AN ABSTRACT OF THE THESIS OF

Lorne Sullivan Curran for the degree of Master of Science in Fisheries Science presented on July 26, 2021.

Title: Population Differentiation in two Northeastern Pacific Marine Species

Abstract approved:

______________________________________________________________________________

Michael A. Banks

Population genetic techniques are now preeminent in differentiating wild populations. Natural resource managers rely on them in their efforts to restore viable populations of fish and wildlife. Overfishing adversely impacted Yelloweye Rockfish (Sebastes ruberrimus) on the U.S. West Coast in the late 20th century. Management actions included shutting down the fishery and curtailing others where Yelloweye are bycatch. The ability to manage separate stocks may bring relief to coastal communities where Yelloweye stock have recovered. The sea otter (Enhydra lutris) was extirpated in Oregon by the turn of the 20th century. The prospect of translocating sea otters to repopulate the Oregon nearshore environment excites some. Other stakeholders may perceive the uncertainty of change as a risk. To test for population structure in the former, I analyzed DNA sequences from Yelloweye across its range, from Southern California to Alaska U.S.A. My findings confirm that a distinct stock exists in the British Columbia inside waters but that outer coastal Yelloweye are relatively panmictic. To inform the Oregon sea otter dialogue, I document the precontact Oregon sea otter as evidenced in the shell middens of First Nations people. I review the efforts of researchers using these
archaeological artifacts to establish the taxonomic status of the precontact Oregon population. Finally, I develop rationale for translocation acknowledging the concerns of stakeholders uncertain about the changes that sea otters restored in Oregon waters might bring to the nearshore coupled natural-human system.
Population Differentiation in two Northeastern Pacific Marine Species

by

Lorne Sullivan Curran

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented July 26, 2021
Commencement June 2022
Master of Science thesis of Lorne Sullivan Curran presented on July 26, 2021

APPROVED:

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Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Lorne Sullivan Curran, Author
ACKNOWLEDGEMENTS

The author expresses sincere appreciation to:

Alaska Dept. of Fish and Game, Sitka and Seward offices

International Pacific Halibut Commission

Michigan State University: Fitzpatrick Lab

National Science Foundation

NOAA: Alaska Sablefish Longline Survey

NOAA: U.S. West Coast Groundfish Bottom Trawl Survey

NOAA: Southern California Shelf Rockfish Hook and Line Survey

NOAA: Northwest Fisheries Science Center

NOAA: Southwest Fisheries Science Center

Oregon Dept. of Fish and Wildlife Marine Resources Program, Groundfish Team

Oregon State University: Center for Quantitative Life Sciences

Oregon State University: Marine Fisheries Genomics Lab

Oregon State University: Risk and Uncertainty Quantification in Marine Science

Oregon State University: State Fisheries Genomics Lab

Oregon State University: Vining Lab

St. Anselm College: O’Lab

University of California: Miller Lab

University of Cambridge: Willerslev Lab

Washington Dept. of Fish and Wildlife
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Chapter 1: General Introduction

Diverse marine taxa along the rim of the Northeastern Pacific Ocean (NEP) share a commonality. Despite the broad latitudinal expanse from Baja California, Mexico, to the Aleutian Islands, Alaska USA, the lack of physical barriers to dispersal would lend credence to an argument against the likelihood of significant genetic population structure. Nonetheless, management for conservation purposes and for commercial/recreational uses requires consideration of the potential for genetically distinct, regional stock structures. Here, I investigate 2 such taxa, the Yelloweye Rockfish across its full range and the once-extant population of sea otters in Oregon. With the former—an economically important fish—I advance the state of knowledge in regards to their population structure using next-generation genomic sequencing methods. With the latter—a marine mammal under the full protection of the U.S. Marine Mammal Protection Act—I synthesize historical research to inform a potential reintroduction into Oregon.

Yelloweye Rockfish (*Sebastes ruberrimus*) are long-lived (max. 140 yrs.) and iteroparous marine fish with a range that spans the NEP. Many groundfish stocks were severely overfished in the 1990s, resulting in fishery closures that remain in effect to this day. Yelloweye fisheries have been closed since 2002, but as bycatch they constrain otherwise viable fisheries. The U.S. West Coast from the Mexican border to the Strait of Juan de Fuca, Washington USA, has been understudied with respect to the population genetics of Yelloweye. Only recently have the reduced representation methods of genetic work, such as the RADseq employed here, been applied to this taxa. While the National Marine Fishery Service (NMFS) closed the fishery, the Pacific Fishery Management Council (Council) sets regional fishery quotas, now limited to commercial bycatch and to
scientific take. In the absence of compelling genetic information to the contrary, the Council arbitrarily divides stocks by political boundaries. A scientific basis for managing any differentiated stocks would be timely.

Sea otters for millennia formed a key part of northeastern Pacific nearshore ecosystems. Fur hunters of the 17-19th centuries decimated stocks of the northern subspecies of sea otter (*Enhydra lutris kenyoni*) and the southern ssp. (*E. l. nereis*). Sea otters were extirpated in Oregon before hunting was halted. Public sentiment has been growing for an attempt to reintroduce sea otters to the Oregon coast. Proposed species translocations now go through a NEPA-mandated process (National Environmental Policy Act) to evaluate for a spectrum of factors in the affected coupled natural-human system. For productive discussions on translocation to take place, a scoping of the considerations of a sea otter reintroduction needs to be developed, and so this study is also timely.

My research into Yelloweye Rockfish was funded in the greater part by the Oregon State University (OSU) Marine Fisheries Genomics Lab. Research to support the sea otter translocation discussion is the product of a National Science Foundation fellowship (1545188 NRT-DESE). Myself and 2 other team members in the OSU Risk and Uncertainty Quantification Program’s 2018-19 cohort brought together diverse disciplinary expertise to address a broader range of the aspects of Oregon sea otter reintroduction than I address here. In doing so, we developed the transdisciplinary skills that I now use to inform both chapters of this thesis.
Chapter 2: Yelloweye Rockfish, S. California To Cook Inlet, Alaska USA

2.1 Yelloweye and the Northeastern Pacific

The evolutionary hotspot of the rockfish genus *Sebastes* centers in the North Pacific and Gulf of California with 96 species (Love, Yoklavich, & Thorsteinson, 2002). Rockfish trend in body shape toward pelagic streamlined forms or heavy and squat bodies suited for a benthic existence. Yelloweye Rockfish fall in the latter category. They are one of the largest of the rockfish, reaching a maximum of 91 cm and 11.3 kg. The latitudinal limits for rockfish in general seem dependent on sea temperatures. The pelagic larval phase does not survive above 18°C, and sea surface temperatures run 20°C at Cabo San Lucas, Mexico. In the Gulf of Alaska, sea surface temperatures can be 4°C in late winter and early spring when Yelloweye females are likely to release young, too cold for the larvae to survive. Historically, Yelloweye’s greatest abundance centers offshore from central California to Southeast Alaska.

*Yelloweye ecology in the subduction zone*

Specialization in habitat is one of the Yelloweye’s defining characteristics. Solitary as adults, they typically restrict themselves to a small home territory in complex offshore habitat: boulder fields, rocky reefs, and caves. Most common between 90 and 180 meters in depth, adults have been found as shallow as 11 m and as deep as 550 m. They are a significant component of the benthic shelf and and shallow slope species assemblages.

The eastern Pacific shelf in the center of Yelloweye range can be defined geologically as the strike zone between the Juan de Fuca Plate and the North American Plate, or the Cascadia Subduction Zone (Yeats, Kulm, Goldfinger, & McNeill, 1998). Underwater topography can be highly rugose. As the Juan de Fuca Plate pushed under the North American Plate, it buckled the crust at Stonewall Banks, 35 km offshore of Newport, Oregon USA, into an anticline. Broadly speaking, this is a dip in the middle with the edges pushed up. The deep rocky reef structure of the Stonewall Banks is now designated a Yelloweye Rockfish Conservation Area.
During the last glacial maximum 21 kya, land erosional processes were at work in present-day Yelloweye habitat, cutting the thalweg of the now-Yaquina River across the Stonewall Banks. The geologic history of the West Coast extends to the emergent coastal landforms that are altering the flow of nearshore currents.

Relics of glacial history also define the Puget Sound/Georgia Basin. Shallow sills at the outlets of the straits were formed where retreating glaciers deposited the product of their erosion as terminal moraines (Thomson, 1981). This holds true for many of the smaller fiords in the region, such as Hood Canal off of Puget Sound and Bute Inlet, opening into the Strait of Georgia. These sills constrain water exchange, create unique marine habitat, and, potentially, restrict genetic flow.

*Northern Pacific Ocean circulation*

Within the North Pacific Gyre, waters move west along the equator in response to wind forcing. In coming up against the Asian land mass, waters deflect north by way of the Kuroshio Current and circulate back to the North American continent as the North Pacific Current. This cross-Pacific current bifurcates west of Vancouver Island, resuming northward as the Alaska Current and to the south as the California Current. The nearshore California Undercurrent travels in counterflow to the California Current at depths below 200 m. While winter southeasterly winds bring downwelling and poleward surface flow to the shelf break region, the northeastern Pacific coast as a whole experiences a spring transition between February and early May. During the spring transition, prevailing offshore winds shift to the north. The Coriolis effect of the spinning earth moves surface waters obliquely away from shore in accordance with the Eckman transport mechanism, and deep, nutrient-rich waters come to the surface, creating an upwelling regime. Nutrients thus supplied to the photic zone, along with the summer sun, promote blooms in the phytoplankton community, and the effect of abundant resources cascades through the food chain.
The West Coast of Vancouver Island Canada is a highly productive fishing region known for demersal, benthic, and pelagic fish stocks (McFarlane, Ware, Thomson, Mackas, & Robinson, 1997). Several oceanographic inputs foster this productivity, but one of the most intriguing one is the cyclonic Juan de Fuca Eddy (JFE). First identified by Tully (1941), the JFE functions as an upwelling center near the initiation of the California Current. California Undercurrent waters are forced through the large, submarine Tully and San Juan Canyons that bisect the shelf to create a dome of nutrient-rich, cold and saline water at the core of the eddy. As measured by positive saline anomalies, the JFE begins with a 17 km near-surface radius in June, growing to a 25 - 35 km radius in September (MacFadyen, Hickey, & Cochlan, 2008). Below 100 m, the extent eddy is more extensive and more seasonally stable. The JFE region is at the weaker end of magnitudes for the summertime north wind and the consequent coastal upwelling. During its semi-permanent residence offshore of the Juan de Fuca Strait separating Canada and the U.S. Olympic Peninsula, it facilitates entrainment of nutrients into regional waters. The ability of this system to retain nutrients and plankton has been implicated in toxic blooms of the *Pseudo-nitzschia* spp. diatom, such as those of 1998 and 2002 that shut down shoreside razor clam harvests (Trainer et al., 2009).

While the Columbia River plume can bring fresh water to the southern Washington shelf, it does not appreciably impact the JFE region. Fresh water to the region instead comes primarily from the Fraser River. At 3500 m$^3$s$^{-1}$, the Fraser has half the output of the Columbia but drives an estuarine circulation pattern wherein much of the tidally-driven intrusion of oceanic waters is entrained into riverine outflow for a nutrient input to regional waters an order of magnitude greater than that of the fresh water alone. The Strait’s buoyant Vancouver Island coastal current first follows the south shore of Vancouver Island, then advects cross-shelf to follow the margin of the San Juan Eddy to varying extents. An estuarine counter-flow replaces much of the outflow with nutrient-rich waters. Key to making use of observations and the models developed is projecting the extent of JFE retention and escape. During periods of strong upwelling, the pattern is only broadly cyclonic with leakage to the south in company
with the southeasterly shelf-break currents. During wind reversals, with the wind to the north, escape is more likely to the northwest. The eddy is the most retentive during periods of wind relaxation. Other West Coast geographic areas have comparable features to the JFE and have been implicated in *Pseudo-nitzschia* blooms. These include waters near Point Conception and the Farallon Islands off of California as well as Heceta Banks offshore of central Oregon. These West Coast topographies favor surface plankton retention and, potentially, Yelloweye larvae as well.

The Strait of Georgia (SG) is relatively shallow, 155 m on average, and constrained to 222 x 28 km in length and width (Thomson, 1981). To the south, its only outlet is the Strait of Juan de Fuca discussed above. To the north, several long, narrow channels open onto a broad expanse of open water between Vancouver Island and Haidaa Gwaii. Temperature and salinity distributions fall into a two-layer structure, with the upper 50 m experiencing strong seasonal patterns. Yelloweye are broadly distributed in the SG, occupying 32% of the total surface area and are caught at all depths (Yamanaka et al., 2006). Models developed by Crean (1976) show that the greatest magnitude of the tidal streams affecting the SG pass through the Strait of Juan de Fuca.

*Life history strategies*

Instead of the mass spawning events common in the marine world, rockfish engage in courtship dances leading to internal fertilization events (Love, Yoklavich, & Thorsteinson, 2002). Females retain sperm and take advantage of the upwelling season to build up fat reserves. Eggs are fertilized and hatch within the female. Off of California, most larval release occurs from March to July. Off of British Columbia, it peaks between May and June, and for Alaska most release events happen between April and September. Older females release larvae earlier in the season, possibly having been better provisioned to support ovarian development than younger females still devoting energy to somatic growth.
Yelloweye are fecund, with between 1.2 and 2.7 million eggs in a season. Bet-hedging occurs in the timing of larval release, with the full span of release potentially running from March to September. Long-term oceanographic trends, such as the Pacific Decadal Oscillation and El Nino, influence the strength of the California Current. These can work to reduce or redirect the California Current and the strength of upwelling. Upon parturition, larval transport is dependent on ocean currents. Flexion, or the development antecedent to the development of fins, happens within 1 - 2 months and marks the beginning of the pelagic juvenile stage.

Pelagic rockfish young are a significant part of the ichthyoplankton assemblage. They have more motility than larvae and may move more independently of ocean currents. Juveniles recruit to nearshore complex habitat with an ontogenetic shift to deeper habitat.

**Potential for Yelloweye population structure**

Adult Yelloweye inhabit limited home ranges in complex rocky habitat with a discontinuous distribution along the continental shelf. Adult dispersal, then, would be the exception rather than the rule. The pelagic larval phase is subject to disruptions in prevailing currents at headlands and capes that may restrict their transport along the coast. Further, Stonewall and Heceta Banks are located in areas of seasonally sluggish currents. As discussed above, the Juan de Fuca Eddy similarly retains its euphotic layer during the summer upwelling period.

Strong upwelling jet systems such as at Point Conception, Cape Mendocino, and Cape Blanco alter the flow of the California Current and block the alongshore transport of some pelagic larvae, e.g., Vermillion Rockfish at Point Conception (Hyde & Vetter, 2009). Stretches of unsuitable habitat may restrict the settlement or long term survival of distant stocks (Johansson, Banks, Glunt, Hassel-Finnegan, & Buonaccorsi, 2008). Larval retainage in areas of seasonally sluggish currents could be effective for much of the pelagic phase. During the infrequent summer storms, the change in wind direction may drive pelagic juveniles to the nearshore. Sufficient numbers of juveniles would then likely recruit there and travel back to the offshore source as adults to form a genetically distinct stock.
2.2 Yelloweye Genetic Research

Partly on the basis of indications of subtle population structure in Yelloweye in British Columbia by Yamanaka et al. (2006), the Committee on the Status of Endangered Wildlife in Canada designated both the inside and outside populations of Yelloweye rockfish as Species of Special Concern.

Siegle employed the genetic technique of random fragment length polymorphisms on substantive sample sizes from British Columbia with minor contributions from Alaska and Oregon (Siegle, 2011). He found limited genetic structure, in part due to technical artifacts incurred during library preparation. Working later with the 9 microsatellite loci developed by Yamanaka and an average of 18.9 alleles per locus, Siegle assayed 2862 individuals from 13 sample locations (Siegle, Taylor, Miller, Withler, & Yamanaka, 2013). A single SG sample site was employed. Sampling was limited outside of British Columbia waters. Pairwise $F_{ST}$ values reached a maximum of 0.0193 between SG and west of Haidaa Gwaii. All but one of the 12 significant findings occurred in comparison with SG. $F_{ST}$ for these averaged 0.0169 (s.d. 0.0018).

In 2010, NOAA (National Oceanic and Atmospheric Administration) listed Yelloweye in Puget Sound/Georgia Basin (PSGB) as a distinct population segment (DPS) under the Endangered Species Act (Tonnes et al., 2016). There was no genetic data at the time to support this. Studies of other rockfish (e.g., Copper Rockfish, *S. caurinus*; Seeb, 1998) across the inner-basin/outer-oceanic divide at the Juan de Fuca supported the inference of genetic distinctiveness for PSGB Yelloweye. While listing decisions may be made on other criteria, population genetics is ascending to a factor of central importance (Kelly, Oliver, Sivasundar, & Palumbi, 2010).

Recently, NOAA confirmed that the DPS status for Yelloweye is supported by genetics using SNPs, (Andrews et al., 2018). Yelloweye Rockfish samples spanned California to Alaska but were concentrated in the PSGB and totaled 151 individuals. They employed a variation on
RADseq to sample 100-bp (base pair) sections of DNA fragmented by the restriction enzyme $SbfI$ and sequenced in a single direction (“single-ended reads”). Hood Canal, a glacial fiord with limited exchange of seawater, contrasted most sharply with outer-coastal individuals ($F_{ST}$, 0.0276). Other inland-water samples had an average pairwise $F_{ST}$ comparison of 0.0191 with outer-coastal. Using samples from the San Francisco Bay area as a point of comparison, pairwise $F_{ST}$ for oceanic sites ranged from zero to 0.015.

**Current goal**

The eventual purpose of this effort is to elucidate potential Yelloweye Rockfish stocks on the U.S. West Coast. At the end of the 20th century, the depleted state of many groundfish stocks led to crisis conditions in the fishing community. The Sustainable Fisheries Act of 1996 required the rebuilding of overfished stocks. Three stocks were declared overfished in 1999, and by 2002 there were 7 species overfished. In 2002, NMFS added Yelloweye Rockfish to the list of overfished species. With an initial 2084 target for rebuilding to sustainable levels, Yelloweye will be perhaps the slowest species to recover. Despite there being no directed fishing, the slow recovery of Yelloweye Rockfish has resulted in restrictions on other fisheries. Yelloweye can be caught accidentally during long-lining for halibut, bottom-trawling, and recreational hook and line. Strict limits on Yelloweye as bycatch can close a fishery before the quota on the target species has been met. The 2017 stock assessment moved the target rebuilding date to 2027 (Thom, 2018). In the absence of biological data to support a different approach, the Council divides management for Yelloweye at the California/Oregon border.

However, with guidance from NOAA Fisheries (Nichols, K., personal communication, September 2018) to expand the breadth of the work, I sought out representative samples from Alaska and British Columbia. It is this larger breadth of potential population structure, spanning the full range of Yelloweye Rockfish that I concern myself within this manuscript.
2.3 Methods

Sample collection

The NOAA U.S. West Coast Groundfish Bottom Trawl Survey has taken Yelloweye Rockfish fin clips since 2005 along the length of the U.S. West Coast in biannual runs. Their Southern California Shelf Rockfish Hook and Line Survey targets rugose habitat inaccessible to trawl habitat, with genetic sampling begun in 2004. The Washington Department of Fish and Wildlife (WDFW) conducts longline and hook and line surveys for groundfish and has taken genetic samples since 2011. The International Pacific Halibut Commission (IPHC) conducts longline surveys, with Yelloweye as bycatch. From 2017-2019, I worked with Oregon Department of Fish and Wildlife (ODFW) port biologists to sample dockside. WDFW at this time began taking genetic samples from this survey effort as well. ODFW also collected fishery-dependent samples out of Astoria on my behalf in 2018 (Table 2.1).

For Yelloweye habitat north of Vancouver Island, I sought samples from both Alaska and British Columbia agencies. I coordinated with the NOAA Alaska Sablefish Survey in the Gulf of Alaska for their 2018 survey work and received dried samples. The Alaska Department of Fish and Game offices in Sitka and Seward agreed to take fishery-dependent samples (also dried). Seward trawl samples were made in the vicinities of Cook Inlet and Prince William Sound. Fisheries and Oceans Canada made available fin clips stored in ethanol from surveys spanning from the north tip of Vancouver Island (Triangle Island) to the northern tip of Haidaa Gwaii. Samples for British Columbia inside waters, also referred to here as Strait of Georgia (SG), came from the vicinity of Texada Island north to Campbell River and up Bute Inlet.

Fin clips stored in dried form were typically stored on absorbent filter paper. Fin clips stored in 95-100% ethanol by agencies had been left in the original ethanol. for those collected by myself, I changed the ethanol twice in the first month of storage. All were stored at room temperature. Diverse data sources were reconciled and compiled in R, now 4.1.0 (R Core Team, 2021).
**DNA extraction**

The OSU Marine Fisheries Genomics lab at the Hatfield field station was used to extract 9 96-well microplates of Yelloweye samples, with some duplication to boost DNA quantities. The standard lab protocol (Ivanova, Dewaard, & Hebert, 2006) employs proteinase-K to digest tissue and glass fiber plates with which to bind and then elute DNA using site-made wash buffers. Without a history of extracting DNA for next-generation sequencing, I failed to anticipate the quantity and quality of DNA required for the types of library preparation and sequencing chosen.

Benchwork was then relocated to the Vining lab, Dept. of Botany and Plant Pathology, on the OSU main campus. This allowed ready access to the OSU Center for Quantitative Life Sciences (CQLS) core lab and its appropriate user-operated equipment. Initial library preparation attempts showed the Ivanova extraction results to be variable in DNA quantity and quality, with guanidine salt contamination likely interfering with downstream enzymatic reactions. With some of the Yelloweye samples not otherwise recoverable, Zymo Genomic Clean and Concentrator kits were employed on 4 plates of these extractions.

I then transitioned to Qiagen DNeasy Blood and Tissue kits for all subsequent extractions (n = 1123). Ten to 15 mg of tissues were lysed overnight at 56°C on a thermomixer set to agitate every twenty minutes. Qiagen employs industry-standard silica-based spin columns to bind, then elute DNA. Proprietary wash buffers removed contaminants provided that undigested tissue was not transferred to the membrane of the column. While the majority of the NOAA samples had been consumed by the Ivanova process, additional S. California samples were obtained. These fin clip punches only amounted to 2 mg each. In concert with carrier RNA, micro spin-columns (Qiagen) were employed to maximize DNA quantities from these samples. Absorbance ratios of 260/280 and 260/230 quantified DNA purity on a sampling of extractions using a DS-11 DeNovix spectrometer/fluorometer. All samples were quantified by
fluorescence on a Biotek Synergy 2 microplate reader. I evaluated extractions for sufficiently high molecular weight by electrophoresis using 0.55% agarose gels.

Library preparation, sequencing

Library preparation involves multiplexing DNA samples to run in single lanes of the sequencing machine employed to obtain the nucleotide identities of the desired fragments while still retaining the identity of the samples. Failing to account for artifacts from library and lane effects can obscure or distort the sequencing results. Samples were therefore grouped by similar average molecular weight of extractions but distributed across libraries to maximize geographic diversity of each library. Samples were either resequenced in a second library or their libraries multiplexed and distributed across three lanes. With negative controls and certain multiplex adaptors dropped because of bioinformatically-weak barcodes, I assembled 10 libraries with ≈90 samples each.

The BestRAD method of Ali et al. (2016) is a simplification of the original Miller, Dunham, Amores, Cresko, & Johnson (2007) RADseq protocol that utilizes restriction enzymes to produce a reduced representation of the genome. “RADtags” are the DNA fragments adjacent to the restriction enzyme cutsite found throughout the genome. These can be reproducibly obtained in multiple samples and their sequences compared for diverse analyses, including the population genetics approach here.

The protocol developed for the application of the Ali et al. method is based on current practices at the Miller lab (O’Rourke, S., personal communication, October 2019) and at the Genetics and Evolution Program in the Conservation Biology Division of the NOAA Northwest Fisheries Science Center (Nichols, K., personal communication, October 2018). Except as noted, library preparation components came from New England Biological Supply. I incorporated into the protocol the NEB instructions for their NEBNext Ultra I DNA Library Prep Kit for Illumina.
With a published range of 50 ng to 200 ng of DNA input, I settled on a target of 150 ng total in 10 ul for a 12 ul reaction. In brief, the restriction enzyme $SbfI$ is used to digest extracted DNA into shorter fragments. T4 DNA ligase attaches the barcoded adaptors that allow multiplexing of up to 96 samples per library. A paramagnetic bead (Mag-Bind, Omega Biotek) purification step is used here, as at several steps in the protocol, to eliminate reaction byproducts. Utilizing the CQLS Bioruptor Pico system, I sheared the fragments to random lengths with nine sonication cycles set to 30 sec. on/90 sec. off. Fragment length was then analyzed by CQLS core lab staff on an Agilent Tapestation. Additional cycles of sonication were used as needed to achieve a targeted size distribution peaking at 350 bp.

Streptavidin beads (Beckman-Coulter) reduce the resultant pool of fragments to RADtags in a manner similar to the paramagnetic bead step by binding to the biotin on biotinylated adaptors. The library is placed on a magnet that collects the beads while undesired fragments are washed away. Then RADtags are separated from the beads by means of another digestion with $SbfI$. A Mag-Bind bead purification step follows. The final steps are standardized Illumina library preparation steps using the NEB kit: adding Illumina-type adaptors and plate identifiers, PCR amplification (12 cycles), size selection by varying proportions of paramagnetic beads, and a final paramagnetic bead cleanup. Adaptors obtained from NEB incorporate dual, combinatorial barcodes to identify libraries. This enabled the multiplexing of libraries to control for sequencing artifacts and still avoid the issue of “index-hopping,” whereby reads from multiplexed samples of one library can be misidentified as those of another library (Costello et al., 2018).

Subsequent steps were performed by CQLS core lab staff. Final library concentration was quantified by qPCR and fragment size distribution characterized on an Agilent Bioanalyzer. The lab HiSeq 3000 processed samples for paired-end 150-bp reads at the rate of one library per lane, though libraries were distributed across 3 lanes. PhiX was initially spiked in at 10%, later at 1%. At times, unrelated projects were sequenced within the lane.
Initial bioinformatics

The FASTQ files from the Illumina HiSeq were processed and analyzed on the CQLS computing infrastructure. The programs FASTQC (Andrews et al., 2010) and MULTIQC (Ewels, Magnusson, Lundin, & Käller, 2016) were employed to visualize the quality of the reads. Flowcell, sequencing lane, and library were tracked such that read groups could be assigned to every demultiplexed sample. When added to the metadata of alignment output, downstream applications can control for artifacts, controlling for the conflation of biological signal with noise. Metadata inserted is then referenced by the read group tag “@RG ID:flowcell.lane PL:ILLUMINA PU:flowcell_barcode.lane.library SM:sample LB:library.” In this way, the metadata retains the unique flowcell identifier, the lane, the Phred quality scoring system, the Illumina barcode, the library name (in lieu of a different treatment), sample identifier, and the library under an additional tag.

My investigations of the demultiplexing software’s assignment of reads to samples brought two issues to the fore. Excessive numbers of reads were assigned to certain barcodes consistently over the initial libraries. Further, under typical defaults of 1-2 mismatches allowed and with the wells for the aforementioned barcodes left dry in subsequent libraries, substantial numbers of reads were assigned to these when the barcodes were left in the key (Figure 2.1). I controlled for this in two steps. In advance of demultiplexing samples, I truncated the leading end of reads by 2 bases in SEQTK 1.3 (Li, 2013b). This eliminates the need to account for errors in the cutsite remnant GG during demultiplexing. Because bestRAD barcodes can be on either of the paired-end reads, I addressed both. Then, in using the STACKS 2.58 (Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013) program PROCESS_RADTAGS to demultiplex samples, I allowed for no mismatches in the barcode.

Minimal filters were applied in PROCESS_RADTAGS. The default setting for quality was kept at Q10 (sliding window of 15% of read length) and RADtag cutsite sequences rescued. Paired-end and bestRAD flags were included and restriction enzyme specified (-q -r --paired --bestrad -e SbfI).
With the release of a chromosome-level reference genome for sister taxa Honeycomb Rockfish (*S. umbrosus*) in November 2020, strategy shifted from de novo assembly to reference genome alignment. NCBI Accession GCA_015220745.1 consists of 138 scaffolds with an N50 of 34,912,176, totaling 801 Mb. I aligned raw, demultiplexed reads to the *S. umbrosus* genome using the BWA-MEM option of BWA 0.7.17 (Li, 2013a). Operator inputs are simple for this program, with the mem option optimized for Illumina output greater than 70 bp long. Read group metadata was inserted at this time.

SAMTOOLS 1.13 utilities by the same group (Li et al., 2009) prepares alignments in SAM format for downstream applications. Fixmate quality controls alignment flags and marks read pairs. Libraries sequenced across several lanes were piped through fixmate (fixmate -u -m), then coordinate-sorted (sort -l). Replicate samples were merged (merge -u) and piped to markdup -r for PCR duplicate marking and removal before being exported as BAM files. PCR duplicates of resequenced samples were removed before merging.

The STACKS pipeline does not handle the above alignments. I returned to use the GSTACKS and POPULATIONS components to build a catalog of loci and call SNPs. Multiple SNPs were allowed per loci, but SNPs occurring on overlapping loci were pruned (--merge-sites). SNPs were exported as vcf (variant call format) files.

**SNP filtering I: quality and missingness**

I follow, in large part, filtering schemes 5 and 6 of the alternatives outlined in O’Leary, Puritz, Willis, Hollenbeck, & Portnoy (2018), whereby quality controls are first applied to SNPs (Table 2.2). Missingness is then controlled for both in SNPs and individuals iteratively and alternatively with increasing stringency. I relied on R and VCFTOOLS 0.1.17 (Danecek et al., 2011) for identification and filtering of sites and individuals. Minimum genotype quality and minimum read depth reduced the count of called genotypes but not that of sites (minGQ 20 --minDP 5). The Unix utility SED was used to adjust the genotype field DP to account for deleted genotypes and a minimum read depth set (--min-meanDP 10). A minor allele
minimum count of 3 allowed for a site to have only a homozygous individual and a heterozygous individual to meet the requirement (-mac 3).

Missingness in SNPs and individuals are interrelated. Filtering for one improves the other, but the inverse is true as well. I plotted missingness for each in R based on reports from VCFTOOLS and assigned a stringency filter at the inflection point in the distribution that did not sacrifice sites or individuals prematurely. With priority given to retaining individuals for this phase, I settled on a 30% missingness rate in sites and 50% in individuals after 3 iterations.

SNP filtering II: population genetics

After dropping a sizable number of individuals, it is advisable to return to GSTACKS (Black, A., personal communication, February 2021). The Bayesian nature of building a catalog in STACKS 2.x by relying on all alignments for a given position would otherwise mean that dropped individuals could affect genotype likelihoods among those retained. Returning to SNP filtering after GSTACKS and POPULATIONS, site quality thresholds were again set. A single set of missingness filters was applied in this second round: 20% missingness for sites and 30% for individuals for most datasets. A secondary dataset (581 samples, see below) employed the more stringent 12.5% loci missingness filter.

Paralogs are a known impediment to accurate SNP calling in many species (McKinney, Waples, Seeb, & Seeb, 2017), whereby loci of tetrasomic inheritance from past genome duplication events are bioinformatically assigned to a single locus. The resultant high proportion of heterozygosity is most obvious when allele frequencies are approximately equal under Hardy-Weinberg equilibrium. McKinney’s HDPLOT illustrates levels of heterozygosity on the x-axis and the metric of D, or deviance from an equal allele balance as expressed in z-scored standard deviations from the expected. While I cannot say that the Yelloweye has had genome duplication events, filtering for paralogous behavior potentially also controls for
heterozygote miscalls (Anderson, 2018) and multiple alignments to the same loci. I plotted
SNPs with the R-based HDPLLOT and filtered in keeping with the publication’s guidance.

Had the call to STACKS and POPULATIONS retained single SNPs per locus, linkage
disequilibrium (LD), or the non-random association of SNPs that violate the test assumptions,
could still be an issue. The relation between scaffolds is not known. To determine an
appropriate window across scaffolds, NGSLD 1.1.1 (Fox, Wright, Fumagalli, & Vieira, 2019),
was used to measure LD decay (--max_kb_distance 0, “infinite”). Data was then imported
into PLINK 1.9 (Chang et al., 2015) and R² was chosen as the metric by which to filter for LD
(--indep-pairwise x 'kb' 1 0.8).

While applications of reduced representation methods enjoy broad access to the target
genome, missing reads or a low rate of reads assigned to a locus inevitably results in uncalled
genotypes. Downstream analysis tools may or may not communicate their method of dealing
with the missing data. Initial analyses were performed under these conditions. For later
iterations, missing data was explicitly imputed or inferred using the program LINKIMPUTER
(Money, Migicovsky, Gardner, & Myles, 2017). Modeling scenarios within the program allow
for differing levels of missingness, minor allele frequency, and read depth threshold. Above the
threshold, statistical inference is made in a typical likelihood-based approach using allele read
depth. Below the threshold, the LD-kNNi approach developed in Money et al. (2015) uses
sites in linkage disequilibrium to impute a genotype. The later version assigns a probability to
each approach. Given these probabilities, a genotype is called. The resultant vcf file goes to
analysis with no missing genotypes.

The inbreeding coefficient F<sub>IS</sub> was employed to identify confounding factors. Metrics for
datasets were generated in VCFTOOLS (-het).

Datasets

While I do not have predetermined populations, for ease in visualization and for a starting
point, samples were grouped by k-means clustering in R using latitude and longitude
normalized to nautical miles (nm). (1 nm:1’ latitude; 1’ longitude:1 nm \cdot \cos(\text{latitude } \theta).) This clustering scheme is admittedly affected by sampling density but comported well with geographic regions. British Columbia and Oregon-California clusters were further subdivided by k-means clustering after initial PCAs suggested the former division.

It was later determined that a more latitudinally-balanced sample set would better suit current purposes. The sampling density north of Vancouver Island was then matched south of the island by subsampling collections on the U.S. West Coast north of Cape Mendocino. This sample set was returned for SNP calling to GSTACKS and POPULATIONS. Filtering was increased to genotypetype quality Q20 to limit inference but not imputation by LINKIMPUTER. Stringency for minor allele frequency threshold was raised to 0.01.

**Analysis tools**

With an abundance of SNPs to inform but also challenge tools for spatial genetic analysis, a number of approaches along similar lines were engaged to identify consistency among findings. Initial analysis employed the R package ADEGENET 2.1.3 (Jombart, 2008). Variant data was imported via vcfR (Knaus & Grünwald, 2017) as genlight objects. Eigenvalues and eigenvectors were generated by what Jombart calls an inter-class approach that maximizes variance between populations. The first 2 principal components were plotted in ADEGENET.

Modeling the number of scenarios that followed was not practical in ADEGENET as the large SNP set unduly taxed its abilities. An alternative method, PLINK 1.9 bases its principle component analysis (PCA) on the variance-standardized relationship matrix. PLINK’s ability to rapidly model scenarios was enhanced by the interactive ability of the R package PLOTLY 4.9.4.1 (Sievert, 2020) to extend the graphing capability of GGPLOT2 3.3.5 (Wickham, 2016). These were implemented in custom R scripting.

Genetic analyses also require testing for significance among relationships. EIGENSOFT (Price et al., 2006) has been cited more often than any other PCA tool in genetic analyses (> 7600 / 15000; Elhaik, 2021). Its PCA approach (SMARTPCA v. 16000) relies on singular value
decomposition. Vcf file conversion for SMARTPCA was implemented in PLINK, with additional custom data conversions to allow non-human variant information into a human-centric program. The option to remove outliers was enabled. SMARTPCA returns significance for its principal components and performs a chi-square test of significance between populations. Pairwise $F_{ST}$ was calculated in STAMPP (Pembleton, Cogan, & Forster, 2013), which employs the (Weir & Cockerham, 1984) method. Bootstrapping was performed 10 000 times across loci to obtain 95% confidence intervals and p-values. Significance of results returned should be evaluated with the Bonferroni multiple comparisons adjustment to an initial $\alpha$ of 0.05, or 0.0024.

2.4 Results

Extracts, library design

Qiagen extractions generally had more than an adequate yield for a 120 - 150 ng reaction. In later repeating extractions initially made using the Ivanova method, the sample tissue quality of those stored in ethanol had often noticeably degraded. With a preferred target of 18 kb fragments, I scored as high those extractions with noticeable bands above 12 kb on agarose gels. Similarly scored extractions were grouped together in libraries but with maximal diversity in sampling location. Of the 4 plates of Ivanova extractions concentrated and cleaned with the Zymo kit, 180 samples had just passable gel scores and were incorporated into libraries. Many of these serve to represent central and northern California. The extractions of dried fin clips that were enhanced with mini-spin columns and carrier RNA performed well. Southern California coverage is due to these. Still, there was a noticeable drop in fragment size relative to the increasing age of samples. More extractions of suitable quality and of considerable scientific interest remained after the time for library preparation was exhausted.

Sequencing output, demultiplexing

Net HiSeq sequencing output ranged from 297 M reads per library (583 M total for the pair) to 325 M, exclusive of PhiX, unrelated projects, and undetermined reads.
PROCESS_RADTAGS assigned a minimum of 27% of reads to individuals and a maximum of 80% on a per-library basis. The upper figure should have been achievable (Black, A., personal communication, February 2021), but it is worth noting others’ experiences. The 10 libraries of Chinook Salmon (Oncorhynchus tshawytscha) developed with ddRAD in (O’Leary, Thompson, & Meek, 2021), for instance, all had a success rate in the 40% range.

The bioinformatics of multiple datasets

Four calls were made to GSTACKS and POPULATIONS for SNPs. These can be loosely grouped into the two primary approaches of the inclusivity of all suitable-quality samples and the selectivity of the latitudinally-balanced strategy. Of the 900 samples that BWA aligned to the reference genome, 815 (call 1) were passed to GSTACKS and POPULATIONS and then evaluated in the first round of SNP filtering. The 815-sample set did not go beyond the first round of SNP filtering. Missingness filters reduced the count of individuals to 614 (call 2, Figure 2.2). These 614, later 613, formed the basis for the initial PCAs in ADEGENET after re-calling SNPs and subsequent filtering in round 2. Poor resolution of clusters in these PCAs might be attributed to the correlation of missingness and $F_{is}$, the inbreeding coefficient (O’Leary, S, personal communication, February 2021). Individuals were accordingly reduced to 581 (3) by identifying the most highly correlated individuals in R plots using the interactive PLOTLY function. Finally, the Washington to Cape Mendocino individuals were subsampled to balance north and south sampling, resulting in a net 239 individuals (4) in the fourth dataset (Table 2.3).

I share representative statistics generated while calling and filtering SNPs during the processing of the 4 datasets. Post-alignment, GSTACKS cataloged a mean of of 78% primary alignments retained (min. 24%, max. 88%) for the 613-sample set. For the 239 individuals, mean percentage of alignments retained was 80% (40 - 80%). With a minor allele frequency of 0.01 and site missingness set at 0.5 for the 613-sample set, POPULATIONS kept 47 164 loci with 93 792 variant sites remaining. With the additional quality filtering criteria of Q20
established at the outset for the 239-sample dataset, the POPULATIONS program kept 47 231 loci with 89 309 variant sites remaining.

For the 613 and for the 239 individuals, loci missingness was set at 0.2 and individual missingness at 0.3 in advance of paralog filtering. With the 239-sample set, this left 60 425 sites and 215 individuals. Missingness did not reduce the number of individuals in the 613-individual dataset. To visualize any correlation between paralogous behavior and loci missingness, I plotted McKinney’s D against heterozygosity and grouped by missingness (Figure 2.3). Missingness in loci increases noise in HDPL0T, and setting limits for D and H involves a judgment call. Filtering for a minimum D of -4 and maximum of 4, as well as heterozygosity in excess of 0.6, left 64 430 SNPs in the 613 sample set. For the now-215 sample set at the same missingness levels, a lesser filter of D ± 7 and H of 0.6, kept 58 589 sites.

Initial analyses in ADEGENET with the 613 individuals were implemented using that program’s imputation of missing data (na.replace()). For PCAs performed in PLINK and SMARTPCA, the imputation was performed in advance. Using the JAVA application LINKIMPUTER, I modeled scenarios of minor allele frequency (MAF) in the 215-individual dataset carried forward, starting from the input of 0.01 and going to 0.05 in increments of 0.01. Read-depth threshold below which imputation (vs. inference) is made for missing genotypes was modeled at steps of 4, 8, and 12. For modeling missingness, the program requires equal increments. I preferred to maintain higher stringency for SNPs relative to that of individuals and so did not apply the option. The highest accuracy score, 97% in each case, was attributed to a MAF of 0.01 with a read-depth threshold of 8. For the 581-individual set, this meant a reduction to 33 073 sites. With the 215 samples, 56 346 sites remained.

Opinions differ as to whether linkage disequilibrium affects PCA analysis (e.g., Price et al., 2006, and Abdellaoui et al., 2013). The first approach to PCAs was performed with and without LD filtering; LD filtering was then incorporated into the balance of analyses.
Examining LD decay in the program NGSLD showed little effect beyond contig length. Within a window length of 100 kb, $R^2$ was modeled for each dataset in PLINK (-indep-pairwise 100 'kb' 1 0.8). A cutoff of 0.8 was chosen in keeping with the inflection point on the graph and with common practice, as in Vaux, Bohn, Hyde, & O’Malley (2021). This reduced the 215-sample set to 42 790 sites.

**Initial analyses**

The larger, 614-individual dataset was initially visualized with ADEGENET tools. The first PCA pointed toward one dried fin clip sample as being a specimen likely mis-cataloged in the field. Dropping this individual allowed some structure to be revealed. British Columbia inside waters (Strait of Georgia, Bute Inlet), strongly separated out from the outer coastal water clusters of the entire northeastern Pacific coast (Figure 2.4). The British Columbia cluster was therefore split along the -128° longitudinal line into BCE and BCW.

To facilitate analysis and the pruning of problematic individuals, PCAs were implemented in PLINK and visualized in R plots with GGPlot2 and PLOTLY. The same general pattern of BC inside waters separating out and limited dispersion of the remaining clusters remained. To look for individuals with a combination of high missingness and homozygosity, $F_{ST}$ values were added to the dataset. Each cluster was examined in turn for non-random association of above-average $F_{IS}$ and missingness relative to the first 2 principal components, resulting in a pruned dataset of 581 individuals. Upon calling and filtering SNPs for these, PLINK PCAs were again created. PLINK PCAs did not vary substantially from those of the EIGENSOFT set. For that reason, I illustrate post-ADEGENET PCAs with just SMARTPCA output below. In addition to the quality checks above, PCAs were inspected for library or survey/sample collection bias, with none found.

**EIGENSOFT SMARTPCA results**

The 281-individual, latitudinally-balanced dataset was analyzed in SMARTPCA. While the program will regress on LD and normalize inputs, I only invoked the outlier analysis. Despite
the selective approach already taken, SMARTPCA dropped two Washington samples.

Examining the PCA with all clusters displayed, northern waters only, and waters from Haida Gwaii to Cape Mendocino reveal regional patterns to the variant dataset underlying analyses. With all clusters displayed, British Columbia inside waters are again distinctively clustered but can share similarities with outer coastal clusters. The set of clusters form a gradient on the first principle component (PC). California, from Cape Mendocino to the south, fits poorly into any clustering scheme (Figure 2.5).

Focusing on the northern clusters, Alaska occurs in a tightly constrained range of PCs within the more diverse British Columbia west and Gulf of Alaska. The gradient along the first PC is maintained (Figure 2.6).

The set of clusters centered on Washington has the weight of samples concentrated in a small range of PCs. Gulf of Alaska has samples that come close to overlapping with British Columbia east. Some Washington samples similarly share more PC1 values with British Columbia east than any other outer coastal samples (Figure 2.7).

EIGENSOFT SMARTPCA found only the first 2 PCs to be significant ($p < 7.5e^{-310}$, $p < 6.2e^{-21}$). The chi-square test for significance in pairwise comparisons found most comparisons significant (Table 2.4) with the exception of Gulf of Alaska and British Columbia east and several comparisons with California.

Pairwise $F_{ST}$

Pairwise $F_{ST}$ values as calculated by the Weir & Cockerham (1984) method in STAMPP often do not retain significance (Table 2.5. British Columbia inside waters retain marked $F_{ST}$ values in comparison with outer coastal waters. Applying the multiple comparison adjustment, all of these still retain significance. While some outer coastal comparisons show significance (AK:OR, AK:WA, WA:GA), $F_{ST}$ values are at least an order of magnitude less than those involving British Columbia inside waters. Comparisons with California are more useful than
the PCAs would suggest but for AK:CA (P = 1.0). There cannot be said to be increasing values with increasing distance between clusters.

2.5 Discussion

The limited sample size here necessitated by lighter sampling densities to the north and southern extents does not strongly indicate any break at Vancouver Island as might be expected from the actions of the North Pacific Current. Pairwise $F_{ST}$ values do not point toward a clinal effect. But the analyses do suggest intriguing regional patterns.

The ADEGENET approach to PCAs most graphically displays the genetic distinctiveness of British Columbia inside waters and the relatively panmictic genetic distribution of the outer coast. Both the PLINK and the SMARTPCA results show a more nuanced organization to the clustering scheme. I include graphs from the latter (Figure 2.5, 2.6, 2.7). British Columbia East and the outer coast distribute in evenly-spaced clusters on the first PC and behave similarly on the second. Alaska retains a distinct identity, even if falling within the footprint of the other northern outer-coastal clusters. Alaskan sample locations were, in fact, taken in the northern Gulf of Alaska. Regarding the central portion of the sampling distribution, portions of British Columbia west overlap with that of Oregon and Washington. Since British Columbia east includes Triangle Island at the tip of Vancouver Island, a measure of overlap here is to be expected.

$F_{ST}$ values support the assertion that the current work broadly confirms that of prior genetic findings on Yelloweye Rockfish. The strongest contrast of Andrews et al. (2018) was that of Hood Canal to outer coastal (0.0276), while the area of the NMFS-designated DPS as a whole averaged 0.0191 in comparison with coastal. The multi-allelic loci of microsatellite data described above can be said to be both statistically and biologically significant at greater than 0.007, per the suggestion put forth by Gomez-Uchida & Banks (2007). Further, such data carries greater statistical weight for a given number of loci at a given value than SNPs. The average $F_{ST}$ of the Siegle et al. (2013) inside:outside comparison of 0.0191 reflects well on the
current result of 0.0168 or higher $F_{ST}$ for British Columbia inside waters versus any point of
comparison on the outside coast.

Given the strong genetic distinctiveness of SG samples, it is possible that this stock must act
as its own source for recruitment. Some Washington samples extend toward the PC values of
British Columbia East in the EIGENSOFT PCAs. Referencing the above discussion of the
Juan de Fuca Eddy and the Strait of Juan de Fuca’s prominence in SG tidal flows, it is
reasonable to suggest that some Washington individuals bear a direct lineage from SG
Yelloweye through larval transport. Not only would SG Yelloweye larvae be carried in the
water column to the head of the California Current, they would also at times be retained by
the JFE until the juvenile stage settled out into the region’s complex, rocky habitat. While no
doubt self-sustaining in its own right, the Washington outer coast likely serves as sink for a
net outflow of larvae from the Strait. With the Fraser River input into the system driving net
outflow for the PSGB, the time of passage during a pelagic larval duration of 2+ months
should easily allow SG larvae to reach the JFE.

Dick, Shurin, & Taylor (2014) found genetic differentiation in Copper Rockfish at replicate
sites between outer coast W. Vancouver Island sites and the heads of adjacent inlets using
microsatellite data. Similar patterns might be determined from the SNP set I developed for
Yelloweye in additional phases of research. There also remains potential for local
differentiation at areas of seasonally sluggish circulation, such as at Stonewall Banks.

SMARTPCA tests found significance for most of the comparisons but for those that include
California. The Weir-Cockerham approach showed slight but significant differences among
many of the outer coastal comparisons. It may be that the current clustering approach divides
potential stocks too finely for stock assessment purposes. Andrews et al. (2018) primarily
reported $F_{ST}$ on just two PSGB clusters and a single clustering of outer coastal samples. That
there is little distinction in Washington and Oregon samples is not surprising on account of
the reduced sample size. The broad overview of coast-wide sampling left most samples out of
consideration. At the current level of detail, there is limited opportunity for distinction across the extent of California Current and attendant upwelling regime. For the current scope of analysis, the prospect for inferences based on pairwise $F_{ST}$ is limited beyond confirming the genetic distinctiveness of British Columbia inside waters. Without a clear biogeographical pattern to other pairwise comparisons, additional conclusions should wait until nested analyses can be carried out with greater depth of samples.

California samples defy characterization relative to other clusters. While samples were distributed across libraries with respect to geographic location to minimize artifacts, and library and survey effect were tested for, the fact remains that all California samples were from dried, most often older, fin clips. These do not appear to be useful in the present analysis.

It is a testament to the increasing importance of population genetic analysis in the management of fishery stocks that diverse agencies have been keeping samples tailored to the same. Some NOAA samples accessed date back to 2004, those of WDFW to 2012. This speaks as well to the initiative of natural resource managers. Intentional collections are far preferred to museum specimens, the genomic potential and challenge of which constitute the equivalent of ancient DNA (McCormack, Rodríguez_Gómez, Tsai, & Faircloth, 2017). Between the efforts of state agencies, NOAA, Fisheries and Oceans Canada, and my sampling of IPHC landings in Oregon, 1649 samples had the potential to be sequenced. In time, Yelloweye collections may lend themselves to longitudinal studies, much as is the practice with otoliths (middle ear structures used for aging; e.g., Tonnes et al., 2016).

Next-generation sequencing, whether by the reduced-representation approach here or the low-coverage whole genome sequencing method coming into its own, offers the researcher tens of thousands more datapoints than prior genetic methods. Their application, however, is seriously impaired by low-quality tissue input relative to the earlier, direct-to-PCR methods. If ethanol preservation is to be employed, keeping ethanol concentrations high and vials in -
80°C for long-term storage may be more effective strategies. If logistics necessitate dried storage, adopting best practices from the plant sciences and lyophilizing samples at the earliest opportunity should improve the quality of their extractions. Chemical stabilization could be pursued, with the caveat of avoiding interference with downstream enzymatic processes. It may well be that with better tissue quality alone, finer-grained population structure along the length of the Northeastern Pacific Ocean could be revealed in future analyses.
Figure 2.1  Certain adaptors are consistently assigned a higher percentage of reads during the demultiplexing stage. Data is for 5 libraries. Barcode adaptors for library construction were ordered in 2 lots and annealed separately.
Figure 2.2  Geographic distribution of sequenced 613-sample set. K-means approach to clustering reflects sampling, latitudinal diversity.
Figure 2.3  Deviance metric (D) of allele read ratio expressed as a z-score set against heterozygosity (H) in SNPs for the now-215 sample set. Loci missingness (lmiss) displayed as gradient of color.
Figure 2.4  ADEGENET PCA for 613-individual sample set, with British Columbia inside waters (BCE) separated from BC outside waters (BCW) and the rest of the outer coast.
Figure 2.5  EIGENSOFT SMARTPCA analysis on 213-sample set. All clusters displayed.

Figure 2.6  EIGENSOFT SMARTPCA analysis on 213-sample set. Northern clusters displayed.
Figure 2.7  EIGENSOFT SMARTPCA analysis on 213-sample set. Clusters from Cape Mendocino to Haidaa Gwaii displayed.
Table 2.1  Yelloweye Rockfish fin clips for genetic research were taken from archival collections of agencies or, in the case of Oregon IPHC landings, by the researcher at the time of landing. IPHC - International Pacific Halibut Commission. Strategy refers to whether samples were taken during agency stock assessment surveys, FI: fishery-independent, or from landings of commercial fishing vessels, FD: fishery dependent.

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<td>ethanol</td>
<td>51</td>
</tr>
<tr>
<td>Washington Dept. of Fish and Wildlife</td>
<td>FI</td>
<td>longline</td>
<td>ethanol</td>
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Table 2.2  SNP filtering scheme for full set of sequenced and aligned samples (n = 815 - 614), working set (n = 614 - 613), and subsequent reduced-sample set (n = 239 - 213). Catalog of loci in each case was built and SNPs called in GSTACKS and POPULATIONS.

<table>
<thead>
<tr>
<th>Step</th>
<th>Aligned</th>
<th>Working</th>
<th>Balanced</th>
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<tbody>
<tr>
<td>genotype quality</td>
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<td>20</td>
<td>20</td>
</tr>
<tr>
<td>minimum read depth</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>minimum-mean read depth</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>minor allele count/freq.</td>
<td>mac 3</td>
<td>mac 3</td>
<td>maf 0.01</td>
</tr>
<tr>
<td>site missingness I</td>
<td>geno ~50%</td>
<td>geno 20%</td>
<td>geno 20%</td>
</tr>
<tr>
<td>individual missingness I</td>
<td>ind ~90%</td>
<td>ind 30%</td>
<td>ind 30%</td>
</tr>
<tr>
<td>site missingness II</td>
<td>geno ~40%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>individual missingness II</td>
<td>ind ~70%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>site missingness III</td>
<td>geno ~30%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Individual missingness III</td>
<td>ind 50%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HDplot</td>
<td>-</td>
<td>D 4.0, H 0.6</td>
<td>D 7.0, H 0.6</td>
</tr>
<tr>
<td>impute missing genotypes</td>
<td>-</td>
<td>-</td>
<td>maf 0.01, depth 8</td>
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<tr>
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<td>0.8</td>
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<tr>
<td>heterozygote miscall rate</td>
<td>-</td>
<td>visualized</td>
<td>-</td>
</tr>
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Table 2.3  Samples clustered by geographic regions for ease in reference during analyses. Additional filtering reduces some counts.

<table>
<thead>
<tr>
<th>cluster</th>
<th>n / 613</th>
<th>n / 239</th>
</tr>
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<tr>
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<td>47</td>
</tr>
<tr>
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<td>Washington</td>
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</tr>
<tr>
<td>British Columbia inside</td>
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<td>25</td>
</tr>
<tr>
<td>British Columbia outside</td>
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<td>16</td>
</tr>
<tr>
<td>Gulf of Alaska</td>
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<td>34</td>
</tr>
<tr>
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<td>26</td>
</tr>
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Table 2.4  EIGENSOFT SMARTPCA chi-square test of significance for difference between clusters.

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<th>pop1</th>
<th>pop2</th>
<th>chisq</th>
<th>p value</th>
<th>pop1 n</th>
<th>pop2 n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alaska</td>
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<td>138.344</td>
<td>0.000</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
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<td>British Columbia outside</td>
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<td>21</td>
<td>55</td>
</tr>
<tr>
<td>Alaska</td>
<td>Washington</td>
<td>122.862</td>
<td>0.000</td>
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</tr>
<tr>
<td>British Columbia inside</td>
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<tr>
<td>British Columbia outside</td>
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<td>British Columbia outside</td>
<td>Gulf of Alaska</td>
<td>10.570</td>
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<td>14</td>
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<td>British Columbia outside</td>
<td>Oregon</td>
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<td>Gulf of Alaska</td>
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<td>Oregon</td>
<td>Washington</td>
<td>36.244</td>
<td>0.000</td>
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<td>34</td>
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</tbody>
</table>
Table 2.5  Pairwise F_{ST} values for the 7 regional clusters as calculated by the Weir-Cockerham method in STAMPP are shown at top right. P-values, as reported by STAMPP, follow on the bottom left.

<table>
<thead>
<tr>
<th></th>
<th>AK</th>
<th>BCE</th>
<th>BCW</th>
<th>CA</th>
<th>GA</th>
<th>OR</th>
<th>WA</th>
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</thead>
<tbody>
<tr>
<td>AK</td>
<td>-</td>
<td>0.01682</td>
<td>0.00000</td>
<td>0.00000</td>
<td>0.00012</td>
<td>0.00095</td>
<td>0.00145</td>
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<td>-</td>
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<tr>
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<td>-</td>
<td>0.00067</td>
<td>0.00021</td>
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<tr>
<td>CA</td>
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<td>0.0000</td>
<td>0.0007</td>
<td>-</td>
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<td>0.00092</td>
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<tr>
<td>GA</td>
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<tr>
<td>OR</td>
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<td>-</td>
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<tr>
<td>WA</td>
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<td>0.0000</td>
<td>0.8983</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0124</td>
<td>-</td>
</tr>
</tbody>
</table>
Chapter 3: The Oregon Sea Otter, Precontact And Future

3.1 Sea Otters and the Northeastern Pacific

The coastal zone of the cold-temperate North Pacific ecoregions extends from California USA northward along the edge of North America, across the Aleutian Islands, and down the edge of the Asian continent to the Sea of Japan. In the late Miocene (5.3 mya), these were subtropical, relatively unproductive, waters beginning to shift to a new state with the onset of the current Quaternary glaciation (Estes, 2015). Now, an upwelling regime brings cold and nutrient-rich waters from the depths into the photic zone, fostering a densely-populated, planktonic trophic level that in turn supports an abundant fauna. Much of the nearshore environment came to be dominated by a kelp ecosystem, anchored by the *Nereocystis* and *Macrocystis* macroalgae that reach the surface from holdfasts some 20 m below. Coming into this nascent environment, the freshwater ancestors of sea otter (*Enhydra lutris*) began specializing in benthic marine invertebrates, including the red sea urchins (*Mesocentrotus franciscanus*) that feed on kelp. The sea otter freed kelp from the limiting effects of herbivores to the benefit of finfish and other associated niche dwellers. Thus, sea otters can serve as keystone predators, in the absence of which kelp ecosystems may give way to comparatively unproductive environments, the so-called “urchin barrens” (Duggins, 1980).

Sea otters are relatively small for marine mammals, less than 45 kg. Unlike the seals that have adapted to the frigidity of the water with a layer of blubber, sea otters depend on their dense fur and a large caloric intake to maintain body temperature. Historically, human interest in sea otters has been economic—their pelage was highly sought after in the fur trade. In the 1700s, Russians began taking sea otters on the Asian continent. Exhausting the stock, they moved across the Aleutian Islands of what is now Alaska USA. With stocks already in decline by 1800, other nations joined in the fur trade, and the full extent of the sea otter’s range, down to Baja California Mexico, was targeted. By the time that the 1911 Treaty for the
Preservation and Protection of Fur Seals had placed a moratorium on their commercial take, the northern subspecies (ssp.) of sea otter (*E. l. kenyoni*) had been extirpated from the North American coast east of the Aleutian Islands but for southcentral Alaska (Jameson, Kenyon, Johnson, & Wight, 1982). The southern ssp. (*E. l. nereis*) was thought to be extinct until a raft of some 50 sea otters was spotted offshore of Big Sur, California in 1938 (Bolin, 1938).

Sea otters were reintroduced to parts of its former range in the late 1960s to early 70s, primarily using Aleutian Islands sea otters as source. Some thirteen attempts were made, including Oregon and Washington USA, Vancouver Island Canada, and southeastern Alaska. Efforts were generally a success but for Oregon. It is important to recognize that reintroductions are a conservation and management strategy to augment the recovery of endangered and threatened species (IUCN/SSC, 2013). In the U.S., two out of the five federally recognized sea otter stocks (i.e., distinct populations) are listed as threatened under the Endangered Species Act. The IUCN Red List rates the global population of sea otters as endangered and declining. Conservation and recovery continue to be a priority for the species. Yet, species reintroductions are inherently risky and involve several sources of uncertainty.

Sea otters have been absent from Oregon for over 100 years (Jameson et al., 1982). Coastal human institutions and practices (e.g., fisheries, recreation, resource management) have developed, and expanded during that time. If sea otters are reintroduced, some of these activities could have impacted reestablishment potential by reducing sea otter habitat or making it inaccessible. However, sea otters also have the potential to affect or influence human practices by changing the ecosystem or reducing prey populations. Some, such as Dungeness crab and red sea urchin, are the target of commercial and recreational nearshore fisheries. Changes or impacts to any of these cultural or economic practices could have implications for management.

Sentiment has been growing for a public discussion over the absence of sea otters in Oregon. The earlier translocation attempts were made prior to the landmark environmental legislation
of the 1970s: the Endangered Species Act (ESA), the Marine Mammals Protection Act (MMPA), and the National Environmental Policy Act (NEPA). Species translocations now go through a NEPA-mandated and publicly-transparent process to evaluate for a spectrum of factors in the affected coupled natural-human system. Oregon has since developed land-use planning goals, including Goal 19: Ocean Resources. Much as Oregon’s marine reserves were sited with local involvement, the locations and magnitude of any potential sea otter relocation will generate intense public input.

Members of introduced species have been deliberately targeted and killed in a number of instances, suggesting that a species’ welfare depends on community tolerance and prevailing values and attitudes (Reading, Clark, & Kellert, 1991). Many species translocations fail (Griffith, Scott, Carpenter, & Reed, 1989). Because of the complexity inherent to reintroductions, greater integration of different disciplines and types of knowledge will likely improve reintroduction outcomes (Reading et al., 1991). It is important that policymakers and wildlife managers understand stakeholder attitudes and dynamics. This allows them to tailor appropriate communication strategies and informs participatory approaches, increasing the likelihood of a successful reintroduction.

The inclusivity of reintroduction decision-making processes can also affect outcomes. For example, the U.S. Fish and Wildlife Service (USFWS) attempted to reintroduce grizzly bears to the Bitterroot Ecosystem on the Idaho/Montana border in the mid-1990s, and despite enjoying widespread public support, the reintroduction was not realized (Smith, 2003). This failure may have been partly attributable to the omission of key stakeholders from reintroduction decision-making processes. Fostering dialogue that potentially builds trust and understanding between potentially adversarial stakeholders may mitigate conflict and nurture compromise, as well as create more social sustainability if and when a translocation occurs. Having insight into stakeholder attitudes and points of commonality and divergence between relevant groups is important in reintroduction decision making. Elucidating select preferences
and perceptions among stakeholders could provide insight to policymakers navigating sea otter reintroduction decisions in Oregon.

How one chooses a source population reveals the nature of the restoration attempt. Do managers simply restore the species to its former range as a coarse fulfillment of the MMPA? Then, the relatively abundant southeastern Alaskan sea otters of the northern ssp. is the stock of convenience. People may advocate instead for the southern ssp. The smaller populations of this Californian sea otter, as compared to those of the northern ssp., currently have limited resilience in the event of pollution in their environment and other threats. Establishing an “outgroup” in Oregon would provide redundancy in a system that must not fail. The focus could be on the populations that carry the alleles most adaptive to the Oregon nearshore environment, fulfilling the MMPA’s more nuanced concern for the marine mammal stock once extant in the area. Surprisingly, despite the regional extirpation of the sea otter, researchers have genetic resources with which to address the question of what ssp. of sea otter or combination thereof once populated the Oregon nearshore. They can employ the ancient DNA (aDNA) in sea otter remains interred at archaeological sites of First Nations people. But the choice of a source stock does not simply rest on the prehistorical record. Without appropriate research to inform management, low genetic diversity in source populations, even in mixed-source translocations, could be problematic. Coupled with a slow rate of population growth, the resultant increased genetic load on nascent Oregon stocks may adversely impact the success of the translocation effort.

3.2 The Archaeological Record

My purpose here is in part to document and communicate the prehistorical evidence for considering the sea otter to have once been an integral part of the Oregon nearshore environment. Secondly, establishing the sea otter’s taxonomic status when it was extant is germane to choosing a source stock for reintroduction. Archaeological resources are critical to this avenue of research.
I focus on coastal Native American archaeological sites with confirmed faunal contents. Archaeological site surveys are inevitably a convenience sample, with the research here restricted to sources such that are readily accessible to researchers outside of the archaeological discipline. Much of the primary sources might otherwise come from the gray literature that Lyman (2011) describes as unpublished or poorly archived and, hence, inaccessible. With the exception of Minor, Toepel, Greenspan, & Barner (1985), all cited publications can be downloaded or found in university libraries. In Oregon archaeological literature, sites are given a three-part designation: “35” for the state of Oregon, a two- or three-letter abbreviation for the county, and a final set of digits reflecting the order in which the site was assigned a number by the Oregon State Historic Preservation Office (OSHPO, 2015). The Cape Creek Shell Midden is then 35LNC27 and located in Lincoln County. I follow best practices in only vaguely specifying site locations in order to preserve them despite problematic artifact-hunting and other trespass.

The Native American Archeological Sites of the Oregon Coast Multiple Property Submission to the National Register of Historic Places (NPS, 2017) has extensive coverage if scant detail. Hall (2009) inventories many faunal sites and has also made sea otter-specific compilations (Hall, 2018). Substantive coverage of coastal First Nations sites can be found in Aikens, Connolly, & Jenkins (2011).

*The precontact sea otter: zooarchaeological evidence*

Native peoples have for millennia created distinctive and enduring landforms with accumulations of snail and bivalve (mussel, clam, oyster) shell in coastal, lacustrine, and riverine environments (Álvarez, Briz Godino, Balbo, & Madella, 2011). These shell middens contain other artifacts of human activity, including faunal remains, tools, and debitage (byproducts of tool manufacture), often with remains of dwellings in close proximity. The 19th century Danish scientist Worsaae initiated the use of shell middens in interdisciplinary research investigating human-environment interaction. Prior to the 1980s, aquatic resource utilization was considered marginal as compared to terrestrial activities until the assumption
was questioned by, among others, Quilter & Stocker (1983). Zooarchaeologists exploit the long-term biological record available in middens to conjecture paleoecological trends. The presence/absence and Number of Identified Specimens (NISP) of remains in different strata can suggest changes in abundance and range. Rick et al. (2009) showed that the Guadalupe fur seal, now largely limited to Baja California, was abundant in southern California through the late Holocene. Sex and age differences between strata can indicate prehistoric rookeries no longer occupied. Investigation of faunal remains is not limited to aDNA and morphometrics. Sclerochronology, the analysis of periodic bone structure, has been applied to marine mammal teeth to determine the seasonality of human site occupation and resource utilization (Quitmyer, Jones, & Arnold, 1997).

First Nations peoples flourished along the thin margin of land of the eastern Pacific coast wherein the biotic richness of the nearshore environment could be accessed. Shell middens are distributed in Oregon both temporally and spatially. Much of the earliest migration and habitation occurred along a shoreline now miles out to sea (Aikens et al., 2011). Terminal Pleistocene/early Holocene (13,000-7,500 ya) sites still extant on headlands have yielded few faunal remains. Middle Holocene (7,500-3,000 ya) faunal remains have been collected from at least 16 sites. For instance, Middle Holocene strata at Tseriadun (35CU7) on the shores of Garrison Lake near Port Orford, once an estuary, and Yaquina Head (35LNC62) both yielded sea otter remains. For phylogenetic work into the relationships among West Coast sea otters in historic times, the primary interest lies in the settlements of the late Holocene (after 3,000 ya) now marked by shell middens. Still, I include earlier sites in the reporting, both because of the length of occupation of many of the sites and because it is advisable to retain sites of uncertain age as a future resource.

Sites include prehistoric quarries, petroglyphs, and fishing weirs. Most are characterized by shell middens. Middens are associated with both permanent settlements and seasonal camps, while permanent settlements are indicated by the presence of housepits. Though faunal remains might be inferred to co-occur with shells in middens, I only record those with faunal
remains that have been documented, typically by excavation. Of the 190 coastal archaeological sites that I recorded, 75 are known to have contained faunal remains (Appendix I). Of those, at least 17 have sea otter remains. In the description below, I call out by name and index these sites but discuss others as appropriate. Further investigation at known faunal sites may increase the number of sites with sea otter artifacts.

Of the 42 sites noted above the 45th parallel, 15 are noted to have held faunal material including five with sea otter (Figure 3.1). Near the Necanicum River and close to the northern border of present-day Oregon, people frequented the site of Palmrose (35CLC47) at least 3,700 ya. But as one of the oldest known Oregon permanent settlements, Palmrose was dated to 2,700 ya by the large plank house excavated there (Aikens et al., 2011). Habitation shifted to nearby Par-Tee (35CLT20) and Avenue Q (35CLT13), likely forced by subsidence events. Middens of all three sites held a large and diverse faunal assembly including 14 species of mammals. Ecola Point (35CLT21) excavations produced 38 sea otter NISP (Lyman 1995). The Netarts Sandspit (36TI1) was also a large community, with house remains dated to 1400-1800 CE. Losey (2002) noted the decrease in the hunting of sea otters and an increase in terrestrial game remains following the 1700 CE earthquake. Immediately north of Oregon on the north side of Cape Disappointment, Fishing Rocks (45PC35) also held sea otter remains (Minor, 1983). Notably, excavations at Columbia River estuarine sites Eddy Point (35CLT33) and Indian Point (35CLT34) found harbor seal (*Phoca vitulina*) remains but not those of sea otters.

Between the 45th parallel and 43.5° north, 27 sites out of 60 tallied are documented to have held faunal remains (Figure 3.2). Sea otter remains have been recovered at 10 of those. Lyman (1988) extensively reviewed the faunal collections from three of the Central Oregon Coast sites in documenting the dramatic changes in distribution and abundance of sea mammals following the 18th and 19th century commercial exploitation of the resource. He used NISP and not the minimum number of individuals (MNI), a more problematic quantitation. Umpqua-Eden (35DO83) on the Umpqua River has shell midden strata dating to 3,100 ya but is best
represented with artifacts from the last 800 years. Lyman records 302 sea otter NISP, 27% of which can be assigned to discrete time spans. The remaining specimen can only be ascribed the full range of 3,000 ya to 50 years before excavation due to disturbances at the site. Sea otter NISP comprises 17% of the NISP for combined sea otter and pinniped. A remarkably full picture of sea otter population dynamics and human utilization can be inferred. Both sexes of sea otters and all ages including newborns, are represented (Lyman, 1991). Bonnot (1951) records the habit of commercial sea otter hunters to kill pups first, as the mother would remain nearby for a second harvest opportunity. The patterns of the striations on found on 17% of the specimen bones offer insight into butchery methods (Lyman, 1991).

The nineteen samples from Whale Cove (35LNC50) span the Late Holocene (Lyman, 1988). An additional six sites with sea otter remains span Yaquina Head (35LNC62) through the Cape Perpetua area. Lyman assigned the 140 sea otter NISP of Seal Rock (35LNC14) to between 400 and 100 ya. Several kilometers inland and across the river from present-day Florence, Siuslaw Dune (35LA25) investigations produced only four sea otter NISP and 14 harbor seal NISP for its marine mammal inventory. Tahkenitch Landing (35DO130), near what is now Tahkenitch Lake, was the scene of intensive marine resource use during the middle Holocene. What was once an estuary was blocked by sand 3,000 ya, leading to the site’s abandonment.

Of some 88 archaeological sites on the Oregon Coast south of 43.5°, 33 have been shown to have held faunal remains. Four of those have identified sea otter remains (Figure 3.3). The people of the Coquille represented the northward extension of the Athapaskans that radiated out of northern California (Aikens et al., 2011 and Hall, 1995). Architecture and customs contrasted with those of the Columbia River and north. Several complexes in the Coquille River valley have been studied in detail, as at Na-so-mah Village (35CS43). Some sites, as in the Bandon Sandspit (35CS35), have since been lost to erosion by the Coquille River. Tseriadun (35CU7), above Port Orford on Garrison Lake, also lost its utility to native marine resource users as estuary turned to lake.
The relative abundance of sea otter remains by NISP relative to combined otters and pinnipeds varies. Sea otter remains are the most common marine mammal on the North Coast at Palmrose and Par-Tee in the north. At Bandon Sandspit, MNI for sea otters has dropped to two (Tveskov 1999). Relative abundance sharply increases in central and southern California (Lyman, 2011). Lyman estimated total NISP for sea otters in Oregon at 1275.

3.3 Taxonomic Research

To demonstrate the importance and need of incorporating genetic considerations into the reintroduction process, I reviewed the research literature into the ssp. status of pre-fur trade sea otter populations.

Information on sea otter genomes was obtained at Genbank (ncbi.nlm.nih.gov), the National Institute of Health’s genetic sequence database. The genetics sections of two recent workshops gave background material: the 2018 Elahka Alliance symposium at Newport Oregon USA and the 2019 Sea Otter Conservation Workshop at the Seattle Aquarium (Washington USA). Taxonomic information comes from the Integrated Taxonomic Information System (itis.gov).

Statistical analyses were performed in R 3.6.1 (R Core Team, 2019). Least cost distance analyses were made in MARMAP (Pante & Simon-Bouhet, 2013). Bathymetry for the above were built from the ETOPO1 Global Relief Model (ngdc.noaa.gov) in a four arc-minute grid. Mapping also employed the R packages GGMAP (Kahle & Wickham, 2013) and GGREPEL 0.8.1 (Slowikowski, 2021). Data exploration involved the packages GGPILOT2 (Wickham, 2016) and GGALLY (Schloerke et al., 2021).

The precontact Oregon sea otter: early work

Taxonomists have employed traditional techniques, in particular skull measurements, to distinguish sea otter ssp. since Linnaeus first described the nominate Asian sea otter (Enhydra lutris lutris) in 1758 (itis.gov). Merriam described the southern sea otter (Enhydra lutris nereis) in 1902. Only in 1991 was the northern sea otter (Enhydra lutris kenyoni) given ssp. status by Wilson, Bogan, Brownell, Burdin, & Maminov (1991), again on the basis of
morphometrics. They ascribed to the contemporary northern sea otter the range of the Aleutian Islands southward to the state of Washington.

The position of the Oregon sea otter along a gradation of sea otter morphological features distributed across the northern Pacific coastline has invited investigation. But with no extant sea otters in Oregon, researchers turn to archaeological artifacts. Lyman (1988) compared teeth from prehistoric Oregon (n = 13) samples with those of historic Californian (n = 10) and Alaskan (n = 20) samples in multiple Student’s t-tests.

He found the lower M1 (molar) width comparison significantly different for California:Alaska (t = 2.486, df = 30, P < 0.01) and Oregon:Alaska (1.791, 33, P < 0.05) but not California:Oregon. Lyman judged differences for the upper M1 significant for California:Alaska (3.391, 28, P < 0.005) and Oregon:Alaska (2.478, 32, P < 0.02). Again, California:Oregon were not significantly different. For the upper P4, the same pattern holds: California:Alaska (1.955, 25, P < 0.05) and Oregon:Alaska (1.897, 24, P < 0.05) are significantly different but not California:Oregon. The lower P4 measurements were commensurate throughout the region. The inference is that the Oregon sea otter largely aligned with the southern ssp. but shared some characteristics in common with the northern ssp..

Unfortunately, the statistical analysis is problematic. The heteroscedacity (unequal variance) among groups precludes Student’s t-test for at least one of the comparisons. I set this issue aside and used tables (Zar, 2010) as Lyman would have done to check p-values. Though an unusual approach, it appears that Lyman employed one-tailed tests in all cases but for the one case that was unequivocally significant (California:Alaska for M1). There is some suggestion in the text that one-tailed tests were the intention. Roest (1973) had found Alaskan sea otter teeth to be larger than Californian teeth, and so an eastward, then southward, gradation in size could be argued. For just the change in analysis of going to two tails, all upper P4 and the Oregon:Alaskan lower M1 significance would be lost. More importantly, given the multiple comparisons made between groups, a Bonferroni or other adjustment is appropriate. Reducing
α to 0.0167 would lose half of the six significant findings. Applying both conditions, exclusive of heteroscedacity concerns, would leave the significance of just the one comparison intact.

Phylogenetic questions, here the taxonomic status of the Oregon sea otter, can also be addressed using genetics. With both systematic zoology and genetics, the power of inference is limited by sample size. For genetics, the number of loci, or markers, along the genome is key to power as well. Molecular work in sea otters has been a story of the limitations of successive generations of genetic markers as much as it has been the story of the understanding of population structure in sea otters. Lewontin & Hubby (1966) (also, Hubby & Lewontin, 1966) revolutionized population genetics by using gel electrophoresis to show polymorphisms, or variation, across dozens of loci in enzymes and proteins for the fruit fly *Drosophila pseudoobscura*. Prior studies had only looked at allozymes at single loci and so lacked power to make species-level inferences. Numerous phylogenetic studies in the 1990s employed allozymes to test for population structure in contemporary sea otter populations. Findings trended toward concluding a lack of genetic diversity, let alone structuring, in the metapopulation across the sea otter’s range. Of 41 loci, Rotterman (1992) only found three to be variable. Similarly, Lidicker & McCollum (1997) showed no geographic clustering in the variation of the five loci with polymorphisms of the thirty that they studied. Given the pervasive lack of variance, though, the findings of no genetic basis for ssp. distinctions could as well be a false negative as they could be a true negative.

Genetic investigations using DNA began with the use of restriction enzymes, “cutters” of DNA strands, in the early technique of random fragment length polymorphism (RFLP). Researchers worked on mitochondrial DNA, a different set of DNA instructions from that contained in nuclear genomic DNA. The sea otter mitochondrial genome is 16,431 bp (base pairs) long versus the 2.4 giga-bp (Gbp) of genomic sea otter DNA (ncbi.nlm.nih.gov). These RLPF studies, such as Cronin, Bodkin, Ballachey, Estes, & Patton (1996), began what became a familiar pattern of contradicting prior work. In contrast to the conclusions of the allozyme studies, their findings supported the northern ssp. designation of Wilson et al. (1991).
Sea otter phylogenetic studies are important for conservation biology and wildlife management. For vertebrates, the ESA’s definition of species for potential listings can extend to the species taxon level, the ssp., or Distinct Population Segments. The Marine Mammal Commission and, at times, USFWS characterizes marine mammals as *stocks*, a Marine Mammal Protection Act (MMPA) term. Gorbics & Bodkin (2001) developed the case for defining three Alaskan stocks, based on geographical distributions, phenotypes, and genetic data (primarily mitochondrial). They espoused a definition of stocks to include a degree of divergent allelic frequencies, reflecting some level of genetic isolation. Of these now-designated stocks, the southwestern Alaskan stock is listed under the ESA as Threatened. (There is a tragic irony here. This stock served as the primary source for the 1960-70s reintroduction efforts in Alaska, Washington, and Oregon.)

*The precontact sea otter: 21st century genetics*

Variation at genetic markers is most frequently assumed to be neutral in effect. They can indicate, by proxy, adaptive variation within genic regions. In small, isolated populations, allelic dropout is more likely to occur due to the stochastic effect of smaller sampling of genomic diversity and fewer recombinations. Deleterious mutations are more likely to become fixed. The affected populations will likely lack the adaptive variation with which to respond to changes in the environment. Thus, monitoring genetic diversity is a prime concern for conservation biologists. Microsatellites in genomic DNA, varying degrees of repetition of short DNA repeats in neutral regions, have been an important metric in measuring diversity. Larson, Jameson, Bodkin, Staedler, & Bentzen (2002) utilized primers developed for mustelids to collect genotypes at six microsatellite loci and developed a novel seventh. Additionally, they found four haplotypes within the control region of mitochondrial DNA. With the primary goal of measuring genetic diversity in translocated and remnant populations, they found no evidence of reduced genetic diversity but significant heterogeneity among populations.

Much of the recent conservation genetics work on sea otters focuses on the status of genetic diversity among contemporary populations and comparisons to pre-fur trade populations. In
the last 20 years, three papers have addressed the taxonomic status of the Oregon sea otter: Valentine et al. (2008), Larson, Jameson, Etnier, Jones, & Hall (2012), and Wellman et al. (2020).

First Nation peoples discarded sea otter bones in the shell middens that are now archaeological sites as described above. The alkalinity of the shells offset the acidity of soils, preserving DNA from complete degradation. Valentine et al. (2008) extracted aDNA from specimens taken from two archaeological sites for a sample size of 16 pre-harvest sea otters (Figures 3.2, 3.3). They began their analysis with the four mitochondrial haplotypes found by Larson et al. (2002). Combining the 151 samples of sea otter mitochondrial DNA from Larson et al. study with their samples gave the Valentine group 6 haplotypes with which to postulate the ssp. status of the Oregon sea otter. Samples from Oregon sea otters \( n = 11 \) matched the C haplotype of southern sea otters and the A haplotype \( n = 2 \) in lesser proportion in the southern sea otter but in greater proportion in northern sea otters. Two haplotypes, W and X, were unique to Oregon. The haplotypes distribute geographically in an intriguing manner. Valentine et al. report two G-tests for significance. They found no significant difference between Oregon and Californian populations \( (P = 0.6) \). The frequency of the C haplotype of these two populations in comparison to the rest was found to be highly significant (Figure 3.4).

Many comparisons do show significant differences. Given the small sample sizes, Fisher’s Exact Test is the more conservative option. In a test for differences between the contemporary southern ssp., precontact Oregon, and northern populations (Washington precontact and contemporary northern ssp. populations pooled), there is a highly significant difference \( (P < 0.0005, \text{Markov simulation with 2000 replicates}) \). In pairwise tests, modern southern ssp. versus old Oregon lacked significance \( (P = 0.0279) \), but old Oregon versus Washington and north is highly significant \( (P < 5.5E-15) \) even with a Bonferroni adjustment. Modern California versus Washington and north is also highly significant \( (P = 3.01E-26) \).
In a study more generally concerned with genetic diversity and population parameters for the species as a whole before the fur trade, Larson et al. (2012) employed 5 microsatellite loci in a study that drew on samples of five pre-fur trade populations and five contemporary populations. From Oregon, 40 samples in total came from five archaeological sites (Figures 3.2, 3.3). In contrast to the Valentine group, Larson et al. concluded that the microsatellite loci of Oregon sea otters are most similar to those of pre-fur trade Washington samples. Finding the inferences from the genomic DNA at odds with Valentine’s findings from (maternally inherited) mtDNA suggested to the authors that pre-fur trade Oregon sea otter population genetics may reflect male northern sea otter input and a female southern sea otter component.

As above, the status of the Oregon sea otter among precontact sea otter populations was only one of the questions that Larson et al. addressed in their 2012 paper and perhaps a minor objective at that. Two of the three metrics given for comparison are $F_{ST}$ and Nei’s genetic distance. The fixation index $F_{ST}$, a genetic measure of population differentiation, here is used in a pairwise comparison between subpopulations. Nei’s genetic distance reflects the degree of divergence between populations or the length of time since they shared a common ancestor.

While not a formal analysis, I can investigate what correlations can be made for swimmable distances between populations and the isolation-by-distance metrics above. The further apart subpopulations are from each other, the greater the $F_{ST}$ should be for those findings to be useful. ATOS, or as the otter swims, is a frequent metric in sea otter literature (Tinker, Doak, & Estes, 2008), referring to the non-linear path an otter might take along the coastline while still remaining within foraging depths. For this comparison, I greatly simplify the path. East and south of southwest Alaska, I only limit the traveling otter to the continental shelf (-200 m deep) and more than 10 m west of the shore. Such passage would be nonetheless be multi-generational. Going east to Russia, I give a still more simplified equivalent that allows for direct passage between the islands of the Aleutian chain. While all $F_{ST}$ but CA:RU (California:Russia) were significant (Figure 3.5), I see no correlation between distances and
F_{ST} for the pairwise comparisons. Similarly, with Nei’s genetic distance (Figure 3.6), any correlation between pairwise distance and the values for genetic distance breaks down after OR:WA, CA:OR, and CA:WA. An additional analysis in the program STRUCTURE (Pritchard, Stephens, & Donnelly, 2000) was used by Larsen et al. to group individuals into clusters, but the assigned groupings lack 16 of 40 Oregon samples. In conclusion, this five-loci microsatellite approach lacks a compelling argument with which to assign the Oregon sea otter to the northern ssp.

The techniques employed to date have largely employed older genetic approaches now falling out of favor. The exception is Wellman et al. (2020). They compared the full mitogenomes of 18 precontact sea otters from the northern Oregon archaeological sites Palmrose and Par-Tee to 16 historical (1850–1990) specimens from more distant sites. These latter came from Russia/Japan (n = 2), the Aleutian Islands (western Alaska, n = 5), south-central Alaska (n = 1), British Columbia (n = 1), Washington (n = 1), Oregon (1 of indeterminate provenance, 1 from Port Orford), and southern California (n = 4). Additionally, 38 contemporary southern Californian mitogenomes and one from Japan from published sources were included in the study. The Wellman group framed their primary research objective to be determining which of the post-fur trade ssp. best matches the historical/archaeological record for precontact Oregon sea otters and, hence, should serve as source stock for a future translocation effort.

Secondarily, they posit that archaeological haplotypes are more diverse than those from historical and contemporary samples.

With 10 of the 27 haplotypes found, the archaeological Oregon sea otter showed greater haplotype diversity (Hd = 0.91) than contemporary California samples (Hd = 0.44) as calculated by the Wellman group in DNASP (Rozas et al., 2017). Their median-joining network analysis of haplotypes, built in POPART (Leigh & Bryant, 2015), is key to evaluating the group’s conclusions (Figure 2, Wellman et al., 2020). I adapt the publication’s diagram to present a simplified view (Figure 3.7). The Wellman group interprets the Oregon samples (n = 20) to cluster with the northern ssp. (n = 7). The Commander Island specimen of the Asian
ssp. groups with these as well. One Japanese specimen of the same ssp. separates strongly; the other shares a haplotype with an Alaskan sample. California samples cluster to one end of the analysis and disproportionately assign to one haplotype of the five identified. A separate analysis truncates these sequences to that of the 222-base control region of Valentine et al. (2008) and incorporates the samples from that study. Similar to the Valentine group’s findings, California haplotypes are associated with archaeological Oregon haplotypes with this approach.

I note a caveat to the construct of the Wellman et al. (2020) study. Groupings of samples processed together for a given sequencing effort are referred to as “libraries.” The authors include mitogenomes from other studies in their analysis. Technical artifacts from different library preparations and sequencing efforts can interfere with haplotype assignments. Nucleotide calls within haplotypes are likelihood-based, both dependent on the count of reads per allele at a given position and the quality of those reads. A fundamental precaution is to mix populations within each sequencing run, something that could not happen here with the contemporary California samples coming from a prior study.

Three factors hamper one’s ability to use the analysis in comparing and contrasting samples on a ssp. basis: (1) the greater haplotype diversity of archaeological specimens than that of historical and contemporary samples, (2) the variance in sample size from stocks (e.g., 2 Asian ssp. to 20 Oregon samples), and (3) the time frame from 2600 BP (Palmrose site) to contemporary samples that spans the genetic bottleneck created by the fur trade’s overexploitation of sea otter stocks.

The greater haplotype diversity of archaeological Oregon samples in combination with their greater sample size makes it difficult to determine if, in fact, these cluster with historical northern ssp. or just distribute more widely because of these factors. The contemporary samples, here, only from California, may simply be more genetically depauperate. We lack a basis of comparison with the northern ssp. As with Larson et al. (2012) discussed above, there
is no indication of isolation by distance. At one extreme, Oregon sea otters are represented by 7 samples; at the other end of the network graphic are the California samples. To accept the network analysis in full, one would also have to dismiss the significance of the Asian ssp. taxonomic designation, as the Russian and Japanese samples fail to cluster together. An additional figure in the publication shows limited commonality in haplotypes between samples from the earlier Palmrose site (2600 -1600 BC) and the Par-Tee site (1850-1150 BC). As the supplementary material has keys that do not transfer between documents, it cannot be determined how the different ages of archaeological Oregon samples distribute in the network analysis.

The authors nicely control for length of mitochondrial sequence in re-examining the inferences made by the Valentine group. They argue that their broader coverage of the mitogenome better represents the taxonomic status of the precontact Oregon sea otter. From this, one would think that they dispute the prior group’s conclusion. Instead, they propose translocations of southern sea otters to the parts of the Oregon coast that the Valentine group sampled, with supplements of northern ssp. on the northern coast. Rather, if one accepts their assignment of Palmrose and Par-Tee sea otters to the northern (and Asian?) ssp., that would be the limit of inference that can be made from this study. Precontact sea otters to the south have yet to be investigated satisfactorily. Significantly, the choice of source stock for a translocation effort does not rest solely on prior taxonomic status as the authors imply but on a host of factors. I discuss some of these additional concerns later in this chapter.

Next-generation sequencing (NGS) is the current standard in genomic investigations. The volume of sequencing data is greatly increased, and, in turn, tens of thousands of markers, e.g., single nucleotide polymorphisms (SNPs), are now routinely obtained. While the restriction-site associated DNA sequencing (RADseq) discussed below can be employed without a reference genome, all methods capitalizing on NGS benefit from one developed for the target or a sister taxon. An annotated genome for the northern sea otter has been published (Jones et al. (2017), Table 3.1). “Elfin,” the Vancouver Aquarium sea otter
individual who contributed his genome, was born in 2002 near Juneau, Alaska. UCLA recently published the southern sea otter genome (Beichman et al., 2019). “Gidget” was a foster mother and resident sea otter at the Monterey Bay aquarium.

BUSCO v2 assembly completeness statistics are given by the Beichman group as southern: 93.3% and northern: 96.2% for complete genes. Annotations for the genome of the northern sea otter call out 19,458 protein-coding gene models. Beichman et al. report more for the southern ssp. (21,909). In contrasting their gene model count for the southern sea otter to that of the South American giant otter (*Pteronura brasiliensis*), the Beichman group suggests that more duplicate calls were made due to the greater fragmentation of the giant otter assembly. While this may also be the case with southern sea otter assembly more fragmented relative to that of the northern, BUSCO statistics also ascribe 0.9% more duplicate gene models to the northern sea otter genome. More likely, the discrepancy is due to different annotation pipelines.

The reference genomes lay the groundwork for low-coverage full genomic sequencing work. While this whole-genome sequencing approach can be appropriate for some aDNA applications, NGS reduced-representation approaches are also established lines of inquiry and more cost-effective (McCormack, Rodriguez_Gómez, Tsai, & Faircloth, 2017). They have yet to be applied to either pre-fur trade or contemporary sea otters. Reduced-representation sequencing methods, such as RADseq, broadly sample the genome at reproducible intervals. RADseq, however, depends on sites in the genome that restriction enzymes recognize remaining unaltered. Across a deep timeline, these sites may mutate, resulting in allelic dropout and, hence, uneven coverage.

Accordingly, the bestRAD approach (Ali et al., 2016) that I used in the research on Yelloweye Rockfish discussed in the prior chapter would not be appropriate for aDNA. Target enrichment methods, however, employ probes designed to target conserved regions of the genome across many taxa. I had intended to trial the ultra-conserved elements (UCEs) of
Faircloth et al. (2012) on previously extracted sea otter aDNA and contemporary samples. My requests for access to these samples, however, were rebuffed.

Probes like UCEs capture neutral (non-coding) portions of the genome. This is appropriate for the taxonomic task at hand. But for the limitation of mutation at cut sites, RADseq methods would otherwise lend themselves to aDNA studies of both neutral variation and of the signs of adaptation that require access to coding regions. Several approaches that combine RADseq and sequence capture may have overcome this limitation. The Ali et al. (2016) protocol Rapture incorporates probes. Hybridization RAD (hyRAD, Suchan et al., 2016) was designed to work with museum specimens.

3.4 Translocations and Society

A possible translocation of sea otters to Oregon would be just one of many attempts to reintroduce carnivores to regions in which they had been extirpated by humans. Past examples serve to illustrate potential societal responses to the prospect. Here, I acknowledge the risks and uncertainties that stakeholders encounter in such a change to the present-day environment. But I also develop the rationale for reintroducing sea otters to Oregon.

A reintroduction example: gray wolves

Eradicated from the western U.S. by 1930, *Canis lupus* was listed as Endangered in 1978 across the contiguous U.S. I focus on its National Rocky Mountains (NRM) Distinct Population Segment (DPS). After the drafting of the 1980 NRM Wolf Recovery Plan, the NEPA-required EIS was completed in 1994 for the reintroduction of wolves into Yellowstone National Park and central Idaho (USFWS, 1994). Some 30 wolves from Alberta, Canada, were introduced into each location in 1995 and 1996. ESA protections were removed in 2017 for the Wyoming’s ≈25 breeding pairs (USFWS, 2017), and Montana’s 15 breeding pairs in 2011 (MFWP, 2015). In each case, state recovery and management plans were approved, then monitored by USFWS for five years. Wolves were not reintroduced into Oregon but migrated there as the Idaho stock expanded. The first wolf to be sighted was captured alive, crated, and
sent back to Idaho (Oregon Wild, 2019). The NRM DPS, which includes the eastern third of Oregon and Washington, was delisted in 2011 (except Wyoming) as the result of a congressional budget rider. Within hours of delisting, the Oregon Department of Fish and Wildlife (ODFW) had killed two wolves and issued permits to landowners to do the same. Effective January 4, 2021, the DPS has been fully delisted (USFWS, 2020).

Local economic interests were not successful in their opposition to the initial reintroduction. These ranchers’ viewpoint deserves special attention. Per the Integrated Taxonomic Information System as curated by the U.S. government, there are 38 recognized ssp. of Canis lupus worldwide (itis.gov). (They include C. l. familiaris, the domestic dog, though the wolf and the dog are sister taxa.) In the 1994 EIS, USFWS acknowledged that early taxonomists working with limited samples called out 24 ssp. in North America. National Park policy states that they will strive to restore native species using that which most closely approximates the extirpated species. The 1978 ESA designation was for the species as a whole, and USFWS set out that any past or present ssp. designation is irrelevant to wolf recovery efforts. Still, USFWS had to address public comments that (1) reintroduced wolves would interfere with the natural recovery of remnant, native wolves (USFWS (1994): there are none), and (2) the genetic mixing of non-native stock with native stock is illegal and lacks scientific integrity (USFWS: there is currently no molecular basis for asserting more than one ssp. in northern North America).

The Oregon Cattlemen’s Association has made numerous attempts to undermine wolf recovery, including (mostly failed) legislative bills. A key argument they employed was that these “Canadian wolves” are non-native, and the public should be able to kill them without restriction. This line of reasoning surfaces in public comments on proposed policies and even in court briefs, as in the challenge to the state’s delisting of wolves in its own list of threatened and endangered species (Intervenor-respondents, 2017). Willamette Weekly explored how deeply this line of reasoning runs. Are these wolves secretly Canadian? Are these
wolves bigger, more predatory than those native wolves extirpated in Oregon? Some claimed that wolves native to Oregon were the size of cocker spaniels (Green, 2017).

Potential negative economic impacts of an Oregon sea otter translocation

Oregon stakeholders have legitimate concerns over potentially adverse impacts of a sea otter translocation. Overlap in likely sea otter habitat and human marine resource usage was projected by Kone, Tinker, & Torres (2021) using a model developed for California. The group predicted that the majority of sea otter occupancy would be on the south coast (Charleston and south) and that sea otters would largely establish residency on the outer coast, as opposed to estuaries. The sea urchin fishery has been conducted as an artisanal fishery with just a few participants landing an annual total of 500,000 pounds (ODFW, 2021a). The commercial Dungeness crab fishery in Oregon is a larger enterprise with 17 million pounds landed annually at an ex-vessel value of $40 million (ODFW, 2021b). The Kone group found that most urchin fishing activity occurs in or close to optimal sea otter habitat. Regarding the Dungeness crab fishery, 9% of high-catch fishing grounds have a comparable proximity.

Why translocate

The International Union of Concerned Scientists defines translocations as “the movement of living organisms from one area with free release into another” (IUCN/SSC, 2013). “Augmentation” supplements an existing population, “introduction” places organisms outside of its historic range, and “re-introduction” returns a species or stock to part of its historic range from which it has been extirpated.

Weeks et al. (2011) delve into the motivations for a translocation. Conservation translocations are species-specific in their focus, creating or maintaining populations with resilience, persistence, and abundance in numbers. Ecological restorations intend to promote an increased biodiversity of indigenous species. The enhanced ecosystem dynamics potentially benefit the human-natural system with greater productivity as well as increased resilience in the face of environmental threats.
Conservation translocations are inevitably motivated by the desire to preserve focal species. But the genetic motivations behind translocation efforts and their implications are less well-defined (Weeks et al., 2011). Genetic capture can capture the majority of a source population’s genetic variation and take advantage of a reintroduction’s site abundant resources to grow the population’s effective size, its breeding population, to levels that will preserve genetic diversity. At this point in population growth, most genetic variation will have been preserved and continue from this point to be maintained. The rationale is applicable when source populations are highly constrained or when the source is a small captive colony, as was the case with California Condors in 1991 (Roach & Patel, 2019). Genetic rescue, as narrowly defined in Weeks et al. (2011), would introduce genetic diversity to mask the expression of deleterious alleles. It assumes that there is a recipient population suffering from inbreeding depression but with locally adaptive alleles (genic varieties) to be preserved. Conservation measures often have short to medium time frames and are applicable when saving an existing population is paramount.

Writers have attempted to expand the usage of genetic rescue (e.g., Whiteley, Fitzpatrick, Funk, & Tallmon, 2015, and Bell et al., 2019) to incorporate more genetic aspects to extinction risk. Hedrick (2005) suggests the more-inclusive term genetic restoration to address the effects of gene flow on not only deleterious alleles but genic neutral and adaptive variation. Remnant and translocated sea otter populations are characterized as low in genetic diversity owing to the population bottleneck caused by the fur trade (e.g., Beichman et al., 2019). With a contraction in effective population size comes increased inbreeding depression, or the reduction in fitness caused by the interbreeding of related individuals (Hedrick & Garcia-Dorado, 2016). This inbreeding load is evidenced by the expression of deleterious alleles otherwise hidden in heterozygotes. Other components of the genetic load carried by small populations are mutational load, the failure to segregate deleterious mutations, and drift load, the random fixation of deleterious mutations.
While genetic rescue, sensu Weeks et al. (2011), can succeed in the short term due to the increased vigor of the consequent hybrids, the hybrids’ relative success can reduce the standing neutral variation and the adaptive variation in the recipient population (Hedrick & Garcia-Dorado, 2016). At the extreme, we run the risk of genetic swamping (Lenormand, 2002). Should the source population be insufficiently diverse, translocated animals will contribute to the genetic load of the source population with an excessive load of their own. There may also be an optimal time to augment a declining population. As population size decreases, homozygosity increases. Selection works against segregating deleterious alleles of major effect. For a time, purging of recessive lethals reduces the inbreeding load more quickly than the population drops. Too early of an augmentation fails to allow the purging of the effects of inbreeding depression (Hedrick & Garcia-Dorado, 2016).

Ecological restorations, by contrast, have an underlying expectation of local adaptation (Broadhurst et al., 2008). Even when a small recipient population persists in an environment, its genetic variability may be limited, and the attempt to recruit from it exclusively may further depress its viability. Rather, the focus is on bringing in high-quality stock that will generate genetic variability, some of which will prove adaptive to the local environment. This approach is well-suited for keystone species that have the potential to dramatically enhance local ecosystems. Time frames are necessarily greater in scope with this strategy, with long-term persistence the goal. The 25-year window that Alaskan sea otter recovery plans (USFWS, 2013) employ in determining listing status are appropriate.

3.5 Discussion

The precontact Oregon sea otter: identity

To what ssp. did the Oregon sea otter belong? The southern ssp. has had a qualified recovery in California, and the northern ssp. is reestablished as far south as Washington. Oregon may have been a hybrid zone between the two, or one ssp. may have dominated. The constellation of recognized ssp. is expanded to the west by the Asian sea otter, ranging from the Kuril
Islands north of Japan to the Commander Islands in the northwestern Pacific Ocean. Choosing the appropriate source population may supply the founding population with genetics more adapted to the Oregon marine environment. Public policy considerations factor into both the taking of sea otters from the source population and the status of the reintroduced population. In the face of criticism from stakeholders inclined to oppose reintroduction efforts, aligning the source population genetics, or combination thereof, to that once native to Oregon is more scientifically defensible.

In hindsight, it is unfortunate that the greater sample size of Larsen et al. (2012) was not leveraged to include the mitochondrial approach of Valentine et al. (2008). Statistical power, again, is multifaceted. The microsatellites of Larson et al. are multi-allelic, yet only a few loci were employed. The Valentine group worked with few samples and slight differences in a short mitochondrial sequence. But the Wellman group demonstrated that their broader mitogenomic strategy offered a different perspective on the Valentine group’s findings. Studies to date have not convincingly answered the question of the ssp. status of the sea otter once found over much of the length of the now-Oregon coastline.

Most of the genetics employed to date for sea otter phylogeny lack the statistical power that contemporary genetics offer. NGS, the current trend in diverse fields of genetic study, utilizes thousands of variations across the genome. With sea otters, researchers gain access to the 2.4-Gbp genome versus a few microsatellite loci or a 222-bp mitochondrial segment. Full genome sequencing is possible but may be overly ambitious for substantive population sampling. Several techniques (e.g., Suchan et al., 2016) instead employ reduced representation sequencing and are optimized for aDNA.

Precontact sea otter genomics would seem to be an irresistible lure for genetics workers, myself included. In researching the genetic position of precontact Oregon sea otters within the continuum of sea otter ssp., two lines of inquiry are possible. Genetic markers can be compared amongst pre-fur trade populations. Or, those, such as the Beichman group, could
take the whole genomic sequencing approach further. Either requires the informed participation of West Coast tribes in several states. The Makah of the Olympic Peninsula, by way of example, curate the faunal remains from their ancestral village of Ozette.

Several questions of ssp. status are at play as well. Answering the question of precontact sea otter distributions entails using all archaeological specimens. The approach would lead to a better understanding of precontact population genetics. Secondly, what contemporary spp. was the precontact Oregon sea otter most similar to? Samples from contemporary southern and northern ssp. need to be compared to the Oregon samples taken from late Holocene strata of First Nations middens. Again, that Oregon artifacts are part of the heritage of existing tribes conditions their use to the tribal councils’ assent.

Oregon may have supported both ssp. in some form of secondary contact after a period of allopatry. While the ssp. status is not settled, neither are there strong contraindications to the hypothesis that southern ssp. influenced the genetic composition of sea otters along the southern portion of the Oregon coastline and the northern ssp. dominated that of, at least, far northern Oregon. It may be that other considerations related to choice of source stock and the current momentum toward a translocation effort will outweigh the desire to wait on a better resolution to the question.

Reintroduction issues
I have returned to Oregon archaeological literature and restated the case for considering sea otters to have been an part of the nearshore ecosystem, as were the First Nations peoples that hunted them. A translocation would restore a part of Oregon, not make a novel introduction. But, public concerns and perceptions factor into policy decisions. Public deliberations must accommodate the voices of diverse stakeholders. NEPA requires federal agencies planning or funding actions to consider the possible environmental effects and posit alternatives to the proposed action (42 USC Chapter 55). Further, agencies must disclose these effects to the public and solicit the public’s opinions on its assessments. To plan for a balanced, fair
discussion on the issues surrounding a potential sea otter reintroduction, the lessons from prior translocations might be revisited.

Should a stakeholder feel threatened—in their economic sense of security, in their sense of community, or in their life values—but not have reasoned arguments with which to counter the perceived threat, they may resort to employing logical fallacies in opposition to a change in the status quo. Much of the objection to gray wolf reintroduction in Idaho and Montana, and their protected status as they moved into Oregon, falls under this category. To counter in advance claims of non-native status for sea otters in Oregon, participants in the reintroduction discussion might be (a) advocating for research into the ssp. status of the Oregon sea otter using current genetic and archaeometric techniques and (b) broadening present-day collective knowledge of the history of sea otters in pre- and post-contact Oregon. Archaeological remains provide tangible evidence of the sea otter’s part in the human-natural system prior to Russo-European exploitation.

It is reasonable on the part of Oregon marine resource users to experience uncertainty over the competition that sea otters may have for fishery target species. The Kone group has addressed foreseeable negative impacts. There is, however, currently no comparable study to quantify the positive effects that sea otters may have on the nearshore ecosystem. Such a study could incorporate the indirect benefits coastal communities could derive from the change brought to the coupled human-natural system by sea otters as well as the direct benefits from recreational users attracted by sea otter occupancy on the Oregon coast.

*Rationale for translocation*

Suggested benefits of sea otter reintroduction have touched on both conservation and ecological restoration purposes. The IUCN Red List rates the global population of sea otters as threatened and declining (iucnredlist.org). A successful Oregon sea otter reintroduction would bolster sea otter numbers. As a keystone species in other parts of its range, sea otters have the potential to reset the Oregon nearshore ecosystem to a more biologically productive
and biodiverse state. Thus far, sea otter conservation has been the dominant theme in reintroduction discussions, and ecological restoration has been the supporting argument.

Modern approaches to genomic investigations could also be applied to characterizing the genetic diversity of contemporary sea otter populations with the goal of informing an Oregon translocation. The improved understanding of levels of diversity and inbreeding load would allow for more informed decisions on mixed-sourcing of subspecies in the re-establishment of Oregon sea otter populations and subsequent augmentation.

I also introduced several genetic motivations for translocations. The expansion of the California sea otter north of its last refuge at Point Sur has been stalled near Monterey Bay. The ssp. is both depressed in effective population size and lacks resilience to hazards (Gagne et al., 2018). The limited population size, much of it located in proximity to major cities, is particularly susceptible to oil spills. But explicitly performing a genetic capture of the southern ssp. may mean establishing an ESA-designated essential experimental population in Oregon. This would entail considerable policy and management considerations. By using the southern spp. simply as a source stock, we also provide a landing spot for the northern male individuals that not infrequently stray into Oregon. This circumstance may prove to facilitate both a genetic rescue of the southern ssp. and the genetic adaptation of translocated stock as selection promotes the alleles most adaptive to the Oregon nearshore environment.
Figure 3.1  Northern Oregon coastal archaeological sites. Faunal remains in 15 out of 42 tallied. Labeled sites are known to have held sea otter remains.
Figure 3.2 Central Oregon coastal archaeological sites. Faunal remains known to occur in 27 out of 60 tallied. Labeled sites are known to have sea otter remains. Genetic studies in red.
Figure 3.3  Southern Oregon coastal archaeological sites with confirmed faunal sites \( n = 33 \) out of 88 sites tallied.
Figure 3.4  Valentine mitochondrial evidence.
Figure 3.5  \(F_{ST}\) from Larson et al. (2012) for pre-fur sea otter subpopulations versus distance between subpopulations. CA:RU comparison lacked significance.

Figure 3.6  Nei’s genetic distance from Larson et al. (2012) for pre-fur trade sea otter subpopulations versus swimmable distance.
Figure 3.7  Simplified view of network analysis by Wellman et al. (2020, original figure available by open access). AK: historical Alaska, aOR: archaeological Oregon, hOR: historical Oregon, RU: Russia, JP: Japan, CA: California, WA: Washington. hap: haplotype count, n: number of specimens across haplotypes. Known northern ssp. are in gray, Asian ssp. in gold, Oregon specimens in yellow, and known southern ssp. in blue. Single haplotypes: individuals depicted by circles.
Table 3.1  Scaffold-level genome assemblies for the northern and southern sea otters compared. Source: ncbi.genome.gov.

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<td>55 496</td>
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Chapter 4: General Conclusion

Yelloweye Rockfish are distinguished within the *Sebastes* genus by both being in the niche of benthic dwellers with small home ranges and one of the most long-lived rockfish. Other rockfish more closely tied to the nearshore environment are often shown to be genetically differentiated at biogeographic breaks, e.g., Yellowtail Rockfish (*S. flavidus*) at Cape Mendocino, California (Hess, Vetter, & Moran, 2011). Yelloweye do not follow that pattern in an obvious fashion on the outer coast. Given iteroparous adults with episodic recruitment success, the larval stage of Yelloweye likely disperse widely on both the California Current to the south of Vancouver Island and to the north of the Island on the Davidson. The reverse flow of undercurrents may facilitate distribution as well. The diverging currents at the tip of Vancouver Island would not seem to be an impediment to gene flow. There is no justification at this time to declare genetically distinct stocks on the U.S. West Coast. I believe this approach allows the Council maximal flexibility in balancing the rebuilding of Yelloweye stock and the needs of West coast fishing communities.

In contrast, there is consensus on the taxonomic status of sea otter ssp., just no agreement on where they intersected latitudinally. The possibilities are fascinating. The hybrid zone could have been limited or a gradual cline. A tension zone may have existed where the loss of adaptive alleles in hybrids rendered them less fit. It is certain, however, that anthropogenic factors will impact its reestablishment. What we can do is plan for its impact on the coupled human-natural system (CNH). Translocation has the potential for at least temporary disruption to ecosystems bereft of sea otter presence for one hundred years and the fisheries that have taken advantage of the prey items left to flourish in their absence. These are the uncertainties and risk I can acknowledge here.

The fate of both taxa are dependent on the goodwill of local coastal communities and community cooperation with policy makers. Communities are in turn dependent on the health of the nearshore environment. The rebuilding of Yelloweye Rockfish stock to sustainable levels
will greatly benefit both commercial fishermen and recreational anglers. Current sensibilities focus attention on sea otters for their contribution to biodiversity, potential conflicts with fisheries, susceptibility to pollution, and charismatic appeal to a broad segment of society. While the tools of governance will support restoring sea otters to their former range, CNH dynamics still apply, and the impetus for action will likely need to come from the public. I hope that the results from my thesis work will inform stakeholders and contribute to the dialogue.
Bibliography


Bonnot, P. (1951). *The sea lions, seals and sea otter of the California Coast.* California Department of Fish; Game.


Hall, R. L. (2009). Background resources and references for “The Oregon Coast before the arrival of Europeans.” Portland, Ore. Retrieved from https://ir.library.oregonstate.edu/downloads/1g05fc48c


Appendix
Oregon Faunal Archaeological Sites

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