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Variability in expression of anadromy by female *Oncorhynchus mykiss* within a river network

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Abstract We described and predicted spatial variation in marine migration (anadromy) of female *Oncorhynchus mykiss* in the John Day River watershed, Oregon. We collected 149 juvenile *O. mykiss* across 72 sites and identified locations used by anadromous females by assigning maternal origin (anadromous versus non-anadromous) to each juvenile. These assignments used comparisons of strontium to calcium ratios in otolith primordia and freshwater growth regions to indicate maternal origin. We used logistic regression to predict probability of anadromy in relation to mean annual stream runoff using data from a subset of individuals. This model correctly predicted anadromy in a second sample of individuals with a moderate level of accuracy (e.g., 68% correctly predicted with a 0.5 classification threshold). Residuals from the models were not spatially autocorrelated, suggesting that remaining variability in the expression of anadromy was due to localized influences, as opposed to broad-scale gradients unrelated to mean annual stream runoff. These results are important for the management of *O. mykiss* because anadromous individuals (steelhead) within the John Day River watershed are listed as a threatened species, and it is difficult to discern juvenile steelhead from non-anadromous individuals (rainbow trout) in the field. Our results provide a broad-scale description and prediction of locations supporting anadromy, and new insight for habitat restoration, monitoring, and research to better manage and understand the expression of anadromy in *O. mykiss*.

Keywords Steelhead trout · Rainbow trout · Migration · Partial migration · Anadromy · Otolith microchemistry · Life history

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Introduction

Partial migration is common in salmonid fishes, and includes relatively short migrations between freshwater

habitats, as well as longer-distance migrations between freshwater and marine environments (anadromy; Jonsson and Jonsson 1993). Spatial variability in migratory behavior is often linked to environmental gradients within river networks. For example, brown trout (*Salmo trutta*; Olsson and Greenberg 2004), Dolly Varden char (*Salvelinus malma*; Koizumi et al. 2006), and rainbow trout/steelhead (*Oncorhynchus mykiss*; McMillan et al. 2007; Narum et al. 2008) exhibit a basic pattern of increasing tendency for residency with increasing upstream distance, which is related to a variety of changes in river ecosystems (Vannote et al. 1980; Montgomery et al. 1999). Marine migratory behavior is variably heritable (e.g., brook trout, *S. fontinalis*; Thériault et al. 2007), but in all species it appears to be a flexible response to variability in environmental conditions (Jonsson and Jonsson 1993; Aubin-Horth et al. 2006). The implication is that environmental variability could provide a useful means for predicting the spatial distribution of migratory behaviors across a landscape.

Prediction of migratory behavior also depends on the sex of individuals because males and females have substantially different costs and benefits of migration (Jonsson and Jonsson 1993; Northcote 1997; Hendry et al. 2004). Females benefit more from greater size resulting from migration and often predominate among migrants (Jonsson and Jonsson 1993; Thériault and Dodson 2003; Koizumi et al. 2006). On their return from the ocean, females may home to specific spawning habitats, whereas males may exhibit little spatial patterning (Neville et al. 2006). Given these differences between sexes, it seems appropriate to consider expression of migratory behavior in salmonids separately for males and females, at least when considering factors that may predict or explain patterns of migration.

Here, we examine whether there is predictable broad-scale variation in marine migratory behavior (anadromy) in female *O. mykiss* within the John Day River, a large river basin located in northeast Oregon. We sampled juvenile *O. mykiss* from streams throughout the John Day River and applied otolith microchemical analysis (Kalish 1990) to determine the migratory life histories of their mothers. This approach assumes that juveniles are captured close to their natal site and that their mothers spawned near their respective natal sites. Accordingly, we described the spatial pattern of female anadromy across the riverscape and predicted the presence of anadromy from patterns of environmental variability. Specific objectives of this study were to: 1) establish accurate measures of female anadromy across a broad spatial area with a protocol that minimized sacrificial sampling; 2) predict patterns of female anadromy in relation to broad-scale environmental gradients; and 3) assess the potential importance of local environmental variability in determining the expression of anadromy.

Methods

Study area

The John Day River (45°44'N, 120°39'W) is a free-flowing tributary to the Columbia River draining approximately 20 500 km^2 of north-central Oregon (Fig. 1). Elevation in the catchment basin ranges from 80 m near the mouth to 2700 m in the headwaters, and annual precipitation varies from about 25 cm to 145 cm (Anonymous 2006a). Most precipitation occurs during the winter and spring and peak stream flows tend to be in the spring, linked to snowmelt. Climate, vegetation, geology, and hydrology vary widely across the basin, including heavily wooded montane forests in the uplands of the northeastern part of the basin (southern Blue Mountains), high desert in the central basin, and the Ochoco and Strawberry Mountain ranges along the southern edge of the basin.

Both anadromous and resident (freshwater, non-anadromous) *O. mykiss* occur throughout the basin, though spawning and rearing of both is generally restricted to areas with relatively cool water, upstream of the lower river mainstem of the John Day River. All *O. mykiss* in the John Day River are the result of natural reproduction with minimal influences from fish of hatchery origin. This relatively unique characteristic of the John Day River makes it an ideal laboratory for understanding natural processes without the confounding influences of hatchery fish. Anadromous individuals are protected as a threatened species under the U.S. Endangered Species Act (Busby et al. 1996). Other salmonine species in the basin include Chinook salmon (*O. tshawytscha*), westslope cutthroat trout (*O. clarkii lewisi*), bull trout (*Salvelinus confluentus*), and introduced brook trout (*S. fontinalis*). To reduce confusion, hereafter "*O. mykiss*" is used to refer to the species as a whole and the common names

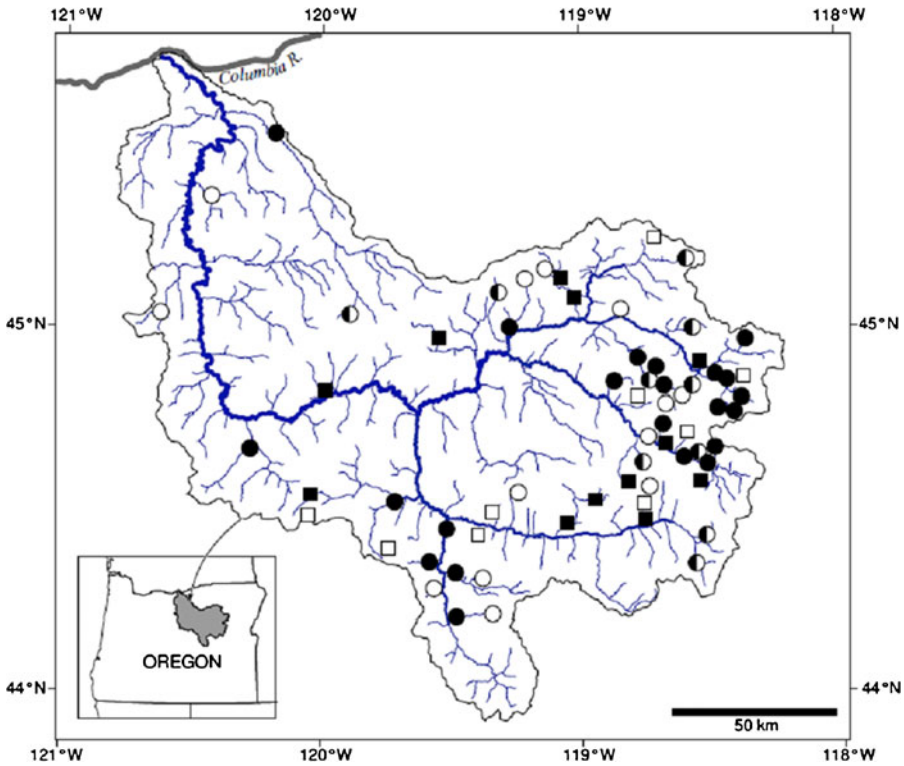

Fig. 1 Fish collection locations and maternal types identified for juvenile *O. mykiss* at study sites in the John Day River in 2007. Sites where two or more juveniles were sampled are indicated with circles (○/●); those where only one juvenile was sampled are indicated with squares (□/■). Hollow markers (○/□) indicate sites where all juveniles were the offspring of rainbow trout, solid markers (●/■) indicate those with all steelhead offspring, and mixed markers indicate at least one offspring of each type (◑)

are used for the migratory life histories: steelhead for anadromous individuals and rainbow trout for resident individuals (Nelson et al. 2004).

Study design

It is increasingly clear that "real world" constraints in applied ecology do not lend themselves to conventional study designs or methods of analysis (Miao et al. 2009). Such constraints do not justify inappropriate study designs or statistical inferences. Rather the challenge is to explore the validity of new approaches that can be applied in real situations. This was the perspective we adopted in designing this study.

We faced two practical constraints in this work. First, the only reliable method available to determine anadromy required sacrificial sampling. Given that anadromous individuals (steelhead) are listed as a threatened, the number of sacrificial samples we could collect was very limited. Second, we needed to develop a predictive model of anadromy in *O. mykiss* that could apply across the vast landscape of the John Day River. It was not possible to simultaneously remedy these conflicting constraints with a conventional approach—for example, simply increasing numbers of individuals sampled at specific locations would limit the number of locations that could be sampled.

After consideration of the alternatives, we elected to sample just two individuals in each location, which allowed us to maximize spatial representation (many locations) across the vast landscape drained by the John Day River. An obvious limitation in such an approach is that our characterization of anadromy at a given location is uncertain, due to imperfect detectability of anadromous individuals. We addressed this issue in two ways. First, we applied a novel test to detect non-random clustering of individuals in each location to identify the probability that both sampled individuals represented the same life history. Strong clustering of individuals would suggest greater detectability of different life histories, based on the limited sampling we were able to conduct. Second, we developed a simple predictive model of anadromy based on samples of single individuals collected at all locations. Predictions from this model were applied to predict the probability of anadromous origin for independently sampled individuals collected from the same locations. If predictions from the original model were transferable to new individuals, our

confidence in the validity of model predictions was improved and concerns regarding our limited ability to sample large numbers of individuals at a given location were reduced.

Our objective was not to develop a detailed model of multiple factors that could be associated with expression of anadromy. Rather, we wished to develop a useful predictive model based on a surrogate for multiple environmental gradients that exist within the John Day River. We used stream size as this surrogate, based on its fundamental influences on key ecosystem processes (Vannote et al. 1980). This work represents a first step toward understanding spatial variation in the expression of anadromy in *O. mykiss*, as well as an evaluation of the efficacy of the novel approaches we developed to gain such insights.

Data collection

We characterized occurrence of anadromy by determining the maternal origins of juvenile *O. mykiss* as an indicator of the presence of spawning steelhead (i.e., identifying individuals with anadromous mothers). Maternal origin was determined via otolith microchemistry. Otolith microchemistry provides a means for determining the maternal origin of individuals through comparing the ratio of strontium (Sr) to calcium (Ca) within different regions of their otoliths (Kalish 1990; Volk 1999; Zimmerman and Reeves 2002). Otoliths are primarily composed of calcium carbonate ($CaCO_3$), and Sr is substituted for Ca relative to the concentration in the environment (Kalish 1990; Zimmerman 2005). Freshwater Sr concentration tends to be much lower than seawater. By comparing Sr to Ca ratios in otolith primordia and subsequent freshwater growth regions it is possible to assign individuals to mothers that resided in marine or freshwater environments. This is because otolith primordia begin forming during the egg stage and strongly reflect the Sr:Ca ratio of the maternal environment during formation of yolk precursors (Kalish 1990). Zimmerman and Reeves (2002) successfully used this method to determine maternal life-history of juvenile *O. mykiss* progeny of both anadromous and non-anadromous females in the Deschutes River, Oregon.

We focused our sampling at sites throughout the study area used for monitoring of steelhead by the State of Oregon (J. Ruzycki, Oregon Department of Fish and Wildlife, pers. comm.). These sites (Fig. 1) were selected using generalized random tessellation stratified sampling (Stevens and Olsen 2004). This sampling process uses hierarchical randomization to produce a spatially balanced equal probability sample, optimized for efficient environmental sampling. Our sampling frame included only streams appearing on a 1:100 000-scale digital streams layer that were accessible to anadromous fish and presumed to be suitable for use by *O. mykiss* by local fisheries biologists (Lloyd and Bowers 2002, 2004). We did not sample stream reaches known to have populations of cutthroat trout due to the difficulty of differentiating their juveniles from *O. mykiss* juveniles (Pollard et al. 1997).

Whenever possible we collected two fish by electrofishing at each site between June–September 2007, one between 50 and 75 mm fork length (FL) and the other between 75 and 100 mm FL. The separate size ranges were collected to reduce the chance of sampling siblings and to avoid bias due to variable spawn timing (Zimmerman and Reeves 2000) or growth rate (Chernoff and Curry 2007) related to maternal origin (anadromous vs. non-anadromous mothers). If either size was unavailable after 30–45 min of sampling, we substituted one from the other size range, or a fish over 100 mm FL if no others were captured. We killed each fish with an overdose of tricaine methanesulfonate (MS-222) and removed both sagittal otoliths, storing them dry in polyethylene vials until returning to the lab, where the vials were filled with ethanol (95% EtOH), We collected a maximum of two fish per location to avoid impacts to threatened steelhead.

Otoliths were prepared following methods in Zimmerman and Reeves (2000) and Donohoe et al. (2008). We cleaned and mounted at least one otolith from each fish, with the sulcus side facing up. Otoliths were mounted on glass microscope slides, or a cover slip attached to a slide using Crystal Bond 509 thermoplastic resin (Structure Probe, Inc, West Chester, Pennsylvania[1]). Mounted otoliths were ground on 1200 and 2000 grit aluminum-oxide sandpaper to the level of the primordia and subsequently polished using a slurry of 0.05 μm alumina

[1] Use of trade or firm names is for reader information only and does not constitute endorsement of any product or service by the U.S. Government

paste in deionized water. After polishing, each fish was aged by examination of otolith banding. The age of each fish was determined by counting alternating translucent and opaque regions. Under reflected light, annuli correspond to the translucent zone (Kalish et al. 1995). Groups of polished otoliths were mounted on petrographic slides for microprobe analysis following the methods of Donohoe and Zimmerman (2010). Each slide was washed with deionized water and mild detergent before air drying and coating with a 40 nm carbon layer.

Elemental analysis was conducted with a Cameca SX-100 wavelength dispersive electron microprobe (Cameca SAS, Gennevilliers, France). For all analyses, the incident electron beam had an accelerating voltage of 15 kV, a 50 nA current, and a 10 μm beam diameter. We measured Sr and Ca simultaneously for all otoliths, using strontianite ($SrCO_3$—USNM R10065) and calcite ($CaCO_3$—USNM 136321) as standards, respectively. Sr was measured using a large TAP (Thallium Acid Phthalate) and regular TAP crystal simultaneously; Ca was measured with a large PET (Pentaerythritol 002) crystal. Counting time for the first 42 otoliths was 60 s on peak, 60 s on background, and 40 s on peak, 40 s on background for the remainder. The average lower detection limit for the 60 s counting time for Sr was 140 ppm, and 171 ppm for 40 s. On these otoliths, we probed three (on the 60 s otoliths) or four points (on the 40 s otoliths) in the maternal growth region near the primordia, and an equal number of points in the freshwater growth region along a roughly proximal transect. Probed points with total weight percent below 90% or above 110% were discarded.

Some freshwater areas have high Sr:Ca ratios that may obscure maternal origin (Rieman et al. 1994; Donohoe et al. 2008). To avoid misidentification of maternal origin resulting from unknown freshwater microchemistry, we tested Sr:Ca ratios in water samples from juvenile sampling sites. We collected water samples at all sample sites except those with restricted access and those directly upstream of another site where water was collected, with no major tributaries in between. Similar to the methods of Wells et al. (2003), we collected two water samples and vacuum-filtered them with 0.45-μm polyethersulfone membranes pre-washed with deionized water, then stored 125 ml of each sample in acid-rinsed HDPE (high density polyethylene) bottles pre-preserved with 1 ml ultrapure nitric acid. After transporting samples from the field, we stored samples in a 40° cold room until analysis with a Varian AA240FS flame atomic absorption spectroscope (Varian, Inc, Palo Alto, California). We calculated mean ionic concentration and molar ratios of Sr:Ca for each site, and compared the results for each site to an anadromy detection threshold of 4.75 mmol/mol: roughly half the Sr:Ca ratio in the Pacific Ocean (de Villiers 1999; Zimmerman 2005; Donohoe et al. 2008). We did not use fish samples from sites with water Sr:Ca ratios over this threshold.

Determination of maternal origin

We assigned maternal origin to each fish by comparing Sr:Ca ratios in the maternal and freshwater growth regions of each otolith. A fish was determined to be of anadromous maternal origin if the Sr:Ca ratio in the maternal growth region was significantly higher than in the freshwater growth region, based on a one-tailed *t*-test with $\alpha=0.05$ (Zimmerman and Reeves 2002). To the full extent possible we used juveniles in their first or second summer (age 0+ or 1+, respectively) after emergence to avoid potential bias from out-migrating smolts and the effects of within-basin movement of juveniles (Tattam 2006).

Data analysis

We first tested for non-random distribution of individuals with different maternal origins within sites, to evaluate the degree to which juveniles with similar life histories were clustered within samples. Then, we divided our sample data into two sets by randomly drawing individuals from all sites where more than one individual was collected. With these data we developed a predictive model of the relationship between stream size and the probability of anadromy at each site. Finally, we tested for spatial autocorrelation to determine if the variability remaining in the model residuals was related to broad-scale gradients unrelated to stream size or more likely a result of local conditions.

Tests for non-random distribution of life histories

With only two individuals sampled at each site (to minimize the number of sacrificial samples), we could not estimate proportions of juveniles of anadromous

versus freshwater maternal origin. Given that the fecundity of an anadromous female exceeds that of a freshwater resident female by an order of magnitude (Jonsson and Jonsson 1993), we assumed that sites where steelhead females had spawned would harbor a relatively large number of their offspring relative to those from rainbow trout females. This also assumes that numbers of steelhead and rainbow trout females are not drastically different (e.g., a large number of rainbow trout females could equal the production of offspring by only a few anadromous females). Under these assumptions, we expected that samples of two juveniles would be more likely to contain two steelhead progeny where steelhead females were present. Accordingly, we expected that relatively few sites would harbor a mix of rainbow trout and steelhead offspring (one of each in our sample of two). Alternatively, if rainbow trout and steelhead females were spatially segregated, we expected a similar pattern: clustering of steelhead and rainbow trout offspring sampled at sites. If neither of these processes was important, we expected to find random associations or lack of clustering between juveniles with different maternal origins. We tested for non-random distribution of juveniles with contrasting maternal origins within sites by using a χ^2 test of independence (Table 1).

Prediction of anadromy To relate occurrence of steelhead to broad-scale environmental gradients, we developed a logistic regression model to predict the probability of individuals with anadromous maternal origin in relation to stream size. We tested model accuracy with several cross-validation techniques. Stream size, indicated for this study by mean annual runoff (m^3/s), is an indicator representing a variety of associated environmental gradients, described below. We estimated mean annual stream runoff (m^3/s) for the 1:100 000-scale NHDPlus (Anonymous 2005) reach containing each sampling site. Because measured stream runoff information was not available for most sites, we used a regression model for variable flow regime streams developed by Sanborn and Bledsoe (2006). This model estimates runoff based on basin area contributing to the sampling site stream reach (km^2) and area-weighted mean annual precipitation (mm) across the basin (Anonymous 2006b).

For the purposes of model development and evaluation we divided the fish data into a fitting data set—containing one fish drawn randomly from each site—and a validation data set containing the remaining fish from each site. At sites with only one fish available, that fish was only included in the fitting data set. To predict probability of anadromy among sites in relation to stream size (as indicated by mean annual runoff), we developed a logistic regression model and fit it with the fitting data set. Natural $\log_e$ transformation of mean annual runoff resulted in the best fitting models (using the score statistic; Hosmer and Lemeshow 2000). Unless further specified, all further reference to this variable refers to the transformed value. We tested variable significance with Wald's X^2, and model significance with the likelihood ratio chi-square, using α=0.05 for both tests.

The models were evaluated for sensitivity and specificity relative to their ability to make predictions about presence or absence of anadromy. It is important at this point to clarify that we could not interpret absence of anadromy as presence of residents, due to asymmetries in probability of sampling residents described above (see Tests for non-random distribution of life histories above). To evaluate model predictive performance, we used receiver-operating characteristic (ROC) plots and area-under-the-curve (AUC) analysis with a 10,000-iteration bootstrapping algorithm to estimate 95% confidence intervals for the AUC (Gönen 2007). To aid in predicting the occurrence of anadromy, we also determined the classification threshold for each model that maxi-

Table 1 Calculations used to conduct $\chi 2$ test for clustering of juvenile *O. mykiss* maternal life histories within sites in the John Day River in 2007. Only sites with two juveniles >2 years old were included. The observed number of sites where both individuals were rainbow trout or steelhead progeny is represented by RbtRbt or StSt, respectively, and the number of sites where one individual was each type is represented by RbtSt. The expected proportions of each combination under the null hypothesis of no association were calculated from the observed proportions of rainbow trout and steelhead progeny across these sites (Rbt and St, respectively), where n is the number of sites. The approach here is analogous to testing for independence of genotypes in genetic data (Hardy-Weinberg Equilibrium; Hartl and Clark 2007)

Combination	Observed	Expected
Same maternal type	RbtRbt + StSt	$(Rbt^2 + St^2) \times n$
Different maternal type	RbtSt	$2 \times Rbt \times St \times n$

mized kappa (Manel et al. 2001; Freeman and Moisen 2008).

Spatial variability of predictions Unexplained or residual variability in the distribution of anadromy remained after our model analysis, so we tested for spatial autocorrelation of model residuals as an indicator of spatial dependence remaining after the influence of stream size was accounted for (Legendre 1993). If we omitted influential broad-scale landscape variables that were unrelated to stream size from the model, spatial autocorrelation could result from the effects of these underlying spatial gradients in conditions among sites. This result would also indicate non-independence among observations, which would violate a key assumption of logistic regression and result in inflated model degrees of freedom. Lack of spatial autocorrelation would support the assumption of independence and suggest that variability in local conditions is responsible for model residuals.

We conducted Mantel tests to determine if model residuals were spatially autocorrelated. First, we calculated pairwise Euclidean geographic distances between sampling sites using the ArcGIS Spatial Analyst extension, and pairwise stream network distances using the 1:100 000-scale medium-resolution National Hydrography Dataset (NHD). We used the PASSaGE software package (Rosenberg 2005) to generate distance matrices for model residuals and perform Mantel tests. We used both Euclidean distances and pairwise stream network distances because both could indicate underlying gradients that directly or indirectly influence the expression of anadromy. For example, geology, terrestrial vegetation, topography, or climate could be associated more closely with Euclidean than stream network distances. Alternatively, stream network distances should be more strongly associated with connectivity of locations within streams due to transport of materials within streams or fish movement.

Significance for Mantel tests was determined using a permutation test that held one matrix constant while randomly reordering the other, then calculating the correlation coefficient. This was repeated 9999 times for each model being tested and the correlation coefficient for the measured data was compared to the null distribution to determine significance (α=0.05, one-tailed). A significant result would indicate the model residuals were spatially autocorrelated in relation to Euclidian or stream network distance.

Results

Determination of maternal origin

We determined maternal origin for 149 juveniles at 72 sites (Fig. 1). The sites we sampled were almost entirely on federal lands, due to limited access to private land. Because of this constraint, we collected few samples from the lower mainstem portions of the river basin near the mouth.

Ninety-one (60.3%) *O. mykiss collected* were steelhead progeny and 58 (39.7%) were rainbow trout progeny. Steelhead progeny occurred at 52 of 72 sites. Overall, most juveniles were <2 years old (Table 2), but at three sites we collected only fish >2 years old, so we excluded those sites from further analysis. There was clear separation in the distribution of fork length between ages (Table 2). The proportion of steelhead progeny was not different between ages (for fish under 3 years old Pearson χ^2=0.86, *df*=2, *P*=0.65).

The mean water Sr:Ca ratio across all sampling sites (n=64) was 2.58 mmol/mol (95% CI 2.36, 2.81). Increasing water Sr:Ca ratio was correlated with increasing longitude (Spearman's ρ=0.47, n=116, P=0.34). One site (#115, on Hideaway Creek) had a mean Sr:Ca ratio of 5.61, high enough to warrant exclusion of otolith microchemistry results from this site (even though Sr concentration was below reliable detection limits).

Tests for non-random distribution of life histories When considering only sites with two juveniles <2 years old (n=47 sites), the maternal life histories of both juveniles tended to be same more often than expected from the overall proportion of maternal origins (Table 3; χ^2=11.15, df=1, P<0.001). Both juveniles had the same maternal life histories at 36 (77%) of these sites.

Prediction of anadromy Increasing stream size was associated with increasing probability of anadromy (Fig. 2). The logistic regression model using stream size correctly classified 78% of the observations of anadromy and non-anadromy in the fitting data set

Table 2 Count, mean lengths (mm; with 95% confidence intervals), and proportion of steelhead progeny (P_a) among all juveniles analyzed (n=149) from the John Day River in 2007

Age	n	Mean length (mm)	95% CI		P_a	95% CI	
			Lower	Upper		Lower	Upper
0	48	56.1	53.6	58.5	0.69	0.55	0.80
1	74	79.4	76.4	82.3	0.55	0.44	0.66
2	24	94.0	88.6	99.4	0.63	0.43	0.79
3	3	85.7	45.7	125.6	0.67	0.20	0.94

(n=69; Table 4) and 68% of the validation data set observations (n=47; Table 5) with a probability cutoff of 0.50. At this probability threshold, the model correctly classified 32 of 40 observations of anadromy (80%) and 21 of 29 observations of non-anadromy (72%) for the fitting data. The optimal threshold for the fitting data (0.51, K=0.56) was substantially lower than that of the validation data (0.62, K=0. 32). There was no correlation between absolute value of model residuals and fish length (Spearman's ρ=0.09, n=116, P=0.34) or fish age (Spearman's ρ=0.058, n=116, P=0.54). The ROC area-under-the-curve (AUC) score was high for the fitting data set (0.82; 95% confidence interval 0.71, 0.91), but moderate for the validation data set (0.69; 95% CI 0.53, 0.84).

Spatial variability of predictions Mantel tests with Euclidian and stream network measures of pairwise geographic distances indicated that model residuals were not spatially autocorrelated among sites (Table 6). Plots of pairwise model residual distances in relation to geographic distances showed no obvious spatial patterns. Thus, the stream size gradient included in the predictive model accounted for the broad-scale variation in the expression of anadromy. However, there was remaining unexplained variability, pointing to the potential importance of local factors influencing expression of anadromy.

Table 3 Count (proportions) of combinations of juvenile *O. mykiss* maternal life histories at sites (n=47 sites) in the John Day River with two juveniles <2 years old collected in 2007

Combination	Observed	Expected
Same maternal type	36 (0.77)	24.6 (0.52)
Both steelhead	23 (0.49)	17.3 (0.37)
Both rainbow trout	13 (0.28)	7.3 (0.15)
Different maternal type	11 (0.23)	22.4 (0.48)

Discussion

Our sampling approach was intended to characterize female anadromy across a broad spatial range while minimizing sacrificial sampling of a threatened species. By foregoing more intensive sampling at each site, we were able use a relatively small total sample size to develop a robust predictive model across a broad spatial extent encompassing large environmental gradients. Our intent was to describe and predict the occurrence of anadromy, rather than to predict the prevalence of steelhead and rainbow trout at each site. Estimated proportions of steelhead offspring are unlikely to correspond to the proportion of steelhead females supported by the site because fecundity of large steelhead may be an order of magnitude greater than small rainbow trout (Jonsson and Jonsson 1993).

Maternal life history

Chemical analysis of ambient freshwater Sr:Ca ratios supported broad-scale use of otolith Sr:Ca ratios to

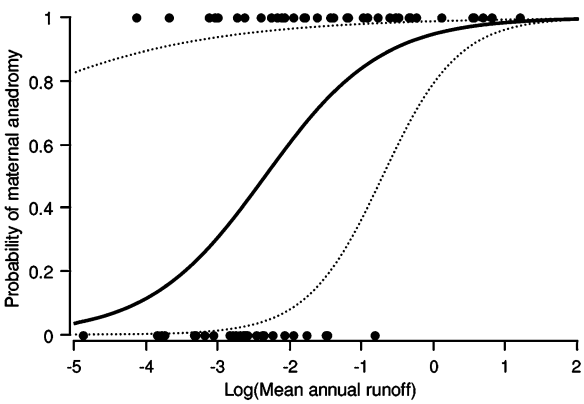

Fig. 2 Probability of anadromy versus stream size (represented by $\log_e$-transformed mean annual runoff (m^3/s)) for *O. mykiss* sampled in the John Day River basin in 2007. Dashed lines indicate upper and lower 95% confidence bounds (see Table 4)

Table 4 Results from logistic regression analysis of the effects of mean annual runoff on the probability of maternal anadromy for fish in the fitting data set (n=69).

Predictor	β	SE	95% CI (Wald's)		Wald's χ^2	df	P	Odds ratio	95% CI (Wald's)	
			Lower	Upper					Lower	Upper
Intercept	2.90	0.79	1.34	4.46	13.34	1	0.0003			
Mean annual runoff	1.24	0.33	0.58	1.90	13.71	1	0.0002	3.45	1.79	6.66

identify maternal origin of individuals. The strong spatial gradient of increasing Sr:Ca concentration with increasing longitude likely reflects underlying geologic gradients, a key concern when we began our sampling effort. However, only one site in our study was rejected for having a Sr:Ca ratio likely to mask maternal origin, and we experienced little difficulty in accurately identifying the offspring of stream-maturing fish. Other studies observed similar spatial variability in freshwater Sr:Ca ratios (Rieman et al. 1994; Wells et al. 2003; Donohoe et al. 2008).

Relatively few sites yielded a mix of steelhead and rainbow trout offspring, suggesting steelhead offspring numerically dominated the sites where they were present or that there was spatial separation between steelhead and rainbow trout spawning. It is difficult to identify which scenario is more plausible without further evidence. In either case, our small sample may not have detected anadromy at all sites where steelhead progeny were present. However, we were still able to estimate the relative odds of occurrence of anadromy within our sample (Johnson et al. 2006).

The relationship between maternal origin of juveniles and their subsequent migratory behavior (Tattam 2006) is unclear, but by sampling fish below 100 mm and focusing on 0+ and 1+ age fish, we minimized the bias associated with potential emigration of 2+ fish as steelhead smolts from our sampled locations. Similarly, we found no bias toward or against anadromous origin associated with fish size.

When analyzing the relationship between anadromy and environmental variability, we assumed the juveniles were captured within the stream reach where their mothers spawned. Whereas some *O. mykiss* may migrate long distances in freshwater (Tattam 2006), there is no evidence available to suggest the <2 year old juveniles used in this study would do so (see also Kahler et al. 2001). Juvenile density is correlated to redd density in both *S. salar* (Beland 1996) and *S. trutta* (Beard and Carline 1991), indicating limited movement from natal areas is a reasonable assumption. During the summer juveniles may move frequently within a stream reach, but they are unlikely to move more than a few hundred meters (Kahler et al. 2001). This limited range of movement is quite small relative to the vast extent of our study area (Fig. 1). Therefore, we conclude that movement of juvenile *O. mykiss* is unlikely to affect our analysis of the distribution of anadromy, and that sampling bias by size or age is probably negligible. If it was true that young juveniles moved extensively over long distances within the John Day River, our findings would represent patterns of habitat use or selection by juveniles, rather than the distribution of their mothers (steelhead or rainbow trout). Given the evidence available regarding movements of juvenile *O. mykiss* in the John Day River (Tattam 2006), this seems less likely, but this alternative interpretation is a possibility.

Table 5 Results from logistic regression analysis of the effects of mean annual runoff on the probability of maternal anadromy for juvenile *O. mykiss* in the validation data set (n=47)

Predictor	β	SE	95% CI (Wald's)		Wald's χ^2	df	P	Odds ratio	95% CI (Wald's)	
			Lower	Upper					Lower	Upper
Intercept	1.57	0.63	0.33	2.82	6.17	1	0.013			
Mean annual runoff	0.56	0.26	0.04	1.08	4.45	1	0.035	1.75	1.04	2.94

Table 6 Mantel test matrix correlations of pairwise differences in model residuals in relation to two measures of between-site distance. Probabilities are for a one-tailed test (to test alternative hypothesis that geographically proximate sites are more similar)

	Stream network distance		Euclidean distance	
Data set	*r*	*P*	*r*	*P*
Fitting data	0.02	0.31	−0.03	0.71
Validation data	0.01	0.38	−0.01	0.58

Predicting anadromy

Female anadromy was predictable across the study area, and we found no evidence of serious bias from assignment of maternal origin, juvenile movement, or sampling that could be attributed to our approach. However, it is important to clarify that model predictions should be interpreted as indicating the relative odds of anadromy. Due to the chance that we failed to detect anadromous individuals when indeed they were present at some sites, the absolute probability of anadromy overall is likely underestimated and thus we can only make inferences about relative odds (Johnson et al. 2006). For example, at sites where the two sampled juveniles were of different maternal origin, only one data set indicated anadromy at a site, thus the other set is known to under-represent anadromy. Furthermore, we cannot infer anything about the relative odds or absolute probability of occurrence of freshwater resident individuals (McMillan et al. 2011). These issues likely contributed to the variable model performance between the fitting and validation data, as these mixed sites were randomly distributed among the data sets. Regardless, the model explained much of the spatial variability in expression of anadromy and provides a useful picture of the occurrence of this life history across a large stream network.

Processes affecting anadromy

Based on work with other salmonines (e.g., Jonsson and Jonsson 1993), it is likely that expression of anadromy in *O. mykiss* in our system is driven by environmental variation that is manifested across a range of spatial and temporal scales. Our model based on stream size used data from a short temporal scale: sampling within 1 year and confined largely to two juvenile cohorts. Furthermore, whereas stream size was a useful predictor of anadromy, the exact processes associated with stream size, and ultimately anadromy, remain unclear.

Stream size is an indicator of a range of ecological and physical processes (Vannote et al. 1980). It is often associated with gradients of hydrologic and biological organization, but is only loosely linked to the physiological processes governing migration. For example, large fish are less vulnerable to predation in large streams and deep pools (Power 1984; Quinn and Kinnison 1999), so large anadromous fish may avoid spawning in small or shallow streams. Furthermore, stream discharge can be used to directly estimate sediment transport (Barry et al. 2004), which is a key component of stream habitat disturbance regimes and development of in-stream habitat (Reice et al. 1990; Reeves et al. 1995). Patterns of scour and sediment transport may govern broad-scale distribution of steelhead spawning (Montgomery et al. 1999), providing a simple process-based explanation to the relative effectiveness of our model. In addition, gradients of stream size may also match gradients in water temperature regime (Torgersen et al. 1999). This has direct bioenergetic consequences for growth, survival, and smolting (Thorpe and Metcalfe 1998; Tattam 2006; McMillan et al. 2011), as well as affecting how fish communities respond to local geomorphology (Torgersen et al. 2006).

Broad-scale environmental gradients were predictably associated with the expression of anadromy, but we also found evidence to suggest the importance of local influences. Our analysis of spatial autocorrelation of the residual variability in anadromy after removing the influence of stream size provided no additional evidence of the influence of spatial gradients, leaving the possibility that localized conditions may play an important role. Within localities, it is likely that variability in thermal conditions, food availability, and population density could be important (Tattam 2006; McMillan et al. 2011). For example, brown trout given restricted food rations develop migratory morphology more frequently than those on a more liberal diet (Olsson et al. 2006; Wysujack et al. 2009). Brown trout in high density stream segments migrated at a much higher rate than those in segments with low population density (Olsson and Greenberg 2004). Ecological processes and species responses to them are scale-dependent, and it is impossible to fully

understand and describe these relationships without multi-scale sampling and modeling beyond the scope of this study (Boyce 2006).

Conclusions

Our results show that female anadromy occurred widely across the John Day River basin, and that variation in stream flow predicted much of the spatial pattern of female anadromy. Our analysis of otolith and water microchemistry successfully identified maternal life histories at almost all sites. Within sites, both juveniles had the same maternal origin more frequently than expected, though we were not able to estimate the proportion of steelhead or rainbow trout progeny overall. A logistic regression model predicting anadromy based on stream size showed moderate accuracy as indicated by cross-validation. Lack of spatial autocorrelation among model residuals indicated the simple stream size model explained most variability in anadromy at the broad spatial scale we sampled, implying remaining variation is due to local conditions and associated influences.

In spite of the practical and unavoidable constraints we faced in designing this study, we were able to produce useful insights into quantifying patterns of anadromy in *O. mykiss* within the John Day River. Each step of the analysis included an evaluation of the validity of the various approaches employed, ranging from determination of otolith microchemistry to predicting landscape patterns of occurrence. When possible, we recommend that more rigorous approaches should be used (e.g., greater sample sizes, more years of study, etc.), but in the present case, these alternatives were simply unavailable. This is certainly the case for many imperiled fishes and we hope this study can provide a useful example.

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