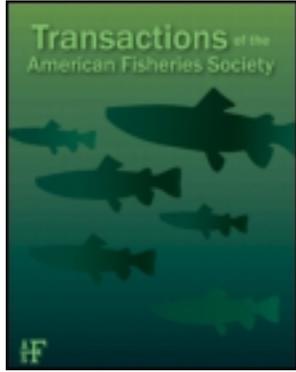


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Parental Effect as a Primary Factor Limiting Egg-to-Fry Survival of Spring Chinook Salmon in the Upper Yakima River Basin

Christopher L. Johnson^a, Philip Roni^b & George R. Pess^b

^a Washington Department of Fish and Wildlife, 600 Capitol Way North, Olympia, Washington, 98501, USA

^b National Marine Fisheries Service, Northwest Fisheries Science Center, Fish Ecology Division, Watershed Program, 2725 Montlake Boulevard East, Seattle, Washington, 98112, USA

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ARTICLE

Parental Effect as a Primary Factor Limiting Egg-to-Fry Survival of Spring Chinook Salmon in the Upper Yakima River Basin

Christopher L. Johnson*

Washington Department of Fish and Wildlife, 600 Capitol Way North, Olympia, Washington 98501, USA

Philip Roni and George R. Pess

National Marine Fisheries Service, Northwest Fisheries Science Center, Fish Ecology Division, Watershed Program, 2725 Montlake Boulevard East, Seattle, Washington 98112, USA

Abstract

Few field estimates of egg-to-fry survival of Chinook salmon *Oncorhynchus tshawytscha* exist, although it is one of the major factors thought to limit freshwater production and recovery of Chinook salmon populations. This is likely due to the challenges of estimating survival at this life stage, which is further complicated by the variety of methods that have been employed. Our study objectives were to (1) develop a method by which spring Chinook salmon egg-to-fry survival could be estimated at a large spatial scale, and (2) investigate the primary factors affecting survival in the natural environment. We conducted a field experiment using 81 artificial redds to test our proposed method for evaluating egg-to-fry survival at a basin scale and to evaluate the effects of parentage (adult mating), river reach, and fine sediment infiltration on survival in the upper Yakima River basin, Washington. Egg-to-fry survival and preemergent Chinook salmon fry developmental stage were significantly different among matings, but were not detectably different among reaches. Fine sediment accumulation in egg boxes from artificial redds was largely below published threshold levels, explained less than 6% of the variation in survival, and was not correlated with developmental stage. In contrast, survival of individual matings in the natural environment and those same matings incubated under controlled hatchery conditions were highly correlated. Our study suggests that in years of low scour and potentially ideal incubation conditions, parental effects play an important role in determining in situ egg-to-fry survival, and that extensive replication and tracking of gamete viability is needed to separate parental effects from environmental factors affecting survival. We provide standardized methods for collecting egg-to-fry survival data and outline a number of potential biases that should be addressed in future research.

Egg-to-fry survival is one of the major factors thought to limit freshwater production and recovery of Chinook salmon *Oncorhynchus tshawytscha* and other salmon populations (Reiser and White 1988; Peterson and Quinn 1996; DeVries 1997). Despite being critical data that is needed to assist with efforts to recover threatened and endangered salmonid populations, adequate estimates of egg-to-fry survival for Chinook salmon do not currently exist (Healey 1991; Bradford 1995). This lack of information is likely due to the difficulty in measuring Chinook salmon and other salmonid egg-to-fry survival in

the field because they deposit their eggs in the gravel during periods that make extensive field research difficult (Quinn 2005). This is particularly true for Chinook salmon, which spawn in large rivers with deeper and swifter water than most other salmon, and for which developing embryos remain in the gravel throughout the winter and early spring when it is difficult to sample (Quinn 2005). Therefore, many of the existing studies of Chinook salmon egg-to-fry survival have occurred in a laboratory setting (Jensen et al. 2009) and may not adequately represent egg-to-fry survival in the natural environment.

*Corresponding author: johnsclj@dfw.wa.gov
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The challenges of producing and comparing estimates of survival at this life stage for Chinook salmon are further complicated by the variety of methods that have been employed. Published field methods include the use of egg boxes, direct planting of gametes, and the capping or excavation of naturally produced redds, each method having different sources and degrees of potential bias (Claire and Phillips 1968; Chapman 1988; Young et al. 1990; Rubin 1995; Reiser et al. 1998). Further, field studies of egg-to-fry survival for both Chinook salmon and other salmonids have almost exclusively been either very intensive studies on one or a few stream reaches and a small number of redds (e.g., Fast et al. 1991; Merz et al. 2004), or very general approaches which compare the total number of adults into a reach with the total number of out-migrants (Healey 1991; Bradford 1995). While such studies have shed some light on the factors limiting survival, they do not allow for comparisons across a basin or among populations. Standardization of protocols is necessary for universally comparable data among researchers and localities (Chilcote 2007), which in turn are necessary to make informed decisions to guide salmon recovery and habitat restoration efforts. As important as the ability to provide comparable estimates of survival is the ability to determine what factors are potentially limiting to the population. This includes the ability to segregate environmental factors (e.g., sedimentation, lack of dissolved oxygen, entombment, scouring of redds) from parental effects, both of which have the potential to limit survival and development of incubating alevin. Differential gamete viability between stocks and individual spawners has long been considered a potential bias when estimating egg-to-fry survival both spatially and temporally (Young et al. 1990; Rubin 1995). More recently, parental effects have been shown to play an important role in both survival and growth, and to vary in magnitude among populations (Heath 1999; Evans 2010). This emphasizes the need to account for potential differences in gamete viability in studies designed to estimate natural egg-to-fry survival.

Numerous environmental factors, including those mentioned above, have been suggested as potentially limiting to Chinook salmon egg-to-fry survival and development. Among these, temperature, fine sediment infiltration, and substrate scour are perhaps most often suggested as the primary factors affecting survival or development (or both) of spring Chinook salmon alevin throughout the incubation period. For instance, temperature has long been cited as a regulating factor in both alevin development and subsequent emergence timing (Alderdice and Velsen 1978; Heming 1982; Beacham and Murray 1990), and both may directly influence postemergence survival (Quinn 2005). Further, decreased survival associated with temperature intolerance has been demonstrated (Murray and McPhail 1988), occurring most frequently in areas such as interior or inland rivers where temperatures drop to near freezing for extended periods (Quinn 2005). Fine sediment infiltration has been shown to limit survival by filling interstitial space between substrate particles and reducing the delivery of dissolved oxygen to developing embryos

(Chapman 1988; Reiser 1998; Greig et al. 2005; cited by Sear et al. 2008), threshold values of approximately 25% percent having been linked to detrimental effects to Chinook salmon survival (Jensen et al. 2009). Lastly, gravel bed disturbance (or "scour"), although often overlooked in the past (Nawa and Frisell 1993), has been suggested more recently as a potentially major factor directly affecting survival of incubating eggs and developing alevin (DeVries 1997, 2008), partially due to a high sensitivity of salmonid populations to variations in scour depth during incubation (Montgomery et al. 1996). Additionally, both gravel scour and deposition have been shown to directly affect fine sediment levels in the egg pocket (Montgomery et al. 1996; DeVries 1997).

We hypothesized that differences in spring Chinook salmon spawning habitat (e.g., temperature, fine sediment infiltration, scour) among reaches of the Upper Yakima River basin would result in observable differences in egg-to-fry survival, alevin development, or both, and that differential survival or alevin development (or both) attributable to parental effects might also be detectable across a variety of spawner habitats in the natural environment. Our study objectives were to (1) develop a method by which spring Chinook salmon egg-to-fry survival could be estimated at a large spatial scale, and (2) investigate the primary factors affecting survival in the natural environment. We provide a standardized method for collecting egg-to-fry survival data and outline a number of potential biases that should be addressed in future research.

METHODS

Study basin.—The Yakima River is a large tributary of the Columbia River in central Washington State with a drainage area of approximately 4,125 km² (USGS 2011; Figure 1). Elevation of the study area, between the downstream and upstream boundaries of Roza Dam and Easton Dam, respectively (Figure 1), ranges from 470 to 640 m above sea level. Basin geology is variable, primarily composed of metamorphic, sedimentary, and intrusive and extrusive igneous rock (Jones et al. 2006). Mean annual precipitation is approximately 69 cm, the majority of which is accumulated as snowfall in the winter months (Vaccaro et al. 2009). The Yakima River is regulated for agricultural irrigation by three primary headwater storage reservoirs: Keechelus, Kachess, and Cle Elum. Flows in the Yakima River generally peak in midsummer, when irrigation demands are high, and then drop dramatically over the course of a few weeks in the early fall. This sizable annual reduction in flow, termed "flip flop," is designed to protect eggs and incubating alevin by forcing redd construction into deeper areas of the channel, thereby decreasing the amount of water necessary to keep redds watered throughout the winter (SOAC 1999). Flow conditions throughout the winter and early spring average approximately 24 m³/s (USBR 2012), increasing only in response to seasonal weather events until flows are again increased in early summer to supply downstream irrigation needs.

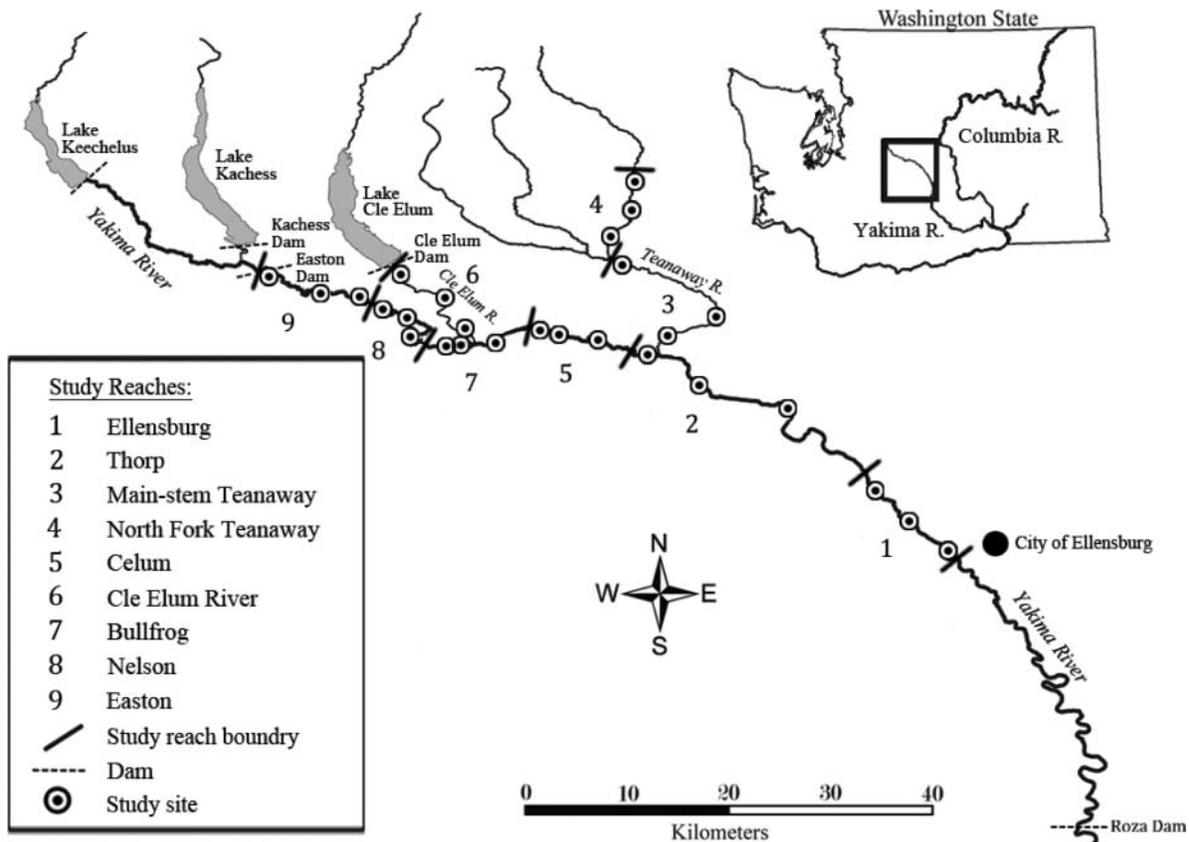


FIGURE 1. Map of the nine contiguous study reaches and three sites per reach in the upper Yakima River basin, 2009. Reach borders are denoted by solid black lines perpendicular to the river channel or the confluence of tributaries with the main-stem Yakima River. Yakima River reaches, beginning with the most downstream, were Ellensburg, Thorp, Celum, Bullfrog, Nelson, and Easton. Tributary reaches were the main-stem Teanaway (MST), the North Fork Teanaway (NFT), and Cle Elum River (CER). Each of the nine study reaches contained upper (most upstream), middle, and lower (farthest downstream) study sites.

Approximately 2,200 (mean = 2,173; SD = 1,099) spring Chinook salmon redds are observed in the upper Yakima River basin annually (2000–2010; YKFP 2010). On average, more than 94% of the annual spring Chinook salmon spawning in the upper Yakima River basin occurs between the town of Ellensburg at river kilometer (rkm) 246, and Easton Dam (~rkm 326), and also in two large tributaries of the Yakima River: the CER and the Teanaway River (Yakima–Klickitat Fisheries Project [YKFP] redd counts 1981–2009, unpublished data; Figure 1). Natural spawning of spring Chinook salmon in the Upper Yakima River is supplemented by the annual release of approximately 700,000 hatchery smolts (Sampson et al. 2010). These smolts are progeny of naturally produced spring Chinook salmon, collected and spawned annually as a part of the YKFP spring Chinook salmon supplementation program.

Study area and design.—The study area consisted of nine total reaches: six contiguous reaches in the main-stem Yakima River, two in the Teanaway River, and one in the CER. Each of the nine reaches contained upper, middle, and lower study sites (Figure 1). Three redds were constructed in each of the study sites over a 3-week period, for a total of 81 artificial redds.

Reach boundaries were based on preexisting Washington Department of Fish and Wildlife (WDFW)–YKFP adult and juvenile survey areas, and also reach morphology (i.e., stream channel gradient and valley confinement). Main-stem Yakima River reaches, beginning downstream at approximately rkm 246.2, were Ellensburg, Thorp, Celum, Bullfrog, Nelson, and Easton. Tributary reaches, beginning at Yakima River rkm 283.2, consisted of MST, NFT, and CER from Yakima River at rkm 299.3 to the base of Cle Elum Dam (Figure 1). A list of study reaches and their respective lengths are provided in Table 1. Channel types were primarily identified as confined or island braided using the Beechie et al. (2006) channel classification criteria (Table 1). Upper Yakima basin redd survey GPS coordinates (A. H. Dittman, National Oceanic and Atmospheric Administration [NOAA], unpublished data) were used to establish areas within each study reach that had been utilized by spring Chinook salmon spawners in the previous year. We then divided each reach into upper, middle, and lower segments, and selected one accessible study site in each of the three segments. In areas where no redds were documented in the previous year, sites were selected in areas where naturally constructed redds had

TABLE 1. Summary of reach characteristics including length (km), channel type (Beechie et al. 2006), spawning substrate size (Wolman pebble counts), and fine-sediment infiltration into redds; D_{50} represents the median particle size or size at which 50% of particles are smaller, D_{84} the size at which 84% of the particles are smaller in size. Fine-sediment infiltration is the percent of total sample that was less than 0.5 or 0.2 mm; ND = no data.

Reach	Length (km)	Channel type	Surface substrate size (mm)				Fine-sediment infiltration (%)					
			D_{50}		D_{84}		<0.5 mm		<0.2 mm		Scour (mm)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Easton	11.7	Island braided	57.3	20.6	101.2	21.2	14.2	11.7	15.5	12.0	0.0	0.0
Nelson	6.8	Island braided	44.8	8.5	80.7	13.9	6.3	4.7	7.9	5.7	0.8	0.7
Bullfrog	12.9	Island braided	61.2	18.7	100.4	36.4	3.9	2.7	4.7	3.2	0.8	0.7
CER	12.9	Island braided	44.8	9.4	77.0	9.5	1.8	1.6	2.5	2.3	0.0	0.0
Celum	11.3	Island braided	48.8	8.3	87.8	15.3	3.2	2.3	4.5	3.2	2.0	1.8
NFT	10.5	Island braided	58.0	9.2	137.3	33.4	14.5	6.1	17.5	7.1	7.2	1.2
MST	19.3	Island braided	61.8	3.2	119.2	18.4	15.7	6.8	18.9	8.3	ND	ND
Thorp	23.3	Confined	54.2	6.0	86.1	1.6	3.8	3.0	4.7	3.3	2.8	2.5
Ellensburg	13.7	Island braided	50.8	5.3	79.5	13.4	7.3	4.7	8.6	5.0	2.8	4.8

been observed in multiple years during WDFW surveys (A. Fritts, WDFW, personal communication). Locations for artificial redd construction in each site were then selected near active, naturally constructed redds. In the absence of active spawning, redds were constructed in areas of the channel in which naturally constructed redds had been documented the previous year. Eggs from a specific mating were placed in either the upper, middle, or lower site of each study reach, each week (Table 2). Three unique matings were made each week over the 3-week study and subsequently classified as matings A through I (Table 2). Due to a shortage of available gametes from one of the available females, an alteration was made in the distribution of gametes in the Teanaway reaches in week 3 (Table 2).

Concurrent research conducted at the Cle Elum Supplementation and Research Facility (CESRF) in Cle Elum, Washington, provided an opportunity to compare survival between eggs incubated in our egg boxes and an independent estimate of survival from naturally spawning adults in a seminatural stream channel. The man-made channel, a 127.0-m-long and 37.9-m-wide

U-shaped structure located on the hatchery grounds, was designed with substrate composition and flow velocities patterned after Chinook salmon spawning preferences (Schroder et al. 2010). As part of the concurrent study, adult Chinook salmon were introduced into the stream channel, where they subsequently constructed redds, selected mates, and spawned naturally. Progeny from these spawning events were then captured in downstream traps following their emergence the next spring, and a portion of these genetically assigned back to their respective parents. A full description of the study design and methods is presented by Schroder et al. (2008). For our comparison, we placed three egg boxes in an unoccupied and isolated, centrally located area of the channel on September 16, 2009. Each box contained fertilized eggs from one of the three September 15 matings. Adults were released into the channel later the same day.

Adult and gamete collection.—Gametes were obtained from the CESRF. Natural-origin spring Chinook salmon adults are collected annually at Roza Dam (rkm 208; Figure 1) for use as broodstock in the YKFP spring Chinook salmon supplementation program (Knudsen et al. 2006; Fast et al. 2008). Adults were collected throughout the run and transported to the CESRF, where they were held until ready to spawn. A small number of hatchery-origin adults were also collected for use in a separate study (Schroder et al. 2010). These adults, being hatchery reared progeny of natural-origin parents, were first-generation hatchery fish. Due to the nature of the supplementation program, progeny from these adults could not be reared and released into the natural environment. Due to their availability, gametes from these adults were utilized for our study, and natural-origin gametes were used only when hatchery-origin gametes were not available. Egg-to-fry survival rates from natural-origin and hatchery-origin adults have been shown to be comparable under hatchery culture conditions (Knudsen et al. 2008). Under

TABLE 2. Within-reach study design by site and stocking week. Each site received eggs from a unique mating weekly (A–C, D–F, and G–H) for the 3-week duration of the stocking period. This allowed equal distribution of a specific cross among all reaches for each stocking event. The total study design was composed of nine reaches, each with three study sites and three artificial redds per site ($n = 81$ artificial redds).

Site	Week 1	Week 2	Week 3 ^a
Upper	A	D	G
Middle	B	E	H
Lower	C	F	I

^aDue to a shortage of available gametes in week 3, adult crosses in the Teanaway reaches were slightly inconsistent with the standardized cross order used in the remainder of the study area; mating H was used in the lower NFT and mating I in the upper MST during the week 3 stocking event.

seminatural conditions, survival rates have been found to be similar between natural-origin and hatchery-origin males (Schroder et al. 2010), but slightly lower (5.6%) in hatchery-origin females when compared with natural-origin spawners (Schroder et al. 2008). However, these differences appear to have been due to adult behavior, morphology, or both rather than gamete viability (Schroder 2008). Based on these findings, we assume any effect on survival due to adult origin is negligible with respect to this study.

Three unique matings were required in each of the three study weeks. Gametes for this study were collected on September 15, 22, and 29, 2009. Eggs were collected from three females each week and individually counted into lots of 100. Eggs and a small amount of ovarian fluid were placed into individual 0.5-L Whirl-Pak bags, filled with oxygen, and kept on ice in a large beverage cooler. Approximately 0.3 mL of milt was also collected from the same number of males each week and stored in individual 0.1-L bags. Moist paper towels were used as a buffer between the gametes and ice while in the cooler. Collected gametes were then held overnight in a walk-in cooler at 5°C. The bags were recharged with fresh oxygen the following morning, sorted by desired mating and stocking order, placed in a smaller cooler with ice, and transported to their respective sampling reaches. In order to help confirm any detectable parental effects in the natural environment, we compared mean survival observed among matings in the natural environment with survival from the same matings held in the hatchery environment. Eggs from each mating were fertilized at the time of gamete collection, placed in individual containers, and incubated under standardized hatchery conditions. Incubation temperatures were adjusted such that the developing embryos collected over the 3-week period would have a similar hatch date. A full description of in-hatchery incubation protocol is presented by Knudsen et al. (2008). Mor-

talities were counted on March 11, 2010, at approximately 953 accumulated thermal units. One of the nine matings was lost due to an unrelated hatchery mishap and was therefore removed from the analysis. We considered a significant correlation between mean survival in the natural environment and survival of in-hatchery groups as supporting evidence that observed differences in the natural environment were attributable to adult spawner fitness.

Redd construction, egg fertilization, and placement.—Preconstruction of redds was necessary to allow the stocking of all 27 sites on the same day. Artificial redds were constructed at each of the Yakima River basin sites on September 14, 21, and 28, 2009, the day before gamete collection. Egg pocket depth was standardized at 30 cm (Figure 2), our estimate of mean Chinook salmon egg burial depth following review of Healey (1991) and DeVries (1997). A covered bottomless bucket was inserted into each artificial redd to prevent backfilling until fertilized eggs could be stocked. The construction of artificial redds and use of modified WV egg incubation boxes (Figure 3) were selected as the most practical methodology given the scale of the study. Egg boxes were first modified by removing the egg tray to increase its effectiveness in evaluating sediment infiltration (Wesche et al. 1989) and to more closely reflect natural conditions. Slots on the top and center of the boxes were then covered with 3.2-mm mesh netting to prevent surviving fry from escaping the enclosure after hatching. A small vial containing a PIT tag was glued to the inside edge of the box, and another was tethered to a 20-cm nylon string to assist in egg box location and recovery (Figure 3). Fertilization of the eggs and box placement occurred on September 16, 23, and 30, 2009. Egg boxes were first filled with gravels that had been collected from the artificial redds and agitated in a perforated bucket to remove fines. Boxes were filled to within approximately 2 cm of the box lid.

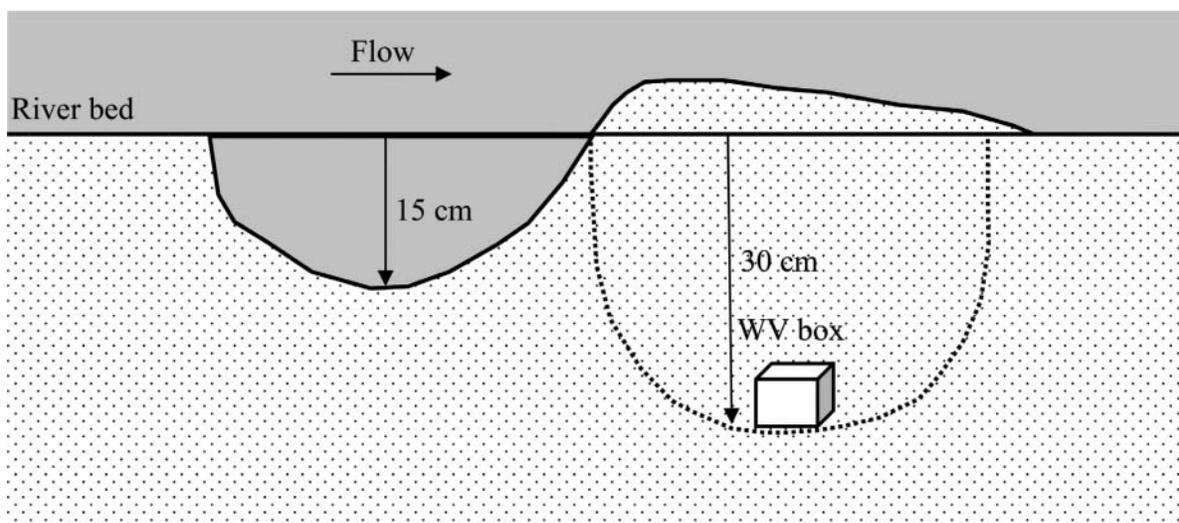


FIGURE 2. Artificial spring Chinook salmon redd bowl and egg pocket depth. Artificial redds were constructed on September 14, 21, and 28, 2009, in the upper Yakima River basin; WV = Whitlock-Vibert.

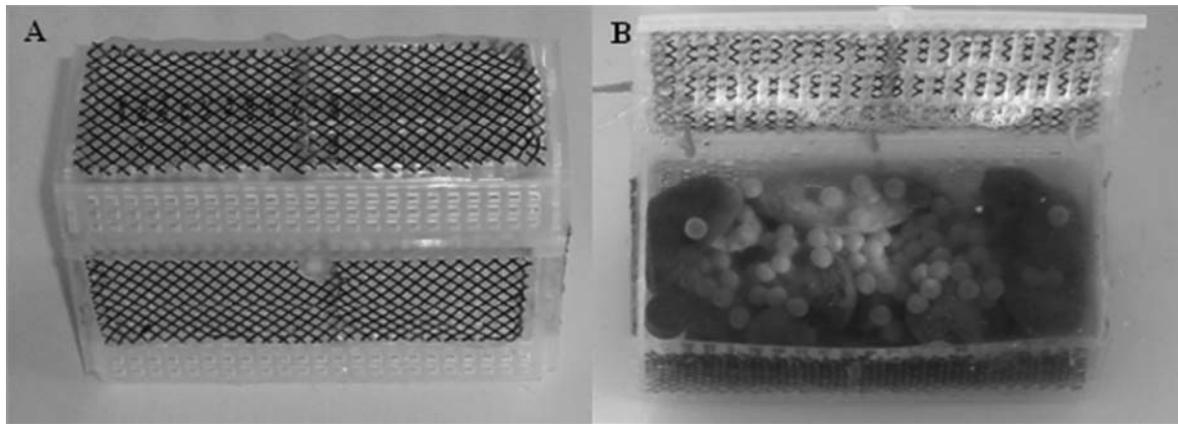


FIGURE 3. (A) Whitlock-Vibert egg box modified by the addition of 3.2-mm mesh netting over areas where fry might escape. (B) The same egg box (submerged in river water) with the top tray removed to allow the addition of gravels from on site. Newly fertilized eggs were allowed to drift into the interstitial spaces of gravels obtained from each artificial redd.

This allowed sufficient interstitial space for the eggs and enough room to prevent the box lid from coming into contact with the eggs when secured. Boxes were then placed in a small, plastic tray filled with river water such that the egg box would be submerged. Due to potential light sensitivity of the gametes (Dey and Damkaer 1990; Flamarique and Harrower 1999), fertilization was conducted in an area shaded from direct sunlight.

Milt was first suspended in a small amount of river water and then poured into the 0.5-L bag containing the eggs. The newly mixed gametes were then set aside for approximately 2 min to allow water hardening (Clark and Hirano 1995) before being gently poured evenly over the gravels of the submerged egg box. Submerging the egg box prior to the addition of fertilized eggs facilitated the consistent distribution of eggs as they were introduced into the egg box. Some difference in the distribution of eggs within the boxes likely occurred due to the size and shape of the gravels collected from the site of the artificial redd. We make the assumption that gravel characteristics within our egg boxes were representative of the study sites. The egg box was then gently transferred to the preconstructed redd and held in place at the bottom of the egg pocket while the bottomless bucket, still in place from the original construction, was filled with the previously excavated and agitated gravels. The bottomless bucket was then removed and the area was excavated or backfilled (or both) as necessary to resemble the bowl and tail-out of a naturally constructed redd (Figure 2). Egg boxes were placed in each artificial redd no more than 2 d following redd construction.

Redd excavation and survival estimates.—Retrieval dates were estimated based on a target developmental stage where 50% of the spring Chinook salmon fry would be expected to have emerged from the gravel. Published values for Chinook salmon average approximately 1,000 accumulated thermal units, measured using the Celsius scale (Alderdice and Velsen 1978; McMichael 2005; Geist 2006). However, in order to decrease

the probability of encountering high-flow conditions during retrieval and of adverse density effects within the boxes, we selected a target value of 900 accumulated thermal units. To monitor the accumulation of thermal units at each of the study sites, temperature loggers were deployed at 23 locations throughout the study area. Temperature loggers were placed in flowing water along the banks, where they could be accessed regularly throughout the incubation period. Correlation equations of daily water temperatures among sites were developed as necessary to aid in forecasting retrieval dates and to provide a secondary method of monitoring in the event that a temperature logger was lost. A buffer of 30 accumulated thermal units was permitted in the recovery protocol to allow for site access, scheduling difficulties, or both.

Egg boxes were recovered between January 6 and June 14, 2010. Artificial redd locations were identified by triangulation from the bank and through the use of a handheld PIT tag detector. Once located, a bottomless polyethylene barrel, with a prow affixed to one side, was placed over the artificial redd to divert flow during excavation. This prevented the loss of fine sediment during recovery and reduced the likelihood of a differential loss of fines among study sites. Egg boxes were visually located using a submersible Aqua Scope and gentle excavation around the area of the egg pocket. Once an egg box was found, gravel was removed from around its perimeter until it could be lifted without excessive agitation. Snorkeling methods were used to locate and recover egg boxes when flows were too high to use the bottomless barrel. In these instances, additional technicians reduced flow by standing in front of the artificial redd until the recovered box was brought to the surface. Snorkeling methods were used in 15 of 81 collection events. All 15 collection events requiring the use of snorkeling methodology were within the two Teanaway River reaches: five in the NFT, and 10 in the MST. Regardless of the extraction method used, all egg boxes were placed directly into a plastic tray while still submerged

to protect the box from direct flow and avoid excess loss of fine sediment to the extent possible. The tray and box were then transported to the bank, where a count was made of the survivors and also any dead eggs or alevin. One recovered egg box was removed from the analysis due to the observation of a detached portion of screening on the egg box, which may have allowed fry to escape the enclosure. Surviving fry were transported live to the laboratory, where length to the nearest millimeter and wet weight to the nearest milligram was measured for each individual. Measurements were made on sacrificed, unpreserved fry 1–2 h following collection in the field. Developmental indices (k_D) were calculated for each of the surviving fry using the equation (Bams 1970)

$$k_D = \frac{10 \cdot \sqrt[3]{\text{Weight in mg}}}{\text{Length in mm}}$$

The Bams equation provided a standardized index of developmental stage, where index values decrease with progressive growth. The index is not reflective of condition but gives insight into the relative stage of development among study reaches.

Spawning substrate and fine sediment infiltration.—Wolman pebble counts (Wolman 1954) were used to characterize surface particle size of spawning gravels at each site. Spawning gravels were compared by median particle size (D_{50}) and the size at which 84% of the particles are smaller (D_{84} ; Kondolf et al. 2008). Fine-sediment infiltration into artificial redds was estimated from the egg boxes following excavation of artificial redds. Sediment which had accumulated in the egg boxes was dried at 80°C for 24 h. Dried samples were then sieved into 4,000-; 2,000-; 500-; 250-; 125-; and 63- μm size categories and weighed to the nearest 0.01 g. Particles less than 2,000 μm in size were considered fines (Lisle 1989; Fudge et al. 2008). Scour chains following the design presented by Nawa and Frisnel (1993) were installed at each of the study sites, one per site, in close proximity to the artificial redds. The scour chains consisted of a 0.8-m length of cable threaded through 50 plastic beads, each 12 mm in diameter. The cable was driven into the substrate using a handheld fence post driver until the last of the beads was within the top layer of the gravel bed substrate. In instances where the cable could not be driven to the desired depth, the number of exposed beads was recorded. As scour occurred throughout the deployment period, beads were exposed to the current and pushed to the end of the cable. Upon retrieval of the egg boxes, the scour chain was located and the number of beads that had slid to the end of the cable was recorded. Due to high-flow conditions at the time of egg box recovery, primarily in the MST, 4 of the 27 scour chains could not be recovered.

Data analysis.—Data collection, handling, and proofing methods were consistent with those recommended by Johnson et al. (2009). A nested ANOVA design was used as the basic design to evaluate potential differences in survival or developmental stage attributable to study reaches or adult mating. Study reach, site, and adult mating were designated independent vari-

ables; site was nested within study reach. The same design was used to identify potential sampling bias due to differences in survival attributable to relative site location, stocking order, or stocking crew (or a combination thereof); a separate test was performed for each of these potential biases by including each variable as an additional predictor to the basic design. A two-factor ANOVA design was used to evaluate potential differences in the number of thermal units accumulated between stocking and recovery among study reaches and adult crosses. A single-factor ANOVA design was used to evaluate potential differences in the number of days required in the gravel to meet target thermal units, percent of fines accumulated throughout the incubation period, scour depth, and substrate size, among study reaches. This design was also used to evaluate potential differences in survival and days in the gravel among study weeks. Pearson's product-moment statistic was used to evaluate potential correlation between survival and developmental stage, days in the gravel, percent fines, substrate size, and between in-hatchery and in-river survival. Survival data were arcsine-transformed, and percent fines data were Box–Cox transformed to meet assumptions of normality. Data transformation was not sufficient to meet test assumptions in the evaluation of scour depth. In this instance, a (nonparametric) Kruskal–Wallis ANOVA was used as an alternative. When significant differences were detected, post hoc Tukey tests were then used to identify specific differences within the design. Descriptive statistics were back-transformed, where appropriate, for clarity. All tests were performed in STATISTICA version 8.0 (StatSoft 2007) or PopTools version 3.0.6 (Hood 2008) with $\alpha = 0.05$.

RESULTS

Survival and Development

Survival among reaches in the upper Yakima River basin ranged between 60% and 87%, and averaged 72.5% (SD = 14.0). We did not detect a significant difference in survival among reaches (Table 3; Figure 4) but did detect differences among the specific matings (Table 3; Figure 5). Similarly, mean developmental indices were not significantly different among study reaches but did differ among the specific matings (Table 3; Figure 6). Survival and developmental indices were not significantly correlated ($R^2 = 0.02$, $P = 0.14$). Survival and developmental indices from egg boxes placed in the artificial spawning channel averaged 58.9% (SD = 26.1) and 1.9 (SD = 0.1), respectively. Neither metric differed significantly from our in-river study reaches containing the same matings (Tukey's honestly significantly difference [HSD]: $P > 0.41$). Survival of individual matings in the natural environment and those same matings incubated in the hatchery environment were highly correlated ($R^2 = 0.71$, $P < 0.01$). Survival was greater in week 3 than in weeks 1 and 2 (Table 4; Tukey's HSD: $P < 0.05$), but was not detectably different among sites within our study reaches (Table 3). We did not detect a significant difference in survival attributable to the order in which reaches were

TABLE 3. Results of ANOVA for comparisons of percent survival (arcsine-transformed) and developmental stage (k_D) of spring Chinook salmon fry from egg boxes recovered from upper Yakima River basin study reaches in 2009. Asterisks denote significant differences among study reaches, study sites (nested by study reach), or parental crosses (or a combination thereof; ANOVA: $P \leq 0.05$).

Source	Effect	df	Mean square	F	P
Survival					
Study reach	Fixed	8	0.162	2.158	0.094
Study site (nested in reach)	Random	18	0.074	1.117	0.368
Parental cross	Fixed	8	0.266	4.006	0.001*
Error		45	0.066		
Developmental stage					
Study reach	Fixed	8	0.003	2.124	0.094
Study site (nested within reach)	Random	18	0.001	1.946	0.038**a
Parental cross	Fixed	8	0.006	8.086	< 0.001*
Error		43	0.001		

*Significant differences in developmental stage were detected in 2 of 27 reach-nested study sites but were not detectable at the reach scale.

stocked, or among survey crews placing fertilized eggs within the artificial redds (Table 4).

Incubation Conditions

Accumulated thermal units at the time of recovery (target = 900) ranged between 894 and 945 (mean = 911.2; SD = 10.4), and were not significantly different among reaches or adult matings (Table 5). However, the number of days required to meet our target number of 900 accumulated thermal units was different among study reaches (Table 5; Figure 7), ranging between 112 and 257 d. Days in the gravel was also different between stocking weeks 1 (mean = 169.6; SD = 36.6) and 3 (mean = 194.7, SD = 26.2; Table 5; Tukey's post hoc: $P = 0.01$). Fine-sediment infiltration ranged between 0.4% and 34.0% (mean = 7.8; SD = 7.6) and was significantly different

among study reaches (Table 5; Figure 8). Fine-sediment intrusion was significantly correlated with survival ($R^2 = 0.06$, $P = 0.03$; Figure 9) and was not correlated with alevin developmental stage ($P = 0.37$). Conversely, the number of days spent in the gravel was not significantly correlated with survival ($P = 0.03$). Scour ranged between 0.0 and 8.4 cm among all study sites ($n = 23$). Scour among study reaches (mean = 2.1; SD = 2.9) was not significantly different (Table 5). Gravel composition (median grain size) for the 84th percentile (D_{84}) was significantly different among reaches but was not different for the 50th

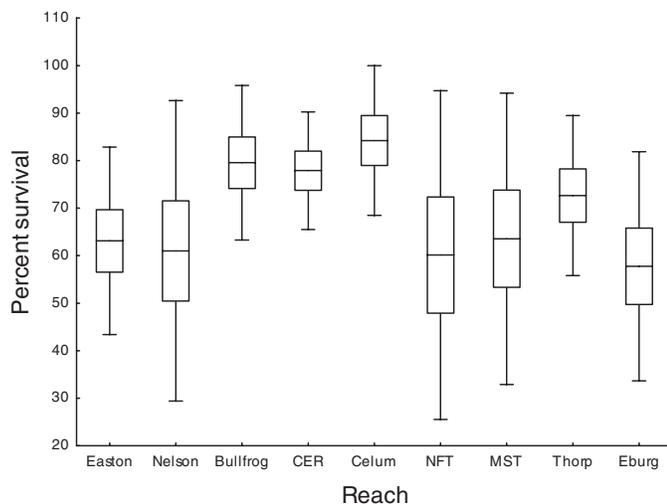


FIGURE 4. Mean egg-to-fry survival (horizontal lines) within study reaches in the upper Yakima River basin 2009–2010. Study reaches are presented in order of upstream to downstream location. Error bars represent the reach mean \pm SD, and boxes represent the reach mean \pm SE.

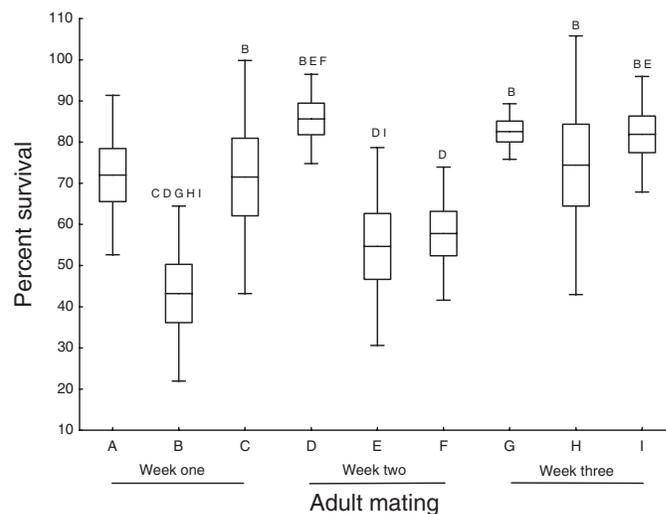


FIGURE 5. Mean egg-to-fry survival (horizontal lines) by adult matings (A–I). Matings A–C, D–F, and G–I were stocked in weeks 1, 2, and 3, respectively. Error bars represent the reach mean \pm SD, and boxes represent the reach mean \pm SE. Detected differences in survival among adult matings are identified above each mean. Survival of individual matings in the natural environment (shown here) and the same matings incubated under hatchery conditions were highly correlated ($R^2 = 0.71$, $P < 0.01$), further supporting detection of a parental effect in the natural environment.

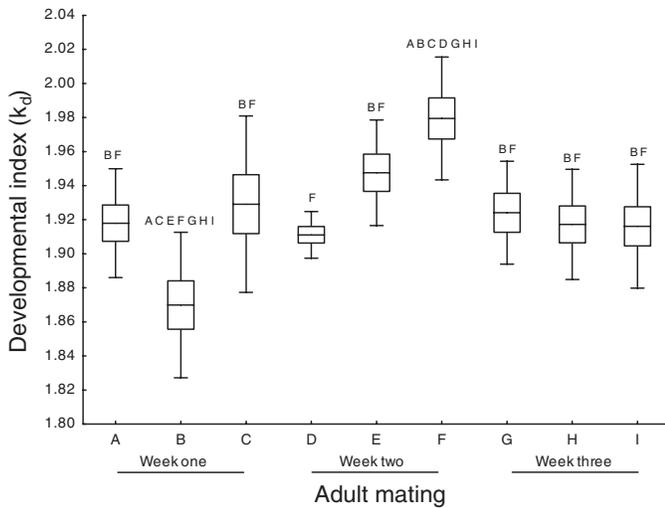


FIGURE 6. Mean developmental index (horizontal lines; k_D ; Bams 1970) by adult mating. Matings A–C, D–F, and G–I were stocked in weeks 1, 2, and 3, respectively. Error bars represent the reach mean \pm SD, and boxes represent the reach mean \pm SE. Detected differences in developmental stage among adult matings are identified above each mean.

percentile (Tables 2, 5). Neither was significantly correlated with survival ($P > 0.40$) or our index of developmental stage ($P > 0.12$).

DISCUSSION

We observed differences in survival and developmental stage among matings, and also a significant correlation in survival be-

tween specific matings in the natural environment and those same matings incubated in the hatchery environment. Our results suggest that parental effects can play a significant role in egg-to-fry survival and alevin development at emergence, which highlights the necessity to account for these potential effects in similar studies. Other authors have also suggested gamete viability as an important factor in estimates of egg-to-fry survival. For example, Young et al. (1990), commenting on the work of Chapman (1988), suggested that differential egg viability and deposition among individuals and stocks could result in inappropriate analysis of survival to emergence, and Rubin (1995) pointed to potential bias in estimates of egg-to-fry survival after detecting differences in survival among control groups incubated under identical conditions. Parental effects on survival have been demonstrated in a number of studies performed in both the hatchery environment and under seminatural conditions (Knudsen 2008; Schroder et al. 2008, 2010), but we are unaware of any studies that have directly accounted for these effects in a field setting. Although our study was successful in detecting differences in both survival and developmental stage among matings over a very large spatial scale, many of our comparisons suggested a high probability of differences among variables but were outside the range of our predetermined α level of 0.05. Among these were potential differences in survival ($P = 0.09$) and development ($P = 0.09$) among study reaches (i.e., environmental influences), correlation between survival and developmental stage ($P = 0.14$), and differences in scour among our study reaches ($P = 0.14$). Having appropriate power to detect existing differences in the natural environment is often

TABLE 4. Main effects and nested ANOVA results for potential indicators of sampling bias. Asterisks denote significant differences among study reaches, study sites (nested by study reach), or parental crosses (or a combination thereof; ANOVA: $P \leq 0.05$).

Source	Effect	df	Mean square	F	P
Survival by study week					
Study week		2	0.458	4.538	0.014*
Error		77	0.101		
Developmental stage by study week					
Study week		2	0.012	7.000	0.002*
Error		75	0.002		
Survival by stocking order					
Study reach	Fixed	8	0.148	2.088	0.110
Study site (nested in reach)	Random	17	0.071	0.986	0.492
Parental cross	Fixed	8	0.240	3.321	0.006*
Stocking order	Fixed	7	0.035	0.483	0.841
Error		38	0.072		
Survival by field crew					
Study reach	Fixed	7	0.090	1.227	0.320
Study site (nested in reach)	Random	18	0.074	1.043	0.441
Parental cross	Fixed	7	0.179	2.525	0.032*
Field crew	Fixed	9	0.049	0.686	0.717
Error		36	0.071		

TABLE 5. Main effects and one-way ANOVA results for comparison of incubation conditions. Asterisks denote significant differences among study reaches, parental crosses, or both (ANOVA: $P \leq 0.05$); na = not applicable.

Source	df	Mean square	F	P
Accumulated thermal units at recovery				
Study reach	8	45.965	0.407	0.912
Parental cross	8	115.084	1.020	0.430
Error	64	112.810		
Days in the gravel (by reach)				
Study reach	8	9,102.028	50.802	<0.001*
Error	71	179.166		
Days in the gravel (by week)				
Study week	2	4,185.281	4.296	0.017*
Error	75	974.170		
Fine-sediment accumulation				
Study reach	8	9.068	8.172	<0.001*
Error	69	1.110		
Scour (Kruskal–Wallis ANOVA)				
Study reach	7	na	(H) 11.221	0.129
Gravel composition (D_{84})				
Study reach	8	1,206.071	2.671	0.042*
Error	17	451.582		
Gravel composition (D_{50})				
Study reach	8	127.686	0.945	0.507
Error	17	135.076		

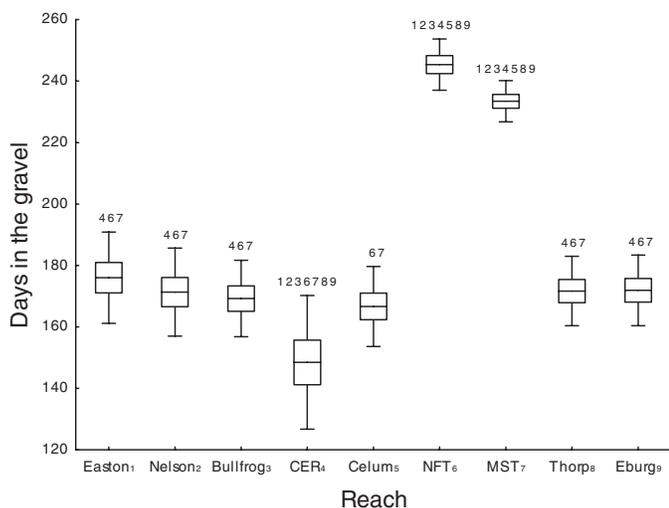


FIGURE 7. Mean number of days in the gravel within study reaches in the upper Yakima River basin 2009–2010 (horizontal lines). Study reaches are presented in order of upstream to downstream location. Error bars represent the reach mean \pm SD, and boxes represent the reach mean \pm SE. Detected differences in the number of days egg boxes were deployed are identified above each mean by corresponding reach subscript numbers.

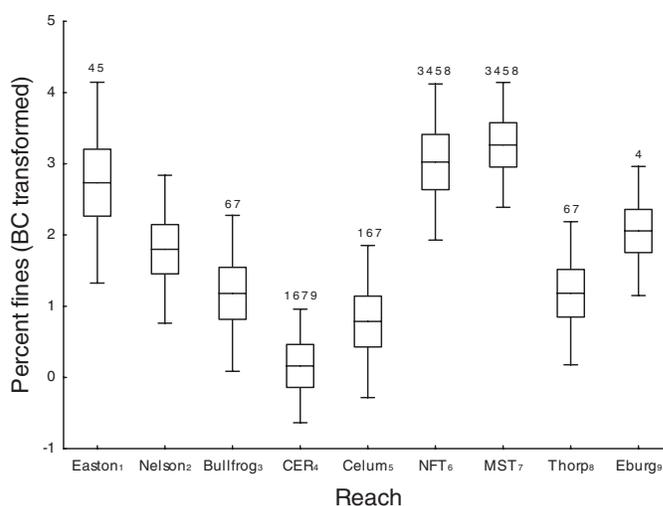


FIGURE 8. Mean fines (horizontal lines) within study reaches in the upper Yakima River basin 2009–2010. Study reaches are presented in order of upstream to downstream location. Error bars represent the reach mean \pm SD, and boxes represent the reach mean \pm SE. Detected differences in the level of fines among reaches are identified above each mean by corresponding reach subscript numbers.

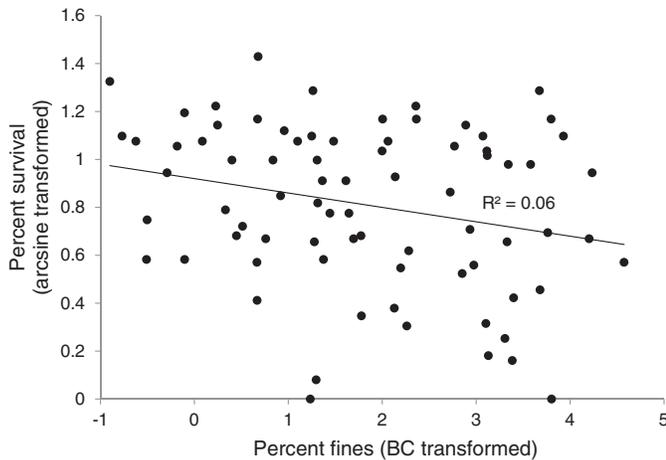


FIGURE 9. Relationship between percent fine sediment (<2 mm) and survival of spring Chinook salmon eggs planted in WV egg boxes in the Upper Yakima River basin 2009–2010 ($R^2 = 0.06$, $P = 0.03$).

a challenge due to multiple sources of variation, and it is quite possible that differences in these metrics and possibly others are more prevalent in some years, or would be detectably different with a larger sampling design. The Yakima basin experienced flow conditions throughout the 2009–2010 incubation period that were lower and less variable than average (Figure 10). These conditions may have resulted in a relaxation of environmental influences on survival, thereby increasing our power to detect differences in survival attributable to the various matings, and simultaneously decreasing our ability to detect what may

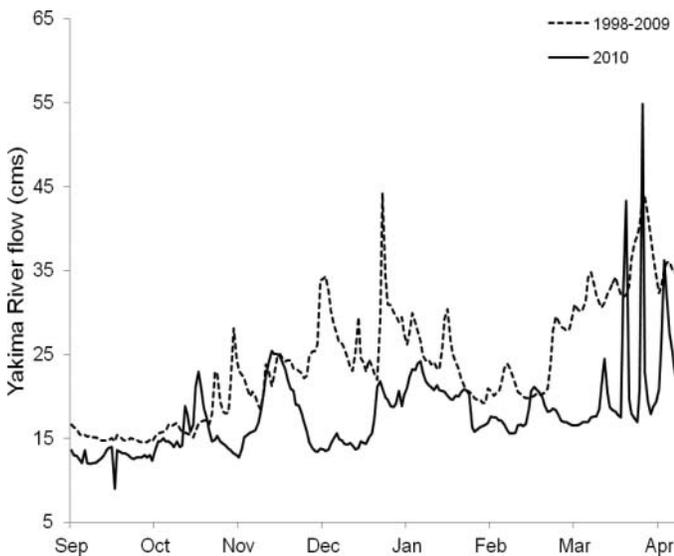


FIGURE 10. Mean daily flow through the incubation period (September 15–April 30) in the Upper Yakima River near Cle Elum, Washington, from 1998 to 2009 (dashed black line), and 2010 (solid black line). Flows are expressed in cubic meters per second (m^3/s). Mean monthly flows through the incubation period averaged $23.6 m^3/s$ between 1998 and 2009, and averaged $17.5 m^3/s$ in 2010. Data obtained from the USBR Hydromet database (USBR 2012).

be substantial environmental effects among reaches under more normative flow conditions. We detected a significant difference in the number of days required to reach target temperature units among our study reaches, retrieval events differing by as much as 145 d. Egg boxes in the CER reach were the first to be recovered, requiring an incubation time significantly less than the remainder of the study reaches (Figure 7). Water temperature in the CER is heavily influenced by the volume of Lake Cle Elum, which is the largest of the three upriver reservoirs and therefore does not experience fall and early winter temperature declines that are present in the remainder of the study area. Conversely, Teanaway River reaches required as much as 60 additional days to reach target temperature units when compared with main-stem Yakima River reaches (Figure 7). Despite large differences in the number of days spent in the gravel, we did not detect a difference in the developmental stage of survivors among reaches. This result is consistent with relationships between temperature accumulation and alevin developmental rates found in other studies (Murray and McPhail 1988; Beacham and Murray 1990). However, despite similar development at emergence, the time at which alevin leave the gravel is also of considerable interest. This is especially true if competition for limited resources exists among individuals originating from different study reaches. Differential emergence timing, even within individual redds, can have significant impacts on growth and survival (Quinn 2005). Earlier emergence can result in greater opportunity for growth and therefore a competitive advantage, as even small size differences can influence territorial disputes, food acquisition, and susceptibility to predation following emergence (Murray and McPhail 1988; Quinn 2005). Given the sizable difference in the accumulation of temperature units observed, it seems likely that differential emergence is present among our study reaches. This is of particular interest in the Yakima River basin, where storage reservoirs and water management practices influence water temperatures throughout the incubation period.

Infiltration of fine sediment explained only 6% of the variation in survival (Figure 9). Less than 4% of our egg boxes contained greater than 25% fines, a value suggested as a threshold over which detrimental effects to incubating Chinook salmon might occur (Jensen et al. 2009). These relatively low mean levels of fine-sediment infiltration may be due in part to the uncharacteristically low and stable flow conditions in the upper Yakima River previously mentioned. Our study reaches experienced mean daily flows of less than $25 m^3/s$ (Figure 10) and little or no substrate scour (Table 1), suggesting stable incubation conditions with little bed load movement. Conversely, we experienced the highest rate of fine-sediment infiltration in our Teanaway River reaches (Table 1; Figure 8). The Teanaway is largely unregulated and experienced the highest flow conditions throughout the study. Although our fines data from Teanaway River sites do not explain a greater proportion of the variation in survival than those in the remainder of the basin, it is possible that under more normative flow conditions and a corresponding

increase in fine-sediment transport, a greater number of sites may experience an increase in sediment accumulation above the proposed threshold. Additionally, differences in habitat conditions among reaches that were detectable in a low-flow year (e.g., median substrate size) may have a larger effect on survival in years where flow conditions are more normative.

Our results are consistent with two of three upper Yakima River studies that included some evaluation of Chinook salmon egg-to-fry survival. Fast et al. (1991) estimated mean survival of 59.6% from a total of 14 capped redds in the Easton–Nelson area of the upper Yakima between 1985 and 1986. Our estimates from the same areas were similar, averaging 65.7%. The two estimates are remarkably close considering the different sources of potential bias of each method and also the potential for temporal environmental differences, although mean flow conditions during the incubation period do appear to have been similar among years (mean = 6.7, 9.1, and 7.6 cms in 1985, 1986, and 2010 respectively; USBR 2012). Our estimate of mean survival from egg boxes stocked in the CESRF artificial channel (58.9%) was also similar to best estimates of survival from naturally spawning Chinook salmon in the channel, which averaged between 55% and 60% in previous years (S. Schroder, WDFW, personal communication), suggesting that survival from the egg boxes is consistent with survival of natural spawners in similar habitats. In contrast, our estimates are considerably lower than those of Major and Mighell (1969), who reported mean egg-to-smolt survival of 84–95% in the Yakima River. However, this apparent discrepancy may be attributable to sampling bias if, as suggested by Healey (1991), Major and Mighell underestimated egg deposition when multiplying average female fecundity of Columbia River Chinook salmon by Yakima River basin redd counts.

Studies estimating salmonid egg-to-fry survival normally utilize one of three primary field methods: excavation of naturally produced redds prior to first emergence, trapping of emergent fry from naturally produced redds, or planting known numbers of eggs in artificially constructed redds (Rubin 1995). Potential biases in the estimation of survival have been well documented for each of these methodologies due to various factors such as adult fecundity, egg deposition, fertilization rate, siltation, flow dynamics, escapement, and predation (Claire and Phillips 1968; Chapman 1988; Young et al. 1990; Rubin 1995; Reiser et al. 1998). Due to the scale and replication required, we chose to use egg boxes to estimate survival. Egg boxes have been shown to provide representative results in both sedimentation and egg incubation studies (Wesche et al. 1989; Garrett and Bennett 1996), and allow a greater number of replicates than comparable methods using the same effort (TEC 1993). However, although egg boxes appear to be effective at measuring survival to near emergence, they do not allow adequate measurement of other potentially significant factors such as predation by fish or aquatic insects, superimposition of redds, egg pocket depth, bed load movement, or the proportion of eggs swept from the redds during spawning. A specific example can be made from our own study with regard to the measure of invertebrate predation.

Stonefly nymphs can prey heavily on salmonid eggs and alevin when present in large numbers (Claire and Phillips 1968). Although we did not generally observe invertebrates within our egg boxes at the time of recovery, the screened egg boxes themselves could have protected eggs from invertebrate predation, resulting in an overestimation of survival. Alternatively, invertebrates inadvertently introduced into the egg boxes at the time of deployment may grow too large to escape the enclosure, thereby increasing predation on the egg lot. This may result in an underestimation of survival relative to a naturally constructed redd at the same location. Additional research will be required to fully evaluate the effect and magnitude of factors potentially affecting egg-to-fry survival that cannot be directly assessed using our proposed methodology.

There are a number of potential sampling biases that must be addressed in order to support our finding of a detectable parental effect on survival in the natural environment. The first is that of environmental- or sampling-induced mortality among the 3 weeks of our study. A review of Figure 5 does suggest that mean survival was greater in the third week of our study (matings G through I), and slight but detectable environmental differences were apparent among study weeks. For instance, we did detect a greater number of days required in the gravel to reach our target accumulation of temperature units between weeks 1 and 3. Because survival was similar among matings made in week 3, we cannot exclude the possibility that observed differences among week 3 matings and matings from weeks 1 and 2 were solely due to a week 3 temporal effect on survival. However, we did not detect a relationship between the number of days in the gravel and survival, and have no evidence to suggest that greater observed survival in week 3 was due to any factor other than the odds of selecting and spawning three adult pairs with similar gametic viability. Further, due to the detectable within-week differences in survival observed among matings in both weeks 1 and 2 (Figure 5), temporal effects in week 3 (if present) would not influence the overall findings of this study. Additionally, the suggestion of an observed parental effect is further supported by the strong correlation of survival observed between specific matings in the natural environment and those same matings incubated under controlled hatchery conditions. A second concern is that survival among matings may appear to be different if the relative location of a study site is more or less conducive to survival. Our study design placed a specific mating in the same relative location within each reach during each stocking week. Had we detected a significant difference in survival among site locations as well as among matings, it may have affected our ability to segregate environmental and genetic effects on survival. A random distribution of crosses within our reaches within a stocking week would likely have improved our design. A third potential bias is the possibility that the order in which reaches were stocked, or the teams that stocked artificial redds, differentially influenced our estimates of egg-to-fry survival. Due to the size of our study, it was not possible to equally distribute the effort of each crew within the

study design. Each team was responsible for the construction and stocking of artificial redds in a particular geographic area each week. We did not detect a significant difference in survival that could be attributed to either stocking crew or the order in which our sites were stocked when accounting for adult mating. Additionally, as with the other two prominent potential biases, correlation between in-hatchery controls and survival in the natural environment suggests that misinterpreting differences in survival among matings due to either stocking order or a crew effect is unlikely. Another source of potential bias exists in the multiple methods used when recovering our egg boxes. In instances of high flow, snorkeling methods were required for extraction due to our inability to hold the bottomless barrel in place against the force of the water. We would expect a potentially higher percentage of fines to be lost using snorkeling methods than with the use of a bottomless barrel due to a more direct exposure to flow during extraction as the box was moved up through the water column. We attempted to minimize these potential effects by directing personnel to stand directly in front of the redd location, thereby diverting flow to the sides as the egg box was extracted. This method was effective but likely less efficient than the solid surface of the barrel. Therefore, we may have underestimated the level of fines where these methods were necessary. However, due to the low percentage of variation in survival explained by the presence of fines among sites in which the bottomless barrel was used, the low number of sites in which snorkeling was necessary (7 of 81), and the high relative level of fines present in the Teanaway reaches compared with others (Table 1), we propose that if such a bias was present, it was negligible.

In summary, our results suggest that both extensive replication and tracking of gamete viability is needed to separate parental effects from environmental factors when estimating egg-to-fry survival. Our extensive use of artificial redds and egg boxes allowed measurement of survival and development among reaches at a basin scale. Our design also allowed comparisons between survival and development, and a number of the primary factors thought to affect and or limit Chinook salmon productivity during incubation (e.g., temperature, fine sediment infiltration, scour). Additional research will be required to examine other factors that may greatly influence survival, such as predation, swim-up mortality, and redd superimposition.

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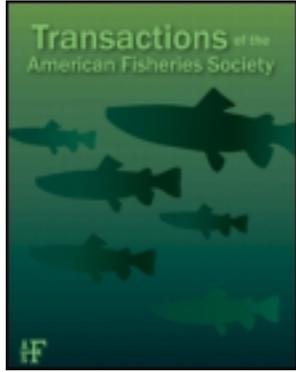
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Matthew R. Campbell^a, Christine C. Kozfkay^a, Timothy Copeland^b, William C. Schrader^b, Michael W. Ackerman^c & Shawn R. Narum^d

^a Idaho Department of Fish and Game, 1800 Trout Road, Eagle, Idaho, 83616, USA

^b Idaho Department of Fish and Game, 1414 East Locust Lane, Nampa, Idaho, 83686, USA

^c Pacific States Marine Fisheries Commission, 1800 Trout Road, Eagle, Idaho, 83616, USA

^d Columbia River Inter-Tribal Fish Commission, 3059-F National Fish Hatchery Road, Hagerman, Idaho, 83332, USA

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ARTICLE

Estimating Abundance and Life History Characteristics of Threatened Wild Snake River Steelhead Stocks by Using Genetic Stock Identification

Matthew R. Campbell* and Christine C. Kozfkay

Idaho Department of Fish and Game, 1800 Trout Road, Eagle, Idaho 83616, USA

Timothy Copeland and William C. Schrader

Idaho Department of Fish and Game, 1414 East Locust Lane, Nampa, Idaho 83686, USA

Michael W. Ackerman

Pacific States Marine Fisheries Commission, 1800 Trout Road, Eagle, Idaho 83616, USA

Shawn R. Narum

Columbia River Inter-Tribal Fish Commission, 3059-F National Fish Hatchery Road, Hagerman, Idaho 83332, USA

Abstract

Assessments of threatened wild Snake River steelhead *Oncorhynchus mykiss* have historically been limited due to a lack of stock-specific information and difficulties in field sampling efforts. We used genetic stock identification (GSI) to estimate the composition of wild adult steelhead migrating past Lower Granite Dam on the Snake River between August 24 and November 25, 2008. Further, we combined genetic data with information on sex, length, age, and run timing to examine for differences in life history or demography among stocks. In total, 1,087 samples collected at the dam were genotyped with 13 standardized steelhead microsatellite loci and a new modified Y-chromosome-specific assay that differentiates sex. A genetic baseline of 66 populations was used to complete GSI of unknown-origin samples from Lower Granite Dam. Large differences in reporting group (stock) contributions were observed for the run as a whole; the Snake River–lower Clearwater River reporting group had the largest single contribution of 36.1% (95% confidence interval [CI] = 30.2–39.7%). Other large contributions were 15.4% (12.8–18.7%) from the upper Clearwater River reporting group and 13.9% (12.5–18.7%) from the lower Salmon River reporting group. Smaller contributions came from the other six reporting groups (Imnaha River: mean = 9.5%, 95% CI = 6.8–13.6%; upper Salmon River: 9.2%, 5.1–11.3%; South Fork Clearwater River: 7.6%, 4.3–8.9%; Middle Fork Salmon River: 5.1%, 3.5–6.4%; South Fork Salmon River: 2.7%, 1.3–3.6%; Elk Creek: 0.5%, 0.0–1.2%). Significant differences in reporting group contributions were observed when samples were grouped according to length, age, and run timing differences. Of the samples analyzed, 372 (34.9%) were identified as males and 694 (65.1%) were identified as females. Our results demonstrate that the GSI methodologies applied to Snake River steelhead have the potential of providing an efficient, minimally intrusive tool for obtaining stock-specific abundance of this threatened distinct population segment. This technology can assist future viability status assessments of Snake River steelhead by contributing to refinements in population delineations, productivity calculations, and annual stock-specific estimation of life history characteristics (e.g., age structure, sex ratio, and run timing).

*Corresponding author: matthew.campbell@idfg.idaho.gov

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Steelhead *Oncorhynchus mykiss* in the Pacific Northwest, USA, have been in decline for the last several decades. In the Columbia River basin, steelhead belong to five distinct population segments (DPSs), all of which are listed as threatened under the Endangered Species Act (U.S. Office of the Federal Register 2011). To assess the extinction risk of salmonid populations and the viability of DPSs, the National Marine Fisheries Service developed the viable salmonid population concept (McElhany et al. 2000). Under this concept, managers attempt to delineate population structure and spatial boundaries, estimate past and present population abundance and growth, and characterize and quantify the diversity of life history characteristics expressed within each DPS. Life history information includes length of freshwater rearing and ocean residency, run timing, age structure at return, size at age, and sex ratio. These assessments contribute to recovery efforts because they allow a better understanding of the mechanisms that have led to population declines and they provide a knowledge base from which to formulate predictions of stocks' responses to different types of management action.

Assessments of the status of Snake River summer-run steelhead have been particularly challenging. The Snake River DPS was originally listed as threatened under the Endangered Species Act in 1997 and encompasses populations that spawn throughout the basin in central Idaho, northeastern Oregon, and southeastern Washington. Formerly, over half of the steelhead produced in the Columbia River basin spawned in Snake River tributaries (Mallet 1974). Raymond (1988) documented that the survival of steelhead emigrating from the Snake River decreased after the construction of dams on the lower Snake River during the late 1960s and early 1970s. There was a period of recovery in the early 1980s, but adult escapement past Lower Granite Dam (Figure 1) into the Snake River basin declined again. While hatchery returns increased, the returns of naturally produced steelhead remained critically low, especially for stocks with a later run timing (Busby et al. 1996). Spawning escapement estimates (and other demographic information) are unavailable for most Snake River steelhead stocks (Busby et al. 1996; Good et al. 2005), and this lack of information presents a persistent challenge to management of the species. Given that steelhead in the Snake River basin spawn on the peak of the spring snowmelt, flow conditions preclude typical monitoring methods, such as weir trapping, spawning observations, and redd counts.

In lieu of more detailed drainage-level, stock-specific information, steelhead that spawn in the Snake River basin have traditionally been assigned to two groups (A-run and B-run) based on the bimodal timing of passage into the Columbia River (as measured at Bonneville Dam) and based on certain life history characteristics (Busby et al. 1996). By definition, A-run steelhead pass Bonneville Dam before August 25 and tend to return after 1 year in the ocean. The B-run steelhead pass Bonneville Dam after August 25, tend to return after 2 years in the ocean, and are thought to be larger at age than A-run steelhead. Migrating adults do not exhibit a bimodal passage distribution at Lower

Granite Dam, and A-run and B-run adults are therefore differentiated and enumerated based on length (A-run: ≤ 78 cm; B-run: > 78 cm; Schrader et al. 2011). In addition to run timing at Bonneville Dam and size differences, the two stocks are believed to also exhibit differences in spawning distribution. The A-run adult steelhead are thought to spawn throughout the Columbia River basin, whereas the B-run steelhead are believed to originate primarily from the Clearwater, Middle Fork Salmon, and South Fork Salmon rivers in Idaho. Putative migration timing and life history characteristics have been used as surrogates for biodiversity in conservation planning for Snake River steelhead. However, the relationship between life history characteristics and passage timing at Bonneville Dam is uncertain (Good et al. 2005). Furthermore, the passage distribution at Bonneville Dam has shifted from bimodal to unimodal in recent years (Robards and Quinn 2002).

Two principal management issues involving Snake River steelhead have arisen in the last several years. First, B-run populations do not appear to be self sustaining (NOAA 2008), and their presence in the drainage has affected Columbia River hydrosystem operation and lower Columbia River fisheries management. In particular, harvest of fall Chinook salmon *O. tshawytscha* is constrained in order to limit impacts to B-run steelhead that are concurrently present in the Columbia River. Secondly, although Snake River B-run steelhead are currently identified as a biologically significant and distinct component of the Snake River evolutionarily significant unit (NOAA 2003), their management is confounded by the lack of a clear and detailed understanding of their actual spawning distribution and evolutionary structure. Nielsen et al. (2009) found that steelhead in Snake River tributaries within Idaho exhibited a complicated pattern of genetic structure, with populations grouping genetically according to drainage locality rather than simply to A-run and B-run designations.

These types of management issues can potentially be addressed through genetic stock identification (GSI). In GSI analysis, reference populations from all suspected contributing stocks are screened with multilocus genetic markers. By use of statistical algorithms, these populations are then grouped or "clustered" together into reporting groups based on genetic similarities. When mixtures of fish of unknown origin are genotyped at the same sets of genetic markers, it is possible to estimate the proportion of each reporting group represented in the mixture (Shaklee et al. 1999; Anderson et al. 2008). A variety of Pacific salmonids, including Chinook salmon, sockeye salmon *O. nerka*, chum salmon *O. keta*, and steelhead, have been researched and managed by using GSI technologies (Beacham et al. 1999, 2000, 2008a, 2008b; Habicht et al. 2007). Previous genetic studies have indicated that steelhead in the Snake River basin exhibit significant genetic structuring at the drainage level (Moran 2003; Nielsen et al. 2009), and GSI procedures have already been used successfully to identify the origin of postspawn steelhead at Lower Granite Dam (Narum et al. 2008). In the present study, we used similar GSI methods to identify the stock

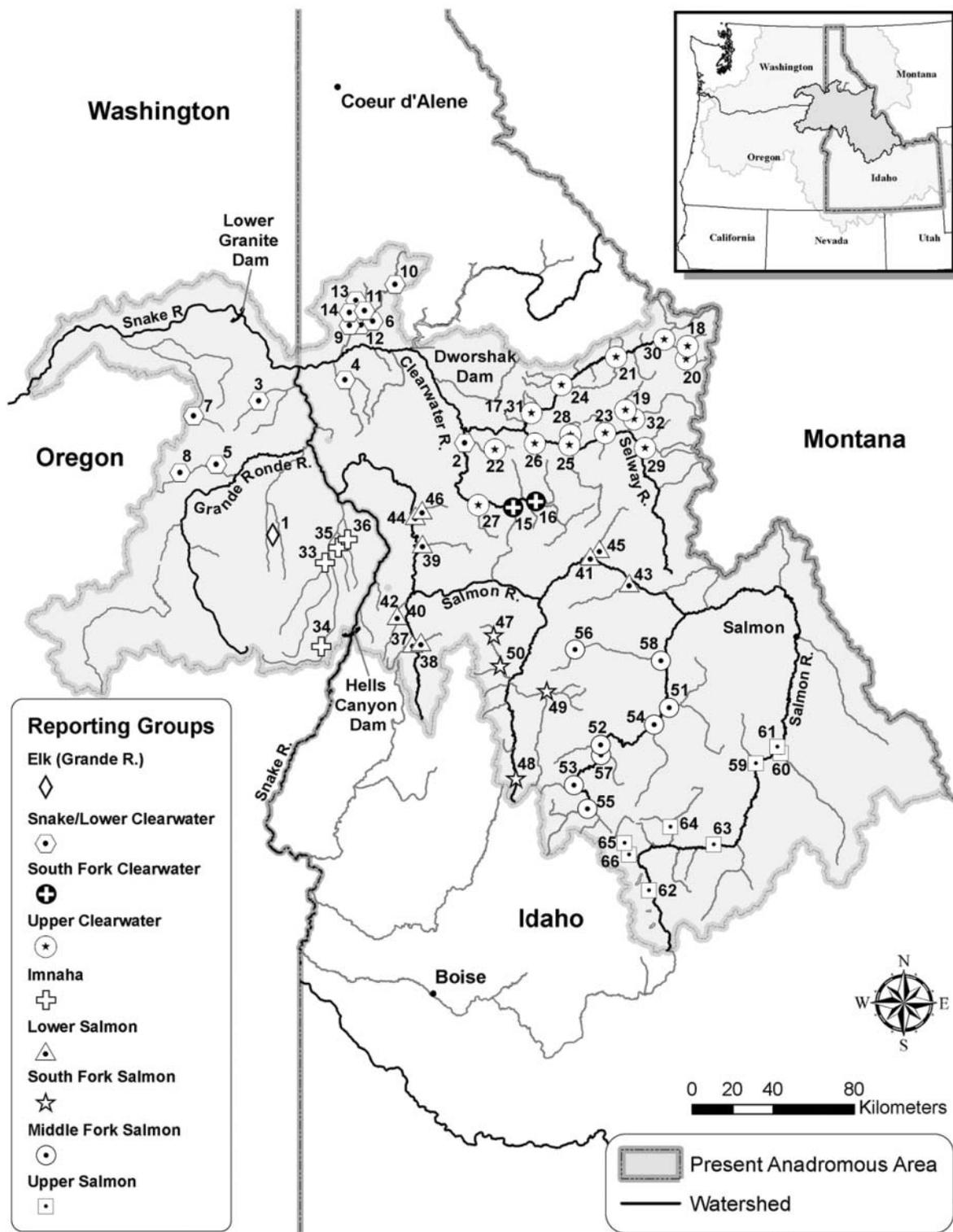


FIGURE 1. The 66 Snake River steelhead populations that served as baseline populations for genetic stock identification (GSI) mixture analyses. Numbers correspond to the site numbers defined in Table 1. Symbols refer to the nine genetic clusters that were identified in Bayesian Analysis of Population Structure software and that were used for GSI reporting groups.

composition of adult wild steelhead migrating past Lower Granite Dam and we combined genetic data with sex, length, age, and run timing information to evaluate demographic similarities and differences among stocks.

METHODS

Snake River genetic baseline.—A genetic baseline of 66 wild, anadromous Snake River basin steelhead collections was available as part of a multilaboratory, collaborative effort to build a standardized coastwide microsatellite baseline for steelhead (Blankenship et al. 2011). All of the collections (3,803 individuals; Table 1; Figure 1) were previously genotyped with a standardized set of 13 microsatellite loci (Stephenson et al. 2009).

To examine genetic relationships among the baseline collections, genetic chord distances (Cavalli-Sforza and Edwards 1967) between all collections were estimated by using GENDIST in PHYLIP version 3.5 (Felsenstein 1993). To help visualize genetic relationships, a neighbor-joining dendrogram was generated from chord distances with the program FITCH in PHYLIP using a bootstrapping algorithm. Bootstrap replicates of 1,000 iterations were attained with SEQBOOT, and a consensus tree was formed with CONSENSE in PHYLIP. The dendrogram was edited and visualized by using TreeGraph 2 (Stöver and Müller 2010).

To assess the appropriate number and population composition of reporting groups for GSI analyses, baseline samples were analyzed with Bayesian Analysis of Population Structure

(BAPS) version 5.3 (Corander et al. 2008). The BAPS software assigns samples to K clusters by using a partition-based mixture model that minimizes deviations from Hardy–Weinberg equilibrium and linkage equilibrium within each cluster. Simulated data sets have shown that BAPS can infer the correct number of subpopulation clusters even at low levels of differentiation (Latch et al. 2006). We used the “clustering of groups of individuals” option in BAPS with a predefined maximum K of 66 (corresponding to the total number of collections). We repeated the run 10 times to check the stability of the results. The best clustering solution (“correct” number of reporting groups) was chosen based on the largest log marginal likelihood value from all runs. To describe genetic differentiation among clusters, we calculated pairwise Nei’s standard distance (Nei 1972) in BAPS.

To evaluate the potential accuracy of selected reporting groups for GSI, we followed the recommended methods of Anderson et al. (2008) in using the program ONCOR (Kalinowski et al. 2007) to perform 100% simulations. These procedures test each population under the scenario that the mixture solely consists of individuals from that population. A population is generally considered to be highly identifiable if allocation to the correct reporting group is 90% or greater (Seeb et al. 2007). The number of mixtures to generate for each population was set at 1,000, with a mixture sample size of 400. Simulated baseline sample sizes were the same as in the actual baseline.

Trapping, sampling, and age assignment.—Wild adult steelhead were captured at the Lower Granite Dam adult trapping facility (Harmon 2003; Figure 1) from August 24 to November 25, 2008 (Figure 2), coinciding with the collection of fall

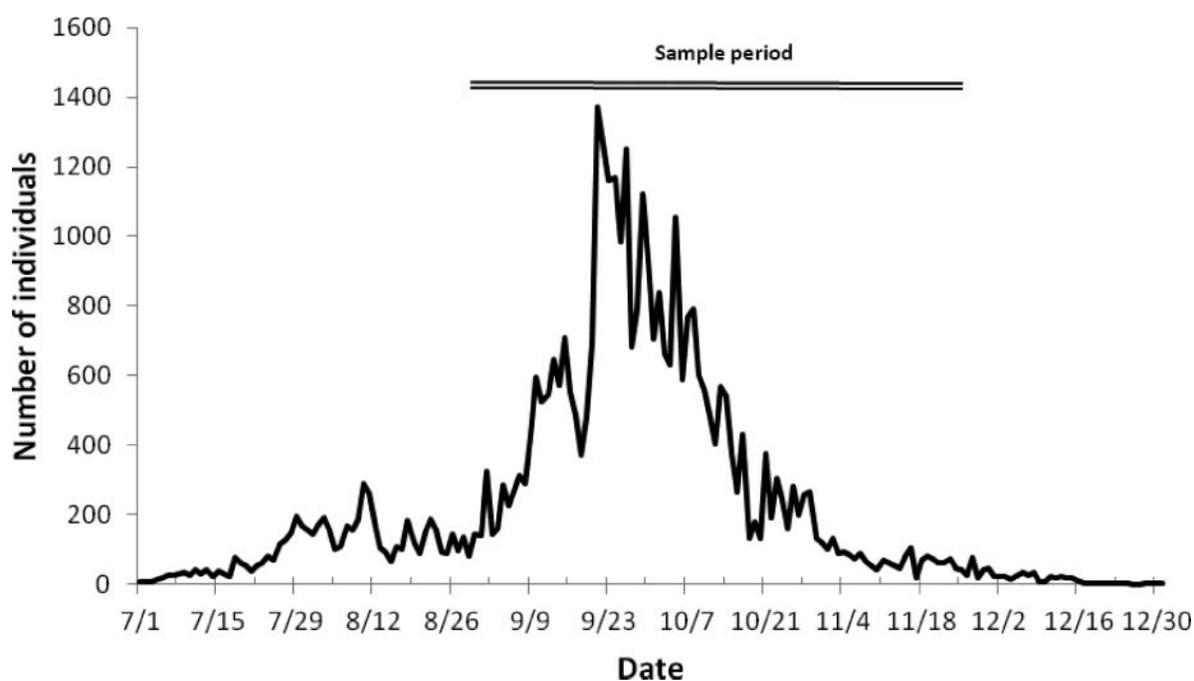


FIGURE 2. Number of wild adult steelhead that passed Lower Granite Dam in 2008. The period when samples were collected (August 24–November 25, 2008) is denoted by the horizontal bar.

TABLE 1. Steelhead populations and corresponding site numbers in the Snake River basin, presented with sample size per population (N), observed heterozygosity (H_O), expected heterozygosity (H_E), and average number of alleles observed per locus (A). For each population, the correct assignment back to reporting group (reporting group accuracy [RGA]) from 100% simulations in ONCOR is presented with lower and upper 95% confidence limits (CLs; RGA = the proportion of simulated fish, in a 100% mixture of fish from a given population, that were correctly assigned back to that population). Genotyping agencies were the Columbia River Inter-Tribal Fish Commission (CRITFC), the Idaho Department of Fish and Game (IDFG), and the Northwest Fisheries Science Center (NWFS, National Oceanic and Atmospheric Administration Fisheries).

Collection	Site number	Agency	N	H_O	H_E	A	RGA	Lower 95% CL	Upper 95% CL
Elk Creek (Grande Ronde River) reporting group									
Elk Creek	1	NWFS	96	0.77	0.76	9.9	0.96	0.93	0.98
Snake River–Lower Clearwater River reporting group									
Cottonwood Creek	2	NWFS	96	0.77	0.78	11.3	0.94	0.91	0.97
Asotin Creek	3	NWFS	110	0.79	0.80	13.1	0.87	0.82	0.92
Mission Creek	4	CRITFC	51	0.76	0.76	10.5	0.96	0.94	0.99
Crooked Creek	5	NWFS	141	0.78	0.78	12.6	0.96	0.93	0.99
Lower East Fork Potlatch River	6	NWFS	41	0.78	0.77	9.5	0.97	0.95	0.99
Tucannon River	7	NWFS	74	0.78	0.79	11.9	0.88	0.83	0.93
Wenaha River	8	NWFS	94	0.77	0.78	11.8	0.91	0.87	0.95
Little Bear Creek	9	IDFG	42	0.75	0.77	10.3	0.90	0.85	0.94
Upper East Fork Potlatch River	10	IDFG	62	0.74	0.75	10.1	0.97	0.95	0.99
Big Bear Creek	11	IDFG	20	0.75	0.76	8.9	0.89	0.84	0.93
Little Bear Creek	12	IDFG	11	0.71	0.76	6.7	0.93	0.89	0.96
Big Bear Creek	13	IDFG	12	0.69	0.73	6.6	0.78	0.71	0.83
Little Bear Creek	14	CRITFC	50	0.80	0.76	9.2	0.98	0.97	1.00
South Fork Clearwater River reporting group									
Tenmile Creek	15	IDFG	47	0.77	0.74	8.5	0.96	0.93	0.98
Crooked River	16	IDFG	80	0.73	0.73	9.6	0.93	0.89	0.96
Upper Clearwater River reporting group									
Canyon Creek	17	CRITFC	34	0.77	0.74	8.2	0.99	0.98	1.00
Storm Creek	18	CRITFC	39	0.75	0.73	8.1	1.00	0.99	1.00
North Fork Moose Creek	19	CRITFC	50	0.73	0.73	9.2	0.99	0.98	1.00
Colt Creek	20	CRITFC	58	0.72	0.71	8.3	1.00	0.99	1.00
Lake Creek	21	CRITFC	52	0.74	0.72	8.8	1.00	0.99	1.00
Clear Creek	22	CRITFC	45	0.74	0.75	9.5	0.91	0.87	0.94
Three Links Creek	23	CRITFC	57	0.78	0.74	8.8	1.00	0.99	1.00
Fish Creek	24	NWFS	80	0.75	0.75	10.2	0.99	0.98	1.00
Gedney Creek	25	NWFS	114	0.76	0.75	10.7	0.98	0.96	1.00
O'Hara Creek	26	IDFG	47	0.75	0.76	9.7	0.97	0.94	0.99
Johns Creek	27	IDFG	31	0.74	0.75	9.5	0.75	0.69	0.80
Gedney Creek	28	IDFG	46	0.73	0.75	9.3	0.98	0.96	1.00
Bear Creek	29	IDFG	45	0.78	0.76	8.5	0.99	0.98	1.00
Crooked Fork Lochsa River	30	IDFG	47	0.75	0.75	8.7	1.00	0.98	1.00
Canyon Creek	31	IDFG	47	0.73	0.73	9.6	0.94	0.91	0.97
North Fork Moose Creek	32	IDFG	47	0.74	0.76	8.6	0.99	0.99	1.00
Imnaha River reporting group									
Camp Creek	33	NWFS	136	0.80	0.77	11.0	0.98	0.96	1.00
Gumboot Creek	34	NWFS	93	0.78	0.77	9.8	0.97	0.94	0.99
Horse Creek	35	NWFS	117	0.77	0.78	11.6	0.91	0.87	0.95
Lightning Creek	36	NWFS	67	0.76	0.78	9.9	0.82	0.77	0.88
Lower Salmon River reporting group									
Boulder Creek	37	IDFG	47	0.77	0.76	10.1	0.93	0.89	0.97
Hazard Creek	38	IDFG	44	0.76	0.78	11.2	0.69	0.63	0.76

TABLE 1. (Continued).

Collection	Site number	Agency	<i>N</i>	<i>H_O</i>	<i>H_E</i>	<i>A</i>	RGA	Lower 95% CL	Upper 95% CL
Slate Creek	39	IDFG	47	0.77	0.79	10.9	0.88	0.83	0.92
Rapid River	40	IDFG	266	0.75	0.76	12.5	0.98	0.96	0.99
Bargamin Creek	41	IDFG	45	0.77	0.78	9.3	0.93	0.90	0.97
Rapid River	42	NWFSC	43	0.76	0.75	9.1	0.99	0.97	1.00
Chamberlain Creek	43	CRITFC	64	0.78	0.76	10.6	0.88	0.82	0.92
Whitebird Creek	44	CRITFC	58	0.76	0.78	9.6	0.96	0.93	0.98
Bargamin Creek	45	NWFSC	45	0.78	0.77	9.3	0.87	0.82	0.92
Whitebird Creek	46	NWFSC	50	0.76	0.77	10.2	0.80	0.74	0.85
South Fork Salmon River reporting group									
Upper Secesh River	47	NWFSC	28	0.70	0.71	6.7	0.99	0.98	1.00
Stolle Meadows	48	NWFSC	44	0.72	0.72	8.2	0.94	0.91	0.97
East Fork South Fork Salmon River	49	IDFG	46	0.77	0.75	8.3	0.91	0.87	0.94
Lower Secesh River	50	IDFG	45	0.70	0.73	8.3	0.95	0.92	0.98
Middle Fork Salmon River reporting group									
Camas Creek	51	CRITFC	52	0.75	0.75	9.5	0.92	0.88	0.95
Pistol Creek	52	CRITFC	23	0.76	0.73	7.6	0.90	0.85	0.94
Sulphur Creek	53	CRITFC	53	0.77	0.72	8.1	0.98	0.96	0.99
Loon Creek	54	CRITFC	59	0.73	0.73	8.8	0.95	0.92	0.98
Marsh Creek	55	CRITFC	57	0.74	0.73	7.7	0.99	0.98	1.00
Upper Big Creek	56	NWFSC	42	0.77	0.77	9.2	0.83	0.78	0.88
Rapid River	57	IDFG	45	0.70	0.72	8.5	0.91	0.87	0.94
Lower Big Creek	58	IDFG	47	0.75	0.74	7.1	1.00	0.99	1.00
Upper Salmon River reporting group									
Morgan Creek	59	IDFG	45	0.80	0.81	11.4	0.86	0.81	0.90
Pahsimeroi River	60	IDFG	41	0.81	0.81	10.8	0.77	0.72	0.83
Pahsimeroi River	61	IDFG	47	0.79	0.80	10.5	0.89	0.84	0.93
Sawtooth Weir	62	IDFG	29	0.77	0.78	9.6	0.76	0.70	0.82
Squaw Creek	63	IDFG	21	0.79	0.79	9.2	0.67	0.60	0.73
West Fork Yankee Fork Salmon River	64	IDFG	47	0.83	0.80	10.2	0.91	0.88	0.95
Upper Valley Creek	65	NWFSC	25	0.78	0.77	7.5	0.92	0.88	0.96
Lower Valley Creek	66	NWFSC	19	0.83	0.79	8.3	0.78	0.72	0.84

Chinook salmon broodstock for the Lyons Ferry Fish Hatchery. The trapping rate for steelhead was dependent upon the trapping rate for fall Chinook salmon, which varied between 10% and 20%. Although most of the hatchery-origin steelhead have a clipped adipose fin, thus allowing for differentiation from wild fish, some are misclipped or are intentionally released unclipped for supplementation purposes. At the adult trapping facility, unclipped hatchery steelhead are identified by the presence of dorsal or ventral fin erosion (Schrader et al. 2011). In 2008, 13.0% of hatchery steelhead passing Lower Granite Dam were unclipped (Schrader et al. 2011). Sampled wild adults were measured for fork length to the nearest centimeter, and scales were collected to determine age. Tissue samples were taken from the anal fin by using a tissue punch and were stored in 100% nondenatured ethanol. Fish were subsampled

from the total number of wild-origin samples collected at the adult trap to maintain an overall sample rate of approximately 5%.

Freshwater and saltwater ages were assigned to each fish based on scale pattern analysis (Davis and Light 1985). Two technicians independently viewed each image to assign ages. Freshwater ages were assigned using a 4 × magnified image, and saltwater ages were assigned using a 1.25 × magnified image. The criterion for a saltwater annulus was the crowding of circuli outside of the check for ocean entry. Freshwater annuli were defined by the “pinching” or “cutting over” of circuli within the freshwater zone in the center of the scale. If there was no age consensus between the two readers, a third reader viewed the image; all readers then collectively examined the image to resolve their differences before a final age was assigned. If a

consensus among the three readers was not attained, the scale sample was excluded from further analysis.

Genotyping and genetic stock identification.—A Nexttec Genomic DNA Isolation Kit was used to extract DNA from tissue samples in accordance with the manufacturer's instructions. Samples were amplified with the 13 standardized microsatellite loci (Stephenson et al. 2009). Specific PCR amplification protocols for all loci, as well as thermal cycling conditions, are available from the corresponding author upon request. Descriptive statistics, including the number of alleles per locus, observed heterozygosity, and expected heterozygosity, were estimated for each baseline collection by using the Microsatellite Toolkit for Microsoft Excel (Park 2001).

In addition to the 13 microsatellite loci, all samples were also screened with Y-chromosome-specific assays that differentiate sex in steelhead. Details of assay configuration and the screening performed on known-sex samples to verify accuracy are described in the Appendix.

To address questions of abundance and demography for the identified stocks, we integrated the genetic data with sex, length, age, and migration timing data from adults sampled at Lower Granite Dam. Putative A-run and B-run steelhead are distinguished on the basis of length (≤ 78 or > 78 cm), age (1 saltwater versus older), and migratory timing (early versus late). Mixture analyses were performed with ONCOR software in different arrangements to estimate stock components under five different scenarios: (1) for the entire wild run of steelhead (all samples grouped together), (2) by sex (males and females separated), (3) by size (mixtures grouped by length: ≤ 78 or > 78 cm), (4) by run timing (mixtures grouped as early [August 24–September 22]; middle [September 23–October 23]; and late [October 24–November 25]), and (5) by total age (3, 4, and 5 years). Separate mixtures were also run with 4-year-old fish separated into two age-classes as defined by years in freshwater and years in saltwater (freshwater: saltwater = 2:2 or 3:1). A 95% confidence interval (CI) for stock composition estimates to each reporting group was estimated by bootstrapping the baseline and mixtures for 1,000 iterations as implemented in ONCOR (Kalinowski et al. 2007).

RESULTS

Snake River Genetic Baseline

Basic descriptive statistics for baseline populations are shown in Table 1. More comprehensive summaries of tests for Hardy–Weinberg equilibrium, linkage disequilibrium, population diversity, and population differentiation were published as part of a larger collaborative effort to describe the influence of landscape on the genetic structure of steelhead throughout the Columbia River basin (Blankenship et al. 2011). The neighbor-joining dendrogram based on Cavalli-Sforza and Edwards' (1967) genetic chord distances generally supported genetic population structuring at the subbasin or drainage scale (Figure 3). Bootstrap support greater than 50% was observed for population

groupings in the Clearwater, Middle Fork Salmon, South Fork Salmon, upper Salmon, Imnaha, and Grande Ronde rivers. Genetic relationships among populations in tributaries to the main-stem Snake, Little Salmon, and main-stem Salmon rivers were less clear, especially between populations that were found lower in these drainages.

Results of group-level mixture analysis on baseline populations with BAPS indicated that the K in the optimal partition was 9, with a log marginal likelihood of 191,524.04 and a posterior probability of 1. The nine clusters were used as reporting groups for subsequent mixed-stock analyses: (1) Elk Creek (Grande Ronde River), (2) Snake River and lower Clearwater River, (3) South Fork Clearwater River, (4) upper Clearwater River, (5) Imnaha River, (6) lower Salmon River, (7) South Fork Salmon River, (8) Middle Fork Salmon River, and (9) upper Salmon River. Clusters generally followed the genetic structuring observed in the neighbor-joining dendrogram and consisted of geographically proximate populations (Figure 1). One exception was Johns Creek (South Fork Clearwater River subbasin), which clustered apart from neighboring populations and instead grouped with populations from the upper Clearwater River (Lochsa River and Selway River drainages). The Snake River–lower Clearwater River cluster encompassed samples from multiple drainages, including the Tucannon River, lower main-stem Clearwater River (below the North Fork Clearwater River), Asotin Creek, and lower Grande Ronde River. The geographic center of this large, multidrainage cluster lies approximately at the confluence of the Snake and Clearwater rivers. Another large, multidrainage cluster was associated with the confluence of the Salmon and Little Salmon rivers and contained samples from the main-stem Salmon River watershed above the Little Salmon River confluence (Bargamin and Chamberlain creeks), from the Little Salmon River (Rapid River, Boulder Creek, and Hazard Creek), and from the main-stem Salmon River below the Little Salmon River confluence (Slate and Whitebird creeks). All but one of the genetic clusters contained multiple populations. The exception was Elk Creek in the Joseph Creek drainage (Grande Ronde River). Pairwise estimates of Nei's genetic distance between clusters ranged from a low of 0.030 (Imnaha River versus Snake River–lower Clearwater River) to a high of 0.192 (South Fork Clearwater River versus Elk Creek; Table 2). The two clusters with the highest average pairwise genetic distances were the upper Clearwater River (0.122) and the South Fork Clearwater River (0.134), and the two clusters with the lowest average pairwise genetic distances were the lower Salmon River (0.072) and the Snake River–lower Clearwater River (0.062).

Results from 100% simulations in ONCOR using the nine reporting groups indicated that seven groups exhibited over 90% mean correct allocation back to reporting group across all populations (Table 3). The two reporting groups that exhibited less than 90% correct allocation were the upper Salmon River (81.8%) and lower Salmon River (88.3%) groups. For the upper Salmon River reporting group, the largest mean misallocation

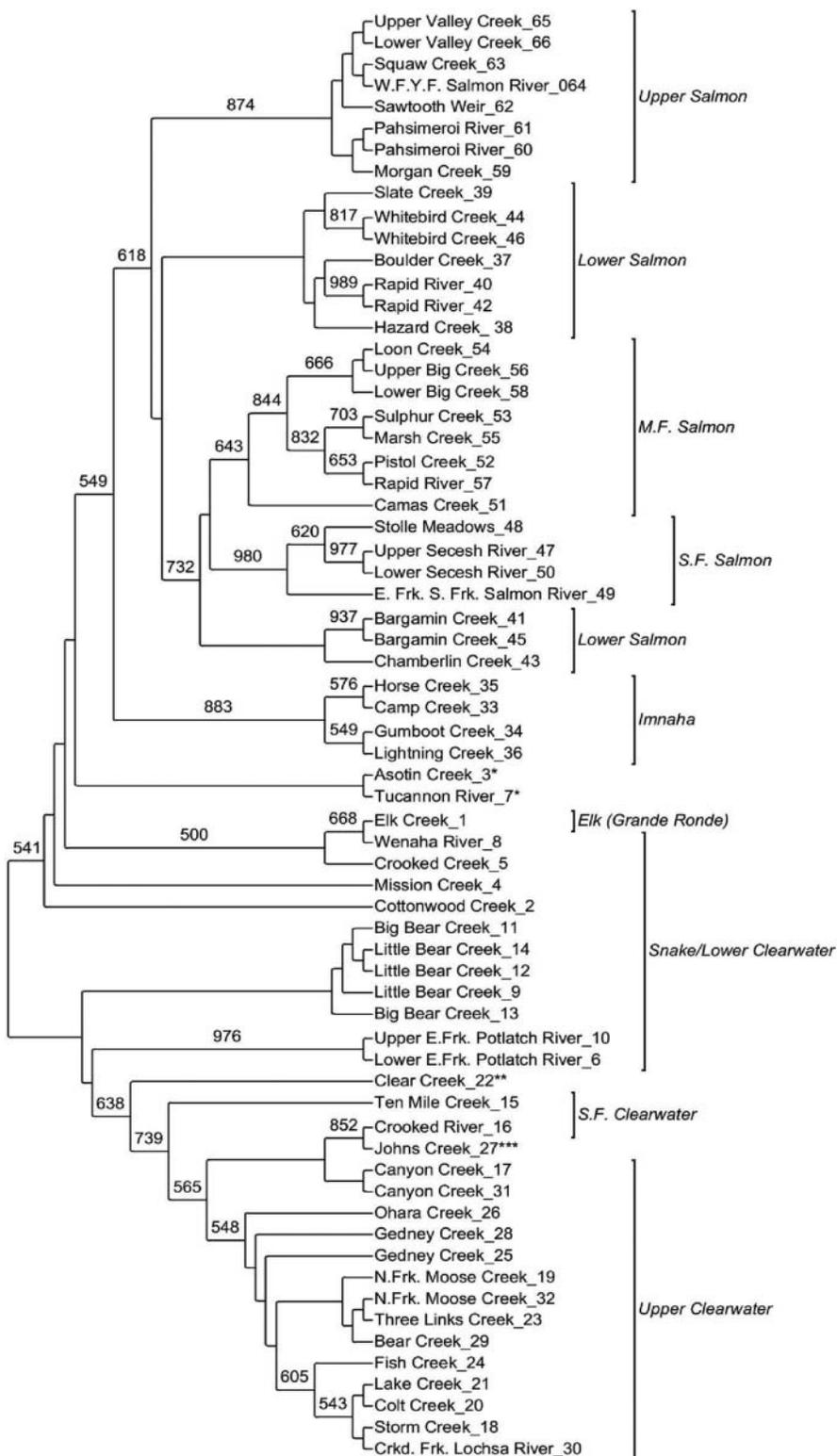


FIGURE 3. Unrooted neighbor-joining dendrogram (Fitch–Margoliash tree based on genetic chord distances [Cavalli-Sforza and Edwards 1967]), showing genetic relationships among the 66 Snake River steelhead baseline sample collections (site number, also defined in Table 1, is presented after each site name). Bootstrap values are only listed if they exceeded 50% of the total iterations (1,000). Italicized names next to brackets refer to cluster (reporting group) designations identified by Bayesian Analysis of Population Structure software (M.F. = Middle Fork; S.F. = South Fork; W.F.Y.F. = West Fork Yankee Fork; Crkd. Frk. = Crooked Fork; * = Asotin Creek_3 and Tucannon River_7 are part of the Snake River–lower Clearwater River reporting group; ** = Clear Creek_22 is part of the upper Clearwater River reporting group; *** = Johns Creek_27 is part of the Snake River–lower Clearwater River reporting group).

TABLE 2. Pairwise Nei's genetic distance estimates for the nine genetic clusters (reporting groups) of steelhead identified in Bayesian Analysis of Population Structure software. Clusters are (1) Elk Creek (Grande Ronde River), (2) Snake River–lower Clearwater River, (3) South Fork Clearwater River, (4) upper Clearwater River, (5) Imnaha River, (6) lower Salmon River, (7) South Fork Salmon River, (8) Middle Fork Salmon River, and (9) upper Salmon River.

Cluster number	Cluster number							
	1	2	3	4	5	6	7	8
2	0.061							
3	0.192	0.094						
4	0.180	0.079	0.062					
5	0.077	0.030	0.137	0.122				
6	0.086	0.044	0.136	0.125	0.039			
7	0.135	0.087	0.185	0.166	0.083	0.064		
8	0.121	0.063	0.149	0.132	0.067	0.035	0.078	
9	0.102	0.038	0.116	0.111	0.048	0.045	0.105	0.086

was to the lower Salmon River reporting group (7.7%). For the lower Salmon River reporting group, the largest mean misallocation was to the Snake River–lower Clearwater River reporting group (4.4%).

Trapping, Sampling, and Age Assignment

Annually, most of the migrating steelhead pass Lower Granite Dam during September and October (Figure 2); during spawn year (SY) 2009, approximately 86% (22,157) of the total

TABLE 3. Results of 100% simulations in ONCOR. The percent allocation of steelhead back to reporting group averaged across all populations is shown. Mean percent correct allocations (in bold italics) are shown along the diagonal.

Actual reporting group	Reporting group allocation								
	Elk Creek (Grande Ronde River)	Snake River–lower Clearwater River	South Fork Clearwater River	Upper Clearwater River	Imnaha River	Lower Salmon River	South Fork Salmon River	Middle Fork Salmon River	Upper Salmon River
Elk Creek (Grande Ronde River)	95.3	3.1	0.0	0.1	0.6	0.5	0.0	0.1	0.2
Snake River–lower Clearwater River	0.4	90.8	0.2	2.6	1.9	2.2	0.1	0.3	1.5
South Fork Clearwater River	0.0	0.7	94.0	4.8	0.1	0.2	0.0	0.1	0.1
Upper Clearwater River	0.0	1.2	1.6	96.3	0.2	0.3	0.1	0.2	0.1
Imnaha River	0.1	5.2	0.0	0.3	91.4	1.7	0.2	0.3	0.8
Lower Salmon River	0.1	4.4	0.0	0.5	1.5	88.3	0.6	1.7	2.9
South Fork Salmon River	0.0	0.5	0.0	0.3	0.6	3.4	94.2	0.9	0.1
Middle Fork Salmon River	0.0	0.7	0.0	0.4	0.3	5.0	0.4	93.1	0.2
Upper Salmon River	0.1	7.3	0.1	0.5	2.3	7.7	0.1	0.2	81.8

TABLE 4. Number of steelhead individuals that were assigned freshwater and ocean ages among fish sampled at Lower Granite Dam during 2008 (X = only the ocean age was assigned).

Ocean age	Freshwater age					
	X	1	2	3	4	5
1	13	5	175	204	27	1
2	35	2	279	158	25	0
3	5	0	20	19	0	0

escapement passed the dam during our sampling period. The estimate of total escapement of wild steelhead that migrated past Lower Granite Dam for the entire run year (between July 1, 2008, and June 30, 2009) was 25,764 (95% CI = 20,301–31,673; Schrader et al. 2011).

In total, 998 scale samples were viewed in an attempt to assign ages (Table 4). Of these, 968 were assigned an ocean age, 915 were assigned both freshwater and ocean ages, and 30 could not be aged. Of 29 fish with known ocean ages from passive integrated transponder tags, 28 fish were aged accurately; thus, the accuracy of age assignments was estimated at 97%. Freshwater ages ranged from 1 to 5 years, and ocean ages ranged from 1 to 3 years (Table 4). More than half (543/968 = 56.1%) of the fish had spent a minimum of 2 years in the ocean, which was previously believed to occur predominantly in B-run stocks. Nearly all of the fish had smolted at 2 or 3 years of age (908/915 = 99.2%). This is in sharp contrast to Snake River hatchery steelhead, which almost exclusively undergo smoltification after 1

year in freshwater (PTAGIS 2011). Total ages at the time of sampling ranged from 2 to 6 years. The length distribution was bimodal, and the proportion of older and larger fish increased over the course of the run (Figure 4).

A total of 1,092 samples were extracted and genotyped. Of these, 1,076 (98.5%) samples yielded complete genotypes (≥ 10 loci), and only those samples were used in GSI analyses. Because biological information (sex, length, and age) for some samples was incomplete, some mixture analyses were run with a total sample size less than 1,076 (all ≥ 914).

The largest contributor to the aggregate run passing Lower Granite Dam was the Snake River–lower Clearwater River reporting group, with a mean of 36.1% (95% CI = 30.2–39.7%; Figure 5a), followed by the upper Clearwater River reporting group (mean = 15.4%; 95% CI = 12.8–18.7%) and the lower Salmon River reporting group (13.9%; 95% CI = 12.5–18.7%). The remaining reporting groups each contributed less than 10% to the overall mixture. Mean contributions were 9.5% (95% CI = 6.8–13.6%) from the Imnaha River, 9.2% (5.1–11.3%) from the upper Salmon River, 7.6% (4.3–8.9%) from the South Fork Clearwater River, 5.1% (3.5–6.4%) from the Middle Fork Salmon River, 2.7% (1.3–3.6%) from the South Fork Salmon River, and 0.5% (0.0–1.2%) from Elk Creek.

Sex ratio was female biased for the 1,066 samples in which sex was identified using the genetic sex assay. Of the 1,066 samples, 372 were males (34.9%) and 694 were females (65.1%). Mixture analyses with samples grouped by sex did not identify any significant differences in reporting group contributions between males and females (all comparisons yielded overlapping 95% CIs; Figure 5b).

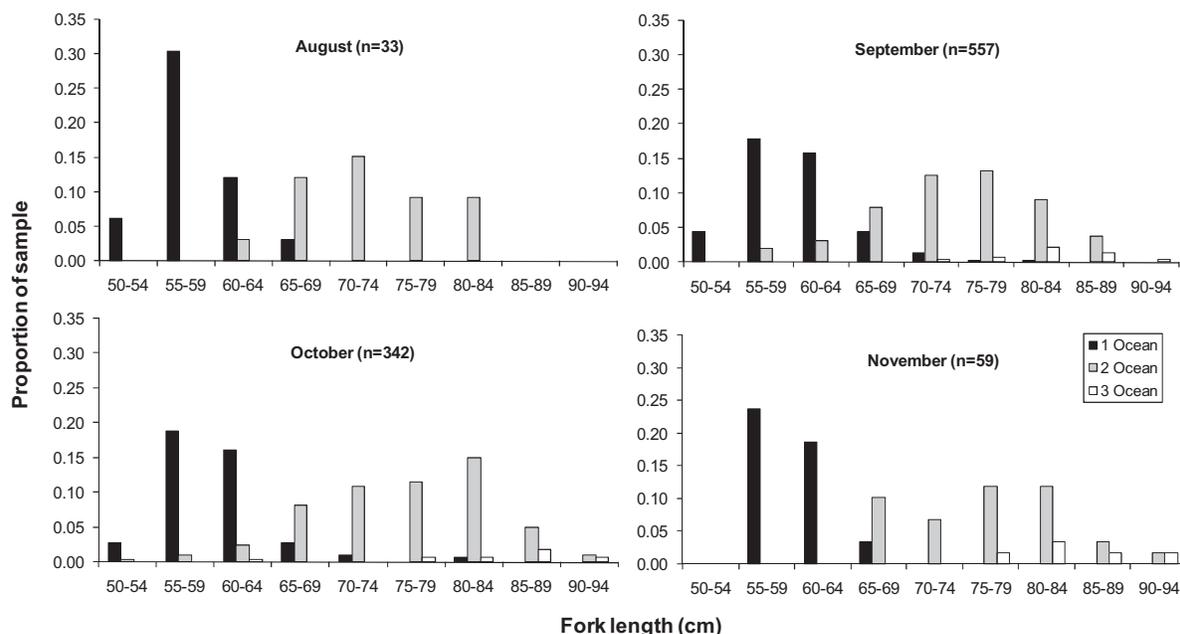


FIGURE 4. Length frequency of steelhead by ocean age for each month of collection at Lower Granite Dam during 2008 (ages were determined from scale samples).

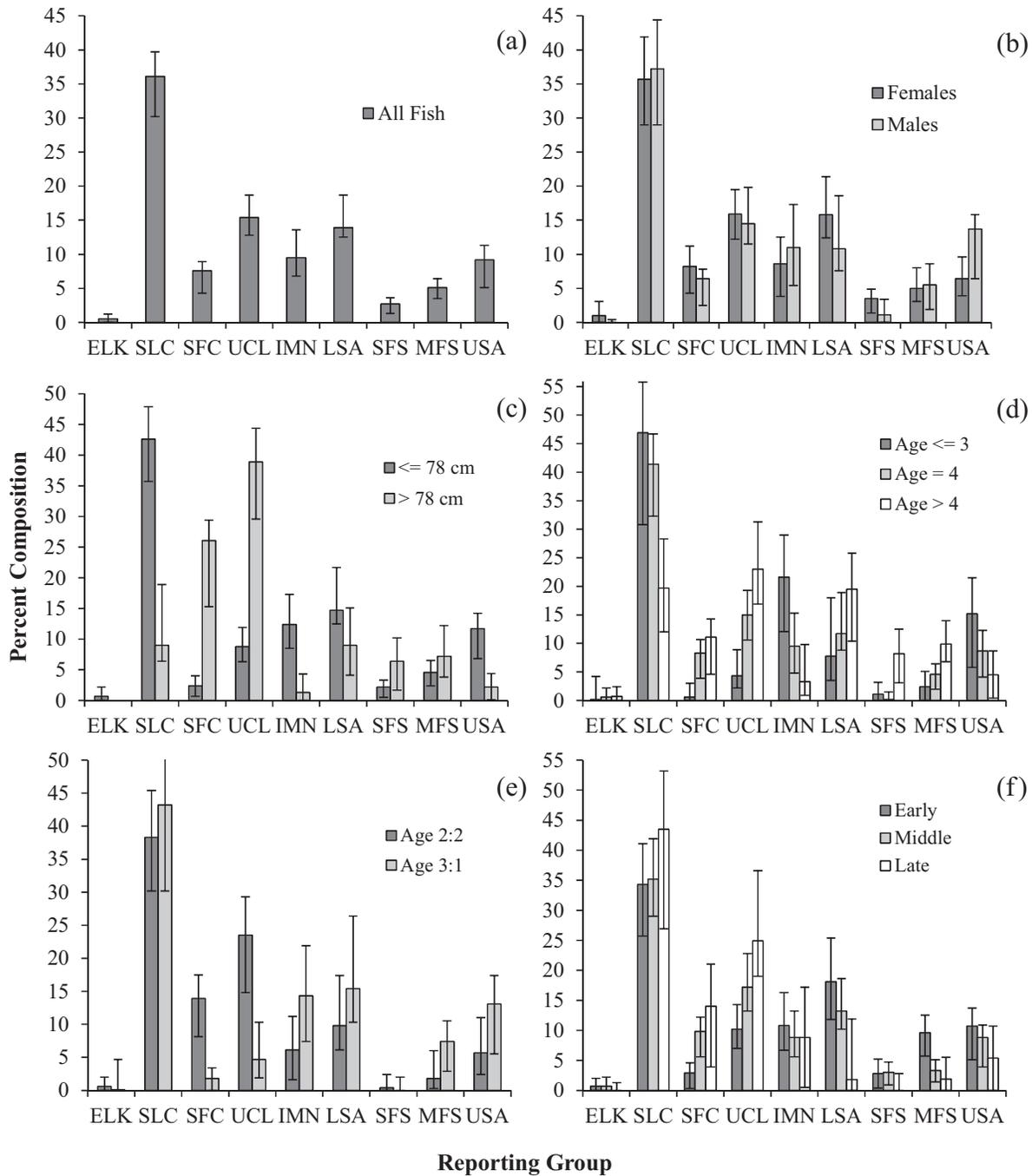


FIGURE 5. Estimated percent contributions ($\pm 95\%$ confidence interval) from nine reporting groups for mixtures of adult steelhead passing Lower Granite Dam: (a) all adults, (b) adults grouped by sex, (c) adults grouped by fork length (≤ 78 or > 78 cm), (d) adults grouped by combined age (years; freshwater age + ocean age), (e) 4-year-old adults grouped by freshwater years: ocean years (age 2:2 or age 3:1), and (f) adults grouped by run timing. Reporting groups are Elk Creek (Grande Ronde River; ELK), Snake River–lower Clearwater River (SLC), South Fork Clearwater River (SFC), upper Clearwater River (UCL), Innaha River (IMN), lower Salmon River (LSA), South Fork Salmon River (SFS), Middle Fork Salmon River (MFS), and upper Salmon River (USA).

Stock composition of each reporting group when analyzed by fork length was noticeably varied across mixtures (Figure 5c). For 78-cm and smaller adults ($N = 767$), the largest contributor to the mixture was the Snake River–lower Clearwater River

reporting group at 42.6% (95% CI = 35.7–47.9%), followed by the lower Salmon River (14.7%; 12.5–21.7%), Innaha River (12.4%; 8.4–17.3%), and upper Salmon River (11.7%; 6.8–14.2%) reporting groups. The remaining reporting groups each

contributed less than 10% to the overall mixture of smaller fish. For adults larger than 78 cm ($N = 229$), the greatest contributor was the upper Clearwater River reporting group (mean = 38.9%; 95% CI = 29.4–44.4%), followed by the South Fork Clearwater River reporting group (26.1%; 15.3–29.4%). All of the remaining reporting groups each contributed less than 10% to the overall mixture of larger adults. Besides the upper Clearwater River and South Fork Clearwater River groups, the South Fork Salmon River and Middle Fork Salmon River were the only other reporting groups for which overall contributions were greater in the B-run mixture (>78 cm) than in the A-run mixture (≤ 78 cm).

We observed different patterns of stock composition when samples were grouped by combined age (freshwater age + ocean age; Figure 5d). The largest contributors to the mixture of age-3 and younger adults ($N = 182$) were the Snake River–lower Clearwater River (mean = 46.9%; 95% CI = 30.8–55.9%), Imnaha River (21.58%; 12.1–29.0%), and upper Salmon River (15.2%; 5.8–21.5%) reporting groups. However, the contribution of these three reporting groups to the mixture of age-4 adults ($N = 483$) was substantially lower, and their contribution to the mixture of age-5 and older adults ($N = 250$) exhibited a further decrease. Moreover, both the Imnaha River (mean = 3.3%; 95% CI = 0.9–9.8%) and the upper Salmon River (4.5%; 4.1–8.7%) reporting groups were among the lowest contributors to age-5 and older adults. The opposite trend was observed among the remaining reporting groups, for which the contributions increased as mixture ages increased. The largest contributor to the mixture of age-5 and older adults was the upper Clearwater River, followed by the Snake River–lower Clearwater River (mean = 19.7%; 95% CI = 12.0%–28.3), lower Salmon River (19.5%; 10.4–25.8%), and South Fork Clearwater River (11.1%; 4.6–14.3%). The remaining reporting groups contributed less than 10% to the overall mixture of age-5 and older adults, although all made their highest contributions to this age-group.

Differences in reporting group contributions were also observed when 4-year-old adults were separated into their two respective age-classes (freshwater years: saltwater years = 2:2 or 3:1; Figure 5e). The South Fork Clearwater River and upper Clearwater River reporting groups contributed significantly more to the mixture of age-2:2 adults (South Fork Clearwater: mean = 13.9%, 95% CI = 7.2–17.0%; upper Clearwater: mean = 23.5%, 95% CI = 15.6–29.7%) than to the mixture of age-3:1 adults (South Fork Clearwater: mean = 1.8%, 95% CI = 0.0–3.3%; upper Clearwater: mean = 4.7%, 95% CI = 2.3–11.0%). Contrasting results were observed among the Imnaha, lower Salmon, Middle Fork Salmon, and upper Salmon River reporting groups, which contributed more to the age-3:1 mixture than to the age-2:2 mixture.

Drainage-specific trends were apparent when separating samples by run timing (Figure 5f). Reporting groups associated with the Clearwater River drainage (Snake River–lower Clearwater River, South Fork Clearwater River, and upper Clearwater River) exhibited a trend of increasing contributions to their

respective mixtures as the run progressed. Conversely, reporting groups associated with the Salmon River (lower Salmon River, South Fork Salmon River, Middle Fork Salmon River, and upper Salmon River) all exhibited a trend of decreasing contributions throughout the run. No clear patterns of increasing or decreasing contributions during the run were observed for the Elk Creek or Imnaha River reporting group.

DISCUSSION

Our results are consistent with more recent genetic investigations indicating that steelhead within and outside the Snake River basin exhibit a complicated pattern of genetic structuring that is partitioned at multiple spatial scales according to environmental and habitat parameters and the influence of hatchery introgression (Nielsen et al. 2009; Blankenship et al. 2011). Our reporting groups were generally correlated with single, terminal river drainages situated at higher elevations and in areas that have been managed for wild populations. However, we also observed large reporting clusters that encompassed main-stem areas and multiple drainages, suggesting interdrainage gene flow. This may be due to similarities in elevation and geology within these areas, leading to similarities in life history timing (emigration and spawning) that would permit successful straying among drainages, thereby reducing population structure. Introgression from hatchery steelhead may have also influenced genetic structure in the main-stem Salmon River, Little Salmon River, and lower Snake River areas (Nielsen et al. 2009), which correspond to the Snake River–lower Clearwater River and lower Salmon River clusters we identified in this study.

The reporting groups we identified were delineated strictly by genetic relationships and in many instances do not follow the populations identified by the Interior Columbia Basin Technical Recovery Team (ICBTRT; ICBTRT 2003). The ICBTRT designations were based largely on a drainage-level geographic hierarchy supplemented with genetic information (Moran and Waples 2004). However, the available genetic data at that time had limited representation from the Idaho portion of the basin, and there was a paucity of data on spawning distributions, natural levels of straying, and hatchery influence within the basin. Results from this study indicate that the construction of fine-scale genetic baselines will contribute to efforts to refine population delineations in the Snake River evolutionarily significant unit for viability assessments.

Using the nine identified reporting groups, we were for the first time able to apportion the adult steelhead escapement to the Snake River basin according to geographic stock structure. Such abundance data were not available to support earlier conservation assessments (Busby et al. 1996; Good et al. 2005). During the SY 2009 escapement period, the largest proportion of adults passing Lower Granite Dam were from the Snake River–lower Clearwater River reporting group, and the remaining contributions ranged from 2.7% (95% CI = 1.3–3.6%; South Fork Salmon River) to 15.4% (12.8–18.7%; upper Clearwater

River). The bulk of the run (65%) consisted of three reporting groups (Snake River–lower Clearwater River, upper Clearwater River, and lower Salmon River), with lesser contributions from the South Fork Salmon River, Middle Fork Salmon River, and upper Salmon River reporting groups. The reason for this disproportionate contribution is not immediately apparent. For example, the habitat of the Middle Fork Salmon River group is largely in protected wilderness with minimal anthropogenic impacts (Thurow 2000), yet the contribution of this group was relatively small (5.1%). Conversely, the Snake River–lower Clearwater River reporting group is from an area with relatively high human population densities and concomitant environmental disturbance. Contributions to the overall mixture were much lower for the Middle Fork Salmon River (mean = 5.1%; 95% CI = 3.5–6.4%) and South Fork Salmon River (2.7%; 1.3–3.6%) reporting groups. With an estimated run size of 25,764 wild steelhead migrating past Lower Granite Dam in SY 2009, approximately 1,314 (95% CI = 902–1,649) adults returned to the Middle Fork Salmon River and 696 (95% CI = 335–928) adults returned to the South Fork Salmon River. These estimates are below the critical population thresholds for these drainages as suggested by the ICBTRT (ICBTRT 2003) and are similar to escapement estimates proposed for these basins in the mid-1980s (Howell et al. 1985).

Beyond providing abundance estimates, our results suggest that the GSI methodologies applied to steelhead at Lower Granite Dam could contribute to documenting and monitoring a variety of diversity traits that are important for the viability of Snake River steelhead, including sex ratio, age and size at return, and run timing. We found that females comprised the majority (>65%) of the adult steelhead run passing Lower Granite Dam. This is not surprising because anadromy should benefit females more than males (Hendry et al. 2004). Sex ratios skewed toward females have been observed in adult steelhead populations throughout the species' range, including California, Alaska, the Columbia River basin, and the Kamchatka Peninsula in Russia (Savvaitova et al. 1997; Hendry et al. 2004; Christie et al. 2011; Hanson et al. 2011). Female-biased sex ratios in steelhead have been attributed to two separate life history behaviors: the predominance of residualization among males and the tendency of anadromous females to spawn more than once (Savvaitova et al. 1997; McMillan et al. 2007). Hydropower dams and distance from the ocean likely prevent most (if not all) successful iteroparous behavior in the Snake River basin (Keefer et al. 2008; Narum et al. 2008). The most likely explanation for the skewed sex ratios that we observed is the residualization of large numbers of males during freshwater rearing. This life history behavior may have been under a higher selective pressure over the last 40 years due to increased mortality associated with anadromy and may have helped to maintain the abundance and diversity of wild steelhead throughout the Snake River basin.

Our results, along with recent population genetic structure analyses (Nielsen et al. 2009), suggest that the reporting and management of Snake River steelhead by using designations

based solely on fish length should be re-evaluated. The use of length criteria for stock delineation is clearly antiquated given the observed variation in freshwater and ocean residence periods and the evidence that all stocks produce both smaller-size or younger-age returning adults (i.e., A-run fish) and larger-size or older-age returning adults (i.e., B-run fish).

We believe that the GSI methodology that was employed to identify steelhead composition at Lower Granite Dam will prove to be an efficient and minimally intrusive tool for obtaining stock-specific abundance and life history information on Snake River steelhead. Small fin tissue samples can be obtained nonlethally from a subsample of returning adult steelhead each year, with minimal handling time and stress. In addition, almost all of the fish that we handled and sampled were successfully genotyped, thus indicating that few fish will undergo handling without ultimately contributing to GSI analyses. These are important considerations for monitoring efforts that involve an Endangered Species Act-listed species. Finally, the addition of an accurate genetic marker for sex provides new opportunities to examine sex-specific demographic processes that may influence population abundance and productivity.

Although the results of this initial study clearly demonstrate the possibilities of GSI technology as a tool for management and conservation of Snake River steelhead, there are still significant opportunities to improve the accuracy, precision, and efficiency of GSI techniques. Bias can be introduced into GSI estimation in several ways. If a significant portion of the escapement originates from populations that are not represented in the baseline, this will lead to misallocation and inaccurate contribution estimates. Because our baseline data set was constructed opportunistically from sampling and genotyping that were not specifically performed for GSI work in the Snake River basin, several important areas or drainages either were not represented or were underrepresented (i.e., North Fork Salmon River, Lolo Creek, Lemhi River, and upper Grande Ronde River). Future sampling should target these areas to determine their potential influence in genetic characterization of existing reporting groups or perhaps in redefining the reporting group delineations. Temporal sampling of the populations that are already included in our baseline will both increase sample sizes (improving allele frequency estimation) and test the stability of the baseline over time. In addition, as more samples become available, there will be increased opportunities for using known-origin individuals for independent testing of the baseline's accuracy beyond the simulation procedures performed here.

In addition to sampling-related issues, we are also interested in the utility of single-nucleotide polymorphic markers (SNPs) for improving GSI analyses in the Snake River basin. The SNPs are amenable for large-scale GSI efforts because they are abundant in the genomes of most organisms and are easily detected with recently developed DNA sequencing technologies (Metzker 2010). In addition, they are generally bi-allelic, which allows highly automated, rapid genotyping (Schlötterer 2004; Van Tassell et al. 2008; Seeb et al. 2009). Further, SNPs can be

found in the coding regions and *cis*—regulatory regions influenced by selection (Helyar et al. 2011), and research has shown that SNPs under diversifying selection may provide increased accuracy and precision in GSI analyses because these loci can exhibit higher differentiation among geographically proximate populations (Habicht et al. 2010; Ackerman et al. 2011).

We are currently working on expanding our sample and genetic marker baselines, and we expect that GSI methods will contribute substantially to future population viability assessments for steelhead in the Snake River basin, providing previously unavailable information on population abundance, productivity, spatial structure, and diversity.

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APPENDIX: Y-Chromosome-Specific Assays (*Omy_SEXY1* and *Omy_SEXY*)

The Y-chromosome-specific assays developed in this study to differentiate sex in steelhead were modified from markers developed by Brunelli et al. (2008) to run in two different configurations: as a TaqMan-based allelic discrimination

assay and as a presence–absence assay in one of the multiplex microsatellite panels screened on steelhead. For the TaqMan-based allelic discrimination assay (*Omy_SEXY1*), we used published primers (Brunelli et al. 2008) and unpublished primers (J. Brunelli, Washington State University, personal communication) to sequence a Y-chromosome region

TABLE A.1. Quantity and concentration of PCR reagents used in the TaqMan-based allelic discrimination configuration of the Y-chromosome-specific assay for steelhead. Primer and probe sequences are also shown (6-FAM = 6-carboxyfluorescein; VIC = 2'-chloro-7'-phenyl-1,4-dichloro-6-carboxyfluorescein; MGB = minor groove binder; NFQ = nonfluorescent quencher).

Quantity (μL)	Concentration	Reagent	Primer or probe sequence
5	10 \times	TaqMan Master Mix	
0.0225	100 μM	<i>OmyY1</i> probe e2	6-FAM-CCT ACC AAG TAC AGC CCC AA-MGB-NFQ
0.0050	100 μM	<i>OmyA</i> probe e500	VIC-GAG GGG TAG TCG TTT GTT CG-MGB-NFQ
0.0513	100 μM	<i>OmyY1.4F</i> primer	5'-CAC AAC ATG AGC TCA TGG G-3'
0.0513	100 μM	<i>OmyY1.4R</i> primer	5'-CGA TTA GAA AGG CCT GCT TG-3'
0.0100	100 μM	<i>OmyA</i> forward primer	5'-GCC TGC TTG CAG AAG TTT TT-3'
0.0100	100 μM	<i>OmyA</i> reverse primer	5'-CTT GAC TGT GTC CAG CTT GC-3'
3.8500	100 μM	Distilled H ₂ O	
1	Unknown	Template DNA	

(*OmyY1*; GenBank accession number EU081756) and to develop a 5' exonuclease assay that amplifies a Y-specific product along with an autosomal product that acts as a control. These products are interrogated using fluorogenic probes (TaqMan chemistry, Applied Biosystems, Inc. [ABI], Foster City, California). Primer and probe sequences and PCR protocols for the TaqMan-based assay are summarized in Table A.1. Thermal cycling conditions were 95°C for 10 min followed by 55 cycles of 92°C for 15 s and 60°C for 1 min. Sex identification is accomplished through analysis of allelic discrimination plots of endpoint fluorescence using an

ABI 7500 Real-Time PCR instrument (Figure A.1). The carboxyfluorescein (FAM) fluorophore (y-axis) is associated with the probe for the Y-specific product (males), while the 2'-chloro-7'-phenyl-1,4-dichloro-6-carboxyfluorescein (VIC) fluorophore (x-axis) labels the autosomal product. Samples that exhibit fluorescence from both FAM and VIC are scored as male. Samples that exhibit VIC fluorescence but not FAM fluorescence are scored as female. Samples that exhibit low or no fluorescence for both FAM and VIC are scored as "no call." The sex typing accuracy for *Omy.SEXY1* was evaluated by genotyping 135 known phenotypic male broodstock and

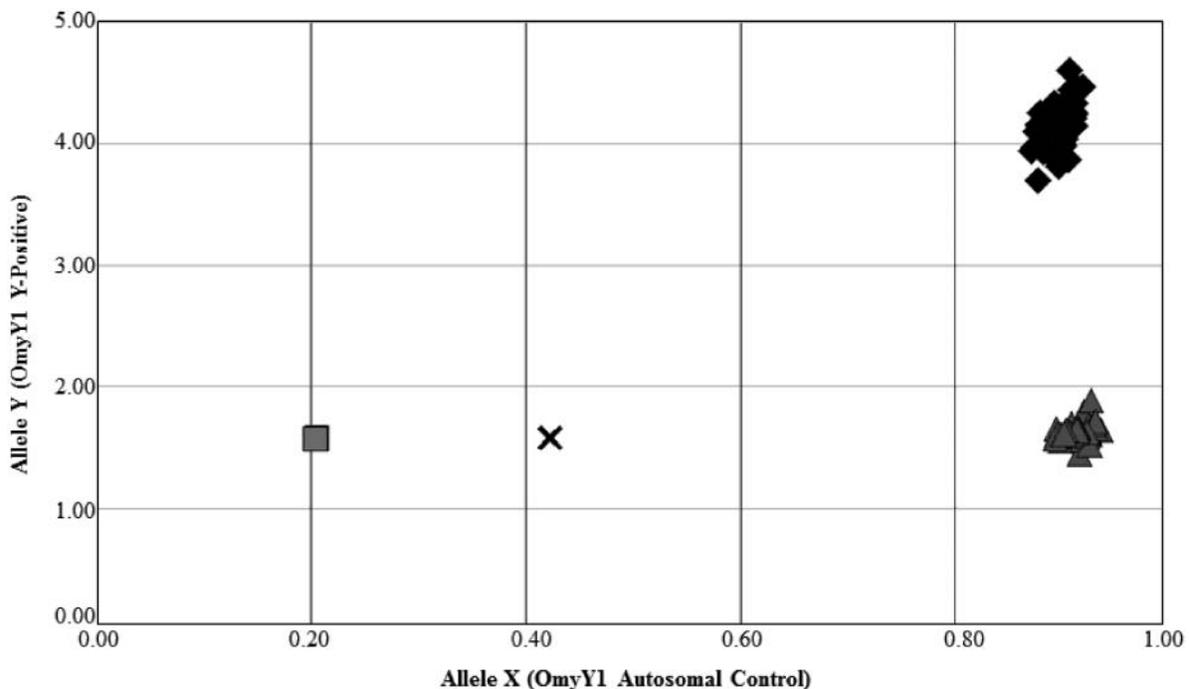


FIGURE A.1. An allelic discrimination plot, showing diagnostic clustering of male (diamonds) and female (triangles) steelhead by using a modified Y-specific assay (*Omy.SEXY1*). Samples identified by triangles amplified the autosomal *OmyA* locus only and are considered females. Samples identified by diamonds amplified both the autosomal *OmyA* locus and the Y-specific locus and are identified as males. The square represents a no-template control sample; the X represents a sample that failed to amplify properly and was not assigned a gender.

144 known phenotypic female broodstock from three Snake River steelhead hatcheries (Dworshak National Fish Hatchery, Sawtooth Fish Hatchery, and Wallowa Fish Hatchery). By following the procedures described above, 1 of the 135 known males was incorrectly identified as female, and the remaining 134 known males were correctly identified as males, thus yielding an overall accuracy of 99.2% (134/135). Of the 144 known females, 1 was scored as no call, 1 was incorrectly identified as male, and 142 were correctly identified as females. Based on the 143 samples scored (i.e., excluding the no-call sample), the overall accuracy for known females was 99.3% (142/143).

For the presence-absence assay (*Omy_SEXY*), we included a 5'-6-FAM fluorescently labeled, unpublished forward primer (OmyY1.2F; J. Brunelli, personal communication) and the reverse primer (OmyY1R) from Brunelli et al. (2008) with three microsatellite loci in a multiplex PCR amplification. Primer sequences, probe sequences, and PCR protocols for this assay are summarized in Table A.2. Thermal cycling conditions were 95°C for 15 min followed by 34 cycles of 94°C for 30 s, 57°C for 1 min 30 s, and 72°C for 60 s, and then a final extension of 60°C for 30 min.

The Y-chromosome-specific product amplified in this multiplex PCR was approximately 465 bp in length and was identified following capillary array electrophoresis using an ABI 3100 genetic fragment analyzer. The following rules were applied when conducting sex discrimination (Figure A.2): (1) any individual that amplified at the other loci in the panel and exhibited

TABLE A.2. Quantity of primer mix (concentration = 100 μ M for all) used in the presence-absence multiplex PCR configuration of the Y-chromosome-specific assay for steelhead. Primer and probe sequences are also shown. Once the primer mix has been made, the PCR is run in a 5- μ L volume on the 7500 Real-Time PCR instrument with 0.12 μ L of primer mix, 2.50 μ L of Qiagen Master Mix (catalog number 206143), 1.38 μ L of distilled H₂O, and 1.00 μ L of template DNA (unknown concentration; NED = 2'-chloro-5'-fluoro-7',8'-fused phenyl-1,4-dichloro-6-carboxyfluorescein; 6-FAM = 6-carboxyfluorescein; VIC = 2'-chloro-7'-phenyl-1,4-dichloro-6-carboxyfluorescein).

Quantity (μ L)	Reagent	Primer or probe sequence
2.82	Omy1001F	5'-NED-GAT TCC ATA ACC TCG CCT TC-3'
2.82	Omy1001R	5'-GTC CTT GTG CTG CCT GCT-3'
2.12	Omy7F	5'-6-FAM-TTA AGT TTT GCC TAG ATA AGG G-3'
2.12	Omy7R	5'-CAA GGA ATG GCA CAG CTT G-3'
0.36	Ogo4F	5'-VIC-GTC GTC ACT GGC ATC AGC TA-3'
0.36	Ogo4R	5'-GAG TGG AGA TGC AGC CAA AG-3'
1.06	OmyY1.2F	5'-6-FAM-GCT AAT GGA CGA CGC TTT TC-3'
1.06	OmyY1R	5'-CGA TTA GAA AGG CCT GCT TG-3'

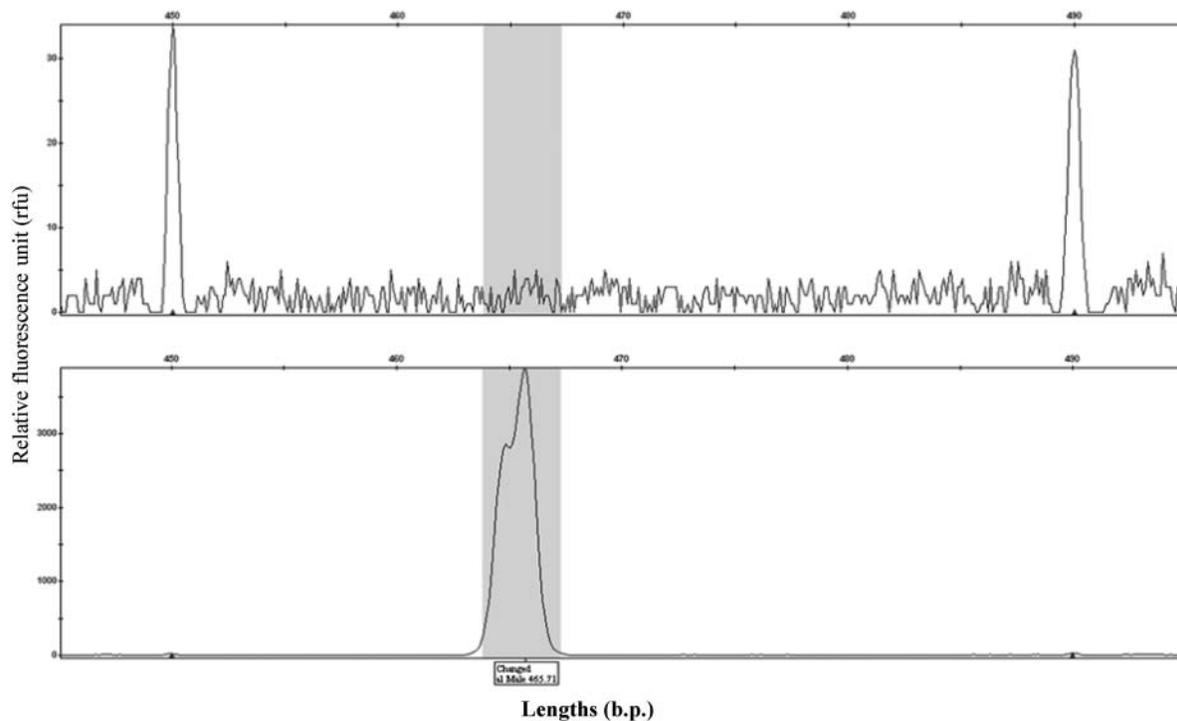


FIGURE A.2. Examples of electropherograms, showing absence (top) and presence (bottom) of the steelhead Y-chromosome-specific product (~465 bp) that was amplified in a multiplex PCR. The y-axis shows relative fluorescence units (RFUs), and the x-axis shows estimated length of the Y-chromosome-specific product (i.e., male "peak"). Samples with an observed peak greater than 1,000 RFUs were scored as male. Samples that exhibited a peak between 100 and 1,000 RFUs were scored as "no call."

an *OmyY1* peak greater than 1,000 relative fluorescence units (RFUs) was scored as male; (2) samples that exhibited a peak between 100 and 1,000 RFUs were scored as no call; (3) samples that failed to amplify at the other loci in the panel were scored as no call regardless of the peak-height RFUs at *OmyY1*; and (4) any individual that amplified at the other loci in the panel and exhibited either no peak or a peak less than 100 RFUs was scored as female. The sex typing accuracy for *Omy_SEXY* was evaluated by genotyping 630 known phenotypic male broodstock and 297 known phenotypic female broodstock from the Oxbow Fish Hatchery. Using the scoring rules described above, 4 of the 630 known males were scored as no call, 5 were incorrectly identified as females, and 621 were correctly identified

as males. Based on the 626 samples scored (excluding the no-call samples), the overall accuracy for known males was 99.2% (621/626). Of the 297 known females, 7 were scored as no call, 4 were incorrectly identified as males, and 286 were correctly identified as females. Based on the 290 samples scored, the overall accuracy for known females was 98.6% (286/290).

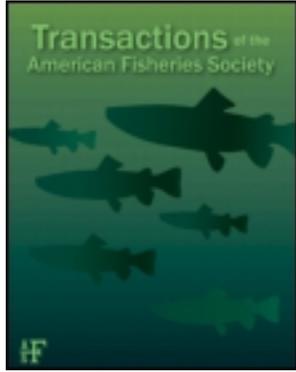
In this study, we screened all adult samples from Lower Granite Dam by using the presence-absence configuration of the Y-chromosome-specific assay. We also screened a total of 327 samples by using the TaqMan-based allelic discrimination configuration of the assay. For the 327 samples in which both assay configurations were run, concordance was high (99.4%; 325/327).

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Effects of Activity and Energy Budget Balancing Algorithm on Laboratory Performance of a Fish Bioenergetics Model

Charles P. Madenjian^a, Solomon R. David^b & Steven A. Pothoven^c

^a U.S. Geological Survey, Great Lakes Science Center, 1451 Green Road, Ann Arbor, Michigan, 48105, USA

^b School of Natural Resources and Environment, University of Michigan, 440 Church Street, Ann Arbor, Michigan, 48109, USA

^c National Oceanic and Atmospheric Administration, Great Lakes Environmental Research Laboratory, Lake Michigan Field Station, 1431 Beach Street, Muskegon, Michigan, 49441, USA

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ARTICLE

Effects of Activity and Energy Budget Balancing Algorithm on Laboratory Performance of a Fish Bioenergetics Model

Charles P. Madenjian*

U.S. Geological Survey, Great Lakes Science Center, 1451 Green Road, Ann Arbor, Michigan 48105, USA

Solomon R. David

School of Natural Resources and Environment, University of Michigan, 440 Church Street, Ann Arbor, Michigan 48109, USA

Steven A. Pothoven

National Oceanic and Atmospheric Administration, Great Lakes Environmental Research Laboratory, Lake Michigan Field Station, 1431 Beach Street, Muskegon, Michigan 49441, USA

Abstract

We evaluated the performance of the Wisconsin bioenergetics model for lake trout *Salvelinus namaycush* that were fed ad libitum in laboratory tanks under regimes of low activity and high activity. In addition, we compared model performance under two different model algorithms: (1) balancing the lake trout energy budget on day t based on lake trout energy density on day t and (2) balancing the lake trout energy budget on day t based on lake trout energy density on day $t + 1$. Results indicated that the model significantly underestimated consumption for both inactive and active lake trout when algorithm 1 was used and that the degree of underestimation was similar for the two activity levels. In contrast, model performance substantially improved when using algorithm 2, as no detectable bias was found in model predictions of consumption for inactive fish and only a slight degree of overestimation was detected for active fish. The energy budget was accurately balanced by using algorithm 2 but not by using algorithm 1. Based on the results of this study, we recommend the use of algorithm 2 to estimate food consumption by fish in the field. Our study results highlight the importance of accurately accounting for changes in fish energy density when balancing the energy budget; furthermore, these results have implications for the science of evaluating fish bioenergetics model performance and for more accurate estimation of food consumption by fish in the field when fish energy density undergoes relatively rapid changes.

Fish bioenergetics models have frequently been applied to problems and issues in fishery science (Hansen et al. 1993; Bajer et al. 2004; Madenjian 2011). Bioenergetics modeling has been instrumental in estimating the strength of the predator-prey trophic link in food webs (Madenjian 2011). For example, Stewart et al. (1981) developed bioenergetics models for salmon and trout and then applied these models to populations in Lake Michigan. Results indicated that each year, the salmonine populations were consuming as much as 33% of the annual pro-

duction of alewives *Alosa pseudoharengus*, the favored prey of the salmonines in Lake Michigan. Stewart et al. (1981) warned fishery managers that the alewife population was headed for a collapse due to predation by salmonines. Heeding the warning, fishery managers began reducing the stocking rates of Chinook salmon *Oncorhynchus tshawytscha* into Lake Michigan during the 1980s (Hansen et al. 1993), and stocking reductions have continued through the 1990s and 2000s (Bence and Smith 1999; Claramunt et al. 2009). Bioenergetics modeling has also been

*Corresponding author: cmadenjian@usgs.gov
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used to assess the effects of various factors on fish growth in lakes (Hayward and Margraf 1987; Madenjian et al. 1998), to assess the role of phosphorus excretion by fish populations in the phosphorus cycling within aquatic ecosystems (Kraft 1993; Bunnell et al. 2005), and to identify the major factors regulating contaminant accumulation in fish (Weininger 1978; Stow et al. 1995).

Despite frequent applications of fish bioenergetics models to fisheries problems, few evaluations of bioenergetics model performance were conducted prior to 1993 (Hansen et al. 1993; Ney 1993). Both Hansen et al. (1993) and Ney (1993) agreed that further testing and evaluation of fish bioenergetics models were needed. Evaluations of fish bioenergetics models, both in the laboratory and in the field, have ensued (Bajer et al. 2003; Chipps and Wahl 2004; Lantry et al. 2008).

Based on results from these fish bioenergetics model evaluations, Bajer et al. (2004) concluded that fish bioenergetics models contained a consumption-dependent systematic error that would cause the models to underestimate consumption when feeding rates were relatively high. These researchers reasoned that the bias was likely due to inaccurate submodels for energy budget components associated with feeding rate. Egestion, excretion, and specific dynamic action (SDA) have typically been modeled as functions of feeding rate in most fish bioenergetics models. Bajer et al. (2004) recommended that additional laboratory work be conducted to measure egestion, excretion, and SDA over broad ranges of consumption level, fish body weight, temperature, and prey type.

Based on findings by Christiansen and Jobling (1990), Madenjian and O'Connor (1999) suggested that fish bioenergetics models' underestimation of consumption at high feeding rates might be an artifact of fish being confined to a laboratory tank and thus having limited swimming activity. Arctic char *Salvelinus alpinus* that were exercised in laboratory tanks exhibited higher gross growth efficiencies (GGEs) than relatively inactive Arctic char (Christiansen and Jobling 1990), and these results indicated that the resting metabolic rate of the inactive fish was actually higher than that of the active fish. To test this idea, Madenjian and O'Connor (1999) proposed that bioenergetics model performance be evaluated in the laboratory for both active and inactive lake trout *S. namaycush* fed an ad libitum ration.

Another factor potentially influencing the laboratory performance of fish bioenergetics models was the model algorithm used to balance the fish's energy budget. To balance the energy budget on day t , Hanson et al. (1997) based their calculations on the fish's energy density on day t (algorithm 1). However, Hewett and Johnson (1987) used the fish's energy density on day $t + 1$ to balance the fish's energy budget on day t (algorithm 2). For the case of a constant fish energy density over time, the two algorithms will yield identical results. However, if the energy density of the fish changes over time, then the two algorithms will yield different results.

The overall goal of this study was to determine whether the effects of fish activity and energy budget balancing algorithm could be responsible for the above-mentioned underestimation of consumption by fish that are fed at a relatively high rate in the laboratory. The specific objective of the study was to determine whether significant bias could be detected in Wisconsin bioenergetics model predictions of consumption and growth based on algorithms 1 and 2 for lake trout at two activity levels (inactive and active) in laboratory tanks. The implications of our findings with regard to fish bioenergetics model evaluation are discussed. We also discuss the importance of properly accounting for changes in fish energy density while generating estimates of food consumption by fish in the field when fish energy density is undergoing relatively rapid changes.

METHODS

Laboratory experiment.—The laboratory experiment was conducted during 16 February–1 July 2010. Lake trout of the Seneca Lake strain were obtained from the Sullivan Creek National Fish Hatchery (U.S. Fish and Wildlife Service, Brimley, Michigan) in September 2009, when average weight of the fish was approximately 600 g (age = 44 months). The fish were fed pelletized commercial trout food at the hatchery, and we continued to administer the same diet during September through November 2009. Beginning in December 2009, the lake trout were acclimated to a diet of bloaters *Coregonus hoyi*, and the acclimation period continued through 15 February 2010. The bloater was selected as the food source because this species has served as a native prey for lake trout in the Laurentian Great Lakes (Madenjian et al. 1998).

Lake trout were maintained in iron-filtered well water at the Great Lakes Science Center in four 2,380-L circular fiberglass tanks (tanks 1–4; water exchange rate = 15 L/min) and four 870-L circular fiberglass tanks (tanks 5–8; water exchange rate = 5 L/min). Using centrifugal pumps, average water velocities in tanks 1–4 were maintained at 16.1, 17.1, 14.4, and 15.6 cm/s, respectively, based on readings from 36 locations in each tank (i.e., used to yield an overall average velocity for each tank). Average water velocities in tanks 5–8 were 2.9, 1.2, 2.1, and 1.5 cm/s, respectively, based on readings from 16 locations in each tank. This contrast in water velocities between the two treatments was selected based on the findings of Christiansen and Jobling (1990), who observed higher GGEs in Arctic char that were subjected to 13–26-cm/s water velocities than in fish that were subjected to 0–7-cm/s water velocities. Ambient well water temperature ranged from 11°C to 13°C, but we used chillers to maintain the water temperature between 8.3°C and 10.0°C, which coincided with the preferred water temperature range for lake trout in the Laurentian Great Lakes (Stewart et al. 1983; Bergstedt et al. 2003). Photoperiod was controlled with fluorescent lighting, which was adjusted seasonally to mimic the duration of daylight for the Laurentian Great Lakes region.

The number of lake trout placed into each tank was 19 fish for tanks 1 and 2; 18 fish for tanks 3 and 4; 14 fish for tanks 5 and 6; 16 fish for tank 7; and 15 fish for tank 8. Each lake trout was weighed on 16 February (the start of the experiment), 24 March, 26 April, 1 June, and 1 July 2010 (the end of the experiment). Lake trout were fed thawed bloaters, which had been caught in Lake Michigan during September 2009 and May 2010, frozen, and stored at -30°C . After thawing, bloaters were cut into pieces, with each piece being between 1 and 5 g in weight. Lake trout in all tanks were fed as much as they would consume during one feeding each day. We chose the ad libitum feeding level because we wanted to test the hypothesis proposed by Madenjian and O'Connor (1999) that fish activity has an effect on bioenergetics model performance for fish that are fed an ad libitum ration. Any food that was not consumed by the lake trout within 1 h after placement into the tank was removed, air dried for about 15 min, and weighed to the nearest 0.1 g.

At the start of the experiment, a subsample of fish was sacrificed from each tank (tanks 1 and 2: $n = 9$; tanks 3 and 4: $n = 8$; tanks 5 and 6: $n = 4$; tank 7: $n = 6$; tank 8: $n = 5$) and stored in plastic bags at -30°C until further processing. All of the 10 lake trout remaining in each tank at the conclusion of the experiment were frozen at -30°C until further processing. Additionally, 10 three-fish composite subsamples of bloaters caught during September 2009 and 10 six-fish composite subsamples of bloaters caught during May 2010 were stored at -30°C for later analysis. More fish were included in the composite subsamples from May because those bloaters were substantially smaller than the bloaters that were caught during September. To determine energy density, lake trout were composited by stage (start or end of experiment) and tank. Each composite (lake trout or bloater) was homogenized in a blender. A 20–30-g portion of each mixture was oven dried for approximately 60 h at 70°C , and energy density was determined for a 1-g subsample of the dried material by using a Parr Model 1261 isoperibol calorimeter.

We calculated the GGE for each tank by subtracting the average weight of lake trout in the tank at the start of the experiment from the average weight of lake trout in the tank at the end of the experiment and then dividing this difference by the average amount of food eaten by a lake trout in the tank during the course of the 135-d experiment. To determine whether GGE differed significantly between the two fish activity levels, a two-sample t -test was applied to the GGE estimates; the GGE estimates for tanks 5–8 served as four low-activity replicates, and the estimates for tanks 1–4 served as four high-activity replicates.

Bioenergetics modeling.—A bioenergetics model for lake trout was developed by Stewart et al. (1983). This model is one of a set of fish bioenergetics models that are commonly referred to as Wisconsin bioenergetics models, as most models were developed by researchers at the University of Wisconsin. We applied the Stewart et al. (1983) model for lake trout to the growth and consumption data from our laboratory experiment. Inputs to the model included (1) water temperature regime experienced by lake trout in the laboratory tanks, (2) diet composition (wet

weight basis) of the lake trout during the experiment, (3) energy densities of bloaters that were fed to the lake trout, and (4) energy densities of the lake trout during the experiment. Thus, our application was slightly different than that used by Stewart et al. (1983) for Lake Michigan lake trout. Rather than estimating energy density of lake trout as a function of lake trout weight per Stewart et al. (1983), we used the initial and final energy densities of lake trout (by tank) as inputs into the bioenergetics model. Predator energy density was linearly interpolated over time between the start and completion of the experiment. In addition, we assumed that lake trout maintained their position within the water flow of the tank; this same assumption was made by Madenjian and O'Connor (1999) in an earlier laboratory evaluation of the lake trout bioenergetics model. Based on visual observations of lake trout in tanks at various times of the day, this assumption appeared to be reasonable. Thus, even though the stocking density (number of lake trout per m^3 of water) in the smaller tanks was nearly three times higher than that in the larger tanks, this difference in stocking density between the two tank sizes did not appear to have an additional influence on lake trout behavior and activity. For bioenergetics modeling purposes, we simulated lake trout at a constant swimming speed equal to the average flow rate within each tank.

We followed the procedure of Madenjian and O'Connor (1999) and used the bioenergetics model in two ways: (1) to predict consumption given the observed starting and ending average weights of lake trout over time interval t and (2) to predict growth given the starting average weight of the lake trout and the observed average consumption over time interval t . Predictions were generated for each test period ($t =$ about 1 month) and for the entire duration of the experiment ($t = 135$ d). All predictions were made on a tank-by-tank basis.

To generate predictions based on algorithm 1, we used the most recent version of the Wisconsin bioenergetics model software (Hanson et al. 1997). According to algorithm 1, the weight of a fish at the start of day $t + 1$, W_{t+1} (g), is calculated as

$$W_{t+1} = \frac{E_t + (ED_t W_t)}{ED_t}, \quad (1)$$

where E_t = net energy (J) gained from the food eaten by the fish during day t , ED_t = energy density (J/g wet weight) of the fish at the start of day t , and W_t = fish weight (g) at the start of day t . To calculate E_t , the sum of the energy allocated to metabolism, egestion, and excretion on day t is subtracted from the energy contained in the food that was consumed on day t . The energy contained in the fish at the end of day t is equal to E_t plus the product of ED_t and W_t . Thus, based on algorithm 1, the weight of the fish at the start of day $t + 1$ is calculated by dividing the energy contained in the fish at the end of day t by the energy density of the fish at the start of day t .

To generate predictions based on algorithm 2, we developed and used a computer program written in PASCAL. According

to algorithm 2, W_{t+1} is calculated by

$$W_{t+1} = \frac{E_t + (ED_t W_t)}{ED_{t+1}}, \quad (2)$$

where ED_{t+1} = energy density (J/g wet weight) of the fish at the start of day $t + 1$. Thus, based on algorithm 2, the fish's weight at the start of day $t + 1$ is calculated by dividing the energy contained in the fish at the end of day t by the energy density of the fish at the start of day $t + 1$. Equation (2) correctly expresses the conservation of energy because to accurately balance a fish's energy budget, the energy contained in the fish at the start of day $t + 1$ (i.e., $W_{t+1} \times ED_{t+1}$) must equal the energy contained in the fish at the start of day t (i.e., $W_t \times ED_t$) plus the net energy gained from the food eaten by the fish during day t . Multiplication of both sides of equation (2) by ED_{t+1} reveals that the use of algorithm 2 leads to an accurate balancing of the energy budget.

Evaluation of bioenergetics model predictions.—To investigate the effects of activity and energy budget balancing algorithm on bioenergetics model performance, we evaluated four sets of monthly predictions of the lake trout bioenergetics model: (1) model predictions based on algorithm 1 for inactive fish (tanks 5–8); (2) predictions based on algorithm 1 for active fish (tanks 1–4); (3) predictions based on algorithm 2 for inactive fish; and (4) predictions based on algorithm 2 for active fish.

We evaluated each set of monthly predictions from the lake trout bioenergetics model in a manner similar to that used by Madenjian and O'Connor (1999). First, we used a t -test for paired comparisons to determine whether the average difference between observed and predicted consumption was significantly different from 0. An average difference that was significantly different from 0 would indicate significant bias in the model predictions. For our application, we subtracted the predicted value from the observed value. In addition, we performed simple linear regression analysis for the predicted values as a function of observed values. If the model predictions were unbiased, then the slope of the regression line would not differ significantly from 1.0 and the intercept of the regression line would not differ significantly from 0. Bonferroni 95% joint confidence intervals were constructed to test the null hypotheses that the slope was equal to 1.0 and the intercept was equal to 0 (Neter et al. 1983). We applied these statistical analyses to the sets of observations and model predictions for monthly consumption and lake trout weight at the end of the monthly test period. As was explained by Madenjian and O'Connor (1999), we expected that in some instances, the paired t -test would be more powerful at detecting bias, whereas in other cases the linear regression analysis would be the more powerful approach. Using the portmanteau test (Madenjian and O'Connor 1999), we failed to detect significant autocorrelation in (1) the residuals from the regression analyses, (2) the differences between observed and predicted consumption, or (3) the differences between observed and predicted final weight. Consequently, we did not expect that re-

sults from our statistical testing would be confounded by serial correlation.

We used two-way ANOVA to determine significance of the effects of activity and energy budget balancing algorithm on the accuracy of the bioenergetics model's 135-d predictions. First, we formed the ratio of predicted : observed cumulative consumption for each tank over the entire 135-d experiment. We then calculated the percent deviation from observed cumulative consumption by taking the absolute value of the difference between this ratio and 1. A two-way ANOVA was then applied to the percent deviation values, with activity and energy budget balancing algorithm as the main effects; the interaction term was also included in the ANOVA model. In a manner analogous to that used for cumulative consumption, we formed the ratio of predicted : observed final weight of lake trout over the 135-d experiment to evaluate the bioenergetics model's predictions for growth. The percent deviation between observed and predicted final weights was calculated by taking the absolute value of the difference between this ratio and 1. A two-way ANOVA, with activity and energy budget balancing algorithm as the main effects and the interaction term included, was applied to the percent deviation values to assess the significance of the main effects for the accuracy of model-predicted cumulative growth over the entire experiment. We set α equal to 0.05 for all statistical testing.

RESULTS

Energy density of lake trout increased in all eight tanks during the experiment. Initial energy densities were 8,585 J/g (wet weight basis) for tank 1; 8,417 J/g for tank 2; 8,101 J/g for tank 3; 9,044 J/g for tank 4; 9,059 J/g for tank 5; 8,671 J/g for tank 6; 8,564 J/g for tank 7; and 8,326 J/g for tank 8. Final energy densities were 10,011 J/g for tank 1; 10,708 J/g for tank 2; 10,904 J/g for tank 3; 10,664 J/g for tank 4; 10,311 J/g for tank 5; 9,285 J/g for tank 6; 9,931 J/g for tank 7; and 10,040 J/g for tank 8. Energy densities of the 10 subsamples of bloaters caught during September ranged from 6,334 to 8,971 J/g, with a mean of 7,871 J/g and SE of 246 J/g. Energy densities of the 10 subsamples of bloaters captured in May ranged from 4,470 to 6,327 J/g, with a mean of 5,479 J/g and SE of 181 J/g.

The GGEs for the eight tanks ranged from 0.194 to 0.293 (Table 1). Mean GGEs for the high-activity and low-activity lake trout were 0.261 and 0.251, respectively. The difference in mean GGE between the two activity levels was not significant (t -test: $t = -0.41$, $df = 6$, $P = 0.6974$).

When algorithm 1 (equation 1) was used to balance the energy budget, the bioenergetics model significantly underestimated monthly consumption for both inactive and active lake trout. For inactive lake trout, results from a paired t -test revealed that the mean difference between observed and predicted monthly consumption was significantly greater than 0 (Table 2). Furthermore, the slope of the regression line of predicted monthly consumption as a function of observed monthly

TABLE 1. Observed and predicted cumulative consumption and cumulative growth by an average lake trout in laboratory tanks (4 tanks per activity level: active or inactive). The experiment was run for 135 d, and the lake trout were fed bloaters. Inactive lake trout were subjected to an average flow rate of 1.9 cm/s, and active lake trout were subjected to an average flow rate of 15.8 cm/s. Observed consumption is the total amount of food eaten by all fish in the tank divided by the number of fish in the tank. Gross growth efficiency (GGE) is the lake trout weight gain divided by the amount of food consumed. For algorithm 1, the energy budget of the lake trout for day t was balanced by using the lake trout energy density on day t . For algorithm 2, the energy budget of the lake trout for day t was balanced by using the energy density on day $t + 1$. The lake trout bioenergetics model developed by Stewart et al. (1983) was used to generate predictions of consumption and growth.

Characteristic	Inactive lake trout				Active lake trout			
	Tank 5	Tank 6	Tank 7	Tank 8	Tank 1	Tank 2	Tank 3	Tank 4
Observed consumption and growth								
Initial weight (g)	694	729	754	729	907	860	890	817
Final weight (g)	1,242	853	1,050	1,092	1,345	1,339	1,518	1,566
Consumption (g)	1,870	641	1,203	1,336	1,734	1,999	2,344	2,649
GGE	0.293	0.194	0.246	0.272	0.252	0.240	0.268	0.283
Predicted consumption and growth based on algorithm 1								
Consumption (g)	1,518	553	942	1,060	1,469	1,596	1,883	2,230
Final weight (g)	1,394	897	1,173	1,222	1,464	1,513	1,715	1,733
Ratio of predicted to observed consumption	0.812	0.863	0.783	0.793	0.847	0.799	0.803	0.842
Ratio of predicted to observed final weight	1.122	1.051	1.117	1.119	1.089	1.130	1.129	1.107
Predicted consumption and growth based on algorithm 2								
Consumption (g)	1,794	654	1,207	1,403	1,824	2,169	2,682	2,693
Final weight (g)	1,272	847	1,048	1,064	1,307	1,276	1,397	1,550
Ratio of predicted to observed consumption	0.960	1.021	1.003	1.050	1.052	1.086	1.144	1.017
Ratio of predicted to observed final weight	1.024	0.992	0.998	0.974	0.972	0.952	0.920	0.990

TABLE 2. Statistical comparison of predicted and observed consumption and growth of lake trout (two activity levels: active and inactive) during a laboratory experiment used to evaluate a lake trout bioenergetics model (N = number of pairs of data). Predictions were based on the model developed by Stewart et al. (1983) and used either algorithm 1 (equation 1) or algorithm 2 (equation 2). Inactive lake trout were subjected to an average flow rate of 1.9 cm/s, and active lake trout were subjected to an average flow rate of 15.8 cm/s. The model was evaluated for its predictions of (1) consumption during a test period of roughly 1 month and (2) weight at the end of a monthly test period. Paired t -tests were used to determine whether the average difference between values (observed value – predicted value) was significantly different from 0. Regression analyses of predicted values as a linear function of observed values were also performed; Bonferroni joint 95% confidence intervals (95% CIs) are shown for the null hypotheses that the intercept (β_0) is equal to 0 and the slope (β_1) is equal to 1.0.

Activity level	Algorithm	N	Mean difference (g)	Attained P for paired t -test	$\beta_0 \pm 95\% \text{ CI (g)}$	$\beta_1 \pm 95\% \text{ CI}$
Food consumption during the test period						
Inactive	1	16	60.8	< 0.0001	20.6 \pm 44.2	0.74 \pm 0.13
Active	1	16	97.7	< 0.0001	51.8 \pm 101.4	0.73 \pm 0.18
Inactive	2	16	-0.7	0.9429	41.3 \pm 60.8	0.87 \pm 0.18
Active	2	16	-40.6	0.0058	77.7 \pm 163.3	0.93 \pm 0.29
Weight at the end of the test period						
Inactive	1	16	-29.9	< 0.0001	15.1 \pm 84.9	1.02 \pm 0.09
Active	1	16	-43.5	< 0.0001	42.7 \pm 86.6	1.00 \pm 0.07
Inactive	2	16	-0.2	0.9698	25.6 \pm 69.7	0.97 \pm 0.07
Active	2	16	16.4	0.0033	11.1 \pm 84.0	0.98 \pm 0.07

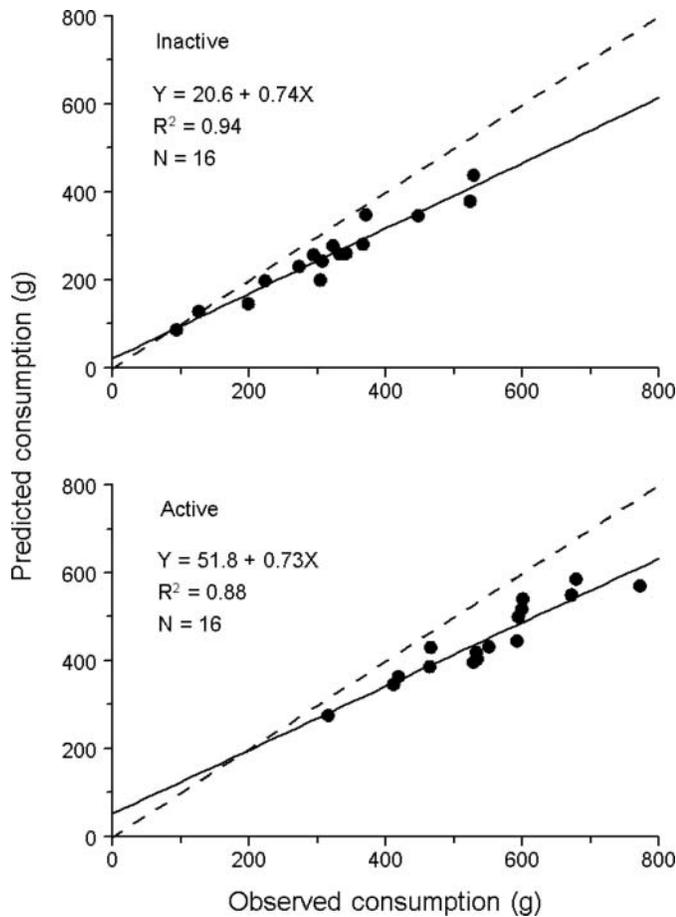


FIGURE 1. Predicted versus observed consumption by an average lake trout in a test tank at two fish activity levels (inactive [average flow rate = 1.9 cm/s] and active [15.8 cm/s]; 4 tanks for each activity level) during each test period (~1 month long; 4 periods/tank). Predictions were made with the bioenergetics model developed by Stewart et al. (1983) and with algorithm 1 (balancing of the energy budget on day t by using lake trout energy density on day t); the model was applied to each combination of tank and test period. The solid line represents the regression line fitted to the points; the dashed line represents the line of 1:1 correspondence between predictions and observations.

consumption was significantly less than 1.0 (Table 2; Figure 1). Similar to the results for inactive lake trout, the mean difference between observed and predicted monthly consumption for active lake trout was significantly greater than 0 (Table 2). Moreover, the slope of the regression line of predicted versus observed monthly consumption was significantly less than 1.0 (Table 2; Figure 1). The degree of underestimation of monthly consumption was similar between the inactive and active lake trout (Figure 1).

When algorithm 2 (equation 2) was used to balance the energy budget, the bioenergetics model predictions of monthly consumption were unbiased for inactive lake trout and slightly biased for active lake trout. Paired t -test results indicated that model predictions were unbiased for inactive lake trout (Table 2). Further, for inactive lake trout, the slope of the regression line of predicted monthly consumption as a function of

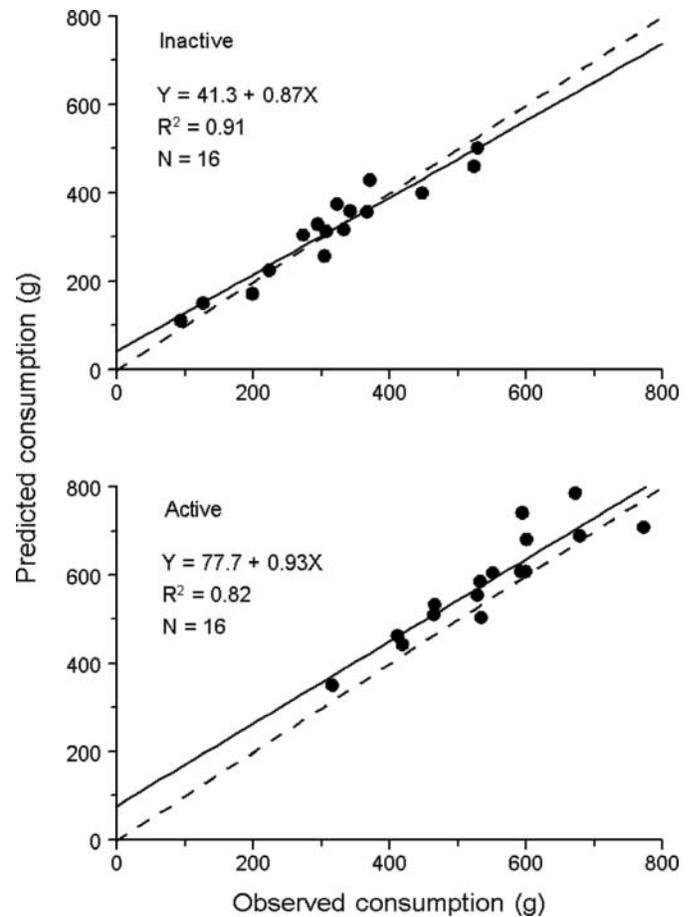


FIGURE 2. Predicted versus observed consumption by an average lake trout in a test tank at two fish activity levels (inactive [average flow rate = 1.9 cm/s] and active [15.8 cm/s]; 4 tanks for each activity level) during each test period (~1 month long; 4 periods/tank). Predictions were made with the bioenergetics model developed by Stewart et al. (1983) and with algorithm 2 (balancing of the energy budget on day t by using lake trout energy density on day $t + 1$); the model was applied to each combination of tank and test period. The solid line represents the regression line fitted to the points; the dashed line represents the line of 1:1 correspondence between predictions and observations.

observed monthly consumption was not significantly different from 1.0, and the intercept was not significantly different from 0 (Table 2; Figure 2). According to paired t -test results for active lake trout, the model slightly overestimated monthly consumption for these fish (Table 2). However, regression analysis did not show significant bias in the model predictions of monthly consumption by active lake trout (Table 2). Overall, model predictions of monthly consumption were more accurate when using algorithm 2 than when using algorithm 1 (Figures 1, 2).

When algorithm 1 was used to balance the energy budget, the paired t -test detected a significant bias in the bioenergetics model's predictions of weight at the end of a monthly test period for both inactive and active lake trout, whereas regression analysis failed to reveal a significant bias in the model predictions. Paired t -test results indicated a significant overestimation

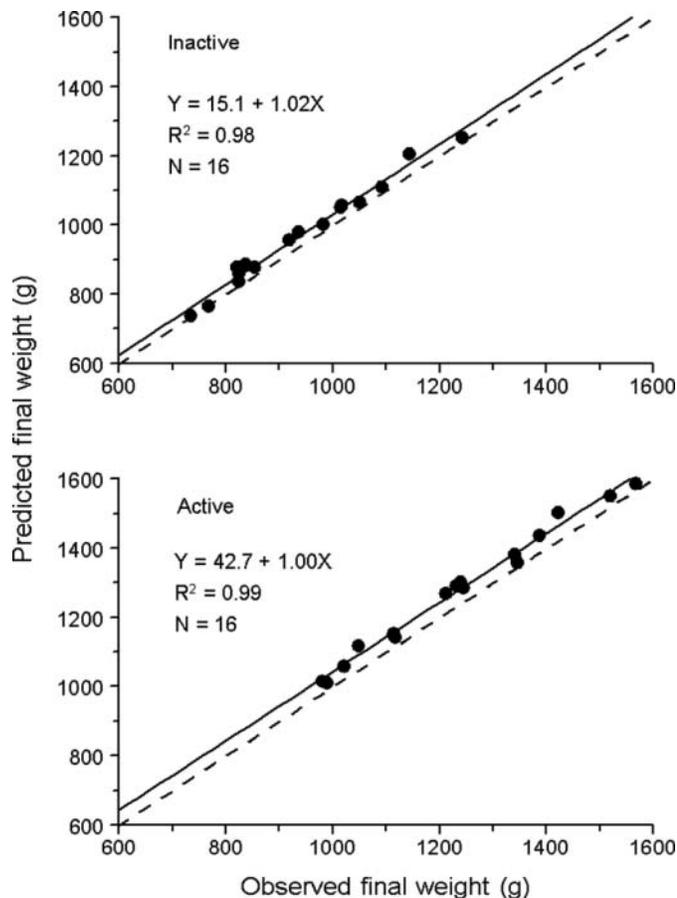


FIGURE 3. Predicted versus observed final weight at the end of each test period (~1 month long) for an average lake trout in a test tank at two fish activity levels (inactive [average flow rate = 1.9 cm/s] and active [15.8 cm/s]; 4 tanks for each activity level; 4 periods/tank). Predictions were made with the bioenergetics model developed by Stewart et al. (1983) and with algorithm 1 (balancing of the energy budget on day t by using lake trout energy density on day t); the model was applied to each combination of tank and test period. The solid line represents the regression line fitted to the points; the dashed line represents the line of 1:1 correspondence between predictions and observations.

of weight at the end of a test period for both activity levels (Table 2). However, regression analysis did not indicate a significant bias in the predictions of weight at the end of a test period for either inactive or active lake trout (Table 2; Figure 3).

When algorithm 2 was used to balance the energy budget, bioenergetics model predictions of lake trout weight at the end of a monthly test period were unbiased for inactive lake trout and were slightly biased for active lake trout. According to the paired t -test results, the model predictions of weight at the end of a test period were not significantly biased for inactive lake trout (Table 2); regression analysis also showed no significant bias in model predictions of weight for inactive lake trout (Table 2; Figure 4). For active lake trout, the paired t -test results indicated that the bioenergetics model significantly underestimated weight at the end of a test period (Table 2). However, regression analysis showed no significant bias in the predictions of weight

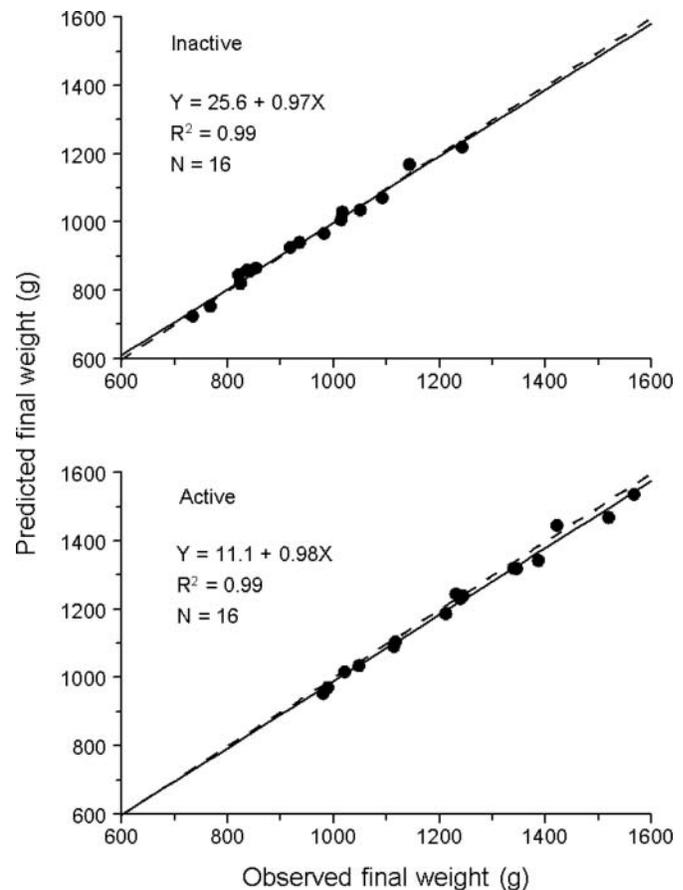


FIGURE 4. Predicted versus observed final weight at the end of each test period (~1 month long) for an average lake trout in a test tank at two fish activity levels (inactive [average flow rate = 1.9 cm/s] and active [15.8 cm/s]; 4 tanks for each activity level; 4 periods/tank). Predictions were made with the bioenergetics model developed by Stewart et al. (1983) and with algorithm 2 (balancing of the energy budget on day t by using lake trout energy density on day $t + 1$); the model was applied to each combination of tank and test period. The solid line represents the regression line fitted to the points; the dashed line represents the line of 1:1 correspondence between predictions and observations.

for active lake trout (Table 2; Figure 4). Overall, bioenergetics model predictions of weight at the end of a test period were more accurate when using algorithm 2 than when using algorithm 1 (Figures 3, 4).

For bioenergetics model predictions of cumulative consumption over the 135-d experiment, algorithm 2 yielded significantly more accurate predictions than algorithm 1 (two-way ANOVA: $F = 51.80$; $df = 1, 12$; $P < 0.0001$). Activity did not have a significant effect on bioenergetics model accuracy ($F = 1.00$; $df = 1, 12$; $P = 0.3381$), and the interaction between activity and energy budget balancing algorithm was not significant ($F = 2.37$; $df = 1, 12$; $P = 0.1494$). When algorithm 1 was used, the bioenergetics model predictions of cumulative consumption over the 135-d experiment were 13–22% lower than the observed cumulative consumption (Table 1). When algorithm 2 was used, the predictions of cumulative consumption were within 5% of

observed values for inactive lake trout and were 1–15% higher than observed values for active lake trout (Table 1).

With regard to bioenergetics model predictions of cumulative growth in weight over the 135-d experiment, algorithm 2 yielded significantly more accurate predictions than algorithm 1 (two-way ANOVA: $F = 39.59$; $df = 1, 12$; $P < 0.0001$). Activity did not have a significant effect on bioenergetics model accuracy ($F = 2.24$; $df = 1, 12$; $P = 0.1606$), and the interaction between activity and energy budget balancing algorithm was not significant ($F = 0.36$; $df = 1, 12$; $P = 0.5588$). When algorithm 1 was used, bioenergetics model predictions of final weight were between 5% and 13% higher than observed final weight (Table 1). When algorithm 2 was used, the bioenergetics model's predictions of final weight were within 3% of observed final weight for inactive lake trout and were 1–8% lower than observed final weight for active lake trout (Table 1).

DISCUSSION

Our results clearly show that algorithm 2 outperforms algorithm 1 in terms of the accuracy of consumption and growth predictions from the bioenergetics model. Monthly consumption was significantly underestimated for both inactive and active lake trout when algorithm 1 was used. In contrast, use of algorithm 2 resulted in no detectable bias in predictions of monthly consumption by inactive lake trout and yielded only a slight overestimation of monthly consumption by active lake trout. Our paired t -test results also indicated significant overestimation of monthly growth for both inactive and active lake trout when algorithm 1 was used, whereas predictions of monthly growth based on algorithm 2 exhibited no significant bias for inactive lake trout. Moreover, for cumulative consumption over the course of the 135-d experiment, the predictions based on algorithm 2 were significantly more accurate than those based on algorithm 1. Predictions of growth in weight over the entire experiment were also significantly more accurate when algorithm 2 was used than when algorithm 1 was used. The superior performance of algorithm 2 can be attributed to its accurate balancing of the fish's energy budget, whereas use of algorithm 1 does not lead to an accurate balancing of the energy budget unless the fish's energy density remains constant over time. Stewart et al. (1983) used the energy density of lake trout on day $t + 1$ in balancing the energy budget of the lake trout on day t , and this same algorithm 2 approach was also used by Stewart (1980) in developing the bioenergetics models for Chinook salmon and coho salmon *O. kisutch*.

The slight bias in bioenergetics model predictions of consumption and growth for active lake trout based on algorithm 2 may be due to energy savings accrued from swimming in groups compared with individual swimming. The lake trout bioenergetics model developed by Stewart et al. (1983) was primarily based on respiration rate measurements of a single lake trout swimming in a respirometer tunnel. However, for certain fish species and at certain ranges of swimming speed, the average respira-

tion rate for a school of fish swimming at a given speed may be lower than the respiration rate of a single fish swimming at that same speed (Blake 2004; Liao 2007). In these cases, swimming in a group at a certain speed affords a lower amount of energy expenditure per fish than the energy expended by a single fish swimming at the same speed in a respirometer tunnel. Consequently, if the active lake trout were saving energy by swimming in a group in our laboratory tanks, then the bioenergetics model would be expected to overestimate consumption by these fish.

Based on our laboratory results, the most plausible explanation for bioenergetics models' underestimation of food consumption when fish feed at a relatively high rate is that the fish's energy density is not taken into account with a sufficient amount of accuracy. Although lake trout were fed ad libitum in our study, bioenergetics model performance was relatively good when algorithm 2 was used to balance the energy budget, whereas bioenergetics model predictions of consumption were biased conspicuously low under algorithm 1. Activity did not have a significant effect on bioenergetics model performance. Therefore, our results provided no evidence that the resting metabolic rate was higher for inactive lake trout than for active lake trout. Consequently, the underestimation of consumption for lake trout feeding at a high rate is probably not attributable to an elevation in the resting metabolic rate of inactive fish compared with active fish. In addition, our results suggest that the components of the lake trout bioenergetics model developed by Stewart et al. (1983) were accurate predictors of egestion, excretion, and SDA, as food consumption did not appear to be underestimated when algorithm 2 was used to balance the energy budget. Of course, laboratory experimentation to specifically quantify resting metabolic rate, egestion, excretion, and SDA will be needed to confirm that these effects were not responsible for the underestimation of consumption at high feeding rates.

Results from our laboratory experiment highlight the importance of properly accounting for changes in fish energy density over time when balancing the fish's daily energy budget. Relatively low feeding rates may lead to a decrease in fish energy density over time, whereas relatively high feeding rates can lead to an increase in fish energy density over time (Madenjian and O'Connor 1999). Use of algorithm 1 will result in (1) the overestimation of food consumption by a fish when that fish's energy density decreases over time and (2) the underestimation of food consumption when the fish's energy density increases over time. The degree of bias in predictions of food consumption increased with increasing magnitude of the rate of change in fish energy density over time. For example, of the eight tanks in our experiment, tank 6 had the smallest relative difference between estimates of cumulative (135-d) consumption based on the two algorithms; the estimates were 553 g for algorithm 1 and 654 g for algorithm 2, and the relative difference was about 15% (using the algorithm 2 consumption estimate as the reference estimate). Coincidentally, the lowest rate of change in lake trout energy density over the 135-d experiment was for tank 6, in which energy density increased at approximately $5 \text{ J} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$.

The greatest relative difference in estimates of cumulative consumption between the two algorithms was for tank 3, with the algorithm 1 consumption estimate being 30% lower than the algorithm 2 estimate. Tank 3 also demonstrated the greatest rate of change in lake trout energy density (increasing at $21 \text{ J}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$) over the entire experiment. Using equations 1 and 2 and assuming that the consumption rate is directly proportional to the estimated weight on day $t + 1$, the ratio of cumulative consumption based on algorithm 1 to that based on algorithm 2 can be approximated by Δ^n , where Δ is the average daily proportional change in fish energy density and n is the number of days in the experiment. Although this is a rough approximation because departures from the assumption can sometimes be substantial, Δ^n may still be useful in gauging the degree of bias imparted by the use of algorithm 1. As previously mentioned, algorithms 1 and 2 will yield identical estimates of consumption and growth when the energy density of the fish is constant over time.

Several examples of fish in lakes increasing their energy density at rates exceeding $5 \text{ J}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ can be gleaned from the literature; therefore, our laboratory results have applicability to the field. Juvenile lake trout and juvenile Chinook salmon from Lake Michigan typically increased their energy density at rates between 5 and $10 \text{ J}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ during the growing season (Stewart et al. 1983; Stewart and Ibarra 1991). Adult alewives in Lake Michigan increased their energy density at rates exceeding $30 \text{ J}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ between August and November (Stewart and Binkowski 1986; Madenjian et al. 2006). It should be kept in mind that in many fish populations, the energy density of the adult fish does not change appreciably as the fish continues to grow (Hanson et al. 1997; Madenjian et al. 2000). In these cases, algorithms 1 and 2 would produce very similar estimates of food consumption. Nonetheless, in some field applications, the two algorithms would yield substantially different estimates of consumption.

Our study illustrates the importance of small details in the algorithm used to balance the fish's energy budget as related to the assessment of fish bioenergetics model accuracy. Evaluation of fish bioenergetics models has been actively pursued during the past 15 years or so (Bajer et al. 2004; Trudel and Rasmussen 2006; Lantry et al. 2008). In laboratory evaluations, fish are typically fed at a variety of rates (including ad libitum) to judge model performance over a broad range of feeding rates. One pattern that has emerged from the set of evaluations to date is that fish bioenergetics models underestimate food consumption when fish feed at a relatively high rate, and this underestimation has been blamed on the models being developed with insufficient data to adequately capture all components of the fish's energy budget at a high level of food intake. Our results indicate that fish bioenergetics models can perform very well at high feeding rates provided that the changes in fish energy density over time are properly taken into account. Our colleagues at the University of Michigan (Yu-Chun Kao and others) have revised the computer code of the bioenergetics model software developed by Hanson

et al. (1997) so that the model predictions are dependent on algorithm 2 rather than on algorithm 1. Plans are being made to make the revised software package available at the website of the Center for Limnology, University of Wisconsin, Madison (limnology.wisc.edu; P. Hanson, personal communication). For future fish bioenergetics model evaluations, we recommend that researchers accurately account for changes in fish energy density over time.

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Genetic Characterization of American Shad in the Edisto River, South Carolina, and Initial Evaluation of an Experimental Stocking Program

Elizabeth Cushman^a, Carolyn Tarpey^a, Bill Post^b, Kent Ware^c & Tanya Darden^a

^a South Carolina Department of Natural Resources, Hollings Marine Laboratory, 331 Fort Johnson Road, Charleston, South Carolina, 29412, USA

^b South Carolina Department of Natural Resources, 217 Fort Johnson Road, Charleston, South Carolina, 29412, USA

^c Bears Bluff National Fish Hatchery, Post Office Box 69, 7030 Bears Bluff Road, Wadmalaw Island, South Carolina, 29487, USA

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ARTICLE

Genetic Characterization of American Shad in the Edisto River, South Carolina, and Initial Evaluation of an Experimental Stocking Program

Elizabeth Cushman* and Carolyn Tarpey

South Carolina Department of Natural Resources, Hollings Marine Laboratory, 331 Fort Johnson Road, Charleston, South Carolina 29412, USA

Bill Post

South Carolina Department of Natural Resources, 217 Fort Johnson Road, Charleston, South Carolina 29412, USA

Kent Ware

Bears Bluff National Fish Hatchery, Post Office Box 69, 7030 Bears Bluff Road, Wadmalaw Island, South Carolina 29487, USA

Tanya Darden

South Carolina Department of Natural Resources, Hollings Marine Laboratory, 331 Fort Johnson Road, Charleston, South Carolina 29412, USA

Abstract

The American shad *Alosa sapidissima* is an anadromous clupeid with once-prolific stocks that have experienced major coastwide declines in abundance over the past century. The American shad spawning run in the Edisto River (South Carolina) has been exhibiting the same decreases as spawning runs in other coastal rivers, and stocking is now being considered as a restoration option for this river system. We utilized a suite of 13 microsatellite loci to provide a baseline genetic characterization of the Edisto River spawning run prior to supplementation and to evaluate the initial success of an experimental stocking program enacted from 2008 to 2010. No significant temporal genetic differentiation was found between sampling years, indicating that the genetic composition of the Edisto River spawning run is temporally stable over short time frames. Estimates of genetic diversity for Edisto River American shad were high (observed heterozygosity = 0.82–0.85) and similar to those observed in other river systems. Estimates of effective population size (3,505–8,379) resembled those reported for other diadromous species and were within the levels recommended for maintaining evolutionary potential. Hatchery-produced individuals were detected within the 2010 year-class of juvenile American shad prior to out-migration (11/314 fish, or 3.5%), demonstrating initial success of the stocking effort (i.e., contribution of hatchery fish to the wild stock). Our results provide valuable information that can be incorporated into management plans for aiding the recovery of American shad in the Edisto River.

The American shad *Alosa sapidissima* (hereafter, “shad”) is an anadromous fish with a native range that spans the eastern coast of North America from the St. Johns River, Florida, to the St. Lawrence River, Quebec (Mansueti and Kolb 1953;

Walburg and Nichols 1967). Ocean-dwelling shad form mixed-stock migratory schools (Talbot and Sykes 1958; Neves and Depres 1979; Dadswell et al. 1987) but move into coastal rivers from late fall through early summer to spawn (November–July,

*Corresponding author: cushmane@dnr.sc.gov
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depending on latitude; Mansueti and Kolb 1953; Walburg and Nichols 1967; Leggett and Whitney 1972). The majority of shad that are native to rivers on the southeastern coast of the United States (i.e., south of Cape Hatteras, North Carolina) die after spawning, whereas those from more northerly rivers may spawn more than once; the degree of repeat spawning increases with latitude (Leggett 1969; Leggett and Carscadden 1978). Juvenile shad typically remain within river systems until the fall of their first year before emigrating seaward, only returning to their natal rivers upon sexual maturity at 3–6 years of age (Mansueti and Kolb 1953; Talbot and Sykes 1958). The shad's predictable life history has played a large role in its importance as a target for commercial and recreational fisheries.

At the turn of the 19th century, nearly 22.7 million kg (50 million lb) of shad were harvested annually, making it one of the most important food fish species on the Atlantic coast of the United States (ASMFC 2007a). However, major coastwide declines in abundance occurred in subsequent decades due to water pollution, overfishing, and the construction of dams with inadequate fish passage (Walburg and Nichols 1967; Bilkovic et al. 2002; Limburg et al. 2003; ASMFC 2007a). As of 2007, many spawning runs of shad on the U.S. Atlantic coast were continuing to decline or were showing no signs of recovery (ASMFC 2007a, 2009).

The Edisto River in South Carolina (Figure 1) supports a commercial shad fishery that has existed for over 100 years and a recreational shad fishery that began in the late 1960s (Mansueti and Kolb 1953; Walburg and Nichols 1967). However, estimates of commercial catch per unit effort (CPUE) in the Edisto River have declined during all available time series, and landings have been below the time series (1979–2005) average for 13 of the last 15 years (ASMFC 2007b), thus prompting interest in restoration efforts. The Atlantic States Marine Fisheries Commission (Washington, D.C.) has determined that assessment, management, and restoration of shad should occur on an individual river basis, as each river system is unique (i.e., life histories, extant fisheries, presence of dams, etc.; ASMFC 2007a). Consequently, obtaining river-specific information, such as migration patterns and genetic diversity, is a vital component of these efforts. The shad CPUE reduction in the Edisto River highlights the need for assembling data that can be used for conservation and management of shad within this river system.

Unfortunately, genetic data for the Edisto River shad spawning run are currently scarce. Previous evaluations have examined genetic diversity and structure within and among rivers throughout the native range of shad (Bentzen et al. 1989; Epifanio et al. 1995; Waldman et al. 1996; Brown et al. 2000; Waters et al. 2000; Hasselman et al. 2010), but no published studies have focused on the Edisto River, although a set of samples was included in dissertation work by Hasselman (2010) as part of a coastwide evaluation of genetic structure between spawning runs. Genetic information, including measures of genetic diversity and effective population size (N_e), reveals vital population characteristics that are essential for conservation and

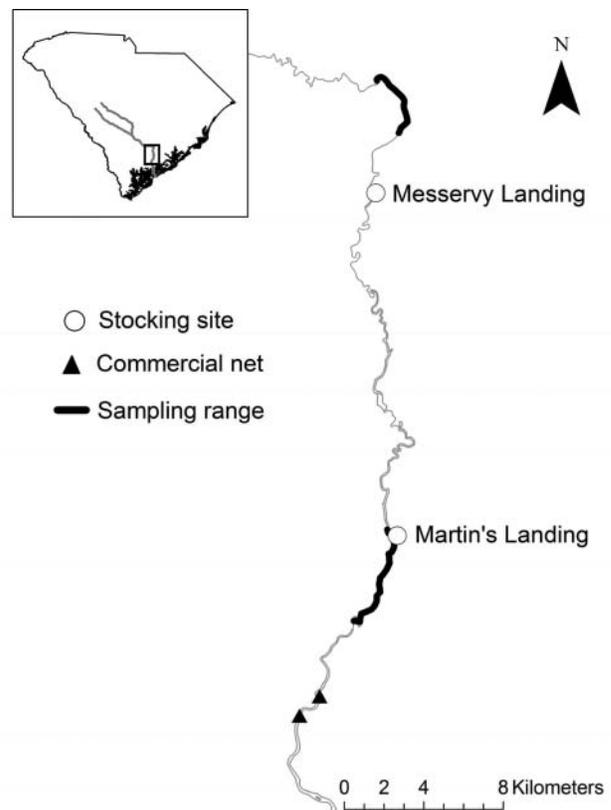


FIGURE 1. Map of the Edisto River, South Carolina, indicating stocking sites for hatchery-produced American shad fry and sampling locations for adult and juvenile American shad. Broodstock (2009 and 2010) and additional adults (2010) were collected from both sampling ranges; 2010 year-class juveniles were collected from the lower sampling range. Commercial adult samples were obtained from commercial set nets during all years (2008–2010).

management. Furthermore, the state of South Carolina is currently considering stocking as a potential restoration option for shad in the Edisto River. Responsible stocking programs require the incorporation of genetic information at all stages of production, and the success of the hatchery program itself must be routinely evaluated to ensure the continued integrity of the endeavor. Critical aspects of a responsible stocking program include comparing the genetics of hatchery broodstock to that of wild populations, monitoring genetic diversity before and after stocking, and measuring the effectiveness of the stocking effort (Blankenship and Leber 1995).

In 2008, the South Carolina Department of Natural Resources (SCDNR), in partnership with the U.S. Fish and Wildlife Service (USFWS), initiated a sampling regime and short-term (i.e., 1–2 years) experimental stocking of shad in the Edisto River, with the goal of providing information that can be used for the conservation and management of shad within this drainage. We utilized a suite of microsatellite loci to (1) estimate the genetic diversity and N_e of shad in the Edisto River prior to stocking, (2) compare the genetic diversity of broodstock with that of wild adults, and (3) determine the contribution of

TABLE 1. American shad sample collections obtained from the Edisto River, South Carolina, in 2008–2010 (N = number of fish sampled; SCDNR = South Carolina Department of Natural Resources; USFWS = U.S. Fish and Wildlife Service; YC = year-class).

Collection year	Description	N	Collector	Collection dates	Gear type
2008	Commercial adult samples	31	Commercial fishermen	12 Mar–23 Apr	Gill net
2009	Commercial adult samples	29	Commercial fisherman	4 Mar–27 Mar	Gill net
	Broodstock	76	SCDNR, USFWS	7 Mar–2 Jun	Electrofisher
2010	Commercial adult samples	193	Commercial fishermen	22 Jan–8 Apr	Gill net
	Broodstock	75	SCDNR, USFWS	9 Mar–30 Mar	Electrofisher
	Additional adults	95	SCDNR	10 Mar–7 Apr	Electrofisher
2010 YC	Juvenile field collections	601	SCDNR	9 Sep–28 Oct	Electrofisher

hatchery-produced juvenile shad to the wild population prior to out-migration as a measure of initial stocking success. These data provide a baseline genetic characterization of the Edisto River shad spawning run and an initial evaluation of stocking as a potential fisheries management tool for Edisto River shad.

METHODS

Hatchery production and stocking.—In spring 2009 (March–June), 76 adult shad (12 females, 59 males, and 5 fish of unknown sex) were collected from the Edisto River by electrofishing and were transported to Bears Bluff National Fish Hatchery (Wadmalaw Island, South Carolina) for use as broodstock. The broodstock were maintained in three round tanks (two 2,700-L tanks and one 4,700-L tank) and were injected with pelleted salmon gonadotropin releasing hormone analog (Ovaplant, Western Chemical, Ferndale, Washington) to induce spawning. The sex ratio and number of fish per tank varied throughout the spawning season to maximize egg production. After successful spawns, fertilized eggs were collected and transferred to 6-L McDonald hatching jars at an average density of 4,000 eggs/jar. The fry were marked with oxytetracycline at 250 mg/L for 4 h in accordance with Atlantic States Marine Fisheries Commission requirements, and the marking technique was verified by examining otoliths from a small subset of the fry (100% absorbance). During April 2009, 12,643 shad fry (age = 7–14 d posthatch) were stocked into the Edisto River. Oxygenated hauling trailers were used to stock 11,364 fry at Messervy Landing (river kilometer 66) and 1,279 fry at Martin's Landing (river kilometer 92; Figure 1).

Similar efforts were conducted in 2010, with a total of 75 broodstock adults (27 females and 48 males) collected from the Edisto River during February and March. Spawning and production resulted in a total of 22,209 oxytetracycline-marked shad fry that were stocked into the Edisto River from April to June 2010 at Messervy Landing. During both production years, the majority of broodstock died after spawning and the surviving individuals were not released or used for further production.

Sample collections.—During spring in 2008–2010, fin clip samples were collected from adult and juvenile shad in the

Edisto River (Figure 1) through a collaborative effort among the SCDNR, USFWS, and cooperating commercial fishermen. Throughout this time period, 1,100 fin clips were taken during a total of seven collections (Table 1). Collections included wild adults sampled in the commercial shad fishery (commercial adult samples; n = 253), wild adults sampled by SCDNR (SCDNR additional adults; n = 95), wild adults collected for broodstock (broodstock; n = 151), and juveniles collected for contribution analysis (65–123 mm total length; n = 601). Juvenile shad were collected during their fall out-migration in 2010 and are hereafter referred to as the 2010 year-class (YC) samples.

Laboratory protocols.—A 95% solution of ethanol was used to preserve all fin clips. Genomic DNA was extracted from a portion of the fin clip tissue by using a Wizard SV Genomic DNA Purification Kit (Promega, Madison, Wisconsin) in accordance with the manufacturer's instructions. Shad samples were genotyped across a suite of 13 *Alosa*-specific microsatellite primers that were developed by Julian and Bartron (2007; Table 2). Markers were combined into three 15- μ L multiplexed PCRs containing autoclaved Milli-Q water (Millipore, Billerica, Massachusetts), 1X HotMaster PCR Buffer (5 Prime, Gaithersburg, Maryland), 3.75-mM magnesium chloride, 0.16-mg/mL bovine serum albumin, 1.28-mM deoxynucleotide triphosphate, 0.05-units/ μ L HotMaster *Taq* (5 Prime), and 1 μ L of DNA (10–50 ng/ μ L). Forward primers were labeled with a fluorescent dye (Life Technologies, Carlsbad, California; Table 2). The PCR amplifications were performed on a Bio-Rad iCycler (Bio-Rad Laboratories, Hercules, California) using protocols modified from Julian and Bartron (2007). Amplifications commenced with an initial denaturation step at 94°C for 2 min; followed by 35 cycles at 94°C for 45 s, 60°C for 45 s, and 64°C for 2 min; and ending with a final extension at 64°C for 10 min. Prior to electrophoresis, PCR products were diluted 1:15 (panels 1 and 2) or 1:18 (panel 3) with autoclaved Milli-Q water, and 0.5 μ L of diluted product was mixed with 0.5 μ L of GeneScan LIZ 500 size standard (Life Technologies) in 9 μ L of formamide. Amplified fragments were separated by capillary electrophoresis on an ABI 3130 Genetic Analyzer, and fragments were scored by two independent readers using GeneMapper Fragment Analysis software (Life Technologies).

TABLE 2. Thirteen *Alosa*-specific microsatellite loci (originally developed by Julian and Bartron 2007) that were used to genotype American shad from the Edisto River. Panel number, fluorescent label (dye), repeat motif, and final PCR concentration (μM) are given for each primer.

Locus	Panel	Dye	Repeat motif	Primer concentration
<i>AsaD030</i>	1	6-FAM	(CTAT) ₂₃	0.13
<i>AsaD031</i>	1	HEX	(CTAT) ₁₄	0.16
<i>AsaC010</i>	1	HEX	(GTAT) ₁₆	0.11
<i>AsaD429</i>	1	NED	(CTAT) ₁₃	0.15
<i>AsaD021</i>	1	NED	(CTAT) ₁₅	0.16
<i>AsaD312</i>	2	6-FAM	(CTAT) ₂₀	0.13
<i>AsaC059</i>	2	6-FAM	(GTAT) ₁₅	0.16
<i>AsaB020</i>	2	HEX	(GAT) ₁₅	0.10
<i>AsaD055</i>	2	NED	(CTAT) ₁₀	0.13
<i>AsaC249</i>	3	6-FAM	(CATA) ₈ (TTCT) ₁₃	0.17
<i>AsaC334</i>	3	6-FAM	(GTAT) ₁₇	0.13
<i>AsaC051</i>	3	HEX	(GAAT) ₇ (GTAT) ₁₃	0.16
<i>AsaD042</i>	3	NED	(CTAT) ₁₂	0.15

Analyses.—Loci were tested for adherence to Hardy–Weinberg equilibrium (HWE), linkage disequilibrium, and the presence of genotyping artifacts for each collection year separately and for all adult collection years combined (2008–2010). Examinations for departures from HWE and for linkage disequilibrium between locus pairs were performed using the program ARLEQUIN version 3.11 (Excoffier et al. 2005) with default parameters. The frequency of any null alleles segregating at each locus was evaluated in CERVUS version 3.0 (Kalinowski et al. 2007). Significance levels for all simultaneous analyses were adjusted by using a sequential Bonferroni correction (Holm 1979; Rice 1989). For each collection year and the combined adult data (2008–2010), the following basic genetic diversity indices were calculated for each locus: number of alleles per locus (N_a), allelic size range (A), observed heterozygosity (H_o), gene diversity (i.e., unbiased expected heterozygosity H_E ; Nei 1987), and inbreeding coefficient (F_{IS} ; Weir and Cockerham 1984). These genetic diversity indices were obtained by using ARLEQUIN and GENEPOP version 4.1 (Raymond and Rousset 1995). Per-locus allelic richness (R) was estimated in FSTAT version 2.9.3.2 (Goudet 1995, 2001) for both separate and combined collection years (2008–2010 adults), with separate collection years standardized to 31 random samples (Leberg 2002).

To evaluate the utility of the marker suite for parentage analysis, loci were examined for the ability to distinguish between related individuals and adherence to the principles of Mendelian inheritance. With the 2009 collection year, CERVUS was used to estimate two probabilities for the loci suite: (1) the average parent pair nonexclusion probability, or the probability that the set of markers will provide an erroneous match of parents to offspring; and (2) the identity nonexclusion probability, or the probability that the set of markers will be unable to distinguish between related individuals. For the Mendelian inheritance tests, 51 shad fry from the 2009 hatchery production were

compared with the 2009 broodstock by using PROBMAX version 3.1 (Danzmann 1997) to identify the contributing parental pairs. The analyzed larval sample included contributions from 3 females and 10 males. The genotypes of the 13 contributing parents were imported into the Family Assignment Program (FAP) version 3.6 (Taggart 2007) to generate all of the possible progeny genotypes associated with these parental crosses. A chi-square (χ^2) test was performed to compare the observed genotypic frequencies from the progeny data set with the expected genotypic frequencies generated by FAP.

To assess the degree of temporal genetic variation within the Edisto River shad spawning run, pairwise comparisons of the genetic differentiation index F_{ST} between all years of sampling (2008–2010 adults and 2010 YC) were performed in ARLEQUIN using 10,000 permutations. Similarly, exact tests comparing allelic (genic) distributions between collection years were conducted in GENEPOP using default parameters. An analysis of molecular variance was also performed to partition genetic variation among years and among individuals within years (F_{ST} -like; 10,000 permutations in ARLEQUIN). Estimates of contemporary N_e for the Edisto River were calculated for each collection year (2008–2010 and 2010 YC) using the single-sample program LDNe version 1.2 (Waples and Do 2008); LDNe analyzes the nonrandom associations between unlinked loci generated by genetic drift (i.e., linkage disequilibrium) to determine contemporary N_e for a single time point and produces three values based on allele frequencies. Allele frequencies were set at default values (<0.01 , <0.02 , and <0.05), but only the <0.02 frequency was reported because it provided estimates for the majority of collections. Waples and Do (2010) also recommended the exclusion of alleles with <0.02 frequency for sample sets larger than 25 individuals. Finally, a random mating model was assumed and confidence intervals (CIs) were calculated using parametric procedures. It should be noted that in a species with

overlapping generations, such as shad, LDNe generates an estimate of the effective number of breeders (N_b) that produced the cohorts from which the samples were taken rather than providing an estimate of N_e , or some value in between N_b and N_e (Waples 2006; Waples and Do 2010). The Edisto River shad spawning run typically consists of four to five cohorts (ages 3–7; see Figure 14.7 in ASMFC 2007b:437), a pattern that was seen in our collections based on comparisons of total length with age-length tables generated for North Carolina shad (Wynne et al. 2009). As our sampled number of cohorts is similar to the generation length of shad (4–5 years; Leggett and Carscadden 1978), our project LDNe estimates for the adult collection years (2008–2010) should approximate N_e (Waples and Do 2010). However, our estimate for the 2010 YC sample does represent N_b (Waples 2006).

Responsible stocking requires the broodstock used for hatchery production and subsequent stocking to be genetically representative of the source population (Blankenship and Leber 1995). Prior to conducting parentage analyses, exact tests comparing allelic (genic) distributions were performed in GENEPOP and pairwise comparisons of F_{ST} were conducted in ARLEQUIN between hatchery broodstock and wild individuals (combined commercial adults and SCDNR additional adults from 2008–2010); these tests were performed for both the 2009 and 2010 production years. Genetic diversity statistics (N_a , R , H_O , and F_{IS}) were also calculated for broodstock and wild individuals, and the broodstock were tested for adherence to HWE and for the presence of genotyping artifacts by using the previously described programs. To determine whether hatchery individuals contributed to the Edisto River spawning run prior to out-migration (i.e., evaluation of stocking success), a subset of 314 of the 601 juvenile shad collected during the fall (August–October 2010) was compared with the 2010 broodstock by using

CERVUS. Approximately 50 fry were subsampled for genotyping from each collection day by using a random design that was stratified by collection event. If fewer than 50 individuals were collected, then all samples from that day were genotyped. Simulations ($n = 5$) for “sexes unknown” parentage analysis in CERVUS consisted of 10,000 offspring and 100 candidate parents (100% sampled) and used allele frequencies that were generated from all samples of adult shad. Critical delta values were determined using 99% confidence for the relaxed criteria and 100% confidence for the strict criteria. All parentage analyses were run with the modal simulation file. The percentage of hatchery contribution was calculated as $[C/(W + C)] \times 100$, where C is the number of cultured individuals and W is the number of wild individuals as designated by CERVUS at the strict confidence level (no additional offspring were identified with the relaxed criteria).

RESULTS

After Bonferroni correction for multiple comparisons ($\alpha = [0.05/13 \text{ comparisons}] = 0.004$), all markers adhered to per-locus HWE within collection years (data not shown) and across all adult collection years combined (all $P > 0.004$; Table 3), except for locus *AsaC334* in the 2008 collection year only. Examinations for linkage disequilibrium found that all loci were physically unlinked and significantly independent ($\alpha = [0.05/156 \text{ comparisons}] = 0.00032$). The frequency of potential null alleles was low (<0.05) for all loci within collections (data not shown) and across collections (Table 3). Average H_O varied between 0.82 and 0.85 for each collection year, and R ranged from 11.8 to 12.9. For the combined collections, H_O was high ($H_O > 0.73$) both for individual loci (Table 3) and for all loci taken together ($H_O = 0.84$), and high levels of polymorphism were

TABLE 3. Genetic diversity statistics for 13 *Alosa*-specific microsatellite loci based on all adult American shad collection years combined (2008–2010; N = sample size; N_a = number of alleles per locus; R = per-locus allelic richness; A = allelic size range in base pairs; H_O = observed heterozygosity; H_E = unbiased expected heterozygosity; F_{IS} = inbreeding coefficient; HWE = Hardy–Weinberg equilibrium P -value, significant at $P \leq 0.004$; Null = frequency of null alleles, where high frequency ≥ 0.050).

Locus	N	N_a	R	A	H_O	H_E	F_{IS}	HWE	Null
<i>AsaD030</i>	495	25	24.99	104–200	0.929	0.923	–0.007	0.853	–0.004
<i>AsaD031</i>	495	13	13.00	182–242	0.828	0.858	0.034	0.091	0.017
<i>AsaC010</i>	497	20	19.98	261–341	0.879	0.902	0.025	0.625	0.012
<i>AsaD429</i>	493	11	11.00	135–179	0.777	0.805	0.035	0.491	0.016
<i>AsaD021</i>	494	15	15.00	251–307	0.864	0.861	–0.004	0.771	–0.003
<i>AsaD312</i>	497	20	19.99	124–212	0.867	0.885	0.019	0.706	0.010
<i>AsaC059</i>	499	17	16.99	267–351	0.810	0.856	0.054	0.047	0.028
<i>AsaB020</i>	499	14	13.99	113–152	0.729	0.745	0.021	0.396	0.011
<i>AsaD055</i>	499	16	15.99	223–283	0.780	0.792	0.016	0.561	0.008
<i>AsaC334</i>	499	31	30.94	104–194	0.874	0.882	0.009	0.648	0.005
<i>AsaC249</i>	494	35	34.99	235–387	0.919	0.908	–0.012	0.429	–0.007
<i>AsaC051</i>	496	20	19.99	242–322	0.810	0.795	–0.019	0.759	–0.010
<i>AsaD042</i>	499	24	23.95	144–240	0.894	0.906	0.014	0.845	0.006

TABLE 4. Results of Mendelian inheritance testing based on 13 *Alosa*-specific microsatellite loci in American shad. Summary statistics (χ^2 values, df, and P -values) are given for each locus.

Locus	χ^2	df	P
<i>AsaD030</i>	6.71	10	0.752
<i>AsaD031</i>	16.20	9	0.062
<i>AsaC010</i>	8.33	10	0.596
<i>AsaD429</i>	8.93	7	0.257
<i>AsaD021</i>	12.35	7	0.089
<i>AsaD312</i>	6.17	11	0.861
<i>AsaC059</i>	13.73	11	0.248
<i>AsaB020</i>	10.82	7	0.146
<i>AsaD055</i>	17.60	12	0.128
<i>AsaC051</i>	10.27	12	0.592
<i>AsaC249</i>	22.27	13	0.051
<i>AsaC334</i>	9.47	13	0.736
<i>AsaD042</i>	9.43	9	0.398

observed ($N_a = 11\text{--}35$ alleles/locus). The values of R in the combined collections ranged from 11.00 to 34.99.

The loci suite provided an average parent pair nonexclusion probability of 2.49^{-13} and an average identity nonexclusion probability of 6.78^{-20} , indicating that the possibility of misassignment in the parentage analysis was substantially less than 0.01% and that individuals could be assigned confidently. Based on analysis of progeny from the 2009 production, it was found that all loci met the expectations of Mendelian inheritance (Table 4).

After Bonferroni correction (adjusted $\alpha = 0.008$), no significant temporal genetic differentiation was found between any of the collection years, as exact tests for genic differentiation ($P > 0.042$) and pairwise comparisons of multilocus F_{ST} estimates ($F_{ST} < 0.001$, $P > 0.127$) were not significant (Table 5). The among-year component of variation produced by analysis of molecular variance was low (-0.01%) and nonsignificant ($F_{ST} = -0.00011$, df = 3, $P = 0.657$). Furthermore, overall estimates of genetic diversity and R were similar between sampling years ($H_O = 0.82\text{--}0.85$; $R = 11.8\text{--}12.9$; Table 6). The LDNe estimates of N_e for the Edisto River 2008–2010 adult

TABLE 5. Results of exact tests for genic differentiation (P -values, above the diagonal) and pairwise F_{ST} values (below the diagonal) between collection years for American shad in the Edisto River (2008–2010 adult collections and 2010 year-class [YC] juveniles; Bonferroni-corrected $\alpha = [0.05/6$ comparisons] = 0.008).

Sample year	2008	2009	2010	2010 YC
2008		0.042	0.096	0.262
2009	0.0009		0.593	0.296
2010	-0.0003	0.0006		0.091
2010 YC	0.0004	-0.0003	-0.0003	

TABLE 6. Genetic diversity statistics (defined in Table 3) averaged across 13 microsatellite loci in American shad adult collections from the Edisto River (2008–2010) and in juveniles of the 2010 year-class (YC).

Statistic	2008	2009	2010	2010 YC
N	31	104	363	314
N_a	12.5	16.1	19.7	18.5
R	12.3	11.8	12.5	12.9
H_O	0.822	0.843	0.845	0.843
H_E	0.856	0.850	0.856	0.856
F_{IS}	0.040	0.009	0.013	0.015

data sets and the estimated N_b for the 2010 YC juvenile data set ranged from 1,749–8,379 (95% CI = 243– ∞ ; Table 7). A value of infinity (∞), which was obtained for the 2010 collection year, is produced by LDNe when there is no evidence for any disequilibrium caused by genetic drift due to a finite number of parents. In this case, the data cannot prove that the population is not “very large” (Waples and Do 2010). Except for the 2010 YC juveniles, the upper confidence limits on all values were unbounded, indicating that N_e is large ($\geq 1,000$). Upper bounds on N_e estimates in LDNe are typically not well defined for large populations, even with robust sample sizes ($n \geq 200$; Waples and Do 2010); however, even with estimates of ∞ , the finite lower confidence limit can still be informative with respect to the limits of N_e .

After Bonferroni adjustment ($\alpha = [0.05/3$ comparisons] = 0.016), no significant genetic difference was detected between broodstock and wild individuals (combined 2008–2010 adults) in either 2009 ($\chi^2 = 29.13$, $P = 0.305$; $F_{ST} = 0.0013$, $P = 0.031$) or 2010 ($\chi^2 = 36.37$, $P = 0.085$; $F_{ST} = 0.0003$, $P = 0.331$), and measurements of genetic diversity were similar (Table 8). All loci met HWE expectations ($P > 0.004$), and the estimated frequencies for genotyping artifacts were low (<0.05) in the broodstock used for both production years. From the random subset of 2010 YC shad juveniles ($n = 314$), 11 individuals were identified as originating from hatchery broodstock, while 303 fish were found to have originated from wild parents. Therefore, the estimated total contribution of hatchery individuals to the 2010 year-class prior to out-migration was 3.5%. The recovered

TABLE 7. Estimates of effective population size (N_e ; 95% confidence interval in parentheses) for each adult collection year (2008–2010) and the estimated effective number of breeders (N_b) for juveniles of the 2010 year-class (YC) of Edisto River American shad. Estimates of infinity (∞) are obtained when N_e is large ($N_e \geq 1,000$). The number of sampled fish (N) is also presented.

Sample year	N_e or N_b	N
2008	3,505 (243– ∞)	31
2009	8,379 (883– ∞)	104
2010	∞ (8,009– ∞)	363
2010 YC	1,749 (1,115–3,811)	314

TABLE 8. Genetic diversity statistics (defined in Table 3) averaged across 13 microsatellite loci for American shad broodstock (2009 and 2010) and wild adult collections (2008–2010 combined) from the Edisto River.

Statistic	2009 brood	2010 brood	Wild
N	76	75	317
N_a	15.2	15.2	19.5
R	15.2	15.2	14.8
H_O	0.849	0.832	0.844
H_E	0.849	0.853	0.857
F_{IS}	-0.001	0.026	0.014

hatchery individuals represented nine unique parental crosses and were collected throughout the fall sampling period (Table 9).

DISCUSSION

The evaluation of genetic diversity and N_e in this study provides important information on the genetic characteristics of the Edisto River shad spawning run before the return and possible contribution of hatchery-produced individuals to the spawning stock. Decreases in population size (i.e., census size) have recently been linked to a reduction in genetic diversity for a number of marine fishes (Hauser and Carvalho 2008). Diminished genetic diversity, along with inbreeding, can increase the risk of extirpation by negatively impacting a species' fitness and capacity to respond to environmental stochasticity (Saccheri et al. 1998; Frankham et al. 2002; Keller and Waller 2002; Reed and Frankham 2003; Frankham 2005), making measurements of genetic diversity valuable indicators of overall genetic "health." Genetic diversity of the Edisto River spawning run, as measured by the degree of polymorphism (N_a and R) and the heterozygosity (H_O) of our loci suite, was high ($N_a = 11$ – 35 alleles/locus; $R = 11.8$ – 12.9 ; $H_O = 0.82$ – 0.85) and similar to the levels of diversity reported to occur in other Atlantic coast spawning runs of shad (Brown et al. 2000; Julian and Bartron 2007; Hasselman

et al. 2010). Our results also resemble previous genetic diversity values obtained for the Edisto River by Hasselman (2010; $R = 11.7$; $H_O = 0.78$). We did not observe temporal differentiation between any of the sampling years (2008–2010 and 2010 YC), indicating that the genetic composition of the Edisto River shad spawning run is temporally stable over at least short time frames (1–2 years). Temporal homogeneity has also been found for the Canadian portion of the species' range (Hasselman et al. 2010). The temporal stability in the Edisto River could be maintained by the large N_e ($>3,000$) and by the presence of multiple generations (4–5 cohorts; ASMFC 2007b) within the spawning run (Waples 1990a).

Our other parameter of interest, N_e , is one of the most important measures in conservation biology (Waples 2002; Frankham 2005), as low N_e has been shown to lead to reduced fitness and an increased likelihood of extinction (Newman and Pilson 1997). Furthermore, loss of genetic variation due to drift is inversely proportional to the N_e , so a low N_e may result in increased inbreeding and, in turn, reduced fitness and a greater risk of extirpation (Gilpin and Soulé 1986). Our method of estimating N_e considers genetic drift to be the sole contributor to the signal in the data (i.e., no selection, mutation, migration, or overlapping generations). Selection and mutation rate should have little influence on our estimates of N_e , as microsatellite markers are considered to be selectively neutral and the mutation rate is not an issue for short-term estimates (Waples and Do 2010). Even though straying between rivers (i.e., migration) has been reported for shad (Melvin et al. 1986; Walther et al. 2008), levels of straying are generally low ($\sim 3\%$) and are unlikely to be of consequence for our calculations. Finally, although shad do exhibit some degree of overlap in generations, our N_e estimations should be minimally influenced because the number of cohorts in our sample resembles the generation length of shad (Waples and Do 2010). Therefore, except for the 2010 YC estimate, which represents a measure of N_b , the estimates obtained in this study are considered to be close approximations of N_e and

TABLE 9. Juvenile American shad from the 2010 year-class (sampled prior to out-migration from the Edisto River) that were identified through parentage analysis as having been hatchery produced (ID = identification number; loci = number of genotyped loci; TL = total length).

Sample ID	Loci	Capture date (2010)	TL (mm)	Parent 1 ID	Parent 2 ID
Asa1847	13	17 Sep	79	AS10039	AS10031
Asa1874	12	17 Sep	78	AS10039	AS10071
Asa1891	13	8 Oct	82	AS10039	AS10071
Asa1894	13	8 Oct	81	AS10039	AS10065
Asa1915	13	8 Oct	86	AS10033	AS10059
Asa1916	13	8 Oct	82	AS10050	AS10057
Asa2180	13	13 Oct	91	AS10050	AS10057
Asa2317	13	21 Oct	96	AS10030	AS10046
Asa2329	13	21 Oct	83	AS10033	AS10055
Asa2332	13	21 Oct	85	AS10050	AS10048
Asa2349	13	28 Oct	93	AS10039	AS10067

were on the order of a few thousand individuals ($N_e = 3,505\text{--}8,379$; 95% CI = 243– ∞). The unbounded upper confidence limits, which were a consequence of large N_e ($\geq 1,000$; Waples and Do 2010), are not as important for conservation purposes as the finite lower bounds, which display the minimum limits of our N_e estimates for shad in the Edisto River. The N_b estimate ($N_b = 1,749$; 95% CI = 1,115–3,811) can be related to N_e by using Waples' (1990b) equation $N_e = gN_b$, where g is the shad generation length (4–5 years; Leggett and Carscadden 1978) under the assumption of semelparity. Using this formula, we obtain N_e values of 6,996–8,745 (95% CI = 4,460–19,055) for the 2010 YC, which are similar to the adult estimates. The values of N_e produced in our study approximate the N_e estimates (hundreds to thousands of individuals) found for other diadromous fishes (e.g., brown trout *Salmo trutta*: Jorde and Ryman 1996; Chinook salmon *Oncorhynchus tshawytscha*: Hedrick et al. 2000; steelhead *O. mykiss*: Ardren and Kapuscinski 2003; Atlantic salmon *S. salar*: Palstra et al. 2009) by various methods of calculation. Furthermore, from a conservation standpoint, N_e estimates for the Edisto River are above the minimum of 50 individuals recommended to avoid significant inbreeding and maintain short-term fitness (Franklin 1980) of a population, and our estimates are within the range of minimum values recommended for maintaining a population's evolutionary potential (i.e., quantitative trait heritability; Frankham 1995) and long-term viability ($N_e = 500\text{--}1,000$: Franklin and Frankham 1998; or $N_e = 1,000\text{--}5,000$: Lynch and Lande 1998). Therefore, in combination with the temporal stability and high diversity measurements, these data suggest that the current Edisto River shad spawning run is genetically "healthy" despite recent declines in CPUE. However, genetic diversity and N_e should continue to be monitored for the Edisto River shad, as recent decreases in abundance are often not immediately reflected in the level of genetic diversity. Furthermore, the baseline characterization generated here provides information on the genetics of the Edisto River shad spawning run prior to experimental stocking. In the future, these data will be used to evaluate any potential genetic influences of the shad restoration effort within this system.

Stocking has become an increasingly important tool for supplementing and restoring wild fish populations (Utter and Epifanio 2002; Miller and Kapuscinski 2003), particularly when the target stock faces extinction. Several states along the U.S. Atlantic coast currently employ or have employed artificial propagation in an effort to preserve and restore depleted spawning runs of shad (Hendricks 1995; ASMFC 2009). However, supplementation of wild spawning runs with hatchery-produced individuals has the potential for negative impacts on the target run's genetic diversity and N_e and can lead to a loss of between-population genetic variation. If the broodstock pool used for hatchery production contains less diversity than the pool of wild individuals, stocking could result in a loss of genetic diversity in the target run that is not reflected in the overall census size, thus having the same detrimental effects on fitness and long-term survival as mentioned earlier (Miller and Kapuscin-

ski 2003; Bert et al. 2007; Hedgecock and Coykendall 2007). Furthermore, broodstock may represent only a small genetic segment of the spawning run and consequently may have a reduced N_e ; thus, stocking may actually result in a decrease in N_e if the released hatchery individuals heavily contribute to the wild spawning run (e.g., the Ryman–Laikre effect; Ryman and Laikre 1991). Finally, the use of broodstock from genetically dissimilar rivers may result in a loss of genetic distinctiveness and cause outbreeding depression (Utter 1998; Utter and Epifanio 2002; Miller and Kapuscinski 2003). Therefore, preserving genetic diversity and delimiting distinct populations are major priorities of responsible stocking, and hatchery programs should strive to collect and maintain broodstock that genetically resemble the source population (Blankenship and Leber 1995; Miller and Kapuscinski 2003). For our study, there was no significant difference between hatchery broodstock and wild individuals, and measures of genetic diversity were similar ($R = 14.8\text{--}15.2$; $H_O = 0.83\text{--}0.85$). Furthermore, at the documented level of contribution ($\sim 4\%$ hatchery; 96% wild), our experimental stocking would be unlikely to exert detrimental effects on wild N_e . Based on the Ryman–Laikre equation (Ryman and Laikre 1991) and an estimated N_e of 5,000 wild shad in the Edisto River (i.e., the approximate average for our study; see Table 7), the total population N_e is only negatively impacted (i.e., drops below 5,000) when the N_e of hatchery-reared fish is extremely low (i.e., $N_e = 20\text{--}50$). However, for full-scale management stocking, specific monitoring of the hatchery N_e and contribution is recommended to prevent unwanted decreases in the N_e of the Edisto River spawning run. Finally, to maintain genetic distinctiveness, broodstock for our experimental stocking project were collected directly from the wild spawning run in the Edisto River, as shad in this watershed are genetically distinct from those in most other rivers on the Atlantic coast of North America (Hasselman 2010). Within-river structure has not been examined for shad in the Edisto River; however, for other river systems, it has been shown that locations within the same drainage do not contain separate spawning runs (Epifanio et al. 1995; Brown et al. 1996; Hasselman et al. 2010). Furthermore, work on geographical patterns of chemical signatures in otoliths suggests that shad do not discriminate among tributaries within natal rivers when spawning (Walther et al. 2008). Thus, our examinations suggest that the broodstock used in this experimental stocking was broadly representative of the wild Edisto River shad spawning run.

The purpose of the hatchery supplementation portion of this experimental project was to determine whether the stocking of shad could be a viable option in the Edisto River by evaluating hatchery contribution to the wild spawning run (i.e., whether hatchery individuals could survive and successfully join their wild counterparts). Evaluating the success of any stocking effort is therefore dependent on the ability to identify hatchery-produced individuals and determine their contribution to the wild population. Identification necessitates the use of a tag or mark to distinguish hatchery fish from wild-produced fish (Blankenship and Leber 1995), and the use of molecular

markers as genetic tags avoids some of the constraints and pitfalls associated with conventional tags because molecular markers require no additional tagging, the mark is never lost, and nonlethal tag recovery is possible. Aside from their use in assessing population genetic parameters, the suite of microsatellite markers presented here is also valuable for hatchery fish identification. These 13 microsatellite loci are polymorphic, adhere to the principles of Mendelian inheritance, and can be used to distinguish between individuals and to correctly match offspring to their parents with a high degree of confidence. Hatchery spawning and production were successful during each year of this experimental project, with 12,000–22,000 fry stocked into the Edisto River during each spring. By using these genetic markers as molecular tags to match broodstock with their offspring, it was determined that although the hatchery contribution was relatively low (~4%), hatchery individuals were present among the Edisto River juvenile shad prior to out-migration. Full-scale stocking of shad in U.S. Atlantic coastal rivers often includes the release of between 100,000 and over 10 million fry, and depending on the location, hatchery contributions of 30% or more have been reported (ASMFC 2007b, 2007c). As an example, juvenile shad collected by lift net at the Holtwood Dam on the Susquehanna River, Pennsylvania, were composed of 39–100% hatchery individuals (determined by otolith analysis) from 1990 to 2004, when between 3 and 13 million fry were stocked annually (see Table 10.3 in ASMFC 2007b:76). Considering the number of the fry that were stocked during our trial project (<1% of those released in the Susquehanna River), a hatchery contribution of approximately 4% is a successful outcome for our experimental-level stocking. Detection of hatchery individuals among the wild fish suggests that stocking could be a viable tool for the management of shad in the Edisto River.

In conclusion, the reduction in Edisto River shad CPUE over the past few decades highlighted the need to obtain river-specific information for this system and to assess the potential for responsible stocking to serve as an effective management tool. Our study generated baseline genetic data for this spawning run, indicating that shad in the Edisto River are genetically diverse and that the N_e for the spawning run is large; however, monitoring of these genetic parameters should continue as hatchery fish return and potentially contribute to the Edisto River spawning run. We recommend that effective population size, especially, be closely monitored for the Edisto River spawning run should full-scale stocking be considered, given that low hatchery N_e could reduce wild N_e at higher contribution levels. Finally, our initial detection of a contribution from stocked hatchery fry prior to out-migration in 2010 provides optimism for the potential of stocking as a viable management option, although the long-term success of this experimental stocking will be further addressed upon the return of hatchery individuals as adult spawners (3–6 years). The results of this project and future work will provide valuable information that can be incorporated into management plans for aiding in the recovery of this important species in the Edisto River.

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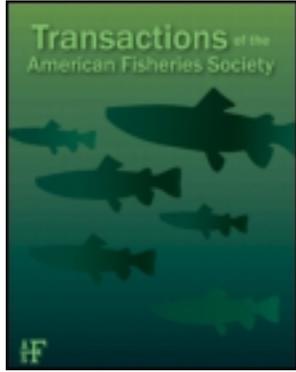
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A Remote-Sensing, GIS-Based Approach to Identify, Characterize, and Model Spawning Habitat for Fall-Run Chum Salmon in a Sub-Arctic, Glacially Fed River

Lisa Wirth^a, Amanda Rosenberger^b, Anupma Prakash^c, Rudiger Gens^c, F. Joseph Margraf^{a e} & Toshihide Hamazaki^d

^a U.S. Geological Survey, Alaska Cooperative Fish and Wildlife Research Unit, Post Office Box 757020, Fairbanks, Alaska, 99775-7020, USA

^b School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Post Office Box 757220, Fairbanks, Alaska, 99775, USA

^c Geophysical Institute, University of Alaska Fairbanks, Post Office Box 7320, Fairbanks, Alaska, 99775, USA

^d Alaska Department of Fish and Game, Division of Commercial Fisheries, 333 Raspberry Road, Anchorage, Alaska, 99518, USA

^e U.S. Geological Survey, Box 25046 MS 406 DFC, Denver, Colorado, 80225, USA

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ARTICLE

A Remote-Sensing, GIS-Based Approach to Identify, Characterize, and Model Spawning Habitat for Fall-Run Chum Salmon in a Sub-Arctic, Glacially Fed River

Lisa Wirth*

U.S. Geological Survey, Alaska Cooperative Fish and Wildlife Research Unit, Post Office Box 757020, Fairbanks, Alaska 99775-7020, USA

Amanda Rosenberger

School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Post Office Box 757220, Fairbanks, Alaska, 99775, USA

Anupma Prakash and Rudiger Gens

Geophysical Institute, University of Alaska Fairbanks, Post Office Box 7320, Fairbanks, Alaska, 99775, USA

F. Joseph Margraf¹

U.S. Geological Survey, Alaska Cooperative Fish and Wildlife Research Unit, Post Office Box 757020, Fairbanks, Alaska 99775-7020, USA

Toshihide Hamazaki

Alaska Department of Fish and Game, Division of Commercial Fisheries, 333 Raspberry Road, Anchorage, Alaska, 99518, USA

Abstract

At northern limits of a species' distribution, fish habitat requirements are often linked to thermal preferences, and the presence of overwintering habitat. However, logistical challenges and hydrologic processes typical of glacial systems could compromise the identification of these habitats, particularly in large river environments. Our goal was to identify and characterize spawning habitat for fall-run chum salmon *Oncorhynchus keta* and model habitat selection from spatial distributions of tagged individuals in the Tanana River, Alaska using an approach that combined ground surveys with remote sensing. Models included braiding, sinuosity, ice-free water surface area (indicating groundwater influence), and persistent ice-free water (i.e., consistent presence of ice-free water for a 12-year period according to satellite imagery). Candidate models containing persistent ice-free water were selected as most likely, highlighting the utility of remote sensing for monitoring and identifying salmon habitat in remote areas. A combination of ground and remote surveys revealed spatial and temporal thermal characteristics of these habitats that could have strong biological implications. Persistent ice-free sites identified using synthetic aperture radar appear to serve as core areas for spawning fall chum salmon, and the importance of stability through time suggests a legacy of successful reproductive effort for this homing species. These features would not be captured with a one-visit traditional survey but rather required remote-sensing monitoring of the sites through time.

*Corresponding author: lmwirth@alaska.edu

¹Present address: U.S. Geological Survey, Box 25046 MS 406 DFC, Denver, Colorado 80225, USA.

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Conducting a habitat study of a species at the extremes of their range can simplify identification of limiting factors; a single environmental condition could be constraining range expansion of the species (Stearns 1977). For example, at northern limits of a species' range, the fundamental habitat requirement and limiting factor may be as simple as liquid water due to severe winter conditions (e.g., freezing, frazil ice, or subzero temperatures). However, this simplification can be compromised by variability in that limiting factor; small changes in environmental conditions could have dramatic consequences. Changing weather conditions from year-to-year, for example, could result in high temporal variability in the presence of liquid water for overwintering fish. Incorporation of long-term data, however, could allow researchers to understand what is 'average' for a particular location and permit detection of long-term trends.

Pacific salmon are anadromous, returning to their natal site to spawn, honing in on hydrological features such as water odor, depth, and velocity, gravel composition, and the presence of cover (Salo 1991). The homing nature of salmon is such that their present distribution and habitat use not only reflect the suitability of habitat at the time of observation, but also the suitability of those areas in years past. The presence of suitable temperatures for egg incubation and juvenile rearing is probably very important, particularly in the northern limits of their range in Alaska. However, the dynamic nature of the Arctic and sub-Arctic environments of Alaska (Milner et al. 1995) highlights the importance of understanding temporal variation in physical processes determining the distribution of salmon species. It is unfortunate, then, that long-term data sets for this region are generally unavailable.

The Yukon River drainage in Alaska possesses two genetically distinct life history forms of chum salmon *Oncorhynchus keta*, summer and fall runs (Seeb and Crane 1999). This species matures and returns to the freshwater environment between ages 3 and 6, and fry emerge and migrate to the ocean in spring during ice-out (Quinn 2005). Fall-run chum salmon begin their spawning migration and enter the Yukon River from late June through early September and spawn in main-stem habitats (Barton 1992). Peak spawning is from mid-October to mid-November, coinciding with decreasing, silty glacial run-off (silt can be detrimental to the survival of salmon eggs; Hausle and Coble 1976; Lapointe et al. 2004; Levasseur et al. 2006) and increased water clarity (Osterkamp 1975). However, late spawning leads to a narrow time window for egg incubation and larval growth prior to spring smolt outmigration. In the northernmost (Arctic and sub-Arctic) regions, areas of upwelling water may offset this disadvantage. Areas with upwelling groundwater or hyporheic exchange provide warmer and more consistent water temperatures for winter incubation and protection from freezing (Reynolds 1997; Fausch et al. 2002; Quinn 2005). A number of studies in other parts of their range have documented the selection of upwelling water for spawning salmon (Reynolds 1997; Geist and Dauble 1998; Baxter and Hauer 2000; Geist et al. 2002).

The goal of our study was to identify and characterize spawning habitat for fall chum salmon and model habitat selection in the main-stem Tanana River, a tributary of the Yukon River at the northern extent of the chum salmon's range, where subzero temperatures and winter conditions are extended and severe. We characterized habitat along an extensive reach of the Tanana River, with particular note of areas of ice-free water during winter, which indicated the presence of groundwater. Given the importance of a legacy of spawning success for this homing species, we also characterized the temporal consistency of these ice-free areas using remotely sensed data collected over the last decade. Finally, to determine the relative importance of groundwater influence on spawning-female reach selection, we related numbers of spawning females to reach characteristics, including the presence of ice-free areas (indicating strong groundwater influence), the permanence of those areas (consistent presence from year to year), and other habitat features that may also play a role in spawning-female habitat selection. Given the potential importance of groundwater-influenced areas (i.e., ice-free) for spawning chum salmon, we collected data to characterize both spatial (through remote-sensing techniques) and temporal (through on-site temperature loggers) thermal characteristics of ice-free areas that persisted over our period of investigation. We anticipated that this approach would demonstrate potential for integrating remote sensing technologies and in-stream data collection for future studies to better elucidate critical habitat characteristics for a commercially and culturally important fish species.

METHODS

Study area.—This study took place in the Tanana River, a large, free-flowing, glacially fed tributary of the Yukon River (Figure 1). This is the largest tributary of the Yukon River, flowing 700 km northwest through a broad alluvial valley, draining an area of 155,250 km². Heavily silted and braided, the Tanana River poses challenges for monitoring and identifying spawning fish and spawning areas. Turbid waters interfere with visual identification of spawning congregations, and the habitat complexity of a large but braided channel can also complicate standard riverine habitat assessments designed for small to medium streams (e.g., Thomson et al. 2001). Runs of fall chum salmon return to the Tanana River and support important subsistence, personal use, and commercial fisheries. However, in recent years, fall chum salmon runs returning to the Yukon River and, consequently, the Tanana River, have been in decline (Borba et al. 2009).

Prior to our study, the only rivers regularly monitored for chum salmon escapements (after the subsidence of glacial waters) were the Toklat (average escapement, 31,000) and Delta (14,000) rivers, tributaries to the Tanana River (Bue et al. 2004). Prior to this study, it was unknown but suspected that chum salmon spawned in the main-stem river, which is the focus of our study. Our study area, located between Fairbanks and Big

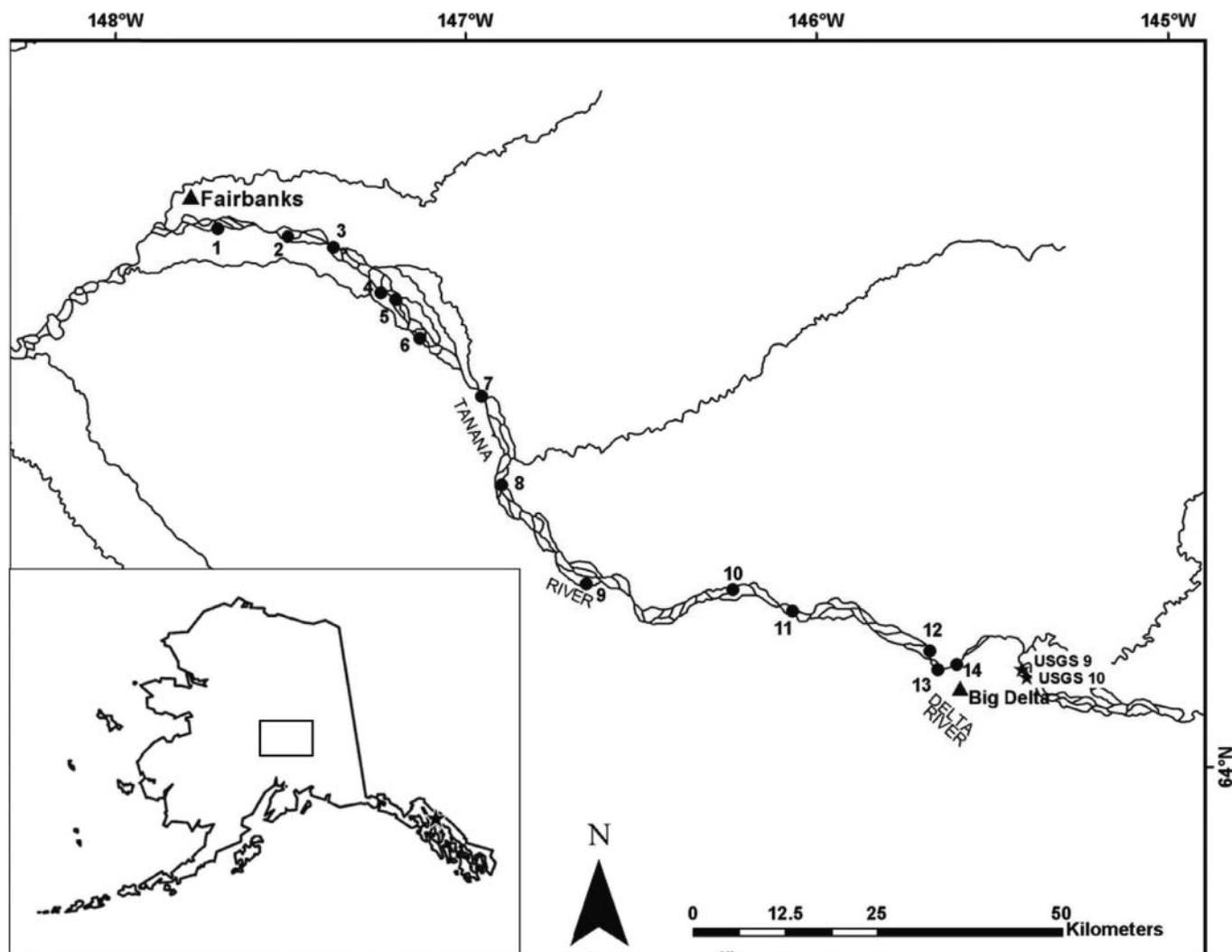


FIGURE 1. Main-stem Tanana River study area between Fairbanks and Big Delta, Alaska. Numbers indicate locations of retrieved surface temperature data loggers.

Delta, Alaska (Figure 1), was 160 river kilometers in length and subdivided into 16 study reaches, each approximately 10 km long (reach length range: 9.4–12.1 km; Table 1). In establishing reaches we took into account smaller tributaries that empty into the Tanana River such that a tributary did not enter in the middle of a reach; this lead to variability in reach length.

Radiotelemetry data.—Adult female fall chum salmon ($N = 328$) were captured with fish wheels upstream of the Kantishna River on the north bank of the Tanana River from 16 August through 30 September 2008 (Yukon River mile 793), between the communities of Manley and Old Minto. Fish were tagged with an internal pulse-coded radio transmitter manufactured by Advanced Telemetry Systems, Inc. (ATS 2008). Transmitters weighed approximately 20 g, were 5.4 cm long and 2.0 cm in diameter, and had a 30-cm transmitting antenna. Surveys to track fish movement were conducted by air at least twice

weekly beginning 2 weeks after initial tag deployment until late December.

Satellite imagery.—Multiple, complementary imagery data sets were used to provide the most accurate and extensive imagery (both in space and through time) possible for the study site and to verify findings and methodology. The RADARSAT-1 images acquired by the Alaska Satellite Facility (ASF) were used to determine persistence of ice-free water. Synthetic aperture radar (SAR) satellites, like RADARSAT-1, image by transmitting electromagnetic signals towards the earth's surface and then receiving the portion of electromagnetic signal that is backscattered towards the satellite. The signal has the capability to penetrate cloud cover, allowing for data collection irrespective of weather (Cumming and Wong 2005), a useful feature for the Alaska environment. The images were collected from the C-band (5.7-cm wavelength) and were taken from the fine beam

TABLE 1. Habitat characteristics and lengths of the 16 study reaches in the Tanana River, Alaska. All habitat variables were used in candidate models of the total number of spawners in the Tanana River.

Reach number	Length (km)	Sinuosity	Braiding	Ice-free surface area (km ²)	Persistent ice-free water	Total number of spawners
1	9.4	1.36	2.19	1.09	1	22
2	10.5	1.20	2.18	0.27	1	14
3	12.1	1.19	2.88	0.57	1	41
4	12.1	1.29	3.49	0.08	0	8
5	10.1	1.32	3.24	0.07	0	5
6	10.3	1.23	2.95	0.01	0	3
7	9.7	1.14	3.01	0.15	1	21
8	9.5	1.28	2.93	0.06	0	14
9	9.6	1.46	3.58	0.00	0	16
10	9.5	1.36	3.84	0.05	0	17
11	9.6	1.30	4.30	0.06	0	14
12	10.3	1.32	3.69	0.13	0	10
13	10	1.19	4.28	0.20	0	5
14	9.8	1.19	4.26	0.14	0	4
15	10	1.14	3.02	0.04	0	1
16	9.8	1.22	2.50	0.02	0	2

and standard beam mode. Fine beam has a spatial resolution of 6.25 m; while the standard beam has a spatial resolution of 12.5 m. Fine-beam images were ideal due to their higher spatial resolution but did not give complete coverage of the study area, resulting in the need to use standard-beam images for the southern reaches. Fine-beam data were collected in March 1996, February 2005, and March 2008, corresponding to late winter when discharge in the river is groundwater-based. Standard-beam images from 1997, 2005, and 2008 were initially examined in conjunction with the fine-beam images to create complete coverage for the study area. A preliminary analysis of ice-free areas for these 3 years identified spatially persistent ice-free areas. To further confirm their persistence through time, additional years of imagery were analyzed (standard beam images for March 1997, 2000–2003, 2005, 2006, and 2008). Yearly observations during the time frame were not available, and these years were selected because they contained the most complete coverage of the study area during late winter (March; Table 2).

Interpretation of SAR images is not immediately intuitive due to the side-looking SAR geometry and the complex interaction of the SAR signal with the target. The intensity of a SAR pixel represents the amount of signal backscattered from the target area (Gens 2009). Among several factors that control the backscatter, two important factors are the amount of moisture (that, in turn, controls the dielectric property) and surface texture of the target (Pietroniro et al. 2005). Dielectric properties for liquid water are different from snow and ice, which can vary greatly depending on the amount of liquid water contained in the snow or ice particles (Fung and Ulaby 1983; Simonett and Davis 1983). The presence of still or slowly flowing water (without surface turbulence) enhances contrast in the radar

signal because it acts as a specular reflector with a low backscatter signal (Pietroniro et al. 2005). Ice-free areas of liquid water surrounded by snow and ice appeared dark, whereas the surrounding snow and ice provided a bright signal on the image. The effect of surface roughness of the ice-free areas in the radar return was negligible.

Images from the advanced visible and near infrared radiometer type 2 (AVNIR-2) onboard the advanced land observation satellite (ALOS) were used as base imagery to identify final spawning location and to calculate the ice-free water surface area

TABLE 2. Synthetic aperture radar (SAR) images used to identify ice-free areas in the Tanana River.

Granule identification	Beam mode	Acquisition date
R1_01952_FN1_F162	Fine beam	Mar 20, 1996
R1_01952_FN1_F161	Fine beam	Mar 20, 1996
R1_06897_ST6_F160	Standard beam 6	Mar 01, 1997
R1_22675_ST6_F160	Standard beam 6	Mar 09, 2000
R1_27877_ST3_F161	Standard beam 3	Mar 08, 2001
R1_33022_ST3_F161	Standard beam 3	Mar 03, 2002
R1_38224_ST1_F161	Standard beam 1	Mar 02, 2003
R1_48600_FN1_F162	Fine beam	Feb 25, 2005
R1_48600_FN1_F161	Fine beam	Mar 25, 2005
R1_48722_ST1_F290	Standard beam 1	Mar 05, 2005
R1_53981_ST7_F290	Standard beam 7	Mar 08, 2006
R1_64435_ST2_F160	Standard beam 2	Mar 09, 2008
R1_64457_FN1_F289	Fine beam	Mar 10, 2008
R1_64457_FN1_F288	Fine beam	Mar 10, 2008

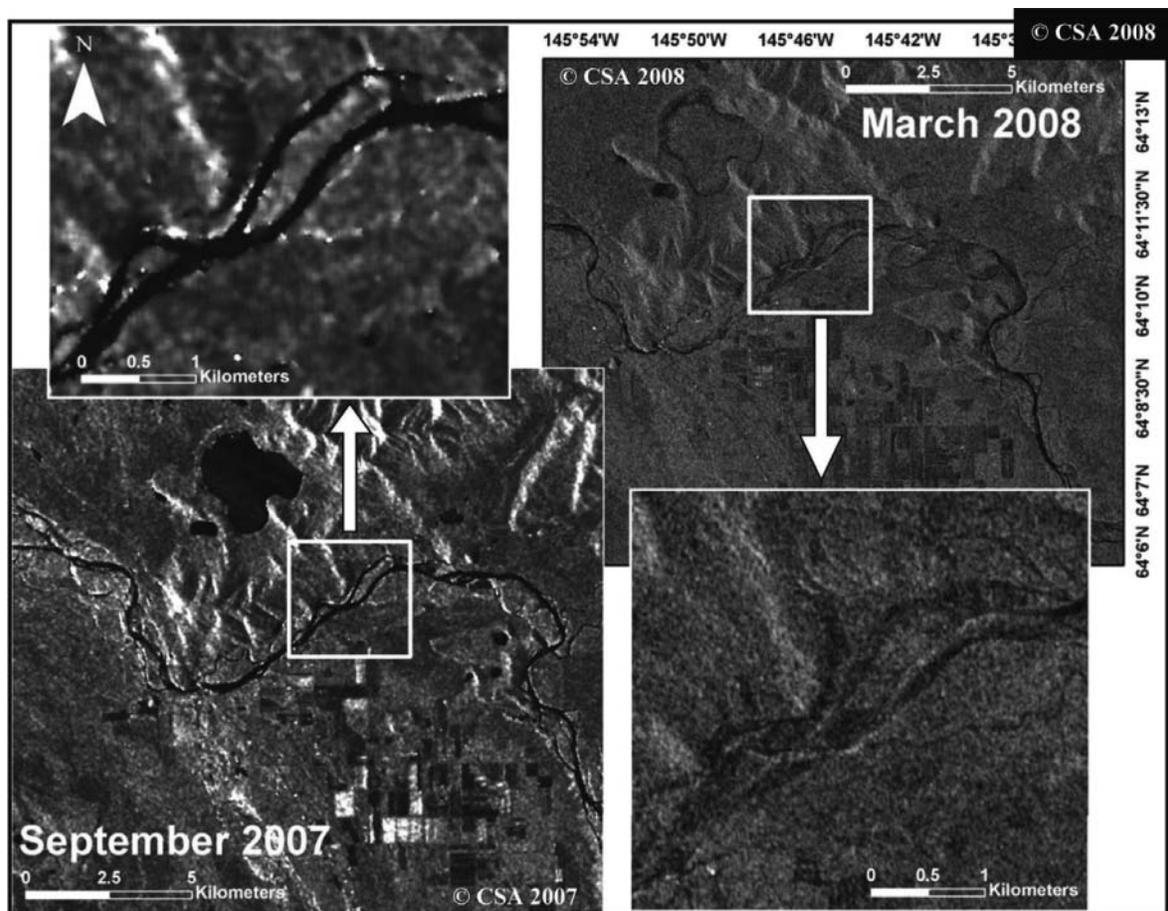


FIGURE 2. Image of the Tanana River illustrating an easily identifiable water signature in September prior to river freeze-up. The frozen river in late winter (March) is less obvious, blending with the surrounding area due to similar radar signatures.

in 2009. The AVNIR-2 sensor acquires four bandwidths, three visible bands (0.42–0.50 μm , 0.52–0.60 μm , 0.61–0.69 μm) and one near-infrared band (0.76–0.89 μm) with 10-m spatial resolutions. The images were acquired in March 2009, and all had 30% or less cloud cover.

Total river surface area, prior to river freeze-up, was calculated by manually digitizing the limits of the water body in ArcGIS (ESRI 2007) on the SAR images acquired from the standard beam mode via a 12.5-m spatial resolution collected on September 30, 2007 (Figure 2). The same SAR images were used to calculate river-braiding index and sinuosity, described below. The AVNIR-2 images of March 2009 (after river freeze-up and during base flow winter conditions; U.S. Geological Survey [USGS] Water Resources Data, Nenana, Alaska; Figure 2) were used for baseline calculations of percent ice-free surface area per reach and to categorize final spawning locations.

Several preprocessing steps were necessary to convert the SAR images from their original data format to a geocoded product amenable to processing and interpretation. The level 1 data acquired from ASF was processed in Mapready (ASF 2009) to convert it from CEOS format to a GeoTIFF format. This

preprocessing step allowed for multi-temporal images to be stacked together in a geographic information system (GIS) for analysis. Ice-free areas were hand-digitized using ArcGIS for all years for which SAR images were available (Figure 3). Once digitizing was complete, layers for the different years were stacked and analyzed for assessing persistence in the occurrence and extent of these areas from year to year. A reach with a persistent ice-free area for each image analyzed was given a value of (1); otherwise, the reach was classified as nonpersistent and given a value of (0). Persistent ice-free areas did not always have the same extent because cold severity affects the degree of ice cover; however, the centroid and overall shape of persistent ice-free areas remained consistent (Figure 4), whereas nonpersistent areas had no overlap from year to year (Figure 3).

Ice-free water classification results for AVNIR-2 imagery give an overall accuracy of 86.2% (Table 3). Unique areas of long-term persistence were identified from the time-series analysis using SAR imagery.

Braiding index and sinuosity.—The braiding index (B) was calculated by the sum of the mid-channel lengths (L_{ctot}) of all the segments of primary channels in a reach and the mid-channel

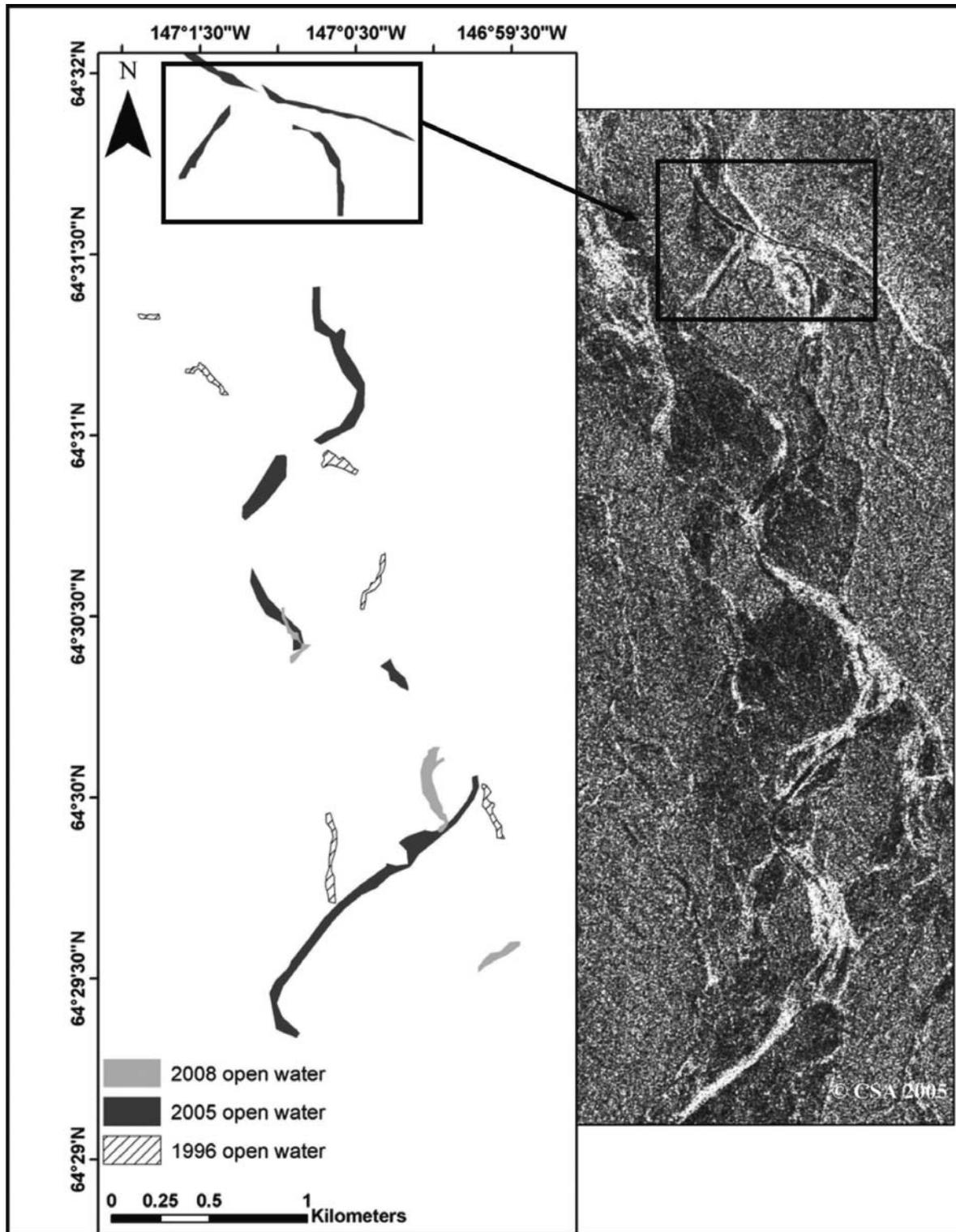


FIGURE 3. Ice-free (open water) areas in the main-stem Tanana River digitized and layered to show a dominant pattern. The location of upwelling areas change with the relocation of the main channel and braiding pattern through time (1996–2008). The arrow is indicating the zone digitized from the 2005 SAR imagery.

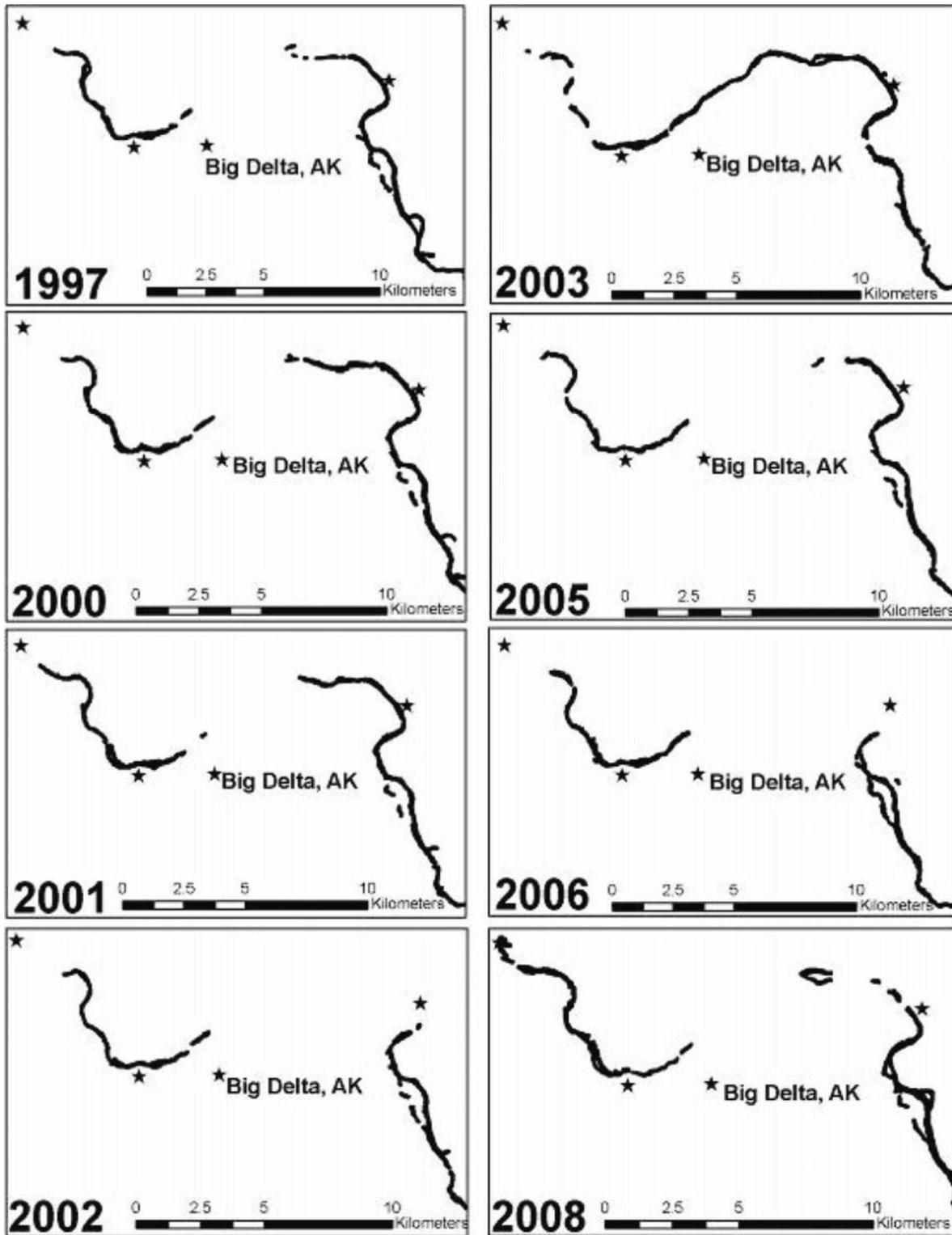


FIGURE 4. Mapped Tanana River ice-free water for each year examined near Big Delta, Alaska. The stars represent fixed locations for each year for location reference. This shows variability in each year for extent of ice-free water and shows two distinct areas that remain open each year studied.

TABLE 3. Accuracy assessment for ice-free (IF) and frozen (FR) classifications of AVNIR-2 imagery.

Reference (ground) data				
Classified data	IF	FR	Total	User's accuracy (%)
IF	5	1	6	83.3
FR	3	20	23	87.0
Total pixels	8	21	29	
Producer's accuracy (%)	62.5	95.2		86.2

length (L_{cmax}) of the widest channel through the reach (Friend and Sinha 1993): $B = L_{\text{ctot}}/L_{\text{cmax}}$.

Sinuosity (P) was calculated by L_{cmax} and the overall length of the channel (L_R), measured along a straight line (Friend and Sinha 1993): $P = L_{\text{cmax}}/L_R$. Ice-free water surface area (km^2) was calculated from AVNIR-2 images from March 2009 by manually digitizing the extents of the water body in ArcGIS (ESRI 2007).

Thermal information.—The optical and SAR imagery provided information to delineate ice-free bodies but did not provide remotely sensed information on water temperatures. Therefore, to address the likely mechanism for that ice-free or the extent of groundwater influence, temperature data loggers were used to characterize water temperatures within areas of ice-free and frozen water. Surface water temperature measurements were collected using HOBO Pro (version 2) water temperature data loggers (Onset 2008) that were deployed in October and November 2008 and continually collected data until retrieval in April 2009. Loggers were placed in eight ice-free locations and 15 frozen locations prior to river freeze-up. These locations were chosen based on visual ice mapping during November–December 2007. Two data loggers were deployed in each ice-free area, one at the upstream end and one at the downstream end. One logger was deployed at each of the frozen water locations.

To obtain quantitative estimates on the water temperatures and gain further insight into the characteristics of areas of groundwater influence, we used Forward-Looking Infrared Radiometer (FLIR) images. In November 2009, FLIR images were collected to describe temperature patterns for selected ice-free areas. The FLIR sensor acquires surface temperature values in a single 7.5–13- μm broadband spectral range. A ThermoVision A320G (FLIR 2009) sensor with a 24° lens was used to capture images at a rate of 30 images/s. Flying at an altitude of 1,300 m provided thermal images with a spatial resolution of 1.7 m. Flying height, ambient temperature, and atmospheric humidity at the time of flight were entered into the FLIR software for automated corrections of the recorded thermal data. The FLIR software uses an inversion of Planck's function to convert spectral radiance to radiant temperature values. We used a uniform

emissivity value of 0.90 during image collection. Most natural substances have emissivity values of 0.80–0.97 (Lillesand et al. 2008), and taking a uniform emissivity did not influence the spatial thermal patterns we obtained. The FLIR images collected for specific ice-free sites were loaded into FLIR-builder software that automatically stitched images together. The images were then geo-referenced via ERDAS Imagine software (Leica Geosystems 2008) using the ALOS AVNIR-2 image from March 2009 as the base image for spatial reference.

Habitat modeling and characterization.—We determined final spawning locations from female fall chum salmon through having at least three telemetry points within a single reach within 1 month of each other. This timeframe was chosen to account for time spent by the female searching for a nest site, digging a redd, spawning, and territorial defense. Tagged individuals that traveled through the study area and showed no site fidelity were not included in analysis. The ALOS -AVNIR-2 images acquired in March 2009 were combined with telemetry data to determine final spawning location.

Using negative binomial regression, we constructed a model of habitat use at the reach scale. The negative binomial is a discrete probability distribution that is used for animal count data and considers zero's, inherent in ecological count data (Power and Moser 1999; Pradhan and Leung 2006; Lewin et al. 2010). The SAS statistical package (SAS 2009) was used for all analyses. The dependent variable was total number of spawning salmon per study reach, determined using methods described above. The independent variables were: braiding, sinuosity, ice-free water surface area (determined from 2009 AVNIR-2 images), and persistent ice-free water (determined from SAR images).

We used the information-theoretic approach, and model selection was based on Akaike's information criterion (AIC; Burnham and Anderson 2002). All variables used to create the model of habitat use were tested for covariance by using Pearson's product-moment correlation coefficient (variables excluded from the same model if $R > 0.60$). Persistent ice-free water (hereafter, persistence) covaried significantly with both braiding and ice-free-water surface area ($R = 0.72$), preventing a test of a global model; we instead tested alternative candidate models. Candidate models included sinuosity, braiding, and ice-free-water surface area; braiding and ice-free-water surface area; persistence; and persistence and sinuosity. Without overwhelming evidence for a single best model, we performed model-averaging using Akaike weights (following Burnham and Anderson 2002).

RESULTS

Chum salmon spawned in reaches that not only contained the greatest areas of ice-free water, but also ice-free water that was persistent over the years monitored. Without overwhelming evidence for a single candidate model of the final spawning reach for fall chum salmon (Table 4), we developed an averaged

TABLE 4. Candidate models of final spawning location for fall chum salmon with intercept and 95% confidence interval in parentheses, R^2 , and corrected Akaike information criterion (AIC_c) values calculated from a negative binomial regression analyses. Also shown is the AIC_c difference (Δ) between candidate models used to calculate weights for model averaging. Due to covariance between ice-free persistence and ice-free area, we were unable to construct a global model.

Model	Variable				R^2	AIC_c	Δ
	P (sinuosity)	B (braiding)	Ice-free area	Persistence			
P , Persistence	4.62 (1.14, 8.10)			-1.51 (-2.17, -0.85)	0.60	68.16	0.00
Persistence				-1.08 (-1.76, -0.42)	0.51	69.52	1.36
B , Ice-free area		0.13 (-0.51, 0.77)	1.62 (0.019, 3.22)		0.33	76.45	8.29
P , B , Ice-free area	1.36, (-2.55, 5.27)	0.06 (-0.59, 0.72)	1.66 (0.021, 3.31)		0.34	78.36	10.20

model of the most plausible models for predicting number of spawning fall chum salmon. It included sinuosity (parameter estimate = 3.06, 95% CI = 1.14–8.10) and persistence (parameter estimate = 1.36, 95% CI = 0.85–2.17). This averaged model had sizably greater correspondence with spawning counts than the most plausible model that included ice-free-water area ($R = 0.57$ versus $R = 0.33$).

Temperature data loggers were retrieved from 14 of 23 locations in April 2009 (Figure 1). Loggers were retrieved from six locations thought to be frozen based on visual aerial surveys conducted in 2007; however, upon retrieval, locations 1, 2, and 12 were ice-free, and sites 3, 4, and 6 had dried and were completely free of ice (Appendix). The most consistent temperatures through the winter season (adequate for egg incubation) were in location 14, a persistent ice-free site (mean = 4.78°C, range = 3.99–5.8°C). Inconclusive results due to logger loss and drying led us to investigate the thermal characteristics of ice-free locations from the FLIR images.

These images were collected from persistent ice-free areas (spanning 1996–2008) to obtain spatially continuous thermal characteristics on a larger, more continuous scale. These images illustrated the thermal heterogeneity of upwelling water areas and the extent of groundwater thermal influence on surface waters. For corroboration, we compared surface temperature loggers from site 14 with FLIR imagery, and data were in agreement. One image from a spawning habitat site near Rika’s Roadhouse displayed warm upwelling water (4.5–6.0°C) entering the system, contrasting with much cooler surface water (1.0°C; Figure 5). A second ice-free area near Bluff Cabin Slough also showed warmer groundwater entering the system. This site contains extensive thermal spatial heterogeneity with strong input of warmer water, presumably from groundwater influx (Figure 6). Two surface water temperature data loggers were retrieved from location 9, which was about 600 m upstream from FLIR image acquisition in the third persistent upwelling site. Both data loggers showed highly variable temperatures, means remaining just above 0.0°C, which suggests limited influence of groundwater in this location.

DISCUSSION

Our observations suggest that spawning chum salmon exhibit strong association with reaches characterized by an increased availability of liquid water in late winter, and the most likely models included information on the spatial and temporal persistence of these habitats, likely driven by groundwater processes. These results suggest that females selected reaches for not only the presence of upwelling water, but also for the stability of groundwater influx. Given that spawning habitat selection is a shorter-term process than groundwater stability over years or decades, this may reflect past reproductive success of previous generations and homing behavior of following generations to reaches with consistent groundwater influences. Alternatively, it could imply that deep groundwater has a different chemical signature that fall chum salmon have evolved to detect and select over areas with groundwater of more shallow and ephemeral origin.

Collection of microhabitat variables for fall chum salmon spawning habitat (e.g., substrate, depth, and velocity) were attempted but unsuccessful due to the dynamic, remote, and logistically challenging nature of a large, glacial river and the season (late fall) that fall chum salmon occupied the habitat. Placement of temperature data loggers based on aerial visual observations resulted in incomplete habitat assessment, where no broad-scale interpretation could be made about fundamental habitat requirements. This led to further examination of temperature patterns within and between upwelling locations using FLIR imagery. Forward-looking infrared images showed extensive thermal heterogeneity within areas influenced by groundwater, but the degree of upwelling influx varied among ice-free locations.

Identifying areas with strong groundwater inputs and complex thermal heterogeneity with FLIR imagery allows for the identification of areas containing a suitable thermal environment for egg incubation and may assist in identification of the first-order control on spawning habitat selection if information on persistent ice-free water is not available. In a concurrent study, the USGS assessed the thermal characteristics of locations in the Tanana River within the area covered by FLIR images.

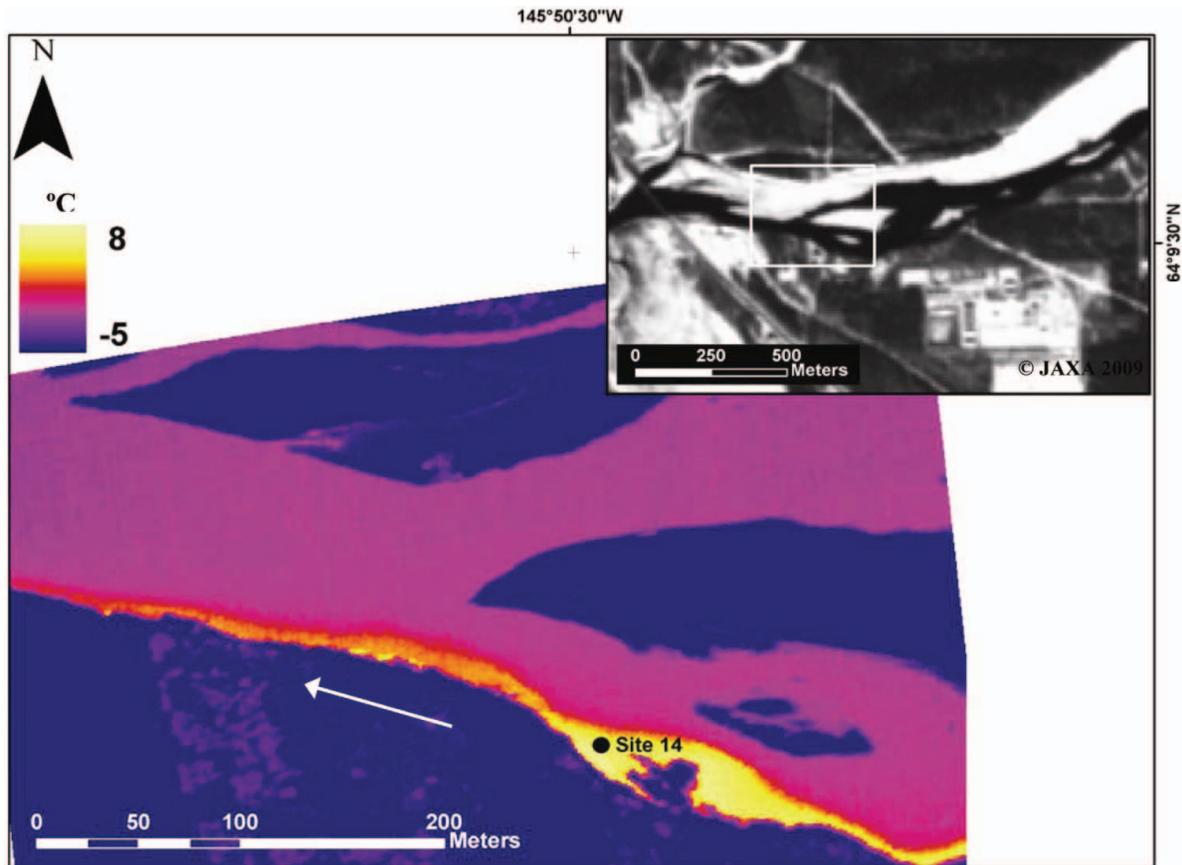


FIGURE 5. Forward-looking infrared (FLIR) image of a fall chum salmon spawning habitat area in the Tanana River near Rika's Roadhouse, showing warm groundwater influx (yellow shaded area) compared with the cooler surface water (pink shaded area). The arrow is flow direction, and the enclosed circle indicates location of surface temperature data logger retrieved from location 14. The inset is showing the AVNIR-2 image used for georeferencing, and in which the white-line box shows the boundaries of the FLIR image. [Figure available in color online.]

Examining the intergravel and surface water temperature characteristics of upwelling habitats, their upwelling habitat locations 9 and 10 had a higher vertical hydraulic gradient and contained stable intergravel temperatures, which provided

sufficient accumulated thermal units (an accumulated effect of temperature over time) for complete egg incubation and fry emergence (Figure 6; Table 5; Burril et al. 2010). Surface water temperatures directly in an area of groundwater input were

TABLE 5. List of locations monitored by USGS scientists, reporting vertical hydraulic gradient (VHG), type of logger used, minimum–maximum range, and mean temperatures and accumulated thermal units (ATU) for each site (from Burril and Zimmerman 2010). No temperature data could be retrieved from location 5.

USGS reach location number	VHG (cm)	Data logger	Temperature (°C)			ATU
			Range	Mean		
5	0.2					
9	0.6	Surface	–0.0	5.5	3.1	467
		40 cm 1	1.2	5.8	5.6	842
		40 cm 2	–0.1	5.5	5.2	783
10	1.0	Surface	0.0	4.0	1.0	158
		40 cm 1	5.0	5.4	5.2	779
		40 cm 2	4.7	5.2	5.0	749

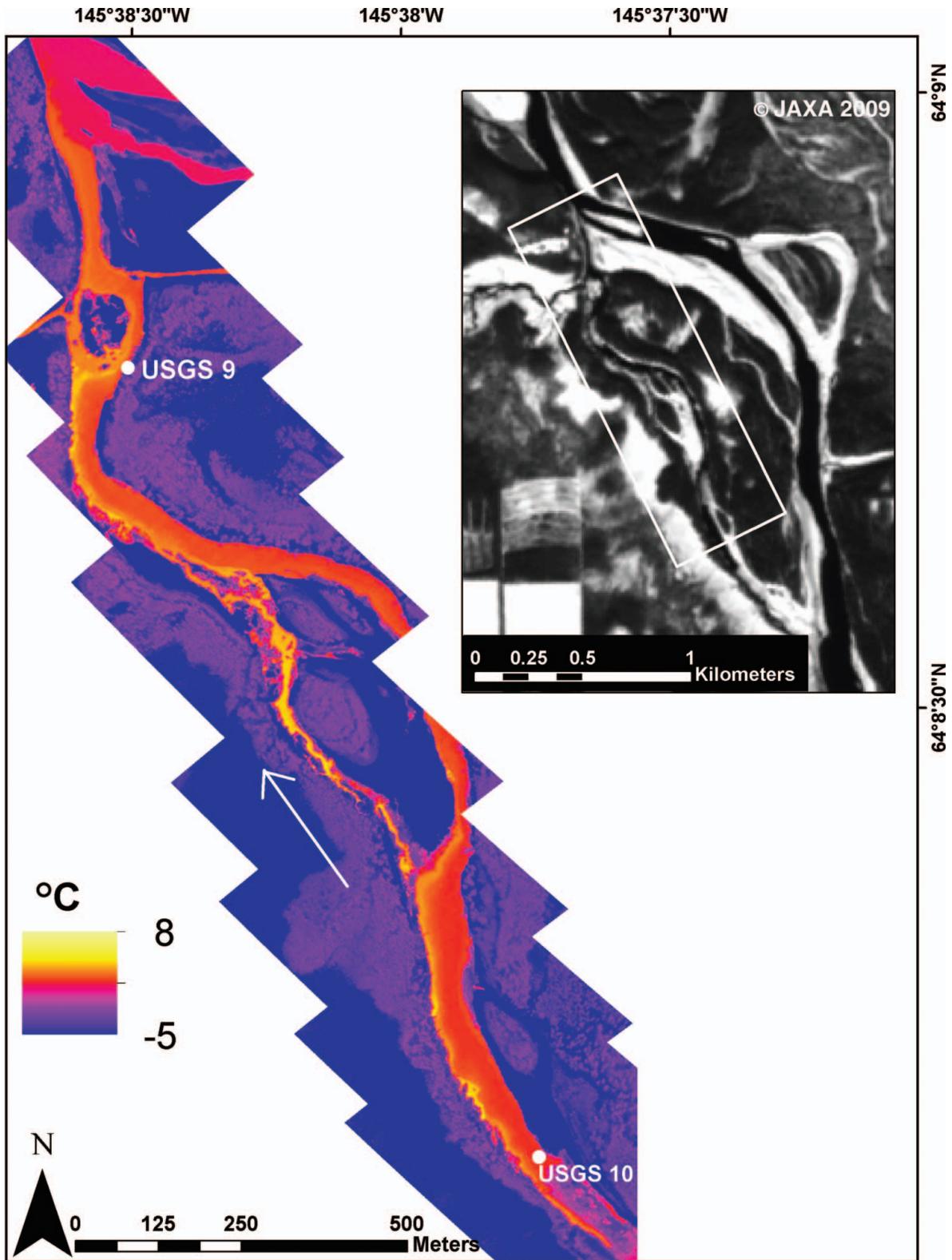


FIGURE 6. Forward-looking infrared (FLIR) image of the Tanana River near Bluff Cabin Slough, displaying spatial thermal heterogeneity of upwelling groundwater influx and the location of Burrell's et al. (2010) U.S. Geological Survey sites 9 and 10, which provided corroborating information. The arrow is flow direction. The inset is the AVNIR-2 image used for georeferencing within which the white-line box shows the boundaries of the FLIR image. [Figure available in color online.]

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stable and similar to intergravel temperatures. Surface water temperatures not directly in an area of groundwater influx, but in close proximity, varied significantly, though intergravel temperatures remained stable. These data illustrate the utility of integrating remote sensing and in-stream data collection to identify critical habitat for spawning fall chum salmon and to investigate the role of the abiotic environment in habitat selection. This would only apply to areas of the river with sufficient mixing and turbulence to permit groundwater-influenced waters to thermally mix with surface waters.

It has been argued that traditional habitat assessment methods employed in a stochastic (snapshot) manner only examine small spatial and temporal phenomena and fail to capture important ecological and physical processes important to fish productivity (Geist and Dauble 1998; Baxter and Hauer 2000; Fausch et al. 2002). Applying traditional habitat-assessment methods for small streams to the large, glacially fed Tanana River proved infeasible, necessitating the acquisition of remotely sensed data. Recent advancements in technology have allowed for the use of optical remote sensing of in-stream habitat features important for juvenile salmon at intermediate scales (Carbonneau et al. 2005a; Carbonneau et al. 2005b; Carbonneau et al. 2006; Smikrud and Prakash 2006; Marcus and Fonstad 2008; Smikrud et al. 2008; Woll et al. 2011). However, in the arctic regions, high solar incidence angles due to the high latitude and frequent cloud cover are common problems. The use of SAR imagery circumvented these problems and allowed for the identification of persistent ice-free habitats important in the two most likely models. However, it was also crucial to include optical data with greater spectral contrast (when available for our study location) to verify and substantiate our visual interpretation of SAR imagery.

The strong selection of spatially and temporally persistent ice-free areas identifies the role of both the presence and temporal stability of this critical habitat for this species. Ephemeral ice-free areas in the main-stem Tanana River are probably dominated by hyporheic flow with smaller, less stable groundwater input. The hyporheic zone where surface water and groundwater interact to influence ecological processes in rivers and streams (Boulton et al. 1998) is controlled by geomorphology (Poole et al. 2006) and can have a strong influence on riverine landscapes (Wiens 2002). Geomorphology and complex patterns of hyporheic exchange and upwelling are important for spawning bull trout *Salvelinus confluentus* (Baxter and Hauer 2000) and brook trout *Salvelinus fontinalis* (Curry et al. 1995). Ice-free areas have provided rearing habitat for juvenile Chinook salmon *Oncorhynchus tshawytscha* during winter in British Columbia (Levings and Lauzier 1991). Trout population densities are generally highest in streams with high groundwater discharge (Fausch et al. 1988).

Now that the surface water and groundwater interaction has been identified in critical habitat areas, the next step is to conduct in-stream analysis to identify water chemistry characteristics within and between upwelling areas. It would be illus-

trative to connect the characteristics of groundwater directly to reproductive success via direct investigation of chum salmon redds. Generally, groundwater may be low in dissolved oxygen and perhaps detrimental to salmon embryo survival (Sowden and Power 1985; Malcolm et al. 2004). However, complex hydrological patterns with strong upwelling and localized downwelling can lead to higher oxygen concentrations suitable for spawning salmonids (Baxter and Hauer 2000). Use of FLIR imagery to identify areas of groundwater influx with complex patterns will aid in targeted in-stream analysis to identify localized downwelling. Additional water chemistry analyses of complex surface water and groundwater mixing environments, identified from FLIR imagery, could potentially identify important chemical cues that spawning fall chum salmon are using to home to spawning grounds. These types of studies can help biologists hone in on localized and important habitats, while elucidating reach and site-scale variables for salmon and better determine physical processes creating and maintaining these habitats.

Although the highest densities of fish were in persistent ice-free habitats, many individuals spawned in areas that eventually froze over. This does not necessarily imply that these individuals were ultimately unsuccessful in their reproductive effort or that they did not select areas where upwelling was taking place. The presence of ice-free water in winter merely indicated sufficient upwelling influence to prevent surface freezing. Alternatively, a lack of ice cover only indicated, but did not directly measure, the presence of groundwater; however, we are confident that any unfrozen water prior to spring thaw in March is the result of groundwater influence, given the severity and length of the sub-zero temperatures of the Alaska winter. However, the fact remains that we did not have the means to observe upwelling phenomena directly at a large spatial extent.

With these caveats in place, we present strong evidence that spatially and temporally persistent upwelling areas with complex surface water and groundwater interactions provide core habitats essential for fall chum salmon population productivity and persistence. Reaches with groundwater influence but lower stability and influence may alternatively offer reproductive habitat for straying fish or fish that are competitively excluded from the core areas. In unstable zones, the continued presence of spawning chum salmon in consecutive years may be due to the influx of individuals into potential sinks from core habitats (Dunning et al. 1992). Further analysis of groundwater input in nonpersistent upwelling areas is necessary to better explain fall chum salmon spatial distributions and determine whether they are truly sink habitats. A common practice in habitat conservation is to prioritize protection of core productive habitats. Typically, these habitats have the largest densities of individuals, are resistant to declines, and offer a source for future emigration to unoccupied habitats (Isaak and Thurow 2006). Although these core areas are clearly important, this conservation strategy can have long-term consequences by constraining populations in the event that core habitats are lost

and no peripheral populations are available to recolonize core areas.

Hubbs and Trautman (1935) drew attention to the need for winter investigations of freshwater fish populations. Although winter studies have increased since that call, they remain challenging and underrepresented in the ecological literature. Winter research is not only particularly challenging, but particularly important in high-latitude regions like Alaska, where winter conditions are severe and small areas of the landscape can have disproportionate importance for the persistence and productivity of fish populations (Reynolds 1997). Transferring the methods used in this study across high latitude river systems has the potential to alleviate logistical challenges associated with working outdoors in the arctic environment.

According to current climate change predictions, arctic freshwater systems will warm more rapidly than the global average, particularly during winter (Prowse et al. 2006). A warming climate is likely to create hydrologic shifts in freshwater river systems, changing seasonal flow, ice cover, and the severity of freeze-up and break-up (Prowse et al. 2006). Studies using winter stream flow as a proxy for groundwater inputs in the Yukon River basin are indicating shifts in surface water and groundwater interactions, with an increase in groundwater contributions to overall stream flow due to permafrost thawing, increasing dissolved inorganic carbon and nitrogen (Striegl et al. 2007; Walvoord and Striegl 2007). It is essential to understand the current chemistry, particularly oxygen levels, of upwelling areas important for salmon spawning if warming events cause a change in groundwater processes and, therefore, the chemical environment of the river. Our study demonstrates that combining remote sensing and in-stream evaluations will allow researchers to create baseline information for monitoring salmon spawning habitat in areas that may be most vulnerable to climate change but have received little attention due to inaccessibility and inadequate survey methods.

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APPENDIX: DATA LOGGER INFORMATION

TABLE A.1. Basic data for surface temperature data loggers retrieved from the Tanana River in April 2009. Location identification numbers 5, 7, 9, and 13 contained two loggers.

Location number	Latitude	Longitude	Start date	Stop date	Temperature (°C)		
					Range	Mean	
1	64.787	-147.761	Oct 25, 2008	Apr 8, 2009	0.02	2.50	0.78
2	64.769	-147.563	Nov 3, 2008	Apr 6, 2009	1.13	6.36	2.92
3	64.747	-147.438	Nov 3, 2008	Apr 6, 2009	-10.44	2.77	0.84
4	64.686	-147.311	Oct 31, 2008	Apr 8, 2009	-4.90	0.85	-1.20
5	64.677	-147.283	Nov 25, 2008	Apr 6, 2009	-12.31	4.95	-0.22
5	64.677	-147.283	Nov 25, 2008	Apr 6, 2009	-9.44	1.97	0.02
6	64.629	-147.228	Nov 3, 2008	Apr 6, 2009	-8.36	6.97	0.08
7	64.548	-147.066	Nov 25, 2008	Apr 6, 2009	-5.67	2.29	0.82
7	64.548	-147.067	Nov 25, 2008	Apr 6, 2009	-9.37	1.72	0.08
8	64.441	-147.031	Nov 25, 2008	Apr 6, 2009	-4.38	3.14	0.20
9	64.309	-146.828	Nov 25, 2008	Apr 6, 2009	-0.06	3.04	0.79
9	64.309	-146.829	Nov 25, 2008	Apr 6, 2009	-0.12	3.62	0.58
10	64.283	-146.412	Nov 25, 2008	Apr 7, 2009	0.02	0.36	0.05
11	64.249	-146.263	Nov 25, 2008	Apr 9, 2009	0.08	3.85	1.18
12	64.176	-145.888	Nov 3, 2008	Apr 7, 2009	-0.06	3.78	2.24
13	64.156	-145.875	Nov 25, 2008	Apr 7, 2009	-3.72	1.48	0.17
13	64.156	-145.874	Nov 25, 2008	Apr 7, 2009	-9.51	1.51	-1.40
14	64.157	-145.841	Dec 5, 2008	Apr 4, 2009	3.99	5.80	4.78

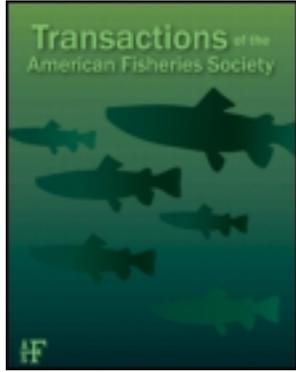
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Estimating and Predicting Collection Probability of Fish at Dams Using Multistate Modeling

John M. Plumb^{a,c}, William P. Connor^b, Kenneth F. Tiffan^c, Christine M. Moffitt^a, Russell W. Perry^c & Noah S. Adams^c

^a Idaho Cooperative Fish and Wildlife Research Unit, University of Idaho, Moscow, Idaho, 83844, USA

^b U.S. Fish and Wildlife Service, Idaho Fishery Resource Office, 276 Dworshak Complex Drive, Orofino, Idaho, 83544, USA

^c U.S. Geological Survey, Western Fisheries Research Center, 5501A Cook-Underwood Road, Cook, Washington, 98605, USA

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ARTICLE

Estimating and Predicting Collection Probability of Fish at Dams Using Multistate Modeling

John M. Plumb*¹

Idaho Cooperative Fish and Wildlife Research Unit, University of Idaho, Moscow, Idaho 83844, USA

William P. Connor

U.S. Fish and Wildlife Service, Idaho Fishery Resource Office, 276 Dworshak Complex Drive, Orofino, Idaho 83544, USA

Kenneth F. Tiffan

U.S. Geological Survey, Western Fisheries Research Center, 5501A Cook-Underwood Road, Cook, Washington 98605, USA

Christine M. Moffitt

Idaho Cooperative Fish and Wildlife Research Unit, University of Idaho, Moscow, Idaho 83844, USA

Russell W. Perry and Noah S. Adams

U.S. Geological Survey, Western Fisheries Research Center, 5501A Cook-Underwood Road, Cook, Washington 98605, USA

Abstract

Dams can be equipped with a bypass that routes a portion of the fish that enter the turbine intakes away from the powerhouse into flumes, where they can be counted. Daily passage abundance can be estimated by dividing the number of fish counted in the bypass by the sampling rate and then dividing the resulting quotient by the collection probability (i.e., the proportion of the fish population passing the dam that is bypassed). We used multistate mark–recapture modeling to evaluate six candidate models for predicting the collection probabilities of radio-tagged subyearling fall Chinook salmon ($n = 3,852$) as a function of 1–2-d time periods (general model), four different combinations of outflow (i.e., the total volume of water passing the dam) and turbine allocation (i.e., the proportion of outflow directed through the turbines), and a null (intercept only) model. The best-fit model was the additive combination of turbine allocation and outflow, which explained 71% of the null deviance. Cross validation of the best-fit model accounted for the variation that may arise from different data sets and the ensuing parameter values on the collection probability estimates and yielded a standard error of 0.613 that can be used to construct approximate 95% prediction intervals in nonstudy years. Such estimates have been unavailable and will be useful anywhere estimates of daily passage abundance at dams with bypasses are needed to manage migratory fishes.

*Corresponding author: jplumb@usgs.gov

¹Present address: U.S. Geological Survey, Western Fisheries Research Center, 5501A Cook-Underwood Road, Cook, Washington 98605, USA.

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Nearly all of the large rivers in North America have been impounded (Benke 1990; Graf 1999), resulting in the fragmentation of river corridors and consequences for the viability of both resident and migratory fish and their ecosystems (Sedell et al. 1989; Sheehan and Rasmussen 1999; Arthington et al. 2010). The primary routes of passage at dams used by downstream-migrating juvenile fish are the turbine intakes and spillways. The potential for increased fish mortality associated with turbine passage is of particular concern (see reviews by Coutant 2001 and Roscoe and Hinch 2010). Many dams are equipped with bypasses composed of screens in the turbine intakes that divert downstream migrating juvenile anadromous salmonid smolts away from the powerhouse into flumes that empty into raceways or the dam tailrace (e.g., Matthews et al. 1977).

In addition to preventing smolts from directly passing into the powerhouse and turbines, bypasses essentially function as fish traps and can be used to collect the data needed to estimate fish abundance. One equation used to estimate abundance, \hat{N}_i , is

$$\hat{N}_i = \hat{Y}_i / \hat{C}_i,$$

where \hat{Y}_i is number of fish in group i estimated to pass the dam via the bypass over a given time period (i.e., the sample count divided by the sample rate) and \hat{C}_i is the collection probability estimated for that time period (after Giorgi and Sims 1987). Collection probability is the proportion of the total number of fish that pass a dam by all passage routes combined that is diverted into the bypass. Outflow is the total volume of water that passes the dam. Turbine allocation is the proportion of outflow passed through the turbines intakes. Decreases in turbine allocation caused by passing water over the spillways decreases collection probability (Giorgi and Sims 1987; Wilson et al. 1991; Coutant and Whitney 2000).

Giorgi and Sims (1987) were the first to develop a method for estimating collection probability, predicting it from turbine allocation and estimating daily passage abundances at a dam with a bypass. They freeze-branded groups of yearling Chinook salmon *Oncorhynchus tshawytscha* and steelhead *O. mykiss* smolts and then released them 32 km upstream of the dam studied. Mark-recapture data collected from the bypass over several days by systematic sampling were used to estimate collection probability assuming a mortality rate of 10% prior to dam passage. Collection probability was then predicted by fitting a regression equation from the collection probability estimates and turbine allocations (range, 0.28–0.76) measured over the first 3 d of recapture of each branded group of fish. Applying this regression equation to the levels of turbine allocation observed during fish passage to predict collection probability, combined with the collection of sample count data in the bypass, provided daily passage abundance estimates during nonstudy years. Though their method was useful, Giorgi and Sims (1987) recommended that future research include an evaluation of their mortality assumption and the collection of additional data at turbine allocations above 0.76.

We built on the findings of Giorgi and Sims (1987) by developing a method to estimate and predict collection probability of migratory fish at dams equipped with bypasses. We selected radiotelemetry technology for developing our method because the route-specific survival and passage of radio-tagged fish can be estimated accurately and precisely over short time intervals with multistate mark-recapture models (e.g., Skalski et al. 2002; Buchanan et al. 2009; Perry et al. 2010). Our objectives were to (1) develop a general model to estimate collection probability at 1–2-d intervals, (2) identify a best-fit model for predicting collection probability from combinations of outflow and turbine allocation, and (3) conduct a cross validation to quantify the prediction error expected when using the best-fit model in nonstudy years.

METHODS

Study location.—We developed the model for application to Snake River basin fall Chinook salmon subyearlings (hereafter referred to as subyearlings) passing Lower Granite Dam. The dam is located on the Snake River in the state of Washington 173 km upriver from the confluence with the Columbia River and 748 km from the Pacific Ocean. The dam is 975 m long and has six primary structures: six turbine intakes that lead into the powerhouse, an eight-bay spillway, navigation lock, earthen dam, bypass, and an adult ladder. The navigation lock and adult fish ladder are not considered significant routes of passage for these juvenile fish due to their intermittent operation, low flow, and allocation (<1% of outflow). Each of the turbine intakes is fitted with extended-length submersible bar screens (1996 to present) that are installed by March 24–April 1 and kept in place until December 15 unless they are damaged (in rare cases) or the air temperature falls below -6.7°C for 24 consecutive hours after late November (USACE 2012). A turbine unit is not operated until damaged screens are repaired. Outflow and turbine allocation are measured every 5 min, and daily mean outflows and turbine allocations are reported on the Internet in real time (DART 2012). Daily fish sampling and counts are made in the bypass on run-of-the river subyearlings (FPC 2012), whereas detection systems within the bypass flumes record the passage of subyearlings implanted with passive integrated transponder (PIT) tags 24-h/d (Prentice et al. 1990a, 1990b; PTAGIS 2012; PTOC 2012).

Estimating Collection Probability.—We extended the multistate route-specific survival model of Skalski et al. (2002) to make time-specific estimates of collection probability. We fit the general model to daily observations of route-specific passage provided by radiotelemetry data collected at Lower Granite Dam in 1997–1998 and 2005–2007 as part of other dam passage research (Adams et al. 1998a, 1998b, 1998c, 1999, 2001; Plumb et al. 2003). The size and type of radio tags and the size and number of study fish that were monitored varied over the years (Table 1). The locations of

TABLE 1. Number of radio-tagged subyearling fall Chinook salmon released approximately 21 km upstream of Lower Granite Dam (*N*), mean weight (range), 24-h posttagging mortality, and mean fork length (range), 1997–1998 and 2005–2007. Specifications are also given for the radiotelemetry equipment, including the resulting effects on tag burden (tag weight/fish weight; mean and range).

Year	<i>N</i>	Fish			Tag (transmitter)			Antenna	
		Length (mm)	Weight (g)	24-h posttagging (%)	Size (mm)	Weight (g)	Tag burden (%)	Length (cm)	Type
1997	199	132 (120–154)	26.9 (20.0–42.1)	10 (4.8)	7.3 × 18.0	1.40	5.4 (3.2–7.3)	31	Sava
1998	295	136 (118–154)	31.3 (20.0–48.5)	2 (0.7)	7.3 × 18.0	1.40	4.6 (2.9–7.3)	31	Sava
2005	1,067	111 (95–157)	14.8 (10.0–45.4)	4 (0.4)	5.6 × 13.9	0.43	3.0 (0.9–4.3)	31	Sava
2006	1,089	111 (97–140)	13.9 (10.0–26.8)	10 (0.9)	5.6 × 13.9	0.43	3.7 (1.9–5.0)	16	S1
2007	1,202	111 (97–143)	14.0 (10.0–35.5)	4 (0.3)	5.6 × 13.9	0.43	3.7 (1.4–5.0)	16	S1

receivers (and consequently the passage route coverage) varied by year, but coverage was sufficient to identify tagged fish passing via bypass and nonbypass routes during all years and to maintain consistently high detection probability (see Results). We compiled and reviewed the detection history of individual fish as described by Skalski et al. (2002) and Perry et al. (2010).

To estimate time-specific collection probabilities, each fish passing the dam was assigned a detection history code indicating (1) the 1–2-d interval when fish were detected passing the dam, (2) whether fish were detected in bypass or nonbypass routes, and (3) whether fish were detected at monitoring sites downstream of the dam. The probability of observing each detection history was then derived from the following underlying probabilities: (1) ϕ_i , apparent survival to the dam in year *i*; (2) τ_{it} , the probability of passing the dam during time interval *t* in year *i*, (3) C_{it} , the probability of passing the dam via the bypass (i.e., collection probability) during time interval *t* in year *i*, (4) p_{itB} and p_{itNB} , the probability of detecting a tagged fish that passed via bypass (B) or nonbypass routes (NB) during time interval *t* in year *i*, and (5) λ_{itB} and λ_{itNB} , the joint probability of surviving and being detected at monitoring sites downstream of the dam for fish passing via bypass (B) and nonbypass (NB) routes during interval *t* in year *i* (Figure 1). For example, the probability of observing detection histories (π) of fish that survived to the dam, passed the dam during interval *t*, and were detected in the bypass but not detected below the dam was expressed as

$$\pi = \phi_i \cdot \tau_{it} \cdot C_{it} \cdot p_{itB} \cdot (1 - \lambda_{itB}).$$

We considered using a second likelihood model to estimate detection probabilities within the bypass (p_{itB}) based on redundant detection systems (see Skalski et al. 2002). However, redundant detection systems within the bypass resulted in all $p_{itB} = 1$, and thus we set all p_{itB} to 1. Because redundant detection arrays were not implemented for the nonbypass routes, we could not estimate time-specific detection probabilities. Instead, we assumed that the detection probabilities for nonbypass routes were constant within a given year.

The detection histories of individual radio-tagged fish were treated as multiple outcomes under a multinomial distribution, and we used maximum likelihood methods to estimate model parameters from the observed frequency of each detection history, i.e.,

$$L(\theta | R_i, n_{ij}) \propto \prod_{i=1}^I \prod_{j=1}^{J_i} \pi_{ij}^{n_{ij}}$$

where $L(\theta | R_i, n_{ij})$ is the likelihood of the parameters (θ) given the data (R_i, n_{ij}), R_i is the number of radio-tagged fish released

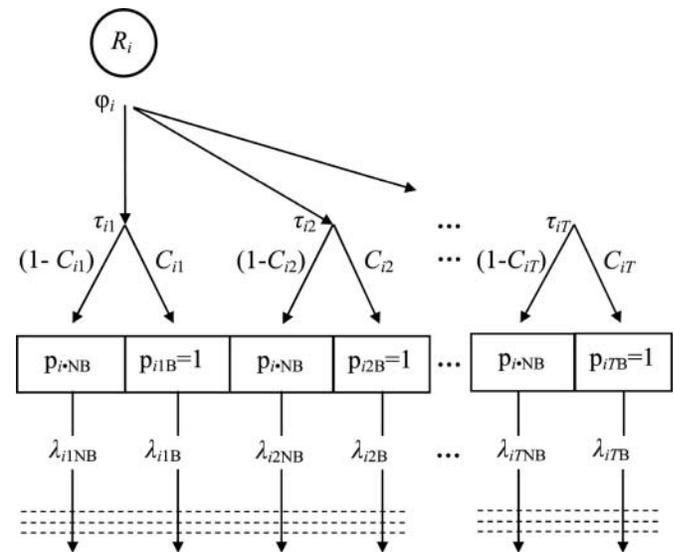


FIGURE 1. Structure of the general multistate mark–recapture model used to estimate collection probability at Lower Granite Dam for annual release groups (R_i) of radio-tagged subyearling fall Chinook salmon. The estimated parameters include apparent survival from release to the dam in year *i* (ϕ_i), the probability of passing the dam during time interval *t* (τ_{it}), collection probability during time interval *t* (C_{it}), within-route detection probabilities for bypass (B) and nonbypass (NB) routes (p_{itNB} and p_{itB}), and joint probabilities (λ_{itNB} and λ_{itB}) of surviving, migrating, and being detected below the dam for the fish that passed through bypassed and nonbypassed routes. The boxes represent Lower Granite Dam, and the dashed horizontal lines represent all detection locations downstream of the dam.

in the i th year ($i = 1, \dots, I$ years), n_{ij} is the number of fish having the j th detection history ($j = 1, \dots, J_i$ detection histories in the i th year), and π_{ij} is the probability of observing the j th detection history in the i th year as described above. The likelihood function was numerically maximized using Powell's algorithm as implemented by Program USER (Lady et al. 2008). We used the inverse of the Hessian matrix to estimate the variance-covariance matrix and standard errors. The main assumptions of the general model are (1) the fates of individuals are independent, (2) fish in a 1–2-d cohort have equal survival and detection probabilities, and (3) the route of passage does not influence detection probabilities at sites downstream of the dam. Additional assumptions common to all mark–recapture studies also apply and are discussed in detail by Burnham et al. (1987) and Skalski et al. (1998, 2002). There are formal ways proposed by Burnham et al. (1987) to test some of these assumptions; however, these tests require three downstream detection sites, which were not available or consistently located across the study years. Nonetheless, others have found that estimates generated under Cormack–Jolly–Seber models are robust to many violations of these assumptions (Skalski et al. 1998).

Predicting collection probability.—Using the framework of generalized linear models, we fitted six candidate models to predict C_{it} during nonstudy years as a logistic function of the predictor variables in each model (McCullagh and Nelder 1989; Lebreton and Pradel 2002). The models and predictor variables were as follows: (1) the time-specific or general model, (2) outflow, (3) turbine allocation, (4) outflow and turbine allocation, (5) outflow and turbine allocation with the outflow \times turbine allocation interaction, and (6) a null or intercept-only model. Data collected in 5-min intervals at Lower Granite Dam were used to calculate mean outflow and turbine allocation for each value of C_{it} . We calculated a likelihood-based r^2 value (r_L^2) for each candidate model similar to that defined by Nagelkerke (1991):

$$r_L^2 = \frac{1 - (L_0/L_m)^{2/n}}{1 - L_0^{2/n}}$$

where L_m is the maximum likelihood for the candidate model and L_0 is the maximum likelihood for the intercept-only model. Nagelkerke (1991) defined n as the total number of binomial occasions; however, herein n is defined as the total number of unique detection histories. To determine the plausibility of the model we used Akaike's information criterion for small sample sizes (AIC_c; Akaike 1973, 1983; Burnham and Anderson 1998).

Cross validation and prediction error.—We conducted a v -fold cross validation to quantify the prediction error associated with using the best-fit predictive model in nonstudy years (Kohavi 1995; Molinaro et al. 2005) and the consequences of different and independent data sets and parameter values on the collection probability estimates. To do this, we first randomly

assigned the 1–2-d cohorts of radio-tagged fish to one of nine partitions of equal size, resulting in seven cohorts per partition. For each of the nine partitions, the best model was fit to all data except that of the v th partition, and the resulting parameter estimates were used to predict the collection probabilities for the cohorts in the v th partition. For each partition, we then calculated the root mean square error between the predicted collection probability and the C_{it} as estimated by the general model, on the logit scale. The standard error of the prediction was estimated as the average root mean square error over the nine partitions. Approximate prediction intervals can then be estimated as

$$\text{logit}^{-1}(\text{logit}(\hat{C}_{it}) \pm z_{1-\alpha/2}\text{SE}),$$

where SE is the prediction error, z is the standard normal quantile for confidence level $1 - \alpha$, $\text{logit}(x) = \ln(x/[1 - x])$, and $\text{logit}^{-1}(x) = e^x/(1 + e^x)$. This prediction interval represents the average error associated with predicting collection probability in nonstudy years.

RESULTS

Test Fish and Tagging

Annual totals of 199–1,202 subyearlings ($N = 3,852$) were radio-tagged and released upstream of Lower Granite Dam during 1997–1998 and 2005–2007 (Table 1). The annual posttagging mortality rates over the 24-h recovery periods prior to releasing the radio-tagged fish ranged from 0.3% to 4.8% (i.e., from 2 to 10 fish). Annual mean tag burden decreased between 1997–1998 and 2005–2007 as tag size decreased (Table 1). The year with the highest 24-h posttagging mortality (1997; 4.8%) was also the year associated with the highest mean tag burden (5.4%).

Fit of the General Model to the Data

The fit of our general model to the radio tag data was nearly perfect, with predicted counts equal to the observed counts for each detection history category. The good fit of the general model to the radio tag data was largely the result of the following combination of factors: (1) the within-bypass detection probabilities were high and set = 1, (2) the joint probabilities of surviving and being detected below the dam (λ_{iNB} and λ_{iB}) were generally high (grand mean = 0.88, SE = 0.107), many of which (74 of 132) were also set = 1, and (3) the within-route detection probabilities for fish passing through the nonbypass routes were also quite high (>0.94, 95% CI < 0.018; Table 2).

Estimating Model Parameters Other than Collection Probability

The focus of our study was on the estimation of collection probability; however, other parameter values are germane to the estimation and interpretation of collection probability estimates. Annual survival from the point of release to the dam (~21 km) ranged from 0.55 (SE = 0.035) to 0.89 (SE = 0.009; Table 2).

TABLE 2. Annual apparent survival (φ_i) of radio-tagged subyearling fall Chinook salmon as they traveled from Blyton Landing to Lower Granite Dam (a distance of 21 km), and the annual within-route detection probabilities for all nonbypass routes ($p_{i\text{-NB}}$).

Year	φ_i (SE)	$p_{i\text{-NB}}$ (SE)
1997	0.548 (0.035)	0.976 (0.023)
1998	0.834 (0.022)	0.954 (0.018)
2005	0.709 (0.014)	0.945 (0.009)
2006	0.889 (0.009)	0.938 (0.009)
2007	0.756 (0.013)	0.939 (0.009)

The lowest survival probability to the dam ($\varphi_{1997} = 0.55$, SE = 0.035) was measured in the year that also had the highest 24-h posttagging mortality and tag burden (Table 1), suggesting that on average the tagged fish in this year experienced a greater level of stress and mortality.

Annual within-route detection probabilities were quite high (>0.94) for nonbypass routes over all study years, suggesting that our assumption of constant within-route detection probabilities was plausible given the high annual estimates obtained under the general model (Table 2). Incorporating and quantifying the within-route detection probabilities for these nonbypass routes on an annual basis enabled an assessment of the average annual bias (i.e., <0.06, or 6%) that might have been incorporated into the collection probability estimates had these within-route detection probabilities been ignored. Even though the within-route detection probabilities for the nonbypass routes were high, suggesting that simpler regression models might also be considered for the analysis (e.g., see Giorgi and Sims 1987; Evans et al. 2008; Zabel et al. 2008), the multistate framework provided a means to assess and account for nonperfect detection if the within-route detection probabilities was unacceptably low.

The joint probabilities of surviving and being detected downstream of the dam were also quite high. Over all 1–2-d cohorts, 64% (42 of 66) had λ_{itB} values that were set to 1 and 50% (33 of 66) had λ_{itNB} values that were set to 1. The grand mean of the estimable λ_{itBS} was 0.84 (SD = 0.125; range = 0.40–0.95), and the grand mean of the estimable λ_{itNBS} was 0.9 (SD = 0.084; range = 0.59–0.99). Given these generally high values and their overlapping uncertainty, there was little evidence to suggest that the joint probabilities of surviving and being detected below the dam differed between bypassed and nonbypassed fish.

Estimating Collection Probability

Over all years, fish passed the dam during 66 unique time intervals and were assigned one of 274 unique detection histories. Of these 66 time intervals, collection probabilities for three intervals (27, 33, and 64; Table 3) were set to zero because no fish were detected in the bypass. The estimated collection probabilities \pm SEs for the remaining 63 time intervals with the general model ranged from 0.014 ± 0.014 to 0.872 ± 0.119 (Table 3).

Predicting Collection Probability

The mean \pm SE outflows measured during the 66 time intervals ranged from 0.870 ± 0.000 – to 3.540 ± 0.001 thousand m^3/s , and the mean \pm SE turbine allocations ranged from 0.36 ± 0.147 to 1.00 ± 0.000 (Table 3). According to the model selection results, this wide range of dam operations affected much of the null deviance in collection probability observed among time intervals. The model with outflow and turbine allocation had the lowest AIC_c value and explained 71% of the null deviance (Table 4). The model that included an interaction term between these two variables was equivocal to the model that did not ($\Delta AIC_c = 0.23$; Table 4). We therefore selected the model with the lowest AIC_c value as the best model for predicting collection probability, i.e.,

$$\hat{C}_{it} = \text{logit}^{-1}(\hat{\beta}_0 + \hat{\beta}_1 \text{ outflow} + \hat{\beta}_2 \text{ turbine allocation})$$

where $\hat{\beta}_0 = -4.54$ (SE = 0.21), $\hat{\beta}_1 = 0.31$ (SE = 0.08), and $\hat{\beta}_2 = 4.31$ (SE = 0.30). These parameter estimates indicate that collection probability increased as both outflow and turbine allocation increased (Figures 2–4).

The best-fit model had observed and expected frequency counts that were not outside the expectations of a multinomial distribution (Figure 2). The Anscombe residuals (McCullagh and Nelder 1989; Lady et al. 2008) for this model were centered about 0.0, with just 2.6% (7 of 274) falling outside the 2.5 and 97.5 percentiles of the standard normal z distribution (Figure 2). Given random chance, one might expect <5% (i.e., <14) of the residuals to fall outside these percentiles. These findings support the conclusion that our multistate approach did not provide overly dispersed estimates of uncertainty.

Cross validation of the best-fit model yielded a prediction standard error of 0.613 that can be used to construct approximate 95% prediction intervals about the collection probability estimates for subyearlings at the dam in nonstudy years

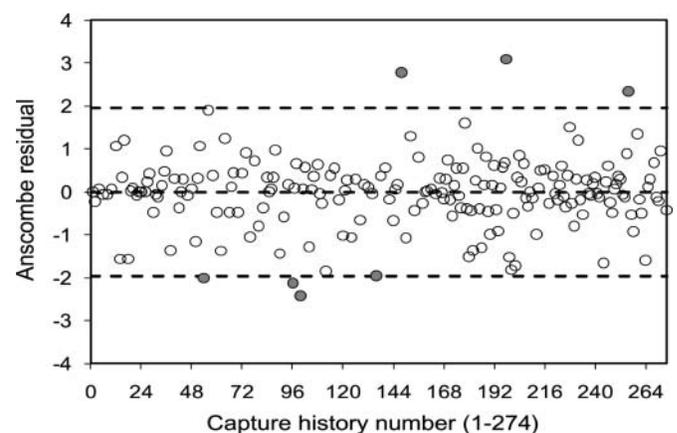


FIGURE 2. Anscombe residuals by capture history number for the lowest-AIC_c model. The dashed lines represent the values -1.96, 0, and +1.96. The gray dots indicate residuals falling outside the 2.5 and 97.5 percentiles of the standard normal z distribution.

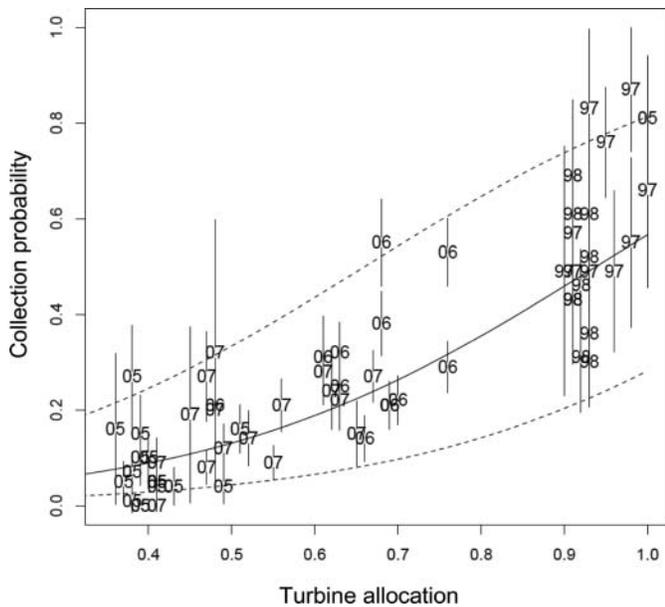


FIGURE 3. Point estimates (error bars = SEs) of collection probabilities estimated for radio-tagged subyearling fall Chinook salmon at Lower Granite Dam with the general model as a function of turbine allocation (1997–1998, 2005–2007; the plotting symbol is the year). The solid line shows the collection probabilities predicted by the best-fit model at a mean outflow of 1.60 thousand m^3/s . The dashed lines show the 95% prediction interval based on a prediction standard error of 0.613 estimated by ninefold cross validation.

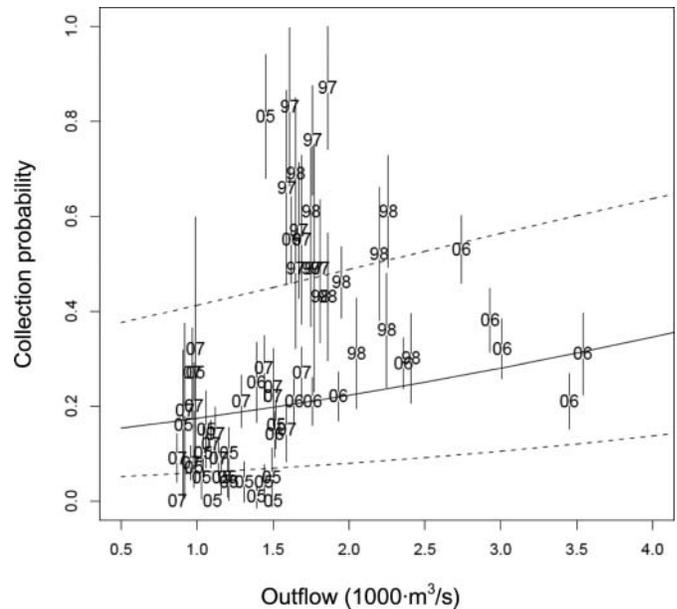


FIGURE 4. Point estimates (error bars = SEs) of collection probabilities estimated for radio-tagged subyearling fall Chinook salmon at Lower Granite Dam with the general model as a function of outflow (1997–1998, 2005–2007; the plotting symbol is the year). The solid line shows the collection probabilities predicted by the best-fit model at a mean turbine allocation of 0.62. The dashed lines show the 95% prediction interval based on a prediction standard error of 0.613 estimated by ninefold cross validation.

(Figures 3 and 4). When models containing only one predictor were compared, turbine allocation ($AIC_c = 1,141$; $r_L^2 = 0.69$) was a stronger predictor of collection probability than outflow ($AIC_c = 1,345$; $r_L^2 = 0.34$; Table 4). Collection probability also increased more rapidly with turbine allocation than with outflow, indicating that turbine allocation drove collection probability more than outflow (Figures 3 and 4). For example, much of the residual variation in the relation between collection probability and outflow was due to the additional effect of turbine allocation. In contrast, there was less residual variation in the relation between collection probability and turbine allocation because the additional effect of outflow was relatively small.

DISCUSSION

The modeling approach that we describe was built on the work of Giorgi and Sims (1987) as a way to estimate collection probability while overcoming three limitations of their approach. First, we overcame the fixed-survival-rate assumption for marked fish because fish survival to the dam was estimated as part of the model. Second, radiotelemetry provided precise time-specific passage route data that cannot presently be acquired with freeze brands, PIT tagging, or many other fish tagging technologies. Third, we were able to estimate subyearling collection probability at turbine allocations as high as 1, which will limit the potential for extrapolation error in future predictions of collection probability.

Few studies have explicitly evaluated fish passage in relation to the operation of large dams, and fewer have provided information over a wide range of dam operations. Wilson et al. (1991), working in the 1980s, present perhaps the first estimates of fish passage using radiotelemetry (on much larger fish [>150 mm fork length]) and a mark–recapture framework for analysis at Lower Granite Dam. They found that spilling 20% or 40% (i.e., turbine allocations of 80% and 60%) of the outflow at the dam resulted in passing 0.41 (SD = 0.06) and 0.61 of the tagged fish via the spillway, respectively. Further, Giorgi et al. (1985) reported a collection probability of about 0.26 at a turbine allocation of 0.58 at John Day Dam. When the fact that direct turbine passage is typically about 0.1 (Adams et al. 1998c, 2001; Puls et al. 2008) is not accounted for, the expected collection probabilities and turbine allocations agree well with the results of this study.

The best-fit predictive model explained 71% of the null deviance in estimated fish collection probability and confirmed that collection probability increased as both outflow and turbine allocation increased. This mechanistic relation supports the theory of passive smolt movement directed by bulk flow (Thorpe and Morgan 1978; Thorpe et al. 1981; Coutant and Whitney 2000). However, the behavioral and physiological responses of fish combined with the hydrodynamic conditions created by passage structures are also plausible factors in the variation in collection probability. For example, juvenile Chinook salmon

TABLE 3. Estimated collection probability under the general model (C_{it}), mean outflow, and mean turbine allocation for each time interval when radio-tagged subyearling fall Chinook salmon passed Lower Granite Dam. The values in parentheses are SEs; NA = not available.

Interval	Start date	C_{it}	Outflow (1,000 m ³ /s)	Turbine allocation
1	Jul 15, 1997	0.494 (0.250)	1.77 (0.07)	0.90 (0.018)
2	Jul 17, 1997	0.494 (0.133)	1.81 (0.03)	0.93 (0.006)
3	Jul 19, 1997	0.872 (0.119)	1.86 (0.03)	0.98 (0.005)
4	Jul 21, 1997	0.760 (0.104)	1.76 (0.04)	0.95 (0.007)
5	Jul 23, 1997	0.494 (0.112)	1.75 (0.04)	0.91 (0.003)
6	Jul 25, 1997	0.550 (0.166)	1.69 (0.03)	0.98 (0.006)
7	Jul 27, 1997	0.566 (0.133)	1.67 (0.04)	0.91 (0.006)
8	Jul 29, 1997	0.494 (0.158)	1.65 (0.04)	0.96 (0.008)
9	Jul 31, 1997	0.830 (0.155)	1.61 (0.05)	0.93 (0.009)
10	Aug 02, 1997	0.661 (0.194)	1.59 (0.03)	1.00 (0.000)
11	Jul 02, 1998	0.522 (0.129)	2.20 (0.04)	0.93 (0.001)
12	Jul 03, 1998	0.358 (0.109)	2.25 (0.04)	0.93 (0.002)
13	Jul 04, 1998	0.300 (0.084)	2.41 (0.05)	0.93 (0.002)
14	Jul 05, 1998	0.608 (0.107)	2.26 (0.02)	0.93 (0.001)
15	Jul 06, 1998	0.306 (0.105)	2.05 (0.03)	0.92 (0.001)
16	Jul 07, 1998	0.464 (0.064)	1.95 (0.03)	0.92 (0.001)
17	Jul 08, 1998	0.430 (0.085)	1.81 (0.02)	0.91 (0.001)
18	Jul 09, 1998	0.690 (0.148)	1.65 (0.11)	0.91 (0.053)
19	Jul 10, 1998	0.614 (0.122)	1.75 (0.04)	0.91 (0.038)
20	Jul 11, 1998	0.426 (0.123)	1.86 (0.04)	0.91 (0.002)
21	Jun 18, 2005	0.810 (0.120)	1.45 (0.01)	1.00 (0.000)
22	Jun 20, 2005	0.053 (0.051)	1.49 (0.01)	0.41 (0.008)
23	Jun 22, 2005	0.036 (0.025)	1.44 (0.01)	0.49 (0.007)
24	Jun 24, 2005	0.014 (0.014)	1.39 (0.00)	0.38 (0.008)
25	Jun 26, 2005	0.040 (0.028)	1.21 (0.01)	0.43 (0.006)
26	Jun 28, 2005	0.159 (0.039)	1.52 (0.01)	0.51 (0.006)
27	Jun 30, 2005	0.000 (NA)	1.51 (0.01)	0.39 (0.008)
28	Jul 02, 2005	0.103 (0.044)	1.21 (0.01)	0.40 (0.006)
29	Jul 04, 2005	0.051 (0.025)	1.16 (0.01)	0.41 (0.006)
30	Jul 06, 2005	0.044 (0.031)	1.31 (0.01)	0.41 (0.007)
31	Jul 08, 2005	0.045 (0.031)	1.20 (0.01)	0.37 (0.006)
32	Jul 10, 2005	0.073 (0.031)	0.98 (0.01)	0.38 (0.003)
33	Jul 12, 2005	0.000 (NA)	1.10 (0.01)	0.39 (0.005)
34	Jul 14, 2005	0.098 (0.046)	1.04 (0.01)	0.39 (0.003)
35	Jul 16, 2005	0.051 (0.035)	1.03 (0.01)	0.41 (0.004)
36	Jul 18, 2005	0.274 (0.096)	0.99 (0.01)	0.38 (0.004)
37	Jul 20, 2005	0.153 (0.070)	1.06 (0.00)	0.39 (0.004)
38	Jul 22, 2005	0.159 (0.147)	0.91 (0.00)	0.36 (0.001)
39	Jun 08, 2006	0.310 (0.075)	3.54 (0.00)	0.61 (0.004)
40	Jun 10, 2006	0.211 (0.047)	3.45 (0.01)	0.48 (0.005)
41	Jun 12, 2006	0.382 (0.056)	2.93 (0.01)	0.68 (0.007)
42	Jun 14, 2006	0.323 (0.052)	3.01 (0.01)	0.63 (0.007)
43	Jun 16, 2006	0.535 (0.060)	2.74 (0.01)	0.76 (0.003)
44	Jun 18, 2006	0.287 (0.043)	2.36 (0.01)	0.76 (0.001)
45	Jun 20, 2006	0.217 (0.040)	1.93 (0.01)	0.70 (0.001)
46	Jun 22, 2006	0.211 (0.040)	1.76 (0.01)	0.69 (0.004)
47	Jun 24, 2006	0.211 (0.036)	1.64 (0.01)	0.69 (0.001)
48	Jun 26, 2006	0.548 (0.080)	1.62 (0.01)	0.68 (0.002)
49	Jun 28, 2006	0.143 (0.037)	1.51 (0.01)	0.66 (0.003)
50	Jun 30, 2006	0.252 (0.073)	1.39 (0.01)	0.63 (0.003)

TABLE 3. Continued.

Interval	Start date	C_{it}	Outflow (1,000 m ³ /s)	Turbine allocation
51	Jun 06, 2007	0.150 (0.057)	1.59 (0.01)	0.65 (0.002)
52	Jun 08, 2007	0.238 (0.070)	1.50 (0.00)	0.62 (0.001)
53	Jun 10, 2007	0.223 (0.051)	1.50 (0.01)	0.63 (0.002)
54	Jun 12, 2007	0.272 (0.043)	1.69 (0.01)	0.67 (0.002)
55	Jun 14, 2007	0.280 (0.058)	1.44 (0.01)	0.61 (0.003)
56	Jun 16, 2007	0.206 (0.044)	1.29 (0.01)	0.56 (0.002)
57	Jun 18, 2007	0.120 (0.040)	1.10 (0.01)	0.49 (0.002)
58	Jun 20, 2007	0.143 (0.047)	1.12 (0.01)	0.52 (0.003)
59	Jun 22, 2007	0.089 (0.025)	1.14 (0.01)	0.55 (0.003)
60	Jun 24, 2007	0.082 (0.025)	0.97 (0.00)	0.47 (0.002)
61	Jun 26, 2007	0.094 (0.040)	0.87 (0.00)	0.41 (0.001)
62	Jun 28, 2007	0.273 (0.083)	0.97 (0.00)	0.47 (0.002)
63	Jun 30, 2007	0.198 (0.080)	0.98 (0.00)	0.48 (0.002)
64	Jul 02, 2007	0.000 (NA)	0.87 (0.00)	0.41 (0.000)
65	Jul 04, 2007	0.190 (0.173)	0.92 (0.00)	0.45 (0.002)
66	Jul 06, 2007	0.320 (0.267)	0.99 (0.00)	0.48 (0.002)

smolts often avoided areas of rapidly accelerating water velocity in laboratory studies (Kemp et al. 2005; Enders et al. 2009), which may explain why fish sometimes accept or reject certain passage structures at dams (e.g., Johnson et al. 2000). Two passage structures—a surface bypass collector (Johnson et al. 2000) and a removable spillway weir (Puls et al. 2008)—were in place at Lower Granite Dam during our study and their periodic operation might explain a portion of the null deviance in collection probability that was not accounted for by turbine allocation and outflow. Physiological status can also affect collection probability, as shown by Giorgi et al. (1988), who studied yearling spring Chinook salmon passing a dam in various stages of smoltification. Thus, changes in fish physiology over time may also explain some of the null deviance in collection probability during our study.

The most essential condition required for predicting the collection probability of subyearlings from turbine allocation and outflow during future nonstudy years is that the configuration and efficiency of the submerged screens in the turbine intakes

at Lower Granite Dam remain unaltered. The most critical assumption when using the predictions of collection probability to estimate daily passage abundances is that radio-tagged fish behave similarly to untagged fish. We recognize that this is arguably a strong assumption, especially in the cases of the run-of-the river and PIT-tagged subyearlings that pass Lower Granite Dam at sizes less than 95 mm (i.e., the minimum length of the radio-tagged fish we studied). Though Zabel et al. (2005) did not account for changes in dam operations as we did, 22 of the 32 analyses they conducted showed that collection probability decreased slightly as the fork length of yearling Chinook salmon and steelhead increased. Zabel et al. (2005) suggested that smaller fish are more likely to enter bypass systems, perhaps because larger fish (with better swimming ability) are better able to avoid the screens. We were not able to analyze fork length as a covariate under the modeling platform. Had we conducted such an analysis, the results would have been confounded because the fish were larger and turbine allocation and outflow were higher in 1997 and 1998 but the fish were smaller and

TABLE 4. Model selection results used to identify the best-fit model for predicting collection probability (C_{it}) for subyearling fall Chinook salmon at Lower Granite Dam during nonstudy years. Abbreviations are as follows: K = the number of parameters; AIC_c = Akaike's information criterion for small sample sizes; ΔAIC_c = the difference in AIC_c value from the that of the lowest- AIC_c model; and r_L^2 = likelihood-based r^2 .

Modeled expression of C_{it}	K	Log-likelihood	AIC_c	ΔAIC_c	r_L^2
Outflow, turbine allocation	131	-427.94	1,127.18	0.00	0.713
Outflow, turbine allocation, and the interaction term	132	-426.98	1,127.41	0.23	0.715
General model (date-specific model)	191	-363.49	1,128.96	1.78	0.825
Turbine allocation	130	-435.46	1,140.06	12.89	0.696
Outflow	130	-537.94	1,345.03	217.86	0.342
Null model	129	-594.36	1,455.73	328.55	NA

turbine allocation and outflow were lower in 2005–2007. We recognize that fish size may have contributed to some of the null deviance in collection probability that was not explained by turbine allocation and outflow and that our best-fit model might underpredict collection probability, leading to overestimation of the abundance of subyearlings that pass Lower Granite Dam at fork lengths less than 95 mm.

Limitations and assumptions notwithstanding, the best-fit predictive model can be used to predict collection probability and estimate daily passage abundance for subyearlings during the period when extended-length submersible bar screens were installed at Lower Granite Dam (i.e., 1996 to the present). Such estimates have been unavailable and will be useful for evaluating the efficacy of measures implemented to recover the Snake River basin fall Chinook salmon listed under the U.S. Endangered Species Act (NMFS 1992). Lower Granite Dam is of particular concern because it is often a starting and ending point for evaluating parr-to-smolt and smolt-to-adult survivals of fall Chinook salmon and other listed Snake River basin populations of anadromous salmonids with respect to dam operations and management actions across the Columbia River basin (e.g., Sandford and Smith 2002; Connor et al. 2003; Achord et al. 2007; Haeseker et al. 2012). Expanding the application of our modeling approach to listed populations of spring Chinook salmon and steelhead, as well as to other dams in the Columbia River basin, would further test the approach and increase its utility. Further, multistate modeling might be useful elsewhere in the world where new dams continue to be constructed and estimates of daily passage abundances are needed to manage native populations of migratory fishes.

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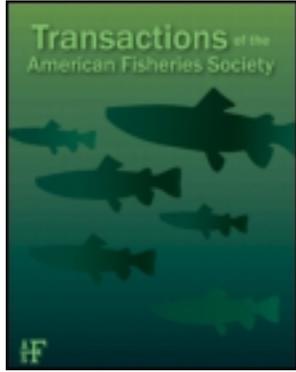
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Genetically Derived Estimates of Contemporary Natural Straying Rates and Historical Gene Flow among Lake Michigan Lake Sturgeon Populations

Jared J. Homola^{a f}, Kim T. Scribner^b, Robert F. Elliott^c, Michael C. Donofrio^d, Jeannette Kanefsky^a, Kregg M. Smith^e & James N. McNair^f

^a Department of Fisheries and Wildlife, Michigan State University, 27 Natural Resources Building, East Lansing, Michigan, 48824, USA

^b Department of Fisheries and Wildlife and Department of Zoology, Michigan State University, 13 Natural Resources Building, East Lansing, Michigan, 48824, USA

^c U.S. Fish and Wildlife Service, Green Bay Fish and Wildlife Conservation Office, 2661 Scott Tower Drive, New Franken, Wisconsin, 54229, USA

^d Wisconsin Department of Natural Resources, 101 North Ogden Road, Peshtigo, Wisconsin, 54157, USA

^e Michigan Department of Natural Resources, 621 North 10th Street, Plainwell, Michigan, 49080, USA

^f Annis Water Resources Institute, Grand Valley State University, 740 West Shoreline Drive, Muskegon, Michigan, 49441, USA

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ARTICLE

Genetically Derived Estimates of Contemporary Natural Straying Rates and Historical Gene Flow among Lake Michigan Lake Sturgeon Populations

Jared J. Homola*¹

Department of Fisheries and Wildlife, Michigan State University, 27 Natural Resources Building, East Lansing, Michigan 48824, USA

Kim T. Scribner

Department of Fisheries and Wildlife and Department of Zoology, Michigan State University, 13 Natural Resources Building, East Lansing, Michigan 48824, USA

Robert F. Elliott

U.S. Fish and Wildlife Service, Green Bay Fish and Wildlife Conservation Office, 2661 Scott Tower Drive, New Franken, Wisconsin 54229, USA

Michael C. Donofrio

Wisconsin Department of Natural Resources, 101 North Ogden Road, Peshtigo, Wisconsin 54157, USA

Jeannette Kanefsky

Department of Fisheries and Wildlife, Michigan State University, 27 Natural Resources Building, East Lansing, Michigan 48824, USA

Kregg M. Smith

Michigan Department of Natural Resources, 621 North 10th Street, Plainwell, Michigan 49080, USA

James N. McNair

Annis Water Resources Institute, Grand Valley State University, 740 West Shoreline Drive, Muskegon, Michigan 49441, USA

Abstract

Natural rates of straying are difficult to quantify over large spatial scales using direct observations, particularly for long-lived fish species characterized by delayed sexual maturity and long interspawning intervals. Using multilocus microsatellite genotypes and likelihood-based statistical methods, we quantified rates of immigration and emigration for six genetically differentiated (mean $F_{ST} = 0.041$) lake sturgeon *Acipenser fulvescens* populations in Lake Michigan based on adults ($n = 437$) captured in tributaries during the spawning season. Estimated rates of straying were high (mean = 0.105), asymmetrical, and highly variable across populations. We found no significant association between the total length (a surrogate measure of age) of individuals that strayed and those that did not. Linear distance between streams was more predictive of straying rates and F_{ST} than least-cost distances estimated based on lakescape features (bathymetry and lake current patterns). Historical rates of gene flow estimated using coalescent analysis indicated a fully parameterized model with variable evolutionarily effective population sizes (θ ; range, 0.684–0.989),

*Corresponding author: homolaj@mail.gvsu.edu

¹Present address: Annis Water Resources Institute, Grand Valley State University, 740 West Shoreline Drive, Muskegon, Michigan 49441, USA.

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and variable and nonsymmetrical migration rates best explained the genetic data. Comparatively high estimates of relative historical gene flow from several numerically depressed populations suggest that these populations were once larger contributors to basinwide gene flow than indicated by estimates of contemporary straying rates. High rates of interpopulation straying contrast with high F_{ST} , suggesting that straying rates are poor indicators of successful reproduction following dispersal.

The propensity for individuals to return to spawn in their natal streams is of ecological and managerial importance. For example, in Pacific salmon *Oncorhynchus* spp. the tendency to stray (i.e., disperse to a stream other than an individual's stream of origin to spawn) is important for the colonization of new or previously extirpated streams (Milner 1987; Milner et al. 2000; Anderson et al. 2008). Straying does not necessarily result in gene flow to the destination population because immigrants may not successfully breed or may be selected against based on prezygotic (e.g., population differences in mate preference) or postzygotic (e.g., reduced offspring fitness via outbreeding depression; Lynch 1991) factors. Source-sink dynamics whereby population numbers are sustained by immigration of individuals from other populations can result in stable populations over generations (Ayllon et al. 2006). Resource managers' concerns about straying are exemplified by conservation hatchery programs (Mobrand et al. 2005), which often adopt strict rearing and release policies to facilitate imprinting to natal waters in order to minimize straying (Scholz et al. 1978; Candy and Beacham 2000). Additionally, in locations where limited harvest of numerically depressed species is permissible, regulations often are established based on assessments of the risk associated with the potential harvest of individuals from numerically less abundant, nontarget populations that may have strayed into the population subject to legal harvest (Policansky and Magnuson 1998; Bott et al. 2009).

Many variables can affect the likelihood of individuals straying. Natal philopatry is observed in many fish species and is believed to result from responses to site-specific olfactory signals detected during migration to spawning areas (Leggett 1977; Quinn 1993). Demographic parameters such as gender, age, and population size also may influence probabilities of straying (Hard and Heard 1999). Individuals originating from populations that are reproductively isolated temporally and spatially have lower expectations of straying than geographically proximal populations that spawn at similar times (Tallman and Healey 1994). Habitat quality also has the potential to affect the tendency of individuals to stray from a particular stream, whether the differences in quality are of anthropogenic (Quinn and Fresh 1984) or natural origin (Leider 1989). Fish produced in hatcheries have been shown to stray more than their wild counterparts (Quinn 1993; Mortensen et al. 2002); however, juveniles that are properly imprinted to target streams are generally more likely to home (Pascual et al. 1995; Dittman and Quinn 1996).

The methods used to quantify straying rates and movement patterns have traditionally included telemetry (Auer 1999) and capture-mark-recapture (Quinn 1993; Mortensen et al. 2002). Tag loss detracts from the usefulness of direct tagging methods when estimating population size and straying rates (Miranda 2002; Smith et al. 2002). Additionally, certain species have life history characteristics that make the implementation of direct straying estimators difficult. For instance, species with long generation times require long-term studies that extend until first reproduction occurs (Wirgin et al. 1997). Extended interspawning intervals and long-distance migrations between reproductive episodes also present challenges for collecting adequate numbers of recaptures from physical tags for accurate assessments of movements.

Molecular techniques can be used to study straying when opportunities to employ direct tagging are limited or impractical (Hansen et al. 2001). Examples of indirect genetic methods being implemented successfully to quantify rates of straying are found in Miller et al. (2001), D'Amelio et al. (2008), and Homola et al. (2010). Moreover, if there is sufficient genetic variation between populations, samples obtained from a single capture can be used to determine an individual's population of origin based on individual assignment testing (Cornuet et al. 1999).

Applications of genetic techniques for species such as lake sturgeon *Acipenser fulvescens* are especially important to elucidate information on straying due to aspects of the species' ecology. Lake sturgeon are a long-lived potamodromous fish species that reach reproductive maturity in 15–25 years, depending on sex (Harkness and Dymond 1961; Houston 1987). Through time, natal philopatry has resulted in genetically distinct lake sturgeon populations throughout the Great Lakes (DeHaan et al. 2006; Welsh et al. 2008) and has facilitated the use of genetic markers as a means of detecting individuals occupying nonnatal habitats (Bott et al. 2009; Homola et al. 2010).

The primary objective of this study was to quantify basinwide straying rates of Lake Michigan lake sturgeon to examine alternative hypotheses about the factors that may contribute to straying rates and directionality, including population demography, lakescape features, and stream environmental features. Given the levels of spatial genetic structure previously documented among Great Lakes lake sturgeon populations (DeHaan et al. 2006; Welsh et al. 2008), we hypothesized that straying among populations would be limited. Furthermore, we expected

that straying would be associated with the age of the straying individuals (i.e., younger fish would stray more frequently) and the geographic proximity of natal and destination streams (closer streams would have a higher likelihood of receiving strays). We also hypothesized that contemporary straying rates would reflect a lower preference for streams with a high degree of anthropogenic disturbance (e.g., dam construction), reflecting recent declines in the amount and quality of spawning habitat available relative to historical levels. To test these hypotheses, the specific objectives of this study were to (1) quantify the direction and rates of straying among lake sturgeon populations ($n = 7$; sample size = 437) that spawn in tributaries to Lake Michigan, (2) evaluate the influence of demographic and environmental variables on straying rates, (3) quantify the relationships among straying rates, genetic variation, and interstream geographic distance, and (4) estimate historic rates of gene flow to facilitate comparisons with the rates of straying in contemporary populations.

METHODS

Sample collection.—Adult lake sturgeon were sampled from Wisconsin tributaries to Lake Michigan, including the lower Fox River during 2000 ($n = 28$), 2001 ($n = 17$), and 2004 ($n = 25$); the Menominee River during 2002 ($n = 23$) and 2005 ($n = 41$); the Oconto River during 2002 ($n = 9$), 2003 ($n = 10$), 2004 ($n = 6$), and 2007 ($n = 2$); and the Peshtigo River during 2001 ($n = 23$), 2002 ($n = 22$), 2004 ($n = 14$), 2007 ($n = 1$), and 2009 ($n = 35$) (Figure 1). Adult lake sturgeon also were sampled from the following Michigan tributaries: the Kalamazoo River during 2004 ($n = 4$), 2005 ($n = 4$), and 2009 ($n = 9$); the Manistee River during 2000 ($n = 30$), 2001 ($n = 17$), 2002 ($n = 36$), 2003 ($n = 16$), and 2004 ($n = 7$); and the Muskegon River during 2002 ($n = 7$), 2003 ($n = 10$), 2004 ($n = 7$), 2005 ($n = 12$), 2008 ($n = 8$), and 2009 ($n = 14$) (Figure 1). Individuals were selected for analysis based on their physical presence at or near spawning areas in each stream during the spawning season (April 15 through June 15). Lake sturgeon were captured using long-handed dip nets, gill nets, seines, or electrofishing. Upon capture, a 1-cm² portion of the dorsal or caudal fin was collected from each individual for genetic analysis and stored in a uniquely marked scale envelope. Sex and maturity status were not apparent for all fish at the time of sampling. Consequently, only individuals of at least the minimum expected size at sexual maturity for either sex (>110 cm) were included in analyses.

Laboratory analysis.—DNA was extracted from fin tissue using QIAGEN DNeasy kits (Qiagen, Inc., Valencia, California) according to manufacturer's specifications. DNA was quantified using a NanoDrop spectrophotometer (NanoDrop Technologies, Thermo Scientific, Wilmington, Delaware) and diluted to a concentration of 20 ng/μL. All individuals were genotyped at twelve microsatellite loci: *AfuG68* (May et al. 1997), *Afu68b* (McQuown et al. 2002), *Spl120* (McQuown et al. 2000), *Aox27* (King et al. 2001), *AfuG9*, *AfuG160*, *AfuG63*, *AfuG74*, *AfuG204*, *AfuG195*, *AfuG56*, and *AfuG112* (Welsh et al. 2003). Polymerase chain reactions (PCR) were conducted in 25-μL

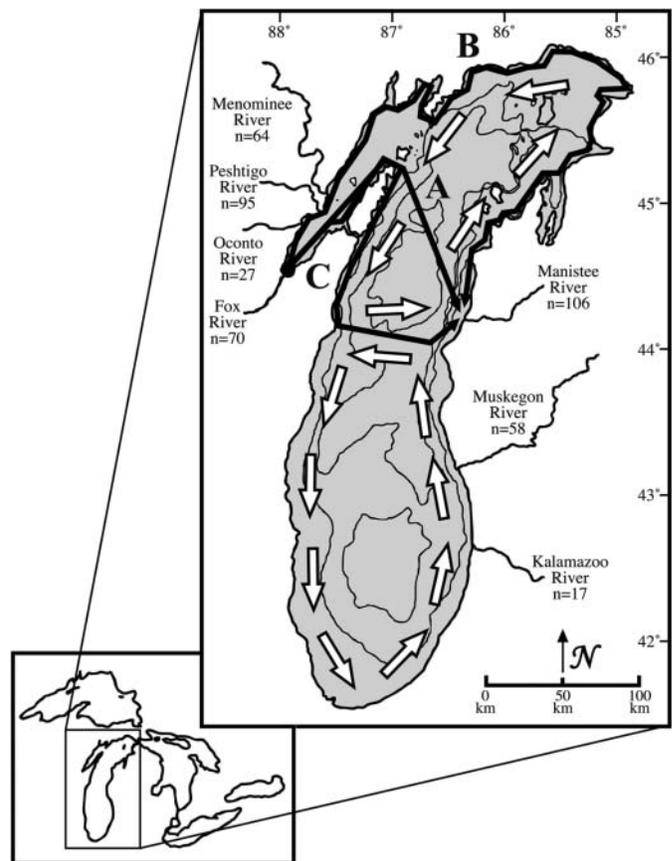


FIGURE 1. Visual representation of the three alternative connectivity scenarios evaluated using genetic data for lake sturgeon from seven remnant spawning populations in the Lake Michigan basin with generalized surface water currents indicated by white arrows. Distance measurements were quantified based on direct open water distance (A), shoreline distance (B), and surface current distance (C).

volumes containing 100 ng of template DNA, 2.5 μL of 10 × PCR buffer (1 M tris-HCl, 1.5 M MgCl₂, 1 M KCl, 10% gelatin, 10% NP-40, and 10% triton X), and 0.8 mM deoxynucleotide triphosphates, 10 pm fluorescently labeled forward and unlabeled reverse primers, sterile water, and 0.5 U *Taq* polymerase. Polymerase chain reactions were performed using Robocycler 96 thermocyclers (Stratagene, Inc., La Jolla, California) under the conditions detailed in Homola et al. (2010). Amplified PCR products were visualized on 6% denatured polyacrylamide gels using an FMBIO II scanner (Hitachi Software Engineering Co., Ltd., Yokohama, Japan). Allele size was determined by comparison with lake sturgeon samples of known genotype and based on molecular size standards. Genotype scores were confirmed by independent scoring by two experienced laboratory personnel.

Statistical analysis.—Measures of genetic diversity were estimated for each population to quantify the degree of differentiation among the lake sturgeon populations. Estimates of allele frequency, exact tests for Hardy–Weinberg equilibrium, and measures of genetic diversity, including allelic richness

(A), observed (H_o) and expected (H_e) heterozygosity, were estimated using the computer program GENEPOP (version 4; Raymond and Rousset 1995). GENEPOP also was used to evaluate significant differences among the genotypic frequencies of each population using a Fisher's exact test. Fixation indexes, including measures of the interpopulation variance in allele frequency (F_{ST}) and the variation among individuals within populations (F_{IS}), were estimated as described by Weir and Cockerham (1984) using the program FSTAT (version 2.9.3.1; Goudet 2001). A Bonferroni correction was used to adjust significance to account for multiple tests. Similarities in allelic frequency prompted the grouping of individuals from the Peshtigo and Oconto rivers for all analyses ($F_{ST} = 0.0006$; DeHaan et al. 2006; Bott et al. 2009). Gametic disequilibrium was assessed to provide estimates of locus independence from other loci in each population using GENEPOP, and a Bonferroni correction was used to adjust alpha levels.

Multilocus microsatellite data were analyzed in program STRUCTURE 2.2 (Pritchard et al. 2000; Falush et al. 2003) to detect the occurrence of population structure without a priori knowledge of putative populations. Data were analyzed using an admixture model assuming correlated frequencies to probabilistically assign individuals to putative genetic clusters using a 100,000 burn-in period, 200,000 Markov chain–Monte Carlo iterations, and a number of possible populations (K) ranging from 1 to 8; this analysis was repeated 10 times to ensure consistency across runs. An upper limit of $K = 8$ was chosen to allow for individuals not originating from one of the seven sampling locations to be placed in a separate group. The Web-based program STRUCTURE HARVESTER (Earl and vonHoldt 2012) was used to summarize estimates of the likelihood of K given the data for each K and replicate to estimate the number of clusters that best fit the data based on the mean likelihood $L(K)$ and its variance and estimates of ΔK (Evanno et al. 2005).

Identification of presumed first-generation migrants (i.e., individuals that strayed from a presumed natal stream to a different stream at the time of spawning) for each population was determined using the program GENECLASS (version 2.0.h; Cornuet et al. 1999). Estimates of emigration and immigration based on individual assignments of first-generation migrants will hereafter be referred to as representing measures of “contemporary rates of straying.” Assignment criteria followed the recommendations in Paetkau et al. (2004) and are detailed in Homola et al. (2010). Mean contemporary rates of straying across all populations were calculated by dividing the total number of individuals that were genetically determined to have strayed by the sum of all individuals sampled from their population of origin. Immigration rates were estimated for each population by dividing the number of strays found in each stream by the total number of individuals sampled in that stream. Emigration rates for each population were estimated by dividing the total number of strays found in each population by the total number of individuals sampled originating from that population regardless of capture location. Disparities between the number of immi-

grating and emigrating individuals for a stream were evaluated using a Fisher's exact test.

Statistical tests to assess potential nonrandomness in the contemporary migration pattern were conducted as follows. Let N_j denote the number of fish in sample j (i.e., the sample from population j), and let N_{ij} denote the number of these fish that originated in population i . Since all sampled fish originated in one of the six populations, $N_j = \sum_i N_{ij}$ for every j . The inferred number of sampled fish N_i' that were in each population i before migrating is given by $N_i' = \sum_j N_{ij}$. To test the null hypothesis that migration was random, we viewed premigration values N_i' as fixed and postmigration values N_{ij} as the result of random sampling from the premigration values. We conducted two such tests, differing in the extent of assumed randomness in migration.

The first test assumes completely random migration. We assumed that all N_i' premigration fish from population i left their natal population and randomly chose one of the six populations to join, with the probability of joining any particular population being $1/6$. The expected number $E(N_{ij})$ of postmigration fish in sample j that originated in population i is given by $E(N_{ij}) = N_i'/6$ for all combinations of i and j . We quantified the overall discrepancy between the observed and expected numbers of fish in cells of sample matrix $\mathbf{N} = [N_{ij}]$ using test statistic $X^2 = \sum_{i,j} [N_{ij} - E(N_{ij})]^2/E(N_{ij})$. For our data, the values of $E(N_{ij})$ in all cells of the expected sample matrix are greater than 1, so X^2 will have an approximately chi-squared (χ^2) distribution with 30 degrees of freedom. We therefore tested the null hypothesis of completely random migration by determining whether the probability that $\chi^2(30) > X_{obs}^2$ is less than 0.05, where X_{obs}^2 is the observed value of X^2 .

The second test permits partially nonrandom migration. Here we assumed that only a fraction m of the premigration fish from each population randomly chose one of the six populations to join, while the remaining fraction $1 - m$ intentionally returned to their natal population. The expected number of postmigration fish in sample j that originated in population i is given by $E(N_{ij}) = mN_i'/6$ for $i \neq j$ and by $E(N_{ii}) = (1 - m)N_i' + mN_i'/6 = (1 - 5m/6)N_i'$ for $i = j$. The value of parameter m is unknown but can be estimated from the data using the estimator $m = (6/5) [1 - \sum_i N_{ii}/\sum_i N_i']$, which for our data yields $m = 0.124$. As in the previous test, we quantified the overall discrepancy between the observed and expected numbers of fish in the cells of the sample matrix using test statistic X^2 . Here, however, the values of $E(N_{ij})$ in several cells of the expected sample matrix are well below 1, so it is not safe to assume that X^2 has an approximately chi-squared distribution. We therefore constructed the complementary cumulative distribution function of X^2 by Monte Carlo simulation and used it to test the null hypothesis of partially nonrandom migration by determining whether the probability that $X^2 > X_{obs}^2$ is less than 0.05.

The relationships between demographic characteristics, the proximity of natal streams relative to other populations, and stream habitat availability and the straying rates of individuals

from each population were quantified. A two-sample *t*-test assuming unequal variances was used to test the null hypothesis that there was no difference in the total body lengths of individuals that strayed and that of those that returned to their stream of origin to spawn. Length was assumed to be a surrogate measure of age (Bruch et al. 2009). Fisher's exact test was employed to evaluate the null hypothesis that there was an equal likelihood to stray for a lake sturgeon native to a Green Bay (Wisconsin) western basin tributary as there was for an individual native to an eastern basin (Michigan) stream. Additionally, Fisher's exact test was used to examine the null hypothesis that a straying individual was equally likely to stray to a stream on the same side of the basin as their natal stream as they were to migrate to a stream on the opposite side of the basin. The effects of the amount of spawning habitat available before the first migration barrier on straying rates were examined using Spearman's rank correlation coefficient. Measures of spawning habitat availability from previous published studies were used (O'Neal 1997; Tonello 2004; Wesley 2005; Daugherty et al. 2009) with the availability for the Oconto and Peshtigo rivers being the mean amount of accessible habitat for each of the two streams.

Linear regression analysis was used to characterize the relationships between degree of interpopulation variance in allele frequency ($F_{ST}/[1 - F_{ST}]$), interpopulation straying rate, and geographic distance between populations. The interpopulation straying rate was calculated as the total number of first-generation migrants between two stream populations divided by the total number of individuals analyzed for both populations. Interstream distances were estimated based on the direct open-water distances between river mouths and using least-cost paths (Spear et al. 2010) based on two river connectivity criteria: (1) shoreline distance based on lake sturgeon depth limitations and (2) distances estimated based on prevailing surface current patterns (Figure 1). Direct open-water distance was estimated as the shortest straight-line distance across water between two streams (Figure 1, segment A). Shoreline distance was the shortest distance between two river mouths following shoreline bathymetric contours (Figure 1, segment B). The depth limitations used to estimate shoreline distances were based on descriptions of the species' depth limitations (approximately 18.2 m; Harkness and Dymond 1961). Water depths were determined using Lake Michigan bathymetric data (U.S. National Oceanic and Atmospheric Administration). A third measure of connectivity was based on water current patterns. Shoreline distance was estimated following two counterclockwise patterns consistent with the prevailing water currents in the northern and southern portions of Lake Michigan (Figure 1, segment C; Beletsky and Schwab 2001). All distances were measured between river mouths using Google Earth (version 5.2.1.1588; earth.google.com).

The relative rates of historical gene flow and evolutionary effective population sizes for the Lake Michigan lake sturgeon populations were based on coalescence analyses from multilocus microsatellite genotypes estimated using the program

MIGRATE, version 3.1.6 (Beerli 2002). Historical rates are referred to as "relative" because they can only be compared with similarly derived rates (i.e., these rates are not directly comparable with contemporary straying rates). The evolutionary effective population size ($\theta = 4N_e\mu$) for nuclear loci was estimated, where N_e is the evolutionary effective total population size and μ is the rate of mutation to new alleles. The number of migrants per generation (N_em) among lake sturgeon populations was calculated based on the model with the best fit among the six evaluated models. Model 1 is an *N*-island model that assumes equal values of θ and equal interpopulation rates of gene flow. Model 2 allows for a variable θ and assumes constant and symmetrical pairwise rates of gene flow among populations. Model 3 assumes equal values of θ and variable and asymmetrical rates of gene flow among populations. Model 4 was designed to evaluate the hypothesis of predominately east-west migration and assumes equal values of θ and variable rates of gene flow among populations on the same or different sides of Lake Michigan. Model 5 also segregates the lake basin into eastern and western sides, assuming that θ is equal and that gene flow rates are equal and symmetrical between populations that spawn on the same side of the basin but possibly different between populations on different sides of the basin. Model 6 is a fully parameterized model, estimating θ for each population and allowing for different and asymmetrical pairwise gene flow rates. A maximum likelihood search of the parameter space included ten short chains (1,000 genealogies per chain), four long chains (10,000 genealogies per chain), and four adaptively heated chains (start temperatures = 1, 1.5, 3, and 10,000; swapping interval = 1). Three independent runs were conducted to evaluate evidence of convergence of all estimated parameters. Empirical estimates of F_{ST} were used for the first run, and output estimates were used during subsequent runs. Each model was evaluated for goodness of fit using a log-likelihood ratio test, and the model best supported by the data was determined based on Akaike's information criterion (Burnham and Anderson 2002). Log-likelihood ratio test statistics are equivalent to a chi-square distribution with degrees of freedom equal to the difference in the number of parameters estimated in the models (Beerli and Felsenstein 2001).

Potential asymmetry in historical gene flow between populations of different effective sizes N_e was assessed as follows: Let m_{ij} ($i \neq j$) denote the relative migration rate from population i to j . Model 6 above allows estimates of m_{ij} and m_{ji} to differ and also allows effective population sizes to differ. For each pair of populations, we calculated the net relative migration rate $m_{ij} - m_{ji}$, where i is the population with the larger effective population size (based on θ , assuming equal mutation rates μ across all populations). This yielded 15 net migration rates. Under the null hypothesis that the direction of net migration is random, there is an equal chance that the sign of $m_{ij} - m_{ji}$ will be positive or negative. A sign test was used to test this hypothesis against the one-sided alternative that positive signs occur more frequently than negative signs, indicating that net migration

TABLE 1. Measures of genetic diversity for seven Lake Michigan breeding populations of lake sturgeon. Abbreviations are as follows: n = sample size, k = mean number of alleles, A = allelic richness, H_o = observed gene diversity within individuals, H_e = expected gene diversity among individuals, and F_{IS} = Wright's inbreeding coefficient (all estimates not significantly different from zero).

Population	n	Diversity measure				
		k	A	H_o	H_e	F_{IS}
Fox River	70	4.83	3.78	0.520	0.517	-0.003
Menominee River	64	4.33	3.66	0.472	0.482	0.021
Oconto-Peshtigo rivers	122	4.83	3.67	0.539	0.534	-0.009
Kalamazoo River	17	3.58	3.41	0.508	0.499	-0.010
Manistee River	106	4.58	3.70	0.509	0.532	0.043
Muskegon River	58	4.42	3.74	0.569	0.549	-0.039
Mean values	72.83	4.43	3.66	0.519	0.519	0.000

tended to be directed from larger to smaller effective population sizes.

Potential dependence of the total migration rate between pairs of populations (sum of migration rates m_{ij} and m_{ji}) on the corresponding interpopulation geographic distance was assessed by linear regression. The 15 estimates of $m_{ij} + m_{ji}$ were regressed against the corresponding interpopulation distances D_{ij} and the null hypothesis that the slope equals zero was tested.

Comparison of measures of interpopulation straying and coalescent-based estimates of historical rates of interpopulation gene flow was conducted using a Mantel test implemented in program PASSAGE, version 2.0 (Rosenberg and Anderson 2011). Significant association between the elements of both matrices was evaluated using a permutation randomization test (Legendre 2000) and reported as a t -test.

RESULTS

Measures of Genetic Diversity within and among Populations

The levels of genetic diversity based on allelic richness (range, 3.41–3.78) and the observed (range, 0.472–0.569) and expected (range, 0.482–0.549) heterozygosity were similar across populations (Table 1). Following Bonferroni corrections, all populations conformed to Hardy–Weinberg expectations at

all loci except for one locus for the Fox River (*AfuG56*), one for the Manistee River (*AfuG160*), and one for the Menominee River (*AfuG56*). The F_{IS} values were not statistically different from zero (range, -0.039 to +0.043; $P > 0.05$). Gametic disequilibrium was found in 3 out of a possible 396 locus combinations (0.76%). Nonindependence was found in the Manistee River population (between *Afu68* and *Afu68b*) and the Oconto–Peshtigo population (between *Afu68b* and *AfuG56* and between *Afu68b* and *Spl120*).

All populations were significantly differentiated genetically based on the estimated interpopulation variance in allele frequency ($F_{ST} = 0.041 \pm 0.006$; Table 2) and pairwise Fisher's exact tests ($P < 0.001$ for all pairings). The F_{ST} -related alpha values were adjusted to 0.003 (0.05/15) following sequential Bonferroni correction (Rice 1989). The greatest level of genetic similarity was found between the Oconto–Peshtigo rivers and the Fox River ($F_{ST} = 0.018$; $P < 0.003$ after sequential Bonferroni correction), which likely is a result of their close geographic proximity (approximate shoreline distance of 60 km). The largest level of interpopulation variance was estimated between the Menominee River and the Kalamazoo River ($F_{ST} = 0.08$; $P < 0.003$), which are located on opposite sides of the basin. It must be noted that lake sturgeon from the lower Fox River were not genetically differentiated from the large Wolf River spawning population that resides upstream in Lake

TABLE 2. Pairwise interpopulation estimates of F_{ST} based on 12 microsatellite loci for six lake sturgeon populations in Lake Michigan, 2000–2009 ($P < 0.003$ for all comparisons). The populations from the Peshtigo and Oconto rivers were combined for analysis because of a lack of significant differences in allele frequency.

River	Fox	Menominee	Oconto-Peshtigo	Kalamazoo	Manistee	Muskegon
Fox		0.044	0.018	0.063	0.044	0.027
Menominee			0.034	0.080	0.060	0.053
Oconto-Peshtigo				0.060	0.044	0.038
Kalamazoo					0.052	0.048
Manistee						0.026
Muskegon						

Winnebago (DeHaan et al. 2006). A portion of the Wolf River–Lake Winnebago population out-migrates into Lake Michigan and returns to spawn in the lower Fox River below the first dam (Elliott and Gunderman 2008). Therefore, fish characterized as being from the lower Fox River also could have originated from the Wolf River spawning population.

Based on the estimated $L(K)$ (mean \pm SD log probability of the data given K over 10 replicates; ΔK method), the best estimate was five genetic clusters. The average estimated posterior probability of individual assignment to each of the five clusters was 0.532. The average membership coefficient for individuals that strayed was 0.104. Based on posterior probabilities of individual assignment, each Wisconsin tributary represented a genetic cluster. On the eastern side of the basin, individuals from the Muskegon River were members of a cluster and individuals from the Manistee and Kalamazoo rivers were members of a cluster. Individuals identified by GENECLASS as strays all had higher posterior probabilities associated with their population of assignment than with the population of capture. Based on ΔK , the estimated number of genetic clusters was two, corresponding to the eastern (Michigan) and western (Wisconsin) basin tributaries, and the relative degree of genetic differentiation among populations from the same side of the basin (mean $F_{ST} = 0.029$; $P < 0.003$) was less than between populations from different sides of the basin (mean $F_{ST} = 0.052$; $P < 0.003$; Table 2). Higher average posterior probabilities of assignment (mean = 0.762) appear to result from higher interpopulation variance in allele frequency among tributaries on different sides of the basin (Table 2). Individuals identified as strays from a different region in the basin (Michigan versus Wisconsin) had a higher posterior probability of cluster assignment to their population by GENECLASS than to the population of capture.

Estimates of Contemporary and Historical Interpopulation Exchange

The contemporary straying rate across all populations was estimated to be 0.105, and asymmetrical patterns of emigration and immigration were estimated for all streams; however, statistical significance was only found for the Oconto–Peshtigo rivers (Table 3). For example, the Manistee River population had an immigration rate (0.085) that exceeded the rate of emigration (0.067), although the rates were statistically indistinguishable ($P = 0.8$). In contrast, the Oconto–Peshtigo rivers were more than three times as likely to receive immigrants from other populations as they were to export individuals (immigration = 0.139, emigration = 0.045; $P = 0.046$; Table 3).

The pattern of contemporary migration was nonrandom. The null hypothesis of random migration was rejected, regardless of whether it was assumed that all fish migrated randomly ($X_{obs}^2 = 1,688.53$, $\text{Prob}[\chi^2(30) > X_{obs}^2] \ll 0.001$) or that only a fraction of each population migrated randomly ($X_{obs}^2 = 62.86$, $\text{Prob}[X^2 > X_{obs}^2] \ll 0.001$). Straying individuals that originated from streams on the eastern side of the basin were equally likely to move into streams on the opposite side of the basin (0.055) as

they were to stray to other eastern basin streams (0.033; $P = 0.44$). Similarly, Green Bay origin lake sturgeon were equally likely to stray to other Green Bay tributaries (0.074) as they were to traverse the basin (0.043; $P = 0.187$). The number of individuals straying from eastern to western tributaries also did not differ significantly from the number of individuals straying from western to eastern tributaries ($P = 0.651$).

Relative rates of historical gene flow based on coalescence analysis indicated that model 6 (the full model with variable θ and different, nonsymmetrical migration) was the model that best fit our genetic data (Table 4). Estimates of evolutionary effective population size (θ) varied from 0.684 in the Kalamazoo River to 0.989 in the Oconto–Peshtigo rivers, although estimates for five of six populations were fairly concordant (0.905–0.989; Table 5). Estimates of relative gene flow from coalescence analysis ranged from 0 (Kalamazoo River to Menominee River) to 22 (Oconto–Peshtigo rivers to Fox River; Table 5). Parameter estimates suggested that historical gene flow between populations was asymmetrical, even among geographically proximal streams. For example, the estimated relative straying rate from the Manistee River to the Muskegon River was 14.77, a value greater than that from the Muskegon River into the Manistee River (5.23; Table 5). The greatest magnitude of asymmetric migration was estimated between the Kalamazoo and Oconto–Peshtigo rivers, with a 19.93 times greater rate of gene flow from the Oconto–Peshtigo rivers into the Kalamazoo River (11.282) than from the Kalamazoo into the Oconto–Peshtigo rivers (0.566). Mantel analyses revealed no evidence for significant association between rates of historical gene flow and contemporary rates of straying ($r = 0.339$, $t = 1.39$, $P = 0.145$).

Impacts of Demographic and Environmental Variables

Analyses indicated no significant relationships between demographic and environmental variables and contemporary rates of straying. On average, the body size of resident individuals exceeded that of strays (straying individuals: 140.9 ± 3.16 cm; resident individuals: 146.6 ± 1.33 cm). However, lake sturgeon body size was not predictive of an individual's likelihood to stray ($t = 1.68$; $P = 0.099$). The proximity (km) of a migration barrier (dam) to the mouth of a tributary, a measure of the amount of spawning habitat available, was not significantly correlated with net rates of straying ($\rho = 0.486$; $P = 0.324$).

Linear regressions to quantify the relationships between the estimated contemporary rates of straying and lakescape features (which were hypothesized to affect the occupancy of open-water lake habitats and the direction of straying) indicated that the direct open-water distance between streams (mean = 235 km, SD = 144 km, range = 27–457 km) was a better predictor of straying rates ($r^2 = 0.316$, $P = 0.029$; Figure 2a) than least-cost distance estimates derived from either of two distance models: shoreline distance (mean = 411 km, SD = 282.5 km, range = 30–740 km; $r^2 = 0.101$, $P = 0.075$) and distance based on surface current patterns (mean = 502 km, SD = 342.7 km, range = 49–1,160 km; $r^2 = 0.061$, $P > 0.190$).

TABLE 3. Results from individual assignment tests estimating the number ($P < 0.05$) of first-generation migrant (straying) lake sturgeon captured during the breeding season within a stream, estimations of immigration and emigration rates for each population, and Fisher's exact test results for immigration versus emigration comparisons.

Presumed population of origin	Total number of individuals across Lake Michigan basin assigned to stream ^a	Individuals strayed into						Emigration versus immigration <i>P</i> -value	
		Green Bay		Eastern basin		Muskegon River	Emigration rate		
		Fox River	Menominee River ^b	Oconto-Peshigo rivers	Kalamazoo River			Manistee River	
Fox River	75 ($n = 70$)		2 (0.029)	5 (0.071)	0 (0.000)	5 (0.071)	0 (0.000)	0.160	0.465
Menominee River	72 ($n = 64$)	1 (0.016)		7 (0.109)	1 (0.016)	0 (0.000)	3 (0.047)	0.167	0.115
Oconto-Peshigo rivers	110 ($n = 122$)	2 (0.016)	1 (0.008)		0 (0.000)	1 (0.008)	1 (0.008)	0.045	0.046
Kalamazoo River	16 ($n = 17$)	0 (0.000)	0 (0.000)	1 (0.059)		1 (0.059)	0 (0.000)	0.125	1.000
Manistee River	104 ($n = 106$)	2 (0.019)	1 (0.009)	2 (0.019)	1 (0.009)		1 (0.009)	0.067	0.800
Muskegon River	60 ($n = 58$)	2 (0.035)	0 (0.000)	2 (0.035)	1 (0.017)	2 (0.035)		0.117	0.765
Immigration rate		0.100	0.063	0.139	0.176	0.085	0.086		

^aThe values in parentheses are the numbers of individuals captured in each river.

^bThe values in parentheses are the proportions of fish from each river listed across the top that strayed into the river in the left column (e.g., for the Menominee River, the proportion is 2/70 = 0.029).

TABLE 4. Summary and quantitative comparison of different coalescence models of interpopulation gene flow (m_{ij}) and evolutionary effective population size (θ) for six Lake Michigan lake sturgeon populations based on 12 microsatellite loci.

Model	Hypothesis	AIC	Δ AIC	Number of parameters
6	Full model, variable θ and m_{ij}	3,753.2	0	36
5	Constant θ , within east–west basin symmetrical m_{ij}	6,292.7	2,539.5	27
4	Constant θ , crossbasin symmetrical m_{ij}	6,477.0	2,723.8	25
3	Constant θ , variable m_{ij}	3,798.5	45.3	32
2	Variable θ , symmetrical m_{ij}	8,789.4	5,036.2	24
1	N -Island model	9,631.0	5,877.8	2

Contemporary interpopulation rates of straying were significantly related to the direct open-water distance between streams ($r^2 = 0.316$, $P = 0.029$), with increased rates of straying estimated for interpopulation pairs in close geographic proximity (Figure 2a). Standardized measures of interpopulation variance in allele frequency ($F_{ST}/[1 - F_{ST}]$) were not significantly related ($r^2 = 0.236$, $P = 0.066$) to stream proximity. However, the positive relationship is likely to be ecologically meaningful (Figure 2b) given the relatively small number of populations in the basin. The standardized variance in allele frequency ($F_{ST}/[1 - F_{ST}]$) was significantly related to interpopulation rates of straying ($r^2 = 0.461$, $P = 0.012$; Figure 2c).

Relative rates of historical gene flow were biased in the direction from larger to smaller effective population size (sign test; $P = 0.018$). Additionally, the total historical rate of gene flow between pairs of populations (i.e., the sum of rates in both directions) showed a statistically significant linear decline with increasing interpopulation distance (slope = -0.026 ; adjusted $R^2 = 0.260$, $P = 0.030$). Thus, historical gene flow tended to be directed from larger to smaller populations and to decline with increasing distance between populations.

DISCUSSION

We combined coalescence analyses estimating historical interpopulation gene flow with assignment tests identifying first-generation migrants to estimate contemporary straying rates in order to characterize rates of lake sturgeon interpopulation exchange. Comparisons of contemporary straying rates and directionality with historical gene flow and directionality provided a means of evaluating the possible effects of population size, age structure, and stream spawning habitat availability that have varied over many decades.

Temporal Variation in Movement of Straying Individuals

Movement patterns among the lake sturgeon populations spawning in Lake Michigan tributaries appear to have varied over time. While attaching specific time frames to coalescence analyses is highly speculative, our estimates provide a cumulative history of gene flow since modern population genetic structuring began to form following the most recent glacial retreat (Bernatchez and Wilson 1998). The relatively high historical

rates of gene flow for lake sturgeon originating in the Oconto–Peshtigo and Manistee rivers suggest that historically the populations that spawn in those tributaries were major contributors to basinwide gene flow (Table 5). However, contemporary estimates of emigration rates for individuals originating in the Oconto and Peshtigo rivers (0.045; Table 3) and Manistee River (0.067; Table 3) are among the lowest for any tributary of Lake Michigan. While the relative rates of emigration appear to be lower than those for other populations (Table 3), the relative rates of immigration to those streams seem to be greater than long-term trends inferred from coalescence analysis (Table 5). Despite the evident temporal stochasticity in specific interstream exchanges, both historic gene flow and contemporary straying rates showed nonrandom movement patterns ($P < 0.05$). This suggests a consistent trend of source–sink dynamics throughout the basin and that populations which were once major sources of straying individuals (net exporters) later became recipients (net importers). Mantel analyses failed to document significant associations between contemporary rates of straying estimates and historical gene flow.

The differences in the relative rates of immigration and emigration are suggestive of changes in numerical abundance or habitat quality and access to spawning sites. For example, the historical estimates of gene flow associated with the Oconto–Peshtigo rivers revealed higher rates of gene flow away from than into the system (Table 5). Based on our contemporary straying estimates, a reversed trend is suggested, with more fish immigrating (0.139) than emigrating (0.045; $P = 0.046$; Table 3). One potential explanation of this difference is that current immigration into the Peshtigo, Oconto, and other rivers from the lower Fox River has increased due to the disruption of upstream movements into the upper Fox River by dams located only 7 km upstream from Lake Michigan (Daugherty et al. 2009); by contrast, in the historical period the upper Fox and Wolf rivers were connected to Lake Michigan. Moreover, because of the close geographic proximity of the Wolf River spawning grounds to the headwaters of the Oconto and Peshtigo rivers, olfactory cues influencing Wolf–Fox River fish that had migrated into Green Bay might attract fish to the Oconto or Peshtigo River. Another potential explanation of the differences between historic gene flow and contemporary straying is alteration of the olfactory cues used for homing by the vast changes in watershed land

TABLE 5. Parameter estimates for migration rates (m_{ij} or m_{ij}/μ) and θ ($4N_e \mu$) with 95% confidence intervals in parentheses for six Lake Michigan lake sturgeon populations for the model of best fit (model 6; see Table 4).

Source population	θ	Migration to							
		Fox River	Menominee River	Oconto–Peshtigo rivers	Kalamazoo River	Manistee River	Muskegon River		
Fox River	0.940 (0.894–0.989)		5.578 (4.939–6.271)	4.350 (4.025–4.692)	4.786 (3.768–5.970)	2.147 (1.868–2.452)	2.847 (2.406–3.338)		
Menominee River	0.905 (0.860–0.953)	7.283 (6.533–8.089)		3.115 (2.841–3.406)	0.650 (0.325–1.146)	1.815 (1.559–2.098)	2.296 (1.899–2.746)		
Oconto–Peshtigo rivers	0.989 (0.963–1.016)	22.000 (20.659–23.399)	13.109 (12.111–14.159)		11.282 (9.660–13.077)	15.539 (14.765–16.339)	14.285 (13.269–15.351)		
Kalamazoo River	0.684 (0.605–0.781)	0.549 (0.363–0.791)	0.000 (0.000–0.039)	0.566 (0.454–0.695)	0.875 (0.700–1.077)	1.653 (1.327–2.029)			
Manistee River	0.921 (0.892–0.953)	11.275 (10.317–12.283)	10.117 (9.247–11.040)	4.873 (4.525–5.242)	15.796 (13.884–17.876)	14.767 (13.738–15.848)			
Muskegon River	0.942 (0.893–0.995)	3.499 (2.984–4.069)	4.224 (3.671–4.829)	2.412 (2.172–2.670)	6.533 (5.322–7.916)	5.234 (4.782–5.702)			

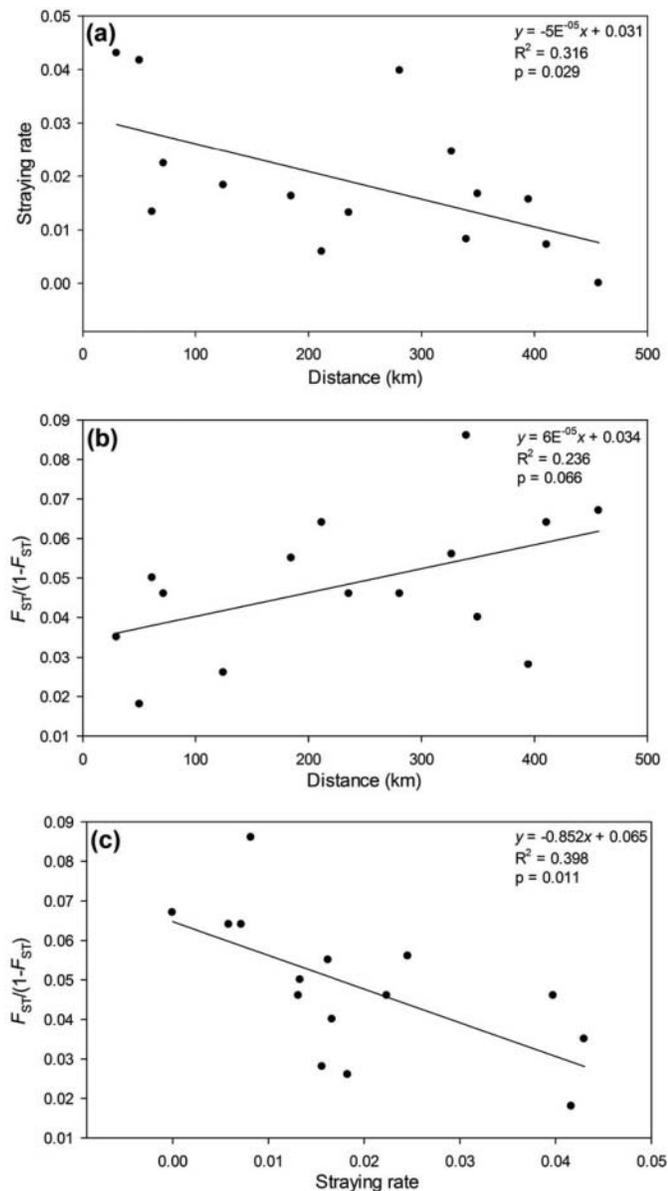


FIGURE 2. Linear regression analyses describing the relationships between measures of interpopulation genetic structure and predictor variables: (a) interpopulation straying rate and Euclidean open-water distance, (b) interpopulation genetic differentiation ($F_{ST}/[1 - F_{ST}]$) and distance, and (c) $F_{ST}/(1 - F_{ST})$ and interpopulation straying rate.

use (currently industrial and agricultural as opposed to historically forest dominated; Cole et al. 1998). Higher contributions by strays originating in the Muskegon and Manistee rivers to the Fox, Oconto, Peshtigo, and Menominee rivers than by those originating in other rivers reinforce the historical importance of Michigan populations to basinwide gene flow (Table 5).

Emigration rate estimates are typically calculated per capita and therefore are difficult to ascertain without accurate population abundance estimates. Considering lake sturgeon biology (i.e., long interspawning intervals and delayed reproductive

maturity), the available abundance estimates for the analyzed populations have prohibitively high levels of uncertainty to be useful in estimating demographic parameters. The method that we used to estimate emigration rates relies on the assumption that sampling effort was equal among streams, which is difficult to achieve when sampling occurs across broad geographic and temporal scales. Ongoing research throughout the Great Lakes is working to improve the accuracy and precision of abundance estimates that then could be used for the estimation of demographic parameters such as immigration, emigration, recruitment, and death rates. Additionally, reliable abundance estimates would benefit our data by adding context to the contemporary straying rates that we estimated. For instance, a population with a relatively low emigration rate might contribute a larger absolute number of strays to the basinwide population than a population with a high emigration rate but low abundance.

Given the evidence of significant interpopulation genetic differentiation across the Great Lakes basin previously reported (DeHaan et al. 2006; Welsh et al. 2008), we anticipated low levels of straying in the populations investigated. However, we documented a relatively high contemporary straying rate (10.45%). This estimate of straying likely underestimates the actual value due to our criterion ($P < 0.05$) for population assignment decisions. For instance, if we were to relax the assignment criterion to $P < 0.10$, the number of strays would increase from 45 ($P < 0.05$) to 87 ($P < 0.10$). The estimated 10.45% straying rate conflicts with the relatively high F_{ST} and nonsignificant F_{IS} values we estimated, suggesting either that the individuals that stray are reproductively unsuccessful (Hendry 2004) or that contemporary straying rates are not reflective of historical rates of gene flow. The natural straying rates that we documented are higher than those in the literature. Notably, a 3.5% straying rate has been documented among two Lake Superior populations of naturally produced lake sturgeon (Homola et al. 2010). Additionally, the straying rates of naturally produced Atlantic salmon *Salmo salar* in the River Imsa, Norway, were estimated to be 6% with no correlation between straying rates and age; however, an increased straying tendency was documented, with longer elapsed time before entering a stream to spawn (Jonsson et al. 2003).

Demographic and Environmental Effects on Straying

Body length was used as a surrogate measure of age to evaluate whether age was a factor contributing to the likelihood of straying since the species' extended time to sexual maturity could result in high straying rates relative to other species. Age and the differences in age structure characterizing different populations likely are not the predominant factors associated with the documented contemporary straying rates. However, the changing size and age structure of each population must be considered, since most Great Lakes lake sturgeon populations are dominated by younger individuals as a result of recent increasing recruitment due to the cessation of harvests and increasing water quality.

Examination of the amount of stream habitat available for spawning revealed little difference between streams that differ in contemporary straying rates. Hay-Chmielewski and Whelan (1997) assessed the suitability of several Michigan tributaries for lake sturgeon spawning using the criteria of population status, discharge, gradient, barriers, spawning habitat, and river temperature. The Kalamazoo, Manistee, Menominee, and Muskegon rivers all were characterized as highly suitable for lake sturgeon reproduction. Additionally, Daugherty et al. (2009) detailed habitat suitability for sturgeon in all of the Green Bay tributaries, allowing comparison of reproductive potential between rivers, and Benson (2006) and Elliott and Gunderman (2008) documented successful reproduction in the Peshtigo, Oconto, and lower Fox rivers. Our analyses detected no significant association between the length of river accessible for spawning and straying rates. In Lake Michigan tributaries, the length of accessible potential spawning habitat has been greatly reduced from historical levels and may contribute to the relatively high levels of straying.

The positive correlation between the length (km) of riverine habitat available for spawning before the first dam and contemporary straying rates, while not statistically significant, is suggestive of an ecologically important correlation. Anthropogenically driven change to spawning areas caused by dam construction may have contributed to incongruities between historical gene flow and contemporary straying rates. Even if a dam is situated upstream of historic spawning grounds, it likely reduces recruitment when downstream habitats are altered by elevated water temperature and changes in flow regimes and substrate composition (Williams and Wolman 1984). Even if rates of gene flow have remained constant over time, low recruitment and numerically depressed population numbers (Hay-Chmielewski and Whelan 1997) likely greatly reduced effective population size, thereby magnifying the effects of genetic drift.

An alternative explanation for the temporal heterogeneity in relative rates of straying versus interpopulation measures of straying is that historical saturation of spawning habitat created density-dependent straying (e.g., Ware and Schweigert 2001). This notion is supported by the general directionality of historical gene flow from populations with higher effective population sizes to ones with lower effective population sizes. Significant reductions in lake sturgeon abundance over the past century may have altered straying propensities across the basin. Once highly abundant throughout the Great Lakes, lake sturgeon are currently far below 1% of their historic levels as a result of habitat degradation, water pollution, and overexploitation (Hay-Chmielewski and Whelan 1997; Elliott 2008).

Linear regression analyses were suggestive of relationships between alternative measures of stream proximity (direct open-water, shoreline, and surface water current straying patterns) and contemporary straying rates. The extended spawning intervals of lake sturgeon (Forsythe et al. 2012) were expected to increase the likelihood of individuals straying along the relatively shallow-

water depth contours that conform to the species' maximum depth threshold (18.2 m; Harkness and Dymond 1961) despite their having to travel a longer distance to remain in shallower waters. However, the relative lack of support for least-cost paths that consider the species' maximum depth threshold (shoreline distance) and surface water current patterns suggests that lake sturgeon traverse deeper waters than previously believed.

The populations analyzed showed a significant relationship between F_{ST} and rates of straying (Figure 2c). Whitlock and McCauley (1999) suggested that equating F_{ST} to the number of individuals in a population (N) multiplied by the rate of straying (m) is unrealistic given that migration rarely occurs as assumed by Sewall Wright's island model. Wright (1943) hypothesized that the variance in gene frequencies among different populations would be related to the number of individuals migrating to or from each population. Model comparisons (Table 4) revealed that the island model, which assumes equal population size and equal and reciprocal migration, was the least supported model evaluated. The significant negative relationship documented between straying rate and F_{ST} provides evidence supporting Wright's isolation-by-distance model (Figure 2c; Wright 1943). Similarly, analyses indicate a negative relationship between straying rate and distance (Figure 2a) and a positive relationship between F_{ST} and distance (Figure 2b), as predicted in Slatkin (1993). This scenario also was documented in chum salmon *Oncorhynchus keta*, which showed a higher degree of genetic dissimilarity than predicted by the relatively high estimated straying rate (Tallman and Healey 1994).

Aspects of sample collection may have influenced study results. However, due to the inherent interannual variability in fish spawning events, we do not believe that these factors reduce the applicability of our findings. There is considerable variation among the years of our sample collection due to the constraints involved in the simultaneous sampling of seven different streams across a large geographic area and several management boundaries. Since sampling occurred over multiple years, the individuals included in our study represent a composite from multiple spawning periods for each population, thereby reducing the bias that would occur if samples were only collected during a single season. Moreover, considering the lake sturgeon's long inter-spawning interval (average 2–3 years for males, 3–7 years for females; Forsythe et al. 2012), using samples obtained over multiple years would be preferable to obtain a representative sample from each population. In addition, efforts were made to ensure that all of the individuals included in our baseline populations were present in each stream for spawning purposes by only analyzing individuals captured in streams during dates of known spawning activity (April 15–June 15). The individuals included in our analyses were > 110 cm in length to ensure that they were at least the minimum expected size at sexual maturity; however, since male lake sturgeon spawn at a younger age than females, some individuals included in the analyses might have been immature females.

Management Implications

Relative rates of historical gene flow estimated by means of coalescence analysis and contemporary rates of straying estimated by assignments of first-generation migrants during the spawning season are not necessarily representative of the degree of demographic independence. The degree to which population growth is affected by straying (demographic connectivity) often is independent of gene flow (Lowe and Allendorf 2010). Long-term tracking using genetic or direct tagging recaptures would be necessary to determine the extent of lake sturgeon straying over longer time periods (i.e., whether individuals return for one or more breeding seasons) to evaluate the importance of demographic connectivity to population stability. Considering the numerically depressed state of all Great Lakes lake sturgeon populations and the asymmetric dispersal of straying individuals from all of the populations we analyzed, changes in the demographic connectivity within the basin could shift source populations to an overall negative growth rate (Lowe and Allendorf 2010). Moreover, low recruitment for Great Lakes lake sturgeon as a result of spawning habitat degradation (Hay-Chmielewski and Whelan 1997) may have increased the dependence of population stability on immigration from other populations (Waples and Gaggiotti 2006).

Improved understanding of lake sturgeon straying will supply the information necessary to enhance the effectiveness of conservation and management efforts. Knowledge of population composition, individual movement tendencies, and historical population characteristics are critical for informing the best possible management practices (Hay-Chmielewski and Whelan 1997; Holey et al. 2000). Determining the contemporary straying rates of Lake Michigan lake sturgeon and comparing them with historic gene flow levels provide the quantitative information necessary for setting long-term management goals aimed at lake sturgeon restoration. Additionally, understanding the straying patterns of lake sturgeon is particularly important considering their reduced population sizes (Hay-Chmielewski and Whelan 1997; Elliott 2008), which may make the species more susceptible to nontarget harvest (Bott et al. 2009) or site-specific pollution events. The correlations analyzed between straying rates, stream geographic proximity, and F_{ST} provide insight into a stage of lake sturgeon life history that is difficult to gain through conventional direct-observation methods.

Population supplementation through the release of hatchery-reared individuals is a common management strategy to augment numerically depressed populations in many fishes (Quinn 1993; Mortensen et al. 2002; Smith et al. 2002), including lake sturgeon (Hay-Chmielewski and Whelan 1997; Schram et al. 1999). Stocking of lake sturgeon could result in elevated levels of straying, as seen in other species (Quinn 1993; Mortensen et al. 2002), including other sturgeon species (Smith et al. 2002). A variation of stocking known as streamside rearing is currently being undertaken across the Lake Michigan basin in hopes of reducing the likelihood of straying for stocked fish (Elliott 2008).

The data presented here represent a valuable baseline for future comparisons when hatchery-reared fish mature.

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Stock Origin of Migratory Atlantic Sturgeon in Minas Basin, Inner Bay of Fundy, Canada, Determined by Microsatellite and Mitochondrial DNA Analyses

Isaac Wirgin^a, Lorraine Maceda^a, John R. Waldman^b, Sierra Wehrell^c, Michael Dadswell^c & Tim King^d

^a Department of Environmental Medicine, New York University School of Medicine, 57 Old Forge Road, Tuxedo, New York, 10987, USA

^b Biology Department, Queens College of the City University of New York, 65-30 Kissena Boulevard, Flushing, New York, 11367-1597, USA

^c Biology Department, Acadia University, Wolfville, Nova Scotia, B4P 2R6, Canada

^d U.S. Geological Survey, Leetown Science Center, 11649 Leetown Road, Kearneysville, West Virginia, 25430, USA

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ARTICLE

Stock Origin of Migratory Atlantic Sturgeon in Minas Basin, Inner Bay of Fundy, Canada, Determined by Microsatellite and Mitochondrial DNA Analyses

Isaac Wirgin* and Lorraine Maceda

Department of Environmental Medicine, New York University School of Medicine, 57 Old Forge Road, Tuxedo, New York 10987, USA

John R. Waldman

Biology Department, Queens College of the City University of New York, 65-30 Kissena Boulevard, Flushing, New York 11367-1597, USA

Sierra Wehrell and Michael Dadswell

Biology Department, Acadia University, Wolfville, Nova Scotia B4P 2R6, Canada

Tim King

U.S. Geological Survey, Leetown Science Center, 11649 Leetown Road, Kearneysville, West Virginia 25430, USA

Abstract

Five distinct population segments of Atlantic sturgeon *Acipenser oxyrinchus* were recently listed (April 2012) as endangered or threatened under the U.S. Endangered Species Act. Atlantic sturgeon are anadromous, spawning occurs in rivers from the St. Lawrence River, Quebec, to the Satilla River, Georgia, and subadults and adults undertake extensive coastal migrations. Bycatch of Atlantic sturgeon in coastal fisheries may have resulted in the slowed or failed rebuilding of many populations despite the imposition of a U.S. federal moratorium on their harvest in 1998. Canada's Bay of Fundy hosts weir and trawl fisheries which bycatch Atlantic sturgeon of unknown origin. Additionally, tidal power development projects for the Bay of Fundy have been proposed which could detrimentally impact migratory sturgeon. We hypothesized that the Atlantic sturgeon that occur in Minas Basin in the Bay of Fundy are of local Saint John River, New Brunswick, origin with little or no U.S. contribution. We used microsatellite DNA (11 loci) and mitochondrial DNA control region sequence analysis along with previously determined characterizations of nine reference spawning populations to quantify their stock origin. We determined that the summer assemblage of Atlantic sturgeon collected within Minas Basin was of mixed origin, with a greater than 60% contribution from the nearby Saint John River but with a substantial (34–36%) contribution from the Kennebec River, Maine, and a smaller (1–2%) contribution from the Hudson River, New York. There was significant genetic heterogeneity between smaller (<130 cm) and larger individuals (≥ 130 cm) in Minas Basin; however, the smaller specimens were not exclusively of proximal Saint John River origin. Our results indicate that Atlantic sturgeon of U.S. origin are vulnerable to anthropogenic impacts in the Bay of Fundy, particularly those of Kennebec River origin.

Atlantic sturgeon *Acipenser oxyrinchus* is a highly migratory, wide-ranging, anadromous species whose historical distribution along the Atlantic coast of North America extended

from Hamilton Inlet, Labrador, to the St. Johns River, Florida. Atlantic sturgeon were once abundant throughout their range. In the 1890s, adult Atlantic sturgeon supported large fish oil and

*Corresponding author: isaac.wirgin@nyumc.org
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caviar-focused fisheries in many rivers along the Atlantic coasts of the United States and Canada. Many, if not all, collapsed at the turn of the 20th century, almost certainly due to overharvesting (Secor and Waldman 1999). U.S. fisheries, which are primarily for flesh and often in coastal waters, have endured at low levels (<5% of historic highs; Smith and Clugston 1997). In Canada, limited fisheries on the Saint John and St. Lawrence rivers have continued to the present (Caron and Tremblay 1999; Dadswell 2006).

In 1998, a U.S. federal moratorium on Atlantic sturgeon harvest was imposed for 20–40 years or when 20 year-classes of mature females are present. In Canada, the allowable take of sturgeon in marine waters was terminated in 2002 (R. Bradford, Department of Fisheries and Oceans, personal communication). These measures probably led to increases in some but not all populations. Recent estimates of adult population size are currently available for only two U.S. populations, those in the Hudson River (870 adults/year; Kahnle et al. 2007) and Altamaha River (343 adults/year; Peterson et al. 2008). In the United States, Atlantic sturgeon were recently afforded protection under the U.S. Endangered Species Act (ESA) by being classified as endangered or threatened (NMFS 2012). The species is not protected in Canada, and commercial fisheries still occur for subadults in the St. Lawrence River, Quebec, and adults in the Saint John River, New Brunswick (Dadswell 2006). There are no estimates of adult population size from either Canadian river.

While historically there were approximately 35 known spawning populations of Atlantic sturgeon, in recent years that number has dwindled to minimally 18 in the United States (ASSRT 2007) and their status is unknown for all but three rivers in Canada (ASSRT 2007). Around the Bay of Fundy, New Brunswick, there is a relatively large population in the Saint John River (annual total allowable catch of 200 adults; R. Bradford personal communication). Spawning is also suspected to occur in the Annapolis, Shubenacadie, and Avon rivers in Nova Scotia (Dadswell 2006).

Depending on their estuary of origin, juvenile Atlantic sturgeon are resident until ages 2–6 in the United States (Dovel and Berggren 1983) and up to 10 years in Canada (Caron and Tremblay 1999; Dadswell 2006), at which time most subadults join adults in marine waters (Dovel and Berggren 1983; Smith and Clugston 1997). There they conduct coastal latitudinal movements until adults return to spawn in their natal estuaries (Dadswell 2006). Age at maturity ranges between 5 and 32 years (Smith and Clugston 1997); maturity occurs at younger ages in individuals from southern populations and in males.

The stock specificity of the migratory patterns of Atlantic sturgeon in coastal waters and to nonnatal estuaries is unknown. It is also unknown whether individual stocks mix or form discrete contingents during these coastal migrations. Migrations of subadult and adult Atlantic sturgeon can be extensive in coastal waters and may include excursions of subadults into nonnatal rivers that may or may not support spawning populations of

Atlantic sturgeon (Dovel and Berggren 1983). Coastal migrations of subadult and adult sturgeon usually follow inshore of the 40-m depth contour between the Bay of Fundy and the Outer Banks of North Carolina (Stein et al. 2004b) and perhaps farther south (Erickson et al. 2011). But on occasion Atlantic sturgeon undertake far longer oceanic movements through much deeper waters (Ludwig et al. 2002; Erickson et al. 2011).

The ESA demands that widely distributed species such as Atlantic sturgeon be managed on the basis of distinct population segments (DPSs) if evidence for such divisions exists. A DPS is defined as the smallest division of a taxonomic species that is permitted protection under ESA and may be proposed based on the discreteness and significance of a population (Waples 1991). Discreteness is often evaluated by quantitative measures of genetic discontinuity. Among other criteria, significance may be demonstrated if a discrete population differs significantly from other populations in its genetic characteristics.

Throughout their range, adult Atlantic sturgeon exhibit strong fidelity to their natal rivers for spawning, as evidenced by both mitochondrial DNA (mtDNA; Wirgin et al. 2000; Grunwald et al. 2008) and microsatellite DNA analyses (King et al. 2001). As a result, most populations are genetically distinct and minimally host separate management units of this species. These genetic data, along with ancillary terrestrial and marine ecoregion mapping information from the Nature Conservancy, were the basis for the designation of five DPS units of Atlantic sturgeon in U.S. waters, including the Gulf Maine (GOM), New York Bight (NYB), Chesapeake Bay (CB), Carolina (CA), and Southeastern U.S. (SE) units (NMFS 2012). All of these units were recommended for listing as endangered under the ESA with the exception of the GOM DPS, which was recommended for listing as threatened. In Canada, the St. Lawrence River and Saint John River populations are viewed as two separate designatable units (DUs). Under the proposed U.S. DPS scheme, the two Canadian populations are considered distinct from the GOM DPS.

Migratory adult and subadult Atlantic sturgeon are vulnerable to anthropogenic disturbances at locales distant from their natal estuaries. Bycatch in entangling gear in coastal fisheries incurs significant mortality that may prolong or prevent the rebuilding of vulnerable populations (Stein et al. 2004a, b). Similarly, Atlantic sturgeon may be susceptible to ship strikes (Brown and Murphy 2010) or compromised water quality (Kennish et al. 1992) in estuaries through which they seasonally move. Atlantic sturgeon have been found dead below the Annapolis Royal tidal turbine in the Annapolis River estuary during numerous summers since the turbine began operation there in 1985. All such fish exhibited characteristic symptoms of mechanical strike by turbine blades (Dadswell and Rulifson 1994; Dadswell 2006).

Minas Basin is an embayment located in the inner reaches of the Bay of Fundy on the Atlantic coast of Canada (Figure 1) that experiences the world's highest tides (Greenberg 1984). At low tide, roughly one-third of its surface area is exposed as mud

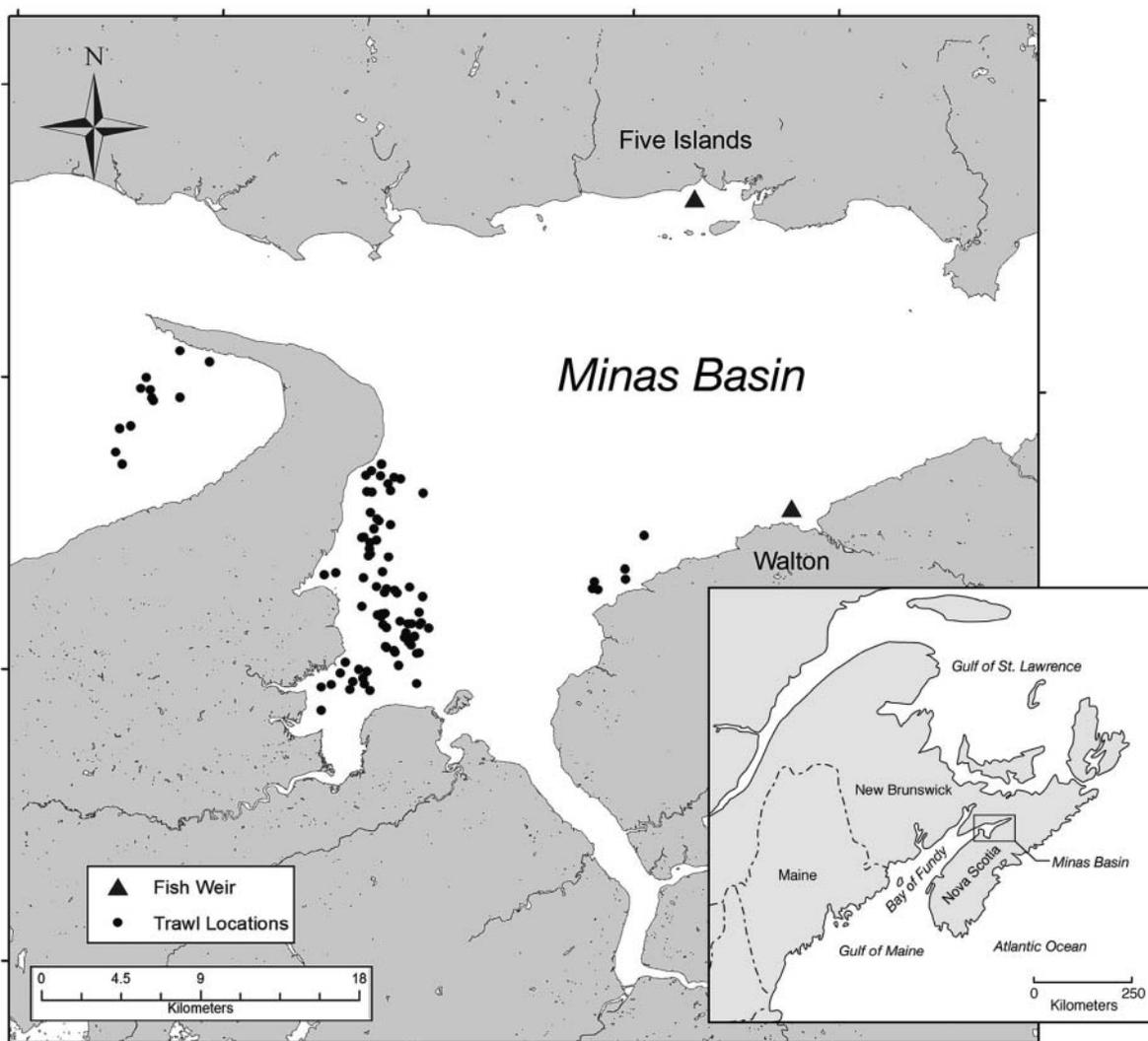


FIGURE 1. Map of Minas Basin showing where subadult and adult Atlantic sturgeon were collected by trawling and weirs.

flats, sand, and salt marshes (Bousfield and Leim 1958). Minas Channel leading into the basin is roughly 50 km long and 24 km wide at the entrance to the Bay of Fundy, narrowing to 5 km at Minas Passage. Tidal currents may reach 4 m/s in the passage but decrease to 1.5 m/s in the basin (Greenberg 1984). Based on tag returns, Atlantic sturgeon from proximal and distant locations (the Saint John River and the Hudson River) migrate to Minas Basin during summer, but the stock composition of this seasonal aggregation is unknown (S. Wehrell, unpublished data). For example, in a recent pop-up satellite archival tag (PSAT) study, one adult Atlantic sturgeon tagged in the Hudson River migrated to the terminal end of the Bay of Fundy within 6 months (Erickson et al. 2011).

In this study, we used previously published and newly derived mtDNA control region sequence haplotypes and microsatellite DNA characterizations of Atlantic sturgeon from nine reference river spawning populations to estimate the stock origin of

individuals and aggregations as bycatch to two fisheries in Minas Basin. Because Atlantic sturgeon currently are protected under the ESA, the stock composition of the fisheries in the Bay of Fundy is of direct relevance to their management in the United States. We report the stock origin of fish on population-specific and DPS-specific bases.

METHODS

Collections of subadult and adult Atlantic sturgeon.—Tissue samples were taken from a subset of subadult and adult Atlantic sturgeon captured as bycatch by commercial fishermen using trawls and intertidal fish weirs from May through August of 2007 ($n = 70$), 2008 ($n = 74$), and 2009 ($n = 37$). During 2007, a total of 51 samples were taken in the Five Islands weir on the north side of the basin and 19 by the trawling in the southern basin (Figure 1). During 2008 and 2009, 49 and 10 samples,

respectively, were taken from the Five Islands weir and 25 and 16 samples from the Walton weir in the southern basin; the remaining 11 sturgeon sampled in 2009 were taken by trawling in the southern basin. Both fishing weirs and trawlers are nonselective capture methods, and the samples from Minas Basin are effectively random since we had no a priori knowledge that any sturgeon would be collected at any site on any day. The captured Atlantic sturgeon from a total bycatch of 574 specimens ranged from 46 to 238 cm total length (TL; S. Wehrell, unpublished data).

The trawler used a stern-towed single-box trawl of 140 mm stretched mesh with modified rock hopper equipment and operated primarily along the south side of Minas Basin (Figure 1). Large, stationary V-shaped fish weirs were constructed on the mudflats on the north shore of Minas Basin near the village of Five Islands and on the south shore near the village of Walton (Figure 1). The fish weirs (2.75 m high and 0.7–1.2 km long) were built near the low-tide mark with wooden posts and covered with small mesh netting and brush. Fish swim inshore behind the weirs at high tide and are captured during ebb tide as the weirs become exposed. At low tide, a small pool persists at the apex of the V from which sturgeon were captured and to which they were returned after sampling. Tissue samples were taken from the caudal fins of a subset of samples and stored in 95% ethanol until analysis.

Reference collections were made from nine rivers (Figure 2) known to host contemporary successful spawning of Atlantic sturgeon and are reported in King et al. (2001; T. King, unpublished data) and a subset of those in Grunwald et al. (2008). Reference collections consisted of either juvenile or adult fish. To ensure that the individuals in this study were natal to the rivers in which they were collected, only those with TLs <50 cm or >130 cm were included. Specimens of these sizes were presumed to be native to their estuarine collection sites because they were either juveniles that are too young to migrate or adults that had returned to their natal rivers to spawn. Specimens between 50 cm and 130 cm TL were considered subadults.

DNA isolations.—Total DNA was isolated from fin clips by incubation in hexadecyltrimethylammonium bromide buffer (Saghai-Marooof et al. 1984) and digestion with proteinase K at 65°C, followed by standard phenol-chloroform extractions and alcohol precipitations. The DNA concentrations and purities were evaluated by using a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware). The DNA concentrations of samples were adjusted to 50 ng/μL for standardization of subsequent procedures.

Sequence analysis of the mtDNA control region.—Atlantic sturgeon-specific primers S1 (5'-ACATTAACCTATTCTCTGGC-3') and G1 (5'-GAATGATATACTGTTCTACC-3'; Ong et al. 1996) were used to amplify an approximately 560-bp portion of the mtDNA control region and to sequence a portion of it. We only report here data on 205 bp of the amplicon to allow for comparison of new and previously reported control region sequence data.

The PCR reactions were in 50-μL volumes that contained 5 μL of 10 × reaction buffer (Roche Applied Science, Indianapolis, Indiana), 0.25 μL of each dNTP (25-mM stocks; Pharmacia, Piscataway, New Jersey), 0.07 μL of S1 primer, 0.05 μL of G1 primer (Integrated DNA Technologies, Coralville, Iowa), 25–50 ng of template DNA, 2 units of *Taq* DNA polymerase (Roche Applied Science), and 43.9 μL of H₂O. The amplification conditions were 94°C for 5 min, followed by 40 cycles at 94°C for 45 s, 56°C for 45 s, and 72°C for 60 s, followed by a final extension at 72°C for 10 min.

Amplicons were dye-terminator cycle sequenced as described by the manufacturer (Beckman Coulter, Fullerton, California). Sequencing conditions were 30 cycles at 96°C for 20 s, 50°C for 20 s, and 60°C for 4 min. The sequencing products were ethanol-precipitated as recommended by Beckman Coulter (except that no EDTA was added), resuspended in 40 μL of Beckman Coulter CEQ sample loading buffer, loaded into a Beckman Coulter CEQ8000 automated capillary-based DNA sequencer, run using the standard long-fast read method, and analyzed with the sequence analysis module of the CEQ8000 Genetic Analysis System.

Microsatellite analysis.—Eleven informative microsatellite loci were scored in all individuals from the Bay of Fundy, including *LS19*, *LS39*, *LS54*, *LS68* (May et al. 1997), *Aox23*, *AoxD45* (King et al. 2001), *Aox44*, *AoxD165*, *AoxD170*, *AoxD188*, and *AoxD241* (Henderson-Arzapalo and King 2002). These loci were selected because they could be reliably scored, they were in Hardy-Weinberg and linkage equilibrium within individual reference spawning populations in previous studies (King et al. 2001; Henderson et al. 2004), and they were effective in distinguishing reference population collections (King et al. 2001; Henderson et al. 2004).

Characterization of microsatellite genotypes in the Bay of Fundy collections was performed using the Beckman Coulter CEQ8000 capillary-based DNA sequencer. Multiplexed PCR reactions were diluted up to 1:3 with Sample Loading Solution (Beckman Coulter). The diluted PCR reactions (0.5–2 μL) were loaded onto 96-well plates along with 0.5 μL of Beckman Coulter CEQ DNA Size Standard-400 and 40 μL of Sample Loading Solution (Beckman Coulter) and run with the FRAG 1 program (Beckman Coulter). MICRO-CHECKER software (Oosterhout et al. 2004) was used to test for the presence of null alleles, errors due to microsatellite stuttering, and large-allele dropout.

Data analyses.—Individual-based assignment (IBA) and mixed-stock analysis (MSA) were used to determine the stock origin of Atlantic sturgeon as bycatch to the two fisheries in Minas Basin. Individual assignment tests using multilocus likelihood functions (after Paetkau et al. 1995) were used to determine the likelihood of each individual's genotype being found in the collection from which it was sampled (without replacement) using the program GeneClass2 (Piry et al. 2004). The IBA tests use Monte Carlo simulations to calculate the likelihood of encountering a particular multilocus genotype from a mixed aggregation in each potential reference source population. The

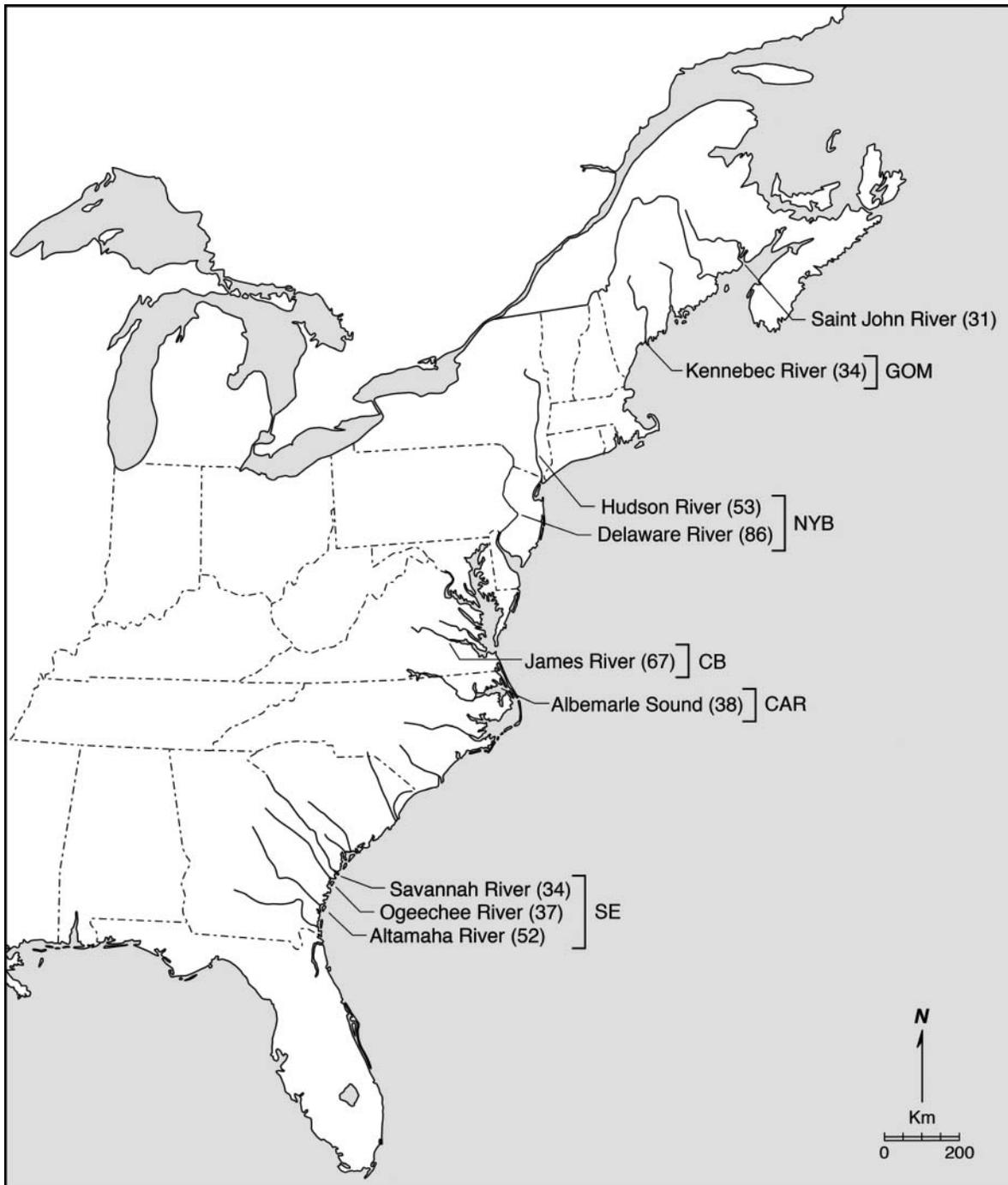


FIGURE 2. Map of Atlantic coast estuaries where nine reference population collections of adult and juvenile Atlantic sturgeon were made. The numbers in parentheses are the numbers of specimens analyzed from the different spawning populations. The five U.S. distinct population segments (DPSs) are indicated to the right of the brackets.

tests were run with 10,000 simulated individuals, a population exclusion threshold of 0.01, and the Bayesian method (Paetkau et al. 2004), which have been shown to perform better than frequency-based and distance methods in cases in which the assumptions of Hardy–Weinberg equilibrium are not met (Cornuet et al. 1999).

Mixed stock analysis with the program ONCOR (Kalinowski et al. 2008; <http://www.montana.edu/kalinowski/Software/ONCOR.htm>) was used to estimate the proportion of each reference population in the mixed sample of fish of unknown origin from Minas Basin and to determine the probability of each individual's belonging to each of the reference collections. In

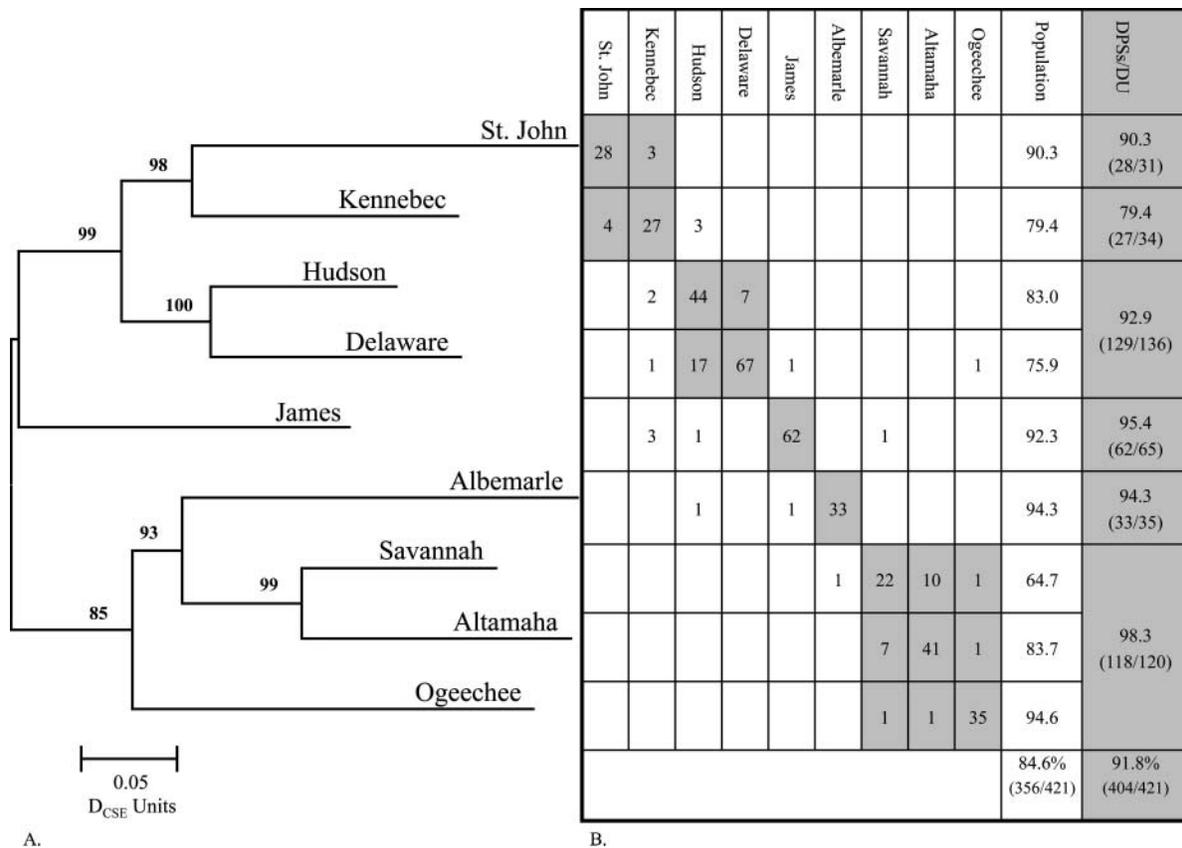


FIGURE 3. Panel (A) shows a neighbor-joining tree depicting the evolutionary relatedness (Cavalli-Sforza and Edwards chord distances) among 421 Atlantic sturgeon specimens representing the nine reference spawning collections surveyed at 11 microsatellite DNA loci. The numbers at the nodes represent the bootstrap support observed at each branch with 5,000 replicates of resampling across loci. Panel (B) shows the results of assignment testing using 11 microsatellite DNA loci and mitochondrial DNA control region sequence haplotypes (scored as a homozygous locus) to determine the likelihood of each individual's multilocus genotype/haplotype being found in the nine reference population collections and five U.S. DPSs and one Canadian designatable unit (DU) from which it was sampled (without replacement) using the program GeneClass2.

contrast to IBA, MSA applies mixture modelling, taking into account the genotypes of individual fish across multiple loci, the multilocus genotype distributions of the reference samples and the multilocus genotype distribution in the mixture samples. The MSA and IBA were conducted on pooled samples from the 3 years of collections. Mixture proportions and their 95% confidence limits were determined based on 10,000 bootstraps. ONCOR was run with a combination of mtDNA haplotypes and the results from the 11 microsatellites or the latter results alone.

The reference data set consisted of multilocus genotypes for 421 fish genotyped at 11 microsatellite loci and the associated mtDNA haplotype coded as a homozygous diploid locus (e.g., haplotype A, 001001). Reference collections were made from the nine spawning estuaries and DPSs depicted in Figure 2: Saint John ($n = 31$; GOM), Kennebec (34; GOM), Hudson (53; NYB), Delaware (86; NYB), James (67; CB), Albemarle Sound (35; SE), Savannah (34; SE), Ogeechee (37; SE), and Altamaha (52; SE).

Analyses were performed to provide estimates of the accuracy of identification of individuals to the nine reference river

collections and reporting groups designated as DPS by NOAA and that from the Saint John River. Raw microsatellite data for the nine reference collections are reported in King (unpublished), and the relationships among the reference populations using this reference microsatellite data are depicted in the neighbor-joining tree in Figure 3A. Mitochondrial DNA control region sequence haplotype characterizations for these reference collections are reported in Grunwald et al. (2008). The results of leave-one-out tests were used to determine the likelihood of each individual's multilocus genotype being found in the individual reference collections and DPS from which it was sampled (without replacement) based on both the mtDNA and the microsatellite data; the tests were done in GENECLASS2 (Cornuet et al. 1999) and the results are shown in Figure 3B.

Hardy-Weinberg equilibrium and linkage equilibrium were evaluated using exact tests implemented in GENEPop version 4.0.10 (Rousset 2008) using Markov chain default parameters. Tests of allelic differentiation among Minas Basin collections made in 2007, 2008, and 2009; among collections made in May-June, July, and August; and between small

(<100 cm and <130 cm) and larger fish (≥ 100 cm and ≥ 130 cm) were conducted using Fisher's exact probability test as implemented in GENEPOP with default Markov chain parameters.

RESULTS

Previously, we demonstrated significant allelic differentiation at these 11 microsatellite loci (King et al. 2001; Henderson et al. 2004; King, unpublished) and mtDNA control region haplotypes (Grunwald et al. 2008) among all nine of the reference spawning collections. Based partially on these results, the nine reference collections were designated as five DPSs under the ESA and the remainder were considered to be distinct management units (ASSRT 2007; NMFS 2012). Grunwald et al. (2008) provided a UPGMA dendrogram based on mtDNA control region sequence haplotype results that depicted the genetic relationships among these nine reference populations. In Figure 3A, we show the evolutionary relationships among these same nine populations in a neighbor-joining tree based on the microsatellites used in this study. Bootstrap support was high, exceeding 85% for all branches of the tree. The assignment accuracy of individuals to their estuaries and DPSs of collection based on the 11 microsatellites and mtDNA control region data is presented in Figure 3B. The accuracy of population assignment based on this data averaged 84.6% across all nine populations, ranging from 64.7% for the Savannah River collection to 94.6% for the Ogeechee River collection. It should be noted that the assignment accuracy to the Kennebec River population was modest at 79.4%, with 4 specimens collected from the Kennebec ($n = 34$) being misassigned to the Saint John River and the remaining 3 specimens being misassigned to the Hudson River. Conversely, assignment accuracy to the Saint John River collection ($n = 34$) was higher at 90.3%, with all 3 misassignments being to the Kennebec River. For the Hudson River collection ($n = 53$), assignment accuracy was 80.3%, with 2 specimens being misassigned to the Kennebec River and the remaining 7 specimens being misassigned to the Delaware River.

As expected, assignment accuracy to individual DPSs was considerably higher, with a mean of 96% across all five DPSs and range of 92.9% for the New York Bight DPS to 98.3% for the Southeastern U.S. DPS. Therefore, there was sufficient genetic differentiation among all nine reference collections to accurately quantify their individual and combined contributions at the DPS level but less so at the population level.

We found highly significant global Hardy–Weinberg disequilibrium across the entire Minas Basin collection. ($\chi^2 = \infty$, $P =$ highly significant) that was due to a highly significant deficit of heterozygotes ($P < 0.001$). Significant ($P < 0.05$) departure from Hardy–Weinberg equilibrium was observed at 5 of the 11 microsatellite loci. In earlier studies of the reference populations, there was no evidence of significant Hardy–Weinberg disequilibria at any of these loci. At all five of these loci, there was a significant deficit of heterozygotes, indicating the pres-

ence of the Wahlund effect. Similarly, we found strong evidence of linkage disequilibrium among many of the locus pairs in this study, with 17 of the 55 loci pairs being in linkage disequilibrium. These analyses suggest that our pooled collection from Minas Basin was comprised of individuals from two or more reference spawning populations.

There was no evidence of temporal heterogeneity of allelic frequencies among the three years in which samples were collected for this study (2007, 2008 and 2009; $\chi^2 = 23.9$, $P = 0.354$). There was no temporal heterogeneity among seasons when allelic frequencies were compared among samples collected in May–June, July, and August across years ($\chi^2 = 15.0$, $P = 0.861$). However, there was highly significant allelic frequency heterogeneity between collections of larger adult (≥ 130 cm TL) and smaller (<130 cm TL) subadult specimens ($\chi^2 = 47.52$, $P = 0.001$). When we extended this analysis to specimens >100 cm TL and smaller than <100 cm TL the difference between size-classes was slightly greater ($\chi^2 = 48.86$, $P < 0.001$). Because of the likely greater propensity of larger individuals to migrate farther along the coast, we hypothesized that most or all of the smaller fish (<100 cm TL) were of local Saint John River ancestry; however, that was not supported by the results. Using assignment results from the IBA tests, we found that smaller individuals (<100 cm TL) were not exclusively assigned to the Saint John River. Of the 15 specimens that were <100 cm TL, 10 were assigned to the Saint John, 3 to the Kennebec, and 2 to the Hudson. Furthermore, there was no significant difference between larger and smaller specimens in the frequency of ancestry assigned to the Saint John River.

The IBA and mixture analyses showed similar results in the quantification of the contributions of the nine reference populations to the mixed bycatch in Minas Basin. The IBA indicated that 61% of the specimens were of Saint John River origin, 34% were from the Kennebec River, 2% were from the Hudson River, and less than 1% were from the James River. No individuals from the other five reference populations were represented in the catch (Figure 4). Analysis of mixture composition using MSA in ONCOR provided very similar results whether considering the mtDNA and 11 microsatellites in combination or the 11 microsatellites alone (Table 1). Mixture analysis indicated that the Saint John contributed 63% (62%), the Kennebec 36% (37%), and the Hudson 1% (2%) of the bycatch in Minas Basin. This approach also did not indicate contributions of more southerly populations to bycatch in Minas Basin (Table 1). Thus, in summary, approximately 35% of the Atlantic sturgeon bycaught in Minas Basin were from the Gulf of Maine DPS, 1–2% from the New York Bight DPS, and perhaps 1% from the Chesapeake Bay DPS.

DISCUSSION

Our results demonstrate that while locally spawned Atlantic sturgeon from the Saint John River provided the bulk of the bycatch in the inner Minas Basin, individuals from U.S. rivers

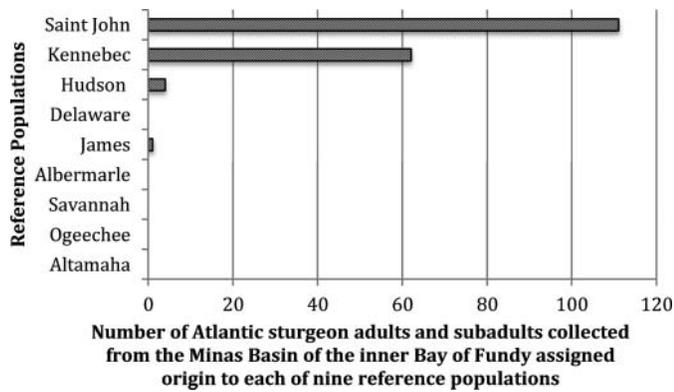


FIGURE 4. Numbers of Atlantic sturgeon specimens captured in the inner Minas Basin that were assigned to the nine reference spawning populations using GeneClass2. The numbers were determined by individual-based assignment tests using combined microsatellite DNA (11 loci) and mtDNA control region sequence analyses.

also made substantial contributions. In total, almost 40% of Minas Basin bycatch was of U.S. origin, with the Kennebec River providing the vast majority of fish (34–36%). The Hudson River also provided a small, 1–2% contribution, while southern U.S. populations made little (<1%) or no contribution. It should be noted, however, that our assignment accuracy at the population level was only a modest 79.4% for the Kennebec River reference collection. Conversely, assignment accuracy to the Saint John River was higher at 90%. It should be noted that mis-assigned specimens from the Kennebec River were assigned to the two most proximal collections (the Saint John River and the Hudson River), thus potentially compromising our accuracy in assigning ancestry among these three populations. However, given the large number of specimens that were assigned to U.S. populations, we feel confident in stating that U.S. populations are a major source of the specimens that were bycaught in Minas Basin.

Previous studies indicated that some Atlantic sturgeon make long coastal migrations (Ludwig et al. 2002; Erickson et al.

2011), but the ultimate destinations of these movements were unknown. Our study suggests that presently those Atlantic sturgeon that migrate to the inner Bay of Fundy during summer are mixtures from at least three (Saint John, Kennebec, and Hudson) and possibly four (James) genetically distinct spawning populations (Grunwald et al. 2008) and from at least two (Gulf of Maine, New York Bight) and possibly three (Chesapeake) different U.S. DPSs and at least one Canadian source, the Saint John River (NMFS 2012). Our results are in part supported by the PSAT tagging study which demonstrated that 1 of 15 adult Atlantic sturgeon marked during the spawning season in the Hudson River migrated during the following year to Cobequid Bay within the Bay of Fundy (Erickson et al. 2011).

Do anthropogenic activities within the inner Bay of Fundy pose significant threats to migratory U.S. sturgeon? It is unlikely that the fisheries within Minas Basin incur significant mortality among their sturgeon bycatch unless poaching occurs, but because of the demands for caviar shipping and processing, poaching is improbable. Previous studies of Atlantic sturgeon bycaught in U.S. coastal waters have determined that high mortality is only seen in entangling sink gill nets and not in trawl fisheries (Shepherd et al. 2007) and the pools which remain in Minas Basin weirs until the tide returns maintain the sturgeon alive.

It is uncertain, however, that if the proposed tidal power development projects in Minas Basin reach fruition their effects will be so benign. Developers have long been interested in harnessing the large tides of the Bay of Fundy to supply energy demands and the first tidal power generating plant in North America was constructed in 1985 at Annapolis Royal in the outer Bay of Fundy (Dadswell and Rulifson 1994). Studies have shown that Minas Channel and Minas Passage have enormous potential power, and the first open tidal turbine prototype was deployed in Minas Passage in 2009 with more prototypes scheduled for deployment in the near future (Greenberg 1984; Percy 2009). Little is known concerning the environmental impact of these types of turbines (Cada et al. 2007). For larger Atlantic sturgeon (>100 cm), the greatest concern is for injury

TABLE 1. Mixed-stock analysis based on 11 microsatellite DNA genotypes and mtDNA control region haplotypes or 11 microsatellite DNA genotypes alone, as implemented in ONCOR to determine the relative contributions of nine reference spawning populations to the bycatch of Atlantic sturgeon in the inner Minas Basin. The values in parentheses are 95% confidence intervals.

Reference population	Estimates based on mtDNA and 11 microsatellites	Estimates based on 11 microsatellites alone
Saint John River	0.626 (0.516–0.719)	0.615 (0.494–0.702)
Kennebec River	0.362 (0.255–0.465)	0.369 (0.254–0.473)
Hudson River	0.012 (0.000–0.059)	0.015 (0.000–0.086)
Delaware River	0.000 (0.000–0.011)	0.000 (0.000–0.023)
James River	0.000 (0.000–0.023)	0.001 (0.000–0.042)
Albemarle Sound	0.000 (0.000–0.000)	0.000 (0.000–0.000)
Savannah River	0.000 (0.000–0.006)	0.000 (0.000–0.011)
Ogeechee River	0.000 (0.000–0.000)	0.000 (0.000–0.000)
Altamaha River	0.000 (0.000–0.000)	0.000 (0.000–0.000)

or mortality caused by mechanical strike (see Dadswell 2006). It is unclear how Atlantic sturgeon navigate through Minas Channel, including their depth of travel and path, or whether they are able to avoid tidal turbines inasmuch as the velocity of the water is greater than they normally encounter (Gilbert 1989; Peake et al. 1997). These tidal turbines could pose a threat to the recovery of some Atlantic sturgeon stocks, such as that in the Kennebec River, because of their utilization of Minas Basin during coastal migrations. Further studies are needed to empirically evaluate the potential effects of these turbines on large benthic fishes such as Atlantic sturgeon.

It is difficult to evaluate the roles of relative population size and geographic location of spawning river on the predominant contribution of the proximal Saint John River and, secondarily, the Kennebec River, to Minas Basin bycatch. Are the large contributions of these two rivers due to the relative robust sizes of these populations or a tendency of Atlantic sturgeon to migrate within the geographic province within which they were spawned? Unfortunately, there is little contemporary data on the status of Atlantic sturgeon populations in general and none for the Saint John and Kennebec rivers. However, it is believed that the Hudson River supports the largest spawning population coastwide in the United States, with an estimated mean annual spawning stock of 870 adults from 1985 to 1992 (Kahnle et al. 2007) and probably a larger size today. It is interesting to note that the only spawning rivers contributing to Minas Basin bycatch were those in closest proximity to the inner Bay of Fundy and that their relative contributions (Saint John > Kennebec > Hudson) were inversely related to geographic distance from capture location. Similar to these results, we found in companion mixed-stock analyses that Atlantic sturgeon collected off the Delaware coast were primarily of Hudson River origin and that those collected off the North Carolina coast were primarily of James River ancestry (I. Wirgin et al., unpublished data). Thus, these preliminary studies suggest that the coastal movements of subadult and adult Atlantic sturgeon usually occur in the coastal province in which they were spawned, as suggested by Waldman et al. (1996).

Several caveats to our analysis should be noted. Accurate mixed-stock analysis requires that most, if not all, of the reference spawning populations potentially contributing to mixed aggregations be adequately characterized, including in terms of their temporal (among and within years) and spatial genetic homogeneity. Analysis of temporal stability is particularly important for threatened or near-extirpated populations in which year-class strength may be supported by a limited number of broodstock, as has been seen for Atlantic sturgeon in the Delaware River (I. Wirgin and M. Fisher, Delaware Division Fish and Wildlife, unpublished data). In this study, we sampled only 9 of at least 18 known extant reference spawning populations. Thus, it is possible that individuals from uncharacterized populations contributed to the mixed Minas Basin aggregation. For example, it has been suggested that rivers around the Bay of Fundy other than the Saint John host contemporary spawning of

Atlantic sturgeon (Dadswell 2006). The potential contribution of these rivers to our mixed collection was suggested by the presence of smaller specimens in our sample that were not assigned to the Saint John River. Furthermore, our characterization of the Kennebec River reference population was based on a relatively small number of reference specimens that were collected several decades ago. Given the likely small size of the Kennebec River adult population and their intermittent spawning success, it is possible that temporal instability of genotype frequencies occurs there. Finally, our characterizations of collections from the reference rivers rarely included analysis of the temporal stability of their allelic and haplotypic frequencies. Despite these caveats, it is certain that Atlantic sturgeon spawned in U.S. rivers (particularly the Kennebec River) frequent the inner Bay of Fundy in coastal migrations and are potentially vulnerable to anthropogenic threats there. This potential threat to the viability of the Gulf of Maine DPS should be considered in future management recommendations for this resource.

In summary, we have demonstrated that U.S. rivers make major contributions to the summer aggregations in Minas Basin on the basis of latitudinal proximity and that a significant number of U.S.-spawned individuals may be vulnerable to anthropogenic threats, including harvest and mortality from power development projects within the inner basin.

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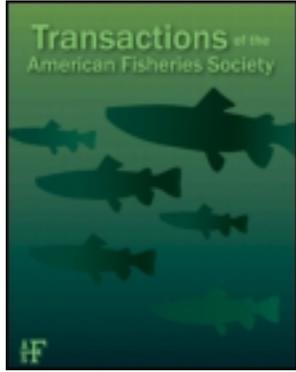
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Conservation Genetics of Remnant Coastal Brook Trout Populations at the Southern Limit of Their Distribution: Population Structure and Effects of Stocking

Brendan Annett ^a, Gabriele Gerlach ^b, Timothy L. King ^c & Andrew R. Whiteley ^d

^a Boston University Marine Program, Marine Biological Laboratory, Woods Hole, Massachusetts, 02543, USA

^b Marine Resources Center, Marine Biological Laboratory, Woods Hole, Massachusetts, 02543, USA

^c U.S. Geological Survey, Leetown Science Center, Aquatic Ecology Branch, Kearneysville, West Virginia, 25430, USA

^d Department of Environmental Conservation, University of Massachusetts-Amherst, Amherst, Massachusetts, 01003, USA

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ARTICLE

Conservation Genetics of Remnant Coastal Brook Trout Populations at the Southern Limit of Their Distribution: Population Structure and Effects of Stocking

Brendan Annett

Boston University Marine Program, Marine Biological Laboratory, Woods Hole, Massachusetts 02543, USA

Gabriele Gerlach

Marine Resources Center, Marine Biological Laboratory, Woods Hole, Massachusetts 02543, USA

Timothy L. King

U.S. Geological Survey, Leetown Science Center, Aquatic Ecology Branch, Kearneysville, West Virginia 25430, USA

Andrew R. Whiteley*

Department of Environmental Conservation, University of Massachusetts–Amherst, Amherst, Massachusetts 01003, USA

Abstract

We examined genetic variation within and among a group of remnant coastal brook trout *Salvelinus fontinalis* populations along the coast of the northeastern United States. These populations occur at the southern limits of anadromy for this species and could form the foundation of a restored anadromous metapopulation. We also tested for genetic introgression between these populations and the hatchery source that has been used to stock these sites. The overall F_{ST} for the natural populations at 12 microsatellite loci was 0.145 (95% confidence interval, 0.108–0.183), and D was 0.225 (0.208–0.243). On average, 94.6% of individuals were correctly assigned to the population where they were collected. Our results suggest that there is little gene flow even between geographically proximate populations. We found little evidence that repeated historic stocking from a known hatchery source has led to genetic introgression into these wild coastal brook trout populations. One hybrid individual appeared to be a backcross between an F_1 and a hatchery individual. Another hybrid individual could not be classified. Our results suggest that nonintrogressed and potentially locally adapted populations of brook trout persist in several small coastal New England streams. These populations should be the focus of future efforts to restore anadromous brook trout in this region.

Knowledge of the genetic composition of the populations of a particular species is a prerequisite for conservation prioritization, genetic monitoring, and population restoration (Schwartz et al. 2007; Laikre et al. 2008). Information about a species' genetic composition includes the amount of genetic variation within and the genetic divergence among populations and, if relevant, the degree of introgression with

anthropogenically introduced individuals (e.g., Laikre et al. 2008). This type of information is necessary for conservation goals ranging from the prevention of further erosion of genetic diversity in the most vulnerable of a series of extant populations (Ellstrand and Elam 1993), genetic rescue of extant populations suffering from inbreeding depression through translocation (Tallmon et al. 2004), or reintroduction of

*Corresponding author: awhiteley@eco.umass.edu
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individuals to habitats where extirpation has occurred (Hansen et al. 2001).

Genetic introgression between wild and anthropogenically introduced individuals has been extensively examined in fish (Hindar et al. 1991; Hansen et al. 2001). Introduced individuals are often the product of captive breeding and are introduced to boost population size (Fraser 2008) or result from species invasion (Allendorf et al. 2004). Captive-bred individuals can be maladapted to the natural environment following rearing in an artificial environment (Fraser 2008; Araki et al. 2009). Hybridization between captive-bred individuals and native local populations may swamp local adaptations in the native populations and cause genetically based loss of fitness (Reisenbichler and McIntyre 1977; Reisenbichler and Rubin 1999; Araki et al. 2007; Fraser 2008). Here we define hybridization as the interbreeding of individuals from distinct populations, regardless of taxonomic status (Rhymer and Simberloff 1996). Recent work has demonstrated positive stocking-pressure-dependent introgression in salmonid populations (Marie et al. 2010, 2011). Alternatively, other studies have demonstrated that hybridization between captive-bred and wild fish may not occur at all or may occur only at low levels (Hansen et al. 2002; Matala et al. 2008; Hansen and Mensberg 2009). In the latter case, genomes may remain intact and population restoration efforts can focus on nonhybridized populations or (if all populations show some level of introgression) on those that are the least affected by hybridization.

The native range of brook trout *Salvelinus fontinalis* extends from the shores of Canada's Hudson Bay south through the Great Lakes and Appalachian Mountains to inland streams in northern Georgia (Power 1980). Like other salmonids, brook trout can be anadromous (so-called sea-run brook trout) wherever there is free access to the sea and marine or freshwater habitats remain sufficiently cool throughout the summer. Adoption of a resident or anadromous life history appears to be highly environmentally sensitive, and growth rate and growth rate efficiency appear to be the most important proximate factors linked to their expression (Morinville and Rasmussen 2003; Thériault et al. 2007). Anadromous brook trout have historically occurred in coastal waters north of New York City (Power 1980). Coastal brook trout populations along the coast of southern New England and Long Island, New York, have been greatly reduced in number by habitat alteration and overfishing during the past century (MacCrimmon and Gots 1980). A survey of 74 coastal streams by the Massachusetts Division of Fisheries and Wildlife identified only 17 coastal brook trout populations that may contain anadromous individuals remaining in this state as of the 1970s (Bergin 1984). Currently, wild-reproducing coastal populations occur in a few tributaries of Nantucket Sound, Buzzards Bay, and Narragansett Bay in southern New England (Hartel et al. 2002) and at least one coastal stream on Long Island, (Ryther 1997). The degree to which the individuals in these remaining coastal populations use the ocean is unclear, but those in some of these populations reach larger sizes and have faster growth

rates than resident brook trout in the same stream (Ben Letcher, U.S. Geological Survey, unpublished results). The remaining small coastal brook trout streams on Cape Cod, Massachusetts, were heavily stocked with domesticated hatchery trout between the 1940s and the 1980s. The cumulative effects of this stocking might have caused widespread hatchery introgression into the remaining coastal brook trout populations (Bigelow 1963; Ryther 1997). Alternatively, the remaining wild-reproducing populations might have resisted hatchery introgression and be an important focus of future restoration efforts.

In this paper, we genetically analyzed five remaining coastal brook trout populations on Cape Cod ($N = 4$) and Long Island ($N = 1$) with 12 microsatellite markers. First, we determined the genetic variation within and divergence among these populations. Second, because there is a history of hatchery stocking in these populations, we estimated the degree of introgression between the hatchery source and the remaining populations. This work provides a foundation for future restoration efforts of the sea-run brook trout at its southern limits.

METHODS

Study Area and Sampling

We sampled wild brook trout from four coastal streams on Cape Cod (Santuit River [SA], Mashpee River [MA], Quashnet River [QU], and Red Brook [RB]) and one coastal stream on Long Island (Connetquot River [CO]) in 2002 and 2003 (Table 1; Figure 1). All of these streams are small (average flows = 0.1–0.5 cubic meters per second), low-gradient, first-order streams fed by coldwater springs along their entire length and draining directly into their estuaries. Each stream is connected to a freshwater pond at its headwaters. The streams flow through oak and pine forests over coarse sandy soils from a glacial outwash plain from elevations not higher than 23 m above sea level.

The Connetquot River, about 300 km southwest of the Cape Cod streams, is the southernmost location where anadromous brook trout occur (Ryther 1997). This 10-km stream's geohydrology is similar to that of the Cape streams. The Connetquot River has been intensively managed for trout fishing since the 1860s, first by an exclusive private club and since 1973 by the state of New York. The Connetquot River has its own specific hatchery located immediately on the stream, which does not maintain a broodstock. Returning brook trout are selected and spawned from the river each year. The hatchery then raises and releases the fish directly back to the river as adults. The hatchery has always used native returning fish for reproduction and has not introduced other brook trout stocks into the river (Gil Bergin, manager, Connetquot Hatchery, Oakdale, New York, personal communication). Natural brook trout reproduction reportedly occurs in some areas of the river.

Hatchery brook trout (hereafter HA) from the Sandwich Fish Hatchery (Sandwich, Massachusetts; Figure 1) have been released directly into the SA, MA, and QU since the 1940s

TABLE 1. Brook trout collection locations with their abbreviations, latitude–longitude coordinates, and number of fish sampled from each location (*N*).

Region and river	Abbreviation	Latitude (N)	Longitude (W)	<i>N</i>
Cape Cod				
Santuit River	SA	41°37.672	70°27.062	29
Mashpee River	MA	41°37.300	70°28.823	43
Quashnet River	QU	41°35.533	70°30.463	82
Red Brook	RB	41°45.915	70°38.035	49
Sandwich Hatchery	HA	41°45.159	70°29.381	37
Long Island				
Connetquot River	CO	40°45.783	73°09.166	40

(Table 2). The hatchery fish are from the Sandwich strain eastern brook trout broodstock, a strain registered with the National Fish Strain Registry. The registry reports the original source of the animals as the Montague, Massachusetts, state fish hatchery and various field sites (Kincaid et al. 2002). This broodstock has always been maintained using spawners from the hatchery

itself, and brook trout from other locations have not been mixed with this hatchery broodstock (Craig Lodowsky, manager, Massachusetts Division of Fisheries and Wildlife, Sandwich Fish Hatchery, personal communication). The fourth Cape Cod site (RB) has been privately owned and managed as an anadromous brook trout fishing camp since the 1860s. Brook trout

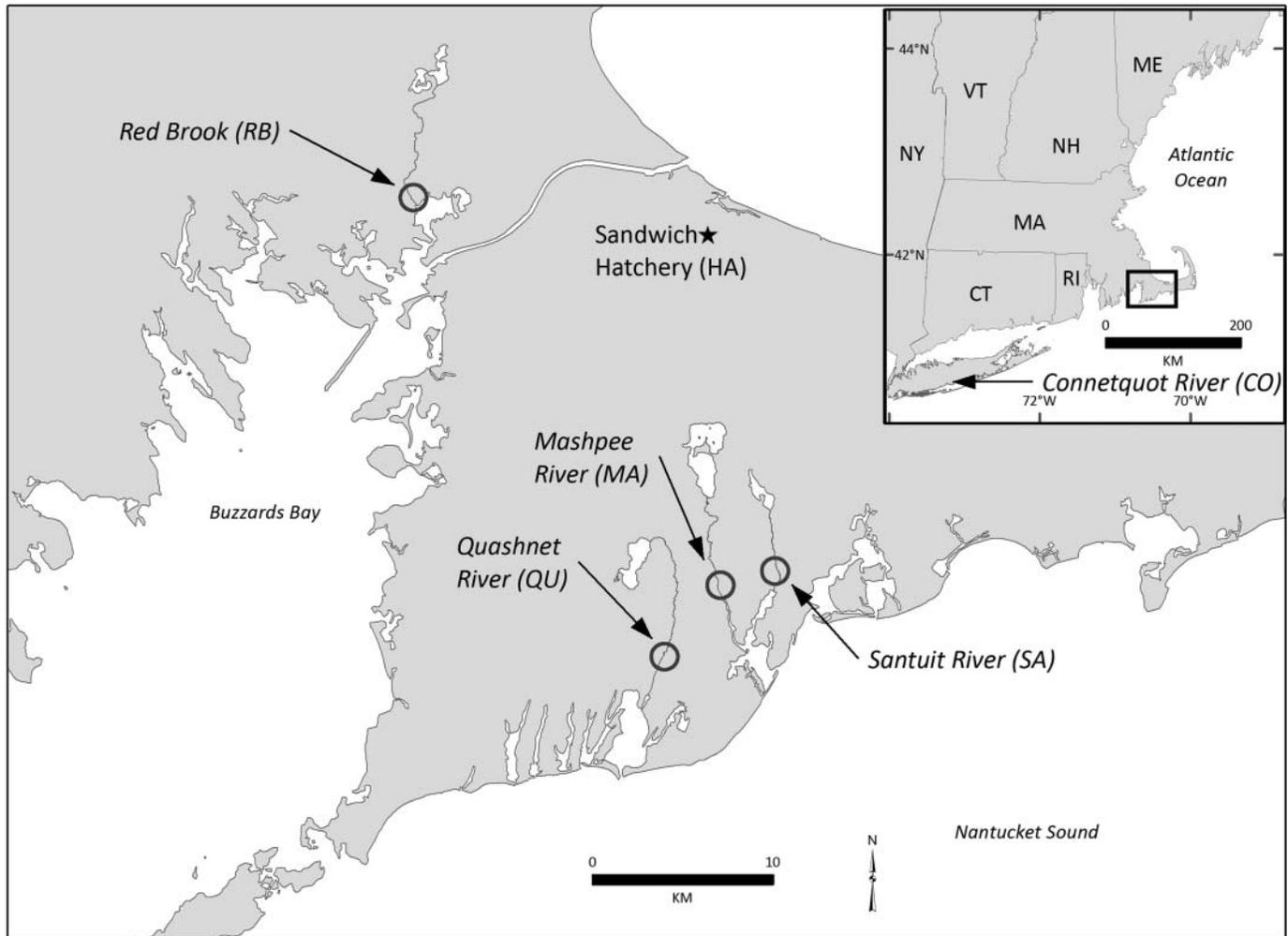


FIGURE 1. Map showing the locations of the coastal streams where brook trout were sampled. Also shown is the location of the Sandwich Fish Hatchery, from which the hatchery strain was obtained.

TABLE 2. Stocking pressure for three of the study sites examined. The entries are the numbers of Sandwich Fish Hatchery adult brook trout stocked into the Santuit River (SA), the Mashpee River (MA), and the Quashnet River (QU) during each decade from 1940 to 2000. Every decade spans the 10 years following the year listed (e.g., 1950s = 1951–1960). The numbers are based on stocking records kept by the Massachusetts Division of Fisheries and Wildlife.

Stream	Decade							Total
	1940s	1950s	1960s ^a	1970s	1980s	1990s	2000s ^b	
SA	0	23,400	2,400	800	2,850	0	0	29,450
MA	18,000	37,150	6,750	400	0	0	0	62,300
QU	0	30,000	4,600	800	0	0	0	36,000
Total	18,000	90,550	13,750	2,000	2,850	0	0	127,750

^aIn 1965, 5,000 1-in (2.54-cm) brook trout fry were stocked into each stream from the Montague State Fish Hatchery. The Montague hatchery is reported as one of the original sources of animals for the Sandwich broodstock. Records indicate that between the years 1959 and 1963 between 600 and 2,350 adult brook trout were stocked in each stream from sources other than the Sandwich Fish Hatchery.

^bThrough 2011.

from the Sandwich Fish Hatchery and other sources have been stocked into RB during this time period. Since the 1990s, hatchery fish are no longer stocked directly into any of the streams. However, thermally stratified headwater ponds at the headwaters of each stream are still stocked with hatchery fish. It is likely that thermal barriers (warmer surface waters) limit the movement of hatchery individuals into stream habitat (Steve Hurley, Massachusetts Division of Fisheries and Wildlife, personal communication).

Wild adult fish ($N = 247$) were collected by pulsed-DC electrofishing (400 V, 0.3–0.5 A, and 60 Hz) or seining in freshwater reaches of the coastal streams within 500 m of tidewater during the summer and fall months before spawning. Population sample sizes ranged between 29 and 82 (Table 1). To reduce the risk of collecting closely related individuals that may be schooling together, fish were sampled from multiple locations separated by approximately 100 m within each stream, except in the Connetquot River, where up-migrating fish were seined from multiple locations below a small dam. Tissue was sampled from 37 individuals from the Sandwich Fish Hatchery in the fall of 2003. Adipose fin tissue was collected from live wild fish prior to release and stored in 95% ethanol until subsequent laboratory analysis.

Genetic Data Analysis

Microsatellites.—Laboratory analysis was conducted at the U.S. Geological Survey, Biological Resources Division, Leetown Science Center, Aquatic Ecology Laboratory in Kearneysville, West Virginia. Genomic DNA was isolated from fin tissue with the Puregene DNA extraction kit (Gentra Systems, Inc., Minneapolis, Minnesota) according to the manufacturer's guidelines. Isolated DNA was resuspended in 100 μ L of 10 mM tris-HCl, pH 8.0, and 1 mM EDTA before use in polymerase chain reactions (PCR). A group of 12 microsatellite loci (King et al. 2012) were selected for their demonstrated polymorphism in other brook trout population studies and examined in all fish (see the supplemental table available in the online version of this article). Each PCR consisted of 100–200 ng of genomic DNA, 0.875 \times DMD multiplex PCR buffer (58 mM tris-HCl

[pH 8.8], 15 mM $(\text{NH}_4)_2\text{SO}_4$, 5.9 mM MgCl_2 , 8.8 mM β -mercapthoethanol, and 6 mM EDTA), 0.32 mM dNTPs, 0.075–0.250 μ M forward and reverse primers (the forward primer labeled with TET, FAM, or HEX; Life Technologies Corporation, Carlsbad, California), and 0.1 U/ μ L *Taq* DNA polymerase (Promega, Madison, Wisconsin) in a total volume of 15 μ L. Amplifications were carried out on a 96-well thermal cycler using the following procedure: initial denaturing at 94°C for 2 min; 35 cycles of 94°C for 45 s, 56°C for 45 s, and 72°C for 2 min; and a final extension at 72°C for 10 min. Fragment electrophoresis and scoring were performed according to the protocols described by King et al. (2001).

Genetic diversity within populations.—Allele frequencies, deviations from Hardy–Weinberg expectations, gametic disequilibrium, observed (H_O) and expected (H_E) heterozygosity (per locus and per population), mean within-population expected heterozygosity (H_S), and the fixation index F_{IS} were calculated with GENEPOP version 4.0.10 (Rousset 2008). Mean allelic richness per population (AR; i.e., the mean number of alleles scaled to the smallest sample size; $N = 29$) was calculated with FSTAT version 2.9.3.2 (Goudet 2001). We corrected for multiple tests for Hardy–Weinberg expectations and gametic disequilibrium with the sequential Bonferroni procedure (Rice 1989). We used an initial α value of 0.05/ k , when k is the number of comparisons. We conducted tests for excess homozygosity at each locus in each population with MICROCHECKER version 2.2.3 (Van Oosterhout et al. 2004) as a test for the presence of null alleles.

Genetic divergence among populations.—Pairwise exact tests for genic differentiation were calculated with GENEPOP (Rousset 2008). F -statistics were calculated with FSTAT (Goudet 2001). We used θ analogues (Weir and Cockerham 1984) for overall and pairwise estimates of F_{ST} . We used the DEMETICS version 0.8-3 (Gerlach et al. 2010) package for R version 2.12 (R Development Core Team 2006) to estimate Jost's D (Jost 2008). We used 1,000 permutations to calculate 95% confidence intervals or P -values for both measures, and we applied a sequential Bonferroni correction to adjust for multiple tests (Rice 1989). We used PHYLIP version

3.5 (Felsenstein 1993) to calculate Cavalli-Sforza and Edwards' (1967) genetic distance (CSE) between each pair of populations with the GENDIST module and constructed an unrooted neighbour-joining dendrogram with the NEIGHBOR module. We used TreeViewX version 0.5.0 (Page 1996) to visualize the dendrogram. The PHYLIP module CONSENSE was used to generate a consensus tree with bootstrap values from 4,000 replicate data sets created in SEQBOOT. We performed maximum-likelihood assignment tests to further test the genetic relationships among populations. GENECLASS version 2.0 (Piry et al. 2004) was used to calculate probabilities of individuals belonging to populations following Rannala and Mountain (1997).

We tested the relationship between genetic and geographic distances between populations (isolation by distance [IBD]) to further examine the factors structuring populations. We used CSE chord distance for the genetic distances. Coastal geographic distances were measured in ArcView 3.2a (ESRI, Redlands, California) as the shortest distance from river mouth to river mouth through the estuaries and the ocean, around headlands and islands. This measure of distance is consistent with previous observations of brook trout movement patterns in the ocean (White 1942). We performed analyses with and without the Long Island population because it is geographically highly removed from the Cape Cod populations (the mean \pm SD pairwise geographic distance for the Cape Cod populations was 32.0 ± 23.3 km; the mean \pm SD distance including the Long Island population was 136.7 ± 136.4 km). We performed Mantel tests with Isolation By Distance Web Service version 3.21 (Jensen et al. 2005).

Hatchery introgression.—We used STRUCTURE version 2.3.2 (Pritchard et al. 2000; Falush et al. 2003) to test for hybridization between the hatchery strain (HA) and each of the Massachusetts wild populations (MA, SA, QU, and RB). Each hatchery–wild population pair was examined separately without prior information for sample location. We used 500,000 replicates and 100,000 burn-in cycles under an admixture model in which we estimated a separate α parameter (i.e., the Dirichlet parameter for degree of admixture) for each population and an initial α of 1.0. We used the correlated allele frequencies model with an initial λ of one. We allowed F to assume a different value for each population, which allows for different rates of drift among populations. We performed 10 runs for $K = 1$ and 2 for each hatchery–wild population pair. The proportion of loci within each individual that were assigned to either the wild population or the hatchery strain (q) was used as an estimate of individual-level hybridization. The Sandwich strain that we examined was the sole known source of individuals introduced into MA, SA, and QU. Other unknown and unavailable sources of fish may have been introduced to RB, and therefore our analysis with the Sandwich strain as the hatchery source could represent an underestimate of introgression rates at this site.

We further examined the probability that individuals belonged to one of five distinct genetically defined categories (pure wild, pure hatchery, F_1 , F_2 , and backcross to either wild

or hatchery fish) with the software NEWHYBRIDS version 1.1 Beta3 (Anderson and Thompson 2002). We performed a separate analysis for each of the four Massachusetts wild–Sandwich Fish Hatchery population pairs and specified the expected genotype frequency of each category. Each run of the Markov chain consisted of a burn-in period of 100,000 iterations followed by 250,000 iterations. We provided prior information on the identity of the hatchery individuals. Individuals belonging to a category with posterior probabilities $>70\%$ were considered correctly assigned (Gunnell et al. 2008).

RESULTS

Genetic Diversity within Populations

The total number of alleles observed at a locus ranged from 2 at *SfoC79* to 15 at *SfoC115* (Table 3). Mean allelic richness ranged from 4.5 to 5.3 (Table 3). Mean expected heterozygosity (H_S), ranged from 0.495 to 0.608 (Table 3). Tests of deviation from Hardy–Weinberg proportions were significant in 6% of the cases (4 of 69 tests; $P < 0.05$), where 3.5 were expected by chance at $\alpha = 0.05$ (Table 3). None of the tests for deviation from Hardy–Weinberg proportions was significant following sequential Bonferroni correction ($\alpha = 0.05$), either for the approximately 12 tests within each population sample or the approximately 6 tests per locus. However, 3 of the 4 significant tests occurred at locus *SfoD91* (in SA, MA, and CO; Table 3). Positive F_{IS} values for this locus in these three populations were consistent with the presence of null alleles (Table 3). Furthermore, tests for excess homozygosity with MICROCHECKER indicated that null alleles may occur at *SfoD91* in CO. Subsequent analyses were performed with and without *SfoD91*, but none of the inferences changed. We therefore report results from the complete 12-locus data set. Significant gametic disequilibrium was detected in 11% of the cases (41 of 374 tests; $P < 0.05$). Upon sequential Bonferroni correction for the approximately 66 locus pairs in each population, three tests remained significant ($\alpha = 0.05$), two of which occurred in QU and one in MA.

Genetic Divergence among Populations

There were 18 population-specific alleles, and qualitative differences in allele frequencies were observed at many loci (Figure 2). One hundred and sixty-nine of the 180 (94%) pairwise exact tests for genic differentiation were significant ($P < 0.05$). Six of the 11 (55%) nonsignificant pairwise exact tests involved the locus with the fewest alleles (*SfoC79*, $N = 2$ alleles). The overall F_{ST} was 0.159 (95% CI, 0.125–0.195), and Jost's D was 0.257 (0.241–0.274). Excluding the hatchery population, the overall F_{ST} was 0.145 (0.108–0.183) and Jost's D was 0.225 (0.208–0.243). Pairwise F_{ST} ranged from 0.05 between RB and CO to 0.22 between SA and QU (Table 4). Pairwise Jost's D ranged from 0.07 between RB and CO to 0.396 between HA and QU (Table 4). All pairwise F_{ST} and Jost's D values were significant following sequential Bonferroni adjustment for multiple tests.

TABLE 3. Summary of genetic variation within brook trout populations from Cape Cod (SA, MA, QU, and RB), a Massachusetts hatchery (HA), and Long Island (CO). The number of alleles (A), observed heterozygosity (H_o), expected heterozygosity (H_e), and F_{IS} are shown for each locus. Significant results for tests of Hardy-Weinberg expectations are indicated by asterisks in the F_{IS} column ($P < 0.05$). Following the locus-specific estimates, the mean values across loci of A , allelic richness (AR; standardized to $N = 29$), H_S (mean H_e across loci), and F_{IS} are shown for each population.

Population	<i>SfoB52</i> (12 alleles)				<i>SfoC24</i> (5 alleles)				<i>SfoC38</i> (4 alleles)				<i>SfoC79</i> (2 alleles)				<i>SfoC86</i> (5 alleles)			
	A	H_o	H_e	F_{IS}	A	H_o	H_e	F_{IS}	A	H_o	H_e	F_{IS}	A	H_o	H_e	F_{IS}	A	H_o	H_e	F_{IS}
SA	5	0.379	0.388	0.022	3	0.679	0.557	-0.219	3	0.483	0.435	-0.110	1	0.000	0.000	NA	3	0.552	0.428	-0.289
MA	6	0.548	0.628	0.128	5	0.558	0.519	-0.076	2	0.023	0.023	0.000	2	0.047	0.046	-0.012	4	0.628	0.587	-0.069
QU	7	0.476	0.511	0.068	3	0.537	0.512	-0.049	4	0.329	0.290	-0.136	1	0.000	0.000	NA	4	0.469	0.435	-0.078
RB	7	0.694	0.663	-0.046	4	0.592	0.517	-0.146	3	0.122	0.118	-0.040	2	0.041	0.040	-0.011	3	0.449	0.512	0.123
HA	5	0.460	0.453	-0.013	3	0.472	0.531	0.111	2	0.460	0.434	-0.059	2	0.297	0.328	0.094	5	0.676	0.652	-0.037
CO	6	0.650	0.748	0.131	3	0.475	0.583	0.185	3	0.300	0.268	-0.120	2	0.275	0.240	-0.147	4	0.325	0.382	0.149

Population	<i>SfoC88</i> (5 alleles)				<i>SfoC113</i> (9 alleles)				<i>SfoC115</i> (15 alleles)				<i>SfoC129</i> (7 alleles)				<i>SfoD75</i> (14 alleles)			
	A	H_o	H_e	F_{IS}	A	H_o	H_e	F_{IS}	A	H_o	H_e	F_{IS}	A	H_o	H_e	F_{IS}	A	H_o	H_e	F_{IS}
SA	3	0.552	0.428	-0.289	4	0.621	0.514	-0.209	5	0.586	0.568	-0.033	3	0.586	0.511	-0.147	8	0.621	0.735	0.155
MA	4	0.628	0.587	-0.069	6	0.744	0.822	0.094	8	0.571	0.645	0.114	5	0.512	0.491	-0.041	10	0.721	0.734	0.017
QU	4	0.469	0.435	-0.078	5	0.561	0.595	0.057	6	0.622	0.655	0.050	5	0.402	0.385	-0.044	8	0.866	0.840	-0.031
RB	3	0.449	0.512	0.123	6	0.531	0.551	0.037	7	0.694	0.754	0.080	6	0.633	0.650	0.027	9	0.816	0.809	-0.009
HA	5	0.676	0.652	-0.037	5	0.676	0.689	0.020	8	0.784	0.837	0.064	4	0.806	0.702	-0.148	7	0.541	0.663	0.185
CO	4	0.325	0.382	0.149	5	0.450	0.588	0.234*	10	0.825	0.760	-0.086	3	0.550	0.519	-0.060	9	0.600	0.553	-0.085

Population	<i>SfoD91</i> (14 alleles)				<i>SfoD100</i> (10 alleles)				Mean across loci			
	A	H_o	H_e	F_{IS}	A	H_o	H_e	F_{IS}	A	AR	H_S	F_{IS}
SA	7	0.448	0.587	0.237*	7	0.931	0.798	-0.167	4.3	4.5	0.520	-0.065
MA	8	0.581	0.660	0.119*	7	0.738	0.755	0.022	5.6	5.3	0.554	0.035
QU	7	0.817	0.766	-0.066	7	0.317	0.354	0.104	5.1	4.5	0.495	0.002
RB	7	0.735	0.807	0.089	6	0.531	0.472	-0.125	5.3	5.0	0.521	0.004
HA	7	0.784	0.828	0.053	5	0.730	0.693	-0.053	4.8	4.6	0.608	0.021
CO	9	0.575	0.826	0.304*	7	0.650	0.583	-0.116	5.4	5.1	0.539	0.073

Cavalli-Sforza and Edwards chord distances also revealed strong genetic divergence among populations (Figure 3). The results of individual assignment tests followed the general pattern observed in pairwise F_{ST} , Jost's D , and CSE comparisons. On average, 94.6% of individuals were correctly assigned to the population from which they were collected (Table 5). Pairwise genetic and geographic distances were not significantly correlated when all of the wild populations were included in the analysis ($z = 209.1$, $r = -0.242$, $P = 0.677$) or when the geographically removed Long Island population was excluded ($z = 36.5$, $r = 0.356$, $P = 0.175$).

Hatchery Introgression

The models from STRUCTURE revealed that introgression between the hatchery strain and each of the four Massachusetts wild populations was low (Figure 4). For each STRUCTURE run and each hatchery–wild population pair, the $K = 2$ model had far greater likelihood estimates. The vast majority of point estimates of q were close to 1.0, which represents a “pure” indigenous brook trout (Figure 4). The median q -values were 0.991 for SA, 0.994 for MA, 0.995 for QU, and 0.995 for RB. Only three wild-caught individuals had point estimates of individual q -values less than 90%. These included single wild-caught individuals from MA ($q = 19.7\%$), SA (61.4%), and QU (85.4%). The 90% credible intervals for q -values included 1.0 for all but the one individual from MA. This individual appeared to be a later-generation hybrid with more hatchery than wild ancestry (90% credible interval, 0.0–0.458; Figure 4).

The results from NEWHYBRIDS allowed further inferences regarding putative hybrid individuals and were generally consistent with those from STRUCTURE. The individual from MA was assigned as a backcross between an F_1 and a hatchery fish (posterior probability = 0.747). The ancestry of the hybrid individual from SA could not be resolved. For this fish, the category with the highest posterior probability (0.381) was F_1 , though all cross-type categories had nonzero posterior probabilities. We did not detect any evidence of hybridization in RB (mean posterior probabilities of pure wild fish = 0.989) or QU (mean posterior probabilities of pure wild fish = 0.991) with NEWHYBRIDS. Based on these results, the analyses of genetic structure were repeated with the MA and SA hybrid individuals removed. The overall inferences did not change (data not shown).

DISCUSSION

A combination of enhanced drift in populations with small effective size and restricted gene flow likely explains the genetic differentiation that we observed. The genetic differentiation of the Cape Cod populations was similar to that observed ($F_{ST} = 0.107$) in a study of 59 anadromous brook trout populations to the north of our study region (Castric and Bernatchez 2003). Castric and Bernatchez (2003) found greater differentiation and weaker IBD among southernmost populations (Gulf of Maine,

USA, and Bay of Fundy, Canada) and lower genetic differentiation and greater IBD among more northern Canadian sites. Our study sites occurred to the south of all of the sites examined by Castric and Bernatchez (2003), and therefore our work extends their results further to the south. That is, our results extend the pattern of increased genetic differentiation and weak IBD at the southern limits of anadromy for coastal populations of brook trout. This pattern is consistent with reduced rates of anadromy among more southern coastal brook trout populations. Individuals at southern sites may be more likely to remain as residents in their natal streams, and gene flow may thus be lower in southern coastal brook trout populations. Acoustic tagging research currently under way suggests that the brook trout in our study sites use the ocean environment (Andy Danylchuck, University of Massachusetts–Amherst, personal communication). Furthermore, inter-river movement of brook trout has been observed between the MA and SA sites (Mullan 1958), which drain to the same estuary and are separated by only 3 km at their mouths. However, the rates of anadromy in our study populations remain poorly understood. A nonmutually exclusive alternative is that southern populations have smaller effective population sizes than northern populations, and therefore enhanced drift without a reduction in gene flow may explain the increased genetic divergence in the south. The range of heterozygosity that we observed at 12 microsatellites (0.495–0.608) was lower than that observed at 6 microsatellites (0.600–0.780) among coastal brook trout populations to the north of our study sites (Castric and Bernatchez 2004), but not dramatically lower. Therefore, drift is not likely to be solely responsible for the genetic divergence observed. We cannot further distinguish the relative influences of drift and gene flow on genetic divergence in these populations with the data in hand.

We found little evidence that introgression between hatchery and wild individuals has occurred in the Cape Cod populations. These streams received heavy hatchery stocking for many years, up until the last 5–10 brook trout generations. More recent stocking has occurred only in headwater ponds, where introduced individuals are confined to cold, deeper waters and are unlikely to have an opportunity to reach the streams. Based on other studies of brook trout with heavy stocking pressure (Marie et al. 2010), we might have expected widespread introgression instead of the low levels observed. Captive-bred individuals can be maladapted to the natural environment following rearing in an artificial environment (Fraser 2008; Araki et al. 2009). The Sandwich Fish Hatchery breeds fish to grow quickly in the hatchery environment and these fish appear to be highly susceptible to angling upon release to natural streams (Craig Lodowsky, personal communication). Poor survival in the wild due to the effects of domestication selection along with high angling mortality could explain the low rates of introgression observed. Another factor contributing to the poor survival of Sandwich Fish Hatchery fish in coastal streams could be that the hatchery fish originated in inland streams (in western

Massachusetts) and thus had low survival rates in the coastal stream habitat. It should be noted that one of our study streams, RB, has been stocked with fish from sources other than the Sandwich broodstock and therefore that our results for this site may underestimate hatchery introgression. We also lack historical samples that would allow us to examine the change over time in the genetic makeup of these populations and would provide definitive evidence for a lack of introgression. For example, historical samples have been used to reveal introgression in brown

trout *Salmo trutta* populations (Hansen and Mensberg 2009). However, the strong genetic divergence between the hatchery source and each of the wild populations and the lack of evidence for introgression suggest that mating between wild and hatchery fish has occurred infrequently in the Cape Cod populations.

The populations we considered are threatened by a variety of stressors, including estuarine eutrophication, water withdrawals, invasive species introductions, and continued habitat loss due to

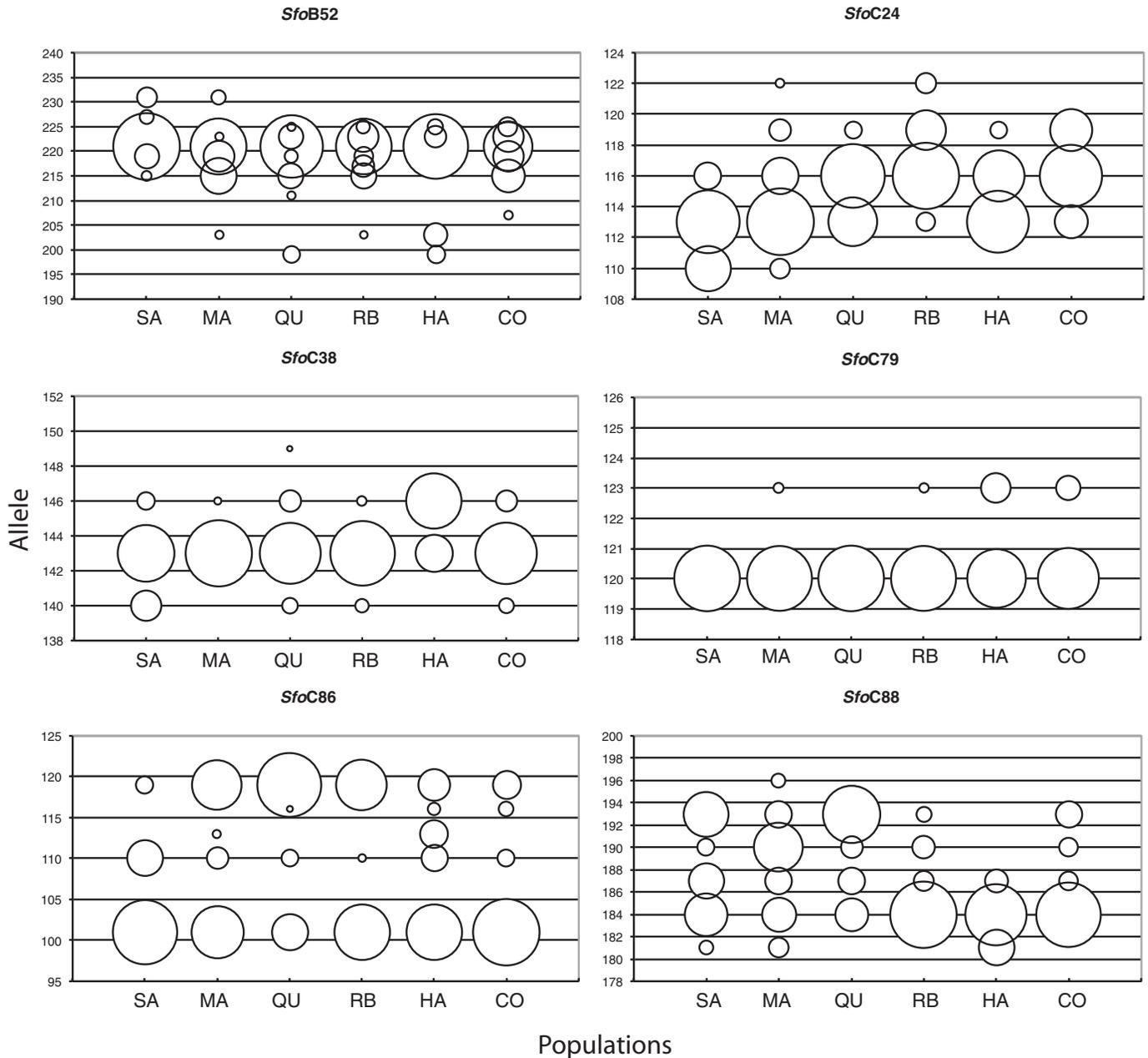


FIGURE 2. Bubble histograms illustrating the allele frequency differences among the six populations (see Table 1 and Figure 1) for each of the 12 microsatellite loci studied. The relative size of each bubble is proportional to the frequency of the corresponding allele in that population.

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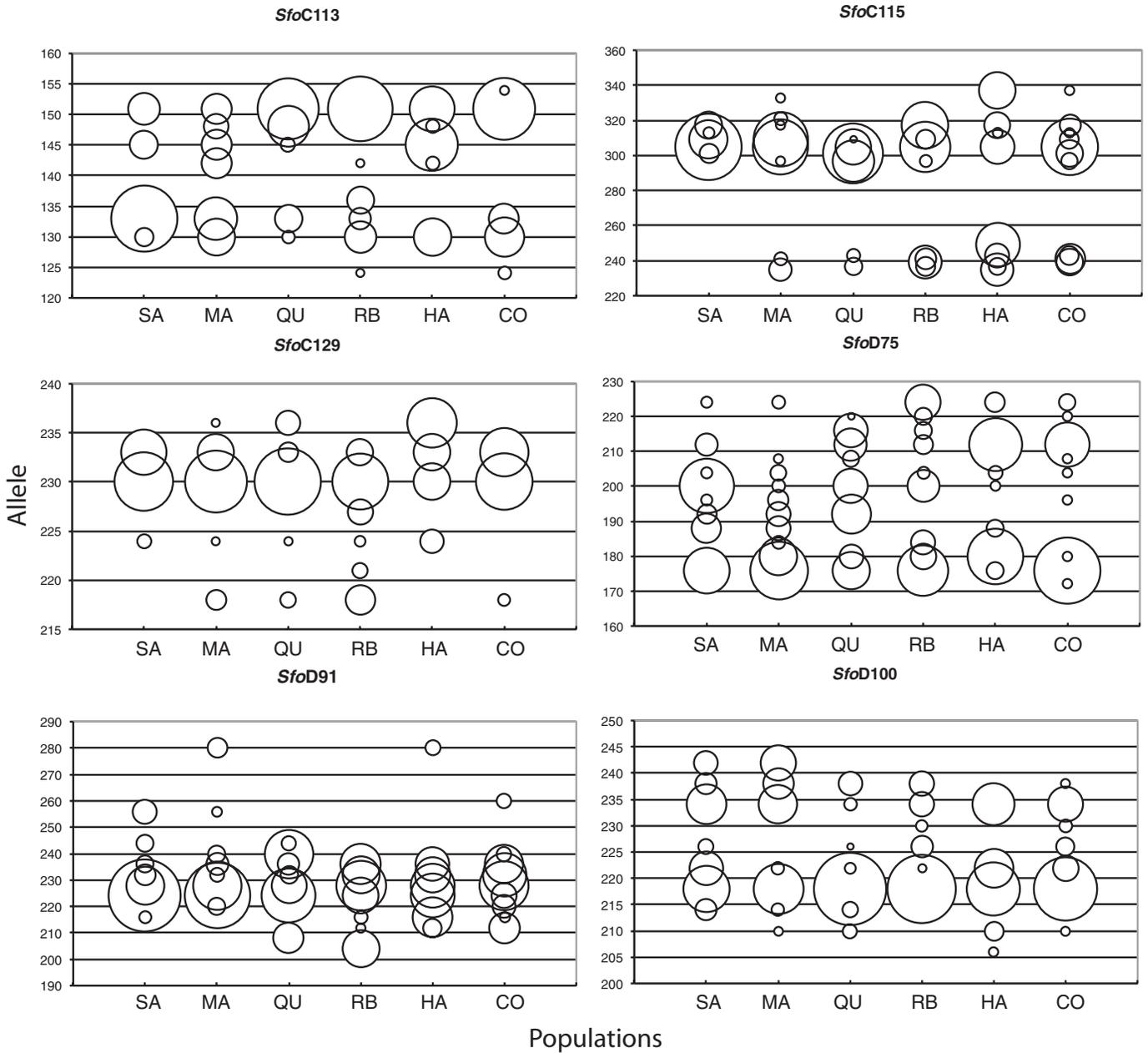


FIGURE 2. Continued.

TABLE 4. Genetic differentiation between pairs of brook trout populations from Cape Cod (SA, MA, QU, and RB), a Massachusetts hatchery (HA), and Long Island (CO). Estimates of pairwise F_{ST} are shown below the diagonal, estimates of pairwise Jost's D are shown above the diagonal. All estimates were significant following sequential Bonferroni adjustment for multiple tests ($\alpha = 0.05$).

Population	SA	MA	QU	RB	HA	CO
SA		0.140	0.287	0.290	0.345	0.254
MA	0.105		0.262	0.241	0.333	0.229
QU	0.215	0.137		0.228	0.396	0.263
RB	0.159	0.129	0.105		0.309	0.071
HA	0.183	0.183	0.228	0.160		0.274
CO	0.152	0.130	0.168	0.050	0.149	

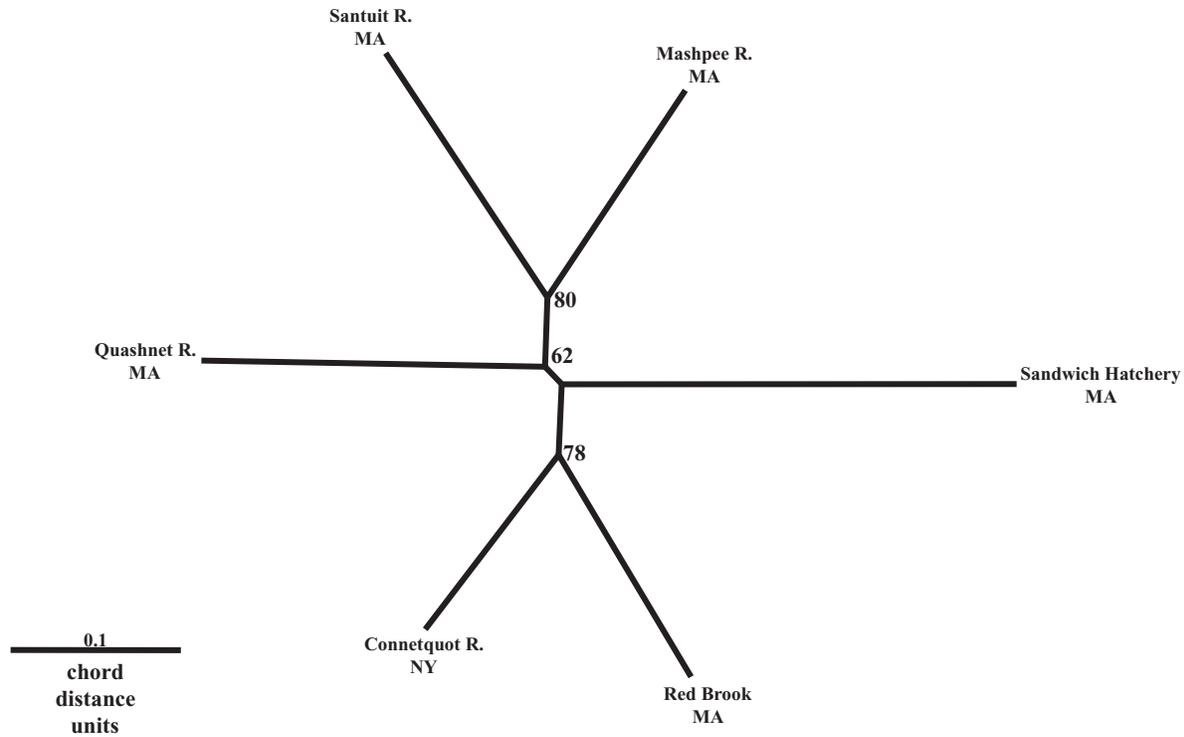


FIGURE 3. Neighbor-joining phenogram depicting the genetic distance (chord distance; Cavalli-Sforza and Edwards 1967) among six brook trout populations from Cape Cod (MA) and Long Island (NY). The numbers indicate the bootstrap support for the nearest node with 4,000 permutations.

urbanization. Restoration efforts aimed at restoring anadromous coastal brook trout populations in Massachusetts are currently under way. Future efforts will involve habitat improvement and fish translocation, either among the extant coastal populations or to currently vacant habitat in an effort to establish new coastal populations with access to the ocean. Our research provides a baseline analysis of extant coastal populations to guide these efforts. Maintenance of genetic diversity in these extant populations is critical to their future potential for adaptive response to environmental changes (Jump and Peñuelas 2005). The strong genetic divergence observed among populations at this

geographic scale suggests that each of these populations might be locally adapted to environmental conditions (Lenormand 2002). The overall lack of introgression from hatchery fish further suggests that native gene pools worthy of conservation have persisted. Further, if in fact the rates of anadromy in our study sites are suppressed relative to historic levels, restoration of connectivity through expression of the migratory anadromous life history is an important conservation goal. Restoration of connectivity could allow the group of Massachusetts populations to form the foundation for a metapopulation of sea-run brook trout at the southern limit of anadromy for this species.

TABLE 5. Population assignment analysis confirming strong genetic differentiation of brook trout populations from Cape Cod (SA, MA, QU, and RB), a Massachusetts hatchery (HA), and Long Island (CO). The rows designate the populations from which individual brook trout were sampled, the columns the populations to which the individuals from those populations were subsequently assigned.

Population	Population					
	SA	MA	QU	RB	HA	CO
SA	0.931	0.069	0.000	0.000	0.000	0.000
MA	0.000	0.977	0.000	0.000	0.023	0.000
QU	0.000	0.037	0.939	0.000	0.000	0.024
RB	0.000	0.000	0.000	0.959	0.000	0.041
HA	0.000	0.000	0.000	0.000	1.000	0.000
CO	0.000	0.050	0.000	0.050	0.000	0.900

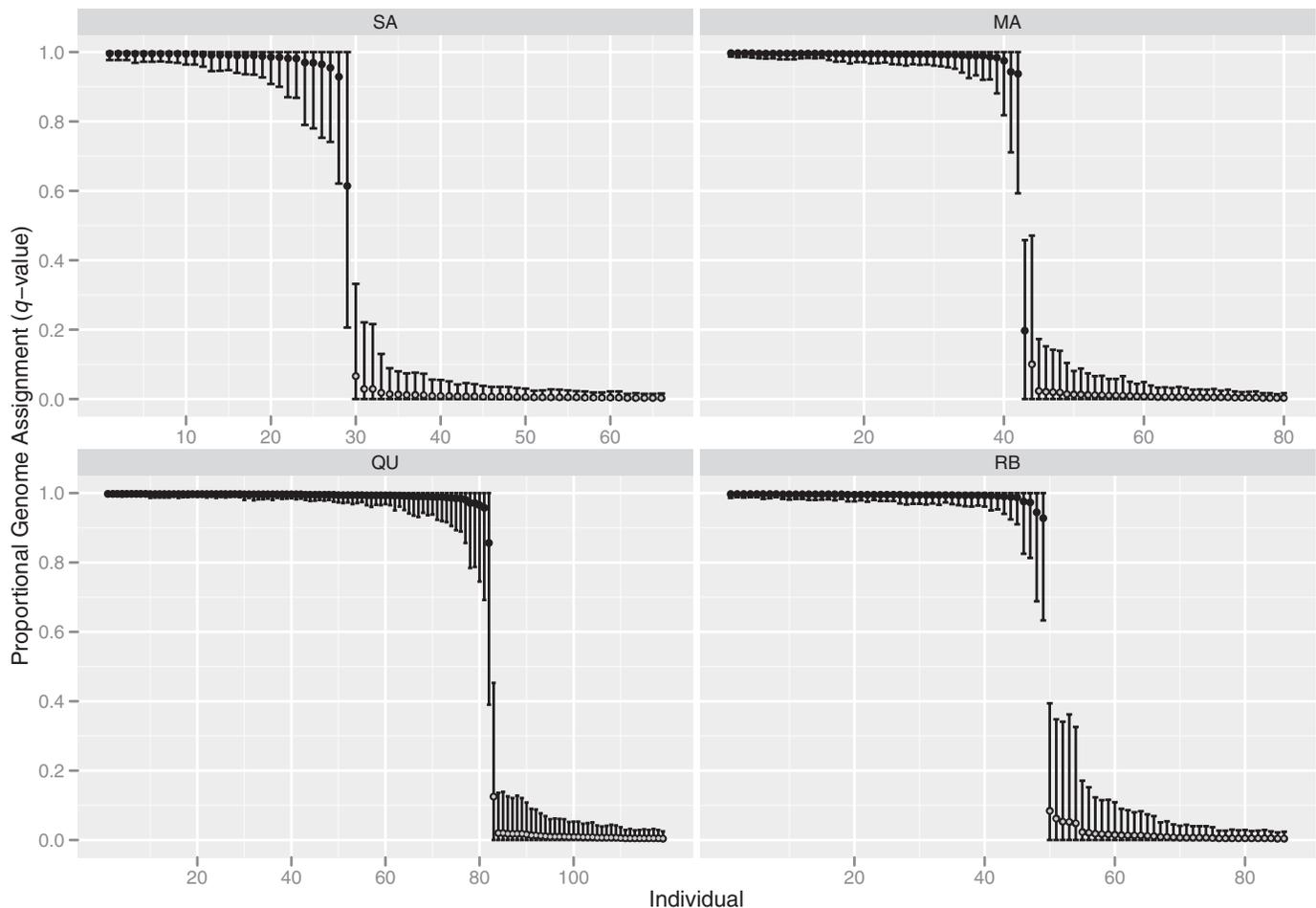


FIGURE 4. Proportions of loci within individuals (q -values; 90% credible intervals shown) assigned to either a wild population or the hatchery strain based on a STRUCTURE model for four Cape Cod brook trout populations. A q -value of 1.0 corresponds to a “pure” wild brook trout, and a q -value of 0.0 corresponds to a “pure” hatchery trout. Each point on the x -axis represents an individual. Wild-caught fish are represented by black points, hatchery fish by grey points. The same sample of hatchery fish from the Sandwich Fish Hatchery was used in each pairwise comparison with wild-caught population samples.

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Loren M. Miller^{a b}, Steven W. Mero^c & Jerry A. Younk^d

^a Minnesota Department of Natural Resources, 1980 Folwell Avenue, St. Paul, Minnesota, 55108, USA

^b Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota, St. Paul, 55108, Minnesota

^c Minnesota Department of Natural Resources, 1201 East Highway 2, Grand Rapids, Minnesota, 55744, USA

^d Minnesota Department of Natural Resources, 2114 Bemidji Avenue, Bemidji, Minnesota, 56601, USA

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ARTICLE

The Impact of Stocking on the Current Ancestry in Twenty Native and Introduced Muskellunge Populations in Minnesota

Loren M. Miller*

Minnesota Department of Natural Resources, 1980 Folwell Avenue, St. Paul, Minnesota 55108, USA; and Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota, St. Paul 55108, Minnesota

Steven W. Mero

Minnesota Department of Natural Resources, 1201 East Highway 2, Grand Rapids, Minnesota 55744, USA

Jerry A. Younk

Minnesota Department of Natural Resources, 2114 Bemidji Avenue, Bemidji, Minnesota 56601, USA

Abstract

Fish stocking, often from multiple source populations, is a common management practice frequently conducted without the means or effort to determine the reproductive contributions of stocked fish. Historically, the Minnesota Department of Natural Resources (MNDNR) has stocked four strains of muskellunge *Esox masquinongy*, but the contribution of these strains to current populations was unknown. Two strains came from Minnesota lakes, Shoepack Lake and Leech Lake, and the other strains came from Wisconsin and Iowa hatcheries and were of uncertain origin. The MNDNR discontinued stocking the Shoepack strain in the 1980s when that strain displayed poor growth in stocked waters. Managers were concerned that ancestry from this strain might be limiting the genetic potential for muskellunge to attain trophy size in stocked populations. Using 13 microsatellite DNA markers, we determined the ancestry of muskellunge in 10 supplemented native populations and 10 introduced populations. The ancestry from each of the four stocked strains of muskellunge was detected in some populations, but the level of ancestry was unrelated to the amount of stocking of a strain. Ancestry from native populations persisted in six of the supplemented populations despite years of stocking. The potential effects of Shoepack strain ancestry on fish size were limited in most lakes because of its low persistence. All stocked strains reproduced in at least some of the lakes, but some lakes had no evidence of reproduction by any stocked strain. Our results will help MNDNR manage genetic diversity among muskellunge populations and direct efforts toward appropriate actions to improve size structure. This study reinforces how genetic data are often useful for evaluating ancestry in stocked fish populations, whereas stocking histories may be poor indicators of current genetic composition.

For decades, billions of fish have been stocked in the United States (Halverson 2008), often with fish from multiple source populations being stocked into given systems over time. Comparisons of stocking success among strains have usually been based on the growth and survival of physically marked fish (e.g.,

Crozier et al. 1997; Wills 2006; Bronte et al. 2007). Genetic markers have distinguished stocked individuals or populations when physical marking was not efficient or feasible (Eldridge et al. 2002; DeKoning et al. 2006). Furthermore, genetic markers allow evaluation of reproductive contributions by stocked

*Corresponding author: lmm@umn.edu
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fish. Recent advances in genetic techniques and analysis have allowed assessment of stocking even in the absence of baseline samples from the stocking source and recipient populations prior to stocking (Halbisen and Wilson 2009; Miller et al. 2009). These genetic tools allow evaluation of the survival and reproductive success of stocked fish from different strains, sometimes many generations after stocking (Piller et al. 2005; Finnegan and Stevens 2008). Where stocking has supplemented native populations, genetic assessments may determine which strains made reproductive contributions and where native populations experienced introgression by genes from stocked strains.

Stocking is a common management tool for muskellunge *Esox masquinongy*, even in regions with abundant native populations (e.g., Margenau 1999; Kerr 2007; Wingate and Younk 2007). Stocking has been used to restore or enhance native populations or create new muskellunge waters to expand sport fisheries. Researchers have evaluated the factors associated with stocking success for muskellunge, success usually being measured as the growth and survival of stocked fish (reviewed in Margenau 1999; Wahl 1999). Success improved with increased size at stocking, the abundance of soft-rayed prey, and reduced predator abundance, especially largemouth bass *Micropterus salmoides* and northern pike *Esox lucius*. Success also depended on abiotic factors, especially temperature (Wahl 1999). Relatively few studies have compared stocking success among strains of muskellunge (Younk and Strand 1992; Margenau and Hanson 1996). Clapp and Wahl (1996) found differences in physiological traits among six populations of muskellunge and suggested that these could lead to differences in performance depending on the thermal environment where they were stocked. No studies have evaluated the reproductive contributions of stocked muskellunge, in particular, success by strain or in relation to native populations.

The Minnesota Department of Natural Resources (MNDNR) has used four main strains of muskellunge for stocking waters throughout the state (see Wingate and Younk 2007 for a review of the MNDNR muskellunge management program). The earliest attempts to spawn and rear muskellunge from Mississippi River drainage waters were deemed unsuccessful (MDC 1934). In the 1950s, the MNDNR utilized a strain derived from Shoepack Lake in the Hudson Bay drainage of northern Minnesota and stocked their descendants in many Minnesota lakes for over 30 years. In the early 1980s, the MNDNR discontinued the use of the Shoepack strain because evidence suggested that the stocked fish were not growing as large as fish from other muskellunge populations (Wingate and Younk 2007). Managers then began developing another Minnesota muskellunge strain derived from Leech Lake in the upper Mississippi basin of north-central Minnesota. Meanwhile, stocking continued with fish obtained from out-of-state suppliers. From the late 1970s to the mid-1980s, fish were obtained from a private grower in Wisconsin, whose source was unknown but was likely the nearby Wisconsin River drainage. In 1984, a few lakes were stocked with muskellunge from the Iowa

Department of Natural Resources' Spirit Lake fish hatchery. The MNDNR began to use the Leech strain widely in the late 1980s and has continued to stock this strain in state muskellunge waters.

The apparent inability of the Shoepack strain to attain large sizes (Younk and Strand 1992; Frohnauer et al. 2007) was a concern because muskellunge primarily support trophy fisheries (Margenau and AveLallemant 2000; Wingate and Younk 2007). Factors that might affect growth and size structure in muskellunge include prey characteristics (Wahl and Stein 1988, 1993), water temperature (Bevelhimer et al. 1985; Wahl and Stein 1991; Clapp and Wahl 1996), angling (Margenau and Hanson 1996), and genetics (Margenau and Hanson 1996; Younk and Strand 1992; Clapp and Wahl 1996). High levels of ancestry from a strain with slow growth or smaller maximum size would require different management responses than the other factors.

We investigated muskellunge ancestry in Minnesota populations in lakes stocked with one to four different strains either to supplement native populations or to introduce muskellunge to create fisheries. The main objectives of this study were to determine the contribution of each strain to the ancestral composition of stocked populations and whether any of the native populations had retained the genetic diversity found in the region prior to stocking. We were particularly interested in the prevalence of ancestry from the slow-growing Shoepack strain because of concerns that it may limit the size attained by Shoepack descendants in stocked populations (Miller et al. 2009). We also evaluated factors that may have affected the level of ancestry from stocked strains. We first tested for a relationship between the amount of stocking and ancestry from each strain. A discordance between stocking history and subsequent ancestral composition has been observed for a number of fish species (Larsen et al. 2005; Finnegan and Stevens 2008; Halbisen and Wilson 2009), but it has not been studied for muskellunge. Finally, we verified natural reproduction by stocked strains. The persistence of ancestry from discontinued strains depends on their ability to reproduce in the stocked lakes.

METHODS

Sample collection.—Scales archived from previous MNDNR spring trap-net assessments of spawning muskellunge were used for genetic analysis. Sample sizes were determined by scale availability from the most recent assessment or that from multiple years if needed to increase sample size (Table 1). Sample sizes ranged from 21 to 76 (mean = 45) for all but two lakes, which had large sample sizes of 174 and 246 from special intensive assessments of these muskellunge populations.

Source population samples were obtained for three of the four stocked strains. We obtained samples directly from Shoepack Lake and Leech Lake. We included a Wisconsin sample from Tomahawk Lake in northeastern Wisconsin, thought to be the region from which the private grower who supplied fish for Minnesota obtained broodstock (data provided by B. Sloss, USGS

TABLE 1. Sample information and stocking history for three lakes that provided source populations and 20 stocked lakes that had native muskellunge populations prior to stocking (N) or now have introduced (I) populations. Sample information includes the year(s) sampled and the sample size (*n*). The stocking history indicates the strains used, the time period stocked (P = stocking continues to the present time) and the total years (Yrs) stocked for each strain (prior to the year of sampling for the Leech strain). Big Mantrap Lake was sampled at two time periods.

Lake	Status	Sample		Stocked strains							
		Year(s)	<i>n</i>	Shoepack		Iowa		Wisconsin		Leech	
				Period	Yrs	Period	Yrs	Period	Yrs	Period	Yrs
Source populations											
Shoepack Lake	N	1993	40								
Tomahawk Lake, Wisconsin	N	2006	49								
Leech Lake	N	1987–1988	29								
Stocked populations											
1. Lobster Lake	I	2009	80	1970–1981	10	1984	1	1983–1988	3	1990–P	15
2. Mille Lacs Lake	I	2006	246	1970–1978	7	1984	1	1985–1989	3	1989–P	9
3. Big Mantrap Lake	N	1984–1988 2004	42 47	1969–1983	15			1987	1	1988–P	9
4. Beers Lake	I	2002	42	1977–1984	3			1981–1988	3	1986–P	6
5. French Lake	I	2007	53	1974–1985	12			1985–1988	4	1989–P	16
6. Rush Lake	I	2006–2007	56	1969–1982	10			1983–1985	3	1989–P	15
7. Sugar Lake	I	2003	37	1970–1978	7			1983–1988	5	1989–P	12
8. Lake Vermilion	I	2005–2006	44	1969–1984	3			1985	1	1987–P	13
9. West Battle Lake	I	2003	22	1969–1984	10			1979–1988	5	1990–P	7
10. Big Lake	N	2008	29	1969–1981	10					1987–P	14
11. Little Boy Lake	N	2000	50	1972–1977	6					1987–1993	4
12. Lake Wabedo	N	2000	43	1972–1981	8					1987	1
13. Deer Lake	N	2003	76	1971–1983	5			1985	1		
14. Moose Lake	N	2008	174	1971–1983	8			1985	1		
15. Lake Bemidji	N	1998	44					1978	1	1982–P	6
16. Lake Miliona	I	2008	51					1982–1987	5	1989–P	10
17. St. Louis River	N	2007–2008	64					1983–P	17 ^a	1989–2005	9
18. Baby Lake	N	1995, 2005	21	1971–1979	8						
19. Cass Lake	N	1991–1997	51	1969–1975	5						
20. Spider Lake	I	2007	27	1969–1979	9						

^aIncludes stocking by the Wisconsin Department of Natural Resources.

Cooperative Fisheries Research Unit, University of Wisconsin–Steven's Point). No sample was available from the source of the fourth stocked strain that was acquired from the Iowa Department of Natural Resources.

We evaluated 20 lakes stocked with one to four different strains of muskellunge (Figure 1; see Table 1 for the stocking history of strains in each lake). Ten lakes had native populations prior to stocking and the other 10 lakes had no muskellunge present before introductions (Table 1). Big Mantrap Lake was stocked 15 times with Shoepack strain fish to establish a brood-stock lake to produce offspring for stocking in other lakes. We included a 1984–1988 sample from Big Mantrap Lake because it had a native muskellunge population prior to stocking with Shoepack Lake fish, so stocked progeny could have contributed Shoepack strain and Big Mantrap Lake ancestry to other popu-

lations. Spider Lake has a self-sustaining introduced population established with only the Shoepack strain.

Genotyping.—We genotyped 1,417 muskellunge using the procedures described in Miller et al. (2009), except that we discontinued using microsatellite locus *EmaA5* so that the remaining 13 loci from Sloss et al. (2008) would combine together in a single electrophoresis run. An additional 50–106 repeated reactions per locus were scored to assess genotyping error (sample size was variable because it included positive controls and samples repeated to fill in a few missing genotypes at individual loci).

Genetic diversity analysis.—Observed and expected heterozygosities and the inbreeding coefficient F_{IS} were estimated for each locus in each sample. Conformance with Hardy–Weinberg expectations was tested using the exact test procedures

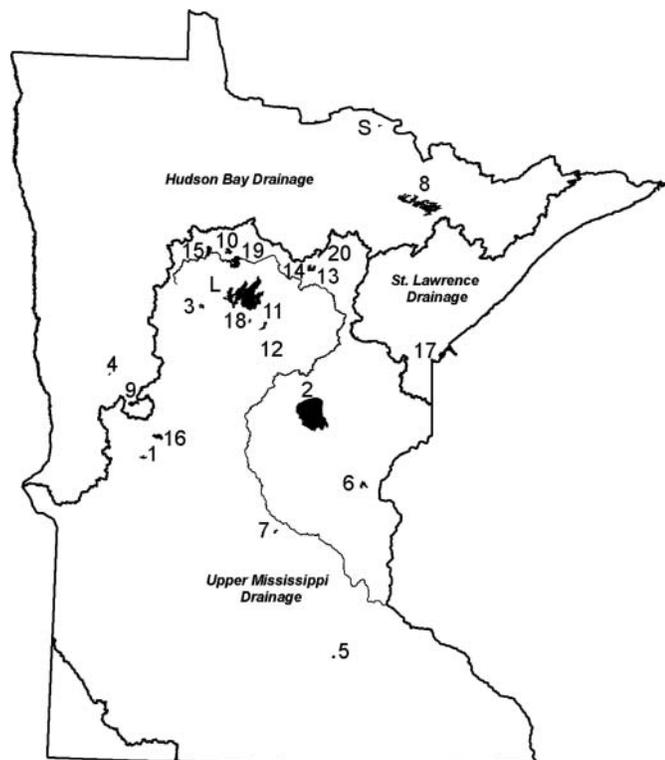


FIGURE 1. Sampling locations for two muskellunge broodstock sources, Leech Lake (L) and Shoepack Lake (S), and 20 stocked lakes (numbered as in Table 1) in Minnesota. Additional stocked muskellunge came from hatcheries in Wisconsin and Iowa. The thick lines delineate major drainage boundaries, and the thin line indicates the Mississippi River.

of Guo and Thompson (1992), as implemented by GENEPOP version 3.4 (Raymond and Rousset 1995) using 10,000 dememorizations, 20 batches, and 500 iterations per batch. GENEPOP was also used to test for linkage disequilibrium between pairs of loci. Significance values were adjusted within each sample using sequential Bonferroni procedures (Rice 1989), but the number of individual tests with P -values < 0.05 is also reported because the Bonferroni correction is conservative. Data were evaluated in MICROCHECKER version 2.2.3 to detect evidence of null alleles (nonamplifying alleles that lead to heterozygotes being scored as homozygotes) or scoring errors due to stuttering or large-allele dropout (Van Oosterhout et al. 2004).

The measure of population differentiation, pairwise F_{ST} , was calculated in FSTAT (Goudet 1995) for samples from stocking source populations and from lakes that had native populations prior to stocking. Differentiation among stocked strains is necessary to distinguish ancestry in the stocked lakes. Differentiation between stocked populations and the source strains may indicate the retention of native ancestry; conversely, lack of differentiation may signify stocking impacts. The St. Louis estuary, which had a native population, was excluded because it receives ongoing stocking with the Leech and Wisconsin strains.

Ancestry analysis.—To determine the number of genetically distinct populations contributing to our samples and the ancestry of individual fish, we used the Bayesian clustering algorithm implemented in the program STRUCTURE (version 2.3.3; Pritchard et al. 2000; also refer to <http://pritch.bsd.uchicago.edu>). We ran STRUCTURE analyses separately on four groups of populations that shared similar stocking histories to avoid falsely assigning ancestry that could not be present in a population. Each group included the appropriate strain source samples and lakes stocked with those strains. We made a few exceptions to avoid excessively dividing the data: Cass Lake and Spider Lake, stocked only with the Shoepack strain, were included with populations stocked with the Shoepack and Leech strains, and Lake Bemidji, Lake Milnora and the St. Louis River estuary, which were stocked only with the Leech and Wisconsin strains, were included with populations stocked with the Shoepack, Leech, and Wisconsin strains. To determine the number of distinct clusters (K), we ran five simulations at each K ranging from 1 to 10 (higher than the potential number of native and stocked populations) using 250,000 Markov chain–Monte Carlo simulations after a burn-in of 50,000 simulations. The model assumed possible admixture (i.e., individuals with mixed ancestries due to mating between strains) and correlated allele frequencies, with no prior population information. For each analysis group, a K value was chosen based on a plateau in the likelihood values and a correspondence of assigned ancestry with known sample information, i.e., clusters corresponding to known stocked strains or to individual lakes that had native populations (Pritchard et al. 2010).

Ancestry and amount of stocking.—We assessed the relationship between the amount of stocking and the percentage of ancestry from each of the two major strains no longer stocked, Shoepack and Wisconsin. Linear regressions were conducted for the average ancestry within each sample on the number of years stocked and separately on the stocking intensity (total number stocked in all years/lake area). Only data for fingerlings were analyzed for stocking intensity because managers will typically stock with greatly different intensities depending on the life stage stocked. We removed one adult and three fry stocking events for the Shoepack strain and three adult stocking events for the Wisconsin strain. The relationship between ancestry and stocking was not evaluated for the Iowa strain because it was stocked just one year and in only two lakes or for the Leech strain because it has been continually stocked in many populations, so samples likely included recently stocked fish.

Reproduction by stocked strains.—STRUCTURE results were also used to identify lakes in which the stocked strains have reproduced. Simply observing natural reproduction (e.g., age-0 fish from unstocked years) could not enable us to determine which strains contributed because all but one lake was stocked with multiple strains or had a prior reproducing population. We used admixed individuals as an indicator of natural reproduction because individuals admixed between stocked strains or a stocked strain and a native population could only result from

reproduction in the lakes. We used the estimated 90% probability intervals for each individual's ancestry estimates and assumed that an individual was admixed when the probability interval exceeded zero for more than one ancestry. This approach may fail to identify some admixed individuals because probability intervals are typically wide in STRUCTURE analyses. We tolerated some error because our interest was verifying reproduction by strains rather than accurately quantifying the numbers of admixed individuals. Testing this approach on source population samples (i.e., nonadmixed individuals) indicated that it was unlikely to falsely indicate reproduction by stocked strains. No individual from a source population sample was identified as admixed with a stocked strain using the probability interval criterion (data not shown). Natural reproduction could not be evaluated based on admixed individuals for several stocked populations that had ancestry from only a single strain.

RESULTS

Genetic Diversity

The 13 microsatellite loci displayed a wide range of variation across sources and stocked populations (see Table A.1 in the appendix, which appears in the online version of this article). They showed no indications of null alleles, stuttering, or allelic dropout. One to three Hardy–Weinberg tests had P -values <0.05 in each of the source population samples, but none were significant after Bonferroni correction (Table A.1). For linkage disequilibrium, zero to nine tests had P -values <0.05 in the source population samples, but only locus pair (*EmaD5*–*EmaD116*) was significant after Bonferroni correction and only for the Wisconsin sample (Table A.1). These results indicate that the loci satisfy the genetic equilibrium assumptions for STRUCTURE analysis of population structure and ancestry. The error rates for repeated samples averaged 0.7% (range,

0.0–2.5% per locus). All errors involved a single mismatched allele and no fish had errors at multiple loci, so errors likely had little effect on ancestry estimates.

Many loci were out of Hardy–Weinberg and linkage equilibrium in stocked populations (Table A.1). These deviations are consistent with the presence of multiple, distinct populations and recent stocking and do not indicate marker deficiencies (e.g., null alleles) or nonindependence of loci. A mixture of stocked strains or stocked and native fish may cause deviation from Hardy–Weinberg expectations due to the Wahlund effect (Hedrick 2005). Although random mating following stocking would establish Hardy–Weinberg equilibrium in a single generation, linkage disequilibrium persists for more generations. Minimizing Hardy–Weinberg and linkage disequilibrium is the process STRUCTURE uses to identify distinct genetic clusters (Pritchard et al. 2000).

Population differentiation was high and statistically significant among the source populations ($F_{ST} = 0.24$ – 0.43), and many of the stocked populations that had native muskellunge ($F_{ST} = 0.09$ – 0.54 , except for those mentioned below) (Table 2). The Shoepack Lake population was highly differentiated from all others except for the population in Big Mantrap Lake, the Shoepack broodstock lake ($F_{ST} = 0.01$ [not significant]). Low but significant F_{ST} values were also found between Leech Lake and Big Lake ($F_{ST} = 0.02$) and Lake Bemidji ($F_{ST} = 0.03$), two lakes that receive ongoing Leech strain stocking. Other populations with low but significant F_{ST} values included geographically proximate pairs that likely have incomplete isolation: Moose Lake and Deer Lake ($F_{ST} = 0.02$) and Little Boy Lake and Lake Wabedo ($F_{ST} = 0.06$).

Distinct Ancestries Identified by STRUCTURE

STRUCTURE identified eight different genetic clusters, corresponding to ancestry from stocked strains and several

TABLE 2. Population differentiation (pairwise F_{ST} ; Goudet 1995) between stocking source populations from Shoepack Lake, Leech Lake, and Wisconsin and populations from nine Minnesota lakes that had native muskellunge prior to stocking. The St. Louis estuary was excluded because it receives ongoing stocking with Leech and Wisconsin strain muskellunge. Values in bold italics indicate tests that were not significant following sequential Bonferroni correction (Rice 1989) for multiple testing ($\alpha = 0.05$, $k = 66$).

	Shoepack	Leech	Wisconsin	Big Mantrap	Moose	Deer	Little Boy	Wabedo	Cass	Baby	Bemidji
Shoepack											
Leech	0.43										
Wisconsin	0.30	0.24									
Big Mantrap	0.01	0.39	0.27								
Moose	0.36	0.16	0.21	0.33							
Deer	0.37	0.16	0.19	0.33	0.02						
Little Boy	0.54	0.17	0.30	0.51	0.25	0.25					
Wabedo	0.47	0.14	0.24	0.44	0.21	0.21	0.06				
Cass	0.35	0.09	0.22	0.31	0.14	0.15	0.22	0.18			
Baby	0.40	0.16	0.19	0.36	0.16	0.16	0.18	0.12	0.15		
Bemidji	0.44	0.03	0.27	0.41	0.22	0.21	0.20	0.18	0.14	0.21	
Big	0.48	0.02	0.29	0.45	0.22	0.21	0.21	0.19	0.13	0.22	0.00

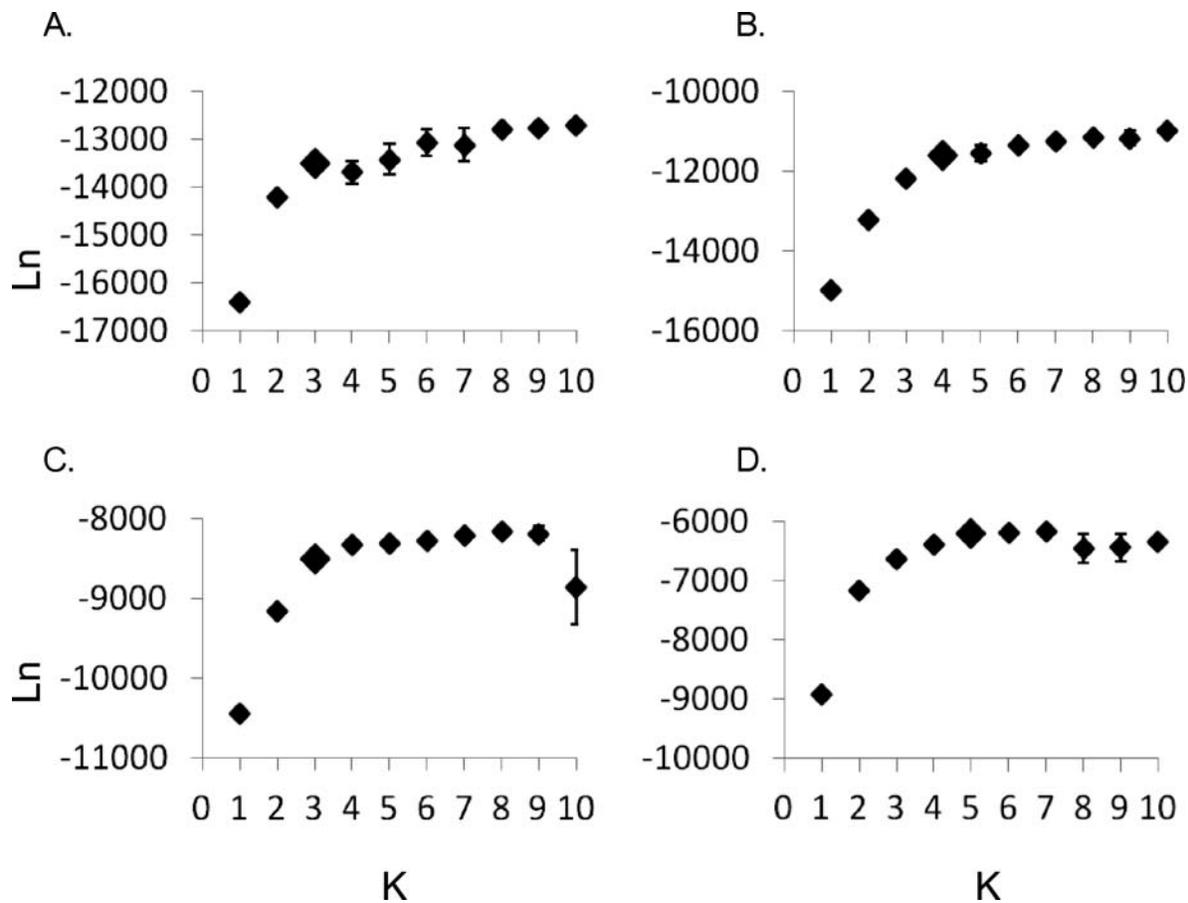


FIGURE 2. Mean posterior probabilities of the data given K clusters ($\ln \text{Pr}[X|K]$) across five replicate simulations with K -values of 1–10; the error bars = SDs. Separate STRUCTURE analyses (Pritchard et al. 2000) were run for each of (A)–(D) four groups of samples combined by common stocking histories. The corresponding results are shown in Figure 3A–D. The value of K chosen from each analysis is indicated by the enlarged symbol.

native populations. Changes in likelihoods (Figure 2) and lake stocking histories were used to identify the number of clusters (K) for each of the four groups of populations analyzed. STRUCTURE reliably distinguished ancestry from the stocked strains. Individuals within each of the three source population samples were assigned to distinct clusters with an average ancestry estimate exceeding 0.95 (Figure 3). Spider Lake, founded with the Shoepack strain, had an estimated 0.99 Shoepack ancestry, indicating that Shoepack ancestry was highly identifiable despite possible bottlenecks and isolation from the source population (Figure 3; Table 3). In the first group of populations, STRUCTURE identified three clusters ($K = 3$; Figure 2A) corresponding to the known stocked strains and assigned varied levels of ancestry to these three strains in the stocked populations (Figure 3A). In the second group of populations, four distinct ancestries ($K = 4$; Figure 2B) were identified in Mille Lacs and Lobster lakes, the fourth ancestry likely corresponding to the Iowa strain (Figure 3B). Additional data support the identification of this cluster as Iowa strain. Samples collected in Mille Lacs Lake in 1991 and 1992 and aged to the 1984 year-class (the only year-class of Iowa strain muskellunge stocked in Minnesota) were

strongly assigned to this fourth cluster (data not shown). Twenty-six of 27 individuals that had estimated ancestry >0.80 were assigned to the presumed Iowa cluster. Also, Mille Lacs and Lobster lakes had the only two introduced populations that showed ancestry from a strain other than the three sampled strains, and these were the only study lakes stocked with the Iowa strain.

For several supplemented native populations there were strong indications of remnant native ancestry in addition to ancestry from stocked strains (Table 3; Figure 3). STRUCTURE results supported $K = 3$ (Figure 2C) for lakes stocked with only the Shoepack and Wisconsin strains. The third cluster corresponded to native ancestry from Moose Lake and Deer Lake, which are located <1 km apart (Figure 3C). Five distinct ancestries ($K = 5$) were identified in the final group of populations, corresponding to the two stocked strains and three distinct native ancestries. Here, the plateau in likelihood values was not as sharply defined as in the previous analyses (Figure 2D), but the distinct ancestry assignments corresponded closely with specific lakes (Figure 3D). Little Boy Lake and Wabedo Lake had primarily individuals with a distinct native ancestry that was

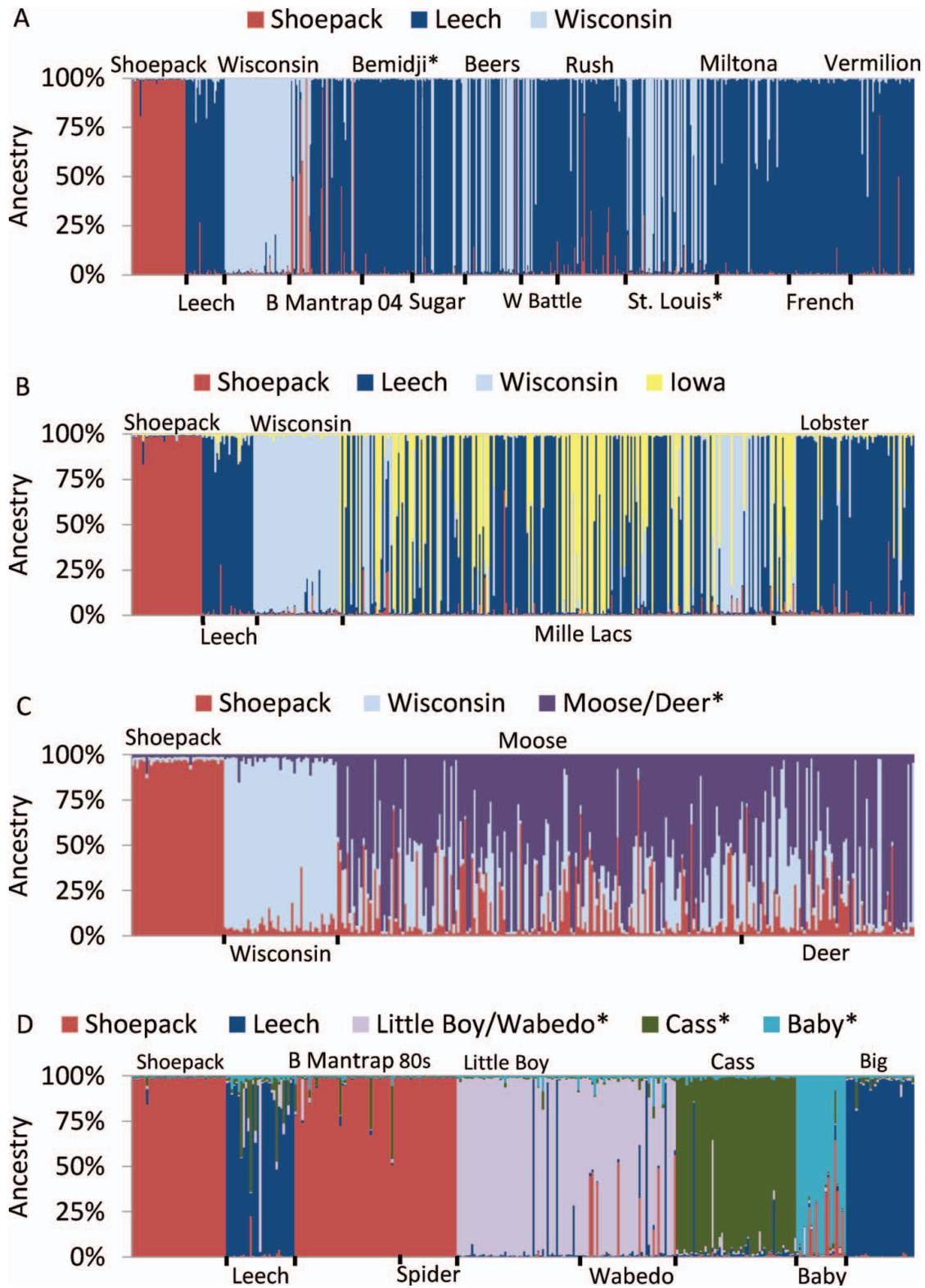


FIGURE 3. Ancestry estimated by STRUCTURE (Pritchard et al. 2000) for individuals from stocked strains and recipient lakes. Panels (A)–(D) show the results from separate analyses with samples (listed immediately above and below each figure) grouped according to common stocking histories. Samples of the known source populations stocked into a given group of lakes (i.e., Shoepack, Leech, and Wisconsin; no sample was available for the Iowa strain) were included in each run. Each vertical line represents an individual fish with its ancestral composition indicated by the different colors. The legend above each figure indicates ancestry corresponding to stocked strains or four distinct native ancestries (indicated by asterisks). [Figure available online in color.]

TABLE 3. Average ancestry in 20 stocked Minnesota muskellunge populations grouped by (1) type of lake, i.e., those with native muskellunge populations prior to stocking versus those with introduced populations and (2) the strain(s) stocked. Big Mantrap Lake was sampled twice—in the 1980s following its use as a Shoepack strain broodstock lake, and in 2004 after additional stocking with the Leech and Wisconsin strains. Dashes indicate that the corresponding ancestry was not a component of the STRUCTURE run in which the sample was included.

Strain stocked and lake	Ancestry				
	Shoepack	Leech	Wisconsin	Iowa	Native
Native populations					
Shoepack–Leech					
Big Lake	0.01	0.96	–	–	0.03
Little Boy Lake	0.00	0.07	–	–	0.92
Lake Wabedo	0.08	0.07	–	–	0.85
Shoepack–Wisconsin					
Deer Lake	0.11	–	0.32	–	0.55
Moose Lake	0.14	–	0.25	–	0.60
Leech–Wisconsin					
Lake Bemidji	0.01	0.98	0.01	–	–
St. Louis River	0.02	0.55	0.43	–	–
Shoepack					
Baby Lake	0.14	0.01	–	–	0.84
Big Mantrap Lake 1980s	0.95	0.01	–	–	0.05
Cass Lake	0.01	0.04	–	–	0.95
Shoepack–Leech–Wisconsin					
Big Mantrap Lake 2004	0.19	0.60	0.22	–	–
Introduced populations					
Shoepack–Leech–Wisconsin–Iowa					
Lobster Lake	0.03	0.77	0.07	0.13	–
Mille Lacs Lake	0.02	0.50	0.20	0.29	–
Shoepack–Leech–Wisconsin					
Beers Lake	0.03	0.52	0.45	–	–
French Lake	0.01	0.98	0.01	–	–
Rush Lake	0.06	0.89	0.05	–	–
Sugar Lake	0.01	0.80	0.19	–	–
Lake Vermilion	0.04	0.93	0.04	–	–
West Battle Lake	0.01	0.75	0.24	–	–
Leech–Wisconsin					
Lake Miliona	0.01	0.90	0.09	–	–
Shoepack					
Spider Lake	0.99	0.00	–	–	0.00

indistinguishable between samples from these interconnected lakes. The Cass Lake sample had its own distinct native ancestry, with no ancestry from the stocked Shoepack strain. Baby Lake fish also showed their own distinct native ancestry despite some admixture with the Shoepack strain. Other lakes known to have had native populations showed little or no indications of a distinct remnant ancestry. The 1980s Big Mantrap Lake population had 0.95 Shoepack ancestry following 15 years of stocking. This lake was used as a broodstock lake, so stocking into other lakes would have contributed predominantly Shoepack ancestry rather than ancestry from the native population. The populations in Big Lake and Lake Bemidji appeared to have

all Leech ancestry (Figure 3A, 3D). The St. Louis estuary had Leech and Wisconsin ancestry (Figure 3A). Few muskellunge were thought to remain in the St. Louis estuary prior to stocking and most of the Wisconsin ancestry was likely due to ongoing stocking by the Wisconsin DNR, but the presence of some remnant ancestry cannot be ruled out by our data.

Amount of Ancestry from Stocked Strains

Overall Shoepack strain ancestry in each lake was generally low and unrelated to the amount of Shoepack strain stocking. Linear regressions of percentage Shoepack ancestry on the

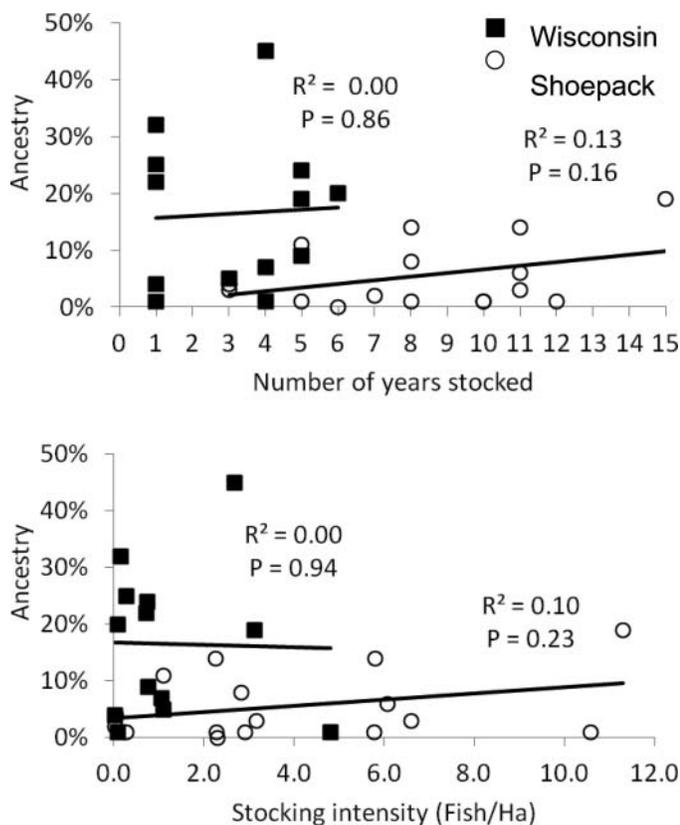


FIGURE 4. Ancestry in stocked populations derived from the Shoepack and Wisconsin strains in relation to the degree to which the strains were stocked in each lake. Regressions were conducted for average ancestry in each population on the number of years stocked (top panel) and stocking intensity measured as total fish per lake area (bottom panel).

number of years stocked or stocking intensity were not significant ($P > 0.05$; Figure 4). Except for the introduced Shoepack-strain population in Spider Lake, the highest Shoepack ancestry was found in the 2004 Big Mantrap sample. Big Mantrap Lake was a broodstock lake swamped by fish with Shoepack ancestry (1984–1988 sample estimate = 0.95) that has since been reduced following the stocking of Wisconsin and Leech strains. Six samples with a history of Shoepack strain stocking apparently had no fish with Shoepack ancestry (introduced populations in West Battle Lake, French Lake, and Sugar Lake and supplemented native populations in Cass Lake, Big Lake, and Little Boy Lake); the low estimates for these populations were likely errors, as indicated by similar estimates for the three populations (Lake Bemidji, Lake Miltona, and the St. Louis River) that were never stocked with Shoepack strain fish (Table 3).

Wisconsin strain ancestry was often relatively high despite only 1–5 years of stocking, but it was not detected in all of the lakes in which it was stocked (Table 3). Linear regressions of percentage Wisconsin ancestry on the number of years stocked or stocking intensity were not significant ($P > 0.05$; Figure 4). Both native and introduced populations were among the samples with high and low Wisconsin ancestry. The St. Louis River estuary

has had ongoing stocking by the Wisconsin DNR, so many of the pure Wisconsin individuals could be recently stocked fish.

The percentages of Leech and presumed Iowa strain ancestries were not regressed against the amount of stocking because of inadequate data. The Iowa strain was stocked in Lobster Lake and Mille Lacs Lake for only 1 year and made substantial contributions in each lake (13% and 29%). Leech strain ancestry was prevalent in most lakes where stocking is ongoing but was only 7% in the two lakes in which Leech strain stocking has stopped (Little Boy Lake and Lake Wabedo) (Table 3).

Reproduction by Stocked Strains

Admixed individuals were identified in many populations, thus confirming reproduction by stocked strains. All stocked strains had detectable reproduction in some of the lakes in which they supplemented native muskellunge populations (Table 4). The proportions of these lakes in which the different strains reproduced differed, but the varied stocking histories made any patterns difficult to interpret. Reproduction by stocked strains was detected in 6 of the 10 lakes with introduced populations.

TABLE 4. Reproduction by stocked muskellunge strains in lakes that had native muskellunge populations prior to stocking and those with introduced populations. Dashes indicate that a strain was not stocked in the given lakes.

Lake	Stocked strain			
	Shoepack	Wisconsin	Leech	Iowa
Native populations				
Big Lake	No	–	? ^a	
Little Boy Lake	No	–	No	
Lake Wabedo	Yes	–	No	
Moose Lake	Yes	Yes	–	
Deer Lake	Yes	Yes	–	
Lake Bemidji	–	No	? ^a	
St. Louis River	–	Yes	Yes	
Baby Lake	Yes	–	–	
Cass Lake	No	–	–	
Big Mantrap Lake	Yes	Yes	Yes	
Introduced populations				
Lobster Lake	Yes	Yes	Yes	Yes
Mille Lacs Lake	Yes	Yes	Yes	Yes
Beers Lake	No	No	No	–
French Lake	No	No	? ^a	–
Rush Lake	Yes	Yes	Yes	–
Sugar Lake	No	No	No	–
Lake Vermilion	Yes	Yes	Yes	–
West Battle Lake	No	No	No	–
Lake Miltona	–	Yes	Yes	–
Spider Lake	Yes ^b	–	–	–

^aBig Lake, Lake Bemidji, and French Lake have all Leech strain ancestry, so reproduction cannot be verified based on admixed individuals.

^bAll Shoepack strain ancestry; reproduction verified by the presence of a self-sustaining population (MNDNR, unpublished data).

Introduced populations had more similar stocking histories and provided no evidence for strain differences in ability to reproduce (Table 4). Reproduction was verified for either all of the strains or none of the strains stocked within a given lake. Admixed individuals were identified between all of the stocked strains and between stocked strains and native populations, showing that the absence of admixed individuals in populations with multiple ancestries was likely due to the lack of reproduction and not assortative mating among strains.

DISCUSSION

Detection of Distinct Ancestries from Stocked and Native Populations

STRUCTURE analyses were able to delineate distinct genetic groups among stocking source populations and native populations, making assignment of ancestry in stocked muskellunge waters of Minnesota feasible even without samples prior to stocking. Ancestry from each of the four stocked strains of muskellunge was detected in some of the lakes in which they were stocked. In lakes that had native populations, analyses identified ancestry consistent with the known stocked strains and remnant native ancestry in six lakes. The differentiation among native populations, even among nearby lakes in the upper Mississippi region, was likely enhanced by limited gene flow and rapid genetic drift in the populations of relatively low abundance typical for muskellunge (Hanson 1986; Margenau and AveLallemant 2000). Greater gene flow may have been possible until relatively recently for many of these populations connected by the Mississippi River or its tributaries, but several dams built in the last century have since barred fish movements. The few other studies of muskellunge genetic structure have not shown strong differentiation on small geographic scales, but they were based on lower-resolution allozyme markers. Koppelman and Philipp (1986) delineated three population cluster—from the St. Lawrence River, Ohio River, and upper Mississippi River—but resolved little differentiation among several Wisconsin populations within the upper Mississippi River group. Fields et al. (1997) delineated several clusters among Shoepack Lake and Mississippi River basin populations in Wisconsin and Minnesota, but this was based on only two polymorphic allozyme loci.

Genetic impacts of stocking prior to the 1950s on what we have called native populations cannot be entirely ruled out; however, records indicate that early attempts to spawn and rear muskellunge in Minnesota were limited and had little success (MGFC 1912; MDC 1934). If an unknown strain was stocked successfully, there should have been unaccountable ancestry and it should have been shared by some of the lakes. Instead, all of the ancestry detected in lakes with introduced populations was consistent with known stocked strains. We found only one ancestry other than that associated with the recorded stocked strains in lakes known to have had native populations. The presumed native ancestry in each lake was distinct with the exception of the

nearby and interconnected pairs of lakes, Moose and Deer lakes and Little Boy Lake and Lake Wabedo. Together, the records and data suggest that the ancestries not associated with the four known stocked strains likely derived from the populations native to the respective lakes.

Amount of Ancestry from Stocked Strains

Ancestry from the stocked strains varied widely among lakes and was not related to the amount of stocking for the discontinued Shoepack and Wisconsin strains. Shoepack ancestry within lakes was usually lower than Wisconsin or Iowa ancestry despite the Shoepack strain's being stocked for more years. Varied levels of introgression by stocked fish into native populations have been documented (Madeira et al. 2005; Spies et al. 2007; Finnegan and Stevens 2008), including by studies in central North America near Minnesota. Halbisen and Wilson (2009) found that four of eight supplemented lake trout *Salvelinus namaycush* populations in Ontario showed little or no introgression from hatchery strains. They found that levels of introgression did not correlate with the amount of stocking, a result that is consistent with our findings for muskellunge. Piller et al. (2005) detected little introgression in two populations stocked for decades with lake trout in the neighboring state of Wisconsin. Wilson et al. (2007) found evidence for the persistence of probable native populations of walleye *Sander vitreus* in Lake Superior, Ontario, and varying degrees of introgression by different stocked strains. In other regions, studies of northern pike (an esocid related to muskellunge) suggested high levels of introgression by stocked fish in France (Launey et al. 2006) but little introgression in a brackish-water population in Denmark (Larsen et al. 2005).

Stocked strains have apparently displaced former native populations or replaced extirpated populations in some lakes. The populations in Big Lake and Lake Bemidji showed little differentiation from the Leech Lake population ($F_{ST} = 0.02$ and 0.03 , respectively). These two lakes were historically connected to Leech Lake via the Mississippi River (dams now act as barriers to upstream movement), and they could presumably have had genetically indistinguishable native populations. However, Cass Lake is located between these two lakes and Leech Lake and has a genetically differentiated population ($F_{ST} = 0.09$), as do two other interconnected lakes, Little Boy Lake and Lake Wabedo ($F_{ST} = 0.17$ and 0.14 , respectively), indicating that Leech strain stocking is the most likely explanation for the genetic similarity between the Big Lake, Lake Bemidji, and Leech Lake samples. The muskellunge in Big Mantrap Lake appeared to have entirely Shoepack strain ancestry after 15 consecutive years of stocking this strain. The separation of Big Mantrap Lake and Shoepack Lake into separate major drainages makes it likely that the similarity of these populations ($F_{ST} = 0.01$) resulted from stocking and not from a lack of differentiation between the native Big Mantrap Lake and Shoepack Lake populations. Few native muskellunge were thought to remain prior to stocking in Big Lake and Lake Bemidji, and the abundance in Big Mantrap

Lake was uncertain (MNDNR, unpublished data). The low productivity of these populations may have made them vulnerable to displacement, which has been shown for other fish species (Evans and Willox 1991), but it is also possible that muskellunge populations were extirpated from these lakes prior to stocking.

Reproduction by Stocked Strains

Natural reproduction by stocked fish (as evidenced by admixed individuals) depended on the lake and not the strain stocked, especially for introduced populations. Dombeck et al. (1986) and Rust et al. (2002) found that the variables explaining the levels of muskellunge reproduction in Midwestern lakes included limited northern pike abundance, water level changes during spawning season, high alkalinity, a high shoreline development factor, drainage-lake systems, and woody debris. Northern pike were present in all of our stocked lakes at various densities. We did not attempt to evaluate habitat as part of this study, but we did observe that lakes in which introduced populations had no indications of natural reproduction were small (79–411 ha), while those with natural reproduction were large (529–53,627 ha). Lake size is clearly not a limiting factor, however, as 24 of the 44 Minnesota lakes considered to have had native populations are less than 411 ha in area (Younk and Pereira 2003).

The lack of reproduction in several introduced populations explains their lack of Shoepack strain ancestry. Shoepack strain stocking had ended 18–25 years prior to sampling, so most of the stocked individuals of this strain had likely died out in these lakes. Three introduced populations had relatively high Wisconsin strain ancestry but no indications of natural reproduction. The Wisconsin strain was stocked more recently than the Shoepack strain, so the nonadmixed individuals were likely stocked fish. Wisconsin ancestry will diminish as the stocked fish die out and the population is maintained through stocking of the Leech strain.

Management Implications

Remnant native ancestry persisted in several stocked lakes, so management designed to conserve genetic structure and possible local adaptations should not be dismissed as “too late” (see Hansen and Mensberg [2009] for a similar conclusion concerning a brown trout *Salmo trutta* population in Denmark). The MNDNR no longer stocks the lakes Baby, Moose, Deer, Cass, Little Boy, and Wabedo, whose populations retain an estimated 55–95% native ancestry. We suggest continuing this policy, but if stocking is deemed necessary that supplementation using broodstock from the lake itself be considered. As a second option, the Leech strain has the advantage of being a broodstock derived from a nearby lake in the same local drainage, so similar forces of selection may have shaped its genetic composition. The MNDNR should consider the feasibility of establishing other broodstocks to support stocking in the Hudson Bay or Lake Superior drainages (Wingate and Younk 2007) or stock where interactions with native muskellunge populations would

be minimal. The low Shoepack strain ancestry in many lakes despite years of stocking provides evidence supporting a cautious approach to stocking into naturally reproducing native populations. Some nonlocal broodstocks may be poorly adapted in the recipient environment, and introgression may lead to the reduction in fitness known as outbreeding depression.

Prior to this study, there was cause for managers to be concerned that Shoepack ancestry could be limiting the size attained by muskellunge in numerous lakes. The Shoepack strain had been stocked into 17 of our 20 study lakes, which included many of the popular muskellunge fisheries in Minnesota. The first population we studied, that of Moose Lake, had substantial Shoepack ancestry and there was evidence that Shoepack strain descendants were not attaining large sizes (Miller et al. 2009). In contrast, this study showed that Shoepack ancestry is likely having limited effects on size structure in most of our study populations because of its low persistence. We did not further evaluate the effect of Shoepack ancestry on fish size because relatively few fish had Shoepack ancestry and older muskellunge are difficult to age from scales (Fitzgerald et al. 1997). But where low persistence rules out possible effects of Shoepack ancestry, MNDNR biologists can focus on other biotic and abiotic factors if size structure is a concern. Studies have shown that muskellunge grow more slowly depending on the species (Wahl and Stein 1988) and size (Wahl and Stein 1993) of available prey. Abiotic factors, especially temperature, have been shown to affect muskellunge growth (Bevelhimer et al. 1985; Wahl and Stein 1991; Clapp and Wahl 1996). Lake size, which may affect temperatures and other abiotic factors, ranges from as small as 23 ha to as large as 53,627 ha across Minnesota’s muskellunge waters (Younk and Pereira 2003). Angler harvest truncated the size distributions of muskellunge in parts of Minnesota by the 1940s (Olson and Cunningham 1989) and may still affect size structure, although muskellunge now support mainly a catch-and-release trophy fishery (Wingate and Younk 2007).

The information we provided in this study will help MNDNR manage genetic diversity among muskellunge populations and allays concerns that Shoepack strain stocking had imposed persistent and widespread limitations on the size potential of muskellunge. Our study identifies the few lakes in which efforts might address Shoepack ancestry in attempts to improve size structure. For example, in one lake managers have decided to remove individuals with high Shoepack strain ancestry during a multiyear marking study of population dynamics. For introduced populations with natural reproduction, managers could intensify monitoring to determine the contribution of natural reproduction to recruitment and adjust stocking accordingly. Our study reinforces the findings that genetic data are often needed to determine ancestry in stocked fish populations, as stocking histories alone may be a poor indication of current genetic composition (e.g., Larsen et al. 2005; Wilson et al. 2007; Finnegan and Stevens 2008). As managers increasingly consider genetic differences among populations when making stocking decisions (e.g., Rider 2006; Welsh et al. 2010), including for muskellunge

(Jennings et al. 2010), genetic tools will help to identify where native genetic diversity persists despite past stocking or to select nonadmixed individuals for broodstocks. Fish geneticists often express concern about disrupting genetic structure and causing outbreeding depression (Hindar et al. 1991; Utter 2003), but stocking may affect traits of direct concern to anglers. The potential to alter heritable performance traits like growth and angling vulnerability (Philipp et al. 2009) should also be considered when making stocking decisions.

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The Impact of Stocking on the Current Ancestry in Twenty Native and Introduced Muskellunge Populations in Minnesota

Loren M. Miller^{a b}, Steven W. Mero^c & Jerry A. Younk^d

^a Minnesota Department of Natural Resources, 1980 Folwell Avenue, St. Paul, Minnesota, 55108, USA

^b Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota, St. Paul, 55108, Minnesota

^c Minnesota Department of Natural Resources, 1201 East Highway 2, Grand Rapids, Minnesota, 55744, USA

^d Minnesota Department of Natural Resources, 2114 Bemidji Avenue, Bemidji, Minnesota, 56601, USA

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ARTICLE

The Impact of Stocking on the Current Ancestry in Twenty Native and Introduced Muskellunge Populations in Minnesota

Loren M. Miller*

Minnesota Department of Natural Resources, 1980 Folwell Avenue, St. Paul, Minnesota 55108, USA; and Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota, St. Paul 55108, Minnesota

Steven W. Mero

Minnesota Department of Natural Resources, 1201 East Highway 2, Grand Rapids, Minnesota 55744, USA

Jerry A. Younk

Minnesota Department of Natural Resources, 2114 Bemidji Avenue, Bemidji, Minnesota 56601, USA

Abstract

Fish stocking, often from multiple source populations, is a common management practice frequently conducted without the means or effort to determine the reproductive contributions of stocked fish. Historically, the Minnesota Department of Natural Resources (MNDNR) has stocked four strains of muskellunge *Esox masquinongy*, but the contribution of these strains to current populations was unknown. Two strains came from Minnesota lakes, Shoepack Lake and Leech Lake, and the other strains came from Wisconsin and Iowa hatcheries and were of uncertain origin. The MNDNR discontinued stocking the Shoepack strain in the 1980s when that strain displayed poor growth in stocked waters. Managers were concerned that ancestry from this strain might be limiting the genetic potential for muskellunge to attain trophy size in stocked populations. Using 13 microsatellite DNA markers, we determined the ancestry of muskellunge in 10 supplemented native populations and 10 introduced populations. The ancestry from each of the four stocked strains of muskellunge was detected in some populations, but the level of ancestry was unrelated to the amount of stocking of a strain. Ancestry from native populations persisted in six of the supplemented populations despite years of stocking. The potential effects of Shoepack strain ancestry on fish size were limited in most lakes because of its low persistence. All stocked strains reproduced in at least some of the lakes, but some lakes had no evidence of reproduction by any stocked strain. Our results will help MNDNR manage genetic diversity among muskellunge populations and direct efforts toward appropriate actions to improve size structure. This study reinforces how genetic data are often useful for evaluating ancestry in stocked fish populations, whereas stocking histories may be poor indicators of current genetic composition.

For decades, billions of fish have been stocked in the United States (Halverson 2008), often with fish from multiple source populations being stocked into given systems over time. Comparisons of stocking success among strains have usually been based on the growth and survival of physically marked fish (e.g.,

Crozier et al. 1997; Wills 2006; Bronte et al. 2007). Genetic markers have distinguished stocked individuals or populations when physical marking was not efficient or feasible (Eldridge et al. 2002; DeKoning et al. 2006). Furthermore, genetic markers allow evaluation of reproductive contributions by stocked

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fish. Recent advances in genetic techniques and analysis have allowed assessment of stocking even in the absence of baseline samples from the stocking source and recipient populations prior to stocking (Halbisen and Wilson 2009; Miller et al. 2009). These genetic tools allow evaluation of the survival and reproductive success of stocked fish from different strains, sometimes many generations after stocking (Piller et al. 2005; Finnegan and Stevens 2008). Where stocking has supplemented native populations, genetic assessments may determine which strains made reproductive contributions and where native populations experienced introgression by genes from stocked strains.

Stocking is a common management tool for muskellunge *Esox masquinongy*, even in regions with abundant native populations (e.g., Margenau 1999; Kerr 2007; Wingate and Younk 2007). Stocking has been used to restore or enhance native populations or create new muskellunge waters to expand sport fisheries. Researchers have evaluated the factors associated with stocking success for muskellunge, success usually being measured as the growth and survival of stocked fish (reviewed in Margenau 1999; Wahl 1999). Success improved with increased size at stocking, the abundance of soft-rayed prey, and reduced predator abundance, especially largemouth bass *Micropterus salmoides* and northern pike *Esox lucius*. Success also depended on abiotic factors, especially temperature (Wahl 1999). Relatively few studies have compared stocking success among strains of muskellunge (Younk and Strand 1992; Margenau and Hanson 1996). Clapp and Wahl (1996) found differences in physiological traits among six populations of muskellunge and suggested that these could lead to differences in performance depending on the thermal environment where they were stocked. No studies have evaluated the reproductive contributions of stocked muskellunge, in particular, success by strain or in relation to native populations.

The Minnesota Department of Natural Resources (MNDNR) has used four main strains of muskellunge for stocking waters throughout the state (see Wingate and Younk 2007 for a review of the MNDNR muskellunge management program). The earliest attempts to spawn and rear muskellunge from Mississippi River drainage waters were deemed unsuccessful (MDC 1934). In the 1950s, the MNDNR utilized a strain derived from Shoepack Lake in the Hudson Bay drainage of northern Minnesota and stocked their descendants in many Minnesota lakes for over 30 years. In the early 1980s, the MNDNR discontinued the use of the Shoepack strain because evidence suggested that the stocked fish were not growing as large as fish from other muskellunge populations (Wingate and Younk 2007). Managers then began developing another Minnesota muskellunge strain derived from Leech Lake in the upper Mississippi basin of north-central Minnesota. Meanwhile, stocking continued with fish obtained from out-of-state suppliers. From the late 1970s to the mid-1980s, fish were obtained from a private grower in Wisconsin, whose source was unknown but was likely the nearby Wisconsin River drainage. In 1984, a few lakes were stocked with muskellunge from the Iowa

Department of Natural Resources' Spirit Lake fish hatchery. The MNDNR began to use the Leech strain widely in the late 1980s and has continued to stock this strain in state muskellunge waters.

The apparent inability of the Shoepack strain to attain large sizes (Younk and Strand 1992; Frohnauer et al. 2007) was a concern because muskellunge primarily support trophy fisheries (Margenau and AveLallemant 2000; Wingate and Younk 2007). Factors that might affect growth and size structure in muskellunge include prey characteristics (Wahl and Stein 1988, 1993), water temperature (Bevelhimer et al. 1985; Wahl and Stein 1991; Clapp and Wahl 1996), angling (Margenau and Hanson 1996), and genetics (Margenau and Hanson 1996; Younk and Strand 1992; Clapp and Wahl 1996). High levels of ancestry from a strain with slow growth or smaller maximum size would require different management responses than the other factors.

We investigated muskellunge ancestry in Minnesota populations in lakes stocked with one to four different strains either to supplement native populations or to introduce muskellunge to create fisheries. The main objectives of this study were to determine the contribution of each strain to the ancestral composition of stocked populations and whether any of the native populations had retained the genetic diversity found in the region prior to stocking. We were particularly interested in the prevalence of ancestry from the slow-growing Shoepack strain because of concerns that it may limit the size attained by Shoepack descendants in stocked populations (Miller et al. 2009). We also evaluated factors that may have affected the level of ancestry from stocked strains. We first tested for a relationship between the amount of stocking and ancestry from each strain. A discordance between stocking history and subsequent ancestral composition has been observed for a number of fish species (Larsen et al. 2005; Finnegan and Stevens 2008; Halbisen and Wilson 2009), but it has not been studied for muskellunge. Finally, we verified natural reproduction by stocked strains. The persistence of ancestry from discontinued strains depends on their ability to reproduce in the stocked lakes.

METHODS

Sample collection.—Scales archived from previous MNDNR spring trap-net assessments of spawning muskellunge were used for genetic analysis. Sample sizes were determined by scale availability from the most recent assessment or that from multiple years if needed to increase sample size (Table 1). Sample sizes ranged from 21 to 76 (mean = 45) for all but two lakes, which had large sample sizes of 174 and 246 from special intensive assessments of these muskellunge populations.

Source population samples were obtained for three of the four stocked strains. We obtained samples directly from Shoepack Lake and Leech Lake. We included a Wisconsin sample from Tomahawk Lake in northeastern Wisconsin, thought to be the region from which the private grower who supplied fish for Minnesota obtained broodstock (data provided by B. Sloss, USGS

TABLE 1. Sample information and stocking history for three lakes that provided source populations and 20 stocked lakes that had native muskellunge populations prior to stocking (N) or now have introduced (I) populations. Sample information includes the year(s) sampled and the sample size (*n*). The stocking history indicates the strains used, the time period stocked (P = stocking continues to the present time) and the total years (Yrs) stocked for each strain (prior to the year of sampling for the Leech strain). Big Mantrap Lake was sampled at two time periods.

Lake	Status	Sample		Stocked strains							
		Year(s)	<i>n</i>	Shoepack		Iowa		Wisconsin		Leech	
				Period	Yrs	Period	Yrs	Period	Yrs	Period	Yrs
Source populations											
Shoepack Lake	N	1993	40								
Tomahawk Lake, Wisconsin	N	2006	49								
Leech Lake	N	1987–1988	29								
Stocked populations											
1. Lobster Lake	I	2009	80	1970–1981	10	1984	1	1983–1988	3	1990–P	15
2. Mille Lacs Lake	I	2006	246	1970–1978	7	1984	1	1985–1989	3	1989–P	9
3. Big Mantrap Lake	N	1984–1988 2004	42 47	1969–1983	15			1987	1	1988–P	9
4. Beers Lake	I	2002	42	1977–1984	3			1981–1988	3	1986–P	6
5. French Lake	I	2007	53	1974–1985	12			1985–1988	4	1989–P	16
6. Rush Lake	I	2006–2007	56	1969–1982	10			1983–1985	3	1989–P	15
7. Sugar Lake	I	2003	37	1970–1978	7			1983–1988	5	1989–P	12
8. Lake Vermilion	I	2005–2006	44	1969–1984	3			1985	1	1987–P	13
9. West Battle Lake	I	2003	22	1969–1984	10			1979–1988	5	1990–P	7
10. Big Lake	N	2008	29	1969–1981	10					1987–P	14
11. Little Boy Lake	N	2000	50	1972–1977	6					1987–1993	4
12. Lake Wabedo	N	2000	43	1972–1981	8					1987	1
13. Deer Lake	N	2003	76	1971–1983	5			1985	1		
14. Moose Lake	N	2008	174	1971–1983	8			1985	1		
15. Lake Bemidji	N	1998	44					1978	1	1982–P	6
16. Lake Miliona	I	2008	51					1982–1987	5	1989–P	10
17. St. Louis River	N	2007–2008	64					1983–P	17 ^a	1989–2005	9
18. Baby Lake	N	1995, 2005	21	1971–1979	8						
19. Cass Lake	N	1991–1997	51	1969–1975	5						
20. Spider Lake	I	2007	27	1969–1979	9						

^aIncludes stocking by the Wisconsin Department of Natural Resources.

Cooperative Fisheries Research Unit, University of Wisconsin–Steven’s Point). No sample was available from the source of the fourth stocked strain that was acquired from the Iowa Department of Natural Resources.

We evaluated 20 lakes stocked with one to four different strains of muskellunge (Figure 1; see Table 1 for the stocking history of strains in each lake). Ten lakes had native populations prior to stocking and the other 10 lakes had no muskellunge present before introductions (Table 1). Big Mantrap Lake was stocked 15 times with Shoepack strain fish to establish a brood-stock lake to produce offspring for stocking in other lakes. We included a 1984–1988 sample from Big Mantrap Lake because it had a native muskellunge population prior to stocking with Shoepack Lake fish, so stocked progeny could have contributed Shoepack strain and Big Mantrap Lake ancestry to other popu-

lations. Spider Lake has a self-sustaining introduced population established with only the Shoepack strain.

Genotyping.—We genotyped 1,417 muskellunge using the procedures described in Miller et al. (2009), except that we discontinued using microsatellite locus *EmaA5* so that the remaining 13 loci from Sloss et al. (2008) would combine together in a single electrophoresis run. An additional 50–106 repeated reactions per locus were scored to assess genotyping error (sample size was variable because it included positive controls and samples repeated to fill in a few missing genotypes at individual loci).

Genetic diversity analysis.—Observed and expected heterozygosities and the inbreeding coefficient F_{IS} were estimated for each locus in each sample. Conformance with Hardy–Weinberg expectations was tested using the exact test procedures

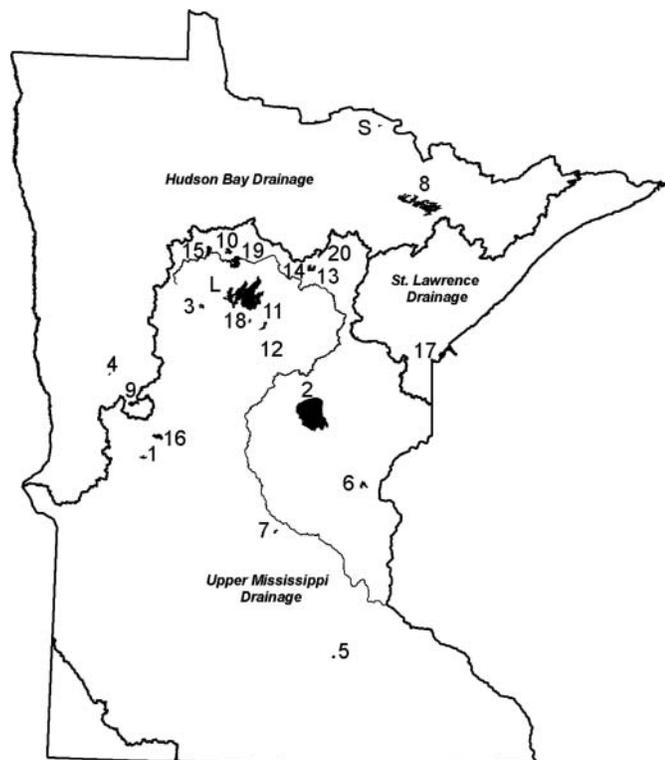


FIGURE 1. Sampling locations for two muskellunge broodstock sources, Leech Lake (L) and Shoepack Lake (S), and 20 stocked lakes (numbered as in Table 1) in Minnesota. Additional stocked muskellunge came from hatcheries in Wisconsin and Iowa. The thick lines delineate major drainage boundaries, and the thin line indicates the Mississippi River.

of Guo and Thompson (1992), as implemented by GENEPOP version 3.4 (Raymond and Rousset 1995) using 10,000 dememorizations, 20 batches, and 500 iterations per batch. GENEPOP was also used to test for linkage disequilibrium between pairs of loci. Significance values were adjusted within each sample using sequential Bonferroni procedures (Rice 1989), but the number of individual tests with P -values < 0.05 is also reported because the Bonferroni correction is conservative. Data were evaluated in MICROCHECKER version 2.2.3 to detect evidence of null alleles (nonamplifying alleles that lead to heterozygotes being scored as homozygotes) or scoring errors due to stuttering or large-allele dropout (Van Oosterhout et al. 2004).

The measure of population differentiation, pairwise F_{ST} , was calculated in FSTAT (Goudet 1995) for samples from stocking source populations and from lakes that had native populations prior to stocking. Differentiation among stocked strains is necessary to distinguish ancestry in the stocked lakes. Differentiation between stocked populations and the source strains may indicate the retention of native ancestry; conversely, lack of differentiation may signify stocking impacts. The St. Louis estuary, which had a native population, was excluded because it receives ongoing stocking with the Leech and Wisconsin strains.

Ancestry analysis.—To determine the number of genetically distinct populations contributing to our samples and the ancestry of individual fish, we used the Bayesian clustering algorithm implemented in the program STRUCTURE (version 2.3.3; Pritchard et al. 2000; also refer to <http://pritch.bsd.uchicago.edu>). We ran STRUCTURE analyses separately on four groups of populations that shared similar stocking histories to avoid falsely assigning ancestry that could not be present in a population. Each group included the appropriate strain source samples and lakes stocked with those strains. We made a few exceptions to avoid excessively dividing the data: Cass Lake and Spider Lake, stocked only with the Shoepack strain, were included with populations stocked with the Shoepack and Leech strains, and Lake Bemidji, Lake Milnora and the St. Louis River estuary, which were stocked only with the Leech and Wisconsin strains, were included with populations stocked with the Shoepack, Leech, and Wisconsin strains. To determine the number of distinct clusters (K), we ran five simulations at each K ranging from 1 to 10 (higher than the potential number of native and stocked populations) using 250,000 Markov chain–Monte Carlo simulations after a burn-in of 50,000 simulations. The model assumed possible admixture (i.e., individuals with mixed ancestries due to mating between strains) and correlated allele frequencies, with no prior population information. For each analysis group, a K value was chosen based on a plateau in the likelihood values and a correspondence of assigned ancestry with known sample information, i.e., clusters corresponding to known stocked strains or to individual lakes that had native populations (Pritchard et al. 2010).

Ancestry and amount of stocking.—We assessed the relationship between the amount of stocking and the percentage of ancestry from each of the two major strains no longer stocked, Shoepack and Wisconsin. Linear regressions were conducted for the average ancestry within each sample on the number of years stocked and separately on the stocking intensity (total number stocked in all years/lake area). Only data for fingerlings were analyzed for stocking intensity because managers will typically stock with greatly different intensities depending on the life stage stocked. We removed one adult and three fry stocking events for the Shoepack strain and three adult stocking events for the Wisconsin strain. The relationship between ancestry and stocking was not evaluated for the Iowa strain because it was stocked just one year and in only two lakes or for the Leech strain because it has been continually stocked in many populations, so samples likely included recently stocked fish.

Reproduction by stocked strains.—STRUCTURE results were also used to identify lakes in which the stocked strains have reproduced. Simply observing natural reproduction (e.g., age-0 fish from unstocked years) could not enable us to determine which strains contributed because all but one lake was stocked with multiple strains or had a prior reproducing population. We used admixed individuals as an indicator of natural reproduction because individuals admixed between stocked strains or a stocked strain and a native population could only result from

reproduction in the lakes. We used the estimated 90% probability intervals for each individual's ancestry estimates and assumed that an individual was admixed when the probability interval exceeded zero for more than one ancestry. This approach may fail to identify some admixed individuals because probability intervals are typically wide in STRUCTURE analyses. We tolerated some error because our interest was verifying reproduction by strains rather than accurately quantifying the numbers of admixed individuals. Testing this approach on source population samples (i.e., nonadmixed individuals) indicated that it was unlikely to falsely indicate reproduction by stocked strains. No individual from a source population sample was identified as admixed with a stocked strain using the probability interval criterion (data not shown). Natural reproduction could not be evaluated based on admixed individuals for several stocked populations that had ancestry from only a single strain.

RESULTS

Genetic Diversity

The 13 microsatellite loci displayed a wide range of variation across sources and stocked populations (see Table A.1 in the appendix, which appears in the online version of this article). They showed no indications of null alleles, stuttering, or allelic dropout. One to three Hardy–Weinberg tests had P -values <0.05 in each of the source population samples, but none were significant after Bonferroni correction (Table A.1). For linkage disequilibrium, zero to nine tests had P -values <0.05 in the source population samples, but only locus pair (*EmaD5–EmaD116*) was significant after Bonferroni correction and only for the Wisconsin sample (Table A.1). These results indicate that the loci satisfy the genetic equilibrium assumptions for STRUCTURE analysis of population structure and ancestry. The error rates for repeated samples averaged 0.7% (range,

0.0–2.5% per locus). All errors involved a single mismatched allele and no fish had errors at multiple loci, so errors likely had little effect on ancestry estimates.

Many loci were out of Hardy–Weinberg and linkage equilibrium in stocked populations (Table A.1). These deviations are consistent with the presence of multiple, distinct populations and recent stocking and do not indicate marker deficiencies (e.g., null alleles) or nonindependence of loci. A mixture of stocked strains or stocked and native fish may cause deviation from Hardy–Weinberg expectations due to the Wahlund effect (Hedrick 2005). Although random mating following stocking would establish Hardy–Weinberg equilibrium in a single generation, linkage disequilibrium persists for more generations. Minimizing Hardy–Weinberg and linkage disequilibrium is the process STRUCTURE uses to identify distinct genetic clusters (Pritchard et al. 2000).

Population differentiation was high and statistically significant among the source populations ($F_{ST} = 0.24–0.43$), and many of the stocked populations that had native muskellunge ($F_{ST} = 0.09–0.54$, except for those mentioned below) (Table 2). The Shoepack Lake population was highly differentiated from all others except for the population in Big Mantrap Lake, the Shoepack broodstock lake ($F_{ST} = 0.01$ [not significant]). Low but significant F_{ST} values were also found between Leech Lake and Big Lake ($F_{ST} = 0.02$) and Lake Bemidji ($F_{ST} = 0.03$), two lakes that receive ongoing Leech strain stocking. Other populations with low but significant F_{ST} values included geographically proximate pairs that likely have incomplete isolation: Moose Lake and Deer Lake ($F_{ST} = 0.02$) and Little Boy Lake and Lake Wabedo ($F_{ST} = 0.06$).

Distinct Ancestries Identified by STRUCTURE

STRUCTURE identified eight different genetic clusters, corresponding to ancestry from stocked strains and several

TABLE 2. Population differentiation (pairwise F_{ST} ; Goudet 1995) between stocking source populations from Shoepack Lake, Leech Lake, and Wisconsin and populations from nine Minnesota lakes that had native muskellunge prior to stocking. The St. Louis estuary was excluded because it receives ongoing stocking with Leech and Wisconsin strain muskellunge. Values in bold italics indicate tests that were not significant following sequential Bonferroni correction (Rice 1989) for multiple testing ($\alpha = 0.05$, $k = 66$).

	Shoepack	Leech	Wisconsin	Big Mantrap	Moose	Deer	Little Boy	Wabedo	Cass	Baby	Bemidji
Shoepack											
Leech	0.43										
Wisconsin	0.30	0.24									
Big Mantrap	0.01	0.39	0.27								
Moose	0.36	0.16	0.21	0.33							
Deer	0.37	0.16	0.19	0.33	0.02						
Little Boy	0.54	0.17	0.30	0.51	0.25	0.25					
Wabedo	0.47	0.14	0.24	0.44	0.21	0.21	0.06				
Cass	0.35	0.09	0.22	0.31	0.14	0.15	0.22	0.18			
Baby	0.40	0.16	0.19	0.36	0.16	0.16	0.18	0.12	0.15		
Bemidji	0.44	0.03	0.27	0.41	0.22	0.21	0.20	0.18	0.14	0.21	
Big	0.48	0.02	0.29	0.45	0.22	0.21	0.21	0.19	0.13	0.22	0.00

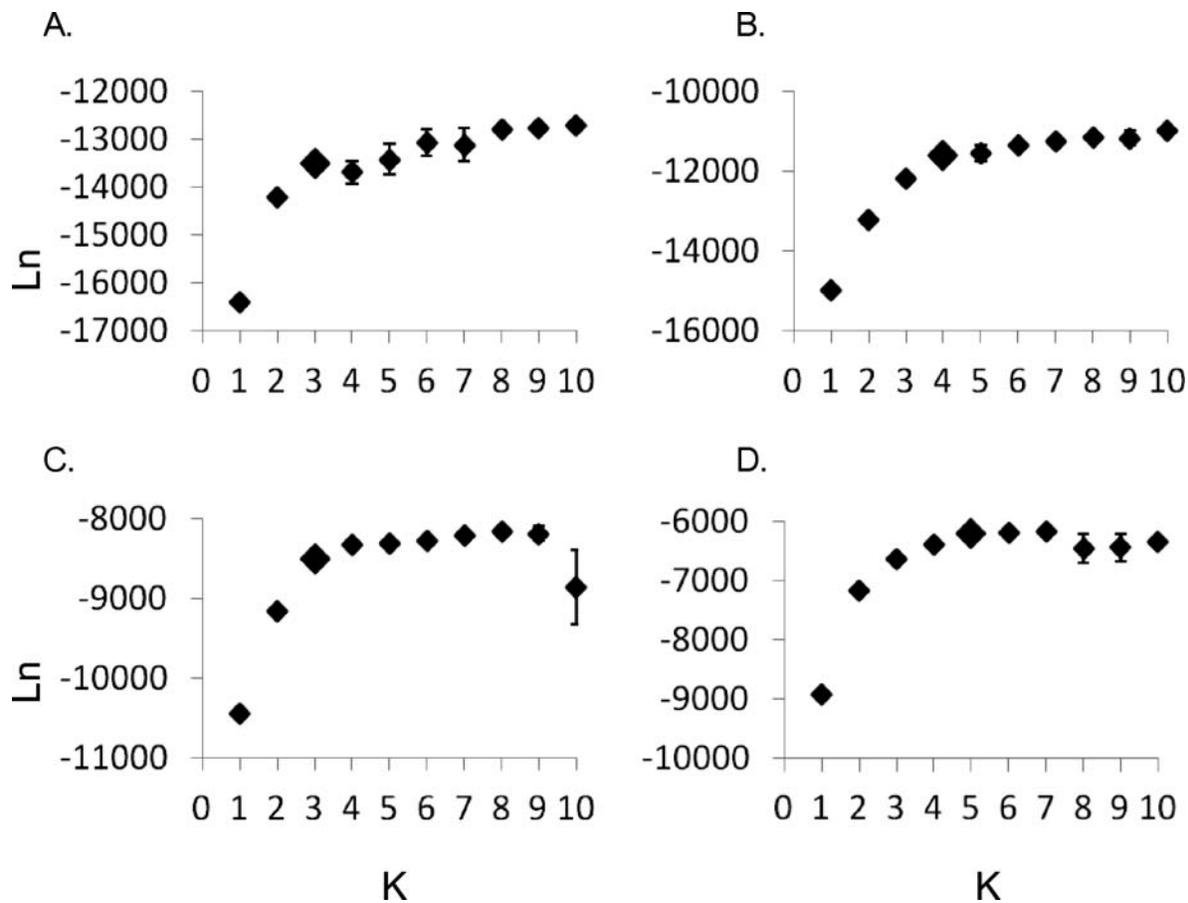


FIGURE 2. Mean posterior probabilities of the data given K clusters ($\ln \Pr[X|K]$) across five replicate simulations with K -values of 1–10; the error bars = SDs. Separate STRUCTURE analyses (Pritchard et al. 2000) were run for each of (A)–(D) four groups of samples combined by common stocking histories. The corresponding results are shown in Figure 3A–D. The value of K chosen from each analysis is indicated by the enlarged symbol.

native populations. Changes in likelihoods (Figure 2) and lake stocking histories were used to identify the number of clusters (K) for each of the four groups of populations analyzed. STRUCTURE reliably distinguished ancestry from the stocked strains. Individuals within each of the three source population samples were assigned to distinct clusters with an average ancestry estimate exceeding 0.95 (Figure 3). Spider Lake, founded with the Shoepack strain, had an estimated 0.99 Shoepack ancestry, indicating that Shoepack ancestry was highly identifiable despite possible bottlenecks and isolation from the source population (Figure 3; Table 3). In the first group of populations, STRUCTURE identified three clusters ($K = 3$; Figure 2A) corresponding to the known stocked strains and assigned varied levels of ancestry to these three strains in the stocked populations (Figure 3A). In the second group of populations, four distinct ancestries ($K = 4$; Figure 2B) were identified in Mille Lacs and Lobster lakes, the fourth ancestry likely corresponding to the Iowa strain (Figure 3B). Additional data support the identification of this cluster as Iowa strain. Samples collected in Mille Lacs Lake in 1991 and 1992 and aged to the 1984 year-class (the only year-class of Iowa strain muskellunge stocked in Minnesota) were

strongly assigned to this fourth cluster (data not shown). Twenty-six of 27 individuals that had estimated ancestry >0.80 were assigned to the presumed Iowa cluster. Also, Mille Lacs and Lobster lakes had the only two introduced populations that showed ancestry from a strain other than the three sampled strains, and these were the only study lakes stocked with the Iowa strain.

For several supplemented native populations there were strong indications of remnant native ancestry in addition to ancestry from stocked strains (Table 3; Figure 3). STRUCTURE results supported $K = 3$ (Figure 2C) for lakes stocked with only the Shoepack and Wisconsin strains. The third cluster corresponded to native ancestry from Moose Lake and Deer Lake, which are located <1 km apart (Figure 3C). Five distinct ancestries ($K = 5$) were identified in the final group of populations, corresponding to the two stocked strains and three distinct native ancestries. Here, the plateau in likelihood values was not as sharply defined as in the previous analyses (Figure 2D), but the distinct ancestry assignments corresponded closely with specific lakes (Figure 3D). Little Boy Lake and Wabedo Lake had primarily individuals with a distinct native ancestry that was

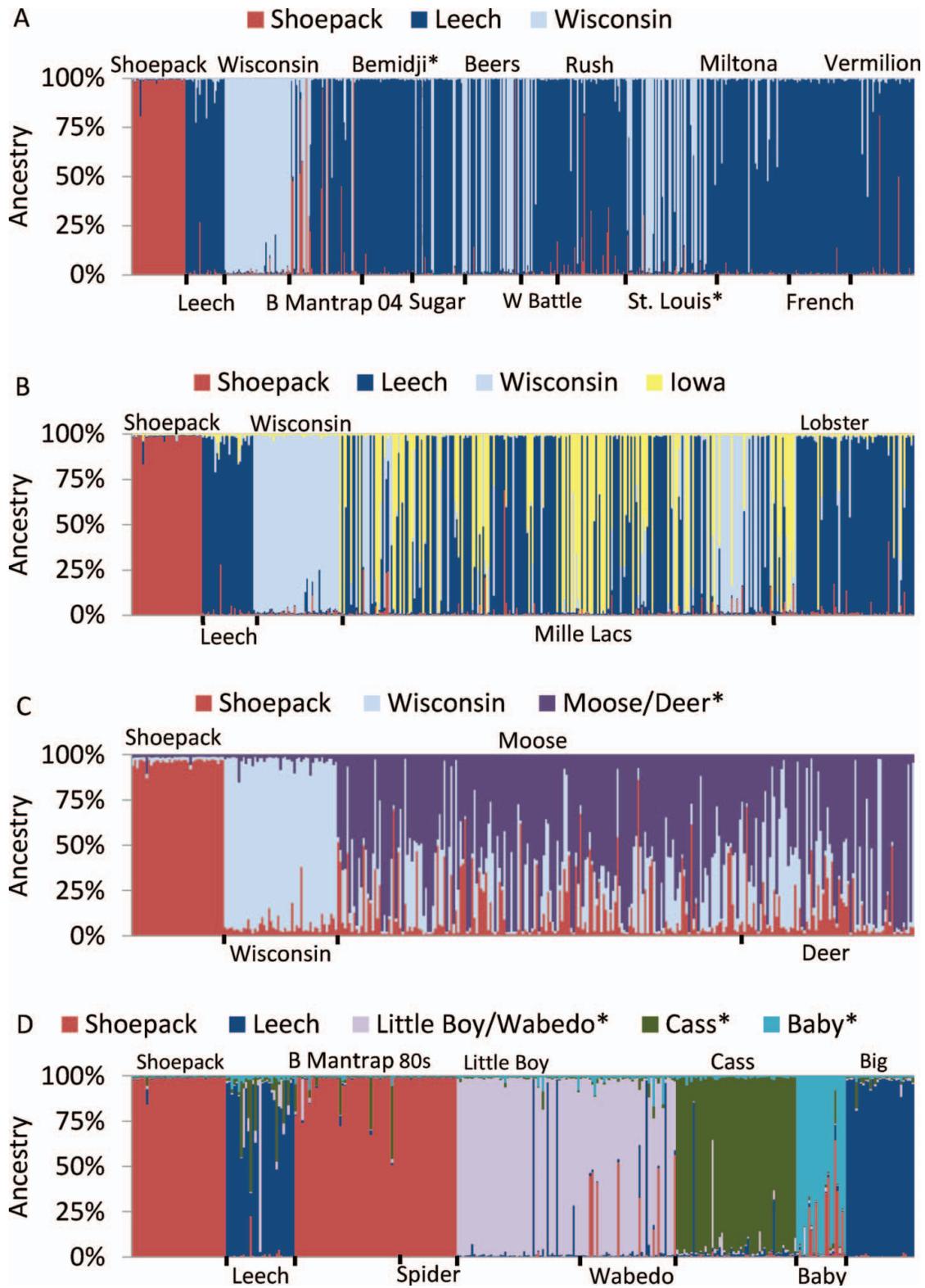


FIGURE 3. Ancestry estimated by STRUCTURE (Pritchard et al. 2000) for individuals from stocked strains and recipient lakes. Panels (A)–(D) show the results from separate analyses with samples (listed immediately above and below each figure) grouped according to common stocking histories. Samples of the known source populations stocked into a given group of lakes (i.e., Shoepack, Leech, and Wisconsin; no sample was available for the Iowa strain) were included in each run. Each vertical line represents an individual fish with its ancestral composition indicated by the different colors. The legend above each figure indicates ancestry corresponding to stocked strains or four distinct native ancestries (indicated by asterisks). [Figure available online in color.]

TABLE 3. Average ancestry in 20 stocked Minnesota muskellunge populations grouped by (1) type of lake, i.e., those with native muskellunge populations prior to stocking versus those with introduced populations and (2) the strain(s) stocked. Big Mantrap Lake was sampled twice—in the 1980s following its use as a Shoepack strain broodstock lake, and in 2004 after additional stocking with the Leech and Wisconsin strains. Dashes indicate that the corresponding ancestry was not a component of the STRUCTURE run in which the sample was included.

Strain stocked and lake	Ancestry				
	Shoepack	Leech	Wisconsin	Iowa	Native
Native populations					
Shoepack–Leech					
Big Lake	0.01	0.96	–	–	0.03
Little Boy Lake	0.00	0.07	–	–	0.92
Lake Wabedo	0.08	0.07	–	–	0.85
Shoepack–Wisconsin					
Deer Lake	0.11	–	0.32	–	0.55
Moose Lake	0.14	–	0.25	–	0.60
Leech–Wisconsin					
Lake Bemidji	0.01	0.98	0.01	–	–
St. Louis River	0.02	0.55	0.43	–	–
Shoepack					
Baby Lake	0.14	0.01	–	–	0.84
Big Mantrap Lake 1980s	0.95	0.01	–	–	0.05
Cass Lake	0.01	0.04	–	–	0.95
Shoepack–Leech–Wisconsin					
Big Mantrap Lake 2004	0.19	0.60	0.22	–	–
Introduced populations					
Shoepack–Leech–Wisconsin–Iowa					
Lobster Lake	0.03	0.77	0.07	0.13	–
Mille Lacs Lake	0.02	0.50	0.20	0.29	–
Shoepack–Leech–Wisconsin					
Beers Lake	0.03	0.52	0.45	–	–
French Lake	0.01	0.98	0.01	–	–
Rush Lake	0.06	0.89	0.05	–	–
Sugar Lake	0.01	0.80	0.19	–	–
Lake Vermilion	0.04	0.93	0.04	–	–
West Battle Lake	0.01	0.75	0.24	–	–
Leech–Wisconsin					
Lake Miliona	0.01	0.90	0.09	–	–
Shoepack					
Spider Lake	0.99	0.00	–	–	0.00

indistinguishable between samples from these interconnected lakes. The Cass Lake sample had its own distinct native ancestry, with no ancestry from the stocked Shoepack strain. Baby Lake fish also showed their own distinct native ancestry despite some admixture with the Shoepack strain. Other lakes known to have had native populations showed little or no indications of a distinct remnant ancestry. The 1980s Big Mantrap Lake population had 0.95 Shoepack ancestry following 15 years of stocking. This lake was used as a broodstock lake, so stocking into other lakes would have contributed predominantly Shoepack ancestry rather than ancestry from the native population. The populations in Big Lake and Lake Bemidji appeared to have

all Leech ancestry (Figure 3A, 3D). The St. Louis estuary had Leech and Wisconsin ancestry (Figure 3A). Few muskellunge were thought to remain in the St. Louis estuary prior to stocking and most of the Wisconsin ancestry was likely due to ongoing stocking by the Wisconsin DNR, but the presence of some remnant ancestry cannot be ruled out by our data.

Amount of Ancestry from Stocked Strains

Overall Shoepack strain ancestry in each lake was generally low and unrelated to the amount of Shoepack strain stocking. Linear regressions of percentage Shoepack ancestry on the

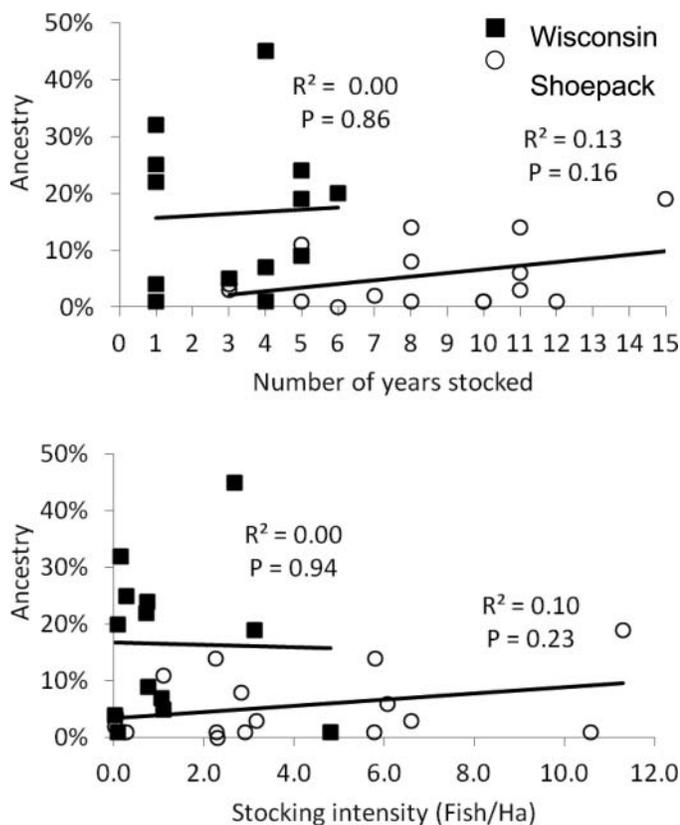


FIGURE 4. Ancestry in stocked populations derived from the Shoepack and Wisconsin strains in relation to the degree to which the strains were stocked in each lake. Regressions were conducted for average ancestry in each population on the number of years stocked (top panel) and stocking intensity measured as total fish per lake area (bottom panel).

number of years stocked or stocking intensity were not significant ($P > 0.05$; Figure 4). Except for the introduced Shoepack-strain population in Spider Lake, the highest Shoepack ancestry was found in the 2004 Big Mantrap sample. Big Mantrap Lake was a broodstock lake swamped by fish with Shoepack ancestry (1984–1988 sample estimate = 0.95) that has since been reduced following the stocking of Wisconsin and Leech strains. Six samples with a history of Shoepack strain stocking apparently had no fish with Shoepack ancestry (introduced populations in West Battle Lake, French Lake, and Sugar Lake and supplemented native populations in Cass Lake, Big Lake, and Little Boy Lake); the low estimates for these populations were likely errors, as indicated by similar estimates for the three populations (Lake Bemidji, Lake Miltona, and the St. Louis River) that were never stocked with Shoepack strain fish (Table 3).

Wisconsin strain ancestry was often relatively high despite only 1–5 years of stocking, but it was not detected in all of the lakes in which it was stocked (Table 3). Linear regressions of percentage Wisconsin ancestry on the number of years stocked or stocking intensity were not significant ($P > 0.05$; Figure 4). Both native and introduced populations were among the samples with high and low Wisconsin ancestry. The St. Louis River estuary

has had ongoing stocking by the Wisconsin DNR, so many of the pure Wisconsin individuals could be recently stocked fish.

The percentages of Leech and presumed Iowa strain ancestries were not regressed against the amount of stocking because of inadequate data. The Iowa strain was stocked in Lobster Lake and Mille Lacs Lake for only 1 year and made substantial contributions in each lake (13% and 29%). Leech strain ancestry was prevalent in most lakes where stocking is ongoing but was only 7% in the two lakes in which Leech strain stocking has stopped (Little Boy Lake and Lake Wabedo) (Table 3).

Reproduction by Stocked Strains

Admixed individuals were identified in many populations, thus confirming reproduction by stocked strains. All stocked strains had detectable reproduction in some of the lakes in which they supplemented native muskellunge populations (Table 4). The proportions of these lakes in which the different strains reproduced differed, but the varied stocking histories made any patterns difficult to interpret. Reproduction by stocked strains was detected in 6 of the 10 lakes with introduced populations.

TABLE 4. Reproduction by stocked muskellunge strains in lakes that had native muskellunge populations prior to stocking and those with introduced populations. Dashes indicate that a strain was not stocked in the given lakes.

Lake	Stocked strain			
	Shoepack	Wisconsin	Leech	Iowa
Native populations				
Big Lake	No	–	? ^a	
Little Boy Lake	No	–	No	
Lake Wabedo	Yes	–	No	
Moose Lake	Yes	Yes	–	
Deer Lake	Yes	Yes	–	
Lake Bemidji	–	No	? ^a	
St. Louis River	–	Yes	Yes	
Baby Lake	Yes	–	–	
Cass Lake	No	–	–	
Big Mantrap Lake	Yes	Yes	Yes	
Introduced populations				
Lobster Lake	Yes	Yes	Yes	Yes
Mille Lacs Lake	Yes	Yes	Yes	Yes
Beers Lake	No	No	No	–
French Lake	No	No	? ^a	–
Rush Lake	Yes	Yes	Yes	–
Sugar Lake	No	No	No	–
Lake Vermilion	Yes	Yes	Yes	–
West Battle Lake	No	No	No	–
Lake Miltona	–	Yes	Yes	–
Spider Lake	Yes ^b	–	–	–

^aBig Lake, Lake Bemidji, and French Lake have all Leech strain ancestry, so reproduction cannot be verified based on admixed individuals.

^bAll Shoepack strain ancestry; reproduction verified by the presence of a self-sustaining population (MNDNR, unpublished data).

Introduced populations had more similar stocking histories and provided no evidence for strain differences in ability to reproduce (Table 4). Reproduction was verified for either all of the strains or none of the strains stocked within a given lake. Admixed individuals were identified between all of the stocked strains and between stocked strains and native populations, showing that the absence of admixed individuals in populations with multiple ancestries was likely due to the lack of reproduction and not assortative mating among strains.

DISCUSSION

Detection of Distinct Ancestries from Stocked and Native Populations

STRUCTURE analyses were able to delineate distinct genetic groups among stocking source populations and native populations, making assignment of ancestry in stocked muskellunge waters of Minnesota feasible even without samples prior to stocking. Ancestry from each of the four stocked strains of muskellunge was detected in some of the lakes in which they were stocked. In lakes that had native populations, analyses identified ancestry consistent with the known stocked strains and remnant native ancestry in six lakes. The differentiation among native populations, even among nearby lakes in the upper Mississippi region, was likely enhanced by limited gene flow and rapid genetic drift in the populations of relatively low abundance typical for muskellunge (Hanson 1986; Margenau and AveLallemant 2000). Greater gene flow may have been possible until relatively recently for many of these populations connected by the Mississippi River or its tributaries, but several dams built in the last century have since barred fish movements. The few other studies of muskellunge genetic structure have not shown strong differentiation on small geographic scales, but they were based on lower-resolution allozyme markers. Koppelman and Philipp (1986) delineated three population cluster—from the St. Lawrence River, Ohio River, and upper Mississippi River—but resolved little differentiation among several Wisconsin populations within the upper Mississippi River group. Fields et al. (1997) delineated several clusters among Shoepack Lake and Mississippi River basin populations in Wisconsin and Minnesota, but this was based on only two polymorphic allozyme loci.

Genetic impacts of stocking prior to the 1950s on what we have called native populations cannot be entirely ruled out; however, records indicate that early attempts to spawn and rear muskellunge in Minnesota were limited and had little success (MGFC 1912; MDC 1934). If an unknown strain was stocked successfully, there should have been unaccountable ancestry and it should have been shared by some of the lakes. Instead, all of the ancestry detected in lakes with introduced populations was consistent with known stocked strains. We found only one ancestry other than that associated with the recorded stocked strains in lakes known to have had native populations. The presumed native ancestry in each lake was distinct with the exception of the

nearby and interconnected pairs of lakes, Moose and Deer lakes and Little Boy Lake and Lake Wabedo. Together, the records and data suggest that the ancestries not associated with the four known stocked strains likely derived from the populations native to the respective lakes.

Amount of Ancestry from Stocked Strains

Ancestry from the stocked strains varied widely among lakes and was not related to the amount of stocking for the discontinued Shoepack and Wisconsin strains. Shoepack ancestry within lakes was usually lower than Wisconsin or Iowa ancestry despite the Shoepack strain's being stocked for more years. Varied levels of introgression by stocked fish into native populations have been documented (Madeira et al. 2005; Spies et al. 2007; Finnegan and Stevens 2008), including by studies in central North America near Minnesota. Halbisen and Wilson (2009) found that four of eight supplemented lake trout *Salvelinus namaycush* populations in Ontario showed little or no introgression from hatchery strains. They found that levels of introgression did not correlate with the amount of stocking, a result that is consistent with our findings for muskellunge. Piller et al. (2005) detected little introgression in two populations stocked for decades with lake trout in the neighboring state of Wisconsin. Wilson et al. (2007) found evidence for the persistence of probable native populations of walleye *Sander vitreus* in Lake Superior, Ontario, and varying degrees of introgression by different stocked strains. In other regions, studies of northern pike (an esocid related to muskellunge) suggested high levels of introgression by stocked fish in France (Launey et al. 2006) but little introgression in a brackish-water population in Denmark (Larsen et al. 2005).

Stocked strains have apparently displaced former native populations or replaced extirpated populations in some lakes. The populations in Big Lake and Lake Bemidji showed little differentiation from the Leech Lake population ($F_{ST} = 0.02$ and 0.03 , respectively). These two lakes were historically connected to Leech Lake via the Mississippi River (dams now act as barriers to upstream movement), and they could presumably have had genetically indistinguishable native populations. However, Cass Lake is located between these two lakes and Leech Lake and has a genetically differentiated population ($F_{ST} = 0.09$), as do two other interconnected lakes, Little Boy Lake and Lake Wabedo ($F_{ST} = 0.17$ and 0.14 , respectively), indicating that Leech strain stocking is the most likely explanation for the genetic similarity between the Big Lake, Lake Bemidji, and Leech Lake samples. The muskellunge in Big Mantrap Lake appeared to have entirely Shoepack strain ancestry after 15 consecutive years of stocking this strain. The separation of Big Mantrap Lake and Shoepack Lake into separate major drainages makes it likely that the similarity of these populations ($F_{ST} = 0.01$) resulted from stocking and not from a lack of differentiation between the native Big Mantrap Lake and Shoepack Lake populations. Few native muskellunge were thought to remain prior to stocking in Big Lake and Lake Bemidji, and the abundance in Big Mantrap

Lake was uncertain (MNDNR, unpublished data). The low productivity of these populations may have made them vulnerable to displacement, which has been shown for other fish species (Evans and Willox 1991), but it is also possible that muskellunge populations were extirpated from these lakes prior to stocking.

Reproduction by Stocked Strains

Natural reproduction by stocked fish (as evidenced by admixed individuals) depended on the lake and not the strain stocked, especially for introduced populations. Dombeck et al. (1986) and Rust et al. (2002) found that the variables explaining the levels of muskellunge reproduction in Midwestern lakes included limited northern pike abundance, water level changes during spawning season, high alkalinity, a high shoreline development factor, drainage-lake systems, and woody debris. Northern pike were present in all of our stocked lakes at various densities. We did not attempt to evaluate habitat as part of this study, but we did observe that lakes in which introduced populations had no indications of natural reproduction were small (79–411 ha), while those with natural reproduction were large (529–53,627 ha). Lake size is clearly not a limiting factor, however, as 24 of the 44 Minnesota lakes considered to have had native populations are less than 411 ha in area (Younk and Pereira 2003).

The lack of reproduction in several introduced populations explains their lack of Shoepack strain ancestry. Shoepack strain stocking had ended 18–25 years prior to sampling, so most of the stocked individuals of this strain had likely died out in these lakes. Three introduced populations had relatively high Wisconsin strain ancestry but no indications of natural reproduction. The Wisconsin strain was stocked more recently than the Shoepack strain, so the nonadmixed individuals were likely stocked fish. Wisconsin ancestry will diminish as the stocked fish die out and the population is maintained through stocking of the Leech strain.

Management Implications

Remnant native ancestry persisted in several stocked lakes, so management designed to conserve genetic structure and possible local adaptations should not be dismissed as “too late” (see Hansen and Mensberg [2009] for a similar conclusion concerning a brown trout *Salmo trutta* population in Denmark). The MNDNR no longer stocks the lakes Baby, Moose, Deer, Cass, Little Boy, and Wabedo, whose populations retain an estimated 55–95% native ancestry. We suggest continuing this policy, but if stocking is deemed necessary that supplementation using broodstock from the lake itself be considered. As a second option, the Leech strain has the advantage of being a broodstock derived from a nearby lake in the same local drainage, so similar forces of selection may have shaped its genetic composition. The MNDNR should consider the feasibility of establishing other broodstocks to support stocking in the Hudson Bay or Lake Superior drainages (Wingate and Younk 2007) or stock where interactions with native muskellunge populations would

be minimal. The low Shoepack strain ancestry in many lakes despite years of stocking provides evidence supporting a cautious approach to stocking into naturally reproducing native populations. Some nonlocal broodstocks may be poorly adapted in the recipient environment, and introgression may lead to the reduction in fitness known as outbreeding depression.

Prior to this study, there was cause for managers to be concerned that Shoepack ancestry could be limiting the size attained by muskellunge in numerous lakes. The Shoepack strain had been stocked into 17 of our 20 study lakes, which included many of the popular muskellunge fisheries in Minnesota. The first population we studied, that of Moose Lake, had substantial Shoepack ancestry and there was evidence that Shoepack strain descendants were not attaining large sizes (Miller et al. 2009). In contrast, this study showed that Shoepack ancestry is likely having limited effects on size structure in most of our study populations because of its low persistence. We did not further evaluate the effect of Shoepack ancestry on fish size because relatively few fish had Shoepack ancestry and older muskellunge are difficult to age from scales (Fitzgerald et al. 1997). But where low persistence rules out possible effects of Shoepack ancestry, MNDNR biologists can focus on other biotic and abiotic factors if size structure is a concern. Studies have shown that muskellunge grow more slowly depending on the species (Wahl and Stein 1988) and size (Wahl and Stein 1993) of available prey. Abiotic factors, especially temperature, have been shown to affect muskellunge growth (Bevelhimer et al. 1985; Wahl and Stein 1991; Clapp and Wahl 1996). Lake size, which may affect temperatures and other abiotic factors, ranges from as small as 23 ha to as large as 53,627 ha across Minnesota’s muskellunge waters (Younk and Pereira 2003). Angler harvest truncated the size distributions of muskellunge in parts of Minnesota by the 1940s (Olson and Cunningham 1989) and may still affect size structure, although muskellunge now support mainly a catch-and-release trophy fishery (Wingate and Younk 2007).

The information we provided in this study will help MNDNR manage genetic diversity among muskellunge populations and allays concerns that Shoepack strain stocking had imposed persistent and widespread limitations on the size potential of muskellunge. Our study identifies the few lakes in which efforts might address Shoepack ancestry in attempts to improve size structure. For example, in one lake managers have decided to remove individuals with high Shoepack strain ancestry during a multiyear marking study of population dynamics. For introduced populations with natural reproduction, managers could intensify monitoring to determine the contribution of natural reproduction to recruitment and adjust stocking accordingly. Our study reinforces the findings that genetic data are often needed to determine ancestry in stocked fish populations, as stocking histories alone may be a poor indication of current genetic composition (e.g., Larsen et al. 2005; Wilson et al. 2007; Finnegan and Stevens 2008). As managers increasingly consider genetic differences among populations when making stocking decisions (e.g., Rider 2006; Welsh et al. 2010), including for muskellunge

(Jennings et al. 2010), genetic tools will help to identify where native genetic diversity persists despite past stocking or to select nonadmixed individuals for broodstocks. Fish geneticists often express concern about disrupting genetic structure and causing outbreeding depression (Hindar et al. 1991; Utter 2003), but stocking may affect traits of direct concern to anglers. The potential to alter heritable performance traits like growth and angling vulnerability (Philipp et al. 2009) should also be considered when making stocking decisions.

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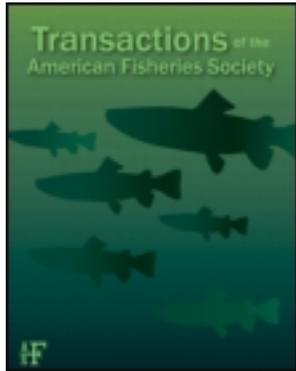
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The Effects of Neutrally Buoyant, Externally Attached Transmitters on Swimming Performance and Predator Avoidance of Juvenile Chinook Salmon

Jill M. Janak^a, Richard S. Brown^a, Alison H. Colotelo^a, Brett D. Pflugrath^a, John R. Stephenson^a, Z. Daniel Deng^b, Thomas J. Carlson^c & Adam G. Seaburg^d

^a Pacific Northwest National Laboratory, Ecology Group, Post Office Box 999, Richland, Washington, 99352, USA

^b Pacific Northwest National Laboratory, Hydrology Group, Post Office Box 999, Richland, Washington, 99352, USA

^c Pacific Northwest National Laboratory, Marine Sciences Laboratory, 1520 West Sequim Bay Road, Sequim, Washington, 98382, USA

^d Columbia Basin Research, School of Aquatic and Fishery Sciences, University of Washington, 1325 Fourth Avenue, Suite 1820, Seattle, Washington, 98101-2509, USA

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ARTICLE

The Effects of Neutrally Buoyant, Externally Attached Transmitters on Swimming Performance and Predator Avoidance of Juvenile Chinook Salmon

Jill M. Janak, Richard S. Brown,* Alison H. Colotelo, Brett D. Pflugrath, and John R. Stephenson

Pacific Northwest National Laboratory, Ecology Group, Post Office Box 999, Richland, Washington 99352, USA

Z. Daniel Deng

Pacific Northwest National Laboratory, Hydrology Group, Post Office Box 999, Richland, Washington 99352, USA

Thomas J. Carlson

Pacific Northwest National Laboratory, Marine Sciences Laboratory, 1520 West Sequim Bay Road, Sequim, Washington 98382, USA

Adam G. Seaburg

Columbia Basin Research, School of Aquatic and Fishery Sciences, University of Washington, 1325 Fourth Avenue, Suite 1820, Seattle, Washington 98101-2509, USA

Abstract

Migrating juvenile salmonids experience rapid decompression that could result in injury or mortality due to barotrauma as they pass turbines at hydropower facilities. Recent research indicates that the risk of injury or mortality due to barotrauma is higher in fish bearing surgically implanted transmitters. Since tagged fish are used to represent the entire population, this tag effect potentially leads to inaccuracies in survival estimates for fish passing turbines. This problem led to development of a novel transmitter, the use of which may eliminate bias associated with the passage of transmitter-bearing fish through turbines. Juvenile Chinook salmon *Oncorhynchus tshawytscha* were tagged with two different neutrally buoyant, externally attached transmitters (types A and B). The effects of transmitter presence on swimming performance were examined by comparing critical swimming speeds (U_{crit} ; an index of prolonged swimming performance) of externally tagged fish, untagged individuals, and fish that received surgically implanted Juvenile Salmon Acoustic Telemetry System acoustic transmitters. Fish tagged with external transmitters had lower U_{crit} than untagged individuals. However, there was no difference in U_{crit} between fish with external transmitter type A or B and fish with surgically implanted transmitters. Testing was conducted to determine whether predator avoidance was affected by the presence of type A transmitters compared with untagged fish. No difference in predation mortality was detected between tagged and untagged fish. Although results suggest that U_{crit} was affected by externally attached transmitters in comparison with untagged fish, the overall impact as reflected by survival was similar; field-based survival studies involving juvenile salmonids passing through hydroturbines are recommended. The absence of swimming performance effects in fish with external tags relative to fish with internally

*Corresponding author: rich.brown@pnnl.gov
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implanted transmitters and the lack of an increased predation risk relative to untagged fish suggest that an externally attached, neutrally buoyant transmitter is a viable option for telemetry studies in estimating survival of juvenile salmonids passing through hydroturbines.

Juvenile salmonids that migrate through hydropower facilities can be exposed to rapid changes in pressure, leading to swim bladder expansion and associated barotrauma that is characterized by swim bladder rupture, hemorrhaging, emboli in the gills, and exophthalmia (Brown et al. 2009, 2012a, 2012c). Recent research indicates that juvenile salmon bearing an internally implanted tag or transmitter are more likely to suffer injury or mortality than untagged fish (Carlson et al. 2012). This could be due to (1) an increased air volume in the swim bladder as the fish compensates for the additional excess mass of the transmitter or (2) the area for swim bladder expansion being limited by the transmitter's presence in the body cavity.

Carlson et al. (2012) surgically implanted acoustic transmitters into juvenile Chinook salmon *Oncorhynchus tshawytscha* and subjected the fish to simulated turbine passage. Tag burden (i.e., weight of the transmitter expressed as a percentage of fish body weight) ranged from 0.0% to 6.6%. The rate of mortality and injury in fish increased not only with the magnitude of pressure change but also with tag burden, indicating that the likelihood of injury or mortality during rapid decompression was increased by the additional mass of the transmitter, the volume of the transmitter inside the body cavity, or both factors. Carlson et al. (2012) suggested that this tag bias likely leads to inaccuracies in estimating the survival of fish as they pass through turbines. These results led to the investigation of whether a neutrally buoyant (tag burden in water = 0.0%), externally attached transmitter could provide more accurate estimates of survival during turbine passage (Brown et al. 2012b; Deng et al. 2012).

In addition to the reduction in barotrauma during turbine passage, externally attached transmitters are commonly used in fisheries research and have many other possible advantages over internal implantation, including reduced time for attachment and handling, the potential for being less invasive, and the ability to be easily shed from the fish once the study has concluded (Lucas et al. 1993; Bégout Anras et al. 1998; Jepsen et al. 2002; Cooke et al. 2003; Deng et al. 2012). However, the presence of an externally attached transmitter is often associated with the possibility of impaired swimming performance (i.e., snags and drag) as well as increased susceptibility to predation, especially for smaller fish.

Many studies have examined the swimming performance of fish with surgically implanted transmitters (Adams et al. 1998; Anglea et al. 2004; Brown et al. 2006), but few have examined the influence of externally attached transmitters (Table 1). Thorstad et al. (2000) found no differences in swimming performance among groups of adult Atlantic salmon that received externally attached radio transmitters, surgically implanted

transmitters, or no transmitters (controls). Peake et al. (1997) compared swimming performance of wild and hatchery-reared Atlantic salmon smolts with externally attached, internally implanted, and gastrically implanted radio tags. Those authors found no difference between externally and internally tagged fish; however, swimming performance was lower for externally and internally tagged fish than for untagged controls.

Increased rates of predation on tagged fish can be attributed to trauma from the tagging procedure, tag visibility to predators, and impaired swimming performance due to drag associated with the transmitter or antenna (Ross and McCormick 1981). Several studies of tagging effects on juvenile salmonids' ability to avoid predators (Jepsen et al. 1998; Anglea et al. 2004; Table 2) have found no difference in predation rates between tagged and untagged fish. However, Adams et al. (1998) reported increased rates of predation on juvenile Chinook salmon into which radio transmitters were surgically and gastrically implanted relative to untagged controls.

Although previous studies have found that external attachment of transmitters can alter the swimming performance and behavior of fish, there is a paucity of research on the effects of externally attached acoustic transmitters on juvenile salmonids. Recent technological advances have led to a reduction in the size of acoustic transmitters, making it possible to study smaller fish. With the decrease in transmitter size resulting in lower tag burdens, external attachment of acoustic transmitters to juvenile salmonids has become a more plausible option for biotelemetry studies. The objective of this research was to determine whether the swimming performance and predator avoidance ability of juvenile Chinook salmon would be compromised by the external attachment of a neutrally buoyant acoustic transmitter that was developed for monitoring the survival of juvenile salmonids passing through hydroturbines.

METHODS

Fish acquisition and holding.—Juvenile fall Chinook salmon were originally obtained as eyed eggs from Priest Rapids Hatchery (Washington Department of Fish and Wildlife) in December 2009. Fish were reared at the Aquatic Research Laboratory (ARL), Pacific Northwest National Laboratory, Richland, Washington. During the study period, all test fish were held inside the ARL in 650-L circular tanks. All holding and test tanks were supplied with 15.0–17.8°C well water. Fish within the rearing and test population were fed an ad libitum ration of Bio Vita Starter (Bio-Oregon, Longview, Washington). Fish that were selected for testing were unfed for 24 h prior to

TABLE 1. Summary of studies examining the effects of transmitters on swimming performance of salmonids (CS = Chinook salmon; RT = rainbow trout *Oncorhynchus mykiss*; SS = sockeye salmon *O. nerka*; AS = Atlantic salmon *Salmo salar*; GI = gastric implantation; SI = surgical implantation; EX = external attachment). Values for length, mass, and tag burden are means or ranges of means (values in parentheses are full ranges of values). Tag burden is the transmitter weight in air expressed as a percentage of fish weight in air. Externally attached transmitters used in the present study were neutrally buoyant, thus applying no tag burden to the fish when in water. Data presented are for all fish tested, including controls and shams.

Reference	Species	<i>n</i>	Tag type	Method of attachment	Length (mm)	Mass (g)	Tag mass in air (g)	Tag burden (%)
Adams et al. (1998)	CS	128	Radio	GI, SI	(95–160)		1.0	(2.2–10.4)
Brown et al. (1999)	RT	38	Radio	SI	88–89	(5.0–10.0)	0.6	(6.0–12.0)
Anglea et al. (2004)	CS	156	Acoustic	SI	(122–198)	(22.2–99.0)	1.5	(1.4–6.7)
Brown et al. (2006)	CS	150	Acoustic	SI	108–110 (94–125)	13.1–13.8 (6.7–23.1)	0.7	(3.1–10.7)
Brown et al. (2006)	SS	150	Acoustic	SI	113–114 (101–133)	11.2–11.5 (7.0–16.0)	0.7	(4.5–10.3)
Thorstad et al. (2000)	AS	168	Radio	EX, SI	(450–590)	(1,021–2,338)	15.1, 25.0	<1.0 (in water)
Peake et al. (1997)	AS	126	Radio	SI, GI, EX	185–208	54.0–112.5	2.6	(1.8–6.0)
Robertson et al. (2003)	AS	80	Radio	SI	143–144	29.2–31.9	0.75	2.4–2.5
Present study	CS	102	Acoustic	EX, SI	124 (98–135)	22.0 (9.0–30.7)	0.53 ^a	2.3 (1.9–2.6) ^a

^aRelates only to SI.

TABLE 2. Summary of studies examining predator avoidance by salmonids (CS = Chinook salmon; AS = Atlantic salmon; BT = brown trout *Salmo trutta*; GI = gastric implantation; SI = surgical implantation; EX = external attachment). Values for length, mass, and tag burden are means or ranges of means (values in parentheses are full ranges of values). Tag burden is the transmitter weight in air expressed as a percentage of fish weight in air. Externally attached transmitters used in the present study were neutrally buoyant, thus applying no tag burden to the fish when in water. Data presented are for all fish tested, including controls and shams.

Reference	Species	<i>n</i>	Tag type	Method of attachment	Length (mm)	mass (g)	Tag mass in air (g)	Tag burden (%)
Adams et al. (1998)	CS	384	Radio	GI, SI	(95–160)		1.0	(2.2–10.4)
Anglea et al. (2004)	CS	160	Acoustic	SI	(122–198)	(22.2–99.0)	1.5	(1.4–6.7)
Jepsen et al. (1998)	AS	50	Radio	SI	(160–180)		1.4, 1.7	
Jepsen et al. (1998)	BT	24	Radio	SI	(160–240)		1.4, 1.7	
Present study	CS	113	Acoustic	EX, SI	125–143 (105–155)	24.0–33.3 (13.2–40.4)	NA	NA

tagging or testing. Fish in both test groups (swimming performance and predator avoidance) ranged from 98 to 155 mm in fork length (FL) and from 9.0 to 40.4 g in weight (Tables 3, 4).

The adult rainbow trout that were used as predators were obtained from Trout Lodge Hatchery (Soap Lake, Washington) in November 2010. All predators were held outside the ARL in two 2,000-L circular tanks prior to the study period. Holding

tanks were supplied with 15–16°C well water. Predators ranged from 300 to 460 mm FL and from 400 to 1,200 g in weight.

Tagging procedures.—Four treatment groups were used in the swimming performance tests: (1) fish that were tagged with an external transmitter anterior to the dorsal fin (type A; Figure 1a), (2) fish that were tagged with a two-part external transmitter beneath the dorsal fin (type B; Figure 1b), (3) fish

TABLE 3. Mean \pm SD and range of fork length (FL) and weight for each treatment group of juvenile Chinook salmon evaluated for swimming performance (JSATS = Juvenile Salmon Acoustic Telemetry System; PIT = passive integrated transponder).

Treatment	<i>n</i>	FL (mm)		Mass (g)	
		Mean \pm SD	Range	Mean \pm SD	Range
External transmitter type A (anterior to dorsal fin)	30	123 \pm 6.4	111–135	21.2 \pm 4.2	14.2–30.4
External transmitter type B (two-part transmitter, beneath dorsal fin)	31	126 \pm 7.3	102–135	22.6 \pm 4.5	11.9–30.3
Internal transmitter (JSATS tag + PIT tag)	10	125 \pm 4.4	119–132	23.1 \pm 2.4	20.3–27.7
Control (untagged)	31	124 \pm 8.6	98–135	21.8 \pm 5.5	9.0–30.7
All treatments	102	124 \pm 7.2	98–135	22.0 \pm 4.6	9.0–30.7

TABLE 4. Mean \pm SD (range in parentheses) fork length (FL) and weight of juvenile Chinook salmon used in predator avoidance trials. Several trials show results for fewer than 10 fish because some fish jumped out of the tank during testing.

Trial	Tagged fish			Untagged fish		
	<i>n</i>	FL (mm)	Mass (g)	<i>n</i>	FL (mm)	Mass (g)
1	7	136 \pm 8 (117–145)	29.8 \pm 5.0 (18.9–35.9)	10	137 \pm 12 (106–152)	31.0 \pm 6.9 (13.2–39.7)
2	7	140 \pm 6 (127–149)	30.5 \pm 4.2 (22.2–38.8)	10	143 \pm 7 (128–155)	33.3 \pm 4.4 (25.2–40.4)
3	10	125 \pm 7 (113–135)	24.0 \pm 4.2 (16.1–31.2)	10	129 \pm 6 (114–135)	25.1 \pm 3.8 (18.5–32.4)
4	10	129 \pm 5 (120–134)	25.9 \pm 3.1 (19.1–29.4)	9	128 \pm 6 (120–135)	25.1 \pm 4.7 (14.8–31.4)
5	10	130 \pm 5 (115–135)	28.9 \pm 3.3 (19.4–32.1)	10	130 \pm 4 (125–135)	28.6 \pm 2.6 (23.5–31.9)
6	10	128 \pm 8 (105–135)	28.4 \pm 5.5 (14.1–35.0)	10	130 \pm 5 (118–134)	29.7 \pm 3.4 (22.6–34.4)
Overall	54	131 \pm 8 (105–149)	27.7 \pm 4.7 (14.1–38.8)	59	133 \pm 9 (106–155)	28.6 \pm 5.2 (13.2–40.4)

that received an internally implanted Juvenile Salmon Acoustic Telemetry System (JSATS; McMichael et al. 2010) acoustic transmitter and a passive integrated transponder (PIT) tag (Destron Technologies, St. Paul, Minnesota), and (4) untagged controls (Table 3). Information on the dimensions and characteristics of the transmitters used in the present study is detailed by Deng et al. (2012).

Deng et al. (2012) found that fish receiving type A transmitters attached using Monocryl 5-0 absorbable monofilament sutures (Ethicon, Inc., Somerville, New Jersey) exhibited better growth than fish that were tagged with type B transmitters. Because of these differences, only two groups were used for predation trials: fish that received type A transmitters (attached with Monocryl 5-0 absorbable sutures) and untagged controls.

To eliminate tagging or handling bias, all tagging was performed by one person (Deters et al. 2010). The daily order in which tagging was performed (i.e., type A or type B transmitter) was randomized. An 80-mg/L solution of tricaine methanesulfonate (MS-222) buffered with an 80-mg/L solution of sodium bicarbonate was used to anesthetize the fish until

they reached stage 4 anesthesia (as described by Summerfelt and Smith 1990). The FL (mm) and mass (g) of each fish were measured while the fish were anesthetized. Fish were placed on a foam rubber pad and were oriented dorsal side up for external attachment or ventral side up for internal implantation. A small tube was inserted into the fish's mouth during tagging to provide a constant maintenance flow of 40-mg/L MS-222 buffered with a 40-mg/L solution of sodium bicarbonate.

External transmitter attachment was performed as described in detail by Deng et al. (2012). Type A transmitters were attached anterior to the dorsal fin by using two sutures that were threaded through the dorsal musculature and secured by a $2 \times 2 \times 2 \times 2$ knot (as described by Deters et al. 2012) that rested in grooves on the top of the transmitter. Type B transmitters were attached using two 25-gauge, 2.22-cm (0.875-in) hypodermic needles (Becton, Dickinson, and Company, Franklin Lakes, New Jersey) to guide the wires (attached to the battery side of the transmitter) through the dorsal musculature. The needles were then removed, the wires were threaded through the transducer side of the transmitter, and the excess wire was trimmed.

Internal transmitters were surgically implanted by making a 6–7-mm incision on the linea alba, inserting a JSATS tag and a PIT tag, and closing the incision with two simple interrupted sutures (Monocryl 5-0 absorbable monofilament) using a $1 \times 1 \times 1 \times 1$ knot (similar to Panther et al. 2011; Deters et al. 2012).

After all tagging procedures (or handling for controls) were completed, fish were allowed to recover in a 20-L bucket containing oxygenated water. After recovery, fish were placed in a floating 20-L bucket (perforated to allow flow-through of water), which was placed in a 650-L circular tank inside the ARL; fish were held in the tank for approximately 24 h prior to testing. Lights inside the ARL were controlled automatically to follow the natural photoperiod.

Swimming performance tests.—A Blazka-type respirometer was used to conduct swimming performance tests. The relationship between water velocity in the swim chamber and motor speed was calibrated using a type S pitot tube (United Sensor Corp., Amherst, New Hampshire). Flow straighteners at the upstream end of the tube were used to achieve uniform water



FIGURE 1. Juvenile Chinook salmon with external transmitters attached: (a) type A transmitter, painted with a green base coat and dark-green spots (used for predation trials and swimming performance tests); and (b) type B transmitter (used for swimming performance tests only). [Figure available online in color.]

velocity within the swim chamber. The swim chamber had an electrified grid at the downstream end. A black shade was placed at the upstream end of the swim chamber during testing to provide shelter and orientation. Flow-through well water (16.8–17.8°C) was supplied to the swim chamber during the tests.

Swimming performance tests were conducted during November 8–December 17, 2010. For each trial, one fish was selected at random and placed inside the swim chamber. Fish were given a 30-min acclimation period during which the respirometer velocity was set at 1 body length (BL)/s. Thereafter, the velocity was increased by 0.5 BL/s every 15 min. When a fish stopped swimming and fell back to the downstream end of the swim chamber, the shocking grid was activated to emit a 6–12-V shock. The fish received a 1-s shock if it came in contact with the grid. If the fish did not swim away from the grid, the fish was shocked consecutively at 1-s intervals for 10 s. If the fish remained on the grid at the end of 10 s, the motor was stopped to allow the fish to swim away from the grid. The velocity was set back to the acclimation speed and was increased gradually to the last velocity setting. If the fish did not swim away from the grid, the fish was considered to be fatigued and received no further shocks. If the fish continued to swim, the procedure was continued until the fish became fatigued. When the fish was considered fatigued, it was removed from the swim chamber and euthanized with MS-222 (250 mg/L). Critical swimming speed (U_{crit}) was calculated based on the formula of Brett (1964):

$$U_{crit} = u_1 + [(t_i/t_{ii}) \times u_{ii}], \quad (1)$$

where u_1 = the highest velocity (cm/s) maintained for the prescribed period, u_{ii} = the velocity increment (cm/s), t_i = time (min) for which the fish swam at the “fatigue” velocity, and t_{ii} = prescribed period of swimming (min).

Predator avoidance tests.—Juvenile fall Chinook salmon were randomly designated as treatment fish (tagged; type A external transmitter) or control fish (untagged) for the predation trials. Type A transmitters were air-brushed with a mixture of green, black, white, and blue paint (CS Coatings, Wausau, Wisconsin) before attachment. The paint camouflaged the transmitter by mimicking the coloring of Chinook salmon (Figure 1a). Sample size for both groups combined was between 17 and 20 fish/trial. Several trials had fewer fish because some fish jumped out of the tank during testing.

Rainbow trout were chosen as predators because of their performance as test predators in previous studies and the ease with which they acclimate to the test environment (Neitzel et al. 2000; Anglea et al. 2004). Ten rainbow trout were held in the 2,000-L circular test tank for an acclimation period of 8 weeks prior to the start of the predation trials. During the acclimation period, predators were conditioned to prey on live fish (as described by Anglea et al. 2004) by feeding them juvenile Chinook salmon (~130 mm FL; 30 g).

Predation trials lasted from December 7, 2010, to January 12, 2011. Trials were at least 7 d apart, and predators were not

fed between trials. To begin the trial, 10 tagged fish and 10 untagged fish were placed in 20-L buckets and were introduced into the 2,000-L circular predation tank by emptying the buckets directly into the tank. Trials started approximately 24 h after the fish were tagged.

Video cameras were set up above the tank to remotely monitor the rates of predation and minimize outside disturbances. Observations from the live video feed were made at 15-min intervals, and observations at the tank were made every hour until the end of the trial. Trials ended when 50% of the prey were consumed or after 8 or 24 h if less than 50% of the prey were consumed. If injuries from predation attempts were serious (e.g., fish lying on the tank bottom), fish were categorized as “consumed” based on the assumption that those fish would not survive the trial. At the end of the trial, all remaining juvenile Chinook salmon were removed from the tank and euthanized with a 250-mg/L solution of MS-222. All fish were externally examined for injuries related to predation attempts.

Statistical analysis.—Differences in U_{crit} among transmitter treatment groups were tested using ANOVA. The first analysis included three groups (type A, type B, and control). The analysis was performed again with the addition of the fourth treatment group (fish with internally implanted transmitters). In addition to transmitter type, the influence of fish length on U_{crit} was examined. The ANOVA was also used to compare each pair of transmitter treatments. To control for the increased probability of a type I error, a Šidák correction was used to adjust the rejection region, depending on the number of pairwise tests:

$$\alpha_{family} = 1 - (1 - \alpha_{comparison})^{1/t}, \quad (2)$$

where t = the number of pairwise tests, $\alpha_{comparison} = 0.05$, and α_{family} = the new familywise error rate.

For swimming performance, power curves were constructed to show the sample size needed for comparing any pair of tagging treatments. Assuming homogeneous variances, the mean square error from the overall ANOVA test was used as an estimate of variance in making calculations involving power. Assuming that the mean square error and sample mean difference between two treatments do not change with increased sample size, we calculated the estimated power and percentage of detectable difference for different levels of n . This was done for the observed sample mean differences.

For the predator avoidance trials, ANOVA was used to test whether tagged (type A) and untagged groups differed in the proportion of fish surviving. All assumptions of parametric tests were met (i.e., independence, normality, and homogeneity of variance). A significance level of 0.05 was used.

RESULTS

Swimming Performance

Comparison between fish with external transmitters and control fish.—Mean U_{crit} for juvenile Chinook salmon ranged from

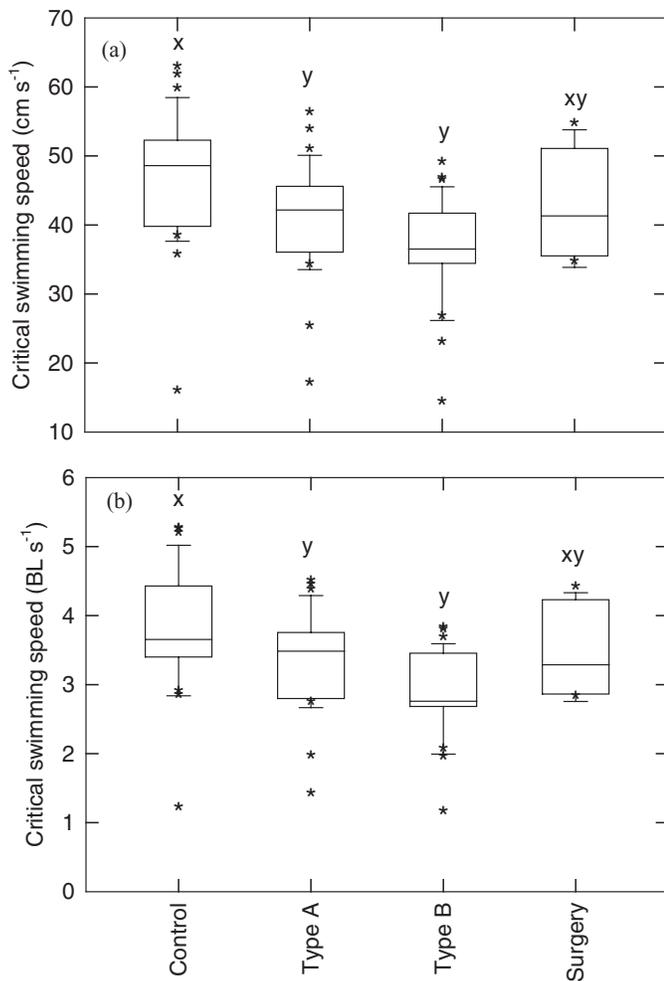


FIGURE 2. Box plots of critical swimming speed in (a) centimeters per second and (b) body lengths (BL) per second for juvenile Chinook salmon that received external transmitters of type A or type B, surgically implanted internal transmitters (Juvenile Salmon Acoustic Telemetry System tag and passive integrated transponder tag), or no tags (control fish). Significant differences ($P < 0.009$) between treatment groups are indicated by differing letters (line within each box = median; lower edge of box = 25th percentile; upper edge of box = 75th percentile; ends of whiskers = $1.5 \times$ interquartile range; asterisks = outliers).

36.7 to 46.7 cm/s (Figure 2). The U_{crit} varied significantly with both fish size ($P < 0.0001$; decreasing with increasing fish size) and transmitter type ($P < 0.0001$). Control fish had significantly higher U_{crit} (mean $U_{crit} = 46.7$ cm/s) than fish with external transmitters of type A (mean $U_{crit} = 41.2$ cm/s; $P = 0.0087$) or type B (mean $U_{crit} = 36.7$ cm/s, $P < 0.0001$). The U_{crit} did not significantly differ between fish tagged with type A transmitters and those tagged with type B transmitters ($P = 0.038$).

The sample sizes from these experiments provided high overall power to determine differences among treatment groups. The sample data from experiments showed that the maximum difference in sample means between treatment groups was 0.07 BL/s (the maximum difference between fish with type B transmitters

and control fish). The data obtained were sufficient to detect a 10% difference with a power of 75%, a 15% difference with a power of 97%, and a 20% difference with a power approaching 100%. The mean U_{crit} for control fish was 11.3% higher than the mean for fish tagged with type A transmitters. Data obtained from these experiments were sufficient to detect this difference with a power of 84%. The mean U_{crit} for controls was 22.5% higher than that of fish with type B transmitters; the power to detect this difference was 99.99%. The mean U_{crit} for fish with type A transmitters was 12.6% higher than that of fish with type B transmitters, and the power to detect this difference was 91%.

Comparison between externally tagged fish and control fish or fish with internally implanted transmitters.—When fish that received internally implanted transmitters were added as a pilot-scale comparison, there was also a significant difference in swimming performance related to fish length (decreasing with increasing FL; $P < 0.001$) and transmitter type ($P = 0.001$). Control fish still had significantly higher U_{crit} than fish with external transmitter type A ($P = 0.0087$; Table 5) or external transmitter type B ($P < 0.0001$). However, there was no significant difference between fish with internally implanted transmitters (mean $U_{crit} = 42.9$ cm/s) and control fish ($P = 0.2245$), fish with type A transmitters ($P = 0.512$), or fish with type B transmitters ($P = 0.0317$). The mean U_{crit} of control fish was 9.0% lower than that of fish with internally implanted transmitters; the power to detect this difference was 29%. The mean U_{crit} for fish that received internally implanted transmitters was 2.5% higher than the mean U_{crit} for fish that received type A external transmitters, with a power of 6% to detect this difference. The U_{crit} of fish with internally implanted transmitters was 15% higher than the U_{crit} of fish that received type B external tags, with a power of 55% to detect this difference.

Predator Avoidance

The percentage of juvenile Chinook salmon consumed by predators was not significantly different ($P = 0.2622$) between tagged (type A) and untagged groups. The percentage of fish consumed did not significantly differ ($P = 0.8263$) among the six predation trials conducted. The percentage consumed averaged 38.9% for untagged fish compared with 47.6% for tagged fish (Figure 3); the estimated difference in survival was 8.7% between the two groups.

DISCUSSION

Juvenile Chinook salmon (98–135 mm) that were tagged with external transmitter types A and B exhibited lower swimming performance than untagged fish. Similar results were reported by Peake et al. (1997) in examining the effects of external transmitters on the swimming performance of Atlantic salmon smolts (range of mean lengths, 185–208 mm; Table 1 provides fish size and tag burden details from the Peake et al. [1997] study and other studies).

Swimming performance of fish that received internally implanted acoustic transmitters was similar to the swimming

TABLE 5. Results of ANOVA comparing critical swimming speed (i.e., U_{crit}) scores (with fork length and tag type as covariates) for juvenile Chinook salmon in pairs of tagging treatment groups (fish with external transmitter types A and B; fish that received surgically implanted internal transmitters [Juvenile Salmon Acoustic Telemetry System tag and passive integrated transponder tag]; and control [untagged] fish). Significant P -values are shown in bold italics ($\alpha_{family} = 0.009$ after Šidák correction; see equation 2).

Comparison	Source	df	Sum of squares	Mean square error	F	P
Type A versus type B	Length	1	4.4522	4.4522	11.8299	0.0011
	Tag type	1	1.6902	1.6902	4.4909	0.0384
	Residuals	58	21.8284	0.3764		
Control versus type B	Length	1	7.7723	7.7723	17.568	0.0001
	Tag type	1	9.7116	9.7116	21.952	<0.0001
	Residuals	59	26.1017	0.4424		
Control versus type A	Length	1	7.2809	7.2809	15.5248	0.0002
	Tag type	1	3.4605	3.4605	7.3788	0.0087
	Residuals	58	27.201	0.469		
Control versus internal	Length	1	5.9544	5.9544	11.8974	0.0014
	Tag type	1	0.763	0.763	1.5246	0.2245
	Residuals	38	19.0182	0.5005		
Type A versus internal	Length	1	2.6528	2.6528	6.5527	0.0147
	Tag type	1	0.1775	0.1775	0.4384	0.512
	Residuals	37	14.979	0.4048		
Type B versus internal	Length	1	1.6785	1.6785	4.7378	0.0358
	Tag type	1	1.7626	1.7626	4.9751	0.0317
	Residuals	38	13.4625	0.3543		

performance of control fish. Other researchers have found similar results for the swimming performance of juvenile Chinook salmon with surgically implanted acoustic transmitters (122–198-mm fish: Anglea et al. 2004; 94–125-mm fish: Brown et al. 2006). However, Brown et al. (2006) found that juvenile sockeye salmon (101–133 mm) with surgically implanted

acoustic transmitters had poorer swimming performance than their untagged counterparts.

Swimming performance of juvenile Chinook salmon with internally implanted acoustic tags was also similar to the swimming performance of fish that were externally tagged with type A and type B transmitters. Although the 10 fish in the internally tagged group were initially added on a pilot scale, the difference in U_{crit} was detected with a moderately high statistical power for the comparison of internally implanted transmitters with type B external transmitters (55% power to detect a difference of 15%). However, there was much lower statistical power to detect any potential difference between fish with internally implanted transmitters and fish with type A external transmitters (6% power to detect the 2.5% difference) or control fish (29% power to detect the 9.0% difference). When the two external transmitter types were compared, we found no difference in swimming performance between fish carrying type A external transmitters and fish with type B transmitters.

Swimming performance also decreased with increasing fish length. This trend was also noted in U_{crit} among the control fish tested by Adams et al. (1998). In addition, Brett (1964) stated that the swimming ability of fish decreases as size increases. However, Peake et al. (1997) found no correlation between U_{crit} and fish length for radio-tagged Atlantic salmon smolts (185–208 mm). The results reported by Peake et al. (1997) mirror those of Brown et al. (2006) for acoustic-tagged juvenile Chinook salmon.

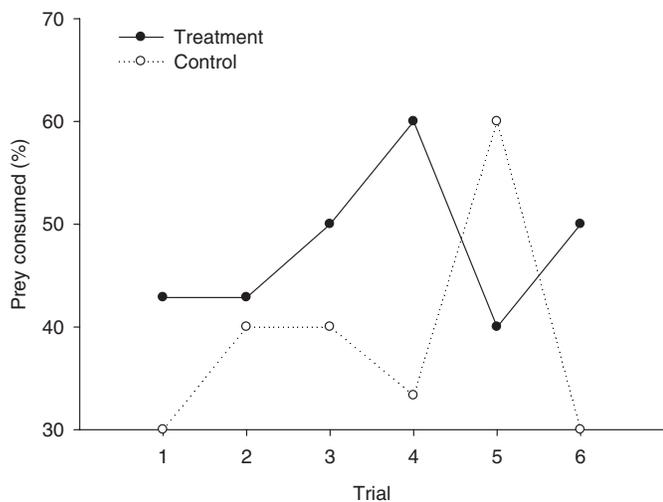


FIGURE 3. Percentages of juvenile Chinook salmon that were consumed by rainbow trout predators during each of six predation trials. Control fish were untagged; treatment fish were tagged with external transmitter type A (i.e., neutrally buoyant). See Table 4 for sample sizes.

Although the swimming performance of externally tagged fish in this study was lower than that of untagged fish, we found no detectable difference in predation rates between tagged and untagged fish. Very few studies have examined the effects of externally attached transmitters on the rates of predation on juvenile salmonids. Although external transmitters have been commonly used in fisheries research, their utility has been somewhat limited to larger fish. The larger size of the fish and the proportionately smaller size of the transmitter could explain why predation effects have not been closely examined. Many factors are involved in a fish's ability to avoid predation; swimming performance, prey conspicuousness, and ability to detect predators may lead to differential predation rates (Bams 1967; Mesa 1994). The presence of an external transmitter has the potential to impair some of these avoidance abilities by possibly creating drag and visible differences among prey. In smaller fish, such as juvenile salmonids, these effects can be magnified and the relative size of the transmitter is imperative. Multiple stressors associated with the tagging process itself may also lead to an increased risk of predation by eliciting physiological and behavioral stress responses, potentially resulting in substandard condition of the prey at the time of their interaction with predators (Temple 1987; Schreck 1990).

The additional mass of a transmitter can cause an increase in fish density, which potentially leads to increased energy expenditure (Lefrançois et al. 2001). This potential increase in energy expenditure could affect both swimming performance and the ability to avoid predation. Although the attachment of an external transmitter adds more surface area to the fish and thus may lead to drag forces, the transmitter used in this study was neutrally buoyant in water. Thus, there was no tag burden for fish bearing external transmitters in our study.

In considering externally attached transmitters, one of the major concerns of researchers is the long-term consequences for the fish. As a juvenile fish grows, a fixed transmitter could have detrimental effects on the fish's well-being, such as inhibited growth and tissue damage. In this study, absorbable monofilament sutures were used for the attachment of type A transmitters. Absorbable monofilament sutures used for surgical implantation of transmitters were expelled in as little as 28 d from juvenile Chinook salmon that were held at 12–17°C (Deters et al. 2012). The acoustic transmitters used in our study have a battery life of approximately 20–70 d. Once the battery has expired, the transmitter and the tagged fish are no longer of use to the researcher. The ability of the external transmitter to be shed after its utility has ended is a major advantage over internally implanted transmitters, which may never be expelled, and over type B transmitters, which were attached by wires and were not designed to be easily shed after conclusion of the research. Deng et al. (2012) found that fish tagged with type B transmitters had significantly lower growth rates after 14 d than fish tagged with type A transmitters and untagged controls. Our swimming performance tests were conducted before those results were available, and the predator avoidance trials were conducted

after those results were obtained. After the growth analysis was completed, only type A transmitters were used for further testing.

Although this research indicates that the swimming performance of externally tagged juvenile Chinook salmon was lower than that of untagged fish, there was no difference in swimming performance between fish with type A or type B external transmitters and fish with internally implanted transmitters. In addition, no difference in predation rates was detected between externally tagged and untagged fish. These results, in combination with the potential advantages of externally attached transmitters (less invasive, transmitter shedding ability, and decreased risk of barotrauma) and the increasingly smaller size of transmitters as technology advances, provide a good indication that an externally attached, neutrally buoyant transmitter may be a viable option for telemetry studies to estimate survival of juvenile salmonids passing through hydroturbines. However, as suggested by Zale et al. (2005), Thorstad et al. (2000), and Brown et al. (2010), conclusive evidence of transmitter effects and the presence of bias resulting from these transmitters will require field studies that involve tagging a wide size range of juvenile salmonids with transmitters and measuring their rates of migration, growth, predation, and survival.

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