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Propagule pressure and stream characteristics influence introgression: cutthroat and rainbow trout in British Columbia

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Abstract. Hybridization and introgression between introduced and native salmonids threaten the continued persistence of many inland cutthroat trout species. Environmental models have been developed to predict the spread of introgression, but few studies have assessed the role of propagule pressure. We used an extensive set of fish stocking records and geographic information system (GIS) data to produce a spatially explicit index of potential propagule pressure exerted by introduced rainbow trout in the Upper Kootenay River, British Columbia, Canada. We then used logistic regression and the information-theoretic approach to test the ability of a set of environmental and spatial variables to predict the level of introgression between native westslope cutthroat trout and introduced rainbow trout. Introgression was assessed using between four and seven co-dominant, diagnostic nuclear markers at 45 sites in 31 different streams. The best model for predicting introgression included our GIS propagule pressure index and an environmental variable that accounted for the biogeoclimatic zone of the site ($r^2 = 0.62$). This model was 1.4 times more likely to explain introgression than the next-best model, which consisted of only the propagule pressure index variable. We created a composite model based on the model-averaged results of the seven top models that included environmental, spatial, and propagule pressure variables. The propagule pressure index had the highest importance weight (0.995) of all variables tested and was negatively related to sites with no introgression. This study used an index of propagule pressure and demonstrated that propagule pressure had the greatest influence on the level of introgression between a native and introduced trout in a human-induced hybrid zone.

Key words: hybridization; introduced species; introgression; Kootenay River, Canada; Oncorhynchus clarki *ssp.;* Oncorhynchus mykiss; *propagule pressure; rainbow trout; westslope cutthroat trout.*

Introduction

Introduced species cause declines in native fauna and flora by affecting reproductive rate, survival rate, net emigration, or disease introductions (Peterson and Fausch 2003). However, when native and introduced species hybridize, other factors may threaten the native species. For example, human-induced hybridization can result in wasted reproductive effort for the native species (Kanda et al. 2002*a*), loss of co-adapted gene complexes (Allendorf and Waples 1996, Gilk et al. 2004), and potentially, extinction (Rhymer and Simberloff 1996). Introgressive hybridization (hereafter introgression) results when native and introduced species breed and produce viable offspring, resulting in the movement of alleles from one genetically distinct entity to another and potentially the creation of a hybrid swarm, where all individuals in the population have alleles from both native and introduced populations (Leary et al. 1995, Allendorf et al. 2001).

In the western North America, introduced rainbow trout (RBT, *Oncorhynchus mykiss*) readily hybridize with native inland cutthroat trout (*O. clarki* ssp.) and the resulting introgression is considered one of the greatest threats to the persistence of most subspecies (Allendorf and Leary 1988, Leary et al. 1995, Behnke 2002). Hundreds of millions of RBT are stocked every year in lakes and streams throughout North America to provide recreational fishing opportunities (Welcomme 1992, Halverson 2008). Stocked RBT often hybridize with native cutthroat trout in the immediate vicinity of where they were stocked (Shepard et al. 2005), but they can also disperse upstream and downstream many kilometers within just a few days of stocking (e.g., 10–30 km; Bettinger et al. 2002, Baird et al. 2006). Recent studies have also demonstrated that F1 and later generation hybrids can disperse large distances (>50 km) from where they were produced and spread RBT alleles into new cutthroat trout populations (Hitt et al. 2003, Rubidge and Taylor 2005, Boyer et al. 2008).

The spatial extent of introgression between native and introduced trout has most often been attributed to environmental variables, especially elevation, gradient, stream width, water temperature, and flow regime

(variously in Fausch 1989, Kruse et al. 1997, Adams et al. 2001, Fausch et al. 2001, Paul and Post 2001, Weigel et al. 2003, De Rito 2004, Ostberg and Rodriguez 2006). In general, more introgression between RBT and native cutthroat trout has been found in low-elevation streams, with warmer stream temperatures and earlier spring runoff than higher elevation streams (Platts 1979, Marotz and Farley 1986, Henderson et al. 2000, Weigel et al. 2003, De Rito 2004, Gunnell et al. 2008).

Although environmental factors are often implicated as limiting the spread of introgression, there is growing evidence that propagule pressure is a significant predictor of successful establishment and spread of many bird (Cassey et al. 2004), fish (Duggan et al. 2006), mammal (Forsyth and Duncan 2001), amphibian (Fitzpatrick and Shaffer 2007), and plant species (Von Holle and Simberloff 2005). We define propagule pressure as a combination of the total number of individuals released, and the number of times and places a species was introduced (Carlton 1996). Studies of cutthroat and RBT hybridization have not fully assessed the role of propagule pressure (but see Weigel et al. 2003), but there is evidence that successful introductions of nonnative trout are stocked more often and in higher numbers (Colautti 2005, Lockwood et al. 2005). This suggests that the role of propagule pressure in introgressed populations may be underestimated.

Using propagule pressure in models to predict levels of introgression is important because if propagule pressure can overwhelm environmental limitations on the ability of an introduced species to disperse, then species invasions and subsequent introgression may expand into areas currently considered unsuitable to the introduced species. Obviously there are limits to this type of expansion, but without understanding the role of propagule pressure, the potential extent of invasions may be significantly underestimated. An example of this type of situation is the stocking of high-elevation lakes with nonnative trout. Often these sites are too cold for the fish to reproduce; however the fish can survive and disperse from these sites, invading other sites tens of kilometers downstream (Bahls 1992, Adams et al. 2001). Models that fail to account for propagule pressure in these situations would confound efforts to model the dispersal of introduced species by assuming environmental limitations to introduced species dispersal play a dominant role (Colautti 2005).

To model propagule pressure, accurate records of species introductions are required. Propagule pressure can be hard to quantify for accidental species introductions (e.g., ballast water transfers; Wonham et al. 2005); however, for species that have been deliberately introduced, propagule pressure is often well documented (Wiltzius 1985, Forsyth and Duncan 2001, Cassey et al. 2004). Salmonid fish introductions provide a good opportunity to test the role of propagule pressure in the establishment and spread of introduced species and introgression with native species, because salmonid

introductions are relatively well documented in terms of the timing, location, and number of propagules introduced (Colautti 2005).

Even if propagule pressure of introduced salmonids can be determined, the effective propagule pressure at any given point in a watershed where native and introduced populations interact must take into account various spatial factors affecting the introduction. These spatial introduction factors include the presence of migration barriers (Rubidge and Taylor 2005, Ostberg and Rodriguez 2006), the distance between native and introduced populations (Gunnell et al. 2008), and the ability of the introduced species to disperse (Lockwood et al. 2005, Rubidge and Taylor 2005, Wonham et al. 2005, Duggan et al. 2006, Lambrinos 2006). The location and frequency of migration barriers (e.g., waterfalls and velocity barriers) can profoundly affect the extent of fish species invasions (Adams et al. 2001) and the extent of hybrid zones (Rubidge and Taylor 2005, Ostberg and Rodriguez 2006) by physically preventing species interactions. When species are not prevented from interacting by physical barriers, invasion theory suggests that introduced species are more likely to establish and spread as propagule pressure increases (Elton 1958, Williamson 1996).

The goal of our research was to test how environmental and spatial variables influence the rate of introgression between native westslope cutthroat trout (WCT, *O. clarki lewisi*) and introduced RBT. Our first objective was to develop a potential propagule pressure index (PPI) based on spatially explicit modeling of dispersal of introduced RBT from stocking points throughout the watershed. Our second objective was to test whether the PPI, spatial variables, and a set of environmental variables could predict the level of introgression between WCT and RBT. We hypothesized that the PPI would be a better predictor of the level of introgression than environmental and/or spatial variables alone because the index explicitly captures the distribution and dispersal potential of RBT within the watershed. We predicted that higher levels of introgression would be found at sites near stocking locations that were stocked more often and with higher numbers of RBT than sites near locations that were not stocked, or stocked with lower numbers of RBT.

STUDY AREA AND BACKGROUND

The study area encompasses the Canadian portion of the Kootenay River from its headwaters in Kootenay National Park down to the Canada/U.S. border near Newgate, British Columbia, Canada (Fig. 1). The Kootenay River is a seventh-order tributary to the Columbia River in southeastern British Columbia with a mean annual discharge of 296 m^3. The study area is ~250 km long and the drainage area is ~18 500 km^2. Like most watersheds throughout western North America, nonnative fish have been stocked extensively throughout the Kootenay drainage, particularly in

low-elevation lakes (MWLAP 2006). Stocking records were available for rainbow trout (RBT; *Oncorhynchus mykiss*) in the Upper Kootenay River from 1915 to present and document stocking of almost 20 million RBT at 114 sites in over 2500 individual stocking events (MWLAP 2006).

The Koocanusa Reservoir, which crosses the borders of British Columbia, Canada and Montana, USA, is a 170 km long reservoir on the Kootenay River that formed after the completion of the Libby dam in 1972 at Libby, Montana (Whatley 1972). The dam is located at a bedrock chute suspected of historically isolating westslope cutthroat trout (WCT, *O. clarki lewisi*) upstream from RBT in the Lower Kootenay River (Behnke 1992). Attempts to establish WCT in the reservoir by both the United States and Canada failed (B. Westover, *personal communication*). A policy was then developed to establish Gerrard strain RBT in the reservoir, and between 1986 and 1998 they were stocked in tributaries to the reservoir and in the reservoir itself (MFWP 2001, MWLAP 2006). Stocking RBT in lakes and reservoirs with potential outlets in the Upper Kootenay River was stopped in 1999 and replaced with WCT stocking or stocking of triploid RBT in all but Premier Lake, British Columbia, Canada (M. MacDonald, *personal communication*).

Leary and others (1987) detected hybridization between WCT and RBT as early as 1986 in the Upper Kootenay River using six allozyme markers. However, they confirmed hybridization at only one tributary (White River) out of the seven sampled. A follow-up study in 1999 using four nuclear DNA markers found hybridization at four of the same seven tributaries sampled in 1986 (Rubidge et al. 2001). Both studies had a similar power to detect hybridization (range from 62% to almost 100% power to detect 1% RBT alleles; Rubidge et al. 2001). The increase in hybridization was attributed to the initiation of a RBT stocking program in the Koocanusa Reservoir in 1988 (Rubidge et al. 2001).

METHODS

Sample site selection and survey design

This project builds on previous sampling by Rubidge (2003) from 1999 to 2001. The raw data she collected were combined with samples we collected from 2002 to 2006 to form a single database used for most analyses. We selected sample streams from third to sixth order tributary streams to the Upper Kootenay River, British Columbia, Canada in a systematic fashion throughout the watershed, at varying distances from Koocanusa Reservoir, which crosses the border of British Columbia, Canada and Montana, USA. We divided selected streams into three reaches of equal length (i.e., lower, mid, and upper elevation), and selected a representative sample site in each reach based on access and location. We chose this basic design because the previous study implicated Koocanusa Reservoir as a source of increased hybridization, and other similar studies suggest-

ed elevation plays a significant role in the distribution of westslope cutthroat trout (WCT, *Oncorhynchus clarki* ssp.), rainbow trout (RBT, *Oncorhynchus mykiss*), and their hybrids (Paul and Post 2001, Rubidge et al. 2001, Weigel et al. 2003, Rubidge and Taylor 2005). Several sample sites were located above known fish migration barriers where presumed pure populations of WCT existed. We classified sites as either open, if introduced RBT could potentially move there from Koocanusa Reservoir (Fig. 1), or closed if isolated from the Koocanusa Reservoir by a fish migration barrier.

Fish capture

We collected and analyzed tissue samples from 2670 fish captured in 31 streams at 45 sample sites from 1999 to 2006. All fish were caught and tissues samples collected as per Rubidge et al. (2001). The majority of the fish (85%) were captured by angling, followed by dip nets (12%), electroshocking (2.5%), and minnow traps (0.5%). Multiple age classes were sampled at each site and sample reaches ranged from 1 to 3 km long, to reduce the likelihood of sampling siblings (Weigel et al. 2003). At each sample site, we attempted to capture a minimum of 30 fish (mean = 59.3, SD = 82.1, range = 12–461; see Fig. 1 for number of samples per site). Sites that were visited multiple times were pooled because a previous study indicated that the level of introgression at a site (i.e., 0% < 10% ≤ 10%) did not change between years (Bennett 2007). We measured each fish to the nearest 0.5 cm and used a length–frequency analysis to estimate the age of each fish (Johnson and Anderson 1974). The overall percentage by each age class was 59.3% adults, 18.4% juveniles, 5.5% fingerlings, and 16.8% fry. Most fry (94%) were collected at one long-term monitoring site at Perry Creek (Bennett 2007). For all fingerling, juvenile, and adult fish, we clipped a small piece of the lower caudal fin placed it in 1.5 mL of 95% ethanol. Fry were collected whole. Sampling occurred during summer low-flow conditions, typically from mid-July to early September.

DNA analysis

Descriptions of the laboratory methods are in Rubidge and Taylor (2004, 2005) for samples collected from 1999 to 2001, and in Bennett (2007) for samples collected from 2002 to 2006. Briefly, we used diagnostic, codominant, nuclear loci to differentiate WCT, RBT, and their hybrids. Four loci were used for data collected from 1999 to 2001, and seven loci for data collected from 2002 to 2006. Loci were either restriction fragment length polymorphisms (RFLPs) or simple sequence repeats (SSR) developed by Baker and others (2002) and Ostberg and Rodriguez (2002, 2004).

Quantifying introgression

We determined the percentage RBT alleles at each sample site as a measure of introgression (Rubidge and Taylor 2004). We calculated the percentage RBT alleles (%RBT) as follows:
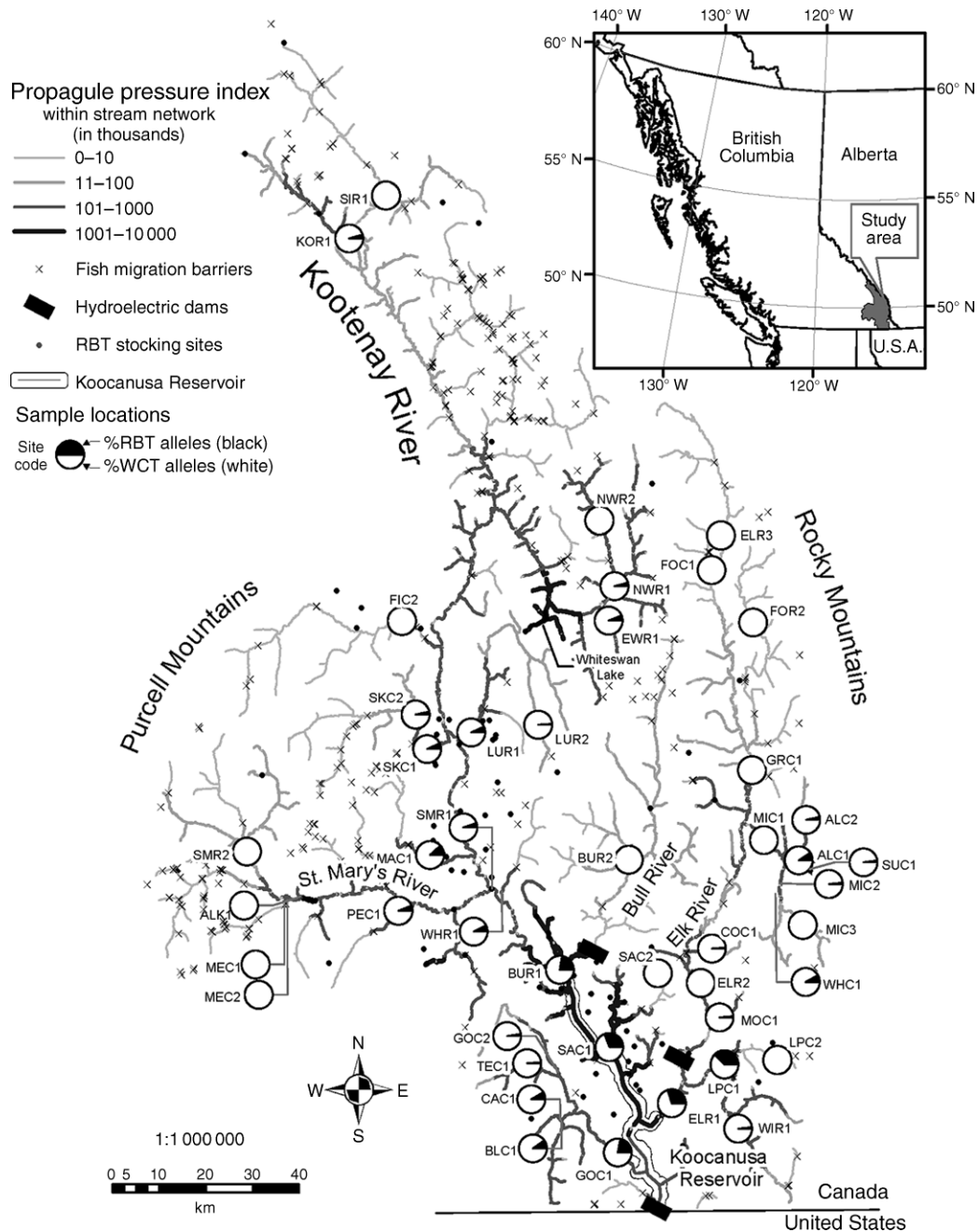
FIG. 1. Study area and location of hybridization monitoring sites ($n = 45$) in the Upper Kootenay River, British Columbia, Canada. Sampling locations are shown as circles with varying sections representing percentages of alleles present in that location's population. The abbreviation %RBT stands for percentage of rainbow trout (*Oncorhynchus mykiss*) alleles and is the black portion of the circles; %WCT stands for percentage of westslope cutthroat trout alleles (*Oncorhynchus clarki* ssp.) and is the white portion of the circles. Sample sites and sample sizes are: lower Alexander Creek (ALC1, $n = 50$), upper Alexander Creek (ALC2, $n = 27$), lower Alki Creek (ALK1, $n = 67$), lower Bloom Creek (BLC1, $n = 40$), lower Bull River (BUR1, $n = 29$), upper Bull River (BUR2, $n = 59$), lower Caven Creek (CAC1, $n = 19$), lower Coal Creek (COC1, $n = 40$), lower Wild Horse River (WHR1, $n = 45$), lower East White River (EWR1, $n = 30$), lower Elk River (ELR1, $n = 28$), mid Elk River (ELR2, $n = 20$), upper Elk River (ELR3, $n = 67$), mid Findlay Creek (FIC2, $n = 32$), upper Fording River (FOR2, $n = 34$), lower Forsyth Creek (FOC1, $n = 23$), lower Gold Creek (GOC1, $n = 127$), upper Gold Creek (GOC2, $n = 30$), lower Grave Creek (GRC1, $n = 20$), upper Kootenay River (KOR1, $n = 60$), lower Lodgepole Creek (LPC1, $n = 30$), upper Lodgepole Creek (LPC2, $n = 28$), lower Lussier River (LUR1, $n = 30$), upper Lussier River (LUR2, $n = 29$), mid Mather Creek (MAC1, $n = 28$), lower Meachen Creek (MEC1, $n = 41$), mid Meachen Creek (MEC2, $n = 24$), lower Michel Creek (MIC1, $n = 29$), mid Michel Creek (MIC2, $n = 149$), upper Michel Creek (MIC3, $n = 25$), lower Morrissey Creek (MOC1, $n = 30$), mid North White River (NWR1, $n = 33$), upper North White River (NWR2, $n = 31$), lower Perry Creek (PEC1, $n = 461$), lower Sand Creek (SAC1, $n = 31$), mid Sand Creek (SAC2, $n = 12$), lower Simpson River (SIR1, $n = 39$), lower

$$\%RBT = (RBT\ alleles/2LN) \times 100 \qquad (1)$$

where RBT alleles are the total number of RBT alleles detected at a site, L is the number of loci used, and N is the total number of fish captured at a site.

Power to detect hybridization

The power of the experimental design to detect the presence of RBT alleles is equal to β or $1 - \alpha$ (Kanda et al. 2002b). The following equation was used to calculate α:

$$\alpha = (1 - q)^{2nx} \qquad (2)$$

where q is the desired frequency of RBT alleles you wish to detect, n is the number of fish sampled, and x is the number of diagnostic markers. The combination of four markers and a sample size of 30 fish per site equates to a 91% probability of detecting 1% RBT alleles (i.e., $1 - \alpha$, where $\alpha = 0.08963$). For seven markers ($n = 30$ fish), the probability of detecting 1% RBT alleles is 98.5%.

When two species have been interbreeding for several generations, there are high misclassification rates associated with distinguishing between backcross and pure individuals (Boecklen and Howard 1997). For example, with four markers the probability of misclassifying first generation hybrids as pure WCT is 0.07, and with seven markers the probability is 0.01. The misclassification rate increases rapidly when classifying later generation backcrosses (i.e., BC-2) to 0.25 and 0.15 when using four markers and seven markers, respectively. This means that our sampling results were probably an underestimate the true number of RBT alleles present.

Variable selection

We selected four environmental and five spatial variables commonly reported in the literature as influencing hybridization between cutthroat and RBT. We also used another environmental variable to describe biologically relevant characteristics of the region where our samples were collected. Broad generalizations about species richness and presence/absence can be made with information about the general biogeographic setting of an area (Vinson and Hawkins 1998, Hawkins et al. 2003). Ecosystems in British Columbia have been classified using the Biogeoclimatic Zone Classification system (BEC; Pojar et al. 1987). The BEC groups ecosystems based on vegetation, soils, topography, and climate factors. We categorized our samples as either occurring in warm and dry (ponderosa pine and interior Douglas-fir) or cool and moist (interior cedar montane spruce, montane spruce, and Englemann spruce/subal-

←
Skookumchuck Creek (SKC1, $n = 65$), mid Skookumchuck Creek (SKC2, $n = 33$), lower St. Mary River (SMR1, $n = 195$), upper St. Mary River (SMR2, $n = 330$), upper Summit Creek (SUC1, $n = 27$), lower Tepee Creek (TEC1, $n = 60$), Wheeler Creek (WHC1, $n = 30$), mid Wigwam River (WIR1, $n = 34$).

pine fir) zones. Table 1 summarizes these variables, their expected influence on the rate of hybridization between cutthroat subspecies and RBT, and summary statistics for each variable are presented in Table 2.

Propagule pressure index (PPI)

We believed that incorporating propagule pressure required a spatially continuous measure that composited the propagule pressure from multiple sites and accounted for both the distance from stocking sites and the existence of any migration barriers. We derived the index from previously described stocking records and barrier data, as well as a 1:50 000 stream layer and 90-m resolution digital elevation model data (DEM). First, a distance raster was created for each stocking site using the cost weighted feature in ArcGIS (version 9.2; ESRI, Redlands, California, USA), based on a stream raster developed from the DEM and stream layer. This raster provided the stream distance from the stocking site to all other points in the watershed. We then incorporated the fish-migration barriers into each distance raster such that barriers upstream of stocking sites prevented fish movement beyond that point (i.e., the cost of moving beyond the barrier was essentially infinite), but barriers downstream of a stocking site would not. We assumed that RBT stocked in streams could move downstream unrestricted and upstream as far as the first known fish-migration barrier (Adams et al. 2001). Lakes with known inlet and/or outlet streams were classified similarly to streams, while unconnected lakes were excluded from this analysis.

We then developed a fish movement model estimating the proportion of stocked RBT that stray a given distance from their original stocking site. We used a decay function to approximate a common stream resident movement strategy, whereby most fish stay close to their natal stream (or stocking site) with less common, but consistent, long-distance migration by some individuals (Fraser et al. 2001, Rodriguez 2002). We derived a constant decay rate of -0.05 for straying fish based on a review of the literature on the movement of stocked RBT by Bjorn and Mallet (1964) and Cresswell (1981), which indicates that ~60% of stocked RBT move ≥ 10 km from their original stocking site, and on typical movement patterns of stream resident salmonids (e.g., Adams et al. 2001, Rodriquez 2002, Schmetterling and Adams 2004).

We created a raster layer of the entire watershed to represent the relative amount of propagule pressure associated with each individual stocking site using the following formula:

$$PP = NoFish\left[\exp\left(-0.05(Dist)\right)\right] \qquad (3)$$

where PP is the relative propagule pressure for a single stocking site, NoFish is the total number of fish stocked at a site (all years combined), -0.05 is the constant decay rate for straying fish, and Dist is the distance to each stocking site in kilometers. This equation was then

TABLE 1. Variables used in multinomial logistic regression to assess introgression between westslope cutthroat trout (WCT, *Oncorhynchus clarki* ssp.) and rainbow trout (RBT, *Oncorhynchus mykiss*), and the hypothesized effect of each variable on the level of introgression.

Variable (units, abbreviation)	Data type	Potential mechanisms influencing introgression
Environmental variables		
Biogeoclimatic zone (0/1, BEC)	categorical (0, warm-dry zone; 1, cool-moist zone)	introgression positively associated with warmer and drier BEC zones because streams in these zones would have more optimal hydrologic conditions for RBT
Elevation (m, Elev)	continuous	introgression negatively associated with elevation because of cooler temperatures and limited spawning period
Mean May water temperature (°C, Temp)	continuous	introgression positively associated with higher mean May water temperature because RBT spawning peaks in May
Mean day of peak flow (date, Flow)	continuous	introgression negatively related to later peak flow dates because of delay in WCT spawning
Width (m, Width)	continuous	introgression positively associated with increasing stream width because RBT spawn in larger streams
Spatial introduction variables		
Propagule pressure index (no. of RBT, PPI)	continuous	introgression positively associated with an increase in the propagule pressure
Above or below migration barriers (0/1, Barrier)	categorical (0, sample site above a barrier; 1, sample site not above a barrier)	introgression positively associated with open sites (i.e., sites that introduced RBT could access from downstream)
Distance to Koocanusa Reservoir (km, DstRes)	continuous	introgression negatively associated with the distance from the Koocanusa Reservoir
Distance to closest stocking site (km, Clst)	continuous	introgression negatively associated with an increase in the distance to the nearest stocking site
No. stocking sites within 10 km (no., StkSt10)	continuous	Introgression positively associated with the number of stocking sites within 10 km

Note: BEC stands for biogeoclimate zone classification.
† ⟨http://www.for.gov.bc.ca/hfd/library/MOE/BOSZ_BOTA.htm⟩

applied to each cell in the stream network to produce a raster where each cell represented the relative number of RBT potentially moving to that location from a single stocking site. For example, the movement model predicted that as much as 60.7% of the stocked RBT could be found as far as 10 km from their original stocking site. Therefore, a site located 10 km from a site stocked with 10 000 RBT (assuming no barriers) would have a relative propagule pressure of 6065. We then summed the relative propagule rasters for all the

TABLE 2. Summary statistics for environmental and spatial independent variables measured at each sample site ($n = 45$).

Variable description (unit)	Code	Mean	Min.	Max.	SD
Environmental					
Biogeoclimatic zone†	BEC	0.4	0.0	1.0	0.5
Elevation (m)	Elev	1120	753	1566	222
Mean May water temperature (°C)	Temp	5.4	3.0	8.5	1.1
Mean day of peak flow (day of year)	Flow	152	134	168	10
Stream width (m)	Width	22.4	1.5	115.0	22.3
Spatial					
Potential propagule pressure (no. RBT)	PPI	270 477	0	1 572 804	340 856
Above or below barrier‡	Barrier	0.6	0.0	1.0	0.5
Distance to Koocanusa Reservoir (km)§	DstRes	80.1	1.0	241.1	54.9
Distance to closest stocking site (km)	Clst	17.2	0.1	66.1	14.8
No. stocking sites within 10 km	Stk10	0.4	0	3	2

Note: The variables were grouped into two categories: environmental site characteristics and spatial introduction characteristics related to potential propagule pressure.
† This binary variable was coded 0 for not warm/dry and 1 for warm/dry.
‡ This binary variable was coded 0 for closed sites and 1 for open sites.
§ Koocanusa Reservoir crosses the border of British Columbia, Canada, and Montana, USA. See Fig. 1.

Table 1. Extended.

Data source	Literature source
British Columbia (BC), Canada government GIS data layer	Pojar et al. (1987), Vinson and Hawkins (1998), Paul and Post (2001), Hawkins et al. (2003)
GIS derived from 90-m digital elevation model	Paul and Post (2001), Weigel et al. (2003)
stream thermographs	Fausch (1988), Griffith (1988), Ennis (1995), Henderson et al. (2000), De Rito (2004)
Canada Environment hydrometric stations	Marotz and Farley (1986), Henderson et al. (2000), Moller and Van Kirk (2003), De Rito (2004)
BC government stream inventory database	Bozek and Hubert (1992), Kruse et al. (1998), Henderson et al. (2000), Weigel et al. (2003)
GIS-derived relative propagule pressure (see *Methods: Propagule pressure index (PPI)*)	Williamson (1996), Kolar and Lodge (2001), Colautti (2005), Lockwood et al. (2005)
BC government stream inventory database	Thompson and Rahel (1998), Adams et al. (2001), Novinger and Rahel (2003), Kondratieff and Myrick (2006)
stream distance using 1:50 000 GIS stream layer	Rubidge and Taylor (2005)
GIS-derived stream distance	Rubidge and Taylor (2005), Ostberg and Rodriguez (2006)
BC government stocking records†	MWLAP (2006)

stocking sites to derive our index in the form of a spatially continuous raster. This final index layer provided us with an estimate of the relative number of RBT that could move to any point in the watershed from all the stocking sites over the entire period of record. We allowed fish to move in both directions simultaneously (i.e., the total number of RBT in the propagule layer was greater than the total number of fish stocked). This index is a relative measure of the contribution of all stocking events at all sites to the overall propagule pressure at any point in the Upper Kootenay River. We tested the accuracy of PPI by using it to predict the percentage of pure RBT (i.e., a fish homozygous for RBT alleles at every loci) at a site using simple linear regression. We predicted a high propagule pressure index would be positively associated with increasing levels of introgression.

Model development and assessment

We used multinomial logistic regression to assess the influence of environmental and spatial introduction variables on the level of introgression between WCT and RBT. We defined three levels of introgression: no RBT alleles present (zero), <10% RBT alleles present

(low), and >10% RBT alleles present (high). We used these levels of introgression in our analysis because they have been proposed as management categories for introgressed cutthroat trout populations (Pritchard et al. 2007) and the frequency distribution of RBT alleles by sample site in our data fit this general trend (Fig. 1). We used the cumulative logit model in SAS (version 9.1; SAS Institute, Cary, North Carolina, USA) to assess the relationship between the response categories and our independent variables because the cumulative logit model assumes response categories are ordered. In our case the response categories are naturally ordered from no RBT alleles present to a high proportion of RBT alleles present. The advantages of using an ordered logit model as compared to an unordered logit model are that the coefficients are easier to interpret and the hypothesis tests are more powerful (Allison 1999). We used the default settings, so the models predicted the probabilities of membership in the lowest category, zero introgression. We tested for multicollinearity between all independent variables using the variance inflation factor (VIF) from a global logistic regression model (Table 3). We also tested for the parallel lines assumption of logistic regression with the score test for the propor-

TABLE 3. A summary of model selection statistics for evaluating the level of introgression between westslope cutthroat trout (WCT) and rainbow trout (RBT) in the Upper Kootenay River, British Columbia, Canada.

		Model selection statistics								
Model	K	AIC	AIC_c	ΔAIC	Rel. like.	w_i	Evid. ratio	% max w_i	r^2	Prop. odds
BEC, PPI	4	62.7	63.7	0.0	1.0	0.4	1.4	100.0	0.62	0.66
GISPP	3	63.7	64.3	0.6	0.7	0.3	3.8	73.7	0.58	0.27
Flow, PPI	4	65.4	66.4	2.7	0.3	0.1	4.2	26.1	0.58	0.08
Temp, PPI	4	65.6	66.6	2.8	0.2	0.1	4.5	24.1	0.58	0.53
Elev, PPI	4	65.7	66.7	3.0	0.2	0.1	4.5	22.1	0.58	0.32
Clst, PPI	4	65.7	66.7	3.0	0.2	0.1	8.4	22.0	0.58	0.31
Clst, DstRes, PPI, Barrier, Stk10	7	65.0	68.0	4.3	0.1	0.0	119.6	11.9	0.66	0.18
Global model	12	70.0	79.8	16.1	0.0	0.0	4555.9	0.0	0.71	0.33

Notes: K is the number of parameters (including two intercepts); AIC, Akaike's information criteria; AIC_c, AIC corrected for small sample size; ΔAIC, the difference between the model with lowest AIC and every other model; Rel. like., $\exp(-0.5 \times \Delta AIC_c)$, which is the likelihood of the model, given the data, relative to the best model; w_i, normalized relative likelihood or Akaike weights; Evid. (evidence) ratio, ratio of the w_i for a given model vs. w_i for the best model; % max w_i, percentage of the maximum w_i reported in the top model; r^2, maximum rescaled r^2, which adjusts generalized r^2 due to the upper bound being <1; Prop. odds, P value for the scored test for the proportional odds assumption test that the grouping of the response variable did not influence the results. For model variables, see Table 1.

tional odds assumption (Allison 1999). The score test assesses the influence of how the dependent variable is categorized on the relationship with the independent variables, and high P values (i.e., >0.05) indicate minimal influence. We dropped two spatial introduction variables (the number of stocking events within 10 km and the total number of fish stocked within 10 km of a site) that had VIFs > 10 (Quinn and Keough 2002).

The propagule pressure variables were heavily left skewed. However, the logistic regression model requires a linear relationship between the logit function and the independent variables and does not require normally distributed error structures in the independent variables (Allison 1999). We chose to transform independent variables based on biological criteria. We expected distance and fish movement variables should have a nonlinear relationship with introgression due to the exponential increase in area (and decrease in propagule pressure) with increasing distance from stocking sites. Therefore, we used natural-log transformation to linearize the effect of the propagule pressure index (PPI), distance to the Koocanusa Reservoir (DstRes), and distance to the closest stocking site (Clst) on the dependent variable. All other variables were not transformed.

We tested 23 candidate models that were a combination of environmental variables only, spatial variables only, single variables, and all variables combined (i.e., the global). The relative fit of candidate models with the data was assessed using the information-theoretic approach (Burnham and Anderson 1998). We used corrected Akaike's information criterion (AIC_c), a second order variant of AIC for small sample sizes, to assess our candidate models (Burnham and Anderson 1998). The model with the lowest AIC_c score was considered the best fitting model; however, the relative plausibility of each model was further assessed by calculating normalized relative likelihood or Akaike weights (w_i). A given w_i can be interpreted as the weight

of evidence that a given model i is the best model given the data and the set of candidate models assessed. We created a composite model from the confidence set of all models with a w_i at least 10% of the highest weight to account for model-selection uncertainty as described in Burnham and Anderson (1998). We calculated the odds ratios and 95% Wald confidence intervals for each variable in the composite model as a measure of the variable's influence on introgression. We also calculated importance weights for each variable by summing the w_i from each model in the confidence set of models that the variable occurred in (Burnham and Anderson 1998). Larger importance weights indicate the variable has a greater influence compared to all other variables in the confidence set of models. We tested for overdispersion of the data and non-independence between sites by approximating the variance inflation factor c (Burnham and Anderson 1998). We estimated c by dividing the goodness of fit χ^2 value for the global model by the degrees of freedom of the global model. Values of $c > 2$ indicate potential overdispersion.

Model validation

We estimated the performance of the top models (as determined by the w_i score) by using a 10-fold cross-validation technique where the data set was divided into 10 groups and the models were run leaving one group out and the logistic regression was calculated on the remaining nine (Efron 1983, Olden et al. 2002). We then assessed the accuracy of the best candidate model by determining the overall classification error rate, based on the proportion of observations that were incorrectly classified (Peterson and Dunham 2001).

RESULTS

Distribution of rainbow trout (RBT) alleles

Introgression (i.e., presence of rainbow trout alleles; RBT, *Oncorhynchus mykiss*) was found at 31 of the 45

sites (68.9%) and was generally greater at lower elevations and closer to the Koocanusa Reservoir, which crosses the border of British Columbia, Canada and Montana, USA (Fig. 1). We found 14 sites where no RBT alleles were detected (i.e., suspected pure westslope cutthroat trout [WCT, *Oncorhynchus clarki* ssp.] populations). However, only four suspected pure sites (upper Bull River, upper Fording River, mid Meachen Creek, and mid Sand Creek) were completely isolated upstream of all documented RBT sources (Fig. 1). Sites without migration barriers between the site and the Koocanusa Reservoir had a significantly higher mean percentage RBT alleles than those with barriers (without barriers, 11.3% ± 19.9% (mean ± SD, $n = 27$); with barriers, 1.5% ± 2.8%, $n = 18$; $t = -2.07$, $P = 0.022$).

Tests for Hardy-Weinberg (H-W) equilibrium and linkage disequilibrium (LD) are presented in Rubidge and Taylor (2004) and Bennett (2007). Briefly, lower elevation sites near the Koocanusa Reservoir had significant heterozygote deficiencies and significant linkage disequilibrium suggesting relatively recent introgression. Higher elevation sites further from the reservoir were in H-W equilibrium and lacked LD, which was attributed to F1 and later generation hybrids spreading RBT alleles to these sites.

Propagule pressure

We found records of 87 RBT stocking sites dating back to 1915 that could be accurately located. Of these, 52 sites were determined to have outlets presumably allowing fish to escape the lake and enter the watershed. A total of 8 283 793 RBT were stocked in lakes with outlets during 1165 individual stocking events. The majority of RBT stocked were fry/eyed egg (60%) and one year olds (28%). Very few (0.4%) age > 2 RBT were stocked and no ages were recorded for 12% of the stocked RBT. The median number of RBT stocked per event was 3500 fish (7110 ± 11 896 RBT, mean ± SD). The majority of stocking sites (70%) and RBT stocked (90%) were below 1200 m elevation (Fig. 2a).

The highest levels of our geographic information system (GIS)-derived propagule pressure index were found in two distinct areas: clustered around and directly upstream of the Koocanusa Reservoir and around Whiteswan Lake (Fig. 1). The propagule pressure index (PPI) ranged from >1 000 000 near the Koocanusa Reservoir and Whiteswan Lake, British Columbia, Canada, to 0 at three pure WCT sites (upper Fording Creek, upper Meachen Creek, and upper Sand Creek; all sites noted here are in British Columbia, Canada). Moderate PPI levels (100 000 to 500 000) existed around St. Mary Lake and Summit Lakes. The PPI was a relatively good predictor of the percentage pure RBT at a site ($r^2 = 0.746$, $P < 0.0001$, $n = 45$ sites), indicating that the index represented the actual RBT propagule pressure as measured by the number of RBT we captured (Fig. 2b).
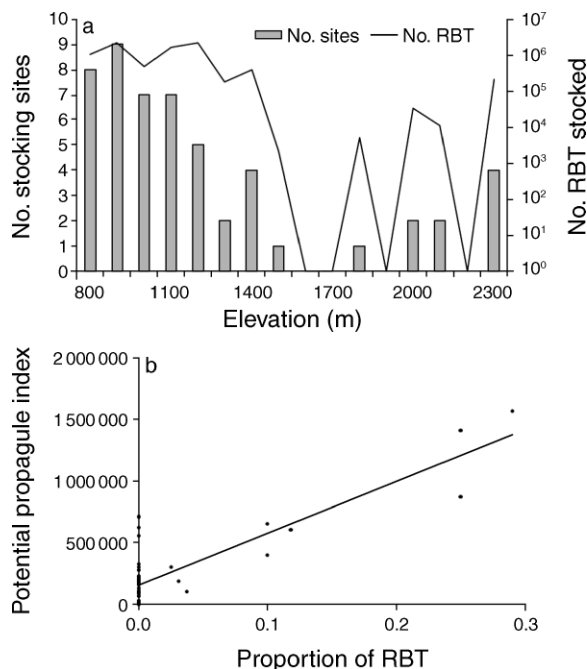

FIG. 2. (a) The number of stocking sites and rainbow trout (RBT) stocked in lakes with outlets, by elevation, within the Upper Kootenay River, British Columbia from 1915 to 2006 and (b) the relationship between the observed proportion of RBT at a site and the predicted propagule pressure index at the site (PPI; the number of fish that could move to any one site from all stocking sites in the watershed).

Model results

Based on the candidate models we tested, the most plausible model for predicting genetic introgression based on Akaike weights (w_i) was the model that included the GIS-derived propagule pressure index (PPI) variable and the biogeoclimatic variable (BEC; Table 3). The BEC and PPI model was 1.4 times more likely to explain introgression than the next most plausible model, based on PPI alone. There were seven models in the confidence set of models (i.e., w_i values at least 10% of the highest ranked model) and PPI was in all of these models (Table 3). Five of the top models contained environmental and spatial variables; however, none of the top models included only environmental variables. The data were not overdispersed, based on our approximation of variance inflation factor (VIF) for the global model ($c = 1.56$), and the global model met the logistic regression parallel lines assumption ($\chi^2 = 15.57$, df = 10, $P = 0.113$).

The composite model created from the top seven models (Table 4) included four spatial variables (Barrier, Clst, PPI, and Stk10) and four environmental variables (BEC, Elev, Flow, and Temp). The propagule pressure index was negatively related to the presence of zero introgression and had the greatest influence on the presence of introgression, shown by an importance

TABLE 4. Model-averaged results for multinomial logistic regression analysis for predicting introgression between WCT and RBT in the Upper Kootenay River, British Columbia, Canada.

Model parameter	Parameter estimate	SE	Odds ratio	Odds ratio, 95% Wald CI		Importance weight
				Upper	Lower	
Intercept 0	12.469	6.011				
Intercept Low	16.613	6.321				
PPI	−1.261	0.394	0.285	0.131	0.613	0.995
BEC	−1.628	0.985	0.196	0.028	1.352	0.356
Flow	0.022	0.038	1.022	0.948	1.101	0.093
Temp	0.869	0.328	2.385	0.457	1.652	0.086
Elev	0.000	0.002	1.000	0.996	1.004	0.078
Clst	0.249	0.497	1.283	0.484	3.401	0.078
Barrier	−0.487	0.858	0.615	0.114	3.301	0.042
DstRes	1.226	0.540	3.407	1.182	9.819	0.042
Stk10	2.206	1.138	9.076	0.975	84.443	0.042

Note: For parameter names and information, see Tables 1 and 2.

weight nearly three times greater than (0.995) the next most influential variable (Table 4). The odds ratio estimate for PPI was 0.285 (95% Wald CI 0.131–0.613). The odds ratio estimate can be interpreted as a 71.5% decrease in the odds of being in the zero introgression category (i.e., no introgression) for every one unit increase in PPI (i.e., $[1 − 0.285] × 100 = 71.5\%$). Since PPI in the composite model is on the natural-log scale, we converted it back to a relative potential number of RBT for ease of interpretation. The predicted probability of a site having zero introgression was >0.95 when PPI was <3000, but once PPI was >750 000 the predicted probability of a high level of introgression was >0.5 (Fig. 3). The next most influential variable was BEC, with an importance weight almost four times larger than any of the lower ranked variables. However, the evidence was uncertain on how BEC and the other variables influence the level of introgression in the composite model or the other top six models because their odds ratio 95% Wald CI all spanned 1 (odds ratio 95% CI only shown for the composite model; Table 4). A 95% Wald CI spanning 1 means that the direction of the relationship cannot be known with a 95% confidence.

Model validation

We tested the cross-validation error rate of the two top models and the composite model. All three models had a relatively low error rate (PPI = 0.307, BEC and PPI = 0.311, composite = 0.326). The most common classification error rate for all three models was predicting zero introgression at a site when it was measured as a low introgression site. No model predicted zero introgression where high introgression was observed.

DISCUSSION

Propagule pressure

Our study demonstrated the strength of propagule pressure as a predictor of the level of introgression

between native westslope cutthroat trout (WCT, *Oncorhynchus clarki* ssp.) and introduced rainbow trout (RBT, *Oncorhynchus mykiss*). All models in the confidence set contained the propagule pressure index (Table 3), and when a composite model was developed, PPI was the most influential variable (Table 4). The role of propagule pressure in species invasions is intuitively appealing and well supported by ecological theory (reviewed in Lockwood et al. 2005). Populations of introduced species will be less susceptible to extinction due to stochastic environmental and genetic effects (e.g., catastrophic disturbance, reduced genetic diversity, and inbreeding) when the population numbers are large, the more often they are introduced, and the more widely they are distributed (Williamson 1996, Kolar and Lodge 2001). Therefore, increased propagule pressure should increase the probability that an introduced species will be successful at all stages of invasion (Marchetti et al.
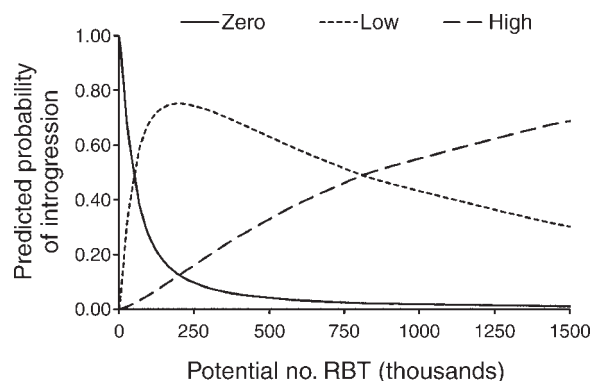

FIG. 3. Predicted probability of introgression level between westslope cutthroat trout (WCT, *Oncorhynchus clarki* ssp.) and rainbow trout (RBT, *Oncorhynchus mykiss*), based on the GIS-derived propagule pressure index (PPI). The "zero" line is the predicted probability of a site having zero introgression, the "low" line is the predicted probability of a site having <10% introgression, and the "high" line is the predicted probability of a site having >10% introgression at the different levels of propagule pressure (i.e., potential number of RBT).

2004, Lockwood et al. 2005, Von Holle and Simberloff 2005). Also, when propagule pressure is not accounted for, it can lead to misinterpretation of more complex models (Colautti 2005). For example, many previously fishless high mountain lakes have been stocked with inland trout; however, these populations are often sustained by continual stocking due to low reproductive success (Bailey and Hubert 2003).

In the Upper Kootenay River, in British Columbia, Canada, the stocking of RBT in lakes with outlets peaked in the 1940s at 1.6 million, and averaged approximately 1.0 million RBT a decade between 1950 and 2000 (MWLAP 2006). If propagule pressure alone had been responsible for the introgression levels we observed, Leary and others (1987) should have found more widespread introgression in the late 1980s. However, Rubidge and Taylor (2005) found evidence that the introgression increased as a result of stocking RBT in Koocanusa Reservoir, which crosses the border of British Columbia, Canada and Montana, USA, which did not begin until 1988. A possible explanation for these findings is that environmental factors prevented most RBT from successfully spawning prior to 1988, or stocking of RBT directly into the Koocanusa Reservoir was more successful at establishing RBT than previous efforts where RBT were typically stocked into small lakes. We do not have data to test these speculations, but changes in environmental factors beneficial to RBT (e.g., reduced flow rates in the spring) have been implicated in recent increases in hybridization between RBT and Yellowstone cutthroat trout despite a long history of RBT stocking without apparent introgression (Henderson et al. 2000, Van Kirk and Jenkins 2005). If environmental factors limit introgression, it does not necessarily weaken the role of propagule pressure, instead continued propagule pressure may increase the probability of invasion when a "window of opportunity" arises.

Environmental and spatial variables

The performance of the composite model provides evidence that the distribution and level of introgression is likely controlled by both environmental and spatial variables. This has also been found for fish species invasions in general (Marchetti et al. 2004). For example, migration barriers limit the movement of introduced salmonids and protect native populations upstream (Harig et al. 2000, Novinger and Rahel 2003, Van Houdt et al. 2005), and we found most of the pure WCT populations above barriers. However, barriers alone are not predictors of introgression because of frequent stocking of RBT above barriers. Elevation is also negatively correlated with the level of introgression (Paul and Post 2001, Weigel et al. 2003), and we found a decrease in the level of introgression with increasing elevation. This suggests that RBT may be prevented from occupying higher elevations by biological limitations. Thermal tolerance tests of RBT and WCT tend to

support this theory, with RBT having higher upper tolerances for water temperature (24°C) compared to WCT (19°C), and appearing to grow over a wider range of temperatures (Bear et al. 2007). The differences in temperature tolerances between RBT and WCT may partly explain why RBT are often restricted to lower elevation sites (Paul and Post 2001, Rubidge et al. 2001, Weigel et al. 2003).

Of equal concern is how environmental factors affect the fitness of the hybrids between native and introduced species (Leary et al. 1995). It is clear that hybrids between WCT and RBT are fertile and their fitness appears to be equal to that of the parentals (Rubidge and Taylor 2004). There is strong evidence that hybrids are spreading RBT alleles in the Upper Kootenay Watershed, British Columbia, Canada (Rubidge and Taylor 2004, 2005) and in other parts of the WCT range (Hitt et al. 2003, Boyer et al. 2008). The implications of these findings are that environmental conditions that restrict RBT to the lower elevation streams are not equally restricting hybrids. This may be due to hybrids having physiological tolerances that are intermediate between the parentals (Arnold 1997, Seiler and Keeley 2007). Presumably the offspring of pure WCT that breed with hybrids (i.e., later generation backcrosses) would have similar physiological tolerances to pure WCT. This could lead to backcrosses being able to spread throughout the watershed if not restricted by migration barriers or selection. Fitzpatrick and Shaffer (2007) found that an interaction between dispersal and selection against introduced salamanders and their hybrids also explained the distribution of introgressed populations.

Model limitations and applicability

Because our propagule pressure index is only an estimate of the relative abundance of RBT, it is of limited usefulness for determining the number of introduced propagules at any one site. However, with the increase in evidence of propagule pressure impact on introgression, models that are more sophisticated should be developed. Our approach can be modified to provide an actual measure of propagule pressure throughout a watershed by accounting for effects of fish preferences for both upstream vs. downstream movement, and larger vs. smaller tributaries. Being able to predict the effects of specific stocking levels of nonnatives on introgression could help resource managers by identifying areas where control or removal programs should be initiated (i.e., high introgression areas most likely to be sources of continued invasion).

It is possible our estimate of propagule pressure from straying RBT failed to account for other sources of RBT alleles. Naturalized RBT populations and hybrids from introgressed populations could also be contributing to propagule pressure in the watershed. In the Flathead River there is evidence of RBT alleles spreading to other sites from introgressed populations via hybrid straying (Hitt et al. 2003, Boyer et al. 2008). Boyer and others

(2008) showed examples of both introgression spreading via hybrids straying to adjacent sites (i.e., the stepping stone model; Kimura and Weiss 1964) and by long-distance (>50 km) dispersal of hybrids (i.e., the continent–island model; Wright 1931) based on their analysis of allele frequencies in a tributary to Flathead Lake, Montana, USA. This indicates that not only are original stocking sites a source of propagule pressure in the watershed, but new "source" pools can be created as hybrid individuals disperse from introgressed populations and the process continues upstream or downstream.

We relied on government stocking records and fish habitat inventory databases to provide data on the number of RBT stocked and the locations of migration barriers within the watershed. We suspect some historic stocking sites were not documented in the databases we used, and we identified several stocking events where the location was not recorded. As an example, Rubidge and Taylor (2005) determined that almost 3 million RBT were stocked in the Upper Kootenay River between 1915 and 1998. However, we determined that almost 20 million RBT were stocked between 1915 and 2006 using the same data source (i.e., BC MWLAP). This discrepancy was likely due to the intensive data synthesis efforts occurring in the late 1990s as the British Columbia Resource Inventory Standards Committee reviewed and combined numerous databases. Also complicating our analysis was our reliance on expert opinion to determine if stocking sites had outlets. We know of at least one site (e.g., Summit Lake) that was wrongly assumed to have no outlet. Upon field inspection, we found a defined channel indicating the lake was frequently connected and we captured RBT fry in the outlet. An inspection of all stocking sites is required to confirm their outlet status and allow for better assessment of each stocking sites potential role in contributing to the overall RBT propagule pressure.

The migration barrier database is likely accurate for larger streams where the majority of our sampling occurred (i.e., greater than third order tributaries). These larger streams have been inventoried numerous times, and it is unlikely that any mainstem barriers have not been identified. However, smaller tributaries are underrepresented in the database, and it is likely numerous migration barriers have not been identified. The effect of this underestimation of barriers in smaller streams will be an overestimation of propagule pressure because of the lack of RBT access to these streams due to migration barriers.

An accurate accounting of propagule pressure is critical in assessing the success and impact of introduced species. Most fisheries agencies in North America have relatively good stocking records especially for salmonids, but it is well recognized that the stocking records are often incomplete (Miller and Alcorn 1946, Nilsson 1972, Bahls 1992, Welcomme 1992, Schindler 2000). It is unknown how many stocking records are missing from

our analyses, but we suspect that the pattern of introductions that were not recorded follows the stocking pattern we observed (Fig. 2a). British Columbia's (Canada) government records go back to 1915 in the database we used, but Schindler (2000) reports stocking in Banff National Park (BNP) as far back as 1901. Therefore, it is possible that we are missing records from at least 1901 to 1914 (assuming that stocking in Kootenay River was taking place at the same time as stocking in BNP which is near the headwaters of Kootenay River). The impact of these missing records on our analysis is also unknown; however, the overall proportion of introduced RBT that were recorded was probably high enough (i.e., >90–95%) that we do not suspect the missing records would change the results of our analyses. For example, if two stocking events a year were missed every year since stocking began (assuming stocking began in 1900 = 106 years) and the mean number stocked per event was 7110 RBT, then this would account for only 9.1% of all the RBT we have records for (i.e., (7110 fish × 106 yr)/8 283 793 recorded stocked). It is unlikely that unrecorded events would be as high as 7110 fish and as often as two per year for the entire period because private entities would not have the resources to sustain this type of effort without it being recorded by provincial agencies.

CONCLUSION

Our estimate of relative propagule pressure demonstrates the importance of this measure in salmonid invasion events and provides further evidence of the general importance of propagule pressure. We agree with those calling for more focus to be placed on assessing the role of propagule pressure, as there is growing evidence that its role in species invasions may have been underestimated (Lockwood et al. 2005, Verling et al. 2005). Despite the importance of propagule pressure, environmental factors do appear to limit the extent of rainbow trout to lower elevation streams in many parts of the westslope cutthroat trout range. However, hybrids appear not to be restricted by environmental factors, and growing evidence suggests that hybrids are now the main vector for spreading introgression. Therefore, despite management agencies reducing or stopping stocking (i.e., decreasing rainbow trout [RBT, *Oncorhynchus mykiss*] propagule pressure), hybrids created in lower elevation sites where RBT have established are now the new source of propagules. Our model results suggest that introgression will spread throughout the watershed unless prevented by migration barriers.

The recent cessation of RBT stocking in the Upper Kootenay River, British Columbia, Canada is certainly a good first step in reducing the threats to the native westslope cutthroat trout (WCT, *Oncorhynchus clarki* ssp.), but it would be naive to think that this alone will prevent the further introgression between RBT and WCT. Monitoring of introgression levels and continued

efforts to determine and eliminate sources of RBT will be essential for the conservation of the remaining pure WCT populations and the potential restoration of those with currently low levels of introgression.

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