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# Exoskeleton dissolution with mechanoreceptor damage in larval Dungeness crab related to severity of present-day ocean acidification vertical gradients

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Exoskeleton dissolution with mechanoreceptor damage in larval Dungeness crab related to

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- 20 Keywords: Dungeness crab, larval sensitivity, global climate change, ocean acidification,
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### 22 Abstract:

Ocean acidification (OA) along the US West Coast is intensifying faster than observed in the 23 global ocean. This is particularly true in nearshore regions (< 200 m) that experience a lower 24 buffering capacity while at the same time providing important habitats for ecologically and 25 economically significant species. While the literature on the effects of OA from laboratory 26 experiments is voluminous, there is little understanding of present-day OA *in-situ* effects on 27 marine life. Dungeness crab (Metacarcinus magister) is perennially one of the most valuable 28 29 commercial and recreational fisheries. We focused on establishing OA-related vulnerability of larval crustacean based on mineralogical and elemental carapace to external and internal 30 carapace dissolution by using a combination of different methods ranging from scanning electron 31 microscopy, energy dispersive X-ray spectroscopy, elemental mapping and X-ray diffraction. By 32 integrating carapace features with the chemical observations and biogeochemical model 33 hindcast, we identify the occurrence of external carapace dissolution related to the steepest  $\Omega$ 34 calcite gradients ( $\Delta\Omega_{cal.60}$ ) in the water column. Dissolution features are observed across the 35 36 carapace, percopods (legs), and around the calcified areas surrounding neuritic canals of 37 mechanoreceptors. The carapace dissolution is the most extensive in the coastal habitats under prolonged (1-month) long exposure, as demonstrated by the use of the model hindcast. Such 38 dissolution has a potential to destabilize mechanoreceptors with important sensory and 39 behavioral functions, a pathway of sensitivity to OA. Carapace dissolution is negatively related 40 to crab larval width, demonstrating a basis for energetic trade-offs. Using a retrospective 41 prediction from a regression models, we estimate an 8.3% increase in external carapace 42 43 dissolution over the last two decades and identified a set of affected OA-related sublethal pathways to inform future risk assessment studies of Dungeness crabs. 44

### 45 Introduction

Since the pre-industrial era, anthropogenic CO<sub>2</sub> uptake along the US West Coast have 46 resulted in rapid intensification OA rate on a global scale (Chavez et al., 2017; Feely et al., 47 2016), resulting in lower carbonate conditions compared to the pre-industrial times. This is 48 because of the low regional buffering capacity, which contributes to low pH and carbonate 49 mineral saturation states for both aragonite ( $\Omega_{ara}$ ) and calcite ( $\Omega_{cal}$ ) (Feely et al., 2018). These 50 changes in carbonate chemistry have resulted in in substantially reduced habitat suitability for 51 marine calcifiers (Bednaršek et al., 2014; Somero et al., 2015). These findings are supported by 52 the field and synthesis work along the North American Pacific demonstrating that calcifying 53 invertebrates will be the ones most impacted by progressive OA (Bednaršek et al., 2017; Busch 54 55 & McElhany, 2016). Apart from evidence of OA impacts on pteropods and other calcifiers caused by the low  $\Omega_{ara}$  conditions in upwelling systems (Bednaršek et al., 2014, 2017) and 56 around CO<sub>2</sub> vent seeps and sites (Manno et al., 2019; Tunnicliffe et al., 2009), there is limited 57 understanding of present-day OA effects on marine life in situ. That is especially relevant for the 58 59 crustaceans since they were considered less sensitive to OA parameters (like pCO<sub>2</sub> or pH) after studies demonstrated their capacity to abate initial hypercapnia and to buffer extracellular acid-60 base disturbances (Melzner et al., 2009; Pane and Barry, 2007) with limited or no change in 61 aerobic metabolism (Paganini et al., 2014). However, restoring internal pH to sustain 62 physiological and biogeochemical processes (Somero, 1986), typically requires activation of 63 buffering, which is energetically expensive process (Cameron, 1985; Michaelidis et al., 2005). 64 Recent experimental findings have demonstrated increased sensitivity to OA-related stressors in 65 66 crustaceans, especially in the early life stages that can be regarded as a potential bottleneck for the population level responses (Schiffer et al., 2014; Small et al., 2015). Regardless of the 67

habitats that different species inhabit, the studies investigating OA effect on larval stages 68 reported lower growth and decreased survival of blue crab (Giltz and Taylor, 2017), delayed 69 metamorphosis in the stone crab (Gravinese et al., 2018), changes in exoskeleton composition 70 (Page et al., 2016) and decreased metabolisms in Tanner crabs (Long et al., 2016) and increased 71 energetic costs in the porcelain crab (Carter et al., 2013), while the pre-larval of the Dungeness 72 crab showed reduced survival and slower progression through the development (Miller et al., 73 74 2016). With annual revenues up to \$220 million (Hodgson et al., 2018; Pacific States Marine Fisheries Commission, 2019), the Dungeness crab (Metacarcinus magister) is one of the most 75 valuable and recreational fisheries in the US coastal waters. Terminal stage of pelagic Dungeness 76 77 crab larvae (megalopae) undergoes long distance transport along the north Pacific coast of North America before settling in suitable benthic settlement site (Shanks, 1995; Sinclair, 1988). Given 78 their diel vertical migration extends down to 60 m depth (Hobbs et al., 1992), megalopae 79 80 encounter steep vertical pH,  $\Omega_{cal}$  gradients in coastal habitats; however, the duration and magnitude of their exposure to these conditions remains largely unknown despite the exposure 81 history notably impacting organismal responses (sensu Bednaršek et al. (2017). 82

To start addressing potential OA vulnerability of the pelagic Dungeness larvae in situ, 83 their spatial distribution must be paired with in situ exposure history (as defined in Bednaršek et 84 al., 2017), understanding of their physiological susceptibility and their structural and 85 mineralogical features. In regards to the later, surprisingly little is known about these features 86 that can predispose an individual to extensive exoskeleton dissolution if the conditions in the 87 external environment are conducive for it. In addition, for detecting environmental clues, the 88 89 decapod exoskeleton contains elongated hair-like structures called setae, which are important chemo- and mechanoreceptors involved in sensory and behavioral responses. While the 90

91 lipoproteic epicuticle-covered exoskeleton consists of two cuticular mineral carbonate layers
92 (Chen et al., 2008) (i.e., the outer exocuticle and the inner endocuticle) in the Dungeness crab
93 adults such structure or its composition is completely unknown for the larvae.

This interdisciplinary study integrates physical, geochemical, biological, and modelling components across the individual and population-level parameters. Identification of the carapace crystalline mineralogical and elemental composition provided an understanding behind the extensive exoskeleton dissolution and mechanoreceptor damage. The latter was linked with the chemical observations made along the North American West Coast to identify the drivers and OA hotspots of *in situ* megalopae vulnerability. The biogeochemical model hindcast was used to determine the impact of *in situ* exposure history in the coastal habitats.

101

### 102 Materials and methods

### 103 Carbonate chemistry, sampling and analyses

For the purpose of this study, the NOAA West Coast Ocean Acidification (WCOA) cruise in 104 May–June 2016 sampled conductivity, temperature, depth, and oxygen. Data on the conductivity, 105 temperature, and pressure of seawater (CTD) were collected along cross-shelf transects, 106 accompanied by biological stations with vertical sections of temperature (T), salinity, nutrients, 107 oxygen, chlorophyll-a (chl-a), dissolved inorganic carbon (DIC), total alkalinity (TA), 108 109 spectrophotometric pH (measured at 25°C and corrected to in situ temperatures, and expressed on the total pH scale, subsequently expressed pH<sub>T</sub>).  $pCO_2$  and calcite saturation state ( $\Omega_{cal}$ ) were 110 calculated using CO2SYS as described by Feely et al. (2016) and Bednaršek et al. (2012). Larvae 111 were collected using Neuston and Bongo nets with a mesh size of 333 µm, which were deployed 112

in an oblique manner at 10 stations during the night in the upper surface waters, an area that encompasses the nocturnal vertical habitat of larval Dungeness crabs of the upper 60 m (Hobbs et al., 1992; Morgan, 1985), with the following environmental parameters along the vertical habitat (Table S1). The duration of tows was 15–20 min. Megalopae were identified and then stored in 100% non-denatured ethanol, and also flash-frozen at -80 °C for later comparison of two different preservation methods.

### 119 Using SEM methods to detect and evaluate carapace dissolution

Megalopae carapaces were investigated using a combination of different methods: 1) scanning 120 electron microscopy (SEM; Hitachi Phenom, USA) to determine potential structural changes on 121 the cuticular surface; 2) energy dispersive X-ray spectroscopy (EDXS at the University of 122 123 Washington) for mineralogical composition; 3) elemental mapping; and X-ray diffraction (University of Washington and Max Planck Institute) for elemental content across mostly 124 lipoproteic carapace (Figure S1, Fig. 1). Here, we define exoskeleton as the cuticle covering the 125 dorsal part of the carapace of the larval crab, and five percopods, with chelae. The carapace 126 epicuticle, which otherwise overlies the crystalline layer and makes dissolution observations 127 impossible, was removed from each megalopa prior to analysis. This was accomplished using 128 sodium hypochlorite, which efficiently removes the epicuticle but does not damage the 129 crystalline layers underneath, even at high concentrations (Bednaršek et al., 2012). On the 130 samples with no hypochlorite treatment, the epicuticle covered the crystalline layer so no 131 investigation of dissolution was possible. Therefore, we tested different concentrations and 132 duration of hypochlorite treatment on the individuals from the same station to ensure that we 133 fully removed the epicuticle, without triggering dissolution of the calcite crystalline layers 134 beneath it. The combination of 1 and 3 % sodium hypochlorite with 15, 30, and 120 min 135

treatments only partially removed the epicuticle, , while 6% hypochlorite treatments for 4 and 6
hours were effective in epicuticle removal, without inducing additional dissolution at the longer
duration.

To determine the treatment does not induce any damage, we have tested the individuals from the same stations with different combination of treatments. We assumed the similarity of exposure history of individuals within the same stations (see modelling section). The results of no difference in dissolution under the different combination of concentration/duration of treatment combination was a confirmation that the treatment did not induce any additional damage, and thus did not confound our field observations.

145 We determined 6% hypochlorite for 3-4 h to be an efficient and effective treatment, similar to the treatment previously described for the removal of the periostracum in pteropods (Bednaršek 146 et al., 2012). After soaking in sodium hypochlorite, samples were rinsed several times in 147 148 Millipore water to remove any organic matter remaining on the surface of the exoskeleton. It is important to note that when examining the presence of setae within the neuritic canals, we did 149 not use any treatment in order to avoid any methodological artifacts. When examining the 150 internal area of the cuticle, we avoided examining the proximity of the gills given that 151 dissolution could be impacted by the processes around. To quantify the dissolution of internal 152 153 carapace cuticle, the percopods and soft tissue of larvae were gently removed from the rest of the 154 body and washed in Millipore water to remove any remaining tissue or organics before treatment with sodium hypochlorite. Each of the five percopod cuticle was examined across the proximal 155 and distal ends, with particular focus on chelae (Figure S2). SEM was used to evaluate the extent 156 and severity of dissolution. We focused on three distinct features: ridging structures, dissolution 157 around setae, and exposed calcite crystals (Figure S2, S3). 158

For estimating the various body parameter change (in mm) of larvae across various 159 vertical OA gradients, we have measured carapace length (CL), total length from rostrum to 160 telson (TL), length from rostrum to dorsal carapace spine (R-DCS), and carapace width (CW). 161 The CW is the most commonly used parameter by various US federal agencies along the US 162 West Coast to regulate crab management catch efforts (Davis et al., 2017). Here, we assume that 163 all the larvae were released at the same time to allow for body parameter comparisons across 164 165 different stations. Using the methodology to characterize the megalopae stages by González-166 Gordillo et al. (2004), we determined that all megalopae were in the intermolt stage, except for those from Station 115, which were transitioning into the premolt stage. We excluded the results 167 168 of the internal dissolution observations from this station in case promoting process changed any features on the internal side that could bias accurate dissolution assessment. 169

### 170 Semi-quantitative dissolution assessment

Altogether, we analyzed 50 individuals from 10 environmental stations across OA-related 171 vertical gradients of varying strength. We used 3-5 individuals per station to determine 172 dissolution extent of the external side of carapace cuticle, as well as the cuticle of the pereopods. 173 We used an additional 2-3 individuals per station to analyze the internal side of the cuticle. 174 175 Approximately 10-20 SEM images were produced per individual on the external and internal sides, with the images being manually examined to detect any signs of dissolution. For crystal 176 exposure characterization, the same categorization of dissolution conditions as previously 177 described in pteropods was used (Bednaršek et al., 2012). We identified three major features of 178 exoskeleton dissolution and developed a categorization scheme for all three features, showing 179 them in their intact forms (Stage 0, Figure S2) and progressively altered forms (Stage 1 and 2; 180 Figure S1; Table S2). These features differentiated damaged surfaces from the intact surfaces 181

182 (Table S2). The cuticular surface of the carapace and the percopods under high  $\Omega_{cal}$  in situ conditions had a smooth, sleek appearance (Figure S2; Stage 0). At greater magnification, 183 individual calcite crystals were visible in these areas (Figure S2). Signs of dissolution tended to 184 be more prevalent and more severe on the surface immediately surrounding setae pores. 185 Consequently, areas around the setae were considered separately from the rest of the 186 exoskeleton. From these observations, a semi-quantitative scoring metric was developed based 187 on previous work on pteropods and used to score the remainder of the samples. For each sample, 188 the three separate features (presence and depth of ridge structures, exposure of individual calcite 189 crystals, and the prevalence of dissolution features around setae pores) were each assigned a 190 191 score. The features were scored on a scale of 0 to 1 based on the severity of dissolution: intact exoskeleton with no dissolution received a score of 0; moderate dissolution received a score of 192 0.5; and substantial dissolution of all examined features was scored 1 (Figures S1–S3). Since the 193 194 crab exoskeleton of the carapace and percopod differ in their chemical composition, these three areas were assigned separate scores. Because of the surface analyses required separately for the 195 external and internal dissolution, both types of analyses could not be conducted on the same 196 individual. All three features displayed similar trends, so the scores were averaged to unitless 197 'relative dissolution', describing internal and external dissolution. Observation of setae 198 presence/absence was included in the exoskeleton observation under SEM on intact specimen 199 before any preparation treatments were conducted to eliminate the possibility of preparation 200 steps affecting setae presence or outrooting them from the carapace. 201

### 202 Mineralogical analyses

203 The mineralogy of selected megalopae was characterized using X-ray diffraction (D8 Discover
204 2D; University of Washington, Seattle). Prior to analysis, carapaces from five megalopae at each

site were coarsely crushed and treated for 10 min using a dilute (3%) sodium hypochlorite solution to minimize interference from organic matter but without compromising mineralized structures. Samples were dried completely and then ground to a fine homogenous powder representing the aggregate of the five individuals from each location. Resulting diffractograms were compared to a catalog of mineral-specific patterns to constrain the primary mineralogy of each sample.

### 211 Elemental analyses

We used energy-dispersive X-ray spectroscopy (EDXS) to estimate elemental composition of the carapace and percopod cross-sections (N = 7) from samples across different natural OA vertical gradients over spatial scales. For elemental analyses, we have not removed the epicuticle from the samples. These gradients analyses were conducted at Max Planck Institute for Marine Microbiology in Bremen, Germany. Prior to analyses, we dehydrated samples using 100 % ethanol and dried them in a critical point dryer. We prepared the sections by fracturing different carapace regions which was followed by the EDXS investigations (Figure 1).

### 219 Statistical analyses

Biological measurements from Dungeness megalopae collected at 10 stations along the North American Pacific Coast (Figure 2) were paired with synoptic environmental data from CTD profiles. Environmental data were summarized as depth-integrated averages from the surface to the maximum depth of each CTD profile to characterize the exposure conditions in the upper water column. In addition,  $\Delta\Omega_{cal,60}$  was estimated as the difference from the observed measurement at each depth bin with that of the surface. This measurement characterized the relative  $\Omega_{cal}$  gradients with increasing depth and accounted for differences in the relative 227 magnitudes of  $\Omega_{cal}$  between stations. Chlorophyll-a observations were highly skewed and so 228 were log-transformed prior to analysis.

Biological responses included dissolution, body parameter, and abundance with various 229 environmental vertical gradients to identify significant associations using generalized linear 230 231 models. For comparison of the biological data to environmental conditions, each depth bin for the depth-integrated values was evaluated to identify at which depth associations between 232 biological response and selected environmental variables were strongest. In addition, carapace 233 dissolution was compared to body parameters to characterize potential linkage between the 234 physiological parameters, growth, and population-level effects (abundance). Comparisons of 235 236 biological measures to each other were also accomplished with generalized linear models.

Gaussian distributions were assumed for all response variable models, excluding 237 238 presence/absence, which was modeled using a binomial logistic response curve. Models and 239 individual parameters were considered significant at  $\alpha = 0.05$ . All models had N = 10 except presence/absence models with N = 24, which included additional stations where tows were 240 conducted but no crabs were found. Finally, all variables were evaluated together to identify 241 pairwise associations using Pearson correlation analysis and redundancy analysis (RDA) to 242 characterize how the biological response measures were jointly explained by the environmental 243 244 variables. For the latter analysis, all input data were standardized to range from 0 to 1 to account 245 for differences in scale between variables. The vegan package for the R statistical programming language was used for standardization and RDA (Oksanen et al., 2019; R Core Team, 2019). 246

For selected predictors, additional models were developed to evaluate the additive effects of two predictors on dissolution. Backward model selection was used to identify the most parsimonious model by sequentially dropping individual predictors and comparing Akaike Information Criterion values (AIC) (Akaike, 1973; Fox and Weisberg, 2011). This allowed us to determine if there was any additional power in combining predictors to explain dissolution, or consequently, if dissolution could be sufficiently explained using only one predictor. For example, the ability of both  $\Omega_{cal}$  and chlorophyll to explain dissolution were evaluated to better understand the relative effects of both.

### 255 *J-SCOPE model outputs of the larval exposure history prior to sampling*

The Joint Institute for the Study of the Atmosphere and Ocean (JISAO)'s Seasonal Coastal 256 Ocean Prediction of the Ecosystem (J-SCOPE, http://www.nanoos.org/products/j-scope/) 257 features dynamical downscaling of regional ocean conditions in Washington and Oregon waters 258 (Siedlecki et al., 2016). Model performance and predictability examined for sea surface 259 260 temperature (SST), bottom temperature, bottom  $O_2$ , pH, and  $\Omega_{ara}$  through model hindcast, reforecast, and forecast comparisons with observations, showing significant measurable skill on 261 seasonal timescales (Kaplan et al.2016; Siedlecki et al., 2016; Norton et al., in revision). 262 Megalopae exposure histories were simulated by releasing 100 representative particles, with 263 vertical migration behavior over 60 m inserted into the predicted circulation field at each of the 264 265 in situ sampling locations and times, and then tracking them backward in time for 30 d following 266 methods described for pteropods in Bednaršek et al. 2017, and for megalopae in Norton et al., in revision. The vertical migration behavior was simulated using the LTRANSv2b larval transport 267 model (North et al., 2008, 2011; Schlag and North, 2012) that has recently been implemented in 268 the J-SCOPE system and adapted for megalopae (Norton et al., in revision). 269

270

271 Results

### 272 Elemental and crystalline characterization of the carapace

The compilation of our results demonstrate that the carapace is highly mineralized and 273 precipitated into a chitin-proteinaceous matrix. XRD identify calcite as a primary polymorph in 274 the carapace. The mineralized exoskeleton of the megalopae intermolt stages consists of the 275 thinner exocuticle on the surface that is less than 2-3 µm thick, and the thicker and more 276 277 compact endocuticle underneath (Figure 1) of approximately 6-7 µm, with the combined thickness up to 10 µm. The carapace surface is extensively covered with setae that are rooted in 278 the calcified neuritic canals each with an average of about 5 µm surface opening (Figures S2 and 279 S3). EDXS investigations characterized detailed elemental structure with average Ca<sup>2+</sup> content of 280 28 % in the carapace and percopods, with much higher Ca<sup>2+</sup> found within in the mid layer and 281 the endocuticle (higher than 50%) compared to less than 20% found in the exocuticle (Figure 1). 282 The carapace endocuticle contains also a high concentration of  $Mg^{2+}$  with some areas of the 283 carapace exceeding 5% content, categorizing it as a more soluble high-Mg calcite. In addition, 284 the internal side contain high concentrations of phosphorus (up to 6%) and strontium (up to 2%) 285 on the inner endocuticle (Figure 1). The percentage of different dissolution features is similar 286 between the carapace and the percopods, however with much less variation in all elements 287 between the carapace and percopods (Figure S1). This elemental composition indicates that other 288 crystalline forms of carbonate could be precipitated into a chitin-proteinaceous matrix, such as an 289 amorphous calcium carbonate (ACC) crystalline layer, but the methods used were not suitable 290 for ACC detection. The strong presence of autofluorescence prevented more precise detection of 291 any other crystalline forms, despite extensive use of Raman spectroscopy for this purpose. 292 Nevertheless, such elemental structure resembles a layer of ACC with Mg<sup>2+</sup>, phosphate and 293

carbonate-rich phase, or ACC with magnesian calcite, as previously demonstrated in the edible
crabs *Cancer pagurus* (Fabritius et al., 2012).

296

### 297 Megalopae habitat characterization with strong vertical and spatial $\Omega_{cal}$ vertical gradients

Crab megalopae were found in both outer-shelf, slope, as well as nearshore (<200 m depth) 298 habitats, with distinctly different vertical environmental gradients in the upper water column. 299 300 Due to the upwelling of deeper, colder, CO<sub>2</sub>-rich waters in the near-shore and coastal habitats, steep gradients in low pH and  $\Omega_{cal}$  values were observed. In comparison, offshore region were 301 characterised with more uniform vertical gradients with lower vertical difference were over the 302 same depth interval (Figure 2). Pronounced steep OA-related vertical habitats were observed in 303 the upper 60 m of the water column, here represented as the difference between the surface and 304 60 m depth ( $\Delta\Omega_{cal.60}$  or  $\Delta pH_{60}$ ), which is within the lower range of megalopae diel vertical 305 migration habitat. Among all tested depths, statistical models comparing biological responses 306 (e.g. exoskeleton dissolution, body parameters, abundance) with environmental conditions had 307 the strongest associations using the 60 m vertical depth integrated value (e.g., external 308 dissolution on body parts vs.  $\Delta\Omega_{cal}$  had the highest R<sup>2</sup> = 0.821 at 60 m). Hereafter, all 309 environmental data are reported using the 60 m depth integrated values. Coastal conditions 310 recorded near-saturation  $\Omega_{cal}$  values down to 1.4, pH down to 7.48, and pCO<sub>2</sub> up to 910 uatm 311 (Table S1; Figure 2).. There were no observations of  $\Omega_{cal} < 1$  or hypoxia, with similar oxygen 312 ranges observed in the onshore and offshore regions, while average temperature that was by 313 about 1.3° C warmer offshore. Food availability was an order of magnitude higher in the onshore 314 regions compared to offshore, with the highest chl-a values recorded at 25  $\mu$ g L<sup>-1</sup> (Figure 2). 315

Multiple environmental parameters co-varied (Figure 3a) as observed in the RDA plot at 316 60 m depth (Figure 3b). The first two axes of the RDA explained approximately 90% of the 317 variation among the biological and environmental parameters. The first RDA axis was 318 characterized by a  $\Delta\Omega_{cal,60}$  vertical gradient and external dissolution, with both having negative 319 loadings along the RDA1 axis. Carapace width was negatively correlated with  $\Delta \Omega_{cal,60}$ , whereas 320 external dissolution was positively correlated, suggesting that larger individuals had less 321 dissolution and were associated with lower gradients in  $\Delta \Omega_{cal,60}$ . While OA parameters (pCO<sub>2</sub>, 322 pH) were all correlated as indicated by alignment with the second RDA axis, the collinearity 323 with temperature was not significant. We found less collinearity among the environmental 324 parameters related to the 60 m vertical gradients, such as  $\Delta\Omega_{cal,60}$ ,  $\Delta O_{2,60}$ , and  $\Delta T_{60}$ . Here, we 325 focused on the mechanistic drivers that are explicitly involved in the external dissolution 326 327 processes, we have examined  $\Delta\Omega_{cal,60}$  in how it relates to external dissolution. Similarly, internal 328 dissolution was negatively correlated with pCO<sub>2</sub> along the second axis with slightly higher loading along the RDA1, and also slightly negatively related with increased temperature. The 329 implications of this association and how they related to model output (Figure 7) will be 330 explained below. 331

## 332 Megalopae carapace dissolution and reduced width as responses to variable OA parameters 333 across vertical scales

Dissolution assessment on the external surface of the exocuticle and internal surface of the endocuticle of the megalopa's carapace and pereopod exoskeleton, was conducted only after confirming that sample preservation did not impact dissolution patterns, i.e. samples preserved in ethanol vs. flash frozen did not exhibit any significant difference in their dissolution features. Using a novel categorization scheme to semi-quantify dissolution features, including ridging

structures, dissolved areas around neuritic canals, and exposed calcite crystals (Figures 4, S1 and 339 S2; Table S2), the individuals demonstrated various extents of these features present on the 340 external side of the carapace and the pereopod exoskeleton (Figure S2 and S3). On the carapace, 341 the front and outer surfaces were the most affected (Figures 4 and S3). On the percopod 342 exoskeleton, the thoracic segments and chelae had the most severe dissolution, while the distant 343 parts were less affected (Figure S3). On all of the examined individuals with external dissolution, 344 we also found evidence for internal endocuticle dissolution, which was, on average, 345 approximately half that observed on the external exocuticle surface. 346

Average dissolution on the exocuticle showed the strongest linear dependence with  $\Delta\Omega_{cal,60}$ (Figure 5; R<sup>2</sup> = 0.866, p < 0.001), demonstrating that the habitats with the steepest 60 m vertical gradients results in the most damaged organisms. Because of the topographic features, there is a spatial variability related to the occurrence of the steepest  $\Delta\Omega_{cal,60}$  gradients, meaning that the lowest exoskeleton dissolution does not always correspond to the offshore gradients.

The internal dissolution showed the most robust evidence, though not statistically significant, of correlation with pCO<sub>2</sub> values (Figure 5;  $R^2 = 0.406$ , p = 0.065) and negative marginal significance with temperature ( $R^2 = 0.435$ , p = 0.053). The internal dissolution rapidly intensified beyond pCO<sub>2</sub> >500 µatm (Figure 5b), with this being a robust threshold. There was no significant correlation between internal and external dissolution (Figure 5; p = 0.18), suggesting decoupling of the two processes.

At sites with a small  $\Delta\Omega_{cal,60}$ , the external surface of the carapace was characterized by predominantly smooth surfaces, the absence of dissolution, and the presence of setae (Figures 7 and S2). Ridging features were present on all examined carapaces but significantly increased at the stations with the greatest  $\Delta\Omega_{cal,60}$  difference (Figures 4 and 5). This presence of ridging

features co-occurred with the increased occurrence of crystal exposure, ranging from increased 362 porosity (Stage 1) to exposed crystals (Stage 2) at the sites with lower  $\Delta \Omega_{cal,60}$  difference, and 363 deeper-protruding dissolution at the sites with greater  $\Delta \Omega_{cal,60}$  difference (Stage 2). Using image 364 analysis, the depth of ridging structures was estimated at approximately 2 µm, around 25% of the 365 cuticle thickness. Given the exocuticle thickness of 2-3µm, the dissolution extended into the 366 endocuticle (Figure 6). The extent of dissolution on percopod exoskeleton was comparable with 367 the external dissolution, especially at the higher dissolution values (Figure S5;  $R^2 = 0.65$ ; p =368 0.0047, slope = 0.901), indicating that both features were reliable metrics for dissolution 369 370 assessment.

There was a distinct pattern of severe dissolution specifically developed around the 371 372 calcified neuritic canals (Figure 6). In megalopae collected at inshore stations (< 200 m bottom depth), the carapace surface around the neuritic canals was markedly dissolved (Stage 2), and 373 mechanoreceptors were often absent. Dissolution around the neuritic canals appeared to alter the 374 morphology of the setae (Figure 6). Setae edges were partially collapsed at the places where the 375 376 mechanoreceptors are anchored in, with the initial ridging features around the canals degenerated into severely dissolved surfaces at the more intense  $\Delta\Omega_{cal.60}$  values (Figure 6). On the megalopae 377 from offshore stations with a smaller  $\Delta\Omega_{cal,60}$  the mechanoreceptors were present with no damage 378 around the neuritic canals and less severe dissolution. Within the region of altered setae, 379 dissolution up to 2-3 µm around the setae (Figure S4) was accompanied by significant canal 380 deformation. This deformation appears to destabilize the attachment of the setae anchor, 381 resulting in the setae 'outrooting'. In some of the calcified neuritic canals, we noted the absence 382 383 of setae but have not yet quantified the frequency of this occurrence.

To examine whether external or internal dissolution affects organismal or even 384 potentially population-level metrics, dissolution measures were compared to megalopae body 385 parameters and abundance. Here, we made an assumption that all the larval were released at the 386 same time to be able to compare different length parameters. We detected a significant negative 387 correlation between external dissolution and width, as indicated by reduced individual carapace 388 width (CW; F =18.61,  $R^2 = 0.823$ , p = 0.013 for the regression of CW against external 389 dissolution on body parts; F = 5.3,  $R^2 = 0.57$ , p = 0.08 for the regression of CW against all 390 external dissolution; Figure 5d), which is particularly strong in the coastal stations. This 391 demonstrates that external OA-related exposure can indirectly result in reduced larval width. 392 393 Carapace width was strongly oriented along the first RDA axis (Figure 3), while being orthogonal to internal dissolution and directly opposed to external dissolution. The latter aligns 394 with previous findings that internal dissolution is uncoupled from carapace width (linear model p 395 396 > 0.05), whereas external dissolution is significantly associated with carapace width (RDA plot). Other length-related parameters (CL, R-DCS, TL) were not affected by OA parameters, 397 demonstrating that only specific body parameters, i.e., width are affected at more severe  $\Delta \Omega_{cal,60}$ 398 vertical gradients. 399

On the higher, population-level response, only chl-a was found to be a significant driver. Abundance was positively correlated with chl-a at 60 m depth for both onshore and offshore habitats ( $R^2 = 0.327$ ; p = 0.008). None of the other environmental parameters had a significant impact. However, in shallow coastal habitats with depth <30 m, temperature was negatively related to larval abundance (for temperature at 10 m,  $R^2 = 0.241$ ; p = 0.02; F = 6.56), although chl-a remained the dominant driver. In addition, neither carapace dissolution nor the width was related to larval abundance, suggesting decoupling of individual- and population-level effects ofenvironmental conditions on larval Dungeness during the present day.

### 408 Megalopae exposure history to coastal OA conditions during the month prior to sampling

Particle back-tracking results with simulated vertical migration between the ocean surface and 60 m depth over a 30-d period from the J-SCOPE simulations showed that megalopae that were released in coastal habitats (<200 m), remained in coastal habitat for nearly a month of simulation regardless of their position in the domain (Figure 7). This retention results in extended exposure to steeper coastal vertical gradients in OA conditions (Figure 2), and consequently, more intense dissolution (Figure 5).

### 415 Discussion

To our knowledge this is the first time that OA-related dissolution of calcite structures in situ has 416 been demonstrated for crustaceans. Our results indicate that it is the exposure to both parameters, 417  $\Delta\Omega_{cal,60}$  (i.e. the difference in calcite saturation depth between the surface and 60 m depth) and 418 pCO<sub>2</sub>, set up by as well as prolonged (<1 month) retention in the coastal waters that characterizes 419 the suite of *in situ* parameters determining the larval crab vulnerability. This primarily 420 421 demonstrates that it is not just the mean state OA conditions, but also the vertical difference in the water column that can induce negative biological responses. Using a retrospective prediction 422 from a regression model (Figure 5a), we estimate an 8.3% increase in the extent of external 423 carapace dissolution over the last two decades. This post-hoc estimate was based on a  $\Delta pH$ 424 changes of 0.02 unit per decade (Carter et al., 2018), comparing current average with the extent 425 of dissolution predicted from our regression model based on the in situ observations (Figure 5a 426 427 with the equation in the figure content) by using the estimated pH conditions two decades prior.

428 This is a reasonable estimate since  $\Delta \Omega_{cal,60}$  is highly correlated with  $\Delta \Omega_{pH,60}$  (F = 204.3, R<sup>2</sup> = 429 0.96, p < 0.001, Figure S6).

What makes this OA-dependent dissolution of megalopae particularly relevant is that the 430 crab samples originated in the supersaturated conditions with respect to calcite (the lowest  $\Omega_{calc}$ 431 =1.41). Since the dissolution reported in other calcifiers has been demonstrated above  $\Omega_{ara}$  of 432 1.4-1.5 (Bednaršek and Ohman, 2015; Bednaršek et al., 2016), we conclude that exoskeleton 433 dissolution is initiated at higher  $\Delta \Omega_{cal.60}$  than predicted based on thermodynamic principles alone. 434 Furthermore, using exposure metrics based on the biogeochemical model output demonstrates 435 that 1-month long exposure in coastal habitats with large  $\Delta\Omega_{cal,60}$  values can result in 436 significantly more dissolution than predicted based on snap-shot observational data. In 437 438 comparison with the chemical observations, particle tracking model output indicates prolonged severity of exposure to the coastal low OA conditions, allowing for more extensive exoskeleton 439 dissolution and reduced larval width in those habitats. It is worth noting that dissolution could be 440 viewed as a physiological strategy to compensate against unfavorable external conditions. 441 442 Dissolution of the outer calcite layer could increase the release of the bicarbonate and hydroxyl ions, raising pH, and providing a rapid alkalinization of the superficial layer (Kunkel et al., 443 2012). This alkaline layer could then provide an additional local protection from exposure to a 444 large  $\Delta\Omega_{cal,60}$  conditions by blocking protons from continuously invading the internal fluid 445 However, as the larvae live in highly dynamic environments, such a layer would be continuously 446 disrupted, explaining the high extent of external dissolution. 447

### 448 Dissolution as a mechanism to offset OA-related extracellular acid-base disturbance?

Species with a developed capacity for ion exchange to maintain extracellular acid-base balance,
are able to compensate for the effects of exposure to high pCO<sub>2</sub> waters and restore extracellular

pH values optimal for physiological and biogeochemical processes (Somero, 1986). They do so via energetically expensive buffering of intra- and extracellular compartments achieved through various mechanisms, such as buffering by seawater-derived bicarbonate sources (Truchot, 1979), and increased respiratory activity to reduce CO<sub>2</sub> loading of the extracellular fluid and nonbicarbonate buffering (Cameron, 1985; Hans et al., 2014; Michaelidis et al., 2005).

However, the downside to the well-established extracellular acid-base control is an energetically 456 demanding process (Hans et al., 2014; Michaelidis et al., 2005; Pane and Barry, 2007; Trigg et 457 al., 2019). Therefore, we hypothesize that the internal carapace dissolution we observed in our 458 study could be a part of a passive ability to buffer reductions in extracellular pH, a feature found 459 460 in a variety of marine invertebrates including bivalves, echinoderms, and crustaceans (Cameron, 1985; Henry et al., 1981; Lindinger et al., 1984; Spicer and Taylor, 1987; Spicer et al., 2007). 461 The narrow neuritic canals around the mechanoreceptors allow communication through secretion 462 across the internal-external cuticle layers (Kunkel et al., 2012). While we currently have no 463 information on the acid-base balance within these larval crabs under prolonged exposure to steep 464 pCO<sub>2</sub> vertical gradients because no controlled experiments have been conducted, we propose 465 466 future studies to examine if internal dissolution could provide some level of bicarbonate ions for buffering at comparatively low cost. 467

Alternative hypothesis for explaining internal dissolution might be based on the severity of external dissolution extending much deeper (Figure S4) to initiate the endocuticle dissolution. Once the dissolution of the external carapace dissolution is initiated, the mineralogical-elemental structure of the mid- and endocuticle can allow for more rapid progression. The presence of high-Mg<sup>2+</sup> content in the endocuticle can cause more rapid dissolution (Andersson et al., 2008), while comparatively lower Ca<sup>2+</sup> content on the outward side presumably results in a weaker

carapace (Chen et al., 2008). In addition, chitin-proteinaceous matrix, such as an amorphous 474 crystalline layer might be present, which could importantly contribute to additional dissolution. 475 Furthermore, while the internal solubility extent may be compensated by phosphorus, it can 476 increase hardness, thereby preventing propagation of fractures, and Sr<sup>2+</sup> because it can replace 477  $Ca^{2+}$  in the mineralization process (Dodd, 1964). In contrast, the observation of less internal 478 dissolution on the endocuticle side compared to the external dissolution could be due to a 479 difference in biomineral composition. For instance, intermixing calcite in the endocuticle with 480 481 organic polymers would create a durable, protective covering, which may prevent the more soluble high-Mg calcite in the endocuticle from dissolving (Chen et al., 2008). However, we 482 483 have no observations of dissolution penetration all the way from the external to the internal side, we thus propose an acid-base balance strategy to be more feasible explanation for the internal 484 dissolution. 485

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### 487 Potential detrimental effect associated with carapace dissolution

One of the most important findings of this study is the correlation between carapace dissolution 488 and the reduction in larval width. This could, overtime, potentially impact population dynamics. 489 We suggest that the dissolution-length linkage could be explained by two different hypotheses: 490 first, pronounced dissolution under severe  $\Delta \Omega_{cal.60}$  vertical gradients results in dissolution rate 491 outpacing calcification rate. In this mismatch of rates of two different processes, calcification 492 493 rate cannot fully compensate for dissolution and results in overall smaller width ('the mismatch' hypothesis). Alternatively, there could be an energetic implication behind the dissolution-494 induced slowdown in width. In this form of the hypothesis, an organism expends additional 495

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energy to increase calcification to counteract dissolution, thus resulting in an energetic trade-off that potentially compromises organismal growth (the '*trade-off*' hypothesis).

Furthermore, for early Dungeness crab life stages in the near-future, the prediction of 498 more frequent and prolonged exposures to more severe  $\Delta \Omega_{cal,60}$  gradients (Turi et al., 2016) could 499 500 have potentially deleterious consequences in terms of behavioral and sensory impairments and chelae function. First, dissolution-affected thinner structures may become too weak to retain their 501 integrity, particularly under more severe conditions and continuous water flow, resulting in 502 ridged, puffed surfaces. Morphological changes may in turn negatively impact larval survival by 503 altering swimming behaviors and competence, including the ability to regulate buoyancy, 504 505 maintain vertical position, and avoid predators (Morgan, 1989; Sulkin, 1984). Similar 506 morphological structures as those observed in our study were noted in the larval form of the European lobster (Agnalt et al., 2013), which under prolonged exposure to OA conditions led to 507 irreparable carapace deformities, and these could lead to an increase in molt-related mortalities 508 (Small et al., 2016). Second, dissolution on both sides of carapace and pereopod exoskeleton will 509 510 inevitably limit the effectiveness of the exoskeleton in providing support for muscles contraction 511 and defense from predators, aiding homeostatic functions, and enabling feeding functions. Third, calcified neuritic canals appear to be one of the dissolution hotspots compromising setae 512 function. Compared with undamaged setae at undissolved surfaces (Figures 6 and S2, S3), 513 dissolved areas may not provide sufficient structural integrity for the setae (Figure 6), potentially 514 impairing their functionality. Given the role of setae as mechanoreceptors directly involved in 515 supporting crustacean sensory and behavior processes, we hypothesize that the absence or 516 517 damage of setae within their neuritic canals may in part provide a mechanistic understanding for potential aberrant behavioral patterns found across various crustacean species under low OA 518

519 conditions, such as slower movement, less tactile recognition, and prolonged searching time, as well as impaired swimming (Alenius and Munguia, 2012; Dissanayake and Ishimatsu, 2011) and 520 behavioral choice (de la Haye et al., 2011). These changes can result in impaired competitiveness 521 and altered predator-prey relationships for crabs (de la Haye et al., 2012; Dodd et al., 2015; 522 Landes and Zimmer, 2012; Wang et al., 2018). Fourth, it is currently unknown whether external 523 dissolution in megalopae could carry over into later life stages, including the reproductively 524 525 active adult stage, and what the potential consequences may be for the population dynamics. 526 However, reduced calcification could result in poor mineralization through the intermolt period that would be especially devastating for larval crabs because of potentially smaller sizes at 527 528 maturity, as well as increased vulnerability to predation during their most sensitive molting 529 stage.

While OA parameters largely affect observed biominerological and organismal 530 responses, population-level responses (i.e. abundances) are driven by food availability, with a 531 lesser role for temperature in the near-shore conditions. Although biological responses at 532 different levels of biological organization appear to be decoupled and responded to different 533 534 drivers across temporal and spatial scales that need to be taken into account to improve biological forecasts and predictions. The only driver that seem to resonate across individual and 535 population level, at least marginally, is the temperature, which might have an opposite effect on 536 both levels. While warmer temperature negatively affects abundances, it also reduces internal 537 dissolution, although the latter is only a marginally significant. 538

To more accurately predict large-scale vulnerability, it is important to consider population connectivity, related to essential population vital rates and affected by dispersal (Lowe and Allendorf, 2010). This can be partitioned into genetic connectivity and demographic

542 connectivity, with our model outputs demonstrating onshore-offshore connectivity along the shelf-coastal and in the northern-southern directions. This implies prolonged exposure to less 543 suitable habitats characterized by low  $\Delta \Omega_{cal,60}$  in the nearshore areas that can exacerbate negative 544 biological effects but some of them could be counteracted by higher food availability. With 545 respect to genetic connectivity, the status of Dungeness crab as a high gene-flow species with 546 low genetic differentiation along the US West Coast and the lack of significant adaptation 547 patterns (Jackson and O'Malley, 2017; Jackson et al., 2018; O'Malley et al., 2017) implies that 548 the genetic pool that might allow for adaptation under future climate scenario will be limited. 549 This points toward the need for more comprehensive population vulnerability assessment that 550 551 can link OA vulnerability with the population genetics.

### 552 Future Directions

Like dissolution in pteropods, larval dissolution observed in Dungeness crab is clear evidence 553 that marine invertebrates are damaged by extended exposure to strong present-day OA-related 554 vertical gradients in their natural environment. The unexplored aspect of OA impacts related to 555 the damaged mechanoreceptors and potentially impaired sensory functions needs to be explored 556 further. Namely, if the sensory functions are impaired, the transitioning from the larval to 557 juvenile stage in their coastal habitat might be compromised under predicted scenarios of steeper 558  $\Delta pH$  and  $\Delta \Omega_{cal,60}$  gradients (Gruber et al., 2012; Turi et al., 2016). Multiple pathways of larval 559 vulnerability should be studied in the context of carry-over effects to the next juvenile benthic 560 stage to explore whether crustacean molting can offset some of the detrimental effects. Such 561 findings should be integrated into a population demographic and exposure history model that 562 could eventually lead to improved management of Dungeness crab stocks (Fernandes et al., 563 564 2017; Lam et al., 2016).

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575 576	References
575 576 577	References Agnalt, AL., Grefsrud, E. S., Farestveit, M., Larsen, M., Keulder, F. (2013). Deformities in
575 576 577 578	References Agnalt, AL., Grefsrud, E. S., Farestveit, M., Larsen, M., Keulder, F. (2013). Deformities in larvae and juvenile European lobster ( <i>Homarus gammarus</i> ) exposed to lower pH at two
575 576 577 578 579	References Agnalt, AL., Grefsrud, E. S., Farestveit, M., Larsen, M., Keulder, F. (2013). Deformities in larvae and juvenile European lobster ( <i>Homarus gammarus</i> ) exposed to lower pH at two different temperatures. <i>Biogeosciences</i> , <i>10</i> , 7883–7895.
575 576 577 578 579 580	References Agnalt, AL., Grefsrud, E. S., Farestveit, M., Larsen, M., Keulder, F. (2013). Deformities in larvae and juvenile European lobster ( <i>Homarus gammarus</i> ) exposed to lower pH at two different temperatures. <i>Biogeosciences, 10</i> , 7883–7895. Akaike, H. (1973). Information theory and an extension of the maximum likelihood principle. In:
575 576 577 578 579 580 581	<ul> <li>References</li> <li>Agnalt, AL., Grefsrud, E. S., Farestveit, M., Larsen, M., Keulder, F. (2013). Deformities in larvae and juvenile European lobster (<i>Homarus gammarus</i>) exposed to lower pH at two different temperatures. <i>Biogeosciences</i>, <i>10</i>, 7883–7895.</li> <li>Akaike, H. (1973). Information theory and an extension of the maximum likelihood principle. In: B. N. Petrov &amp; F. Csaki (Eds.) Second International Symposium on Information Theory (pp.</li> </ul>

- 583 Alenius, B., Munguia, P. (2012). Effects of pH variability on the intertidal isopod, *Paradella*
- *dianae. Marine and Freshwater Behaviour and Physiology, 45*, 245–259.

585	Andersson, A. J., Mackenzie, F. T., Bates, N. R. (2008). Life on the margin: Implications of
586	ocean acidification on Mg-calcite, high latitude and cold-water marine calcifiers. Marine
587	Ecology Progress Series, 373, 265–273.

- 588 Bednaršek, N., Feely, R. A., Reum, J. C. P., Peterson, B., Menkel, J., Alin, S. R., Hales, B.
- 589 (2014). *Limacina helicina* shell dissolution as an indicator of declining habitat suitability
- owing to ocean acidification in the California Current Ecosystem. *Proceedings of the Royal Society B: Biological Sciences*, *281*, 20140123.
- 592 Bednaršek, N., Feely, R. A., Tolimieri, N., Hermann, A. J., Siedlecki, S. A., Waldbusser, G. G.,

McElhany, P., Alin, S. R., Klinger, T., Moore-Maley, B., Pörtner, H. O. (2017). Exposure

- 594 history determines pteropod vulnerability to ocean acidification along the US West Coast.
- *Scientific Reports*, 7, 4526.
- 596 Bednaršek, N., Johnson, J., Feely, R. A. (2016). Comment on Peck et al: Vulnerability of
- 597 pteropod (*Limacina helicina*) to ocean acidification: Shell dissolution occurs despite an
- 598 intact organic layer. *Deep-Sea Research Part II: Topical Studies in Oceanography*, 127, 53–
- 599 56.

- Bednaršek, N., Ohman, M. D. (2015). Changes in pteropod distributions and shell dissolution
  across a frontal system in the California Current System. *Marine Ecology Progress Series*, *523*, 93–103.
- 603 Bednaršek, N., Tarling, G. A., Bakker, D. C., Fielding, S., Cohen, A., Kuzirian, A., McCorkle,
- D., Lézé, B., Montagna, R. (2012). Description and quantification of pteropod shell
- dissolution: A sensitive bioindicator of ocean acidification. *Global Change Biology*, 18,
- 606 2378–2388.

- Busch, D. S., McElhany, P. (2016). Estimates of the direct effect of seawater pH on the survival
  rate of species groups in the California Current Ecosystem. *PLoS One*, *11*, e0160669.
- 609 Cameron, J. N. (1985). Compensation of hypercapnic acidosis in the aquatic blue crab,
- 610 *Callinectes sapidus*: the predominance of external sea water over carapace carbonate as the
- 611 proton sink. *Journal of Experimental Biology*, *114*, 197–206.
- 612 Carter, B. R., Feely, R. A., Williams, N. L., Dickson, A. G., Fong, M. B., Takeshita, Y. (2018).
- 613 Updated methods for global locally interpolated estimation of alkalinity, pH, and nitrate.
- *Limnology and Oceanography Methods, 16*(2), 119–131.
- 615 Chavez, F., Pennington, J. T., Michisaki, R. P., Blum, M., Chavez, G. M., Friederich, J., Messié,
- 616 M. (2017). Climate variability and change: Response of a coastal ocean ecosystem.
- 617 *Oceanography*, *30*, 128–145.
- Chen, P.-Y., Lin, A. Y.-M., McKittrick, J., Meyers, M. A. (2008). Structure and mechanical
  properties of crab exoskeletons. *Acta Biomaterialia*, *4*, 587–596.
- 620 Davis, S., Sylvia, G., Yochum, N., Cusack, C. (2017). Oregon Dungeness Crab Fishery
- 621 Bioeconomic Model: A Fishery Interactive Simulator Learning Tool. Version 5.7. Prepared
- by Coastal Oregon Marine Experiment Station, Oregon State University and The Research
- Group, LLC for the Oregon Dungeness Crab Commission.
- de la Haye, K. L., Spicer, J. I., Widdicombe, S., Briffa, M. (2011). Reduced sea water pH
- disrupts resource assessment and decision making in the hermit crab *Pagurus bernhardus*.
- 626 *Animal Behaviour, 82, 495–501.*

- de la Haye, K. L., Spicer, J. I., Widdicombe, S., Briffa, M. (2012). Reduced pH sea water
- disrupts chemo-responsive behaviour in an intertidal crustacean. *Journal of Experimental*
- 629 *Marine Biology and Ecology, 412,* 134–140.
- 630 Dissanayake, A., Ishimatsu, A. (2011). Synergistic effects of elevated CO<sub>2</sub> and temperature on
- 631 the metabolic scope and activity in a shallow-water coastal decapod (*Metapenaeus joyneri*;
- 632 Crustacea: Penaeidae). *ICES Journal of Marine Science*, 68, 1147–1154.
- Dodd, J. R. (1964). Environmentally controlled variation in the shell structure of a pelecypod
  species. *Journal of Paleontology*, *38*, 1065–1071.
- 635 Dodd, L. F., Grabowski, J. H., Piehler, M. F., Westfield, I., Ries, J. B. (2015). Ocean
- 636 acidification impairs crab foraging behaviour. *Proceedings of the Royal Society B:*
- 637 *Biological Sciences*, *282*, 20150333.
- 638 Fabritius, H. O., Karsten, E. S., Balasundaram, K., Hild, S., Huemer, K., Raabe, D. (2012).
- 639 Correlation of structure, composition and local mechanical properties in the dorsal carapace
- 640 of the edible crab *Cancer pagurus*. *Zeitschrift für Kristallographie*, 227(11), 766–776.
- 641 Feely, R. A., Alin, S., Carter, B., Bednaršek, N., Hales, B., Chan, F., ..., L. Juranek (2016).
- 642 Chemical and biological impacts of ocean acidification along the west coast of North
- 643 America. *Estuarine, Coastal and Shelf Science, 183*, 260–270.
- 644 Feely, R. A., Okazaki, R. R., Cai, W.-J., Bednaršek, N., Alin, S. R., Byrne, R. H., Fassbender, A.
- 645 (2018). The combined effects of acidification and hypoxia on pH and aragonite saturation in
- the coastal waters of the California current ecosystem and the northern Gulf of Mexico.
- 647 *Continental Shelf Research*, *152*, 50–60.

648	Fernandes, J. A., Papathanasopoulou, E., Hattam, C., Queirós, A. M., Cheung, W. W., Yool, A.,
649	Artioli, Y., Pope, E. C., Flynn, K. J., Merino, G., Calosi, P., Beaumont, N., Austen, M. C.,
650	Widdicombe, S., Barange, M. (2017). Estimating the ecological, economic and social
651	impacts of ocean acidification and warming on UK fisheries. Fish and Fisheries, 18, 389-
652	411.
653	Fox, J., Weisberg, S. (2011). An R Companion to Applied Regression. USA: SAGE
654	Publications.
655	Giltz, S. M., Taylor, C.M. (2017). Reduced growth and survival in the larval Blue Crab
656	Callinectes sapidus under predicted ocean acidification. Journal of Shellfish Research, 36,
657	481-485.
658	González-Gordillo, J. I., Rodríguez, A., Queiroga, H. (2004). Characterization of the megalopal
659	premoult stages of the Green crab, Carcinus Maenas (Decapoda, Portunidae), from
660	laboratory culture. Journal of Crustacean Biology, 24, 502-510.
661	Gravinese, P. M., Enochs, I. C., Manzello, D. P., van Woesik, R. (2018). Warming and pCO <sub>2</sub>
662	effects on Florida stone crab larvae. Estuarine, Coastal and Shelf Science, 204, 193–201.
663	Gruber, N., Hauri, C., Lachkar, Z., Loher, D., Frölicher, T. L., Plattner, GK. (2012). Rapid
664	progression of ocean acidification in the California Current System. Science, 337, 220-223.
665	Hans, S., Fehsenfeld, S., Treberg, J. R., Weihrauch, D. (2014). Acid-base regulation in the
666	Dungeness crab (Metacarcinus magister). Marine Biology, 161, 1179–1193.
667	Henry, R. P., Kormanik, G. A., Smatresk, N. J., Cameron, J. N. (1981). The role of CaCO <sub>3</sub>
668	dissolution as a source of $HCO_3^-$ for the buffering of hypercapnic acidosis in aquatic and
669	terrestrial decapod crustaceans. Journal of Experimental Biology, 94, 269-274.

670	Hobbs, R. C., Botsford, L. W., Thomas, A. (1992). Influence of hydrographic conditions and
671	wind forcing on the distribution and abundance of Dungeness crab, Cancer magister, larvae.
672	Canadian Journal of Fisheries and Aquatic Sciences, 49, 1379–1388.

- Hodgson, E. E., Kaplan, I. C., Marshall, K. N., Leonard, J., Essington, T. E., Busch, D. S., 673
- 674 Fulton, E. A., Harvey, C. J., Hermann, A. J., McElhany, P. (2018). Consequences of spatially
- variable ocean acidification in the California Current: Lower pH drives strongest declines in 675
- benthic species in southern regions while greatest economic impacts occur in northern 676

677 regions. Ecological Modelling, 383, 106–117.

- Jackson, T. M., O'Malley, K. G. (2017). Comparing genetic connectivity among Dungeness crab 678 679 (Cancer magister) inhabiting Puget Sound and coastal Washington. Marine Biology, 164(6), 123.
- 680
- Jackson, T. M., Roegner, G. C., O'Malley, K. G. (2018). Evidence for interannual variation in 681 genetic structure of Dungeness crab (Cancer magister) along the California Current System. 682
- *Molecular Ecology*, 27(2), 352–368. 683
- Kaplan, I. C., Williams, G. D., Bond, N. A., Hermann, A. J., Siedlecki, S. A. (2016). Cloudy 684
- with a chance of sardines: Forecasting sardine distributions using regional climate models. 685
- Fisheries Oceanography, 25, 15–27. 686
- 687 Kunkel, J. G., Nagel, W., Jercinovic, M. J. (2012). Mineral fine structure of the American lobster cuticle. Journal of Shellfish Research, 31, 515-526. 688
- Lam, V. W. Y., Cheung, W. W. L., Reygondeau, G., Sumaila, U. R. (2016). Projected change in 689 global fisheries revenues under climate change. Scientific Reports, 6, 32607. 690

- Landes, A., Zimmer, M. (2012). Acidification and warming affect both a calcifying predator and
   prey, but not their interaction. *Marine Ecology Progress Series*, 450, 1–10.
- Lindinger, M. I., Lauren, D. J., Mcdonald, D. G. (1984). Acid-base balance in the sea mussel,
- *Mytilus edulis*. III. Effects of environmental hypercapnia on intra- and extracellular acid-base
  balance. *Marine Biology Letters*, *5*, 371–381.
- Long, W. C., Swiney, K. M., Foy, R. J. (2016). Effects of high pCO<sub>2</sub> on Tanner crab
- reproduction and early life history, Part II: Carryover effects on larvae from oogenesis and
- embryogenesis are stronger than direct effects. *ICES Journal of Marine Science*, *73*(3), 836–
  848.
- Lowe, W. H., Allendorf, F. W. (2010). What can genetics tell us about population connectivity?.
   *Molecular Ecology*, *19*, 3038–3051.
- Manno, C., Rumolo, P., Barra, M., d'Albero, S., Basilone, G., Genovese, S., Mazzola, S.,
- Bonanno, A. (2019). Condition of pteropod shells near a volcanic CO<sub>2</sub> vent region. *Marine Environmental Research*, 143, 39–48.
- 705 Melzner, F., Gutowska, M. A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M. C.,
- 706 Bleich, M., Pörtner, H. O. (2009). Physiological basis for high CO<sub>2</sub> tolerance in marine
- ectothermic animals: Pre-adaptation through lifestyle and ontogeny? *Biogeosciences*, 6(3),
- 708 4693-4738.
- 709 Michaelidis, B., Ouzounis, C., Paleras, A., Pörtner, H. (2005). Effects of long-term moderate
- 710 hypercapnia on acid-base balance and growth rate in marine mussels *Mytilus*
- 711 galloprovincialis. Marine Ecology Progress Series, 293, 109–118.

- Miller, J. J., Maher, M., Bohaboy, E., Friedman, C. S., McElhany, P. (2016). Exposure to low pH
  reduces survival and delays development in early life stages of Dungeness crab (*Cancer magister*). *Marine Biology*, *163*, 118.
- Morgan, S. G. (1989). Adaptive significance of spination in estuarine crab Zoeae. *Ecology*, 70,
  464–482. https://doi.org/10.2307/1937551
- 717 North, E. W., Schlag, Z., Hood, R. R., Li, M., Zhong, L., Gross, T., Kennedy, V. S. (2008).
- 718 Vertical swimming behavior influences the dispersal of simulated oyster larvae in a coupled
- 719 particle-tracking and hydrodynamic model of Chesapeake Bay. *Marine Ecology Progress*
- 720 Series, 359, 99–115.
- 721 North, E. W., Adams, E. E., Schlag, Z., Sherwood, C. R., He, R., Hyun, K. H., Socolofsky, S. A.
- 722 (2011). Simulating oil droplet dispersal from the *Deepwater Horizon* spill with a Lagrangian
- approach. In Y. Liu, A. Macfadyen, Z. Ji, & R. H. Weisberg (Eds.), Monitoring and
- 724 Modeling the Deepwater Horizon Oil Spill: A Record Breaking Enterprise. *Geophysical*

*Monograph Series* (pp. 217–226). USA: American Geophysical Union.

- 726 Norton, E., Siedlecki, S.A., Kaplan, I.C., Hermann, A.J., Fisher, J., Morgan, C., Officer, S.,
- 727 Saenger, C., Alin, S.A., Newton, J.A., Bednaršek, N., and Feely, R.A. (*in revision*). The
- importance of environmental exposure history in forecasting Dungeness crab megalopae
- occurrence using J-SCOPE, a high-resolution model for the US Pacific Northwest. *Frontiers*
- *in Marine Science*
- 731 Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Wagner, H.
- 732 (2019). Vegan: Community Ecology Package. R package version 2.5-5. Available at
- 733 https://CRAN.R-project.org/package=vegan.

734	O'Malley, K. G., Corbett, K., Beacham, T. D., Jacobson, D. P., Jackson, T. M., Roegner, G. C.
735	(2017). Genetic connectivity of the Dungeness crab (Cancer magister) across oceanographic
736	regimes. Journal of Shellfish Research, 36(2), 453-465.
737	Pacific States Marine Fisheries Commission (2019). Species Report: Commercial Land Catch:
738	Metric-Tons (mt), Revenue, and Price-per-pound (Price/lbs). Portland, OR: Pacific States
739	Marine Fisheries Commission. Available at
740	https://reports.psmfc.org/pacfin/f?p=501:1:5808950816361::NO::: Accessed 3/25/2019.

741 Paganini, A. W., Miller, N. A, Stillman, J. H. (2014). Temperature and acidification variability

reduce physiological performance in the intertidal zone porcelain crab *Petrolisthes cinctipes*.

743 *Journal of Experimental Biology, 217*(22), 3974–3980.

Page, T. M., Worthington, S., Calosi, P., Stillman, J. H. (2016). Effects of elevated pCO<sub>2</sub> on crab

survival and exoskeleton composition depend on shell function and species distribution: A

comparative analysis of carapace and claw mineralogy across four porcelain crab species

from different habitats. *ICES Journal of Marine Science*, *74*(4), 1021–1032.

Pane, E., Barry, J. (2007). Extracellular acid-base regulation during short-term hypercapnia is

effective in a shallow-water crab, but ineffective in a deep-sea crab. *Marine Ecology* 

750 *Progress Series, 334*, 1–9.

R Core Team (2019). R: A Language and Environment for Statistical Computing. R Foundation
 for Statistical Computing. Available at https://www.R-project.org/.

- 753 Schiffer, M., Harms, L., Pörtner, H., Mark, F., Storch, D. (2014). Pre-hatching seawater pCO<sub>2</sub>
- affects development and survival of zoea stages of Arctic spider crab *Hyas araneus*. *Marine*
- *Ecology Progress Series*, 501, 127–139.

756	Schlag, Z. R., North, E. W. (2012). Lagrangian TRANSport model (LTRANS v.2) User's Guide.
757	Cambridge, Maryland: University of Maryland Center for Environmental Science, Horn
758	Point Laboratory.
759	Shanks, A. L. (1995). Mechanisms of cross-shelf dispersal of larval invertebrates and fish. In: L.
760	R. McEdward (Ed.), Ecology of Marine Invertebrate Larvae (pp. 324–367). USA: CRC
761	Press.
762	Siedlecki, S. A., Kaplan, I. C., Hermann, A. J., Nguyen, T. T., Bond, N. A., Newton, J. A.,
763	Williams, G. D., Peterson, W. T., Alin, S. R., Feely, R. A. (2016). Experiments with seasonal
764	forecasts of ocean conditions for the northern region of the California Current upwelling

system. *Scientific Reports*, *6*, 27203.

- Sinclair, M. (1988). Marine Populations: An Essay on Population Regulation and Speciation.
  Washington Sea Grant. Seattle, Washington: University of Washington Press.
- 768 Small, D. P., Calosi, P., Boothroyd, D., Widdicombe, S., Spicer, J. I. (2015). Stage-specific
- changes in physiological and life-history responses to elevated temperature and pCO<sub>2</sub> during
- the larval development of the European lobster *Homarus gammarus* (L.). *Physiological and*
- 771 *Biochemical Zoology*, *88*, 494–507.
- Small, D. P., Calosi, P., Boothroyd, D., Widdicombe, S., Spicer, J. I. (2016). The sensitivity of
- the early benthic juvenile stage of the European lobster *Homarus gammarus* (L.) to elevated
  pCO<sub>2</sub> and temperature. *Marine Biology*, *163*, 53.
- Somero, G. N. (1986). Protons, osmolytes, and fitness of internal milieu for protein function.
- American Journal of Physiology–Regulatory, Integrative and Comparative Physiology, 251,
- 777 R197–R213.

778	Somero, G. N., Beers, J. M., Chan, F., Hill, T. M., Klinger, T., Litvin, S. Y. (2015). What
779	changes in the carbonate system, oxygen, and temperature portend for the northeastern
780	Pacific Ocean: A physiological perspective. <i>BioScience</i> , 66, 14–26.
781	Spicer, J. I., Taylor, A. C. (1987). Carbon dioxide transport and acid-base regulation in the blood
782	of the beach-hopper Orchestia gammarellus (Pallas) (Crustacea: Amphipoda). Ophelia, 28,
783	49–61.
784	Spicer, J. I., Raffo, A. (2007). Widdicombe, S., Influence of CO <sub>2</sub> -related seawater acidification
785	on extracellular acid-base balance in the velvet swimming crab Necora puber. Marine
786	<i>Biology, 151</i> , 1117–1125.
787	Sulkin, S. D. (1984). Behavioral basis of depth regulation in the larvae of brachyuran crabs.
788	Marine Ecology Progress Series, 15, 181–205.
789	Trigg, S. A., McElhany, P., Maher, M., Perez, D., Busch, D. S., Nichols, K. M. (1 August 2019).
790	Uncovering mechanisms of global ocean change effects on the Dungeness crab (Cancer

- magister) through metabolomics analysis. BioRxiv, 574798. 791
- 792 Truchot, J. P. (1979). Mechanisms of the compensation of blood respiratory acid-base
- 793 disturbances in the shore crab, Carcinus maenas (L.). Journal of Experimental Zoology, 210, 407-416. 794
- Tunnicliffe, V., Davies, K. T., Butterfield, D. A., Embley, R. W., Rose, J. M., Chadwick Jr, W. 795
- 796 W. (2009). Survival of mussels in extremely acidic waters on a submarine volcano. Nature Geoscience, 2, 344–348. 797

798	Turi, G., Lachkar, Z., Gruber, N., Münnich, M. (2016). Climatic modulation of recent trends in
799	ocean acidification in the California Current System. Environmental Research Letters, 11,
800	014007.
801	Walther, K., Anger, K., Pörtner, H. (2010). Effects of ocean acidification and warming on the
802	larval development of the spider crab Hyas araneus from different latitudes (54° vs. 79°N).
803	Marine Ecology Progress Series, 417, 159–170.
804	Wang, Y., Hu, M., Wu, F., Storch, D., Pörtner, HO. (2018). Elevated pCO <sub>2</sub> affects feeding
805	behavior and acute physiological response of the Brown Crab Cancer pagurus. Frontiers in
806	Physiology, 9,1164.
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814	Figures
815	Figure 1: The cross sections of the Dungeness crab megalopae. Left panel (a): cross section of
816	carapace (with increasing numbers describing the transition from the thinner exo- (1) to thicker
817	endo-cuticle (6). Right panel (b): distribution of various elements (C-carbon-a; N-nitrogen-b;

818  $Sr^{2+}$ - strontium-c;  $Mg^{2+}$ - magnesium-d; P-phosphor-e; S-sulphur-f; K-potassium-g,  $Ca^{2+}$ -

819 calcium-h). The more intense colors depict higher elemental concentration. Spectrum 820 and % content of selected elements in either carapace or percopod exoskeleton (*a*). The numbers in (*a*) coincide with the numbers in (*b*) that indicate the position within the carapace.

**Figure 2:** Interpolated pCO<sub>2,60</sub> (*a*),  $\Delta\Omega_{cal,60}$  (*b*) and chlorophyll (*c*) conditions in the onshore and offshore habitats along the US West Coast in June 2016. pCO<sub>2</sub> reflects the conditions at 60m depth and  $\Delta\Omega_{cal,60}$  indicates the difference between the surface and 60 m depth. *c*) Chlorophyll distribution and concentration (chl-a;  $\mu g/L$ ) demonstrate an order of magnitude difference between the regional nearshore and offshore region. The numbers indicate the stations at which the crabs were collected.

Figure 3: Correlation matrix of environmental variables with biological endpoints for Dungeness
megalopae: (a) Darker green values are strong positive correlations and darker purple values are
strong negative correlations, while dimmer green and purple indicate weaker correlations; and b)
Redundancy analyses (RDAs) for environmental variables used in the analyses with crab
biological measurements (internal and external dissolution, carapace width).

Figure 4: External carapace and percopod exoskeleton of the Dungeness crab megalopae (*a*) in its undamaged form (*b*, *c*) and with dissolution presence ranging from mild (Stage 1; *d*) to severe (Stage 2; *e*, *f*) patterns showing similarity in the structural damages (*g*) or exposed crystals (*h*). Indicated is the scale of the measurements ( $\mu$ m). The undamaged megalopae originated from the offshore or northwards habitats characterized by low  $\Delta\Omega_{cal,60}$  vertical gradients, while the most severely affected megalopae came from the nearshore or coastal habitats with steep  $\Delta\Omega_{cal,60}$ conditions. See more detailed explanation of the exoskeleton dissolution in the Supplementary

840 Figures S2, S3.

841 Figure 5: Estimated linear relationships between ocean-acidification conditions and dissolution. Specifically for a)  $\Delta\Omega_{cal 60}$  and relative external dissolution (R<sup>2</sup> = 0.87; p < 0.001) with the 842 equation of relative external dissolution =  $0.181 \times \Delta \Omega_{cal.60} + 0.215$ ; b) Depth-integrated pCO<sub>2.60</sub> 843 and relative internal dissolution ( $R^2 = 0.41$ ; p = 0.064); c) Comparison of the relative external 844 and internal dissolution ( $R^2 = 0.24$ ; p = 0.18); and d) Relative external dissolution and carapace 845 width ( $R^2 = 0.57$ ; p = 0.08). Dotted lines show the linear regression fit between all points. The 846 solid line in (c) is the 1:1 line and the green line in (d) is the regression fit only through the 847 onshore points. See methods for explanation of the term relative dissolution. Carapace width is in 848 mm. 849

Figure 6: Presence of setae on the percopods (*a*) and carapace surface (*b*) of the megalopae on the intact individuals. The exposure to greater  $\Delta \Omega_{cal,60}$  differences mechanically damages the setae and results in their absence and outrooting (black squares) because of the dissolution around the neuritic canals (*d*, *f*) and damage with the collapsed structure (*e*).

Figure 7: Particle initialization locations (a) and average backtracked locations (b-e) for 7, 14, 854 21, and 30-day simulated particles exhibiting diel vertical migration (DVM) between 0 and 60 m 855 depths. Replicate particles (n=100) were initialized in the model at 51 locations representing the 856 sampling stations for the 2016 West Coast Ocean Acidification Cruise. J-SCOPE's historical 857 858 simulation of ocean conditions for 2016 was used to simulate advection of particles, and each 859 particle exhibited vertical swimming between the ocean surface at night and a maximum daytime depth of 60 m. On a weekly basis, particle locations were averaged for all 100 particles 860 initialized at the same station, which sometimes resulted in the average location being on land. 861 These particles were moved to the nearest shoreline. Station color varies by transect for 862

863 improved resolution of dispersal patterns occurring at different latitudes. The 200 m isobath is864 shown for reference, and land is shaded in grey.

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Figure 2 Click here to down bad Figure







Figure 4 Click here to download high resolution image





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