Title

*Pacific geoduck (*Panopea generosa*) resilience to natural pH variation*

Authors

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Declarations of interest: none

Figures: All figures are provided in color.

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Abstract

Pacific geoduck aquaculture is a growing industry, however little is known about how geoduck respond to varying environmental conditions, or how production might be impacted by low pH associated with ocean acidification. Ocean acidification research is increasingly incorporating multiple environmental drivers and natural pH variability into biological response studies for more complete understanding of the effects of projected ocean conditions. In this study, eelgrass habitats and environmental heterogeneity across four estuarine bays were leveraged to examine low pH effects on geoduck under different natural regimes, using proteomics to assess physiology. Juvenile geoduck were deployed in eelgrass and adjacent unvegetated habitats for 30 days while pH, temperature, dissolved oxygen, and salinity were monitored. Across the four bays pH was lower in unvegetated habitats compared to eelgrass habitats, however this did not impact geoduck growth, survival, or proteomic expression patterns. However, across all sites temperature and dissolved oxygen corresponded to growth and protein expression patterns. Specifically, three protein (trifunctional-enzyme subunit β-subunit, puromycin-sensitive aminopeptidase, and heat shock protein 90-α) abundance levels and shell growth positively correlated with dissolved oxygen variability and inversely correlated with mean temperature. These results demonstrate that geoduck are resilient to low pH in a natural setting, and other abiotic factors (i.e. temperature, dissolved oxygen variability) may have a greater influence on geoduck physiology. In addition this study contributes to the understanding of how eelgrass influences water chemistry.

Key words

Aquaculture, comparative physiology, ocean acidification, Panopea generosa, proteomics

Introduction

The Pacific geoduck, *Panopea generosa,* is native to the North American Pacific Coast and is a burgeoning aquaculture species with strong overseas demand as a luxury commodity (Coan et al. 2000; Shamshak and King 2015; Vadopalas et al. 2010). The largest burrowing clam in the world, cultured geoduck reach upwards of 180mm and are harvested after growing approximately 6-7 years in sub- or intertidal sediment (Vadopalas et al. 2015; WA DNR website 2018; Washington Sea Grant 2013). The long grow-out period and high per-animal value highlights the importance of site selection for farmers to maximize investment, however there remains a paucity of data on the optimal environmental conditions for geoduck aquaculture.

As marine calcifiers, geoduck may be vulnerable to ocean acidification due to their reliance on calcite and aragonite (forms of calcium carbonate) for shell secretion (Orr et al. 2005; Weiss et al. 2002), both of which become less biologically available as seawater pH declines with pCO_2 enrichment (Feely et al. 2008). A broadening body of research on marine calcifiers indicates that, generally, projected low pH will shift organisms' physiology to the detriment of species-wide abundances and distributions (Boyd et al. 2016; DeBiasse and Kelly 2016; Kurihara 2008; Kroeker et al. 2010; Pörtner 2008; Pörtner and Farrell 2008; Ries et al. 2009). However, broad generalizations of how ocean acidification affects calcifiers are few due

42   to varying pH sensitivity between taxa (Clark et al. 2009; Gazeau et al. 2007; Miller et al. 2009;
43   Ries et al. 2009; Talmage and Gobler 2009; Waldbusser et al. 2016) and life stage (Kurihara
44   2008; Kroeker et al. 2010), thus lessons learned from other bivalve species cannot directly be
45   applied to geoduck without investigation.
46   The effect of low pH on cultured geoduck needs to be explored to help the aquaculture
47   industry make informed site selection, selective breeding, and investment decisions. For
48   practical application, geoduck ocean acidification studies should best replicate the natural
49   environment in which they are grown, which is primarily Washington State (90% of global
50   production) in the Puget Sound estuary where environmental drivers vary between subbasin,
51   season, and diurnal cycle (Moore et al. 2008; Shamshak and King 2015). Lessons from more
52   advanced ocean acidification research on other taxa can also be leveraged. For example a
53   significant conclusion is that low pH is not occurring in isolation, but rather in conjunction with
54   changes in other environmental drivers (for example temperature, dissolved oxygen, salinity),
55   and thus single-stressor studies are limited in their predictive capacity (Ban et al. 2014; Byrne
56   and Przeslawski 2013; DeBiasse and Kelly 2016; Gobler et al. 2014; Harvey et al. 2013;
57   Kroeker et al. 2013; Padilla-Gamiño et al. 2013; Przeslawski et al. 2015). Another consideration
58   is the incorporation of naturally-occuring diurnal pH variability into ocean acidification studies, as
59   variable pH can have differing effects on marine calcifiers compared to persistent low pH
60   (Baumann et al. 2015; Boyd et al. 2016; Cornwall et al. 2013; Frieder et al. 2014; Gunderson et
61   al. 2016; Paganini et al. 2014; Reum et al. 2016).
62   To best predict the effect of ocean acidification on geoduck aquaculture this project
63   deployed geoduck in variable environmental conditions and leveraged the natural pH
64   differences between eelgrass and unvegetated habitats in Washington State estuaries. Ocean
65   acidification studies are increasingly exploiting naturally low pH systems to monitor the
66   environmental heterogeneity alongside test organisms (for example, hydrothermal vents,
67   shallow CO_2 seeps, coastal upwelling regions, and eutrophic estuaries) (Duquette et al. 2017;
68   Hofmann et al. 2011; Howarth et al. 2011; Thomsen et al. 2013; Tunnicliffe et al. 2009; Kerrison
69   et al. 2011). Compared to controlled laboratory studies, these deployment studies can uniquely
70   incorporate daily cycles in air exposure, temperature, pH, dissolved oxygen, salinity, and food
71   availability (Chapman et al. 2011; Groner et al. 2018; Middelboe and Hansen 2007; Ringwood
72   and Keppler 2002).
73   Estuaries along the United States Pacific Coast are ideal, natural mesocosms for
74   examining the effect of ocean acidification on commercially vital calcifiers, as they contain
75   dense macroalgae beds (Bulthuis 1995), environmental conditions that vary considerably
76   between subbasins (Babson et al. 2006; Banas et al. 2004; Dethier et al. 2010; Moore et al.
77   2008; Yang and Khangaonkar 2010), and have rich communities of native and cultured shellfish
78   (Dethier et al. 2006; Miller et al. 2009; Washington Sea Grant 2015). Furthermore, coastal
79   estuaries have already shifted towards lower pH, warmer averages, and are projected to
80   continue along this trend (Abatzoglou et al. 2013; Busch et al. 2013; Doney et al. 2007; Feely et
81   al. 2012, 2010, 2008; Mote and Salathé 2010). The buffering capacity of macroalgae (seagrass
82   meadows, kelp forests) allows for block-designed experiments to examine the effect of pH,
83   while controlling for varying background environments and maintaining diurnal fluctuations
84   (Middelboe and Hansen 2007; Palacios and Zimmerman 2007; Wahl et al. 2018).

85   In order to better inform geoduck aquaculture practices, we set out to examine how low
86   pH and other natural variation in environmental conditions influence geoduck growth and
87   physiology, using eelgrass as a primary determinant of water chemistry. Physiology was
88   evaluated with a two-phase proteomics approach using Selected Reaction Monitoring, with
89   targets identified using Data Independent Acquisition. This novel application demonstrates the
90   advances in proteomic research and the potential it has to improve aquaculture production.

Methods

Experimental Design

93   *Panopea generosa* juveniles (14.0 +/- 0.85 mm) from the same hatchery-produced
94   cohort were out-planted in four bays throughout Western Washington State from June 21 to July
95   21, 2016: Fidalgo Bay (FB), Port Gamble Bay (PG), and Case Inlet (CI) in Puget Sound, and
96   Willapa Bay (WB) located off the southwest Pacific Coast of Washington (Table 1, Figure 1). All
97   locations were selected based on the criteria that both eelgrass beds ("eelgrass"), and
98   unvegetated sediment ("unvegetated") habitats were present. Clams were placed in 10 cm
99   diameter polymerizing vinyl chloride (PVC) pipes buried in sediment with 5 cm exposed; this
100 method replicates aquaculture techniques. Five clams were placed in each of the 3 tubes in
101 both the eelgrass and unvegetated habitat, with a total of 30 clams across 6 tubes per bay.
102 Pipes were covered with a protective mesh exclosure to limit predation. The replicate structures
103 surrounded and were equidistant to a suite of water quality sensors capturing pH, salinity,
104 dissolved oxygen, and temperature (via pH and DO probes) for the duration of the 30-day
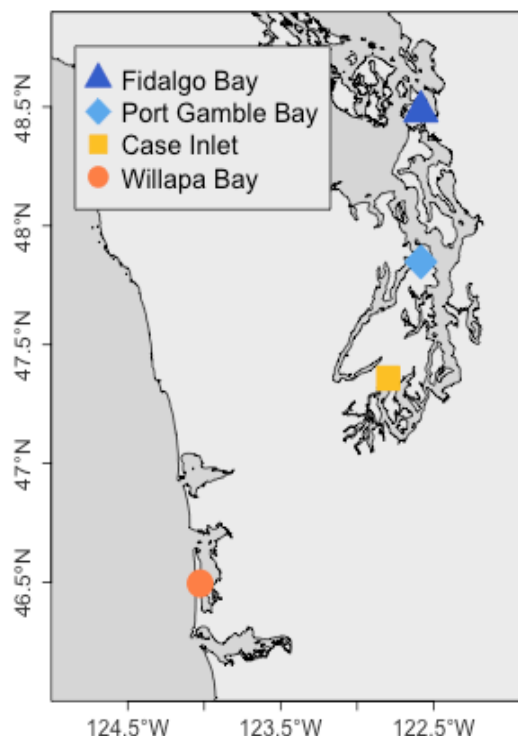105 outplant. Sensors were modified for submersible, autonomous data collection.

Figure 1: Geoduck juveniles were deployed for 30 days in 2 habitats (eelgrass beds, unvegetated) within 4 bays in Western Washington State

Bay	Eelgrass Habitat	Unvegetated Habitat
Fidalgo Bay	48° 28' 52.8312" N 122° 35' 0.7044" W	48° 28' 54.0876" N 122° 35' 0.708" W
Port Gamble Bay	47° 50' 52.7388" N 122° 34' 58.5084" W	47° 50' 33.6336" N 122° 35' 1.7952" W
Case Inlet	47° 21' 30.3808" N 122° 47' 47.2182" W	47° 21' 28.5721" N 122° 47' 44.7457" W
Willapa Bay	46° 29' 42.288" N 124° 1' 35.472" W	46° 29' 40.124" N 124° 1' 34.0882" W

Table 1: Coordinates for geoduck placement.

Collection and Sampling

Animals were collected during low tide and transferred on wet ice to shore where mortality and size were recorded. Live animals were dissected, ctenidia tissue was isolated and flash-frozen in an ethanol-dry ice bath. During sampling all instruments were sterilized between samples via bleach then ethanol, and rinsed with nanopure water. Samples were held on dry ice while transported back to the lab and stored at -80℃.

Environmental and Growth Data

Temperature, pH, salinity and dissolved oxygen data were compared between outplant locations. Outliers for all environmental parameters were removed, as determined using Tukey Interquartile Range (IQR) method (Tukey 1977), excluding data outside the inner fence (1.5*IQR). Tidal charts from WWW Tide/Current Predictor and salinity data (<20ppt) were also used to remove DO and pH data corresponding to periods of tidal exposure. Four probe failures occurred during deployment (Supplemental Table 1, *failed*) and these data were not included in the analysis (pH at Port Gamble-eelgrass, salinity at Port-Gamble unvegetated & Case-Inlet eelgrass, DO in Fidalgo Bay eelgrass). Salinity data from two additional locations was not reliably measured (Willapa Bay-unvegetated, Fidalgo Bay-unvegetated), so habitat comparisons were not performed for salinity data. For each parameter at each location, daily mean and daily standard deviation time series were calculated. Relative growth for each animal was determined as (L_f-L_i) , where L_f= final geoduck shell length, L_i = average initial geoduck shell length within each exclosure (n=5). Differences in growth and environmental parameters between habitat were compared using 2-way analysis of variance (ANOVA) applied to regression models (value ~ habitat*bay). Bays and ad-hoc regions (north vs. south bays) were tested using 1-way ANOVA. Pairwise comparisons were tested with the t-statistic. Significance for all tests was defined as $P < 0.05$, corrected for multiple comparisons using the Bonferroni correction.

Protein Analysis

Protein Preparation

Relative protein abundance was ultimately assessed in a two-phase proteomics approach using Selected Reaction Monitoring (SRM), with targets identified using Data Independent Acquisition (DIA). Tissues were prepared separately for DIA and SRM, both following the protocol in Timmins-Schiffman et al. (2014) with a few exceptions. For DIA, 8 ctenidia tissue samples were analyzed, one sample from each location and habitat: FB-eelgrass (G048), FB-unvegetated (G058), PGB-eelgrass (G077), PGB-unvegetated (G068), CI-eelgrass (G010), CI-unvegetated (G018), WB-eelgrass (G131), WB-unvegetated (G119). For SRM new ctenidia samples were examined, 12 individuals per bay (Fidalgo Bay, Port Gamble Bay, Case Inlet, Willapa Bay), with 6 from each habitat (eelgrass, unvegetated) for 48 samples total. Tissue was homogenized with sterile plastic pestle in 100 µl lysis buffer (50 mM NH_4HCO_3, 6M urea solution) and sonicated with Sonic Dismembrator (Fisher Scientific, Model 120) at 50% amplitude for ten seconds, three times. Protein concentration was quantified via Pierce™ BCA Protein Assay Kit (ThermoFischer Scientific, Waltham, MA USA).

Mini-Trypsin Digestion

Aliquots of protein (30 µg for DIA, 100 µg for SRM) were suspended in Lysis Buffer (50 mM NH_4HCO_3 + 6 M urea solution) to a total volume of 100 µl followed by: 1) a 1 hour incubation at 37°C with 200 mM Tris(2-carboxyethyl)phosphine (2.5µl) and 1.5 M Tris at pH 8.8 (6.6 µl); 2) 1 hour at room temperature in dark with 200 mM iodoacetamide (20 µl); 3) 1 hour at room temperature with 200 mM diothiothreitol (20 µl); 4) 1 hour at room temperature with 2 ug/µl Lysyl Endopeptidase (Lys-C, Wako Chemicals) (3.3 µg); 5) overnight at room temperature in 25 mM NH_4HCO_3 (800 µl) + high pressure liquid chromatography grade methanol (200 µl) + Pierce Trypsin Protease, MS Grade (1 ug/µl, Thermo Scientific) at 1:30 enzyme:protein ratio (3.3 µg). Samples were evaporated to near dryness at 4°C using a CentriVap Benchtop Vacuum Concentrator.

Desalting

Samples were desalted to isolate peptides using MacroSpin Columns (Nest Group, 50-450 µl, Peptide Protein C18). Peptides were reconstituted in 5% acetonitrile + 0.1% trifluoroacetic acid (TFA) (100 µl), then 10% formic acid (70 µl) was added to achieve pH ≤2. Columns were washed with 60% acetonitrile + 0.1% TFA (Solvent A, 200 µl) four times, then equilibrated with 5% acetonitrile + 0.1% TFA (Solvent B, 200 µl) three times. Peptides were bound to the column by running the digest through the column twice, followed by peptide elution with two additions each of Solvent A (100 ul). Columns were spun for 3 minutes at 3000 rpm on VWR Galaxy 16DH digital microcentrifuge at each stage. Samples were evaporated to near dryness at 4°C, then reconstituted in the Final Solvent (3% acetonitrile + 0.1% formic acid) (60 µl for 0.5 µg/µl final concentration of protein, and 50 µl for 2 µg/µl final concentration for DIA & SRM, respectively).

Autosampler preparation and internal standard

172 Final mixtures for mass spectrometry included 3.13 fmol/µl Peptide Retention Time Calibration
173 mixture (PRTC), 0.33 µg/µl and 0.5 µg/µl protein for DIA and SRM, respectively, in Final Solvent
174 for 15µl total volume. To confirm that peptides were quantified correctly in SRM, 10 µg from 5
175 randomly selected geoduck peptide samples were pooled, and 8 dilutions were prepared by
176 diluting with oyster peptides at known concentrations (1x, 1.3x, 2x, 4x, 6x, 8x, 8.7x, 9x) and
177 analyzed alongside other samples.

178 **Data Independent Acquisition**

179 Data acquisition

180 Data Independent Acquisition (DIA) was performed to assess global protein abundance patterns
181 and to identify consistently detectable peptides for SRM targets. Eight samples, one per
182 deployment location, were analyzed in technical duplicates via liquid chromatography tandem
183 mass spectrometry (LC-MS/MS) with the Thermo Scientific™ Orbitrap Fusion Lumos™
184 Tribrid™. Prior to sample analysis, the 30 cm analytical column and 3 cm trap were packed in-
185 house with with C18 beads (Dr. Maisch HPLC, Germany, 0.3 µm). For each sample, 3 µl of
186 protein + PRTC solution (1.0 µg of geoduck protein) was injected and analyzed in MS1 over
187 400-900 *m/z* range, in 5 *m/z* overlapping isolation windows from 450-850 *m/z* with 15K
188 resolution in MS2. Final Solvent blanks were run between each geoduck peptide injection to
189 ensure against peptide carry-over. Lumos MS/MS method and sequence files are available in
190 the project repository (GitHub).
191
192 Protein identification and analysis

193 Proteins were inferred using an assembled, translated, and annotated *P. generosa* gonad
194 transcriptome (combined male and female) (Timmins-Schiffman et al. 2017). Transcriptome
195 peptides were queried against those detected by the Lumos MS/MS using PEptide-Centric
196 Analysis (PECAN) (Ting 2017) to create a peptide spectral library (.blib type file). DIA raw files
197 were first demultiplexed using MSConvert (ProteoWizard version 3.0.10738, 2017-04-12)
198 (Chambers et al. 2012) with filters set to vendor centroiding for msLevels [2,3] (--"peakPicking
199 true 1-2"), and optimizing overlapping spectra ("demultiplex optimization-overlap only"). The
200 transcriptome fasta file was tryptic digested *in silico* in Protein Digestion Simulator (version
201 2.2.6471.25262), set to Fully Tryptic from 400-6000 fragment mass range, 5 minimum residues
202 allowed, 3 maximum missed cleavages and peak matching thresholds set to 5 ppm mass
203 tolerance, and 0.05 ppm NET tolerance. Skyline version 3.7.0.11317 (MacLean et al. 2010)
204 automatically selected transition peaks and quantified peptide abundances using peak area
205 integration. All PRTC peptide peak selections were manually verified and corrected. Skyline
206 peak selection error rate was calculated by manually checking chromatograms from 100
207 proteins across all DIA samples. Auto-selected peaks were assigned correct or incorrect
208 selection based on transition retention time alignment across replicates, using PRTC peptides
209 as a reference. Transition peak area, defined henceforth as abundance, was exported from
210 Skyline for analysis in R version 2.4-3 (R Core Team 2016). Abundance was normalized by the
211 total ion current (TIC) for each injection. Technical replicate, bay and habitat differences were
212 assessed to inform SRM analysis via non-metric multidimensional scaling (NMDS) analysis

213 using `metaNMDS` in the vegan package (Oksanen et al. n.d.) on log(x+1) transformed
214 abundances using a Bray-Curtis dissimilarity matrix. Technical replicate spectral abundances
215 clustered together on NMDS plots, thus were averaged across each sample. Bay and habitat
216 differences in global abundance were visually but not quantitatively analyzed (Supplemental
217 Figure 4).

Selected Reaction Monitoring

Target selection

220 Thirteen proteins were selected for SRM targets (Table 2). First, candidate proteins (~200) were
221 selected based on biological function listed in the Universal Protein Knowledgebase (Apweiler et
222 al. 2004) and scientific literature, emphasizing proteins involved in the cellular response to
223 oxidative stress (Matoo et al. 2013, Tomanek 2011, 2014, 2016). Candidates were screened for
224 detectability using DIA results. Selected proteins were required to have ≥3 high quality peptides,
225 each with ≥3 transitions, present in all DIA biological and technical replicate data. Quality
226 peptides had uniform peak morphology and retention time in Skyline across replicates. A total of
227 49 peptides were selected for SRM: 39 to quantify 13 target proteins (116 transitions), and 10
228 for internal standard (30 transitions). A full list of transition targets are published on
229 panoramaweb.org and available in the project repository (GitHub).

SRM Protein Targets	Top Blast Hit Uniprot SpID (E-value)	Peptide sequences
heat shock protein 90-alpha	P30946 (0)	GVVDSEDLPLNISR EVVQSSAFVER DSSTMGYMAAK
heat shock protein 70	Q91233 (0)	TTPSYVAFNDTER NAVVTVPAYFNDAQR IINEPTAAALAYGLDK
superoxide dismutase	P28757 (1e-57)	THGAPTDEER ISLTGPHSIIGR TIVVHADVDDLGK
catalase	P00432 (0)	AGELGGSDPDYAMR LYSYSDTHR LTANIAGHLIGAQEFIQK
peroxiredoxin-1	Q6B4U9 (2e-95)	ALFIIDDK QITMNDLPVGR LVQAFQFTDK
puromycin-sensitive aminopeptidase	Q11011 (0)	LNSGSVGVYR SLTENFVTEEQAK SIQQSVENIR
protein disulfide-isomerase	P07237 (0)	NNKPSDYQGGR DNVVVIGFFK MDSTANEVEDVK
ras-related protein rab-11B	O35509 (1e-85)	VVLVGDSGVGK STIGVEFATR AQLWDTAGQER
sodium/potassium-transporting ATPase subunit alpha	Q13733 (9e-62)	TVIEPMAGDGLR MVTGDNVNTAR LLDQVWPDLR

glycogen phosphorylase (muscle form)	Q9WUB3 (0)	APNSFNLR VLYPNDNFFEGK TSFDAFPDK
trifunctional enzyme β-Subunit (mitochondrial)	O46629 (0)	AAQDNGLLTDVLAYK ALELGLKPK FNLWGGSLSLGHPFGATGV R
cytochrome P450	P00185 (7e-39)	IITRPFNVNGLLAYDSR WLDESGVFLPEEHPSR QSLLPFGATGPR
arachidonate 5-lipoxygenase	P09917 (2e-94)	APGLPAQIK MDVEGTLPEDLK GLGLGGVPGQNGK

Table 2: SRM proteins targets selected based on biological function and detectability across DIA samples

Data acquisition

SRM samples were analyzed on a Vantage Triple-Stage Quadrupole Mass Spectrometer (Thermo Scientific, San Jose, CA, USA), and injected by a nanoACQUITY UPLC[®] system (Waters, Milford, MA, USA) at random in two technical replicates. For each sample, 2 µl of protein + PRTC solution (1.0 µg of geoduck protein) was injected, trapped on a 3 cm pre-column and separated on a 30 cm analytical column using a chromatography gradient of 2-60% acetonitrile over 60 minutes. Columns were prepared as in DIA (above). Samples were injected in randomized groups of 5, followed by a Peptide Retention Time Calibration (PRTC) plus bovine serum albumin peptides (BSA) standard, then Final Solvent blank. Vantage MS sequence and method files are available in the project repository (GitHub).

Protein identification and quality assurance

Peptides were identified and quantified via Skyline-daily version 3.7.1.11357 (MacLean et al. 2010). Raw SRM files were imported into a Skyline-daily project along with the target protein transitions, and the spectral library (.blib file) created previously in the DIA Protein Identification step. SRM peptides were verified by regressing PRTC peptide retention time (RT) in SRM against retention time in DIA. A fitted model from PRTC peptides predicted RT of protein target peptides. Where necessary, peak selection and boundaries were manually adjusted for SRM peptide chromatograms, and actual RT were regressed against predict RT to confirm correct selection ($F_{(1,38)}$: 5768, p-value: < 2.2e-16, Adjusted R-squared: 0.9933) (Supplemental Figure 5). Transition peak area, defined henceforth as abundance, was exported from Skyline for further analysis in R (R Core Team 2016). Abundance results from the separate serial dilution samples were used to remove peptides that did not adhere to the dilution curve. Briefly, dilution abundances (exported from Skyline) for each transition were normalized by the most dilute sample abundance, then regressed against predicted ratios. Peptides with slope coefficient 0.2<x<1.5 and adjusted R^2 >0.7 were included in analysis. Ten of the 39 peptides were discarded from the dataset based on dilution standards results (Supplemental Figure 6). To determine and remove disparate technical replicates, NMDS analysis was performed as described above. Technical replicates with ordination distance >0.2 were removed, and only samples with two technical replicates were preserved for analysis (Supplemental Figure 7). Thirteen technical replicates from different samples and all replicates from three sample were

261 discarded, for 84% technical replicate and 94% biological replicate retention. Within samples,
262 transitions with coefficients of variation (CV) > 40% between technical replicates were also
263 discarded (2% of all transitions across 21 samples). In final dataset for differential analysis, 10
264 proteins, 26 peptides, and 77 transitions were retained. Mean transition abundance was
265 calculated for replicates, with zero in the place of n/a values, which Skyline generates for
266 replicates without peaks. Transition abundances within each peptide were summed for a total
267 peptide abundance before analyzing for differential abundance.
268
269 **Differential protein analysis**
270 After data quality screening, peptide abundance was analyzed for differences between locations
271 and habitats. NMDS plots visualized patterns in peptide abundances by bay and habitat as
272 described above. Global peptide abundance was compared between bay and habitats using
273 two-way ANOVA on log-transformed abundances. For protein-specific comparisons, peptide
274 abundances were grouped by protein, box-cox transformed (Box and Cox 1964) and normality
275 confirmed via qqplot (Wickham 2017). Two-way ANOVA tested abundances for each protein
276 between eelgrass and unvegetated habitats within and between bays. Pairwise comparisons for
277 differentially abundant proteins were tested with the t-statistic. Peptides within proteins were
278 regressed against each other to confirm stable abundance patterns. For all statistical analyses,
279 significance was defined as alpha ≤ 0.05, corrected for multiple comparisons using the
280 Bonferroni correction.
281
282 **Correlative analysis**
283 To understand how environmental and biometric parameters covaried, Pearson's product-
284 moment correlation and scatter plots were assessed between protein abundances, growth, and
285 environmental summary statistics (mean and variance). Each protein was assessed
286 independently. Due to salinity probe malfunction, salinity data were not included in correlation
287 tests.
288
289 All analyses were performed in RStudio version 1.1.383 (R Core Team 2016). Data and R
290 scripts are available in the project repository, currently located at github.com/RobertsLab/Paper-
291 geoduck-eelgrass-OA, and Skyline project files are published to panoramaweb.org.

Results

293 Environmental & Growth Data
294 Mean pH differed significantly between habitats across all bays ($F(1,346)=325.1$, $p=1.6e-50$)
295 (Figure 2). During the deployment pH was recorded from 6.71 to 8.34, with mean pH 7.86±0.15
296 in eelgrass, and 7.51±0.25 in unvegetated habitats. Variability in pH was significantly different
297 between bays ($F(3,346=52.7$, $p=8.4e-27$), but variability did not differ between habitats. On
298 average across all locations, pH fluctuated daily by 0.46±0.23 pH units. Considerable
299 heterogeneity between bays was observed in the other environmental parameters. Mean
300 temperature was significantly different between all bays ($F(3,396) =192.7$, $p=7.6e-76$), and
301 temperature decreased with latitude (coldest in Fidalgo Bay, warmest in Willapa bay).
302 Temperature did not differ between habitats within bays (Supplemental Figure 1). Dissolved

303   oxygen (DO) varied between bays in both daily standard deviation ($F_{(3,370)}=170.4$, $p=1.9e-71$)

304   and mean ($F_{(3,370)}=97.3$, $p=3.1e-45$). DO variability was substantially higher in the two

305   northern bays (SD was 5.6 and 3.9 mg/L in FB, PGB), as compared to the southern bays (2.5

306   and 1.4mg/L in CI, WB), but variability did not differ between habitats (Supplemental Figure 2).

307   Mean salinity differed by bay ($F_{(3,253)}=456.9$, $p=1.4e-100$), with the largest differences

308   between Fidalgo Bay (mean 29.9 ppt) and the other three bays (mean 23.4-27.0 ppt)

309   (Supplemental Figure 3). Growth significantly differed between northern and southern bays

310   ($F_{(1,97)}=54.8$, $P=4.9-11$), but not between habitats either within or across all bays. Geoduck in

311   Fidalgo Bay and Port Gamble Bay grew larger compared to Willapa Bay, and Case Inlet (Figure

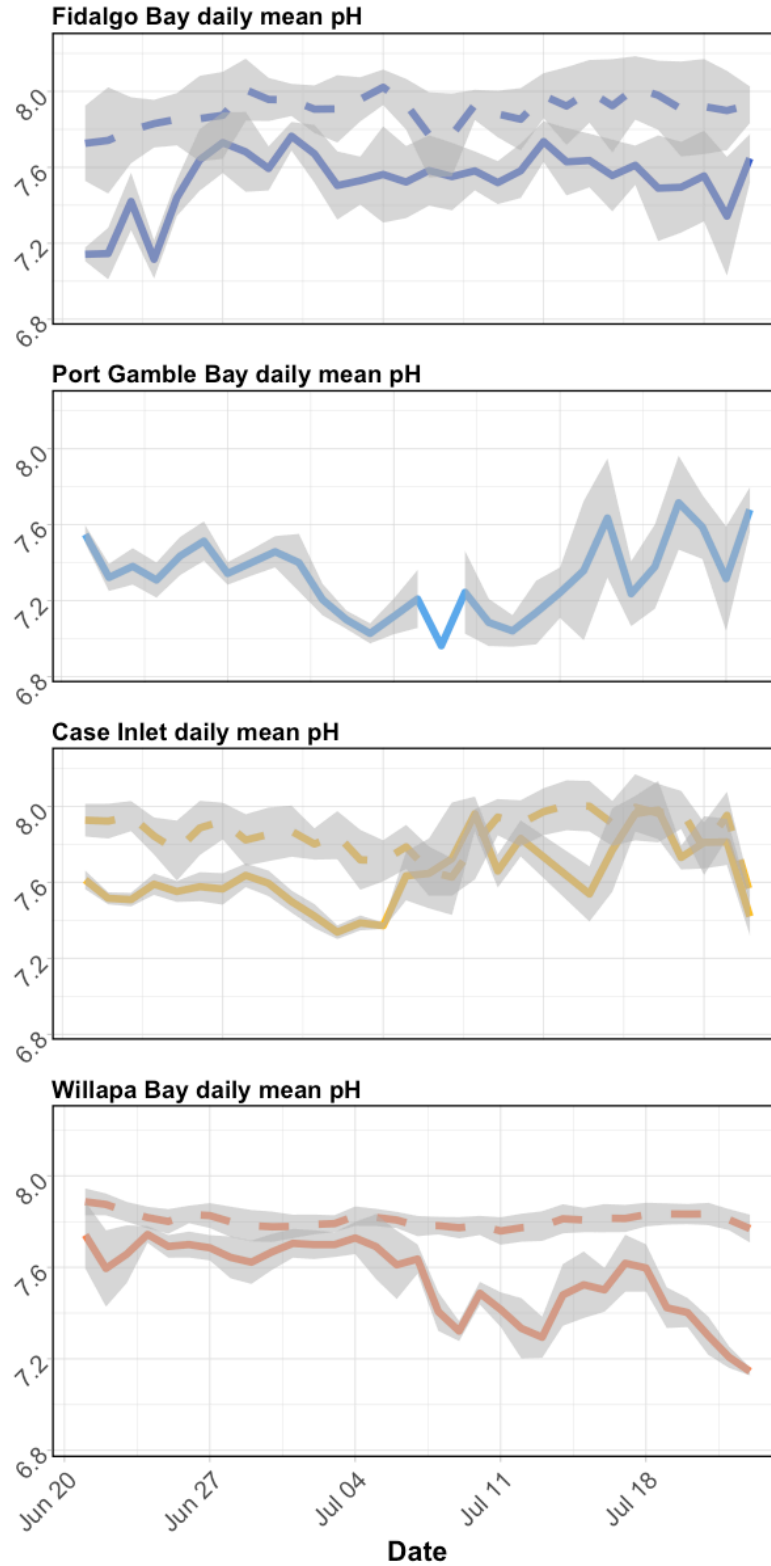312   3). Survival did not differ among locations.

Figure 2: Daily mean pH in eelgrass (dashed lines) and unvegetated (solid lines) across bays during geoduck deployment. Gray ribbons denote daily standard deviation. Data from Port Gamble bay eelgrass are not included due to probe failure.
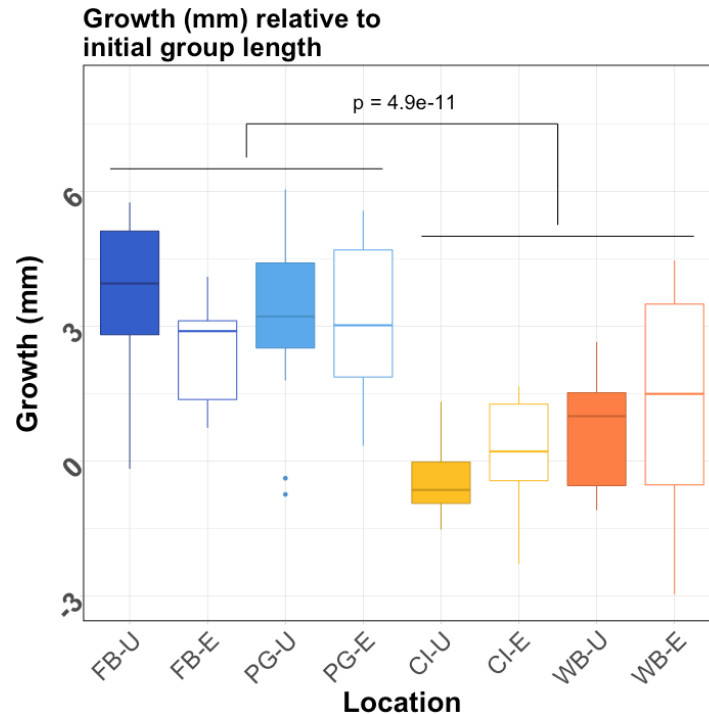
Figure 3: Geoduck shell growth after 30 days across Willapa Bay (WB), Case Inlet (CI), Port Gamble Bay (PG), and Fidalgo Bay (FB), where -U and -E represent unvegetated (filled boxes) and eelgrass habitats (empty boxes), respectively. Growth is relative to the mean initial shell length within deployment groups (n=5 per group, 3 groups per location). Geoduck that did not survive deployment are not included. Growth differed significantly between southern bays (WB, CI) and northern bays (PG, FB).

Protein Detection and Variance

In DIA, a total of 298,345 peptide transitions were detected from 30,659 distinct peptides across the 8 samples (one sample per habitat from each bay). These peptides were associated with 8,077 proteins, and more than half of the proteins (4,252) were annotated using Universal Protein Resource database (UniProt). Automated peak selection (Skyline) success rate was 71%.

In SRM, final dataset after screening included 10 proteins, 26 peptides, and 77 transitions. The 3 proteins fully screened from the dataset were heat shock protein 70, peroxiredoxin-1, and ras-related rab. The SRM mean coefficients of variation (CV) of technical replicate abundances across all transitions decreased from 18.2% to 9.6% after screening. Transition abundance CV within bays ranged from 24.9% to 83.2% with mean 50.1%, and within deployment locations CV ranged from 11.6% to 93.0% with mean 48.9% (Supplemental Table 3). Within proteins, regression analysis indicated that peptide abundances from the same protein differed slightly, however the relative abundances across samples was consistent. This indicates a small degree of background variability in peptide detection, digestion, or stability within proteins that applied to all samples (Pep1xPep2: R^2_A=0.985, coefficient=0.682; Pep1xPep3: R^2_A=0.990, coefficient=0.954; Pep2xPep3: R^2_A=0.990, coefficient=0.954).

Protein Abundance Differences

332 None of the 10 targeted proteins were significantly different between habitats within or across

333 bays (Figure 4, Supplemental Table 2). NMDS plots of all transitions in DIA and those targeted

334 in SRM revealed clustering of overall proteomic response by bay (Supplemental Figures 4 & 8).

335 In SRM, Fidalgo Bay and Port Gamble Bay samples clustered together (henceforth "northern

336 bays"), and some overlap between Case Inlet and Willapa Bay ("southern bays") indicated

337 similar protein abundances within these ad-hoc regions (Supplemental Figure 8). This was

338 verified from the ANOVA results, which detected significant differences between northern and

339 southern bays in three proteins: HSP90-α (HSP90) ($F_{(1,133)}$=20.5, p-adj=1.8e-4), trifunctional-

340 enzyme subunit β-subunit (TEβ) ($F_{(1,88)}$=11.1, p-adj=0.018), and puromycin-sensitive

341 aminopeptidase (PSA) ($F_{(1,130)}$=9.11, p-adj=0.043). HSP90 and TEβ were also significantly

342 different between bays (respectively: $F_{(3,131)}$= 7.80, p-adj=0.0011; $F_{(3,345)}$= 5.19, p-

343 adj=0.034). For these three proteins, abundance was lowest in Case Inlet (southernmost in

344 Puget Sound) followed by WIllapa Bay (southernmost overall), then Port Gamble Bay, and
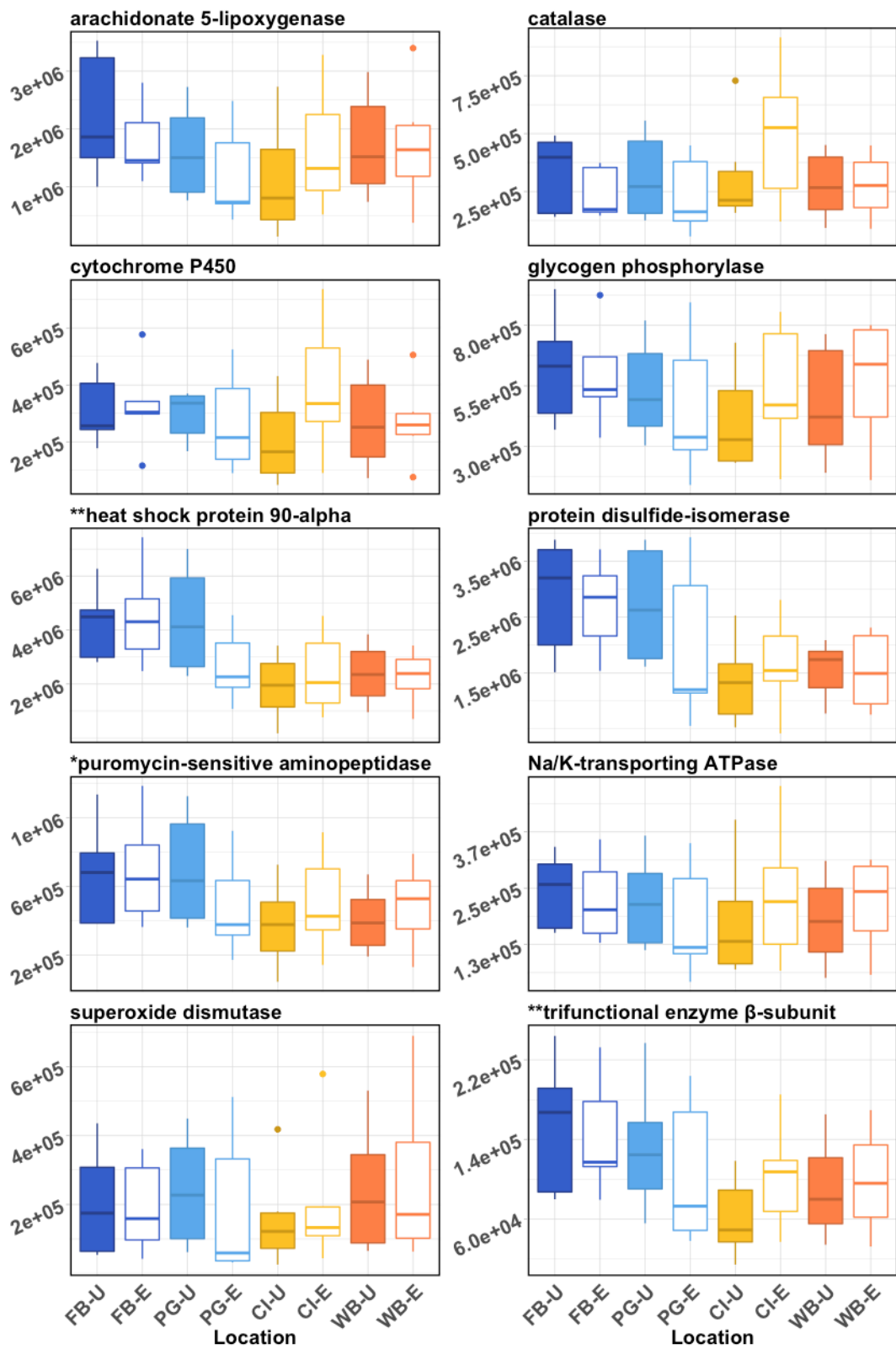
345 highest in Fidalgo Bay (northernmost).

346   Correlation between Environment, Abundance, and Growth

347   Growth positively correlated with peptide abundance for 8 of the 10 targeted proteins, including

348   the three proteins that were differentially abundant between bays (Table 3). Growth also

349   also correlated with most environmental parameters (excluding salinity SD). Heat Shock Protein 90

350   correlated positively with dissolved oxygen SD. Mean and SD pH did not correlate with any

351   peptide abundance patterns or growth. None of the environmental parameters, nor growth,

352   correlated significantly with peptide abundances pooled across all proteins.

	Growth	heat shock protein 90	puromycin sensitive aminopeptidase	trifunctional enzyme β-subunit
Growth	---	**r=0.55** **p=2.15e-11**	**r=0.48** **p=6.67e-9**	**r=0.41** **p=8.59e-6**
T_{mean}	**r= -0.39** **p=8.11e-21**	r= -0.36 p=0.060	r= -0.24 p=0.48	r= -0.21 p=0.70
T_{sd}	**r= 0.39** **p=2.10e-20**	r= 0.24 p=0.42	r= 0.15 p=1	r= 0.11 p= 1
DO_{mean}	**r= 0.45** **p=2.12e-28**	r= 0.26 p=0.34	r=0.18 p=0.91	r=0.21 p=0.66
DO_{sd}	**r= 0.48** **p=2.64e-32**	**r=0.41** **p=0.021**	r=0.31 p=0.16	r=0.31 p=0.15
pH_{mean}	**r= -0.32** **p=1.08e-12**	r= -0.09 p=1	r=-0.02 p=1	r=-0.03 p=1
pH_{sd}	**r= 0.30** **p=8.72e-11**	r= 0.22 p=0.73	r=0.06 p=1	r=0.04 p=1

Table 3: Correlation analysis results between growth, environmental parameters, and peptide abundance (z-transformed). Correlation coefficient r shown with p-values adjusted via Bonferroni correction (number of comparisons). Correlations deemed significant are in bold.

Discussion

354   This study tested the effects of varying pH on geoduck, a valuable aquaculture species

355   in a natural setting, and confirmed that *Zostera marina* eelgrass can effectively alter local pH

356   during warm summer months (June and July). With this design, future ocean acidification

357 research on cultured shellfish can augment findings from controlled laboratory studies with field
358 deployments to incorporate natural variability associated with an organism's habitat. Global and
359 targeted proteomics was assessed alongside growth and environmental data for an integrated
360 view of how geoduck respond to varying environmental conditions. Proteomics is a powerful
361 approach suitable for comparative physiological studies of non-model, cultured fishes. Using a
362 two step method, this study detected substantially more proteins (8,077) compared to the
363 previous geoduck study using Data Dependent Analysis (3,651) (Timmins-Schiffman et al.
364 2017). This produced a valuable protein catalogue for future projects, as researchers can now
365 skip directly to the targeted SRM phase to greatly reduce the cost and time associated with a
366 discovery analysis.
367   Geoduck exhibited no phenotypic differences between pH conditions, counter to our
368 predictions. We predicted that within eelgrass habitats pH would be higher, creating a refuge
369 against the less alkaline surrounding waters and reducing oxidative stress. Concordantly,
370 proteins involved in the oxidative stress response would be less abundant inside eelgrass
371 habitats (such as superoxide dismutase, peroxiredoxin-1, catalase, and HSP), possibly
372 translating to differential growth. While pH in eelgrass habitats was found to be consistently
373 higher in this study, no differences in protein abundance, growth or survival were found between
374 habitats across all four bays. This suggests that juvenile geoduck may tolerate a wide pH range
375 in the context of the natural environment in which they are cultured.
376   Earlier studies on other clam species point to some degree of pH tolerance, but also
377 describe complex responses to low pH that vary between metrics, species, and when secondary
378 stressors are applied (Fernández-Reiriz et al. 2011; Ivanina et al. 2013; Matoo et al. 2013;
379 Range et al. 2011; Ries et al. 2009; Ringwood and Keppler 2002; Waldbusser et al. 2010). For
380 example, juvenile carpet shell clams (*Ruditapes decussatus*) under ambient (8.2) and reduced
381 pH (7.8, 7.5) for 75 days displayed no difference in size, weight, or calcification rate (Range et
382 al. 2011), but other physiological parameters (clearance, ingestion, respiration, ammonia
383 excretion) differed at day 87 (Fernández-Reiriz et al. 2011). In the hard shell clam *Mercenaria
384 mercenaria,* protein oxidation, biomineralization, and standard metabolic rate (SMR) in adults
385 were largely unaffected by hypercapnia alone, but when combined with elevated temperature
386 SMR increased and shell strength decreased (Ivanina et al. 2013; Matoo et al. 2013).
387 Interestingly, the baltic clam (*Macoma balthica*) grew significantly larger in low pH (7.35 vs. 7.85
388 for 29 days), and were largest when combined with low dissolved oxygen (3.0 mg/L vs. 8.5
389 mg/L) (Jansson et al. 2015). Geoduck metrics examined in this study were not affected by
390 varying pH, but other physiological parameters may be affected (metabolic rate,
391 biomineralization, reproductive development, cytoskeleton), and should be examined in future
392 studies.
393   The complex, mixed responses exhibited in clam species may, in part, be a function of
394 local adaptation to varying environmental drivers. Pacific geoduck are native to the Puget
395 Sound, a region that experiences regular episodes of low pH in certain areas (Busch et al. 2013;
396 Feely et al. 2010, 2008), and thus the species may have evolved under selective pressure to
397 withstand periods of low pH. The native Northeast Pacific Coast oyster, *Ostrea lurida,* also
398 shows signs of pH tolerance as veliger larvae compared to non-native *Crassostrea gigas*
399 (Waldbusser et al. 2016), a stage primarily found to be vulnerable in other calcifying species (for
400 reviews see Byrne and Przeslawski 2013; Kurihara 2008). Geoduck are also infaunal

401 organisms, extending their long siphons into the water column for feeding and retreating to deep
402 burrows during low tide or when disturbed (Goodwin and Pease 1987). Sediment and burrow
403 chemistry, while influenced by the overlying water column, can have lower pH due to aerobic
404 microbial activity (Gattuso and Hansson 2011), another potential source of selective pressure
405 shaping this giant clam's pH tolerance (Gattuso and Hansson 2011; Widdicombe and Spicer
406 2008). An important future step is to assess the relative influence of sediment pH and overlying
407 water column pH on burrowing calcifiers. This is particularly applicable when comparing habitats
408 that likely have varying bacterial communities and activity.
409   While pH was not a universal predictor of geoduck phenotype in this study, mean
410 temperature and dissolved oxygen variability correlated significantly with biometric parameters
411 and separated into two groups: northern bays (Fidalgo and Port Gamble Bays), and southern
412 bays (Case Inlet, and Willapa Bay). Geoduck grew less (or not at all, in Case Inlet) with lower
413 protein levels in the southern bays, which were warmer with less variable dissolved oxygen
414 content.
415   Temperatures in the southern bays (16-18°C) during the deployment dates may have
416 been thermally stressful, depleting energy reserves due to elevated metabolism and denatured
417 protein repair, particularly if food availability was limited (Lesser et al. 2010; Newell and Branch
418 1980; Thomsen et al. 2013). Proteomics data from the southern bays did not suggest acute
419 stress as key stress-response proteins, HSP90 and HSP70, were inversely related to
420 temperature, however tissues were collected at day 30 and a stress signal could have been
421 captured with earlier or more frequent samples. Conversely, ectothermic organisms
422 acclimatized to lower temperature can compensate for depressed enzymatic reaction rates by
423 increasing concentrations of proteins (Lesser and Kruse 2004), and this may be at play in the
424 protein data from geoduck placed in northern, colder bays. Temperature also tends to control
425 microbial communities in the ocean, which could have been a factor influencing geoduck growth
426 (Sunagawa et al. 2015).
427   Whether dissolved oxygen (DO) variability could have influenced geoduck in this study is
428 less clear. Less DO fluctuation in the southern bays could be an indication of less phytoplankton
429 biomass, translating to lower food availability (Anderson and Taylor 2001; Bergondo et al. 2005;
430 Winter et al. 1975). While we were unable to monitor chlorophyll during the outplant, both
431 southern bays, Willapa Bay and Case Inlet, may have phytoplankton populations that are
432 controlled by shellfish grazers due to long residence times and aquaculture activity (Banas et al.
433 2007; Washington Sea Grant 2015). It is possible that food availability was different between
434 northern and southern locations during the outplant period (June-July), and could be the
435 underlying cause of higher growth and protein abundances in the northern sites (Carmichael et
436 al. 2004; Liu et al. 2016; Loosanoff and Davis 1963), although this warrants additional data
437 collection.

Conclusion

439 This is the first study to investigate geoduck performance alongside varying pH conditions, and
440 contributes a geoduck peptide database useful for quantifying multiple proteins simultaneously.
441 The primary finding is that geoduck aquaculture may be less impacted by ocean acidification
442 compared to other environmental stressors, for example ocean warming. Geoduck ocean

443 acidification research is in its infancy, and these results are a snapshot into geoduck physiology
444 at one developmental stage, with individuals from one genetic pool, and with present-day pH
445 levels in Washington State. To best inform current and future geoduck aquaculture, further
446 foundational studies are needed to elucidate the species' pH limits in conjunction with more
447 acute environmental stressors.
448   This study also demonstrates applied use of systems such as eelgrass beds in estuaries
449 to test pH effects in a natural system. There is growing interest in using macroalgae as an
450 ocean acidification bioremediation tool, also known as phytoremediation (Greiner et al. 2013;
451 Groner et al. 2018; Hendriks et al. 2014; Washington State Blue Ribbon Panel on Ocean
452 Acidification 2012). Incorporating seagrass into shellfish-pH interaction studies can help
453 evaluate the potential for merging mariculture with shellfish aquaculture to improve growing
454 conditions for vulnerable cultured species.

Reference List

464 Abatzoglou, John T., David E. Rupp, and Philip W. Mote. 2013. "Seasonal Climate Variability
465   and Change in the Pacific Northwest of the United States." *Journal of Climate* 27 (5): 2125–
466   42. https://doi.org/10.1175/JCLI-D-13-00218.1.
467 Anderson, Timothy H., and Gordon T. Taylor. 2001. "Nutrient Pulses, Plankton Blooms, and
468   Seasonal Hypoxia in Western Long Island Sound." *Estuaries* 24 (2): 228–43.
469   https://doi.org/10.2307/1352947.
470 Apweiler, Rolf, Amos Bairoch, Cathy H. Wu, Winona C. Barker, Brigitte Boeckmann, Serenella
471   Ferro, Elisabeth Gasteiger, et al. 2004. "UniProt: The Universal Protein Knowledgebase."
472   *Nucleic Acids Research* 32 (Database issue): D115–19. https://doi.org/10.1093/nar/gkh131.
473 Babson, A. L., M. Kawase, and P. MacCready. 2006. "Seasonal and Interannual Variability in
474   the Circulation of Puget Sound, Washington: A Box Model Study." *Atmosphere-Ocean* 44
475   (1): 29–45. https://doi.org/10.3137/ao.440103.
476 Banas, N. S., B. M. Hickey, P. MacCready, and J. A. Newton. 2004. "Dynamics of Willapa Bay,
477   Washington: A Highly Unsteady, Partially Mixed Estuary." *Journal of Physical
478   Oceanography* 34 (11): 2413–27. https://doi.org/10.1175/JPO2637.1.
479 Banas, N. S., B. M. Hickey, J. A. Newton, and J. L. Ruesink. 2007. "Tidal Exchange, Bivalve
480   Grazing, and Patterns of Primary Production in Willapa Bay, Washington, USA." *Marine
481   Ecology Progress Series* 341: 123–39. https://doi.org/10.3354/meps341123.

482   Ban, Stephen S., Nicholas A. J. Graham, and Sean R. Connolly. 2014. "Evidence for Multiple
483   Stressor Interactions and Effects on Coral Reefs." *Global Change Biology* 20 (3): 681–97.
484   https://doi.org/10.1111/gcb.12453.

485   Baumann, Hannes, Ryan B. Wallace, Tristen Tagliaferri, and Christopher J. Gobler. 2015.
486   "Large Natural pH, CO2 and O2 Fluctuations in a Temperate Tidal Salt Marsh on Diel,
487   Seasonal, and Interannual Time Scales." *Estuaries and Coasts* 38 (1): 220–31.
488   https://doi.org/10.1007/s12237-014-9800-y.

489   Bergondo, Deanna L., Dana R. Kester, Heather E. Stoffel, and Wendy L. Woods. 2005. "Time-
490   Series Observations during the Low Sub-Surface Oxygen Events in Narragansett Bay
491   during Summer 2001." *Marine Chemistry* 97 (1): 90–103.
492   https://doi.org/10.1016/j.marchem.2005.01.006.

493   Box, G. E. P., and D. R. Cox. 1964. "An Analysis of Transformations." *Journal of the Royal
494   Statistical Society. Series B, Statistical Methodology* 26 (2): 211–52.
495   http://www.jstor.org/stable/2984418.

496   Boyd, Philip W., Christopher E. Cornwall, Andrew Davison, Scott C. Doney, Marion Fourquez,
497   Catriona L. Hurd, Ivan D. Lima, and Andrew McMinn. 2016. "Biological Responses to
498   Environmental Heterogeneity under Future Ocean Conditions." *Global Change Biology* 22
499   (8): 2633–50. https://doi.org/10.1111/gcb.13287.

500   Bulthuis, Douglas A. 1995. "Distribution of Seagrasses in a North Puget Sound Estuary: Padilla
501   Bay, Washington, USA." *Aquatic Botany* 50 (1): 99–105. https://doi.org/10.1016/0304-
502   3770(94)00443-P.

503   Busch, D. Shallin, Chris J. Harvey, and Paul McElhany. 2013. "Potential Impacts of Ocean
504   Acidification on the Puget Sound Food Web." *ICES Journal of Marine Science: Journal Du
505   Conseil* 70 (4): 823–33. https://doi.org/10.1093/icesjms/fst061.

506   Byrne, Maria, and Rachel Przeslawski. 2013. "Multistressor Impacts of Warming and
507   Acidification of the Ocean on Marine Invertebrates' Life Histories." *Integrative and
508   Comparative Biology* 53 (4): 582–96. https://doi.org/10.1093/icb/ict049.

509   Carmichael, R. H., Andrea C. Shriver, and I. Valiela. 2004. "Changes in Shell and Soft Tissue
510   Growth, Tissue Composition, and Survival of Quahogs, Mercenaria Mercenaria, and
511   Softshell Clams, Mya Arenaria, in Response to Eutrophic-Driven Changes in Food Supply
512   and Habitat." *Journal of Experimental Marine Biology and Ecology* 313 (1): 75–104.
513   https://doi.org/10.1016/j.jembe.2004.08.006.

514   Chambers, Matthew C., Brendan Maclean, Robert Burke, Dario Amodei, Daniel L. Ruderman,
515   Steffen Neumann, Laurent Gatto, et al. 2012. "A Cross-Platform Toolkit for Mass
516   Spectrometry and Proteomics." *Nature Biotechnology* 30 (10): 918–20.
517   https://doi.org/10.1038/nbt.2377.

518   Chapman, Robert W., Annalaura Mancia, Marion Beal, Artur Veloso, Charles Rathbun, Anne
519   Blair, A. F. Holland, et al. 2011. "The Transcriptomic Responses of the Eastern Oyster,
520   Crassostrea Virginica, to Environmental Conditions." *Molecular Ecology* 20 (7): 1431–49.
521   https://doi.org/10.1111/j.1365-294X.2011.05018.x.

522   Clark, Dana, Miles Lamare, and Mike Barker. 2009. "Response of Sea Urchin Pluteus Larvae
523   (Echinodermata: Echinoidea) to Reduced Seawater pH: A Comparison among a Tropical,
524   Temperate, and a Polar Species." *Marine Biology* 156 (6): 1125–37.
525   https://doi.org/10.1007/s00227-009-1155-8.

526 Coan, Eugene V., Paul Valentich Scott, and Frank R. Bernard. 2000. *Bivalve Seashells of*
527 *Western North America: Marine Bivalve Mollusks from Arctic Alaska to Baja California*.
528 Santa Barbara Museum of Natural History.
529 Cornwall, Christopher E., Christopher D. Hepburn, Christina M. McGraw, Kim I. Currie, Conrad
530 A. Pilditch, Keith A. Hunter, Philip W. Boyd, and Catriona L. Hurd. 2013. "Diurnal
531 Fluctuations in Seawater pH Influence the Response of a Calcifying Macroalga to Ocean
532 Acidification." *Proc. R. Soc. B* 280 (1772): 20132201.
533 https://doi.org/10.1098/rspb.2013.2201.
534 DeBiasse, Melissa B., and Morgan W. Kelly. 2016. "Plastic and Evolved Responses to Global
535 Change: What Can We Learn from Comparative Transcriptomics?" *The Journal of Heredity*
536 107 (1): 71–81. https://doi.org/10.1093/jhered/esv073.
537 Dethier, Megan N., Tom Mumford, Tom Leschine, Kurt Presh, Si Simenstad, Hugh Shipman,
538 Doug Myers, et al. 2006. "Native Shellfish in Nearshore Ecosystems of Puget Sound."
539 WASHINGTON UNIV SEATTLE. http://www.dtic.mil/docs/citations/ADA477852.
540 Dethier, Megan N., Jennifer Ruesink, Helen Berry, Amy G. Sprenger, and Blain Reeves. 2010.
541 "Restricted Ranges in Physical Factors May Constitute Subtle Stressors for Estuarine Biota."
542 *Marine Environmental Research* 69 (4): 240–47.
543 https://doi.org/10.1016/j.marenvres.2009.10.015.
544 Doney, Scott C., Natalie Mahowald, Ivan Lima, Richard A. Feely, Fred T. Mackenzie, Jean-
545 Francois Lamarque, and Phil J. Rasch. 2007. "Impact of Anthropogenic Atmospheric
546 Nitrogen and Sulfur Deposition on Ocean Acidification and the Inorganic Carbon System."
547 *Proceedings of the National Academy of Sciences of the United States of America* 104 (37):
548 14580–85. https://doi.org/10.1073/pnas.0702218104.
549 Duquette, Ashley, James B. McClintock, Charles D. Amsler, Alberto Pérez-Huerta, Marco
550 Milazzo, and Jason M. Hall-Spencer. 2017. "Effects of Ocean Acidification on the Shells of
551 Four Mediterranean Gastropod Species near a CO2 Seep." *Marine Pollution Bulletin* 124
552 (2): 917–28. https://doi.org/10.1016/j.marpolbul.2017.08.007.
553 Feely, Richard A., Simone R. Alin, Jan Newton, Christopher L. Sabine, Mark Warner, Allan
554 Devol, Christopher Krembs, and Carol Maloy. 2010. "The Combined Effects of Ocean
555 Acidification, Mixing, and Respiration on pH and Carbonate Saturation in an Urbanized
556 Estuary." *Estuarine, Coastal and Shelf Science* 88 (4): 442–49.
557 https://doi.org/10.1016/j.ecss.2010.05.004.
558 Feely, Richard A., Terrie Klinger, Jan A. Newton, and Meg Chadsey. 2012. *Scientific Summary*
559 *of Ocean Acidification in Washington State Marine Waters*. US Department of Commerce,
560 National Oceanic and Atmospheric Administration, Office of Oceanic and Atmospheric
561 Research.
562 https://pdfs.semanticscholar.org/5964/ffc14ae680fc855864a55b10351ed86e5935.pdf.
563 Feely, Richard A., Christopher L. Sabine, J. Martin Hernandez-Ayon, Debby Ianson, and Burke
564 Hales. 2008. "Evidence for Upwelling of Corrosive' Acidified' Water onto the Continental
565 Shelf." *Science* 320 (5882): 1490–92.
566 http://science.sciencemag.org/content/320/5882/1490.short.
567 Fernández-Reiriz, Ma José, Pedro Range, Xosé Antón Álvarez-Salgado, and Uxio Labarta.
568 2011. "Physiological Energetics of Juvenile Clams Ruditapes Decussatus in a High CO_2
569 Coastal Ocean." *Marine Ecology Progress Series* 433: 97–105.

570   http://www.jstor.org/stable/24875417.

571   Frieder, Christina A., Jennifer P. Gonzalez, Emily E. Bockmon, Michael O. Navarro, and Lisa A.

572   Levin. 2014. "Can Variable pH and Low Oxygen Moderate Ocean Acidification Outcomes for

573   Mussel Larvae?" *Global Change Biology* 20 (3): 754–64. https://doi.org/10.1111/gcb.12485.

574   Gattuso, Jean-Pierre, and Lina Hansson. 2011. *Ocean Acidification*. OUP Oxford.

575   https://market.android.com/details?id=book-eoxpAgAAQBAJ.

576   Gazeau, Frédéric, Christophe Quiblier, Jeroen M. Jansen, Jean-Pierre Gattuso, Jack J.

577   Middelburg, and Carlo H. R. Heip. 2007. "Impact of Elevated CO2 on Shellfish Calcification."

578   *Geophysical Research Letters* 34 (7): L07603. https://doi.org/10.1029/2006GL028554.

579   Gobler, Christopher J., Elizabeth L. DePasquale, Andrew W. Griffith, and Hannes Baumann.

580   2014. "Hypoxia and Acidification Have Additive and Synergistic Negative Effects on the

581   Growth, Survival, and Metamorphosis of Early Life Stage Bivalves." *PloS One* 9 (1): e83648.

582   https://doi.org/10.1371/journal.pone.0083648.

583   Goodwin, C. Lynn, and Bruce Pease. 1987. *The Distribution of Geoduck (Panope Abrupta)*

584   *Size, Density, and Quality in Relation to Habitat Characteristics such as Geographic Area,*

585   *Water Depth, Sediment Type, and Associated Flora and Fauna in Puget Sound,*

586   *Washington*. State of Washington, Department of Fisheries, Shellfish Division.

587   Grant, Washington Sea. 2013. "Final Report: Geoduck Aquaculture Research Program." *Report*

588   *to the Washington State Legislature. Washington Sea Grant Technical Report WSG-TR*,

589   13–03.

590   Greiner, Jill T., Karen J. McGlathery, John Gunnell, and Brent A. McKee. 2013. "Seagrass

591   Restoration Enhances 'Blue Carbon' Sequestration in Coastal Waters." *PloS One* 8 (8):

592   e72469. https://doi.org/10.1371/journal.pone.0072469.

593   Groner, Maya L., Colleen A. Burge, Ruth Cox, Natalie Rivlin, Mo Turner, Kathryn L. Van

594   Alstyne, Sandy Wyllie-Echeverria, John Bucci, Philip Staudigel, and Carolyn S. Friedman.

595   2018. "Oysters and Eelgrass: Potential Partners in a High pCO2 Ocean." *Ecology*, May.

596   https://doi.org/10.1002/ecy.2393.

597   Gunderson, Alex R., Eric J. Armstrong, and Jonathon H. Stillman. 2016. "Multiple Stressors in a

598   Changing World: The Need for an Improved Perspective on Physiological Responses to the

599   Dynamic Marine Environment." *Annual Review of Marine Science* 8: 357–78.

600   https://doi.org/10.1146/annurev-marine-122414-033953.

601   Harvey, Ben P., Dylan Gwynn-Jones, and Pippa J. Moore. 2013. "Meta-Analysis Reveals

602   Complex Marine Biological Responses to the Interactive Effects of Ocean Acidification and

603   Warming." *Ecology and Evolution* 3 (4): 1016–30. https://doi.org/10.1002/ece3.516.

604   Hendriks, Iris E., Y. S. Olsen, L. Ramajo, L. Basso, A. Steckbauer, T. S. Moore, J. Howard, and

605   C. M. Duarte. 2014. "Photosynthetic Activity Buffers Ocean Acidification in Seagrass

606   Meadows." *Biogeosciences* 11 (2): 333.

607   http://search.proquest.com/openview/dd8bd20d2b762c317796efc36e6dd278/1?pq-

608   origsite=gscholar&cbl=105740.

609   Hofmann, Gretchen E., Jennifer E. Smith, Kenneth S. Johnson, Uwe Send, Lisa A. Levin,

610   Fiorenza Micheli, Adina Paytan, et al. 2011. "High-Frequency Dynamics of Ocean pH: A

611   Multi-Ecosystem Comparison." *PloS One* 6 (12): e28983.

612   https://doi.org/10.1371/journal.pone.0028983.

613   Howarth, Robert, Francis Chan, Daniel J. Conley, Josette Garnier, Scott C. Doney, Roxanne

614   Marino, and Gilles Billen. 2011. "Coupled Biogeochemical Cycles: Eutrophication and
615   Hypoxia in Temperate Estuaries and Coastal Marine Ecosystems." *Frontiers in Ecology and*
616   *the Environment* 9 (1): 18–26. https://doi.org/10.1890/100008.
617   Ivanina, Anna V., Gary H. Dickinson, Omera B. Matoo, Rita Bagwe, Ashley Dickinson, Elia
618   Beniash, and Inna M. Sokolova. 2013. "Interactive Effects of Elevated Temperature and
619   CO2 Levels on Energy Metabolism and Biomineralization of Marine Bivalves Crassostrea
620   Virginica and Mercenaria Mercenaria." *Comparative Biochemistry and Physiology. Part A,*
621   *Molecular & Integrative Physiology* 166 (1): 101–11.
622   https://doi.org/10.1016/j.cbpa.2013.05.016.
623   Jansson, Anna, Joanna Norkko, Sam Dupont, and Alf Norkko. 2015. "Growth and Survival in a
624   Changing Environment: Combined Effects of Moderate Hypoxia and Low pH on Juvenile
625   Bivalve Macoma Balthica." *Journal of Sea Research* 102 (August): 41–47.
626   https://doi.org/10.1016/j.seares.2015.04.006.
627   Kerrison, Philip, Jason M. Hall-Spencer, David J. Suggett, Leanne J. Hepburn, and Michael
628   Steinke. 2011. "Assessment of pH Variability at a Coastal CO2 Vent for Ocean Acidification
629   Studies." *Estuarine, Coastal and Shelf Science* 94 (2): 129–37.
630   https://doi.org/10.1016/j.ecss.2011.05.025.
631   Kroeker, Kristy J., Rebecca L. Kordas, Ryan Crim, Iris E. Hendriks, Laura Ramajo, Gerald S.
632   Singh, Carlos M. Duarte, and Jean-Pierre Gattuso. 2013. "Impacts of Ocean Acidification on
633   Marine Organisms: Quantifying Sensitivities and Interaction with Warming." *Global Change*
634   *Biology* 19 (6): 1884–96. https://doi.org/10.1111/gcb.12179.
635   Kroeker, Kristy J., Rebecca L. Kordas, Ryan N. Crim, and Gerald G. Singh. 2010. "Meta-
636   Analysis Reveals Negative yet Variable Effects of Ocean Acidification on Marine
637   Organisms." *Ecology Letters* 13 (11): 1419–34. https://doi.org/10.1111/j.1461-
638   0248.2010.01518.x.
639   Kurihara, H. 2008. "Effects of CO2-Driven Ocean Acidification on the Early Developmental
640   Stages of Invertebrates." *Marine Ecology Progress Series* 373 (December): 275–84.
641   https://doi.org/10.3354/meps07802.
642   Lesser, Michael P., Meredith A. Bailey, Daniel G. Merselis, and John R. Morrison. 2010.
643   "Physiological Response of the Blue Mussel Mytilus Edulis to Differences in Food and
644   Temperature in the Gulf of Maine." *Comparative Biochemistry and Physiology. Part A,*
645   *Molecular & Integrative Physiology* 156 (4): 541–51.
646   https://doi.org/10.1016/j.cbpa.2010.04.012.
647   Lesser, Michael P., and Valerie A. Kruse. 2004. "Seasonal Temperature Compensation in the
648   Horse Mussel, Modiolus Modiolus: Metabolic Enzymes, Oxidative Stress and Heat Shock
649   Proteins." *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative*
650   *Physiology* 137 (3): 495–504. https://doi.org/10.1016/j.cbpb.2003.10.022.
651   Liu, W., C. M. Pearce, R. S. McKinley, and I. P. Forster. 2016. "Nutritional Value of Selected
652   Species of Microalgae for Larvae and Early Post-Set Juveniles of the Pacific Geoduck Clam,
653   Panopea Generosa." *Aquaculture* 452 (Supplement C): 326–41.
654   https://doi.org/10.1016/j.aquaculture.2015.10.019.
655   Loosanoff, Victor L., and Harry C. Davis. 1963. "Rearing of Bivalve Mollusks." In *Advances in*
656   *Marine Biology*, edited by F. S. Russell, 1:1–136. Academic Press.
657   https://doi.org/10.1016/S0065-2881(08)60257-6.

658   MacLean, Brendan, Daniela M. Tomazela, Nicholas Shulman, Matthew Chambers, Gregory L.
659   Finney, Barbara Frewen, Randall Kern, David L. Tabb, Daniel C. Liebler, and Michael J.
660   MacCoss. 2010. "Skyline: An Open Source Document Editor for Creating and Analyzing
661   Targeted Proteomics Experiments." *Bioinformatics* 26 (7): 966–68.
662   https://doi.org/10.1093/bioinformatics/btq054.

663   Matoo, Omera B., Anna V. Ivanina, Claus Ullstad, Elia Beniash, and Inna M. Sokolova. 2013.
664   "Interactive Effects of Elevated Temperature and CO2 Levels on Metabolism and Oxidative
665   Stress in Two Common Marine Bivalves (Crassostrea Virginica and Mercenaria
666   Mercenaria)." *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative*
667   *Physiology* 164 (4): 545–53. https://doi.org/10.1016/j.cbpa.2012.12.025.

668   Middelboe, Anne Lise, and Per Juel Hansen. 2007. "High pH in Shallow-Water Macroalgal
669   Habitats." *Marine Ecology Progress Series* 338: 107–17.
670   http://www.jstor.org/stable/24871746.

671   Miller, A. Whitman, Amanda C. Reynolds, Cristina Sobrino, and Gerhardt F. Riedel. 2009.
672   "Shellfish Face Uncertain Future in High CO2 World: Influence of Acidification on Oyster
673   Larvae Calcification and Growth in Estuaries." *PloS One* 4 (5): e5661.
674   https://doi.org/10.1371/journal.pone.0005661.

675   Moore, Stephanie K., Nathan J. Mantua, Jan A. Newton, Mitsuhiro Kawase, Mark J. Warner,
676   and Jonathan P. Kellogg. 2008. "A Descriptive Analysis of Temporal and Spatial Patterns of
677   Variability in Puget Sound Oceanographic Properties." *Estuarine, Coastal and Shelf Science*
678   80 (4): 545–54. https://doi.org/10.1016/j.ecss.2008.09.016.

679   Mote, Philip W., and Eric P. Salathé. 2010. "Future Climate in the Pacific Northwest." *Climatic*
680   *Change* 102 (1-2): 29–50. https://doi.org/10.1007/s10584-010-9848-z.

681   Newell, R. C., and G. M. Branch. 1980. "The Influence of Temperature on the Maintenance of
682   Metabolic Energy Balance in Marine Invertebrates." In *Advances in Marine Biology*, edited
683   by J. H. S. Blaxter, Frederick S. Russell, and Maurice Yonge, 17:329–96. Academic Press.
684   https://doi.org/10.1016/S0065-2881(08)60304-1.

685   Oksanen, J., F. Guillaume Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R.
686   Minchin, et al. n.d. *Vegan: Community Ecology Package* (version R package version 2.4-1,
687   2016). Accessed December 18, 2017. https:// CRAN.R-project.org/package=vegan.

688   Orr, James C., Victoria J. Fabry, Olivier Aumont, Laurent Bopp, Scott C. Doney, Richard A.
689   Feely, Anand Gnanadesikan, et al. 2005. "Anthropogenic Ocean Acidification over the
690   Twenty-First Century and Its Impact on Calcifying Organisms." *Nature* 437 (September):
691   681. https://doi.org/10.1038/nature04095.

692   Padilla-Gamiño, Jacqueline L., Morgan W. Kelly, Tyler G. Evans, and Gretchen E. Hofmann.
693   2013. "Temperature and CO2 Additively Regulate Physiology, Morphology and Genomic
694   Responses of Larval Sea Urchins, Strongylocentrotus Purpuratus." *Proceedings of the*
695   *Royal Society of London B: Biological Sciences* 280 (1759): 20130155.
696   https://doi.org/10.1098/rspb.2013.0155.

697   Paganini, Adam W., Nathan A. Miller, and Jonathon H. Stillman. 2014. "Temperature and
698   Acidification Variability Reduce Physiological Performance in the Intertidal Zone Porcelain
699   Crab Petrolisthes Cinctipes." *The Journal of Experimental Biology* 217 (22): 3974–80.
700   https://doi.org/10.1242/jeb.109801.

701   Palacios, Sherry L., and Richard C. Zimmerman. 2007. "Response of Eelgrass Zostera Marina

702   to CO^2 Enrichment: Possible Impacts of Climate Change and Potential for Remediation of
703   Coastal Habitats." *Marine Ecology Progress Series* 344: 1–13.
704   http://www.jstor.org/stable/24871448.
705 Pörtner, Hans O. 2008. "Ecosystem Effects of Ocean Acidification in Times of Ocean Warming:
706   A Physiologist's View." *Marine Ecology Progress Series* 373: 203–18.
707   http://www.jstor.org/stable/24872925.
708 Pörtner, Hans O., and Anthony P. Farrell. 2008. "Physiology and Climate Change." *Science* 322
709   (5902): 690–92. http://www.jstor.org/stable/20145158.
710 Przeslawski, Rachel, Maria Byrne, and Camille Mellin. 2015. "A Review and Meta-Analysis of
711   the Effects of Multiple Abiotic Stressors on Marine Embryos and Larvae." *Global Change
712   Biology* 21 (6): 2122–40. https://doi.org/10.1111/gcb.12833.
713 Range, P., M. A. Chícharo, R. Ben-Hamadou, D. Piló, D. Matias, S. Joaquim, A. P. Oliveira, and
714   L. Chícharo. 2011. "Calcification, Growth and Mortality of Juvenile Clams Ruditapes
715   Decussatus under Increased pCO2 and Reduced pH: Variable Responses to Ocean
716   Acidification at Local Scales?" *Journal of Experimental Marine Biology and Ecology* 396 (2):
717   177–84. https://doi.org/10.1016/j.jembe.2010.10.020.
718 R Core Team. 2016. *R: A Language and Environment for Statistical Computing* (version
719   1.1.383). https://www.R-project.org/.
720 Reum, Jonathan C. P., Simone R. Alin, Chris J. Harvey, Nina Bednaršek, Wiley Evans, Richard
721   A. Feely, Burke Hales, et al. 2016. "Interpretation and Design of Ocean Acidification
722   Experiments in Upwelling Systems in the Context of Carbonate Chemistry Co-Variation with
723   Temperature and Oxygen." *ICES Journal of Marine Science: Journal Du Conseil* 73 (3):
724   582–95. https://doi.org/10.1093/icesjms/fsu231.
725 Ries, Justin B., Anne L. Cohen, and Daniel C. McCorkle. 2009. "Marine Calcifiers Exhibit Mixed
726   Responses to CO2-Induced Ocean Acidification." *Geology* 37 (12): 1131–34.
727   https://doi.org/10.1130/G30210A.1.
728 Ringwood, Amy H., and Charles J. Keppler. 2002. "Water Quality Variation and Clam Growth: Is
729   pH Really a Non-Issue in Estuaries?" *Estuaries* 25 (5): 901–7.
730   https://doi.org/10.1007/BF02691338.
731 Shamshak, Gina Louise, and Jonathan R. King. 2015. "From Cannery to Culinary Luxury: The
732   Evolution of the Global Geoduck Market." *Marine Policy* 55 (May): 81–89.
733   https://doi.org/10.1016/j.marpol.2015.01.014.
734 Sunagawa, Shinichi, Luis Pedro Coelho, Samuel Chaffron, Jens Roat Kultima, Karine Labadie,
735   Guillem Salazar, Bardya Djahanschiri, et al. 2015. "Ocean Plankton. Structure and Function
736   of the Global Ocean Microbiome." *Science* 348 (6237): 1261359.
737   https://doi.org/10.1126/science.1261359.
738 Talmage, Stephanie C., and Christopher J. Gobler. 2009. "The Effects of Elevated Carbon
739   Dioxide Concentrations on the Metamorphosis, Size, and Survival of Larval Hard Clams
740   (Mercenaria Mercenaria), Bay Scallops (Argopecten Irradians), and Eastern Oysters
741   (Crassostrea Virginica)." *Limnology and Oceanography*.
742 Thomsen, Jörn, Isabel Casties, Christian Pansch, Arne Körtzinger, and Frank Melzner. 2013.
743   "Food Availability Outweighs Ocean Acidification Effects in Juvenile Mytilus Edulis:
744   Laboratory and Field Experiments." *Global Change Biology* 19 (4): 1017–27.
745   https://doi.org/10.1111/gcb.12109.

746   Timmins-Schiffman, Emma B., Grace A. Crandall, Brent Vadopalas, Michael E. Riffle, Brook L.
747   Nunn, and Steven B. Roberts. 2017. "Integrating Discovery-Driven Proteomics and Selected
748   Reaction Monitoring To Develop a Noninvasive Assay for Geoduck Reproductive
749   Maturation." *Journal of Proteome Research* 16 (9): 3298–3309.
750   https://doi.org/10.1021/acs.jproteome.7b00288.
751   Ting, Ying Sonia. 2017. "Shifting the Paradigm: Peptide-Centric Analysis of Systematically
752   Sampled Mass Spectrometry Data."
753   https://digital.lib.washington.edu/researchworks/bitstream/handle/1773/38147/Ting_washing
754   ton_0250E_16676.pdf?sequence=1&isAllowed=y.
755   Tomanek, Lars. 2014. "Proteomics to Study Adaptations in Marine Organisms to Environmental
756   Stress." Journal of Proteomics 105 (Supplement C): 92–106.
757   Tomanek, Lars. 2015. "Proteomic Responses to Environmentally Induced Oxidative Stress."
758   The Journal of Experimental Biology 218 (Pt 12): 1867–79.
759   Tomanek, Lars, Marcus J. Zuzow, Anna V. Ivanina, Elia Beniash, and Inna M. Sokolova. 2011.
760   "Proteomic Response to Elevated PCO2 Level in Eastern Oysters, Crassostrea Virginica:
761   Evidence for Oxidative Stress." The Journal of Experimental Biology 214 (Pt 11): 1836–44.
762   Tukey, John W. 1977. "Exploratory Data Analysis."
763   https://pdfs.semanticscholar.org/2f40/fd06bc9fd00a27437e14ed171e96e4fd9326.pdf.
764   Tunnicliffe, Verena, Kimberley T. A. Davies, David A. Butterfield, Robert W. Embley, Jonathan
765   M. Rose, and William W. Chadwick Jr. 2009. "Survival of Mussels in Extremely Acidic
766   Waters on a Submarine Volcano." *Nature Geoscience* 2 (April): 344.
767   https://doi.org/10.1038/ngeo500.
768   Vadopalas, Brent, T. W. Pietsch, C. S. Friedman - Malacologia, and 2010. 2010. "… Proper
769   Name for the Geoduck: Resurrection of Panopea Generosa Gould, 1850, from the
770   Synonymy of Panopea Abrupta (Conrad, 1849)(Bivalvia: Myoida: Hiatellidae)." *BioOne*.
771   http://www.bioone.org/doi/abs/10.4002/040.052.0111.
772   Vadopalas, Brent, Jonathan P. Davis, and Carolyn S. Friedman. 2015. "Maturation, Spawning,
773   and Fecundity of the Farmed Pacific Geoduck Panopea Generosa in Puget Sound,
774   Washington." *Journal of Shellfish Research* 34 (1): 31–37.
775   https://doi.org/10.2983/035.034.0106.
776   Wahl, M., S. Schneider Covachã, V. Saderne, C. Hiebenthal, J. D. Müller, C. Pansch, and Y.
777   Sawall. 2018. "Macroalgae May Mitigate Ocean Acidification Effects on Mussel Calcification
778   by Increasing pH and Its Fluctuations: Biogenic Fluctuations Mitigate OA Effects." *Limnology
779   and Oceanography* 63 (1): 3–21. https://doi.org/10.1002/lno.10608.
780   Waldbusser, George G., Matthew W. Gray, Burke Hales, Chris J. Langdon, Brian A. Haley, Iria
781   Gimenez, Stephanie R. Smith, Elizabeth L. Brunner, and Greg Hutchinson. 2016. "Slow
782   Shell Building, a Possible Trait for Resistance to the Effects of Acute Ocean Acidification."
783   *Limnology and Oceanography* 61 (6): 1969–83. https://doi.org/10.1002/lno.10348.
784   Waldbusser, G. G., H. Bergschneider, and M. A. Green. 2010. "Size-Dependent pH Effect on
785   Calcification in Post-Larval Hard Clam Mercenaria Spp." *Marine Ecology Progress Series*
786   417 (November): 171–82. https://doi.org/10.3354/meps08809.
787   Washington Sea Grant. 2015. "Shellfish Aquaculture in Washington State." *Final Report to the
788   Washington State Legislature* 84.
789   https://pdfs.semanticscholar.org/b833/e0fcb8a0459697f94fd86b9848ee0e59c0a2.pdf.

790   Washington State Blue Ribbon Panel on Ocean Acidification. 2012. "Ocean Acidification: From
791   Knowledge to Action, Washington State's Strategic Response." 12-01-015 . Washington
792   Department of Ecology, Olympia, Washington.
793   https://fortress.wa.gov/ecy/publications/documents/1201015.pdf.
794   "Washington's Wild Geoduck Fishery | WA - DNR." n.d. Accessed November 20, 2017.
795   https://www.dnr.wa.gov/programs-and-services/aquatics/shellfish/washingtons-wild-
796   geoduck-fishery.
797   Weiss, Ingrid Maria, Noreen Tuross, Lia Addadi, and Steve Weiner. 2002. "Mollusc Larval Shell
798   Formation: Amorphous Calcium Carbonate Is a Precursor Phase for Aragonite." *The Journal*
799   *of Experimental Zoology* 293 (5): 478–91. https://doi.org/10.1002/jez.90004.
800   Wickham, Hadley. 2017. "ggplot2 - Elegant Graphics for Data Analysis (2nd Edition)." *Journal of*
801   *Statistical Software, Book Reviews* 77 (2): 1–3. https://doi.org/10.18637/jss.v077.b02.
802   Widdicombe, Stephen, and John I. Spicer. 2008. "Predicting the Impact of Ocean Acidification
803   on Benthic Biodiversity: What Can Animal Physiology Tell Us?" *Journal of Experimental*
804   *Marine Biology and Ecology* 366 (1): 187–97. https://doi.org/10.1016/j.jembe.2008.07.024.
805   Winter, D. F., K. Banse, and G. C. Anderson. 1975. "The Dynamics of Phytoplankton Blooms in
806   Puget Sound a Fjord in the Northwestern United States." *Marine Biology* 29 (2): 139–76.
807   https://doi.org/10.1007/BF00388986.
808   "WWW Tide/Current Predictor - Site Selection." n.d. Accessed December 7, 2017.
809   http://tbone.biol.sc.edu/tide/.
810   Yang, Zhaoqing, and Tarang Khangaonkar. 2010. "Multi-Scale Modeling of Puget Sound Using
811   an Unstructured-Grid Coastal Ocean Model: From Tide Flats to Estuaries and Coastal Waters."
812   *Ocean Dynamics* 60 (6): 1621–37. https://doi.org/10.1007/s10236-010-0348-5.

Supplemental Material

Supplemental Table 1. Outplant location metrics; FB=Fidalgo Bay, PG=Port Gamble, SR=Skokomish River Delta, CI=Case Inlet, WB=Willapa Bay; -E=Eelgrass, -U=Unvegetated. Δ Habitats & Δ Bays: F-statistic and p-adjusted from 2-way ANOVA testing differences between habitats and bays. **Growth did not differ within ad-hoc regions (south=CI & WB, north=FB & PG). *Salinity probes failed fully or intermittently at 4 of the 8 locations; habitat salinities were not compared.									
Deployment location	**FB-E**	**FB-U**	**PG-E**	**PG-U**	**CI-E**	**CI-U**	**WB-E**	**WB-U**	**Δ Habitat**	**Δ Bays**
% Survival	87	80	80	93	67	60	93	100	p= 0.88	p=0.25
Mean Relative % Growth (± SD)	16.2 (±7.3)	23.3 (±12.5)	22.2 (±12.5)	21.1 (±13.3)	1.2 (±8.3)	-2.8 (±5.3)	10 (±16.2)	4.5 (±8.3)	p=1	**p= 2.4e-10	**p= 2.4e-10
Mean pH (TS)	7.90	7.54	*failed*	7.32	7.87	7.63	7.81	7.55	**p= 1.6e-50**	**p= 7.3e-35**
pH Standard Deviation (TS)	0.19	0.23	*failed*	0.25	0.16	0.20	0.06	0.18	p=1	**p= 8.4e-27**
Mean DO (mg/L)	*failed*	10.9	10.8	11.9	10.4	8.3	8.4	8.8	p=1	**p= 3.1e-45**

DO Standard Deviation (mg/L)	*failed*	5.7	3.9	3.9	2.6	1.9	1.5	1.3	p=1	**p= 1.9e-71**
Mean Temp (°C)	15.1	15.1	15.3	15.2	16.6	16.4	18.2	18.2	p=1	**p= 7.6e-76**
Temp Standard Deviation (°C)	1.6	1.6	2.1	2.4	1.7	1.6	1.2	1.2	p=1	p=1
Mean Salinity (ppt)	30.2	*29.1	23.4	*failed*	*failed*	24.7	27.3	*24.4	*Not tested	***p= 1.4e-100**
Salinity Standard Deviation (ppt)	0.4	*0.34	1.3	*failed*	*failed*	1.2	0.70	*1.1	*Not tested	***p= 1.9e-6**
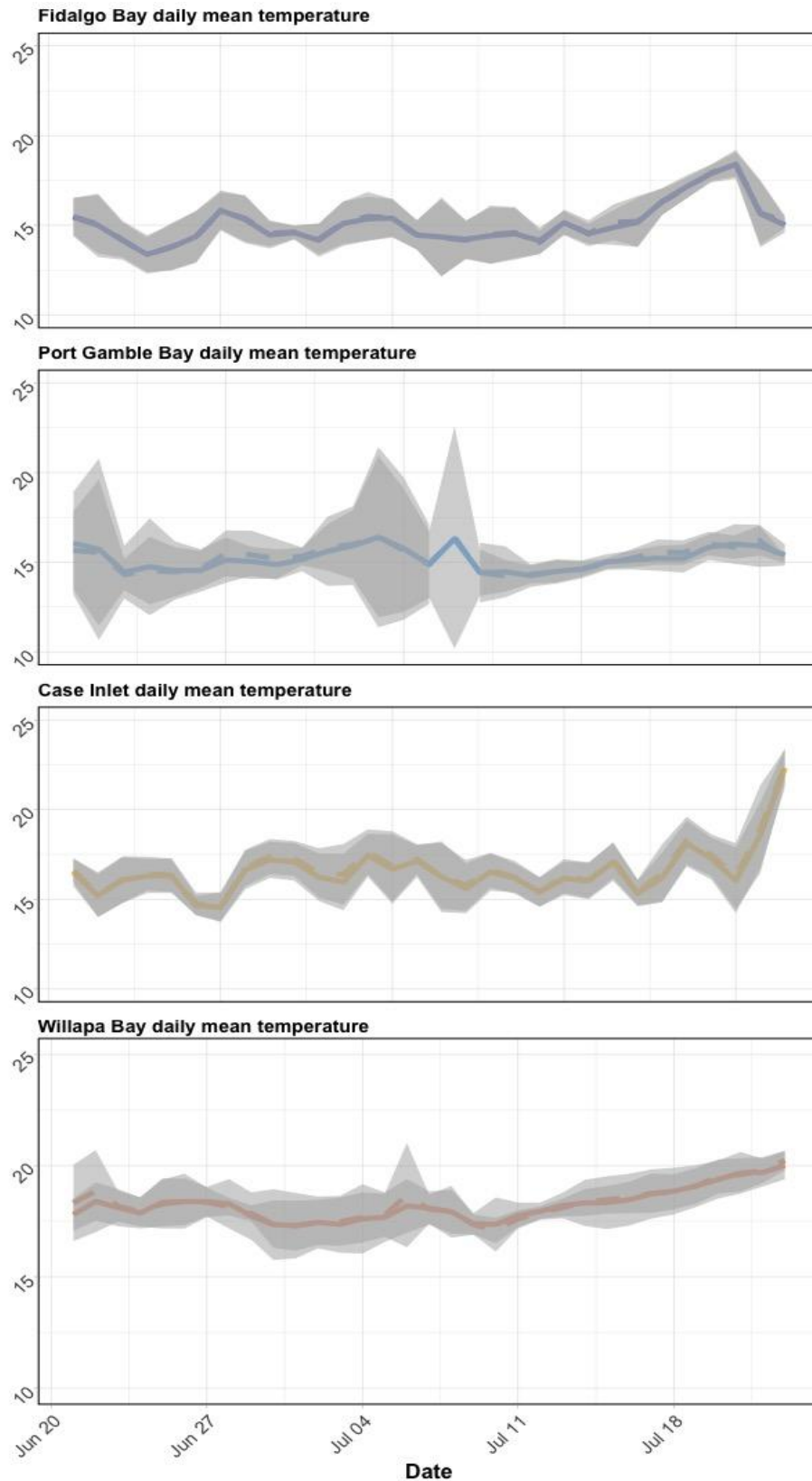
Supplemental Figure 1: Daily mean temperature in eelgrass (dashed lines) and unvegetated (solid lines) across bays during geoduck deployment. Gray ribbons denote standard deviations per day.
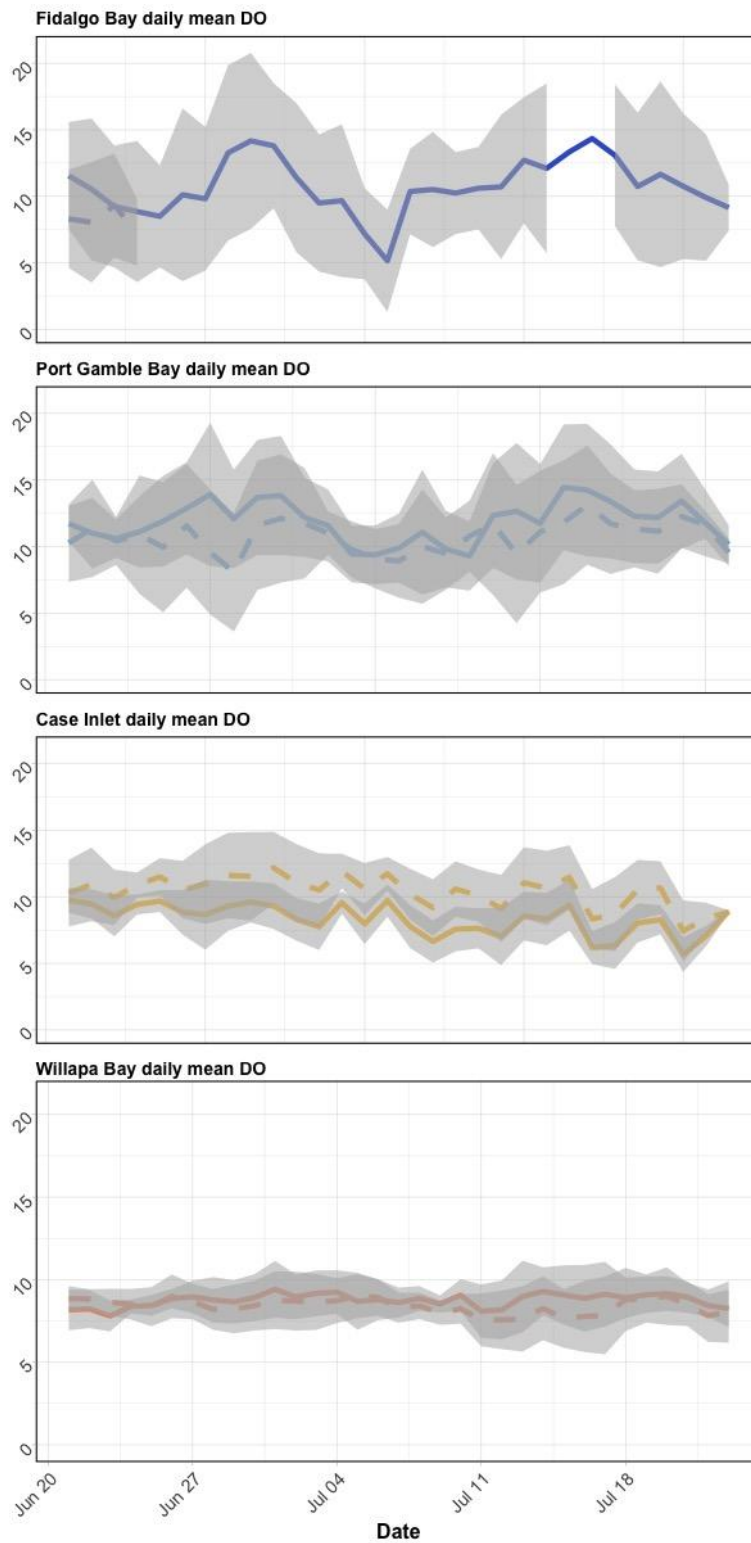
Supplemental Figure 2: Daily mean DO in eelgrass (dashed lines) and unvegetated (solid lines) across bays during geoduck deployment. Gray ribbons denote standard deviations per day. Fidalgo Bay eelgrass probe failed towards the beginning of the outplant period.
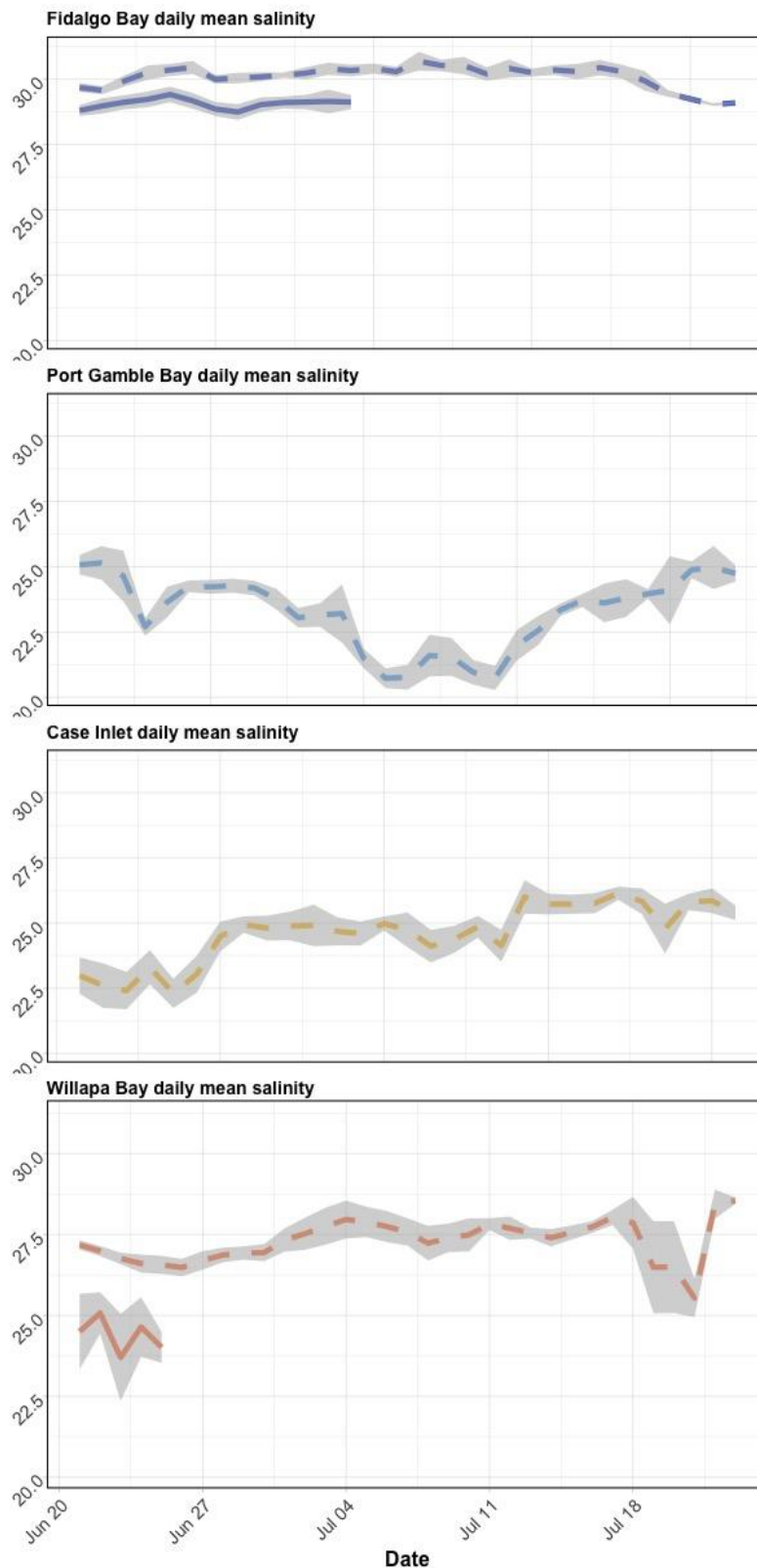
Supplemental Figure 3: Daily mean salinity in eelgrass (dashed lines) and unvegetated (solid lines) across bays during geoduck deployment. Gray ribbons denote standard deviations per day. Salinity probes failed at several sites; no habitat comparisons were made.

Supplemental Table 2: SRM protein ANOVA results by Region (FB/PGB vs. CI/WB), Bay, and Habitat for all proteins combined, then each protein individually, with Pr(>F)-adjusted calculated via the Bonferroni Correction. Bold = significantly different abundance. Habitat was tested using 2-way ANOVA with abundance ~ Bay*Habitat.

	Comparison	Df	Sum Sq	Mean Sq	F value	Pr(>F)	P.adj
all peptides combined	**North vs. South**	**1**	**18.2**	**18.2**	**10.9**	**0.00010**	**0.0014**
	Bay	**3**	**34.2**	**11.4**	**7.0**	**0.00012**	**0.0017**
	Habitat	1	1.2	1.2	0.75	5.4	14
arachidonate	North vs. South	1,133	7.4	7.4	1.3	0.26	3.6
	Bay	3,131	60	20	3.7	0.014	0.20
	Habitat	1,130	2.2	2.2	0.40	0.53	7.4
catalase	North vs. South	1,128	8.6E-05	8.6E-05	0.46	0.50	7.0
	Bay	3,126	0.00019	6.4E-05	0.34	0.79	11
	Habitat	1,125	1.4E-08	1.4E-08	7.4E-05	0.99	14
cytochrome P450	North vs. South	1,133	0.0065	0.0065	0.96	0.33	4.6
	Bay	3,131	0.015	0.0051	0.76	0.52	7.3
	Habitat	1,130	0.0085	0.0085	1.3	0.26	3.7
glycogen phosphorylase	North vs. South	1,133	94	94	1.05	0.31	4.3
	Bay	3,131	459	153	1.7	0.16	2.3
	Habitat	1,130	114	114	1.3	0.26	3.6
HSP90-alpha	**North vs. South**	**1,133**	**421,443**	**421,443**	**21**	**1.3E-05**	**0.00018**
	Bay	**3,131**	**477,740**	**159,247**	**7.8**	**7.83E-05**	**0.0011**
	Habitat	1,130	567	567	0.028	0.87	12
protein disulfide isomerase	North vs. South	1,84	3.8	3.8	5.55	0.021	0.29
	Bay	3,82	4.6	1.5	2.2	0.10	1.4
	Habitat	1,81	0.01	0.006	0.0078	0.93	13

puromycin-sensitive	**North vs. South**	**1,130**	**0.94**	**0.94**	**9.1**	**0.0031**	**0.043**
	Bay	3,128	1.2	0.40	3.87	0.011	0.15
	Habitat	1,127	0.09	0.089	0.87	0.35	4.9
sodium/potassium transporting ATPase	North vs. South	1,88	0.65	0.65	0.42	0.52	7.2
	Bay	3,86	3.2	1.06	0.68	0.56	7.9
	Habitat	1,85	0.85	0.85	0.55	0.46	6.5
superoxide dismutase	North vs. South	1,66	0.0012	0.0012	1.8	0.18	2.5
	Bay	3,64	0.0020	0.00067	1.02	0.39	5.5
	Habitat	1,63	5.4E-05	5.4E-05	0.081	0.78	11
trifunctional enzyme β-subunit	**North vs. South**	**1,88**	**252**	**252**	**11.1**	**0.0013**	**0.018**
	Bay	**3,86**	**345**	**115**	**5.19**	**0.0024**	**0.034**
	Habitat	1,85	22	22	1.0	0.32	4.5

Supplemental Table 3: Mean coefficients of variation of SRM transition abundance for each protein, location

Protein	CI-B	CI-E	FB-B	FB-E	PG-B	PG-E	WB-B	WB-E
arachidonate 5-lipoxygenase	0.84	0.57	0.44	0.33	0.46	0.64	0.48	0.58
catalase	0.61	0.52	0.47	0.42	0.50	0.66	0.50	0.43
cytochrome P450	0.74	0.57	0.38	0.43	0.26	0.58	0.56	0.46
glycogen phosphorylase (muscle form)	0.55	0.49	0.35	0.35	0.37	0.61	0.51	0.47
heat shock protein 70	0.59	0.61	0.20	0.33	0.37	0.44	0.45	0.38
heat shock protein 90-alpha	0.70	0.62	0.32	0.40	0.45	0.52	0.50	0.49
protein disulfide isomerase	0.63	0.50	0.38	0.31	0.38	0.70	0.41	0.43
peroxiredoxin-1	0.56	0.46	0.31	0.33	0.42	0.46	0.40	0.36
puromycin-sensitive aminopeptidase	0.69	0.52	0.39	0.40	0.42	0.51	0.44	0.42
ras-related protein rab-11B	0.61	0.43	0.30	0.33	0.42	0.63	0.57	0.54
Na/K transporting ATPase	0.70	0.58	0.30	0.36	0.40	0.62	0.51	0.44

Superoxide dismutase	0.49	0.53	0.49	0.57	0.30	0.71	0.46	0.44
Trifunctional Enzyme β-subunit	0.61	0.48	0.41	0.38	0.46	0.63	0.52	0.53

Supplemental Figure 4: Non-metric Multi-Dimensional Scaling plot (NMDS) showing patterns of similarity among DIA peptide abundances between technical replicates (same symbol/color), deployment bay (same color), and north/south region, where each point represents one geoduck. Relative proximity of points represents overall degree of peptide abundance similarity.
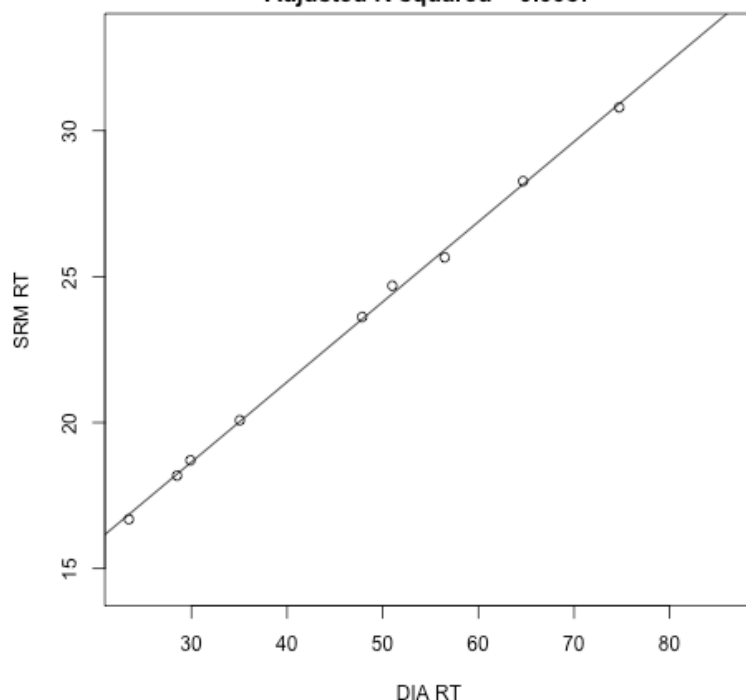

Supplemental Figure 5: Linear regression model fit of PRTC internal standard peptides in SRM against DIA, used to confirm identity of targeted peptides in SRm by calculating adjustment in retention time between SRM and DIA.

Supplemental Figure 6 (Interactive, online): Dilution curve peptide abundance ratios regressed against predicted ratios from serial sample dilutions. Peptides with slope coefficient 0.2<x<1.5 and adjusted R2 >0.7 were included in analysis. Link to interactive figure: http://owl.fish.washington.edu/generosa/Generosa_DNR/Dilution-Curve-Transitions.html

Supplemental Figure 7 (Interactive, online): Non-metric Multi-Dimensional Scaling plot (NMDS) of SRM technical replicates. Link to interactive figure: http://owl.fish.washington.edu/generosa/Generosa_DNR/NMDS-technical-replicate.html
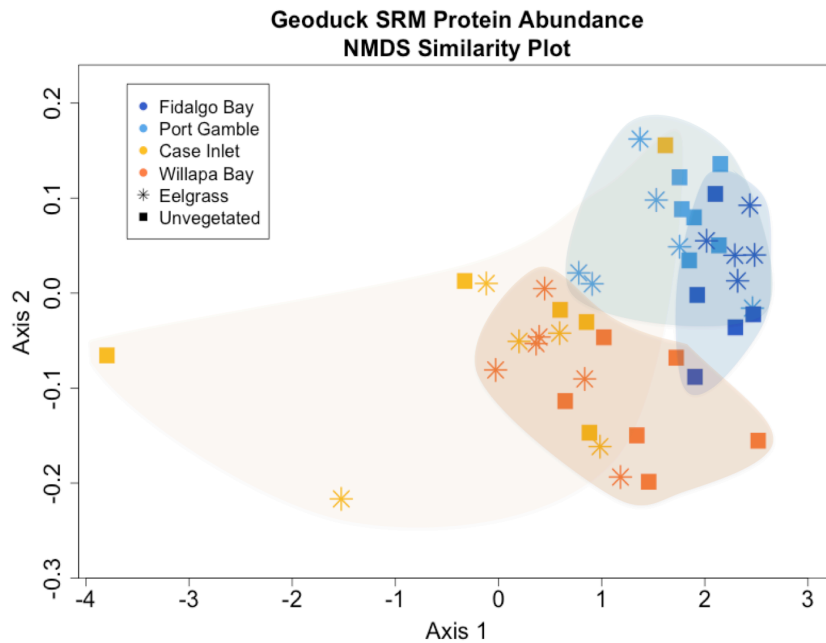
Supplemental Figure 8: Non-metric Multi-Dimensional Scaling plot (NMDS) showing patterns of similarity among targeted SRM peptide abundances between deployment locations, where each point represents one geoduck. Relative proximity of points represents overall degree of peptide abundance similarity.