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Context-dependence of abiotic and biotic factors

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influencing performance of juvenile clams

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19 **Abstract**

20 Post-settlement survival and growth of bivalves can be limited by abiotic and biotic factors,
21 both of which are spatially variable. Rarely has the importance of these factors been tested
22 concurrently in the field. Our study spanned three spatial scales in estuarine waters of
23 Washington state (Region: north vs. south; Within-region: fresher vs. saline; Within-site: mid-
24 vs. low-tidal elevation). Predator access and sediment conditions were manipulated in a
25 crossed experimental design, with juvenile (3-mm and 6-mm) Manila clams (*Ruditapes*
26 *philippinarum*) outplanted in open- or closed-top mesh tubes. We found differences between
27 treatments that appeared only at sites with cancrid crabs, suggesting that predators, rather
28 than emigration, likely reduced numbers of clams in open tubes. We had hypothesized that
29 clams at lower tidal elevations, which experience longer immersion times, would show
30 improved growth but reduced survivorship because of greater exposure to marine predators.
31 However, these patterns were evident at only one of three sites (lower-elevation treatments
32 were lost at the fourth). The larger size class of clams was more tolerant of abiotic stressors at
33 all sites, but the magnitude of difference in survival between size classes was sometimes
34 dependent on other treatments. The maximum predator effect on survival was 74% (north,
35 high salinity, low-intertidal site), whereas the maximum abiotic effect appeared as 62% lower
36 survival and 59% slower growth for 3-mm clams at another site (north, fresher). In laboratory
37 trials, high water temperatures (28-32°C) and low salinity (5-15) acted synergistically to cause
38 juvenile clam (6-12 mm) mortality, whereas clams tolerated each of these stressors alone.
39 Context-dependence in the relative importance of predation and abiotic stressors was apparent
40 in our results, but contrary to expectations, abiotic stressors did not characterize southern or

41 fresher sites. Instead, extreme high temperatures occurred at the site with the wide tidal flat
42 rather than in the south, and the within-region salinity differences appeared not to exceed
43 tolerances of juvenile clams.

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47 **Introduction**

48 Juvenile marine bivalves in soft sediments are subject to a variety of environmental
49 biotic and abiotic forcing processes that impact their growth and survival. Key biotic processes
50 are predation, and to a lesser degree competition and food supply since both living space and
51 planktonic or detrital food are typically readily available (Beal, 2006; Beukema et al., 2017). Key
52 abiotic processes include temperature and salinity extremes for intertidal species (Chew and
53 Ma, 1987; Petersen et al., 2008; Takeuchi et al., 2015). Wave energy or currents may displace
54 individuals (Hunt and Mullineaux, 2002; Takeuchi et al., 2015) and sedimentation may smother
55 them, although mobile taxa such as clams can move up and down in the sediment to avoid
56 these stressors to some degree. All these processes act differentially across life history phases.
57 Newly settled bivalve spat, which are < 0.5 mm and live near the sediment surface, are most
58 vulnerable. Mortality estimates of spat settled in the field exceed 90%, with most studies
59 suggesting predation as the primary process leading to loss of individuals within months of
60 settling (Hunt and Scheibling, 1997; Williams, 1980). However, there are few studies
61 manipulating critical processes that affect post-recruitment stages of infaunal bivalves, or
62 testing how these vary with field context and bivalve age.

63 Larger juvenile clams remain vulnerable to most of the processes discussed above,
64 although types of predators change. Juvenile clams <1 cm must live in the top few cm of
65 sediment because their siphons are short; thus they are buffered from daily environmental
66 stressors relative to new settlers, but are still subject to weather extremes and to surface
67 predators such as crabs, whelks, fishes, and shorebirds (e.g., Wilson, 1991). Studies of juvenile
68 clam survival and growth in ecologically and economically important species have shown both

69 positive and negative effects of environmental change, such as reduced growth and higher
70 mortality with warming climate for *Limecola (Macoma) balthica* in the Netherlands, while more
71 warm-adapted bivalve species thrive (Beukema et al., 2009). Unusual environmental conditions
72 can benefit bivalve populations by reducing predator numbers or foraging efficiency (Altieri,
73 2008; Glaspie et al., 2017). As climate warms, ectotherms on the rising portion of thermal
74 performance curves will experience increases in growth rates if food is not limiting, and larger
75 adults tend to have increased survival and reproductive output (Peters, 1983). However,
76 gradual environmental forcing such as warming of water and/or air may eventually cause
77 tolerance thresholds to be crossed. With climate change, it is likely that this will happen with
78 increasing frequency.

79 Infaunal bivalves are also subject to the emerging environmental stressor of reduced pH
80 caused by ocean acidification (OA), exacerbated in sediment by respiration of infaunal
81 organisms. OA is particularly an issue in estuaries because of the already-low pH of terrestrial
82 runoff (Glaspie et al., 2017). Areas of low pH and low aragonite saturation may be complex
83 spatially because of the local heterogeneity of organisms, and temporally because of diurnal
84 changes (Miller and Waldbusser, 2016). These chemical stressors can weaken shells of juvenile
85 clams and inhibit burrowing and predator-escape behaviors, leaving them more vulnerable to
86 wave energy and to consumption (reviewed in Green et al., 2009, Clements and Hunt, 2017).
87 Adding shell hash (crushed shell) to sediment has shown mixed success in buffering low pH and
88 improving clam settlement and survival (Green et al., 2013; Green et al., 2009; Greiner et al.,
89 2018).

90 A variety of techniques in shellfish aquaculture have been developed to deal with
91 variable settlement and post-settlement survival, which are often unpredictable even when
92 predators are controlled (Cigarria and Fernandez, 2000). Many species are now bred in
93 hatcheries and raised for some length of time under optimal conditions until spat (“seed”) is
94 large enough to be outplanted. Clam farmers then typically use netting and/or graveling of
95 surface sediments to reduce predator access to growing clams (e.g., Beal and Kraus, 2002;
96 Ruesink et al., 2014; Munroe et al., 2015). However, abiotic environmental changes can still
97 affect growth and survival. Mass mortalities of farmed clams have been reported associated
98 with high rainfall or thermal extremes (Nie et al., 2017b; Yan et al., 2006; Zhang and Yan, 2006).

99 Because of the economic importance of bivalves, their physiology has been well studied,
100 especially in adults. Temperature, salinity, food availability, and type of food can all affect
101 filtration rates and assimilation efficiencies of clams (e.g., Nakamura, 2004; Nie et al., 2017b;
102 Specht and Fuchs, 2018); these metabolic changes can vary even among strains in cultured
103 species (reviewed by Nie et al., 2017a). Stressful environmental conditions such as low salinity
104 cause many bivalves to remain closed, thus leading to starvation and/or asphyxiation (Elston et
105 al., 2003; La Peyre et al., 2013). On the other hand, behavioral adaptations such as shell closure
106 can allow bivalves to tolerate brief periods of extremes in salinity or temperature (e.g., Kim et
107 al. 2001).

108 Manila (=Asari) clams (*Ruditapes philippinarum*) were the focus of our study of juvenile
109 clam performance. Manilas are not native to Washington State but are an economically critical
110 farmed species both in the state and worldwide; this species accounts for ca. 25% of the global
111 production of molluscs (4 M tonnes produced in 2016;

112 http://www.fao.org/fishery/culturedspecies/Ruditapes_philippinarum/en). While there is a
113 substantial aquaculture-driven literature on settlement, growth, and physiology of Manilas (see
114 Discussion), few field studies compare clam growth and survival among sites varying both
115 biotically and abiotically, or compare these effects among clam sizes. We tested variation in
116 performance in two sizes of juvenile clams; field experiments involved careful site selection,
117 treatments at two tidal elevations, and manipulation of sediment type and predator access. The
118 potential drivers of clam growth and mortality that we examined were salinity (across sites),
119 temperature (across sites and within sites at different tidal elevations), porewater chemistry
120 (shell hash treatment), and predation (across sites, elevations, and using predator exclusion
121 treatments). We expected: 1) tradeoffs would occur between growth and survival, because
122 clams at lower tidal elevations could feed for longer times but also be exposed to more
123 predation; 2) buffering sediments with shell would improve clam performance more at lower-
124 than higher-salinity sites; 3) summer temperature extremes would reduce clam performance
125 primarily at southern sites and at higher tidal elevations, given regional gradients in water
126 temperature and the timing of mid-day low tides; and 4) all of these stressors would exert
127 weaker effects on larger clams. We also carried out a controlled laboratory study to test for
128 synergistic effects of high temperature and low salinity on juvenile clam survival. Overall, our
129 study design elucidated the context-dependence of both biotic and abiotic drivers of early post-
130 settlement clam performance.

131 **Methods**

132 Juvenile Manila clams for all experiments were obtained from Taylor Shellfish hatcheries
133 and shipped to Washington State under their import permit.

134 *Field Sites*

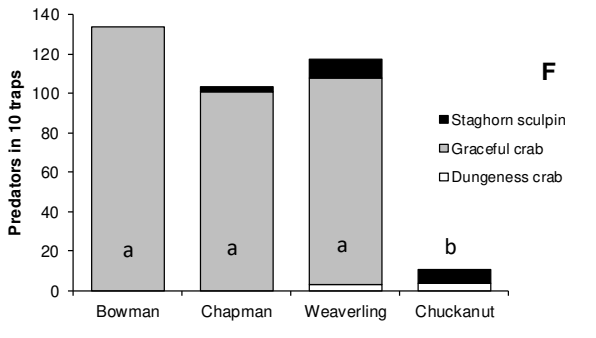
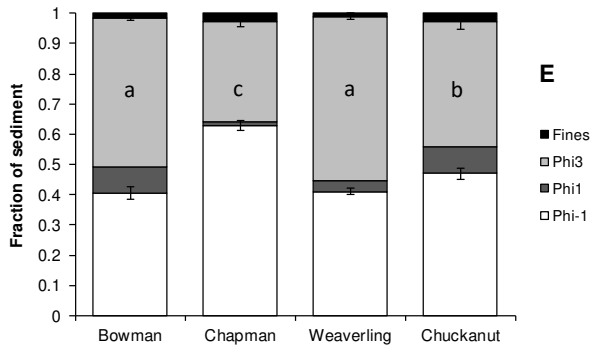
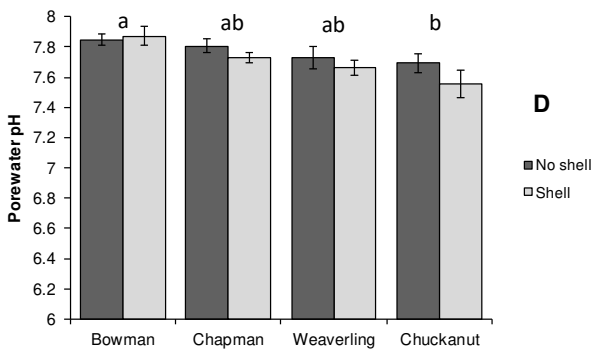
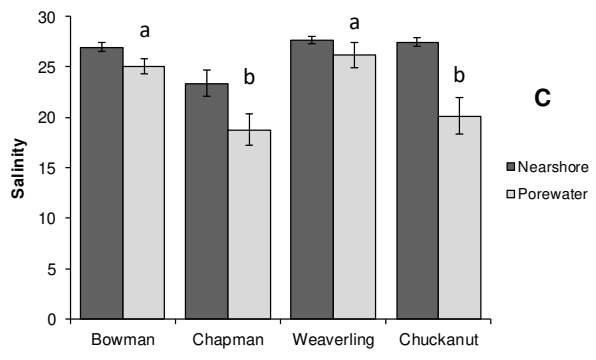
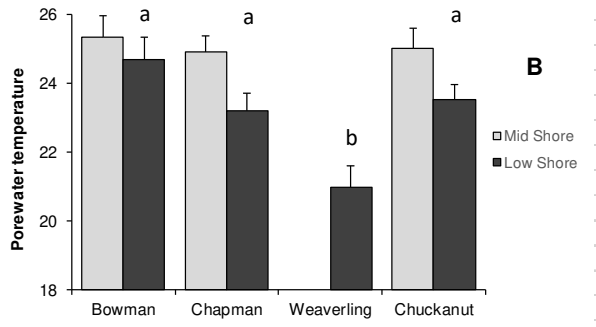
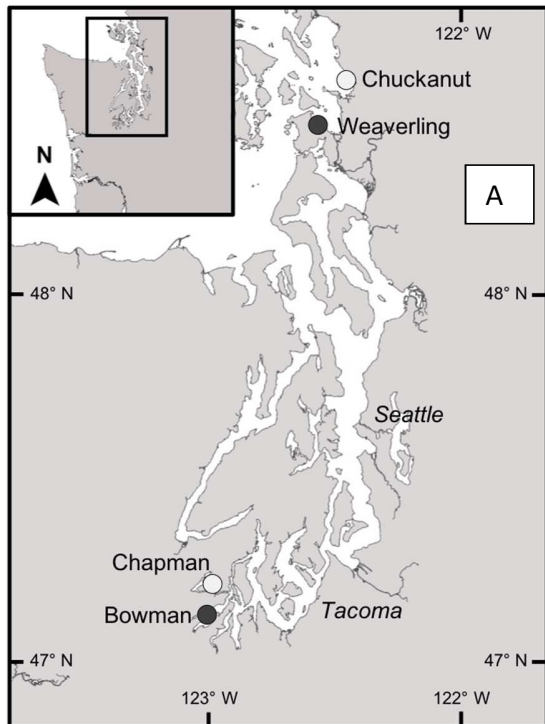
135 We chose paired sites in each of two distinct regions of the Salish Sea (Fig. 1A), in the
136 northwest corner of the contiguous U.S. We expected the regions to differ in temperature,
137 since the timing of summer mid-day low tide is delayed by several hours from the northern to
138 southern region, elevating the sediment temperatures experienced by intertidal organisms in
139 the south (Dethier et al., 2010). Within each region, we selected sites that were near and far
140 from freshwater sources to generate variability in salinity; we term these fresher and saline
141 sites. At each site, we worked at two tidal elevations within the range typically used for
142 aquaculture of Manila clams and where naturally-settled individuals are found: a mid-intertidal
143 elevation (ca. +1.0 m above mean lower low water (MLLW)), and a lower-intertidal elevation
144 (+0.3 m MLLW). Most of our sites have economically important bivalve aquaculture operations,
145 including for our target species. Additionally, they include beaches where there is access for
146 recreational or tribal shellfish harvesting (see <http://wdfw.wa.gov/fishing/shellfish/beaches/>).

147 We measured environmental parameters related to abiotic stressors and predation at
148 all four sites, with methods and statistics provided in Supplemental material. During the
149 experimental period, we had Tidbit temperature loggers deployed at both elevations at all 4
150 sites except at Weaverling (north saline), where the Tidbit at +1 m MLLW was vandalized. As
151 expected from tidal exposure, average daily maximum temperatures at mid-shore exceeded
152 those at low elevation (Fig. 1B). Among sites, Weaverling had cooler daily maximum
153 temperatures than the other three sites (Fig. 1B). The same general pattern can be seen in
154 longer-term temperature data from the four sites (Suppl. Figure 2). Salinity measurements of
155 both porewater and water over the tidal flats confirmed our selection of fresher (porewater

156 salinity ~20) and saline sites (salinity ~25) within each region (Fig. 1C). Southern sites occurred
157 in lower-pH water (7.8-8.0) than northern sites (8.3-8.5) based on shallow water-column
158 (nearshore) samples, a regional difference also noted in oceanographic sampling (Feely et al.,
159 2010). Porewater pH showed trends as expected from salinity, namely fresher sites had lower
160 porewater pH than saline sites, although only Chuckanut was significantly lower than its paired
161 site (Bowman) in post-hoc tests (Fig. 1D). No buffering of pH over 24 hours was detected
162 following localized shell addition to the sediment (450 cm²; Shell $F_{1,31}=2.4$, $P=0.14$; see Suppl.
163 Methods) . Sites differed in sediment type in ways that mirrored salinity, particularly because
164 fine sand predominated at the saline northern and southern sites, but siltier sediments
165 occurred at sites closer to rivers (Fig. 1E). Predators in traps, especially cancrid crabs comprising
166 92% of catch, had similar abundances at three sites but were uncommon at the fresher
167 northern site (Fig. 1F). We were unable to estimate abundances of other potential predators
168 such as shorebirds.

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172 Figure 1. A. Four sites where Manila clams were outplanted at two tidal elevations in summer
173 2017. Open circles = fresher sites, closed circles = saline. B-E. Environmental conditions at the 4
174 sites. Sediment conditions and predator densities were measured at +1.0 m MLLW. Error bars
175 are one s.e.; different letter codes follow results of post-hoc tests among the four sites. (B)
176 Average daily maximum temperatures (°C) recorded in the porewater during the 29 days of
177 clam deployment (July 22 to Aug. 19) when all loggers were functioning. Letter codes refer to
178 comparison of low-shore temperatures. (C) Salinity measured in situ with YSI Ecosense meter in
179 May-Sep 2016 and 2017 (N=8-10 dates). Letter codes compare porewater. (D) pH measured
180 with SeaFet probe on samples collected by piezometer, June 2017 (N=5 with and without shell
181 added). Letter codes compare sites. (E) Sediment collected in June 2017 from mesh tubes
182 present for 11 months (N=9). (F) Predators caught after one day in baited Fukui traps (N=10,
183 half deployed in Jun 2016 and half in Jun 2017). Letter codes compare summed crab numbers.
184 See Supplemental material for details.
185

186 *Field experiment*

187 Fixed numbers and sizes of seed clams were placed in experimental outplanting units
188 consisting of tubes of Teflon window-screening (1.5 mm openings) 8 cm diam. x 20 cm deep
189 including a mesh bottom (Supplement Photo 1). Porewater passed naturally through all tubes.
190 There were four tube types in a two-factor crossed design: Predator access was manipulated
191 with a screen top, giving “open” and “top” treatments. Only predators <2 mm diameter could
192 get into the “top” tubes. Porewater chemistry was manipulated through natural buffering by
193 shell mixed into the tubes, giving “no shell” and “shell” treatments. In “no shell” treatments,
194 smooth pebbles ~3-4 cm wide were used to fill the bottom third of each tube and the
195 remaining space was filled nearly to the top with a 50/50 mixture of clean marine-derived sand
196 and pea gravel. In the “shell” treatments, the fill was equal parts of sand, pea gravel, and
197 crushed-oyster shell hash. Each of the four treatment types was replicated five times at each
198 elevation at each site, for a total of 160 tubes.

199 Tubes were deployed 6-13 Jul 2017 and retrieved after two months (15-19 Sept). We
200 placed five sets along a 50 m transect using random numbers to determine the location of each

201 set along the transect and the treatment order per set. Within a set, tubes were placed roughly
202 15 cm apart and embedded so the top was approximately level with the surrounding sediment.
203 The top few mm of each tube was filled with clean wet sand, 40 Manila clams were placed on
204 top, covered with a small amount of additional sand, and clean sea water was gently squirted
205 over the top. These densities are lower than those likely to result in competition for food or
206 space among clams of this size (Beal et al., 2001). The full-top tubes were then sewn completely
207 shut using monofilament.

208 The outplanted clams were from two size classes, with 20 of each size per tube: small
209 (3.2 ± 0.6 mm sd) and medium (6.2 ± 0.3 mm sd). All outplants had been marked with alizarin
210 red stain in the lab (Peterson et al., 1995). This dye is non-toxic and does not significantly affect
211 growth, at least in scleractinian corals (Holcomb et al., 2012). Clams were soaked in an alizarin-
212 seawater solution for 3 days, after which the entire shell was pink. Field-grown white shell was
213 clearly distinguishable when clams were retrieved after two months.

214 At the end of each experiment, all the sediment in each tube was sieved on 2 mm sieves
215 in the field, and both live and dead clams were returned to the lab for measurement. We
216 measured (using calipers: nearest 0.1 mm) shell length (maximum shell dimension) of the pink-
217 stained portion and the total length. Low-elevation treatments at one site (Bowman) were
218 disturbed by aquaculture activities and not recovered, so we could not test tidal elevation as a
219 factor at this site.

220 Response variables for the statistical analyses focused on the number of recovered live
221 clams and their final size relative to starting size. We use “survival” to refer to the number of
222 live clams retrieved in each tube at the end of the experiment, recognizing that it incorporates

223 mortality and emigration of the original 20 clams of each size. Analyses were carried out for
224 each site separately due to expectations that treatments would have context-dependent
225 effects. For survival analyses, final counts of live clams per tube were tested with a linear
226 mixed-effects model in R, with fixed effects of size (small vs. medium), tidal elevation (low vs.
227 mid), open vs. top, no shell vs. shell, and all two, three, and four-way interactions. Tube was
228 considered a random effect as both small and medium clams were present in each tube. This
229 model structure was also used for growth analyses, except instead of size class, we included
230 initial size as a continuous covariate that did not interact with other factors. Residuals of all
231 models were examined and conformed to Gaussian assumptions. Factors were considered
232 significant when $P < 0.05$.

233 *Temperature and Salinity Tolerances in the Lab*

234 We quantified juvenile Manila clam survival under controlled combinations of
235 temperature and salinity using microcosms at the University of Washington's Friday Harbor
236 Laboratories. Juvenile clams from Taylor Shellfish were kept in running seawater tables at
237 ambient local conditions ($\sim 11^{\circ}\text{C}$, 30 ppt) and fed 1 ml of 10:1 water to algae solution (Reed
238 Mariculture Seroy Shellfish Diet 1800[®] Instant Algae) daily. We divided juvenile clams into three
239 size classes (6-8 mm, 8-10 mm, and 10-12 mm). Microcosms (small plastic tubs) were set at 36
240 unique combinations of salinity and temperature within and beyond local abiotic conditions.
241 We tested six salinity treatments (5, 10, 15, 20, 25, and 30) by diluting ambient seawater with
242 deionized water, verified with a YSI Pro30[®] Instrument. Microcosms containing all three size
243 classes of clams were placed in water baths heated gradually over an hour (with clams in the
244 water) to a specific temperature (10° , 16° , 20° , 24° , 28° , 32°C) using aquarium heaters. Each of

245 the 36 microcosms was kept aerated with a Tetra Whisper Non-UL aquarium pump. We
246 randomly selected 10 individuals of each clam size class for each temperature and salinity
247 combination. Clam mortality was evaluated at 72 hours of exposure. We did not feed clams
248 during these short trials. Clam survival per container was the response variable in a generalized
249 linear model with temperature and salinity as fixed effects (continuous). We were especially
250 interested in the interaction between these effects. Binomial error structure was assumed
251 (each clam was either live or dead).

252

253 **Results**

254 *Field Experiment*

255 Initial size of the clams was an important factor for survival over two months, in
256 complex combinations with other treatments. Overall, there was a significant main effect of
257 initial clam size on survival at all sites (Table 1); more 6-mm (63%) than 3-mm clams (48%) were
258 retrieved alive. At three sites, mesh tops on tubes improved retrieval of clams (Fig. 2, Table 1).
259 Since the only site showing no effect of tops (Chuckanut, north fresher) also was distinctive in
260 lacking cancrid crabs (Fig. 1F), this context-dependence helps confirm predators as a key driver
261 of early clam survival. We had predicted that protection from predators would have a greater
262 impact on survival lower on the shore where predators have longer access, but only
263 experiments at Weaverling (north saline site) showed this expected interaction. With tops on
264 tubes, survival was high at both elevations; in tubes without tops, we recovered far fewer live
265 at the mid-intertidal elevation and even fewer at the lower elevation (Fig. 2, Table 1).
266 Weaverling was one of two sites where an elevation x top interaction was meaningful, since no

267 predator effects were seen at Chuckanut, and no low-elevation tubes were recovered at
268 Bowman (south saline).

269 Clam size appeared as a significant interaction in several other treatment effects on
270 survival. At Bowman, a significant size x top interaction emerged because open tubes had
271 similar counts of small and medium clams (8-9 per tube), but tubes with tops had on average 18
272 medium clams and only 12 small clams (Table 1). Thus either tops protected 6-mm better than
273 3-mm clams, or smaller clams suffered from other mortality sources in tubes with tops. A
274 moderate number (1-15 per tube, avg 3.2) of smaller clams were recovered dead from these
275 tubes (intact empty shells), suggesting they were killed by abiotic stressors (Fig. 2). At
276 Chuckanut we saw the overall lowest clam survival, at 44% relative to 59-61% at other sites. A
277 significant clam size x elevation interaction occurred because 3-mm clams were more likely to
278 be retrieved at the low (mean of 7 per tube) than middle elevation (6), but this pattern was
279 reversed for 6-mm clams (10 low and 12 mid). Thus, smaller clams suffered especially as
280 elevation increased. Many mid-shore clams at Chuckanut were recovered dead and intact (Fig.
281 2), as at Bowman.

282 At Chapman (south fresher) we found the most complex statistical outcome, including a
283 significant size x top x shell interaction (Table 1). Medium clams had excellent survival (96%)
284 when protected by mesh tops, but clams in tubes without tops had lower survival that varied
285 with shell treatment (44% with shell, 32% without). As at Bowman, the mesh tops did not
286 protect 3-mm clams (72%) as well as 6-mm clams (96%), and without tops, these small clams
287 survived better without shell (44%) than with shell (28%). The significant interactions in the
288 tests of how shell influenced clam survival do not enable strong conclusions regarding any

289 benefit of shell. However, poor survival of 3-mm relative to 6-mm clams under tops at this site,
 290 along with Bowman and Chuckanut, points to significant sources of mortality other than
 291 predators.

292

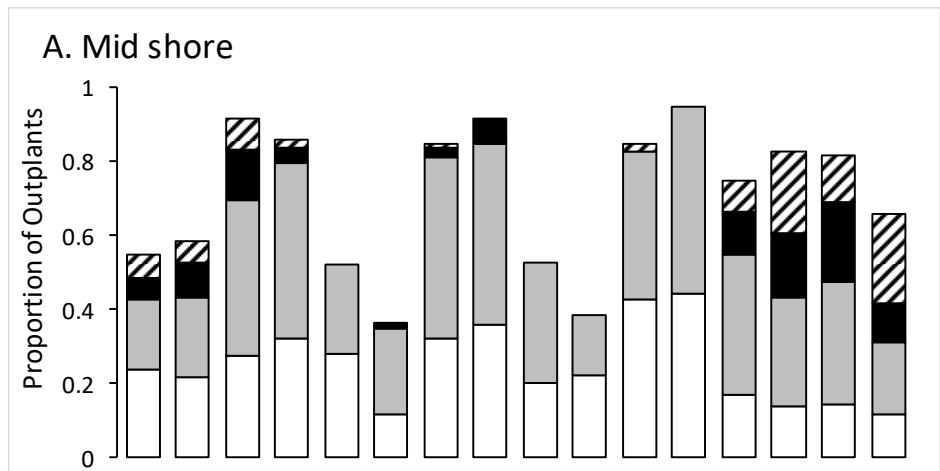
293 Table 1. Analysis of Manila clam survival at each of four sites in Washington State. Results as F-
 294 values (p-values) for fixed effects, with tube as random effect in linear mixed-effects model to
 295 account for small and medium clams deployed in each tube. All factors have one degree of
 296 freedom, with residual degrees of freedom varying among sites due to some missing tubes
 297 (Bowman 14, Chapman 32, Weaverling 20, Chuckanut 31). Significant p values are in bold.
 298

	South/Saline Bowman	South/Fresher Chapman	North/Saline Weaverling	North/Fresher Chuckanut
Initial clam size (small v. medium)	7.46 (0.016)	22.9 (0.0001)	6.00 (0.024)	53.5 (0.0001)
Elevation	--	0.95 (0.34)	4.95 (0.038)	0.13 (0.72)
Shell/no	0.23 (0.63)	0.08 (0.78)	0.08 (0.78)	3.00 (0.09)
Mesh top/no	8.85 (0.010)	78.5 (0.0001)	153 (0.0001)	0.05 (0.83)
Size x Elev.	--	2.54 (0.12)	0.22 (0.64)	5.21 (0.030)
Size x Shell	0.23 (0.64)	4.89 (0.03)	0.48 (0.50)	3.89 (0.058)
Size x Top	14.2 (0.002)	18.0 (0.0002)	0.22 (0.65)	0.34 (0.56)
Elev. x Shell	--	0.86 (0.36)	0.01 (0.91)	0.02 (0.90)
Elev. x Top	--	2.34 (0.14)	4.36 (0.05)	1.25 (0.27)
Shell x Top	0.20 (0.67)	0.01(0.93)	1.85 (0.19)	0.04 (0.85)
Size x Elev. x Shell	--	0.01 (0.93)	1.14 (0.30)	0.03 (0.87)
Size x Elev. x Top	--	0.00 (1.00)	0.04 (0.84)	2.49 (0.12)
Size x Shell x Top	0.47 (0.51)	7.52 (0.01)	1.52 (0.23)	0.03 (0.88)
Elev. x Shell x Top	--	3.44 (0.07)	0.37 (0.55)	0.12 (0.73)
Size x Elev. x Shell x Top	--	0.63 (0.43)	2.47 (0.13)	0.24 (0.63)

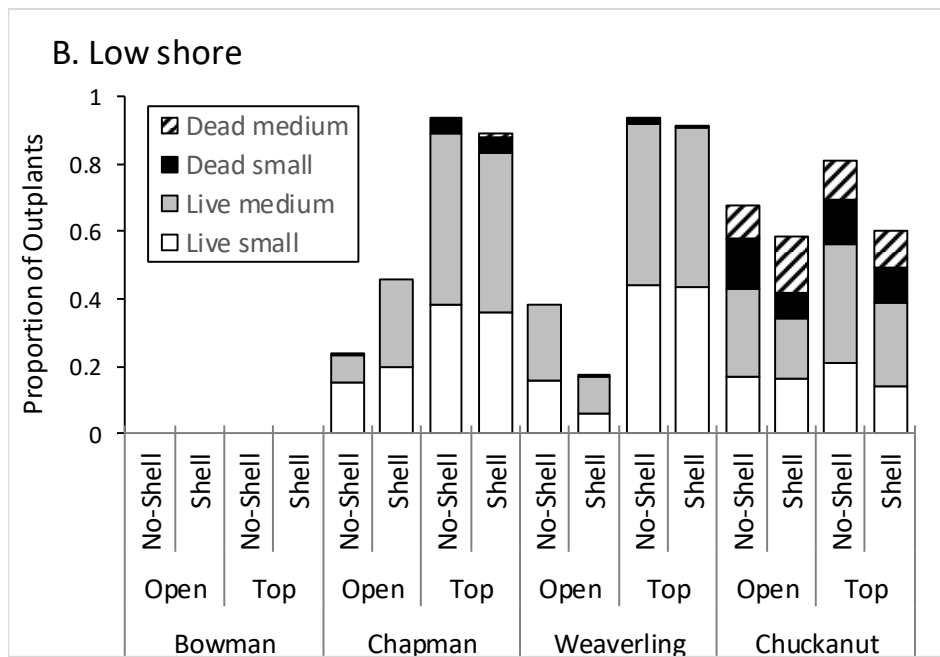
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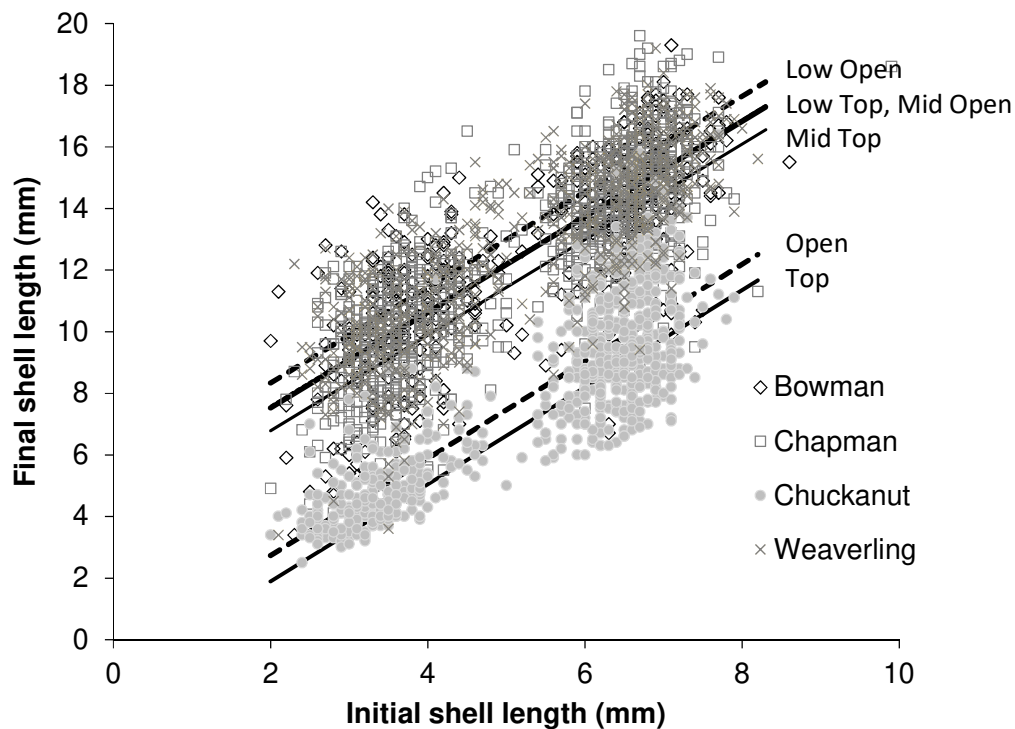
304 Figure 2. Manila clams recovered two months after outplant at four sites in Washington State,
 305 distinguishing live and dead clams. A) Mid-intertidal outplants at ca. +1.0 m MLLW, B) Low-
 306 intertidal outplants at ca. +0.3 m MLLW.

307

308 Growth at all four sites over the two month experiment varied with initial clam size, and
 309 we saw some site differences as well as treatment effects, especially with tube tops (Table 2).

310 Figure 3 shows initial (pink) and final size of each of the 2768 clams recovered alive after two

311 months; the 'small' vs 'medium' initial sizes can be seen as clouds of points. The variation
312 among individuals was striking; some small (3 mm) clams barely grew while some exceeded 14
313 mm after two months, even within a starting size class, site, and treatment.
314



315
316 Figure 3. Initial and final length of Manila clams (N = 2768) outplanted in summer 2017 at four
317 sites in Washington State. Each point represents one clam, but statistical analyses (see
318 Supplement) considered tube as a random effect to avoid pseudoreplication. Lines are based on
319 effect sizes from linear mixed effects models, including only significant factors. Lines for low-
320 intertidal treatments with tops and mid-intertidal treatments without tops (Open) are too
321 similar to be visualized separately. Chuckanut analysis is separate from other three sites.
322

323 Clam growth at Chuckanut (north fresher) was strikingly slow relative to other sites.
324 After two months, clams that initially averaged 3 mm long had grown to only 3.5 mm, but to an
325 average of 8.4 mm at other sites (Fig. 3). In all site-specific analyses, final size was strongly
326 related to initial size. Slopes of this relationship were near 1.5 at all sites, indicating that the

327 amount of shell growth increased with initial size; absolute growth was greater for larger clams.
328 Experimental treatment effects on growth were generally small (<20%) and inconsistent among
329 sites. At Weaverling (north saline), growth did not change with any treatment (Table 2). At
330 Chuckanut and at Bowman (south saline), growth was slowed by tops on tubes. At Chapman
331 (south fresher), we found a significant three-way interaction (elevation x top x shell; Table 2),
332 making it difficult to interpret the ecological significance of singular effects. In keeping with
333 expectations of longer feeding times, growth increased at lower elevation, but only in two of
334 the four combinations of treatments: tops without shell (comparing just this treatment
335 between elevations: $F_{1,8}=10.4$, $P=0.01$), and no tops with shell ($F_{1,8}=17.3$, $P=0.003$). At low
336 elevation in tubes with tops, clams grew slower with shell than without (Shell effect size = -0.96,
337 $se\ 0.37$, $F_{1,8}=2.31$, $P=0.05$), but given the interaction, this may not be statistically significant. For
338 the visualization of growth in Figure 3, we aggregated data from the three sites with rapid clam
339 growth and included both site and tube as nested random effects to generate the trendlines.
340 This analysis (see Supplement Table 2) found a general tendency across these three sites for
341 growth to be slower under mesh tops (9% slower for 3-mm clams, and less as initial size
342 increased) and at higher elevation (7% for 3-mm clams, also less as initial size increased), as
343 these two main treatment effects were significant.

344

345

346 Table 2. Analysis of Manila clam growth at each of four sites in Washington State. Results as F-
 347 values (p-values) for fixed effects, with tube as random effect in linear mixed-effects model to
 348 account for multiple clams deployed in each tube. Initial clam size was a continuous covariate
 349 and not included in interactions with other fixed effects. All factors have one degree of
 350 freedom, with residual degrees of freedom (df) differing by site due to loss of some tubes and
 351 mortality of clams. Residual df for experimental treatments and clam size covariate are:
 352 Bowman 14, 405; Chapman 32, 929; Weaverling 20, 652; Chuckanut 31, 653). Significant p
 353 values are in bold.
 354

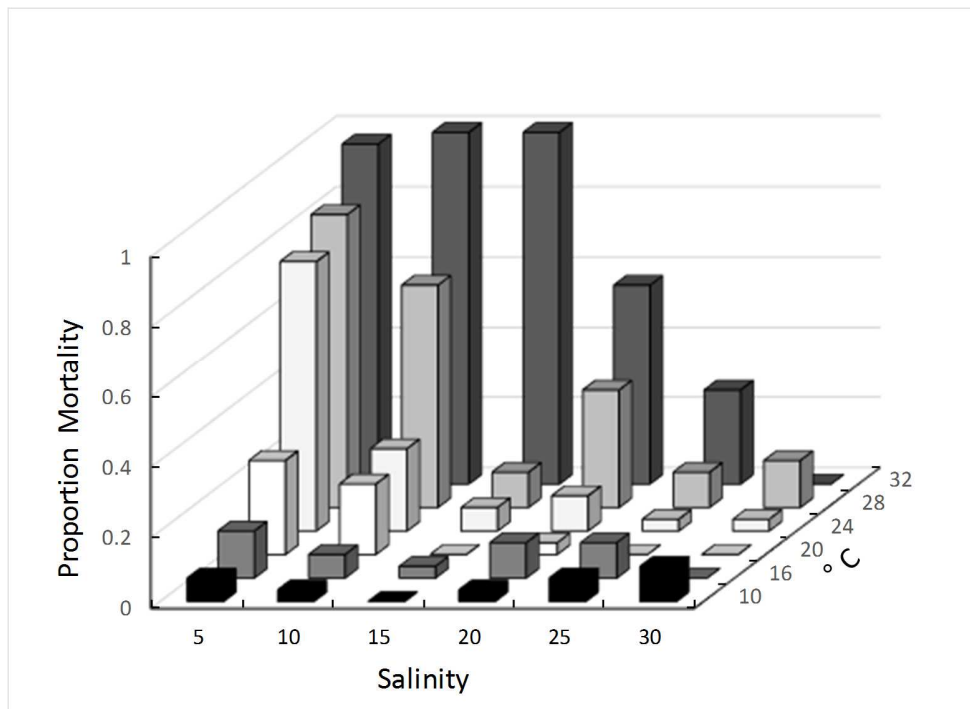
	South/Saline Bowman	South/Fresher Chapman	North/Saline Weaverling	North/Fresher Chuckanut
Initial clam size (mm)	717 (0.0001)	3328 (0.0001)	1507 (0.0001)	3105 (0.0001)
Elevation	--	26.7 (0.0001)	1.30 (0.27)	1.61 (0.21)
Shell/no	0.42 (0.53)	0.00 (1.00)	1.18 (0.29)	4.12 (0.051)
Mesh top/no	6.18 (0.026)	27.7 (0.0001)	0.007 (0.93)	5.38 (0.027)
Elev. x Shell	--	0.0 (0.98)	0.05 (0.83)	0.02 (0.90)
Elev. x Top	--	0.01 (0.92)	4.09 (0.057)	0.03 (0.87)
Shell x Top	2.61 (0.13)	1.61 (0.21)	0.63 (0.44)	1.24 (0.27)
Elev. x Shell x Top	--	5.92 (0.021)	1.71 (0.21)	0.52 (0.48)

355
 356

357 *Laboratory manipulation of temperature and salinity*

358 In our lab microcosms, there were no apparent differences in mortality among clam
 359 sizes; in each treatment, similar proportions of each size class died, so size classes are pooled
 360 for illustration (making N = 30 individuals per combination of factors). Clam mortality over 72
 361 hours varied dramatically among conditions (Fig. 4). None of the 30 control clams in ambient
 362 flowing seawater died. At either high temperatures (20-32 degrees) or low salinities (5-15 ppt),
 363 Manila clams experienced low rates of mortality, but when both parameters were combined,
 364 mortality increased to nearly 100% under extreme conditions. There was a highly significant
 365 interaction between the factors ($Z_{1,32}=-5.3$, $p \ll 0.001$; see Supplement Table 3); at higher

366 temperatures, lower salinities caused more mortality than would be the case if the
367 environmental stressors acted non-additively.
368



369
370 Fig. 4: Proportion of juvenile Manila clam mortality after 72 hours in different temperature and
371 salinity combinations.
372

373 **Discussion**

374 Understanding factors affecting survival and growth of juvenile clams through
375 vulnerable early life stages is critical both for aquaculture efforts and soft-sediment ecology.
376 Our field and lab experiments investigated a variety of environmental forcing functions that can
377 affect performance of juvenile Manila clams, and suggest that the roles of these factors are
378 highly context-dependent. In addition to site-level variability in survival and growth, particularly
379 impaired at our Chuckanut (north fresher) site, factors such as intertidal elevation, predator

380 protection, and initial clam size had different effects across sites. The largest magnitude effects
381 of predators were seen in the low-intertidal zone of Weaverling (74% fewer clams in open than
382 top treatments); there, predation impacts generally seemed to affect 3-mm and 6-mm clams
383 similarly. Abiotic factors were more important to clam performance at other sites, as inferred
384 by survival and growth in tubes with tops, as well as from the numbers of dead clams recovered
385 with intact shells. In our study, 3-mm clams were often more susceptible to abiotic stressors
386 than were 6-mm clams, and conditions at the mid-intertidal elevation appeared more stressful
387 than at the lower elevation. The largest magnitude effects of abiotic stressors occurred for 3-
388 mm clams at the mid-intertidal elevation at Chuckanut, where survival was 62% less and growth
389 59% slower than at other sites. The weakest predation effects were at Chuckanut, consistent
390 with few predators there. At Bowman, there was a more even distribution of predation effects
391 (32% based on difference between top and open treatments relative to initial counts) and other
392 sources of mortality (25% reduction in tubes with tops relative to initial counts). At Chapman,
393 three-way interactions among factors appeared in both survival and growth analyses, with
394 overall 53% of losses from predation and 16% from other sources of mortality.

395 In contrast to our expectations that clams in the southern region might suffer the most
396 from high temperatures, the poorest clam performance was at our low-salinity northern site
397 (Chuckanut). Thermal performance curves for Manila clams vary widely depending on their
398 geographical source and lab acclimation protocols, suggesting substantial adaptability; optimal
399 temperatures reported range from 9 to 35° (Nakamura, 2004; Nie et al., 2017a; Solidoro et al.,
400 2000), but most studies suggest that temperatures over 30° are lethal. Manila clams can survive
401 extended periods of salinity 15 (Elston et al., 2003) which was rarely reached at any of our sites.

402 Correlative data strongly implicate temperature extremes at Chuckanut as the cause of poor
403 clam performance. Detailed temperature data (Suppl. Fig. 3) recorded spikes of porewater
404 temperature during spring tides on hot days in the summer. Over the time period in 2017 when
405 clams were deployed, Weaverling had 2 days when porewater temperature exceeded 28
406 degrees, Chapman 6, Bowman 11, and Chuckanut 15 days. These site differences closely mirror
407 the proportions of clams found dead across all treatments in September (Weaverling <1%,
408 Chapman 5%, Bowman 14%, Chuckanut 34%) (Fig. 2). Perhaps most importantly, about a month
409 after clams were outplanted at Chuckanut, there were 5 consecutive days in August at these
410 high temperatures; back-calculating from the amount of growth that had occurred before
411 mortality (in the clams collected dead in September), it is likely that they died during those
412 August tides. Another metric of heat stress at this site is that unlike at the nearby Weaverling
413 site, porewater temperatures did not fully cool off at each high tide (Suppl. Fig. 3). For example,
414 porewater temperatures at Weaverling dropped to 15-16° each evening, whereas at Chuckanut
415 they never went below 22°. This pattern of high-minimum temperatures persisted until
416 September when low tides began to fall at night, and temperatures at the two sites became
417 very similar (Suppl. Fig. 4). However, predicting and detecting these sorts of extreme thermal
418 events is difficult; extremes at Chuckanut were not visible in either monthly point
419 measurements or average maximum daily temperatures from loggers, which were similar to
420 two other sites (Fig. 1B). Such extreme temperatures may not occur at Chuckanut every
421 summer, since outplants a year earlier did not show unusually poor performance.

422 Local bathymetry at Chuckanut may be responsible for these physical extremes relative
423 to our other sites. The tidal flat there is broad and heats up on warm summer days (with low

424 tides in midday). It is likely that the water draining slowly off this low-slope site never fully
425 mixes with deeper waters before being washed back onto the tidal flat, keeping the
426 temperatures from cooling down overnight. A similar phenomenon appears to occur in some
427 areas of Willapa Bay (Banas et al., 2007; Hickey and Banas, 2003). Shellfish farm managers at
428 the Chuckanut site say that local currents bring cool water to parts of the embayment but not
429 where our outplants were emplaced. If water is 'sloshing' up and down over this tidal flat
430 without thorough mixing with larger masses, as our temperature data indicate, this water could
431 also have reduced food availability for filter feeders (Banas et al., 2007). Wide tidal flats may
432 pose challenges for mobile benthic predators in terms of long-distance movement with the tidal
433 cycle or high temperatures; that is, the same factors contributing to strong abiotic effects on
434 clams at the Chuckanut site may have been stressful for predators as well, keeping them at low
435 abundance (Fig. 1F) and eliminating predation effects there. Only at the Chuckanut site did we
436 find evidence supporting our hypothesis that abiotic conditions would be more stressful for
437 clams with longer emersion; there, small clams in tubes with tops had 36% survival at low
438 elevation but only 28% survival at mid elevation. At all other sites and for medium clams,
439 survival under tops was more similar across tidal elevations.

440 As with juveniles of many organisms, the performance of smaller clams was predicted to
441 be more vulnerable to environmental changes than that of larger clams. Susceptibility of
442 smaller clams to stress has been clearly demonstrated for OA effects (Green et al., 2009; Miller
443 and Waldbusser 2016), and there is a broad literature on vulnerability of smaller clams to other
444 stressors as well as to predators (reviewed by Hunt and Scheibling, 1997). We found that
445 growth rates of medium vs. small clams were both dependent on initial size but were generally

446 unaffected by our manipulations. However, survival was influenced in some cases by clam size.
447 Our smaller clams were recovered less often after two months. Even in tubes with tops that
448 prevented predation and emigration, smaller clams survived less well than medium clams at
449 two of the sites; we suspect they were more likely to be killed by abiotic stressors such as high
450 porewater temperatures. Size-dependent mortality was also noted in Manila clams by Tezuka
451 et al. (2012). Our lab experiments did not show any clear differences in susceptibility of three
452 different size classes to manipulated temperature and salinity, but all those individuals were
453 larger than the field-deployed clams.

454 We also hypothesized that there would be a tradeoff between survival and growth
455 when comparing high and low elevations, with mid-shore being poorer for growth but lower-
456 shore clams more subject to predation by marine consumers such as crabs. Only one site
457 (Weaverling: north saline) showed the expected pattern of predator-exclusion having a greater
458 positive effect on survival lower on the shore. Because having tops on tubes also kept the
459 outplanted clams from being washed out, we cannot prove that this was a predator effect.
460 Contrary to expectations, growth over the summer months was very similar between units
461 placed higher versus lower on the shore. Only at Chapman (south, fresher) did we see the
462 expected increase in growth with longer immersion time for some low-shore outplants (Table
463 2). Other studies have generally found a positive growth effect of greater immersion time
464 (reviewed by Dang et al., 2010) except when continuous immersion results in extensive fouling
465 of bivalve (oyster) shells (Bishop and Peterson, 2006). During the experimental period, clams at
466 0.3 m MLLW were immersed 92% of the time, whereas those at 1.0 m MLLW were immersed
467 82% of the time. This difference may have been insufficient to generate growth differences. In

468 addition, positive impacts of time available for feeding may be obscured by non-consumptive
469 effects of predators. For instance, *Mercenaria* clams close their valves in response to nearby
470 predatory whelks (Irlandi and Peterson, 1991). Similarly, direct disturbance from crabs or a
471 chemical cue could reduce feeding by low-elevation clams in our study, an effect termed “crab
472 fright” (or “drill fright” from predatory whelks) by aquaculture farm managers at our sites.

473 Surprisingly, growth was similar at three of our four sites even though they experience
474 very different water masses, field temperatures, salinities, and probably food resources.
475 Growth in experimental units with mesh tops (to exclude predators) was significantly lower
476 than in the treatments lacking tops, although only by a small percentage (<10% for most sizes
477 and sites; Fig. 3). We were able to show in a longer-term study that there was no difference in
478 sediment accumulation between open units and those with tops (Suppl. Fig. 1), but it is likely
479 that the relatively fine mesh (1.5 mm openings) of tops reduced water flow enough to limit
480 food availability to the clams inside. Manila clams consume diverse food types including
481 phytoplankton, dissolved organic matter, and/or detrital particles (Watanabe et al., 2009). We
482 found that post-larval clams can survive and grow on a diet of kelp detritus particles as well as
483 on phytoplankton (unpubl. data). For some clams, riverine-source POM or detritus is an
484 important diet source (Sakamaki and Richardson, 2008; Seitz et al., 2017). We do not have data
485 on food resources available at our experimental sites, although all four sites have either
486 productive commercial (Chuckanut, Bowman, Chapman) or recreational (Weaverling) bivalve
487 harvests and thus likely have abundant food resources, at least seasonally. Clam farmers at
488 several of our sites commented, however, that local currents lead to substantial variation in
489 growth rates at the scales of tens of meters.

490 Detecting patterns in growth was made more challenging by the enormous per-
491 individual variation in growth rates even under identical field conditions. Coefficients of
492 variation within all sites and treatments were ~ 0.20 (except at Chuckanut, where they exceeded
493 0.5), whereas values in the literature (calculated from reported mean and s.d.) range from
494 0.005 (Beal and Kraus, 2002) or 0.03 (Beal, 2006) to 0.08 (Smith and Langdon, 1998) for final
495 sizes of known clam cohorts. Only a small amount of growth variation in our experiments was
496 explained by our experimental treatments. Even small amounts of growth suppression can
497 result in greatly reduced survivorship and reproduction in other bivalves (Nakaoka, 2000).

498 We expected that sites with lower salinity, or the higher variation in salinity that
499 accompanies proximity to a freshwater source, could also lead to reduced survival or growth,
500 but our data did not show such a consistent pattern. Chuckanut had lower salinity than its site
501 pair but also experienced high temperatures (discussed above), whereas in the southern region
502 Chapman had lower salinity than its site pair but survival and growth were both high. A broader
503 experiment with 4 site pairs varying in salinity also did not find a salinity effect (unpubl. data).
504 Manila clams are tolerant of extended low salinity of 15 (Elston et al., 2003), although below
505 this level clams cannot maintain normal metabolic activity (Kim et al., 2001). Most salinity
506 experiments have been performed with adult clams and at cool temperatures. Our short-term
507 lab experiments with juvenile clams suggested that synergisms between high temperatures and
508 low salinities can cause high mortalities. Mortality that we are attributing to high porewater
509 temperatures at Chuckanut (discussed above) could have been exacerbated by low porewater
510 salinities there, sometimes recorded as low as 12.

511 Field and lab studies on the effects of ocean acidification on bivalves have suggested
512 alarming trends, such as high mortality of oyster larvae in hatcheries (Barton et al., 2012) and
513 'death by dissolution' of hardshell clam spat in low pH sediment (Green et al., 2009). Low pH
514 can also inhibit burrowing in bivalves; this has the advantage of keeping clams out of chemically
515 poor conditions, but leaves them more vulnerable to predators and waves (Clements et al.,
516 2017). Green et al. (2009) found that adding crushed clam shell to a tide flat resulted in larger
517 numbers of clam recruits. Ruesink et al. (2014) showed improved survival of recently recruited
518 clams with shell addition, although gravel addition had a similar positive effect. Greiner et al.
519 (2018) found no effect of added shell hash on recruitment or survival of young Manila clams.
520 Because low-salinity water in estuaries often has reduced pH from terrestrial runoff, we had
521 hypothesized that buffering porewater pH by the addition of crushed shell would improve
522 performance of juvenile clams, with an effect especially clear at our lower-salinity sites.
523 However, neither survival nor growth differed between clams grown in mixed pebble-sand
524 versus units with crushed shell added. Tests of clam shell breaking strengths from a similar 11-
525 month experiment also showed no effect of shell addition (Dethier and Dobkowski, in prep.).
526 Our outplanted clams may have been too large to suffer significant damage from reduced
527 porewater pH, which likely impacts new recruits more severely. In addition, all our porewater
528 pH measurements were >7.5, whereas in the study by Green et al. (2009), in muddier
529 sediments, pH was near 7.0 and even when buffered was only 7.3. Our short-term field
530 experiments adding crushed shell to mixed pebble-sand showed no effects on porewater pH
531 after 24 hours (Fig. 2C) but might have had an impact over longer time scales. Shell addition by
532 Greiner et al. (2018) raised porewater pH by 0.1-0.2 units over ca. one month.

533 Because of the economic importance of Manila clams and other bivalves, there is
534 considerable literature on their physiology. In general, when food is unlimited, filtering rates
535 and growth increase with water temperature although there is a threshold (varying with
536 population) beyond which these rates decline (Solidoro et al., 2000; Nakamura, 2004). When
537 food rations are fixed, high temperatures can cause filtration and growth to stop, as bivalves
538 attempt to compensate for high metabolic costs of filtering activity and respiration (Nakamura,
539 2004; Beukema et al., 2009). Local growers anecdotally comment that they can often see a
540 “check” in farmed clams when growth ceases in the summer because of heat stress (or a
541 combination of heat and insufficient food); under some stressful conditions, Manila clams come
542 to the sediment surface, increasing their vulnerability to heat and to predators (Jason Ragan,
543 Taylor Shellfish, pers. comm.). Thus even in populations adapted to local conditions, high-
544 temperature stress can reduce growth and cause mortality, as appears to have happened at our
545 Chuckanut site. Lab studies with other clams show that lethal temperatures are sometimes only
546 a few degrees warmer than physiologically optimum temperatures in terms of filtration and
547 growth rates (Verdelhos et al., 2015). Extreme events can happen too suddenly for local
548 populations to acclimate.

549 Warm temperatures thus can be a mixed blessing for bivalves. When summer growth is
550 rapid because of accelerated filtering plus seasonal plankton blooms, clams get large more
551 quickly. This in turn increases survival since size-dependent mortality is the standard pattern
552 (Tezuka et al., 2013; Williams, 1980). Rapid growth also gets clams to harvestable size more
553 quickly and allows them to reproduce sooner. However, extremely warm conditions can exceed
554 the clams’ ability to maintain homeostasis (e.g., using heat shock proteins, Nie et al., 2017b),

555 eventually leading to death. Mass mortalities attributed to heat waves have been reported for
556 infaunal bivalves in diverse geographic regions (Verdelhos et al., 2015; Nie et al., 2017b).
557 Freezing-induced mortality is also known for clams (e.g., Elston et al., 2003) although it is
558 probably more common in epifauna such as oysters (John Adams, Skookum Inlet Farm, pers.
559 comm.).

560 As humans seek to adapt to climate change and continue to rely on marine species for
561 protein, what components of change will alter geographic ranges or significantly affect local
562 ecology and physiology? Helmuth et al. (2010) argue that temperature averages are much less
563 important than extremes, such as acute local hot-weather events, and our clam data appear to
564 fit this pattern. For juvenile clams that live in near-surface sediments, acclimation to gradual
565 climate warming is possible, but even short-term local environmental fluctuation may exceed
566 tolerance thresholds. As noted by Helmuth et al. (2010), “to an organism, all relevant
567 environmental changes are very local” (p. 997). Working at multiple sites showed us how
568 unpredictable these events can be spatially and temporally, and how daily maximum
569 temperatures may be of particular relevance to local organisms.

570

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584

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