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Operational Plan: Genetic Connectivity Among Populations of Pacific Razor Clams in Cook Inlet, Alaska

by

Michael D. Booz

Kristen M. Gruenthal

and

Christopher Habicht

January 2022

Alaska Department of Fish and Game **Divisions of Sport Fish and Commercial Fisheries**

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Weights and measures (metric)

centimeter	cm
deciliter	dL
gram	g
hectare	ha
kilogram	kg
kilometer	km
liter	L
meter	m
milliliter	mL
millimeter	mm

Weights and measures (English)

cubic feet per second	ft^3/s
foot	ft
gallon	gal
inch	in
mile	mi
nautical mile	nmi
ounce	oz
pound	lb
quart	qt
yard	yd

Time and temperature

day	d
degrees Celsius	°C
degrees Fahrenheit	°F
degrees kelvin	K
hour	h
minute	min
second	s

Physics and chemistry

all atomic symbols	
alternating current	AC
ampere	A
calorie	cal
direct current	DC
hertz	Hz
horsepower	hp
hydrogen ion activity (negative log of)	pH
parts per million	ppm
parts per thousand	ppt, ‰
volts	V
watts	W

General

Alaska Administrative Code	AAC
all commonly accepted abbreviations	e.g., Mr., Mrs., AM, PM, etc.
all commonly accepted professional titles	e.g., Dr., Ph.D., R.N., etc.
at	@
compass directions:	
east	E
north	N
south	S
west	W
copyright	©
corporate suffixes:	
Company	Co.
Corporation	Corp.
Incorporated	Inc.
Limited	Ltd.
District of Columbia	D.C.
et alii (and others)	et al.
et cetera (and so forth)	etc.
exempli gratia (for example)	e.g.
Federal Information Code	FIC
id est (that is)	i.e.
latitude or longitude	lat or long
monetary symbols (U.S.)	$, ¢
months (tables and figures): first three letters	Jan,...,Dec
registered trademark	®
trademark	™
United States (adjective)	U.S.
United States of America (noun)	USA
U.S.C.	United States Code
U.S. state	use two-letter abbreviations (e.g., AK, WA)

Mathematics, statistics

all standard mathematical signs, symbols and abbreviations	
alternate hypothesis	H_A
base of natural logarithm	e
catch per unit effort	CPUE
coefficient of variation	CV
common test statistics	(F, t, χ^2, etc.)
confidence interval	CI
correlation coefficient (multiple)	R
correlation coefficient (simple)	r
covariance	cov
degree (angular)	°
degrees of freedom	df
expected value	E
greater than	>
greater than or equal to	≥
harvest per unit effort	HPUE
less than	<
less than or equal to	≤
logarithm (natural)	ln
logarithm (base 10)	log
logarithm (specify base)	$\log_2$, etc.
minute (angular)	′
not significant	NS
null hypothesis	H_O
percent	%
probability	P
probability of a type I error (rejection of the null hypothesis when true)	α
probability of a type II error (acceptance of the null hypothesis when false)	β
second (angular)	″
standard deviation	SD
standard error	SE
variance	
population	Var
sample	var

REGIONAL OPERATIONAL PLAN SF.2A.2022.01

OPERATIONAL PLAN: GENETIC CONNECTIVITY AMONG POPULATIONS OF PACIFIC RAZOR CLAMS IN COOK INLET, ALASKA

by
Michael D. Booz
Alaska Department of Fish and Game, Division of Sport Fish, Homer
Kristen M. Gruenthal
Alaska Department of Fish and Game, Division of Commercial Fisheries, Anchorage
and
Christopher Habicht
Alaska Department of Fish and Game, Division of Commercial Fisheries, Anchorage

Alaska Department of Fish and Game
Division of Sport Fish
333 Raspberry Road, Anchorage, Alaska, 99518-1565

January 2022

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Michael D. Booz,
Alaska Department of Fish and Game, Division of Sport Fish,
3298 Douglas Place, Homer, AK 99603-7942, USA

Kristen M. Gruenthal
Alaska Department of Fish and Game, Division of Commercial Fisheries
333 Raspberry Road Anchorage, AK 99518, USA,

and

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

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Area Manager	Michael Booz		1/3/22
Fisheries Geneticist II	Kristen Gruenthal		1/3/22
Biometrician	Bobby Hsu		1/3/22
Research Coordinator	Tim McKinley		1/6/22

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ABSTRACT

This study will explore population genetic connectivity of Pacific razor clam (*Siliqua patula*) from beaches located throughout Cook Inlet, Alaska through restriction-site associated DNA sequencing (RAD-seq) with genotyping approximately 5,000 to 15,000 single nucleotide polymorphisms (SNPs).

Key words: razor clams, *Siliqua patula*, Cook Inlet, population genetics

INTRODUCTION

PURPOSE

Historically, beaches along Cook Inlet have supported commercial, sport, and personal use fisheries for Pacific razor clam (*Siliqua patula*), with beaches along the east providing the largest sport and personal use Pacific razor clam fisheries in Alaska whereas roughly 98% of the annual harvest from beaches on the west are from a commercial fishery. However, east Cook Inlet beaches have been closed to sport and personal use harvests since 2015 due to low abundances at key beaches with concomitant decreases in the observed average lengths and percentages of large-sized clams at all beaches (Booz et al. 2019). This is in contrast to a stable commercial fishery during that period in west Cook Inlet; yet recently, catch per unit effort (CPUE), total harvest, and age and size compositions have shown signs of change in westside stock productivity (Booz et al. 2019). Although the universality of these age and length trends on the east side suggests postsettlement mortality as a primary driver of the decreases, there may be other potential mechanisms influencing both sides of Cook Inlet, such as changes in source-sink population dynamics or oceanographic processes influencing settlement (Booz and Dickson *In prep*).

This study will explore genetic connectivity of Pacific razor clam from beaches located throughout Cook Inlet, Alaska through restriction-site associated DNA sequencing (RAD-seq) with genotyping approximately 5,000 to 15,000 single nucleotide polymorphisms (SNPs). Currently, there is limited biological and population ecology information on razor clams in Cook Inlet despite their importance for supporting sport, personal use, and commercial fisheries. There is only 1 historical population genetic study of the species that covered a broad swathe of coastal North America. Moreover, there is little comparable and consistent information gained from other commercially valuable species in the family Pharidae, such as Chinese razor clam (*Sinonovacula constricta*) and European pod razor shell (*Ensis siliqua*). Patterns of population structure relative to the spatial scale of collection are inconsistent and may be dependent on various anthropogenic influences, local hydrographic processes, and other unidentified mechanisms.

This study will hopefully improve the understanding of what biological processes influence the reproductive ecology and recruitment of Pacific razor clam in east and west Cook Inlet and will provide critical information for current and future harvest strategies for the Cook Inlet sport and commercial razor clam fisheries going forward. Population genetic connectivity, or lack thereof, among known clam beaches is an important consideration for abundance and demographic survey design, assessing differences in stock productivity between areas, and the selection of harvest strategies. Moreover, it may provide timely information for the 2022 Alaska Board of Fisheries statewide shellfish meeting to assess an ADF&G proposal to implement a management plan for east Cook Inlet razor clam sport and personal use fisheries. The data from this study may also improve our understanding on differences in stock productivity between east and west Cook Inlet.

BACKGROUND

Pacific razor clam (hereafter referred to as "razor clams") are large marine bivalves in the family Pharidae found on sandy intertidal beaches along the west coast of North America from Pismo Beach, California to the Bering Sea (Weymouth and McMillan 1931). In Alaska, razor clams are scattered throughout their distribution from the southeast region (Kruzof Island to Yakutat) through southcentral region (Cordova to Cook Inlet) and extending south along the Alaska Peninsula including Kodiak and Aleutian Islands (Nickerson 1975). In Cook Inlet, razor clams are found in a discontinuous distribution with clam beds on both east and west Cook Inlet (Figure 1). Razor clams in Alaska support sport, personal use, subsistence, and commercial fisheries.

Biology

Razor clams are soft-shelled, sexually dimorphic, and phytoplankton filter feeders (Weymouth et al. 1925). Razor clams can burrow into the sediment to a depth of about 25 cm and are found from the intertidal zone to approximately 18 m water depth (Bourne 1969, Jones et al. 1998). It has been noted that juvenile razor clams, which have much thinner shells and may be more likely to live in the top few centimeters of substrate, are at much greater risk than adults to exposure by heavy wave action, temperature fluctuations, salinity changes, and other environmental conditions (Szarzi 1991). At the southern end of the range in California, maximum age is generally 5 years and the maximum recorded length is 129 mm whereas the oldest clams observed in Alaska are 19 years (Weymouth and McMillan 1931). The maximum recorded age for Cook Inlet razor clams is 15 years and the maximum recorded size (184 mm) was found for a razor clam dug in 1987 in the Deep Creek area (ADF&G Division of Sport Fish, Homer, unpublished data). Weymouth and McMillan (1931) found slower growth for clams in the northern extent of the range but also describe a decreased survival rate at southern locations; at Pismo Beach California, razor clams older than 4.4 years had an estimated 5 percent survival, whereas this same low survival did not occur until razor clams were older than 15.6 years at Hallo Bay, Alaska. Within Cook Inlet, razor clam growth performance index values have demonstrated an overall inverse relationship with latitude (Blackmon 2020).

Sexual maturity in razor clams is related more to size than age (Weymouth et al. 1925; Nickerson 1975). Temperature is thought to be the dominant parameter that triggers razor clam spawning (Nickerson 1975). Weymouth et al. (1925) concluded a minimum temperature threshold of 13°C (55°F) was required for spawning. Nelson (*Unpublished*) reported that in Alaska, spawning is thought to occur when water temperature rises to just above 8°C. Females broadcast 6–10 million eggs into the water where they are fertilized indiscriminately by sperm broadcast from males (Kaiser 1977). In Cook Inlet spawning occurs from mid-July through September (McKellar 2014). Larvae drift from 6 weeks to 2 months or more as they metamorphose and then settle to the substrate as juveniles (Weymouth et al. 1925; Nickerson 1975; Nelson *Unpublished*). It is unknown if larval razor clams settle in the same geographical area from where they originated.

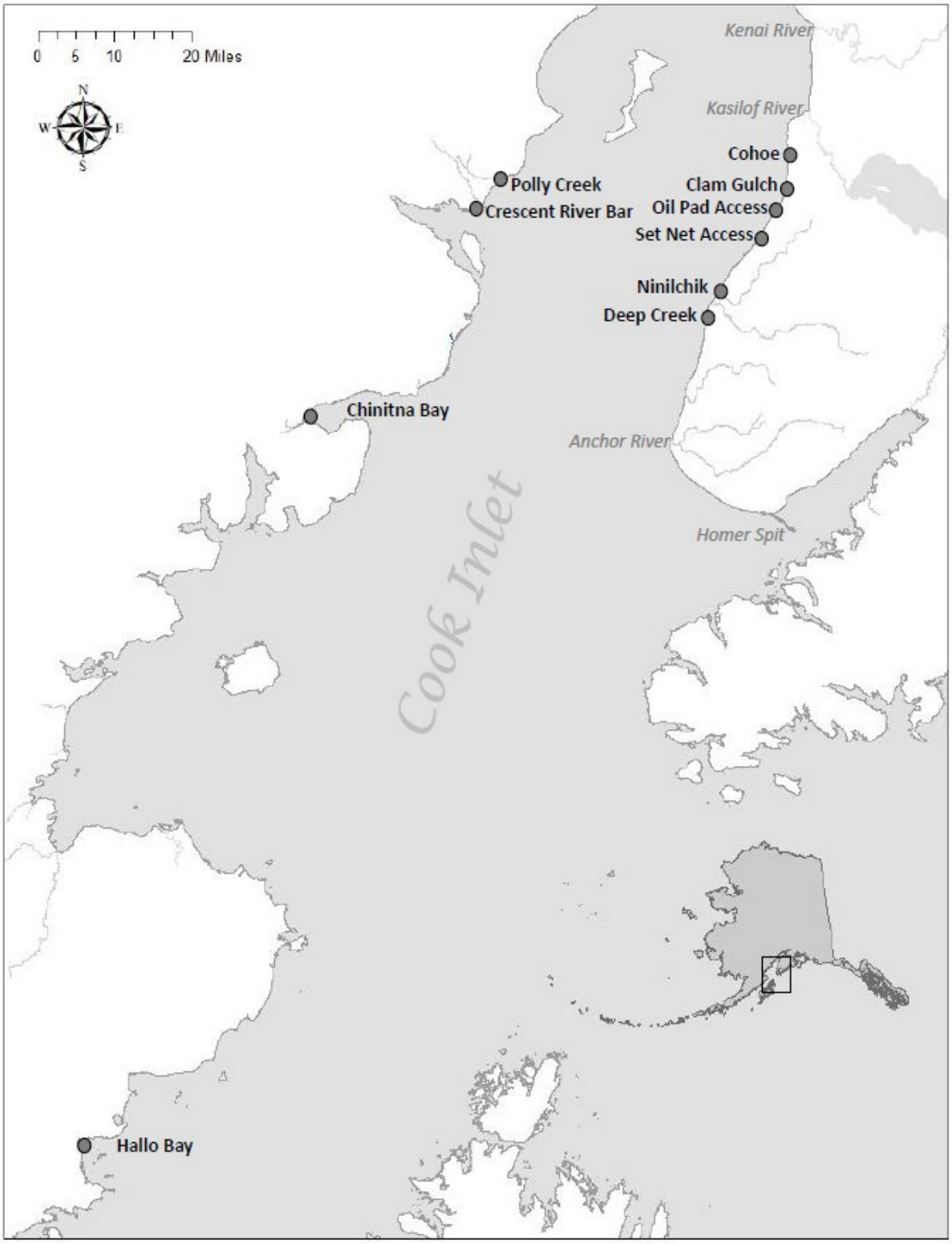

Figure 1.–Map of Cook Inlet and Alaska Peninsula razor clam sampling beaches.

East Cook Inlet

Beaches along east Cook Inlet (Figure 1) historically provided the largest sport and personal use (SPU) fishery for razor clams in Alaska (Szarzi and Hansen 2009). This fishery is defined in regulation to occur from the mouth of the Kenai River south to the tip of Homer Spit but is confined primarily to an 81 km section bounded by the Kasilof River south to the Anchor River. This area is a narrow and continuous sandy intertidal beach with only small gravel intrusions at the mouths of streams. The SPU fishery has been managed by the Alaska Department of Fish and Game (ADF&G), Division of Sport Fish since 1959 when Alaska became a state and the commercial harvest of razor clams was banned on east Cook Inlet beaches. Harvest, effort, and CPUE in the SPU fishery all peaked in the early 1990s and showed significant declines from 2009 through 2014 (Figure 2). The sport and personal use harvest averaged 1.1 million clams annually during its peak from 1984 to 1995 but declined to an average of 360,000 annually from 2004 to 2014. Both the sport and personal use fisheries have been closed by emergency orders annually since 2015 due to low abundances of adult razor clams at Ninilchik and Clam Gulch beaches (Booz et al. 2019).

The Division of Sport Fish has monitored the razor clam stock via hand-dug sampling for age and length composition and from abundance surveys as well as assessed the SPU fishery for digger distribution with aerial surveys and harvest and effort from creel and Statewide Harvest Survey data (Kerkvliet et al. 2021). In 2011 through 2014, abundance surveys were refined with the assistance of the Alaska Pacific University Fisheries, Aquatic Science, and Technology Laboratory (APU FAST Lab) to provide more information on trends in recruitment of juvenile clams to the beaches, recruitment of adult clams to the fishery, and natural mortality of juvenile and adults clams for both Ninilchik and Clam Gulch beaches (Booz and Dickson *In prep*). These were used to develop indices to assess productivity of east Cook Inlet razor clams.

East Cook Inlet razor clams have gone through a substantial decline in productivity, indicated in all indices (Booz and Dickson *In prep*). Initially, age and length composition data detected a downward trend towards younger and smaller clams starting in the mid to late 2000s on all east Cook Inlet beaches (Szarzi and Hansen 2009). This trend continued to decline through 2020, resulting in a larger than average percentage of juvenile clams comprising the samples from most beaches (Booz and Dickson *In prep*). Although juvenile razor clam abundances improved immediately following the SPU fishery closure, adult abundances have not recovered due to an annual natural mortality rate of 40–80%.

ADF&G submitted a proposal to the Alaska Board of Fisheries statewide miscellaneous shellfish meeting in 2022 to implement a management plan for east Cook Inlet razor clams. If adopted, this would divide the east Cook Inlet beach into 2 management areas (Clam Gulch and Ninilchik) with a SPU fishery in the northern beaches managed using data from the Clam Gulch abundance survey and a SPU fishery in the southern beaches managed using data from the Ninilchik abundance surveys. This plan would also establish adult clam abundance thresholds for each management area that would trigger either a new limited SPU fishery or the historical fishery for that management area. The plan would also use stock productivity indices of age and length compositions, recruitment, natural mortality, and growth along with the adult abundance threshold for triggering the historical fishery.

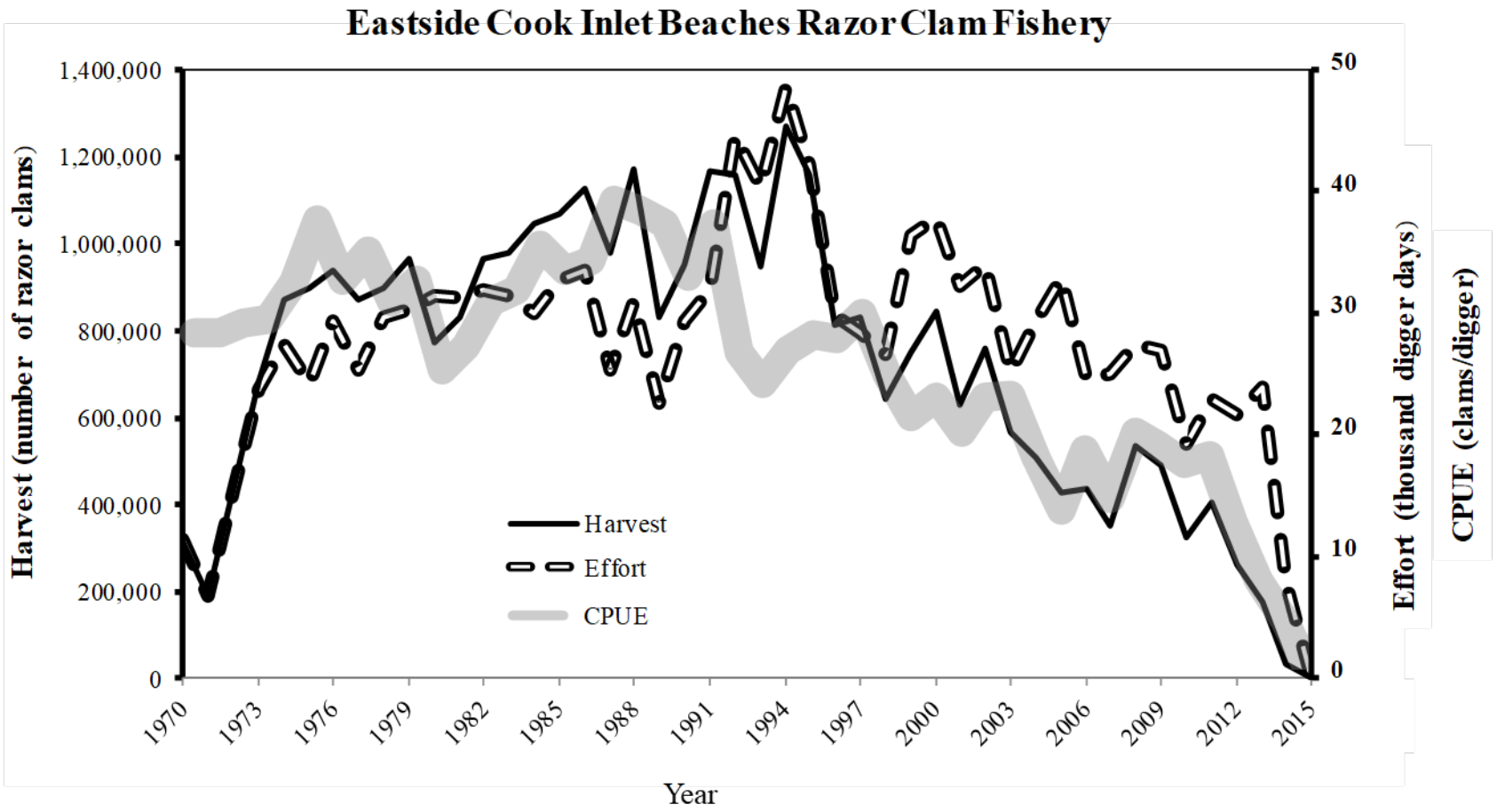

Figure 2.–Total razor clam harvest, effort, and CPUE in the Eastside sport and personal use razor clam fishery, including all Eastside beaches, 1970–2015.

Source: ADF&G creel surveys and Statewide Harvest Survey (Mills 1979-1980, 1981a, 1981b, 1982-1991, 1992a, 1992b, 1993, 1994; Howe et al. 1995, 1996; Alaska Sport Fishing Survey database [Internet]. 1996–present. Anchorage, AK: Alaska Department of Fish and Game, Division of Sport Fish. Available from: http://www.adfg.alaska.gov/sf/sportfishingsurvey/).

West Cook Inlet

Unlike the east Cook Inlet beaches, sandy intertidal areas in west Cook Inlet are not contiguous and razor clam distribution is not well documented (Figure 1). Overall, the amount of razor clam habitat in west Cook Inlet is much greater than east Cook Inlet. The more well-known locations are from Polly Creek to the Crescent River bar, Chinitna Bay, Silver Salmon Creek, and Oil Bay in Kamishak Bay. Both commercial and SPU razor clam fisheries occur in west Cook Inlet. The commercial fishery only occurs in a 19-mile section from Harriet Point to Crescent River Bar. The SPU fishery is open throughout, but the most popular area overlaps with the commercial fishery at Polly Creek and Crescent River bar. These are remote fisheries with access by boat from the tractor launch facilities in Anchor Point and Deep Creek, the City of Kenai boat launch on the Kenai River, and by small fixed-wing aircraft from Cook Inlet communities. Boating to these fisheries requires crossing Cook Inlet for at least 30 miles in open seas, which usually requires a sufficiently large vessel. Access by fixed-wing aircraft requires landing on the intertidal beach in locations of higher elevation with stable substrate. It is assumed most of the effort in the SPU fishery occurs from May through August and on days with larger minus tides. Some charter operators that operate out of the Deep Creek tractor launch offer boat transport to the fishery. Because clam diggers harvest clams unassisted, all harvest is considered unguided and the charter operators are not required to complete a logbook for the trip.

Commercial harvest of razor clams from west Cook Inlet beaches has occurred since 1919 (Marston and Frothingham 2019). No overall commercial harvest limits are in place for any area in regulation; however, ADF&G manages the commercial razor clam fishery to achieve a harvest of no more than 350,000–400,000 lb in the shell annually (guideline harvest level). Virtually all the commercial harvest has come by hand-digging, although regulations prior to 1990 allowed the use of mechanical harvesters (dredges) south of Spring Point, or within a 1-mile section of the Polly Creek beach. Numerous attempts to develop feasible dredging operations were largely unsuccessful due to excessive shell breakage or the limited availability of clams in the area open to this gear. Mechanical means of harvesting is no longer permitted in any area of Cook Inlet.

The statewide harvest survey (SWHS; Alaska Sport Fishing Survey database [Internet] 1996–present. Anchorage, AK: Alaska Department of Fish and Game, Division of Sport Fish http://www.adfg.alaska.gov/sf/sportfishingsurvey/.) has produced SPU fishery shellfish effort and razor clam harvest estimates in numbers of clams in west Cook Inlet since 1986; commercial effort and harvest in pounds is available from Division of Commercial Fisheries fish ticket data (Booz et al. 2019). The west Cook Inlet SPU fishery harvest has been estimated for several locations and based on these, harvest primarily occurs in the Polly Creek to Crescent River area. To facilitate comparisons between the sport and commercial harvest in the Polly Creek to Crescent River area, conversions from numbers to pounds were generated via a razor clam length-weight relationship developed in the late 2000s (McKellar 2014). On average from 1986 through 2019, the SPU fishery in west Cook Inlet harvested approximately 38,000 clams whereas the commercial fishery harvested about 900,000 clams in this area. Roughly 98% of the 1986–2019 average total west Cook Inlet harvest was in the commercial fishery.

Overall, razor clams in west Cook Inlet have not experienced the same declines in productivity that has been observed in east Cook Inlet but some of the trends in the recent fishery data from the SWHS and fish tickets suggest that the stocks may be experiencing some changes. From 2017 through 2019, the commercial fishery annual harvest averaged approximately 171,000 lb and has

not reached the guideline harvest level since 2013. The 2017–2019 average west Cook Inlet SPU razor clam harvest (16,099) was below the historical average harvest, although the recent average annual days fished (1,286) was similar to the historical average. Some declines in age and length compositions of the harvest have also been observed in both the sport and commercial fisheries.

Population Genetic Structure

LeClair and Phelps (1994) conducted the only known population genetic research on Pacific razor clam, genotyping 24 variable allozyme loci (49 total) in 5 samples collected in 1990 from Clam Gulch, Alaska to Seaside, Oregon, along the west coast of North America. The authors found significant allele frequency differences among locations, with pairwise comparisons involving the Clam Gulch sample generating the greatest differences (mean Cavalli-Sforza chord distance D_{CH} = 0.123). This study does provide evidence for broad-scale population structuring, but it also highlights our gap in knowledge regarding diversity and divergences on smaller spatiotemporal scales and the need for contemporary genetic data.

Although there is limited genetic information on Pacific razor clam, more work has been conducted on the related Chinese razor clam, a commercially important species native to the western Pacific. These large Asian bivalves are widely distributed along the coast of China and Japan and are cultured extensively throughout the region (Guo et al. 1999). Chinese razor clams have an ecology and biology similar to that of the Pacific razor clam, inhabiting intertidal mudflats in bays and estuaries. Niu et al. (2012) used 8 microsatellite genetic markers to describe population structure for Chinese razor clams on a broad geographic scale in 10 populations sampled along the coast of China from the Korean border in the north to near Taiwan in the south. Populations were significantly differentiated from one another (F_{ST} = 0.044) and broadly sorted into 2 large regional groupings. Evidence was also found for site-specific cryptic speciation as well as culture-mediated translocations of individuals known to have occurred over the last 800 years. In Japan, Orita et al. (2021) used RAD-seq[1] to generate nearly 140,000 loci in 30 Chinese razor clam (16 wild, 12 from an area experiencing transplantation of artificially produced clam seedlings and two captive) collected from sites spanning less than 25 km of coastline in the northwest portion of Ariake Bay, Saga Prefecture. The authors discovered 2 distinct population groupings (F_{ST} = 0.052), consistent with the heavy presence of aquaculture, but no differences among the wild samples.

Another commercially fished species with similar ecology found in European coastal waters is known as the pod razor shell. Fernández-Tajes et al. (2007) used 61 RAPD[2] loci to document isolation by distance in 6 populations collected on a broad geographic scale along the coasts of Ireland, Spain, and Portugal, although Nei's genetic distances were small (D_A = 0.051–0.065) among the Iberian populations relative to the Irish samples. More recently, Varela et al. (2012) used microsatellite data to document low genetic differentiation among Spanish and Portuguese populations (F_{ST} = 0.000-0.032) and a moderate differentiation between these populations and the Irish coast (F_{ST} = 0.071-0.100).

Overall, population genetic studies among the Pharidae do not reveal consistent patterns relative to the spatial scale of collection and may be dependent on variable anthropogenic influences, local hydrographic processes, and other unidentified mechanisms. We cannot, therefore, make confident assumptions on population structuring, connectivity, and the magnitude of gene flow among populations of Pacific razor clams in Cook Inlet to help answer our questions regarding disjunct

[1] Restriction site associated DNA sequencing.
[2] Random amplified polymorphic DNA.

stock productivity on the east and west sides. This project will provide insight into this important piece of biological information.

OBJECTIVES

PRIMARY OBJECTIVE

Test the null hypothesis that there is panmixia (i.e., random mating, where F_{ST} is not significantly different from zero) among sampling sites on the east and west sides of Cook Inlet with adequate statistical power to have at least a 90% probability of detecting an F_{ST} as low as 0.01, if it exists.

SECONDARY OBJECTIVE

Describe relationships among sampling sites and across age classes using multidimensional scaling and maximum likelihood-based clustering analyses to examine spatial and temporal relationships.

METHODS

SAMPLE COLLECTION

In 2019, razor clam genetic tissue samples were obtained from hand-dug collections throughout east and west Cook Inlet and Hallo Bay on the Alaska Peninsula (Figure 1). Cook Inlet tissue samples were obtained during ADF&G sampling for age and length compositions, and samples from Hallo Bay were collected by National Park Service (NPS) staff. Faculty and students from the APU FAST Lab assisted with the sampling in east Cook Inlet and NPS supported the collection in Chinitna Bay. In east Cook Inlet, razor clams were hand dug on 9 beaches from Deep Creek north to Cohoe, which represents approximately 40 km of area. In west Cook Inlet, hand dug samples were collected from 3 distinct beaches at Polly Creek and Crescent River Bar (which are approximately 22 km apart) and at Clam Cove within Chinitna Bay.

In Cook Inlet, hand-dug razor clams were assumed to represent the harvest (if the fishery had been open in east Cook Inlet) because sampling mimics digger behavior and uses the same gear (shovels) as SPU diggers. Sample sites on the east side of Cook Inlet included Cohoe, Clam Gulch (North and South), Oil Pad Access, Set Net Access, Ninilchik (North, South, and Bar), and Deep Creek, and sites sampled on the west side included Polly Creek, Crescent River, and Chinitna Bay (Figure 1). On each beach, crews were comprised of a variety of skill levels and dug clams from the same general section as sampled in previous years. Samplers walked throughout a beach like a typical clam digger searching for clams, rather than sampling from a relatively small area. A sample size goal of 150 clams was established for each beach and all clams dug by samplers were retained regardless of size or condition. All clams were dug by shovel to minimize shell damage, and samplers also used a dowel rod in the clam "show" (a shallow dimple or depression in the sand) to differentiate clam shows from false shows and to use the rod as a reference to avoid breaking the razor clam shell with the shovel. Sampling occurred in mid-June 2019 on tides ranging from −2.1 to −4.0 ft. For access to Ninilchik Bar, a tide lower than −3.0 was needed.

In Hallo Bay in Katmai National Park and Preserve on the Alaska Peninsula, razor clams were opportunistically dug during other research activities in July 2019. The crew was comprised of 7 to 9 people using clam guns to collect 46 razor clams.

SAMPLE PROCESSING

In the laboratory, 50 razor clams were chosen randomly from the 150-clam sample from each Cook Inlet beach. These samples were comprised of a variety of ages and sizes and were individually numbered to provide corresponding age and length data for each genetic tissue sample, as described in Booz et al. (2020). Razor clams were processed by removing the tissue from the shell using scissors or small knives. For genetic tissue sampling, at least 50 mg of the siphon was removed and placed into a corresponding numbered vial. For the Cook Inlet samples, at least 1 unbroken valve was used for measuring length and aging. Shells were aged using the methods described by Nelson (*Unpublished*) and the recommendations of Coggins (1994). Each sample was aged during processing in the laboratory and the total length was measured using Mitutoyo Digimatic Calipers. For the Hallo Bay samples, only a total length measurement was collected from each clam, but the data were not maintained for each genetic tissue sample.

GENETIC LABORATORY PROCEDURES

DNA Extraction

Whole genomic DNA will be extracted from 46–49 individual razor clams from each site (Table 1) using NucleoSpin 96 Tissue Kits (Macherey-Nagel, Allentown, PA, USA). A total of 200 ng of each razor clam DNA extract will be aliquotted into 96-well PCR plates, dried by evaporation, and used in downstream laboratory processing.

Table 1.–Cook Inlet razor clam sample sizes used for RAD-seq.

Site	Location	n
Cohoe	Cook Inlet East	48
Clam Gulch North	Cook Inlet East	48
Clam Gulch South	Cook Inlet East	48
Oil Pad Access	Cook Inlet East	48
Set Net Access	Cook Inlet East	48
Ninilchik North	Cook Inlet East	47
Ninilchik South	Cook Inlet East	48
Ninilchik Bar	Cook Inlet East	47
Deep Creek	Cook Inlet East	47
Polly Creek	Cook Inlet West	48
Crescent River	Cook Inlet West	49
Chinitna Bay	Cook Inlet West	48
Hallo Bay (Katmai)	Alaska Peninsula	46

Restriction Site–Associated DNA Sequencing (RAD-seq)

RAD-seq will be performed with the restriction enzyme *Sbf*I following the methods of Ali et al. (2016) and Ackiss et al. (2020). Pooled libraries will be sequenced at the University of Oregon Genomics & Cell Characterization Core Facility on 1 shared lane of a NovaSeq 6000 2x150bp S4 flow cell (Illumina, San Diego, CA, USA).

SNP DISCOVERY AND GENOTYPING

The Gene Conservation Laboratory server will house all bioinformatic pipeline components to conduct SNP discovery and genotyping. Stacks v2.55 (Paris et al. 2017; Rochette and Catchen 2017; Rochette et al. 2019) will be used to identify and genotype up to 100,000 single nucleotide

polymorphisms (SNPs), depending on genetic variability and data quality, with genotypes exported in variant call format (vcf). Comprehensive filtering of individuals and loci will be performed in VCFtools v0.1.16 (Danecek et al. 2011) according to the methods of Bootsma et al. (2020). Individuals missing more than 20% of SNPs, SNPs missing in more than 20% of individuals, and SNPs not in the first 140 base pairs of each RAD-tag will be removed. Putatively duplicated or paralogous loci will be identified using HDplot (McKinney et al. 2017), and loci with heterozygosity (H) greater than 0.5 and read-ratio deviations (D) less than −7 or greater than 7 will be removed. Single-SNP F_{IS} will be estimated using R package diveRsity (Keenan et al. 2013), and any SNPs with F_{IS} less than −0.2 or greater than 0.2 will be removed. Target retention is 5,000 to 15,000 SNPs in at least 24 individuals per sample site, even though some sources may advocate for far fewer (e.g., Li et al. 2020; Marandal et al. 2020).

DATA ANALYSIS

Once loci and individuals have passed the filtering steps described above, the final genotype data will be outputted in *.vcf and *.gen formats and any necessary downstream data conversion will be performed using PDGSpider v2.1.1.5 (Lischer and Excoffier 2012). Pairwise relatedness will be estimated among individuals using ML-Relate (Kalinowski et al. 2006) and removing 1 individual from each pair when $r > 95\%$ because this may indicate duplicate tissue samples, pending review of paired demographic data. Then, standard population genetic analyses will be performed to determine genetic structure and infer connectivity. Expected heterozygosity and number of alleles at a locus will be estimated in the R package adegenet v2.1.4 (Jombart 2008). Population differentiation will be assessed using pairwise F_{ST} in Genepop v4.7.5 (Rousset 2008). A chi-square test, where $\chi^2(df = 1) = 2 \times N \times F_{ST}$, will be used to assess the null hypothesis that $F_{ST} = 0$. We will then perform a sequential Bonferroni correction to account for multiple comparisons in the analysis. Two individual-based multidimensional scaling approaches—Principal Coordinate Analyses (PCoA) and Discriminant Analyses of Principal Components (DAPC)—will be conducted in adegenet to visualize patterns of relationship suggesting genetic clustering in the dataset and interconnection among the sampled populations. Finally, fastSTRUCTURE (Raj et al. 2014) will be used to identify the number of distinct genetic clusters in the dataset (i.e., K populations) and to determine the degree of admixture among populations.

SCHEDULE AND DELIVERABLES

Objective	FY2021 Quarters (Q) Q1	Q2	Q3	Q4	FY2022 Quarters (Q) Q1	Q2	Q3	Q4	Status
Sampling									Complete
DNA Extraction				X					Complete
RAD Sequencing					X				Complete
SNP Discovery and Genotyping						X	X		
Data Filtering and Analysis							X	X	
Results Interpretation							X	X	
Report Writing								X	

RESPONSIBILITIES

Mike Booz, Lower Cook Inlet Sport Fish Area Management Biologist, ADF&G
Duties: Coordinate sample collection, prepare operational plan, and write the report.

Kristen Gruenthal, Fisheries Geneticist II, Gene Conservation Laboratory, Commercial Fisheries, ADF&G
Duties: Technically review study design and sampling methods; perform RAD sequencing, SNP discovery and genotyping, and population genetic data analyses; provide results interpretation; and coauthor the report. Provide assistance in drafting the operational plan and technical assistance should changes in the design be necessary.

Christopher Habicht, Principal Geneticist, Gene Conservation Laboratory, Commercial Fisheries, ADF&G
Duties: Technically review study design; provide results interpretation; and coauthor the report.

Brad Harris, PhD, Director, Fisheries Aquatic Science & Technology Laboratory*, Alaska Pacific University*
Duties: Provide samples for genetic analysis and coauthor the report.

Other assisting personnel
Duties: Hand dig to collect razor clams and process razor clams to collect genetic tissue samples, measure length, and estimate age.

BUDGET SUMMARY

Line item	Category	Budget ($K)
100	Personal Services	28.3
200	Travel	0.0
300	Contractual	3.0
400	Commodities	5.8
500	Equipment	0.0
Total		37.1

Personal Services paid through General Funds:

- Mike Booz 1.0 months = $11,447
- Bobby Hsu: 0.25 months = $2,538
- Kristen Gruenthal: 1.00 months = $9,438

- Chris Habicht: 0.25 months = $4,845

Contractual items donated by the NOAA Fisheries Auke Bay Laboratories include DNA sequencing on an Illumina NovaSeq NGS System at the University of Oregon Genomics & Cell Characterization Core Facility (GC3F). Contractual items for equipment maintenance and support must be maintained annually, including for the liquid handling robots, centrifuges, sonicator, and thermal cyclers.

Commodities include biochemical supplies for DNA extraction and the GCL included extraction kits and reagent alcohol. Biochemical supplies for RAD-seq were donated by NOAA Fisheries Auke Bay Laboratories and include reagent alcohol and chemicals including primers, master mix, reaction buffer, AMPure, and NEBNext kits, among other chemicals. DNA extraction and RAD-seq consumable supplies may include gloves and plastics, such as plates, pipette tips, and storage boxes. All supplies are necessary to collect genetics data as required. Laboratory processing cost $10 per sample for 576 samples (six 96-well plates).

There are no equipment or travel costs.

All ADF&G purchases follow State of Alaska purchasing rules and use the State of Alaska contracted vendors. If the item is only available from 1 vendor (sole source), then it is purchased directly from the vendor, again following State of Alaska procurement rules. All amounts are based on historical costs.

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