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JOURNAL OF CRUSTACEAN BIOLOGY, 33(5), 691-701, 2013

Journal of Crustacean Biology

SPATIO-TEMPORAL POPULATION GENETIC STRUCTURE AND MATING SYSTEM OF RED KING CRAB (*PARALITHODES CAMTSCHATICUS*) IN ALASKA

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ABSTRACT

Red king crab, *Paralithodes camtschaticus* Tilesius, 1815, an economically and culturally important species in the state of Alaska, experienced drastic reductions in abundance over large portions of their Alaskan range by 1980. Abundance of crabs in some of the most important historical fishing areas have failed to rebound, some even in the absence of fishing, highlighting the need for additional research to infer genetic structure and reproductive biology of the species that can then be used to inform management efforts. Red king crab samples were collected from eleven locations throughout Alaska ($n = 845$), of these, six locations were sampled at least one generation apart. Results of this study suggest moderate rates of gene flow within the Gulf of Alaska/Western Alaska region. Levels of genetic differentiation among populations within Southeast Alaska were higher than seen elsewhere, and there was strong evidence of multiple distinct populations. Red king crab in Bristol Bay and in two areas in Southeast Alaska show signs of recent population bottlenecks and shifts in allele frequencies not observed in previous studies that used less polymorphic genetic markers. In addition to population genetic structure analyses, 24 female red king crab and their broods were collected for purposes of inferring mating system. There was no evidence of multiple paternity in any brood. The results of this study support continued management of distinct geographic groups within the Gulf of Alaska/Western Alaska region and suggest that finer-scale management may be beneficial in Southeast Alaska.

KEY WORDS: genetic differentiation, management, *Paralithodes camtschaticus*, population, red king crabs

DOI: 10.1163/1937240X-00002173

INTRODUCTION

Red king crab, *Paralithodes camtschaticus* Tilesius, 1815, have been a commercially important species in Alaskan waters since the 1930s when the Japanese fleet began harvesting crab in the eastern Bering Sea and near Kodiak Island in the Gulf of Alaska (Orensanz et al., 1998; Bowers et al., 2008; Bechtol and Kruse, 2009). United States fishing vessels began harvesting red king crab in the eastern Bering Sea in 1947, and while catch per unit effort reached a maximum in 1960, harvest continued to rise and eventually reached an annual peak of 129.9 million pounds in 1980 (Otto and Jamieson, 2001; Bowers et al., 2008; Dew, 2011). The economic value of the red king crab fishery was important to the development of coastal communities, such as Kodiak, and the current cultural and social importance of this species is evident in tourism, festivals, and mainstream media.

The collapse of red king crab populations in Alaska and their subsequent failure to rebound to historic abundances over the past few decades, despite cessation or reduction of commercial fishing (Wooster, 1992; Orensanz et al., 1998), highlight the need for a better understanding of the basic population structure, ecological interactions, and life history of this economically and culturally important species. Abundances of red king crab in Bristol Bay and around Kodiak Island are substantially lower than historic levels and, in the case of Kodiak Island, remain so even though the fishery has been closed since 1983 (Dew and McConnaughey, 2005; Bechtol and Kruse, 2009).

Difficulties associated with the assessment of spatial and temporal patterns of genetic differentiation among populations must be overcome in order to provide insight into the basic life-history of red king crab, such as larval dispersal and the location and approximate geographic scale of distinct mating subunits. Genetic divergence among large populations progresses slowly and can be reduced by the migration of only a few individuals per generation. Historical influences, such as post-glacial colonization, can also obfuscate divergence patterns. Marine fish and shellfish species, such as red king crab, have moderate to extended pelagic larval stages (on the order of months) and relatively large population sizes. These species often exhibit weaker genetic population structure than species with smaller population sizes and more sedentary larvae (Ward et al., 1994; Waples, 1998; Kinlan et al., 2005). This weak genetic structure previously led researchers to believe that many marine populations were essentially open, meaning that individuals were equally likely to mate with each other regardless of the geographic distances between them (Hauser and Carvalho, 2008). Advances in genetic techniques and increased

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DOI:10.1163/1937240X-00002173

knowledge of life history and dispersal patterns have led to the realization that many marine species do not have open populations as was once suspected. Rather, some marine species show high levels of population differentiation (Barber et al., 2002; Palumbi, 2004). Consequently, relationships between management boundaries and distinct genetic stocks may need to be reanalyzed (Palumbi, 1994; Strathmann et al., 2002; Sherman et al., 2008; Palof et al., 2011). The likelihood of detecting weak population structure in marine species may be increased by sampling larger numbers of loci or individuals, by using highly polymorphic markers, e.g., microsatellites, by acquiring temporally and spatially separated samples, and by coupling genetic data with information on life history, oceanography and species ecology (Waples, 1998).

Previous studies of red king crab population structure in Alaska reported low to moderate levels of overall genetic differentiation and suggested grouping populations into two or more regional subunits. Allozyme analyses by Seeb et al. (1989) and Grant et al. (2011) suggested grouping red king crab into three distinct regions: Southeast Alaska, Gulf of Alaska, and the Bering Sea. Grant et al. (2011) observed an overall $F_{ST} = 0.003 \pm 0.016$ and no significant difference among temporally spaced samples within Bristol Bay and three locations within Southeast Alaska (Barlow Cove, Seymour Canal, and Deadman Reach) as determined by homogeneity tests ($p > 0.05$). Seeb et al. (2001) reported genetic differences between crabs sampled in Southeast Alaska and those from other collections in the Gulf of Alaska and the Bering Sea and hinted at some finer-scale structuring within regions, but results were based on observed allele frequency differences among samples at five microsatellite loci that likely included the presence of null alleles. Grant and Cheng (2012) reported differences among three regions of Alaskan red king crab (Norton Sound and the Aleutian Islands, southeastern Bering Sea and western Gulf of Alaska, and Southeast Alaska) based on single nucleotide polymorphisms (SNPs, $F_{CT} = 0.054$) and mitochondrial DNA (mtDNA, $\Phi_{CT} = 0.222$).

While previous studies of red king crab population genetic structure suggest wide-scale differences over the species' Alaskan range, there is an opportunity to examine genetic divergence at a finer scale. Microsatellite markers mutate more rapidly than allozymes, mtDNA, or SNPs, thus providing the possibility of detecting genetic structure at a geographic scale that cannot be detected by these more slowly mutating markers. Microsatellite markers have been demonstrated to be better at distinguishing closely related populations than SNPs (Narum et al., 2008). The higher mutation rates and polymorphism of microsatellite markers also make them a better choice for detecting recent demographic events (Haasl and Payseur, 2011), such as reductions in effective population size, recent dispersal events, and parentage (Luikart and England, 1999).

The genetic mating system of red king crab has not been explored and it is unknown whether female crabs mate with only a single male or are polyandrous during breeding. The mating process in red king crab and the lack of spermathecae in females (Powell and Nickerson, 1965) suggest that female crabs likely remain monandrous during a given mating season, but the true mating system of a species cannot always be predicted by physiology and mating behavior (Birkhead and Hunter, 1990; Chapman et al., 2004). Mating system may vary spatially and temporally (Sainte-Marie et al., 2002; Gosselin et al., 2005; Neff et al., 2008). Gosselin et al. (2005) suggested that spatial variation observed in the rates of multiple paternity for American lobster (*Homarus americanus* H. Milne Edwards, 1837) may be a result of skewed sex ratios that result from sex-selective harvest of large males. Neff et al. (2008) reported that some of the variance observed in rates of multiple paternity of the guppy (*Poecilia reticulate*) could be explained by the levels of predation faced by different populations.

Recently, researchers and regional stakeholders have begun to examine the feasibility of stocking red king crab and blue king crab (*P. platypus* Brandt, 1850) in Alaskan waters as a supplement to natural populations and a potential method for overcoming recruitment limitation (see Swingle et al., 2013). Understanding the mating system as it relates to instances and rates of multiple paternity is then important for addressing issues of acceptable broodstock census size (N_c) and effective size (N_e). Mating system also influences the overall genetic variation present within broodstock family groups. The amount of genetic variation present in a group of individuals influences the number of genetic markers needed to discriminate that group within a larger population, and is vital to any efforts to monitor the survival, migration, and reproductive success of wild populations (Palsboll, 1999).

The first objective of this research is to examine red king crab spatial and temporal population genetic structure with microsatellite loci. By incorporating highly polymorphic microsatellite markers we increase statistical power to detect recent demographic events and to infer population genetic structure at a finer-scale than has been achieved in previous studies. Comprehending patterns of genetic subdivision of a species in space and time is crucial for understanding historic population-level events, as well as predicting future responses to anthropogenic impacts and changing environmental conditions. The second objective is to determine whether female red king crab mate singly or multiply. This information regarding population genetic structure and mating system is particularly important for red king crab in Alaska, because this information may contribute to improved management and subsequent chances of recovery for this commercially and culturally important species.

Materials and Methods

Sample Collection

A total of 845 red king crab individuals were collected from eleven locations spanning from Southeast Alaska to Norton Sound, and temporally spaced collections of individuals were obtained within Norton Sound, Bristol Bay, the Pribilof Islands, Barlow Cove, Gambier Bay and Deadman Reach (Fig. 1). Three broad geographic regions were defined *a priori* for population structure analyses: Western Alaska (including Adak Island, Norton Sound, Bristol Bay, and Pribilof Islands collections), Gulf of Alaska/Kodiak Island (including Kachemak Bay, Chiniak Bay, and Alitak Bay collections) and Southeast Alaska (including Deadman Reach, Gambier Bay, Barlow Cove and Seymour Canal collections). Muscle tissue samples for all individuals collected from 1988-1996 were obtained from the ADFG Gene Conservation Laboratory in Anchorage, and were originally collected from commercial fishing vessels or during stock surveys.

Ovigerous female red king crab for mating system analysis were collected in 2007 and 2008 with pots by a commercial fishing vessel from

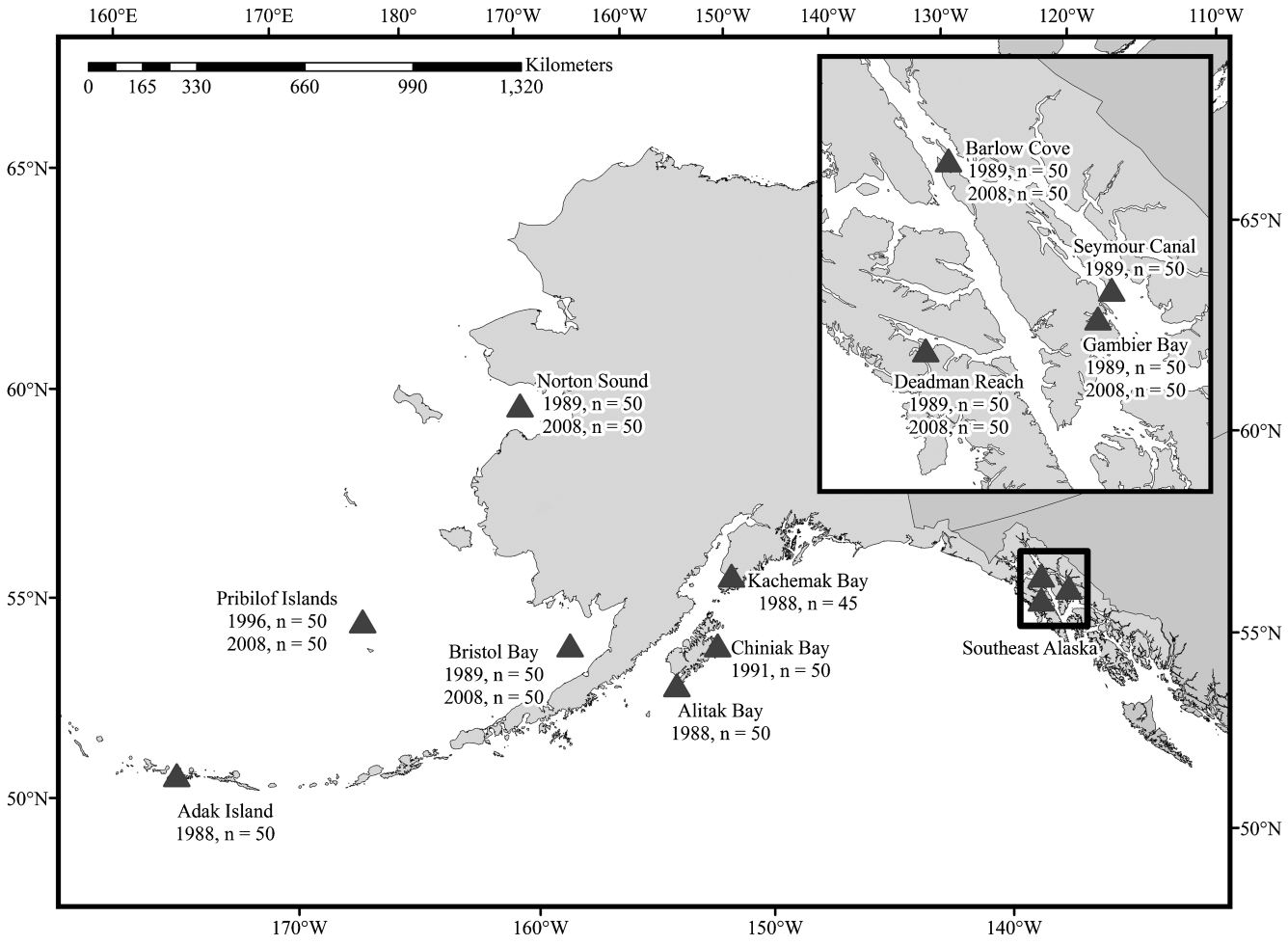

Fig. 1. Red king crab sample sizes, locations, and years for spatial and temporal population genetic structure analyses.

Bristol Bay. Females ranged from 106.7 to 159.6 mm carapace length in 2007 (mean = 132.7 mm) and from 121.5 to 148.3 mm carapace length in 2008 (mean = 129.9 mm); these are representative of the size range of multiparous females in Bristol Bay (Otto et al., 1990; Swiney et al., 2012). Female crabs were housed in individual containers once hatching began to ensure isolation of larvae. Tissue samples were collected from the chelae of 12 female crabs for each year and a haphazard sample of 20 total embryos and zoeal stage 1 larvae (Z1) were taken from the brood of each female for genotyping and paternity analysis. Three previously developed red king crab microsatellite loci (*Pca*101, *Pca*103 and *Pca*107; Seeb et al., 2002) were used to determine mating system.

Microsatellite Analysis

Genomic DNA was extracted from hemolymph samples following the procedure of Ivanova et al. (2006) and stored at −20°C. A proteinase K and ammonium acetate precipitation technique (Puregene DNA™ isolation protocol, Gentra Systems, Minneapolis, MN, USA) was used to extract genomic DNA from tissue samples and resulting samples were stored at −20°C. Six microsatellite loci were amplified for population genetic structure analyses using three previously designed primer pairs (*Pca*101, *Pca*103, and *Pca*107; Seeb et al., 2002), along with two redesigned primer pairs (*Pca*100B and *Pca*104B) and one newly designed primer pair (*Pca*201) (Table 1). Polymerase chain reaction (PCR) conditions and thermal profiles varied by microsatellite locus (Table 1). Reactions included forward primers fluorescently-labeled with IRDye® infrared dye (LI-COR, Lincoln, NE, USA). Fluorescently labeled primers composed 10% of the total forward primer concentration in each PCR reaction. Microsatellite loci were amplified using a DNA Engine Tetrad PTC-225 Peltier thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA) and resulting amplicons were visualized on a LI-COR 4300 DNA Analyzer. Microsatellite allele sizes were determined using Saga Generation 2 microsatellite analysis software v.3.2.1 (LI-COR).

Genetic Diversity and Population Genetic Structure

Collections of red king crab from 11 locations throughout Alaska were analyzed (Fig. 1). The software GENEPOP v.4 (Rousset, 2008) was used to perform pseudo-exact tests for departure from Hardy-Weinberg expectations at each locus (Guo and Thompson, 1992) and estimate gametic disequilibrium. The program FSTAT v.2.9.3.2 (Goudet, 1995) was used to estimate allele frequencies, observed (H_O) and expected (H_E) heterozygosity (Levene, 1949), F-statistics, pairwise F_{ST} values, genetic diversity measures, and to perform pairwise homogeneity tests. Homogeneity tests were performed between temporally spaced collections within locations and collections were pooled for subsequent spatial analyses if no significant difference in allele frequency was detected. Weir and Cockerham's (1984) θ was used for estimates of F_{ST} and 95% confidence intervals for multilocus F_{ST} estimates were established by bootstrap sampling across all loci (Goudet et al., 1996). Additionally, G'_{ST} (Hedrick, 2005) was estimated as a genetic differentiation measure that is corrected for subpopulation homozygosity using the program GenoDive v.2.0 (Meirmans and van Tienderen, 2004). Correcting for within-population diversity can be important when dealing with highly polymorphic markers such as microsatellites, because the maximum values possible for traditional F_{ST} and related measures are constrained by within-population genetic diversity from multiple alleles (Meirmans and Hedrick, 2011). Pairwise and overall estimates of Jost's (2008) D were obtained with the program SMOGD (Crawford, 2010). Red king crab allozyme allele frequencies from Grant et al. (2011) were also used to estimate Jost's (2008) D and to provide a comparison of differentiation levels with microsatellite

Table 1. Red king crab primer characteristics and thermal profiles.

Locus	Primers	Sequence	Thermal profile	GenBank accession no.
*Pca*100B	F: GGTGCTCATCATTACTCAGG R: TGTTGTTTCAGTTCGGGCT	$(TAA)_{11}$	92°C (5 min); 32 cycles of (92°C (30 s) + 56°C (30 s) + 1°C/s to 72°C + 72°C (20 s)); 72°C (30 min)	AY047220
*Pca*101	F: TTTCGGTTACTCGATATAATGC R: TTTTTCTCTGCTTACGAAGG	$(TATC)_{18}AA(TCAA)_4$	95°C (15 min); 32 cycles of (94°C (30 s) + 54°C (90 s) + 72°C (60 s)); 60°C (30 min)	AY047223
*Pca*103	F: AGAAAGGTCAAGTGTATTAGCC R: CAAATACGAGTAAGTTCTTTAGTGC	$(ATT)_{15}(AGT)_4$	95°C (15 min); 32 cycles of (94°C (30 s) + 54°C (90 s) + 72°C (60 s)); 60°C (30 min)	AY047221
*Pca*104B	F: GACACACATACACTTTCTCCATC R: GCTTGCTTCCTTGAGTGT	(TATC)	92°C (5 min); 32 cycles of (92°C (30 s) + 56°C (30 s) + 1°C/s to 72°C + 72°C (20 s)); 72°C (30 min)	AY047225
*Pca*107	F: ACCTCTCGTTGTAACTGTGC R: TACACCTTGCTGTTCAGTCC	(CTAT)	92°C (5 min); 32 cycles of (92°C (30 s) + 56°C (30 s) + 1°C/s to 72°C + 72°C (20 s)); 72°C (30 min)	AY047227
*Pca*201	F: ACTTTCGCTTCTGGGGGCAG R: GGCATGCTTATAACGTGCAG	(CATA)	94°C (2 min); 32 cycles of (94°C (30 s) + 60°C (40 s) + 72°C (60 s)); 72°C (20 min)	KF128983

data. Where appropriate, significance levels were adjusted with a sequential Bonferroni correction (Rice, 1989).

Spatial population genetic structure of red king crab was investigated with Spatial Analysis of Molecular Variance (SAMOVA) using the program SAMOVA v.1.0, which conducts a simulated annealing approach to maximize the proportion of total variance attributed to differences among groups of populations (Dupanloup et al., 2002). This eliminates the need to determine group assignment *a priori* when testing genetic structure. The annealing process was repeated 100 times to ensure the validity of the assignment of populations into K different groups. The process was repeated with values of $K = 2$ to 11. Suggested population groupings for each value of K were then tested in Arlequin v.3.1 (Excoffier et al., 2005) and the significant grouping that maximized the proportion of variance attributed to differences among groups of populations (F_{CT}) was chosen as the best population grouping. Each AMOVA analysis was performed with Arlequin using 10 000 random permutations to test for statistical significance.

Population structure was also inferred using the Bayesian clustering method of Pritchard et al. (2000) as implemented in the program Structure v.2.3.3, under the admixture model with correlated allele frequencies. A burn-in period of 1×10^5 steps, followed by 1×10^5 MCMC iterations, was used to test the likelihood that red king crab collections belonged to K populations. A range of K from 1 to 11 was explored, with three replicates at each value of K.

The statistical software R v.2.10.1 (R Development Core Team, 2011) was used to test for significant differences in genetic diversity, to examine patterns of isolation by distance, and to visualize similarities among sample collections through ordination. Differences in genetic diversity among regions were tested with analysis of variance (ANOVA) and Tukey's HSD tests. Tests for isolation by distance were conducted with a Mantel test in the *ecodist* R package (Goslee and Urban, 2007). Ten thousand permutations of the data were performed and 1×10^4 bootstrap replicates were used to calculate 95% confidence intervals. Principal component analysis (PCA) was performed on arcsine-square root transformed allele frequencies with the *adegenet* R package (Jombart, 2008) (see Table A1 in the Appendix in the online version of this article, which can be accessed via http://booksandjournals.brillonline.com/content/1937240x). Alleles with frequencies less than 0.05 in every sample were excluded to mitigate the effects of these rare alleles, while still maintaining most of the variation in the data. PCA is useful for visualizing relationships among multivariate data, because most of the variability in the data is captured in a small number of principal components without the necessity of adhering to strict and possibly unrealistic population structure model assumptions. Linear discriminant analysis was then performed on the first 50 principal components as a way to maximize among-group variation while minimizing within-group variation (Jombart et al., 2010).

Population Bottlenecks

The program BOTTLENECK v.1.2.02 (Cornuet and Luikart, 1996) was used to test for genetic signals resulting from severe decreases in abundance of red king crab in recent years. When a population experiences a severe bottleneck there is a loss of both heterozygosity and allelic diversity. For a period of time after a bottleneck event the reduction in allelic diversity progresses more rapidly than the decrease in heterozygosity, resulting in heterozygosity that is greater than what would be expected for the same number of loci if the system were at mutation-drift equilibrium (Cornuet and Luikart, 1996; McEachern et al., 2011). A two-phase model (TPM), which is a combination of a stepwise mutation model (SMM) and an infinite alleles model (IAM) was used because it has been shown to fit observed allele frequencies for microsatellite data better than either of the other two individual models (Di Rienzo et al., 1994; McEachern et al., 2011). Three proportions of SMM in the TPM were used (70%, 80%, and 90% SMM) and excess heterozygosity was tested with a Wilcoxon signed-rank test. Populations were pooled as determined previously by homogeneity tests.

Mating System

Power to detect multiple paternity within a sample of embryos and Z1 from a single brood was determined with the program PrDM v.1 (Neff and Pitcher, 2002). The program PrDM uses a Monte Carlo simulation method to determine the probability that multiple mating will be detected given the number of loci used, the number of alleles at each locus, the population allele frequencies, and the number of potential sires and their relative reproductive contributions (Neff and Pitcher, 2002). Population allele frequencies for Bristol Bay red king crab in 2008 were determined with the program FSTAT v.2.9.3.2 (Goudet, 1995). Three loci with relatively high degrees of polymorphism (*Pca*101, *Pca*103 and *Pca*107; Seeb et al., 2002) were selected and simulations were conducted that involved two potential sires with relative reproductive contributions of gametes to zygotes of 1:1 and 9:1. The probability of detecting multiple paternity is also dependent on

the number of offspring sampled, so scenarios involving 10 and 20 offspring were tested for each of the above paternal contribution ratios.

Single paternity would be rejected if three or more non-maternal alleles were present within any given brood at a single locus. Requiring three non-maternal alleles as the condition for rejecting single paternity provides a conservative estimate of the minimum number of potential contributing sires by assuming that sires are heterozygous at each locus (McKeown and Shaw, 2008).

RESULTS

Genetic Diversity

Both within-population heterozygosity (H_S) and allelic richness (AR) showed a great deal of genetic variation across the study area. Mean within-population expected H_S was 0.82 (range: 0.54 to 0.92) and mean within-population AR was 9.62 (range: 5.24 to 14.63). H_S and AR were lower in collections from Southeast Alaska than in those from Western Alaska and the Gulf of Alaska (Fig. 2; H_S: Western Alaska – Southeast Alaska, $p < 0.001$; Gulf of Alaska – Southeast Alaska, $p < 0.001$; AR: Western Alaska – Southeast Alaska, $p = 0.008$; Gulf of Alaska – Southeast Alaska, $p = 0.001$). There were no differences in genetic variation between the latter two regions.

No populations showed departure from Hardy-Weinberg expectations after sequential Bonferroni correction (initial $p > 0.05$). After sequential Bonferroni correction, no locus pair for a collection showed evidence of significant departure from gametic disequilibrium.

Temporal Population Structure

Temporal AMOVA of genetic variation over space and time suggested that temporal genetic variation (0.39%, df = 6, $p < 0.001$) accounted for less variation than was distributed spatially (2.03%, df = 5, $p < 0.001$), but both contributions were still significant. Pairwise homogeneity tests were not significant within Norton Sound, Pribilof Islands, or Barlow Cove (Table 2), which suggested stability of allele frequencies over time. Collections for each of these three locations were pooled across years for subsequent spatial analyses. Deadman Reach showed the most striking difference between temporal collections as indicated by F_{ST} estimate (Table 2) and discriminant analysis of principal components (Fig. 3). Temporally spaced collections within Barlow Cove and Gambier Bay in Southeast Alaska also showed a separation along the first and second discriminant axes, but no such relationship was immediately evident among temporally spaced collections in Western Alaska (Fig. 3).

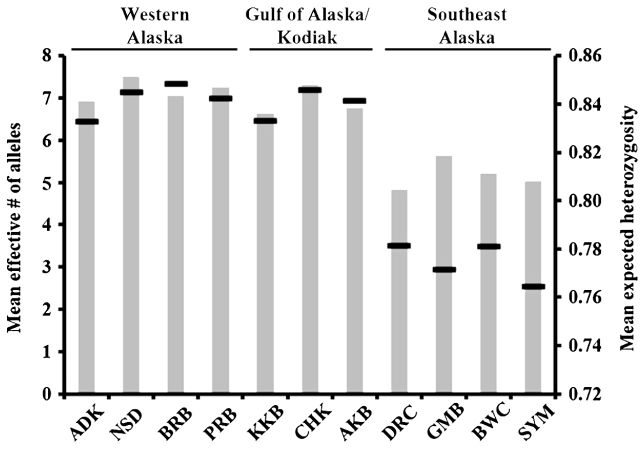

Fig. 2. Mean effective number of alleles (columns) and mean expected heterozygosity (dashes) of red king crab populations. Locations: ADK (Adak Island), NSD (Norton Sound), BRB (Bristol Bay), PRB (Pribilof Islands), KKB (Kachemak Bay), CHK (Chiniak Bay), AKB (Alitak Bay), DRC (Deadman Reach), GMB (Gambier Bay), BWC (Barlow Cove), SYM (Seymour Canal).

Table 2. Pairwise F_{ST} values and p-value of homogeneity tests among pairs of temporally-spaced red king crab samples.

Sample	F_{ST}	p
Norton Sound	0.002	0.150
Bristol Bay	0.009	0.003
Pribilof Islands	0.002	0.830
Deadman Reach	0.028	<0.001
Gambier Bay	0.013	0.001
Barlow Cove	0.007	0.999

Spatial Population Structure

Moderate levels of population differentiation, with an overall $F_{ST} = 0.025$ (SE = 0.009), were observed. After correction for multiple tests, 49 of 55 pairwise homogeneity tests were significant (Table 3). Pairwise comparisons between Southeast Alaska populations and all others were uniformly significant, as indicated by relatively high F_{ST} and D

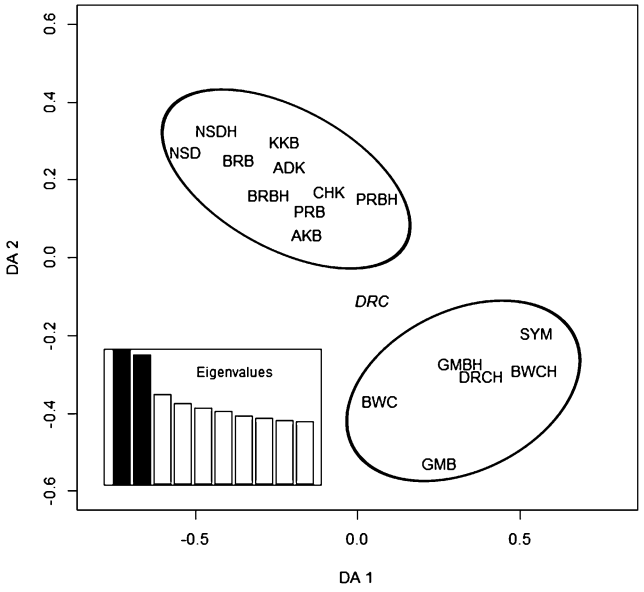

Fig. 3. Linear discriminant analysis of principal components based on arcsine-square root transformed allele frequency data from 11 locations where red king crab were sampled. Ellipses represent the two regions of the Bering Sea/Gulf of Alaska, and Southeast Alaska. Locations: ADK (Adak Island), NSD (Norton Sound), BRB (Bristol Bay), PRB (Pribilof Islands), KKB (Kachemak Bay), CHK (Chiniak Bay), AKB (Alitak Bay), DRC (Deadman Reach), GMB (Gambier Bay), BWC (Barlow Cove), SYM (Seymour Canal). Four letter abbreviations ending in "H" denote historic samples. The inset graph shows the proportion of variation described by the first ten principal components. DRC was excluded from the ellipse enclosing Southeast Alaska because it showed signs that bottlenecks had greatly affected allele frequencies since the first sample was collected at this location (DRCH).

Table 3. Pairwise estimates of F_{ST}/D (Jost, 2008) are given below the diagonal and p-values for homogeneity tests are given on the upper diagonal. Locations: ADK (Adak Island), NSD (Norton Sound), BRB (Bristol Bay), PRB (Pribilof Islands), KKB (Kachemak Bay), CHK (Chiniak Bay), AKB (Alitak Bay), DRC (Deadman Reach), GMB (Gambier Bay), BWC (Barlow Cove), SYM (Seymour Canal).

	ADK	NSD	BRB	PRB	KKB	CHK	AKB	DRC	GMB	BWC	SYM
ADK	–	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
NSD	0.011/0.040	–	<0.001	<0.001	0.021	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
BRB	0.016/0.054	0.007/0.015	–	<0.001	<0.001	<0.001	0.000	<0.001	<0.001	<0.001	<0.001
PRB	0.007/0.027	0.010/0.022	0.014/0.038	–	0.008	0.008	0.458	<0.001	<0.001	<0.001	<0.001
KKB	0.015/0.067	0.004/0.014	0.014/0.060	0.006/0.013	–	0.144	0.229	<0.001	<0.001	<0.001	<0.001
CHK	0.014/0.047	0.008/0.025	0.008/0.021	0.003/0.001	0.004/0.003	–	0.173	<0.001	<0.001	<0.001	<0.001
AKB	0.010/0.034	0.005/0.006	0.009/0.018	0.000/0.000	0.001/0.000	0.000/0.000	–	<0.001	<0.001	<0.001	<0.001
DRC	0.057/0.164	0.045/0.125	0.036/0.079	0.037/0.072	0.044/0.100	0.027/0.031	0.033/0.046	–	<0.001	<0.001	<0.001
GMB	0.052/0.166	0.056/0.123	0.054/0.107	0.033/0.106	0.047/0.134	0.034/0.056	0.027/0.065	0.048/0.044	–	<0.001	<0.001
BWC	0.045/0.163	0.049/0.140	0.05/0.125	0.022/0.063	0.037/0.093	0.022/0.029	0.018/0.038	0.031/0.010	0.011/0.015	–	<0.001
SYM	0.048/0.171	0.054/0.177	0.055/0.152	0.028/0.099	0.042/0.138	0.024/0.047	0.027/0.076	0.034/0.020	0.021/0.030	0.004/0.001	–

estimates and homogeneity tests (Table 3). Hedrick's (2005) correction for within-population genetic diversity, which is useful when dealing with highly polymorphic loci like those used in this study, provided evidence for greater divergence among populations with an overall $G'_{ST} = 0.128$ ($p = 0.001$, SE $= 0.046$). Jost's (2008) D provided an overall estimate of differentiation $D = 0.074$ (SE $= 0.001$). Overall $F_{ST} = 0.010$ (SE $= 0.003$) was observed among Adak Island, Norton Sound, Bristol Bay, and Pribilof Island collections in Western Alaska. Among Chiniak Bay, Alitak Bay, and Kachemak Bay collections in the Gulf of Alaska, overall $F_{ST} = 0.002$ (SE $= 0.002$) was observed. Overall differentiation was higher among Deadman Reach, Barlow Cove, Gambier Bay, and Seymour Canal collections in Southeast Alaska, with $F_{ST} = 0.021$ (SE $= 0.013$).

Splitting all of the red king crab collections into five groups was determined by SAMOVA as the configuration that maximized the proportion of total variation attributed to differences among groups ($F_{CT} = 0.025$, $p < 0.001$; Table 4). The first group consisted solely of Adak Island and the second group was composed of the remaining collections in the Western Alaska and Gulf of Alaska regions. Collections in Southeast Alaska were then divided into three groups, which included Gambier Bay and Deadman Reach as single-population groups, and Barlow Cove and Seymour Canal as a single group. Estimates of F_{CT} decreased for values of K above and below five, however estimates of F_{CT} were significant for values of $K = 3$ through 8 ($p < 0.05$; Table 4).

Bayesian clustering in the program Structure resulted in a maximum likelihood at $K = 2$. The first cluster included the four collections from Southeast Alaska and the second cluster included all others (data not shown). Similarly, linear discriminant analysis of principal components revealed a separation between Southeast Alaska collections and all others (Fig. 3). The first principal component explained 42.3% of the total variation and the second principal component explained 11.7%. The first and second discriminant axes show a clear separation between Southeast Alaska collections and those in Western Alaska and the Gulf of Alaska (Fig. 3). There is no distinct separation between Western Alaska and Gulf of Alaska collections. Discriminant analysis of principal components also shows greater separation among Southeast Alaska collections than is seen for the remaining collections (Fig. 3).

Comparison of pairwise $F_{ST}/(1 - F_{ST})$ and straight-line distance matrices for all pairs of spatial populations (historic collections excluded) resulted in a significant isolation by distance pattern (Mantel $r = 0.618$, df $= 54$, $p < 0.001$). When populations were split into the two regions suggested by the other spatial analyses, the pattern broke down, for both Southeast Alaska (Mantel $r = -0.067$, df $= 5$, $p = 0.541$) and all other populations (Mantel $r = 0.52$, df $= 20$, $p = 0.064$). However, there is little power to detect a pattern within Southeast Alaska with only four collections.

Population Bottlenecks

Tests of heterozygosity excess suggested recent population bottlenecks at three locations, but the results were highly dependent upon the assumed underlying mutational model. The historic collections from Bristol Bay and Gambier

Table 4. Spatial analysis of molecular variance (SAMOVA) groupings that maximized proportion of total variance among groups of populations (F_{CT}) and minimized values representing the extent of differentiation between populations within groups (F_{SC}) for $K = 2$ to 9. Locations: ADK (Adak Island), NSD (Norton Sound), BRB (Bristol Bay), PRB (Pribilof Islands), KKB (Kachemak Bay), CHK (Chiniak Bay), AKB (Alitak Bay), DRC (Deadman Reach), GMB (Gambier Bay), BWC (Barlow Cove), SYM (Seymour Canal).

K	Groupings	F_{SC}	F_{CT}	p
2	(ADK) and (all others)	0.022	0.002	0.542 ± 0.005
3	(ADK), (NSD, BRB, PRB, KKB, CHK, AKB), (DRC, GMB, BWC, SYM)	0.010	0.021	0.001 ± 0.000
4	(ADK), (NSD, BRB, PRB, KKB, CHK, AKB), (GMB), (DRC, BWC, SYM)	0.008	0.022	0.001 ± 0.000
5	(ADK), (NSD, BRB, PRB, KKB, CHK, AKB), (GMB), (DRC), (BWC, SYM)	0.005	0.025	0.000 ± 0.000
6	(ADK), (CHK), (NSD, BRB, PRB, KKB, AKB), (GMB), (DRC), (BWC, SYM)	0.005	0.022	0.001 ± 0.000
7	(ADK), (CHK), (NSD, BRB, PRB, KKB, AKB), (GMB), (DRC), (BWC), (SYM)	0.007	0.020	0.013 ± 0.001
8	(ADK), (CHK), (NSD), (BRB, PRB, KKB, AKB), (GMB), (DRC), (BWC), (SYM)	0.006	0.018	0.036 ± 0.002
9	(ADK), (CHK), (NSD), (BRB, AKB), (PRB, KKB), (GMB), (DRC), (BWC), (SYM)	0.006	0.016	0.067 ± 0.003

Bay, as well as the recent Deadman Reach collection, had significant heterozygosity excess ($p < 0.05$) under the 70% SMM. No collections had significant heterozygosity excess for the 90% SMM or 100% SMM models. These three locations were the only ones to show a significant change in F_{ST} over time (Table 1).

Mating System

The genotyping of 20 offspring (the number assayed in the study) from each of 24 broods provided 99.8% probability of detecting multiple paternity when assuming equal contribution of gametes from two males to each brood, and 85.6% probability when assuming an unequal input of gametes from two males to each brood at a ratio of 9:1. No evidence of multiple paternity was detected in 24 broods of red king crab, because each brood included two or fewer nonmaternal alleles at each locus (Table 5). Each brood consisted of genotypes that could be produced by the known maternal genotype and the contribution of a single male of unknown genotype.

Discussion

Genetic Diversity and Population Structure

The most striking patterns in red king crab population genetic structure come from the differences observed in populations from Southeast Alaska versus those from the rest of Alaska. The populations in Southeast Alaska show the highest levels of within-region genetic divergence, as well as high genetic divergence from populations in the rest of the study area, while also having the lowest within-population genetic diversity. This may be a result of genetic drift acting at a faster rate on relatively smaller red king crab populations in Southeast Alaska or the results of deeper historic patterns such as re-colonization from glacial refugia. Southeast Alaska is dominated by small bay and fjord systems (Weingartner et al., 2009) and localized currents in this region may result in higher degrees of local larval retention than are present in the more open waters of the Bering Sea and Western Alaska.

Results from discriminant analysis of principal components, Bayesian clustering analysis, and pairwise F_{ST} estimates all are consistent with a two-region model of broad genetic differentiation and distinguish Southeast Alaska from all other collections. The large geographic distance between Southeast Alaska and the rest of the sample locations make the potential for larval transfer less likely. Southeast Alaska is also near the southernmost range of red king crab and populations are unlikely to receive larval input from other distant locations due to the predominantly northward flow of the Alaska Current (Stabeno et al., 2004). Complex currents within Southeast Alaska and the Gulf of Alaska may also inhibit larval transport between them, resulting in two regional metapopulations that are genetically distinct from one another.

Larger population sizes and increased connectivity in the Gulf of Alaska and Western Alaska likely account for the decrease in genetic divergence among populations relative to Southeast Alaska. Ocean currents flow predominantly from the western Gulf of Alaska through passes in the Aleutian Islands and northward into the Bering Sea (Stabeno et al., 2001, 2004). These currents may serve as a conduit for larval transport and provide a mechanism for connectivity, in contrast to Southeast Alaska where more complex oceanography may inhibit gene flow.

While the greatest differences exist between Southeast Alaska and the Western Alaska/Gulf of Alaska regions, SAMOVA results suggest some finer-scale genetic structuring within regions. The apparent difference between Adak Island and the other Western Alaska/Gulf of Alaska populations is likely due to the relative geographic isolation of Adak Island. Adak Island is located near the westernmost end of the Aleutian Islands and larval migration to and from the area is likely to be interrupted by the complex ocean currents that exist within the Aleutian Island chain (Stabeno et al., 2001, 2004). Red king crab populations within Southeast Alaska show signs of genetic differentiation over a much smaller geographic scale. Smaller genetic effective sizes and decreased connectivity of populations in Southeast Alaska may account for this genetic structure.

Although samples from before the red king crab population declines of the late 1970s were not available to directly assess before-and-after genetic effects, temporal genetic structure analyses after the collapse suggested heterogeneity within some locations. Significant shifts in allele frequency over time observed within Bristol Bay, Deadman Reach, and Gambier Bay, may be the result of recent population bottlenecks. This pattern of temporal shifts in allele frequencies in multiple populations argues for an important role for genetic drift in at least some of the red king crab

Table 5. Alleles detected at each microsatellite locus within each brood as determined by genotyping offspring and mothers. Multiple paternity would be indicated by the presence of three or more non-maternal alleles at a given locus within a brood. Allele sizes represent the number of base pairs. Maternal alleles are in boldface.

Year	Female and brood	*Pca*101 alleles	*Pca*103 alleles	*Pca*107 alleles
2007	1	**247**, **263**, 267	**245**, 248, **260**, 269	215, **223**, **231**, 235
	2	**247**, 263, **267**	**248**, 251, **257**	**223**, 227, **231**
	3	251, **255**, 259, **263**	**257**, **269**	235, **243**, **263**
	4	247, **251**, **263**, 279	**248**, 254, **257**	**211**, 223, 235, **239**
	5	239, **243**, 259, **263**	**248**, 251, **257**, 260	223, **235**, **259**
	6	243, 251, **259**, **263**	**251**, **257**, 269	239, 247, **259**, **263**
	7	223, **227**	**245**, **254**, 257	**203**, 211, 235
	8	**227**, 235	**257**, 266	**223**, 231, 247
	9	251, 255, **263**, **267**	248, **254**	235, **255**, **263**, 271
	10	243, **247**, **267**	**248**, **257**	**203**, **227**, 235, 259
	11	243, **251**	**248**, **254**, 257, 260	**235**, 239, **255**
	12	**239**, 243, 259, **263**	**245**, 248	231, **239**, **243**, 255
2008	13	**227**, 235, **263**	245, **254**	**223**, 243, **251**, 259
	14	**235**, 239, **251**, 267	**251**, **254**	235, **243**, **259**
	15	251, **259**, **263**	248, 251, **260**, **263**	**235**, 239, 243, **251**
	16	247, **255**, **263**	**248**, **257**	219, **251**, **255**
	17	**243**, **259**, 267	**245**, 254, **260**	**223**, **255**, 257, 263
	18	**223**, 239, **251**, 263	248, **257**, **263**	203, **227**, **235**, 271
	19	239, **243**, 247, **259**	251, **257**, **269**	**235**, **243**, 255
	20	243, 251, **259**	**248**, 251, **257**, 260	**223**, 243, **247**, 255
	21	239, **243**, **259**, 263	**248**, 251, 257	227, **239**, 263, **265**
	22	**243**, 247, **255**, 259	**251**, 254, **257**, 269	**223**, 235, 255, **259**
	23	237, **243**, 255, **263**	**245**, 251, **254**	223, **251**, 259, **271**
	24	**227**, 237, **243**, 263	**242**, 251, **254**	**235**, 239, **247**

populations, and suggests that harvest can drive local abundances to extremely low numbers in some locations.

Bottlenecks occur when a population undergoes a drastic reduction in effective size that reduces genetic variation. This leads to an increase in heterozygosity of sampled selectively neutral loci, compared to what would be expected for the same number of alleles if the population were at mutation-drift equilibrium. This condition may persist for several generations until a new equilibrium is reached (Cornuet and Luikart, 1996). However, false bottleneck signals have recently been observed among single populations within a stepping-stone or island model (Wakeley, 1999; Stadler et al., 2009). Chikhi et al. (2010) demonstrated that this effect is most prevalent when employing markers with high variability or when populations have a large genetic effective size. It is also worth noting that significant bottleneck signals observed in this study (Bristol Bay, Gambier Bay, and Deadman Reach) were also highly dependent on the assumed mutational model. Nevertheless, we are confident that a recent bottleneck in Deadman Reach explains the observed changes in allele frequency and F_{ST} results that show this population has changed dramatically over time. Reports from field biologists that red king crab at Deadman Reach have been at extremely low density during recent stock abundance surveys are consistent with the population bottleneck signals present in our data from this site (G. Bishop, Alaska Department of Fish and Game, personal communication, 2010). Previous studies, including Grant et al. (2011) and Grant and Cheng (2012), did not detect population bottlenecks at any location, but used less polymorphic and less sensitive genetic markers.

Overall levels of genetic divergence among collections suggest some moderate structuring of red king crab populations in Alaska (overall $F_{ST} = 0.025$). The overall estimate of Jost's (2008) D for this study (0.074) was substantially higher than overall D (<0.001) estimated by averaging across allozyme loci using the data of Grant et al. (2011). Levels of differentiation observed in other invertebrate species with planktonic larval periods similar to those of red king crab were often lower than observed in this study. Puebla et al. (2008) reported $F_{ST} = 0.011$ (95% CI: 0.008-0.015) from microsatellite markers for snow crab (*Chionoecetes opilio* O. Fabricius, 1788) in the northwest Atlantic. Merkouris et al. (1998) reported $F_{ST} = 0.0046$ for allozyme data from Tanner crab (*Chionoecetes bairdi* Rathbun, 1924) in Alaska. Gaffney et al. (2010) reported $F_{ST} = 0.004$ (range: −0.005 to 0.0011) from microsatellite data for weathervane scallops (*Patinopecten caurinus* Gould, 1850) in the northeast Pacific. Beacham et al. (2008) reported slightly higher overall differentiation ($F_{ST} = 0.031$, SD = 0.007) from microsatellite data for Dungeness crab (*Metacarcinus magister* Dana, 1852) in British Columbia. Hedrick's (2005) measure of differentiation (G'_{ST}) corrected for the high heterozygosity levels often present in microsatellite data and provided an overall measure of differentiation that is well above that provided by traditional F-statistics ($F_{ST} = 0.025$ versus $G'_{ST} = 0.128$). This number may more accurately describe the degree of differentiation among collections, because it adjusts for the maximum within-population expected heterozygosity, while simultaneously making it easier to compare levels of differ-

entiation among studies that employ markers with differing levels of variability (Meirmans and Hedrick, 2011).

Mating System

Our results from 24 female red king crab and their broods are all consistent with single paternity as the dominant mating system in red king crab. These results are reasonable, given the females' inability to store sperm and the mate-guarding behavior exhibited by male crabs.

The single mating of red king crab females means a greater number of females are needed in order to create broodstock of sufficiently large N_e for an enhancement program than would be needed with multiple paternity, because multiple paternity typically increases N_e (Pearse and Anderson, 2009). Large N_e is important because it decreases the loss of genetic variation and fitness that occur in small populations (Schultz and Lynch, 1997).

Single paternity, however, may make it easier to detect hatchery produced individuals in the wild via genetic tagging. Fewer genetic markers may be employed to assign individuals to hatchery or wild origin if hatchery-reared family groups are less genetically diverse as a result of single paternity. If enhancement of red king crab populations in Alaska takes place, these genetic markers can be used to monitor the survival, migration, and reproductive success of introduced individuals in order to determine the genetic impacts of the stocking program.

General Conclusions and Management Recommendations

Understanding the microevolutionary forces that influence population genetic structure over space and time is critical when dealing with species of commercial importance, such as red king crab in Alaska. Results of this study suggest moderate rates of gene flow within the Gulf of Alaska/Western Alaska region and support management of populations at a scale no smaller than that considered for this study. Other lines of evidence from life history or morphology may provide reason for stock management in these regions to occur at a smaller scale. Levels of genetic differentiation among populations within Southeast Alaska were higher than those in the other regions surveyed, and there was strong evidence of multiple distinct populations, suggesting this region warrants management decisions at a smaller geographic scale than is necessary elsewhere. Red king crab in Bristol Bay and the Deadman Reach and Gambier Bay areas in Southeast Alaska show signs of recent population bottlenecks and shifts in allele frequencies not observed elsewhere, which may be indicative of small numbers of breeders in these locations. Single paternity in red king crab likely results in decreased genetic diversity within family groups and lower N_e. If releases of hatchery-raised crab are used to enhance wild stocks, the mating system impacts on N_e, along with the observed patterns of population divergence, should be used to minimize risks to the genetic integrity of wild populations.

ACKNOWLEDGEMENTS

The authors would like to thank P. Jensen, J. F. Morado, B. Daly, R. Foy, C. Shavey and all those involved in the collection of samples for this project. The authors would also like to thank W. D. Templin, W. S. Grant, and T. Harrington of the Alaska Department of Fish and Game Gene Conservation Laboratory for providing historic samples. G. L. Eckert, A. J. Gharrett, R. P. Kovach, and P. D. Barry all provided comments and constructive reviews throughout the project. Partial funding was provided by Alaska Sea Grant and a NOAA aquaculture grant.

The findings and conclusions in the paper are those of the authors and do not necessarily represent the views of the National Marine Fisheries Service, NOAA. Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

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RECEIVED: 3 December 2012.
ACCEPTED: 24 April 2013.
AVAILABLE ONLINE: 5 June 2013.

APPENDIX

Table A1. Loadings for the first two principal components obtained from arcsine-square root transformed allele frequencies.

Locus	Allele	PC1	PC2
*Pca*100B	280	0.1953	0.1127
*Pca*100B	289	0.0018	−0.0791
*Pca*100B	292	−0.1579	−0.0570
*Pca*100B	295	0.0621	0.0823
*Pca*100B	298	−0.1380	−0.0263
*Pca*100B	301	−0.0332	−0.0211
*Pca*100B	304	−0.1031	−0.1001
*Pca*101	227	−0.1441	0.0244
*Pca*101	235	−0.1257	0.1101
*Pca*101	239	−0.1635	0.0705
*Pca*101	243	−0.0835	0.2538
*Pca*101	247	−0.2725	−0.2598
*Pca*101	251	−0.1470	0.1881
*Pca*101	255	−0.0321	0.0322
*Pca*101	259	0.0339	−0.0522
*Pca*101	263	0.3839	0.2955
*Pca*101	267	0.0159	−0.5875
*Pca*103	242	−0.0027	0.0549
*Pca*103	245	0.0491	0.1880
*Pca*103	248	0.2257	−0.0755
*Pca*103	251	0.0331	0.0524
*Pca*103	254	0.0347	0.1064
*Pca*103	257	−0.2321	−0.0032
*Pca*103	260	−0.0375	−0.1006
*Pca*103	263	−0.0799	−0.1183
*Pca*103	266	−0.1981	−0.0051
*Pca*103	269	0.1337	−0.1031
*Pca*104B	194	0.0404	0.1019
*Pca*104B	202	−0.0030	−0.0398
*Pca*104B	210	−0.0014	−0.0362
*Pca*104B	214	−0.1088	0.0737
*Pca*104B	222	−0.1668	0.1234
*Pca*104B	226	−0.1052	0.1295
*Pca*104B	230	0.1408	−0.0696
*Pca*104B	234	−0.1053	−0.0877
*Pca*104B	238	−0.0227	−0.0261
*Pca*104B	242	0.1827	−0.0559
*Pca*107	207	−0.1121	0.1183
*Pca*107	215	0.2139	0.0581
*Pca*107	223	0.0937	−0.0422
*Pca*107	227	0.0287	−0.0548
*Pca*107	235	−0.1177	−0.0142
*Pca*107	239	−0.1106	−0.0182
*Pca*107	243	0.0398	0.0158
*Pca*107	247	−0.0144	0.0226
*Pca*107	251	−0.0597	0.1428
*Pca*107	255	0.2257	−0.1251
*Pca*107	259	−0.1905	0.1853
*Pca*107	263	−0.1510	0.0732
*Pca*107	267	0.0096	−0.0197
*Pca*107	271	−0.0492	−0.0936
*Pca*201	318	0.0596	0.0660
*Pca*201	322	0.0167	−0.0272
*Pca*201	326	−0.0678	−0.0945
*Pca*201	330	0.0593	−0.0100
*Pca*201	334	−0.0656	0.0696
*Pca*201	338	0.1390	0.0390
*Pca*201	342	0.0740	−0.0567
*Pca*201	346	−0.1200	0.0166
*Pca*201	350	−0.1282	−0.0258
*Pca*201	354	−0.0858	0.1982
*Pca*201	362	−0.0593	0.0799