Regional Operational Plan CF.5J.2015.01

Phylogeography and Management of Golden King Crab populations in Alaska

by

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and

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June 2015

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

SYMBOLS AND ABBREVIATIONS

The following symbols and abbreviations, and others approved for the Système International d'Unités (SI), are used without definition in the following reports by the Divisions of Sport Fish and of Commercial Fisheries: Fishery Manuscripts, Fishery Data Series Reports, Fishery Management Reports, and Special Publications. All others, including deviations from definitions listed below, are noted in the text at first mention, as well as in the titles or footnotes of tables, and in figure or figure captions.

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 CF.5J.2015.01

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by

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Alaska Department of Fish and Game
Commercial Fisheries Division

June 2015

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Signature Page

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Project leader(s):	*W. Stewart Grant, Fishery Biologist IV* *William Templin, Principal Geneticist*
Division, Region and Area	Statewide
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Approval

Title	Name	Signature	Date
Project leader	W. Stewart Grant		4 June 15
Principal Geneticist	William Templin		6/8/15
Chief Marine Fisheries Scientist	Christopher Siddon		6/19/2015

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ABSTRACT

The stock structure of golden king crab (*Lithodes aequispinus*) in the Gulf of Alaska and Aleutian Islands is uncertain, and this uncertainty can introduce considerable error into stock assessments. Populations in the Bering Sea and along the Aleutian Archipelago are jointly managed by the Alaska Department of Fish and Game (ADF&G) and the North Pacific Fishery Management Council (NPFMC), and those in the Gulf of Alaska are managed by ADF&G. Catches are presently regulated by regions defined by harvest registration areas and by State of Alaska larval drift zones. Harvests are limited to males, and quotas are determined from size-age profiles indicating abundance trends and from abundance survey data examined with population models. The accuracies of stock assessment models, however, depend on correctly defining discrete, more or less self-sustaining populations. Two marker types, mitochondrial (mt) DNA sequence polymorphisms and microsatellite allele variants, will be used to test for population structure and for possible historical effects of harvesting on genetic diversity. Previous studies of the closely related red king crab (*Paralithodes camtschaticus*) showed three large genetic subdivisions across the North Pacific, and this pattern serves as the null hypothesis of golden king crab stock structure. Cytochrome oxidase I in the mtDNA genome will be sequenced with universal polymerase chain reaction primers, and cross-species microsatellite loci will be developed from primers designed for other lithodid crabs. mtDNA haplotype and microsatellite allele-frequency data will be examined with standard genetic statistics. We expect to find significant differences among samples, since golden king crab populations inhabit deeper waters than red king crab populations and, hence, may be more isolated from one another. These results will better inform stock assessment procedures and improve the management of golden king crab harvests.

Key words: golden king crab, State of Alaska, commercial fishery, genetics, phylogeography, mtDNA, microsatellite, Aleutian Archipelago, Gulf of Alaska

PURPOSE

The stock structure of golden king crab (*Lithodes aequispinus*) in the Gulf of Alaska and around the Aleutian Islands is uncertain, and this uncertainty can introduce considerable error into stock assessments. The analysis of population structure with genetic markers can help delineate stock boundaries for stock assessments. Populations of golden king crab inhabit deep coastal waters of the North Pacific from northern British Columbia, throughout the Gulf of Alaska and Bering Sea, along the Aleutian Archipelago, into Russian coastal waters and to as far as central Japan (Butler and Hart 1962; Rodin 1970; Hughes 1981; Hiramoto 1985; Somerton and Otto 1986; Otto and Cummiskey 1985). Populations in the Bering Sea and along the Aleutian Archipelago are jointly managed by the Alaska Department of Fish and Game (ADF&G) and the North Pacific Fishery Management Council (NPFMC), and those in the Gulf of Alaska are managed by ADF&G. Catches are limited within regions defined by harvest registration areas and by State of Alaska larval drift zones (Table 1) (Statute 5 AAC 41.295.d). Harvests are limited to males, and quotas are determined from size-age profiles indicating abundance trends and from abundance survey data examined with population models (Zheng et al. 1996). The accuracies of these models, however, depend on correctly defining discrete, self-sustaining populations. In this study geographical surveys of molecular genetic markers will be used to resolve genetic population structure to aid in defining population production units.

In addition to defining the geographical boundaries of self-sustaining population production units, molecular markers can also be used to detect deep genetic partitions between lineages reflecting lineage sorting or divergence in ancient isolations. Deep genetic divergences between lineages often signal components of diversity meriting specific management attention. Molecular markers can also be used to measure levels of genetic diversity within populations. Small populations, isolated in deep depressions or in coastal fjords, may be particularly susceptible to the loss of genetic diversity through random genetic drift. While marine populations are thought to be large (Bunch et al. 1998), because of the large potential for gene flow between populations,

genetic surveys of some marine species show that harvest levels can sufficiently diminish population sizes so that genetic diversity is lost (Hauser et al. 2002). Molecular markers can also be used to test whether the geographical gradient in fecundity (Somerton and Otto 1986) is associated with an environmental gradient that is correlated with allele-frequency differences among populations.

OBJECTIVES

The results of life-history and genetic studies of Tanner and snow crabs (*Chionoecetes sp*.), and especially those of red king crab, set the stage for constructing hypotheses for our proposed study of golden king crab populations.

1. H_O: Golden king crab show large populations that extend over large areas. Population homogeneity is promoted in part by imprecise onshore-offshore breeding migrations and by larval drift in near shore currents.

   H_A: Deep-water populations are isolated and this leads to greater levels of divergence between populations than has been observed for red king crab.

The distributions of haplotype and allele frequencies will be used to estimate standard measures of divergence between populations. Significant allele-frequency differences would indicate genetic isolation among populations. A nested model design will indicate the geographical scales of differentiation.

2. H_O: Golden king crab populations are subdivided into at least three large population groups across the North Pacific that arose through reproductive isolation and divergence in refugia during glacial maxima in the Pleistocene Epoch.

   H_A: Large amounts of dispersal and gene flow have genetically homogenized populations originating from glacial refugia.

Tidal glaciers covered much of the coast of Alaska and British Columbia during glacial maxima, destroying coastal habitats that supported populations of golden king crab. Genetic imprints of isolation are present in most marine species in the Northeast Pacific, but lacking in others. High levels of gene flow, mediated by the movement of pelagic larvae in ocean currents might have homogenized present-day populations.

3. H_O: Golden king crab populations inhabiting physically fragmented deep-water fjord areas show greater amounts of population subdivision than do populations in along the outer coast.

   H_A: Populations within the various regions show similar levels of divergence.

Populations inhabiting deep-water fjords, such as those in Southeast Alaska, are likely to show greater levels of divergence from one another than populations along the outer coast. The

complex shore line in Southeast Alaska and local currents may limit larval dispersal, whereas the swift currents of the Alaska Coastal Current may enhance larval dispersal between areas. This can be tested with comparisons of the level of divergence between regional populations in these two areas. Populations showing fine-scale structure in which local populations are self-sustaining would require different harvest management than populations that are connected to one another through adult migration or larval dispersal.

4. H_O: Golden king crab populations show a geographical gradient in genetic diversity that coincides with gradients in mean size and fecundity.

   H_A: Variability in mean size and fecundity reflects phenotypic plasticity, is shaped by environmental factors and does not reflect genetic differentiation.

An association between genetic markers and life-history variation may indicate that some life-history traits are under genetic control. Such an association would have to be confirmed by breeding studies, but would be important for the development of mariculture techniques to supplement wild populations.

METHODS

RESEARCH APPROACH

The research outlined in this proposal will be used to strengthen stock assessments of golden king crab in Alaskan waters by employing genetic markers to estimate stock structure. The chances of detecting genetic differences between populations, if they exist, depend on three variables: 1) effect size–the level of divergence between populations, 2) sample sizes used to estimate genetic parameters, and 3) the number and nature of the genetic markers. Marine species often show only small amounts of genetic divergence between populations, because of the large potential for larval dispersal in the ocean. Hence, only small divergences are expected in golden king crab in areas where larval dispersal is enhanced by ocean currents. However, strong genetic discontinuities appear between populations of some North Pacific marine species because of historical ice-age isolations in refugia. To detect structure on smaller geographical scales, sample sizes must be adequate to provide statistical power to detect small genetic differences, and simulations can provide guidelines for sample sizes (see below). Lastly, the inheritances, mutation rates, and number of DNA markers can influence the ability of a genetic study to resolve genetic population structure (see below).

SAMPLES

The Gene Conservation Laboratory has archived tissue samples collected from the mid-1990s to 2011, including samples from the Aleutian Islands to Southeast Alaska (Table 2). Additional samples will be collected during commercial cruises at sites along the Aleutian Island Archipelago. Hemolymph or muscle tissue will be collected from golden king crabs and will be stored in 96% ethanol until laboratory analysis.

Simulations with PowSim (Ryman and Palm 2006; Ryman et al. 2006) were used to estimate sample sizes and number of genetic markers needed to provide the statistical power to detect expected levels of divergence (F_{ST} = 0.001 to 0.02) between golden king crab populations. We

estimated the probability of detecting a significant value of F_{ST} for a given level of divergence between three populations with samples sizes ranging from n = 25 to 200 and with numbers of polymorphic loci ranging from 1 to 40. Each simulated locus initially expressed 8 alleles with a frequency distribution of {0.25, 0.25, 0.125, 0.125, 0.0625, 0.0625, 0.0625, 0.0625}. N_e was set to 2,000 to generate a value of F_{ST} with 1,000 repetitions. Values of F_{ST} in Grant and Cheng (2012) and Vulstek et al. (2013) for red king crab were used as expected values for golden king crab.

Results indicate that sample sizes of at least n = 100 and 40 polymorphic microsatellite loci, or n = 200 and only 10 loci, are needed to detected small differences on the order of F_{ST} = 0.001 with a probability of about 0.90 (Figure 3). At higher levels of divergence between populations (F_{ST} = 0.01) samples sizes of 50 crabs examined with at least 10 microsatellite loci should provide statistical power greater than 0.90. While this proposed survey of microsatellite variability is expected to detect fine-scale genetic population structure, a previous study of red king crab (Vulsteck et al. 2013) showed that microsatellites failed to detect deep evolutionary structure that was detected with mtDNA and other nuclear genes (Grant and Cheng 2012). The lack of power for microsatellites to detect deep genetic partitions is likely due to the high mutation rate and allelic convergence. Hence, we have also included mtDNA in this study.

LABORATORY METHODS

Our strategy is to use two kinds of markers with different inheritances and different mutation rates to be able to survey variability arising on long and short time scales. Microsatellites are encoded in diploid genes that are bi-parentally inherited, whereas mtDNA consists of a single sequence copy in an individual and is maternally inherited. Hence, the effective population size of microsatellite genes is four times that for mtDNA. The comparison of allele-frequency patterns between nuclear (microsatellites) and organellar (mtDNA) genes can provide insights into the extent that sex-biased dispersal influences population structure (Prugnolle et al. 2002).

We will examine variation at microsatellite loci and use primers, as a start, that were used for red (4 loci: Vulsteck et al. 2013) and blue (22 loci; Stoutamore et al. 2012) king crabs. Even though golden king crab has been placed in the genus *Lithodes*, it is closely related to both red and blue king crabs, which are in the genus *Paralithodes* (Figure 4). The phylogenetic tree of lithodid crabs in Figure 4 was produced with Bayesian tree analysis of mitochondrial DNA sequences of cytochrome oxidase subunit I (COI) 597 base pairs in length. Bars represent 95% credibility intervals. Sequences were downloaded from GenBank, except those for red king crab, which were taken from Grant and Cheng (2012).We will use microsatellite PCR conditions and primers in Vulsteck et al. (2013) and Stoutamore et al. (2012) in a first-pass attempt to develop microsatellite markers in golden king crab. Preliminary tests show that cross-specific primers successfully amplify microsatellite loci in golden king crab. Previous studies of king crab demonstrated greater resolution of small scale population structure with microsatellites than the mtDNA, but not large-scale structure, which was best resolved with mtDNA markers.

The mtDNA cytochrome oxidase I gene sequences will be surveyed for variation in golden king crab populations. This gene was chosen because it can be amplified with the polymerase chain reaction (PCR) using universal crustacean primers, and because it was the most informative marker of population structure and genetic diversity in red king crab. The mitochondrial DNA COI universal primers LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer et al. 1994) amplify

a fragment used for species 'barcoding'. PCR mixtures will consist of a 50 μL mixture of 2.0 μL template DNA in 1x Colorless GoTaq Flexi Buffer, 2.5 mM $MgCl_2$, 1 mM dNTPs (ABI), 1 μM of forward and reverse primers, and 5 U GoTaq Flexi DNA polymerase (Promega Inc., Madison, WI). PCR amplifications will be conducted in ABI 9700 thermocyclers with an initial denaturation of 1 min at 95° C, 37 cycles of 40 s at 95° C, 40s at primer annealing temperature 41° C, and 1 min at 72° C; the final cycle was at 4° C for 5 min. Cycles will have a ramp speed of 1° C per second. The PCR amplifications will be sequenced in both the forward and reverse directions by the High-Throughput Genomics Unit (Seattle, WA). Sequences will be aligned with sequences for red king crab published in GenBank (JF738153–JF738249) with the MUSCLE algorithm (MEGA6) and adjusted by eye. Polymorphic sites will be confirmed with sequences in both directions.

STATISTICAL ANALYSIS

Standard measures of genetic diversity, including inbreeding (F_{IS}), gene diversity (H, microsatellites and h, mtDNA) and nucleotide diversity (Θ_π, mtDNA) (Hedrick 2011) will be estimated with ARLEQUIN (Excoffier et al. 2005) and GENEPOP (Rousset 2008). Departures from neutrality will be tested with Tajima's D (Tajima 1989), which detects excesses of low-frequency alleles in a sample that might be due to natural selection or to recent demographic growth. A 95% plausible parsimony network of mtDNA haplotypes will be constructed with TCS 1.21 (Clement et al. 2000). Additionally, mismatch analysis (Rogers and Harpending 1992) and Bayesian skyline plot (Drummond and Rambaut 2007) analyses of mtDNA sequences will provide estimates of historical demography. Estimates of contemporary effective population size, N_e, will be made using the extent of linkage disequilibria among microsatellite loci (Waples and Do 2010). All these statistics will provide insights into the historical and recent demographic history of golden king crab populations.

The distributions of haplotype and allele frequencies will be used to estimate standard measures of divergence between populations, including F_{ST} for microsatellites and mtDNA, R_{ST} for microsatellites, and Φ_{ST} for sequences of mtDNA. Unbiased F_{ST} is estimated from allele- or haplotype-frequencies and measures the degree of differentiation between populations at drift-migration equilibrium (Weir and Cockerham 1984). Two additional statistics, R_{ST} (for microsatellites) and Φ_{ST} (for mtDNA), incorporate mutation into estimates of divergence between populations by using both allelic frequencies and divergences between alleles (or haplotypes). These latter two measures of divergence attempt to measure the effect of recent mutations on estimates of divergence, and may be more sensitive to recent population isolation than F_{ST}. F_{ST} is estimated from allele frequencies and can be related to genetically effective migration (gene flow) using $F_{ST} \approx 1/(1 + 4Nm)$ by assuming the island model of migration. While the assumptions of this model may not apply to all population groups, the use of F_{ST}, and F_{ST}-like statistics, allows comparisons with other species.

Population structure will be examined using a priori and a posteriori methods of analyses. We will use the analysis of molecular variation (AMOVA) in ARLEQUIN with an a priori nested sample design to explore geographical and temporal population structure that has been imprinted in the microsatellites and mtDNA. Additionally, a spatially model-free analysis of diploid microsatellite variability will be made with STRUCTURE (Pritchard et al. 2000), which is based on the fit of the genotypic data to single and multilocus Hardy-Weinberg expectations. Individual assignment methods using GENECLASS (Piry et al. 2005) will also be used to estimate recent

movement between areas, but the power of this approach depends on the extent of differentiation between areas; the greater the differentiation the more power to detect migrants (Manel et al. 2005).

If populations are reproductively isolated from one another, identifying the pattern of isolation is of importance to fishery management. When gene flow between populations is limited by restricted movement of larvae or adults, a pattern of isolation by distance (IBD) is expected to arise (Wright 1943). A correlation between genetic distances, corrected for level of heterozygosity [$F_{ST}/(1 - F_{ST})$], and geographic distances would be informative about the genetic structure of golden king crab populations. Possible IBD will be tested for all the molecular markers following Rousset (1997). On the other hand, if a mosaic pattern of genetic patches appears in the analysis, golden king crabs may be structured in a metapopulation, in which subpopulations are instable and vary in abundance (Hanski 1998). Molecular markers can be used to distinguish between these two models (Wang 2005; Waples 2005).

INTERPRETATION AND APPLICATION OF RESULTS

Exploited wild populations can be viewed in two ways (Andrewartha and Birch 1984). First, the *ecological* population is a group of interacting individuals that are affected by density dependent processes and is the focus of fishery management. Second, the *evolutionary* population shows genetic continuity over time and is often the focus of conservation concerns about continued persistence. The temporal perspective of process influencing an ecological population in fishery management is short, reaching back at most decades, or often only from year to year, whereas the evolutionary perspective of a population extends over millennia. Both short-term ecological processes and long-term evolutionary processes influence the genetic variability seen in present-day populations, and the patterns of variability from both processes must be considered in the use of genetic data for fishery management.

On short time scales, decadal and annual shifts in climate influence local population abundances and connectivities between populations (Anderson and Piatt 1999; Loher and Armstrong 2005). While fishery managers are most interested in contemporary processes influencing the demography of a population, the effects of historical processes on genetic patterns must also be recognized. Climate in the North Pacific varies on long and short time scales. On long time scales, periodic coastal glaciations during Pleistocene glacial maxima led to isolations in refugia, producing deep genetic partitions among surviving populations. A biogeographic discontinuity between marine species' distributions occurs along the Aleutian Islands (Hunt and Stabeno 2005). Populations of several species in the western portion of the Aleutian Archipelago show genetic affinities to Asian populations, whereas populations in the eastern portion of the Aleutians show affinities to Gulf of Alaska or Bering Sea populations. For example, Pacific herring (Grant and Utter 1984; Wildes et al. 2011; Beacham et al. 2008), Pacific cod (Canino et al. 2010), and red king crab (Grant and Cheng 2012; Vulstek et al. 2013; Grant et al. 2014) show deep partitions among major regional population groups due to ancient Pleistocene isolations.

Estimates of stock structure from genetic markers have weaknesses that need to be considered in their application to fishery problems. Estimates of migration from genetic data (e.g. F_{ST}) are generally not useful to managers because these estimates assume an equilibrium between random drift and migration, and because estimates consist of the number of migrants (Nm) and not the migration rate (m). Managers are interested in stock structure estimates based on contemporary processes and in the proportion of individuals migrating between stocks that influence

demographic independence. The ideal harvest design is to manage stocks, or production units, that are demographically independent of one another; that is, they are self-sustaining and are not components of a larger stock complex. Over long time scales, only a few migrants can prevent extensive genetic divergence between populations that are demographically independent of one another. Simulations indicate populations with migration rates as large as 10% can still be demographically independent of one another (Hastings 1993; Waples and Gaggiotti 2006). This large rate of migration would produce genetic homogeneity, and hence demographic independence between stocks would not be detectable with genetic markers. Conversely, the finding of genetic differences indicates demographic independence between populations.

Based on previous population genetic studies of marine species in the North Pacific, there is a high probability of finding genetic differences between populations of golden king crab along the Aleutian Archipelago because this area represents a biogeographic transition. In addition, populations of golden king crab are expected to be more isolated than populations of red king crab, because golden king crabs typically inhabit deep canyons and depressions that may inhibit adult migration. The larvae of golden king crab also appear to be less buoyant than red king crab larvae and tend to settle out in deep rather than in shallow areas. These sources of population isolation are expected to produce greater levels of genetic structure in golden king crab than in red king crab. Overall, this study attempts to better incorporate genetic data into the management of golden king crab (Waples et al. 2008).

SCHEDULE AND DELIVERABLES

Date	Activity
June–December 2015	Microsatellite primer development
January-May 2016	Microsatellite analysis Mitochondrial DNA primer optimization
June–December 2016	Sample collection in Aleutian Islands
January–May 2017	Laboratory analyses
June–December 2017	Data analysis and manuscript preparation

RESPONSIBILITIES

PRINCIPAL INVESTIGATORS

Stewart Grant, co-Project leader, Fishery Biologist IV

As co-project leader, is responsible for formulating research objectives to meet project goals, writing operational plan, overseeing budgets, and supervising project technicians. This position will serve as the primary project biologist and will be responsible for training personnel and supervising data collection. The project biologist will be responsible for collating, analyzing data, preparing reports and drafting research manuscripts for publication in a peer-reviewed scientific journal.

William Templin, co-Project leader, Principal Geneticist

As co-project leader, is responsible for formulating research objectives to meet project goals, writing operational plan, overseeing budgets, and supervising project technicians. This position will serve as a project biologist responsible for collating, analyzing data, preparing reports and drafting research manuscripts for publication in a peer-reviewed scientific journal. Additionally, this project biologist submits invoices, manages budgets, and prepares budget requests.

LABORATORY TECHNICIAN

Heather Liller, Laboratory biologist, Fishery Biologist II

This position provides laboratory support for the molecular analysis of golden king crab tissues, and is responsible for generation of microsatellite genotypic data, and mitochondrial DNA sequences. This position also provides support for statistical analysis and will assist in the preparation of reports and manuscripts for publication.

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TABLES AND FIGURES

Table 1.- Larval drift zones in marine waters of Alaska.

1. Southeastern Alaska: Canadian border to Cape St. Elias.
2. South Central Alaska: Cape St. Elias to Cape Igvak, including Kodiak Island.
3. Southern Alaska Peninsula: Cape Igvak to Samalga Pass.[1]
4. Aleutian Islands: All islands west of Samalga Pass.
5. Southeastern Bering Sea and northern Alaska Peninsula: Samalga Pass Kuskokwim River, including Pribilof Islands.
6. Northeastern Bering Sea: Kuskokwim River to Cape Prince of Wales, including islands north of Kuskokwim River.
7. Chukchi Sea: Cape Prince of Wales to Point Barrow. [2]
8. Beaufort Sea: Point Barrow to Canadian border.[3]

[1] This extends the western boundary of larval drift zone 4 defined in Fish & Game regulation 5 AAC 41.245 and is based on the conclusions of G.L. Hunt and P.J. Stabeno (2005).

[2] Larval drift zones 1–6 are defined in Fish & Game regulation 5 AAC 41.245. Larval drift zone 7 is defined here because of recent increases in the abundances of several marine plants and invertebrates, presumably due to climate warming.

[3] Larval drift zones 1–6 are defined in Fish & Game regulation 5 AAC 41.245. Larval drift zone 8 is defined here because of recent increases in the abundances of several marine plants and invertebrates, presumably due to climate warming.

Table 2.- List of archived samples of golden king crab to be used in this study.

Location	North latitude	Longitude	Sample size
Adak Island	51.8627	-176.6607	100
Pribilof Islands	58.2000	-158.6000	100
Bering Sea	–	–	10
Dutch Harbor	53.9010	-166.5128	100
Mid Chatham	56.8258	-134.5330	36
Lower Chatham	56.5541	-134.5730	47
Frederick Sound	57.0500	-134.2500	110
Stikine-Clarence Strait	56.1671	-134.7640	35
North Stephens Passage	58.0795	-134.088	47

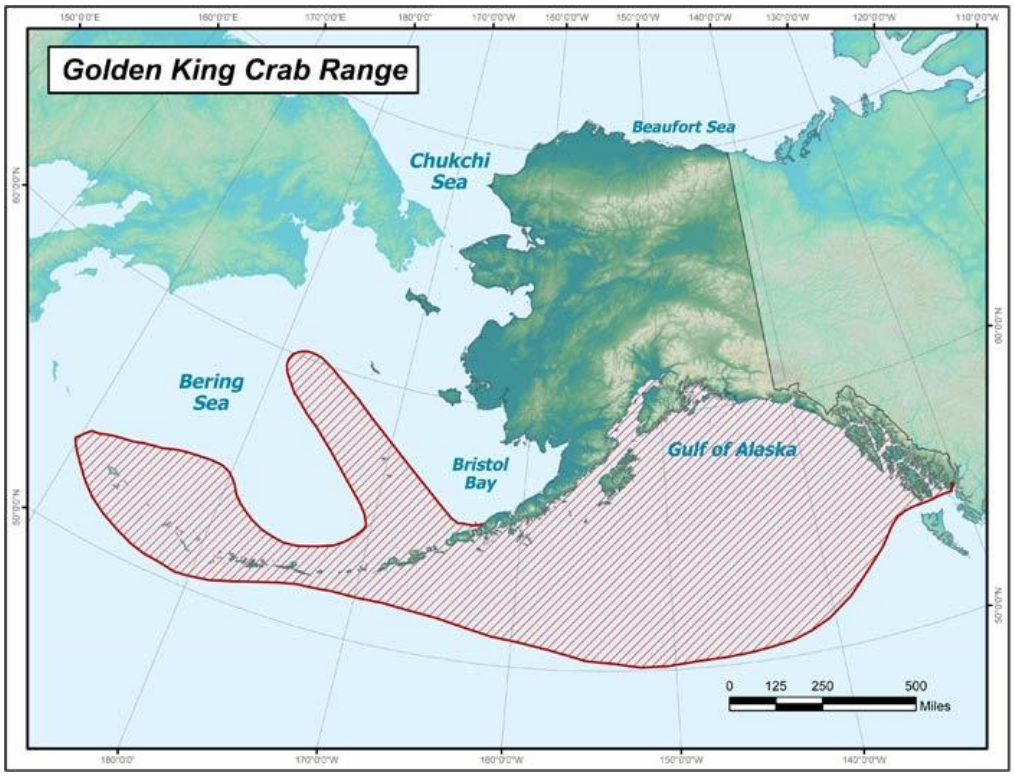

Figure 1.- Map of Northeastern Pacific and Bering Sea showing the distribution of golden king crab in Alaskan waters.

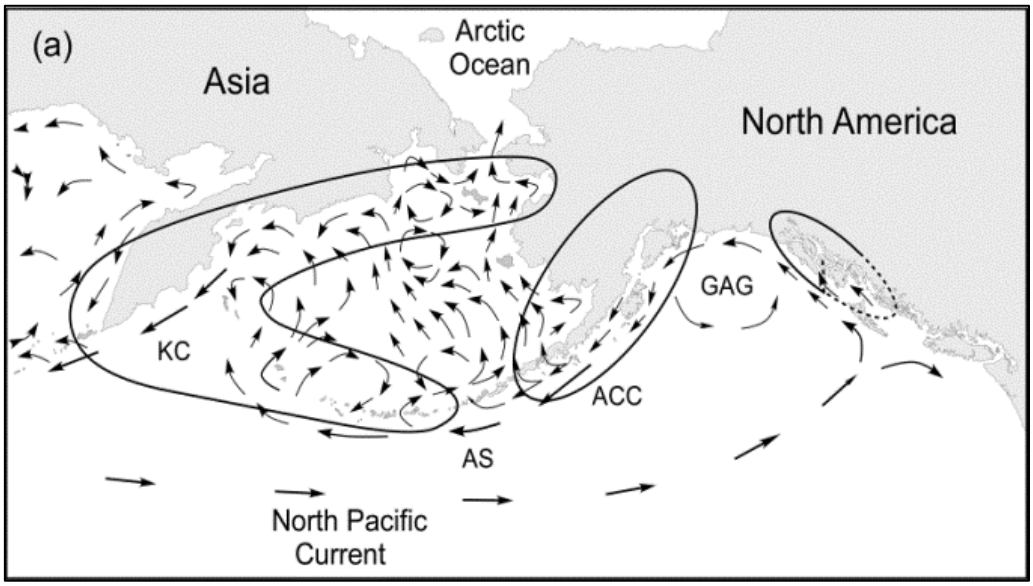

Figure 2.- Large-scale genetic population structure of red king crab in the North Pacific, estimated with allozyme (Seeb et al. 1990; Grant et al. 2011), mtDNA and SNP (Grant and Cheng 2012), and microsatellites (Vulstek et al. 2013) population markers.

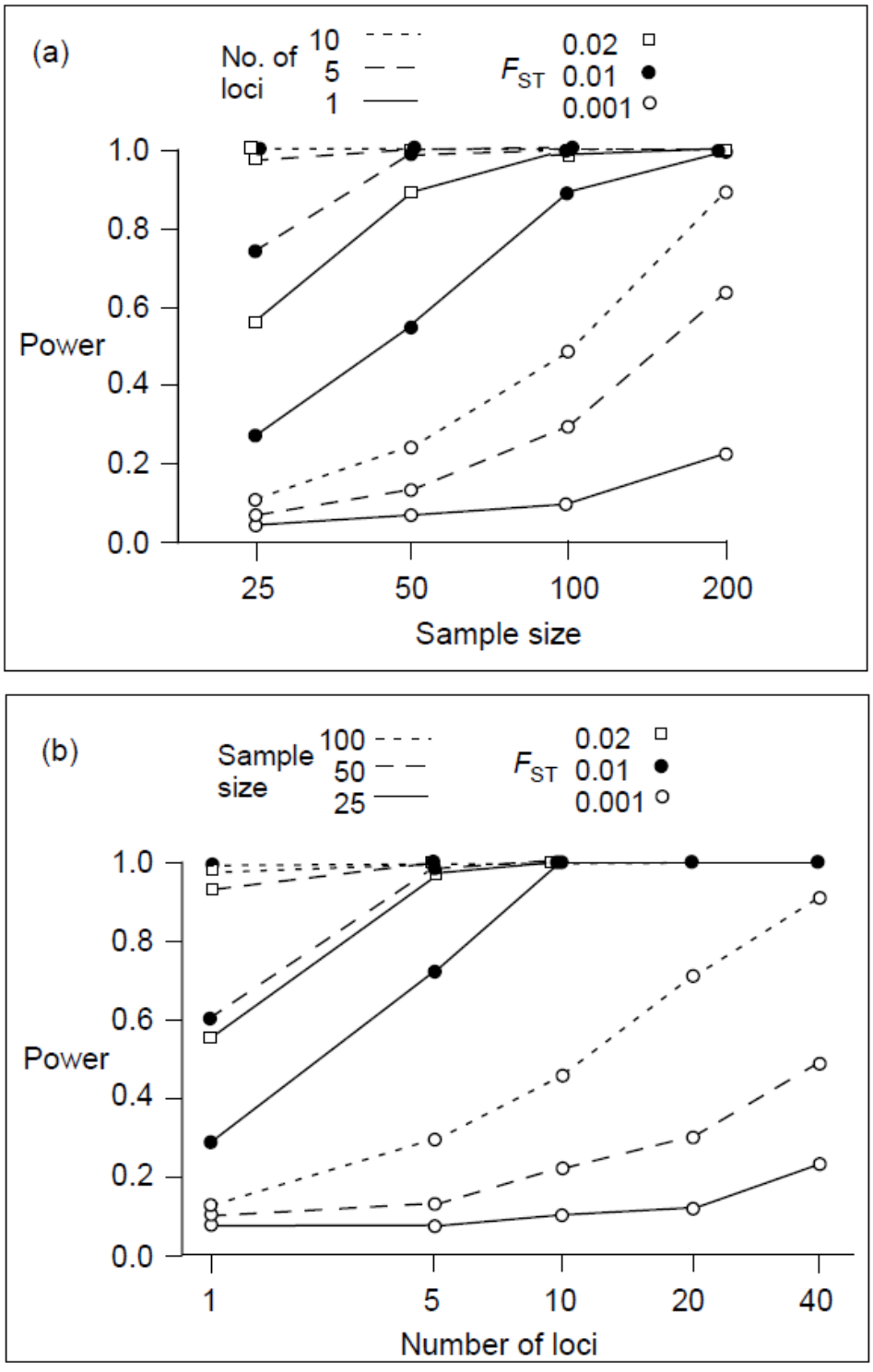

Figure 3.- Probability of detecting a significant value of FST for a given level of divergence between three populations, samples size and number of polymorphic loci generated with PowSim4 (Ryman and Palm 2006).

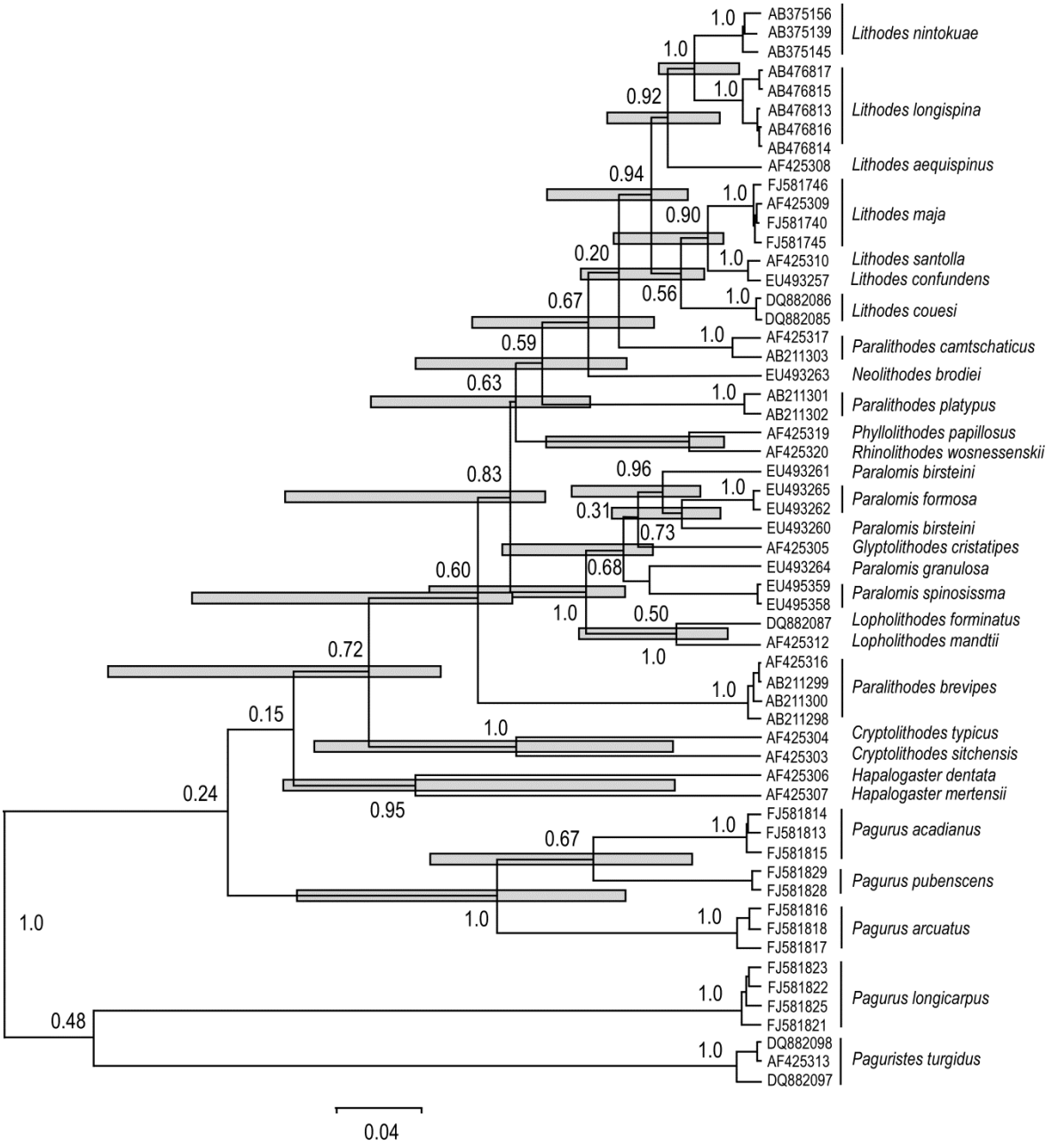

Figure 4.- Phylogenetic tree of lithodid crabs estimated with Bayesian tree analysis of mitochondrial DNA sequences of cytochrome oxidase I 597 base pairs in length. Bars represent 95% credibility intervals.