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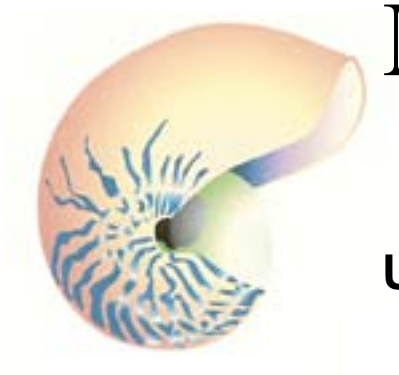

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Defining genetic structure in Alaskan populations of the snow crab, *Chionoecetes opilio*

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Project Summary

Decreasing sea ice in the Arctic is expected to impact marine ecosystems, and to lead to increased human activity in the region in the form of shipping traffic, fishing pressure, and mineral resource exploration and extraction. In the face of these pressures, we examine genetic population structure in the snow crab, *Chionoecetes opilio*, to determine degrees of population connectivity between the Arctic and more southerly portions of the species' range. Snow crab constitutes a valuable commercial resource in the Bering Sea, as well as other areas in the North Pacific and Atlantic Oceans. However, large populations also exist in lesser-studied areas further north, including the Chukchi and Beaufort Seas. Stocks of the snow crab are relatively unexplored north of the Bering Strait, and not yet targeted by fisheries, although commercial-sized individuals were recently reported in areas of the Western Beaufort Sea. Prevailing hydrographic conditions suggest that the long-lived planktonic snow crab larvae might be transported long distances with northerly currents passing through the Bering Strait, into the Chukchi Sea, and along the Beaufort Shelf. Thus, Chukchi and Beaufort populations could well be genetically linked to commercially exploited Bering Sea populations, with gene flow occurring in the direction of water mass movement. Here we examine degrees of genetic population structure in Alaskan snow crab to aid in estimating potential spatial scales of human impacts on this species. Based on data from seven polymorphic microsatellite loci, we find evidence of minimal genetic population structuring throughout the Alaskan region (G_{ST} = 0.001), suggesting snow crab constitute one large, panmictic population in this region. However, one sample site in the Western Beaufort Sea, where larger-sized individuals were found, did differ significantly from all others. Significant linkage disequilibrium in this location is likely indicative of admixture with a highly divergent population that was not otherwise sampled within this study. Thus, stocks in the Beaufort Sea may warrant further investigation, and comparisons between Alaskan and other Arctic and sub-Arctic populations may shed light on the nature of this group of larger-sized individuals in the Beaufort.

Introduction

Snow crabs (Chionoecetes opilio) occur throughout the continental shelf regions of the North Pacific, Northwest Atlantic and Arctic Oceans (Figure 1). The species represents a valuable commercial resource and has historically comprised the largest and most valuable crab fishery in the US (328.6 million pounds harvested in 1991, over $155 million ex-vessel; North Pacific Fishery Management Council 2010). Snow crab harvest increased during the 1980s as catches of its warmer-water relative, the Tanner crab (*C. bairdi*), began to decline. Since that time, the Bering Sea snow crab stock has undergone large fluctuations in population size. Although population dynamics are tracked in two separate subdistricts (divided at 173° W longitude), the Bering Sea snow crab fishery is managed as a panmictic unit with a single harvest quota (Bowers et al. 2008). Relatively little attention has been paid to *C. opilio* stocks in the Chukchi and Beaufort Seas where large aggregations also occur (Paul et al. 1997; Bluhm et al. 2009), including recently-discovered commercial-sized individuals on the Beaufort Sea continental slope (Rand and Logerwell 2011). These unexplored populations may be sources or sinks for genetic exchange with other, more intensively fished populations further south, or further east in Canadian waters. Recent evidence indicates snow crabs are undergoing a northward range contraction, apparently due to changing temperatures (Dionne et al. 2003; Orensanz et al. 2004). This range shift may increase population sizes in the Chukchi and Beaufort Seas, and alter dynamics of dispersal and migration in the region. Current gene flow patterns are thus relevant to predictions of how human activities in the north may impact populations in the south and east.

In crabs and other species with planktonic larval phases, movement away from detrimental habitat conditions and/or re-colonization of an area after a disturbance can be achieved through larval dispersal. Dispersal also constitutes a means of genetic exchange between geographically distant populations. Directionality of dispersal may thus indicate whether areas “upstream” from potential disturbances may help to re-seed an affected population. Hydrographic flow through the Alaskan shelf seas is predominantly northward (Figure 2), with a 6-month transit time recorded for water parcels moving from the Bering Strait up to Barrow (Winsor and Chapman 2004). The planktonic period for snow crab typically lasts at least three to four months (Incze et al. 1984; Incze et al. 1987). Thus, larval dispersal distances could be significant, facilitating homogenization of the regional gene pool and downstream transport of colonists to the north. Indeed, previous investigations into snow crab population structure using allozymes indicate homogeneous population structure in the Bering Sea (Merkouris et al. 1998), although allozymes are less polymorphic than other markers such as microsatellites, and may thus fail to detect structure in large populations. In contrast, microsatellites have indicated population structure over similar spatial scales in Canadian snow crab (Puebla et al. 2008), with regional separations detected between Greenland and Northeast Canada. Genetic tools have not been applied in examining connectivity to northern populations in Alaska. However, complex ontogenetic migrations (Orensanz et al. 2004; Ernst et al. 2005) and possible hydrographic retention of larvae in some areas (Parada et al. 2010) may generate more potential population structure than has been accounted for in current management strategies.

Here we employ microsatellite techniques to examine population structure of snow crab within the Bering, Chukchi and Beaufort Seas to determine whether genetically distinct subpopulations exist despite the absence of obvious physical barriers to dispersal. Specifically, we test the null hypothesis that snow crabs form a panmictic population throughout the Alaska region. We use microsatellite markers developed for similar studies in the Canadian Arctic, which were shown to be sensitive enough to detect regional population structure (Puebla et al. 2003; An et al. 2007; Puebla et al. 2008).

Materials and Methods

Sample collection

Field sampling was conducted in the Beaufort, Chukchi, and Bering Seas between 2008 and 2010 (Figure 2). Collection information for all samples is summarized in Table 1. Beaufort Sea samples (*n =145*) were collected in August 2008 during a US Minerals Management Service expedition aboard the F/V *Ocean Explorer* using an 83-112 eastern otter trawl (for cruise details see Rand and Logerwell 2011). Samples were frozen whole aboard the vessel at -20°C and transferred to -80°C in the home laboratory. Samples from six stations in the Beaufort Sea (MMS stations 2, 4, 22, 23, 24 and 26; Table 1, Figure 2) were combined due to low sample numbers and proximity of the stations to one another. Snow crab samples were not initially collected for the purposes of genetic analysis during this expedition, but as part of a community structure analysis conducted by B. Bluhm (UAF). Frozen tissue samples were later donated to this project for genetic analysis. Thus, due to the opportunistic nature of these collections, genetic sample sizes were low at some sites. Results from samples collected at these six pooled sites are reported here as one site (Beaufort Sea Pooled, BFP), as indicated in Figure 2. However, larger sample sizes were collected at MMS stations 7 and 8. These sites also yielded substantially larger individual body sizes (≥86.2 ±15.2 mm, compared to 34.6±15.2 mm average carapace width (CW)), suggesting they may represent a distinct stock. In summary, samples collected from the Beaufort Sea are represented by three points in our dataset: BFP (6 pooled sites), BC (Barrow Canyon; MMS station 7), and WBF (Western Beaufort Sea; MMS station 8) (see Figure 2, inset).

Chukchi Sea samples (*n = 268*) were collected in September 2009 during the Russian-American Long-term Census of the Arctic (RUSALCA) cruise aboard the R/V *Professor Khromov*, using otter and plumb-staff beam trawls. As many as 50 females were collected from each of seven stations to encompass a broad area (Table 1, Figure 2). Both male (*n = 17*) and female (*n = 7*) crabs were analyzed from the North Chukchi Shelf site (NC). Fewer crabs were obtained at this site, yet it represents an important data point as it is the farthest-north collection location. The majority of Chukchi crabs collected at all Chukchi sites were immature (92.4% females only) and assumed to not have moved great distances since settlement (Lovrich et al. 1995; Ernst et al. 2005).

Bering Sea samples (*n = 200*) were collected in July 2010 aboard the National Marine Fisheries Service annual trawl survey cruises (for details see Chilton et al. 2011). Sampling occurred on a 20-nm grid, and was conducted aboard the F/V *Aldebaran* and the F/V *Alaska Knight* using 83-112 eastern otter trawls. All samples for genetic analysis were collected

aboard the F/V *Alaska Knight*, with the exception of 50 samples from the Pribilof Island station (PI) that were collected aboard the F/V *Aldebaran* within the same month (Table 1, Figure 2). Three sampling sites were chosen in an effort to encompass the Bering Sea distribution of adolescent crabs, which are believed not to have made significant ontogenetic migrations at this stage. Pre-pubescent females (or those one molt prior to maturity) were targeted at each of three sites (*n = 50-100* individuals per site) in order to be consistent with Chukchi sampling and to obtain a snapshot of the female *only* population in the Bering Sea, about which more life-history information is known (Ernst et al. 2005). A 2.5-cm section of the 4th walking leg was sampled from each live crab on board ship, and preserved in 95% ethanol.

DNA extraction, amplification, and sequencing

A subset of tissue samples from the Chukchi (*n* = 80) and Beaufort Sea (*n* = 10) crabs were sent to the Biodiversity Institute of Ontario for sequencing of the mitochondrial gene cytochrome *c* oxidase subunit I (COI), which is commonly used for species "barcodes" (cf., Hebert et al. 2003). DNA extraction, amplification, and sequencing were carried out according to the institute's standard protocols using the primers HCO2198 and LCO1490 (Folmer et al. 1994). Data were used to confirm species identification of adult and larval tissues used in the study, and to determine whether the COI gene would be informative for examining intra-specific relationships. Virtually no variation was found at this locus (data not shown), and we determined that microsatellite methods would be required to examine population structure. COI data are currently available through the Barcode of Life Database (www.boldsystems.org), and will be released to GenBank upon publication of the peer-reviewed manuscript. A total of 24 crab larvae that were collected from live plankton samples during the RUSALCA 2009 expedition to the Chukchi Sea were also analyzed. DNA was extracted using the Qiagen DNeasy® protocol. Mitochondrial (*cyt b*) sequences were generated to confirm genus and species of collected samples. While we initially planned to analyze a more extensive set of larval samples in an effort to attribute larvae to their source population, the microsatellite analysis of adult tissues described below revealed such minimal population structure that it was determined larval analysis would not be informative.

For microsatellite analysis, genomic DNA was extracted from tissues using Omega Bio-Tek E.Z.N.A.® and Qiagen DNeasy® extraction kits. Seven published microsatellite loci were selected and successfully amplified for this analysis: *Cop2*, *Cop3*, *Cop4*, *Cop3-4II*, *Cop24-3* and& *Cop11* (Puebla et al. 2003) and *EC0106* (An et al. 2007). Attempts to amplify five additional loci (*Cop4-1* and *Cop77*; Puebla et al. 2003 and *KC030*, *KC0181* and *KC0235*; An et al. 2007) were either unsuccessful or yielded unusable amplifications (e.g., irregular stuttering).

A total volume of 10 μl was used in polymerase chain reaction (PCR) mixes and consisted of 1 μl milli-Q water, 1 μl 10x forward and reverse primer (diluted with TE buffer), 5 μl Qiagen hot start *Taq* polymerase multiplex kit (2x; containing a final concentration of 3 mM $MgCl_2$), and 3 μl template DNA. PCR conditions consisted of a 30 minute denaturation at 94°C, followed by 40 cycles of 30 seconds denaturing at 96°C, 50 seconds annealing at 55°C, and 1 minute of extension at 72°C with a final extension time of 20 minutes at 72°C. Three PCR

multiplexes consisting of 2 loci each (*Cop113* and *Cop3-4II*, *Cop2* and *ECO106*, *Cop3* and *Cop4*) were used; however, *Cop24*-3 was run independently due to interference when paired with any other locus. Final PCR product was submitted to the Yale DNA Facility (dna-analysis.research.yale.edu) for capillary electrophoresis on a 3730 *x*/ 96 Genetic Analyzer with LIZ500 size standard. All samples were amplified, analyzed, and scored independently a minimum of two times to ensure accurate genotyping with a range of 0-8.5% missing data per locus.

Descriptive statistics and population differentiation

Allele scoring was conducted using GeneMapper® software (version 3.7; Applied Biosystems). Tests for departures from Hardy-Weinberg equilibrium (HWE) and the presence of null alleles were calculated by comparing sets of observed and randomized alleles using a cumulative binomial distribution (Weir 1996). Significance tests for departures from HWE were conducted using Fisher's combined probability test, as implemented in the program MICRO-CHECKER (version 2.2.3; Van Oosterhout et al. 2004). The Hardy-Weinberg Principle states that allele frequencies in a population should remain constant in the absence of disturbing forces, including non-random mating, mutations, selection, and genetic drift. Thus, departures from HWE at particular loci or in particular sampling locations may indicate that the loci are being acted upon by these forces and thus not neutrally inherited; this assumption of neutrality is fundamental to statistical methods of population genetic data analysis. Null alleles are cases in which a particular locus is not amplifying in some individuals, and is indicated by an abundance of homozygotes beyond what would be expected by chance. Loci which show evidence of null alleles must be dropped from further analysis because they will bias results.

All loci were tested for linkage disequilibrium (LD), which is a test for non-random association of alleles at different loci (e.g. physical linkage or selection). LD tests were carried out using a Markov chain method to provide unbiased *p*-values for results of a contingency table analysis performed in GENEPOP (version 4.0; Rousset 2008) with 10,000 batches and 20,000 iterations per batch. Evidence of LD was detected at one station in the Beaufort Sea (WBF; see below), and additional tests were performed to determine the cause of significant tests for LD. The *M*-ratio (Garza and Williamson 2001) was used to test for historical bottlenecks. This metric is calculated as the number of alleles in a given sample divided by the total number of possible allelic states. The program BOTTLENECK (version 1.2.02; Piry et al. 1999) was used to test for a heterozygote excess, as would be expected after a bottleneck, using a Wilcoxon signed-rank test with 10,000 iterations in the WBF sample site only. Two separate tests for relatedness among individuals at WBF were performed using GenAlEx (version 6.1; Peakall and Smouse 2006).

To test the hypothesis of panmixia across the study region, a Bayesian clustering approach was implemented in the program STRUCTURE (version 2.3.1; Pritchard et al. 2000) to assign individuals to populations (clusters) based on their genotypes. All possible numbers of clusters (*K*) from 1 (panmixia) to 13 (distinct population at each sampling site) were tested using the admixture and correlated allele-frequency models. For each value of *K*, a total of five runs were performed with 500,000 Markov Chain Monte Carlo (MCMC) repetitions

following a 500,000 repetition burn-in period, and the most likely number of clusters (i.e., populations) was determined based on the *ad hoc* likelihood measures *L(K)* (Pritchard et al. 2000) and *ΔK* (Evanno et al. 2005).

In addition to Bayesian inference of population differentiation, allele-frequency statistics including the *F*-statistic analogues G_{ST}, G_{IS}, (Nei 1973) and expected heterozygosity (H_e), were calculated using the R statistical-software package DEMEtics (Gerlach et al. 2010) with 1000 bootstrap replicates. Because G_{ST} can perform poorly in high-diversity populations (Gerlach et al. 2010) such as those examined here, Jost's (2008) measure of true differentiation (*D*) was also calculated in DEMEtics with 1000 bootstrap replicates. Calculations for observed heterozygosity (H_o) were performed in GENODIVE (version 2.0; Meirmans and Van Tienderen 2004). Allelic richness, rarefied for the smallest sample size present (n = 24), was calculated using HP-RARE software (Kalinowski 2005) and a Wilcoxon signed-rank test was carried out in the statistical software package JMP (SAS institute, Cary, NC, USA) to test for significance between sampling sites. Pairwise comparisons of allele frequencies among all sample sites and regions were performed using Fisher's exact test as implemented in the software GENEPOP (Raymond and Rousset 1995), with 10,000 batches and 10,000 iterations.

The application of Jost's *D* (2008), likely the most sensitive measure employed here, is appropriate, particularly as a supplement to Nei's (1973) G_{ST}. G_{ST} is strongly based on heterozygosity and mathematically "bounded" by the overall heterozygosity (H_S) within the population (i.e., when H_S is large, G_{ST} cannot reach 1; Nei 1973, Jost 2008). Hedrick (2005) realized this weakness and put forth a new measure (G'_{ST}), which is standardized to overall heterozygosity. However, these two statistics more accurately measure migration rate, which is only a single cause of population differentiation among others (i.e., random sampling of genes at mating, bottlenecks). Jost's *D* accounts for effective allele frequencies and provides an arguably more ecologically relevant and conservative measure of differentiation (Jost 2009). Due to the fact that a great deal of debate exists around this topic (e.g., Meirmans and Hedrick 2011), and for comparability with other studies, both measures are used here.

Quality control: Cross-validation of results by an external laboratory

A randomly-selected subset of 60 of the samples analyzed here was also sent to an external lab for cross-validation of results. This external lab is run by Jean-Marie Sevigny of Department of Fisheries and Oceans Canada, who has co-authored a similar study on snow crab populations in the northwest Atlantic (Puebla et al. 2008). The Sevigny lab genotyped these samples according to their own published protocol. However, only 5 loci were analyzed by both labs due to difficulties with two of the markers. Specifically, the combination of a short repeat motif and irregular allele amplification made allele scoring difficult, and thus complicated the combining of datasets. Consistency between labs in scoring of individuals ranged from 95.7% to 98.9% (Table 5). Scoring discrepancies were likely due to amplification artifacts and/or poor DNA quality.

Average differences in raw allele scores for each locus were used, in conjunction with scatter plots of raw allele sizes from both datasets, to combine datasets and conduct a meta-

population analysis. A total of 1504 samples (613 from the Alaskan region, 891 from the northwest Atlantic) were successfully combined into one dataset and analyzed for population structure at 5 loci (*Cop2*, *Cop4*, *Cop3-4II*, *Cop24-3* and *Cop113*). This dataset will be further augmented with additional samples from our collections, as well as samples from the Barents Sea obtained by the Sevigny lab, and we will collaborate on a future publication on pan-Arctic meta-population structure in snow crab. This larger-scale analysis will examine population connectivity between Alaskan and other Arctic snow crab populations, and determine whether exchange may also be occurring between Pacific and Atlantic populations. We are also interested in determining whether the Barents Sea population may be a source for possible migrants that may be contributing to deeper-living populations in the Beaufort Sea (explained below).

Results

The seven microsatellite markers used in this study were found to be highly polymorphic, with alleles per locus ranging from 12 (*Cop4*) to 41 (*ECO106*; mean = 24.6 ±8.8; Table 2). Rarefied allelic richness (i.e., allelic richness corrected for differences in sample size between locations, based on the smallest sample size *n = 24*) ranged from 11.35-14.41 (mean = 13.42 ± 0.77; Table 3). Allelic richness did not differ significantly between any two sampling sites (Wilcoxon signed-rank test, data not shown, $p = 0.12$). Overall observed heterozygosities (H_O) at each locus ranged from 0.228 (*Cop4*) to 0.908 (*Cop24-3*; mean = 0.773 ±0.244; Table 2). Observed heterozygosity averaged across all loci for each sampling site ranged from 0.721 (at site PH) to 0.815 (at site NC; mean = 0.773 ±0.026; Table 3).

Significant departures from HWE were found in 14 out of 91 possible population-locus pairings (15.4%; Table 3). These departures are most likely due to random error associated with genotyping and null alleles, rather than real population dynamics. All significant tests were attributed to heterozygote deficits and the possible presence of null alleles; however, no more than three significant tests occurred at any site and no more than five were observed for any locus (Table 3). The highest frequencies of departure from HWE at a single locus occurred at *Cop3-4II* and *ECO106* (4 and 5, respectively). Although MICRO-CHECKER did not detect evidence of error due to stuttering, these loci produced stutter patterns that may have contributed to a tendency to miss heterozygotes when being scored due to their wide stutter arrays. In checking for HWE departures due to stuttering, the program MICRO-CHECKER detects significant absences of heterozygotes separated by a single repeat unit, as would be likely if a short stutter band obscured peaks. However, these loci often amplify with wide stutter arrays, which could conceal heterozygotes more than one repeat unit away from each other, making it more likely to incorrectly score as a homozygote. Thus, we employed a standardized method of scoring based on peak morphology, and stutter arrays that were atypical were often re-amplified a number of times, or omitted as missing data. We thus feel confident in our results.

Tests for linkage disequilibrium (LD) revealed 15 significant results out of 273 tests of each locus combination per site (significance level 0.05/13 = 0.0038). All 15 of the significant results occurred at the Western Beaufort Slope (WBF) site, suggesting a site-specific phenomenon rather than a population-wide problem with a particular locus. Significant LD

can indicate a population bottleneck, sampling of closely related individuals, or recent immigration. Genotyping error was ruled out by blind scoring procedures, in which individuals were scored prior to grouping within sampling sites for analysis. Tests for heterozygote excess, as would be expected after a population bottleneck, were performed in the program BOTTLENECK and were not significant (Wilcoxon one-tailed test $p = 0.148$, data not shown). BOTTLENECK, however, can fail to identify known bottlenecks in some cases (e.g., Hundertmark and Van Daele 2010). Therefore, the *M*-ratio was also calculated for the population in question, as it has been shown to more accurately detect bottlenecks (e.g., Garza and Williamson 2001; Spear et al. 2006). However, using the critical value of *M* (0.680) from Garza and Williamson (2001), evidence of a bottleneck in the WBF population was not found ($M = 0.732$). Relatedness metrics (cf., Queller and Goodnight 1989; Ritland 1996), which range from -1 to +1 with larger positive values indicating greater relatedness than expected by chance, yielded values near zero for the WBF samples (-0.013 and -0.027, respectively). Furthermore, the values for both relatedness tests were not significantly different from those of all other populations (Wilcoxon signed-rank test; $p = 0.457$). Therefore, admixture with individuals from an unsampled population appears to be the most likely cause of this result.

Estimates of the posterior likelihood measure *L(K)* indicated the most likely number of clusters (i.e., populations) to be 1 (Figure 3). The highest value for the second-order rate of change between values of *K* (ΔK) was 3 (Figure 4), indicating the value of *K* is not higher than 3. Although *L(K)* has been shown to inaccurately estimate the true value of *K* in some cases (Pritchard et al. 2000, Evanno et al. 2005), ΔK can only be applied to values larger than 1 and therefore is not capable of assessing $K = 1$. Moreover, the decrease in the mean of estimated ln probability between $K = 1$ and $K = 2$ is much larger than that between $K = 3$ and $K = 4$ (Figure 3), so the magnitude of ΔK between $K = 3$ and $K = 4$ would likely be less than between $K = 1$ and $K = 2$, if such a measure could be calculated. We thus interpret $K = 1$ as the best estimate. The graphical representation of individual clustering at $K = 3$ (Figure 5; vertical lines represent individuals and the proportion of their genotype that is assigned to each cluster indicated by color), allele frequency comparisons, and *F*-statistics further support the conclusion of $K = 1$ in this analysis.

The global G_{ST} value was extremely low (0.001; Table 2) compared to other northern crab populations (0.031, Beacham et al. 2008; 0.011, Puebla et al. 2008), and Jost's (2008) measure of true differentiation (*D*) based on effective alleles was equally low (0.004; Table 2). These low values were driven primarily by the WBF sampling site, which showed significant linkage disequilibrium. Pairwise comparisons of G_{ST} values for all sampling sites with the exception of the WBF site were extremely low and resulted in no significant comparisons (Table 4). Allele frequencies also differed significantly (Fisher's method) at the WBF site from all other sites (data not shown). Pairwise *D* values were relatively low; however, the Beaufort Sea Pooled (BFP) site showed significant differentiation from a number of sites (Table 4), signifying that some genetic isolation may be occurring in the region. Nonetheless, allele frequencies, allelic richness, G_{ST} values, and the Bayesian analysis performed in STRUCTURE suggest that this structuring is weak (Tables 3 and 4).

Discussion

Snow crabs show a high level of genetic homogeneity throughout the Alaska region with the exception of the anomalous Western Beaufort Sea (WBF) site (see Figure 2). Significant linkage disequilibrium at this site may reflect the presence of individuals from an unsampled population. The measures of differentiation employed here suggest that genetic differentiation of individuals at this site from other sampled locations is significant, but subtle. It is likely that the long larval phase (>3 months, (Incze et al. 1984; Incze et al. 1987), long-distance adult migrations, and relatively short generation time (4.5-7.5 years; Alunno-Bruscia and Sainte-Marie 1998; Kruse et al. 2007) are homogenizing the gene pool in the Alaska region. Expansion of snow crab from the Bering Sea into the Chukchi and Beaufort Seas relatively recently, following the last glacial maximum, may also explain low divergence, and a similar phenomenon has been proposed in Bering Sea red king crab (Grant et al. 2011).

Genetic population structure in Alaskan snow crab

Failure to detect multiple sub-populations within the Alaska region using STRUCTURE is not surprising given that the algorithm in this program designates population groupings in a way that minimizes linkage disequilibrium (LD), which was found at the most divergent sampling site (Western Beaufort Sea, WBF). Furthermore, the low G_{ST} values found within the region represent population structure below levels at which the program can consistently detect differentiation ($F_{ST} = 0.03$; Latch et al. 2006). Population structure below this threshold can certainly be ecologically relevant, particularly when G_{ST} is affected by high heterozygosity. Here, however, the consensus of all statistical measures employed points to minimal structuring.

Significant Jost's *D* values were found for the pooled Beaufort Sea sites (BFP; Table 4); however, all other divergence measures do not suggest any differentiation at this location. Two sites from the Chukchi Sea as well as the two most geographically distant Bering Sea sites show no significant differentiation from BFP. Thus, these *D* values are unlikely to be ecologically relevant. Significant pairwise comparisons of Jost's *D* were found at three additional sampling sites (CL, NC, BC; Table 4); however, this pattern was not supported by multiple measures, and the spatial distribution of these sites does not indicate an ecologically relevant pattern such as a distinct stock. Significant pairwise allele frequency differences, G_{ST}, and *D* values all suggest that the WBF individuals are genetically distinct to some degree (Table 4). However, evidence for significant linkage disequilibrium at this site warrants further investigation.

While significant linkage disequilibrium can indicate lack of independence of genetic markers, true linkage has been ruled out here based on the finding that significant tests were not randomly distributed throughout the dataset. A bias in genotyping is also unlikely due to the scoring protocol implemented. All Beaufort Sea individuals were scored as a pooled sample, out of which subpopulations have not yet been defined. Other explanations for significant linkage disequilibrium include: 1) a historical population bottleneck; 2) the

collection of closely related individuals (i.e., siblings); 3) recent immigration from a distinct population not sampled here (admixture).

A historical bottleneck selects a small proportion of genotypes from the entire pool at random. Thus, the frequency of some haplotypes may appear in proportions greater than would be expected under an equilibrium scenario. However, tests for heterozygote excesses in BOTTLENECK and the calculation of the *M*-ratio show no evidence of such an event (Table 3). Sampling of closely-related individuals may also produce haplotype frequencies different than expected under random sampling due to the inheritance of a small number of genotypes from similar parents. However, tests for relatedness also showed no evidence of familial relationships among sampled individuals (data not shown). Admixture thus seems the most likely explanation of genetic distinctness at site WBF, given the environmental setting at this particular location. A newly admixed group could contain unique genotypes from each contributing population and the absence of heterozygous genotypes expected from random mating. Alternatively, a similar pattern may result from mating between admixed groups if offspring were not recruiting back into either population. A local recruitment event would, in theory, break down the pattern of linkage disequilibrium by 50% per generation (e.g., Frankham et al. 2002).

Snow crab are known to migrate into deeper waters with ontogeny to form aggregations and mate (Ernst et al. 2005). All crab (36 males, 4 females) collected at the Western Beaufort (WBF) site were morphometrically mature. The WBF site is located in 320 m of water and represents the northernmost observation of snow crab in the Beaufort Sea (Bluhm et al. 2005; Rand and Logerwell 2011). The Beaufort Sea experiences both eastward and cross-shelf (northward) flow (Garrison and Becker 1976; Pickart 2004), which could act to transport larvae into the Canada Basin or the Eastern Beaufort shelf, where snow crab have not been documented. Although samples from the surrounding pooled sites (BFP) show slight differentiation (Table 4), the genetically divergent population required for this scenario may have originated outside the sampling area, such as the Mackenzie River Delta area, where historical (1963 only) snow crab presence has been recorded (Atkinson and Wacasey 1989) and may be ephemeral. An unsampled population is also known to exist in the Barents Sea (Alvsvåg et al. 2009).

To further test the likelihood of this scenario, 20 randomly selected individuals from Greenland (Greenland data from Puebla et al. 2008; found here to be significantly divergent from Western Beaufort Sea crabs) were grouped with 20 randomly selected individuals from site WBF and tested for linkage disequilibrium. This test produced no significant results; however, the unsampled population assumed to be contributing migrants to site WBF could be more genetically distinct from this site than the Greenland population, and thus send a stronger signal detected by the linkage tests. Bayesian clustering failed to identify the WBF sampling site as a distinct population; however, the algorithm implemented in STRUCTURE works to minimize linkage disequilibrium when creating clusters. In theory, an actively reproducing population that is not self-recruiting but is a sink population receiving migrants from surrounding areas could be exploited with little consequence to future population size. Sampling of individuals further to the east would help to confirm this hypothesis. Evidence here does not suggest that the significantly larger-sized crabs found at sites in Barrow

Canyon (BC) and the Western Beaufort (WBF) are from a separate genetic stock, so it may be possible that exposure to warm water that occurs at depth in the area (Pickart et al. 2005) may be responsible for the larger size of these crabs.

Management implications

It is unlikely that distinct snow crab population units remain undiscovered within the Alaskan region, given the spatial coverage of sampling achieved here relative to the potential larval dispersal distances achievable in the 3- to 4-month planktonic period, and modeled recruitment dynamics (Ernst et al. 2005; Parada et al. 2010). Our results indicate extensive gene flow throughout the region, suggesting disturbed areas in the Chukchi Sea should be recolonized either from large local or southerly populations. Observations of gravid females in the Chukchi Sea in this study and others (Paul et al. 1997; Bluhm et al. 2009) indicate reproduction is also occurring locally, and thus recruitment within the Chukchi Sea is possible. Moreover, estimated residence times for water masses in the Chukchi Sea are sufficient to allow for larval retention within this region (Winsor and Chapman 2004). Crab larvae are also well-known to exhibit behavioral control of dispersal and may actively remain in shallow shelf waters despite long-lived larval phases (e.g., Christy 2011). In any case, the genetic homogeneity throughout the Alaska region precludes any direct quantification of larval dispersal distance. We can say only that dispersal dynamics appear to be sufficient to homogenize the gene pool in this region, which suggests long-distance dispersal.

One site in the Beaufort Sea (WBF) where larger-sized individuals were found did show significant genetic differentiation and lower (although non-significant) allelic richness than all other populations, indicating that a separate stock may be present at this location. Our results provide some evidence that the gene pool at this site may be receiving inputs from another as-yet unsampled population. This site was one of the deeper sites sampled in the Beaufort, which could indicate that a distinct population of larger individuals is living at slope depths below the sampled area. Alternatively, migrants or larvae may be entering the Beaufort Sea from some more distant Arctic location. This group of harvestable-sized crabs found in the Beaufort Sea should be explored further in an effort to determine whether an additional unsampled stock is present in deep waters.

The absence of a strict source-sink relationship between regions and evidence of extensive genetic exchange suggests northern stocks could persist through local recruitment even if populations continue to decline in the Bering Sea (North Pacific Fishery Management Council 2010). A widely-distributed panmictic population is likely to be resilient to local disturbance if recolonization can be facilitated by influx from neighboring locations. However, populations with numerous distinct units are also thought to be more resilient to local disturbance than panmictic populations such as those identified here (Schindler et al. 2010). The enhanced genetic diversity found in more structured populations is thought to confer an advantage in that impacts of a disturbance affecting one localized subpopulation might not be felt by other distinct groups within the region that possess a slightly different genetic make-up.

In contrast to snow crab, populations of Tanner (*Chionoecetes bairdi*) and red king crab (*Paralithodes camtschaticus*) show evidence of genetic divergence between Southeast Alaska, Gulf of Alaska, and Bering Sea regions and even within the Bering Sea (Tanner crab only) (Bunch et al. 1998; Merkouris et al. 1998; Grant et al. 2011). Larger-scale differentiation of these stocks is thought to be caused by physical barriers to movement created by the Aleutian Island chain and the southeast Alaskan archipelago, although population structure of Tanner crab within the Bering Sea is unexplained (Merkouris et al. 1998). Current flow and spawning-site fidelity may be responsible, although Merkouris et al. (1998) and this study found no evidence of population structure within Bering Sea snow crab which would also be affected by hydrographic features on the open shelf. No such substantial physical barriers appear to obstruct gene flow in snow crab inhabiting the northern Bering Sea and Chukchi shelf.

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Project Organization

Dr. Hardy was the lead investigator on this project and organized and/or conducted sample collection, served as the primary advisor to the graduate student conducting the analyses (Greg Albrecht), and executed all administrative aspects of the project, including report-writing. Dr. Iken served in an advisory role, contributing both opportunities for field work and expertise in the interpretation of project results. Dr. Hundertmark provided technical and analytical expertise as well as equipment for laboratory work. He further contributed substantially to graduate student training, including serving as a thesis committee member. Mr. Albrecht obtained samples from Bering Sea sites through ADF&G, conducted all laboratory analyses, and analyzed data under the supervision of Dr. Hundertmark. He also coordinated with an outside laboratory to conduct quality-control analyses, contributed substantially to report writing, presented results at state and national meetings including management agency meetings, and produced a manuscript that has been submitted to a peer-reviewed journal.

Study Products

Written reports and publications supported at least in part through this award:

Albrecht, G.T., Hundertmark, K.J., Hardy, S.M. Defining genetic population structure of snow crab (*Chionoecetes opilio*) in the Bering, Chukchi and Beaufort Seas. Can J Fish Aq Sci, Submitted.

Hardy, S.M., Lindgren, M., Konakanchi, H, Huettmann, F. 2011. Predicting the distribution and ecological niche of unexploited snow crab (*Chionoecetes opilio*) populations in Alaskan waters: A first open-access ensemble model. Integr Comp Biol **51**: 608-622.

Hardy, S.M., Iken, K., Hundertmark, K., Albrecht, G.T. 2011. Population connectivity in Bering, Chukchi and Beaufort Sea snow crab populations: Estimating spatial scales of disturbance impacts. University of Alaska Coastal Marine Institute Annual Report No. 17, pp. 56-66.

Hardy, S.M., Iken, K. 2010. Population connectivity in Bering, Chukchi and Beaufort Sea snow crab populations. University of Alaska Coastal Marine Institute Annual Report No. 16, pp. 68-72.

Oral and poster presentations:

Albrecht, G.T. June 2011. Genetic population structure and historical connectivity of snow crab (*Chionoecetes opilio*). University of Alaska, Fairbanks, MS Thesis Defense.

Albrecht, G.T., Hardy, S.M., Hundertmark, K., Lopez, J.A. 2011. Defining genetic population structure of snow crab (*Chionoecetes opilio*). Alaska Marine Science Symposium, Anchorage, AK.

Hardy, S.M., Albrecht, G.T., Hundertmark, K., Huettmann, F., Konakanchi, H., Lindgren, M., Bluhm, B., Sevigny, J.-M. 2011. Assessing distribution and structure of snow crab (*Chionoecetes opilio*) populations in Alaskan waters. Society for Integrative and Comparative Biology Annual Meeting, Salt Lake City, UT.

Albrecht, G.T., Hardy, S.M., Hundertmark, K. 2010. Defining genetic population structure in snow crab. University of Alaska Coastal Marine Institute, Annual Research Review, Fairbanks, AK.

Albrecht, G.T., Hardy, S.M., Hundertmark, K. 2010. Defining genetic population structure in snow crab (*Chionoecetes opilio*). Alaska Interagency Crab Meeting, Anchorage, AK.

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Table 1. Summary of trawl station and sample collection information. Station abbreviations correspond to those in Figure 2; (*N*) number of males (*m*) and females (*f*) analyzed from each station; depth in meters at each station; latitude and longitude at each station; (Avg. CW) average carapace widths of individuals collected at each sampling site ± standard deviation.

Site Name	**Abbreviation**	***N***	**Depth (m)**	**Lat (N): Long (W)**	**Avg. CW (mm)**
Pribilof Islands	PI	77*f*, 23*m*	71	57.50 : -168.75	50.5±6.1
Bering Sea	BR	50*f*	47	60.01 : -169.33	*
Saint Matthew Island	SMI	50*f*	61	60.66 : -172.12	43.9±4.0
Chukotka Coast	CH	50*f*	53	66.56 : -170.59	33.5±5.7
Point Hope	PH	*29f*	57	67.88 : -168.31	46.3±5.1
Cape Lisburne	CL	39*f*	47	68.57 : -166.55	35.4±4.5
SW Chukchi Shelf	SWC	50*f*	54	69.41 : -174.51	44.1±5.9
Wrangel Island	WI	26*f*	86	71.24 : -174.47	45.5±5.6
NW Chukchi Shelf	NWC	50*f*	146	73.21 : -175.34	35.6±4.4
N Chukchi Shelf	NC	7*f*, 17*m*	350	74.30 : -165.57	36.1±15.3
W Beaufort Slope	WBF (*station: 8*)	4*f*, 36*m*	320	71.72 : -152.84	82.1±14.8
Barrow Canyon	BC (*station: 7*)	8*f*, 65*m*	334	71.98 : -154.41	88.5□15.0
	BFP (*station:2)*	4*m*	478	71.89 : -154.95	25.4±3.1
	BFP (*station: 4)*	1*f*, 4*m*	356	71.90 : -153.91	20.5±3.4
	BFP (*station: 22)*	5*m*	182	71.51 : -152.20	34.6±14.4
Beaufort Sea Pooled	BFP (*station: 23)*	3*f*, 3*m*	45	71.58 : -155.05	37.1±11.5
	BFP (*station: 24)*	2*f*, 4*m*	49	71.68 : -154.48	30.4±6.8
	BFP (*station: 26)*	3*f*, 3*m*	53	71.55 : -153.48	59.5±6.1
	BFP (*all stations*)	9f, 23m			34.6±15.2

*Average carapace width is not available for this station; however, all females collected within the Bering Sea were between 30-65mm carapace widths and considered to be pre-pubescent/immature.

Table 2. Loci specific data. Number of alleles per locus; size range (base pairs); (H_O) observed heterozygosity; (H_S) gene diversity; (G_{IS}) the measure of individual diversity relative to its subpopulation (in this case sampling site); (G_{ST}) the measure of subpopulation diversity relative to the total; (*D*) Jost's (2008) measure of differentiation; ± standard deviation.

Locus	**Alleles**	**Size Range (bp)**	H_O	H_S	G_{IS}	G_{ST}	*D*
Cop2	24	291-341	0.835	0.838	0.003	0.000	0.000
Cop3	21	210-318	0.791	0.861	0.081	0.001	0.005
Cop4	12	211-259	0.228	0.267	0.145	0.000	0.000
Cop3-4II	22	119-209	0.865	0.911	0.050	0.002	0.016
Cop24-3	29	145-253	0.908	0.925	0.019	0.003	0.032
Cop113	23	114-166	0.885	0.892	0.007	0.000	0.000
ECO106	41	187-271	0.898	0.959	0.064	0.003	0.080
Mean/Global	24.6 ±8.8	-	0.773 ±0.244	0.807 ±0.242	0.043	0.001	0.004

Table 3. Summary of descriptive statistics at each station for Alaska region data. (*A*) allelic richness over all loci and rarefied to the smallest sample size; (H_O) observed heterozygosity ± standard deviation; significant departures from Hardy-Weinberg equilibrium (HWE)* marked with an X, based on comparisons of observed and randomized allele frequencies.

Sample site	***A***	***H_o***	***Cop 2***	***Cop 3***	***Cop 4***	***Cop 3-4II***	***Cop 24-3***	***Cop 113***	***ECO 106***
Pribilof Islands (PI)	13.9	0.762		X					X
Bering Sea west of Nunivak Island (BR)	13.9	0.790							
Sainte Mathews Island (SMI)	13.5	0.736		X		X			X
North of Chukotka (CH)	13.6	0.757				X			
Southwest of Point Hope (PH)	13.7	0.721			X	X			
Northwest of Cape Lisburne (CL)	14.0	0.765							X
Southwest Chukchi Sea (SWC)	13.7	0.786				X			
Wrangel Island (WI)	14.4	0.793							
Northwest Chukchi Sea (NWC)	13.4	0.793		X					
Northern Chukchi Sea (NC)	13.5	0.815							
Western Beaufort Slope (WBF)	11.4	0.779			X				
Barrow Canyon (BC)	13.2	0.776							X
Beaufort Sea Pooled (BFP)	12.5	0.772							X
Mean	13.42 ±8.8	0.773± 0.026							

*All significant departures from HWE were due to heterozygote defecits.

Table 4. Pairwise G_{ST} (top) and D (bottom) values for Alaskan region sampling sites. Negative values presented as zeros; bold values indicate significance after Bonferroni correction for multiple tests.

	PI	**BR**	**SMI**	**CH**	**PH**	**CL**	**SWC**	**WI**	**NWC**	**NC**	**WBF**	**BC**	**BFP**
BFP	0.0008	0	0.0011	0.0014	0.0017	0.0018	0	0	0.0015	0.0014	**0.0030**	0.0011	-
BC	0.0005	0.0016	0	0.0009	0	0.0009	0	0.0004	0	0	**0.0028**	-	**0.0397**
WBF	**0.0036**	**0.0024**	**0.0016**	**0.0030**	**0.0035**	**0.0026**	**0.0016**	**0.0026**	**0.0031**	**0.0024**	-	**0.0556**	**0.0685**
NC	0	0.0001	0	0.0006	0	0	0	0	0	-	**0.0598**	0	**0.0685**
NWC	0.0007	0.0009	0.0003	0.0013	0	0	0	0	-	0	**0.0729**	0	**0.0574**
WI	0.0018	0	0	0.0012	0	0	0	-	0	0	**0.0670**	0	0.0251
SWC	0.0009	0.0010	0	0	0	0	-	0	0	**0.0356**	**0.0578**	0.0062	0.0226
CL	0.0010	0	0.0004	0.0010	0	-	0.0121	0	0.0049	**0.0430**	**0.0659**	0.0282	**0.0706**
PH	0	0.0012	0	0	-	0	0.0140	0	0.001	0	**0.0849**	0	**0.0800**
CH	0.0006	0	0	-	0	**0.0357**	0	0.0097	0.0161	0.0222	**0.0560**	0.0092	**0.0485**
SMI	0.0008	0	-	0	0	0.0240	0	0.0034	0.0119	0.0097	0.0296	0	**0.0484**
BR	0.0016	-	0.0114	0.0006	0.0334	0.0104	0.0278	0	0.0256	0.0289	**0.0528**	**0.0361**	0.0085
PI	-	0.0197	0	0.0004	0	0.0267	0.0008	0	0.0057	0.0076	**0.0670**	0	0.0182

Table 5. Scoring consistency (%) between laboratory groups analyzing the same samples for the purpose of dataset validation.

Locus	Number compared	Allele scoring consistency (% similar)
Cop2	48	95/96 = 98.9%
Cop4	48	92/96 = 95.8%
Cop3-4II	44	87/88 = 98.8%
Cop 24-3	49	95/98 = 96.9%
Cop113	46	88/92 = 95.7%

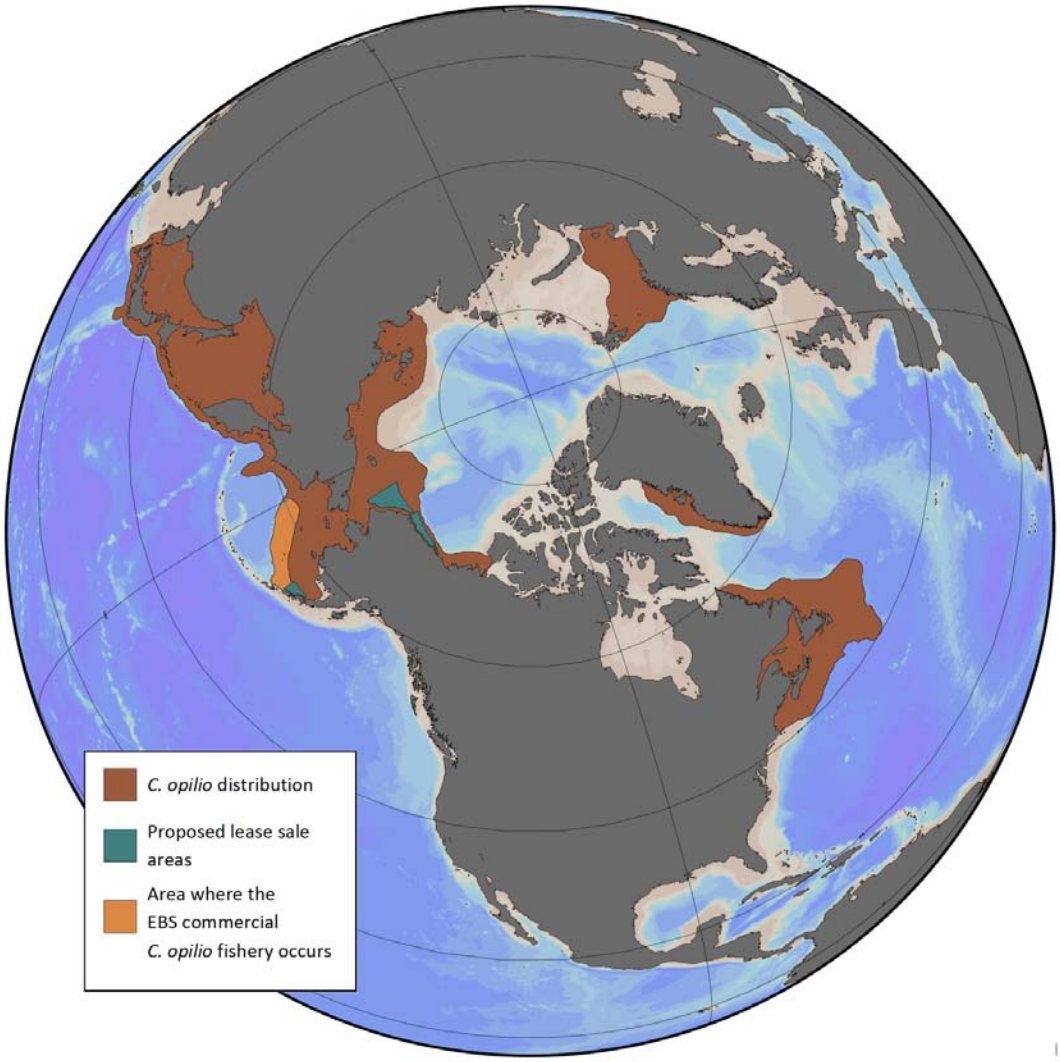

Figure 1. Known distribution of snow crab (maroon) with oil and gas lease areas (teal) and the Bering Sea commercial fishery (tan) highlighted (Rathbun 1925; Atkinson and Wacasey 1989; Squires 1990; Alvsvåg et al. 2009, www.boemre.gov).

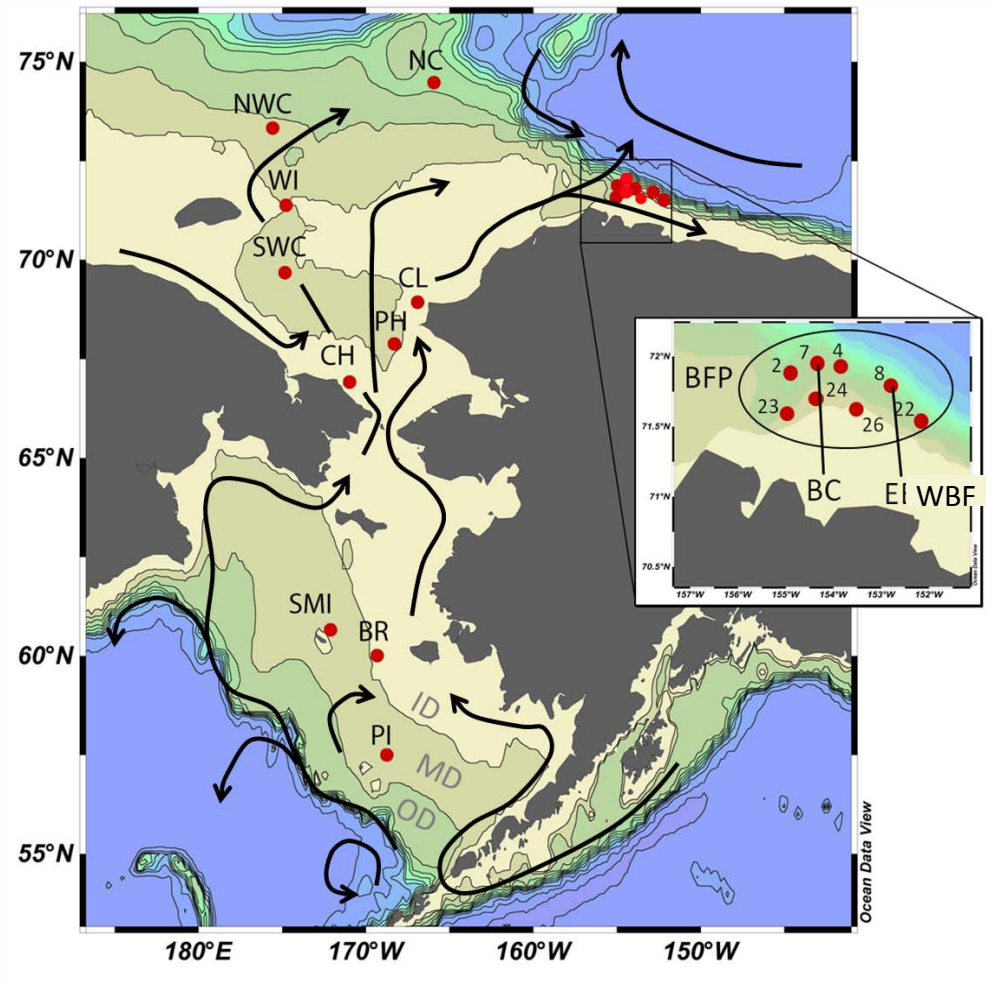

Figure 2. Map showing sample locations (red dots). Station BFP (inset) in the Beaufort Sea represents samples pooled from sites 2, 4, 22, 23, 24 & 26 (site numbers correspond to Rand and Logerwell 2011). Idealized oceanographic flow through the study area (based on Stabeno et al. 2001; Weingartner et al. 2005; Parada et al. 2010) shown by black arrows.

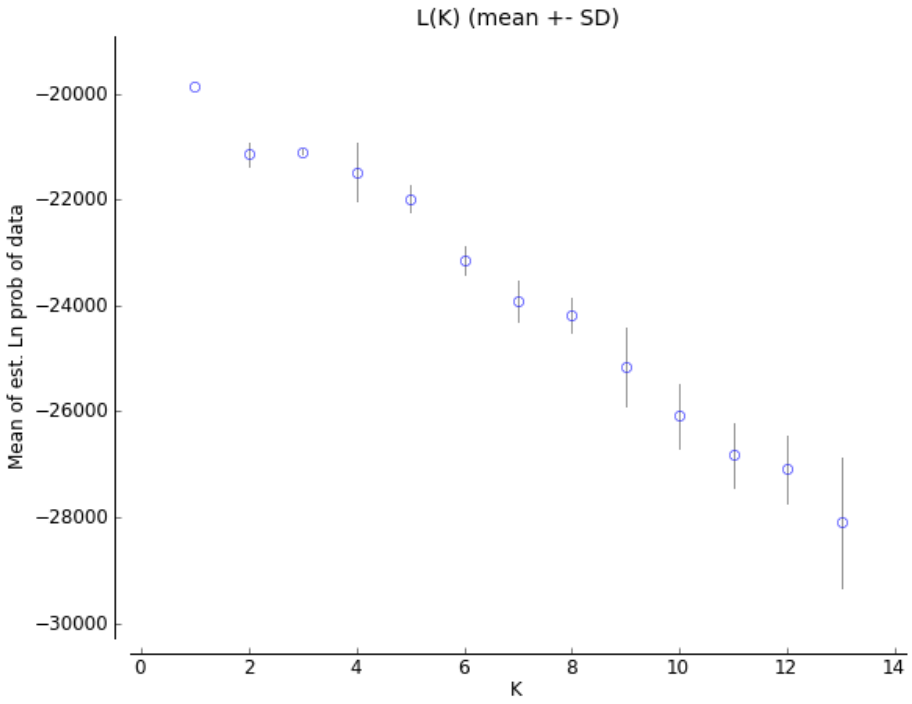

Figure 3. Mean of estimated ln-likelihood probabilities for all possible values of *K* (clusters) from 1 to 13. Probabilities were estimated using a Bayesian analysis method implemented in the program STRUCTURE and plotted using STRUCTURE Harvester.

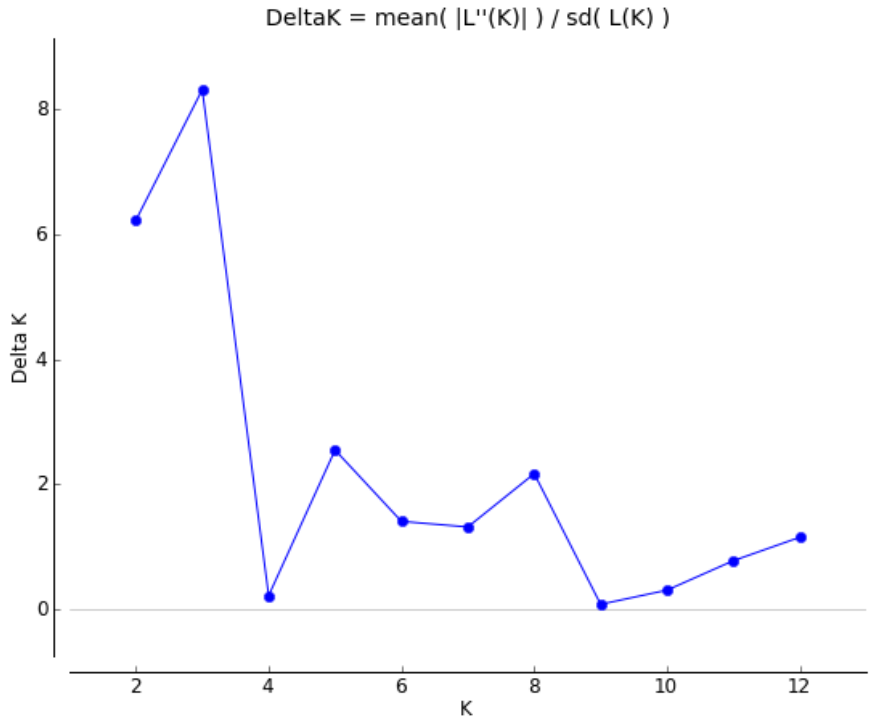

Figure 4. Second-order rate of change (Δ*K*) for cluster values of 2-13. Data were generated using a Bayesian analysis method implemented in the program STRUCTURE and plotted using STRUCTURE Harvester.

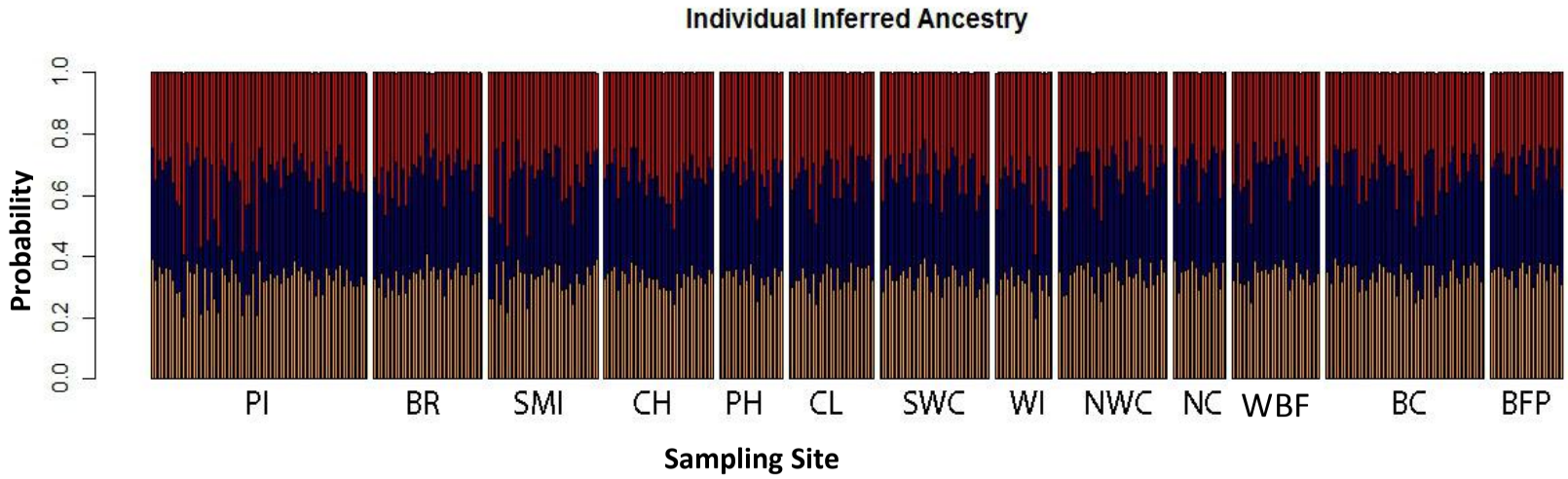

Figure 5. Graphical representation of Bayesian clustering analysis of the Alaska region from STRUCTURE. Each vertical line represents the probability that an individual's genotype corresponds to each cluster (K = 3).

The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.