exhibits good predictive performance.
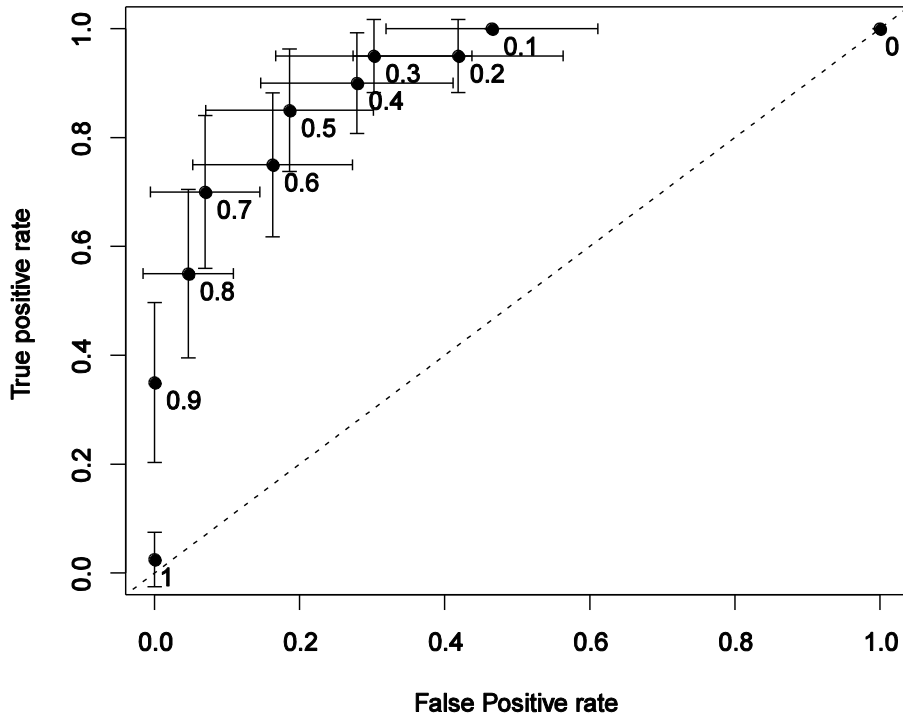
Figure 7: ROC plot for the Nooksack dace presence or absence model for a range of decision thresholds (i.e., predicted probabilities of occurrence above which Nooksack dace are considered present at a site). The diagonal dashed line represents the 1:1 line, where the true positive rate equals the false positive rate indicating poor predictive performance. The error bars reflect the variability (%) in the true positive rate and false positive rate based on bootstrap sampling of observations and predictions of Nooksack dace presence or absence.

4.5   Variation in abundance estimates with sample size

Understanding the trade-off between sample size and precision in abundance estimates is important for developing recovery-monitoring protocols. I plotted the relationship between the coefficient of variation (CV) for bootstrapped abundance and sample size for Brunette River, Bertrand Creek, and Pepin Creek (Fig. 8). For all study creeks, a sample of size of 15 – 25 sites appears adequate to obtain an abundance estimate within CV ≤ 0.35 of the estimated abundance.

Sample sizes below 5 sites will likely result in very imprecise (CV > 0.7) estimates

of Nooksack dace abundance in Bertrand Creek and Brunette River.
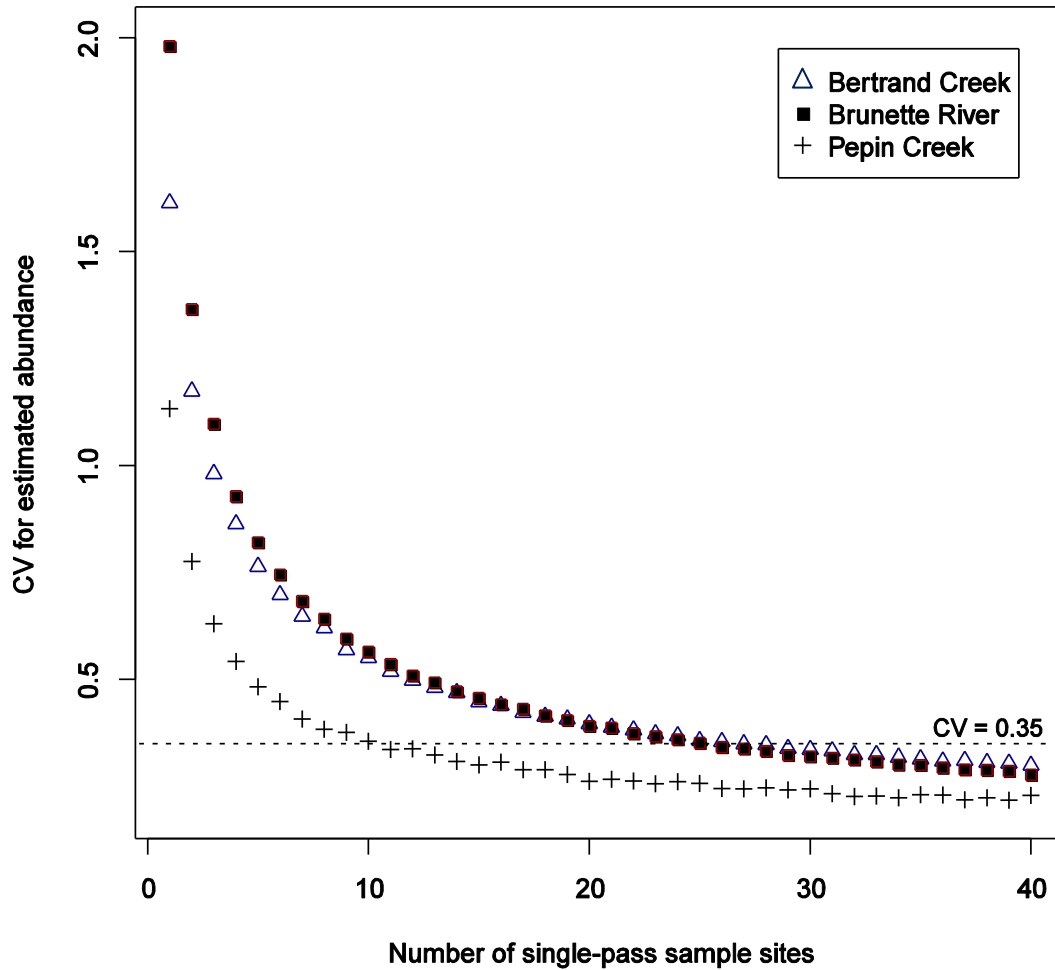
Figure 8: Coefficient of variation for bootstrapped abundance with increasing sample size in Bertrand Creek, Brunette River, and Pepin Creek. The dashed line represents a CV of 0.35.

5: DISCUSSION

5.1 Nooksack dace abundance

My estimates of total adult Nooksack dace abundance suggest that population sizes in all streams are below target levels in the recovery strategy (Table 13) (Pearson et al. 2008). In two of these streams, Pepin and Fishtrap Creek, Nooksack dace are virtually extirpated. The mean total estimated abundance in Bertrand Creek from my analysis is at 76% of target levels, and the target falls within the 95% confidence interval of my estimate. A target abundance estimate is not published for Brunette River in the recovery strategy; therefore, it is not possible to gauge population status in relation to current population levels for this stream. However, if target abundances for Brunette River are estimated in the same way as target levels for other streams (Pearson et al. 2008), then given the current available riffle habitat area in Brunette River, and an average fish density of 1.9 fish per m^2 of riffle area (Inglis et al. 1994), I obtain an estimated target abundance of 13,800 fish. My estimated abundance in the Brunette River is thus substantially lower than this target level.

Table 13: Recovery strategy target abundance levels for Nooksack dace in the year 2015, and riffle areas in each study stream used to calculate target abundance levels, assuming an average Nooksack density of 1.9 fish per m^2 of riffle area (Pearson et al. 2008).

Study Stream	Recovery strategy target abundance (# of fish)	Riffle area used to set target abundance levels in the recovery strategy (m^2)
Brunette River	Not Published	Not Published
Bertrand Creek	5,700	3,000
Pepin Creek	4,400	2,300
Fishtrap Creek	3,900	2,030

Target abundances in the recovery strategy (Pearson et al. 2008) were calculated using an estimated average riffle density of 1.9 fish per m^2 (Inglis et al. 1994). However, my estimates of mean Nooksack dace density were substantially lower than this average density estimate. For instance, Bertrand Creek had the highest mean density of fish during the 2009 sampling season, and the 95% confidence interval for this estimate did not overlap with the 95% confidence interval around Inglis et al.'s (1994) average density estimate (CL: 1.21 - 2.59 fish per m^2 of riffle area assuming a normal error distribution).

The significant difference between Inglis et al.'s (1994) average density estimate and my mean density estimates could be a result of the former study's small sample size of 20 sites (including pools, riffles, and glides) from 7 locations in Bertrand Creek, or due to differences in habitat quality of riffles sampled. Alternatively, mean density of Nooksack dace in riffles may have declined since 1994 due to degradation of instream and riparian habitat from rapid urbanization (Pearson et al. 2008). For instance, in the Township of Langley, where three of the study streams are located, between 1996 and 2006 the resident human population has increased by 17% (an increase from 80,179 to 93,726 people),

and the number of occupied private dwellings has increased by 25% (an increase from 26,645 to 33,335 dwellings) (BC Stats 1996 and 2006). This rapid pace of urbanization has increased pressure on local streams and resident fish, in the form of increased pollution and water extraction, stream bank erosion, and sedimentation (Pearson et al. 2008, Hall and Schreier 1996, Hall et al. 1998).

My work provides 2009 estimates of Nooksack dace density in each stream. Using this information, I could develop new recovery targets representative of the current available riffle habitat in each stream. However, without a clear understanding of the reasons for the significant difference between my density estimates and those of Inglis et al. (1994), using my estimates to develop recovery targets could be considered a shifting baseline view (Pauly, 1995) of changes in Nooksack dace abundance with time. In other words, using my density estimates as reference points for setting targets may shift the perception of what actual densities of Nooksack dace were in each stream (in about 1994) prior to current levels of urbanization. New target abundance levels calculated using my density estimates would be significantly lower than those in the recovery strategy, and may be insufficient to maintain a viable population in each stream. Alternatively, the recovery strategy targets may be overly optimistic or unattainable because these targets were calculated using the density of dace in high-quality riffle habitats (Pearson et al. 2008, Inglis et al. 1994). Since the quality of riffle habitats in each stream ranges from low to high, applying this density estimate uniformly across all riffle habitats may have produced target abundance levels that are biased high. In fact, in two of the streams, Pepin and

Fishtrap Creek, the availability of riffle habitat has decreased since 1999 (Table 3 vs. 13), suggesting that without restoration of critical riffle habitat, the recovery strategy objective "to increase Nooksack dace abundance to target levels in *all watersheds* by 2015" (Pearson et al. 2008) may not be achievable.

Nooksack dace likely occur in non-riffle habitats, and potentially in areas outside identified critical habitat in each stream. Adult fish were found in one pool and two glide habitats sampled in Bertrand Creek, however, I did not include these habitat types when determining total adult Nooksack dace abundance in the stream. Therefore, my estimates of total adult Nooksack dace abundance are likely biased low. I was unable to estimate the abundance of Nooksack dace in pool and glide habitats because I did not conduct mark-recapture experiments in those habitat types to assess the capture efficiency of single-pass electrofishing. When I sampled the pool containing Nooksack dace in Bertrand Creek, fish were captured only on the armoured left bank of the stream, where large cobbles and boulders were present in the water. Similarly, I also observed Nooksack dace remaining relatively stationary on large cobbles and boulders that were used to armour bridge pillars and banks at crossings, which I did not sample to avoid any effects from man-made travel routes (e.g., runoff from roads). Thus, armoured areas in each stream and habitats (pools, riffles and glides) outside identified critical habitat may contain significant numbers of Nooksack dace that are not included in my abundance estimates. Future sampling should endeavour to quantify Nooksack dace abundance in all habitat types and at road crossings

along the full length of each stream to obtain a more accurate estimate of total Nooksack dace abundance in each stream.

5.2 Capture-efficiency modeling and estimation

Mean capture efficiency from my mark-recapture experiments (29%) is almost identical to the mean measured efficiency calculated by Peterson et al. (2004) (28%) in similar mark-recapture experiments conducted on salmonid species. Other studies using an unbiased estimator (like mark recapture) have also similarly estimated low electrofishing capture efficiency (Rosenberger and Dunham 2005, Bayley and Peterson 2001). Price and Peterson (2010) found that capture efficiency of single-pass electrofishing is generally less than 35% in warm-water streams like those in my study, and differs significantly depending on species groups and habitat characteristics.

Modeled capture efficiency had a strong negative relationship with the proportion of instream vegetation and was positively related to the proportion of substrate composed of cobble. Increasing the proportion of instream vegetation has a negative effect on modeled capture efficiency because it likely makes the observation and capture of individual fish more difficult. Nooksack dace capture efficiency appeared to increase with the proportion of substrate composed of cobble. It is unclear why capture efficiency was higher at sites with cobble substrate, but it is possible that because sites with larger substrate have larger pore spaces between substrate particles, stunned fish are carried by stream flow more easily from these pore spaces into the open where they are captured. If this were the case, we could also expect an increase in the proportion of boulders at a

site concurrent with a decline in the proportion of cobble, which would result in a higher capture efficiency. This scenario runs contrary to my capture-efficiency model, which predicts an increase in capture efficiency as the proportion of cobble increases. Such a scenario, however, should be rare in the four streams, because only a few sample sites (12 of 83 riffle sites) in my study had boulder substrate, and at a vast majority of sites (69 of 83 riffle sites) finer substrates (i.e., sand and gravels) and bedrock were the dominant alternatives to cobble. Thus, at sites with a greater proportion of cobble (as opposed to boulders), I would expect average pore size to be greater and capture efficiency to be higher.

Mark-recapture sites used to develop my capture-efficiency model were located in Bertrand Creek and Brunette River only, and capture efficiency estimates for single-pass sites sampled in Pepin Creek were estimated using this capture-efficiency model. Capture efficiency estimates for Pepin Creek could therefore be biased. However, I attempted to account for the full range of the available proportion of instream vegetation and the proportion of substrate composed of cobble (i.e., the model covariates) in all streams when selecting my mark-recapture sites. Therefore, model capture-efficiency estimates generated using this habitat data from Pepin Creek should be reliable.

My results suggested that capture-efficiency predictions were relatively accurate, and that my capture-efficiency model was almost unbiased. Unexplained variability in model predictions may be due to (1) variables unaccounted for in the model, (2) behavioural biases of fish and/or samplers, (3)

natural variation in the environment, or (4) violation of mark-recapture assumptions (Price and Peterson 2010).

Double-block nets have been used in previous studies (Rosenberger and Dunham 2005, Peterson et al. 2004) to assess the escape rate of fish from mark-recapture sites and to examine potential violation of the closed-site assumption required for mark recapture. In my mark-recapture experiments, I assumed a closed population without explicitly testing the closed-site assumption. However, Rosenberger and Dunham (2005) and Peterson et al. (2004) found escape rates of less than 1 fish per site during a 24-hour period when sampling salmonids. Since fish in my study were given only a 3-hour re-acclimation period, fish losses from escape should be minimal. A 3-hour period for re-acclimation and random redistribution of fish throughout each mark-recapture sites was assumed to be adequate; however, this assumption remains untested, and could have resulted in some error in the capture-probabilities between marking and recapture events.

I examined whether capture efficiency differed by size class. My results showed no difference in capture probability among size classes for Nooksack dace (Fig. 5), even though other work (Anderson 1995, Bayley and Dowling 1993, Buttiker 1992) has shown electrofishing to be a size-selective sampling method. A large proportion of adult Nooksack dace (65%) captured in my study fell within a narrow size range (67 – 87 mm), reducing the potential for size-based bias.

The equal capture probability assumption between marked and recaptured fish may have been violated as well, due to changes in fish behaviour after release (i.e., from handling, marking, and shocking stress), translocation of fish at

2 mark-recapture sites, and random sampling variation. However, the 24-hour

recovery period used in this study has been shown to be adequate to minimize

the effects of these factors for other species (Carrier et al. 2009, Rosenberger and

Dunham 2005, Mesa and Scherek 1989, Petersen and Cedarholm 1984). If these

factors did adversely affect fish behaviour, it is uncertain what effect they had on

site-scale capture efficiency and resulting estimates of abundance in each stream.

5.3 Habitat features affecting Nooksack dace presence or absence

Many of the habitat co-variates in my Nooksack dace presence-absence

model are commonly reported in the literature for other freshwater fish species in

similar analyses (Jowette et al. 2007, Rashleigh et al. 2005, Turgeon and

Rodriguez 2005, Ault and White 1994). The effect of these habitat features on the

predicted probability of occurrence of a species varies by species. More

importantly, however, the probability of detection (or capture) for a given species

can bias estimates of its predicted probability of occurrence (MacKenzie 2006, Gu

and Swihart 2004). A low probability of detection can result in false negatives

(i.e., not detecting the species when it is actually present at a site). To obtain

unbiased estimates of the predicted probability of occurrence, it is necessary to

quantify and account for the false negative rate in the sample (Delaney and Leung

2010, MacKenzie and Royle 2005, Tyre et al. 2003, MacKenzie et al. 2002). I did

not estimate the false negative rate for my sample, and therefore cannot quantify

the associated bias in my model predictions. However, because the mean

capture probability in my study was low, false negatives were likely present in my

sample, and therefore, my presence-absence model most likely underestimates the true probability of Nooksack dace occurrence.

My presence-absence model only provides information regarding use or non-use of habitat, and does not indicate habitat preference. However, information from the model can also serve as a very rough index of habitat suitability for Nooksack dace because presence or absence is most likely strongly correlated with habitat preference. For instance, the level of substrate embeddedness had a substantial influence on the probability of Nooksack dace occurrence, but it is likely also important in determining habitat suitability for Nooksack dace. Previous work (Pearson 2004, Inglis et al. 1994) has shown that Nooksack dace prefer riffle sites with loose cobbles and gravels, where interstitial spaces between substrate particles are larger, allowing the fish to hide from predators and disturbances in the stream. Sites with high levels of embeddedness have more fine sediment (i.e., >75% sand, clay and silt) in these pore spaces resulting in less space for fish to escape into when a predator is sighted or a disturbance is detected in the stream. Furthermore, embedded riffle sites have been shown to have lower macro-invertebrate species diversity and abundance (Pedersen and Friberg 2006), decreasing prey availability, and likely reducing habitat suitability for Nooksack dace.

Gravel and cobble substrates (i.e., substrate sizes between 0.2 cm - 25.6 cm, Table 2) have been shown to be the most preferred substrate types for Nooksack dace (Pearson et al. 2008, Inglis et al. 1994). I captured over 95% of Nooksack dace at single-pass riffle sites with a mean substrate size in this range,

suggesting gravels and cobble substrates are preferred over finer substrates.

Inglis et al. (1994) developed habitat suitability curves for the Nooksack dace

showing suitable water velocities and depths for the species. Their work indicated

that highest fish densities occurred at water velocities between 0.10 m/sec to 0.30

m/sec. However, in my single-pass sampling, over 90% of fish caught were found

in riffle habitats with velocities greater than 0.30 m/sec. My result is more in line

with Pearson et al. (2008), who suggested that Nooksack dace typically occur in

riffles with water velocities greater than 0.25 m/sec. Inglis et al. (1994) showed

that Nooksack dace prefer water depths between 0.05 m to 0.30 m. I caught 97%

of Nooksack dace at single-pass riffles sites at mean depths in this same range,

providing further support for the suitability of these depths for Nooksack dace.

Suitable habitat features like those described above should be considered

when planning and conducting habitat restoration for the Nooksack dace.

However, consideration should also be given to the needs of salmonid species,

because cutthroat trout (*Oncorhynchus clarkii*), rainbow trout (*O. mykiss*), coho

(*O. kisutch*), chum (*O. keta*), and chinook (*O. tshawytscha*) salmon are also found

in these streams. Salmonids depend on coarse substrate (gravels and cobbles),

low-gradient habitats with good flow for building redds during spawning, and on

deep pool habitats for rearing as fry and smolts in freshwater streams. For the

most part, habitat requirements for salmon are complementary with those of the

Nooksack dace, however, because Nooksack dace are riffle specialists and prefer

warmer water, they likely have less need for good instream habitat complexity

(e.g., coarse woody debris) and stream cover than salmonids do.

Potential critical habitat reaches for Nooksack dace are currently identified based on reaches of stream that are greater than 10% riffle by length (Pearson et al. 2008). This scale of critical habitat identification may be suitable for management purposes at the watershed level, however, at the reach scale, my presence-absence model may be more suitable. For instance, mapped critical habitat reaches in the recovery strategy (Pearson et al. 2008) provide information on potential riffle habitat for the Nooksack dace, but do not provide accurate information on the current distribution of Nooksack dace. My presence-absence model can help identify riffle sites within critical habitat likely to contain Nooksack dace, and these sites can then be prioritized for restoration efforts. Pepin Creek serves as a good example, where identified critical habitat extends north from the US-Canada border for approximately 4 km, but Nooksack dace were only detected in the lower 570 m of the creek, suggesting that restoration efforts should be focused on this lower section. My model accurately predicted Nooksack dace presence in this section, and absence of fish through the remainder of the creek. Thus, my model can be an effective tool in determining which areas within identified critical habitat should be prioritized for restoration or recovery actions.

5.4 Recovery Monitoring for the Nooksack dace

5.4.1 Rationale

The development of an action plan is currently underway for the Nooksack dace. The action plan is a document that outlines the recovery measures (i.e., recovery projects and activities) that should be implemented to meet the goals

and objectives outlined in the recovery strategy for the Nooksack dace. The

Species at Risk Act (2002) requires monitoring protocols be designed to assess

recovery of the species once recovery measures are put in place. Section 49 (1)

(d.1) of *SARA* (2002) states:

> "*An action plan must include, with respect to the area to which the action
> plan relates, the methods to be used to monitor the recovery of the
> species and its long-term viability.*"

Recovery monitoring protocols for the Nooksack dace must therefore be

included in the action plan, to monitor trends in abundance in each stream.

Furthermore, the recovery strategy (Pearson et al. 2008) identifies the

development of 'a monitoring protocol for population abundance' as one of the

process performance measures in achieving the recovery objective of increasing

Nooksack dace abundance to target levels by 2015. My two-stage single-pass

sampling procedure sets the stage for developing recovery-monitoring protocols

for the Nooksack dace by providing an efficient and reliable method for assessing

Nooksack dace abundance.

The action plan aims to meet recovery strategy objectives, and therefore

recovery-monitoring protocols should assess how well objectives outlined in the

recovery strategy are met. The recovery objectives for the Nooksack dace

include: (1) for all currently and historically suitable habitats in native streams to

be occupied by 2015, (2) to increase Nooksack dace abundance to target levels in

all watersheds by 2015, and (3) to ensure that at least one reach in each

watershed supports a high density of Nooksack dace (Pearson et al. 2008).

My presence-absence model, which predicts the probability of Nooksack dace occurrence based on habitat features, may serve as a good index for potential habitat occupancy. However, I did not sample all areas of current and historically suitable habitat in each watershed, and therefore, I cannot assess occupancy as defined in recovery objective 1 above. Future work to assess habitat occupancy could use a single-pass sampling method similar to the one I used. To minimize the costs and effort associated with sampling for fish throughout current and historical suitable habitats, I recommend using the presence-absence model from this study to select a smaller subset of sample sites within these suitable habitat areas, where the probability of Nooksack dace occurrence is high. This subset of sites can then be sampled to determine Nooksack dace occupancy.

The success of recovery measures in meeting recovery objective 2 (attaining target abundances in all watersheds by 2015) can be assessed by monitoring trends in population abundance in each stream, to determine whether Nooksack dace abundance is increasing or decreasing in relation to target levels.

It is difficult to assess whether recovery objective 3 (to ensure at least one reach in each watershed supports a high density of Nooksack dace) will be achieved because the objective does not provide a quantitative measure for 'high density of Nooksack dace,' or a definitive timeline within which this objective must be achieved. The biological performance measure in the recovery strategy for this objective is the number of reaches where catch-per-unit-effort (CPUE) exceeds 0.8 Nooksack dace per standard Gee-trap (24 hr set, $n \geq 10$) (Pearson et

al. 2008). This measure does not provide an estimate of the density of fish per m^2 of habitat area, and therefore, it cannot be compared with current estimates of Nooksack dace density, or with the previously estimated average density of 1.9 fish per m^2 of riffle area (Inglis et al. 1994). Managers should consider developing an alternate quantitative measure of 'high density of Nooksack dace,' and a definitive time line for accomplishing this objective so that monitoring protocols can adequately assess whether this objective has been met.

Four monitoring questions can be posed to assess the success of recovery objectives 2 and 3, which respectively aim to increase abundance to target levels in each watershed by 2015, and ensure that at least one reach in each watershed supports a high density of Nooksack dace (Pearson et al. 2008). These are:

(1) Is Nooksack dace abundance in each watershed above or below recovery strategy target levels?

(2) By what quantity has Nooksack dace abundance increased or decreased in relation to previous measures of abundance in each watershed?

(3) Does at least one reach of identified critical habitat in each watershed contain a 'high density of Nooksack dace'?

(4) How does the estimate of Nooksack dace density in the sampled identified critical habitat reach compare with the highest observed average density of 1.9 fish per m^2 of riffle area?

5.4.2 Monitoring Sampling Design

I developed a model to predict the capture efficiency at each riffle site using just the proportion of instream vegetation, and the proportion of substrate composed of cobble at each site. This model serves to calibrate the capture efficiency of single-pass sampling, therefore, future sampling requires only Stage 1 of the procedure (i.e., single-pass sampling) to be completed at riffle sites in each stream, along with measurements of the proportion of instream vegetation and the proportion of substrate composed of cobble at each site. These habitat measurements can then be used in the model to predict capture efficiency at sample sites, which in turn can be used to estimate abundance (Fig. 4). It is important to note however, that my capture-efficiency model was based on the natural range of riffle habitat characteristics observed in 2009. If future habitat use of the Nooksack dace changes, or sampling equipment or techniques change, or the observed range of habitat characteristics occurs outside the range of characteristics encompassed in my capture-efficiency model, predictions from the model will be inaccurate, and should not be used to calibrate single-pass sampling.

5.4.2.1 Sampling locations

Recovery strategy objectives aim to ensure recovery of Nooksack dace in all native streams. Riffle sites should therefore be sampled in all four streams, Bertrand Creek, Pepin Creek, Fishtrap Creek, and Brunette River. Sample sites for monitoring can be selected using the procedures I described in the Methods section, or via other means. However, attempts should be made to sample

representatively throughout identified critical habitat (Fig. 1 and 2), and potentially

outside critical habitat in each stream. Sampling sites outside critical habitat will

permit assessment of expansion in the distribution of Nooksack dace in each

stream, and provide more accurate estimates of total abundance in study

streams. Sample sites selected in each stream on each monitoring cycle (i.e.,

each time monitoring is conducted) should be systematically varied to accurately

assess changes in abundance, instead of using index sites, which may not reflect

changes in abundance or distribution over time.

5.4.2.2   Sampling timing

Sampling should be conducted before April 30th or after July 15th, which are

periods outside of the spawning and incubation window for Nooksack dace

(Pearson 2009). Sampling within the spawning period will likely result in harm to

the fish population. Consideration should also be given to spawning windows for

other fish species (e.g., salmon and the Salish sucker (*Catostomus catostomus*)).

DFO timing and location restrictions may apply, and will need consideration when

choosing the Nooksack dace sampling window.

5.4.2.3   Sampling method

The single-pass sampling method described in the Methods section of this

paper (Fig. 3) should be followed when conducting sampling for recovery

monitoring. At present, only riffle habitats should be sampled because single-pass

capture efficiency has not been modeled for other habitat types. However, once

single pass capture efficiency has been modeled for pool and glide habitats, these

habitats should also be sampled to obtain more accurate estimates of abundance in each stream. Because my Nooksack dace abundance and density estimates were based only on adults (fork length >54mm), fish lengths need to be measured at sample sites. The only habitat characteristics that require measurement are the proportion of instream vegetation, the proportion of substrate composed of cobble, site length (m), and wetted width (m). Site length and wetted width are used to calculate the riffle area at each site. The spatial location of each sample site should be recorded using a hand-held GPS unit.

5.4.2.4   Data analysis

Catch at sample sites must be examined for spatial autocorrelation to ensure independence in catch between sites. This can be accomplished using the spatial location data collected during sampling. Capture efficiency at each site can be estimated using equation (1), substituting in model-averaged co-efficient values from Table 8 for β_m, and habitat data collected at each site for X_m (Fig. 4, Step 2-4). Abundance and density can then be calculated without bootstrapping by using the abundance estimation pathway on the right side of Figure 4 (Steps 5-7), or with bootstrapping sample data to obtain error in the total estimated abundance and the mean density in each stream ($N_t \pm SE$ and $r \pm SE$, respectively).

5.4.3 Monitoring Costs

A two-stage single-pass sampling method is cost-effective because a small crew can sample more sites per sampling day in comparison to multi-pass removal methods. My single-pass sampling method required a 3-person crew

with electrofishing training and certification. The current daily rate for such a crew

is approximately $300-$400 per person. Costs for renting the electrofisher, the

pole-seine, a vehicle, and other equipment (including incidentals) can be

approximated at $350-$500 per day. Thus, overall cost for an 8-hour sampling

day can be expected to range from $1250 to $1700.

My examination of how precision in abundance estimates changes with

sample size (Fig. 7) demonstrated that approximately 25 riffle sites would need to

be sampled in Brunette River and Bertrand Creek, and approximately 15 riffle

sites would need to be sampled in Pepin Creek to obtain adequate precision (CV

< 0.35) in estimates of abundance. Based on these sample sizes, the

characteristics of each stream, and my experience sampling in each stream, I

would expect 2 sampling days for Pepin Creek and 4 sampling days each for

Bertrand Creek and Brunette River. Sampling in Fishtrap Creek should require no

more than 1 day, because significant amount of riffle habitat was only present

upstream of Marshall Road (Fig. 1) during the 2009 sampling season. However, if

habitat restoration introduces new riffle habitat in downstream sections of Fishtrap

Creek, an additional sampling day may be required. Because no Nooksack dace

were found in Fishtrap Creek during 2009, approximately 15 sites along the creek

should be adequate to identify re-colonization if it occurs. I estimate the maximum

overall costs for annual sampling to be $16,500 (Table 15). Factoring in the costs

associated with data analysis and report writing, total overall costs for each

monitoring event can be approximated at $23,700.

Table 14: Expected maximum annual cost of monitoring given approximate number of sites to be sampled in each stream, the number of sampling days required, and the maximum approximate cost of an 8-hour sampling day of $1,700.

Study Stream	Samples sites to obtain precise estimates of abundance (CV < 0.35)	Number of sampling days	Expected maximum sampling cost ($)
Brunette River	25	4	6,000
Bertrand Creek	25	4	6,000
Pepin Creek	15	2	3,000
Fishtrap Creek	15	1	1,500
		Field Costs:	16,500
		Analysis and Reporting:	5,000
		TOTAL COSTS:	**23,700**

5.4.4 Monitoring Time Frame

Selecting an interval between monitoring events depends not just on the needs of a species, but also on the resources available to monitor it. The recovery strategy suggests a minimum of 5-years between monitoring events for evaluating Nooksack dace abundance in each stream (Pearson et al. 2008). I am unaware of the resources available at the disposal of managers to monitor Nooksack dace, therefore, I can only make subjective recommendations based on my knowledge and experience.

The most recent assessment of Nooksack dace population trends was conducted in 1999 (Pearson 2004), 10 years before my 2009 sampling season. Since then, the Nooksack dace has been nearly extirpated from 2 of 4 natal streams in Canada. I therefore conclude that a decadal monitoring cycle is too infrequent; a 5-year monitoring cycle as suggested in the recovery strategy seems appropriate to allow for adequate time between implementation of restoration measures and evaluation of monitoring objectives.

5.5 Nooksack dace as a Peripheral Species

Peripheral (or marginal) species are those species that have no more than 10% of their range in British Columbia (Bunnell et al. 2004). The Nooksack dace in Canada can be considered a peripheral species because it is only found in 4 streams in BC, whereas it occurs in over 20 streams just south of the Canada-US border in the state of Washington. Bunnell et al. (2004) further distinguished peripheral populations of species as being continuous or disjunct. Continuous (or ecologically marginal) populations are at the edge of their range, which expands or contracts depending on the favorability of environmental conditions. These ecologically marginal populations are less likely to be genetically isolated from the core population, but may, however, struggle to survive on the edge of their range, which can sometimes lie across international borders. In contrast, disjunct (or geographically marginal) populations are separated by barriers or long distances that isolate them from the core population, increasing the odds of genetic isolation and speciation (Gould 2002). These populations often have higher genetic variability, occupy less suitable environments, have lower abundances and ranges, and exhibit local rarity (Bunnell et al. 2004). Geographically marginal populations are of higher conservation importance because they are more likely to result in genetically divergent and evolutionary significant species (Millar and Libby 1991, Beardmore 1983).

The four Nooksack dace populations in BC can be considered disjunct populations because they are isolated from the core population in the US and from one another by long distances of severely degraded or unsuitable habitat

(Pearson, 2000). Natural recolonization of habitat from which Nooksack dace have been extirpated is highly unlikely (Pearson et al. 2008), and therefore, odds of recolonization of natal streams in BC from populations south of the border are very low. Given these barriers to genetic exchange, Nooksack dace populations in BC are virtually genetically isolated from populations in the U.S., and from each other. The very short generation time of the species (2 years) (Pearson 2004), along with genetic isolation of each population, increases the potential for genetic divergence. Not all peripheral species, however, are divergent and fewer still are likely to evolve into new taxa, but there is merit in sustaining populations that have been reproducing in isolation long enough to be divergent (Bunnell et al. 2004). The Nooksack dace populations in BC (especially in Brunette River and Bertrand Creek) are self-sustaining disjunct peripheral populations that have been reproductively isolated from populations in Washington state, and therefore warrant conservation concern and resources to maintain their potential for evolutionary divergence.

5.6 Conclusion

Nooksack dace population sizes in all natal streams in BC are below recovery-strategy target levels, reaffirming the endangered designation for the species. Availability of riffle habitat appears to be a limiting factor in at least two streams, Pepin Creek and Fishtrap Creek, where the quantity and quality of riffle habitat has declined since 1999, and the species is now virtually extirpated. Habitat restoration is needed in these two streams along with measures to mitigate against anthropogenic stressors (i.e., pollution, water extraction, removal

of riparian vegetation, etc.) to prevent further population loss. I am uncertain of population trends in Bertrand Creek and Brunette River because available riffle area in these streams is relatively high, but the quality of habitat is likely declining due to rapid urbanization (Pearson et al. 2008). Restoration actions in these two streams should primarily focus on preventing loss of riffle habitat and improving the quality of available riffle habitat. Important habitat features to consider when planning habitat restoration for Nooksack dace include: mean water depths between 0.05 – 0.30 m, mean water velocities greater than 0.25 m/sec, coarse substrate types (i.e., gravels and cobbles) and low to medium substrate embeddedness. Urgent restoration action is necessary in all study streams, in order to successfully accomplish the recovery objective of increasing Nooksack dace abundance to target levels in all watersheds by 2015.

Electrofishing is the most effective method of sampling Nooksack dace, as less stressful methods like minnow trapping have proved ineffective at capturing these fish (Pearson, 2004). However, to minimize impacts on the fish, single-pass electrofishing is preferable over multi-pass electrofishing, and can provide accurate estimates of abundance when sampling capture efficiency is calibrated using an unbiased estimation method (e.g. mark recapture). Calibrating single-pass sampling using mark recapture is initially more effort-intensive, however, once calibrated, subsequent sampling only requires non-intensive single-pass sampling. Such a two-stage process is cost-effective and efficient when developing monitoring protocols, where multiple iterations of sampling are

required over time to examine population trends and assess changes in abundance.

Monitoring protocols for the Nooksack dace should endeavour to assess population abundance in all four streams every 5 years. Longer intervals between sampling events may result in substantial declines in population abundance before a declining trend is detected. Results from monitoring should inform recovery actions and allow for efficient allocation of resources (funding, staff time, etc.) to each stream. Climate-change, rapid urbanization, introduced predators, and natural disturbances (i.e., storm events) continue to hinder recovery of the Nooksack dace. Long-term recovery of this species will depend on consistent monitoring, appropriate recovery actions (including community stewardship), and commitment to protecting this species even in the face of an unfavourable political climate.

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