



# Fine-scale spatial and temporal genomic variation among Dungeness crab *Cancer magister* larval recruits in the California Current Ecosystem

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**ABSTRACT:** Dynamic marine environments can shape complex spatial and temporal patterns in the population connectivity of marine species, and this is often exemplified in species with long larval phases. Here, we used a genotyping-by-sequencing (GBS) approach to examine fine-scale spatial and temporal genomic variation among Dungeness crab *Cancer magister* larval recruits sampled in the California Current Ecosystem. Specifically, we compared samples collected during expected- and late-season recruitment time periods within 2 consecutive years (2017 and 2018) at 2 sites in Oregon, USA (Yaquina Bay and Coos Bay). Evidence was found for high gene flow between the expected- and late-season recruits within each year and at both sites based on 1389 neutral loci. In contrast, strong genetic differentiation was observed between these 2 groups within each year and at both sites based on variation at 2 putatively adaptive loci. Contrary to prediction, the magnitude of genetic differentiation between these 2 seasonal groups was greater in 2017 when the Pacific Decadal Oscillation was stronger, upwelling was weaker, and the spring transition was later. Spatial genetic variation was not observed within 2017 or 2018. Comparing across years, expected- and late-season groups were differentiated at putatively adaptive loci. Interestingly, strong genetic differentiation was also observed between late-season groups across years. We found no evidence for cohesive larval dispersal among recruits based on genetic relatedness estimates. Overall, our findings provide evidence for high connectivity within Dungeness crab, but suggest that selective pressures and ocean conditions influence the genetic composition of larval recruits both intra- and inter-annually.

**KEY WORDS:** Genotyping-by-sequencing · Larval dispersal · Molecular ecology · Ocean conditions · Pelagic larval duration · Population connectivity · Population genomics

## 1. INTRODUCTION

Dynamic marine environments can produce complex spatial and temporal patterns of population connectivity in marine species (Hellberg et al. 2002, Selkoe et al. 2008). Population connectivity within a species can be defined by the movement of individuals among groups or locations (demographic connectivity) and by gene flow between groups of individuals (genetic connectivity) (Cowen et al. 2007, Hedgecock et al. 2007, Lowe & Allendorf 2010, Kool

et al. 2013). For benthic marine species with pelagic larval phases, population connectivity is determined by both larval exchange throughout the species' range and by the ability of migrants to survive and reproduce at recruitment locations (Palumbi 2003, Hedgecock et al. 2007, Pineda et al. 2007, Cowen & Sponaugle 2009).

Larval dispersal patterns of marine species are difficult to study in vast and variable ocean environments; however, genomic methods can be used to make inferences about these patterns (Pineda et al.

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2007, Selkoe & Toonen 2011, Marko & Hart 2018). Dispersal and survival of migrants influence the scale and rate at which genetic drift and natural selection occur (Grosberg & Cunningham 2001). Therefore, investigating the population genetic structure of a species provides an evolutionary supported framework for organism–environment interactions occurring within marine ecosystems and offers insight into larval dispersal patterns (Grosberg & Cunningham 2001, Hellberg 2009). Moreover, the recent development of genotyping-by-sequencing (GBS) methods has enabled a shift in how population genetic structure is studied in marine species: from using only presumably neutral loci to also incorporating putatively adaptive loci and testing for divergent selection (Angeloni et al. 2012, Narum et al. 2013, Li & Wang 2017). In some species, evidence for weak population genetic structure was found based on neutral loci while variation at putatively adaptive loci indicated divergent selection among populations (Nielsen et al. 2009, Gagnaire et al. 2015).

The Dungeness crab *Cancer (Metacarcinus) magister* is a valuable fishery species distributed throughout 3 large marine ecosystems along the west coast of North America: the California Current Ecosystem (CCE), the Salish Sea Ecosystem (SSE), and the Gulf of Alaska Ecosystem (GOA) (Wild & Tasto 1983, Rasmuson 2013). The North Pacific Current, which stems from the North Pacific Gyre, bifurcates into the northern-flowing Alaska Current and the southern-flowing California Current at the junction of these 3 ecosystems (Checkley & Barth 2009). Reduced genetic connectivity between these ecosystems has been observed in marine species with long pelagic larval durations (PLDs) (e.g. shore crab *Hemigrapsus nudus*, hermit crab *Pagurus hirsutiusculus*; Kelly & Palumbi 2010). In previous population genetic studies of adult benthic stage Dungeness crab from the CCE, SSE, and GOA, genetic differentiation was observed within and between ecosystems based on variation at neutral loci (microsatellites) (Jackson & O'Malley 2017, O'Malley et al. 2017, Jackson et al. 2017).

Larval dispersal promotes genetic connectivity within Dungeness crab, since movement of the benthic invertebrate is restricted (Diamond & Hankin 1985, Hildenbrand et al. 2011, Rasmuson 2013). The PLD of the Dungeness crab is documented as 3–4 mo long (Wild & Tasto 1983). After hatching, the Dungeness crab develops through 5 pelagic zoeal stages into a final pelagic megalopa stage, which recruits to the nearshore benthic environment (Wild & Tasto 1983). During the PLD, dispersal is influenced by

ocean conditions (including physical oceanography) and behavior of the megalopae (Rasmuson & Shanks 2014). The influence of ocean conditions on recruitment success has been studied by monitoring megalopae recruitment timing and abundance in Coos Bay, Oregon, USA, (CCE) for over 2 decades (Shanks & Roegner 2007, Shanks et al. 2010, Rasmuson 2013, Shanks 2013, Rasmuson & Shanks 2020). Based on this long-term timeseries, the researchers observed that years characterized by stronger negative Pacific Decadal Oscillation (PDO) indices, stronger upwelling, and earlier physical spring transitions were correlated with higher levels of megalopae recruitment (Shanks & Roegner 2007, Shanks et al. 2010, Shanks 2013). These ocean conditions impact northern and southern alongshore transport and transport across the continental shelf.

Dungeness crab larval hatch timing varies with latitude and occurs later in the year within the northern range of the species, resulting in recruitment timing differences between ecosystems (i.e. CCE, SSE, GOA) (reviewed by Rasmuson 2013). The previously mentioned long-term studies on megalopae recruitment in Coos Bay have consistently documented recruitment later into the year than expected for the CCE (Shanks & Roegner 2007, Shanks et al. 2010, Shanks 2013). Since the timing of this late-season recruitment matches that of northern ecosystems (GOA and SSE), researchers hypothesize that the April through July ('expected-season') megalopae recruits originate from within the CCE, but the August through September ('late-season') megalopae recruits originate from the GOA or SSE (Shanks 2013). This hypothesis is supported by the finding that higher abundances of late-season recruits were observed during years with a stronger negative PDO index, which has been correlated with a stronger southward transport in the CCE (Keister et al. 2011, Shanks 2013). Since genetic differentiation has been observed between adult Dungeness crabs from different ecosystems (CCE, SSE, and GOA) (Jackson & O'Malley 2017, O'Malley et al. 2017, Jackson et al. 2017), genetic approaches can be used to test the hypothesis that late-season recruits in Coos Bay originate from a northern ecosystem; specifically, by quantifying the magnitude of genetic differentiation between expected-season and late-season recruits.

In a prior study, we used a GBS approach to examine genetic variation at both neutral and adaptive loci within the 2014 Dungeness crab megalopae recruits in Coos Bay. We found evidence for weak but significant genetic differentiation between expected-

season recruits (May) and late-season recruits (September) based on variation at 1913 neutral loci and strong genetic differentiation based on variation at 2 putatively adaptive loci (Lee & O'Malley 2020). The inference of the findings was limited due to only 1 year of data from a single location. However, the established methods and intriguing findings warranted further examination of fine-scale spatial and temporal patterns of genetic differentiation among Dungeness crab recruits using both neutral and adaptive loci.

Here, we again used a GBS approach, and tested for genetic differentiation among Dungeness crab megalopae recruits in 2 consecutive years and at 2 sites in the CCE. Our first objective was to test for intra-annual genetic differentiation between recruiting Dungeness crab megalopae in Coos Bay and Yaquina Bay, Oregon. We hypothesized that expected-season recruits would be genetically differentiated from late-season recruits at both neutral and putatively adaptive loci if they originated from different ecosystems with divergent selective pressures. Our second objective was to test if the magnitude of intra-annual genetic differentiation among the recruiting megalopae at the 2 sites varied across years, 2017 and 2018. We hypothesized that intra-annual genetic differentiation would be greater in 2018 when the positive PDO was weaker (Table 1), indicating the southern-flowing California Current was stronger, resulting in higher abundances of late-season recruits. Our third objective was to test for relatedness among individual Dungeness crab megalopae within the 2 CCE sites. We hypothesized that collections of recruiting Dungeness crab megalopae would exhibit higher relatedness than expected by chance and would contain full-siblings or half-siblings if cohesive larval dispersal occurred during the 3–4 mo larval phase.

## 2. MATERIALS AND METHODS

### 2.1. Sample collection

In 2017 and 2018, Dungeness crab megalopae recruits were collected from 2 sites within the CCE: Coos Bay (43.34°N, 124.33°W) and Yaquina Bay (44.62°N, 124.05°W), Oregon, USA (Fig. 1). At each site, a light trap was fished daily from 1 April through 31 October in 2017 and from 1 March through 31 October in 2018, following the methodology of Shanks et al. (2010). The daily abundance of megalopae caught in each trap was recorded.

For each site and year, 47 Dungeness crab megalopae were randomly selected for sequencing from the first day when more than 50 megalopae were collected and from the last day when more than 50 megalopae were collected (i.e. 8 collection timepoints; Fig. 2, Table 2). Collection timepoints were categorized as expected-season (April–July) or late-season (August–September), based on CCE life history timing documented in the literature (Rasmuson 2013, Shanks 2013). Megalopae from these 8 collection timepoints were preserved in 95% ethanol for genetic analyses.

### 2.2. DNA sequencing, loci identification, and single nucleotide polymorphism (SNP) calling

Individual megalopae were homogenized using a TissueLyser II (Qiagen), and genomic DNA was extracted from each megalopa using a DNeasy Blood and Tissue Kit (Qiagen). A modified version of the Elshire et al. (2011) GBS method was used to construct 4 libraries, each composed of 96 uniquely bar-coded individual megalopae (Lee & O'Malley 2020). The DNA of each megalopa was cut into fragments through a double digestion with 2 high-fidelity restriction enzymes, *SbfI* and *MspI*. A unique barcode was then ligated to the DNA fragments of each sample, which allows for the identification of each sample when pooled. The DNA samples were pooled into groups (libraries) of 94 samples with 2 sample replicates, and the multiplexed DNA was amplified with Illumina sequencing primers using polymerase chain reaction (PCR). No size selection was performed on the libraries in order to capture a larger number of sequences for downstream analyses. The libraries were purified using a QIAquick PCR Purification Kit (Qiagen). Prior to sequencing, an Agilent 2100 Bioanalyzer (Agilent Technologies) was used to assess the library fragment sizes and infer the quality of the libraries. After quality assessment, each GBS library

Table 1. Ocean conditions in the California Current Ecosystem in 2017 and 2018: Pacific Decadal Oscillation (sum of monthly index January–July; JISAO 2019), upwelling (sum of daily upwelling index March–September; units: Mt s<sup>-1</sup> per 100 m of coastline; PFEL 2019), and physical spring transition (day of the year; Bograd et al. 2009)

Ocean condition	Mean (1998–2016)	2017	2018
Pacific Decadal Oscillation	1.38	5.18	1.31
Upwelling	3827	3205	4377
Spring transition	104	115	104

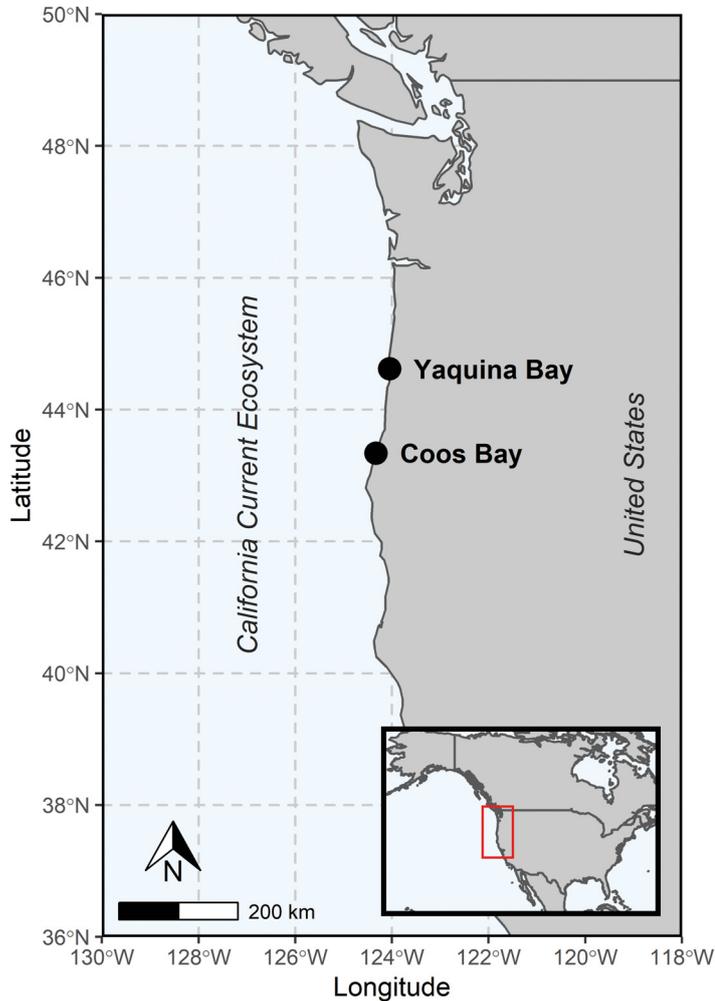


Fig. 1. Location of study sites in the California Current Ecosystem: Yaquina Bay and Coos Bay, Oregon, USA

was sequenced on a single lane of an Illumina HiSeq 3000 using 150 bp paired-end sequencing chemistry. Following the bioinformatic methods (documented in Text S1 in the Supplement at [www.int-res.com/articles/suppl/m649p067\\_supp.pdf](http://www.int-res.com/articles/suppl/m649p067_supp.pdf)), the raw sequence reads were assessed and filtered for quality and then the 'stacks' v. 2.2 program (Catchen et al. 2011, 2013, Rochette & Catchen 2017) was used to identify a set of polymorphic loci within the samples and call SNPs between individuals.

### 2.3. Calculating summary statistics

Population genetic summary statistics were calculated among megalopae within each collection timepoint based on SNP genotypes of each sample. Expected heterozygosity ( $H_e$ ), observed heterozygosity

( $H_o$ ), inbreeding coefficient ( $F_{IS}$ ), and allelic richness ( $A_R$ ) were calculated using the program R v. 3.5.0 (R Core Team 2019) with the R package 'hierfstat' v. 0.4.22 (Goudet 2005). To test if  $F_{IS}$  was significantly different from 0 (excess or deficit of heterozygotes) within each collection timepoint, a 95% confidence interval was calculated with 10 000 bootstraps across all loci. The R package 'whoa' v. 0.0.1 (Anderson 2018) was used to examine the loci and estimate heterozygote miscall rate. The R package 'pegas' v. 0.10 (Paradis 2010) was used to determine if any loci within each collection timepoint departed from Hardy-Weinberg proportions (HWPs) with an exact test (Wigginton et al. 2005), and p-values were adjusted for multiple testing by applying a false discovery rate (FDR) correction (q-value < 0.05). The R package 'adegenet' v. 2.1.1 (Jombart et al. 2010) was used to conduct a discriminate analysis of principal components (DAPC) of all loci.

### 2.4. Identifying putatively adaptive loci

We tested for putatively adaptive differences between the expected-season and late-season megalopae recruits. Three outlier detection programs were used to identify loci with high  $F_{ST}$  (fixation index) estimates. The statistical approaches and error rates differ among the 3 programs (Narum & Hess 2011). The program 'bayescan' v. 2.1 (Foll & Gaggiotti 2008) was used with a prior of 100 and an FDR threshold of 0.05 (q-value). The program 'outflank' v. 0.2 (Whitlock & Lotterhos 2015) was used with an FDR threshold of 0.01 (q-value). The program 'fsthet' v. 1.0.1 (Flanagan & Jones 2017) was used with a 95% confidence interval. To conservatively identify outlier loci and reduce false positives, only loci identified by all 3 outlier detection programs were categorized as putatively adaptive loci for downstream analyses. The allele frequencies of the putatively adaptive loci were determined for each collection timepoint using the program 'vcftools' v. 0.1.13 (Danecek et al. 2011). The consensus sequences of the putatively adaptive loci were compared to the National Center for Biotechnology Information (NCBI) database using BLASTN and the 'somewhat similar sequences' parameter (Altschul et al. 1990).

### 2.5. Genetic differentiation analyses

The R package 'stampp' v. 1.5.1 (Pembleton et al. 2013) was used to calculate pairwise  $F_{ST}$  estimates

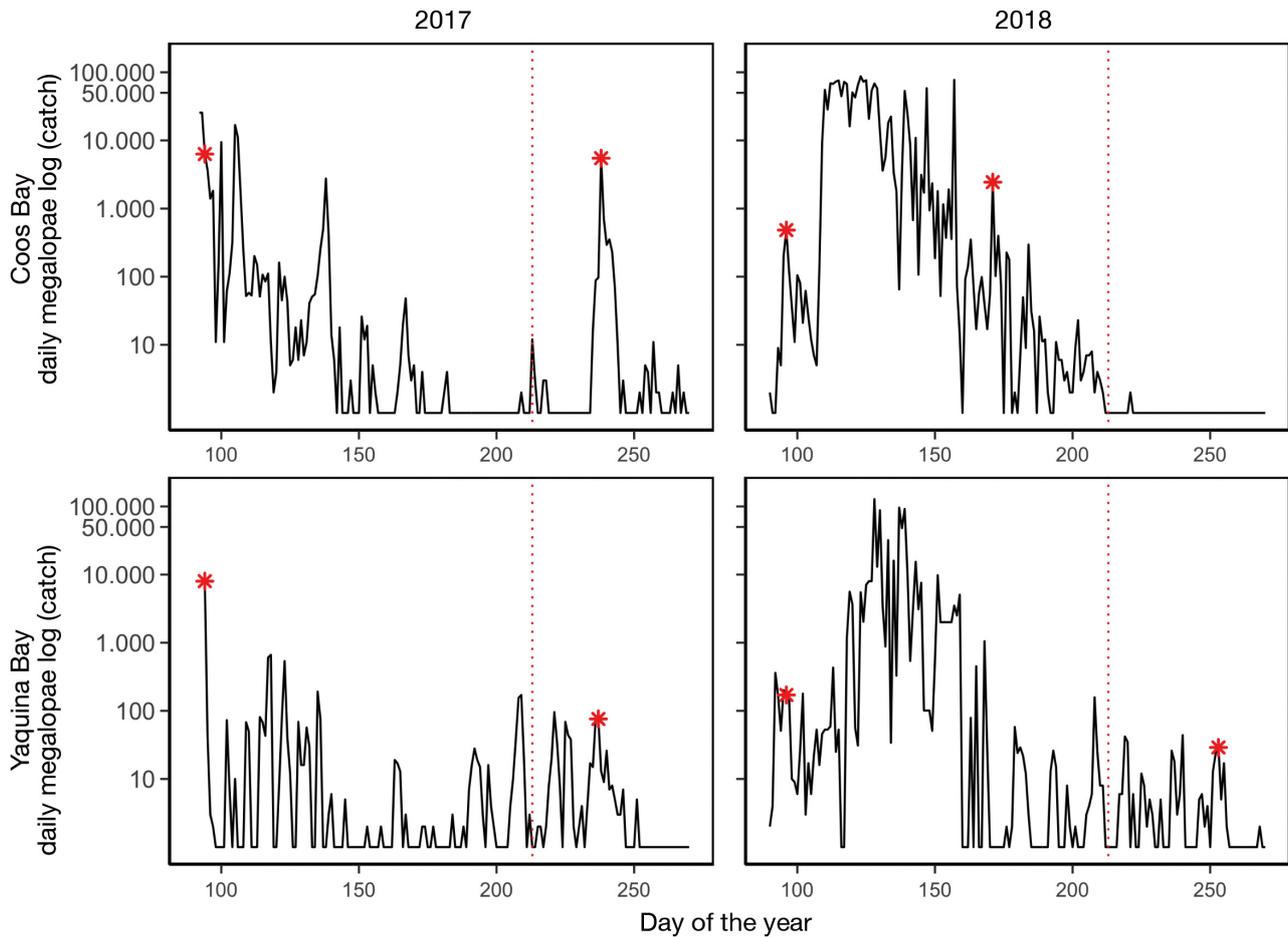


Fig. 2. Daily catch (log) of Dungeness crab megalopae from light traps fished in Coos Bay and Yaquina Bay, Oregon, in 2017 and 2018. Red dotted line indicates the divide between expected-season and late-season recruits (day of the year 213: 1 August). The red asterisks indicate the collection timepoints for genomic analysis within each year and site

(Weir & Cockerham 1984) between each collection timepoint, site, and year using presumably neutral loci, putatively adaptive loci, and combined neutral and adaptive loci. Significance of the pairwise  $F_{ST}$  estimates based on neutral loci were quantified using 10 000 bootstraps across all loci to determine 95% confidence intervals and associated p-values. The p-values were adjusted for multiple testing with an FDR correction ( $q$ -value < 0.05).

## 2.6. Estimating the effective number of breeders

The effective number of breeders ( $N_b$ ) of each collection timepoint and each year was estimated using 'NeEstimator' v. 2.1 (Do et al. 2014) with the single-sample linkage disequilibrium method (Waples & Do 2010). Only neutral loci were used in the analyses. A p-critical value of 0.02 was used for analysis of each collection timepoint and a p-critical value of 0.01 was used for analysis of each year.

## 2.7. Relatedness analyses

To test for evidence of cohesive larval dispersal among the Dungeness crab megalopae recruits, relatedness ( $r$ ) among individuals within each collection timepoint was measured. Only neutral loci were used in the relatedness analyses. All 7 relatedness estima-

Table 2. Number of Dungeness crab megalopae sampled at 2 sites in Oregon, USA, at each collection timepoint

Year	Site	Month	Day	Recruitment season	n
2017	Coos Bay	April	4	Expected	47
		August	26	Late	47
	Yaquina Bay	April	4	Expected	48
		August	26	Late	48
2018	Coos Bay	April	6	Expected	47
		June	20	Expected	44
	Yaquina Bay	April	6	Expected	45
		August	27	Late	45

tors included in the 'coancestry' v. 1.0.1.9 (Wang 2011) program were implemented with 1000 pairs of simulated dyads and 6 defined relationship types (including half siblings and full siblings) using 100 simulated datasets. For each method, a Pearson's correlation coefficient was calculated between the simulated data and the observed kinship to determine the accuracy of each method (Table S1). The dyadic likelihood estimator of Milligan (2003) was chosen as the best estimator to test for relatedness among Dungeness crab megalopae recruits because it had the highest Pearson's correlation coefficient. Based on the 'coancestry' (Wang 2011) simulations with defined kinship pairs, it was determined that an  $r$ -value  $>0.25$  between dyads was the appropriate threshold for identifying true half-siblings and full-siblings with  $0 \pm 0$  (mean  $\pm$  SD) false positives and  $1.2 \pm 2.7$  false negatives per 100 datasets simulated. Using the empirical data, relatedness values were estimated for every megalopae dyad within each collection timepoint. Any dyads with  $r$ -values indicating half or full siblings ( $r > 0.25$ ) were reported. Additionally, the overall relatedness value of each collection timepoint was calculated, and significant differences between the relatedness estimates were determined using 1000 permutations and 95% confidence intervals.

### 3. RESULTS

#### 3.1. Intra-annual variation in megalopae recruitment within Coos and Yaquina Bays

Megalopae recruitment varied between the 2 seasons (expected and late) and between the 2 sites (Coos Bay and Yaquina Bay) (Fig. 2, Table 3). In 2017, over 10 times more total megalopae were trapped in Coos Bay than Yaquina Bay, and more late-season megalopae were trapped in Coos Bay than Yaquina Bay. In 2018, over twice as many megalopae were trapped in Coos Bay than Yaquina Bay (Fig. 2, Table 3); however, only 1 late-season megalopa recruit was trapped in Coos Bay and 306 were trapped in Yaquina Bay.

The timing of megalopae recruitment varied between the 2 sites (Fig. 2, Table 3). In 2017, recruitment in Coos Bay continued for 17 d longer than in Yaquina Bay. However, the

highest daily recruitment abundance in 2017 occurred in early April at both sites, and recruits were observed from April through September. In 2018, recruitment in Yaquina Bay lasted 35 d longer than in Coos Bay. At both sites, the first megalopae recruits of the year were observed on 30 March, and similarly, the highest daily abundance of the year was recorded on 8 May at both sites. However, the last recruits were caught on 9 August in Coos Bay, whereas recruits were caught in Yaquina Bay through 13 September.

#### 3.2. Inter-annual variation in megalopae recruitment within Coos and Yaquina Bays

Ocean conditions (i.e. PDO, upwelling, and spring transition) differed between 2017 and 2018 (Table 1). Although both years were characterized by a positive PDO (defined by the sum of the monthly PDO index from January through July), the positive PDO was stronger in 2017, suggesting weaker southward transport. Annual upwelling (defined by the sum of the daily upwelling index from March through September) was lower in 2017 than 2018. Lastly, the physical spring transition occurred 11 d later in 2017 than 2018. The expectation based on previous research of these ocean conditions and recruitment abundances (Shanks & Roegner 2007, Shanks et al. 2010, Shanks 2013) was that the total number of recruits and the number of late-season recruits would be higher in 2018 than 2017.

At both sites, an order of magnitude fewer megalopae were trapped in 2017 than in 2018 (Table 3). Moreover, inter-annual comparisons of megalopae abundances within each site showed differences in recruitment patterns. In Coos Bay, fewer total megalopae were trapped in 2017 than in 2018; however, more late-season megalopae were trapped in 2017

Table 3. Historic mean Dungeness crab megalopae abundances recorded in 1998–2001 and 2007–2016 in Coos Bay (Shanks 2013, A. Shanks unpubl. data) and megalopae abundances in Coos Bay and Yaquina Bay in 2017 and 2018

Year	Site	Total megalopae	Expected-season megalopae	Expected-season % of total	Late-season megalopae	Late-season % of total
Historic mean	Coos Bay	719 182	677 493	94.20	41 689	5.80
2017	Coos Bay	117 359	109 983	93.72	7 376	6.28
	Yaquina Bay	11 960	11 385	95.19	575	4.81
2018	Coos Bay	1 501 314	1 501 313	100.00	1	0.00
	Yaquina Bay	635 485	635 179	99.95	306	0.05

than in 2018, only 1 late-season megalopa was trapped in 2018. The Coos Bay recruitment season was 47 d shorter in 2017 than in 2018. In Yaquina Bay, fewer megalopae were trapped in 2017 than in 2018, but in 2017, slightly more late-season megalopae were trapped than in 2018 (Table 3). The length of the Yaquina Bay recruitment season was 17 d shorter in 2017 than in 2018.

### 3.3. Genetic sampling

The 2017 megalopae used for sequencing were collected on the same day at both sites: 4 April (expected-season) and 26 August (late-season) (Table 2). Since the lengths of the recruitment seasons differed between the 2 sampling sites in 2018, only the expected-season collection timepoints were from the same day at both sites: 6 April. The late-season collection timepoint from Yaquina Bay was 27 August. Late-season megalopae were not available from Coos Bay in 2018; instead, a second expected-season collection timepoint in Coos Bay was 20 June.

### 3.4. Calculating summary statistics

After filtering the sequence reads of the 4 GBS libraries (Text S2 and Fig. S1), 371 megalopae and 1391 loci remained for analysis (Table S2). Minimal differences were observed in genetic diversity measures among the 8 collection timepoints (Table 4). The range of allelic richness was 1.491–1.501. Furthermore,  $H_e$  (range: 0.2682–0.2745) was greater than  $H_o$  (range: 0.2377–0.2490), and all collection timepoints were significant for heterozygote deficiency with a 95% confidence interval ( $F_{IS}$  range: 0.0837–0.1047). With the exception of the Yaquina Bay samples from 2017, the expected-season collection timepoints had lower inbreeding coefficients ( $F_{IS}$ ) than the late-season collection timepoints. A range of 54–90 loci within each collection timepoint were found to deviate from HWP (Table S3). However, these loci were not removed from the dataset because substructure may be present within collection timepoints; adult reproduction occurs among groups of individuals within limited geographic areas, and

the groups of larvae mix offshore prior to recruitment and sampling. Filtering the sequences and loci reduced the chance that deviations from HWP were a result of genotyping errors or null alleles. The DAPC indicated that 2 groups could be differentiated among 1391 loci using more than 10 principal components (Fig. S2).

### 3.5. Identifying putatively adaptive loci

To identify putatively adaptive loci between expected- and late-season recruits, all April collection timepoints ( $n = 4$ ) were grouped together to represent expected-season recruits, and all August collection timepoints ( $n = 3$ ) were grouped together to represent late-season recruits. The June 2018 expected-season collection timepoint in Coos Bay was excluded from the dataset when testing for outliers. Two putatively adaptive loci (CLocus\_24 and CLocus\_3063) were identified by all 3 outlier detection programs (Tables S4 & S5). These 2 loci (>200 bp) did not align with the 2 putatively adaptive loci (>200 bp) identified by Lee & O'Malley (2020). The remaining 1389 loci were categorized as presumably neutral.

The 2 putatively adaptive loci had varying allele frequencies at each collection timepoint (Table 5). In 2017, the allele frequencies at the 2 putatively adaptive loci differed between the expected- and late-season collection timepoints at both sites. In 2018, the allele frequencies at CLocus\_3063 were different between expected- and late-season collection timepoints, and the allele frequencies at CLocus\_24 were more similar between expected- and late-season collection timepoints. One of the 2 putatively adaptive

Table 4. Genetic summary statistics for 2017 and 2018 expected-season and late-season Dungeness crab megalopae from Coos Bay and Yaquina Bay, Oregon, based on variation at 1391 loci. Number of individuals genotyped ( $n$ ), expected heterozygosity ( $H_e$ ), observed heterozygosity ( $H_o$ ), inbreeding coefficient ( $F_{IS}$ ), and allelic richness ( $A_R$ ) are listed for the expected-season and late-season megalopae at each site for each year

Year	Site	Month	Recruitment season	$n$	$H_e$	$H_o$	$F_{IS}$	$A_R$
2017	Coos Bay	April	Expected	47	0.2697	0.2451	0.0877	1.494
		August	Late	47	0.2689	0.2377	0.1047	1.492
	Yaquina Bay	April	Expected	48	0.2712	0.2415	0.1022	1.496
		August	Late	48	0.2682	0.2400	0.0962	1.491
2018	Coos Bay	April	Expected	47	0.2724	0.2474	0.0909	1.498
		June	Expected	44	0.2731	0.2461	0.0950	1.499
	Yaquina Bay	April	Expected	45	0.2732	0.2490	0.0837	1.499
		August	Late	45	0.2745	0.2481	0.0946	1.501

Table 5. Allele frequencies at the 2 putatively adaptive loci (Clocus\_24 and Clocus\_3063) identified within the 2017 and 2018 expected-season and late-season Dungeness crab megalopae from Coos Bay and Yaquina Bay

Year	Site	Month	Recruitment season	n	Clocus_24		Clocus_3063	
					A Frequency	T Frequency	C Frequency	T Frequency
2017	Coos Bay	April	Expected	47	0.9545	0.0455	0.6316	0.3684
		August	Late	47	0.4545	0.5455	0.2702	0.7297
	Yaquina Bay	April	Expected	48	0.9268	0.0732	0.7368	0.2632
		August	Late	48	0.3780	0.6220	0.2632	0.7368
2018	Coos Bay	April	Expected	47	0.9047	0.0953	0.8461	0.1539
		June	Expected	44	0.9000	0.1000	0.7142	0.2858
	Yaquina Bay	April	Expected	45	0.8536	0.1464	0.7875	0.2125
		August	Late	45	0.9744	0.0256	0.4054	0.5946

loci (Clocus\_24) aligned to a sequence in the NCBI database with a low Expect value (E-value:  $2 \times 10^{-8}$ ), indicating a significant match to a predicted transcription factor, SOX-1-like in Pacific white shrimp *Litopenaeus vannamei* (NCBI Sequence ID: XM\_027382823.1).

### 3.6. Genetic differentiation

Across sites, seasons, and years, the  $F_{ST}$  estimates based on variation at neutral loci were low ( $F_{ST}$  range: 0.0000–0.0012) and nonsignificant after applying an FDR correction (q-value < 0.05; Table 6). Similarly,  $F_{ST}$  estimates were nonsignificant when including both presumably neutral and the 2 putatively adaptive loci (Table S6).  $F_{ST}$  estimates based on variation at the 2 putatively adaptive loci (Table 7) were notably higher compared to those based on the neutral loci.

In 2017,  $F_{ST}$  estimates between expected- and late-season recruits based on the putatively adaptive loci ranged from 0.3411–0.4209. The genetic differentia-

tion was slightly lower in Coos Bay ( $F_{ST} = 0.3411$ ) compared to Yaquina Bay ( $F_{ST} = 0.4209$ ). However, low genetic differentiation ( $F_{ST} = 0.0000$  and 0.0048) was observed when comparing seasonal groups between the 2 sites (e.g. expected-season from Yaquina Bay vs. expected-season from Coos Bay).

In 2018, the  $F_{ST}$  estimate between expected- and late-season recruits in Yaquina Bay based on the putatively adaptive loci was 0.2071 (Table 7). Due to low recruitment, there was no late-season collection timepoint in Coos Bay. When comparing the 2 expected-season collection timepoints (April and June) from Coos Bay, the  $F_{ST}$  estimate was 0. However, a comparison of the 2 Coos Bay expected-season collection timepoints (April and June) to the Yaquina Bay late-season collection timepoint resulted in  $F_{ST}$  estimates >0 (April  $F_{ST} = 0.1124$  and June  $F_{ST} = 0.1333$ ) but lower than the intra-annual  $F_{ST}$  estimates within Yaquina Bay. Similar to 2017, there was no genetic differentiation when comparing seasonal groups between the 2 sites. Comparison of the 2018 expected-season recruits between Coos

Table 6. Pairwise  $F_{ST}$  estimates based on variation at the 1389 neutral loci identified within Dungeness crab megalopae collected in 2017 and 2018. No comparisons were significant after false discovery rate (FDR) correction. Late-season collection timepoints (i.e. August) are shaded in gray. Pairwise  $F_{ST}$  comparisons between expected- and late-season samples are in **bold**. Intra-annual  $F_{ST}$  estimates are shaded blue while inter-annual  $F_{ST}$  estimates are not shaded

			2017				2018			
			Coos Bay		Yaquina Bay		Coos Bay		Yaquina Bay	
			April	August	April	August	April	June	April	August
2017	Coos Bay	April								
		August	<b>0.0011</b>							
	Yaquina Bay	April	<b>0.0004</b>	<b>0.0006</b>						
August		<b>0.0009</b>	<b>0.0005</b>	<b>0.0004</b>						
2018	Coos Bay	April	0.0000	<b>0.0009</b>	0.0007	<b>0.0005</b>				
		June	0.0008	<b>0.0009</b>	0.0000	<b>0.0003</b>	<b>0.0000</b>			
	Yaquina Bay	April	0.0000	<b>0.0012</b>	0.0004	<b>0.0008</b>	<b>0.0000</b>	<b>0.0007</b>		
		August	<b>0.0000</b>	0.0007	<b>0.0007</b>	0.0000	<b>0.0008</b>	<b>0.0000</b>	<b>0.0000</b>	<b>0.0000</b>

Table 7. Pairwise  $F_{ST}$  estimates based on variation at the 2 putatively adaptive loci identified within Dungeness crab megalopae collected in 2017 and 2018. Late-season collection timepoints (i.e. August) are shaded in gray. Pairwise  $F_{ST}$  comparisons between expected- and late-season samples are in **bold**. Intra-annual  $F_{ST}$  estimates are shaded blue while inter-annual  $F_{ST}$  estimates are not shaded

		2017				2018			
		—Coos Bay—		—Yaquina Bay—		—Coos Bay—		—Yaquina Bay—	
		April	August	April	August	April	June	April	August
2017	Coos Bay	April							
		August	<b>0.3411</b>						
	Yaquina Bay	April	<b>0.0048</b>	<b>0.3777</b>					
		August	<b>0.3896</b>	<b>0.0000</b>	<b>0.4209</b>				
2018	Coos Bay	April	0.0000	<b>0.3273</b>	0.0000	<b>0.3736</b>			
		June	0.0000	<b>0.3381</b>	0.0000	<b>0.3833</b>	<b>0.0000</b>		
	Yaquina Bay	April	0.0399	<b>0.3569</b>	0.0000	<b>0.3978</b>	<b>0.0024</b>	<b>0.0000</b>	
		August	<b>0.0687</b>	0.2776	<b>0.1590</b>	0.3352	<b>0.1124</b>	<b>0.1333</b>	<b>0.2071</b>

Bay and Yaquina Bay resulted in low  $F_{ST}$  estimates (0.0000 and 0.0024).

The magnitude of genetic differentiation between expected- and late-season recruits based on putatively adaptive loci was higher in 2017 ( $F_{ST}$  range: 0.3411–0.4209) compared to 2018 ( $F_{ST}$  range: 0.1124–0.2071; Table 7). Furthermore, greater differentiation was observed between 2017 and 2018 recruits ( $F_{ST}$  range: 0.0687–0.3978) than within the 2018 recruits ( $F_{ST}$  range: 0.1124–0.2071; Table 7). Interestingly, the 2018 Yaquina Bay late-season recruits were differentiated from the 2017 late-season recruits in both Yaquina Bay ( $F_{ST} = 0.3352$ ) and Coos Bay ( $F_{ST} = 0.2776$ ).

### 3.7. Effective number of breeders

The estimated  $N_b$  was high within each year and across all 8 collection timepoints, with the exception of the 2017 Coos Bay expected-season recruits (Table 8). Overall, recruits in 2017 had a lower  $N_b$

(13 611) than the recruits in 2018 (49 855). In 2017, the  $N_b$  estimates were lower for Coos Bay ( $N_b = 2772$  and 9390) compared to Yaquina Bay ( $N_b = \text{infinite}$ ). Although the  $N_b$  estimates for the 2018 late-season recruits from Yaquina Bay and the June (expected-season) recruits from Coos Bay were high ( $N_b = 21489$  and 12 900, respectively), these values were lower than the  $N_b$  calculated at both sites for the April 2018 (expected-season) recruits ( $N_b = \text{infinite}$ ).

### 3.8. Relatedness analyses

Using the dyadic likelihood estimator of Milligan (2003), neither full-siblings nor half-siblings ( $r > 0.25$ ) were observed within the megalopae sampled. Relatedness among megalopae dyads was low and never exceeded  $r = 0.1208$ ; the mean relatedness across all collection timepoints was 0.0172 ( $n = 8$ , Table 9). However, when comparing overall relatedness between collection timepoints, the relatedness estimate for the 2017 late-season recruits from Coos Bay ( $r = 0.0198$ )

Table 8. Estimates of effective number of Dungeness crab breeders ( $N_b$ ) with range of 95% confidence interval (CI) for each collection timepoint and year. Estimates based on the single-sample linkage disequilibrium method of Waples & Do (2008) with a p-critical value of 0.02 for collection timepoints and a p-critical value of 0.01 for years

Year	Site	Month	Recruitment season	$N_b$	95% CI	$N_b$	95% CI
2017	Coos Bay	April	Expected	2772	(1710–7252)	13611	(7940–47117)
		August	Late	9390	(2978–Infinite)		
	Yaquina Bay	April	Expected	Infinite	(6687–Infinite)		
		August	Late	Infinite	(8606–Infinite)		
2018	Coos Bay	April	Expected	Infinite	(6516–Infinite)	49855	(13010–Infinite)
		June	Expected	12900	(3141–Infinite)		
	Yaquina Bay	April	Expected	Infinite	(4693–Infinite)		
		August	Late	21489	(3641–Infinite)		

Table 9. Estimated relatedness ( $r$ ) of Dungeness crab megalopae within each collection timepoint using the dyadic likelihood estimator of Milligan (2003). The relatedness among megalopae within the 2017 Coos Bay August timepoint (\*) was significantly greater than all other timepoints (95% confidence interval). Relatedness among megalopae within the 2018 Coos Bay April timepoint (†) was significantly less than the 2017 Yaquina Bay April and August timepoints (††) (95% confidence interval)

Year	Site	Month	Recruitment season	$r$
2017	Coos Bay	April	Expected	0.0168
		August	Late	0.0198*
	Yaquina Bay	April	Expected	0.0175††
		August	Late	0.0176††
2018	Coos Bay	April	Expected	0.0159†
		June	Expected	0.0169
	Yaquina Bay	April	Expected	0.0162
		August	Late	0.0166

was significantly greater than all other collection timepoints ( $r$  range: 0.0159–0.0176) at a 95% confidence interval. The relatedness estimate for the 2018 Coos Bay April expected-season recruits ( $r = 0.0159$ ) was significantly lower than the relatedness estimates for the 2017 Yaquina Bay expected- and late-season recruits ( $r = 0.0175$  and  $0.0176$ , respectively).

#### 4. DISCUSSION

We found evidence for spatial and temporal genetic homogeneity among Dungeness crab megalopae recruits in the CCE based on variation at neutral loci. However, based on variation at 2 putatively adaptive loci, genetic differentiation between expected-season (April and June) and late-season (August) recruits was detected across sites and years. Contrary to our hypothesis that genetic differentiation would be greater when the positive PDO was weaker, the magnitude of genetic differentiation between the 2 seasonal recruitment groups based on putatively adaptive loci was greater in 2017 than 2018. Furthermore, megalopae recruits within our sampling groups did not exhibit relatedness measures high enough to indicate cohesive larval dispersal.

##### 4.1. Genetic differentiation between expected- and late-season megalopae recruits

Low genetic differentiation between expected- and late-season recruits based on variation at neutral

loci was detected in this study as well as in 2014 recruits (Lee & O'Malley 2020). While the  $F_{ST}$  estimate was the same in 2014 and 2017 ( $F_{ST} = 0.0011$ ), the  $F_{ST}$  estimate was significant in 2014 but not in 2017 and 2018. This is attributed to the inclusion of an additional sampling site and 2 years of data, which required correction for multiple testing using FDR. Previous studies on adult Dungeness crab in the CCE used only neutral loci (microsatellites), and low genetic differentiation was observed among adults within the CCE and higher genetic differentiation was observed between adults in the CCE and other ecosystems (GOA and SSE) (Jackson & O'Malley 2017, O'Malley et al. 2017, Jackson et al. 2017). Therefore, the finding of low genetic differentiation between expected- and late-season recruits at neutral loci across 3 years suggests that both seasonal groups of recruits originate from within the CCE.

However, the GBS method used in this study allowed us to identify putatively adaptive loci and detect genetic differences between the expected- and late-season recruits, thus providing evidence for divergent selection. These genetic differences may be attributed to (1) pre-settlement selection whereby ocean conditions differentially influenced the genetic composition of successful recruits within the 2 seasonal groups or (2) the 2 seasonal groups originated from different breeding populations that experienced divergent selective pressures. Dungeness crab pelagic larvae are exposed to ocean conditions that vary both intra- and inter-annually (Lough 1976), such that selective pressures influence which individuals survive and thus may result in adaptive differences between recruitment groups. However, successful recruits must also survive to reproductive age to influence future generations. In the CCE, a greater number of megalopae recruit to the nearshore than can be supported by the ecosystem; therefore, only a portion of the annual megalopae recruits survive to reproductive age (Galloway et al. 2017). Reduced survival of maladapted migrants would preserve adaptive genetic variation within and between ecosystems, since population connectivity is determined by both larval exchange and the ability of migrants to survive and reproduce at recruitment locations (Palumbi 2003, Hedgecock et al. 2007, Pineda et al. 2007).

##### 4.2. Examination of putatively adaptive loci

The strong genetic differentiation based on variation at putatively adaptive loci was similar to findings from the 2014 study (Lee & O'Malley 2020); however,

the 2 putatively adaptive loci identified in the 2014 study were different from the 2 loci identified among the 2017 and 2018 megalopae recruits. While these loci might be located in the same region of the genome, we cannot confirm this without a reference genome. The difference in putatively adaptive loci between the studies may be due to the use of a reduced-representation sequencing approach (i.e. GBS), as well as 'stacks' de novo alignment (Catchen et al. 2011, Catchen et al. 2013). While this is a cost-effective method of loci discovery in species without a reference genome, it is not specifically designed to identify the same loci across different experiments (Andrews et al. 2016).

Outlier loci identified in genome-wide scans do not necessarily represent adaptive loci (Shafer et al. 2015), and the lack of reference genomes for crustaceans makes it difficult to annotate outlier loci (Rotllant et al. 2018). However, 1 putatively adaptive locus (CLocus\_24; Table S5) matched a SOX-1-like transcription factor in Pacific white shrimp (Zhang et al. 2019). In humans, SOX-1 is a transcription factor involved in regulating embryonic development (NCBI Gene ID: 6656). Interestingly, the development timing differs between expected- and late-season recruits (Rasmuson 2013, Shanks 2013). This could support the hypotheses that (1) the late-season recruits originate from a northern ecosystem, given that hatch timing differs between northern and southern ecosystems (Rasmuson 2013) or (2) the late-season recruits represent megalopae with an extended PLD, and subsequently, a longer development time than expected-season recruits (Wild & Tasto 1983).

In Dungeness crab megalopae, sex cannot be determined morphologically. Therefore, the sex ratios in our samples were unknown, and it is possible that the putatively adaptive loci may represent sex-linked variation (Benestan et al. 2017). However, by using large sample sizes, randomly sampling megalopae at 2 sites and across 2 years, and then combining data in an inter-annual analysis, we reduced the potential of uneven sex ratios influencing genetic differentiation measurements.

### 4.3. Inter-annual differences among megalopae recruits

Although intra-annual genetic differentiation based on putatively adaptive loci was observed within Dungeness crab recruits in both 2017 and 2018, the magnitude of genetic differentiation varied inter-

annually. The results did not support our hypothesis that stronger intra-annual genetic differentiation would be detected in 2018 compared to 2017 due to ocean conditions. In contrast, genetic differentiation between the 2017 recruits was stronger ( $F_{ST}$  range = 0.3411–0.4209) than the genetic differentiation between the 2018 recruits ( $F_{ST}$  = 0.1124–0.2971). This finding deviates from predictions based on ocean conditions which indicated greater potential for southward larval transport from the GOA or SSE to the CCE in 2018 than in 2017 (Shanks 2013).

Jackson et al. (2017) detected inter-annual variation in population genetic structure of adult Dungeness crabs sampled within the CCE based on neutral loci, and these findings were attributed to differences in ocean conditions (i.e. PDO, upwelling, and spring transition) during the PLD of each year-class. Similarly, the variation in magnitude of intra-annual genetic differentiation across years (2014, 2017, 2018) may be attributed to differences in ocean conditions (Lee & O'Malley 2020). When comparing ocean conditions, 2014 had the strongest positive PDO, weakest upwelling, and latest spring transition, followed by 2017 and then 2018 (Table 1). These 3 ocean parameters have been correlated with lower overall abundances of megalopae recruits in Coos Bay (Shanks & Roegner 2007, Shanks et al. 2010, Shanks 2013). True to the correlations, the total abundances of megalopae recruits in 2014 and 2017 were below average and the total abundance of megalopae recruits in 2018 was well above average. Based on these ocean conditions, we hypothesized that years with stronger positive PDOs would have the weakest intra-annual genetic differentiation due to a weaker southern-flowing California Current in the CCE, which would reduce southward larval transport of Dungeness crabs. However, the year with the strongest positive PDO (2014) had the highest intra-annual genetic differentiation, and the year with the weakest positive PDO (2018) had the lowest genetic differentiation based on putatively adaptive loci. Since only years with positive PDOs were examined, future studies should examine negative PDO years to test if this pattern is consistent across different regimes. Furthermore, other ocean conditions besides PDO likely influence larval dispersal, such as El Niño/Southern Oscillation (ENSO) events, which can strengthen the northward California Undercurrent and northward Davidson Current within the CCE (Lynn & Bograd 2002).

Few late-season Dungeness crab megalopae recruits were observed in 2018. In Coos Bay, only 1 late-season megalopa recruit was sampled while a

few hundred were sampled in Yaquina Bay. Prior to 2018, the lowest number of late-season megalopae recruits recorded in Coos Bay during sampling years spanning both expected-season and late-season time periods (16 yr dataset) was just over 3000 megalopae in 2016 (Shanks & Roegner 2007, Shanks et al. 2010, Shanks 2013, 2019, Rasmuson & Shanks 2020, A. Shanks unpubl. data). Our results suggest that local ocean conditions may have differed between the 2 sites (Hickey & Banas 2003), or that regional ocean conditions (e.g. PDO) limited the southward transport distance of pelagic larvae via the California Current (Coos Bay is 144 km south of Yaquina Bay). Interestingly, the 2018 late-season recruits in Yaquina Bay were genetically differentiated from the late-season recruits in 2017 (both Coos and Yaquina Bays), a finding that may be attributed to differences in larval dispersal distances between years.

The inclusion of a second expected-season timepoint (June) in Coos Bay in 2018 provided an opportunity to further test the hypothesis that intra-annual genetic differentiation exists specifically between the expected- and late-season groups based on life history timing. Based on our hypothesis, any megalopae recruits sampled from April through July (expected-season) should be genetically similar to each other yet genetically different from late-season recruits (August through September). In 2018, the expected-season April (Yaquina and Coos Bays) and June (Coos Bay) megalopae recruits were not genetically differentiated based on variation at putatively adaptive loci, but they were all genetically differentiated from the late-season August 2018 recruits in Yaquina Bay. The genetic similarity between April and June recruits provides evidence against the alternative hypothesis that any 2 collections of megalopae recruits sampled on different days throughout the season are genetically differentiated. Furthermore, despite differences in megalopae recruitment abundances between Coos Bay and Yaquina Bay in 2018, seasonal genetic differentiation was still observed across both sites.

#### 4.4. Relatedness of megalopae recruits

Offspring from a female Dungeness crab are released together but influenced by ocean conditions during the long PLD prior to recruitment (Wild & Tasto 1983, Rasmuson 2013). Therefore, larval ecology suggests that cohesive larval dispersal of related individuals (kin) is less likely because of larval mixing within the water column (Victor 1984, Selkoe et

al. 2008). However, kinship aggregation has been documented in several other marine species with long PLDs using genetic relatedness measures (Iacchi et al. 2013, Adrian et al. 2017, Riquet et al. 2017), and higher than expected relatedness has been observed among adult Dungeness crab at several locations within the CCE (Jackson et al. 2017). We hypothesized that related megalopae would be observed within collection timepoints, indicating that Dungeness crab pelagic larvae exhibit cohesive larval dispersal. However, the relatedness analysis of the 2017 and 2018 megalopae indicated that Dungeness crab megalopae recruits did not exhibit cohesive larval dispersal. Additionally,  $N_b$  was not notably reduced within collection timepoints or years.

#### 4.5. Changing ocean conditions and implications for the fishery

With our findings of neutral genetic homogeneity and evidence for divergent selection, we cannot reject our hypothesis that late-season recruits represent larvae from a northern ecosystem (SSE or GOA) (Shanks 2013). However, further evidence is needed to confirm the origin of the late-season recruits, such as genetic assignment of CCE megalopae recruits to adult samples from northern ecosystems or development of biophysical models. Moreover, our results suggest that the timing of Dungeness crab life history events in the CCE should be studied more thoroughly to better understand temporal differences observed among recruits. The late-season recruits may represent a group of megalopae with an extended PLD, a longer developmental timing, or a later hatching time (Wild & Tasto 1983). More importantly, phenology of marine species life histories are shifting with changing ocean conditions (Asch 2015). Documenting life history and developmental changes in Dungeness crab remains important to the management of the fishery. Currently, the CCE Dungeness crab commercial fishery is managed under the 3-S system whereby only males of a specific size are harvested during a defined season (Rasmuson 2013). This management strategy assumes that males have the opportunity to reproduce at least once before being harvested. Moreover, it is estimated that in the last 2 decades, an average of 79% of legal sized males were harvested each year in the CCE (Richerson et al. 2020). Under this management strategy, it is important to maintain a current and complete understanding of the timing of maturation, mating, and development of this species.

The Dungeness crab megalopae recruitment abundance and timing patterns in 2018 were anomalous. Considering data collected over the past 2 decades, 2018 had the highest number of recruits ever observed and was the first time a negligible number of late-season recruits was observed in Coos Bay (Shanks & Roegner 2007, Shanks et al. 2010, Shanks 2013, 2019, A. Shanks unpubl. data). Lower abundances of late-season recruits are often observed in years with stronger positive PDOs, which is hypothesized to be attributed to weaker southward transport by the California Current (Shanks 2013). However, 2017 had a stronger positive PDO than 2018, but more late-season recruits. Moreover, greater intra-annual genetic differentiation was observed in 2017 than 2018, contrary to the observed ocean conditions. Considering the timing of Dungeness crab life history, the megalopae recruiting within the CCE during the marine heatwave and ENSO events of 2015–2016 (i.e. 'the Blob'; Peterson et al. 2017) composed a majority of the breeding population for the 2018 megalopae recruits. This may have impacted the recruits (either through physical oceanography, ecological conditions, or physiological tolerance of the species) and thus resulted in atypical patterns. With changing ocean conditions, it is becoming important to understand genetic variation, between and within ecosystems, due to selective pressures such as increasing water temperatures and marine heat waves.

The complex relationship between larval dispersal and ocean conditions during the PLD is still poorly understood. Future studies combining genetic analyses with physical transport models (Segura-García et al. 2019) or oceanographic models (Norton et al. 2020) may be informative toward understanding why both intra- and inter-annual differences in Dungeness crab megalopae were observed based on variation at putatively adaptive loci. Here, we examined a vulnerable early life history stage of a marine species in the context of larval dispersal and 3 well-studied ocean parameters (i.e. PDO, upwelling, and spring transition) (Shanks & Roegner 2007, Shanks et al. 2010, Shanks 2013); however, other ocean parameters impact Dungeness crab larvae (e.g. temperature, salinity, dissolved oxygen, nutrients, nitrate, chlorophyll) (Miller et al. 2016, Bednaršek et al. 2020, Norton et al. 2020). Future studies implementing interdisciplinary approaches to examine an array of physical, biological, chemical, and ecological variables would be informative (Bashevkin et al. 2020), since Dungeness crab population connectivity is shaped by both larval dispersal and survival of re-

cruits to reproductive age. With changing ocean conditions, it is likely that atypical patterns in larval dispersal and larval recruitment will be observed more often. Variations in larval recruits due to changing ocean conditions may eventually impact fishery harvests, fishery-reliant communities, and the Dungeness crab industry. Therefore, studying this vulnerable, early life history stage is vital to understanding population connectivity within this socially and economically valuable marine species.

*Data accessibility.* Sequence data are archived in NCBI SRA, BioProject accession: PRJNA587891.

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