OWET
OREGON WAVE ENERGY TRUST

Evaluating the population genetic structure of Dungeness crab (*Cancer magister*) off the Oregon coast

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On behalf of Oregon Wave Energy Trust

And the Oregon Dungeness Crab Commission

Oregon Wave Energy Trust (OWET) is a nonprofit public-private partnership funded by the Oregon Innovation Council. Its mission is to support the responsible development of wave energy in Oregon. OWET emphasizes an inclusive, collaborative model to ensure that Oregon maintains its competitive advantage and maximizes the economic development and environmental potential of this emerging industry. Our work includes stakeholder outreach and education, policy development, environmental assessment, applied research and market development.

Table of Contents

Introduction

The Dungeness crab (*Cancer magister*) fishery is the most valuable 'single-species' fishery in Oregon. In the 2012-2013 season, commercial fishery landings totaled more than 18 million pounds (lbs) at a value of nearly $49 million (Oregon Department of Fish and Wildlife). Over the past six decades, there have been large fluctuations in landings of Dungeness crab off the west coast of the United States. In Oregon, for example, there were 3.1 million lbs of Dungeness crab landed during the 1972-73 commercial season compared to 33.7 million lbs in 2004-05 (Figure 1). Given the continuous increase in number of crab pots fished throughout this period (Figure 2), it is believed that the inter-annual variability in landings is not a result of harvest patterns but likely due to large-scale oceanographic features affecting larval delivery (i.e. timing of the spring transition) and density-dependent effects influencing new recruits (Shanks & Roegner 2007; Shanks 2013).
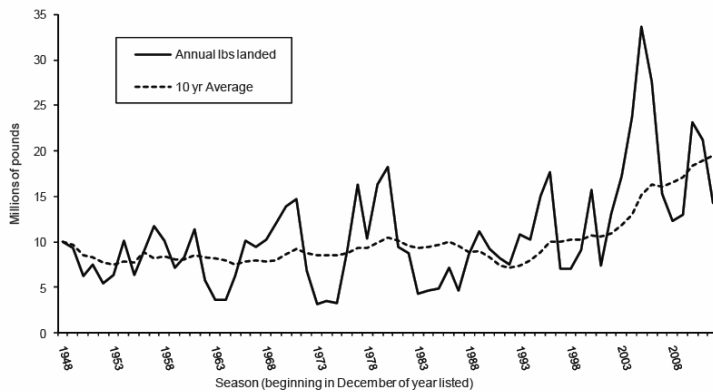

Figure 1. Landings (millions of lbs) per Dungeness crab season compared to the 10 year average.
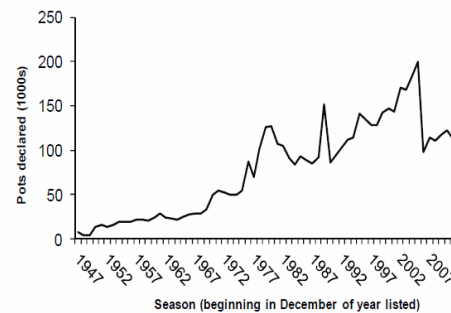

Figure 2. Pots declared by year in the Oregon Dungeness crab fishery. Boat pot limits were implemented in the 2006-07 crab season.

Despite the high economic and social value of Dungeness crab, no stock assessment work has been conducted on coastal Dungeness crab populations in Oregon. The fishery is currently managed based on a sex, size, and season strategy (e.g. males of a minimum size fished during specified months). It is estimated that a majority of all legal-sized male crabs (typically one or two year classes) are captured annually in the fishery, but studies suggest this does not cause into decreased mating success for females (Hankin et al. 1997). The fishery is assumed to be stable given the long term trends in commercial landings.

While there is no evidence to suggest an immediate threat of fisheries collapse, there is, however, increasing interest to collect more biological data to better understand this species life history. For instance, the Oregon Nearshore Strategy Report (ONS)(2006) recommended the development of

conservation and harvest plans for both commercially and recreationally harvested shellfish to ensure sustainable management of Oregon's shellfish. The recent Marine Stewardship Council (MSC) certification of Oregon Dungeness crab also identified important information gaps concerning the fishery that are required to be fulfilled within a five year period in order to retain certification. In response to these developments, the Oregon Department of Fish and Wildlife (ODFW) developed the Oregon Dungeness Crab Research and Monitoring Plan (2013). As the resource management agency, ODFW is currently working to help fill the information gaps identified through the MSC certification process as well as in the ONS report. The new ODFW plan outlines historical and current biological, ecological and socio-economic research and monitoring efforts on the Oregon Dungeness crab resource and future plans to address information gaps in each of these areas.

Motivation for Study

This study was initiated to contribute to the biological (i.e stock structure) and soci-economic (i.e spatial planning) research efforts identified in the new ODFW plan. Firstly, sustainable management of Dungeness crab should include an understanding of how patterns of larval distribution, abundance, and dispersal regulate adult population dynamics. This depends on explicit knowledge of population genetic stock structuring for which information is currently lacking. Dungeness crabs are managed jointly by the Tri-State (California, Oregon and Washington) Dungeness crab committee, but from a biological perspective, considered a single continuous population. However, the possibility of subpopulations based on genetic diversity exists (Rassmusson 2012). Selective harvest of subpopulations can cause genetic changes within a population, which could result in reduced productivity of a stock (Allendorf et al. 2008).

Secondly, Marine Spatial Planning (MSP) is a topic that has garnered much attention at the local, state, and federal level in recent years, and is likely to continue to do so for several more. In Oregon, the recent interest from ocean renewable energy developers (e.g. nearshore wave and offshore wind energy) and establishment of marine reserves has resulted in legislative and commission actions to define how the state's territorial sea (shoreline to 3 nautical miles offshore) is allocated. Because 'real estate' (i.e. fishing grounds) is as important to the crab fishery as crabs are, proposed wave energy parks and marine protected areas have created concern within the local commercial fishing fleet. Fishermen are concerned that both of these proposed uses of Oregon's nearshore waters may eliminate some of the traditional fishing areas (ODCC).

The goal of this study was to evaluate the genetic diversity and population genetic structure of Dungeness crab off the Oregon coast and provide baseline data that could be used to help inform decisions on marine

spatial planning. From a conservation and management standpoint, it is critical to determine the population genetic structure and genetic diversity within subpopulations to ensure the long term viability of a species.

Methods: Sample collection

In collaboration with ODFW, 801 Dungeness crab samples were collected during the 2011Pre-Season Test Fishery. The sampling design consisted of 12 latitudinal transects where six crab pots were fished at each of three different depths (15, 30, and 45 fathoms)(Figure 3). Muscle tissue from the hind leg of each female and sub-legal size male (< 6 ¼ inch carapace width) crab caught during the Pre-Season Test Fishery was stored in individual 50mL vials filled with 95% ethanol and transported to K.G. O'Malley's genetics laboratory at the Hatfield Marine Science Center in Newport, Oregon (Table 1). All legal size male crab, regardless of shell condition, were collected for meat recovery and transported to the processors and were not available for sampling.

Table 1. List of sampling ports, 12 latitudinal transect sites and number of female and sub-legal size male Dungeness crab collected during the 2011 Pre-Season Test Fishery.

| Area/Port	Site	Latitude	Longitude	Males	Females	Total
Astoria	North	46° 11'N	-124° 10'W	49	30	79
	South	46° 01'N	-124° 03'W	50	52	102
Garibaldi	North	45° 42'N	-124° 02'W	73	25	98
	South	45° 24'N	-124° 03'W	24	4	28
Newport	North	45° 03'N	-124° 06'W	24	36	60
	South	44° 23'N	-124° 12'W	50	65	115
Coos Bay	North	43° 44'N	-124° 14'W	25	4	29
	South	43° 27'N	-124° 20'W	25	4	29
Port Orford	North	42° 55'N	-124° 35'W	48	39	87
	South	42° 39'N	-124° 30'W	50	49	99
Brookings	North	42° 16'N	-124° 27'W	25	12	37
	South	42° 02'N	-124° 21'W	25	13	38
			Totals	468	333	801
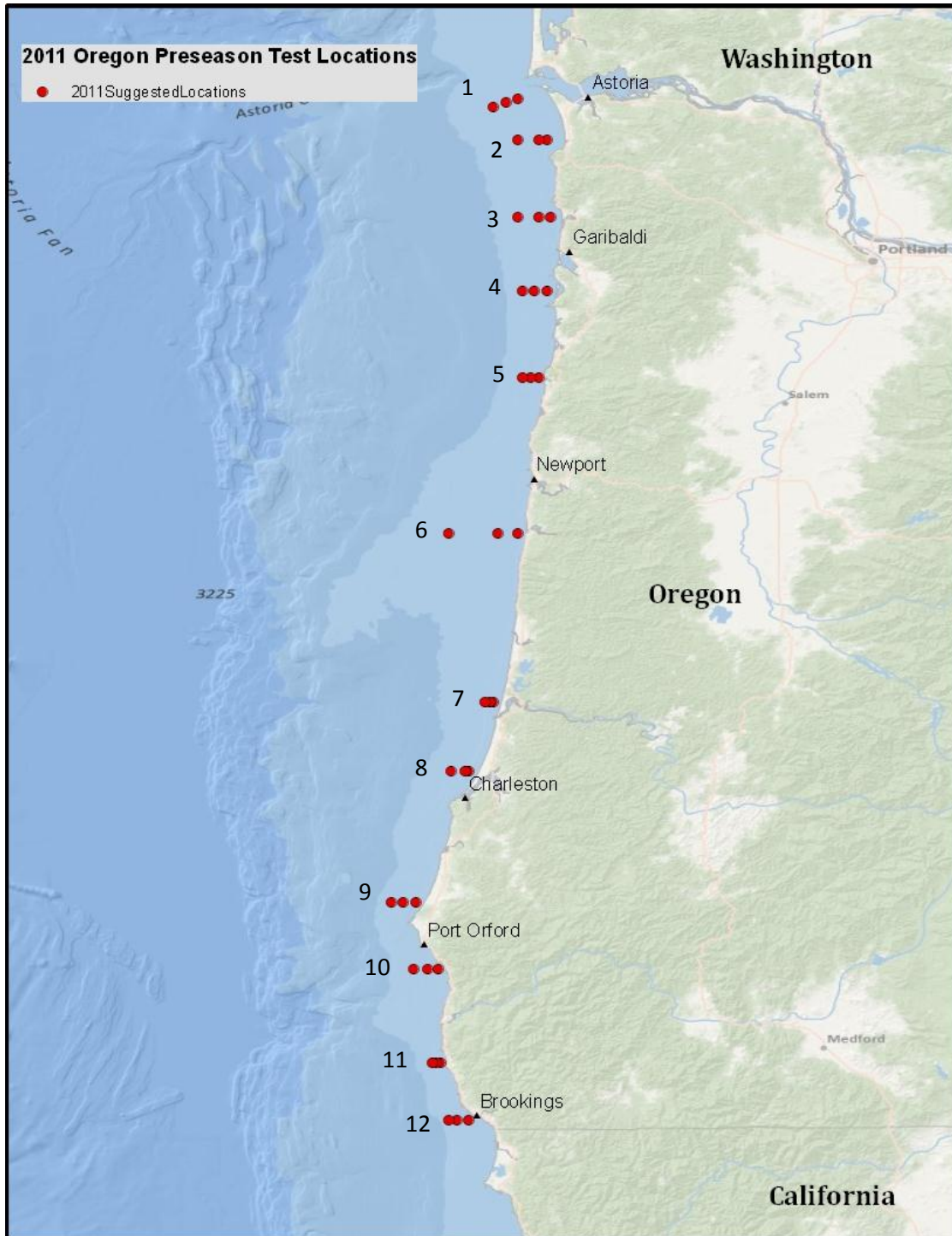
Figure 3. The 12 latitudinal transect lines sampled for female and sub-legal size male Dungeness crab during the 2011 Pre-Season Test Fishery.

Methods: Sample processing

Genomic DNA was extracted from Dungeness crab muscle tissue using the protocol derived by Ivanova et al. (2006). Individuals were genotyped at 10 microsatellite loci using previously developed oligonucleotide primers (Table 2). Polymerase chain reaction (PCR) was performed in 5μL reactions according to the authors' protocols. Amplified PCR products were electrophoresed on an ABI 3730XL DNA Fragment Analyzer and scored using GENEMAPPER® software.

Table 2. List of ten microsatellite loci used to genotype Dungeness crab samples. Primer sequences, total number of alleles observed, accession number, and reference are provided for each locus.

Locus	Primer Sequence		No. Alleles	Accession No.	Reference
Cma33	Forward	AGG AAG CAC GCG ATG GGA AG	49	AY359597	§
	Reverse	GGA TTG GTT GGA AAA ATT ACT CTT TGC TC			§
Cma41	Forward	ATA CTG GAC TCC AAC CGA CG	116	AY359600	§
	Reverse	GGA TCT AAA CAG ACG ATT TAT TGT TTT			§
Cma102	Forward	TTC AGC TGC ACT TCA GTG AT	12	AY521552	¥
	Reverse	CTG TAG TGA ACT AAA TTA CTG TT			¥
Cma103	Forward	GTT CCA AAT ACA GTT GAC C	8	AY521553	¥
	Reverse	GTC TTC CTA TGT CCT CCT T			¥
Cma107	Forward	GCG TTC AAG GAT TAT TAC TGA GT	47	AY521554	¥
	Reverse	GTT TCC CCT GAC TCA TCC CCT C			¥
Cma108a	Forward	GCA GTA GGA ACA GCA GCT GAT	23	AY521555	¥
	Reverse	GTT TAT TTC GTC ACC AGA GAG A			¥
Cma108b	Forward	CAG GTG TGG TTG TGT CCC TTT A	9	AY521556	¥
	Reverse	GTT CAG TTG AAC CCA GAG TGA CA			¥
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			¥

§ Beacham et al. (2008); ¥ Kaukinen et al. (2004)

Methods: Statistical analyses

Genetic diversity is often described as the combination of different genes found within a population of a single species, and the pattern of variation found within different populations of the same species. To examine the genetic diversity within and among Oregon Dungeness crab samples, we calculated observed

(H_o) and expected (H_e) heterozygosities using the software program GENETIX version 4.02 (Belkhir 2000). At a single microsatellite marker, heterozygosity is the proportion of individuals that have two different alleles (i.e. heterozygous); heterozgosity (H) ranges between zero and one. H_o is the observed proportion of heterozygotes whereas H_e is the expected proportion of heterozygotes if the population is mating at random. H_e provides a better measure than H_o to compare the relative amount of variation in different populations, as long as the populations are mating at random (Nei 1977). We also examined conformance to Hardy–Weinberg equilibrium (HWE) and linkage equilibrium using the software program Genepop version 3.3 (Raymond & Rousset 1995). Deviation from Hardy-Weinberg proportions or linkage equilibrium can be indicative of population substructure or inbreeding. To correct for multiple testing when calculating HWE and linkage equilibrium, we employed false discovery rate corrections (Benjamini & Hochberg 1995). All of the above analyses were conducted to examine genetic diversity both within each of the 12 sample locations as well as among all samples collectively.

The total number of alleles at a genetic marker is a valuable complementary measure of genetic variation because it is indicative of the long-term evolutionary potential of populations (Allendorf 1986). The major drawback of this measure, unlike heterozygosity, is that it is highly dependent on sample size. That is, there is a tendency to detect more alleles at a marker as sample sizes increase. To avoid this problem, we calculated allelic richness (AR), which is a measure of allelic diversity that takes into account sample size (El Mousadik & Petit 1996). In addition to calculating AR, we determined the number of private alleles (PA) within each of the 12 sample locations using GeneAlEx version 6.501 (Peakall & Smouse 2006, 2012). Private alleles are those alleles that are present in only one of the 12 sample locations.

To test for evidence of population structure from our coastwide sample which spans approximately 462 km from north to south, we performed a Principal Coordinate Analysis (PCoA) based on a pairwise, individual-by-individual genetic distance matrix as well as a sample location-by-sample location genetic distance matrix (using GeneAlEx). We calculated global and pairwise F_{st} estimates among sample locations using Weir and Cockerham's θ (Weir and Cockerham 1984)(using GENETIX). F_{st} is a measure of allele frequency divergence among subpopulations and ranges from zero, when all subpopulations have equal allele frequencies, to one, when all subpopulations are fixed for different alleles. We also performed exact tests for differences in genic (i.e. allele) and genotypic (i.e. genotype) frequencies among sample locations (using Genepop). Exact tests were conducted with specified Markov chain parameters of 5000 dememorization steps followed by 500 batches of 5000 iterations per batch. This exact test for population differentiation is accurate and unbiased even for very small samples or low-frequency alleles (Raymond & Rousset 1995).

Population substructure can result from limited gene flow between groups of individuals within a metapopulation. This phenomenon is known as isolation-by-distance (IBD) and is a simple consequence of limited dispersal across space. IBD was evaluated by testing for a statistically significant association between genetic differentiation as measured by $F_{st}/(1 - F_{st})$(Rousset 1997) and geographic distance among the 12 sample locations (using Isolde in Genepop). Geographic distances between latitude/longitude coordinates points were calculated using the following website: http://www.movable-type.co.uk/scripts/latlong.html.

Effective population size (N_e) is the number of individuals in a population who contribute offspring to the next generation. In an ecological sense, the census size of a population (N_c) can be measured by simply counting the number of adults in a locality. However, from the theory of population genetics what really matters is the chance that two copies of a gene will be sampled as the next generation is produced, and this is affected by the breeding structure of the population. In practice, N_e is usually lower than N_c. We estimated effective population size (N_e) using the linkage disequilibrium method of Waples & Do (2008), as implemented in NeEstimator V2 (Do et al. 2014). We report our estimate of $N_e \pm 95\%$ confidence intervals determined using the jackknife re-sampling method of Waples & Do (2008).

Results: Genetic variation among all individuals

We genotyped 791 individuals across 12 sample locations at 10 microsatellite loci, with the number of alleles per locus ranging from seven to 116. Ten individuals had low quality DNA and thus were excluded from the analyses. Expected heterozygosity (H_e) was between 0.591 and 0.979, while observed heterozygosity (H_o) exhibited a slightly narrower range from 0.577 to 0.917 (Table 3). These results are similar to those reported by Beacham et al. (2008) for Dungeness crab in British Columbia, Canada.

Three loci showed significant deviation from HWE after correcting for multiple tests: *Cma107*, *Cma41* and *Cma33*. *Cma107* was previously reported to show significant deviation from HWE in 4 out of 8 Dungeness crab populations in British Columbia and this result was attributed to the wide range in allele size and reduced amplification of larger sized alleles (Beacham et al. 2008). Given this finding, we excluded *Cma107* from further analyses. We identified 116 alleles at *Cma41* which is 2.5 times the number of alleles identified at this locus for 130 Dungeness crabs off the coasts of CA, OR, WA, BC and AK (Toonen *et al.* 2004) and therefore likely represents spurious allele identification. In contrast, the number of alleles identified at *Cma33* (N = 33) among these 130 samples was similar to the number of alleles identified in this study (N = 49). Therefore, we chose to retain *Cma33* for further population genetic structure analyses but excluded *Cma41* due to the spurious allele identification. Tests for linkage

disequilibrium were significant in only 1of the 36 comparisons (2.7%) after correcting for multiple tests (*Cma103/Cma108b*).

Table 3. Number of alleles per locus (NA), expected heterozygosity (H_e), and observed heterozygosity (H_o) for each of the 10 microsatellite loci. Significant deviation from Hardy-Weinberg equilibrium is denoted with an asterisk.

Locus	NA	H_e	H_o
*Cma33**	49	0.932	0.915
*Cma41**	116	0.979	0.917
Cma102	12	0.764	0.762
Cma103	8	0.686	0.682
*Cma107**	47	0.945	0.608
Cma108a	23	0.703	0.725
Cma108b	9	0.685	0.687
Cma114	11	0.608	0.581
Cma117	7	0.591	0.577
Cma118	17	0.854	0.850

Results: Genetic variation within sample location

Expected heterozygosity (H_e) within sample location based on data from eight microsatellite loci ranged between 0.707 and 0.737, while observed heterozygosity (H_o) exhibited a slightly wider range from 0.661 to 0.758 (Table 4). Again, these results are comparable to what has been reported for eight Dungeness crab subpopulations located off the coast of British Columbia (Beacham et al. 2008). The average number of alleles per locus within sample location varied from 8.75 for Coos Bay South where the fewest number of crab were sampled (N = 29) to 12.75 for Newport South where the largest number of crabs were sampled (N = 115).   However, when we measured allelic richness and accounted for differences in sample size among locations, we found that the number of alleles per locus was similar among all the 12 locations (Table 5). We identified private alleles at two of the eight microsatellite loci in all sample locations except Charleston North and Brookings North. Six sample locations had a single private allele at *Cma33* with frequencies from 0.005 to 0.018 while two sample locations each had two private alleles at this locus with frequencies from 0.005 to 0.26 (Table 6). Seven sample locations had a single private allele at *Cma108a* with frequencies from 0.004 to 0.018. Determining the number and frequency of alleles within populations is critical baseline information since allelic diversity is more sensitive to reduction in population size than heterozygosity (Allendorf 1986).

Tests for linkage disequilibrium (i.e. nonrandom association) were significant in 12 of 336 comparisons (3.5%) after correcting for multiple tests. Port Orford South and Newport South had the highest percentage of LD comparisons at ~18% and 14% respectively. *Cma103* and *Cma108b* were in linkage disequilibrium in all sample locations except Coos Bay North. Possible factors that could lead to nonrandom association of loci include selection, non-random mating, or population structure. There were no significant deviations from HWE out of the 96 comparisons after correcting for multiple comparisons.

Table 4. Expected (H_e) and observed (H_o) heterozygosity and average number of allele per locus for each of the 12 sample locations. Results are based on data from eight microsatellite loci.

Population	ID #	H_e	H_o	Avg No. Alleles/Locus
Astoria N	1	0.717	0.727	11.875
Astoria S	2	0.714	0.725	12.625
Garibaldi N	3	0.737	0.709	12.250
Garibaldi S	4	0.716	0.710	9.250
Newport N	5	0.729	0.711	10.875
Newport S	6	0.728	0.725	12.750
Coos Bay N	7	0.718	0.661	8.750
Coos Bay S	8	0.707	0.747	9.000
Port Orford N	9	0.718	0.724	12.000
Port Orford S	10	0.722	0.758	12.375
Brookings N	11	0.731	0.728	9.500
Brookings S	12	0.721	0.686	9.750

Table 5. Allelic richness per locus based on minimum sample size of 23 individuals for each of the 12 sample locations.

Locus	1	2	3	4	5	6	7	8	9	10	11	12
Cma33	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	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	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	7.76	7.75	7.72	8.46	9.84	9.17	8.47	11.04	7.68	7.77	7.57	8.22
Cma108b	5.61	4.48	6.06	5.79	5.16	5.95	3.96	5.00	5.40	5.26	4.90	5.43
Cma114	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	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	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	64.98	65.10	68.17	69.54	66.91	68.49	66.40	68.34	66.15	65.83	66.04	67.84

Table 6. The number of private alleles identified within sample location. Population ID #, locus, and frequency are listed for each private allele.

Population	Locus	# Private alleles	Frequency
1	*Cma33*	1	0.006
2	*Cma33*	2	0.005
	Cma108a	1	0.005
3	*Cma33*	1	0.005
	Cma108a	1	0.005
4	*Cma33*	1	0.018
5	*Cma108a*	1	0.008
6	*Cma108a*	1	0.004
8	*Cma33*	1	0.018
	Cma108a	1	0.018
9	*Cma33*	1	0.006
	Cma114	1	0.006
	Cma108a	1	0.006
10	*Cma33*	1	0.005
	Cma108a	1	0.005
12	*Cma33*	2	0.26, 0.013

Results: Genetic differentiation among individuals and sample locations

A Principal Coordinate Analysis (PCoA) based on a pairwise, individual-by-individual genetic distance matrix explained only 25.86% of the total variation with axis 1 accounting for 11.32%, axis 2 accounting for 7.78%, and axis 3 explaining 6.76%. This result indicates that there is no distinct clustering among the 791 Dungeness crab samples based on individual genetic variation. In contrast, a PCoA based on a pairwise, sample location-by-sample location genetic distance matrix explained 96.21% of the overall variation among the 12 locations. Axis 1 accounted for 63.61% while axes 2 and 3 accounted for 18.84% and 13.76% respectively (Figure 2). Axis 1 separates Astoria North (Pop 1) and Coos Bay South (Pop 8). This result indicates that Astoria North and Coos Bay South are genetically divergent.
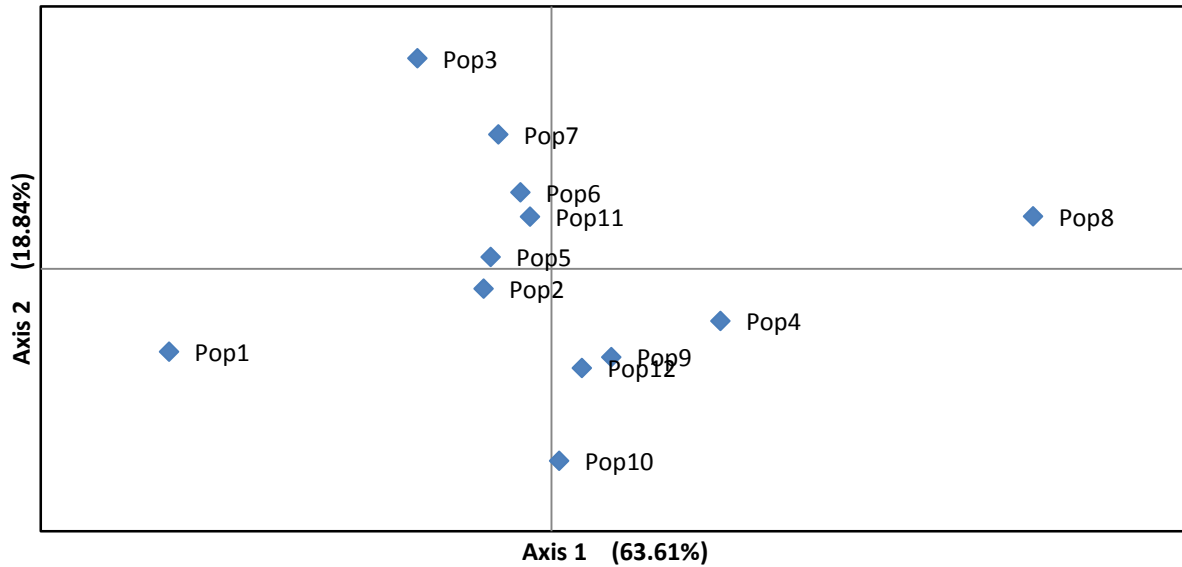
Figure 4. Principal coordinate analysis (PCoA) of genetic distances among the 12 sample locations (Pop). The three axes combined accounted for 96.21% of the total variation.

Findings based on pairwise F_{st} estimates for the 12 sample locations correspond to those from the sample-location by sample-location PCoA illustrated in Figure 2. Astoria North and Coos Bay South were significantly differentiated based on variation at the eight microsatellite loci ($F_{st} = 0.014$, $P < 0.05$) after correcting for multiple tests (Table 7). Similarly, Astoria North and Coos Bay South were significantly distinct based on genic ($P = 0.001$) and genotypic ($P = 0.001$) exact tests after correcting for multiple tests. Two pairwise comparisons (Astoria South and Coos Bay South, Garibaldi North and Coos Bay South) were also marginally significant prior to correcting for multiple tests.

We found no evidence for isolation by distance among the 12 locations based on the test for an association between genetic differentiation and geographic distance was not significant (Figure 5). This result indicates that there is gene flow among Dungeness crab along the coast of Oregon.
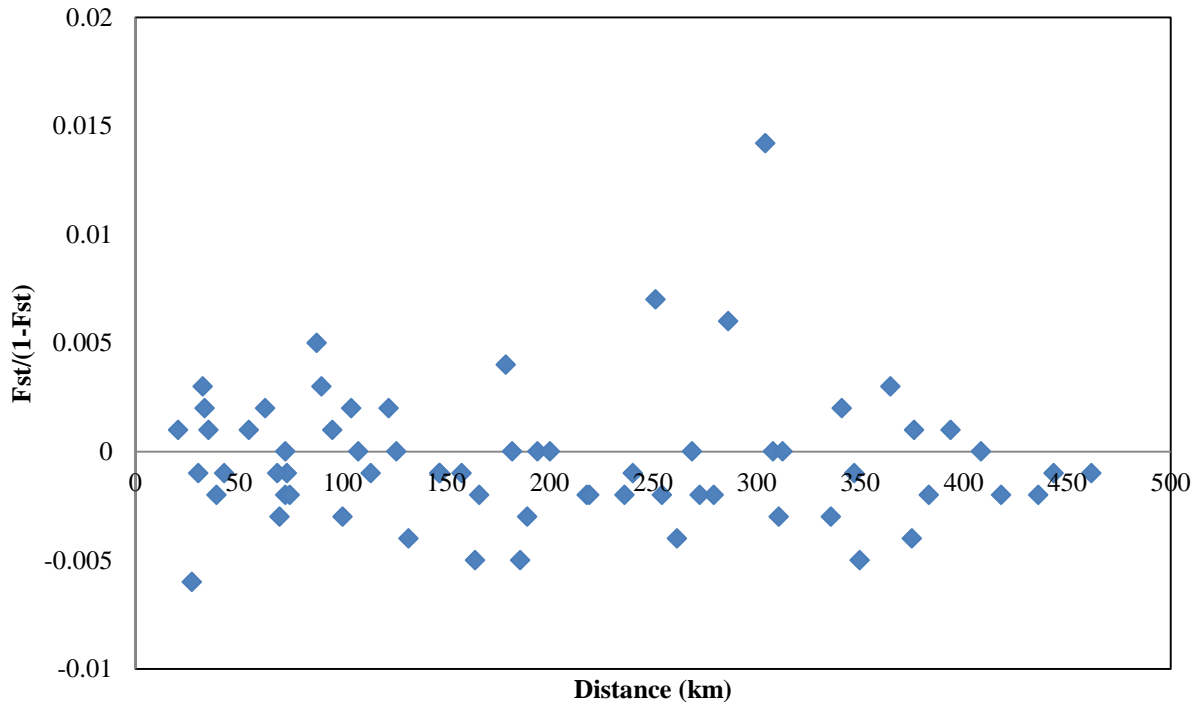
Figure 5. Pairwise relationship of genetic distance, as measured by $F_{st}/(1 - F_{st})$, and geographic distance (km) among the 12 Dungeness crab sample locations.

Results: Effective population size

We estimated effective population size, an important demographic metric used to assess the short and long-term viability of a population using estimates of linkage disequilibrium (LD) found among the 791 Dungeness crab samples. The principal of the LD method implemented in the software program NeEstimator is based on random linkage disequilibrium that arises by chance each generation in finite populations (Laurie-Ahlberg & Weir 1979; Hill 1981). Given that we found two loci to be in linkage disequilibrium among all 12 sample locations (*Cma103 and Cma108b*), we excluded one (*Cma108*) prior to estimating N_e. Our estimate of N_e based on seven microsatellite loci was infinite (95% C.I. = 3854 – infinite). The LD method assumes discrete generations but since our Dungeness crab sample consists of overlapping generations, our results should be interpreted as an estimate of the effective number of breeders (N_b) that produced these cohorts.

Discussion and Conclusions:

Larval dispersal plays a fundamental role in structuring populations in the marine environment, however, quantifying dispersal scales by tracking large numbers of individuals is challenging. Alternatively, genetic markers are an effective tool to help understand how patterns of larval dispersal regulate adult

14

population dynamics. Using this approach, the spatial and temporal scale of population structuring can be delineated to help inform conservation and management decisions. Here, we report on the first coastwide population genetics study of Oregon Dungeness crab based on microsatellite loci. These genetic data provide baseline information on the genetic diversity and population structure of Oregon Dungeness crab and can be used to help inform decisions on (1) ocean spatial planning (i.e. wave energy devices) and (2) sustainable fishery management.

Genetic diversity has been defined as the variety of alleles and genotypes present in a population that is reflected in morphological, physiological and behavioral differences between individuals and populations (Frankham et al. 2002). Conservation of genetic diversity is essential to the long-term survival of any species, particularly when considering changing environmental conditions. Reduced genetic diversity has the potential to negatively impact the adaptive potential of a species and lead to an increased risk of deleterious inbreeding effects. We estimated the genetic diversity of Oregon Dungeness crabs based on eight microsatellite loci and found that levels of heterozygosity were similar to those reported for eight Dungeness crab subpopulations in British Columbia (Beacham et al. 2008). Furthermore, comparison of the number of alleles at each microsatellite locus reveal that Oregon Dungeness crabs have a greater number of observed alleles at 6 of the 8 loci compared to Dungeness crabs in British Columbia. Our findings indicate that the Oregon Dungeness crab population sample does not show evidence of decreased genetic diversity.

Thorough knowledge of population genetic structure is crucial for the biologically sustainable management of a species to avoid reducing genetic diversity within populations and overharvest (Laikre et al. 2005). Many marine species have the capacity to disperse over large geographical areas, either by larvae behaviorally interacting with large scale ocean currents, or by seasonal migration of juveniles and adults. The implication is that these marine species with wide distributions, large population sizes, high fecundity, and a long pelagic larval phase would show weak or no population structure (Waples 1998). However, a number of genetic studies have challenged this traditional view by demonstrating population subdivision in marine species on a limited geographical scale ranging from tens to a few hundred kilometers (e.g. Nielsen et al. 2004, Miller et al. 2006, Olsen et al. 2008). Although genetic differentiation tends to be much smaller in marine than in freshwater and terrestrial species (Waples 1998), and is often barely statistically significant, its biological relevance has been demonstrated by correlation with environmental factors (Bekkevold et al. 2005), and by temporal and spatial replication (Cimmarutaet al. 2008).

Based on allele frequency differences at the eight microsatellite loci, we found little evidence for population structuring among Oregon Dungeness crabs. Among the 12 sample locations, only one pairwise comparison was significant indicating that Astoria North is genetically distinct from Coos Bay South. Two other pairwise comparisons were marginally significant prior to correcting for multiple tests (Astoria South and Coos Bay South; Garibaldi North and Coos Bay South). We did not find evidence for the isolation by distance model of population genetic structure of Dungeness crab sampled off the Oregon coast. In contrast to our study, Beacham et al. (2008) reported significant local population differentiation consistent with high level of retention of larval crabs within Alison Sound, British Columbia as well as differentiation between a west coast Vancouver Island population and a population adjacent to the southern Strait of Georgia. Similar to our study, however, this population genetic structuring did not follow the isolation by distance model. Further research is required to determine the biological relevance, if any, of the population differentiation detected between Dungeness crabs sampled in northern Oregon compared to south central Oregon especially since the Coos Bay South sample location is proximate to current and proposed wave energy development sites. Ocean Power Technologies' wave energy PowerBuoy is positioned in an approximately 33 acre triangular area about 2.5nm west of Reedsport wile a proposed offshore wind facility would be located approximately 15nm offshore of Coos Bay in 200fm depth. The proposed ocean spatial planning further increases the need for additional study of the genetic diversity and population structure of Dungeness crab in this region.

Recent data now indicate that genetically effective population sizes (N_e) in marine species, especially those characterized by high fecundity and high larval mortality, are typically 2–6 orders of magnitude smaller than census population sizes (N_c)(Hauser and Carvalho 2008). Such insights from genetic data have profound implications for both our understanding of the demography of marine species and the management and conservation of harvested populations. The estimated N_e of Oregon Dungeness crab was infinite, indicating there is little evidence for linkage disequilibrium due to drift among individuals. Therefore, the number of effective breeders (N_b, see Results) is larger than 10^3, and likely much larger. The recommendation for conservation managers is to maintain a minimum N_e of 50 to avoid inbreeding depression and 500 to maintain the long term evolutionary potential of a population (Jamieson & Allendorf 2012). Based on our one-sample N_e estimate for Oregon Dungeness crab, there is no immediate threat to population persistence. However, additional sampling is required to assess the temporal stability in allele frequencies and provide robust estimates N_e for this population.

Here we provide an estimate of genetic diversity and population structuring of Oregon Dungeness crab based on data from one year. The next step is to examine temporal changes in genetic diversity and

structure over a broader geographic range and understand how it relates to changing ocean conditions. This approach, referred to as Genetic Monitoring (GeM), can be used to detect genetic changes induced by harvest or other anthropogenic activities that might affect long-term sustainability (Schwartz et al. 2007). GeM involves genetic analysis of a temporal series of samples to detect genetic changes over time. While genetic changes cannot be avoided, we have the ability to detect them, measure their magnitude, and respond by modifying management accordingly (Allendorf et al. 2014).

Future Research:

To expand on the current study and examine temporal changes in genetic diversity and structure of Dungeness crab over a broader geographic, we have collaborated with the Oregon Department of Fish and Wildlife (ODFW), Washington Department of Fish and Wildlife (WDFW), and California Department of Fish and Wildlife (CDFW) to conduct a multi-year population genetic study of Dungeness crab. As of result of the coordination efforts of Kelly Corbett (ODFW), Dan Ayres (WDFW), Pete Kalvass (CDFW), and participation of the commercial crabbing fleet, we were able to collect tissue samples from 4,084 female and sub-legal size male Dungeness crab during the 2012 Pre-Season Test Fishery. The sampling design consisted of 32 latitudinal transects with the most northern transect located off Westport, WA and the most southern located off Half Moon Bay, CA. Six crab pots were fished at three different depths (15, 30, and 45 fathoms) and three rounds of sampling were conducted in Washington and Oregon and one round in California. Carapace width data was collected for every crab sampled during Washington's Pre-Season Test Fishery while carapace width data was collected for a subset of the crab caught during Oregon's 2012 Pre-Season Test Fishery. In addition to the ocean sampling effort, we collaborated with the ODFW Shellfish program to collect tissue samples of Dungeness crab from Yaquina (N = 187) and Alsea bays (N = 133) in August 2012. During the quarterly sampling events, ODFW biologists fish a single pot at 12 sites in Yaquina Bay and 9 sites in Alsea Bay. Biological information collected for each sample included: sex, carapace width, and shell condition (e.g. hard vs. soft).

K.G. O'Malley was recently awarded funds by Oregon Sea Grant to conduct the genetic and statistical analyses on the ocean and bay Dungeness crab samples collected in 2012 as well as to conduct another round of ocean and bay sampling in 2014 following the same methods implemented in 2012. K.G. O'Malley is currently recruiting a graduate student to work on this project with an anticipated start date in fall 2014. The total budget for this project is $302,062.

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