

Title:

Genetic stock diversity of juvenile Coho Salmon in selected at-risk freshwater habitats of the lower Kenai River watershed.

Principal Investigator(s):

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Andy Barclay, Gene Conservation Laboratory, Alaska Department of Fish and Game.

Objective

1. Characterize the seasonal and spatial genetic stock diversity of juvenile Coho Salmon in selected at-risk freshwater habitats of the lower Kenai River watershed using mixed-stock analysis with genetic characters, such that the estimated proportional stock compositions for each temporal and geographic stratum are within 10 percentage points of the true values 95% of the time.

Tasks

- a. Augment the genetic stock baseline for Kenai River Coho Salmon to further refine the identifiable stock structure;
- b. Sample and compare the genetic stock composition of juvenile Coho Salmon in selected habitats during the open water period and
- c. Describe habitat characteristics that predict the diversity of stocks utilizing areas that are at-risk of habitat degradation.

Justification

The Kenai River watershed has been heavily impacted by high rates of recreational use and development. As a result, an extensive effort to protect, restore, and reconnect Kenai River freshwater habitats has been underway for the better part of 30 years (Johnson and Pyper 2010; Loshbaugh 2014). The restoration and conservation of these habitats is intended to increase and maintain the capacity of the river by providing food, refuge, and other crucial ecosystem functions for salmon. Although, Coho Salmon are expected to benefit from these activities, the realized benefit to the stock complex is poorly understood, inhibiting the strategic implementation of habitat restoration and conservation efforts.

Commonly, the relative value of a particular river reach or tributary to the overall productivity and capacity of the system has been gaged by the production from spawning adults in a particular area. However, assessing the potential benefits that conservation and restoration activities might provide for Kenai River Coho salmon requires an evaluation of not only when and how juveniles use the conserved or restored habitats but also whether the activity primarily benefits the local population or serves to supplement the wider watershed. Coho Salmon populations in the Kenai River exhibit a complex genetic and geographic structure affecting patterns of juvenile migration and habitat use. Juveniles from discrete sub-stocks are known to

migrate throughout the watershed and rear in various non-natal habitats (Massengill 2013; Burger et al. 1983; Elliott and Finn 1984). An understanding of this re-distribution by discrete stocks is critical to understanding the importance of specific reaches or tributaries to effectively design conservation plans to maintain complex, interconnected habitats to support the entire salmon life cycle..

Age-specific freshwater habitat use patterns for juvenile Coho Salmon in Southcentral Alaska have indicated a general migration of age-0 fish from spawning and incubation areas to habitats more conducive to rearing that are generally characterized by wetland inputs of fine particulate organics and nutrients, grassy over-hanging banks, deep slow moving water, little or no woody debris, and peat or mud substrate bottoms (Bradley et. al., 2016; Neher et al., 2013; and King et al., 2012). The mechanisms driving the selection of these habitats has been attributed to their conduciveness to macrophyte and macroinvertebrate production, optimal water temperatures and refugia from seasonal habitat restrictions (McCormick and Harrison 2011; and Elliott and Finn 1984). Although the Kenai River watershed consists of expansive habitats that possess these attributes many are at risk of being degraded or lost through development activities. In fact, the lower Kenai river is estimated to have lost an average of ~16 acres of wetland a year since 1950 and the quality of many remaining lower river areas has been degraded from development through increased runoff, sedimentation, and fragmentation (Hall and Kratzer 2001; Loshbaugh 2014). The degradation of the freshwater and estuarine habitats that juvenile salmon use for rearing and migration can result in a decline in life history diversity (Bottom et al. 2005) reducing the resiliency of Coho Salmon in the watershed to future habitat change (Schindler et al., 2015).

Monitoring the redistribution of juvenile Coho Salmon during their freshwater residence will provide land managers and policymakers' insights into how management actions, development activities, and other habitat change may affect not only locally produced stocks but the larger stock complex. In this study, we propose to augment a recently developed single nucleotide polymorphism (SNP; Barclay et al. 2017) data set for spawning populations of Coho Salmon in the Kenai River and use standard genetic stock identification techniques to examine the stock origins of rearing Coho Salmon occupying sites most at-risk of habitat degradation. In addition, we will investigate which habitat characteristics may predict stock diversity using linear mixed-effects models. These objectives will help to identify areas and attributes that act to supplement the rearing capacity of the entire Kenai River, thus, aiding in the selection and design of restoration and conservation actions for at-risk habitats that support the rearing requirements for the greatest diversity of Coho Salmon stocks.

Study Area

The glacially turbid Kenai River originates in Cooper Landing at the outlet of Kenai Lake and flows 132 km before entering Cook Inlet. The watershed drains approximately 5,210 km² of mountains, glaciers, forests, and the Kenai Peninsula's second and third largest lakes, Skilak and Kenai lakes. The upper Kenai River main-stem flows approximately 22.5 river kilometers between Kenai and Skilak lakes. The lower main-stem river meanders 80.4 river kilometers below Skilak Lake before intersecting Cook Inlet. A large portion of the watershed is within the boundaries of the Chugach National Forest and Kenai National Wildlife Refuge, but the lower 72 rkm of the main-stem river transects federal, state, municipal, tribal, and private lands and is the

largest developed area in the watershed (Figure 1). The lower watershed consists of a number of tributaries that are dominated by the attributes thought to optimize Coho Salmon freshwater production, yet are subjected to risks posed by urbanization. Field sampling will be focused on lower Kenai River tributaries, which are thought to have the greatest intrinsic potential and are at the highest risk of the cumulative damage related to development, including Soldona Creek, Beaver Creek, Slikok Creek, and a number of small tributaries in the lower Kenai River Estuary.

Beaver Creek

Beaver Creek enters the Kenai River at river mile (RM) 10.25. The first stream mile (SM) is in a residential subdivision, experiences a large amount of boat traffic and has a large number of docks, moorings and walkways. The creek originates from a wetland that includes Beaver, Ootka, Tern and Ermine lakes. The main channel is crossed by two roads; the Kenai Spur Highway (SM 2.6) and marathon road (SM 13.3). A small tributary enters the main channel at SM 2.4 and flows under Beaver loop Road. The culverts at the Kenai Spur Highway and Beaver loop Road crossings were considered partial fish barriers until their replacements in 2009 and 2014 respectively (King and Clark. 2004). The portion of Beaver creek above SM 5.0 falls within the Kenai National Wildlife refuge but is not devoid of anthropogenic impacts. The creek divides into two branches at SM11.9. The east fork is the lesser channel and is also crossed by Marathon Road (SM 3.1) and the road to drill pad 7 (SM 2.1). The Beaver Creek watershed encompasses the Beaver Creek oil and gas exploratory unit and has been heavily impacted by exploration activities since 1967 (<https://www.blm.gov/programs/energy-and-minerals/oil-and-gas/about/alaska/Cook-Inlet-Units>).

Slikok Creek

Slikok Creek divides into two branches at SM 6.5. The north fork is considered the lesser channel and originates from wetlands that include Headquarters, Nordic and Slikok lakes. The main channel originates from bog meadows within the Kenai National Wildlife Refuge and includes a small tributary originating at Tote Lake. Four major roads cross Slikok Creek; College loop Road (SM 0.8), Kalifornsky Beach Road (SM1.1), the Sterling Highway (SM 5.1) and Arc Loop Road (SM 5.3). The major road crossings were acting as fish passage barriers until inadequate culverts were replaced in the late 1990s (King and Clark. 2004). A number of gavel driveways also transect the creek. The Slikok drainage includes residential subdivisions, farms (mostly hay fields), a large junk yard, gravel mines and a large municipal landfill. The drainage has been damaged by grazing livestock) and extensive off-road vehicle activity (Loshbaugh 2014). An area adjacent to the creek was used as a fire training site, and petroleum contamination was found in the ground there. Remediation work was done there from 1992 through 2012, but as of 2019, ADEC still lists it as an active toxic site (http://dec.alaska.gov/Applications/SPAR/CCReports/Site_Report.aspx?Hazard_ID=454). Where the creek flows into the main river (RM 18.75) was set aside as Slikok Creek Recreation Site, and the campus of Kenai Peninsula College. Anglers seeking Kenai River access caused widespread erosion due to bank trampling (Johnson and Pyper 2010) . Efforts to protect both habitat and access included the construction of platforms and walkways and bank closures. Areas within the drainage are infested with invasive reed canary grass and have eradication efforts have begun (Loshbaugh 2014).

Soldotna Creek

Soldotna Creek drainage originates from wetlands northwest of Sevena Lake and enters the Kenai River at RM 22.1. Tree and Dirk's Lake outlets are the major tributaries to Soldotna Creek and enter the creek at SM 9.6 and 7.25, respectively. The watershed is dominated by private property, and development has occurred throughout the drainage. The lower creek is in the center of the city of Soldotna and a large portion of the headwaters are in a large residential subdivision around Mackey Lakes. Five major roads cross Soldotna Creek, three single lane gravel roads (SM 0.25, 2.7, and 4.7), the Sterling Highway (SM 2.25) and East Redoubt Road (0.51). A private road crosses Tree Lake outlet at SM 2.1. The culverts at the Sterling Highway and East Redoubt Road crossings were considered obstacles to salmon passage and subsequently replaced (King and Clark. 2004). The drainage has been damaged by widespread gravel mining and other excavation activities. Northern pike were illegally introduced into Derks Lake in the 1970s and spread into adjacent Lakes within the drainage during high water. Pike were eradicated from the drainage by the end of 2016 (R. Massengill, ADF&G, personal communication, 2019). The City of Soldotna established Soldotna Creek Park on the south side of the creek's mouth in 1988.

Kenai River Estuary

A number of small tributaries enter the estuary between RM 0 and 7. Shqit (Boraas 2009b), Campground (Elliott and Finn 1984) or "No-name" Creek (Orejuela 2015) empties right at the river mouth (RM 0). Airport projects radically transformed its east branch. In 1965 the upper portion of the east branch was dredged and channelized to form a floatplane basin. Ryan Creek runs from the wetlands north of the city of Kenai through the Airport Industrial Park Subdivision into a ravine before entering the river near RM 0.9. During airport construction it was channelized to improve drainage. In the late 1970s, a portion was covered over by the Spur Highway; and about 135 m of it now flows underground (Loshbaugh 2014). Home Creek (Elliott and Finn 1984) and associated wetlands were altered through the development of a gravel pit north of Beaver Loop road and empties into the main river near RM 6.8. The lower creek was dredged and adjacent wetlands were filled (Resource Development Council for Alaska 1982). Kalifonsky Creek (Elliott and Finn 1984), has two branches. Kalifornsky Beach Road partly dammed the western fork near its junction with Bridge Access Road, creating a pond. The smaller, eastern branch headwaters have been dammed by a driveway causeway (Loshbaugh 2014).

Kenai River Mile 12 to 22

The mainstem Kenai River from mile 12 to 22 has a handful of small unnamed tributaries and side channels. Two small tributaries drain the wetlands sounding Sports Lake and enter the river at about RM 15 and one drains lands sounding the Ciechanski subdivision. The off channel habitat is limited in this area and is primarily confined to the lowest section of the reach.

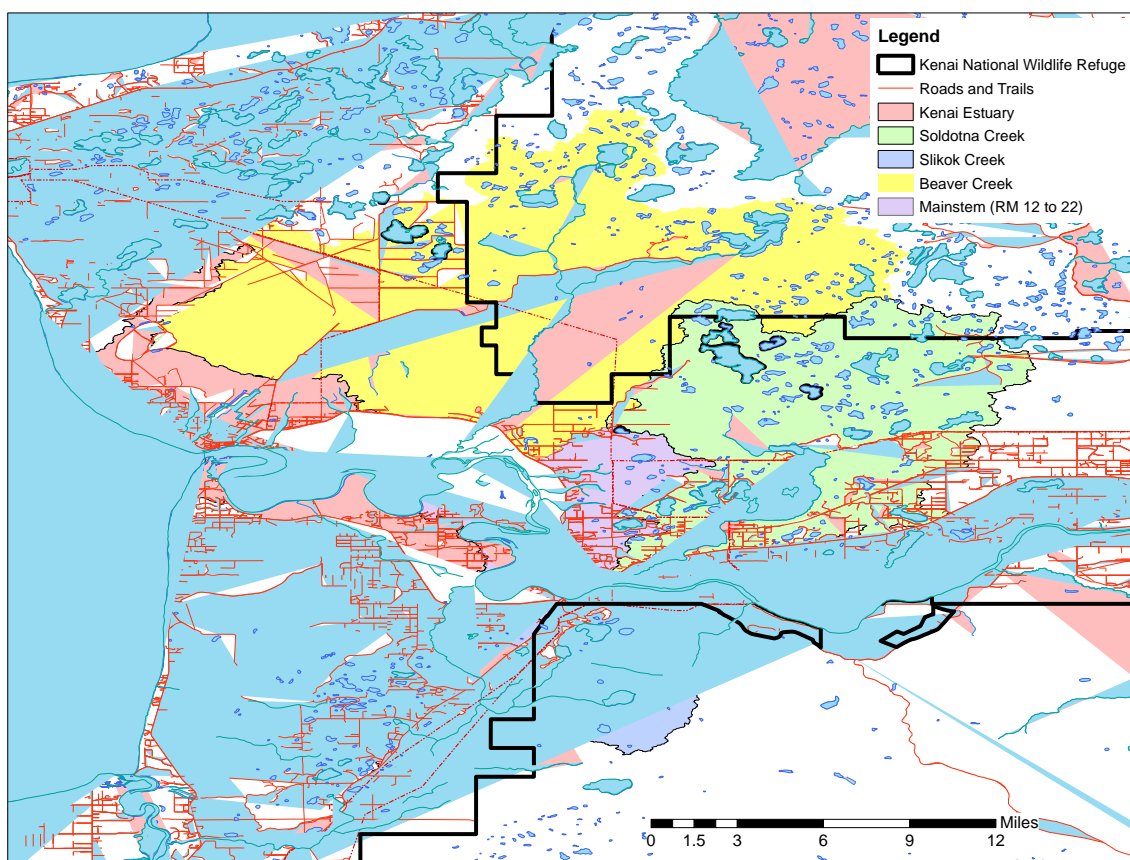


FIGURE 1.– Map of the lower Kenai River watershed illustrating developed areas within each of the 5 study reaches.

Methods

Baseline Augmentation

As a pre cursor to characterizing the seasonal and spatial distribution of juvenile Coho Salmon stocks we will first need to augment the genetic stock baseline for spawning populations of Coho Salmon in the Kenai River. The baseline currently comprises allele frequency estimates for 82 SNP loci surveyed in 1360 samples from 14 locations (Table 1; Barclay et al. 2017). A simulation study was conducted to determine how well mixed-stock analysis is likely to perform with the current baseline and stock groups defined in Table 1. Using the R package *rubias* (Moran and Anderson 2019), 200 mixture samples of N=200 fish were generated using the program defaults to simulate a variety of mixing proportions. Stock compositions were estimated using the Bayesian conditional approach (Moran and Anderson 2019) with leave-one-out cross-validation by gene copies (Anderson et al. 2008). A second simulation study was done, with 200 test mixtures of N=200 created to on average represent a realistic scenario for what might be expected in a reach (Booth 1990; Table 2). With this mixture sample size for these stock groups, stock proportion estimates fell within 10% points of the true value 95% of the time (Figures 2,3).

Table 1. Locations, year sampled and sample size for Coho Salmon collected in the Kenai River for genetic baseline (from Barclay et al. 2017).

Stock Group	Location	Year	N
Tributaries-Upper Kenai Lake	Trail Creek	2006	108
	Grant Creek	2013	95
	South Fork Snow River	1998	71
		2002	24
Tributaries-Lower Kenai Lake	Summit Creek	2002	50
	Quartz Creek	1998	73
	Tern Lake	2002	95
Kenai River Mainstem	below Kenai Lake	2014	95
	below Skilak Lake	1999	78
		2014	95
Tributaries-Middle Kenai River	Russian River	2013	93
		2014	47
	Skilak River	2003	94
Moose River	East Fork Moose River	2002	93
Tributaries-Lower Kenai River	Killey River	2000	67
		2002	25
	Funny River	2006	92
	Slikok Creek	2008	65

Mixture proportions are founded on the probability of mixture genotypes originating from the set of baseline populations. An additional six spawning populations have been identified as potential important contributors to the mixed stock and are proposed to be added to the genetic baseline. These populations include the mainstem River below Naptown rapids, Beaver Creek, Soldotna Creek, King County Creek, the Moose River at the Swan Lake outlet, and the Upper Killey River (Table 3). Further refinement of the baseline will improve representation of the diversity of Kenai River Coho salmon to provide greater accuracy and precision in our stock of origin estimates and thus better describe the stock diversity in rearing areas.

Table 2. Estimated spawning contributions and standard errors by reporting group.

Reporting Group	Contribution ^a	S.E.
Tributary-Upper Kenai Lake	1.8%	0.1%
Tributary-Lower Kenai Lake	2.6%	2.8%
Tributary-Middle River	4.4%	2.4%
Mainstem	77.2%	2.3%
Moose River	6.1%	3.0%
Tributary-Lower River	7.9%	5.7%

^a adapted from Booth 1990

The sampling of spawning fish will be conducted by a variety of means, depending on their location, run timing, and relative abundance. Due to their relatively small spawning populations, dispersed spawning locations and protracted run timings Beaver, Soldotna and King County Creeks will all be sampled with a weir and fish trap. The Moose River at the Swan Lake outlet, and the Upper Killey River will be primarily sampled with a combination of entanglement gill, seines, and dip nets. The mainstem River below Naptown rapids will be sampled by soliciting samples from fisherman targeting known spawning locations and entanglement gill nets. Sampling efforts will coincide with reported peak spawn timing outlined in Table 3. A target sample size of 95 fish per spawning population has been determined to be sufficient to achieve efficient estimates of stock proportions in mixed-stock analysis applications (Kalinowski 2004). Fin clips will be collected from each fish sampled, and stored in either silica desiccant beads or non-denatured ethanol until genetic analysis.

Table 3. Kenai Coho genetic baseline sampling locations, dates, and sample sizes

Sample population	Target Sample Size ^a	Spawn Timing			Proposed Sampling Dates
		Sept.	Oct.	Nov.	
Upper Killey River/ Benjamin Creek	95	— — —	^{b, c}		Sept. 25 to Oct. 1, 2019
Moose River (Swan lake outlet)	95		— — — —	^{b, d}	Oct. 3 to Oct. 8, 2019
King County Creek	94	— — —	^b		Aug. 26 to Oct. 1, 2019
Mainstem Kenai River (Below Naptown Rapids)	95	— — — — — —	^b		Sept. 15 to Nov. 15, 2020
Soldotna Creek	45	— — — — —	^e		Sept 1 to Oct. 1, 2020
Beaver Creek	93	— — —	^e		Sept 1 to Oct. 1, 2020

— denotes a calendar week

^a Sample sizes are the remaining number of samples needed to meet the established goal of 95 fish per population

^b Spawn timing reported by Booth 1990

^c Spawn timing reported by Burger et al 1983

^d Spawn timing reported by Booth and Otis 1996

^e Spawn timing reported by Elliott and Finn 1984

Juvenile Tissue Sample Collection

Stock-specific patterns of juvenile habitat use are expected to vary spatially (dependent on the amount and proximity of optimal habitat to spawning areas), temporally (summer vs winter), and during ontogenetic development (fry, yearling, etc.). Therefore, we propose to sample and compare the genetic stock diversity of juvenile Coho Salmon among supposed at-risk rearing habitats during the spring (April to June), summer (June to September) and fall (September to November) periods. The temporal frequency of sampling is intended to capture the seasonal patterns of stock-specific habitat use previously identified in the lower River (Elliott and Finn 1984). The areas selected for sampling include areas that are both at risk of anthropogenic impacts (Loshbaugh 2014) and have a suspected high intrinsic habitat potential (Bradley et. al., 2016; Neher et al., 2013; and King et al., 2012). The sample size selections required to ensure accurate and precise mixed-stock analysis by time and area was determined to be a total of 200 tissue samples for each stratum (Figures 2, and 3).

We will collect juvenile Coho Salmon in tributaries and estuarine wetlands of the lower mainstem Kenai River using passive two way traps to catch fish entering and leaving each tributary and estuarine reach (Elliott and Finn 1984). Mainstem and off channel sampling will be conducted using minnow traps, or seining. Sampling will be conducted with a rotating sample design so that each of the 15 time (spring, summer and fall) and area (Soldotna Creek, Beaver Creek, Slikok Creek, the Kenai River Estuary, and the Kenai river from RM 12 to 22) strata will be sampled once a week ensuring a sample size of at least 200 fish per sample site and period. This effort will result in a total of 3000 samples taken during the 32 week sampling event (Table 4). We will measure all genetically sampled fish to the nearest millimeter (fork length [FL]), obtain a tissue sample from the caudal fin, and release the fish. To classify Coho Salmon from multiple brood years, we will use the FL frequency distributions to categorize juveniles as age 0 and 1+ (Sethi et al., 2017). Tissue samples will be preserved in non-denatured ethanol. Non-target species and Coho Salmon caught once sample sizes have been reached will be enumerated and released to quantify relative abundances.

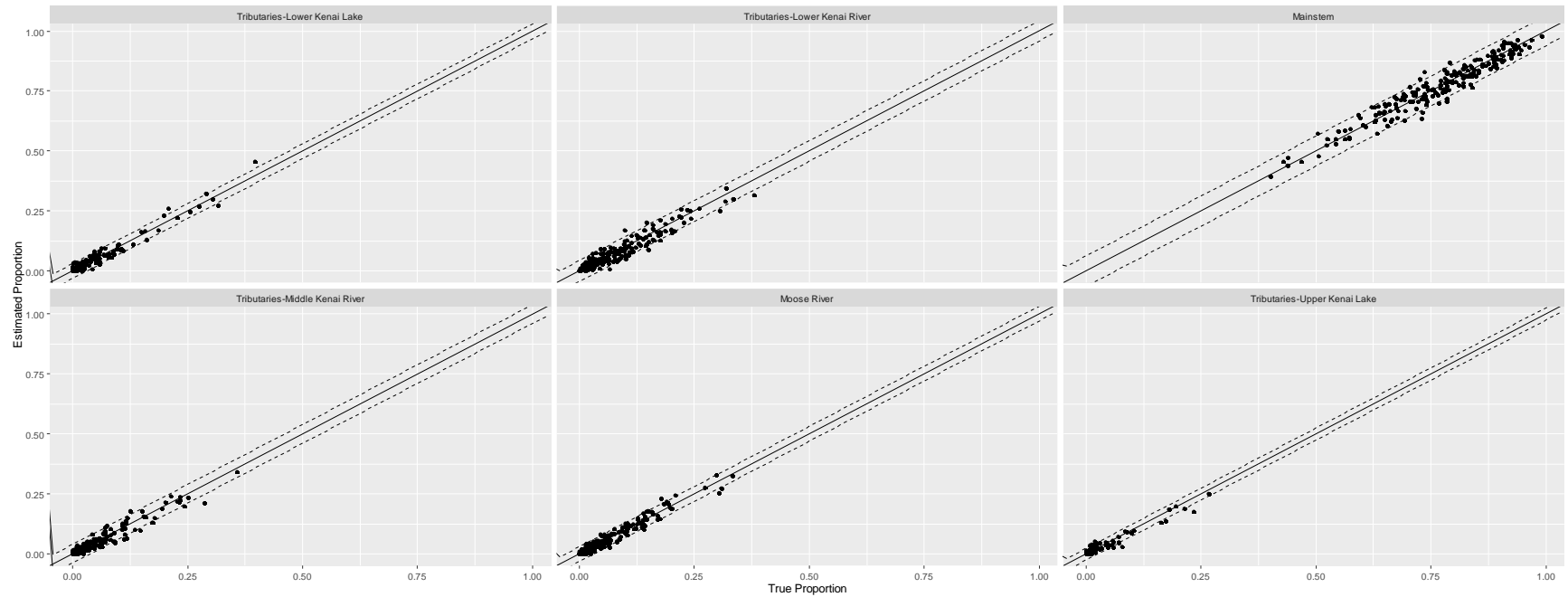


Figure 3. Plots of estimated mixture proportion versus true proportion for 200 simulated mixture samples of 200 fish each for six stock groups of Kenai River Coho Salmon. On average, mixture proportions should approximate those shown in Table 2. Estimates fall within 10% points of the true value (solid line) 95% of the time (dashed lines).

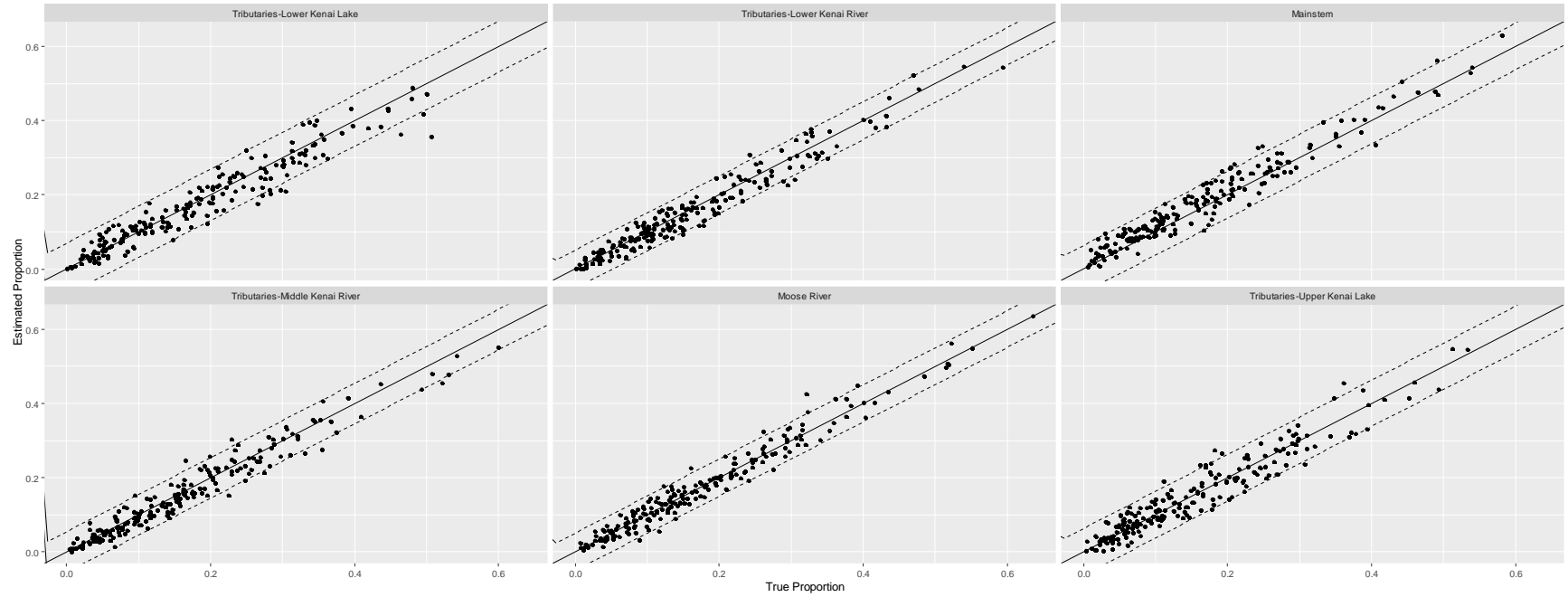


Figure 2. Plots of estimated mixture proportion versus true proportion for 200 simulated mixture samples of 200 fish each for six stock groups of Kenai River Coho Salmon. Estimates fall within 10% points of the true value (solid line) 95% of the time (dashed lines).

Table 4. Juvenile sampling locations, times and sample sizes.

Spring											
	Week 1							Week 8			
Sample Location	Apr. 20	Apr. 21	Apr. 22	Apr. 23	Apr. 24		Jun. 8	Jun. 9	Jun. 10	Jun. 11	Jun. 12
Estuary	<i>n</i> =25				...		<i>n</i> =25				
Beaver Creek		<i>n</i> =25				...		<i>n</i> =25			
Slikok Creek			<i>n</i> =25						<i>n</i> =25		
Soldotna Creek				<i>n</i> =25		...				<i>n</i> =25	
Mainstem (RM 12 - 22)					<i>n</i> =25		...				<i>n</i> =25
Summer											
	Week 9							Week 20			
Sample Location	Jun. 15	Jun. 16	Jun. 17	Jun. 18	Jun. 19		Aug. 31	Sept. 1	Sept. 2	Sept. 3	Sept. 4
Estuary	<i>n</i> =17				...		<i>n</i> =17				
Beaver Creek		<i>n</i> =17				...		<i>n</i> =17			
Slikok Creek			<i>n</i> =17						<i>n</i> =17		
Soldotna Creek				<i>n</i> =17		...				<i>n</i> =17	
Mainstem (RM 12 - 22)					<i>n</i> =17		...				<i>n</i> =17
Fall											
	Week 21							Week 32			
Sample Location	Sept. 5	Sept. 6	Sept. 7	Sept. 8	Sept. 9		Nov. 23	Nov. 24	Nov. 25	Nov. 26	Nov. 27
Estuary	<i>n</i> =17				...		<i>n</i> =17				
Beaver Creek		<i>n</i> =17				...		<i>n</i> =17			
Slikok Creek			<i>n</i> =17						<i>n</i> =17		
Soldotna Creek				<i>n</i> =17		...				<i>n</i> =17	
Mainstem (RM 12 - 22)					<i>n</i> =17		...				<i>n</i> =17

Assaying Genotypes

Baseline samples: Baseline samples will be genotyped at 82 SNP loci following the laboratory methods described in Barclay et al. (2017).

Juvenile mixtures: Total genomic DNA will be isolated from tissue samples using 96-well Qiagen DNeasy® kits. Genomic DNA will be normalized to a final concentration of 30ng/ml. Samples will be genotyped at 82 SNP loci described in Barclay et al. (2017) and an additional SNP locus that can distinguish Coho and Chinook Salmon (Habicht et al. 2019) via custom Taqman® SNP Genotyping Assays. Genomic DNA and TaqMan™ OpenArray™ Genotyping Master Mix will be loaded onto TaqMan® OpenArray®Real-Time PCR plates and run on a QuantStudio™ 12K Flex Real-Time PCR thermocycler as described in the TaqMan®OpenArray®Genotyping Getting Started Guide, but substituting an Open Array™AccuFill™System for the OpenArray® AutoLoader. Genotype cluster plots for all runs will be combined and scored with TaqMan®Genotyper Software Version 1.

Laboratory Failure Rates and Quality Control

Samples lacking genotypes for greater than 80% of the loci and any individuals identified as a Chinook Salmon will be deleted from subsequent analysis. Quality control and estimation of genotyping error will be implemented by isolating DNA a second time from 8% of the samples (1 row per 96-well plate) and re-assaying genotypes. Laboratory errors that are detected will be corrected for, and genotyping error rates will be calculated according to Barclay et al. (2017).

Mixed Stock Analysis and Stock Compositions

The stock compositions of the time and area mixtures will be estimated using BAYES (<http://www.abl.afs.noaa.gov/sida/mixture-analysis/bayes>; Pella and Masuda 2001). Values for initial stock proportions for four Markov Chain Monte Carlo chains of 10,000 iterations each will be 95% from a single stock group, with 5% distributed evenly among the remaining populations. Values for genetic prior parameters will be as described in Pella and Masuda (2001). The Raftery and Lewis (1996) diagnostic will be used to verify the number of iterations was sufficient. Convergence among chains will be determined using Gelman and Rubin (1992) shrink factors; shrink factors of less than 1.2 will be used to indicate convergence. Mean stock composition estimates and 95% credible intervals will be generated after a discard of the first half of the samples.

Spatial and Temporal Genetic Stock Diversity and Evenness

We will calculate a reach and period specific diversity index (Shannon and Wiener 1949) as a measure of diversity that combines both stock richness (the number of stocks in a given area) and heterogeneity (their relative abundances). The Shannon-Wiener diversity index (H), found in each reach and (r) period (t) combination, will be calculated as:

$$H_{r,t} = \sum_{i_{r,t}=1}^{s_{r,t}} (p_{i_{r,t}}) [\ln(p_{i_{r,t}})]$$

Where $H_{r,t}$ is the Shannon-Wiener diversity index, s is the number of stocks in a given area (r) at time t , and $p_{i_{r,t}}$ is the proportion the total abundance represented by the i^{th} stock.

The Shannon evenness index is easier to interpret and is obtained by dividing the Shannon Diversity Index by its maximum, constraining the index between 0 (when one stock is dominant) and 1 (when all stocks are equally present).

Physical Habitat Characterization

Fortunately the lower Kenai River has been the focus of numerous efforts to describe variables thought to be important for Salmon rearing. The National Hydrography Dataset (NHD; <https://www.sciencebase.gov/catalog/item/5d0b04d5e4b0e3d311602852>) supplies us with variables such as, reach topography (Digital Elevation Model DEM), Cumulative drainage area and mean annual flow (National Watershed Boundary Dataset; WBD). Other efforts provide reach specific water temperatures (thermal network mapping; Jones in prep), Riparian vegetation classification (National Wetlands Inventory; <https://www.fws.gov/wetlands/>), water quality measures (Orejuela 2015) and an inventory of the area affected by various development activities (Hall and Kratzer 2001; and Gracz in prep).

Relating Habitat Variables to Stock Diversity

We propose to consolidate, summarize and synthesize the aforementioned habitat variables to determine which variables are important for predicting seasonal and reach specific stock diversity.

Logistics and Schedule

The project will operate out of and be administered by the Kenai Fish and Wildlife Conservation Office. Adult sampling will require helicopter access and short (1 to 5 day) duration field excursions to sample remote spawning sites. Juvenile sample sites will be accessed via the road system and by river boat and will not require a field camp. All sample locations will be accessed using the most direct route possible and permission from landowners will be secured in advance when accessing private property. Habitat data consolidation, and summarization will include much of the currently available information for the lower Kenai River and its tributaries. Assaying the genotypes and mixed stock analysis will be completed collaboratively by the USFWS's Conservation Genetics Laboratory and ADF&G's Gene Conservation Laboratory. All data analysis will be supported by the USFWS's Fisheries and Ecological Services biometric staff. The proposed timeline and tasks for the project are detailed in Table 5.

Table 5. Proposed timeline and schedule of tasks.

Task	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Logistics/Planning					X ²⁰¹⁹	X ²⁰¹⁹	X ²⁰¹⁹					
Adult Sampling								X ²⁰¹⁹	X ²⁰¹⁹	X ²⁰¹⁹	X ²⁰¹⁹	
Juvenile Sampling				X ²⁰²⁰	X ²⁰²⁰	X ²⁰²⁰	X ²⁰²⁰	X ²⁰²⁰	X ²⁰²⁰	X ²⁰²⁰	X ²⁰²⁰	
Amass and summarize physical habitat Data												X ²⁰²⁰
Assaying Genotypes	X ²⁰²¹	X ²⁰²¹	X ²⁰²¹									
Data Analysis				X ²⁰²¹	X ²⁰²¹							
Report Writing						X ²⁰²¹	X ²⁰²¹					
Final Report (2021)								X ²⁰²¹				

Cooperators

This project is supported by the Soldotna office of the Alaska department of fish and game, the leading State agency with expertise and authority in salmon stock assessment, protection and management. The ADF&G will contribute technical expertise and sampling assistance on an as-needed basis. Tony Eskelin (907-262-9368) is the primary contact.

This project is also supported by the Kenai Watershed forum (KWF), a local Non-governmental organization with expertise in conducting water quality and watershed assessments on the Kenai River. The KWF will be conducting complementary investigations to identify the current status and trends of wetlands in the lower Kenai River, and will provide technical assistance and sampling assistance on an as-needed basis. Mike Gracz (907-235-3788) and Maggie Harings (907-260-5449) are the primary contacts.

Deliverable Products

This project will characterize the genetic stock diversity of juvenile Coho Salmon in selected at-risk freshwater habitats of the Kenai River watershed. Results will be published in FWS Alaska Fisheries Technical Reports or peer-reviewed literature, and the information will be available for presentation and distribution to interested parties.

Responsibilities

Principal investigator: Jim Boersma, Fishery Biologist

Duties: As lead biologist, responsible for hiring and training personnel, supervising data collection, analyzing data, and transferring tissue samples and associated data to the Conservation Genetics Laboratory for mixed stock analysis. Serves as the primary or co-author on any reporting and publications.

Co-principle investigator: Penny Crane, Geneticist

Duties: Represents the Conservation Genetics Laboratory and is responsible for the analysis of tissue samples for mixed stock analysis and providing estimates. Serves as the primary or co-author on any reporting and publications.

Co-principle investigator: Andy Barclay, Geneticist

Duties: Represents the Gene Conservation Laboratory and is responsible for the analysis of the tissue samples for adult baseline collections and assisting with statistical analysis. Serves as a co-author on any reporting and publications.

Consulting Biometrician: Anna-Marie Benson, Biometrician

Duties: Provides guidance on sampling design and data analysis, and assists with the preparation and editing of the operational plan and any reports. Serves as a co-author on any reporting and publications.

Sampling Crew: a GS-5, and a GS-6, Fisheries Technician

Duties: Operates vehicles and watercraft and sampling equipment, adheres to sampling schedule, records data accurately, enters data into a computerized database in a timely manner, and completes miscellaneous duties as assigned.

Budget Narrative and Table

Budget narrative and table: The Kenai Fish and Wildlife Conservation Office will receive and administer the funds and will be responsible for completing the project. The requested salary amount reflects the salary and benefits for a seasonal technician, needed to carry out the bulk of the field work. The requested materials amount will purchase the required supplies for collecting juvenile samples. Matching funds include the salary costs for time spent on the project by additional biological technicians, the field biologist, geneticists and biometrician. The CGL will provide all juvenile sample genotype assaying equipment and supplies. The matching funds provided by the KFWCO for contractual services are intended for the helicopter operations needed to access remote spawning locations.

Description of Service	Total
ADF&G geneticist, FB III, 4 weeks	\$6,935
USFWS geneticist, GS-12, 6 weeks	\$16,206
USFWS Fish Biologist, GS-11, 12 weeks	\$20,805
Field sampling crew leader salary, GS-6, 32 weeks (includes overtime)	\$33,734
Field sampling technician salary, GS-4, 32 weeks (includes overtime)	\$27,050
Conservation Genetics Lab, juvenile assay staff time.	\$15,520
Misc. field equipment (waders, raingear, seines, nets, minnow traps, etc...)	\$4,500
Juvenile sample genotype assaying supplies	\$55,000

Contractual services, 7 hours of helicopter flight time	\$12,300
Total Budget:	\$192,050

This proposal represents an efficient leveraging of resources to identify areas and attributes that act to supplement the rearing capacity of the entire Kenai River, thus, aiding in the selection and design of restoration and conservation actions for at-risk habitats that support the rearing requirements for the greatest diversity of Coho Salmon stocks.

Submitted by:	_____	Date:	May 4, 2020
	Principal Investigator		_____
Approved by:	_____	Date	_____
	Field Office Supervisor		
Reviewed by:	_____	Date	_____
	Biometrician		

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