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5 Article type : Original Article

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8 Evidence for inter-annual variation in genetic structure of Dungeness crab (*Cancer magister*)

9 along the California Current System

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18 Keywords: Genetic connectivity; Isolation by distance; Inter-annual variability; Kin aggregation;

19 Marine invertebrate; California Current System

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25 Running Title: Genetic connectivity of Dungeness crab

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/mec.14443

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26 **Abstract**

27 Using a combination of population- and individual-based analytical approaches, we
28 provide a comprehensive examination of genetic connectivity of Dungeness crab (*Cancer*
29 *magister*) along ~1,200 km of the California Current System (CCS). We sampled individuals at
30 33 sites in 2012 to establish a baseline of genetic diversity and hierarchal population genetic
31 structure, and then assessed inter-annual variability in our estimates by sampling again in 2014.
32 Genetic diversity showed little variation among sites or across years. In 2012, we observed
33 weak genetic differentiation among sites (F_{ST} range = -0.005 – 0.014) following a pattern of
34 isolation by distance (IBD), and significantly high relatedness among individuals within nine
35 sampling sites. In 2014, pairwise F_{ST} estimates were lower (F_{ST} range = -0.014 – 0.007), there
36 was no spatial autocorrelation, and fewer sites had significant evidence of relatedness. Based on
37 these findings, we propose that inter-annual variation in the physical oceanographic conditions of
38 the CCS influence larval recruitment and thus gene flow, contributing to inter-annual variation in
39 population genetic structure. Estimates of effective population size (N_e) were large in both 2012
40 and 2014. Together, our results suggest that Dungeness crab in the CCS may constitute a single
41 evolutionary population, though geographically limited dispersal results in an ephemeral signal of
42 isolation by distance. Furthermore, our findings demonstrate that populations of marine
43 organisms may be susceptible to temporal changes in population genetic structure over short time
44 periods, thus inter-annual variability in population genetic measures should be considered.

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49 **Introduction**

50 Understanding the spatial scales over which populations interact is a fundamentally
51 important theme of marine conservation and ecology. Nevertheless, estimating population
52 connectivity, or the exchange of individuals among populations, in the marine environment can
53 be a difficult task, since dispersal primarily occurs during a planktonic larval phase in many
54 species (Cowen et al. 2007; Cowen and Sponaugle 2009). In lieu of efficient direct methods (e.g.
55 mark-recapture, telemetry tagging), genetic markers have become an increasingly popular

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56 indirect tool for evaluating connectivity (Hellberg et al. 2002; Waples and Gaggliotti 2006). As a
57 result, delineation of population sub-units and the design of management strategies has relied
58 extensively on estimates of genetic connectivity (i.e. the effect of gene flow on populations)
59 (Palumbi 2003; Palsbøll et al. 2007; Lowe and Allendorf 2010).

60 Estimates of genetic connectivity have traditionally been inferred from analyses that
61 assess population genetic structure at a single point in time, providing a snapshot of the amount
62 of diversity (e.g. heterozygosity, allelic richness) and degree of differentiation (e.g. F_{ST}, G_{ST}, D)
63 within and among populations (Slatkin 1985; Waples and Gaggliotti 2006; Lowe and Allendorf
64 2010). Using this approach, numerous studies have revealed an array of different spatial patterns
65 of population genetic structure which often contradict the assumption that a lengthy pelagic larval
66 duration leads to strong genetic connectivity (Weersing and Toonen 2009). Others have
67 demonstrated the advantage of complementary, 'individual-based' measures such as kinship
68 (Palsbøll et al. 2010; Iacchei et al. 2013; Selwyn et al. 2016; Treske et al. 2016; Truelove et al.
69 2017), parentage analyses (Jones et al. 2005; 2009; Christie et al. 2010; Pusack et al. 2014;
70 Christie et al. 2017) and assignment tests (Manel et al. 2005; Thomas and Bell 2013; Benestan et
71 al. 2015; Christie et al. 2017). These analyses focus on how genetic variation within populations
72 is distributed among individuals, and have contributed to a growing paradigm that local processes
73 (e.g. kin aggregation, retention) play important roles in regulating genetic connectivity alongside
74 large scale processes (e.g. dispersal) (Christie et al. 2010; Underwood et al. 2012; Pusack et al.
75 2014; Klein et al. 2017).

76 Despite the wealth of knowledge surrounding factors that influence genetic connectivity
77 in the marine environment, including evolutionary processes (e.g. gene flow, natural selection)
78 (Slatkin 1985; Hillbish 1996; Hellberg 2009; Nielsen et al. 2009; Nayfa and Zenger 2016),
79 ecological processes (e.g. dispersal, recruitment) (Weersing and Toonen 2009; Selkoe and
80 Toonen 2011; Chust et al. 2016), and environmental variables (e.g. habitat continuity, circulation)
81 (Selkoe et al. 2008; Galindo et al. 2010; White et al. 2010; Selkoe et al. 2016), there have been
82 proportionately few studies that evaluate temporal trends in population genetic structure. This
83 disparity is somewhat surprising, since conservation and management strategies that are based on
84 knowledge of population genetic structure are inherently reliant on the assumption that such
85 patterns are temporally consistent. Assessing inter-annual variation in population genetic

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measures provides a means to monitoring population responses to a changing environment or anthropogenic pressures such as harvest or habitat loss (Schwartz et al. 2007). Sampling at multiple time points can also provide an analytical advantage over single sample study designs since population genetic measures of 'high gene flow' marine organisms are often confounded by greater statistical uncertainty due to the elevated influence of small biases associated with sampling design, marker choice, or conformance to statistical assumptions (Waples 1998). For instance, Knutsen et al. (2011) assessed genetic connectivity of Atlantic cod (*Gadus morhua*) in coastal Norway and observed very weak, yet significant, levels of population genetic structure ($F_{ST} < 0.01$), bringing to question the biological relevance of such minute genetic differences. However, the authors observed a consistent pattern of weak population genetic structure over a ten year time series which emphasized the importance of their findings. In contrary, several studies have found evidence for inter-annual variation in population genetic structure, which as a result uncovered the interaction among genetic structure and temporally variable ecological processes such as sweepstakes reproductive success and kin aggregation (Hedgecock 1994a; Christie et al. 2010), as well as physical processes such as oceanographic conditions and ocean circulation patterns (Forin and Höglund 2007; Hogan et al. 2012; Kamin et al. 2014; Klein et al. 2016; Pascual et al. 2016). These processes play an influential role in genetic connectivity, but would have gone undetected by only analyzing data from a single time point.

The majority of studies that consider inter-annual variability have employed single cohort sampling designs that provide the fine scale resolution necessary for disentangling the mechanisms of inter-annual variability, but fail to capture variability in the population as a whole (but see Pusack et al. 2014). Alternatively, evaluating mixed cohorts provides less detail on mechanisms of inter-annual variation, but more clarity on how inter-annual variability in genetic connectivity (i.e. gene flow) influences genetic structure of the population over time (see findings of Forin and Höglund 2007; Hogan et al. 2010; Klein et al. 2016), which may have more direct implications for fishery management.

The California Current System (CCS) is a large-scale oceanographic regime of the northeastern Pacific Ocean which spans the North American coast from Vancouver Island, British Columbia to the Baja California Peninsula, Mexico. The CCS is highly productive and supports a wide ranging biodiversity of fishes and invertebrates. Population genetic structure of organisms

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116 with planktonic larvae in the CCS are variable, ranging from those which are genetically
117 homogenous across broad geographic ranges (Addison et al. 2008; Kelly and Palumbi 2010,
118 Sivasundar and Palumbi 2010), to those that are characterized by weak genetic differentiation
119 (Buonaccorsi et al. 2004; White et al. 2010; Iacchei et al. 2013), chaotic genetic patchiness
120 (Hedgecock 1994a; Selkoe et al. 2006; Selkoe et al. 2010; Cornwell et al. 2016), or discrete
121 population genetic structure (Cope 2004; Kelly and Palumbi 2010; Sivasundar and Palumbi 2010;
122 Hess et al. 2011; Sanders and Palumbi 2011). Despite well documented intra- and inter-annual
123 variation of physical oceanographic conditions within the CCS (reviewed in Hickey and Banas
124 2003; Rebstock 2003; King et al. 2011; McClatchie 2014) and the knowledge that these
125 conditions play a role in shaping genetic connectivity (White et al. 2010; Iacchei et al. 2013),
126 their influence on temporal patterns of genetic connectivity is poorly understood. Moreover, to
127 our knowledge no population genetic study of marine fishes or invertebrates in the CCS has
128 evaluated inter-annual variation in genetic connectivity of a mixed-cohort population.
129 The Dungeness crab (*Cancer magister*) is an iconic CCS species, and supports the most
130 valuable commercial fishery along the west coast of the United States (Van Voorhees et al. 2016).
131 The commercial fishery in the CCS is restricted to only males of carapace widths greater than 158
132 mm, during a limited entry season that primarily occurs in winter months (i.e. avoiding the
133 molting cycle). Fishing pressure is immense, harvesting an estimated 90% of legal sized male
134 crab on an annual basis (Methot and Botsford 1982). Dungeness crab in the CCS have been
135 considered a single open population for management purposes, though knowledge of population
136 connectivity or stock delineation to guide decision making is lacking. Previous population genetic
137 studies of Dungeness crab have provided evidence for genetic structure in partially enclosed
138 waters of British Columbia (Beacham et al. 2008), as well as homogeneity along 585 km of the
139 Oregon coast and differentiation between the Oregon coast and two sites in British Columbia
140 (O'Malley et al. 2017). Further, there is evidence of reduced connectivity between Dungeness
141 crab inhabiting partially enclosed waters (i.e. Puget Sound, Washington) as compared to the open
142 coast (Jackson and O'Malley 2017). These findings demonstrate the need to examine genetic
143 connectivity within this species at a coast wide scale.
144 In this study we use Dungeness crab in the CCS as a model system to investigate inter-
145 annual variation in population genetic structure of a widely distributed marine invertebrate. Our

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146 primary objectives were to 1) characterize genetic variation of Dungeness crab throughout ~1,200
147 km of the CCS and 2) evaluate inter-annual variability in population genetic structure. Using both
148 population- (e.g. AMOVA, F_{ST}) and individual-based analyses (e.g. relatedness, assignment
149 analysis) within a hierarchal analytical framework, we first establish a baseline of population
150 genetic structure in 2012 to test the null hypothesis of panmixia. We then test for inter-annual
151 variability by repeating these analyses in 2014. Lastly, we discuss our findings regarding genetic
152 connectivity of this species in relation to changing oceanographic conditions.

153 **Methods**

154 Study species

155 The Dungeness crab is distributed continuously from the Pribilof Islands, Alaska to Santa
156 Barbara, California (Rasmuson 2013). In this study, we focus on the CCS from northern
157 Washington to Half Moon Bay, California (Fig. 1). Dispersal primarily occurs during the
158 planktonic larval phase, as adult migrations in the CCS tend to be localized on a scale of 20-50
159 km (Snow and Wagner 1965; Gotshall 1978; Collier 1983; Diamond and Hankin 1985;
160 Hildenbrand et al. 2011). The planktonic larval phase lasts approximately four months and
161 consists of five zoeal stages and one megalopal stage (Poole 1966; Moloney et al. 1994). Early
162 stage zoea are released during winter months, and transported northward and seaward by the
163 Davidson Current (Lough 1976; Riley 1983). At the time of the “spring transition” from
164 downwelling to upwelling conditions, the Davidson current weakens and late stage zoea are
165 typically found off the continental shelf in the southward flowing California Current. Here, zoea
166 molt into megalopae and migrate inshore to settle (Jamieson and Phillips 1988; reviewed in
167 Rasmuson 2013). The timing of megalopae settlement is known to vary both within years and
168 among years, and the magnitude of larval recruitment has been correlated with oceanographic
169 indices such as the timing of the spring transition, Pacific Decadal Oscillation (PDO), and the
170 amount of upwelling during the settlement season (i.e. spring and summer) (Shanks and Roegner
171 2007; Shanks et al. 2010; Shanks 2013). These indices relate to variation in the wind-forced,
172 along- and cross-shelf circulation thought to influence larval trajectories, and likely gene flow.

173 Sample collection

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174 In collaboration with the Washington, Oregon, and California Department of Fish and
175 Wildlife agencies (WDFW, ODFW, and CDFW, respectively) and the commercial fishing fleet,
176 Dungeness crab were collected in November 2012 and 2014 during the Tri-State Pre-Season Test
177 Fishery (Pacific States Marine Fisheries Commission 2014) (Fig. 1). A string of six pots was
178 fished at three depths (15 m, 30 m, 45 m) along 33 latitudinal transects. Each transect represents a
179 single sampling site. Muscle tissue was sampled from adult females and sub-legal sized males (≤
180 158 mm carapace width) by removing a hind walking leg and preserving it in 95% ethanol. Legal
181 sized males were not available for genetic analysis since they were retained by the test fishery for
182 meat recovery. Prior to release, carapace width was recorded for individuals sampled in
183 Washington. The two most southern transects, Duxbury Reef and Half Moon Bay, were not
184 sampled in 2014.

185 We collected tissue samples from $N = 4{,}041$ crab in 2012 and $N = 1{,}804$ crab in 2014.
186 Individuals were subsampled for genotyping by including all females, and randomly selecting
187 males to achieve a sample size of up to 100 individuals per site (Table S.1, Supporting
188 Information). We genotyped a greater proportion of males (78%) than females (22%) overall,
189 though this ratio varied among sites. To evaluate the potential for sex bias in our results we
190 conducted a principle components analysis (PCA) based on allele frequencies of individuals by
191 sex in the package 'adegenet' (Jombart 2008) in R version 3.2.1 (R Core Team 2016) for both
192 2012 and 2014 (Benestan et al. 2016; Jackson and O'Malley 2017). We did not observe any
193 clustering among sexes in either year (Fig. S.1, Supporting Information), and thus chose not to
194 analyze males and females separately.

195 Laboratory methods

196 Genomic DNA was extracted from Dungeness crab muscle tissue as described by Ivanova
197 et al. (2006). DNA was amplified by Polymerase Chain Reaction (PCR) at 10 microsatellite loci
198 using previously developed oligonucleotide primers (Kaukinen et al. 2004; Toonen et al. 2004)
199 (Table S.2, Supporting Information). PCR was carried out in 6 µL reactions containing 25 mM
200 MgCl, 10 mM dNTPs, 10 µM forward and reverse primers, 5x colorless PCR buffer, 5 U/µL Taq
201 polymerase, double distilled water (ddH2O), and 1 µL of DNA template. Thermocycling
202 protocols consisted of 25-35 cycles at 95° C for 30 s, followed by 48 – 61.2° C for 30 s, and 70°

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203 for 45 s, with number of cycles and annealing temperature varying for each locus. PCR amplicons
204 were electrophoresed on an ABI 3730XL DNA Fragment Analyzer and scored using
205 GeneMapper® software. Duplicate genotypes found within the same site in a given year were
206 assumed to be error and removed.

207 Analysis of genetic diversity

208 Conformance to Hardy-Weinberg proportions (HWP) was evaluated for each locus using
209 the probability test option of the software program Genepop version 4.2 (Raymond and Rousset
210 1995; Rousset 2008). Linkage equilibrium was also evaluated for each pair of loci in all
211 populations separately using probability tests in Genepop. Markov chain parameters for tests of
212 HWP and linkage equilibrium included 1,000 dememorization steps and 100 batches of 1,000
213 iterations per batch. False Discovery Rate (FDR) corrections using $\alpha = 0.05$ (Benjamini and
214 Hochberg 1995) were applied to *P*-values of tests for linkage equilibrium. The presence of null
215 alleles was estimated using the software program FreeNA (Chapuis and Estoup 2007). FreeNA
216 estimates the frequency of null alleles in each sampling site across all loci, and calculates global
217 and pairwise F_{ST} estimates (Weir 1996) using observed data with and without the addition of null
218 alleles. To assess the degree of departure from HWP, the inbreeding coefficient (F_{IS}) (Weir and
219 Cockerham 1984) was computed for each site using the software program GENETIX version
220 4.02 (Belkhir 2004). We then tested if F_{IS} values significantly deviated from zero by performing
221 10,000 permutations, and applying FDR corrections ($\alpha = 0.05$). Number of alleles per locus and
222 expected heterozygosity were also calculated for each site using GENETIX. Since sample sizes
223 varied considerably among sites, we also calculated allelic richness using FSTAT version 2.9.3.2
224 (Goudet 2001). Allelic richness is the number of alleles per locus, corrected to the smallest
225 sampling size ($N = 10$).

226 Analysis of population genetic structure

227 *AMOVA and pairwise* F_{ST}

228 To test for evidence of genetic structure, we performed an analysis of molecular variance
229 (AMOVA) (Excoffier et al. 1992) under the assumption of the infinite allele model as

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230 implemented in GenoDive version 2.0b23 (Meirmans et al. 2004; 2006). Within a hierarchal
231 framework, we assessed the variance in allele frequencies among individuals, sampling sites, and
232 regions in both 2012 and 2014. Regions were defined *a priori* based on prominent topographical
233 features that are associated with strong upwelling fronts and eddies in the CCS (e.g. Cape Blanco,
234 Cape Mendocino, and Point Arena) (Lagerloef 1992; Barth et al. 2000; Marchesiello et al. 2003).
235 Local oceanographic features are thought to strongly influence planktonic larval dispersal (Pineda
236 et al. 2007), and as such, these physical prominences are also associated with biogeographic
237 breaks and population genetic structure in several CCS species (Cope 2004; Kelly and Palumbi
238 2010; Hess et al. 2011; Lotterhos et al. 2014). Our analysis consisted of four regions: 1) North
239 California Current (North CC), 2) Mid California Current (Mid CC), 3) Ft. Bragg, and 4) South
240 California Current (South CC) (Fig. 1).
241 Following the AMOVA, we calculated pairwise F_{ST} estimates (θ; Weir and Cockerham
242 1984) among individual sampling sites and regions for both 2012 and 2014 using GENETIX. F_{ST}
243 estimates were tested for significance by performing 10,000 permutations, and applying FDR
244 corrections ($\alpha = 0.05$). When effective population size is large and genetic differentiation is low,
245 there is a greater amount of statistical uncertainty associated with measures of genetic divergence
246 (e.g. F_{ST}) than when genetic differentiation is greater (Kalinowski 2005). We assessed the
247 relationship between sample size and statistical power for detecting genetic differentiation as
248 measured by F_{ST} in our dataset using the simulation software POWSIM (Ryman and Palm 2006).
249 POWSIM uses observed sample sizes, the number of microsatellite loci, and allele frequencies at
250 each locus to simulate sampling of individuals from a given number of sub-populations that have
251 diverged to a user-defined level of genetic differentiation (F_{ST}). POWSIM then tests for
252 significant differentiation among samples using Fisher's exact test. The proportion of significant
253 tests after all iterations (in this case 1,000) is the power to detect genetic differentiation at the
254 given value of F_{ST}. Simulations based on observed allele frequencies indicated that there was a
255 high probability of type I error when F_{ST} was very small ($F_{ST} < 0.001$). Based on further rounds
256 of simulations, we concluded that low statistical power was likely attributed to small sample sizes
257 at some sites, and that at least 50 samples for each site were needed to gain high statistical power
258 (> 95%) to detect genetic differentiation. We also evaluated how smaller sample sizes in 2014
259 influenced estimates of F_{ST} by resampling our 2012 dataset using sample sizes observed in 2014

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260 and recalculating pairwise F_{ST} estimates among sites 100 times. When the sample size at a given
261 site was greater in 2014 than 2012, we did not resample and all individuals were included;
262 Duxbury Reef and Half Moon Bay were not included in this analysis as they were only sampled
263 in 2012.

264 *Spatial autocorrelation*

265 To evaluate the relationship between genetic differentiation and geographic proximity for
266 both 2012 and 2014, we conducted a simple Mantel test using pairwise matrices of F_{ST} estimates
267 and Euclidean (i.e straight line) distance between sampling sites in GenoDive. Significance was
268 tested using 1,000 permutations. Significant Mantel tests cannot always be interpreted as
269 evidence of isolation by distance (IBD) (Wright 1943), since abrupt (even random), non-
270 continuous changes in genetic variation can still produce significant results (Meirmans 2012).
271 Furthermore, IBD may not be uniform throughout the entire the study range. To examine the
272 presence of IBD more closely, we constructed a Mantel correlogram (Oden and Sokal 1986) in
273 GenoDive. This approached allows for a test of spatial autocorrelation at specific distances, and
274 clarifies interpretation of Mantel results when correlation is weak. Distance observations were
275 divided into seven continuous, non-overlapping 'distance classes' which had ranges of 0 – 120,
276 121 – 240, 241 – 360, 361 – 480, 481 – 600, 601 – 720, and ≥ 721 km (Table S.3, Supporting
277 Information). These seven distance classes were designed to maintain consistency in the
278 geographic range of each class, while including a sufficient number of pairwise comparisons
279 within each class to accurately test for a correlation (Diniz-Filho et al. 2013). We then computed
280 Mantel's r (r_m; similar to Pearson's correlation coefficient) for the relationship between F_{ST} and
281 geographic distance within each class, and constructed a correlogram by plotting Mantel's r and
282 the mean geographic distance of each class (Oden and Sokal 1986; Diniz-Filho et al. 2013).
283 Mantel's r for each distance class was tested for significance using 1,000 permutations. We also
284 constructed a distogram (i.e. a plot of the mean of pairwise F_{ST} estimates and the mean
285 geographic distance among sites within each distance class) (Diniz-Filho et al. 2013) to illustrate
286 the trend of genetic differentiation among sites within each distance class.
287 Lastly, since regional site groupings were based on *a priori* hypotheses regarding
288 topographical features, it is possible that regional boundaries may not represent true biological

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289 boundaries, or genetic breaks. Instead, the presence of IBD can result in a pattern of genetic
290 structure which is misinterpreted as a genetic break (Meirmans 2012). We investigated the role of
291 IBD as a driver of regional genetic structure by conducting a partial Mantel test in GenoDive
292 (Smouse et al 1986). A partial Mantel test is similar to a simple Mantel test, but controls for the
293 statistical influence of a third matrix. In the context of this study, we tested the correlation
294 between pairwise F_{ST} estimates among sites and region membership, while accounting for
295 geographic distance as a covariate. In other words, we asked: Are sites located within the same
296 region more similar than expected by chance, after we account for the geographic distance
297 between sites? To describe region membership, we constructed a pairwise matrix of 1s, when
298 sites were located within the same region, and 0s, when they were not (Meirmans 2012). This
299 relationship was tested for significance using 1,000 permutations.

300 *Assignment tests*

301 We calculated the proportion of individuals that could be correctly assigned to the region
302 in which they were sampled to further test the null hypothesis of panmixia. In assignment
303 analyses, if a high proportion of individuals can be correctly assigned based on their genotypes,
304 there is evidence of population genetic structure (Manel et al. 2005). We determined the
305 proportion of individuals that could be correctly assigned to the site or region (i.e. location) in
306 which they were sampled using the software program GENECLASS2 (Piry et al. 2004).
307 Assignment was determined according to the Bayesian method of Rannala and Mounatin (1997)
308 as this method been shown to out-perform frequency and distance based measures (Cornuet et al.
309 1999). Individuals were excluded from a location if the probability of their genotype occurring in
310 that site was below a threshold of 0.05 (i.e. Type 1 error), and assigned to the location in which
311 their genotype had the highest probably of occurrence. Expected frequency probabilities for
312 genotypes within each location were based on a simulated distribution generated by the Monte-
313 Carlo resampling algorithm of Paetkau et al. (2004) (N = 1,000). To avoid biased assignments,
314 individuals being assigned were removed from their home region before calculating allele
315 frequencies for that region (Efron 1983).

316 *Relatedness*

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317 Relatedness analyses have been useful in disentangling biological processes contributing
318 to genetic differentiation (Iacchei et al. 2013; Treske et al. 2016). We evaluated relatedness in
319 Dungeness crab by computing the Lynch and Ritland (1999) relationship coefficient (r) for each
320 pair of individuals using the package ‘related’ (Pew et al. 2015). To validate our use of Lynch and
321 Ritland’s (1999) r as a relatedness estimator, we conducted several rounds of simulations in
322 ‘related’ using Lynch and Ritland’s (1999) r, the triadic likelihood estimator (Wang 2007), and
323 the dyadic likelihood estimator (Milligan 2003). During simulations, genotypes of individuals
324 were generated based on the allele frequency distribution of our observed data and all
325 relationships were known. Simulations indicated that Lynch and Ritland’s (1999) r had a similar
326 separation between the distributions of relatedness values for non-relatives and relatives as the
327 two commonly used likelihood estimators (Fig. S.2, Supporting Information).

328 Based on this finding, we then calculated mean r ($\overline{r}$) among all pairs of individuals
329 within a given site. Given the absence of individual demographic data (e.g. carapace width, age)
330 to provide support for putative half- or full- siblings, we did not attempt to identify sibling pairs.
331 Instead, we identified sites that had a higher $\overline{r}$ than expected in a randomly associated population.
332 Significance of $\overline{r}$ was tested by permuting individuals among sites 1,000 times and re-calculating
333 $\overline{r}$ after each iteration to generate a null distribution of $\overline{r}$ for each site (Fig. S.3, Supporting
334 Information). We then compared the observed $\overline{r}$ of each site to its respective distribution and
335 obtained a pseudo P-value (Pew et al. 2015). Observations of genetic differentiation among
336 sampling sites may be associated with pairwise relatedness among individuals within those sites
337 (Selkoe et al. 2006; Iacchei et al. 2013; Pusack et al. 2014). Examining this relationship is
338 important for interpreting results in the context of contemporary ecological processes (i.e. kin
339 aggregation) or multi-generational evolutionary processes (i.e. genetic drift) appropriately. We
340 assessed the association between $\overline{r}$ and the mean of pairwise F_{ST} estimates for each site by
341 estimating the Pearson’s correlation coefficient.

342 Effective population size

343 Effective population size (N_e) is an important parameter to understanding genetic
344 connectivity, as it places genetic variation in the context of microevolutionary processes (i.e.
345 adaptation, genetic drift) (Hare 2011). N_e was estimated by computing the single-sample linkage

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346 disequilibrium estimator (Waples and Do 2008) implemented in NeEstimator version 2.01 (Do et
347 al. 2014) for both 2012 and 2014. We excluded low frequency alleles from calculation of N_e by
348 choosing a P-critical value of 0.01.

349 **Results**

350 Analysis of genetic variation

351 Several sites were found to significantly deviate from Hardy-Weinberg proportions
352 (HWP) in either 2012 (*N* sites = 9) or 2014 (*N* sites = 5), but only Brookings South deviated from
353 HWP in both years (Table S.4, Supporting Information). Loci which deviated from HWP at these
354 sites were inconsistent, but included *Cma102*, *Cma114*, *Cma118*, *Cma17*, *Cma33*, and *Cma43*
355 (Table S.4, Supporting Information). F_{IS} estimates indicated that departures from HWP included
356 both heterozygote excess and deficiency in 2012 (F_{IS} range: -0.061 – 0.099), though only Astoria
357 North ($F_{IS} = 0.099$, $P < 0.001$) and Port Orford South ($F_{IS} = 0.050$, $P < 0.001$) significantly
358 deviated from zero after correcting for multiple tests. F_{IS} estimates in 2014 again indicated both
359 heterozygote excess and deficiency (F_{IS} range: -0.055 – 0.111), though no site significantly
360 deviated from zero after correcting for multiple tests (Fig. 1) (Table S.5, Supporting Information).
361 Estimated null allele frequencies averaged 1.4% and 1.2% across all loci in 2012 and 2014,
362 respectively, and did not affect global or pairwise F_{ST} across all markers. Significant linkage
363 disequilibrium (LD) was found in eight pairs of loci in 2012 and five pairs of loci in 2014, though
364 no locus pair was in LD across years. Further examination revealed that for most pairs of loci the
365 overall significance of LD could be attributed to a single site having a *P*-value of zero, and no
366 site-specific pattern of LD was present. Given the inconsistency of observed patterns of LD, these
367 results suggest that loci used in this study are not physically linked.

368 There was little distinguishable spatial pattern of genetic diversity present among sites or
369 across years. The number of alleles per locus was variable among sites in both 2012 (NA range:
370 7.3 – 13.5) and 2014 (NA range: 6.0 – 13.6), though allelic richness had a much narrower range
371 in both 2012 (AR range: 5.5 – 6.2) and 2014 (AR range: 5.5 – 6.0). Expected heterozygosity was
372 also similar between years (2012 H_e range: 0.622 – 0.712; 2014 H_e range: 0.637 – 0.714) (Fig.1)
373 (Table S.5, Supporting Information).

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374 Analysis of population genetic structure

375 *AMOVA and pairwise F_{ST}*

376 In 2012, AMOVA indicated that nearly all genetic variation was attributed to variation
377 among individuals within sampling sites (99.7%). No significant proportion of genetic variation
378 was explained by variance among sampling sites (0.1%, $P = 0.067$); however, eight pairwise F_{ST}
379 estimates were significant after applying False Discovery Rate corrections. Eel River was
380 significantly differentiated from Buoy 3 ($F_{ST} = 0.010$, $P < 0.001$), Grayland ($F_{ST} = 0.007$, $P =$
381 0.001), Seaview ($F_{ST} = 0.007$, $P < 0.001$), and Garibaldi South ($F_{ST} = 0.009$, $P < 0.001$). Russian
382 River and Half Moon Bay were both significantly differentiated from Buoy 3 ($F_{ST} = 0.009$, $P <$
383 0.001; $F_{ST} = 0.007$, $P = 0.001$, respectively) and Garibaldi South ($F_{ST} = 0.010$, $P < 0.001$; $F_{ST} =$
384 0.008, $P < 0.001$, respectively) (Fig. 2a). Low pairwise F_{ST} estimates are likely due to the inverse
385 relationship between the maximum attainable F_{ST} and H_e (Meirmans and Hedrick 2011).

386 AMOVA provided evidence for significant regional differentiation in 2012, despite it only
387 accounting for a very small proportion of total genetic variance (0.2%, $P < 0.001$). Pairwise F_{ST}
388 estimates indicated that North CC was significantly differentiated from Mid CC ($F_{ST} = 0.001$, P
389 < 0.001) and South CC ($F_{ST} = 0.002$, $P < 0.001$). South CC was also significantly differentiated
390 from Ft. Bragg ($F_{ST} = 0.003$, $P = 0.002$). North CC and Mid CC were not significantly
391 differentiated from Ft. Bragg ($F_{ST} = 0.001$; 0.001, respectively), and Mid CC was not
392 significantly differentiated from South CC ($F_{ST} = 0.001$) (Table 1a).

393 In 2014, AMOVA indicated that all genetic variation was attributed to variation among
394 individuals within sampling sites (~100%), and no sites were significantly differentiated based on
395 pairwise comparisons (F_{ST} range: -0.014 – 0.007) (Fig. 2b). Furthermore, there was no evidence
396 of significant pairwise differentiation at the regional level (F_{ST} range: -0.001 – 0.002) (Table 1b).
397 Recalculating pairwise F_{ST} estimates after resampling the 2012 data set using 2014 sample sizes
398 did not yield much contrast to F_{ST} estimates based on the full 2012 data set (Fig. S.4, Supporting
399 Information), therefore it is unlikely that inter-annual variation in estimates of population genetic
400 structure is due to differences in sample sizes between years.

401 *Spatial autocorrelation*

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402 Genetic differentiation (as measured by pairwise F_{ST}) was significantly correlated with
403 geographic distance between sampling sites in 2012 ($r_m = 0.232$, $P = 0.006$). While this
404 relationship was significant using all sites together, it was not significant for every distance class.
405 Sites that were less than 120 km apart were more similar than expected by chance ($r_m = 0.136$, P
406 $= 0.001$), and sites that were greater than 720 km apart were more different than expected by
407 chance ($r_m = -0.17$, $P = 0.025$). Despite the lack of statistical significance, other distance classes
408 (121 – 240, 241 – 360, 361 – 480, 481 – 600, and 601 – 720 km) showed a steady increase in
409 genetic differentiation with increasing geographic distance, indicative of isolation by distance
410 (Fig. 3a and 3b) (Table S.3, Supporting Information). In 2014, there was no significant
411 correlation between genetic differentiation and geographic distance across all sites ($r_m = 0.032$, P
412 $= 0.281$). No relationship emerged after evaluating separate distance classes, though sites
413 between 481 – 600 km apart were more different than expected by chance ($r_m = -0.089$, $P =$
414 0.014) (Fig. 3a) (Table S.3, Supporting Information).

415 A partial Mantel test indicated that genetic differentiation among sites was not related to
416 region membership after accounting for geographic distance between sites in either 2012 or 2014
417 ($r_m = -0.212$; 0.112, $P = 0.500$; 0.523, respectively). Therefore, sites located within the same
418 region are more likely to be genetically similar due to their geographic proximity as opposed to a
419 break in gene flow.

420 *Assignment tests*

421 In 2012, the proportion of correct assignments in most sites was lower or slightly greater
422 than what would be expected if assignment was random (1/33 = 0.03), but greater in Buoy 3
423 (0.26), Grayland (0.11), and Jack Ass (0.39). Ft. Bragg had the greatest proportion of correct
424 assignments (0.58) among regions, likely owing to the greater assignment success found at Jack
425 Ass. North CC, Mid CC, and South CC were near or below random assignment (1/4 = 0.25). The
426 proportion of correct assignments among sites was similar in 2014, with Seaview (0.18),
427 Cranberry (0.15), and Astoria South (0.15) have the greatest proportion of individuals correctly
428 assigned. Assignment success increased for North CC (0.55), likely due to higher proportions of
429 correct assignments in Seaview, Cranberry, and Astoria South. The proportion of correct
430 assignments for Mid CC, Ft. Bragg, and South CC was approximately random (Table 2). Low

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431 assignment success among sites and regions further supports the finding of very weak genetic
432 differentiation in 2012 and 2014.

433 *Relatedness*

434 In 2012, $\overline{r}$ was significantly greater than expected in nine sites: Astoria South ($\overline{r}$ = 0.005,
435 pseudo-P < 0.001), Garibaldi South ($\overline{r}$ = 0.004, pseudo-P = 0.009), Newport North ($\overline{r}$ = 0.003,
436 pseudo-P = 0.016), Port Orford South ($\overline{r}$ = 0.003, pseudo-P = 0.031), Brookings North ($\overline{r}$ =
437 0.003, pseudo-P = 0.025), Eel River ($\overline{r}$ = 0.029, pseudo-P < 0.001), Russian River ($\overline{r}$ = 0.007,
438 pseudo-P = 0.011), Duxbury Reef ($\overline{r}$ = 0.013, pseudo-P < 0.001), and Half Moon Bay ($\overline{r}$ = 0.015,
439 pseudo-P < 0.001). In 2014, the only sites with significantly greater than expected $\overline{r}$ were
440 Grayland ($\overline{r}$ = 0.002, pseudo-P = 0.032), Garibaldi South ($\overline{r}$ = 0.007, pseudo-P = 0.032), and
441 Brookings North ($\overline{r}$ = 0.003, pseudo-P = 0.041) (Fig. 1) (Table S.5, Supporting Information).
442 Note that $\overline{r}$ represents mean pairwise calculations of r among all individuals within each site
443 including both relatives and non-relatives, and unlike other estimators, r does not conform to the
444 typical 0 – 1 scale (i.e. non-relative pairs may attain negative values). Therefore, $\overline{r}$ is considerably
445 less than what would be expected for true half-siblings (r = 0.25) and full-siblings (r = 0.5)
446 (Lynch and Ritland 1999). We also found a significant positive correlation between $\overline{r}$ and mean
447 pairwise F_{ST} in both 2012 (Pearson's r = 0.70, P < 0.001), suggesting that genetic differentiation
448 among sites may be in part driven by fine scale genetic structure within sites. This correlation was
449 also significant in 2014 (Pearson's r = 0.36, P = 0.046), though the association was very weak
450 and likely not biologically relevant.

451 Effective population size

452 Based on the lack of clearly defined genetic structure in either 2012 or 2014, we pooled
453 all sites in respective years to most accurately estimate N_e of a single population. N_e estimates in
454 2012 and 2014 were large, and remained relatively consistent across years (2012 N_e = 29,711,
455 95% CI = 9,970 – infinity; 2014 N_e = 31,106, 95% CI = 7,642 – infinity) (Table 3). The wide
456 range of 95% confidence intervals indicated that precision of estimates was low in both years,

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457 therefore it is difficult to determine whether true N_e is large (i.e. thousands) or very large (i.e.
458 infinite) (Waples and Do 2010).

459 **Discussion**

460 Evidence for strong connectivity and geographically limited gene flow

461 Our first objective in this study was to establish a baseline of population genetic structure
462 of Dungeness crab in the California Current System (CCS). Throughout our ~1,200 km study
463 range, we observed that a substantial amount of the total genetic variation was found within sites,
464 indicating high genetic diversity and relatively weak genetic structure. Previous population
465 genetic studies of Dungeness crab have also found similar levels of genetic diversity in coastal
466 Oregon (O'Malley et al. 2017) and British Columbia (Beacham et al. 2008). Our findings are also
467 comparable to other marine invertebrates in the CCS (acorn barnacle *Balanus glandula*,
468 Hedgecock 1994b; kelp bass *Paralabrax clathratus*, Selkoe et al. 2006; California spiny lobster
469 *Panulirus interruptus*, Iacchei et al. 2013), as well as other crustacean species with long lived
470 planktonic larvae (southern rock lobster *Jasus edwardsii*, Thomas and Bell 2010; European spiny
471 lobster *Palinurus elephas*, Palero et al. 2011). Our observation of weak genetic differentiation
472 among sites is consistent with the findings of O'Malley et al. (2017) along the Oregon coast.
473 Weak genetic differentiation across broad geographic ranges is also fairly common among fishes
474 and invertebrates with planktonic larval stages in the CCS (grass rockfish *Sebastes rastrelliger*,
475 Buonaccorsi et al. 2004; California mussel *Mytilus californianus*, Addison et al. 2008; several
476 invertebrates, Kelly and Palumbi 2010). Often times, weak genetic differentiation is attributed to
477 widely dispersing planktonic larvae, and it is assumed that populations are homogenous. Low
478 proportions of correct assignments among sites in both 2012 and 2014 may be construed as
479 evidence of panmixia. However, we caution against that interpretation since the ability to detect
480 fine-scale structuring using assignment analyses decreases when genetic structure is weak (Jones
481 and Wang 2012). In contrary, we observed a correlation (though not significant coast wide)
482 between increasing genetic differentiation and geographic distance between sites in 2012,
483 indicating the presence of isolation by distance (IBD) (Wright 1943). This finding suggests that
484 in at least some generations Dungeness crab maintain a pattern of gene flow that follows

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485 geographically limited dispersal. In this manner, gene flow occurs within spatially restricted
486 'neighborhoods' which are linked together as described by the one dimensional stepping stone
487 model (Kimura and Weiss 1964). However, neighborhoods appear to be continuous as opposed to
488 discrete, thereby lacking well defined boundaries. This pattern has been observed in the CCS
489 among grass rockfish (*Sebastes rastrelliger*) and copper rockfish (*S. caurinus*) (Buonaccorsi et al.
490 2002; 2004), as well as red drum (*Sciaenops ocellatus*) inhabiting estuaries of the northern Gulf
491 of Mexico (Gold et al. 2001). The result is strong genetic connectivity among Dungeness crab
492 throughout the CCS, which is likely achieved by gene flow that occurs over several generations,
493 as opposed to broad scale panmixia.

494 In combination with geographically limited gene flow, coancestry among individuals
495 within sites may contribute to genetic differentiation of Dungeness crab in the CCS. We found
496 that several sites in both years had higher mean relatedness ($\overline{r}$) than would be expected in a
497 randomly associated population, particularly Eel River, Duxbury Reef and Half Moon Bay (in
498 2012). This finding is specifically noteworthy since we sampled the adult population, which is
499 subject to increasing admixture over time with successive recruitment events. Unfortunately, our
500 study design limits the level of detail we can provide regarding kin aggregation in Dungeness
501 crab, though considering early life history provides some insight to potential mechanisms which
502 would lead to higher than expected relatedness within several sites. For instance, shelf/slope
503 species of the CCS, such as Dungeness crab, have evolved specific life history traits such as
504 timing of larval release and length of pelagic larval duration in order to employ the seasonal
505 change in oceanographic conditions (i.e. circulation), and thus limit the latitudinal displacement
506 of larvae relative to their parental population (Shanks and Eckert 2005). Granted Shanks and
507 Eckert (2005) were referring to parental populations at a very coarse scale (e.g. northern and
508 southern CCS), evidence of isolation by distance and relatedness within several sites suggests that
509 local recruitment may occur to some degree on a smaller scale. It is less likely that the relatedness
510 found in this study is driven by sweepstakes reproductive success, which is typically followed by
511 chaotic genetic patchiness or otherwise unpatterned population genetic structure not observed
512 here (Christie et al. 2010; Hedgecock and Pudovkin 2011). Lastly, it is possible that cohesive
513 dispersal of kin throughout the planktonic larval phase could result in relatedness in the adult
514 population, though this would be somewhat remarkable, given that Dungeness crab larvae leave

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515 the continental shelf and return inshore over a roughly four month period. Turbulence from wind
516 and swell would likely diffuse kin; however, this behavior has been hypothesized with
517 compelling evidence in other species having lengthy PLDs (kelp bass *Paralabrax clathratus*,
518 Selkoe et al. 2006; Miry's demoiselle *Neopomacentrus miryae*, Ben-Tzvi et al. 2012; domino
519 damselfish *Dascyllus trimaculatus*, Bernardi et al. 2012; California spiny lobster *Panulirus*
520 *interruptus*, Iacchei et al. 2013; splitnose rockfish *Sebastes diploproa*, Ottman et al. 2016). Future
521 studies could assess demographic processes of larvae (e.g. larval dispersal trajectory, mortality
522 rate, interaction with fine-scale oceanographic conditions), as well as genetic variation within and
523 among settlement cohorts to better understand how early life history characteristics contribute to
524 possible kin aggregation of Dungeness crab in the CCS.

525 Regional genetic structure in the CCS has been observed in rockfishes *Sebastes spp*.
526 (Cope 2004; Gomez-Ulchia and Banks 2005, Johansson et al. 2008, Hess 2011), and a variety of
527 rocky intertidal species (Kelly and Palumbi 2010; Sander and Palumbi 2011). In our study,
528 regional genetic structure only accounted for a very small proportion of the total genetic
529 variation, and was found to covary with geographic distance between sampling sites. Assignment
530 success was found to be greater than would be expected if assignment were random within Ft.
531 Bragg in 2012 and North CC in 2014, but these assignment successes were likely driven by the
532 greater assignment successes of few sites within those regions. Based on these findings, it appears
533 that the topographical features we used to define regions (Cape Blanco, Cape Mendocino, and
534 Point Arena) do not represent genetic breaks for Dungeness crab. Instead, our results demonstrate
535 subtle shifts in allele frequencies over a broad range that would result from geographically
536 limited gene flow.

537 Inter-annual variability in genetic connectivity

538 Our second objective in this study was to evaluate inter-annual variability in population
539 genetic structure. Both years were characterized by weak genetic differentiation, but pairwise F_{ST}
540 estimates among sites in 2014 were noticeably lower than in 2012, with no evidence of IBD.
541 There were also fewer sites having significantly high mean relatedness in 2014 than 2012. We
542 hypothesize that inter-annual variability in population genetic structure is in part driven by
543 oceanographic conditions which may influence larval dispersal. The relationship between

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544 carapace width and molt increment (i.e. a proxy for age) (Roegner unpublished data) for crab
545 sampled in Washington provides a crude approximation for the recruitment cohorts present in this
546 study. These data suggest that crab sampled in 2012 completed their larval phase in 2008, 2009,
547 or 2010, and those sampled in 2014 likely did so in 2010, 2011, or 2012. Based on oceanographic
548 measurements within those years, the 2008, 2009, and 2010 larval cohorts would have
549 experienced different physical conditions in the CCS compared to the 2010, 2011, and 2012
550 larval cohorts. For example, Pacific Decadal Oscillation (PDO) entered a brief positive (El Niño)
551 phase during late-2009, lasting until mid-2010 (Bjorkstedt et al. 2010) (Fig. 4a). During El Niño
552 events the flow of the California Current weakens, and as a result there is likely less southward
553 larval advection (Shanks 2013). Unfortunately, it is difficult to hypothesize on how the 2009-
554 2010 El Niño event influenced gene flow, since the cohort that underwent its larval stage during
555 that period is probably represented in both sampling years in some proportion. Timing of the
556 spring transition between 2010 and 2012 (measured by the method of Shanks and Roegner 2007)
557 was late relative to other years (Fig. 4b), and coincided with a decrease in the amount of
558 upwelling during the spring and summer, which reached a minimum in 2011 (upwelling index
559 data was downloaded from http://www.pfeg.noaa.gov/pfel) (Fig. 4c). The spring transition marks
560 the seasonal switch to upwelling favorable winds, which are thought to play a role in the
561 advection of megalopae back on to the continental shelf, where they are transported to the
562 nearshore via internal tides (Shanks 2013). It is possible that a delayed spring transition and less
563 upwelling during the spring and summer may have contributed to increased dispersal by
564 prolonging latitudinal transport in the California Current (as well as reduced overall recruitment).
565 As a result, displacement of larval cohorts between 2010 and 2012 may have been further from
566 points of larval release than in other years. This hypothesis is consistent with the lack of IBD and
567 fewer sites having significantly high mean relatedness in 2014, as compared to 2012. It is
568 important to note, however, that the degree of temporal variation in physical oceanographic
569 conditions can be variable throughout the CCS, and the effect of such variation on larval
570 trajectories may not be ubiquitous coast wide. Fluctuation in dispersal trajectory has also been
571 hypothesized to influence inter-annual variation in population genetic structure notably among
572 shore clingfish (*Lepadogaster lepadogaster)* (Klein et al. 2016) along the Iberian Peninsula. This
573 has also been observed among turbot (*Psetta maxima*) in the Baltic Sea (Florin and Höglund

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574 2007) and bicolor damselfish (*Stegates patitus*) in the Mesoamerican Barrier Reef System (Hogan
575 et al. 2010).

576 Effective population size

577 Estimates of effective population size (N_e) were imprecise in both years, though we can
578 nevertheless conclude that true N_e must be large. Genetic methods for estimating N_e lose
579 precision as the signal of genetic drift becomes clouded by background noise (i.e. inherent
580 sources of error caused by sampling design, marker choice, violation of statistical assumptions)
581 (Waples and Do 2010), which is common in genetic estimates for high gene flow species (Waples
582 1998). It is therefore difficult to differentiate between a true N_e that is large (i.e. thousands) or
583 very large (i.e. tens of thousands) (Waples and Do 2010). A similar finding was reported for the
584 commercially harvested western rock lobster (*Panulirus cygnus*), also owing to the effect of the
585 low signal to noise ratio (Kennington et al. 2013). Estimating N_e based on sibship frequency may
586 provide a more precise calculation of N_e (Wang 2009). However, due to the complex,
587 polygamous mating system of Dungeness crab (Worton et al. 2010; Jensen and Bentzen 2012),
588 sibling relationships are more complex, which downwardly biases estimates of N_e when true N_e
589 is large (Wang 2009; Wang 2016). Though N_e estimates appear to be large, continued monitoring
590 of N_e may still be warranted as intense commercial harvest can result in a loss of genetic diversity
591 (i.e. reduction in N_e) even when the census population size is large (Hauser et al. 2002).

592 Conclusions

593 In this study, we have provided the most detailed assessment of genetic connectivity of
594 Dungeness crab to date. Based on genetic data collected in 2012, our findings suggest that
595 Dungeness crab are at least weakly geographically limited in dispersal, resulting in a long,
596 continuous gradient of genetic differentiation over our ~1,200 km study range. In 2014, no spatial
597 pattern was present and our results provided greater support for broad scale panmixia. So is
598 dispersal, and for that matter gene flow, geographically restricted in the CCS? The answer likely
599 depends on the generation in question. Dungeness crab larvae are known to be accomplished
600 swimmers relative to other planktonic organisms (Fernandez 1994; Rasmuson and Shanks 2014).
601 However, they are limited by the duration of their larval development, and influenced by physical

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602 oceanographic conditions such as currents and winds (i.e. both northward and southward
603 advection). It is well documented that many marine organisms do not reach the full dispersal
604 potential that is suggested by their pelagic larval duration (Cowen et al. 2000; Shanks 2009), and
605 Dungeness crab are likely no exception. Since Dungeness crab life history may employ physical
606 oceanographic processes to limit the latitudinal displacement of larvae (Shanks and Eckert 2005),
607 it is possible that variation in those processes may promote wider dispersal (and thus gene flow).
608 Dungeness crab dispersal and gene flow is likely geographically limited, though the extent of
609 limitation may vary depending on oceanographic conditions which influence larval transport.
610 Therefore, both local recruitment and large scale dispersal may play distinct roles in shaping
611 genetic connectivity of this species, as in Klein et al. (2016).

612 Overall, our results show that populations of coastal marine organisms are capable of
613 undergoing temporal changes in population genetic structure over short time periods. Even within
614 the short time span of two years, we observed measurable differences in population genetic
615 structure that were preceded by a brief change in oceanographic conditions. Our findings support
616 that sampling at multiple time points augments the study of genetic connectivity of marine
617 organisms, as inter-annual variability in population genetic structure may reveal important
618 biological processes that may have otherwise gone unnoticed (e.g. ephemeral patterns of genetic
619 structure). This study also further demonstrates that evidence of local population genetic structure
620 (e.g. IBD, relatedness) can be observed among a large scale pattern of genetic homogeneity,
621 which would not have been feasible without sampling a broad study range and employing fine-
622 scale sampling design. Since there are several commonalities in life history traits of shelf/slope
623 species in the CCS (Shanks and Eckert 2005), our findings are likely applicable to other CCS
624 species beyond Dungeness crab. Even though we now have a greater understanding of genetic
625 connectivity for this species, we can only hypothesize about the demographic processes that
626 regulate gene flow (e.g. dispersal). Future studies that examine larval dispersal trajectory of
627 Dungeness crab larvae could be used to test our hypotheses regarding geographically limited
628 dispersal, and provide greater detail on how connectivity changes with varying ocean conditions.

629 **Acknowledgements**

630 This report was prepared by Oregon Sea Grant under grant number NA14OAR4170064
631 (CFDA No. 11.417) (Project Number R/RCF-33) from the National Oceanic and Atmospheric

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632 Administration's National Sea Grant College Program, US Department of Commerce, and by
633 appropriations made by the Oregon State Legislature. The statements, findings, conclusions, and
634 recommendations are those of the authors and do not necessarily reflect the views of these
635 funders. We thank the Washington Department of Fish and Wildlife (WDFW), Oregon
636 Department of Fish and Wildlife (ODFW), California Department of Fish and Wildlife, and the
637 Dungeness crab commercial fishing fleet for cooperation and assistance during sample collection.
638 We specifically acknowledge the field coordination efforts of Daniel Ayres (WDFW), Kelly
639 Corbett (ODFW), and Peter Kalvass (CDFW).

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Author Contributions

Laboratory analysis, data management, data analysis, and writing were performed by Tyler Jackson to fulfill requirements for a Master of Science degree from Oregon State University. Curtis Roegner and Kathleen O'Malley were co-principal investigators for this project and provided support for data analysis and writing. Grant funding for this project was awarded to Kathleen O'Malley.

Data Accessibility

Genotypes of Dungeness crab at all ten loci used in this study can be accessed on Dryad at DOI: http://dx.doi.org/10.5061/dyad.1g62f.

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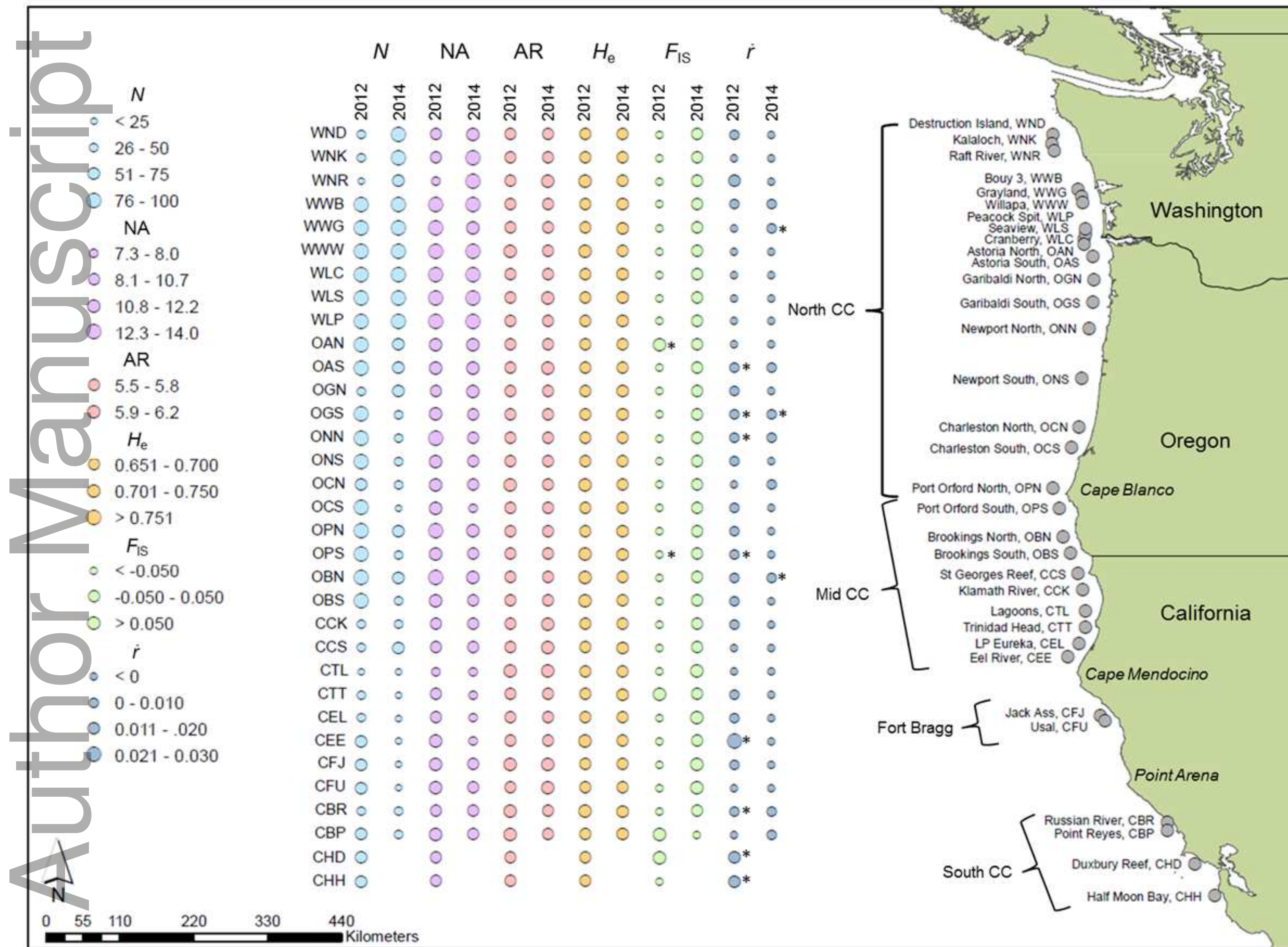

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1026 **Figure 1**. Map of the 33 sites sampled during the 2012 and 2014 Tri-State Pre-Season Test Fishery in Washington, Oregon, and
1027 California and summary statistics based on variation at ten microsatellite loci. Sites were subdivided into four regions: North CC,
1028 Mid CC, Ft. Bragg, and South CC, based on three topographical features including Cape Blanco, Cape Mendocino, and Point
1029 Arena. CHD and CHH were only sampled in 2012. Summary statistics include the number of individuals genotyped (N), number
1030 of alleles per locus (NA) (smallest $N = 10$), allelic richness (AR), expected heterozygosity (H_e), inbreeding coefficient (F_{IS}), and
1031 mean pairwise relatedness ($\overline{r}$).Significant F_{IS} values after applying FDR corrections ($\alpha = 0.05$) and $\overline{r}$ values greater than
1032 expected (pseudo-$P < 0.05$) are denoted (*).

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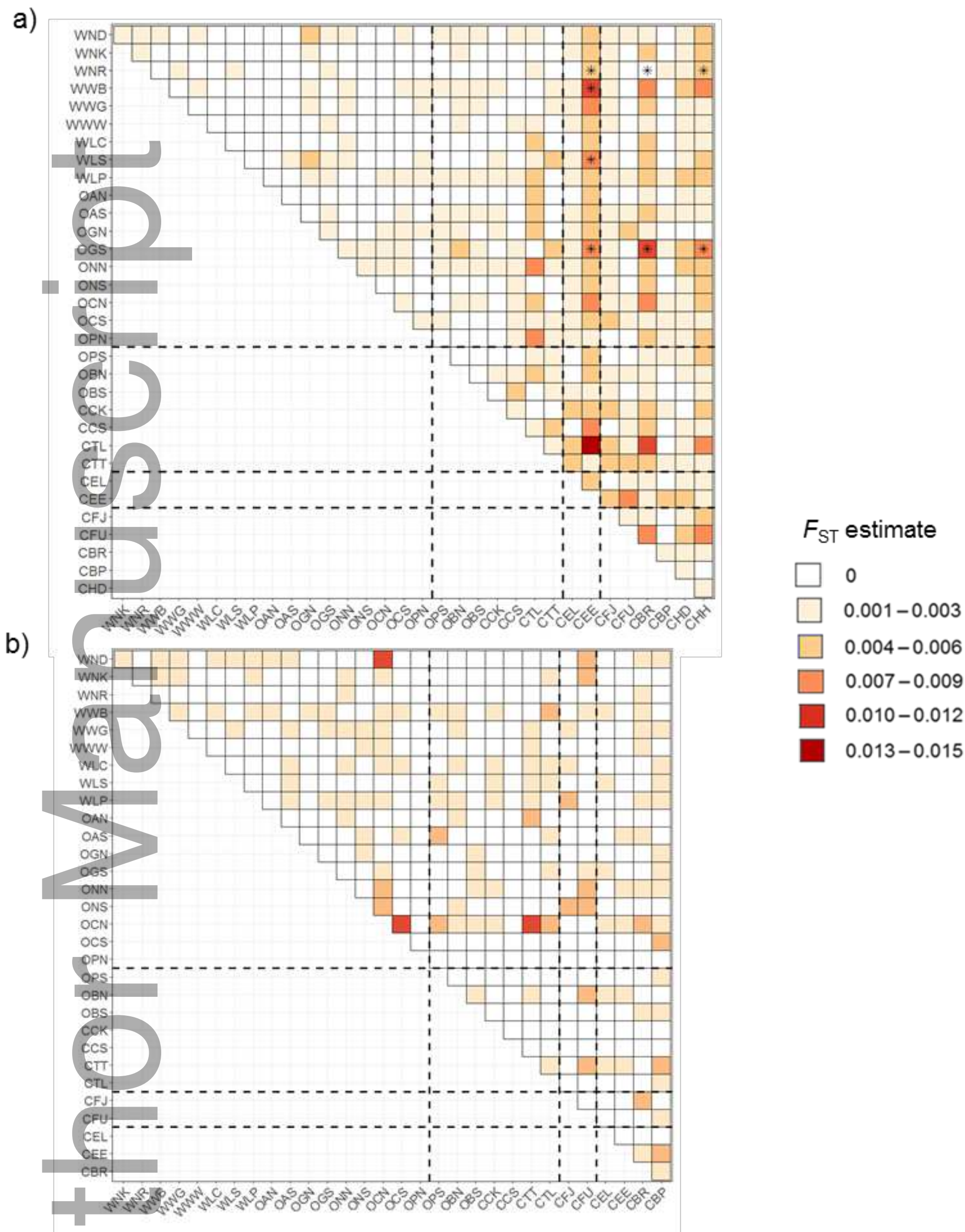

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1034 **Figure 2**. Heat map based on pairwise F_{ST} estimates among sampling sites in a) 2012 and b)
1035 2014. Each pairwise comparison is color coded based on the F_{ST} estimate. Significant F_{ST}
1036 associated *P*-values after applying False Discovery Rate corrections are denoted (*). Dashed lines
1037 represent boundaries of *a priori* defined regional for geographical reference.

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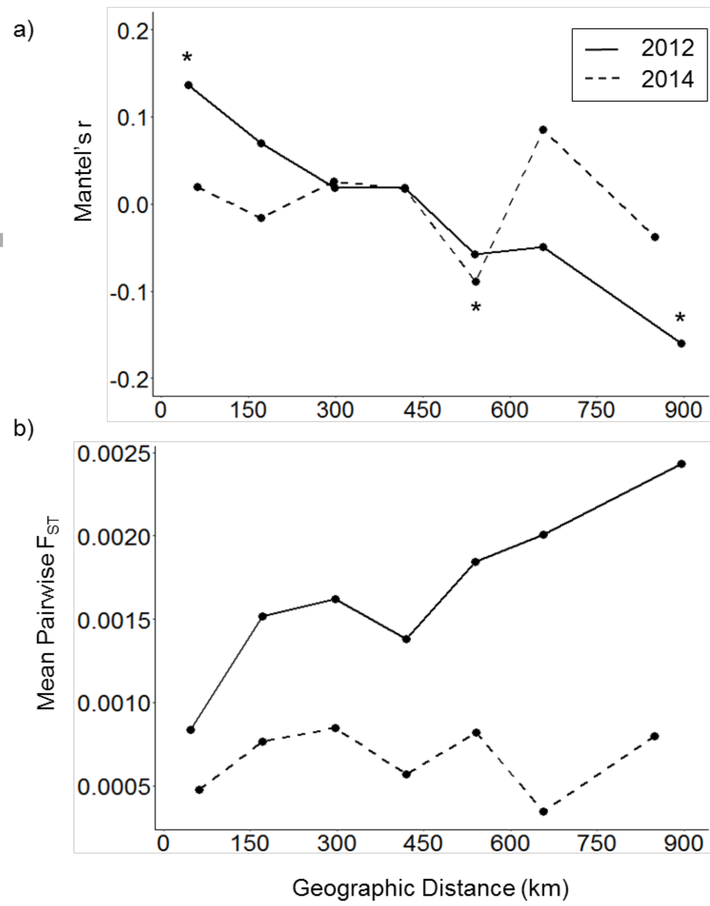

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1067 **Figure 3**. a) Mantel correlogram and b) distogram illustrating the relationship between genetic
1068 differentiation (as measured by pairwise F_{ST}) and mean geographic distance among sites grouped
1069 into seven distance classes in 2012 (solid lines) and 2014 (dashed lines). Significant correlations
1070 (Mantel's r, r_m) are denoted (*) above for 2012 and below for 2014. Mean pairwise F_{ST} in the
1071 distogram is calculated as the mean value of pairwise F_{ST} estimates among sites within each
1072 distance class.
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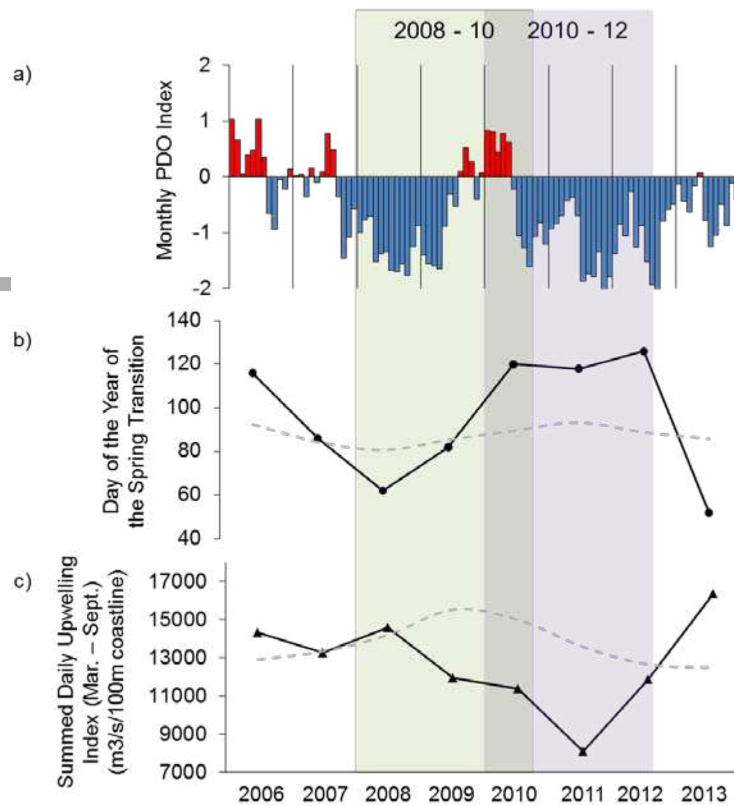

1104 **Figure 4**. Eight year (2006 –
1105 2013) trend in inter-annual
1106 variation in physical
1107 oceanographic conditions
1108 including a) monthly Pacific
1109 Decal Oscillation
1110 index, b) day of the year of the spring transition based on sea level data from Crescent City,
1111 California (starting at January 1), and c) summed daily upwelling index at 42° N during the
1112 megalopae settlement season in the California Current System (Mar. – Sept.) measured as cubic
1113 meters per second for every 100 m of coastline. Grey dotted lines indicate ten year moving
1114 averages. Potential cohorts years for crab sampled in 2012 (2008 - 2010) and 2014 (2010 – 2012)
1115 are shaded in light green and purple, respectively.

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Table 1. Pairwise F_{ST} estimates based on variation at ten microsatellite loci among the four regions in (a) 2012 and (b) 2014. Significant values after applying False Discovery Rate corrections are denoted (*). F_{ST} estimates based on a sample size less than 50 individuals per site are italicized.

	Mid CC	Ft. Bragg	South CC
(*a*)			
North CC	0.001*	0.001	0.002*
Mid CC		0.001	0.001
Fort Bragg			0.003*
(*b*)			
North CC	0.000	*0.000*	0.000
Mid CC		*-0.001*	0.000
Ft. Bragg			*0.002*

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Table 2. Proportion of individuals correctly assigned to the a) site or b) region in which they were sampled based on the expected frequency of an individual's genotype within a location (Rannala and Mountain 1997; Paetkau et al. 2004).

		Prop. Correctly Assigned	
	Site	2012	2014
a)	WND	0.00	0.02
	WNK	0.00	0.02
	WNR	0.00	0.03
	WWB	0.26	0.01
	WWG	0.11	0.00
	WWW	0.08	0.05
	WLP	0.04	0.02
	WLS	0.00	0.18
	WLC	0.01	0.15
	OAN	0.01	0.01
	OAS	0.01	0.15

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	OGN	0.00	0.03
	OGS	0.00	0.00
	ONN	0.00	0.00
	ONS	0.01	0.00
	OCN	0.03	0.03
	OCS	0.00	0.00
	OPN	0.01	0.05
	OPS	0.05	0.03
	OBN	0.02	0.02
	OBS	0.02	0.05
	CCK	0.00	0.04
	CCS	0.00	0.02
	CTL	0.00	0.00
	CTT	0.00	0.05
	CEL	0.00	0.00
	CEE	0.00	0.00
	CFJ	0.39	0.00
	CFU	0.00	0.00
	CBR	0.05	0.00
	CBP	0.00	0.00
	CHD	0.07	
	CHH	0.04	
b)	North CC	0.27	0.55
	Mid CC	0.22	0.21
	Ft. Bragg	0.58	0.22
	South CC	0.17	0.22

Table 3. Estimates of effective population size (N_E) and range of 95% confidence intervals for each region using the Waples and Do (2008) single-sample linkage disequilibrium method with a P-critical value of 0.01 for (a) 2012 and (b) 2014.

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Year	N_E estimate	95% Confidence Interval
2012	29,711	9,970 – infinity
2014	31,106	7,642 – infinity

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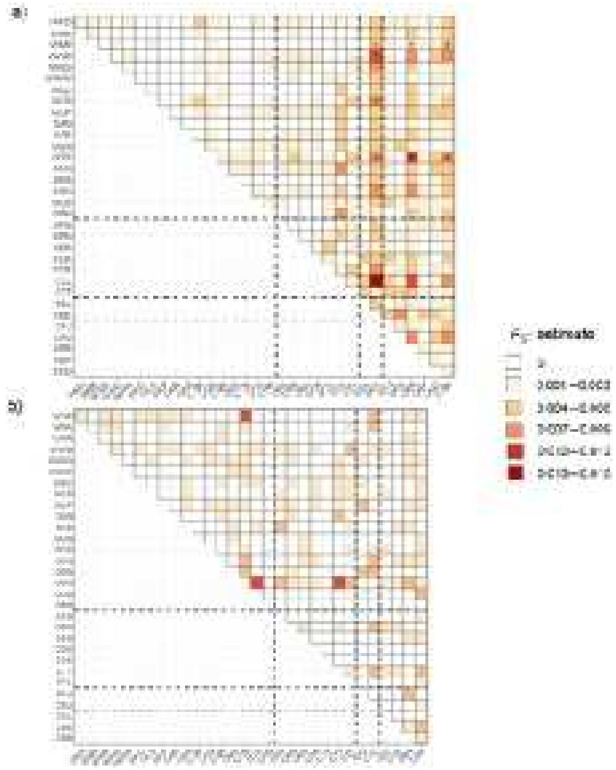

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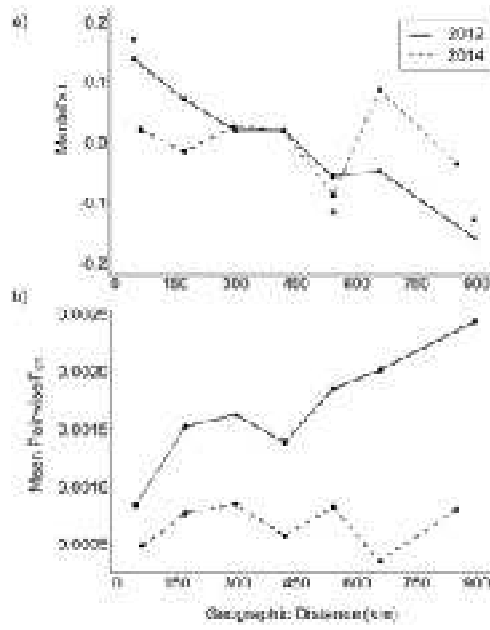

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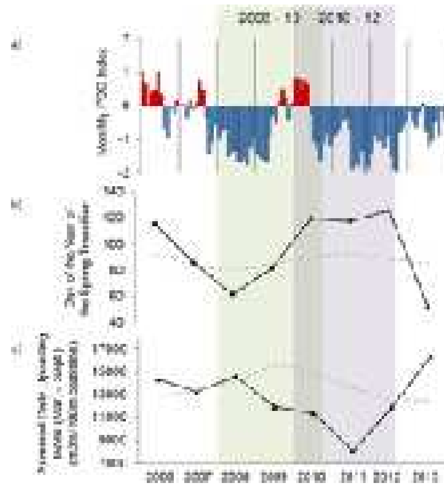

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