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# GENETIC CONNECTIVITY OF THE DUNGENESS CRAB (*CANCER MAGISTER*) ACROSS OCEANOGRAPHIC REGIMES

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***ABSTRACT*** Limited approaches exist for studying population connectivity in widely dispersing marine benthic invertebrates. Genetic techniques can provide important insights toward identifying recruitment trajectories. Here, 10 microsatellite loci were used to examine connectivity among Oregon Dungeness crabs (*Cancer magister,* Dana, 1852) in the California Current System (CCS) ($n = 801$) as well as between Oregon and two British Columbia populations, Alison Sound ($n = 54$) and Boundary Bay ($n = 48$). Using population-based methods (F-statistics), evidence for weak genetic differentiation was found among 12 sites in Oregon that did not conform to a pattern of isolation by distance. Whereas individual-based methods (kinship analyses) indicated higher than expected relatedness in two Oregon sites, this finding did not help interpret the pattern of genetic differentiation observed among sites in the CCS. Extending our analyses to British Columbia, it was determined that genetic diversity within the Boundary Bay population was comparable to that observed for Oregon, whereas genetic diversity within Alison Sound was considerably lower. Furthermore, genetic connectivity between Oregon and British Columbia was reduced as Alison Sound was genetically distinct from all Oregon sites, whereas Boundary Bay was genetically differentiated from several Oregon sites. In accordance, a Bayesian clustering approach provided support for two genetic groups: (1) Oregon and Boundary Bay and (2) Alison Sound. Kinship analysis revealed a high degree of relatedness within Alison Sound which helps explain the observed pattern of population differentiation. By combining population-based and individual-based approaches, these results demonstrate that connectivity between ocean and fjord-like areas is reduced and may lead to elevated kinship in isolated populations.

***KEY WORDS:*** Dungeness crab, *Cancer magister*, gene flow, isolation by distance, kinship, microsatellites, population structure

## INTRODUCTION

Population connectivity is based on the dispersal of individuals (e.g., larvae, juveniles, or adults) among geographically separated subpopulations that comprise a metapopulation (Cowen & Sponaugle 2009). Population connectivity is a broad term that can be partitioned into genetic connectivity which describes the degree to which gene flow affects evolutionary processes within populations, and demographic connectivity which explains the extent to which population growth and vital rates are affected by dispersal (Lowe & Allendorf 2010).

In the marine environment, dispersal often occurs during a pelagic larval phase such that estimates of population connectivity are difficult to achieve through direct observation or mark and recapture approaches (Selkoe & Toonen 2011). Indirect approaches (e.g., otolith microchemistry, stable isotope analysis, and genetic methods), however, have proven quite useful in estimating connectivity, and there is accumulating evidence that larvae rarely reach their full dispersal potential (Marko 2004, Cowen et al. 2006, Becker et al. 2007). Furthermore, pelagic larval duration (PLD) may not be a reliable predictor of connectivity as a number of genetic studies have documented population structure among species with long larval durations as well as little structure in species with short pelagic periods (reviewed in Weersing & Toonen 2009, Selkoe & Toonen 2011, but see Faurby & Barber 2012). Together, these findings have resulted in a paradigm shift away from the notion that most marine populations are genetically homogenous across broad geographic scales.

For marine crustaceans, estimates of connectivity rely almost exclusively on genetic methods, thereby providing insight on the evolutionary consequences of dispersal but generally limited information regarding demographic connectivity. To study genetic connectivity, gene flow is assessed and often estimated by calculating $F_{st}$, a measure of allele frequency divergence among populations. In general, the level of genetic differentiation among populations of marine species has been found to be less than those of freshwater and anadromous species (Ward et al. 1994, Waples 1998). This finding has been attributed to a lack of physical boundaries in the ocean and subsequent increased dispersal potential of the large amount of pelagic eggs and larvae commonly produced by marine species (reviewed in Nielsen & Kenchington 2001). Furthermore, the large effective population sizes ($N_e$; Wright 1931) of many marine species are expected to lower the impact of genetic drift and subsequently the accumulation of allele frequency differences at neutral loci among populations (Waples 1998).

A potentially more informative approach is coupling population-based methods to estimate gene flow (i.e., *F*-statistics) with an individual-based method such as kinship. Kinship analyses provide an index of the relative relatedness of all genotyped individuals and have been used to identify recruitment patterns that cannot be understood using traditional

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*F*-statistics. For instance, Iacchei et al. (2013) combined kinship estimates with *F*-statistics to explain contemporary drivers of population genetic differentiation in the California spiny lobster (*Panulirus interruptus*, Latreille, 1802), a species with a lengthy 8–11-mo PLD. Initial findings revealed significant population structure that did not correlate with distance between sampling locations. Rather, pairwise $F_{st}$ estimates between adjacent sites exceeded that among geographically distant locations. Instead of attributing this result to unexplainable "chaotic genetic patchiness" (Johnson & Black 1982), the authors conducted a kinship analysis and found a higher proportion of kin within sites that strongly correlated to the greater differentiation among sites. These findings suggest that the lobsters have substantial localized recruitment and/or maintain planktonic larval cohesiveness whereby siblings are more likely to settle together than disperse across sites (Iacchei et al. 2013). This is a rather remarkable finding considering the dispersive physical environment experienced by larvae during their long planktonic development.

The Dungeness crab (*Cancer magister*) is a decapod crustacean that is widely distributed along the Pacific coast in North America from the Aleutian Islands in Alaska to southern California, inhabiting the continental shelf, estuaries, and inland fjords (Rasmuson 2013). The Dungeness crab undergo a lengthy PLD (74–163 days) during which they transition through five zoea stages and one megalopae stage before settling to the benthos (Poole 1966, Moloney et al. 1994). Given that the majority of adults have been reported to migrate only 2–20 km over a 9-mo period (Diamond & Hankin 1985, Hildenbrand et al. 2011), larvae appear to be the primary means of long-range dispersal for this species.

The member/vagrant hypothesis of Sinclair (1988) postulates that patterns of larval ecology have evolved to ensure life cycle closure within dominant oceanographic circulatory systems. Dungeness crabs are an interesting organism in this regard because of their long PLD and the three large-scale oceanographic regimes that compose their species range. These are the California Current System (CCS) off the West Coast of the USA, the Gulf of Alaska Gyre off British Columbia, Canada and Alaska, and the Salish Sea (Puget Sound and Strait of Georgia). Currents of the CCS and Gulf of Alaska Gyre are eastern boundary currents separated by the eastward flowing North Pacific Current. The border of the three regimes occurs where the North Pacific Current impinges at the North American continental mass between 48–50°N (Vancouver Island). Variation in the strength and location of currents at this "leaky" boundary, due to climatic forcings such as the Pacific Decadal Oscillation (PDO), may determine whether larvae from different regimes are retained or exchanged (Shanks 2013).

To date, only one published study has examined the genetic connectivity of the Dungeness crab and it focused on adults sampled from eight locations off the coast of British Columbia, Canada. Based on data from eight microsatellite loci, Beacham et al. (2008) found that the Alison Sound location was genetically distinct from all other seven locations ($F_{st}$ range: 0.119–0.145), a result consistent with the hypothesized high level of retention of larval crabs within the sound. Furthermore, the authors found evidence for subdivision among four other locations, although the $F_{st}$ estimates were a magnitude smaller ($F_{st}$ range: 0.002–0.017). Overall, evidence for population genetic structure did not correspond to a pattern of isolation by distance (IBD; Wright 1943) in which genetic differentiation increased with distance.

In the CCS, the Dungeness crab is the most valuable commercial fishery (Rasmuson 2013). Whereas the fishery is managed separately by each state (e.g., Washington, Oregon, and California), the state regulatory systems are based on the same principles and biologically, it is considered one panmictic population. For instance, all three states use the 3-S management technique which controls the sex (i.e., only males), the minimum size, and the season of individuals harvested (Rasmuson 2013). Although no formal stock assessment is conducted for Dungeness crabs, there has been increasing interest among stakeholders to collect more data on this species, particularly off the Oregon coast given the developments in marine spatial planning (e.g., ocean energy testing sites and marine reserves). Therefore, the objectives of this study were to (1) provide the first estimates of genetic diversity and population structure for any shelf/slope benthic crustacean, the Dungeness crab, off the Oregon coast, (2) test for evidence of reduced genetic connectivity between Oregon and two previously reported Dungeness crab populations from different parts of the species range off the coast of British Columbia, and (3) test for evidence of kin aggregation among the adults sampled off the coasts of Oregon and British Columbia.

## MATERIALS AND METHODS

### *Sample Collection*

In collaboration with the Oregon Department of Fish and Wildlife and the commercial crab fishing fleet, Dungeness crabs (*Cancer magister*) were sampled off the Oregon coast during the 2011 Preseason test fishery (Pacific States Marine Fisheries Commission 2014). The sampling design consisted of 12 latitudinal transects with six crab pots fished at three different depths (27, 55, and 82 m) (Fig. 1). Each transect line represents a sampling site for a total of 12 sampling sites in Oregon. Muscle tissue from the hind leg of each female and sublegal size male (<158 mm carapace width) crab was collected and stored in a 50-mL vial filled with 95% ethanol ($n = 801$; Table 1). All legal size male crabs from the test fishery were transported to seafood processors for meat recovery and were not available for genetic analysis. In addition, DNA samples from two sites (Alison Sound $n = 54$ and Boundary Bay $n = 48$; Fig. 1, Table 1) sampled in 2002 off the coast of British Columbia (Beacham et al. 2008) were included in the study.

### *Genetic Analyses*

Genomic DNA was extracted from muscle tissue using the protocol derived by Ivanova et al. (2006). Individuals were genotyped at 10 microsatellite loci; eight loci previously used by Beacham et al. (2008) and two loci developed by Toonen et al. (2004) (Table 2). Polymerase chain reaction was performed in 5-μL reactions according to the authors' protocols. Amplified polymerase chain reaction products were electrophoresed on an ABI 3730XL DNA Fragment Analyzer and scored using GeneMapper software (Applied Biosystems, Foster City, CA). Individuals failing to amplify at six or more microsatellite loci were excluded from the analyses, with 893 individuals included in the analyses.

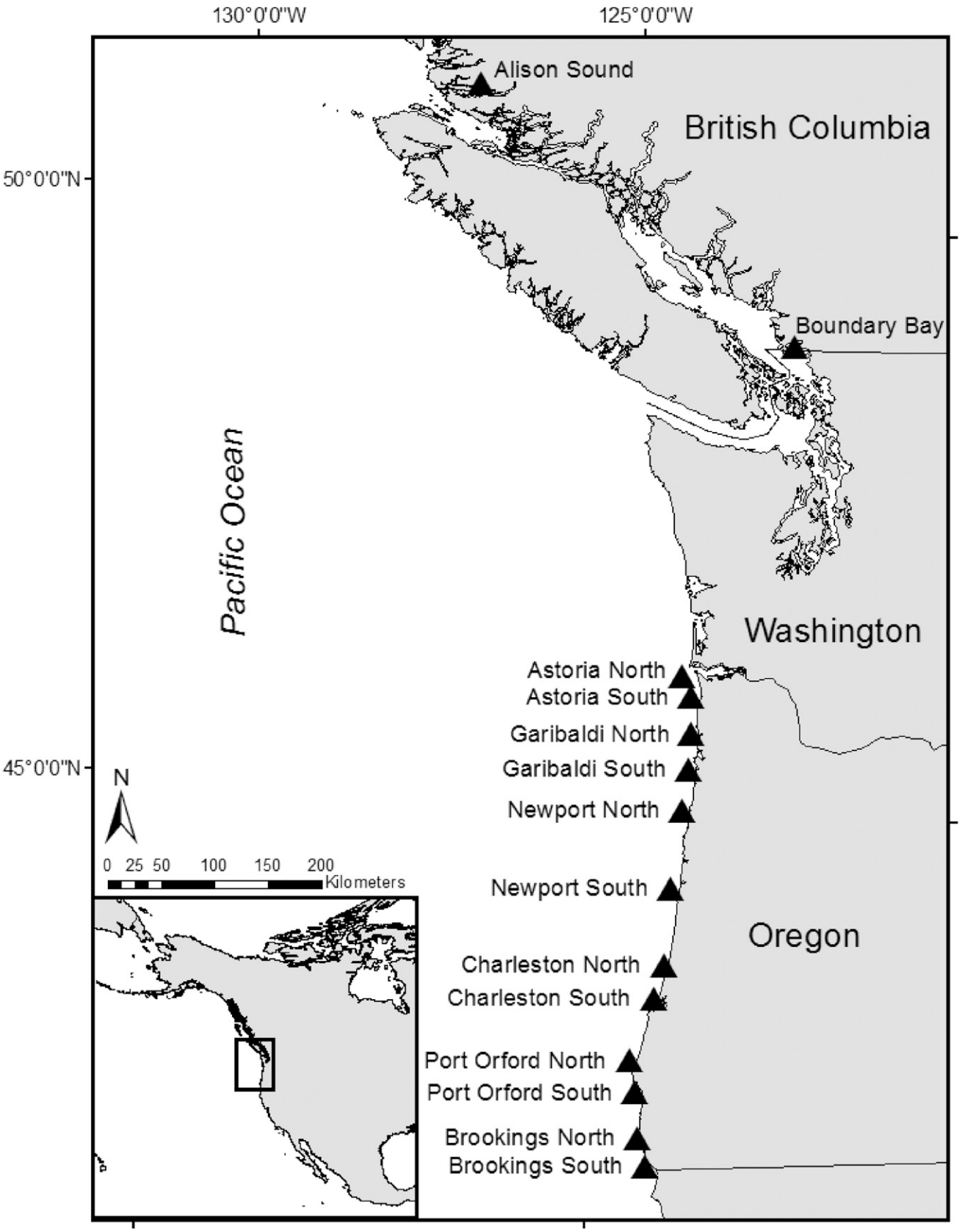


**Figure 1. Map of the Dungeness crab sampling sites off the coast of British Columbia and Oregon. Site names correspond to the sites listed in Table 1.**

**TABLE 1.**

**List of areas and sites from which adult Dungeness crabs were sampled for genetic analyses.**

| British Columbia | Area | Site | Latitude | Longitude | Males | Females | Total |
|---|---|---|---|---|---|---|---|
| 1 | Alison Sound | Alison Sound | 51° 40′ N | 127° 05′ W | NA | NA | 54 |
| 2 | Boundary Bay | Boundary Bay | 49° 06′ N | 123° 00′ W | NA | NA | 48 |
| **Oregon** | **Area** | **Site** | **Latitude** | **Longitude** | **Males** | **Females** | **Total** |
| 3 | Astoria | North | 46° 11′ N | 124° 10′ W | 49 | 30 | 79 |
| 4 | | South | 46° 01′ N | 124° 03′ W | 50 | 52 | 102 |
| 5 | Garibaldi | North | 45° 42′ N | 124° 02′ W | 73 | 25 | 98 |
| 6 | | South | 45° 24′ N | 124° 03′ W | 24 | 4 | 28 |
| 7 | Newport | North | 45° 03′ N | 124° 06′ W | 24 | 36 | 60 |
| 8 | | South | 44° 23′ N | 124° 12′ W | 50 | 65 | 115 |
| 9 | Charleston | North | 43° 44′ N | 124° 14′ W | 25 | 4 | 29 |
| 10 | | South | 43° 27′ N | 124° 20′ W | 25 | 4 | 29 |
| 11 | Port Orford | North | 42° 55′ N | 124° 35′ W | 48 | 39 | 87 |
| 12 | | South | 42° 39′ N | 124° 30′ W | 50 | 49 | 99 |
| 13 | Brookings | North | 42° 16′ N | 124° 27′ W | 25 | 12 | 37 |
| 14 | | South | 42° 02′ N | 124° 21′ W | 25 | 13 | 38 |
| – | – | – | – | – | – | – | 903 |

British Columbia sites were sampled in 2002 as part of a previously published study by Beacham et al. (2008). Oregon sites were sampled in 2011 during the preseason test fishery).

### *Statistical Analyses*

#### Genetic Variation within Sites

Conformance to Hardy–Weinberg proportions (HWP) and linkage equilibrium were examined using Genepop version 3.3 (Raymond & Rousset 1995). Number of observed alleles per locus, expected and observed heterozygosity, and fixation index (level of inbreeding) were calculated using GenAlEx version 6.5 (Peakall & Smouse 2006, 2012). Allelic richness for each locus at each sample site was estimated with FSTAT (Goudet 2002).

#### Genetic Differentiation among Sites

To test the null hypothesis of panmixia, genetic differentiation among the sampling sites was calculated with pairwise $F_{st}$

**TABLE 2.**

**List of 10 microsatellite loci used to genotype Dungeness crab samples.**

| | Primer | | | Accession | |
|---|---|---|---|---|---|
| Locus | Sequence | | No. Alleles | No. | Reference |
| Cma33 | Forward | AGG AAG CAC GCG ATG GGA AG | 49 | AY359597 | * |
| | Reverse | GGA TTG GTT GGA AAA ATT ACT CTT TGC TC | | | * |
| Cma102 | Forward | TTC AGC TGC ACT TCA GTG AT | 13 | AY521552 | † |
| | Reverse | CTG TAG TGA ACT AAA TTA CTG TT | | | † |
| Cma103 | Forward | GTT CCA AAT ACA GTT GAC C | 10 | AY521553 | † |
| | Reverse | GTC TTC CTA TGT CCT CCT T | | | † |
| Cma108a | Forward | GCA GTA GGA ACA GCA GCT GAT | 23 | AY521555 | † |
| | Reverse | GTT TAT TTC GTC ACC AGA GAG A | | | † |
| Cma114 | Forward | CAA GTA AGA GAA TGG AAT CGT ATT | 11 | AY521557 | † |
| | Reverse | GTT TGC CAA AGA GCA TCA GTG ACA A | | | † |
| Cma117 | Forward | GTC TGA GAC GAG CCA ACA TC | 7 | AY521558 | † |
| | Reverse | GTT TCA ACA GGA AAC ATG AAA TAG GA | | | † |
| Cma118 | Forward | GGA GAG GGA GCG ACT GTC | 17 | AY521559 | † |
| | Reverse | GTT TGG TGT ATT ACA AAA CAA CCA GTA A | | | † |
| Cma41 | Forward | ATA CTG GAC TCC AAC CGA CG | 118 | AY359600 | * |
| | Reverse | GGA TCT AAA CAG ACG ATT TAT TGT TTT | | | * |
| Cma107 | Forward | GCG TTC AAG GAT TAT TAC TGA GT | 47 | AY521554 | † |
| | Reverse | GTT TCC CCT GAC TCA TCC CCT C | | | † |
| Cma108b | Forward | CAG GTG TGG TTG TGT CCC TTT A | 9 | AY521556 | † |
| | Reverse | GTT CAG TTG AAC CCA GAG TGA CA | | | † |

Primer sequences, number of alleles, accession number, and reference are provided for each locus.
* Toonen et al. (2004).
† Kaukinen et al. (2004).

values (Weir & Cockerham 1984) and a permutation test with 1,000 iterations was used to assess the statistical significance (Genetix Version 4.02). A Genic exact test for differences in allele frequencies among samples was used with specified Markov chain parameters of 1,000 dememorization steps followed by 100 batches of 1,000 iterations per batch (Genepop version 3.3). Genic exact tests for population differentiation are accurate and unbiased even for very small samples or low-frequency alleles (Raymond & Rousset 1995). The false discovery rate (FDR) correction procedure of Benjamini & Hochberg (1995) was used to correct for multiple testing. Isolation by distance (Wright 1943) was evaluated by examining the association between genetic differentiation as measured by $F_{st}/(1 - F_{st})$ (Rousset 1997) and geographic distance among sites with Isolde in Genepop. Geographic distances between latitude/longitude coordinates were calculated using the following website: http://www.movable-type.co.uk/scripts/latlong.html.

Population structure was evaluated with the Bayesian clustering approach implemented in STRUCTURE v. 2.3.3 (Pritchard et al. 2000). A hierarchical approach was adopted as suggested by Rosenberg et al. (2002) for cases involving large data sets. STRUCTURE was initially run on the whole data set (i.e., British Columbia and Oregon). Four independent runs were performed for each $K$ (number of clusters) between 1 and 15 with no prior information on sampling location, using the admixture and correlated allele frequencies model. Burn-in and length of simulation were set at 50,000 and 200,000 iterations, respectively. The sampling location prior option was subsequently used for the British Columbia and Oregon sites following the approach mentioned earlier to assist in the identification of clustering as was suggested for cases of subtle population structure (Hubisz et al. 2009). Samples from British Columbia were subsequently excluded and four independent runs were performed with the admixture and correlated allele frequencies model for each $K$ between 1 and 13 with no prior information on sampling location. Burn-in and length of simulation were set at 50,000 and 200,000 iterations, respectively. The sampling location prior option was subsequently used for the 12 Oregon sites following the approach mentioned earlier to assist in the identification of clustering.

Results from each of the four STRUCTURE runs (i.e., OR and BC with no location prior, OR and BC with location prior, OR with no location prior, and OR with location prior) were processed in STRUCTURE HARVESTER v. 0.6.93 (Earl & vonHoldt 2012). This web-based program plots the mean of the likelihood values per $K$ and the ad hoc $\Delta K$ (Evanno et al. 2005) to estimate the most likely value of $K$. The results were plotted in Excel.

#### Kinship

To understand how alleles are shared between individuals rather than just among populations, the Lynch & Ritland (1999) relationship coefficient $r$ was calculated for each pair of individuals in the data set using the R package *Related* (Pew et al. 2015). To identify the sites that had higher mean $r$ than expected in a randomly associated population, a null distribution of expected mean relatedness was generated for each site by performing 1,000 random permutations (i.e., of individuals from all 14 sites), and a pseudo $P$ value was calculated.

## RESULTS

### *Microsatellite Loci Evaluation*

Two microsatellite loci, *Cma107* and *Cma41*, showed significant deviation from HWP in 14 and six of the sample sites, respectively. *Cma107* was previously reported to show significant departure from HWP in four of the eight Dungeness crab (*Cancer magister*) populations in British Columbia that was attributed to reduced amplification of larger-sized alleles (Beacham et al. 2008). There were 116 alleles identified at *Cma41* which is more than twice the number of alleles previously identified for 130 Dungeness crabs sampled off the west coast of North America (Toonen et al. 2004) and therefore likely represents spurious allele identification. Given these findings, both *Cma107* and *Cma41* were excluded from further analyses. Although *Cma33* showed significant departure from HWP in both the Alison Sound and Boundary Bay populations, this locus was retained because a single allele was found at high frequency (41%) in Alison Sound, whereas in Boundary Bay, three homozygote genotypes were found at high frequency.

Linkage disequilibrium was observed between *Cma103* and *Cma108b* in all 14 sample sites, and *Cma108b* was excluded from further analyses. The first seven loci listed in Table 2 were subsequently used to assess the genetic variation within and among the 12 sample sites in Oregon and the two sites in British Columbia.

### *Genetic Variation within the Oregon Sites*

Expected heterozygosity for Oregon sample sites ranged from 0.715 for Charleston South to 0.740 for Garibaldi North (Table 3). The fixation index was –0.062 (excess heterozygotes) for Charleston South and 0.042 (excess homozygotes) for Garibaldi North. Allelic richness ranged from 59.37 (Astoria North) to 63.75 (Garibaldi South) (Table 4).

**TABLE 3.**

**Sampling sites with standard genetic parameters: ($N$) number of individuals; ($N_A$) mean number of alleles per locus; ($H_o$) observed and ($H_e$) expected heterozygosity; and fixation index ($F$).**

| ID # | Sites | $N$ | $N_A$ | $H_o$ | $H_e$ | $F$ |
|---|---|---|---|---|---|---|
| 1 | Alison Sound | 54 | 5.29 | 0.570 | 0.566 | –0.011 |
| 2 | Boundary Bay | 48 | 11.29 | 0.696 | 0.706 | 0.004 |
| 3 | Astoria N | 78 | 12.43 | 0.730 | 0.721 | –0.009 |
| 4 | Astoria S | 98 | 13.43 | 0.738 | 0.724 | –0.022 |
| 5 | Garibaldi N | 97 | 12.86 | 0.713 | 0.740 | 0.042 |
| 6 | Garibaldi S | 28 | 9.71 | 0.714 | 0.724 | 0.024 |
| 7 | Newport N | 60 | 11.43 | 0.717 | 0.736 | 0.028 |
| 8 | Newport S | 115 | 13.29 | 0.727 | 0.732 | 0.007 |
| 9 | Charleston N | 29 | 9.43 | 0.662 | 0.727 | 0.096 |
| 10 | Charleston S | 28 | 9.57 | 0.757 | 0.715 | –0.062 |
| 11 | Port Orford N | 86 | 12.43 | 0.728 | 0.725 | –0.003 |
| 12 | Port Orford S | 97 | 13.14 | 0.756 | 0.728 | –0.042 |
| 13 | Brookings N | 37 | 10.14 | 0.736 | 0.738 | –0.001 |
| 14 | Brookings S | 38 | 10.29 | 0.703 | 0.726 | 0.027 |

Results are based on data from seven microsatellite loci.

### *Genetic Differentiation among the Oregon Sites*

In Oregon, one of the 66 pairwise $F_{st}$ comparisons was significant as Astoria North was significantly differentiated from Charleston South ($F_{st} = 0.009$, $P$ value $= 0.018$) (Table 5A). Results from the Genic exact tests corroborated this finding and also provided evidence for significant genetic differentiation between Garibaldi North and Brookings South ($P = 0.020$), Newport North and Brookings South ($P = 0.037$), and Port Orford North and Brookings South ($P = 0.0353$) (Table 5B). The $F_{st}$ estimate and Genic exact test results, however, were not statistically significant after correcting for multiple tests using the FDR method. No evidence for IBD along the Oregon coast was observed (Fig. 2).

### *Genetic Variation within the Two British Columbia Populations*

In British Columbia, measures of genetic variation for Boundary Bay were similar to those for the Oregon sites. The expected heterozygosity was 0.706 whereas allelic richness was 60.21 (Tables 3 and 4). In contrast, both measures were considerably lower for the Alison Sound site, with an expected heterozygosity of 0.570 and allelic richness of 33.11 (Tables 3 and 4). The fixation index for Boundary Bay was 0.004 and –0.011 for Alison Sound (Table 3).

### *Genetic Differentiation among the Oregon Sites and British Columbia Populations*

Within British Columbia, Alison Sound and Boundary Bay were genetically distinct from one another ($F_{st} = 0.2217$, $P$ value $= 0$, Genic exact test $P = 0$) based on the data from seven microsatellite loci, a finding consistent with Beacham et al. (2008). After correction for multiple tests using the FDR method, the Alison Sound population was genetically distinct from all 12 Oregon sites. Pairwise estimates of $F_{st}$ ranged from 0.1655–0.1884 ($P$ value $= 0$) with equivalent results for all of the pairwise Genic exact tests ($P = 0$) (Table 6A, B). A significant genetic differentiation was observed between the Boundary Bay population and several sites in Oregon based on the $F_{st}$ estimates, but the findings did not correspond to an "IBD" pattern. For instance, Boundary Bay was significantly differentiated from Astoria North and South, Newport North and South, and Port Orford North and South. The significant $F_{st}$ estimates ranged from 0.005–0.0083 (Table 6A). Lack of genetic differentiation between Boundary Bay and the remaining sites in Oregon was likely attributed to small samples sizes because results from the Genic Exact tests showed that both Alison Sound and Boundary Bay were significantly differentiated from all 12 Oregon sites (Table 6B).

The Alison Sound site was more genetically differentiated from the Boundary Bay site ($F_{st} = 0.2217$) than it was from the 12 Oregon sites ($F_{st}$ range: 0.166–0.208). Three alleles from three loci were not present in the Boundary Bay population but were present in the Alison Sound population and some of the Oregon sites. In addition, the Boundary Bay population displayed 32 alleles from six loci that were not present in the Alison Sound population but were present in one or more of the sites in Oregon.

Results from the STRUCTURE analyses of both Oregon and British Columbia Dungeness crab sites showed that the highest posterior likelihood was for $K = 2$ clusters; Alison Sound versus all of the remaining Dungeness crab samples. This finding was consistent with and without location prior (mean Ln $P$(D) = –21,417.750, and –21,920.825, respectively, Fig. 3) and corroborated by the $\Delta K$ method (Evanno et al. 2005). Within Oregon, results from the STRUCTURE analyses with and without the location prior showed that the highest posterior likelihood was for $K = 1$ cluster (mean Ln $P$(D) = –1,948.05, and –1,944.48, respectively).

### *Kinship*

Mean observed relatedness among individuals within each sampling site ranged from –0.004 to 0.367 (Table 7). Two sites in Oregon, Astoria North (mean $r = 0.006$, pseudo $P = 0.001$) and Port Orford South (mean $r = 0.004$, pseudo $P = 0$) and both populations in British Columbia, Alison Sound (mean $r = 0.367$, pseudo $P = 0$) and Boundary Bay (mean $r = 0.027$, pseudo $P = 0$) had greater observed mean relatedness than would be expected in a randomly associated population (Table 7).

## DISCUSSION

### *Genetic Connectivity within Oregon*

Genetic diversity of the Dungeness crab (*Cancer magister*), as measured by expected heterozygosity and allelic richness,

**TABLE 4.**

**Allelic richness per locus based on a minimum sample size of 23 individuals for each of the 14 sample sites.**

| Locus | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Cma33* | 8.44 | 18.12 | 19.79 | 19.98 | 19.46 | 20.18 | 18.73 | 19.72 | 20.15 | 19.27 | 20.68 | 19.07 | 19.73 | 21.14 |
| *Cma102* | 3.49 | 6.37 | 6.82 | 7.52 | 7.41 | 7.00 | 7.26 | 7.33 | 6.99 | 7.00 | 7.16 | 7.15 | 7.43 | 7.06 |
| *Cma103* | 4.98 | 6.22 | 5.76 | 4.31 | 5.97 | 5.64 | 5.25 | 5.78 | 3.97 | 5.00 | 5.17 | 5.28 | 4.83 | 5.40 |
| *Cma108a* | 4.89 | 8.42 | 7.76 | 7.75 | 7.72 | 8.46 | 9.84 | 9.17 | 8.47 | 11.04 | 7.68 | 7.77 | 7.57 | 8.22 |
| *Cma114* | 3.97 | 5.76 | 5.53 | 6.42 | 6.10 | 5.46 | 4.74 | 5.66 | 5.71 | 6.43 | 5.87 | 6.55 | 6.12 | 4.96 |
| *Cma117* | 1.46 | 3.73 | 4.66 | 4.58 | 5.27 | 5.64 | 5.07 | 5.38 | 5.84 | 4.00 | 5.49 | 5.44 | 4.94 | 3.60 |
| *Cma118* | 5.88 | 11.59 | 9.06 | 10.07 | 10.20 | 11.37 | 10.87 | 9.50 | 11.32 | 10.61 | 8.71 | 9.31 | 10.52 | 12.03 |
| Total | 33.11 | 60.21 | 59.37 | 60.62 | 62.11 | 63.75 | 61.75 | 62.54 | 62.44 | 63.34 | 60.75 | 60.57 | 61.14 | 62.41 |

Site numbers correspond to the sites listed in Table 1.

**TABLE 5.**

**Test statistics for measures of genetic differentiation among the 12 sample sites based on variation at seven microsatellite loci.**

**(A)**

| | Astoria S | Garibaldi N | Garibaldi S | Newport N | Newport S | Charleston N | Charleston S | Port Orford N | Port Orford S | Brookings N | Brookings S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Astoria N | 0.0001 | 0.0003 | 0.0030 | –0.0001 | –0.0004 | –0.0015 | 0.0090* | 0.0010 | 0.0004 | –0.0016 | –0.0011 |
| Astoria S | – | 0.0006 | –0.0010 | 0.0009 | –0.0005 | –0.0013 | 0.0049 | –0.0010 | 0.0012 | –0.0009 | –0.0004 |
| Garibaldi N | – | – | 0.0007 | 0.0001 | –0.0010 | –0.0023 | 0.0043 | –0.0005 | 0.0014 | –0.0022 | 0.0008 |
| Garibaldi S | – | – | – | –0.0021 | –0.0020 | –0.0060 | –0.0027 | –0.0021 | 0.0007 | –0.0054 | –0.0040 |
| Newport N | – | – | – | – | –0.0013 | –0.0006 | 0.0028 | –0.0009 | 0.0007 | –0.0027 | –0.0020 |
| Newport S | – | – | – | – | – | –0.0017 | 0.0005 | –0.0017 | 0.0002 | –0.0015 | –0.0030 |
| Charleston N | – | – | – | – | – | – | –0.0002 | 0.0009 | 0.0025 | –0.0039 | –0.0014 |
| Charleston S | – | – | – | – | – | – | – | 0.0013 | 0.0015 | 0.0023 | –0.0032 |
| Port Orford N | – | – | – | – | – | – | – | – | –0.0017 | –0.0006 | –0.0027 |
| Port Orford S | – | – | – | – | – | – | – | – | – | –0.0003 | –0.0019 |
| Brookings N | – | – | – | – | – | – | – | – | – | – | –0.0052 |

**(B)**

| | Astoria S | Garibaldi N | Garibaldi S | Newport N | Newport S | Charleston N | Charleston S | Port Orford N | Port Orford S | Brookings N | Brookings S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Astoria N | 0.5236 | 0.4445 | 0.3666 | 0.3041 | 0.4407 | 0.4241 | 0.0438* | 0.0452* | 0.1731 | 0.5445 | 0.5128 |
| Astoria S | – | 0.0812 | 0.4345 | 0.2905 | 0.5099 | 0.8070 | 0.1890 | 0.4586 | 0.3105 | 0.6127 | 0.0878 |
| Garibaldi N | – | – | 0.3553 | 0.1690 | 0.3453 | 0.3568 | 0.3477 | 0.3033 | 0.1658 | 0.6032 | 0.0200* |
| Garibaldi S | – | – | – | 0.6175 | 0.8388 | 0.6958 | 0.4854 | 0.6064 | 0.2488 | 0.8541 | 0.4514 |
| Newport N | – | – | – | – | 0.2723 | 0.4504 | 0.0739 | 0.0518 | 0.0680 | 0.3026 | 0.0337* |
| Newport S | – | – | – | – | – | 0.7178 | 0.4555 | 0.8940 | 0.4994 | 0.5459 | 0.4164 |
| Charleston N | – | – | – | – | – | – | 0.4757 | 0.3578 | 0.1957 | 0.8518 | 0.2021 |
| Charleston S | – | – | – | – | – | – | – | 0.2129 | 0.6967 | 0.2980 | 0.5891 |
| Port Orford N | – | – | – | – | – | – | – | – | 0.5473 | 0.3167 | 0.0353* |
| Port Orford S | – | – | – | – | – | – | – | – | – | 0.5177 | 0.3972 |
| Brookings N | – | – | – | – | – | – | – | – | – | – | 0.5528 |

Both the (A) $F_{st}$ estimates with an associated $P$ value $< 0.05$ and (B) Genic exact test $P < 0.05$ are denoted significant (*). None of the values were significant after correcting for FDR.

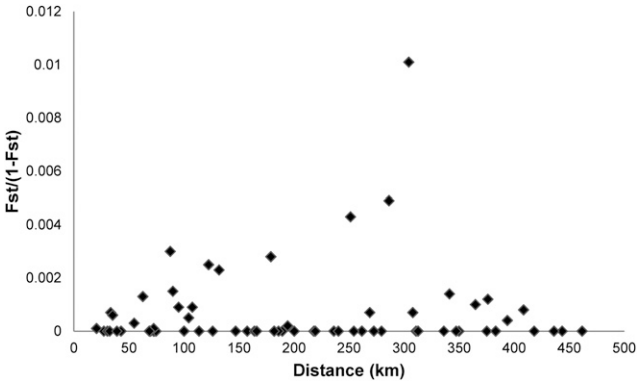


**Figure 2. Pairwise relationship of genetic distance, as measured by $F_{st}/(1 - F_{st})$, and geographic distance (kilometer) among the 12 Dungeness crab sample locations.**

was moderately high along the 585-km Oregon coastline and comparable with what has been observed in other marine crustaceans (Kenchington et al. 2009, Thomas & Bell 2013). Whereas there was some evidence for weak genetic differentiation within Oregon (*F*-Statistics and Genic exact tests), it did not correlate with the distance between sampling sites. Instead, the most northern site (Astoria North) was differentiated from Charleston South, and three sites north of Cape Blanco (Garibaldi North, Newport North, and Port Orford North) were genetically differentiated from the most southern site (Brookings South). It is possible that Cape Blanco may inhibit gene flow between the northern and southern areas because there is a strong coastal upwelling jet that moves offshore upstream of the Cape during the upwelling season (Barth et al. 2000). If acting as passive particles, recruiting megalopae would be preferentially advected back onto the continental shelf either north or south of the Cape. This phenomenon has been observed in intertidal invertebrates where significantly higher recruitment rates have been reported north of Cape Blanco than anywhere south of the Cape (Connolly et al. 2001). Furthermore, in a recent study evaluating connectivity among rockfish conservation areas in the U.S. and Canada, Lotterhos et al. (2014) reported a genetic break for black rockfish (*Sebastes melanops*, Girard, 1856) near Cape Blanco. The genetic break corresponds to a known shift in upwelling dynamics and may subsequently affect reproductive success and larval retention. Based on observations for both invertebrates and rockfish, it would be appropriate to test the temporal stability of our results for the Dungeness crab to determine if Cape Blanco acts as a barrier to larval dispersal.

Using the model-based clustering method of Pritchard et al. (2000), no evidence for genetic subdivision was found among the Dungeness crab sampled along the Oregon coast. Instead, individuals constituted a single panmictic population. Whereas Bayesian clustering methods have the advantage of requiring just the individual genotype and no reference to sample origin, their performance depends on the levels of population differentiation. For example, Latch et al. (2006) used simulated data to evaluate the performance of STRUCTURE and found that it could not detect more than one population at an $F_{st}$ of 0.01 but did infer the correct number of populations at an $F_{st}$ range: 0.02–0.03. Given the inconsistent results from the pairwise genetic estimates and Bayesian clustering approach, it is important to evaluate the patterns of genetic connectivity among Dungeness crabs across years. Previous studies have observed significant correlations between megalopae abundance and the timing of spring transition (Shanks & Roegner 2007), PDO (Shanks 2013), upwelling index (Shanks 2013), and subsequent fishery harvest rates. Knowing how these variables influence not only megalopae abundance, but also interannual variation in genetic connectivity among the adult recruits will improve the understanding of the population dynamics of the Dungeness crab.

#### *Genetic Connectivity between Oregon and British Columbia*

Extending the analyses to include two populations from British Columbia enabled the examination of genetic connectivity between the open ocean and a fjord-like area. Whereas genetic diversity within Boundary Bay and the Oregon sites was similar, genetic diversity within Alison Sound was considerably lower compared with Boundary Bay and all sites in Oregon. This finding is likely attributed to the narrow, shallow channel leading into Alison Sound

**TABLE 6.**

**Test statistics for measures of genetic differentiation between the Oregon and British Columbia sampling sites based on variation at seven microsatellite loci.**

| **(A)** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
| 1 | 0.2217* | 0.1868* | 0.1803* | 0.1835* | 0.1884* | 0.1655* | 0.1721* | 0.2081* | 0.1883* | 0.1776* | 0.1816* | 0.1810* | 0.1762* |
| 2 | – | 0.0066* | 0.0076* | 0.0047 | 0.0042 | 0.0083* | 0.0057* | –0.0034 | 0.0052 | 0.0066* | 0.0060* | 0.0065 | 0.0050 |
| **(B)** | | | | | | | | | | | | | |
| | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
| 1 | 0* | 0* | 0* | 0* | 0* | 0* | 0* | 0* | 0* | 0* | 0* | 0* | 0* |
| 2 | – | 0* | 0* | 0* | 0.0034* | 0* | 0* | 0.0215* | 0.0003* | 0* | 0* | 0.0010* | 0.0018* |

Numbers (1–14) correspond to the site names listed in Table 1. Both the (A) $F_{st}$ estimates and (B) Genic exact test results that were significant after correcting for FDR are denoted as *.

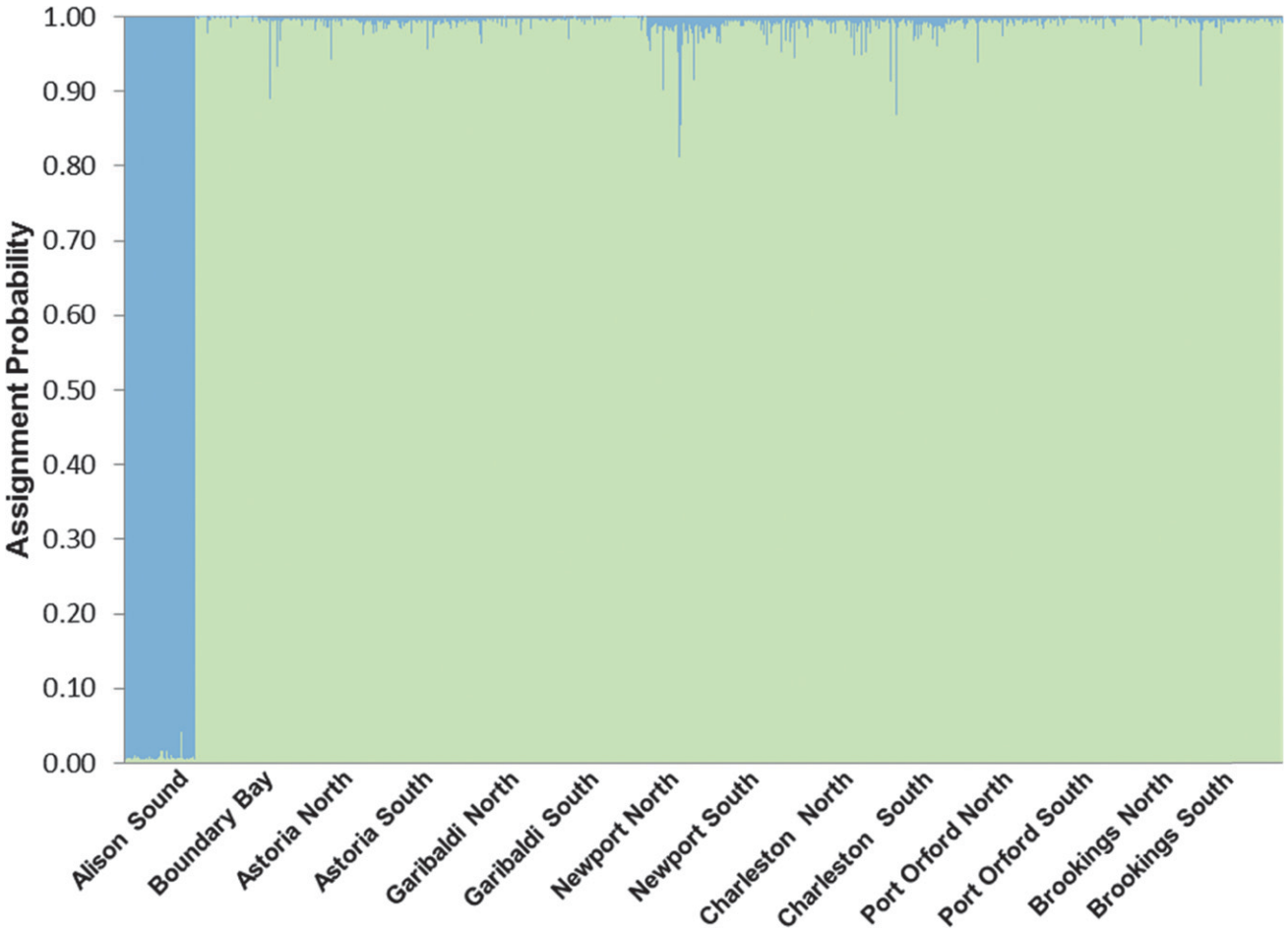


**Figure 3. STRUCTURE bar plot for $K = 2$. Each vertical line represents the assignment probability of a single individual to one (or more) of the genetic clusters.**

and subsequent reduced water volume exchange between the sound and outside waters. Consequently, there is likely a high level of retention of larval crabs within Alison Sound and reduced immigration of larval crabs from other areas (Beacham et al. 2008). Accordingly, the Alison Sound population was significantly genetically differentiated, as measured by $F_{st}$, from all 12 Oregon sites whereas the Boundary Bay population was only genetically differentiated from some of the Oregon sites and the $F_{st}$ estimates were at least a magnitude smaller. Furthermore, slightly greater genetic differentiation was observed between the Alison Sound and Boundary Bay populations ($F_{st} = 0.2217$) than between the Alison Sound population and the Oregon sites ($F_{st}$ range: 0.1655–0.2081), indicative of higher genetic connectivity between each British Columbia population and Oregon than between the two British Columbia populations. Results from the model-based clustering method, considering all 14 sampling locations, were consistent with this finding and provided support for two groups: (1) Alison Sound and (2) the Oregon coast and Boundary Bay.

### *Kinship as a Potential Explanatory Variable of Genetic Differentiation*

Individual-based methods, such as kinship analyses, have the potential to complement traditional population-based methods (i.e., *F*-statistics) which are often blind to relatedness of individuals. For example, determining how alleles are shared among individuals can reveal which locations have comparatively little ongoing genetic exchange when, in fact, low $F_{st}$ values suggest high contemporary population connectivity (Iacchei et al. 2013). Whereas some evidence for increased relatedness within two Oregon sites (Astoria North and Port Orford South) was observed, it did not correlate to the weak genetic differentiation detected between some Dungeness crab sampling sites. In contrast, mean relatedness was considerably higher in Boundary Bay and remarkably higher in Alison Sound and thus likely explains the strong genetic differentiation observed between British Columbia and Oregon Dungeness crab sites. Greater than expected relatedness within sites could result from (1) recruitment pulses of kin (i.e., planktonic larval cohesiveness), (2) sweepstakes recruitment whereby the recruiting cohort consists of offspring from only a few individuals, and/or (3) in the case of Alison Sound, larval retention. Previous studies with other organisms have detected high levels of relatedness within cohorts of larval recruits (Selkoe et al. 2006, Bernardi et al. 2012) and thus it would be interesting to test the hypothesis of kin aggregation in the Dungeness crab by sampling recruiting megalopae both offshore and in the nearshore. It has been well established that vertical migration behaviors can affect

**TABLE 7.**

**Mean observed relatedness (*r*) within sites.**

| ID # | Sites | Mean *r* | Pseudo *P* value |
|---|---|---|---|
| 1 | Alison Sound | 0.367* | 0.000 |
| 2 | Boundary Bay | 0.027* | 0.000 |
| 3 | Astoria N | 0.006* | 0.001 |
| 4 | Astoria S | 0.001 | 0.098 |
| 5 | Garibaldi N | 0.001 | 0.069 |
| 6 | Garibaldi S | –0.004 | 0.666 |
| 7 | Newport N | –0.002 | 0.620 |
| 8 | Newport S | –0.001 | 0.430 |
| 9 | Charleston N | –0.004 | 0.639 |
| 10 | Charleston S | 0.000 | 0.427 |
| 11 | Port Orford N | 0.001 | 0.131 |
| 12 | Port Orford S | 0.004* | 0.000 |
| 13 | Brookings N | 0.002 | 0.295 |
| 14 | Brookings S | –0.001 | 0.502 |

* Denotes observations significantly greater than expected after 1,000 random permutations.

larval trajectories on the continental shelf and nearshore zone (Morgan 2014), and that Dungeness crab megalopae in particular are not dispersed as simple passive particles (e.g., Roegner et al. 2013).

#### *Evidence of Larval Connectivity from Plankton Studies?*

Studies of Dungeness crab reproductive timing, larval ecology, and oceanography provide evidence for limited larval connectivity across the junction of the three main oceanographic regimes occupied by this species. In estuaries of Oregon and Washington, located in the center of the CCS, larval recruitment is generally pulsed during spring-early summer correlating to a winter release of larvae into the plankton (Roegner et al. 2007, Shanks 2013). The source of these spring-summer recruits is thought to be within the CCS (e.g., a self-recruiting population). During development, larvae are transported north and offshore during the winter downwelling period, and then move back south and onshore during current reversals as the spring transition generates upwelling conditions (Reilly 1985, Shanks & Roegner 2007); however, smaller pulses of larvae in the CCS estuaries are also found as late as November (Roegner et al. 2007, Shanks 2013), and these autumn recruits are likely derived from sources north that have a later larval release date. Analogously, in SE Alaska, *Cancer magister* megalopae are sometimes observed in June, when local populations are just undergoing larval release (Fisher 2006). Because larval recruitment in SE Alaska usually occurs from August through October, these early recruits likely originated from populations in the CCS or Salish Sea (Park et al. 2007).

In contrast to the comparatively simple coastline of the CCS, the structurally complex fjord topography comprising the Salish Sea and the coasts of British Columbia and SE Alaska produces conditions that can facilitate both local retention (i.e., Alison Sound) or wider dispersal (i.e., Boundary Bay) (Fisher 2006, Weingartner et al. 2009, Smith & Eckert 2011). The Salish Sea, with its convoluted basins and limited mechanisms of exchange, would appear to present opportunities for genetic isolation from coastal stocks. Yet, researchers have found size and behavioral differences between Salish Sea and coastal stocks that suggest episodic larval connectivity of coastal stocks through the Strait of Juan de Fuca (Dinnel et al. 1993). Within the Salish Sea, crab larvae have been found to be widely dispersed in surface layers thus decreasing opportunity for isolation (Sorochan & Quijon 2014). These lines of evidence support the possibilities of genetic mixing of coastal and interior stocks. In contrast, the very reduced physical connection and remote location of Alison Sound likely explains the lower genetic connectivity with both coastal Oregon and British Columbia stocks.

Findings from population genetic studies of fish species which also inhabit the shallow shelf and slope are varied. Wishard et al. (1980) reported no evidence for population structure in the Chilipepper rockfish (*Sebastes goodie*, Eigenmann & Eigenmann, 1890) whereas Withler et al. (2001) found evidence of distinct populations of Pacific ocean perch (*Sebastes alutus*, Gilbert, 1890) along the continental shelf of the northeastern Pacific Ocean. But, the major bifurcation in oceanic currents near the entrance of Puget Sound which separates the Oregon and British Columbia sites has been suggested to be responsible for genetic differences in a number of marine species, including the rosethorn rockfish (*Sebates helvomaculatus*, Ayres, 1859) (Rocha-Olivares & Vetter 1999).

At present, it is not clear how often larval (and hence genetic) exchange occurs between circulatory regimes in the Northeast Pacific. Directionality and magnitude of transport likely depend on large-scale physical drivers such as the PDO and the El Niño–Southern Oscillation cycle which affect the timing, direction, and strength of coastal currents. For example, native mole crab (*Emerita analoga*, Linnaeus, 1767) and invasive green crab (*Carcinus maenas*, Linnaeus, 1758) both exhibit northward range extensions during El Niño events (Sorte et al. 2001; Yamada & Kosro 2009), when winter northward coastal currents are intensified. Because populations with large $N_e$ sizes diverge more slowly through genetic drift than those with small $N_e$, a smaller fraction of migrants (i.e., larval recruits) are needed to counteract the effects of drift (i.e., reduce $F_{st}$). In contrast, genetic divergence will occur more quickly in isolated basins such as Alaskan fjords, where a large number of recruits are needed to counteract genetic drift. To our knowledge, many of these populations have not yet been genetically evaluated. In addition, these results may be applicable to other crustacean species with similar life history strategies, such as *Cancer gracilis* (Dana, 1852) and *Cancer oregonensis* (Dana, 1852), for which information on population genetic structure is currently lacking.

## SUMMARY

Our study provided the first estimates of genetic diversity and connectivity for the Dungeness crab, a shelf/slope crustacean in the central California Current System. By including samples from British Columbia, it was able to compare estimates of genetic diversity and connectivity between outer coast and interior basin regions and demonstrate reduced connectivity, particularly between the fjord-like Alison Sound and outer coast. Results from the first kinship analyses

conducted for adult Dungeness crab which supports the hypothesis posited by Beacham et al. (2008) of larval retention within Alison Sound are also presented. Furthermore, evidence of higher mean relatedness within two outer coast sites in the California Current are also reported. Additional research is necessary to determine whether these results can be attributed to planktonic larval cohesiveness and/or sweepstakes recruitment. Further research should also target estimates of genetic connectivity along a latitudinal gradient north of the divergence of the Alaska Current and the California Current.

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