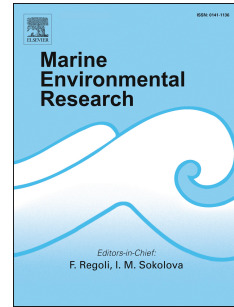


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Differential effects of ocean acidification and warming on biological functioning of a predator and prey species may alter future trophic interactions

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1 **Title:** Differential effects of ocean acidification and warming on biological functioning of a predator
2 and prey species may alter future trophic interactions

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8
9 **ABSTRACT**

10 Independently, ocean warming (OW) and acidification (OA) from increased anthropogenic
11 atmospheric carbon dioxide are argued to be two of the greatest threats to marine organisms.
12 Increasingly, their interaction (ocean acidification and warming, OAW) is shown to have wide-
13 ranging consequences to biological functioning, population and community structure, species
14 interactions and ecosystem service provision. Here, using a multi-trophic experiment, we tested the
15 effects of future OAW scenarios on two widespread intertidal species, the blue mussel *Mytilus edulis*
16 and its predator *Nucella lapillus*. Results indicate negative consequences of OAW on the growth,
17 feeding and metabolic rate of *M. edulis* and heightened predation risk. In contrast, *Nucella* growth
18 and metabolism was unaffected and feeding increased under OAW but declined under OW
19 suggesting OA may offset warming consequences. Should this differential response between the two
20 species to OAW, and specifically greater physiological costs to the prey than its predator come to
21 fruition in the nature, fundamental change in ecosystem structure and functioning could be
22 expected as trophic interactions become disrupted.

23 **Keywords –** Climate Change; Predation; Stressor Interaction; Biotic Interaction; Physiology

24 1. INTRODUCTION

25 Ocean warming (OW) and acidification (OA) are arguably two of the greatest threats facing marine
26 organisms as a result of increasing atmospheric carbon dioxide (CO₂) from anthropogenic sources
27 (Shukla et al., 2019). Increasingly, their interaction (i.e. ocean acidification and warming (OAW)) has
28 been shown to have wide-ranging consequences to the biological functioning of organisms including
29 changes to physiology (Lemasson et al., 2018; Li et al., 2015), morphology (Knights et al., 2020), and
30 behaviour (Manríquez et al., 2021) resulting in changes to population and community structure
31 (Lemasson et al., 2018; Manríquez et al., 2021), inter- and intra-species interactions (Sadler et al.,
32 2018), and the provision of ecosystem services (Listiawati and Kurihara, 2021).

33 Exposure to multiple stressors like OAW has been shown to be more biologically costly than a single
34 stressor (e.g., temperature or pH; Gunderson et al., 2016); a scenario arguably more reflective of
35 expected change in marine environments (Wernberg et al., 2012) than independent fluctuations in
36 these metrics. While organisms can adapt to changes in the abiotic and biotic conditions where they
37 occur (Alley, 1982; Jupe et al. 2020), this can come at a biological cost such as change in metabolic
38 performance and fitness (e.g., Braby and Somero, 2006; Lemasson et al., 2018; Breitberg et al., 2015;
39 Clements and Comeau, 2019). These costs may be detectable as an upregulation of metabolism
40 (Lemasson et al., 2018; Matoo et al., 2013) or through increased O₂ consumption rates as individuals
41 attempt to maintain homeostasis through physiological (e.g. cardio-circulation and the 'oxygen and
42 capacity dependent thermal tolerance' concept, Pörtner 2012) or behavioural compensation (Giomi
43 et al., 2016; Lemasson et al., 2018).

44 Increasing feeding may be one behavioural mechanism available to an organism to negate the
45 negative effects of OAW (Clements and Darrow, 2018). But what remains unclear is the extent to
46 which organisms can 'upregulate' feeding in response to associated increases in the metabolism, and
47 whether this regulation can be maintained (Harvey and Moore, 2017; Lord et al., 2017). Indeed, in
48 some cases, increasing energy intake may not be a viable option, such as when animals reduce feeding
49 as an anti-predator response (Naddafi and Rudstam, 2013) which itself can indirectly result in modified
50 biomineralization processes (Bibby et al., 2007), or changes in body size and reproductive output
51 (Harvey and Moore, 2017; Lemasson and Knights, 2021). Predation is well known to be an essential
52 driver of ecosystem dynamics (e.g., Sherker et al. 2017; Sadler et al. 2018) influencing prey population
53 dynamics via both consumptive effects (CEs) and non-consumptive effects (NCEs)(Orrock et al., 2008).
54 Under OAW, changes in the magnitude of NCEs (Bibby et al., 2007; Clements and Comeau, 2019) and
55 CEs (Sadler et al., 2018) during predator-prey interactions are predicted (see reviews: Briffa et al.,
56 2012; Clements and Hunt, 2015), affecting physiological, morphological and behavioural mechanisms,

57 as well as feeding strategies and induced defences (Lemasson and Knights, 2021; Manríquez et al.,
58 2021; Sadler et al., 2018). However, the biological cost of reduced physiological performance in
59 metrics like O₂ consumption and acid-base regulation may ultimately affect the extent to which
60 organisms can respond to an external input and individuals may autonomously prioritise maintenance
61 of internal homeostasis over a behavioural response (Bibby et al., 2007; Briffa et al., 2012; Harvey and
62 Moore, 2017; Lord et al., 2017).

63 Susceptibility to OAW may be dependent on species and taxa (Briffa et al., 2012; Clements and
64 Comeau, 2019) although calcifying species are shown to be particularly sensitive (Lemasson & Knights,
65 2021; Knights et al., 2020; Li et al., 2015; Sadler et al., 2018). *Mytilus edulis* (blue mussel) is a marine
66 intertidal and subtidal bivalve most commonly distributed in the Atlantic Ocean in temperate regions
67 (Knights, 2012), forming highly complex reef structures which support a multitude of other organisms.
68 In the UK, *Mytilus* spp. is an integral part of UK aquaculture and the national fisheries economy. The
69 species is resilient to environmental perturbations but stressors like OAW may reduce their nutritional
70 quality and fitness (Lemasson et al., 2019; Li et al., 2015). A major predator of *M. edulis* is the
71 gastropod whelk, *Nucella lapillus*, (Hunt and Scheibling, 1998). Unlike bivalves, gastropods have been
72 shown to be physiologically more resilient to OAW (Clements and Comeau, 2019) and mis-match in
73 response to environmental change could lead to predator-prey relationships becoming unbalanced
74 (Harvey and Moore, 2017; Sadler et al., 2018).

75 Given ocean acidification and temperature conditions are predicted to drastically change by end-of-
76 century (Shukla et al., 2019) with potential consequences for the physiology and morphology of
77 species and trophic interactions, here we evaluate the effects of elevated temperature and
78 acidification scenarios on the performance and trophic interaction of *Mytilus edulis* and *Nucella*
79 *lapillus*. Specifically, we test the effect of future climate scenarios on (1) individual physiological
80 responses of *M. edulis* and *N. lapillus* including standard metabolic rate, feeding rate, changes in shell
81 and somatic growth, and condition index of mussels (CI); and (2) the strength of trophic interactions
82 between *M. edulis* and *N. lapillus* to assess potential changes in ecosystem functioning under future
83 climate scenarios.

84

85 **2. MATERIALS AND METHODOLOGY**

86 2.1. Animal collection and husbandry

87 Adult blue mussels (*M. edulis*) and adult dog whelks (*Nucella lapillus*) of a similar size were collected
88 from a mid-shore intertidal site in Sidmouth, UK (50°40'41.1" N, 3°14'05.1" W) in April 2021. All
89 animals were cleaned of epibiota and individually marked using a water-based non-toxic nail varnish

90 (Acquarella (USA) which excludes toluene, formaldehyde, Dibutyl phthalate (DBP) and other solvents)
 91 and a permanent marker to allow for identification. Animals were acclimated for 2-weeks under
 92 standard laboratory conditions (12:12 h Light:Dark cycle, 15 °C, Salinity = 34 - 36, pH 8 (with natural
 93 variation due to fluctuations in atmospheric pressure; see Lemasson et al. 2018 and Knights et al. 2020
 94 for a full description)). Throughout acclimation and treatment, not including experimental starvation
 95 periods, mussels and whelks were fed twice weekly. Full water changes were conducted post feeding
 96 to maintain water quality ($\text{NH}_3 < 0.5 \text{ mg L}^{-1}$). Each *N. lapillus* was fed one opened mussel (*M. edulis*) (<
 97 10 mm) and given 12 h to feed. Each *M. edulis* was given 1 h to feed on cultured *Isochrysis galbana* at
 98 a cell density of 24000 to 30000 cells mL^{-1} .

99 2.2. Experimental design

100 Post acclimation, *M. edulis* ($37 \pm 5 \text{ mm}$ in length) were randomly selected for one of eight treatment
 101 combinations with temperature (ambient; 15 °C, and elevated; 20 °C), $p\text{CO}_2$ (ambient; 500 ppm, and
 102 elevated; 1000 ppm) and predator cues (present or absent). The eight treatments were: [1] **Ambient**
 103 **control** '15 °C × 500 ppm $p\text{CO}_2$ × predator absent', [2] **predator control** '15 °C × 500 ppm $p\text{CO}_2$ ×
 104 predator present', [3] **OA** '15 °C × 1000 ppm $p\text{CO}_2$ × predator absent', [4] **OA + predator** '15 °C × 1000
 105 ppm $p\text{CO}_2$ × predator present', [5] **OW** '20 °C × 500 ppm $p\text{CO}_2$ × predator absent', [6] **OW + predator**
 106 '20 °C × 500 ppm $p\text{CO}_2$ × predator present', [7] **OAW** '20 °C × 1000 ppm $p\text{CO}_2$ × predator absent', and
 107 [8] **OAW + predator** '20 °C × 1000 ppm $p\text{CO}_2$ × predator present'. Animals were exposed for 8-weeks.
 108 Temperature and $p\text{CO}_2$ treatments were chosen to simulate future predicted IPCC OAW scenarios
 109 (Shukla et al., 2019). There were 3 tanks per treatment (*M. edulis*; $n = 3$). *N. lapillus* ($25 \pm 3 \text{ mm}$ in
 110 length) were exposed to the same conditions as the mussels but without a predator cue; ambient
 111 control, OA, OW, and OAW again with 3 tanks per treatment (*N. lapillus*, $n = 3$). *N. lapillus* were
 112 identifiable by number and kept fully submerged in perforated containers to control for treatment
 113 exposure. The size of *M. edulis* and *N. lapillus* did not differ between tanks (*M. edulis*: $p = 0.981$, $F_{23,48}$
 114 $= 0.445$; *N. lapillus*: $p = 0.872$, $F_{11,22} = 0.517$) or treatments (*M. edulis*: $p = 0.95$, $F_{3,72} = 0.117$; *N. lapillus*:
 115 $p = 0.225$, $F_{3,30} = 1.54$).

116 2.3. OA and Temperature Design

117 For the controls and OW treatments, air stones gently bubbling ambient air under atmospheric
 118 pressure were present in each tank. For OA, pure CO_2 was slowly released into a Buchner flask mixed
 119 with dry air ($\approx 500 \text{ ppm } p\text{CO}_2$) using multistage CO_2 regulators (EN ISO 7291; GCE, Worksop, UK). $p\text{CO}_2$
 120 levels were monitored using a CO_2 analyser (LI-820; LI-COR, Lincoln, NE, USA). pH was measured twice
 121 a week using a microelectrode (InLab® Expert Pro-ISM; Mettler- Toledo Ltd, Beaumont Leys, UK)

122 attached to a pH meter (S400 Seven Excellence; Mettler- Toledo Ltd, Beaumont Leys, UK), calibrated
123 with Mettler Toledo buffers.

124 The experiment took place in a 15°C controlled temperature laboratory. Tanks under elevated
125 temperature were kept in a water bath, with the temperature kept constant using aquarium heaters
126 (thermocontrol °200, EHEIM Jager GmbH and Co. KG, Stuttgart, Germany).

127 For predator cue treatments, two *N. lapillus* were placed in an individual perforated polypropylene
128 plastic container to prevent predation of mussels and submerged in each tank for the duration of the
129 experiment. Whelk density per tank is representative of *Nucella lapillus* densities on U.K. intertidal
130 shores (Knights, *unpublished data*) and similar to densities found elsewhere (e.g. Hunt and Scheibling,
131 1998).

132 2.4. Carbonate chemistry

133 Total alkalinity (TA) was measured weekly using a calibrated potentiometric titrator (TitraLab
134 AT1000© series HACH Company, USA). Weekly, a 50 mL sample was taken from each tank and tested
135 to calculate TA. Temperature and salinity were taken *in situ* using a temperature probe (HH806AU,
136 Omega, U.K.) and a handheld refractometer (S/Mill, Atago, Tokyo, Japan) respectively. TA, salinity,
137 and temperature data were recorded to calculate calcite and aragonite saturation, and $p\text{CO}_2$
138 concentration in each treatment tank using CO2SYS software (Lewis and Wallace, 1998) using
139 Mehrbach solubility constants (Mehrbach et al., 1973), refitted by Dickson and Millero (1987).
140 Seawater chemistry data are shown in Appendices Tables 1 and 2.

141 2.5. Morphological and physiological metrics

142 2.5.1. Body measurements and dry mass equation

143 Body metrics and mass were recorded at three time points: (1) prior to experimental treatment
144 exposure; (2) week 4; and (3) week 8. For *M. edulis*, length, width, and height were recorded. Wet
145 weight was recorded using an analytical balance (Mettler Toledo, ML, Germany) after placing animals
146 on paper towel for 15 min. Dry mass of *M. edulis* was estimated for each time point using the equation
147 (eq. 1) from Knights (2012) as follows:

$$148 \text{ eq. (1) } y = 0.0508e^{0.9441x}$$

149 where x is shell length and y is total dry mass.

150 After 8-weeks, *M. edulis* were dissected and biometrics (length, width, height, total wet weight, wet
151 tissue weight, shell weight, and dry tissue weight) were measured (see condition index).

152 For *N. lapillus*, wet weight was recorded by leaving animals out of water for 5 min and drying, then
 153 recording total weight to the nearest 1/100th g using an analytical balance (Mettler Toledo, ML,
 154 Germany). Length from apex to siphonal canal was recorded using callipers.

155 2.5.2. *M. edulis* condition index

156 Body condition index (CI) of *M. edulis* was calculated using the following equation (eq. 2) after
 157 Davenport and Chen (1987; BCI, eq. (1):

$$158 \quad \text{eq. (2)} \quad (1) \quad \frac{\text{Dry tissue weight}}{\text{shell length}} \times 100$$

159 The shell length of *M. edulis* was measured to the nearest 0.05 mm using callipers. Animals were
 160 dissected to remove all tissue from shell, which was placed into a pre-weighted plastic weighing boat
 161 to dry in an oven at 60°C. Tissue was weighted at 48 h and 72 h to ensure a constant mass (dry tissue)
 162 had been achieved and CI calculated from eq. 2.

163 2.5.3. O₂ consumption rate

164 Respiration rate was used as a proxy for Standard Metabolic Rate (SMR). Respiration rate was
 165 recorded using microfibre optic oxygen sensors (Fibox 4, PreSens Germany). Temperature and salinity
 166 were recorded prior to each set of data collection and barometric pressure was obtained from the
 167 Plymouth Live weather Station (<http://www.bearsbythesea.co.uk>). Each was input into the PreSens to
 168 allow O₂ measurements to be corrected for fluctuations in temperature, salinity, and pressure.

169 All *M. edulis* (n = 72; 9 per treatment) and *N. lapillus* (n = 36, 9 per treatment) were placed in 250 mL
 170 and 120 mL sealed jars, respectively. For the first respiration data point, sea water (salinity = 34 - 36)
 171 was filtered to 2 µm and then autoclaved and aerated at 15°C. For time point 4 and 8, water was pre-
 172 equilibrated to the appropriate treatment conditions. To maintain stable temperature, during data
 173 collection, jars were kept in a water bath at 15°C or 20°C. All animals were starved for ~8 days prior to
 174 data collection to eliminate any change in respiration due to digestion and alter respiration rates (Sejr
 175 et al. 2004, Ansell & Sivasdas 1973). Within the jar, water was mixed using a magnetic stir bar for the
 176 duration of the experiment (400 rpm). Data collection started when jars were closed. For *M. edulis*,
 177 data points were only counted if the animal was visibly open. All data points before 15 min were
 178 discounted for both animals to allow for acclimation. O₂ (mg L⁻¹) was recorded every 5 min for 40 min
 179 or until O₂ saturation reached 75 % to avoid exposure to hypoxic conditions. O₂ measurements were
 180 corrected for background bacterial respiration or primary productivity by offsetting respiration rate
 181 with O₂ changes in jars without an animal in them. Respiration rate was also normalised to 1 g of
 182 calculated dry weight (Knights, 2012). SMR was calculated using the following equation (eq. 3).

183
$$\text{eq. (3)} \quad SMR = \frac{V \times r}{t \times DM}$$

184 where v is volume of jar (L), r is change in O_2 in jar (mg L^{-1}), t is time (min), and DM is dry mass (g)
 185 calculated using the relationship defined in Knights (2012).

186 2.5.4. *M. edulis* clearance rate

187 The same individuals used for respirometry were also used for clearance rate (CR). *M. edulis* were
 188 starved for 24 to 72 h. The CR assay followed methodology in Lemasson *et al.* (2018). Individuals were
 189 placed in 300 mL of UV treated and filter sea water (15°C , 500 ppm $p\text{CO}_2$, salinity = 34 – 36) and
 190 subsequent data points were recorded in water pre-equilibrated to treatment conditions. A dilution
 191 of 1:100 mL shellfish diet (Shellfish diet 1800, Reed Mariculture, USA) was used as feed. *M. edulis* were
 192 given up to 20 min to open and algae added once opened. Any animals which closed during the assay
 193 were discounted and re-done the following day. Once open, 700 μL of stock solution was used per
 194 beaker at a concentration of 24,000 to 30,000 cell mL^{-1} . In each beaker, a magnetic stirrer bar (400
 195 rpm) was used to keep the water well-mixed. A 20 mL sample (t_0) was taken 2 min after stock solution
 196 was added to allow for adequate mixing of algae. Another 20 mL sample (t_1) was taken after 20 min
 197 of filtering. Counts of the algae in the water were done in triplicate by a Coulter Counter (Beckman
 198 Coulter, Z2). CR was calculated using the following equation (eq. 4).

199
$$\text{eq. (4)} \quad CR = \left(\frac{v \times 60}{t}\right)(\ln t_0 - \ln t_1)$$

200 where CR is clearance rate (L h^{-1}), v is volume (L), t_0 is the initial sample (cell L^{-1}) and t_1 is the sample
 201 (cell L^{-1}) taken after 20 min. CR was then normalised to 1 g by dividing by calculated dry mass of
 202 individual (Knights, 2012).

203 2.6. Feeding behaviour

204 Feeding behaviour of *N. lapillus* was assessed under treatment conditions to look at both predator risk
 205 of *M. edulis* and feeding rate of *N. lapillus*. After 8-weeks of exposure to the experimental treatments,
 206 *N. lapillus* were starved for 7 to 9 d. *M. edulis* used in the experiment were pre-acclimated for 8 weeks
 207 in each of the experimental treatments. Five pre-acclimated *M. edulis* (20 to 45 mm length) were
 208 placed in each tank, with 11-12 tanks in each of the four treatments. There were control tanks ($n = 3$)
 209 included in each treatment which contained only *M. edulis*. *N. lapillus* were placed into tanks and
 210 mortality was measured every 24 h over 8 d. Mussels were considered dead when they gaped open
 211 and did not respond when physically disturbed (Lupo *et al.*, 2021). In tanks without predators there
 212 was one mortality in the elevated temperature and $p\text{CO}_2$ treatment (6.7 % mortality).

213 2.7. Statistics

214 Data were tested for assumptions of normality, bias and homoscedasticity of residuals. Data were log-
215 transformed or square-root transformed if data did not meet assumptions. All data were analysed
216 using R (version 4.1.1, R Core Team, 2021) and all graphs were produced using the 'ggplot2' package
217 (Wickham, 2016). Where significance was identified Tukey HSD post-hoc pairwise comparison was
218 used to find differences between groups. 'Tank' was included as a random factor in all analyses.

219 2.7.1. Shell, body metrics and CI

220 Mussel shell length, width, height, wet weight, CI, and whelk shell length, and wet weight were all
221 analysed using the same linear mixed effects model (Package 'nlme'; Pinheiro et al., 2021). For
222 everything except CI, change in metric from week 0 to week 8 was used in the analysis. 'Temperature'
223 (two levels: 15°C; ambient, 20°C; elevated) and 'pCO₂' (two levels: 500 ppm; ambient, 1000 ppm;
224 elevated) were considered fixed factors for all analyses. Mussel metrics had an additional factor, 'cues'
225 (two levels: present, absent).

226 2.7.2. SMR and CR

227 SMR and CR were analysed with a linear mixed effects model with temporal autocorrelation.
228 'Temperature' (two levels: 15°C; ambient, 20°C; elevated) and 'pCO₂' (two levels: 500 ppm; ambient,
229 1000 ppm; elevated) were considered fixed factors for all SMR and CR analyses. Mussel SMR and CR
230 had an additional factor, 'cues' (two levels: present, absent). 'Time (in treatment)' (3 time points: week
231 0, 4, and 8) was also included in the analysis.

232 2.7.3. Whelk feeding rate

233 A two-factor linear mixed effects model was used to analyse differences in proportional mortality (or
234 whelk predation rate) of mussels at day 8 of the experiment. 'Temperature' (two levels: 15°C; ambient,
235 20°C; elevated) and 'pCO₂' (two levels: 500 ppm; ambient, 1000 ppm; elevated) were considered fixed
236 factors.

237 **3. RESULTS**

238 3.1. Mortality and growth

239 3.1.1. *M. edulis*

240 Mortality was 23 % under OAW, 6 % under OW and control (ambient) conditions, and 0 % under OA.
241 *M. edulis* mortality was significantly higher in the OAW treatment over all other treatments ($p < 0.01$,
242 $F_{4,67} = 4.382$).

243 There was a significant interaction between OW, OA and predator presence on shell length ($p < 0.05$,
 244 $F_{1,61} = 5.635$) and a significant reduction in growth when predators were present (Fig 1). Shell length
 245 increased by 159 %, from an average increase of 0.24 mm, in the presence of predators, to 0.63 mm,
 246 when predator cues were absent (Tukey HSD; $p < 0.001$). There was no effect of OW alone (Tukey
 247 HSD; $p = 0.438$) or predator presence alone (Tukey HSD; $p = 0.300$) on growth in length.

248 There was no effect of OW ($p = 0.176$, $F_{1,65} = 1.870$), OA ($p = 0.998$, $F_{1,65} = 0.000$), or cues ($p = 0.107$,
 249 $F_{1,65} = 2.664$) on width change over the 8-week exposure ($p = 0.051$, $F_{7,61} = 2.149$).

250 There was a significant interaction between OW and OA on height ($p < 0.01$, $F_{1,53} = 7.420$). There was
 251 a 113 % increase in shell height compared to the control under OA increasing by 0.40 mm under OA,
 252 versus just 0.19 mm under ambient conditions (Tukey HSD; $p < 0.01$). There was no effect of OAW on
 253 height (Tukey HSD; $p = 0.415$). There was also an interaction between cue presence and OA ($p < 0.05$,
 254 $F_{1,53} = 5.420$) on mussel height with a 175 % increase in height under OA compared to the control
 255 (Tukey HSD; $p < 0.01$). There was no effect of OA on height when predators were present (Tukey HSD;
 256 $p = 0.574$).

257 Median wet weight decreased by 55% under OA from 0.59 g to 0.26 g after 8-week regardless of OW
 258 and cue presence (Fig. 4; $p < 0.05$, $F_{1,61} = 4.988$).

259 3.1.2. *Nucella lapillus*

260 There was 12 % mortality in the OW treatments and 0 % in all other treatments. However, there was
 261 no significant effect of OW ($p = 0.167$, $F_{1,32} = 2.000$) or OA ($p = 1.000$, $F_{1,32} = 0.000$) on survival in *N.*
 262 *lapillus*.

263 There was no effect of OW ($p = 0.666$, $F_{1,30} = 0.190$) or OA ($p = 0.678$, $F_{1,30} = 0.176$) on length change
 264 in *N. lapillus* after 8 weeks. There was no effect of OW ($p = 0.849$, $F_{1,30} = 0.037$) or OA ($p = 0.738$, $F_{1,30}$
 265 $= 0.114$) exposure on wet weight change in *N. lapillus* after 8 weeks exposure to treatments. There
 266 were no interactions.

267 3.2. Condition index

268 CI of *M. edulis* increased by 8.1 % from 3.07 to 3.32 when predator cues were present ($p < 0.01$, $F_{1,63}$
 269 $= 7.225$). OW ($p = 0.819$, $F_{1,63} = 0.053$) and OA ($p = 0.875$, $F_{1,63} = 0.025$) had no effect on CI.

270 3.3. Metabolic rate

271 3.3.1. *M. edulis*

272 There was a significant interaction between OW and OA on SMR in *M. edulis* ($p < 0.05$, $F_{1,194} = 4.44$)(Fig.
 273 2). O_2 consumption rates increased in an additive fashion by 18.8 % under OA. OW increased the SMR

274 of *M. edulis* by 33 %. However, there was no effect of OA on SMR under OW. There was also an
275 interaction between time in treatment and cue presence ($p < 0.05$, $F_{(2,194)} = 3.399$). In the absence of
276 cues, SMR decreased by 16 % from week 0 to 4 and remained the same from week 4 to 8. SMR in
277 response to predator presence was maintained until week 4, and from week 4 to week 8, SMR reduced
278 by 31 %.

279 3.3.2. *N. lapillus*

280 There was an effect of OW (figure 7; $p < 0.001$, $F_{1,88} = 18.631$) but not OA ($p = 0.808$, $F_{1,88} = 0.059$) on
281 whelk O_2 consumption rates, with OW increasing whelk O_2 consumption rates by 42 % (Fig. 2).

282 3.4. Clearance rate

283 There was a four-way interaction between OW, OA, predator presence, and time in treatment ($p <$
284 0.01 , $F_{2,194} = 6.184$, Fig. 3). At 15°C, when predators were absent, CR stayed the same through time
285 in the control (Tukey HSD; $p = 0.929$) and OA treatments (Tukey HSD; $p = 0.939$). When predators were
286 present, CR reduced by 35 % over the 8-week exposure for both the control (Tukey HSD; $p < 0.05$) and
287 OA treatment (Tukey HSD; $p < 0.01$). When cues were absent, OW had no effect on CR (Tukey HSD; p
288 $= 0.450$). OAW significantly reduced CR by week 8 (Tukey HSD; $p < 0.001$). OW with predator presence
289 did not affect CR until after week 4 of exposure. There was no difference in CR of mussels by week 4
290 (Tukey HSD; $p = 0.480$), by week 8, CR in the presence of predators was 31 % less than in the absence
291 of predators (Tukey HSD; $p < 0.01$). Under OAW, when predators were absent, CR increased 21 % by
292 week 4 (Tukey HSD; $p = 0.100$) then decreased 44 % from week 4 to week 8 (Tukey HSD; $p < 0.001$),
293 with an 33 % overall decrease in CR (Tukey HSD; $p < 0.01$). When predators were present, CR decreased
294 31% by week 4 (Tukey HSD; $p < 0.05$), then increased 48 % from week 4 to week 8 (Tukey HSD; $p <$
295 0.05), with no overall change in CR (Tukey HSD; $p = 0.942$).

296 3.5. Whelk feeding rate

297 There was clear trend, if not significant interaction, between OW and OA on mussel mortality as a
298 result of whelk predation (Fig. 4; $p = 0.056$, $F_{1,30} = 3.961$). At 15°C, percentage mortality of mussels
299 decreased 9% from 33 % to 24 % (Tukey HSD; $p = 0.170$) under OA. At 20°C (OW), mussel mortality
300 increased by 10% from 48 % to 58 % under OAW (Tukey HSD; $p = 0.146$) (Fig. 4).

301 4. DISCUSSION

302 OAW impacts are being documented ubiquitously across marine taxa and marine ecosystems with
303 wide ranging variable effects and complex interactions between pH and temperature stressors (e.g.,
304 Clements and Hunt, 2015; Knights et al., 2020; Kroeker et al., 2013). In this study, the impacts of future

305 predicted OAW on growth and physiology have been highlighted in two major marine invertebrate
306 taxa, mussels and dog whelks. Further investigation elucidated the impacts to the predator response
307 of *M. edulis* and effects of OAW on the predator-prey relationship between these species. Results
308 indicate significant effects of OA, OW, OAW, and predator presence on growth, CR and SMR in *M.*
309 *edulis*. Less pronounced effects on growth and SMR were seen in *N. lapillus*, alongside an increase in
310 predation rate under OAW, indicating increased predation risk to *M. edulis*.

311 4.1. Growth and condition

312 The effect of OAW on shell and somatic growth in marine invertebrates appears highly species
313 dependent (Gazeau et al., 2013; Kroeker et al., 2013; Lemasson et al., 2018; Lemasson and Knights,
314 2021). For *M. edulis*, OA was found to increase shell growth (length and height) alongside an increase
315 in SMR. In *N. lapillus*, there was no effect of OAW or individual effects of OA or OW on growth in shell
316 length or growth in wet weight (but see Mayk et al. 2022 where shell growth was shown to increase
317 under OA). Increased shell growth in *M. edulis* may be explained in terms of carbonate chemistry. For
318 example, *M. edulis* biomineralize using two different forms of calcium carbonate, a mixture of calcite
319 (~17 %) and aragonite (~83 %) (Hubbard et al., 1981). Aragonite has a greater dissolution rate to calcite
320 under OA conditions (Feely et al., 2004). Therefore, dissolution of the shell under lower pH may lead
321 to mineralogical plasticity in biomineralization, as seen in this study, despite some evidence for a net
322 decrease in calcification rate under OA (Leung et al., 2017; Li et al., 2015). There are variable effects
323 of OAW on shell growth in the literature with the majority of the literature reporting negative impacts
324 on growth (e.g., Fitzer et al., 2015; Lemasson and Knights, 2021). Despite this, we observed an increase
325 in length of *M. edulis* under OA. However, the literature shows that animals calcifying under OA may
326 prioritise investment in lower quality shell structure (i.e. greater size, weaker shell; Leung et al. 2022),
327 which consequently may increase predation risk (Gazeau et al., 2013; Li et al., 2015; Sadler et al.,
328 2018).

329 Environmental stressors can interact to influence the overall effect of a stressor on an organismal trait
330 (Kroeker et al., 2017). The increase in growth under OA was counteracted under elevated temperature
331 or in the presence of predator cues indicating an antagonistic relationship between these variables
332 and biomineralization traits. In the presence of predators, mussels can induce calcification to increase
333 shell thickness as an anti-predator response. This upregulation of calcification is a common non-
334 consumptive effect (NCE) of predators within a prey population (Freeman, 2007). However, under
335 environmental stress the cost of upregulating calcification increases, particularly under OA as shell
336 dissolution increases (Nienhuis et al., 2010). Mussels may be calcifying at the same rate but
337 reallocating the energy used to prioritise shell thickness over shell size as an anti-predator defence

338 strategy. Shell thickness has been shown to decrease under OA conditions over a relatively short time
339 scale (8 weeks) in *M. edulis* (Fitzer et al., 2015; Sadler et al., 2018). Alongside this, net calcification rate
340 has been reported to decrease in mussels under elevated $p\text{CO}_2$ (Li et al., 2015). Within this study, shell
341 thickness and net calcification rate were not recorded, however, based on the literature, we predict
342 there was likely a trade-off between structural integrity and size of shell mussels exposed to OA (Fitzer
343 et al., 2015; Knights et al., 2020; Sadler et al., 2018).

344 4.2. Condition index

345 CI is used to comparatively assess the reproductive condition of mussels between treatments (Knights,
346 2012). In this study, perhaps surprisingly, CI increased under predator presence. Given the CI
347 calculation uses shell length and dry tissue weight, this suggests the animals are investing more in
348 somatic tissue than length as length change did not differ between OAW treatments when cues were
349 present. OAW had no effect on CI in mussels despite evidence suggesting otherwise in the literature.
350 For example, temperature increase (Sunila, 1981) and enhanced food availability (Hatcher et al., 1997)
351 both led to an increased metabolism which resulted in a greater CI of *Mytilus* sp.. Low pH was also
352 found to increase condition index in *M. californianus* (Rose et al., 2020). On the other hand, Lemasson
353 and Knights (2021) found effects of OAW on CI to be species-specific and found no effect of OAW on
354 CI in European flat oysters (*Ostrea edulis*). The results suggest that *M. edulis* may be prioritising
355 reproduction and fecundity over long term survival. A similar finding was shown in *Daphnia magna*,
356 which displayed greater investment in fecundity under size selective predation pressure (Zhang et al.,
357 2016) and also in *M. edulis*, where gonad development was accelerated when exposed to starfish cues
358 (Reimer, 1999).

359 4.3. Metabolism

360 Maintaining metabolic rate in response to energetic demand is essential for survival and basic
361 functions like growth and feeding (Gazeau et al., 2013). Metabolism is closely linked to temperature,
362 particularly in marine ectotherms (Seibel and Walsh, 2003). O_2 consumption rates (SMR), increased
363 under OAW by 33 % for *M. edulis*, in concordance with previously reported increases in SMR in bivalves
364 under OAW (Lemasson et al. 2018). The upregulating effect of low pH on SMR in mussels at 15 °C was,
365 however, masked by elevated temperature. pH had no additional effect on SMR alongside elevated
366 temperature. Similar results have been found in *M. edulis*, where temperature is the dominant factor
367 in influencing SMR and addition of low pH stress does not affect the SMR response (Lemasson et al.,
368 2018; Matoo et al., 2021). However, the increased SMR of *M. edulis* in response to OA at 15 °C is not
369 well documented. OA exposure puts physiological stress on the internal homeostasis on an organism;
370 energetic demand for acid-base regulation increases as pH of internal fluids lowers (Gazeau et al.,

371 2013). The metabolic upregulation seen in *M. edulis* was not evident in *N. lapillus*, indicating a greater
372 resilience of *N. lapillus* to OA and supports the suggestion that some species of gastropod are more
373 resilient to OA than bivalves (Clements and Comeau, 2019).

374 Change in physiology in response to an external stimulus (i.e. predator presence or OAW) can result
375 in metabolic depression in animals over time (Gazeau et al., 2013; Seibel and Drazen, 2007). In this
376 study, time in treatment and predator presence interacted to induce metabolic depression in *M. edulis*
377 after just 4 weeks of exposure and may be explained by anti-predator response strategies. Animals
378 respond in different ways to predators depending on their mobility. Mobile animals may upregulate
379 the metabolism to escape a predator, immobile animals, such *M. edulis*, may downregulate the
380 metabolism to reduce predator contact through processes such as feeding (Gazeau et al., 2013; Seibel
381 and Drazen, 2007). Alongside these findings, metabolic depression resulting from predator exposure
382 under OA has been observed in mussels (*Brachidontes pharaonis*) exposed to crab predator cues
383 (*Eriphia verrucosa*) (Dupont et al., 2015). The metabolic depression of *M. edulis* seen in this study may
384 have resulted from a reduced feeding rate when predator cues were added (i.e. reduced energy
385 acquisition), coupled with increased physiological stress of exposure conditions (i.e. offsetting shell
386 dissolution and maintaining acid-base homeostasis) (Gazeau et al., 2013; Seibel and Drazen, 2007). *M.*
387 *edulis* may have the capacity over short time scales (< 4 weeks) to maintain physiological performance
388 under climate change stressors thereby compensating using trade-offs. However, over a longer time
389 scale (> 4 weeks), in the presence of predators, *M. edulis* have a reduced metabolic performance which
390 may be unsustainable and fitness-reducing as less energy is available for other physiological processes
391 (Gazeau et al., 2013).

392 4.4. Clearance rate

393 Clearance rate (CR) is a semi-quantitative measurement and can be used as a measure of physiological
394 or behavioural performance (Lemasson et al., 2018) and is closely linked to metabolic processes so
395 that it can be used to balance energy acquisition and expenditure (Giomi et al., 2016). Increase in
396 metabolic rate, from OAW, can be an issue if energy acquisition does not also increase. Here, a
397 complex interaction was found between temperature, $p\text{CO}_2$, predator presence, and time in
398 treatment. Despite increases seen in SMR as a result of OA and OW exposure, food intake (CR) did not
399 increase under the same scenarios. Food availability or intake is a known limiting factor of animal
400 resilience to OAW stressors (Clements and Darrow, 2018). Therefore, energetic requirements may not
401 have been met, resulting in the decreasing trends seen over time in SMR.

402 From a behavioural perspective, feeding is a behaviour that increases the predation risk of an animal
403 and can be downregulated by the animal accordingly (Křivan and Eisner, 2003). Here, cue presence

404 led to much greater change in CR than OAW scenario, resulting in reduced feeding of the animals
405 when cues were present in all treatments but elevated temperature. Under elevated temperature,
406 with predators present, feeding rate of *M. edulis* did not decrease as expected, suggesting a potential
407 trade-off or 'decision' to prioritise physiological demand over predator behavioural response (Briffa
408 et al., 2012). While this may reduce fitness in relation to predation risk, it has potential to work as a
409 compensatory mechanism for OW as more energy is acquired to offset negative impacts of OW (Giomi
410 et al., 2016). Animals may upregulate feeding to maintain physiological processes despite greater
411 predation risk. This removes or alleviates food intake as a limiting factor for animal wellbeing under
412 OAW scenarios (Clements and Darrow, 2018).

413 In *M. edulis*, reduced feeding rate as an anti-predator response in conjunction with the increased
414 energy requirement observed in individuals exposed to OAW illustrates a clear juxtaposition between
415 behavioural and physiological responses when relating to fitness maintenance. When exposed to
416 OAW and predation, energy intake falls short of energy expenditure as seen in eventual metabolic
417 depression. On the one hand, reduced feeding rate when exposed to cues, under OAW, indicates the
418 mussels behavioural response (e.g., cue perception) is not impaired (Clements and Comeau, 2019).
419 On the other, this may be detrimental to future adaption as it shows the animal is prioritising
420 behavioural rather than physiological mechanisms of survival.

421 4.5. Species-specific differences and interactions

422 Molluscs, as a taxa, have been shown to be particularly sensitive to OAW in terms of survival,
423 calcification, growth, and development, compared to crustaceans, fish, and algae (see review: Kroeker
424 et al., 2013). Within the taxa, the effects of OAW exposure are often species-specific as illustrated in
425 the growth and mortality differences found here. *N. lapillus* had no significant response to OAW
426 scenarios either in length or wet weight whereas growth rate increased for *M. edulis*, in length and
427 height, and decreased for wet weight. Mortality was also significantly greater in *M. edulis* under OAW
428 conditions, whereas *N. lapillus* mortality was unaffected. The disparity between OAW responses in
429 bivalves and gastropods has been documented in behavioural defences (see review by Clements and
430 Comeau, 2019) with bivalves more sensitive than gastropods to OAW illustrated by predator
431 avoidance behaviour (e.g bivalves: Clements et al., 2017; gastropods: Queirós et al., 2015).
432 Behavioural responses to OAW can be indicative of physiological underlying effects of OAW, such as
433 impacts to metabolism or growth (Clements and Comeau, 2019; Gazeau et al., 2013). An increased
434 susceptibility of bivalves to OAW over their predators may lead to incongruity in their biotic
435 relationships. That is, if there is greater biological cost to the bivalve and no change in cost to the

436 gastropod predator as shown here, this may suggest potential for modification of predator-prey
437 dynamics and wider trophic impacts.

438 Predation rate is a key driver in ecosystem dynamics (Holling, 1959). Change in a predator's feeding
439 rate has potential to destabilise lower trophic levels (Kroeker et al., 2017). In this study, elevated
440 temperature increased *N. lapillus* feeding rates by 82 %. In the literature, Quieros et al. (2015)
441 reported that *N. lapillus* foraging distance and foraging time increased under OA suggesting an
442 increased feeding rate. However, this may increase their own susceptibility to predation from higher
443 trophic levels (Křivan and Eisner, 2003). Nevertheless, increased feeding of *N. lapillus* as a result of
444 elevated temperature coupled with the negative impacts of OAW on *M. edulis*, and increased SMR
445 but reduced feeding rate, could negatively affect *M. edulis* populations. Predation risk of prey animals
446 is reported to increase in bivalves under OA regardless of predator exposure to OAW stress (Sadler et
447 al., 2018; Sanford et al., 2014). Increased predation leading to greater consumptive effects of *N.*
448 *lapillus* on *M. edulis* may have knock-on consequences to ecosystem services and wild mussel fisheries
449 (Lemasson and Knights, 2021; Sadler et al., 2018). However, local ecosystem effects may vary
450 depending on functional redundancy (i.e. biodiversity) within a community and plasticity of the
451 populations affected (Kroeker et al., 2017). Investigating OAW with predation as a stressor adds
452 ecological relevance to a study and help elucidate the interacting effects of OAW in an ecologically
453 relevant setting (Kroeker et al., 2013).

454

455 5. CONCLUSION

456 The relative biological cost of OAW impacts individual animal fitness and will reflect into the
457 population. The two species, *M. edulis* and *N. lapillus*, had contrasting responses in terms of growth,
458 metabolism, and feeding to OAW exposure. In addition to this, the species chosen are ecologically
459 linked in marine ecosystems, therefore impacts to one will affect trophic relationships (Holling, 1959).
460 Here, *M. edulis* demonstrated greater effect sizes from OAW exposure than *N. lapillus*. The interaction
461 between the two species also changed under OAW exposure, exhibited though change in both NCEs
462 (e.g., reduced CR in *M. edulis*) and CEs (e.g., increased predation rate of *N. lapillus*). The differential
463 responses of the two species and the increased feeding rate seen in *N. lapillus* indicates that under
464 future climate change scenarios, *M. edulis* may experience greater predation risk alongside
465 physiological implications whereas in contrast, *N. lapillus* may largely be unaffected if food is not
466 limited. This could lead to shifts in ecosystem functioning and services depending on the functional
467 redundancy within the ecosystem and susceptibility of different species to OAW (Kroeker et al., 2017).
468 This area of investigation would benefit from different multi-trophic interactions being explored under

469 OAW to further comprehend the extent of change to ecosystem services with particular emphasis on
470 stressor interactions. The singular and interactive effects of the stressors (pH, temperature, predator
471 presence, and time) on the metrics observed highlighted a mitigation of the effects of OA when
472 stressors interact. This supports the necessity of research prioritising interactive effects of multiple
473 stressors over single stressor experiments. The combination of temperature, pH and predator
474 presence is a far more holistic and ecologically relevant analysis of the effects of climate stressors on
475 organisms and clearly the interaction between stressors may mitigate the reported effects of any one
476 stressor on an organism. Current literature investigating singular stressors on an animal, when those
477 stressors are not environmentally relevant or likely (e.g., OA) has clear methodological drawbacks and
478 efforts should be made to rectify or enhance the current knowledge base.

479

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484

485 **REFERENCES**

- 486 Alley, T.R., 1982. Competition theory, evolution, and the concept of an ecological niche. *Acta*
487 *Biotheor.* 31. <https://doi.org/10.1007/BF01857239>
- 488 Ansell, A.D. and Sivadas, P., 1973. Some effects of temperature and starvation on the bivalve *Donax*
489 *vittatus* (da Costa) in experimental laboratory populations. *JEMBE* 13(3), 229-262.
- 490 Bibby, R., Cleall-Harding, P., Rundle, S., Widdicombe, S., Spicer, J., 2007. Ocean acidification disrupts
491 induced defences in the intertidal gastropod *Littorina littorea*. *Biol. Lett.* 3, 699–701.
492 <https://doi.org/10.1098/rsbl.2007.0457>
- 493 Braby, C.E., Somero, G.N., 2006. Following the heart: Temperature and salinity effects on heart rate
494 in native and invasive species of blue mussels (genus *Mytilus*). *J. Exp. Biol.* 209.
495 <https://doi.org/10.1242/jeb.02259>
- 496 Breitberg, D., Salisbury, J., Bernhard, J., Cai, W.-J., Dupont, S., Doney, S., Kroeker, K., Levin, L., Long,
497 W.C., Milke, L., Miller, S., Phelan, B., Passow, U., Seibel, B., Todgham, A., Tarrant, A., 2015. And on
498 top of all that... Coping with ocean acidification in the midst of many stressors. *Oceanography* 25,
499 48–61. <https://doi.org/10.5670/oceanog.2015.31>
- 500 Briffa, M., de la Haye, K., Munday, P.L., 2012. High CO₂ and marine animal behaviour: Potential
501 mechanisms and ecological consequences. *Mar. Pollut. Bull.* 64, 1519–1528.
502 <https://doi.org/10.1016/j.marpolbul.2012.05.032>

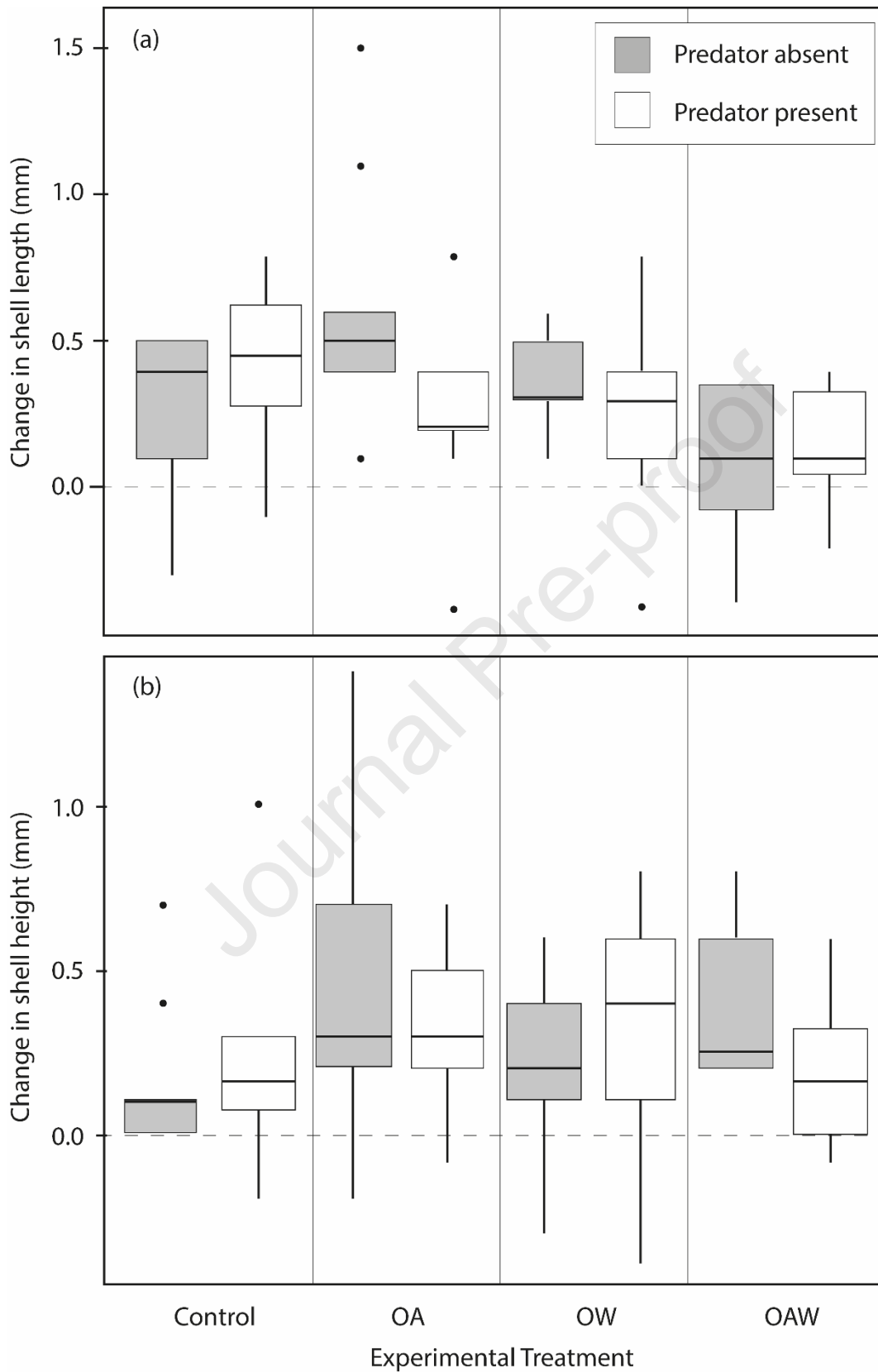
- 503 Clements, J.C., Bishop, M.M., Hunt, H.L., 2017. Elevated temperature has adverse effects on GABA-
504 mediated avoidance behaviour to sediment acidification in a wide-ranging marine bivalve. *Mar. Biol.*
505 164, 56. <https://doi.org/10.1007/s00227-017-3085-1>
- 506 Clements, J.C., Comeau, L.A., 2019. Behavioral defenses of shellfish prey under ocean acidification. *J.*
507 *Shellfish Res.* 38, 725–742. <https://doi.org/10.2983/035.038.0324>
- 508 Clements, J.C., Darrow, E.S., 2018. Eating in an acidifying ocean: a quantitative review of elevated
509 CO₂ effects on the feeding rates of calcifying marine invertebrates. *Hydrobiologia.*
510 <https://doi.org/10.1007/s10750-018-3665-1>
- 511 Clements, J.C., Hunt, H.L., 2015. Marine animal behaviour in a high CO₂ ocean. *Mar. Ecol. Prog. Ser.*
512 <https://doi.org/10.3354/meps11426>
- 513 Davenport, J., Chen, X., 1987. A comparison of methods for the assessment of condition in the
514 mussel (*Mytilus edulis* L.). *J. Molluscan Stud.* <https://doi.org/10.1093/mollus/53.3.293>
- 515 Dickson, A.G., Millero, F.J., 1987. A comparison of the equilibrium constants for the dissociation of
516 carbonic acid in seawater media. *Deep Sea Res. Part A, Oceanogr. Res. Pap.* 34.
517 [https://doi.org/10.1016/0198-0149\(87\)90021-5](https://doi.org/10.1016/0198-0149(87)90021-5)
- 518 Dupont, S., Mercurio, M., Giacoletti, A., Rinaldi, A., Mirto, S., D'Acquisto, L., Sabatino, M.A., Sara, G.,
519 2015. Functional consequences of prey acclimation to ocean acidification for the prey and its
520 predator. *PeerJ Prepr.* 3. <https://doi.org/10.7287/peerj.preprints.1438>
- 521 Feely, R.A., Sabine, C.L., Lee, K., Berelson, W., Kleypas, J., Fabry, V.J., Millero, F.J., 2004. Impact of
522 anthropogenic CO₂ on the CaCO₃ system in the oceans. *Science* 305.
523 <https://doi.org/10.1126/science.1097329>
- 524 Fitzer, S.C., Vittert, L., Bowman, A., Kamenos, N.A., Phoenix, V.R., Cusack, M., 2015. Ocean
525 acidification and temperature increase impact mussel shell shape and thickness: problematic for
526 protection? *Ecol. Evol.* 5, 4875–4884. <https://doi.org/10.1002/ECE3.1756>
- 527 Freeman, A.S., 2007. Specificity of induced defenses in *Mytilus edulis* and asymmetrical predator
528 deterrence. *Mar. Ecol. Prog. Ser.* 334, 145–153. <https://doi.org/10.3354/meps334145>
- 529 Gazeau, F., Parker, L.M., Comeau, S., Gattuso, J.-P., O'Connor, W.A., Martin, S., Pörtner, H.-O., Ross,
530 P.M., 2013. Impacts of ocean acidification on marine shelled molluscs. *Mar. Biol.* 2013 1608 160,
531 2207–2245. <https://doi.org/10.1007/S00227-013-2219-3>
- 532 Giomi, F., Mandaglio, C., Ganmanee, M., Han, G.-D., Dong, Y.-W., Williams, G.A., Sarà, G., 2016. The
533 importance of thermal history: costs and benefits of heat exposure in a tropical, rocky shore oyster.
534 *J. Exp. Biol.* 219, 686–694. <https://doi.org/10.1242/JEB.128892>
- 535 Gunderson, A.R., Armstrong, E.J., Stillman, J.H., 2016. Multiple stressors in a changing world: The
536 need for an improved perspective on physiological responses to the dynamic marine environment.
537 *Ann. Rev. Mar. Sci.* 8, 357–378. <https://doi.org/10.1146/annurev-marine-122414-033953>
- 538 Harvey, B., Moore, P., 2017. Ocean warming and acidification prevent compensatory response in a
539 predator to reduced prey quality. *Mar. Ecol. Prog. Ser.* 563, 111–122.
540 <https://doi.org/10.3354/meps11956>

- 541 Hatcher, A., Grant, J., Schofield, B., 1997. Seasonal changes in the metabolism of cultured mussels
542 (*Mytilus edulis* L.) from a Nova Scotian inlet: The effects of winter ice cover and nutritive stress. J.
543 Exp. Mar. Bio. Ecol. 217. [https://doi.org/10.1016/S0022-0981\(97\)00042-7](https://doi.org/10.1016/S0022-0981(97)00042-7)
- 544 Holling, C.S., 1959. Some characteristics of simple types of predation and parasitism. Can. Entomol.
545 91. <https://doi.org/10.4039/Ent91385-7>
- 546 Hubbard, F., McManus, J., Al-Dabbas, M., 1981. Environmental influences on the shell mineralogy of
547 *Mytilus edulis*. Geo-Marine Lett. 1. <https://doi.org/10.1007/BF02462445>
- 548 Hunt, H.L. and Scheibling, R.E., 1998. Effects of whelk (*Nucella lapillus* (L.)) predation on mussel
549 (*Mytilus trossulus* (Gould), *M. edulis* (L.)) assemblages in tidepools and on emergent rock on a wave-
550 exposed rocky shore in Nova Scotia, Canada. J. Exp. Mar. Bio. Ecol., 226(1), pp.87-113.
- 551 Jupe L.L., Bilton D.T., Knights A.M., 2020. Do differences in developmental mode shape the potential
552 for local adaptation? Ecology 101(3): e02942
- 553 Knights, A.M., 2012. Spatial variation in body size and reproductive condition of subtidal mussels:
554 Considerations for sustainable management. Fish. Res. 113, 45–54.
555 <https://doi.org/10.1016/j.fishres.2011.09.002>
- 556 Knights, A.M., Norton, M.J., Lemasson, A.J., Stephen, N., 2020. Ocean acidification mitigates the
557 negative effects of increased sea temperatures on the biomineralization and crystalline
558 ultrastructure of *Mytilus*. Front. Mar. Sci. 7, 773. <https://doi.org/10.3389/fmars.2020.567228>
- 559 Křivan, V., Eisner, J., 2003. Optimal foraging and predator-prey dynamics III. Theor. Popul. Biol. 63.
560 [https://doi.org/10.1016/S0040-5809\(03\)00012-1](https://doi.org/10.1016/S0040-5809(03)00012-1)
- 561 Kroeker, K.J., Kordas, R.L., Crim, R., Hendriks, I.E., Ramajo, L., Singh, G.S., Duarte, C.M., Gattuso, J.P.,
562 2013. Impacts of ocean acidification on marine organisms: Quantifying sensitivities and interaction
563 with warming. Glob. Chang. Biol. 19, 1884–1896. <https://doi.org/10.1111/gcb.12179>
- 564 Kroeker, K.J., Kordas, R.L., Harley, C.D.G., 2017. Embracing interactions in ocean acidification
565 research: confronting multiple stressor scenarios and context dependence. Biol. Lett. 13, 20160802.
566 <https://doi.org/10.1098/rsbl.2016.0802>
- 567 Lemasson, A.J., Hall-Spencer, J.M., Fletcher, S., Provstgaard-Morys, S., Knights, A.M., 2018.
568 Indications of future performance of native and non-native adult oysters under acidification and
569 warming. Mar. Environ. Res. 142, 178–189. <https://doi.org/10.1016/J.MARENRES.2018.10.003>
- 570 Lemasson, A.J., Hall-Spencer, J.M., Kuri, V., Knights, A.M., 2019. Changes in the biochemical and
571 nutrient composition of seafood due to ocean acidification and warming. Mar. Environ. Res. 143.
572 <https://doi.org/10.1016/j.marenres.2018.11.006>
- 573 Lemasson, A.J., Knights, A.M., 2021. Differential responses in anti-predation traits of the native
574 oyster *Ostrea edulis* and invasive *Magallana gigas* to ocean acidification and warming. Mar. Ecol.
575 Prog. Ser. 665, 87–102. <https://doi.org/10.3354/MEPS13687>
- 576 Leung, J.Y.S., Russell, B.D., Connell, S.D., 2017. Mineralogical plasticity acts as a compensatory
577 mechanism to the impacts of ocean acidification. Environ. Sci. Technol. 51, 2652–2659.
578 <https://doi.org/10.1021/acs.est.6b04709>

- 579 Leung, J.Y., Zhang, S. and Connell, S.D., 2022. Is ocean acidification really a threat to marine
580 calcifiers? A systematic review and meta-analysis of 980+ studies spanning two decades. *Small*,
581 18(35), p.2107407.
- 582 Lewis, E., Wallace, D., 1998. Program developed for CO₂ system calculations. Ornl/Cdiac-105.
- 583 Li, S., Liu, C., Huang, J., Liu, Y., Zheng, G., Xie, L., Zhang, R., 2015. Interactive effects of seawater
584 acidification and elevated temperature on biomineralization and amino acid metabolism in the
585 mussel *Mytilus edulis*. *J. Exp. Biol.* 218, 3623–3631. <https://doi.org/10.1242/jeb.126748>
- 586 Listiawati, V., Kurihara, H., 2021. Ocean warming and acidification modify top-down and bottom-up
587 control in a tropical seagrass ecosystem. *Sci. Rep.* 11. <https://doi.org/10.1038/s41598-021-92989-0>
- 588 Lord, J., Barry, J., Graves, D., 2017. Impact of climate change on direct and indirect species
589 interactions. *Mar. Ecol. Prog. Ser.* 571, 1–11. <https://doi.org/10.3354/meps12148>
- 590 Manríquez, P.H., Jara, M.E., González, C.P., Seguel, M.E., Domenici, P., Watson, S.A., Anguita, C.,
591 Duarte, C., Brokordt, K., 2021. The combined effects of climate change stressors and predatory cues
592 on a mussel species. *Sci. Total Environ.* 776. <https://doi.org/10.1016/j.scitotenv.2021.145916>
- 593 Matoo, O.B., Ivanina, A. V., Ullstad, C., Beniash, E., Sokolova, I.I., 2013. Interactive effects of elevated
594 temperature and CO₂ levels on metabolism and oxidative stress in two common marine bivalves
595 (*Crassostrea virginica* and *Mercenaria mercenaria*). *Comp. Biochem. Physiol. - A Mol. Integr. Physiol.*
596 164. <https://doi.org/10.1016/j.cbpa.2012.12.025>
- 597 Matoo, O.B., Lannig, G., Bock, C., Sokolova, I.M., 2021. Temperature but not ocean acidification
598 affects energy metabolism and enzyme activities in the blue mussel, *Mytilus edulis*. *Ecol. Evol.* 11,
599 3366–3379. <https://doi.org/10.1002/ECE3.7289>
- 600 Mayk, D., Peck, L.S., Backeljau, T. and E.M. Harper, 2022. Shell thickness of *Nucella lapillus* in the
601 North Sea increased over the last 130 years despite ocean acidification. *Communications Earth &*
602 *Environment*, 3(1), pp.1-12.
- 603 Mehrbach, C., Culbertson, C.H., Hawley, J.E., Pytkowicz, R.M., 1973. Measurement of the apparent
604 dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnol. Oceanogr.* 18.
605 <https://doi.org/10.4319/lo.1973.18.6.0897>
- 606 Naddafi, R., Rudstam, L.G., 2013. Predator-induced behavioural defences in two competitive invasive
607 species: The zebra mussel and the quagga mussel. *Anim. Behav.* 86.
608 <https://doi.org/10.1016/j.anbehav.2013.09.032>
- 609 Nienhuis, S., Palmer, A.R., Harley, C.D.G., 2010. Elevated CO₂ affects shell dissolution rate but not
610 calcification rate in a marine snail. *Proc. R. Soc. B Biol. Sci.* 277, 2553–2558.
611 <https://doi.org/10.1098/RSPB.2010.0206>
- 612 Orrock, J.L., Grabowski, J.H., Pantel, J.H., Peacor, S.D., Peckarsky, B.L., Sih, A., Werner, E.E., 2008.
613 Consumptive and non-consumptive effects of predators on metacommunities of competing prey.
614 *Ecology* 89. <https://doi.org/10.1890/07-1024.1>
- 615 Pinheiro J, Bates D. 2022. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-
616 157, <https://CRAN.R-project.org/package=nlme>
- 617 Portner, HO. 2012. Integrating climate-related stressor effects on marine organisms: unifying
618 principles linking molecule to ecosystem-level changes. *Mar. Ecol. Prog. Ser.* 470, 273-290.

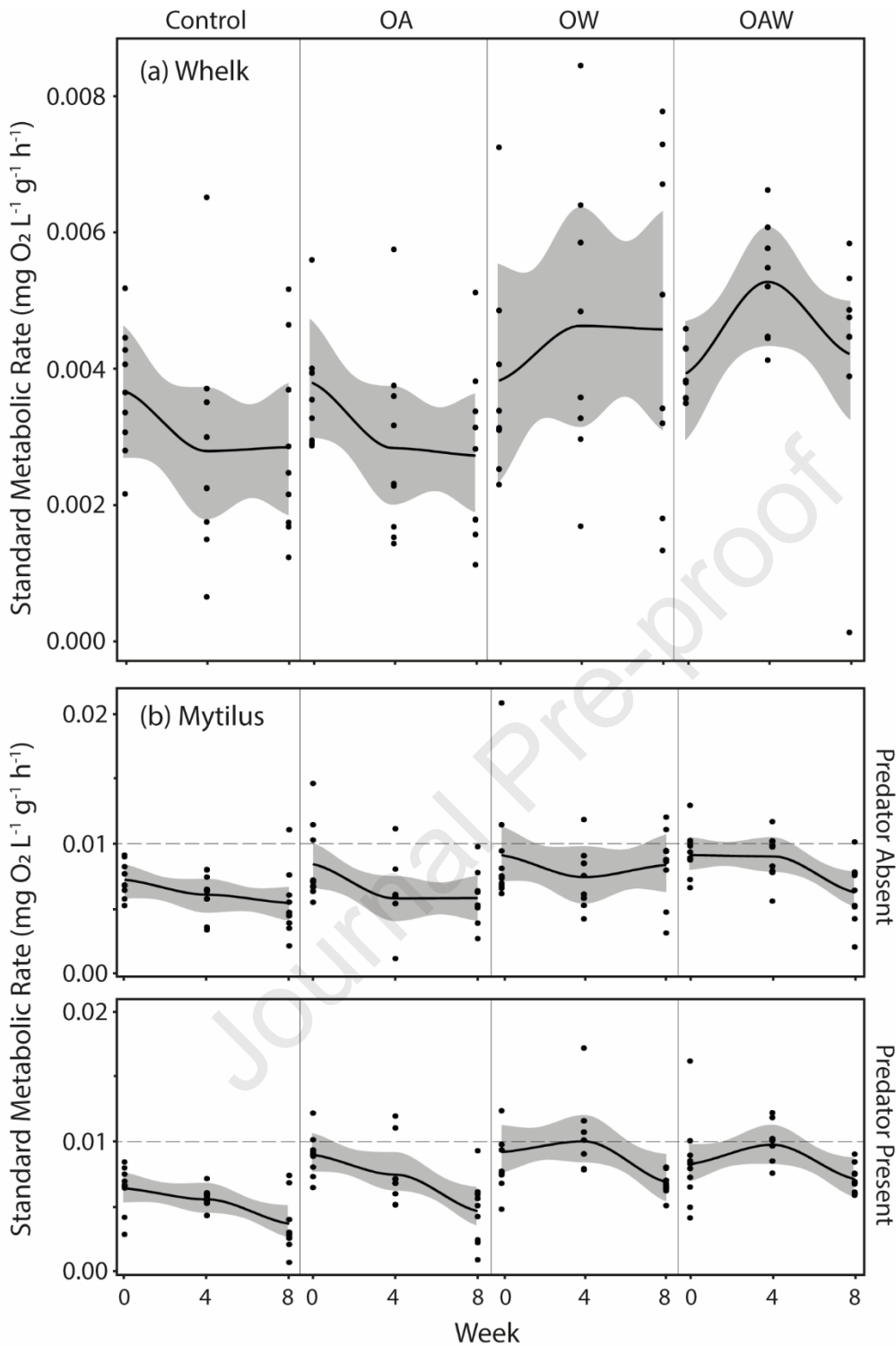
- 619 Queirós, A.M., Fernandes, J.A., Faulwetter, S., Nunes, J., Rastrick, S.P.S., Mieszkowska, N., Artioli, Y.,
620 Yool, A., Calosi, P., Arvanitidis, C., Findlay, H.S., Barange, M., Cheung, W.W.L., Widdicombe, S., 2015.
621 Scaling up experimental ocean acidification and warming research: From individuals to the
622 ecosystem. *Glob. Chang. Biol.* 21. <https://doi.org/10.1111/gcb.12675>
- 623 Reimer, O., 1999. Increased gonad ratio in the blue mussel, *Mytilus edulis*, exposed to starfish
624 predators. *Aquat. Ecol.* 1999 332 33, 185–192. <https://doi.org/10.1023/A:1009946526175>
- 625 Rose, J.M., Blanchette, C.A., Chan, F., Gouhier, T.C., Raimondi, P.T., Sanford, E., Menge, B.A., 2020.
626 Biogeography of ocean acidification: Differential field performance of transplanted mussels to
627 upwelling-driven variation in carbonate chemistry. *PLoS One* 15, e0234075.
628 <https://doi.org/10.1371/JOURNAL.PONE.0234075>
- 629 Sadler, D.E., Lemasson, A.J., Knights, A.M., 2018. The effects of elevated CO₂ on shell properties and
630 susceptibility to predation in mussels *Mytilus edulis*. *Mar. Environ. Res.* 139, 162–168.
631 <https://doi.org/10.1016/j.marenvres.2018.05.017>
- 632 Sanford, E., Gaylord, B., Hettinger, A., Lenz, E.A., Meyer, K., Hill, T.M., 2014. Ocean acidification
633 increases the vulnerability of native oysters to predation by invasive snails. *Proc. R. Soc. B Biol. Sci.*
634 281. <https://doi.org/10.1098/rspb.2013.2681>
- 635 Seibel, B.A., Drazen, J.C., 2007. The rate of metabolism in marine animals: environmental
636 constraints, ecological demands and energetic opportunities. *Philos. Trans. R. Soc. B Biol. Sci.* 362,
637 2061–2078. <https://doi.org/10.1098/RSTB.2007.2101>
- 638 Seibel, B.A., Walsh, P.J., 2003. Biological impacts of deep-sea carbon dioxide injection inferred from
639 indices of physiological performance. *J. Exp. Biol.* <https://doi.org/10.1242/jeb.00141>
- 640 Sejr, M.K., Petersen, J.K., Jensen, K.T. and Rysgaard, S., 2004. Effects of food concentration on
641 clearance rate and energy budget of the Arctic bivalve *Hiatella arctica* (L) at subzero temperature.
642 *JEMBE* 311(1), 171-183.
- 643 Sherker, Z., Ellrich, J., Scrosati, R., 2017. Predator-induced shell plasticity in mussels hinders
644 predation by drilling snails. *Mar. Ecol. Prog. Ser.* 573, 167–175. <https://doi.org/10.3354/meps12194>
- 645 Shukla, P.R., Skea, J., Buendia, E.C., Masson-Delmotte, V., Pörtner, H.-O., Roberts, D.C., Zhai, P.,
646 Slade, R., Connors, S., Diemen, R. van, Ferrat, M., Haughey, E., Luz, S., Neogi, S., Pathak, M., Petzold,
647 J., Pereira, J.P., Vyas, P., Huntley, E., Kissick, K., Belkacemi, M., Malley, J., 2019. IPCC, 2019: Climate
648 Change and Land: an IPCC special report on climate change, desertification, land degradation,
649 sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems.
- 650 Sunila, I., 1981. Reproduction of *Mytilus edulis* L. (Bivalvia) in a brackish water area, the Gulf of
651 Finland. *Annales Zoologici Fennici*, 121-128
- 652 Wernberg, T., Smale, D.A., Thomsen, M.S., 2012. A decade of climate change experiments on marine
653 organisms: Procedures, patterns and problems. *Glob. Chang. Biol.* 18(5), 1491-1498
654 <https://doi.org/10.1111/j.1365-2486.2012.02656.x>
- 655 Wickham, H. 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.
- 656 Zhang, C., Jansen, M., Meester, L. De, Stoks, R., 2016. Energy storage and fecundity explain
657 deviations from ecological stoichiometry predictions under global warming and size-selective
658 predation. *J. Anim. Ecol.* 85, 1431–1441. <https://doi.org/10.1111/1365-2656.12531>

659 FIGURES



660

661 **Figure 1.** Change in mussel (a) shell length and (b) shell height in the presence (white bars) and
 662 absence (grey bars) of whelks. Box and whiskers show: median (thick line); hinges = ± 1 IQR beyond
 663 the median; whiskers = ± 1 SD beyond the IQR; and dots indicate outliers.



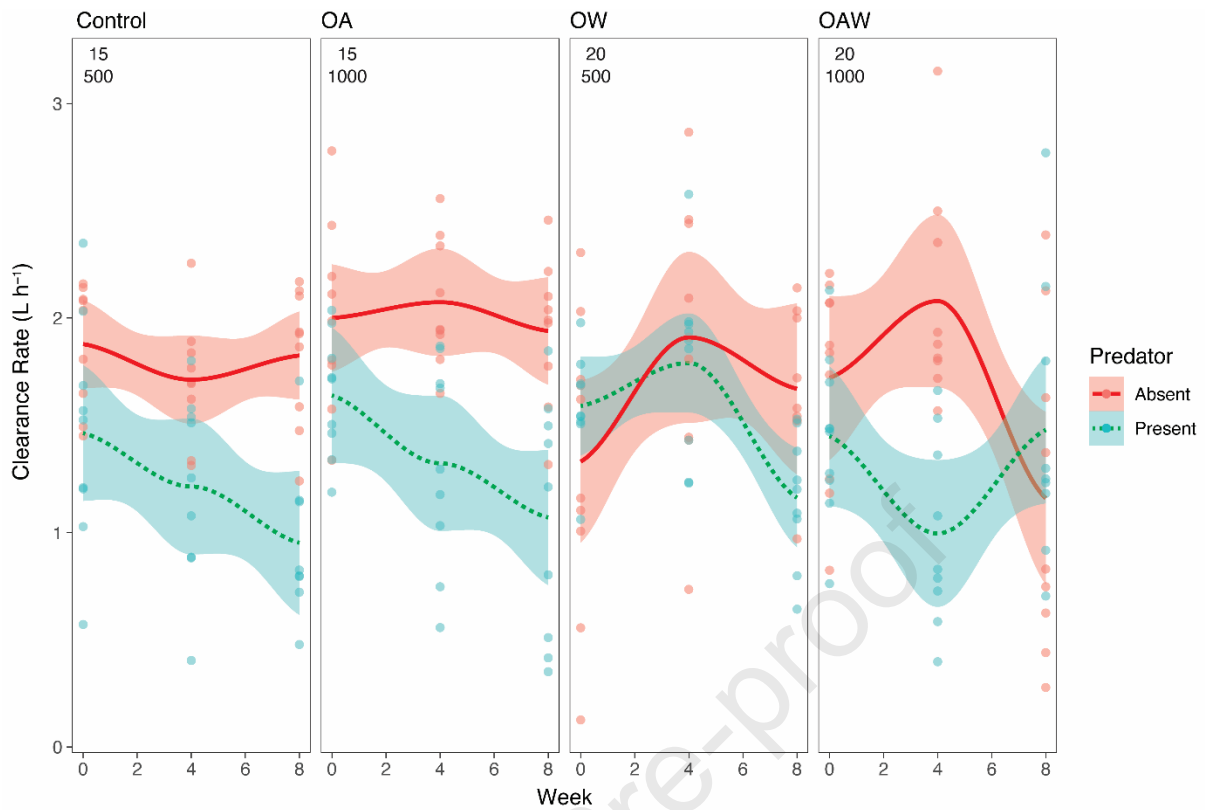
664

665 **Figure 2.** Localised regression (loess) of change in standard metabolic rate (SMR; $\text{mg O}_2 \text{ L}^{-1} \text{ g}^{-1} \text{ h}^{-1}$)
 666 over time in (a) whelks, and (b) mussels with/without predators (whelks) exposed to control
 667 conditions and combinations of ocean acidification (OA), ocean warming (OW), and ocean
 668 acidification and warming (OAW). Shading indicates 95% confidence intervals around the mean.

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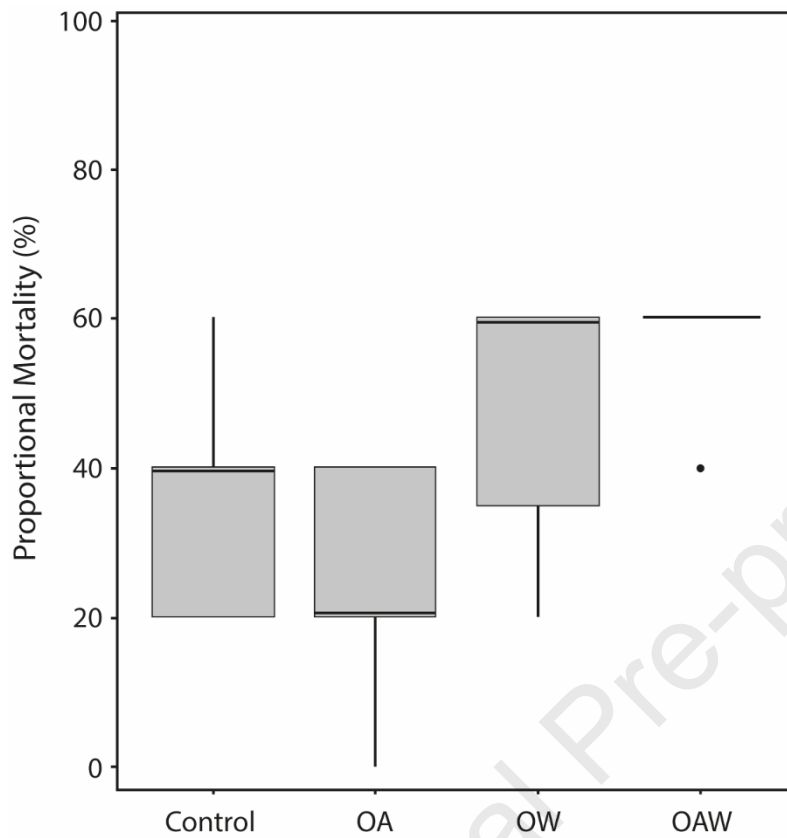


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673 **Figure 3.** Localised regression (loess) of mussel clearance rate ($L h^{-1}$) with (present) and without
 674 (absent) a predator under current, ocean acidification (OA), ocean warming (OW) and ocean
 675 acidification and warming (OAW) scenarios. Shading indicates 95% confidence intervals around the
 676 mean.

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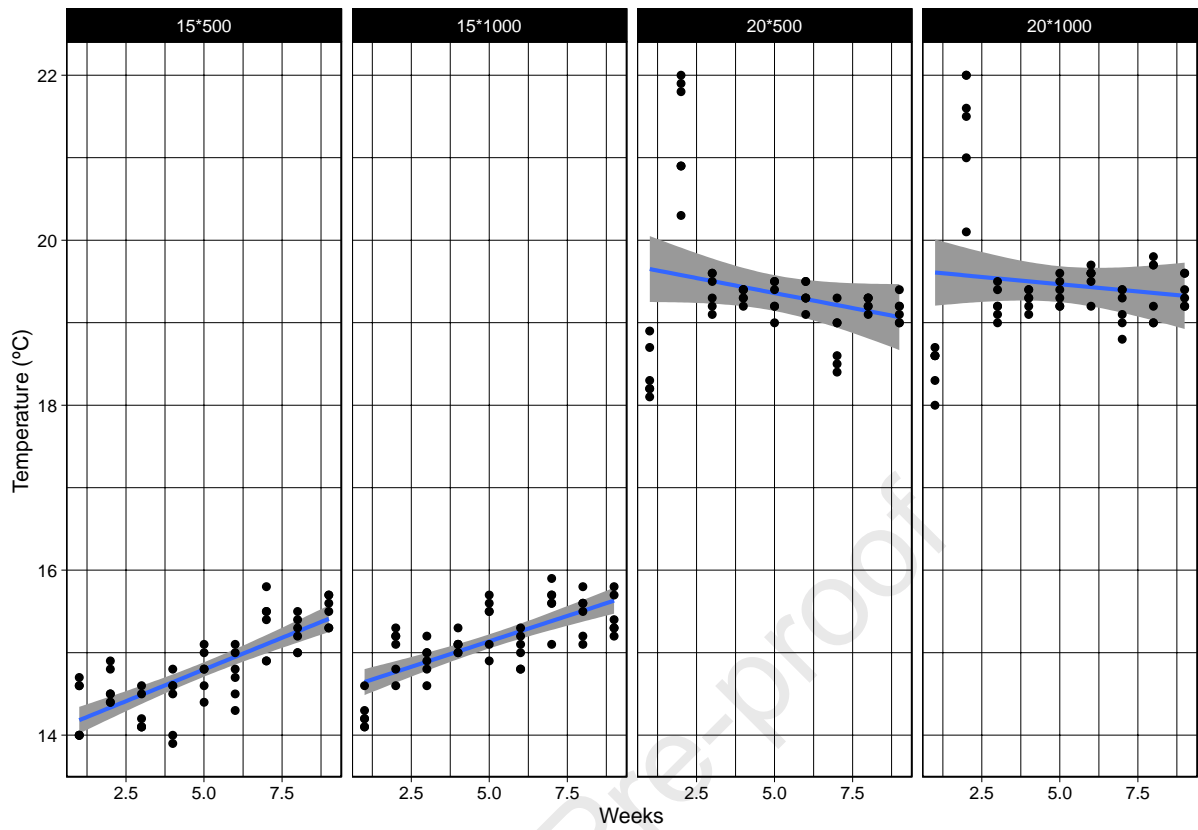
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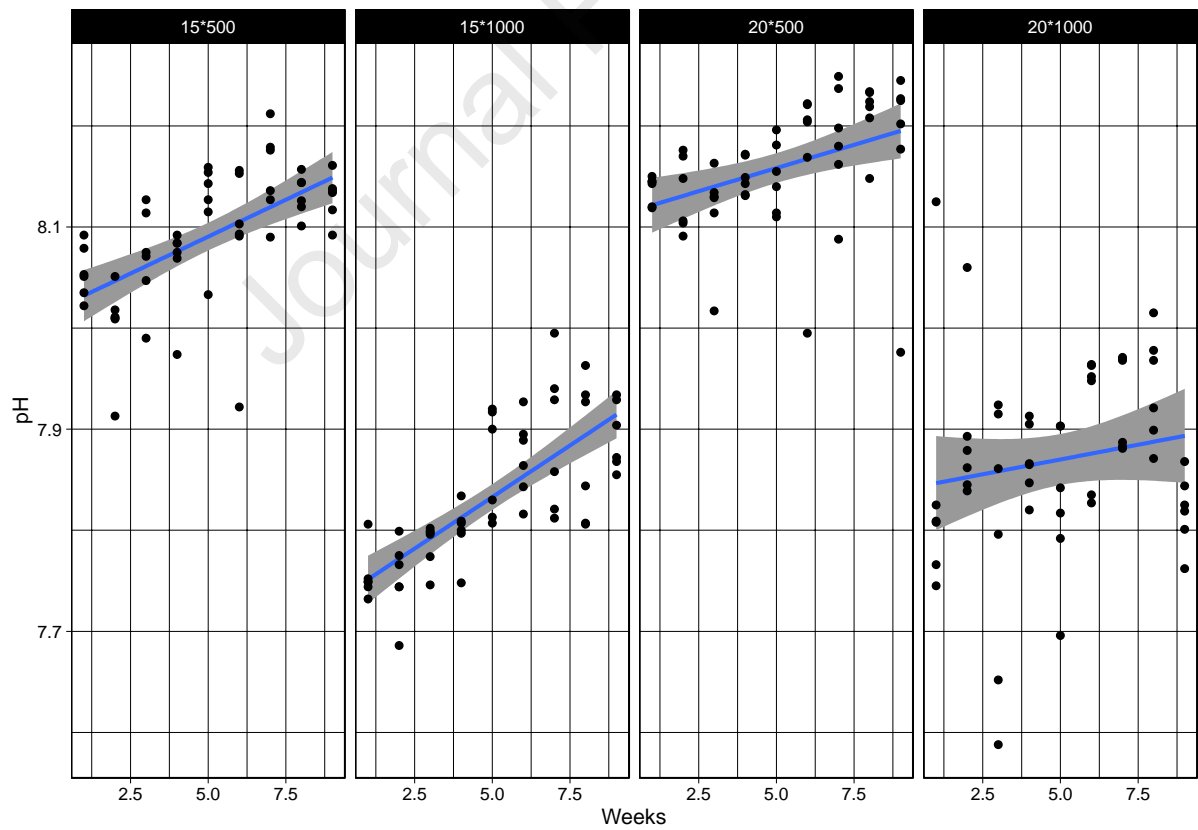
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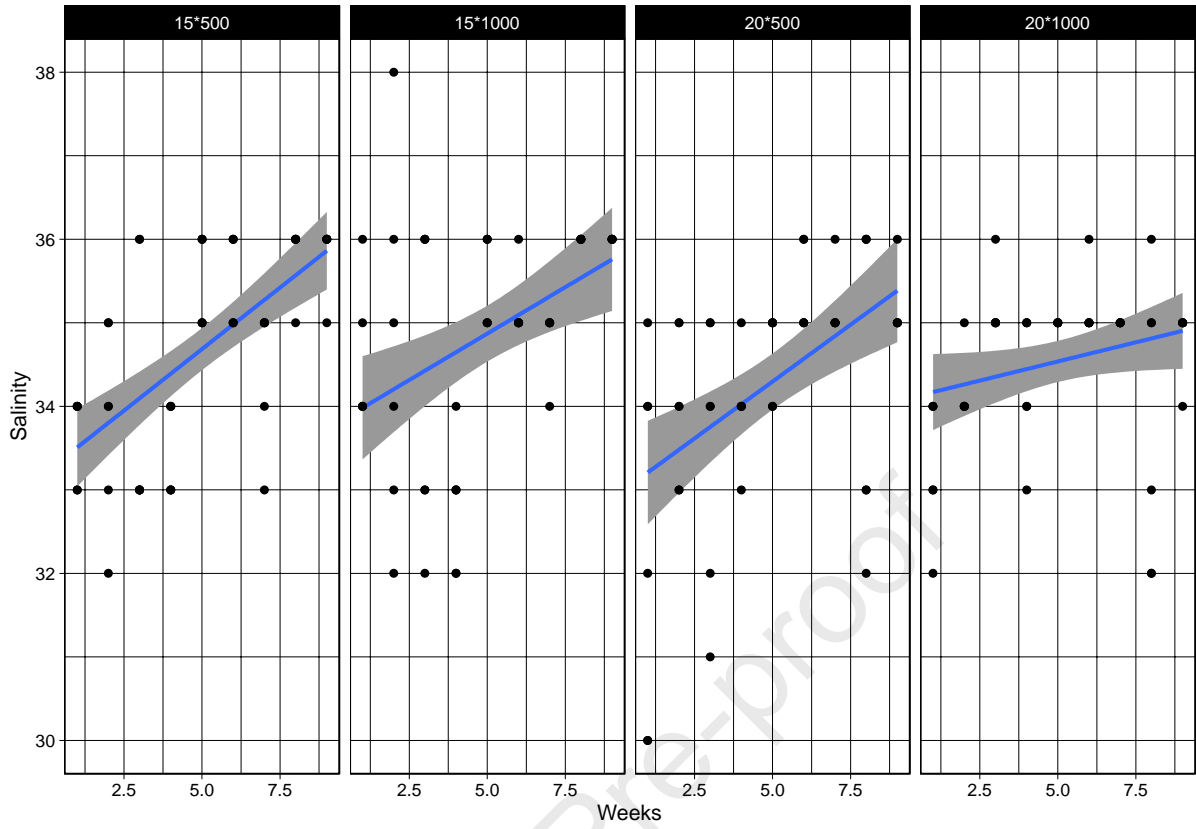
681 **Figure 4.** Percentage mortality of mussels after 8 days of whelk feeding. Whelks and mussels were
682 exposed to control conditions, and combinations of ocean acidification (pCO₂) and ocean warming
683 (temperature) scenarios for 8-weeks. Box and whiskers show: median (thick line); hinges = ± 1 IQR
684 beyond the median; whiskers = ± 1 SD beyond the IQR; and dots indicate outliers.



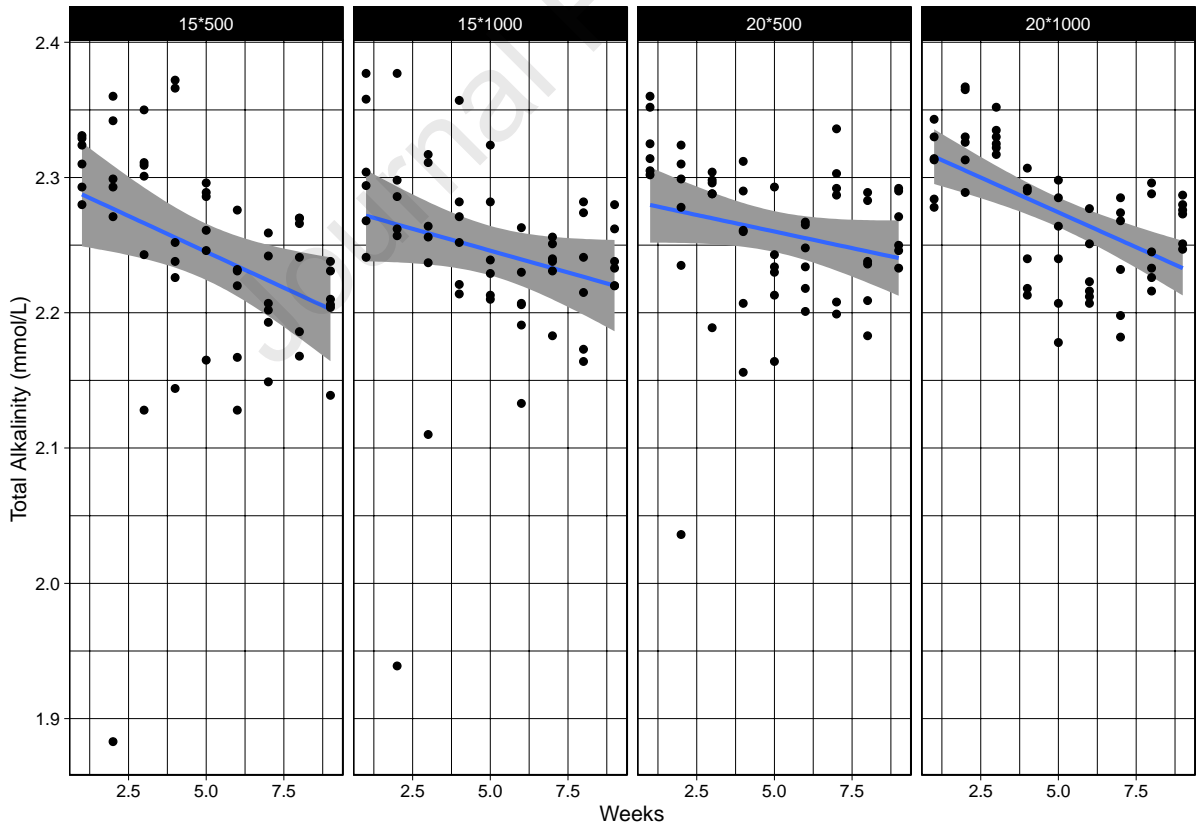
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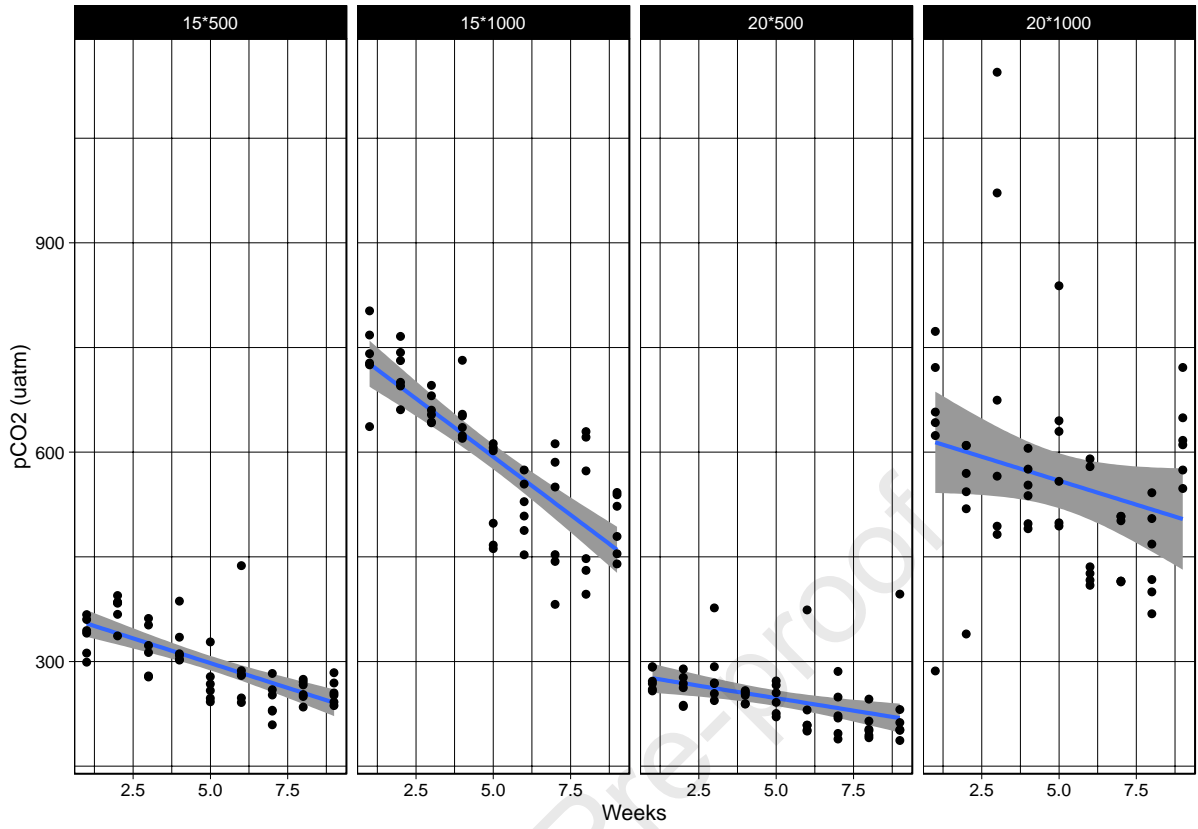
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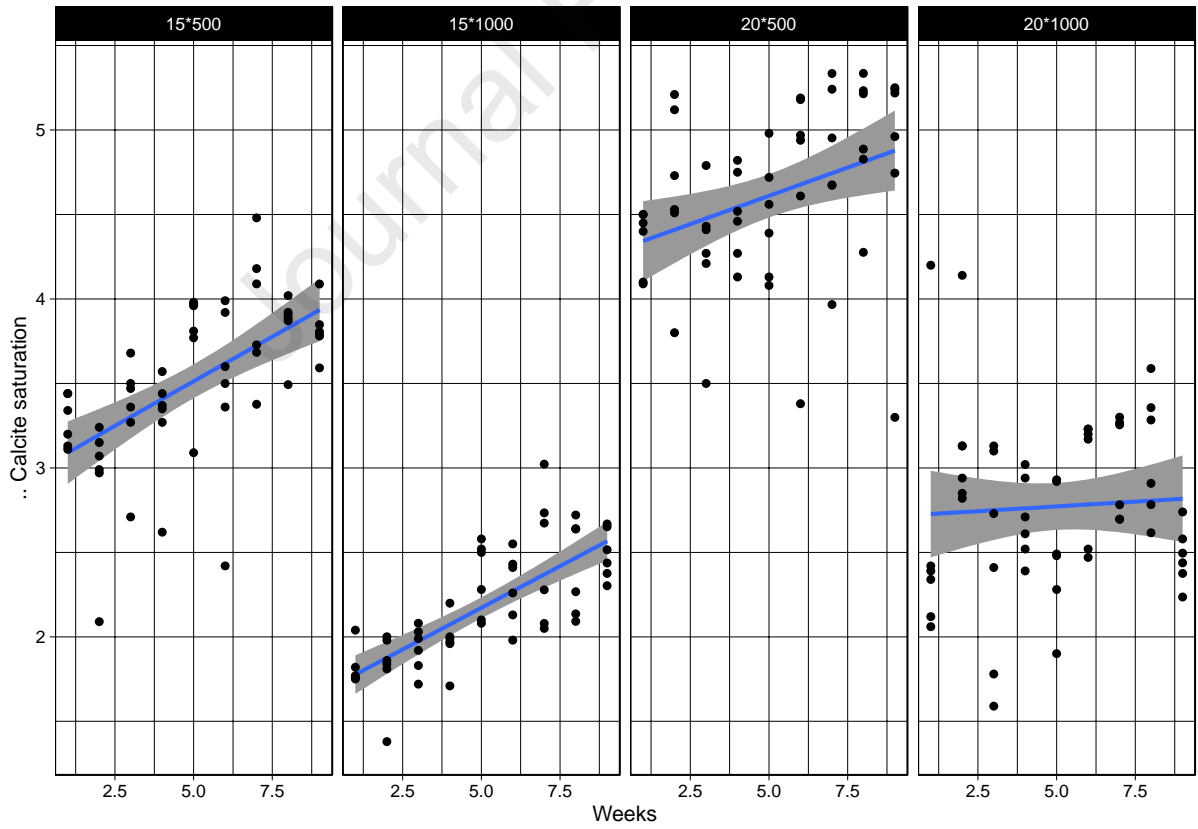
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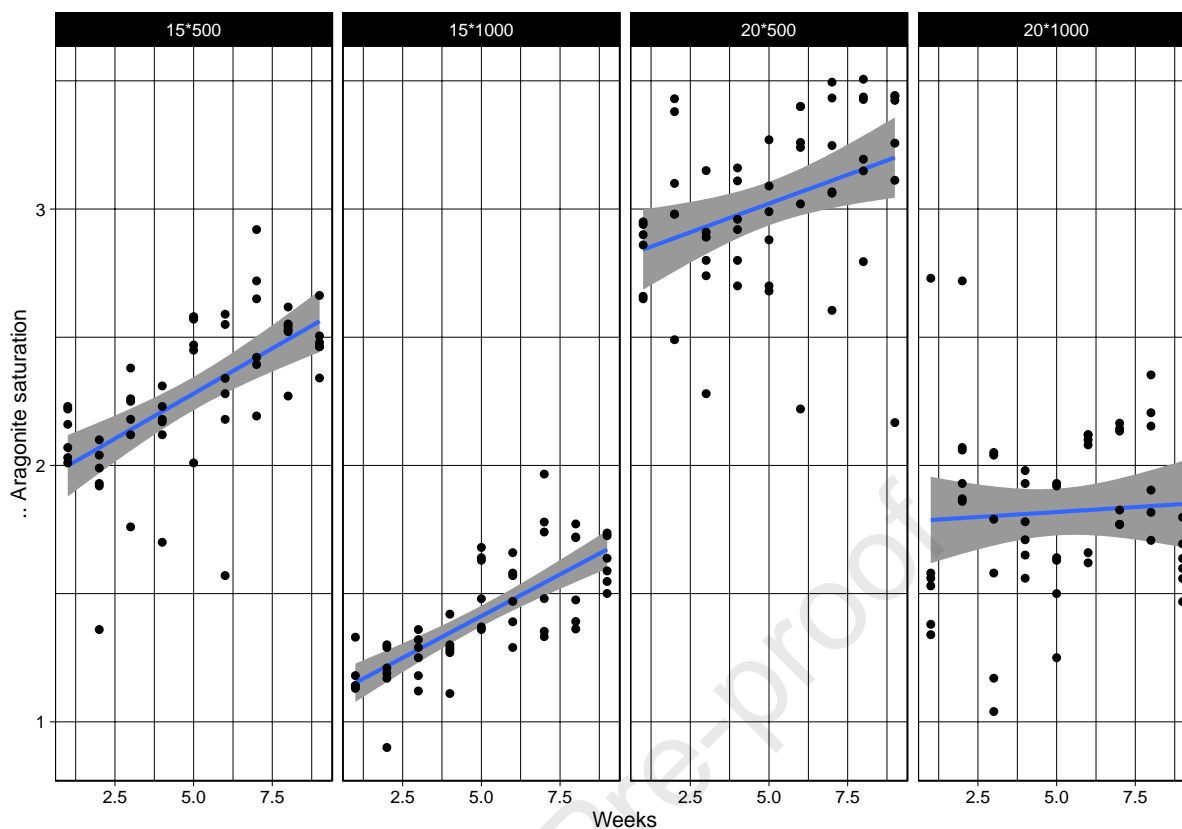
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692 Appendix 1- Carbonate chemistry parameters monitored throughout the 8-week exposure in each treatment.
 693 Treatments; 15*500 = 15°C x 500 ppm pCO₂, 15*1000 = 15°C x 1000 ppm pCO₂, 20*500 = 20°C x 500 ppm pCO₂,
 694 20*1000 = 20°C x 1000 ppm pCO₂. (a) temperature (°C), (b) pH, (c) salinity (psu), (d) TA- total alkalinity (mmol L⁻¹)
 695 ¹), (e) pCO₂ -partial pressure of carbon dioxide in seawater (µatm), (f) calcite saturation in seawater, (g) aragonite
 696 saturation in seawater. Confidence intervals (95%) around linear regression lines are shown.

697 Table A1- Seawater carbonate chemistry average values +/- standard deviation throughout each treatment over 8 weeks. Temperature, Salinity, pH, Total alkalinity (TA),
 698 partial pressure of CO₂ (pCO₂), calcite saturation state (Ω calcite), aragonite saturation state (Ω Aragonite).

Exposure	Temperature (°C)	Salinity	pH	TA (mmol L ⁻¹)	pCO ₂ (μ atm)	Ω calcite	Ω aragonite
Control	14.80	34.69	8.09	2.25	297.73	3.51	2.28
± SD	0.51	1.18	0.06	0.08	52.35	0.45	0.30
Elevated CO ₂ (OA)	15.14	34.87	7.83	2.25	593.47	2.17	1.41
± SD	0.44	1.33	0.07	0.07	107.43	0.34	0.22
Elevated Temperature (OW)	19.36	34.30	8.16	2.26	247.51	4.61	3.02
± SD	0.80	1.40	0.06	0.06	44.43	0.49	0.33
Elevated CO ₂ and temperature (OAW)	19.47	34.54	7.87	2.27	559.15	2.77	1.82
± SD	0.79	0.92	0.09	0.05	145.97	0.50	0.33

699

700 Table A2- Seawater carbonate chemistry average values +/- standard deviation throughout each treatment during *Nucella lapillus* feeding experiment (8 d). Temperature
 701 (°C), Salinity, pH, Total alkalinity (TA), partial pressure of CO₂ (pCO₂), calcite saturation state (Ω calcite), aragonite saturation state (Ω Aragonite).

Exposure	Temperature (°C)	Salinity	pH	TA (mmol L ⁻¹)	pCO ₂ (μ atm)	Ω calcite	Ω aragonite
Control	15.55	35.86	8.13	2.28	266.84	3.97	2.58
± SD	0.25	0.64	0.05	0.04	36.87	0.26	0.17
Elevated CO ₂ (OA)	15.76	36.00	7.75	2.31	738.49	1.97	1.28
± SD	0.30	0.76	0.05	0.04	91.53	0.21	0.14
Elevated Temperature (OW)	19.01	35.85	8.17	2.30	241.06	4.84	3.18
± SD	0.39	0.53	0.08	0.03	32.37	0.60	0.39
Elevated CO ₂ and temperature (OAW)	18.98	35.54	7.79	2.36	711.38	2.47	1.62
± SD	0.24	0.50	0.11	0.05	171.52	0.57	0.37

702

Highlights

- Multiple environmental stressors act upon multiple trophic levels
- Mollusc predator and prey respond differently to future climate scenarios
- Prey are negatively impacted physiologically and behaviourally
- Predators unaffected resulting in elevated predation risk for prey
- Potential for fundamental change in trophic interactions affecting biodiversity

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Author Statement

Rebecca Greateorex: Conceptualization; Investigation; Writing – Original

Antony Knights: Conceptualization; Formal analysis; Writing – Review & Editing; Supervision

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Declaration of interests

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

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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