RESEARCH/PD ANNUAL REPORT - PROGRESS REPORT

2015 annual report - progress
Brian Kemp
Genetic Analysis of Chinook Salmon Using Ancient, Historic and Modern DNA
R/HCE/PD-3
Submitted On: 04/13/2016 12:40:28 PM

METRICS & MEASURES

Metric/Measure	Value	Note
Acres of coastal habitat	0	
Fishermen and seafood industry personnel	0	
Communities - economic and environmental development	0	
Stakeholders - sustainable approaches	0	
Informal education programs	0	
Stakeholders who receive information	40	presentations
Volunteer hours	0	
P-12 students reached	0	
P-12 educators	0	

REQUESTED INFORMATION

Publications

No **Publications** information reported

Students Supported

Bobbi Johnson (Continuing Student)
bobbi.johnson@wsu.edu
Washington State University, School of Biological Sciences

Field of Study: Biology
Advisor: Gary Thorgaard and Brian Kemp
Degree Type: PhD
Degree Year: 2016

Student Project Title:

Involvement With Sea Grant This Period (capstone, fellow, intern, etc.):

Post-Graduation Plans (employer, grad school, etc.):

Was this thesis/dissertation supported by Sea Grant?: Yes

Thesis / Dissertation:

New or Continuing?: continuing

Degree awarded this reporting period?: No

Financially supported?: Yes

Narratives

Genetic analysis of Chinook salmon using ancient, historic and modern DNA
Uploaded File: Narrative_Kemp.pdf

Partners This Period

No **Partners This Period** information reported

STANDARD QUESTIONS

Impacts and Accomplishments

(1)

Type	accomplishment
Title	Washington Sea Grant researchers develop techniques for use of ancient DNA in genetic analysis of Chinook salmon
Relevance	Collecting DNA from ancient fish samples for comparing genetic diversity of past and present salmon populations presents unique challenges for researchers. Most derive from the low amount of available or usable DNA in ancient samples, making it important to maximize the number of samples that can be analyzed for gathering such population data. In addition, coexisting molecules tend to interfere with lab methods such as use of polymerase chain reaction (PCR) designed to prepare DNA for analysis.
Response	Washington Sea Grant-supported researchers sought to develop and evaluate improved methods for amplifying DNA from degraded samples. They developed a modified PCR protocol dubbed “Rescue PCR” and systematically tested it.
Results	The team developed a cost- and time-efficient protocol that improves the success rate for recovering DNA from archeological samples, which are often difficult to process. This protocol can be implemented in the lab without specialized equipment or supplies and is useful for population-based studies of ancient samples.
Recap	Washington Sea Grant researchers developed cost-efficient tools for reconstructing genetic diversity of

Recap	ancient salmon populations that can be useful for future population-based studies.
Comments	
Primary Focus Area	Healthy Coastal Ecosystems
Secondary Focus Areas	Sustainable Fisheries and Aquaculture
Goals	Ocean and coastal habitats are protected, enhanced and restored.|Fisheries are safe, responsibly managed and economically and culturally vibrant.
Partners	Washington State University
PI Draft	* Type impact * Title The development of a simple method to Polymerase Chain Reaction (PCR) amplify DNA from challenging ancient samples * Relevance Obtaining DNA from ancient samples presents unique challenges relative to contemporary samples. Most such challenges are related to reductions in the amount of available and/or useable DNA and coexisting molecules that interfere with laboratory methods designed to prepare the DNA for analysis (such as PCR). * Response A modified laboratory process, specifically an enhanced recipe of reagents, was designed that improves success rates in PCR amplifying DNA recovered from archeological samples. The method is time and cost efficient, and can be implemented in the lab without the need for additional equipment or specialized supplies. Sea Grant provided the funds used to develop and systematically test the protocol which, in turn, led to an improved recovery rate of DNA for the project as initially proposed.. * Results This method expanded the sample size in an ongoing ancient salmon DNA study and will be useful for population-based studies utilizing ancient samples. In such studies, maximizing the number of samples that can be analyzed is important for the generation of population-level data. * Recap A simple, cost-effective method was developed that helps overcome challenges common in ancient samples and increases the probability of obtaining usable genetic material for population level comparisons of ancient and contemporary samples. Comments Primary Focus Area Healthy Coastal Ecosystems Secondary Focus Areas Goals Fisheries are safe, responsibly managed and economically and culturally vibrant. Partners ---------- * Type impact * Title Baseline genetic data for ancient Chinook salmon populations spawning above Grand Coulee Dam * Relevance An inter-agency investigation into the feasibility of reintroducing anadromous salmon above Grand Coulee Dam is currently being pursued. One tenet of this evaluation is the identification of candidate fish stocks for use in reintroduction. However, no genetic data exists for populations of Chinook salmon that spawned upstream of the current location of the

	Grand Coulee prior to its construction. * Response Mitochondrial DNA haplotypes, or maternal genetic lineages, were determined for 49 Chinook salmon samples which pre-date European contact. All samples were from areas above the Grand Coulee Dam. Simple measures of genetic diversity were also determined at the single and mixed-stock level. Sea Grant provided the funds to extract, amplify, and sequence the genetic material from 15 of the 49 samples. These 15 samples were from a terminal fishing location near Spokane Falls used by Native Americans 2500 – 7500 years ago, likely representing a single-stock of Chinook salmon over a long period of time. * Results These data provide the first characterization of genetic composition for Chinook salmon prior to European contact. Moreover, these data may be broadly useful in the selection of stocks for reintroduction by providing data that is comparable to that available for contemporary populations. * Recap Genetic data was collected from ancient Chinook salmon samples collected above the current location of Grand Coulee Dam. Comments Primary Focus Area Sustainable Fisheries and Aquaculture Secondary Focus Areas Goals Fisheries are safe, responsibly managed and economically and culturally vibrant. Partners

Tools, Technologies, Information Services / Sea Grant Products

(1)

Description	Mitochondrial DNA haplotypes, or maternally inherited genetic lineages, for ancient and contemporary samples from the Columbia River Basin.
Developed (in the reporting period)?	Yes
Used (in the reporting period)?	No
Used for EBM?	Yes
ELWD product?	No
Number of managers	0
Description/Names of managers	

(2)

Description	Modified PCR protocol (“rescue PCR”) developed to obtain useable DNA from difficult to process ancient samples.
Developed (in the reporting period)?	Yes

Used (in the reporting period)?	Yes
Used for EBM?	Yes
ELWD product?	No
Number of managers	0
Description/Names of managers	

Economic Impacts

No **Economic Impacts** information reported

Community Hazard Resilience

No **Community Hazard Resilience** information reported

Meetings, Workshops, Presentations

(1)

Type of Event	Public or professional presentation
Description	Meeting: American Fisheries Society Annual Meeting Type: Platform presentation Title: Seven Millennia of Change: Comparison of Ancient and Modern Columbia River Chinook Salmon Using Mitochondrial DNA Authors: Bobbi Johnson, Brian Kemp, Gary Thorgaard Location: Portland, OR Note: Selected as Best Student Paper
Event Date	08-17-2015
Number of Attendees	40

Leveraged Funds

No **Leveraged Funds** information reported

Award Information

Title: Genetic analysis of Chinook salmon using ancient, historic and modern DNA
Project Number: R/HCE/PD-3
UW award number: 762754
WSU home account number: 13A-2430-0453
WSU sub-account number: 13A-2482-0268

Our project has two main objectives: (1) to expand on our previous work using mitochondrial DNA (mtDNA) by incorporating additional samples and (2) to develop and evaluate methods for pursuing nuclear DNA.

In addition to samples from pre-European arrival, we had hoped to incorporate additional samples from the early-European arrival and subsequent development periods (AD 1800-1900 and AD 1930-1950). Most of these samples were formalin-preserved fish specimens held by the Burke Museum at the University of Washington. However, after extensive trials we feel that it is unlikely that we will be able to include these samples due to the DNA sequence damage we observed, likely induced by formalin treatments. Although we were able to obtain amplifiable DNA from a few specimens (N=4), in each case this DNA was from a non-target species and may be due to cross-contamination and the reuse of jars in museum collections. This observation has important implications for museum curation practices.

Although the formalin-preserved samples will not likely be included in the final project, we will still be able to complete the first objective of expanding on our previous work by including two additional samples of ancient salmonids, ones collected from the Spokane River and Snake River systems. The Spokane River samples consist of three chronological groups, dated to approximately 7500, 3250, 2500 years before present (YBP). The Snake River samples are estimated to range from 10000 – 200 YBP. These two sample groups will provide a complement to the ancient and contemporary samples already sequenced and allow us to quantify and compare pre- and post-European genetic diversity and structure in the Columbia River Basin at multiple scales.

We have cataloged (photographed and measured) and initiated DNA recovery methods from all of the 115 samples from the Spokane River and 113 samples from the Snake River. To-date, 33 of the Spokane River samples have yielded mtDNA sequences used to identify them to the species level. All except one were confirmed to be Chinook salmon (*Oncorhynchus tshawytscha*). The non-Chinook sample was identified as rainbow trout/steelhead (*O. mykiss*). From the 32 Chinook samples, we have determined haplotypes for 15 of them (Table 1).

For the Snake River sample group, 42 vertebrae have produced amplifiable DNA, all of which has been sequenced to confirm species. From these, 22 were confirmed to be Chinook and haplotypes have been successfully determined for 18 (Table 1). Interestingly, the samples we acquisitioned from the Snake River were all classified by the WSU Museum of Anthropology as “fish remains, likely salmonid”. However, during our genetic confirmation we found that this classification was incorrect almost 50% of the time (20 of 42 were misclassified), frequent species found using genetic data were northern pikeminnow (*Ptychocheilus oregonensis*) and longnose sucker (*Catostomus catostomus*), among others. This highlights the importance of genetic confirmation for species identification of archeological specimens, especially those from areas where species are likely to be diverse. Our genetic species identifications will be provided to the WSU Museum of Anthropology and will help refine the fish assemblage information for these archeological sites in future studies utilizing these samples.

Using these Chinook mitochondrial haplotypic data we have also estimated basic genetic diversity statistics to compare the diversity in the ancient sample groups versus that observed in contemporary groups (Table 1). The diversity in the ancient samples for both the Upper Columbia (previously collected data) and Snake River groups is much higher than that in the contemporary counterparts. Haplotype diversity, defined as the number of unique haplotypes found relative to total number of samples under study decreased from 0.731 to 0.242 in the upper Columbia populations and from 0.601 to 0.244 in the Snake River populations. Nucleotide diversity, which quantifies on average how different two random haplotypes are from each other and is indicative of the presence of long-term genetic diversity in a population, was reduced from 0.21 to 0.10 in the upper Columbia populations and from 0.21 to 0.08 in the Snake River populations. The Spokane River does not have a contemporary counterpart, as anadromous fish were extirpated from this portion of the basin in the early 1900s.

We also constructed mtDNA haplotype networks to compare the changes observed from ancient to contemporary times in the upper Columbia and Snake River populations (Figure 1). Haplotype networks are a visual depiction of the specific relationship between haplotypes. Chinook exhibit four major mtDNA haplotypes with several, less common haplotypes derived by one or two genetic steps from them. In the upper Columbia, ancient populations exhibited the four main lineages, along with two rarer, derived lineages. Three of these linages, including one of the main types, appear to have been lost in the contemporary populations. The most prevalent haplotype (TSA 17) accounts for only 38% of the ancient population, yet is observed in 87% of the contemporary Chinook population. In contrast, only one lineage present in the ancient Snake samples was not observed in the contemporary group. The most common haplotype (again, TSA 17) was initially more frequent in the Snake River than the upper Columbia (56% of the ancient samples) and now accounts for a similar proportion (86% of the contemporary lineages) of this group. Thus, although a single haplotype defines approximately the same proportion of each group and the overall values for genetic diversity are similar for these groups, the Columbia River appears to have actually experienced a greater degree of haplotype loss from ancient to contemporary times.

Taken together, both the haplotype diversity indices and the haplotype network indicate that the Snake River populations have experienced lower overall losses in genetic diversity relative to the Columbia River populations. However, the Columbia River has maintained slightly more of the long-term genetic diversity (indexed by nucleotide diversity), indicating that the lineages that do remain in these populations are somewhat more diverse from each other than those in the Snake River populations.

As discussed above, species identification was better for the Spokane samples (with only one of 33 misidentified) but the initial overall success rate in recovering DNA was limited to 7% of the samples. In response, we developed a modified PCR protocol, one that we have dubbed “rescue PCR”, which increased the success rate to 31%. We also applied this method to the Snake River group and increased the success rate from 20% to 37%. In addition to using the protocol to obtain DNA for the proposed study we also generated sufficient data for publication of a separate methods paper. We plan to submit this for publication before the end of May 2016 and will include this in our final project report.

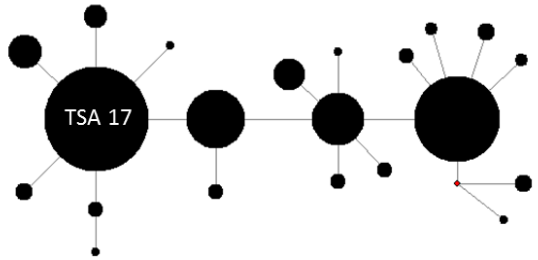
Related to our second objective (to develop and evaluate methods for pursuing nuclear DNA) we have completed the preliminary work for this objective, including selecting a protocol and obtaining the necessary supplies and reagents to test and optimize that protocol for our ancient samples. We will report on this objective fully in our final report.

Tables and Figures

Table 1. Haplotype (lineages) composition, haplotype diversity (h), and nucleotide diversity (x100) (π) for sample groups.

		Haplotype: TSA___											
		Previously Identified					Newly Identified						
Group	N	1A	1B	10	12	17	22	23	24	25	26	*h*	π (x100)
Spokane River - Ancient	15	--	--	8	--	5	--	--	--	1	1	0.562	0.22
Upper Columbia - Ancient*	34	3	9	6	--	13	--	2	1	--	--	0.731	0.21
Upper Columbia - Contemporary*	240	--	5	20	1	208	6	--	--	--	--	0.242	0.10
Snake River - Ancient	18	3	--	2	--	10	--	2	--	1	--	0.601	0.21
Snake River - Contemporary*	96	8	1	2	--	83	--	--	--	2	--	0.244	0.08

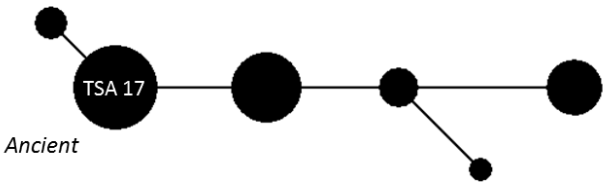
Figure 1. Haplotype networks for all known types as well as the ancient and contemporary sample groups for this project. Each circle represents a distinct haplotype, lines indicate relationships between types. Circle size is proportional to haplotype frequency in the sample group. Haplotype networks all follow identical layout (types, if sampled, are in the same location throughout all networks).



All documented comparable haplotypes for Chinook. Four main types with 17 less frequent types separated by one or two genetic mutations.

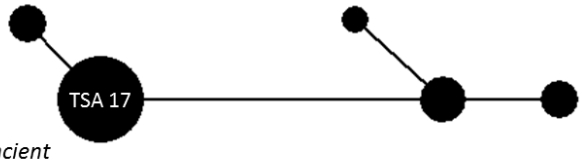
Generated using dataset in Martin et al. 2010 our previous data, and the data generated from this project. Frequency was determined for relative frequency within a group to reduce bias from larger sample size in Martin et al. 2010 and contemporary groups. Small red circle is unsampled type presumed to exist.

Upper Columbia River

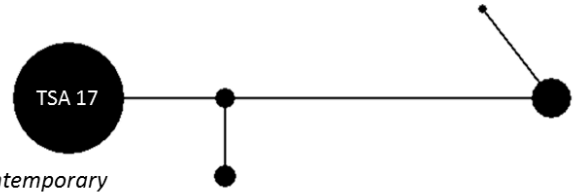


Ancient

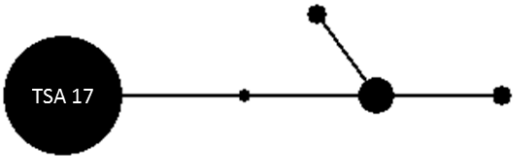
Snake River



Ancient



Contemporary



Contemporary