PHYSIOLOGICAL RESPONSES OF SCALLOPS AND MUSSELS TO

Lindsay Alma*[1,2], Courtney J. Fiamengo[1], Simone R. Alin[3], Molly Jackson[1,4], Kris Hiromoto[1], Jacqueline L. Padilla-Gamiño[1]

[1] School of Aquatic and Fishery Sciences, University of Washington, 1122 NE Boat St., Seattle, WA 98195, USA, [2] Bodega Marine Laboratory, College of Biological Sciences, University of California, Davis, 2099 Westshore Rd., Bodega Bay, CA, 94923, USA [3] National Oceanic & Atmospheric Administration/Pacific Marine Environmental Laboratory (NOAA/PMEL), 7600 Sand Point Way NE, Seattle, WA 98115, USA, [4] Taylor Shellfish Hatchery,

Corresponding authors: L. Alma, lalma@ucdavis.edu

Abstract

Puget Sound (Washington, USA) is a large estuary, known for its profitable shellfish aquaculture industry. However, in the past decade, scientists have observed strong acidification, hypoxia, and temperature anomalies in Puget Sound. These co-occurring environmental stressors are a threat to marine ecosystems and shellfish aquaculture. Our research assesses how environmental variability in Puget Sound impacts two ecologically and economically important bivalves, the purple-hinge rock scallop (*Crassodoma gigantea*) and Mediterranean mussel (*Mytilus galloprovincialis*). Our study examines the effect of depth and seasonality on the physiology of these two important bivalves to gain insight into ideal grow-out conditions in an aquaculture setting, improving the yield and quality of this sustainable protein source. To do this, we used Hood Canal (located in Puget Sound) as a natural multiple-stressor laboratory, which allowed us to study acclimatization capacity of shellfish in their natural habitat and provide the aquaculture industry information about differences in growth rate, shell strength, and nutritional sources across depths and seasons.

Bivalves were outplanted at two depths (5 and 30 m) and collected after 3.5 and 7.5 months. To maximize mussel and scallop growth potential in an aquaculture setting, our results suggest outplanting at 5 m depth, with more favorable oxygen and pH levels. Mussel shell integrity can be improved by placing out at 5 m, regardless of season, however, there were no notable differences in shell strength between depths in scallops. For both species, $\delta^{13}C$ values were lowest at 5 m in the winter and $\delta^{15}N$ was highest at 30 m regardless of season. Puget Sound's combination of naturally and anthropogenically acidified conditions is already proving to be a challenge for shellfish farmers. Our study provides crucial information to farmers to optimize aquaculture grow-out as we begin to

Keywords: bivalve, acclimatization, temperature, oxygen, ocean acidification, aquaculture

1. Introduction

Coastlines are ideal habitats for most bivalves, where water is shallow, primary production is high, and there is substrate to settle onto (Borges and Gypens, 2010). The natural complexity and variability of coastal systems has allowed bivalves to evolve a wide tolerance to changing environmental conditions over millennia. However, anthropogenic disturbances at global to watershed scales across the last two centuries have caused dramatic changes in temperature, fluctuations in the thermocline, shoaling of the aragonite saturation horizon, and reduced dissolved oxygen (DO) with depth in marginal seas (Feely et al., 2012). Rising atmospheric CO_2 concentration is predicted to result in warmer ocean temperatures, hypoxia, ocean acidification (OA), and extreme weather (Gruber et al., 2012; Melzner et al., 2011; Moritsch et al., 2022; Rykaczewski and Dunne, 2010). OA is the process in which increased levels of atmospheric CO_2 dissolve into the ocean resulting in a more acidic environment (Jiang et al., 2023). This reduction

alters carbonate chemistry making it difficult for calcifiers to build their shells and results in the dissolution of existing shell (Ekstrom et al., 2015; Melzner et al., 2020). Hypoxia, or lower dissolved oxygen levels, often increase with depth and stratification, and can also be the result of increased nutrient loads from runoff and upwelling, resulting in extreme algal blooms called eutrophication (Gobler and Baumann, 2016). Local anthropogenic nutrient load from agriculture, sewage, runoff, and other human activities have increased eutrophication, which exacerbates hypoxia and OA in coastal areas (Borges and Gypens, 2010; Wallace et al., 2014).

In Washington state, USA, the shellfish aquaculture industry is an vital economic driver with an estimated annual income of $270 million (Barton et al., 2015). Puget Sound, Washington, is the second largest estuary in the USA and home to numerous shellfish farms. Organisms living in the Puget Sound have experienced acidified conditions, temperature anomalies, and hypoxia levels that exceed levels predicted by the Intergovernmental Panel on Climate Change (IPCC, RCP 8.5) global climate models for the end of the century over the past decade (Alin et al., 2023; IPCC, 2014; Wallace et al., 2014). Hood Canal, a large fjord-like channel on the west side of Puget Sound, has recorded some of the most extreme oceanographic conditions in the Pacific Northwest (Alin et al., 2021; Feely et al., 2010). Our study was conducted in Hood Canal, just offshore of the Taylor Shellfish Hatchery (Figure 1), a large commercial bivalve aquaculture farm. In Hood Canal, environmental variability is influenced by seasonal upwelling, snowmelt, riverine freshwater inputs, anthropogenic activity, and relatively high water residence time. During colder months, the water is well mixed, and the pycnocline is weakly defined (Feely et al., 2010). In contrast, during warm months of the year the water column is characterized by a defined pycnocline, warm upper layer, and cold, hypoxic, acidified bottom-waters. It is predicted that global warming will

strengthen and lengthen stratification in Puget Sound and may affect bivalve populations (Moore et al., 2015). We chose Hood Canal to conduct our study because Taylor Shellfish Farm has experienced challenges with bivalve production and survival since 2007 due to ocean acidification (Barton et al., 2015). Currently the hatchery buffers incoming hatchery seawater to raise carbonate ion availability for shellfish larvae and combat larval mortality due to acidification of the surrounding waters (Barton et al., 2015; Hoegh-Guldberg et al., 2015). By studying acclimatization (or the change in an organism's physiology based upon changes in the environment) of shellfish at this commercial hatchery, we provide important information that can assist with the optimization of shellfish aquaculture in the face of rapid ocean and climate change. In our study, we examined shellfish acclimatization potential by looking at the effects of environmental variability on the physiological performance of the purple-hinge rock scallop (*Crassodoma gigantea*) and the Mediterranean mussel (*Mytilus galloprovincialis*). These species are considered ecologically important because of their ability to filter water, sequester nitrogen and carbon, and their shells form reefs and provide hard surfaces for other organisms to settle, thus increasing biodiversity (Gutiérrez et al., 2003). *C. gigantea* is a native species to the North American Pacific Coast, and the aquaculture industry has great interest in the potential commercial profitability of this species (Culver et al., 2006; Leighton and Phleger, 1977; Walker, 2016). Its large edible adductor muscle is considered a delicacy that is expected to sell at a high market value. Although there is much interest in this species in the aquaculture industry, research on this species is very sparse when compared to many other bivalves, and we are only beginning to understand its responses to oceanographic stressors (Alma et al., 2020; Jackson, 2021). *M. galloprovincialis* is an edible mussel which is extensively cultured in the aquaculture industry and is well-studied due to its ecological and economic importance worldwide. This species of mussel is native to the

104 Mediterranean Sea and Atlantic Ocean but was introduced to Puget Sound in the early 20th century
105 by the aquaculture industry.
106
107 We seek to explore an important question: what is the acclimatization potential of bivalves when
108 subjected to dynamic environmental conditions? Our study provides a snapshot of potential
109 product quality in a long-line aquaculture setting, which may assist the aquaculture industry in
110 optimally placing their shellfish for grow-out. The aquaculture industry has the ability to harness
111 the scope for acclimatization in bivalves and select for favorable characteristics by modulating *in*
112 *situ* grow-out conditions through space and time. They may be able to take advantage of this
113 plasticity to continue producing optimal product as conditions change into the future. Maximizing
114 growth and shell integrity is important to aquaculture because it can optimize profit and
115 marketability.
116
117 By holding the two ecologically and economically important species at either 5 m or 30 m depths
118 for 3.5 or 7.5 months in the inland fjord of Hood Canal, we used this dynamic "natural laboratory"
119 with multiple co-occurring climate change-related stressors. Our experiment spanned December
120 to June at two depths, allowing us to capture both seasonal mixing patterns (well-mixed and
121 stratified). While the quantity of climate change-related multiple stressor experiments has been
122 increasing in the literature, many experiments are performed in the laboratory within carefully
123 controlled conditions that fail to effectively represent the complexity of real-world scenarios where
124 multiple stressors interact and fluctuate (Hofmann et al., 2011; McElhany, 2017; Reum et al.,
125 2014). It is, therefore, critical to study physiological performance in the naturally variable
126 environment, where numerous parameters (e.g. temperature, pH, salinity, dissolved oxygen)

127 fluctuate simultaneously and interact with each other at various spatiotemporal scales to affect
128 organismal performance (Wernberg et al., 2012). To assess bivalve field acclimatization potential,
129 we measured physiological metrics including growth rate, shell strength, and isotopic composition,
130 and we associated their responses to estuarine conditions experienced during their deployment.
131 We hypothesized larger differences in physiological response metrics between depths in spring,
132 when there is less vertical mixing, as opposed to the well-mixed water column in the winter.
133 Climate change-related parameters like OA, hypoxia, and warming temperatures are expected to
134 vary across depths and seasons and affect the growth rate, shell strength, and stable isotope profiles
135 of mussels and scallops. We hypothesize that both growth rate and shell strength will be greater at
136 the 5 m depth in spring due to higher temperatures and more favorable oxygen and carbonate
137 chemistry conditions. Isotopic signatures can reflect subtle changes in physiology based on the
138 environment and can thus provide important information regarding bivalve grow-out placement.
139 We expect to see higher $\delta^{15}N$ and $\delta^{13}C$ at the deeper depths where the nutrient supply is rich, and
140 terrestrial food sources are not as prominent (Fry, 2006). Understanding how *in situ* environmental
141 variability affects bivalve skeletal properties, growth rate, physiological performance, and changes
142 in biochemistry is vital to accurately predicting the acclimatization potential of these economically
143 and ecologically important species.
144

145 **2. Methods**

146 *2.1. Field Conditions*

147 Our study site was located just offshore of Taylor Shellfish Farms in Hood Canal, Puget Sound,
148 Washington (47.820°, -122.833°, Figure 1). *M. galloprovincialis* is often grown on long-lines,
149 which usually span 20–100 m depth and can experience a large breadth of oceanographic

conditions, leading to differences in aquaculture product quality along the long-line with depth (Araújo et al., 2020; Aure et al., 2007; Smart, 2019). Furthermore, the depth range in our study is consistent with the purple hinge rock scallop habitat, which spans the low-intertidal to 80 m (Bourne, 1987; Whyte et al., 1990).

Chlorophyll-*a* (chl-*a*) data, used to quantify phytoplankton biomass, was not directly measured at our field site, but we used fluorometer data measured *in situ* by the buoy located 2.3 km from our study site. The ORCA buoy was not functioning during our study period, so we averaged by day of year all available chl-*a* data from 2010 to 2021 at 5 and 30 m depth to approximate chl-*a* levels in the area.

2.2. Seawater Chemistry

Our two depths were chosen because they correspond to the shallow (5 m) and deep-water (30 m) intake pipes at Taylor Shellfish Hatchery. At these depths, we were able to deploy cages and measure environmental variables simultaneously. Water from the two depths was brought up to a fixed shore platform, and weekly oceanographic data were recorded throughout the experiment (temperature, salinity, and dissolved oxygen). Discrete water samples from each depth were collected from the intake lines weekly and preserved with mercuric chloride for carbonate chemistry analysis in accordance with ocean carbon community standard operating procedures (Dickson et al., 2007). Carbonate chemistry bottle samples were processed at NOAA's Pacific Marine Environmental Laboratory (PMEL), Seattle, Washington. Dissolved inorganic carbon (DIC) concentrations were measured on analytical systems consisting of a coulometer (UIC, Inc.) coupled with a Single Operator Multiparameter Metabolic Analyzer (SOMMA) developed to

extract DIC from seawater. Total alkalinity (TA) samples were analyzed according to the open-cell titration standard operating procedure (SOP 3b in Dickson et al., 2007), using a custom analytical system built at Scripps Institution of Oceanography (SIO). DIC instruments were calibrated via gas loops. Instrument accuracy and precision for DIC and TA analyses were monitored at regular intervals using Certified Reference Materials (CRMs), consisting of filtered and UV-irradiated seawater supplied by the Dickson Lab (SIO). Uncertainty for DIC and TA measurements is ± 0.1% of measured values (roughly ±2 µmol/kg). More complete description and references on DIC and TA analytical methods can be found in the metadata for (Alin et al., 2021). Using DIC, TA, temperature, and salinity data, we calculated the saturation state of the aragonite form of calcium carbonate (Ωara), partial pressures of CO_2 (pCO_2), and pH_T (pH on the total scale) values using the CO_2SYS program (Pelletier et al., 2007) with Lueker et al. (2000) dissociation constants.

2.3. Field Experiment and Growth

We obtained eight-month-old, purple-hinge rock scallops (*C. gigantea*) and one-year old Mediterranean mussels (*M. galloprovincialis*) from Taylor Shellfish Farm. Scallops were bred in the hatchery from wild broodstock. The non-native mussels are from an aquaculture hatchery line, bred using ~1000 individuals from existing Taylor Shellfish farmed populations. We measured shell height (from hinge to apex) using a caliper (0.1 mm precision) and tagged individuals by adhering numbered "bee tags" (Betterbee, Greenwich, New York) to their shells with superglue (Pacer Technology Zap-A-Gap Adhesives). At the beginning of the experiment, December 10, 2016 (T_0), we dissected n = 10 individuals from each species, flash froze their tissue, and placed them in a -80 °C freezer for storage. All individuals were measured before the start of the field

196 experiment to define starting size. For mussels (n = 54), average shell length ± S.E. was 49.7 ±
197 0.40 mm, shell width was 27.40 ± 0.22 mm, and weight was 9.80 ± 0.31 g. For scallops (n = 46),
198 average shell length was 40.85 ± 0.34 mm, shell width was 40.05 ± 0.36 mm, and weight was 9.41
199 ± 0.24 g. For the field experiment, n = 300 shellfish were placed into mesh oyster bags made of
200 semirigid HDPE plastic and secured with zip ties to form a bag ~ 0.4 m^3. On December 11, 2016,
201 shellfish (n = 600 shellfish per species) were deployed to our study sites at 5 and 30 m below the
202 surface using SCUBA. We collected subsets of scallops and mussels 3.5 and 7.5 months after
203 deployment (March 22, 2017, and June 27, 2017, respectively). We quantified growth rate by
204 measuring the shell height of individuals, subtracting the initial shell height, and dividing by time
205 deployed (Gobler et al., 2017; Hiebenthal et al., 2012; Kim et al., 2013; Riascos and Guzman,
206 2010). We cleaned all remaining tissue off shells with terrycloth and stored them at room
207 temperature for future shell strength analysis. We then flash froze tissue samples and stored them
208 at -80 °C for further analysis.
209
210 *2.4. Shell Strength*
211 We measured shell thickness and point-crushed the shell with a hydraulic press to quantify the
212 force it took to puncture the shell. Dry shells (n = 50 for mussels, n = 46 for scallops) were
213 rehydrated in seawater for 24 hours prior to crushing. We used a micro-caliper to measure shell
214 thickness to the nearest 0.01 mm. An Instron Universal Testing Machine measured the force (in
215 newtons, N) needed to create a hole in the shell (Wilkie and Bishop, 2012). We punctured two
216 holes into the shell (one at 1 cm from the edge of the shell and the other in the middle of the shell)
217 at 30 mm/min using a steel pin with a diameter of 2.5 mm. We averaged the puncture forces for
218 each individual shell and calculated *S*, which is shell strength expressed in megapascals (N mm^{-2}).

219 *S* can be calculated by normalizing *F* (the maximum penetrating force, in N), by *t* (shell thickness,
220 in mm), and *d* (diameter of the punch, in mm) (Carnarius et al., 1996; Ikejima et al., 2003; Tyler,
221 1961).
222
223 *2.6. Isotopic Signatures*
224 We freeze-dried (VirTis Co.) the visceral mass of scallops and mussels and homogenized them
225 using a ball-mill (Wig-L-Bug Model MSD). To measure $\delta^{13}C$ and $\delta^{15}N$ stable isotope values, we
226 weighed 600 ± 10 µg of freeze-dried and ground shellfish tissue (n = 10 per cohort) using a
227 microbalance (sensitivity 10 µg) and packed it into a small tin. Glutamic Acid I, II (0.42 µg) and
228 Bristol Bay salmon (0.339 µg) standards of known isotopic composition were packed into tins and
229 interspersed with our samples. Samples were processed at University of Washington's IsoLab on
230 a Finnigan MAT253 mass spectrometer connected to a Costech elemental analyzer in continuous-
231 flow mode (https://isolab.ess.washington.edu/laboratory/solid-CN.php) in accordance with
232 methods highlighted in Fry et al. (1992). $\delta^{13}C$ and $\delta^{15}N$ results are reported as parts per thousand
233 relative to the reference standard Vienna PeeDee Belemnite (VPDB) and atmospheric air,
234 respectively.
235
236 *2.7. Statistical Analysis*
237 Prior to analysis, a Shapiro-Wilks Test was performed to assess normality, and if needed, values
238 of analysis were $\log_{10}$, arcsine, or square root transformed to achieve normality assumptions before
239 a model was run. We ran ANOVA and Tukey-HSD tests in *R* version 1.0.53 to determine
240 significant differences in growth, shell strength, and isotopic signatures among collection times,
241 depths, and species. A correlation matrix was created to determine co-varying oceanographic

242 conditions using the *corrplot* package in *R* (Taiyun, 2014). Data for this project can be accessed at
243 https://doi.org/10.6084/m9.figshare.21809631.v1 (Alma, 2023).
244

245 **3. Results**

246 *3.1. Field Conditions and Seawater Chemistry*

247 Hood Canal seawater chemical and physical properties varied greatly by depth and season (Table
248 1). Throughout the winter (December 11, 2016, to March 22, 2017), water at 30 m depth was ~
249 1.5 °C warmer than water at 5 m depth; however, in the spring (March 23 to June 27, 2017), water
250 at the shallow depth was ~ 3.8 °C warmer (Figure 2A, Table 1) than at 30 m. We found higher
251 variability in temperature at 5 m than 30 m (Figure S1). Water temperature at 5 m fluctuated
252 between 6.8 and 18.8 °C throughout the seven-month study, while temperatures at 30 m remained
253 more consistent at 7.8–11.3 °C. The minimum and maximum temperatures recorded in this study,
254 6.8 and 18.8 °C, were obtained at the shallow depth in January and June, respectively (Figure 2A).
255 Salinity was lower and more variable at 5 m depth (22.7–29.4) than at 30 m (28.3–30.3) (Figure
256 2B, Figure S1). Salinity fluctuations at both depths were more prominent in winter, likely a
257 reflection of storm mixing or runoff events. Dissolved oxygen levels were higher at 5 m than 30
258 m throughout the experiment; however, we observed more stable and consistently low dissolved
259 oxygen readings at 30 m during the spring due to increased water column stratification preventing
260 vertical mixing (Figure 2C, Figure S1, Figure S2). Chl-*a*, data collected from the Dabob Bay
261 ORCA monitoring buoy over the last ten years was used as a proxy for phytoplankton biomass
262 (Figure S3). There are several years of data missing, and in some instances, there were no data or
263 only one year worth of data for a particular day of the year, namely in December and January. The
264 spike in chl-*a* at 5 m in January may be an artifact of a single year of data and may not be

representative of the annual patterns. Overall, 30 m often has lower chlorophyll than 5 m, and most spikes (representing potential algal blooms) were seen in April and May. pCO_2 levels were consistently higher at the 30 m depth, especially during the spring when levels reached 3738 µatm (pH_T = 7.09, Ωara = 0.20) at 30 m and 1002 µatm (pH_T = 7.62, Ωara = 0.70) at 5 m on May 30, 2017. pCO_2 levels were lower at 5 m than 30 m during both seasons (Figure 2D, Figure S1). Both pH_T and aragonite saturation state remained higher throughout the experiment at 5 m depth than 30 m (Figure 2E and 2F, Table 1). pH_T at the 5 m depth ranged between 7.62 to 8.42, and at 30 m depth pH_T ranged between 7.09 to 7.81. Ωara ranged between 0.7 and 2.7 at 5 m and between 0.2 and 0.7 at 30 m.

3.2. Growth

Growth rates based on shell height were 129% and 125% higher across seasons at 5 m than that at 30 m depth in mussel and scallops, respectively ($F_{2,213}$ = 316.3, $F_{2,182}$ = 231, $p < 0.001$, $p < 0.001$, Figure 4A and 4B). Mussels and scallops at 5 m depth showed higher growth rates in the spring compared to the winter ($p = 0.022$, $p = 0.017$, Figure 4A and 4B) and no seasonal differences in growth rates were observed at 30 m depth ($p = 0.99$, $p = 0.35$). Growth rates in mussels were 124% and 132% greater at the 5 m depth than at the 30 m depth in winter and spring. Growth rates in scallops were 136% and 115% greater at 5 m depth than at the 30 m depth in winter and spring.

3.3. Shell Strength

Shell strength differed based on seasons and depth for mussels (season – $F_{1,247}$ = 152.49, $p < 0.001$, depth – $F_{1,247}$ = 52.34, Figure 4C). Mussel shells acclimatized to 5 m depth were 40% and 22% stronger than deep-acclimatized mussels in the winter and spring, respectively ($p < 0.001$, $p <$

0.001, Figure 4C). In scallops, shell strength differed between seasons but not depths (season – $F_{1,231} = 6.08$, $p = 0.002$, depth – $F_{1,231} = 2.31$, $p = 0.13$, Figure 4D). Shell strength in scallops only differed between two cohorts, i.e., organisms collected at 30 m in the spring were 37% stronger than those collected from 5 m in the winter ($p = 0.013$, Figure 4D).

3.5. Isotopic Signatures

Isotopic signatures of mussels changed with both season and depth for $\delta^{13}C$, but only with depth for $\delta^{15}N$ ($\delta^{13}C$ season – $F_{2,45} = 128.1$, $p < 0.001$, depth – $F_{1,45} = 407.8$, $p < 0.001$; $\delta^{15}N$ season – $F_{2,45} = 1.17$, $p = 0.2$, depth – $F_{1,45} = 241.9$, $p < 0.001$, Figure 5A, Table S1, Figure S4). Mussels at 5 m in spring had higher $\delta^{13}C$ values than at 5 m in the winter. Overall higher $\delta^{13}C$ values were found at 30 m than 5 m. $\delta^{15}N$ values in mussels differed between depths; higher $\delta^{15}N$ values were found at 30 m than 5 m (both seasons, Figure S4). At 5 m, $\delta^{15}N$ values were higher in the spring than the winter; however, at 30 m, $\delta^{15}N$ values were higher in the winter than the spring ($p < 0.001$, $p = 0.03$, respectively, Figure 5C, Table S1, Figure S4). $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures of scallops placed at 30 m were not different from isotopic signatures measured at the beginning of the experiment (T_0, December 10, 2016). C:N ratios (Figure S4) were affected by both depth and season (depth – $F_{1,36} = 215.2$, $p < 0.001$, season – $F_{1,36} = 222.7$, $p < 0.001$), and had the highest ratio in the 5 m spring cohort.

Scallops showed similar patterns in $\delta^{13}C$ levels to mussels (season – $F_{2,45} = 96.73$, $p < 0.001$, depth – $F_{1,45} = 180.50$, $p < 0.001$, Figure 5B, Table S1, Figure S4). Scallops from 5 m in spring had higher $\delta^{13}C$ values than at 5 m in the winter ($F_{2,45} = 96.73$, $p < 0.001$). Higher $\delta^{13}C$ values were found at 30 m than 5 m. $\delta^{15}N$ values in scallops differed between seasons and depth (season – $F_{2,45}$

311 = 11.41, $p < 0.001$, depth – $F_{1,45} = 196.01$, $p < 0.001$). Similar to $\delta^{13}C$, higher values of $\delta^{15}N$ were
312 observed at 30 m than 5 m ($p = 0.003$, $p < 0.001$, respectively, Figure 5B, Table S1, Figure S4).
313 T_0 scallops had similar signatures to the 30 m depths for $\delta^{15}N$, and for $\delta^{13}C$ signatures were similar
314 to all cohorts except 5 m winter. C:N ratios were higher in the spring than winter (season – $F_{1,45}$ =
315 14.09, $p < 0.001$,) and were higher at 5 m than 30 m in both seasons (depth – $F_{1,45} = 20.57$, $p <$
316 0.001).
317

318 **4. Discussion**

319 *4.1. Seawater Chemistry*

320 Puget Sound's Hood Canal experiences seasonal hypoxia and strong stratification due to warming,
321 upwelling, riverine, and anthropogenic nutrient inputs (Khangaonkar et al., 2018). Our results
322 show a relatively well-mixed water column in the winter and increasing stratification as the water
323 warms in the spring. Temperatures experienced by shellfish in our experiment were as high as 18.8
324 °C and as low as 6.8 °C. In Hood Canal, salinity is typically affected by external freshwater input
325 and precipitation (Reum et al., 2014). The lower salinity values at 5 m in spring are likely due to
326 the outflow of snowmelt and terrestrial runoff, which creates a freshwater lens containing high
327 nutrient load (Khangaonkar et al., 2018). Strong spring stratification and warmer surface waters in
328 Hood Canal can lead to increased phytoplankton blooms on the surface and metabolic influences
329 on oxygen concentrations and carbonate chemistry at all depths (Lowe et al., 2019).
330

331 For the majority of the experiment, pCO_2 values were above 1000 µatm (Ωara = 0.2 to 0.6, pH_T =
332 7.09 to 7.60) at the 30 m depth, which exceeds the global surface ocean average pCO_2 levels
333 projected by IPCC (2014) for year 2100. An aragonite saturation state less than 1 is of concern

because bivalves biomineralize aragonite to form their hard shells and prolonged undersaturation may lead to shell corrosion, and deployed mussels and scallops experienced these conditions at 5 m in winter and at 30 m throughout the experiment (Feely et al., 2008; Miller et al., 2009). Oxygen concentrations were relatively high at 5 m in both season, while they were more variable but tended to be lower at 30 m during winter, although levels observed never fell to values where widespread shellfish mortality might be expected (Vaquer-Sunyer and Duarte, 2008).

4.2. Growth and Shell Strength

Growth of mussels and scallops acclimatized to 5 m was > 100% higher than those from the 30 m depth. In a similar study, the giant scallop, *Placopecten magellanicus,* held at different depths in Newfoundland, Canada, had higher growth rates at the shallow 10 m depth when compared to deeper depths (20 and 30 m) (MacDonald and Thompson, 1985). In our study, higher growth at shallow depths is likely due to a combination of multiple factors such as higher aragonite saturation state, DO, food availability, and overall warmer temperatures, especially during the spring. Similarly, *P. magellanicus* had faster growth rates in shallower water (10 m) where temperatures and food availability were higher. If growers seek fast shellfish growth, it is advisable to place bivalves for grow-out in the top few meters of the water column, where temperatures and carbonate chemistry are more favorable. In our study, shellfish from "spring" season had acclimatized longer than shellfish from the "winter" season, potentially confounding the interaction between acclimatization time and season.

With unfavorable carbonate chemistry, more energy may be expended on shell formation and there may be a disruption in extra- and intracellular acid-base equilibria, causing a trade-off of metabolic

357 energy away from growth (Michaelidis et al., 2005; Pörtner et al., 2005; Stevens and Gobler, 2018;
358 Wittmann and Pörtner, 2013). Low DO levels at deeper depths (generally around 2 – 3 mg/L for
359 bivalves) may reduce the ability of shellfish's ctenidia to extract oxygen from the water to sustain
360 basic cellular function, possibly redirecting energy away from growth and toward acclimatory and
361 somatic maintenance processes (Carrington et al., 2015; Froehlich et al., 2016; Moullac et al.,
362 2007; Sokolova et al., 2012; Stevens and Gobler, 2018). A similar study found that scallop
363 (*Argopecten irradians)* experienced significantly lower growth rates when exposed to water
364 collected from Forge River Estuary, New York, which has naturally low DO and pH levels (Gobler
365 et al., 2014).
366
367 Mussels acclimatized to 5 m depth had considerably stronger shells than those from 30 m depth.
368 This is possibly due to favorable aragonite saturation states at the surface. These results are
369 consistent with previous studies in which calcifying species, a snail (*Austrocochlea porcata*) and
370 the blue mussel (*Mytilus edulis*), had weaker shells when exposed to acidified conditions (Coleman
371 et al., 2014; Li et al., 2015). In Washington, it is predicted that increased uptake of anthropogenic
372 CO_2 in the future will cause the aragonite saturation horizon to shoal further than it already has,
373 making suitable habitat for calcifiers scarce (Feely et al., 2012). As suggested by Green et al.
374 (2009), "death by dissolution" is a very real possibility for bivalves as climate change progresses.
375
376 In contrast to mussels, the strongest scallop shells were found at 30 m depth in the spring, whereas
377 the most fragile shells were found at 5 m during the winter. Similar results have been seen in the
378 gastropod mollusk, *Subninella undulata,* whose shell strength was not directly related with pH
379 treatments of 8.2 and 7.7 after 65 days of exposure (Coleman et al., 2014). A previous study

subjected *C. gigantea* to 1050 µatm and 365 µatm pCO_2 for six weeks and shells were subsequently CT-scanned to measure shell density (Alma et al., 2020). Scallops from this study had a significantly lower periostracum density in the high pCO_2 treatments suggesting that this outer layer dissolves first when compared to inner shell layers. We did not measure the thickness of the periostracum (organic outermost layer of the shell) layer in our study, however, it is probable that the scallops from our experiment were protected from dissolution due to their thick periostracum, which may explain the compromised growth rates but not shell strength (Gazeau et al., 2013). *C. gigantea* is known to be more abundant at deeper depths, up to 80 m deep, where aragonite saturation in Hood Canal reaches levels of ~0.5 in the colder, well-mixed months, and ~0.6 in the warmer highly stratified months (Feely et al., 2010; Whyte et al., 1990). In comparison, *M. galloprovincialis* can be found up to 40 m deep where aragonite saturation in Hood Canal can reach ~0.7 in the cold months and ~0.8 in the warm months (CABI, 2020; Feely et al., 2010), so it is possible that the native *C. gigantea* has evolved better biomineralization mechanisms to cope with acidified conditions than the more shallow-adapted *M. galloprovincialis*.

4.4. Isotopic Signatures

Analysis of stable isotope signatures can provide a time-integrated assessment of an individual's diet origin and insight into the influence of the environment on their assimilation rate (Gaillard et al., 2017; Galimany et al., 2017; Lowe et al., 2019). A small change in the C:N ratio may be indicative of environmental stress and alterations in the system's food web dynamics (Patterson and Carmichael, 2018). $\delta^{13}C$ can be used as an indicator of primary production sources, while $\delta^{15}N$ can be used as a proxy for trophic position and sub-lethal stress (Michener and Lajtha, 2008). Differences in $\delta^{13}C$ manifested as lower (more negative) $\delta^{13}C$ levels in both species at the 5 m

403 depths, especially in winter, likely as a reflection of increased terrestrial C3 plant input, which has
404 a lower $\delta^{13}C$ signature. Similar seasonal differences have also been seen in wild-sampled Hood
405 Canal Pacific oysters, *Crassostrea gigas,* who exhibited lower $\delta^{13}C$ in the winter (November–
406 December) when compared to summer (June–August) (Conway-Cranos et al., 2015), suggesting
407 that the oysters collected in summer had less terrestrial-based organic food sources than those
408 collected in winter. The isotopic pattern seen in the shallow winter cohort may point to increased
409 runoff due to snowmelt or precipitation, directing more terrestrial food sources into Hood Canal
410 (Simenstad and Wissmar, 1985). Differences across depths can be seen in both $\delta^{13}C$ and $\delta^{15}N$, in
411 which the 30 m depth had higher isotopic values than the 5 m depth, for both species. A similar
412 isotopic signature to our study was seen in the oyster *C. gigas,* where those grown close to the
413 bottom near a seagrass bed had higher $\delta^{13}C$ and $\delta^{15}N$ when compared to oysters grown offshore
414 (Hori et al., 2019). Oysters grown offshore had a more pelagic-based diet, while those grown on
415 tidal flats ingested a diet of both benthic and pelagic matter, resulting in higher nitrogen content
416 and higher quality protein. *C. gigas* acclimated to Hood Canal conditions (15 km from our site)
417 are suggested to have relied on a diet of predominantly salt marsh vegetation-derived carbon,
418 resulting in a reduced $\delta^{13}C$ signature (Conway-Cranos et al., 2015). It can also be postulated that
419 cohorts with higher $\delta^{13}C$ were acclimatized to environments with more terrestrial C4 plant
420 particulates (e.g., grasses), marsh grass, algal primary productivity, eelgrass, and marine
421 particulate organic matter (POM), all of which are relatively enriched in ^{13}C compared to terrestrial
422 C3 plants (e.g., trees). Consumers integrate the carbon isotopic composition of their diet into their
423 bodies, and can thus reflect changing food sources through time (Hama et al., 1983; Michener and
424 Lajtha, 2008). This is a plausible explanation for the carbon isotopic signatures observed in
425 mussels and scallops, as conditions at 5 m in the spring likely had higher phytoplankton

426 productivity than in the winter, and the 30 m locations had a high abundance of marine POM,
427 while the 5 m location in the winter had mainly terrestrial POM.
428
429 Higher $\delta^{15}N$ values at 30 m may be correlated with higher denitrification at depth, in which
430 microbial metabolic activity preferentially uses lighter nitrogen isotopes, leaving the surroundings
431 enriched in $\delta^{15}N$ (isotopic fractionation) (Schlesinger and Bernhardt, 2013). Lower dissolved
432 oxygen at depth may have created a more favorable environment for denitrification and further
433 increased $\delta^{15}N$. Subsequently when phytoplankton uptake nutrients associated with denitrification,
434 their $\delta^{15}N$ signature increases which may be reflected in bivalves who consume them due to the
435 trophic enrichment factor (Zhang et al., 2010). Bivalves at deeper depths may also consume a
436 higher proportion of POM, which is comprised largely of denitrified particles and organic debris,
437 which will often contain an enriched $\delta^{15}N$ signature (Michener and Lajtha, 2008). Additionally,
438 sub-lethal environmental stress responses in bivalves can prompt an increase in metabolic
439 processes and disrupt nitrogen processing resulting in increased preferential excretion of light
440 nitrogen isotopes into the environment leaving tissues enriched with $\delta^{15}N$ (Patterson and
441 Carmichael, 2018). For example, in the Eastern oyster *Crassostrea virginica,* those who were field-
442 acclimatized to 3.66 mg/L DO had lower growth and a higher $\delta^{15}N$ signature when compared to
443 those acclimatized to 6 mg/L. This suggests excess excretion of lighter $\delta^{14}N$ by bivalves due to
444 stressful conditions may be also be correlated with changes in metabolic processes like growth or
445 starvation (Patterson and Carmichael, 2018). Changes in environmental $\delta^{15}N$ may also be
446 attributed to a multitude of factors including changes in agricultural runoff, increased storm
447 activity, or anthropogenic pollution (Piola et al., 2006); therefore, it is difficult to trace changes in
448 organismal $\delta^{15}N$ without measurements of source end-members. A longer- term study of tissue-

specific and environmental end-member isotopic signatures may provide further insight into our

5. Conclusion

We identified major differences in shellfish growth, shell strength, and stable isotopes across seasons and depths. Both of our study species showed faster growth at 5 m than 30 m. Shell strength changed with depth in mussels (higher at 5 m than 30 m) but did not change with depth in the scallops. Both mussels and scallops had low $\delta^{13}C$ and $\delta^{15}N$ levels at the 5 m depth in winter. The acclimatization capacity of scallops and mussels has been shown in this study, and this information may inform shellfish farmers to optimize marketable attributes, especially as climate change progresses. Our results are especially relevant for the burgeoning rock scallop aquaculture market, farms who grow mussels on long lines, and aquaculture locations that are already starting to experience the effects of climate change. Future research that acclimatizes bivalves for a longer period of time, and that examines transgenerational effects, metabolism, isotopic endmembers, and genetic expression should be implemented.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the Victor and Tamara Loosanoff Endowed Fellowship awarded to LA, The Royalty Research Fund Fellowship, the Sloan Foundation Fellowship, and the NOAA-Saltonstall-Kennedy Grant Program (No. 12493539) awarded to JLPG. We would like to graciously thank our collaborators: Rhonda Elliot, Benoit Eudeline, and Gordon King of Taylor Shellfish for their expertise, donation of shellfish, and assistance in the field. This research would not have been possible without the help of our esteemed colleagues and friends, and volunteers: Abigail Ames, Jeremy Axworthy, Eileen Bates, Joth Davis, Evan Fiorenza, Julia Hart, Mike Maher, Ruben Pimentel, Miranda Roethler, Laura Spencer, Angel Sar, Lily Stair, Brent Vadopalas, Jon Wittouck, and the entire Padilla-Gamiño lab. We would like express gratitude for mentoring and editing support from Gordon Holtgrieve, Paul McElhany, Steven Roberts, and Parker McCready. We thank Andy Schauer of UW Earth and Space Sciences for assistance with isotope processing, Ryan Newell from UW Applied Physics Lab for monitoring buoy chlorophyll data, and Bill Kuykendall of UW Mechanical Engineering for assistance with the Instron machine. This is PMEL contribution #5248.

References

Alin, S.R., Newton, J., Greeley, D., Curry, Herndon, B., Kozyr, J., Alex; Feely, A., R., 2021. A compiled data product of profile, discrete biogeochemical measurements from 35 individual cruise data sets collected from a variety of ships in the southern Salish Sea and northern California Current System (Washington state marine waters) from 2008-02-. NOAA Natl. Centers Environ. Information. Dataset.

Alin, S.R., Newton, J.A., Feely, R.A., Greeley, D.J., Siedlecki, S., Curry, B., Herndon, J., Warner, M., 2023. Physical and biogeochemical conditions in the southern Salish Sea across a decade-long cruise time-series (2008–2018): Seasonality and response of ocean acidification and hypoxia to major environmental anomalies. Earth Syst. Sci. Data Discuss. [preprint]. https://doi.org/https://doi.org/10.5194/essd-2023-239

Alma, L., 2023. Mussels and Scallops Data 21809631. https://doi.org/https://doi.org/10.6084/m9.figshare.21809631.v1

Alma, L., Kram, K.E., Holtgrieve, G.W., Barbarino, A., Fiamengo, C.J., Padilla-Gamiño, J.L., 2020. Ocean acidification and warming effects on the physiology, skeletal properties, and microbiome of the purple-hinge rock scallop. Comp. Biochem. Physiol. -Part A Mol.

Integr. Physiol. 240. https://doi.org/10.1016/j.cbpa.2019.110579
Araújo, J., Soares, F., Medeiros, A., Bandarra, N.M., Freire, M., Falcão, M., Pousão-Ferreira, P., 2020. Depth effect on growth and fatty acid profile of Mediterranean mussel (*Mytilus galloprovincialis*) produced on a longline off south Portugal. Aquac. Int. 28, 927–946. https://doi.org/10.1007/s10499-019-00504-0
Aure, J., Strohmeier, T., Strand, Ø., 2007. Modelling current speed and carrying capacity in long-line blue mussel (*Mytilus edulis*) farms. Aquac. Res. 38, 304–312. https://doi.org/10.1111/j.1365-2109.2007.01669.x
Barton, A., Waldbusser, G.G., Feely, R.A., Weisberg, S.B., Newton, J.A., Hales, B., Cudd, S., Eudeline, B., Langdon, C.J., Jefferds, I., King, T., Suhrbier, A., McLaughlin, K., 2015. Impacts of coastal acidification on the Pacific Northwest shellfish industry and adaptation strategies implemented in response. Oceanography 28, 146–159. https://doi.org/10.5670/oceanog.2015.38
Borges, A. V., Gypens, N., 2010. Carbonate chemistry in the coastal zone responds more strongly to eutrophication than to ocean acidification. Limnol. Oceanogr. 55, 346–353. https://doi.org/10.4319/lo.2010.55.1.0346
Bourne, N., 1987. Scallop culture in British Columbia. Fourth Alaska Aquac. Conf.
CABI, 2020. *Mytilus galloprovincialis* (Mediterranean mussel): Invasive Species Compendium Datasheet report. Wallingford, UK CAB Int. www.cabi.org/isc. 2020, 1–20.
Carnarius, K.M., Conrad, K.M., Mast, M.G., Macneil, J.H., 1996. Relationship of eggshell ultrastructure and shell strength to the soundness of shell eggs. Poult. Sci. 75, 656–663. https://doi.org/10.3382/ps.0750656
Carrington, E., Waite, J.H., Sarà, G., Sebens, K.P., 2015. Mussels as a model system for integrative ecomechanics. Ann. Rev. Mar. Sci. 7, 443–469. https://doi.org/10.1146/annurev-marine-010213-135049
Coleman, D.W., Byrne, M., Davis, A.R., 2014. Molluscs on acid: Gastropod shell repair and strength in acidifying oceans. Mar. Ecol. Prog. Ser. 509, 203–211. https://doi.org/10.3354/meps10887
Conway-Cranos, L., Kiffney, P., Banas, N., Plummer, M., Naman, S., MacCready, P., Bucci, J., Ruckelshaus, M., 2015. Stable isotopes and oceanographic modeling reveal spatial and trophic connectivity among terrestrial, estuarine, and marine environments. Mar. Ecol. Prog. Ser. 533, 15–28. https://doi.org/10.3354/meps11318
Culver, C.S., Richards, J.B., Page, H.M., 2006. Plasticity of attachment in the purple-hinge rock scallop, *Crassadoma gigantea*: Implications for commercial culture. Aquaculture 254, 361–369. https://doi.org/10.1016/j.aquaculture.2005.10.022
Dickson, A.G., Sabine, C.L., Christian, J.R., 2007. Guide to Best Practices for Ocean CO_2 measurements. PICES Special Publication, Guide to Best Practices for Ocean CO_2 measurements. PICES Special Publication.
Doney, S.C., Fabry, V.J., Feely, R.A., Kleypas, J.A., 2009. Ocean acidification: The other CO_2 problem. Ann. Rev. Mar. Sci. 1, 169–192. https://doi.org/10.1146/annurev.marine.010908.163834
Ekstrom, J.A., Suatoni, L., Cooley, S.R., Pendleton, L.H., Waldbusser, G.G., Cinner, J.E., Ritter, J., Langdon, C., Van Hooidonk, R., Gledhill, D., Wellman, K., Beck, M.W., Brander, L.M., Rittschof, D., Doherty, C., Edwards, P.E.T., Portela, R., 2015. Vulnerability and adaptation of US shellfisheries to ocean acidification. Nat. Clim. Chang. https://doi.org/10.1038/nclimate2508

550 Feely, R.A., Alin, S.R., Newton, J., Sabine, C.L., Warner, M., Devol, A., Krembs, C., Maloy, C.,
551 2010. The combined effects of ocean acidification, mixing, and respiration on pH and
552 carbonate saturation in an urbanized estuary. Estuar. Coast. Shelf Sci. 88, 442–449.
553 https://doi.org/10.1016/j.ecss.2010.05.004
554 Feely, R.A., Sabine, C.L., Byrne, R.H., Millero, F.J., Dickson, A.G., Wanninkhof, R., Murata,
555 A., Miller, L.A., Greeley, D., 2012. Decadal changes in the aragonite and calcite saturation
556 state of the Pacific Ocean. Global Biogeochem. Cycles 26.
557 https://doi.org/10.1029/2011GB004157
558 Feely, R.A., Sabine, C.L., Hernandez-Ayon, J.M., Ianson, D., Hales, B., 2008. Evidence for
559 upwelling of corrosive "acidified" water onto the continental shelf. Science (80-.). 320,
560 1490–1492. https://doi.org/10.1126/science.1155676
561 Froehlich, H.E., Gentry, R.R., Halpern, B.S., 2016. Synthesis and comparative analysis of
562 physiological tolerance and life-history growth traits of marine aquaculture species.
563 Aquaculture 460, 75–82. https://doi.org/10.1016/j.aquaculture.2016.04.018
564 Fry, B., 2006. Stable isotope ecology, Encyclopedia of Ecology. Springer.
565 https://doi.org/10.1016/B978-0-12-409548-9.10915-7
566 Fry, B., Mersch, F.J., Tholke, K., Garritt, R., Brand, W., 1992. Automated Analysis System for
567 Coupled $\delta^{13}C$ and δ Measurements. Anal. Chem. 64, 288–291.
568 https://doi.org/10.1021/ac00027a009
569 Gaillard, B., Meziane, T., Tremblay, R., Archambault, P., Blicher, M.E., Chauvaud, L.,
570 Rysgaard, S., Olivier, F., 2017. Food resources of the bivalve *Astarte elliptica* in a sub-
571 Arctic fjord: A multi-biomarker approach. Mar. Ecol. Prog. Ser.
572 https://doi.org/10.3354/meps12036
573 Galimany, E., Lunt, J., Freeman, C.J., Reed, S., Segura-García, I., Paul, V.J., 2017. Feeding
574 behavior of eastern oysters *Crassostrea virginica* and hard clams *Mercenaria mercenaria* in
575 shallow estuaries. Mar. Ecol. Prog. Ser. 567, 125–137. https://doi.org/10.3354/meps12050
576 Gazeau, F., Parker, L.M., Comeau, S., Gattuso, J.P., O'Connor, W.A., Martin, S., Pörtner, H.O.,
577 Ross, P.M., 2013. Impacts of ocean acidification on marine shelled molluscs. Mar. Biol.
578 160, 2207–2245. https://doi.org/10.1007/s00227-013-2219-3
579 Gobler, C.J., Baumann, H., 2016. Hypoxia and acidification in ocean ecosystems: Coupled
580 dynamics and effects on marine life. Biol. Lett. 12. https://doi.org/10.1098/rsbl.2015.0976
581 Gobler, C.J., Clark, H.R., Griffith, A.W., Lusty, M.W., 2017. Diurnal fluctuations in
582 acidification and hypoxia reduce growth and survival of larval and juvenile bay scallops
583 (*Argopecten irradians*) and hard clams (*Mercenaria mercenaria*). Front. Mar. Sci.
584 https://doi.org/10.3389/FMARS.2016.00282
585 Gobler, C.J., DePasquale, E.L., Griffith, A.W., Baumann, H., 2014. Hypoxia and acidification
586 have additive and synergistic negative effects on the growth, survival, and metamorphosis
587 of early life stage bivalves. PLoS One 9. https://doi.org/10.1371/journal.pone.0083648
588 Green, M.A., Waldbusser, G.G., Reilly, S.L., Emerson, K., O'Donnell, S., 2009. Death by
589 dissolution: Sediment saturation state as a mortality factor for juvenile bivalves. Limnol.
590 Oceanogr. 54, 1037–1047. https://doi.org/10.4319/lo.2009.54.4.1037
591 Gruber, N., Hauri, C., Lachkar, Z., Loher, D., Frölicher, T.L., Plattner, G.K., 2012. Rapid
592 progression of ocean acidification in the California Current System. Science (80-.). 337,
593 220–223. https://doi.org/10.1126/science.1216773
594 Gutiérrez, J.L., Jones, C.G., Strayer, D.L., Iribarne, O.O., 2003. Mollusks as ecosystem
595 engineers: The role of shell production in aquatic habitats. Oikos 101, 79–90.

https://doi.org/10.1034/j.1600-0706.2003.12322.x
Hama, T., Miyazaki, T., Ogawa, Y., Iwakuma, T., Takahashi, M., Otsuki, A., Ichimura, S., 1983. Measurement of photosynthetic production of a marine phytoplankton population using a stable $\delta^{13}C$ isotope. Mar. Biol. 73, 31–36. https://doi.org/10.1007/BF00396282
Hiebenthal, C., Philipp, E., Eisenhauer, A., Wahl, M., 2012. Interactive effects of temperature and salinity on shell formation and general condition in Baltic Sea *Mytilus edulis* and Arctica islandica. Aquat. Biol. https://doi.org/10.3354/ab00405
Hoegh-Guldberg, O., Bruno, J.F., 2010. The impact of climate change on the world's marine ecosystems. Science (80-.). https://doi.org/10.1126/science.1189930
Hoegh-Guldberg, O., Cai, R., Poloczanska, E.S., Brewer, P.G., Sundby, S., Hilmi, K., Fabry, V.J., Jung, S., 2015. The ocean. In climate change 2014 – Impacts, adaptation and vulnerability: Part B: Regional aspects: Working group II contribution to the IPCC fifth assessment report. Cambridge Cambridge Univ. Press.
Hofmann, G.E., Smith, J.E., Johnson, K.S., Send, U., Levin, L.A., Micheli, F., Paytan, A., Price, N.N., Peterson, B., Takeshita, Y., Matson, P.G., de Crook, E., Kroeker, K.J., Gambi, M.C., Rivest, E.B., Frieder, C.A., Yu, P.C., Martz, T.R., 2011. High-frequency dynamics of ocean pH: A multi-ecosystem comparison. PLoS One 6. https://doi.org/10.1371/journal.pone.0028983
Hori, M., Lagarde, F., Richard, M., Derolez, V., Hamaguchi, M., Makino, M., 2019. Coastal management using oyster-seagrass interactions for sustainable aquaculture, fisheries and environment. Bull. Japan Fish. Res. Educ. Agency 35–43.
Ikejima, I., Nomoto, R., McCabe, J.F., 2003. Shear punch strength and flexural strength of model composites with varying filler volume fraction, particle size and silanation. Dent. Mater. 19, 206–211. https://doi.org/10.1016/S0109-5641(02)00031-3
IPCC, 2014. Impacts, Adaptation, and Vulnerability: Contribution of working group II to the fifth assessment report of the Intergovernmental Panel on Climate Change. Intergov. Panel Clim. Chang. 1–44.
Jackson, M., 2021. Reproductive biology of farmed purple-hinged rock scallop (*Crassadoma gigantea*). Univ. Washingt.
Jiang, L.Q., Dunne, J., Carter, B.R., Tjiputra, J.F., Terhaar, J., Sharp, J.D., Olsen, A., Alin, S., Bakker, D.C.E., Feely, R.A., Gattuso, J.P., Hogan, P., Ilyina, T., Lange, N., Lauvset, S.K., Lewis, E.R., Lovato, T., Palmieri, J., Santana-Falcón, Y., Schwinger, J., Séférian, R., Strand, G., Swart, N., Tanhua, T., Tsujino, H., Wanninkhof, R., Watanabe, M., Yamamoto, A., Ziehn, T., 2023. Global Surface Ocean Acidification Indicators From 1750 to 2100. J. Adv. Model. Earth Syst. https://doi.org/10.1029/2022MS003563
Khangaonkar, T., Nugraha, A., Xu, W., Long, W., Bianucci, L., Ahmed, A., Mohamedali, T., Pelletier, G., 2018. Analysis of Hypoxia and Sensitivity to Nutrient Pollution in Salish Sea. J. Geophys. Res. Ocean. 123, 4735–4761. https://doi.org/10.1029/2017JC013650
Kim, T.W., Barry, J.P., Micheli, F., 2013. The effects of intermittent exposure to low-pH and low-oxygen conditions on survival and growth of juvenile red abalone. Biogeosciences. https://doi.org/10.5194/bg-10-7255-2013
Laing, I., 2000. Effect of temperature and ration on growth and condition of king scallop (*Pecten maximus*) spat. Aquaculture. https://doi.org/10.1016/S0044-8486(99)00262-8
Leighton, D.L., Phleger, C.F., 1977. the Purple-Hinge Rock Scallop: a New Candidate for Marine Aquaculture. Proc. Annu. Meet. - World Maric. Soc. 8, 457–469. https://doi.org/10.1111/j.1749-7345.1977.tb00136.x

642 Li, S., Liu, C., Huang, J., Liu, Y., Zheng, G., Xie, L., Zhang, R., 2015. Interactive effects of
643 seawater acidification and elevated temperature on biomineralization and amino acid
644 metabolism in the mussel Mytilus edulis. J. Exp. Biol. 218, 3623–3631.
645 https://doi.org/10.1242/jeb.126748
646 Lowe, A.T., Bos, J., Ruesink, J., 2019. Ecosystem metabolism drives pH variability and
647 modulates long-term ocean acidification in the Northeast Pacific coastal ocean. Sci. Rep. 9,
648 1–11. https://doi.org/10.1038/s41598-018-37764-4
649 Lueker, T.J., Dickson, A.G., Keeling, C.D., 2000. Ocean pCO_2 calculated from dissolved
650 inorganic carbon, alkalinity, and equations for K1 and K2: Validation based on laboratory
651 measurements of CO_2 in gas and seawater at equilibrium. Mar. Chem. 70, 105–119.
652 https://doi.org/10.1016/S0304-4203(00)00022-0
653 MacDonald, B., Thompson, R., 1985. Influence of temperature and food availability on the
654 ecological energetics of the giant scallop Placopecten magellanicus. I. Growth rates of shell
655 and somatic tissue. Mar. Ecol. Prog. Ser. 25, 279–294. https://doi.org/10.3354/meps025279
656 McElhany, P., 2017. CO_2 sensitivity experiments are not sufficient to show an effect of ocean
657 acidification. ICES J. Mar. Sci. 74, 926–928. https://doi.org/10.1093/icesjms/fsw085
658 Melzner, F., Mark, F.C., Seibel, B.A., Tomanek, L., 2020. Ocean Acidification and Coastal
659 Marine Invertebrates: Tracking CO_2 Effects from Seawater to the Cell. Ann. Rev. Mar. Sci.
660 https://doi.org/10.1146/annurev-marine-010419-010658
661 Melzner, F., Stange, P., Trübenbach, K., Thomsen, J., Casties, I., Panknin, U., Gorb, S.N.,
662 Gutowska, M.A., 2011. Food supply and seawater pCO_2 impact calcification and internal
663 shell dissolution in the blue mussel mytilus edulis. PLoS One 6.
664 https://doi.org/10.1371/journal.pone.0024223
665 Michaelidis, B., Ouzounis, C., Paleras, A., Pörtner, H.O., 2005. Effects of long-term moderate
666 hypercapnia on acid-base balance and growth rate in marine mussels M*ytilus*
667 *galloprovincialis.* Mar. Ecol. Prog. Ser. 293, 109–118. https://doi.org/10.3354/meps293109
668 Michener, R., Lajtha, K., 2008. Stable Isotopes in Ecology and Environmental Science: Second
669 Edition, Stable Isotopes in Ecology and Environmental Science: Second Edition.
670 https://doi.org/10.1002/9780470691854
671 Miller, A.W., Reynolds, A.C., Sobrino, C., Riedel, G.F., 2009. Shellfish face uncertain future in
672 high CO_2 world: Influence of acidification on oyster larvae calcification and growth in
673 estuaries. PLoS One 4. https://doi.org/10.1371/journal.pone.0005661
674 Moore, S.K., Johnstone, J.A., Banas, N.S., Salathé, E.P., 2015. Present-day and future climate
675 pathways affecting *Alexandrium* blooms in Puget Sound, WA, USA. Harmful Algae 48, 1–
676 11. https://doi.org/10.1016/j.hal.2015.06.008
677 Moritsch, M.M., Byrd, K.B., Davis, M., Good, A., Drexler, J.Z., Morris, J.T., Woo, I.,
678 Windham-Myers, L., Grossman, E., Nakai, G., Poppe, K.L., Rybczyk, J.M., 2022. Can
679 Coastal Habitats Rise to the Challenge? Resilience of Estuarine Habitats, Carbon
680 Accumulation, and Economic Value to Sea-Level Rise in a Puget Sound Estuary. Estuaries
681 and Coasts. https://doi.org/10.1007/s12237-022-01087-5
682 Moullac, G. Le, Quéau, I., Souchu, P. Le, Pouvreau, S., Moal, J., Coz, J.R. Le, Samain, J.F.,
683 2007. Metabolic adjustments in the oyster *Crassostrea gigas* according to oxygen level and
684 temperature. Mar. Biol. Res. https://doi.org/10.1080/17451000701635128
685 Patterson, H.K., Carmichael, R.H., 2018. Dissolved oxygen concentration affects δ15 N values
686 in oyster tissues: implications for stable isotope ecology . Ecosphere 9, e02154.
687 https://doi.org/10.1002/ecs2.2154

688 Pelletier, G.J., Lewis, E., Wallace, D.W.R., 2007. CO_2SYS.XLS: a calculator for the CO_2 system
689 in seawater for Microsoft Excel/VBA. Washingt. State Dep. Ecol. Natl. Lab. Olympia,
690 WA/Upton, NY, USA - Open Access Libr.
691 Piola, R.F., Moore, S.K., Suthers, I.M., 2006. Carbon and nitrogen stable isotope analysis of
692 three types of oyster tissue in an impacted estuary. Estuar. Coast. Shelf Sci. 66, 255–266.
693 https://doi.org/10.1016/j.ecss.2005.08.013
694 Pörtner, H.O., Langenbuch, M., Michaelidis, B., 2005. Synergistic effects of temperature
695 extremes, hypoxia, and increases in CO_2 on marine animals: From Earth history to global
696 change. J. Geophys. Res. C Ocean. 110, 1–15. https://doi.org/10.1029/2004JC002561
697 Reum, J.C.P., Alin, S.R., Feely, R.A., Newton, J., Warner, M., McElhany, P., 2014. Seasonal
698 carbonate chemistry covariation with temperature, oxygen, and salinity in a fjord estuary:
699 Implications for the design of ocean acidification experiments. PLoS One 9.
700 https://doi.org/10.1371/journal.pone.0089619
701 Riascos, J.M., Guzman, P.A., 2010. The ecological significance of growth rate, sexual
702 dimorphism and size at maturity of Littoraria zebra and L. variegata (Gastropoda:
703 Littorinidae). J. Molluscan Stud. https://doi.org/10.1093/mollus/eyq011
704 Rykaczewski, R.R., Dunne, J.P., 2010. Enhanced nutrient supply to the California Current
705 Ecosystem with global warming and increased stratification in an earth system model.
706 Geophys. Res. Lett. 37. https://doi.org/10.1029/2010GL045019
707 Schlesinger, W.H., Bernhardt, E.S., 2013. Biogeochemistry: An Analysis of Global Change,
708 Third Edition, Biogeochemistry: An Analysis of Global Change, Third Edition.
709 https://doi.org/10.1016/C2010-0-66291-2
710 Simenstad, C., Wissmar, R., 1985. $\delta^{13}C$ evidence of the origins and fates of organic carbon in
711 estuarine and near-shore food webs. Mar. Ecol. Prog. Ser. 22, 141–152.
712 https://doi.org/10.3354/meps022141
713 Smart, D.C., 2019. Local Adaptation of the Blue Mussel (*Mytilus galloprovincialis*) in Southern
714 New Zealand.
715 Sokolova, I.M., Frederich, M., Bagwe, R., Lannig, G., Sukhotin, A.A., 2012. Energy
716 homeostasis as an integrative tool for assessing limits of environmental stress tolerance in
717 aquatic invertebrates. Mar. Environ. Res. 79, 1–15.
718 https://doi.org/10.1016/j.marenvres.2012.04.003
719 Stevens, A.M., Gobler, C.J., 2018. Interactive effects of acidification, hypoxia, and thermal
720 stress on growth, respiration, and survival of four North Atlantic bivalves. Mar. Ecol. Prog.
721 Ser. 604, 143–161. https://doi.org/10.3354/meps12725
722 T. Walker, 2016. Rock scallop: Viable candidate for aquaculture? - Aquaculture North America
723 [WWW Document]. Aquac. North Am. URL
724 https://www.aquaculturenorthamerica.com/rock-scallop-viable-candidate-for-aquaculture-
725 1051/ (accessed 6.4.21).
726 Taiyun, W., 2014. Visualization of a correlation matrix. CRAN.
727 Tyler, C., 1961. Shell Strength: Its Measurement and its Relationship to other Factors. Br. Poult.
728 Sci. https://doi.org/10.1080/00071666109382385
729 Vaquer-Sunyer, R., Duarte, C.M., 2008. Thresholds of hypoxia for marine biodiversity. Proc.
730 Natl. Acad. Sci. U. S. A. https://doi.org/10.1073/pnas.0803833105
731 Wallace, R.B., Baumann, H., Grear, J.S., Aller, R.C., Gobler, C.J., 2014. Coastal ocean
732 acidification: The other eutrophication problem. Estuar. Coast. Shelf Sci. 148, 1–13.
733 https://doi.org/10.1016/j.ecss.2014.05.027

734 Wernberg, T., Smale, D.A., Thomsen, M.S., 2012. A decade of climate change experiments on
735 marine organisms: Procedures, patterns and problems. Glob. Chang. Biol.
736 https://doi.org/10.1111/j.1365-2486.2012.02656.x
737 Whyte, J.N.C., Bourne, N., Hodgson, C.A., 1990. Nutritional condition of rock scallop,
738 *Crassadoma gigantea* (Gray), larvae fed mixed algal diets. Aquaculture 86, 25–40.
739 https://doi.org/10.1016/0044-8486(90)90219-D
740 Wilkie, E.M., Bishop, M.J., 2012. Differences in shell strength of native and non-native oysters
741 do not extend to size classes that are susceptible to a generalist predator. Mar. Freshw. Res.
742 63, 1201–1205. https://doi.org/10.1071/MF12078
743 Wittmann, A.C., Pörtner, H.O., 2013. Sensitivities of extant animal taxa to ocean acidification.
744 Nat. Clim. Chang. 3, 995–1001. https://doi.org/10.1038/nclimate1982
745 Zhang, J., Gilbert, D., Gooday, A.J., Levin, L., Naqvi, S.W.A., Middelburg, J.J., Scranton, M.,
746 Ekau, W., Peña, A., Dewitte, B., Oguz, T., Monteiro, P.M.S., Urban, E., Rabalais, N.N.,
747 Ittekkot, V., Kemp, W.M., Ulloa, O., Elmgren, R., Escobar-Briones, E., Van Der Plas, A.K.,
748 2010. Natural and human-induced hypoxia and consequences for coastal areas: Synthesis
749 and future development. Biogeosciences. https://doi.org/10.5194/bg-7-1443-2010
750