Oral Presentations

Historical demography and population genomics of adaptive radiations in Great Lakes salmoinds

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Understanding the origins and maintenance of biodiversity are a crucial challenge for fisheries biologists. Studies of adaptive radiations have shed light on some of the potential mechanisms driving diversification, including cases where ecological opportunities have led to resource partitioning and differentiation. In most cases, studies focus on a single clade of radiating lineages, but to understand the full context of diversification it is valuable to understand the diversification of species with close interactions. For example, the Laurentian Great Lakes are home to a species flock in the genus Coregonus ("ciscoes") and several morphotypes of Lake Charr (*Salvelinus namaycush*). These groups have a predator-prey relationship, occupy diverse habitats, and span ecological depth gradients. To better understand the evolutionary history of these groups in the Great Lakes, we used coalescence-based demographic modeling of whole-genome sequence data to reconstruct the history of effective population size fluctuations. By tracing the trajectories of effective population size through time, we were able to identify the temporal context of diversification for the cisco species complex, which was during the Last Interglacial period (80-90 ka). Demographic analyses of genomes in Lake Charr lineages revealed that this predator likely co-evolved with its ciscoes (i.e., their prey) while following its food source into novel lake habitats created by glacial meltwaters. In addition, a comprehensive population genomic analysis of nearly 300 individuals across five Coregonus species has allowed us to improve our understanding of underlying features that contribute to adaptive differentiation. Collectively, these data firmly support the Great Lakes as a primary driver of adaptive diversification across two co-evolving salmonid radiations.

Dispersal of salmon eDNA from net pens in nearshore Southeast Alaska

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Environmental DNA (eDNA) is increasingly used to track animals in aquatic habitats, but uncertainty remains about the present location of an animal relative to eDNA detections. In marine environments, physical characteristics influence the distribution of eDNA and likelihood for species detection. Additionally, the taxonomic specificity of the genetic assay used — quantitative PCR (qPCR), species-specific primers, or metabarcoding – impacts eDNA detection. In this study, we make use of hatchery pens containing 46 million juvenile chum salmon (*Oncorhynchus keta*) in nearshore Southeast Alaska and sample every 80 m along a 2 km transect to test the attenuation of eDNA over surface distance during incoming and outgoing tides. Further, we evaluate how detection of chum salmon differs between qPCR, a salmonid-specific primer set, and a 12S fish metabarcoding primer set. We find that chum salmon are detected throughout the 2 km transect with all three genetic assays, and that the number of sequencing reads in the metabarcoding data show a semi-quantitative relationship over increased distance. Chum eDNA concentration remains consistent across tides in the qPCR data; however,

12S metabarcoding data show a stronger background eDNA signal during outgoing tide likely attributable to the compositional nature of the data rather than an actual reduction in the amount of chum eDNA present. Results from this study support the idea that eDNA is transported ≥2 km away from its source and that the distance over which a signal is detected is related to the strength/biomass of the initial source. This horizontal transport is more readily quantified using qPCR whereas metabarcoding data are subject to variable eDNA inputs from transient organisms that compete with the point-source for sequencing read representation.

Spatiotemporal dynamics of chum salmon bycatch in the Bering Sea

Patrick Barry, Chris Kondzela, Jordan Watson, Ellen Yasumiishi, Wes Larson, Megan McPhee

Chum salmon are a vital cultural and food resource for the people of western Alaska. Recently, large population declines have resulted in subsistence and commercial fishery closures. The Bering Sea is a productive ecosystem where chum salmon from western Alaska as well as other North Pacific stocks feed and mature. Large numbers of chum salmon are periodically taken as bycatch in the Bering Sea walleye pollock fishery and concern residents of western Alaska in the face of these declines. Management of the pollock fishery continually evolves in response to salmon bycatch, but the ability to anticipate and mitigate bycatch risk is complicated by the interactions between the abundance and distribution dynamics of chum salmon and the dynamic behavior of the pollock fleet. We used genetic data from chum salmon bycatch from 2011 to 2023 to estimate the contribution of 6 reporting groups (SE Asia, NE Asia, Coastal Western Alaska, the Upper/Middle Yukon, Southwest Alaska, and the Eastern Gulf of Alaska/Pacific Northwest) to bycatch mixtures. We investigated stock-specific distributions of chum salmon bycatch in response to a suite of environmental factors hypothesized to affect both biological processes (abundance and distribution of chum salmon stocks) and pollock fleet behavior. On average Western Alaska comprises 22% of the bycatch while Asia and Gulf of Alaska/Pacific Northwest comprise 50% and 28%, respectively. Asia reporting groups comprise the largest proportion of catches in the western Bering Sea and decrease in proportion closer to the Alaska Peninsula while all other groups show the opposite trend. SE Asia and Coastal Western Alaska reporting groups had lower contributions and the Eastern Gulf of Alaska/Pacific Northwest reporting group had higher contributions later in the fishing season. Later fishing behavior and increased catches of more southern stocks was negatively correlated with sea ice extent anomaly.

High genetic differentiation in three salmonid species from the Skagit River Hydroelectric Project Area, Washington, USA

Daniel Bingham, Scott Blankenship, Erin Lowery, Jeff Fisher

We analyzed a combination of GT-Seq SNPs, microsatellites, and genotyping by sequencing data to describe the diversity, structure, and effective population size of Bull Trout (*Salvelinus confluentus*), Dolly Varden (*S. malma*), and Rainbow Trout (*Oncorhynchus mykiss*) in the Skagit River Hydroelectric Project Area, located in Washington, USA (Project Area). The Project Area's hydrogeological history, which includes both ancient connections and blockages due to

glaciation and recent isolation from the construction of the Skagit Hydro Project, makes the interpretation of the colonization and evolution of the three species complex. Hybridization between Bull Trout and Dolly Varden and between Rainbow Trout and Cutthroat Trout (*O. clarkii*) was common. The differentiation between Project Area Bull Trout and downstream populations was high (F_{ST} = 0.28), like that observed between the coastal and interior lineages. Project Area Dolly Varden was distinct within the southern subspecies (*S. m. lordi*) genetic cluster and was characterized by a pattern of isolation by distance. Nearly all Rainbow Trout were highly differentiated from both the coastal (*O. m. irideus*) and interior (*O. m. gairdneri*) genetic lineages and genetic structure was generally associated with contemporary watershed boundaries. However, some genetic clusters were widely distributed across multiple watersheds, and a population from Pyramid Creek, which is isolated upstream of a barrier, clustered with the coastal subspecies, indicating potential human introduction. Effective population size in Bull Trout and Dolly Varden was small (Ne < 50). In conclusion, except for Pyramid Creek Rainbow Trout, all three species from the Project Area were highly genetically distinct from nearby conspecific populations, suggesting a prolonged history of isolation predating construction of the Skagit Hydro Project.

Observed effects of habitat restoration on juvenile Chinook Salmon recruitment rates using parentage methods

Scott Blankenship, Avery Scherer, Cheryl Dean, Kirsten Sellheim, Jamie Sweeney, Joseph Merz

Information is limited about quantifiable effects of salmonid habitat restoration on reproductive performance, although this information is necessary for evaluating whether population responses follow restoration actions (support population recovery). We used close-kin mark-recapture methods to investigate juvenile recruitment from Chinook Salmon naturally reproducing in gravel augmented (restored) and nonaugmented (unrestored) reaches of a highly managed California River. Genetic parentage techniques linked adult females that had spawned in augmented and nonaugmented spawning habitat with juvenile Chinook Salmon sampled the following spring at a trap located below the natal rearing area. Successful recruitment was documented from both augmented and nonaugmented habitats, with no statistical difference between the two habitat types. The recruitment rate per adult female was low (0.19-0.24 juveniles/female) compared to that observed in other systems using similar techniques. Within the juvenile collections, most females in the study had 0 or 1 offspring observed; however, one female that spawned in restored habitat had 25 offspring recovered at the trap. We modeled recruitment success in relation to a range of biological and environmental variables including spawning habitat site, spawning habitat treatment (augmented, nonaugmented), annual spawner abundance, year spawned, female fork length, spawning and hatch day, and flow maximum and variance. There was an inverse relationship between annual adult abundance (escapement obtained from carcass surveys) and probability of successful reproduction (observed recruits), suggesting habitat limitation may be having density-dependent effects. Female body size, spawning day, and mean daily temperature were all statistically associated with recruitment, suggesting that both biological and environmental factors independent of habitat influenced reproduction potential. This study provides direct evidence of reproductive success from

augmented spawning habitat (restoration sites) and insights into factors driving reproductive success.

Comparative genomics of rainbow trout (*Oncorhynchus mykiss*): is the genetic architecture of migratory behavior conserved among populations?

Catherine I. Clare, Krista M. Nichols, Frank P. Thrower, Ewann A. Berntson, Matthew C. Hale

Rainbow trout (*Oncorhynchus mykiss*) are a partially migratory species wherein some individuals undergo long-distance anadromous migrations, and others stay as residents in their native freshwater streams. The decision to migrate is known to be highly heritable, and yet, the underlying genes and alleles associated with migration are not fully characterized. Here we used a pooled approach of whole genome sequence data from migratory and resident trout of two native populations - Sashin Creek, Alaska and Little Sheep Creek, Oregon - to obtain a genome-wide perspective of the genes and alleles associated with both resident and migratory life history. We calculated estimates of genetic differentiation and selection between the two phenotypes to locate regions of interest and then compared these associations between populations. We identified numerous regions associated with life history development in the Sashin Creek population with a notable area on chromosome 8 that may play a critical role in the development of the migratory phenotype. However, very few alleles appeared to be associated with life history development in the Little Sheep Creek system, suggesting population specific genetic effects are likely important in the development of anadromy. Our results indicate that a migratory life history is not controlled by a singular gene or region, but that there are many independent ways for a migratory phenotype to emerge in a population. Therefore, promoting genetic diversity in migratory individuals is paramount to conserving these populations. Ultimately, our data add to a growing body of literature that suggests that population specific genetic effects, likely mediated through environmental variation, contribute to life history development in rainbow trout.

Evaluating intraspecific diversity in Central Valley Chinook salmon over the past 20 years

Erin E. Collins, T. Thompson, M.H. Meek

Intraspecific diversity allows populations to contend with stochastic and extreme environmental conditions. One system that is representative of notable intraspecific diversity is Chinook salmon in the Central Valley of California, USA. It is the only place in the species range where four adult migration timings (Winter, Spring, Fall, Late-fall) co-occur. These populations are declining and are of serious conservation import; with Winter run listed as Endangered and Spring run listed as Threatened under the Endangered Species Act (ESA). To assess intraspecific diversity of the different runs in the Central Valley, we used RAD-sequencing to genotype outmigrating juveniles every year for over 20 years of sampling. Individuals were assigned to migration-timings and subpopulations of origin using a baseline of samples with known origins. We aimed to determine changes in genetic health over time by estimating effective population size (N_e) and the effective number of breeders per year (N_b) for each population. We also conducted Redundancy analyses (RDA) to improve our understanding of the environmental

variables that may be driving observed changes in genetic diversity. This study provides managers with insights into how hatchery practices and changing environmental conditions have impacted the genetic diversity of ESA listed Chinook salmon populations over the past 20 years, to inform future management decisions in a changing climate.

A Cutthroat trout chromosome-level genome assembly, transcriptomes, and low-coverage Whole genome sequencing

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Native Cutthroat Trout of the Rocky Mountains are the focus of intensive conservation efforts aimed at preserving what remains of this declining iconic species. Those efforts can be furthered with a reference genome to inform molecular studies supporting those conservation goals. A chromosome-level genome assembly for the Greenback Cutthroat Trout (*Oncorhynchus clarkii ssp*) was produced using Illumina, Pacific Biosciences, Hi-C, and transcriptome data from a Bear Creek, Colorado population male. The assembly spans 2.3 Gb with a contig N50 of 11.2 Mb and 97% of the genome assigned to 33 chromosomes. The observed high rate of duplication for Actinopterygii universal single-copy orthologs (BUSCO) of 37% is consistent with the autotetraploid origin of the Oncorhynchus genome. Phylogenetic reconstructions based on the transcriptomes of Cutthroat Trout subspecies are confounded by tetraploidy. Low coverage whole genome sequencing and this reference genome for Cutthroat Trout will allow for the ready implementation of next-generation sequencing-based, conservation-directed analyses of individuals and populations across the species' entire native North American range.

The genomics of an isolated lake bull trout population in Idaho

Alexandra Fraik, James J. Nagler, Paul Hohenlohe

Multiple life history forms of bull trout (*Salvelinus confluentus*) are known across their native range. These include anadromous, fluvial, adfluvial and non-migratory stream-residents. Although a few bull trout populations located in small, sub-alpine lakes have been reported, the biology of this life history form is incomplete. We undertook a genomic and morphometric characterization of a subalpine lake population of bull trout in the Clearwater mountains in Idaho to determine whether a fifth, non-migratory, lake resident life history form is possible. Bull trout were sampled from Fish Lake (elevation = 1,812 m) its outflow, Lake Creek, in the North Fork Clearwater River watershed as well as Lake Pend Oreille to serve as an outgroup. Using > 30,000 single nucleotide polymorphisms (SNPs), we estimated patterns of diversity and divergence between the lake and creek populations. We found significant genetic divergence between Fish Lake and Lake Creek bull trout providing strong support for reproductive isolation. Additionally, we found evidence suggesting Fish Lake and Lake Pend Oreille bull trout shared more recent common genetic ancestry than Lake Creek bull trout had with either population. Morphometric analysis of bull trout from Fish Lake and Lake Creek identified a singular difference: one linear segment from the back of the head to the origin of the pelvic fin explained 94.5% of the variance in a principal components analysis between the two populations. Although

questions remain, the evidence to date implies that Fish Lake bull trout are non-migratory and are genetically distinct from Lake Creek. The morphometric analysis provides evidence for a unique bull trout phenotype from Fish Lake compared to fish from Lake Creek. The implication of these findings is important because there are conservation concerns for bull trout in the US, and life history diversity and small population persistence are key features of recovery plans for this species.

Genotyping at sea informs in-season fisheries management in real-time

Tyler Dann, Natura Richardson, Jodi Estrada, Heather Hoyt, Michael Link, Sara Gilk-Baumer

Bristol Bay sockeye salmon are one of America's most valuable natural resources and define the economy, ecology, and culture of the region. The record 79 million sockeye salmon that returned to Bristol Bay in 2022 were worth a record $351M to fishermen and billions to the economy after accounting for multiplier effects. The large and temporally compressed run is managed to meet Alaska Department of Fish and Game (ADF&G) escapement goals for the nine major drainages of the bay. Mixed stock analysis (MSA) using 24 SNP markers is used to inform in-season management. MSA is conducted on samples from a test fishery that captures fish about seven days before they arrive in fishing districts. Historically, samples taken for genetic analysis were shipped from Port Moller to Anchorage where genotyping was performed at the ADF&G Gene Conservation Laboratory (GCL). The time and effort sailing to port to transport samples takes time away from fishing and increases costs. Here we describe a novel approach of genotyping at sea to circumvent lost time, costs, and weather delay issues. In 2021, samples were processed at sea in tandem with samples at the GCL to validate the approach. A comparison of 38,255 genotypes showed a 98.51% concordance rate between the two labs. The at sea lab was fully implemented and improved upon in 2022. We discuss improvements to the timeline of results, how well at sea estimates predicted inshore run, and potential improvements and other applications for the at sea lab.

Efficient population representation with more genetic markers increases performance of a genetic stock identification baseline

John Hargrove, Thomas Delomas, John Powell, Jon Hess, Shawn Narum, Matthew Campbell

Genetic stock identification (GSI) is an important fisheries management tool to identify the origin of fish harvested in fisheries or sampled via routine monitoring. Long-term monitoring using GSI can benefit from periodic updates of the genetic baseline to ensure allele frequencies are representative of contemporary populations. We used an updated baseline to evaluate how population representation, marker number, and marker type affected the performance and accuracy of genetic stock assignments (self-assignment, bias, and holdout group tests) for steelhead (*Oncorhynchus mykiss*) in the Snake River basin. First, we compared the performance of the existing genetic baseline (representing 22 of 24 existing populations) with a newly developed one which had a reduced number of individuals distributed across more populations (24) using the same set of markers. While self-assignment and bias rates did not differ

significantly in this comparison, there was a substantial improvement in performance for the new baseline in holdout results (mean increase of 24.7%). Second, we compared the performance of the new baseline with increased numbers of genetic markers (~2x increase of single nucleotide polymorphisms; SNPs) for the same set of baseline individuals. In this comparison, self-assignment results produced significantly higher rates of self-assignment ($p < 0.001$; +10.0%) but did not significantly affect bias or leave-one-out results beyond those gained from population representation (yet increased markers substantially improved holdout performance over the original baseline by 22.6%). Third, we compared 334 SNPs versus opportunistically discovered microhaplotypes from the same amplicons and showed the latter produced significantly higher rates of self-assignment ($p = 0.01$; +2.4%), lower bias ($p < 0.01$), and slight increase in holdout performance (0.6%). Combined, we show the performance of genetic baselines can be improved via representative and efficient sampling, that increased marker number consistently improved performance over the original baseline, and that opportunistic discovery of microhaplotypes can lead to small improvements in GSI performance.

Temporal shifts in adaptive haplotypes associated with age-at-maturity in Dworshak National Fish Hatchery steelhead

Audrey C. Harris, Stuart C. Willis, Shawn R. Narum, Matthew R. Campbell

Recently discovered candidate regions in anadromous salmonids provide a genetic tool to potentially predict phenotypes of great interest to managers, such as run timing and age-at-maturity. One such region, the SIX6 gene on chromosome 25, is associated with age-at-maturity in steelhead and may drive some life history variation exhibited by hatchery broodstocks. Previous research has demonstrated that ten single nucleotide polymorphisms (SNPs) on chromosome 25 are associated with ocean duration and body length, with individuals possessing either "short" or "long" haplotypes. Individuals with "short" haplotypes tend to spend one year in the ocean and return at shorter body lengths, while those with "long" haplotypes tend to spend two or more years in the ocean and return at longer body lengths. Steelhead returning to Dworshak National Fish Hatchery (NFH) have historically been older at return and attained larger maximum body sizes than other steelhead hatchery broodstocks in the Snake River basin In Idaho. However, haplotype and genotype frequencies for candidate markers on chromosome 25 have not been previously assessed for this for this hatchery stock. We used ten SNPs associated with age-at-maturity to statistically phase adaptive haplotypes and then calculate haplotype and genotype frequencies for Dworshak NFH broodstock. To assess potential temporal shifts in haplotype and genotype frequencies, we included individuals from the 1969 founding wild stock, subsequent broodstocks from 1970-1976, and contemporary broodstocks from recent spawn years (2014-2016; 2019-2022). Our results demonstrate that Dworshak NFH broodstocks possess adaptive haplotypes previously observed in other interior-lineage steelhead, but frequencies for the "long" haplotype are generally greater in magnitude than those observed elsewhere in the Columbia River basin. Our work constructing adaptive haplotypes for an iconic broodstock with high environmental, cultural, and economic value provides information for managers regarding the genetic basis of life history diversity in steelhead.

Visual and genetic stock identification of a test fishery forecast Columbia River spring Chinook stocks 2 weeks into the future

Jon E. Hess, Bethany M., Michelle W. Rub, John M. Whiteaker, Jeff K. Fryer, Shawn R. Narum

Test fisheries provide data on strength and timing of a run of anadromous fishes in advance of scheduled fisheries. Greatest benefits of test fisheries are realized when predictions comprise the highest levels of resolution and accuracy of stock-specific data and with adequate timing to allow for fisheries planning. The Spring Chinook Test Fishery on the lower Columbia River mainstem has ideal characteristics for powerful applications. First, this drift net fishery located near the Columbia River mouth intercepts fish at least 163 Rkm before they can migrate to one of two major routes, Willamette River vs Bonneville Dam, providing data 2 weeks in advance based on average migration rates (~12 Rkm/day). Second, visual stock identification (VSI) can quickly (minutes) distinguish Spring Chinook into two major stocks (lower and upriver) that utilize different migration routes with routine handling and modest accuracy (>80%). Third, improved accuracy and resolution of stocks is afforded by collection of tissue and expedited genotyping analysis. In this study, we used five years (2017 – 2019, 2021 – 2022) of data to evaluate the level of accuracy, resolution, and advance timing of predictions for the stock-specific run timing and abundance of Spring Chinook arriving at Willamette River and Bonneville Dam. VSI provided consistent predictive ability for estimating the timing and magnitude of upriver Spring Chinook abundance at Bonneville Dam; and GSI improved this predictive ability even at increased resolution to the hatchery broodstock level. Lower river stocks could be predicted accurately with GSI but with less resolution within stock. Overcoming some minor logistical challenges could allow VSI and GSI to be used in combination to provide fisheries managers with timely information to help plan Columbia River fisheries.

Whole genome re-sequencing of Chinook salmon to estimate standing variation across populations

Rebekah L. Horn, Shawn R. Narum

Chinook salmon (*Oncorhynchus tshawytscha*) populations in the Columbia River Basin represent multiple distinct genetic lineages that exhibit diverse life history traits. Previous studies have elucidated neutral population structure and signals of adaptive variation including a genomic region underlying adult migration timing (GREB1L/ROCK1). In this study, we extended tests of neutral and adaptive genomic variation with low coverage whole genome re-sequencing data from 53 populations of Chinook salmon representing the three genetic lineages (iST, interior stream-type; iOT, interior ocean-type; and LC, Lower Columbia). With the filtered dataset of 13 million SNPs, we contrasted patterns of genomic variation within and among lineages and examined the extent of a selective sweep at a major effect region on Chr28 associated with adult migration timing. Allele frequency variation in the GREB1L/ROCK1 region on Chr28 was highly correlated with mean migration, however, the extent of selection within the genomic region controlling run timing was much narrower in iST populations compared to iOT or LC populations. Variation in sequence read depth in GREB1L/ROCK1 also provided evidence that a duplication may be responsible for reduced recombination in this portion of the genome. Lastly,

SNP positions across GREB1L/ROCK were assessed for their utility in discriminating run-timing among lineages and compared to markers used in previous studies to assist with identifying early and late run fish among populations. These results extend knowledge of genomic variation in this species of conservation concern and provide estimates of standing genetic variation within populations for candidate genes such as GREB1L/ROCK1.

Genomic evidence for domestication selection in three hatchery populations of Chinook salmon, *Oncorhynchus tshawytscha*

Author List- Natasha Howe, Charles D. Waters, Matthew C. Hale, Wesley A. Larson

Salmon hatcheries are widely used across the Pacific Northwest to enhance fisheries and supplement declining wild populations. However, substantial evidence suggests that hatchery fish have reduced fitness compared to their wild counterparts. Domestication selection, or adaptation to the hatchery environment, poses a potential risk to wild populations if introgression between hatchery and wild fish occurs. While few studies have investigated domestication selection on a genomic level, none have done so in parallel across multiple hatchery-wild population pairs. In this study, we examined three separate hatchery populations of Chinook salmon, *Oncorhynchus tshawytscha*, and their corresponding wild progenitor populations using low-coverage whole genome sequencing. We sequenced 192 individuals from populations across Southeast Alaska and estimated genotype likelihoods at over six million loci. Each hatchery population, which was reared in a hatchery for approximately seven generations, was then compared to its wild progenitor population using multiple metrics of genomic divergence. While evaluating population-level genomic differentiation (F_{ST}), we discovered numerous outlier peaks in each hatchery-wild pair, although no outliers were shared across the three comparisons. Further analyses indicated that these relatively small (6 - 60 kilobase) peaks are likely due to genetic hitchhiking on hatchery-selected alleles, though the effects of these peaks on fitness are unknown. Overall, our genome-wide analyses demonstrate that domestication selection is prevalent in all hatchery facilities, but the genetic pathways differ across populations, possibly due to a polygenic basis of fitness related traits. These results provide fine-scale genetic evidence for domestication and highlight the need to assess if certain management practices, such as integration of wild broodstock, can universally mitigate genetic risks despite multiple pathways of domestication.

Harnessing the power of regional baselines for broad-scale genetic stock identification: A multistage, integrated, and cost-effective approach

Bobby Hsu, Chris Habicht

Genetic stock identification (GSI) estimates the contribution of each population to a mixture and these analyses are usually conducted regionally using genetic baselines specific to the stocks expected in that region. Often these regional baselines cannot be combined to produce broader geographical baselines. In cases where the mixture contains stocks spanning across a wide area a broad-scale baseline is created, but these baselines often are unable to resolve among regional

stocks. Here, we introduce a new GSI method to harness the resolution capabilities of baselines developed for regional applications in the analyses of mixtures containing fish from a broad geographic range. This multistage process allows for disparate baselines to be used in a single integrated process that estimates the propagated errors from each stage. The baselines used by this model do not require any overlap in markers or in populations representing the broad-scale or regional baselines. The integrated multistage framework allows GSI of a wide geographic area without first developing a large scale, high resolution genetic baseline, or dividing a mixture sample into smaller regions beforehand. This approach is more cost-effective than updating range-wide baselines with all critical regionally important markers.

Understanding Chinook salmon life history variation in the Yuba River, California

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Chinook salmon (*Oncorhynchus tshawystcha*) display incredible life history diversity, underpinning their ability to adapt to environmental change. In the Yuba River in California, there is a concerted effort to understand federally listed Chinook populations. Unfortunately, we lack a complete understanding of the life history diversity present in the system. Of particular importance is understanding the timing of returning adult spring and fall run in the river and how that relates to their natal origin and juvenile emigration strategy (size/timing). We set out to understand this diversity by combining the power of genetic information with otolith microchemistry on adults Chinook salmon collected from the Yuba River post-spawning. Genomic variation in the GREB1L region is tightly linked with run timing in Chinook and will be helpful for understanding distributions of spring and fall run Chinook across the migration window. Otoliths analyses from these same fish provided paired age estimates, natal origin assignments, and juvenile emigration size. We found all GREB1L genotypes (homozygous early, homozygous late and heterozygous) in Yuba River spawners, with the homozygous early fish tending to arrive during the spring and the homozygous late fish during the fall. The otolith strontium isotope analyses revealed a variety of juvenile migratory strategies, with fry emigrants being most common across all years studied. However, higher contributions of late migrating smolts were observed in wetter years, presumably because of increased upstream rearing habitat and\/or improved survival through a cooler Delta. Otolith age reconstructions also showed diversity in return ages, with 2- 3-, and 4-year-old spawners present, and natural-origin fish tending to return age-4. This study confirms that ESA-listed spring run Chinook salmon are spawning in the Yuba River, and that there is still a diverse portfolio of phenotypes across all life stages, highlighting the need to support this diversity both within and among populations.

Using environmental DNA in water samples to monitor the distribution and abundance of salmonids

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The use of environmental DNA (eDNA) in water samples has become a popular, inexpensive, and noninvasive method for monitoring aquatic biodiversity. However, the application of eDNA methods requires careful consideration of advantages and disadvantages to ensure proper inference for conservation and management. This paper presents three case studies on the use of eDNA for monitoring salmonids, comparing conventional fisheries monitoring approaches, including rotary screw traps, underwater visual surveys, and bottom trawling, to eDNA monitoring. The first study compared standard and environmental DNA methods for estimating Chinook salmon smolt abundance in the Klamath River, California. Our results suggest that eDNA may potentially be a useful predictor of weekly abundance of out-migrating Chinook salmon smolts but when estimates of abundance were less than 13,500 smolts per week, the concentration of eDNA was too low to accurately estimate smolt abundance. In another study we compared eDNA and underwater visual surveys for detecting juvenile coho salmon (*Oncorhynchus kisutch*) in small streams. We show that eDNA methods and underwater visual surveys provided nearly identical patterns of detection and non-detection of coho salmon in small pools, but that eDNA concentrations were a were poor predictors of coho salmon counts in small pools. In the last example, we compared eDNA and bottom trawling for detection of coho salmon in Humboldt Bay, California. We found that eDNA detected coho salmon whereas trawl surveys never detected coho salmon. We emphasize that while eDNA approaches are effective for monitoring, future applications should carefully consider study objectives to ensure proper inference for conservation and management.

A current review of epigenetic effects associated with salmonid supplementation and domestication

Ilana J. Koch, Hayley M. Nuetzel, Shawn R. Narum

Several studies have demonstrated lower fitness of salmonids born and reared in a hatchery setting compared to those born in nature, yet broad-scale genome-wide genetic differences between hatchery-origin and natural-origin fish have remained largely undetected. Recent research efforts have focused on using epigenetic tools to explore the role of heritable changes outside of genetic variation in response to hatchery rearing. We synthesized the results from salmonid studies that have directly compared methylation differences between hatchery-origin and natural-origin fish. Overall, the majority of studies found substantial differences in methylation patterns and overlap in functional genomic regions between hatchery-origin and natural-origin fish which have been replicated in parallel across geographical locations. Epigenetic differences were consistently found in the sperm of hatchery-origin versus natural-origin fish along with evidence for maternal effects, providing a potential source of multigenerational transmission. While there were clear epigenetic differences in gametic lines between hatchery-origin and natural-origin fish, only a limited number explored the potential mechanisms explaining these differences. We outline opportunities for epigenetics to inform salmonid breeding and rearing practices and to mitigate for fitness differences between hatchery-origin and natural-origin fish. We then provide possible explanations and avenues of future epigenetics research in salmonid supplementation programs, including: 1) further exploration of the factors in early development shaping epigenetic differences, 2) understanding the functional genomic changes that are occurring in response to epigenetic changes, 3) elucidating the

relationship between epigenetics, phenotypic variation, and fitness, and 4) determining heritability of epigenetic marks along with persistence of marks across generations.

Is structural variation necessary to create islands of divergence in moderate gene flow species? A case study in sockeye salmon

Wes Larson, Peter Euclide, Yue Shi, Kristen Gruenthal, Kris A. Christensen, Jim Seeb, Lisa Seeb

Local adaptation is often facilitated by loci clustered in relatively few regions of the genome, termed genomic islands of divergence. However, the mechanisms that create, mold, and maintain these islands are poorly understood. Here, we use sockeye salmon as a model species to investigate the mechanisms responsible for creating islands of divergence linked to adaptive variation. Previous research suggests that multiple islands are involved in adaptive radiation of sockeye salmon. However, these studies were based on low-density genomic methods that genotyped tens to thousands of loci, making it difficult to elucidate the mechanisms responsible for islands. We used whole genome resequencing to genotype millions of loci to investigate these mechanisms. We discovered 64 islands, 16 of which were shared between two isolated drainages; these 16 islands were clustered in four genomic regions. Characterization of the shared regions suggested that three of four were likely created by chromosomal inversions, while the other was created by processes not involving structural variation. Additionally, all four regions were relatively small (< 600 kb), suggesting inversions and other low recombination regions do not have to span megabases to be important for adaptive divergence. In sum, our study demonstrates that heterogeneous selection can lead to a mosaic of islands created by different mechanisms within the same genome. Future studies should continue to investigate how gene flow, selection, and the architecture of genetic traits interact to influence the genomic landscape of adaptive divergence.

Genetic variation underlying dispersal in Sockeye salmon

Samuel May, David Paez, Jeffrey Hard, Peter Westley, Daniel Schindler, Ray Hilborn, Lorenz Hauser, Kerry Naish

Dispersal mediates the flow of individuals and genes between habitats and breeding populations, and thus has profound implications for ecology and evolution. Extensive theoretical work has examined how dispersal and philopatry may evolve in variable habitats, but these studies make a key assumption that natural selection can act on genetic variation underlying dispersal phenotypes. In fact, few studies have quantified heritability values for dispersal in any species, and there exists no evidence for genetic variation in dispersal propensity in any aquatic species. This study quantified heritability values using animal models and molecular-based pedigrees of two adjacent Sockeye salmon populations in Bristol Bay, Alaska, comprising nearly 10,000 individuals across two generations of returning adult spawners. Narrow-sense heritability was significant on the observed scale (heritability = 0.12; 95% credible interval 0.06 - 0.16), the scale on which the phenotype is expressed and on which natural selection operates. On the underlying

latent scale, which is useful to compare heritability estimates across study species and traits, the heritability estimate was 0.64 and also significant (95% credible interval 0.43 - 0.79). Compared to other published heritability values for important life history traits in salmonids, these values are remarkably high. These results indicate that dispersal can evolve in response to natural selection. We discuss how anthropogenic activities may therefore affect the evolution of dispersal in nature, with potential consequences for adaptive evolution and demographic connectivity.

Y-chromosome haplotype diversity and male age at maturity in Chinook salmon

Garrett McKinney, Paul Moran

Variation in size and age at maturity is an important component of life history diversity in salmonids. In male, variation in size and age at maturity in males can be associated with different reproductive strategies. Larger males gain a reproductive advantage by monopolizing access to females while younger smaller males may gain reproductive success by sneaking among mating pairs. Distinct haplotype blocks on the Y-chromosome have been previously associated with variation in male age at maturity throughout Alaska and in the Wenatchee River. The relationship between Y-chromosome haplotypes and age at maturity is complicated not just by different haplotype identities among geographic regions, but by translocation of the sex determining region among chromosomes, which could change patterns of linkage between sex and genes influencing age at maturity. Here we present evidence of new y-chromosome haplotypes in additional populations of Chinook salmon and discuss broader patterns of Y-chromosome haplotype diversity across the range of Chinook salmon.

Experimental integrated sockeye salmon enhancement reveals demographic benefits but phenotypic costs

Megan McPhee, Pat Barry, Sara Gilk-Baumer, Chris Habicht, Scott Vulstek, John Joyce, William Smoker, Anthony Gharrett

Transboundary (Alaska-Canada) joint enhancement of sockeye salmon pursuant to the Pacific Salmon Treaty follows an integrated hatchery model. Broodstock are taken from the wild return, eggs are fertilized in situ then transported to the Snettisham Hatchery near Juneau, Alaska for incubation, and resulting fry are stocked into recipient lakes to rear and emigrate alongside wild-born fish. Understanding the demographic efficacy of this form of enhancement, along with its effects on genetic and phenotypic diversity, is critical to assessing its performance. In this study, we took advantage of the weir on Auke Creek, Juneau, Alaska that allowed us to genetically sample all returning adult sockeye salmon and quantify hatchery and wild productivity (adult offspring/spawner) in the first generation. We calculated relative productivity over three brood years of experimental enhancement (2011-2013) by assigning returning adult offspring to individual hatchery or wild-spawning parents. Hatchery productivity greatly exceeded that of wild spawners, with annual relative productivity ranging from 6.0 to 48.9 in females and 8.9 to 58.7 in males. Hatchery and wild-born salmon had similar adult run timing and size at age, but

hatchery-born fish tended to mature at a younger age, and this effect persisted after accounting for the potential relationship between return date and parent age. In systems such as Auke Lake, where spawning and\/or incubation habitat is limited, supplementation can be an effective way to demographically boost wild sockeye salmon populations. However, our study showed that even a single generation of enhancement can cause undesirable phenotypic changes in the recipient population. Ongoing work will examine the effects of the short-term experimental enhancement of Auke Lake sockeye salmon on relative reproductive success, genetic diversity, and life-history traits in the second generation.

The Fast and the Fishy: Advancements in genomic run identification for Chinook salmon management in the Central Valley

Mariah Meek, Sarah Brown, Scott Meyer, Brett Harvey, Emily Funk, Andrea Schreier, Melinda Baerwald

Genomics have revolutionized our ability to identify cryptic biodiversity. One species where this is particularly true, with large management implications, is Chinook salmon of the Central Valley of California. The Central Valley of California is the southern-most end of the native species range and only place in the world where four runs co-occur. These four runs have differences in their conservation status, with Winter run being Endangered under the Endangered Species Act, Spring run being Threatened, and Fall and Late-fall not being ESA listed. Due to differences among listing status and the need for accurate tools for run identification, we now have several methods for determining run identification of Central Valley Chinook salmon. This includes a brand new CRISPR-based approach that is fast, accurate, and can be completed in the field. Despite the various methods in use today, little work has been done to compare results across methods and evaluate their level of concordance or determine what situations each method is ideally suited to address. In this talk, we will provide a summary of each method in use today and present new data on the concordance among methods. We will also discuss the implications of these findings for management of Chinook in the Central Valley and make recommendations for moving forward.

Genetic research, monitoring, and evaluation in US West Coast fishery management and the conservation of protected marine resources

Paul Moran

The field of molecular genetics has advanced far more quickly than the fishery management community has been able to exploit these powerful new methods. Moreover, long traditions, conventions, and legal frameworks have additionally constrained the exploitation of genetic and genomic methods. Basic research surrounding the molecular basis of life-history variation is forging ahead, yet applications of genetic methods in management have lagged. There seems to be confusion, even among geneticists and fishery biologists, as to exactly how genetic data are used by NOAA and the Pacific Fishery Management Council (PFMC). This paper gives several

examples of the use of genetic stock identification as well as some of the challenges and limitations we 've experienced in supporting management. I describe the statistical challenge of compositional forecasting for rare bycatch events, as well as the unique difficulty of making fishery recommendations based on insufficient data, where those represent the “best available science”. A sweeping replacement of the current coded-wire tag system with parentage-based tagging has not been forthcoming. Nor has broad re-definition of NOAA ESUs based on adaptive alleles. However, in collaboration with the NOAA 's West Coast Region and the PFMC, our group continues to make a variety of unique contributions to management, including independent evaluation of the re-defined coded-wire tag base period, biological opinions for groundfish fisheries and southern resident killer whales, and multiple consultations on proposed rule changes in midwater and bottom trawl fisheries. Change has been slow, but demand for genetic and genomic information has increased steadily.

Back to the Future: 2,000 years of Skagit River Salmonidae species and life-history diversity

Remi Murdoch, Joanna Elmore, Taylor Wilcox, Ross Salerno, Molly Carney, Alex Fraik

The construction of dams and resulting habitat fragmentation has disrupted the migratory paths and life histories of anadromous fish, particularly salmonids. Historical records in the Skagit River basin in northwestern Washington have captured recent declines in numerous salmonid species ' populations due to anthropogenic stressors such as dams, hatcheries, and industrial harvesting. However, there are no written records of pre-colonization salmonid species ' distributions, abundances, and life-history forms. To fill this gap, we are conducting a pilot study to identify species, life-history forms, and relative abundances from archaeological salmonid vertebrae excavated along the Skagit River. Combining ancient DNA extractions and high-throughput qPCR assays we will create a cost and resource efficient method for species identification. Second, we will validate species identification from high-throughput qPCR with mitochondrial DNA sequencing. We additionally will call haplotypes from the mitochondrial DNA data to coarsely assess genetic diversity within species and compare to publicly available contemporary data. This method provides increased sensitivity of species identification for highly degraded ancient DNA samples while conserving valuable and finite DNA. Finally, combining these data with analysis of isotopic signatures of vertebrae annuli will provide life-history data regarding the ocean-migrating phenotype. The interdisciplinary approach presented in this study could provide tribes, stakeholders, and conservation managers with valuable deep-time baseline data for salmonid species in the Skagit River.

Genetic evaluation of trojan YY brook trout treatments in New Mexico during 2019-2022

Steven M. Mussmann, Melissa C. Nehmens

Trojan YY male (MYY) Brook Trout provide a targeted method for treatment and potential eradication of invasive Brook Trout populations. Since 2019, these fish have been stocked into four streams in New Mexico (Leandro Creek, Placer Creek, Rito de los Piños, and Rio Bonito) with the intent of eradicating non-native Brook Trout populations. Here, we provide the genetic

evaluation of MYY Brook Trout performance in the four treatment streams and one untreated control stream (Little Vermejo Creek) from 2019 through 2022. Genetic samples (N = 3,610) representing young-of-year (YOY) and adult samples from five streams and each MYY cohort from 2018 through 2022 were amplified using a 223-locus GTseq panel. Genetic sex identification was performed for all individuals, and MYY Brook Trout offspring were identified using NEWHYBRIDS. Male-favored sex ratios and increasing MYY offspring proportions were found among YOY fish in all four treatment streams. However, these demographic changes have been more slowly accrued in adult population segments. Additional findings could impact genetic monitoring of MYY actions in New Mexico and elsewhere. For example, sex-specific genotyping biases were detected for two of four evaluated sex-identifying markers (BrunelliSex_GTseq and SalveSDYU4sep15_GTseq). MYY offspring with XX sex genotypes were also detected in Placer Creek and Rio Bonito, indicating hatchery escapement of MXY Brook Trout among the MYY fish. Despite these issues, we observed positive performance of MYY Brook Trout as a potential management tool. However, possible MXY hatchery escapement and slow recruitment of MYY offspring to reproductive age provide potential areas of study for future monitoring efforts.

Genetic variation associated with migration timing in lineages of Chinook salmon and steelhead in the Columbia River Basin

Shawn Narum

With the discovery of a major effect region of the genome (GREB1L, ROCK1) for migration timing in both Chinook salmon and steelhead, several subsequent studies have investigated the effect size and distribution of early and late migration alleles among populations in the Columbia River. Here, I synthesize results of these studies for the major lineages of Chinook salmon and steelhead that include highly distinct groups in the interior Columbia River that exhibit atypical life histories from most coastal lineage populations of these two species. Whole genome studies with high marker density have provided extensive insight to SNPs most associated with migration timing, and suites of markers for each species have been genotyped in large numbers of individuals to further validate phenotypic effects. This combination of results has extended our understanding of genetic variation associated with life history diversity in unique populations of the Columbia River, however much research remains necessary to determine the causal mechanism for this major effect region on migration timing.

The genomics of the re-establishing summer and winter steelhead in the Elwha River following large scale dam removal

Krista M. Nichols, Alexandra K. Fraik, Michael L. McHenry, Paul Hohenlohe, George R. Pess

Dam construction and river habitat fragmentation disrupt important life histories and movement of aquatic species. This is especially true for salmonids such as *Oncorhynchus mykiss* that exhibit numerous, diverse life history strategies including the propensity and timing for juvenile and adult migration. River and habitat connectivity play crucial roles for these life-history forms,

influencing the physical and temporal migratory habitat required to support survival and reproduction in heterogeneous river systems. When habitats are blocked by river barriers, and this habitat becomes unavailable, selection may remove these diverse life-history forms, diminishing the phenotypic and genetic variation of the species overall. While barrier removal is becoming a more common occurrence for re-establishing this connectivity, we still lack a clear understanding of how migratory habitat restoration impacts recovery of not just a species, but its distinct, life-history forms. In this study, we investigate the contributions of different evolutionary lineages to the resurgence of the diverse life history forms of Elwha River *O. mykiss*. Utilizing multiple longitudinal genomic data sets, we characterize the population genetics of *O. mykiss* across the river. First, we test whether population genetic structure and diversity change as anadromous steelhead begin to re-establish in the upper watershed following dam removal. Second, we identify potential ancestral sources for putatively adaptive alleles underlying the early adult ocean-return timing lineage of *O. mykiss*. Finally, we investigate the impact that sympatric coastal cutthroat trout populations introgressing with native *O. mykiss* lineages have on re-establishing steelhead populations.

The progression of naturalization: Using Parentage-Based Tagging to monitor the reintroduction of spring Chinook salmon to Lookingglass Creek, OR

Hayley Nuetzel, Peter F. Galbreath, Benjamin A. Staton, Carrie A. Crump, Leslie M. Naylor, Gene E. Shippentower

Supplementation of depressed salmonid populations with hatchery production has been questioned due to domestication effects, which may reduce reproductive fitness. However, for extirpated populations, reintroduction essentially requires use of hatchery stocks. We evaluated this strategy by monitoring the naturalization of spring Chinook salmon reintroduced to Lookingglass Creek, OR (Grande Ronde Basin) from a captive brood, hatchery stock. We compared the reproductive success (RS) of naturally spawning natural-origin (NOR) relative to hatchery-origin (HOR) adults across nine brood years. Individual RS (the number of progeny produced) was estimated by pedigree reconstruction analyses, and then analyzed by generalized linear models to estimate the effect of parental origin, while controlling for potentially confounding covariates. When evaluating RS by juvenile progeny, NOR spawners were more likely to be reproductively successful and, when successful, produced more progeny on average than successful HOR counterparts. We found a similar advantage when evaluating RS by adult progeny, although the origin effect was not as important among successful spawners. Results suggest fish reintroduced from a hatchery stock possess the adaptive capacity to positively contribute to natural productivity and recovery goals.

A single generation in the wild increases fitness for descendants of hatchery Chinook salmon (*Oncorhynchus tshawytscha*)

Kathleen G. O'Malley, David I. Dayan, Nicholas M. Sard, Marc A. Johnson, Cristí-n K. Fitzpatrick, Ryan Couture

Reintroduction is an important tool for salmon recovery. These programs often use hatchery salmon from a nearby source to re-establish populations in vacant, historically occupied habitat. However, this approach is challenged by the relatively low reproductive success that hatchery-origin (HOR) salmon experience when they spawn in the wild, relative to their natural-origin (NOR) counterparts. In this study, we used genetic parentage analysis to compare the reproductive success of three groups of Chinook salmon (*Oncorhynchus tshawytscha*) reintroduced above the Cougar Dam on the South Fork McKenzie River, Oregon: HOR salmon from an integrated stock; their first generation of wild-born offspring (hereafter F1s); and NOR salmon that were born elsewhere. We found that F1s produced nearly as many adult offspring as NOR salmon, and 1.7-fold more adult offspring than their hatchery parents. This result suggests that, for the South Fork McKenzie reintroduction program, a single generation in the wild increases fitness for the descendants of hatchery salmon. However, even with elevated fitness, successful reintroduction remains demographically constrained by extrinsic factors.

Genetic parentage reveals the (un)natural history of Central Valley hatchery steelhead

Laura Goetz, Hayley Nuetzel, David Vendrami, Anne Beulke, Eric Anderson, John Carlos Garza, Devon Pearse

Effective management of conservation-reliant species requires understanding both biological and anthropogenic influences. We considered hatchery production of steelhead in the Central Valley of California and investigated how differences among genetic lineages and management practices impact life-history traits. We genotyped 23,753 steelhead returning to the four California Central Valley hatcheries over nine years from 2011-2019, confidently assigning parentage to 13,683 individuals to determine age of spawning and rates of iteroparity and repeat-spawning within each year. We found differences among hatcheries in genetic lineage, management practices, and environmental factors were all reflected in significant differences in spawner life-history traits among hatchery steelhead. Differences between coastal and central valley steelhead lineages contributed to significant differences in age at return, timing of spawning, and rates of iteroparity. Adaptive genomic variation associated with anadromy varied among hatchery programs and was also associated with age of steelhead spawners. Hatchery management practices likely drive differences in the distribution of return timing and frequency of repeat-spawning among hatcheries, while environmental impacts including drought influenced among-year variation in number of returns.

Applying genome editing technology to advance salmon functional genomics and conservation

Michael Phelps

Genetic analysis is now routinely performed on thousands of returning salmon each year in the Pacific Northwest and the genetic differences between many stocks have been established. There is a strong interest in employing salmonid genetics expertise to gain an understanding of the genomic factors that drive local adaptation, and which can provide insight into key life history

traits and phenotypes of conservation concern. A limitation of modern salmon genomics is often the difficulties associated with experimentally validating genetic discoveries to confirm causation or to establish insights into the biological mechanisms underlying key salmon physiological processes. In the age of genome editing technology, evaluating genomic discoveries is no longer a limitation since it is now possible to modify the genome of salmonid model systems to test genetic questions under controlled conditions. Our ability to edit the genome of Pacific salmonid species is rapidly advancing and has progressed to a point where multi-loci editing can be performed routinely with CRISPR technology. Two applications of genome editing technology that will be discussed include the development of genetic barcodes to genetically differentiate hatchery stocks as well as using genome editing technology to evaluate genetic findings linked to core salmon life history traits, such as run timing and thermal tolerance. The salmonid model systems that have been developed are just the beginning of a new frontier in our ability to understand the genomes of these magnificent animals, hopefully leading to new discoveries that can improve salmon conservation efforts across the Pacific Northwest.

Efficient species identification for large-scale Pacific salmon genetic monitoring programs

Zachary Robinson, Jeff Stephenson, Kim Vertacnik, Stuart Willis, Rebekah Horn, Jesse McCane, Katharine Coykendall, Shawn Narum

Genetic monitoring of Pacific salmon in the Columbia River Basin provides crucial information to fisheries managers that is challenging to obtain through traditional methods. Programs such as genetic stock identification (GSI) and parentage-based tagging (PBT) involve genotyping hundreds of thousands of individuals each year using Genotyping-in-Thousands by sequencing (GT-seq). Although rare, these sample collections inevitably include misidentified species, which exhibit low genotyping success on species-specific GT-seq panels. Additionally, juvenile salmonids sampled at early life stages are more difficult to identify to the species level, and these collections often include higher rates of off-target species. For laboratories involved in large-scale genotyping efforts, diagnosing off-target species and reassigning them to the appropriate monitoring program can be costly and time-consuming. To address this problem, we identified 19 primer pairs that that exhibit consistent cross-species amplification among salmonids and contain 53 species informative variants. These genetic markers reliably discriminate among the 12 salmonid species tested and have been included in species-specific GT-seq panels for Steelhead, Sockeye, Coho, and Chinook Salmon. We then incorporated a species-calling script into our standard GT-seq genotyping pipeline, which automates the identification of off-target species. Following extensive testing, we demonstrate that the genetic markers and accompanying script accurately identify species and are robust to missing data and common genotyping errors. Finally, we used these tools to identify Coho Salmon incidentally caught in the Columbia River Chinook Salmon sport fishery and used PBT to determine their origin. These species-informative genetic markers and computing resources provide an efficient means of species identification for ongoing genetic monitoring in the Columbia River Basin.

Parentage-based tagging at-scale: Applications in Canadian mixed-stock fisheries and broodstock monitoring

Eric B. Rondeau, Terry D. Beacham

Amplicon-based DNA sequencing is powering a revolution, delivering a cost-effective way to enable the incorporation of genetic data to fisheries resource management. Perhaps most commonly enabled through genetic stock identification methods (GSI), amplicon based panels can be extended to related techniques, such as Parentage-based tagging, or PBT Parentage-based tagging. PBT is a method of assigning genetic parentage to individuals of unknown origin by comparison to a database of parental genotypes, predominately hatchery broodstock, thus recovering not only specific parentage but stock and age of origin. On the Pacific coast of Canada, the application of PBT has been focused on Chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon, species for which significant hatchery augmentation efforts are in place. To enable PBT, the last few years has seen an intensive effort in the collection of greater than 160 thousand Chinook and 60 thousand coho brood between 2013-2022 - this year 's collection is expected to encompass >99% of broodstock utilized in BC and Yukon hatcheries. Genotyping of the samples have been performed utilizing amplicon panels consisting of hundreds of markers, and the incorporation of the resulting genotypes into a coastwide genetic baseline allows for the dual application of GSI and PBT to maximize efforts in determining mixed-stock fishery proportions. An update on the use of PBT in Canada will provide examples of performance relative to coded-wire tags (CWT), the expanding role in broodstock monitoring, and new methods and species on which PBT monitoring is expanding in the coming years.

Reliability of trans-generational genetic mark-recapture (tGMR) for enumerating Pacific salmon

Sam Rosenbaum, Sam May, Kyle Shedd, Randy Peterson, Brian Elliot, Megan McPhee

As Pacific salmon continue to decline across much of their range, it is imperative to further develop reliable tools to quantify trends in population abundance. Estimating the number of spawning adult salmon (escapement) is a fundamental but difficult task for fishery managers. Escapement monitoring of salmon typically requires intensive sampling across the return migration, often in remote locations, and is subject to changing environmental conditions that can reduce data quality. Alternative methods that increase the efficiency, accuracy, and precision of escapement monitoring are desirable, particularly during times of shrinking budgets for fisheries management. With the advent of high-throughput genotyping, biologists can now combine molecular pedigree information with life-history data to make robust inferences about the size of wild populations using trans-generational genetic mark-recapture (tGMR). Non-lethal genetic samples 'mark' adults and 'capture' juveniles, then multi-locus genotypes are used to identify parent-offspring pairs ('recaptures'), provided that these kinship relationships can be identified and that certain mark-recapture assumptions such as equal capture probability are met. Our project aims to help optimize the emerging tGMR methodology by comparing tGMR estimates to a traditional mark-recapture project for Chinook salmon from the Chilkat River in Southeast Alaska. We further identify potential biases arising from violations of the equal probability of capture assumption using an individual-based simulation model to evaluate the accuracy and precision of tGMR under varying demographic and sampling scenarios. We leverage empirical values to parameterize simulations exploring the influence of age-specific

reproductive success and sampling selectivity on tGMR estimates. Finally, we determine how adult sampling location and timing can impact tGMR estimates by comparing adult samples collected from the mainstem of the Chilkat River in June from samples collected in the upriver tributaries in August. Our assessment of the reliability of tGMR informs this method's potential for accurate and precise monitoring of population declines as well as recovery.

Hybridization decreases native Cutthroat Trout reproductive fitness

William Rosenthal, John Fennell, Elizabeth Mandeville, Jason Burckhardt, Annika Walters, Catherine Wagner

Examining the action of natural selection in wild populations presents many challenges, but also sheds light on important ecological and evolutionary processes. Additionally, in hybridizing populations, natural selection may be an important determinant of the eventual outcome of hybridization. We characterized several components of relative fitness in hybridizing populations of Yellowstone Cutthroat Trout and Rainbow Trout in an effort to better understand the prolonged persistence of both parental species despite predictions of extirpation. Thousands of genomic loci enabled precise quantification of hybrid status in adult and subsequent juvenile generations; a subset of those data also identified parent-offspring relationships that we used to assess the effects of ancestry on reproductive output and mate choice decisions. We found a relatively low number of late-stage (F3+) hybrids and saw an excess of F2 juveniles relative to the adult generation, which suggests the presence of hybrid breakdown decreasing the fitness of F2+ hybrids later in life. Assessments of reproductive output showed that Yellowstone Cutthroat Trout females are more likely to successfully reproduce and produce slightly more offspring than their Rainbow Trout and hybrid counterparts. Mate choice was highly variable, though we did find statistical support for slight female preference for males of similar ancestry and higher Yellowstone Cutthroat Trout ancestry. Together, these results show that native Yellowstone Cutthroat Trout are able to outperform Rainbow Trout in terms of reproduction and suggests that management action to exclude Rainbow Trout from spawning locations may bolster the now-rare Yellowstone Cutthroat Trout.

Calibrating eDNA sampling strategies for monitoring endemic salmonids in Western US drought-stricken ecosystems

Gregory Schumer, Yekaterina Karpenko, Michelle Leung, Myfanwy Johnston, Bobbie Flores, Jamie Sweeney, Kirsten Sellheim, Joe Merz

The ongoing drought in the Western United States has taken a severe toll on the endemic Chinook salmon (*O. tshawytscha*) and Steelhead/Rainbow Trout (*O. mykiss*), leading to acute and chronic stress levels. To carefully monitor the adverse impacts of rapidly fluctuating ecosystems on the distribution of salmonids, an effective technique for data collection is required. Systematic sampling for environmental DNA (eDNA) coupled with species-specific quantitative PCR (qPCR) analyses presents an ideal way to survey species distribution across large systems, especially when shorter or no permitting processes and rapid deployment are

crucial. Since eDNA sampling does not affect the target species, extensive permits and licenses are not required. However, to ensure maximum efficiency with a limited sampling timeframe, prior experience with a study location is recommended. In situations where prior knowledge is insufficient, tools such as the publicly available artemis modeling framework can be used to optimize the sampling design within days. By utilizing artemis and positive control experiments, we were able to calibrate our eDNA sampling strategy in advance of actual sampling. Our optimized sampling strategy entailed collecting 3 liters of water, through 3 filters, at 1 km intervals, and analyzing each filter 9 times for the presence of eDNA from both *O. tshawytscha* and *O. mykiss*. When we applied this sampling approach over a 22km study area on the Lower American River in Northern California, we discovered a higher concentration of *O. tshawytscha* eDNA compared to that of *O. mykiss* eDNA. Overall, the combination of artemis modeling and live car experiments provides a systematic way to calibrate and optimize eDNA sampling strategies, allowing for early identification of target species and better conservation outcomes.

Frequency and fitness consequences of close-kin inbreeding in wild Sockeye salmon populations

Emily Schwabe, Samuel May, Charles Waters, Kerry Naish

Fitness reductions associated with inbreeding (i.e., inbreeding depression) have been documented across a variety of taxa, typically from experimental studies of captive populations. However, rates of inbreeding in wild populations and subsequent consequences to individual fitness and population productivity remain comparatively understudied. In particular, there are few examples of the effects of close-kin inbreeding on fitness in wild populations. This study quantified the frequency and fitness consequences of inbreeding using molecular-based pedigrees comprising nearly 10,000 individuals from two wild Sockeye salmon (*Oncorhynchus nerka*) populations in Bristol Bay, Alaska. Inbreeding coefficients were quantified and regressed against lifetime reproductive success and fitness-linked traits. Yet, adult-to-adult pedigrees only provide information on matings that produced offspring who returned to spawn. Therefore, we also compared the observed frequency of inbreeding to the frequency expected by chance alone, predicted by a temporally-explicit individual-based model parameterized with empirical data. We found no effect of inbreeding on lifetime reproductive success. However, results indicated that inbreeding decreased adult body size and reproductive lifespan and delayed return timing to spawning streams. Simulation results indicated that inbreeding was detected less frequently than expected by chance, suggesting that inbred fish may have lower marine survival than non-inbred fish. We discuss the benefits and drawbacks of using pedigrees to study inbreeding in the wild and the utility of our results to the management and captive breeding of salmonids.

Comparative riverscape genetics of Elwha River salmonid species and life-history forms following dam removal

Travis Seaborn, Kimberly Ledger, Yingxin Su, Jong Yoon Jeon, Aimee Fullerton, David Kuligowski, Todd Bennett, Keith Denton, Michael McHenry, John McMillan, Joseph H. Anderson, Todd R. Seamons, George Pess, Krista M. Nichols, Garrett McKinney, Alexandra K. Fraik

Barriers such as hydroelectric dams inhibit migratory pathways essential to many aquatic species, resulting in significant losses of species, their unique life-histories and genetic diversity. Understanding the impacts of dam removal and monitoring recovery of species, life-history and population diversity is crucial to fully understand the restoration response. We used the removal of two large dams on the Elwha River as an opportunity to characterize how restored connectivity impacts the re-establishment of two fish species Chinook salmon (*Oncorhynchus tshawytscha*), and Steelhead/rainbow trout (*Oncorhynchus mykiss*) and their unique ocean migration return-timing life-history forms. In this study, we employed riverscape genetics to understand how restoration and the environment influence the distribution of neutral and return-timing genetic variation underlying the migratory life-history forms and species at- and between-sampling sites. We genotyped fish sampled over time and sample in the Elwha River using GTSequencing loci for both species at neutral and putatively adaptive loci in and near the major effect genic region GREB1L/ROCK1 putatively associated with migration timing. We observed little evidence for changes in neutral genetic structure for either species over time or space. We observed a statistically significant increase in the early-return time alleles in above-dam *O. mykiss* population post-dam removal. For *O. tshawytscha*, at-site genetic variation was shaped by river distance, at-site habitat differentiation, and a combination of at-site environmental factors; while the between-site genetic variation was mainly shaped by river distance. For all *O. mykiss*, at-site and between-site genetic variation is primarily explained by river distance. Genetic variation in just juvenile or adult Steelhead, respectively, were influenced by at- and between-site environmental factors and habitat difference. Our study illustrates, despite the differences across species and types of genetic variation, river distance and environmental factors strongly explained their response to dam removal.

Haplotype association analyses for fine-mapping of QTLs for IHNV resistance in two commercial lines of rainbow trout

Christopher Setzke, Nobu Masaki, Yniv Palti, Roger Vallejo, Sixin Liu, Maureen Purcell, Kyle Martin, Kerry Naish

Genome-wide association studies (GWAS) can identify quantitative trait loci (QTLs) associated with complex traits. However, trait-associated SNPs rarely detect the casual variants, especially when GWAS is performed using lower-density marker panels. Recombination events between markers and variants can diminish the utility of marker-assisted selection in aquaculture, or in tracking fitness traits in wild populations. Therefore, higher resolution mapping is frequently employed to identify markers more closely linked to causal variants or candidate SNPs for further investigation. In large mapping populations, it is often more efficient and cost-effective to sequence individuals that are segregating at a QTL, rather than attempt to perform high resolution genotyping on all individuals used in the initial GWAS. Here we describe an analytical approach to achieve this objective in two commercial lines of rainbow trout. QTLs for infectious hematopoietic necrosis virus (IHNV) resistance were previously detected using 57K SNP array genotype data from 100 (N=1,867) and 103 (N=1,772) full-sib families from the two lines respectively. Here, QTLs that were shared between commercial lines or explained a considerable proportion of the additive genetic variance within lines were prioritized for further

statistical analysis. Chromosomes were phased and 3-SNP haplotypes spanning >0.5Mb within these QTL regions were constructed. Haplotype inheritance error was measured, and association analyses were performed to identify specific haplotypes associated with resistance and susceptibility to IHNV. The Pearson correlation coefficient was then calculated between the number of resistance haplotypes in the two parents and IHNV survival rate in their offspring in order to estimate the combined phenotypic effects of haplotypes at several QTL. Offspring of parents segregating at resistant and susceptible haplotypes were then selected for future high-density sequencing. This approach can help inform future fine-mapping studies from lower-density SNP arrays and improve marker-assisted selection in commercial aquaculture.

Status and future plans for coastwide genetic baselines used by ADF&G

Kyle Shedd, Heather A. Hoyt, Tyler H. Dann, Andy W. Barclay, Elizabeth M. Lee, Chase S. Jalbert, Kristen M. Gruenthal, Wei Cheng, Sara E. Gilk-Baumer

Genetic mixed stock analysis (MSA) or genetic stock identification (GSI) remains one of the primary applications used by the Alaska Department of Fish & Game, Gene Conservation Laboratory (GCL). The baselines we use are the foundational infrastructure for all our MSA projects, ranging in size from small, regional baselines with just a few hundred individuals to large, coastwide baselines with over 65K individuals. These large, coastwide baselines represent massive investments in collaboration, time, energy, and money, but are critical for stock identification in highly mixed-stock fisheries and marine surveys. This presentation aims to give an Alaska-centric overview of coastwide baselines currently in use by the GCL for all five species of salmon, status of baseline development, and opportunities for collaboration.

Southern Appalachian Brook Trout Reintroductions: Does Genetics matter?

Rebecca Smith, David Kazyak, Barbara Lubinski, Matt Kulp, Ben Fitzpatrick

Wildlife reintroductions are a conservation tool to re-establish native species to their historical ranges. However, identifying appropriate source populations for reintroductions can be a challenge because introduced genotypes may not be well suited for the target environment. In practice, reintroductions are rarely preceded by detailed genetic evaluation. Brook Trout (*Salvelinus fontinalis*) are imperiled within the Southern Appalachian Mountains, and have been extirpated from much of its historic range due to anthropogenic impacts and competition with non-native species. In Great Smoky Mountains National Park (GRSM), Brook Trout are the only native salmonid and have been isolated in remote headwater systems since the early 1900s, exchanging little to no gene flow between populations. State and federal agencies have been strategizing to restore the genetically unique Southern Appalachian Brook Trout to their native range and reintroduction efforts are ongoing in GRSM. Currently there are over 48 kilometers of reintroduced Brook Trout habitat in the National Park, which encompasses 13 different streams, and management has plans to restore six additional streams this year. To prevent depleting source populations, managers use native Brook Trout from multiple source populations for reintroductions into a single habitat. Previous studies indicated that multiple source populations do not readily admix as a consequence of being too genetically differentiated and/or a mismatch

in spawning phenology. This reported nonrandom admixture raises concerns about assortative mating or outbreeding depression. My research addresses critical admixture issues by (1) creating a null model of unconstrained mixture for comparison against observed data collected at restoration sites and (2) investigating any source bias that may be present in restoration sites. To generate a null distribution of expected outcomes, we assume random mating, even mixture (no selection favoring once source over another), and use realistic assumptions about Brook Trout life history and demographics. We then compare observed versus predicted outcomes. Future research will utilize high-throughput sequencing to assess the genetic composition of additional reintroduced populations. Our research aims to improve our understanding of past restoration outcomes and provide decision-relevant information to guide future reintroductions.

A large, recently evolved supergene facilitates rapid adaptation of an introduced fish

Morgan M. Sparks, Avril M. Harder, Claire E. Schraidt, Lisa W. Seeb, Mark R. Christie

Numerous and sometimes complex genetic mechanisms may promote rapid adaptation to novel or changing environments. Chromosomal inversions—which are characterized by the rearrangement of chromosomal segment that reverses its original 5 prime to 3 prime position—form the genetic basis for many divergent life history strategies. Moreover, inversions can act as "supergenes" because they are inherited as a large, non-recombinant haplotype blocks that maintain suites of coadapted genes underlying adaptive phenotypes. Here, we present evidence for the role of a supergene in the recent adaptation of northern Pacific Ocean pink salmon that were introduced to the Great Lakes 31 generations ago. We use 134 samples from the native and invaded range and medium-coverage (~14X) whole-genome resequencing to identify loci facilitating adaptation to the Great Lakes. These data indicate a large non-recombinant block containing a putative inversion—29 Mbp or 1.1% of the genome—is present on chromosome 10 of pink salmon in the Great Lakes. We show that this inversion appears exclusively in the Great Lakes (i.e., was not found in an additional 1,098 pink salmon that ranged from Japan to British Columbia, Canada) and contains key osmoregulatory genes significantly overrepresented within the inversion, which matches expected adaptation of an obligate anadromous fish to a wholly freshwater environment. Furthermore, this inverted region has significantly lower absolute differentiation relative to the rest of the chromosome on which it is found in Great Lakes samples, suggesting it recently evolved. Together, our results present a compelling example of how a recently evolved supergene aids in the contemporary adaptation of a population to a novel environment.

Conservation of the Paiute cutthroat trout: discovering genetic markers to monitor multiple refuge populations and translocations

Yingxin Su, Amanda Finger, Matthew A. Campbell, Jeff Rodzen

The Paiute cutthroat trout (*Oncorhynchus clarki seleniris*, PCT) is an extremely rare subspecies of cutthroat trout that is currently threatened by small population sizes and isolation. Its native habitat is a stretch of lower Silver King Creek, a tributary of the east fork Carson River,

California, bounded by fish barriers. In 1912, PCT were moved above the barrier into Upper Silver King Creek. Subsequently, non-native salmonids were introduced into lower Silver King Creek, eradicating PCT due to out-competition and hybridization. Meanwhile, the pure PCT were translocated multiple times over decades, resulting in nine isolated refuge populations today: five of which are within the SKC watershed while four are outside. Translocations may be an essential component of PCT management in the future in order to boost effective population sizes (Ne) and reduce the loss of genetic diversity in each population and the species as a whole. This requires the creation of baseline genetic data in addition to monitoring the genetic changes after the translocation. Yet, PCT presents a challenge for marker discovery given their extremely low genetic diversity, whole genome duplication, and unshared local genetic drift in each refuge population. Here we discuss the method of discovering the markers across PCT populations that have low shared polymorphism. We then used these markers to explore population structures, genetic diversity, and the estimation of Ne in individual refuge populations over time. We also evaluated a translocation event from the out-of-basin North fork Cottonwood Creek refuge population to upper Silver King Creek, using parentage analysis. We found the combination of the SNP markers and the genome-wide dataset is likely the optimal solution for this subspecies. This work is relevant to the conservation of species with multiple genetically depauperate refuge populations where managers are considering long-term monitoring and translocations.

Genomic insights into the demographic history and conservation status of Danish whitefish (*coregonus* spp.) populations

Aja Tengstedt, Shenglin Liu, Camilla Gundlund, Dorte Bekkevold, Magnus W. Jacobsen, Michael Møller Hansen

Integrating genomics in conservation has frequently been highlighted as a way of improving insight into the evolutionary dynamics of threatened populations and species, yet critics have voiced concern regarding the utility of conservation genomics. Here, we demonstrate how genomics approaches shed light on the intricate demographic history of the morphologically diverse European lake whitefish species complex (*Coregonus lavaretus*, ELW) in Denmark. This includes the North Sea houting (*Coregonus oxyrhynchus*, NSH), which by some has been considered a separate species due to its pronounced morphological divergence and unique ability to tolerate oceanic salinities. Our results support a previously hypothesized scenario of rapid post-glacial population expansion following whitefish colonization of Denmark but contradicted estimates of a shallow evolutionary history and recent divergence time between NSH and ELW. Rather, our models indicated that population divergence occurred shortly after the last glaciation ca. 13,000 BP. Analysis of runs of homozygosity (ROH) revealed that inbreeding has occurred recently and to a moderately high extent in the NSH. Finally, we found significant enrichment for gene ontology (GO) terms relating to osmoregulation among the candidate genes under selection in NSH to an extent unparalleled by any ELW population. This suggests that mitigating the future risk of inbreeding depression by establishing gene flow between NSH and ELW populations involves a risk of inducing outbreeding depression. In addition to demonstrating the advantages of utilizing genomic data in conservation, our results confirm that the NSH is morphologically, genetically, and adaptively divergent from ELW. While its species status may

be questioned, the North Sea houting is unique among whitefishes and merits conservation as an independent unit.

A range-wide catalog of whole genomes reveals broad importance and complexity of GREB1L

Tasha Thompson, Matthew Sloat, Mariah Meek

Technological advances are facilitating the identification of markers for adaptive variation in wild salmon. However, new markers are typically discovered in one or a small number of populations, and evaluating the efficacy of the markers at a broader scale is time and resource intensive. Here, we present a catalog of nearly 500 individual whole genomes from Chinook salmon sampled across the species' range that can serve as a resource for evaluating adaptive diversity at a range-wide scale. To demonstrate the utility of the catalog, we present a case study with GREB1L, a locus strongly associated with adult migration phenotypes. We use the whole genome catalog to evaluate the architecture of the GREB1L locus across the species ' range, predict the range-wide efficacy of previously-published markers, identify potential new markers, and generate hypotheses about GREB1L's influence in previously untested populations. We then apply markers vetted through the range-wide catalog to novel populations. Our results indicate the GREB1L locus modulates adult migration characteristics across the range, including within northern populations with relatively compressed migration timing. Furthermore, we identify and characterize variation within the GREB1L locus that facilitates fine-tuning of migration phenotypes beyond what is observed in populations with simple bimodal spring/fall timing.

Using terminal genetic stock identification (GSI) and upriver mark-recapture to estimate total systemwide escapement of Pacific salmon

Ryan Whitmore, David Hankin

Estimation of total escapement for Pacific salmon is particularly problematic for large river systems that contain a multitude of component stocks. It is typically impossible, for both logistical and financial reasons, to carry out independent stock-specific escapement estimation programs in all major tributaries, and combine them to produce an estimate of total escapement. To address this typical problem, we generate an estimate of total system-wide escapement by combining an estimate of stock-specific escapement with a lower river GSI estimate of the stock-specific proportion for Coho Salmon (*Oncorhynchus kisutch*) in the Skeena River watershed, British Columbia. Our approach utilizes a mark-recapture estimate of escapement to the Bulkley River watershed above Witset ($\hat{E}_B$) and a terminal test fishery GSI estimate of the proportion of Bulkley River fish in the run ($\hat{p}_B$) at Tyee, 24 km above the river entrance. The estimate of total escapement, E_T, is $\hat{E}_T = \hat{E}_B/\hat{p}_B$. Our approach also allows estimation of stock-specific escapements, E_i, at either Conservation Unit (CU) or "Genetic Unit" (GU) levels as $\hat{E}_i = \hat{p}_i \hat{E}_T$, where i denotes a particular CU (or well-identified GU). In addition, we calculate errors of estimation of total escapement and CU or GU escapements based on delta method approximations.

Contrasting patterns of sequence variation in steelhead populations reflect distinct evolutionary processes

Stuart Willis, D. Katharine Coykendall, Matthew R. Campbell, Shawn Narum

Multiple evolutionary processes influence genome-wide allele frequencies and quantifying effects of genetic drift and multiple forms of selection remains challenging in natural populations. Here we investigate variation at major effect loci in contrast to patterns of neutral drift across a wide collection of steelhead populations that have declined in abundance due to anthropogenic impacts. Whole genome resequencing of 74 populations of steelhead revealed genome wide patterns (~8 million SNPs) consistent with expected neutral population structure. However, allelic variation at major effect loci associated with adult migration timing (chromosome 28: GREB1L/ROCK1) and age at maturity (chromosome 25: SIX6) was consistent with how selection has acted on phenotypic variation. Variation at major effect loci was influenced by evolutionary processes with differing signals between the strongly divergent Coastal and Inland lineages, while allele frequencies within and among populations within the Inland lineage have been driven by natural selection as well as recent anthropogenic influences. Recent anthropogenic effects appeared to have influenced the frequency of major effect alleles including artificial selection for specific traits in hatchery stocks with subsequent gene flow into natural populations. Selection from environmental factors at various scales has also likely influenced variation for major effect alleles. These results reveal evolutionary mechanisms that influence allele frequencies at major effect loci that are critical for conservation of phenotypic traits and life history variation of this protected species.

Poster Presentations

Understanding Yellowstone cutthroat trout hybridization and connectivity within the Teton river system, a combined genomics and modeling approach

Meggan Alston, Michael Youngwirth, Alexandra Fraik, Paul Hohenlohe, Travis Seaborn

Connectivity restoration efforts are an important strategy to assist native trout persistence across Western US river systems. However, increased connectivity may have unintended negative consequences, such as increasing hybridization rates between native and invasive species. This is particularly relevant for migratory species with multiple life history strategies. Here we use SNP genotyping data from single-digest RAD sequencing to assess population structure and estimate existing rates of hybridization and strength of assortative mating between Yellowstone cutthroat trout (*Oncorhynchus clarkii boulveri*) and non-native rainbow trout (*Oncorhynchus mykiss*) from the Teton River Basin. Samples were collected from approximately 470 individuals from several locations on the river mainstem and four tributaries. These include fish visually identified as cutthroat trout, rainbow trout and putative hybrids. Results from genetic analyses will be incorporated into demographic-genetic individual-based models to predict how different stream connectivity scenarios identified from stakeholder engagement may alter Yellowstone cutthroat trout life history variation and future hybridization outcomes. Understanding which restoration actions can facilitate native trout persistence while also limiting increased hybridization with heterospecifics will be key to informing future conservation and management decisions.

Using a foreign DNA tracer to calibrate natural environmental DNA signals of coho salmon (*Oncorhynchus kisutch*) in small streams

Gavin Bandy, Andrew Kinziger, Andre Buchheister, Eric Bjorkstedt

Environmental DNA (eDNA) is rapidly developing as an efficient, cost-effective and noninvasive approach for determining the presence of rare species. To date, there has been relatively little evaluation of the utility of eDNA for determining local-occupancy at small scales within streams. Determining local scale-occupancy with eDNA poses challenges owing to particle transport from upstream and the potential for detection of eDNA originating outside of the study reach. Using an exogenous eDNA tracer presents a method to correct for this potential bias. In this research, we evaluated the utility of using a foreign eDNA (FeDNA) tracer to determine local-scale occupancy of coho salmon (*Oncorhynchus kisutch*). A suspension of FeDNA, sourced from species not naturally occurring in sampled systems, was created to mimic naturally-shed eDNA in an aquatic system, and was introduced into streams at a known concentration and rate. The dynamics of FeDNA within a site was used to calibrate the movement of native eDNA and to improve inferences into local scale patterns of coho salmon occupancy. Concurrent snorkel surveys were conducted as an independent estimate of coho salmon abundance and distribution as a basis for evaluating eDNA estimates of coho salmon occupancy. We developed a ratio estimator to evaluate the changes in eDNA concentration between naturally occurring and foreign eDNA sources. Preliminary analysis demonstrates the

potential for this methodology to provide insights into the scales at which eDNA can be used to indicate local-scale occupancy. The creation of a novel methodology for determining local occupancy will hopefully provide a new tool for monitoring endangered and threatened species at small scales within streams.

The importance of place; Fish diversity observed using DNA metabarcoding

Scott Blankenship, Katie Karpenko, Cheryl Dean

The importance of place cannot be overstated in biology, as place often drives the principal partition in genetic variance, is correlated with abundance, and underlies biodiversity. This project describes fish biodiversity observed using DNA metabarcoding within an evolving tidal wetland engineered as a living laboratory for habitat restoration (Dutch Slough Tidal Marsh Restoration Project). DNA metabarcoding approaches were incorporated into the Dutch Slough project performance assessment, as reticulated tidal habitat is challenging to survey effectively with nets. An EDNA sampling designed was executed pre and post breaching of levees surrounding the engineered habitat exposing the habitat to the San Francisco Estuary. The fish community observed at each survey event contributes to base condition assessment of 1) fish community trends as habitat evolves over time, 2) the presences of protected, native, and non-native species, and 3) seasonal differences in habitat use. Comparing pre and post breach eDNA surveys, fish biodiversity increased within restored habitat post breach. Fish in the project vicinity appeared to colonized habitat once access was provided. Non-native species outnumbered native species by approximately 2-to-1, reflecting the background condition of the Estuary. Among the 42 fish species observed during ongoing eDNA surveys, five protected native fish species have been observed within restored habitat (Chinook Salmon, Green Sturgeon, Longfin Smelt, Pacific Lamprey, and Rainbow Trout).

Comparing eDNA and juvenile salmon trap passage estimates on the Sacramento River

Jacqueline Bridegum, Andrew Kinziger, Gavin Bandy, Bill Poytress, Mark Henderson

The Sacramento River supports four different ecotypes of Chinook salmon, *Oncorhynchus tshawytscha*, including spring, fall, late fall, and winter runs. Due to various anthropogenic actions (e.g., hydropower dams and habitat modifications) the spring and winter runs are listed as threatened and endangered, respectively. Monitoring for juveniles in these imperiled populations on the Sacramento River uses a network of monitoring sites with varying reliability for producing population-level abundances due to the unreliability of run identification (e.g., length at date), low juvenile catch rates, and the subsequent large variance in gear efficiency estimates. One sampling method that has been proposed to improve salmon monitoring efforts throughout the Sacramento River is Environmental DNA (eDNA). eDNA is genetic material that is shed from an organism into the surrounding environment in various forms (e.g., mucus, urine, tissue). In rivers, these particles are collected in water samples and the species-specific concentrations of DNA are assayed and used to draw inference on species occupancy or abundance. To determine if it was feasible to quantify the abundances of outmigrating juveniles from different runs, we

collected water samples near a rotary screw trap in the upper Sacramento River. Water samples were collected weekly in the fall and spring to quantify the abundances of spring and winter run salmon. We preliminarily found a positive correlation between the quantity of eDNA in the water samples and the abundance of outmigrating juvenile Chinook salmon. This positive relationship could be applied to facilitate a better understanding of the timing, survival, abundance, and movement of juvenile Chinook Salmon throughout the entire Sacramento River.

Characterization of the significance and distribution of a large chromosomal inversion in native populations of rainbow trout

Matthew C. Hale, Matthew A. Campbell

Chromosomal inversions (CIs) have long been recognized as a major driving force behind local adaptation. Failure of crossing over in heterozygous individuals results in substantial recombination suppression allowing for adapted alleles to be inherited as distinct haplotype blocks. Maintenance of both the ancestral and derived forms of CIs suggest the underlying effects of balancing selection that may be maintained over long evolutionary time scales. A large (~55 MB) pericentric inversion on chromosome five has been well described and documented throughout the North American range of rainbow trout (*Oncorhynchus mykiss*). The different forms of this inversion have been linked to a diverse set of adaptive phenotypes including development rate, migratory behavior, and phototransduction. An additional large (~11MB) CI is present on chromosome 20 in rainbow trout, however, this CI has been far less studied with regard to linked phenotypes, geographic and taxonomic distribution of the different forms of the inversion, and its age. Here we use genomic techniques to characterize the chromosome 20 CI in populations of rainbow trout from the Pacific Northwest and Alaska. We focused our analyses to document the different functions of genes within the chromosome 20 inversion and describe how the different forms of the inversion segregate across North America. We also use phylogenomic techniques to document the age of the inversion and evolutionary relationships of the different forms of the inversion in the major north American subspecies of rainbow trout.

Genome-wide association study for precocious maturation of two-year-old male spring Chinook Salmon (*Oncorhynchus tshawytscha*)

Nick F. Hoffman, Stuart C. Willis, Hayley M. Nuetzel, Shawn R. Narum, Andrew L. Pierce, James J. Nagler

Male spring Chinook Salmon (*Oncorhynchus tshawytscha*) that precociously mature at two years of age are referred to as minijacks. For hatcheries that are designed to conserve and increase abundances of anadromous males, high incidences of minijacks detract from production goals. The Cle Elum Supplementation & Research Facility (CESRF, Cle Elum, Washington) has reported high minijack rates across generations suggesting precocious maturation may be subject to some level of genetic control. Identifying regions in the genome associated with the minijack phenotype would be the first step towards understanding genetic factors influencing this trait. To

test for genomic regions associated with the minijack phenotype, we performed low-coverage whole genome re-sequencing (lcWGS) using juvenile male fish from CESRF with known maturity phenotypes. Data from lcWGS were separated into replicated phenotypic groups based on an individual's position within a bimodal distribution of plasma 11-ketotestoersone concentrations, which indicate maturation status and were measured previously. Sequence alignment to the reference genome and variant calling was done with the PPalign module in the Poolparty pipeline. After filtering the reads, mean depth of coverage ranged from 16.0 to 25.7 X (mean = 22.2 ± 2.8 X SD) per group and the proportion of the genome covered ranged from 82.2% to 65.5%. The intended approach for analyses is to utilize the ~ 22.36 million single nucleotide polymorphisms retained after alignment for association tests. Allele frequency differences will be compared between phenotypes using: F_{ST}, sliding F_{ST}, Local Score, and CMH genome-wide association tests. Significant signals found in multiple association tests will be considered candidate regions for the minijack phenotype for further validation.

Utility of parentage-based tagging for monitoring Coho salmon (*Oncorhynchus kisutch*) in the interior Columbia River basin

Rebekah L. Horn, Becky Johnson, Cory Kamphaus, Jon Lovrak, Kraig Mott, Todd Newsome, Hayley M. Nuetzel, Shawn R. Narum

After decades of diminishing abundance in the mid 1900's, Coho salmon (*Oncorhynchus kisutch*) were considered extirpated from the interior Columbia River by the 1980s. In the mid-1990s, the Confederated Tribes of the Umatilla Indian Reservation, the Confederated Tribes and Bands of the Yakama Nation, and the Nez Perce Tribe began successful reintroduction programs of Coho salmon upstream of Bonneville Dam, which were initially sourced from lower Columbia River hatcheries. While genetic sampling for parentage-based tagging (PBT) is not implemented at all Coho salmon hatchery programs in the interior Columbia River basin, a PBT baseline including Coho salmon hatchery broodstock was first instituted in 2012 with two participating programs and has since expanded to include eight programs. Here we present the first Coho salmon PBT baseline from seven hatchery programs located in the interior Columbia River basin, and two sites at or downstream of Bonneville Dam, composed of over 32,000 broodstock samples. Analyses of baseline collections revealed genetic structure followed a temporal pattern based on three-year broodlines rather than geographic location or stocking history, and similar levels of genetic diversity across hatchery programs. The effective number of breeders per hatchery and spawn year ranged from 110 (CI: 58-745) to 410 (CI: 384-437). The PBT baseline provided multiple direct applications such as detecting the distribution of hatchery-origin fish on the spawning grounds in the Methow River basin and identifying the origin for Coho salmon collected in a mixed stock at Priest Rapids Dam (PRD). In two collection years at PRD, 93% of sampled Coho salmon could be assigned to a hatchery of origin, providing total age, location, and sex information. The Coho salmon PBT baseline requires additional yearly samples from lower river hatcheries to be used for basin-wide applications, but with wide-spread support, can be a useful tool in the management of Coho salmon in the Columbia River basin.

Effects of depth, distance to shore, and water velocity on organismal and extra-organismal environmental DNA concentrations in a large river

Dylan Keel, Andrew Kinziger

Environmental DNA (eDNA) is a sensitive tool for detection of aquatic species and concentrations of eDNA in water samples have been useful for estimating abundance. This study evaluated the effects of depth, distance to shore, and water velocity on the concentration of organismal and extra-organismal eDNA concentrations in the Klamath River, California (basin area = 40,000 km^2). At each of six river cross-sections 32 water samples were collected, including surface-grabs and depth-grabs evenly distributed across the cross-section, and eDNA concentrations were determined for the parasite *Ceratonova shasta* and Chinook salmon, *Oncorhynchus tshawytscha*, using droplet digital PCR. *Ceratonova shasta* eDNA concentrations varied widely from non-detectable levels to 257,222 copies per liter, with an average of 52,187 copies per liter. In contrast, *O. tshawytscha* eDNA concentrations were much lower, ranging from non-detectable levels to 3,733 copies per liter, with an average of 540 copies per liter. Within individual cross-sections variation, expressed as the coefficient of variation, of eDNA concentration spanned from 0.40 to 1.43 for *C. shasta* and from 0.45 to 0.80 for *O. tshawytscha*. A semivariogram analysis of spatial Pearson residuals of non-spatial models revealed that eDNA concentration in water samples taken more closely together showed a pattern of spatial autocorrelation. Spatial autocorrelation represented a substantial portion of the variance in eDNA concentrations in *C. shasta*, accounting for 60.3% but less for *O. tshawytscha*, accounting for 33.3%. Generalized linear mixed-effects models were used to investigate the factors that might be contributing to the variability in eDNA concentrations, while accounting for spatial autocorrelation present in the data. The analysis showed that for *C. shasta*, variables such as depth, distance to shore, or velocity did not explain the observed variability in eDNA concentrations. In contrast, for *O. tshawytscha*, the top model identified a pattern where eDNA concentrations decreased with each 48 cm increase in depth resulting in an 8-23% decrease in eDNA concentration (95% credible interval), consistent with the known depth preference of juvenile *O. tshawytscha*. Investigators intending to estimate mean eDNA concentration along river cross-sections should consider pilot studies to assess eDNA concentration variability; this study found that in order to estimate the mean eDNA concentration at a location in a river, assuming a level of statistical power (e.g., coefficient of variation of 0.2), on average 15 water samples were necessary for *C. shasta* and 11 for *O. tshawytscha*.

Evolutionary diversification of rainbow trout from Western North America

Janet L. Loxterman, Tyler Breech, Ernest R. Keeley

Rainbow trout (*Oncorhynchus mykiss*) are one of the most widely distributed freshwater fish species in North America. Natural biogeographic barriers and geological history have led to extensive diversification of rainbow trout. Numerous subspecies have been identified and are

managed as such; however, no study has examined phylogenetic diversity over the species range. In order to better understand diversification within rainbow trout, we sampled populations from across their native range and used mitochondrial DNA sequence divergence at the ND2 gene to examine phylogenetic patterns. Describing important patterns of diversification will aid in conservation efforts by identifying unique lineages and their geographic extent.

DNA sampling of smolts improves early estimates of adult sockeye returns for fisheries management

Stephen Latham, Catherine Michielsens, Angela Phung

Under increasingly volatile climate conditions, forecasting of Fraser River sockeye run size has been particularly challenging in recent years. During the migration of returning adults, the estimation of total escapement using time-density models improves as more data as acquired. Early in the migration period, there is a data scarcity and heavier reliance on an uncertain forecast. During this period of uncertainty, one alternative approach is to use the "Smolt Method of Updating Run Forecasts" (SMURF). The SMURF approach requires juvenile DNA samples in the year of ocean entry to estimate the abundance of returning adults. Juvenile DNA samples have been collected by the Department of Fisheries and Oceans Canada and other small research programs which played a greater role for the 2022 return due to COVID preventing regular DFO juvenile sampling operations. The estimated ratio of Early to Late Shuswap post-smolt juveniles has been informative of the true ratio of Early to Late Shuswap adults. In the last three dominant years (2014, 2018, 2022), the SMURF approach provided a more precise estimate of the Late Shuswap run size compared to the pre-season forecast earlier in the management season.

Reproductive success of reconditioned kelt steelhead in the Yakima River Basin

Jeff J Stephenson, Shawn R. Narum, Ryan Branstetter, Jeremiah Newell, Tim Resseguie, David E. Fast, William J. Bosch, Joseph W. Blodgett, Andrew L Pierce, Douglas R. Hatch

Reconditioning of kelt (post-spawned) steelhead may be an effective strategy to mitigate for high mortality inflicted on this ESA listed species by the hydrosystem. We evaluated the effectiveness of this strategy by testing whether reconditioned kelts provide a demographic boost to the natural population in the Yakima River Basin. Specifically, we compared nine spawn years of relative reproductive success of reconditioned kelts to that of anadromous adult steelhead captured as either upstream migrants at Prosser Dam or downstream post spawn fish captured at the Chandler Juvenile Monitoring Facility. Successful reproduction by the reconditioned kelts has been confirmed and appears sufficient to provide a demographic boost for this species and to retain genetic diversity and its iteroparous life history.

Assessing population structure and hatchery introgression within Skagit River Basin *Oncorhynchus mykiss*

Craig Wells, Catherine Austin, Todd Seamons, Krista Nichols, Paul Hohenlohe, Alex Fraik

The Skagit River Basin of northwestern Washington state has historically supported robust populations of *Oncorhynchus mykiss*, including both the anadromous Steelhead and resident Rainbow Trout life history forms. Anthropogenic activities within the basin over the last century—particularly the use of hatchery systems and hydrological damming—have contributed to the decline of numerous salmonid species' populations and have led to the listing of Skagit River Steelhead as "Threatened" under the Endangered Species Act (ESA), but their effects on population structure, genetic diversity, or gene flow within species have yet to be assessed with the power of genomic data. In this study, we generated SNP genotypes from restriction site-associated DNA sequencing (RADSeq) data from ~1,200 fin clips from fish sampled across the watershed to assess the population genetic diversity and structure of Skagit River *O. mykiss*. We will use these data to identify the population genetic structure between and gene flow among natural origin populations that occur in tributary streams downstream of the major hydroelectric projects within the Skagit River Basin. We will then leverage genomic data generated from two prominent hatchery stocks of Steelhead—Chamber's Creek and Skamania River—to determine the extent of genomic introgression within natural origin populations that may have resulted from the Skagit River's former hatchery program. These results will be crucial for assessing the influence of modern anthropogenic activities on Rainbow Trout and ESA-listed Steelhead populations within the Skagit River Basin—information that is necessary to identify the severity of these threats to these imperiled salmonids and inform future conservation measures.

Analysis of iteroparous spawning phenology in steelhead trout (*Oncorhynchus mykiss*) using low-coverage whole genomic resequencing

Stuart Willis, Jeff Stephenson, Doug Hatch, Shawn Narum

Steelhead have arguably the most diverse array of life history variations among Pacific salmonids, including individuals, almost invariably females, that do not die after spawning but rather return to the ocean ("kelting") and may spawn again in the following year ("consecutive") or later years ("skip"). Recognizing this, several hatchery programs have been developed to recondition post-spawn females for release. However, many of these reconditioned fish may not spawn the subsequent year, consuming resources for fish that may not survive to spawn again. Thus, to identify genetic markers associated with spawning phenology, we examined low-coverage whole genomic resequencing data from several year-classes of 'consecutive' and 'skip' spawning female steelhead from two facilities: Chandler Juvenile Monitoring Facility at Prosser Dam (Prosser, WA) on the Yakima River and Dworshak Fish Hatchery (Orofino, ID) on the Clearwater River. Sequence data were processed with POOLPARTY, which identified and retained 10,214,054 variants for analysis. Very few regions of notable genomic divergence were identified from these data, and while the local score analysis of CMH test p-values revealed a handful of regions above the threshold FDR of 0.05, these were orders of magnitude lower in score than other regions associated with life history traits with known heritable components.

While some hormonal indicators have been found useful in predicting if a fish will be a consecutive or skip spawner, and it is conceivable that spawning phenology is mostly condition-dependent. Although some heritable factors likely contribute to spawning phenology through metabolism, disease resistance, or other physiological processes that contribute to condition at or after spawning, these likely-polygenic traits will require very precise phenotype data and analytical design to detect with genome wide association analyses.
