1   **Ocean acidification alters zooplankton communities and increases top-down pressure of a**

2   **cubozoan predator**

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ABSTRACT

The composition of local ecological communities is determined by the members of the regional community that are able to survive the abiotic and biotic conditions of a local ecosystem. Anthropogenic activities since the industrial revolution have increased atmospheric CO_2 concentrations, which have in turn decreased ocean pH and altered carbonate ion concentrations: so called ocean acidification (OA). Single-species experiments have shown how OA can dramatically affect zooplankton development, physiology and skeletal mineralization status, potentially reducing their defensive function and altering their predatory and anti-predatory behaviours. This means that increased OA may indirectly alter the biotic conditions by modifying trophic interactions. We investigated how OA affects the impact of a cubozoan predator on their zooplankton prey, predominantly Copepoda, Pleocyemata, Dendrobranchiata, and Amphipoda. Experimental conditions were set at either current (pCO$_2$ 370 µatm) or end-of-the-century OA (pCO$_2$ 1100 µatm) scenarios, crossed in an orthogonal experimental design with the presence/absence of the cubozoan predator *Carybdea rastoni*. The combined effects of exposure to OA and predation by *C. rastoni* caused greater shifts in community structure, and greater reductions in the abundance of key taxa than would be predicted from combining the effect of each stressor in isolation. Specifically, we show that in the combined presence of OA and a cubozoan predator, populations of the most abundant member of the zooplankton community (calanoid copepods) were reduced 27 % more than would be predicted based on the effects of these stressors in isolation, suggesting that OA increases the susceptibility of plankton to predation. Our results indicate that the ecological consequences of OA may be greater than predicted from single-species experiments, and highlight the need to understand future marine global change from a community perspective.

INTRODUCTION

The composition of ecological communities is determined by the members of the regional species pool that are able to pass through a series of ecological filters (Lawton 2000). Initially, to become part of a community, species from the regional pool have to overcome the "dispersal filter", i.e. they need to physically enter the local community. Having arrived, the species then has to pass through the "abiotic filter", i.e. it has to be physiologically capable of tolerating the abiotic conditions present in the local area. After clearing the dispersal and abiotic filters, a species must finally clear the "biotic filter". In order to pass through the biotic filter a species must be able to co-exist with the other members of the regional species pool, species who themselves have already been able to pass through both the dispersal and abiotic filters. Co-existing with these other community members means a species must not be competitively excluded, or exploited to extinction by predators. It is impossible for a species to exert competitive or consumptive pressure (i.e. become part of the biotic filter) if it cannot pass through the dispersal or abiotic filters; thus, the filters concept is often depicted as a linear process (Fig. 1, Lawton 2000). Although the filters concept was first proposed to describe the presence or absence of species, community composition can also change through changes in species' relative abundances (Clarke, 1993; Roemmich & McGowan, 1995; Forbes & Hammill, 2013). Changes in the strength of the different filters may not necessarily lead to the exclusion of species, but community composition may still be altered through reductions in their abundance.

Marine zooplankton communities are often dominated by Copepoda. Of the copepods, calanoids are the most abundant and alone can constitute up to 80 % of total zooplankton biomass (Beaugrand *et al.*, 2002; Lavaniegos & Ohman, 2007). The dominance of calanoid copepods, combined with the size of the world's oceans, suggests that they constitute the largest biomass of a single group of organisms on earth and play a pivotal position in the food web (Gallienne & Robins, 2001; Stibor *et al.*, 2004; Turner,

69   2004). This high biomass makes marine copepods a crucial link between the ocean's primary producers

70   and higher trophic levels (Garzke *et al.*, 2016). This crucial role within pelagic marine food webs means

71   that any reduction in copepod abundance could decrease the survival of their consumers (Cross *et al.*,

72   2009), and have far-reaching consequences for marine communities (Frederiksen *et al.*, 2006).

73   The biological consequences of increased pCO$_2$ levels (ocean acidification - OA) in the marine

74   environment appear detrimental for many species (Fabry et al. 2008, Byrne et al. 2011, Kroeker et al.

75   2013, Przeslawski et al. 2015). Laboratory experiments and *in situ* observations at high pCO$_2$ sites (e.g.

76   CO$_2$ vents) have revealed that for calcifying organisms such as echinoderms, molluscs, corals, and

77   crustaceans, OA can broadly lead to reduced survival and developmental success (Hall-Spencer *et al.*,

78   2008; Sheppard Brennand *et al.*, 2010; Gaylord *et al.*, 2011; Kroeker *et al.*, 2011) and alter shell

79   mineralization (Byrne *et al.*, 2013; Wolfe *et al.*, 2013; Taylor *et al.*, 2015). However, some of the most

80   abundant and ecologically important species (e.g. copepods) in pelagic environments possess chitinous

81   exoskeletons that will not be as impacted by the dissolution status of sea water compared to calcifying

82   organisms (Kroeker *et al.*, 2013). Nevertheless OA has been associated with reduced adult and juvenile

83   survival (Lewis *et al.*, 2013; Cripps *et al.*, 2014), altered reproductive rates (Fitzer *et al.*, 2012; Thor &

84   Dupont, 2015), and reduced growth (Fitzer *et al.*, 2012) in copepods. These changes may be due to

85   changes in copepod metabolism in response to OA (Thor & Dupont, 2015)**,** which then lead to the

86   reallocation of resources away from reproduction. These detrimental effects of OA on chitinous species

87   suggest that although these taxa may not suffer the same impacts on shell morphology, they are not

88   immune to the negative effects of OA.

89   The total effects of OA on individual taxa may also be magnified through indirect interspecific

90   interactions, as shown in experiments using pairs of grazers and herbivores (Poore *et al.*, 2013), or

91   predators and prey (Nagelkerken & Munday, 2016). Across a range of different biomes, changes in

92   pairwise consumer-resource interactions have been shown to scale up to the community level, leading to

93   changes in overall composition (Estes & Palmisano, 1974; Osman & Whitlatch, 2004; Hammill *et al.*,

94   2015a). Within marine ecosystems cubozoa ("box jellyfish"), are zooplanktivorous predators that

95   consume large volumes of zooplankton, including copepods (Buskey, 2003). Previous investigations into

96   the effects of OA on cubozoa have shown that they are capable of surviving and asexually reproducing at

97   pH levels as low as 7.6, although rates of asexual propagation through budding are reduced (Klein *et al.*,

98   2014). The ability of cubozoans to survive and reproduce (albeit at a reduced rate) under OA conditions

99   suggests an ability to tolerate potential future ocean conditions. What is not clear is whether OA reduces

100   the ability of cubozoans to capture prey, therefore reducing the effect of predation, or whether the

101   physiological effects of OA on their zooplankton prey makes escape harder, therefore increasing the

102   effect of predation. In terms of the ecological filters proposed by Lawton (Lawton, 2000), potential

103   changes in predation strength may lead to OA having indirect effects on the strength of the biotic filter,

104   potentially increasing the impact of OA on community structure (Fig. 1).

105   Here we investigate whether future OA conditions will alter plankton communities by changing

106   the strength of the abiotic filter, or by also increasing predation risk, thereby indirectly increasing the

107   strength of the biotic filter (Lawton, 2000). Using marine zooplankton communities and a natural

108   predator, a cubozoan jellyfish, we conducted a microcosm experiment in which the presence of predators

109   and OA was crossed in a 2 x 2 factorial design: $p\mathrm{CO}_2$ 370 or 1100 µatm crossed with predator

110   presence/absence. We predicted that in isolation, OA conditions or the presence of cubozoan predators

111   would alter the composition of zooplankton communities by reducing the abundances of multiple taxa,

112   including dominant copepods. However, we also predicted that when OA and predators were present

113   simultaneously, their combined impacts on community structure, driven by changes in copepod

114 abundances would be greater than would be predicted from combining their isolated effects, as OA is

115 likely to increase susceptibility to predation.

116

117 **MATERIALS AND METHODS**

118 *Organism collections*

119 Zooplankton were collected from Chowder Bay in Sydney Harbour, Australia (-33° 50' 59.6394", 151°

120 15' 0.36") using a 250 μm plankton net. Many zooplankton taxa swarm in the presence of light, a

121 behavioral tactic that has been exploited by cubozoans (Buskey, 2003). Plankton were therefore collected

122 at night by illuminating the water surface using 200 lumen lamps (Icon© Headlamp, Black Diamond

123 Equipment LTD, Salt Lake City, UT, USA), then towing the plankton net 20 times through the

124 illuminated water. After each tow the contents of the net were emptied into a 20 L white plastic

125 bucket. The net was emptied carefully after each tow to minimize damage to organisms. While

126 illumination can alter the relative abundances of zooplankton, a previous investigation in temperate

127 waters showed that the use of illumination led to the exclusion of only 1 out of 43 taxa, as opposed to

128 excluding 9 out of 43 taxa in the absence of illumination (McConnell *et al.*, 2010). We therefore opted to

129 use illumination as it facilitated rapid collection of large numbers of the most abundant taxa, due to the

130 positive phototaxis characteristic of many zooplankton (Buskey, 2003; Porter *et al.*, 2008; Martynova &

131 Gordeeva, 2010). This method also substantially reduced the handling time needed to obtain sufficient

132 populations for the experiment. Following collection, fresh sea water was added to the bucket to make

133 the volume up to 15 L. The contents of the bucket were then thoroughly mixed through gentle stirring for

134 30 sec, and divided into 75 subsamples each of 200 mL. Twelve plankton kreisels were then inoculated

135 with four of the 200 mL subsamples, selected at random. We did not identify the organisms introduced

136 into each kreisel prior to the start of the experiment to avoid stress associated with microscopic

137   identification. To assess the initial composition of the plankton, we collected a second zooplankton

138   sample that was handled in an identical manner (eg. collection, 75 x 200 mL subsamples), where the

139   subsamples were immediately fixed in 75 % ethanol for later identification. These preserved subsamples

140   were identified to ascertain initial composition and to quantify the likelihood that significant differences

141   existed among the experimental replicates at the start of the experiment. After the zooplankton

142   communities within these subsamples were quantified, we performed numerical simulations in which

143   every possible combination of subsamples were randomly assigned to the different experimental kreisels,

144   and looked for significant differences among treatments. This simulation exercise revealed that there was

145   a < 5 % chance that significant differences existed among the treatments at the start of the experiment

146   (*Supplementary Materials*).

147   The cubozoan *Carybdea rastoni* naturally occurs in and around Sydney Harbour (The

148   Australian Museum, 2016). *Carybdea rastoni* have a broad diet consisting of zooplankton and small fish

149   (Larson, 1976). Due to its ease of identification and capture, we used this species as the gelatinous

150   predator in the experiment. Because of their delicate body structure, *C. rastoni* were individually

151   collected from the water using a 2 L bucket rather than a plankton net. For the treatments containing

152   predators, a single individual *C. rastoni* was placed in each experimental kreisel at the start of the

153   experiment. All *C. rastoni* used in the experiment measured between 20 and 25 mm across the widest

154   part of the bell, and we found no differences in size among treatments ($t_{(4)} = 0.35$, $P = 0.74$). We were

155   able to capture sufficient *C. rastoni* for the experiment in less than 1 hour, suggesting they were relatively

156   abundant in Chowder Bay at the time of collection.

157

158   *Experimental vessels*

159   Custom-built plankton kreisels (Greve, 1968) were used as the experimental vessels (n = 12). Plankton

160   kreisels allow pelagic zooplankton to be cultured in flow-through systems, while preventing individuals

161   adhering to solid surfaces. Each kreisel consisted of a tank measuring 260 mm x 400 mm x 73 mm, with

162   the circular component being a 73 mm-wide section of PVC tubing with a diameter of 210 mm (Fig. S1).

163   Placing the circular component within the tank generates a circular current, preventing adhesion of

164   zooplankton. A "slip" was inserted immediately adjacent to the inflow in order to create a more laminar

165   flow. An outflow tube was installed in the circular component and covered with 100 μm mesh to prevent

166   any organisms escaping. We used three kreisels in each of the four experimental treatments, and the

167   experiment was ran for 10 d. Throughout the experiment, 150 μL of Instant Algae (Shellfish Diet 1800[©],

168   Reed Mariculture, Campbell CA, USA) was added to each kreisel daily. As the water was only filtered to

169   20 μm, the flow through system also supplied phytoplankton as an additional source of food (Byrne,

170   *pers. obs.*).

171

172   *Experimental conditions*

173   The kreisels were supplied with filtered sea water (FSW; 20 μm filtered) from four 60 L header tanks.

174   Manipulating pCO_2 within these four header tanks allowed them to supply different OA treatments to the

175   experimental kreisels. Two header tanks were assigned as containing ambient sea water (pCO_2 370

176   μatm), and two contained elevated pCO_2 sea water (pCO_2 1100 μatm, see table 1). Each header tank

177   consisted of a 60 L plastic tub with a tight fitting lid. Filtered sea water entered each of the header tanks

178   at a rate of 1 L min^{-1}. The OA treatment was achieved by bubbling CO_2–enriched air vigorously through

179   sea water using 20 cm ceramic diffusers. In the two header tanks supplying current conditions, ambient

180   air was bubbled through the diffusers. To achieve target pCO_2 for the OA treatments, ambient air was

181   scrubbed of CO_2 and then mixed with industrial-grade CO_2 (BOC, Sydney, Australia) through a VSO®

182   thermally compensated low flow controller valve (Parker Hannifin, USA). A software-controlled,

183   proportional–integral–derivative (PID) device ensured pCO_2 concentration in the gas mix at the level

184   predicted for the year 2050 (CSIRO and Bureau of Meteorology 2016). Header tank temperatures were

185   maintained using software-controlled solenoid valves that mixed warm and cold water to achieve a

186   constant temperature of 21 °C, representing ambient temperature at the time of collection of the plankton.

187   Each header tank then supplied three of the experimental kreisels, *via* gravity, at a rate of 300 mL min^{-1}.

188   Excess water within the header tank drained out via an overflow.

189   Treatment stability was monitored every second day using a handheld multimeter (Multi 3430 Set G,

190   Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Oberbayern, Germany) equipped with a

191   temperature/pH sensor (SenTix 940, Wissenschaftlich-Technische Werkstätten GmbH). On each

192   monitoring occasion, pH measurements were taken from each of the three experimental kreisels. The pH

193   electrode was calibrated prior to use with high precision NIST buffers pH 4, 7 and 10 (ProSciTech,

194   Townsville, QLD, Australia). Total alkalinity (TA) was determined from water samples collected every

195   second day through potentiometric titration (907 Titrando, Metrohm) using certified reference standards

196   (Dickson et al., 2007). Full physico-chemical parameters including bicarbonate and carbonate ion

197   concentration ($[HCO_3^-]$ and $[CO_3^{2-}]$ and calcite and aragonite saturation states (Ω_{cal} and Ω_{ara}) were

198   subsequently calculated using CO_2SYS (Pierrot *et al.*, 2006), using CO_2 disassociation constants K_1 and

199   K_2 from Mehrbach et al. (1973) refit by Dickson and Millero (1987), and KSO_4 from Dickson et al.

200   (2007). Values are provided in Table 1. Within each of the OA treatments, predators were randomly

201   allocated to the three kreisels being fed by each of the header tanks. As each OA treatment was fed by

202   two header tanks, each of which supplied water to three kreisels, one header tank would supply two

203   predator replicates, and the other would supply one. A coin toss was used to establish which of the header

204   tanks in each OA treatment would supply two predator replicates. Following this initial allocation, the

205   individual kreisels that would house communities containing predators were identified using a random

206   number generator. This process was undertaken to produce a random allocation of predators across

207   header tanks, while also ensuring one header tank in each OA treatment did not also supply all predator

208   treatments.

209   *Data collection, visualization, and Statistical Analyses*

210   At the termination of the experiment, all zooplankton were collected from each of the experimental

211   kreisels and preserved in 75 % ethanol. Zooplankton abundances were calculated by identifying each

212   individual using a Leica EZ4 stereo dissecting microscope. Differences in zooplankton community

213   composition between treatments were visualized using multidimensional scaling (MDS) plots, a method

214   that implements ordination methods to illustrate differences in multidimensional data. Abundance of each

215   zooplankton taxon was used in the MDS plots, and visualized in 2-dimensions. Within this 2-

216   dimensional space, communities that have compositions similar to each other are clustered together,

217   whereas communities with different compositions are spaced further apart. Formal analysis of differences

218   in the zooplankton community composition data required a simultaneous analysis of multiple response

219   variables (abundances of each zooplankton taxa) using two explanatory variables (OA and predator

220   presence). We therefore quantified differences in overall community composition using PERMANOVA

221   within the vegan package, using the R statistical programming language (R Foundation for Statistical

222   Computing, 2015). PERMANOVA analyses incorporate distance matrices, and are considered a robust

223   metric to analyze differences in community composition (Forbes & Hammill, 2013; Hammill *et al.*,

224   2015a). As our data had two independent descriptive variables (OA and presence/absence of a cubozoan

225   predator), we performed a 2-way PERMANOVA including an interaction term. The contributions of

226   different taxa to community level differences were calculated using SIMPER (Warton *et al.*, 2012).

227   While PERMANOVA indicates differences in multivariate data, it does not describe how

228   communities differ, or demonstrate if a significant interaction represents an antagonistic or synergistic

229   effect. To better understand how community composition is altered due to OA and predator presence, we

230   performed 2-way ANOVAs on each zooplankton taxon to analyze differences in abundance. Within each

231   of the ANOVA tests, we included an OA x predator interaction term. To understand whether a significant

232   interaction term indicated an antagonist or synergistic interaction between OA and predators, we

233   generated multiplicative risk models (Soluk & Collins, 1988; Atwood *et al.*, 2014), then compared the

234   result of these null models to our observed data. The general form of the multiplicative risk null model

235   (MRNM) is described by the following equation:

236

237   (Eq. 1) $MRNM = \left(E_{OA} + E_{predators}\right) - \left(E_{OA} * E_{predators}\right)$

253

238   where *MRNM* is the predicted effect of the combined stressors (i.e. the "null model" or the abundance we

239   would expect if predators and OA acted independently of each other), as a proportional change in the

240   metric of interest (e.g. the abundance of each zooplankton). E_{OA} and $E_{predators}$ represent the respective

241   observed proportional changes due to OA and the presence of a cubozoan predator. These multiplicative

242   null models are useful as they never predict reductions in abundances greater than 100%. Should our

243   observed reductions in zooplankton abundance due to both OA and the presence of predators be greater

244   than predicted by the null model, this would indicate these two factors act synergistically. If reductions in

245   abundance are less than predicted by a null model, this indicates an antagonistic interaction. We also

246   performed numerical simulations for the taxa where a significant OA x predator interaction was found to

247   compare predator consumption rates under ambient and elevated pCO_2 conditions (*Supplementary*

248   *Materials*).

249   Within the PERMANOVA and ANOVA tests, "header tank ID" was included as a blocking term.

250   In all analyses, we found no significant effect of header tank ID, and no significant interactions with

251   either predator presence/absence or OA condition (all $P > 0.05$). We therefore removed all terms

252   involving header tank ID and re-ran the analyses.

254

Results

Plankton community

At the end of the experimental trials, all members of the zooplankton community belonged to the

subphylum Crustacea. This dominance of Crustacea is consistent with the analysis of the initial

community composition, and all taxa appeared to survive well in the kreisels during the experiment

(*Supplementary Materials*). Adult copepods were the most abundant members of the community,

accounting for 91.8 % of the total number of individuals. Of these copepods, the vast majority (99.6 %)

were members of the order Calanoida, with the remainder being members of the order Cyclopoida. After

adult copepods, the second most abundant members of the community were copepod nauplii (3.1 %). The

remainder of the community consisted of members of the orders Amphipoda (2.5 %), Pleocymata (1.7

%), Dendrobranchiata (0.6 %) and Mysida (0.4 %).

Community-level effects of experimental stressors

We found that community-level changes in the presence of both drivers were different from what would

be expected given the results of these stressors in isolation, as indicated by the presence of a significant

OA x predator interaction ($F_{(1,8)} = 28.99$, $P < 0.001$, PERMANOVA, Fig. 2). In isolation, OA was

associated with significant changes in community composition ($F_{(1,8)} = 59.81$, $P < 0.001$, Fig. 2). The

addition of a cubozoan predator in isolation also led to significant changes in the zooplankton community

($F_{(1,8)} = 27.34$, $P < 0.001$, PERMANOVA, Fig. 2). Within the zooplankton community, calanoid

copepods were the greatest contributor to differences between treatments, accounting for 88.52 ± 2.30 %

of the differences between treatments (SIMPER).

276

Effects of experimental drivers on individual zooplankton taxa

Reductions in calanoid abundances due to a combination of cubozoan predation and OA were $27.00 \pm$ 1.50% greater than would be expected based on the results of these drivers in isolation (OA x predator interaction, $F_{(1,8)} = 45.61$, $P < 0.001$, ANOVA, Fig. 3a). Predation rates appeared to increase under OA conditions, with cubozoan predators consuming 36.7 % (95% CI: 23.02 % - 50.08 %) of available calanoids at ambient pCO$_2$, and 82.7 % (95% CI: 62.50 % - 97.98 %) under elevated pCO$_2$ (*Supplementary Materials*). Abundances of pleocyematids were also reduced by 61.34 ± 7.80% more through a combination of cubozoan predators and OA than would be predicted by the effect of these stressors in isolation, demonstrated by a significant interaction term and comparison against the multiplicative null model ($F_{(1,8)} = 6.25$, $P = 0.033$, Fig. 3b). Cubozoan predators consumed 0 % (95 % CI: -28.60 % - 28.60 %) of pleocyematids at ambient pCO$_2$, but the rate of consumption increased to 71.5 % (95% CI: 42.09 % - 100.1 %) under elevated pCO$_2$ (*Supplementary Materials*). These results indicate that predation rates on calanoids and pleocyematids increased under OA conditions.

In addition to the significant OA x predator interactions, abundances of certain zooplankton taxa were also reduced by each of our experimental drivers when applied in isolation. Abundances of calanoids were 59.4 % $\pm$ 8.6 % lower under OA conditions compared to controls ($F_{(1,8)} = 251.65$, $P <$ 0.001, ANOVA Fig. 3a). In addition, copepod nauplii were completely absent under OA conditions ($F_{(1,8)}$ = 110.09, P < 0.001, Fig. 3c). Under current ambient conditions, the presence of a cubozoan predator led to a 36.6 % $\pm$ 9.5 % reduction in the abundance of calanoids ($F_{(1,8)} = 127.46$, $P < 0.001$, ANOVA Fig. 3a). Dendrobranchids were also entirely absent in the presence of cubozoan predators, in both ambient and OA conditions ($F_{(1,9)} = 12.00$, $P = 0.007$, Fig. 3d).

298   For all other zooplankton orders and sub-orders (Cyclopoida, Amphipoda, and Mysida), we found

299   no significant changes in abundance following exposure to OA conditions, the presence of cubozoan

300   predators, or an interaction between the two (all $P > 0.05$, Fig. 3e-g). All cubozoan predators survived,

301   and were observed to be freely moving around the kreisels at the termination of the experiment.

302

303   **Discussion**

304   Our data revealed that ocean acidification (OA) can lead to substantial changes in the composition of

305   zooplankton communities through direct and indirect mechanisms. Crucially, when compared to

306   multiplicative null models, the changes in abundance we observed for multiple taxa under a combination

307   of OA and predators were greater than would be expected based on the effects of these stressors in

308   isolation. This synergistic effect indicates that OA will not only directly affect community composition

309   by changing the strength of the abiotic filter, but also by altering the strength of the biotic filter through

310   increasing predation risk (Lawton, 2000).

311   In isolation, OA and the presence of cubozoan predators were associated with significant changes

312   in community composition, driven by reductions in calanoid copepods and copepod nauplii. These

313   changes in copepod abundance indicate that OA can alter the strength of the abiotic filter to such a degree

314   that it can detrimentally affect the most abundant member of the zooplankton community. As predicted,

315   under current conditions, exposure to cubozoan predators also altered the composition of the zooplankton

316   community, through reductions in abundances of Calanoida and Dendrobranchiata. These predator-

317   mediated changes in overall community composition indicate that cubozoan predators represent a current

318   and important component of the biotic filter.

319   Although the exoskeletons of chitinous organisms are less affected by OA than calcified taxa

320   (Cohen *et al.*, 2009; Byrne *et al.*, 2011; Chan *et al.*, 2011; Gaylord *et al.*, 2011), previous investigations

321 show that copepods can be affected through changes in metabolic rates (Thor & Dupont, 2015), which

322 can be compensated for by increased grazing rates (Li & Gao, 2012). These metabolic changes have been

323 associated with reallocation of energetic resources, and may lead to a reduction in the amount of energy

324 available for escape. Calanoid copepods have been shown to escape gelatinous predators by initiating

325 multiple escape jumps, which can lead to less than 1% of predator encounters resulting in capture

326 (Suchman, 2000). Because of this reliance on an energetically expensive escape strategy, any abiotic

327 change that reduces energy available for escape could increase predation on copepods. We specifically

328 provided an over-abundance of food to increase the chances that individuals had sufficient resources for

329 growth and reproduction. However, we cannot rule out that under OA conditions, changes to resource

330 requirements (Li & Gao, 2012), and metabolism (Thor & Dupont, 2015) limited the ability of

331 zooplankton to survive in the presence of a predator. Although untested here, this may represent a series

332 of additional physiological mechanisms by which OA affects copepod susceptibility to predation and

333 represents an important area for future investigations.

334   To our knowledge, no studies have been conducted on the foraging mode of *C. rastoni.* However,

335 similar species of cubozoan jellyfish are considered to be largely "semi-passive hunters" (although see

336 Courtney *et al.*, (2015)), using low resolution vision to seek out habitats with high prey densities and then

337 passively collecting prey on their extended tentacles as they swim (Buskey, 2003; Garm *et al.*, 2011).

338 This type of foraging mode most resembles filter feeding, which is characterized as having a Type I

339 functional response (Titelman & Hansson, 2006; Møller & Riisgård, 2007). Within a Type I functional

340 response, predators should consume a constant proportion of their prey regardless of prey density.

341 However, in the current study, cubozoan predators consumed a greater proportion of the available

342 copepods under OA conditions compared with ambient (*Supplementary Materials),* suggesting OA

343 increased the proportion of food available for capture. Conversely, OA may increase the food

344   requirements of cubozoans, causing them to dedicate more effort to prey collection. This increase in prey

345   collection effort could mean that copepods and other taxa need not become more susceptible to predation

346   under OA conditions. The increased susceptibility of prey to predation and increased food requirements

347   of predators may not be mutually exclusive, meaning that the observed reduction in copepods may be a

348   byproduct of their additive effect. We therefore cannot conclusively determine whether the increased

349   predation rates observed under OA conditions were the result of increased prey susceptibility, or

350   increased predation effort. Future analyses into how OA alters the metabolism and behavior of both

351   predators and prey may aid in teasing apart the individual-level or physiological-level mechanisms at the

352   base of our results, and provide insight into whether it is OA-mediated changes in predators, prey, or both

353   that are driving the patterns we observe here. Nonetheless, our results do demonstrate that OA increases

354   the top-down pressure exerted on zooplankton communities by individual cubozoan predators,

355   highlighting the importance of considering predator-dependent mortalities when considering the full

356   impacts of OA.

357   All *C. rastoni* survived until the experiment was terminated after 10 days, and were observed

358   freely moving around the kreisels. This 100% survival rate implies that *C. rastoni* are relatively tolerant

359   of OA conditions, at least in terms of survival over a relatively short time period. Physiological

360   investigations into the effects of OA on cubozoans have indicated that they are able to cope with near

361   future OA conditions, showing no significant changes in survival, and maintaining asexual reproduction

362   (although at a lower rate) (Klein *et al.*, 2014). The reduced reproduction rates observed under OA for

363   cubozoans in previous experiments (Klein *et al.*, 2014) may mean that although each individual cubozoan

364   exerts more top-down pressure, there are fewer cubozoans in the ecosystem. The total net effect of the

365   cubozoan population on their prey will therefore depend on whether the decrease in reproduction is

366   greater than, equal to, or less than the increase in predation rates. Short-term predator-prey experiments

367 such as ours provide insights into the factors that alter interaction strength, such as the relationships

368 between biodiversity and strength of trophic links (Kratina *et al.*, 2007). However, complementary

369 longer-term (> 2 predator generations) experiments are necessary to understand if these short-term

370 experiments translate to changes in overall community dynamics, rather than composition at a single time

371 point (compare Kratina et al. 2007 to Hammill et al. 2015b). As the experiment we present here was

372 conducted over a relatively short time frame (< 1 predator generation), we do not know if these changes

373 in the strength of trophic interactions translate into long-term alterations in the relative abundances of

374 predators and prey.

375   At the population level, several studies have reported OA affecting life history parameters that

376 influence population growth rates (Albright *et al.*, 2010; Fitzer *et al.*, 2012). Given the rapid generation

377 time of copepods (Gillooly, 2000), nauplii introduced at the start of our experiment could have reached

378 the adult stages by the end of the experiment. However, it is unlikely that our reduced copepod nauplii

379 abundances were caused by an increase in the number of individuals transitioning to the adult stages, as

380 OA was also associated with a reduction in the number of adult copepods. Furthermore, predation alone

381 did not decrease copepod nauplii. Our observation of reduced nauplii abundances under OA conditions is

382 therefore best explained by previously reported factors such as reduced reproduction (Fitzer *et al.*, 2012;

383 Thor & Dupont, 2015), reduced hatching success (Mayor *et al.*, 2007), or increased nauplii mortality

384 (Pedersen *et al.*, 2014). Given the substantial reduction in nauplii and adult copepods we observed, OA

385 could have significant consequences for the population trajectory of copepods, and their roles within

386 ecosystems. We observed no nauplii under elevated pCO$_2$, suggesting that copepod reproduction was

387 dramatically reduced, potentially causing their populations to crash. Copepods are a dominant member of

388 zooplankton communities (Beaugrand *et al.*, 2002; Lavaniegos & Ohman, 2007) and exert significant

389 grazing pressure on primary producers (Sommer & Sommer, 2006). A reduction in top-down control

390   associated with an OA-mediated drop in copepod populations, coupled with the ability of phytoplankton

391   to capitalize on the increased pCO$_2$ associated with OA (Schippers *et al.*, 2004), may lead to an increase

392   in magnitude of phytoplankton blooms under future ocean conditions.

393   The challenges that OA poses for the production and maintenance of heavily calcified skeletons

394   means that much previous research has focused on organisms possessing these structures (Gaylord *et al.*,

395   2011; Coleman *et al.*, 2014). While the chitinous skeletons of copepods and other planktonic crustaceans

396   may make this group less susceptible physiologically to OA (Poore *et al.*, 2013), the large decrease in

397   copepod nauplii production we observed could lead to substantial reductions in copepod populations.

398   Copepods have been proposed to account for the highest biomass of a single group of organisms on earth

399   (Gallienne & Robins, 2001; Turner, 2004), and also form the important link between pelagic primary

400   producers and economically important fisheries (Frederiksen *et al.*, 2006). Reductions in copepod

401   numbers have also been associated with declines in members of higher trophic levels such as salmon

402   (Cross *et al.*, 2009), highlighting that OA-mediated changes low in the food chain could influence many

403   species in marine food webs. Understanding how OA affects lower trophic levels in a community-wide

404   context is therefore critical to elucidating the total effects of changing oceanic conditions.

405   While our experiment indicates that zooplankton communities are altered through a combination

406   of OA and predators, it was designed and conducted as a proof of concept. As our study was conducted in

407   a single geographic location, and at only one point in time, we are cautious about generalizing our results

408   to a wider geographic area. We can, however, speculate about how the geographic location affected our

409   experiment, and how we may expect the results to differ in other areas. Previous studies indicate that

410   species experiencing fluctuating conditions have been shown to be more tolerant to changes in abiotic

411   conditions than species that experience greater stability (Maas *et al.*, 2012; Lewis *et al.*, 2013). The

412   temperate nature of Chowder Bay is associated with annual fluctuations in mean temperatures between

413   17 and 25 °C (http://www.seatemperature.org/). While data on pH fluctuations in Chowder Bay are not

414   available, the near shore, temperate nature of the region would suggest that pH levels are variable

415   (Hofmann *et al.*, 2011). These fluctuations in pH may provide a buffering effect on zooplankton

416   communities, we therefore need to understand the interplay between magnitudes of OA and the

417   susceptibility of different communities to determine the full community-level consequences of OA on

418   marine communities.

419   We have demonstrated that OA may directly increase the strength of the abiotic filter, and

420   indirectly increase the strength of the biotic filter in a marine plankton community. As a consequence of

421   the indirect increase in the strength of the biotic filter, OA may not need to cause mortality in isolation to

422   substantially change the composition of pelagic communities. Our results highlight the need to consider

423   direct and indirect effects if we are to understand the total impacts of OA on marine communities in the

424   coming decades (Kroeker *et al.*, 2013; Poore *et al.*, 2013; Gaylord *et al.*, 2015).

425

433

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588   *erythrogramma*). *Global Change Biology*, **19**, 2698–2707.

589

590

591   **Table 1**

|	Ambient treatment						OA treatment					
Header Tank	**1**			**2**			**3**			**4**		
Predator present	**Yes**	**No**	**No**	**Yes**	**No**	**Yes**	**Yes**	**Yes**	**No**	**No**	**No**	**Yes**
Kreisel number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Temperature (^oC)	21.11	21.09	21.08	21.11	21.06	21.14	21.08	21.1	21.09	21.1	21.11	21.12
($\pm$S.E)	0.1	0.07	0.09	0.05	0.05	0.1	0.07	0.08	0.08	0.06	0.06	0.07
Salinity	34.62	34.68	34.65	34.66	34.64	34.68	34.65	34.67	34.65	34.64	34.65	34.63
($\pm$S.E)	0.04	0.06	0.05	0.03	0.05	0.05	0.05	0.06	0.06	0.07	0.06	0.07
pH_{NIST}	8.20	8.23	8.22	8.19	8.20	8.19	7.79	7.79	7.8	7.77	7.77	7.77
($\pm$S.E)	0.02	0.04	0.02	0.03	0.02	0.03	0.02	0.03	0.03	0.05	0.05	0.05
TA ($\mu Eq\ kg^{-1}$)[a]	2294.96	2295.64	2293.39	2298.66	2295.11	2297.55	2290.43	2298.74	2293.47	2290.37	2296.11	2294.16
($\pm$S.E)	10.35	10.18	5.19	7.53	4.57	6.78	9.5	5.43	6.7	9.65	14.55	7.97
pCO_2 (μatm)[a]	367.81	356.1	353.6	379.16	367.76	364.2	1096.82	1090.78	1079.88	1161.53	1157.7	1157.56
($\pm$S.E)	22.49	22.5	16.28	27.02	23.38	37.95	56.38	87.37	88.44	90.2	83.84	84.17
DIC ($\mu mol\ kg^{-1}$)[a]	2013.82	2001.72	2004.79	2012.6	2014.08	2013.4	2195.45	2192.62	2195.62	2204.28	2207.86	2205.92
($\pm$S.E)	17.01	16.37	9.89	17.03	11.98	18.84	12.92	15.45	15.21	13.85	16.12	12.54
HCO_3^- ($\mu mol\ kg^{-1}$)[a]	1801.97	1782.39	1788.72	1803.57	1802.33	1805.46	2072.23	2069.02	2071.3	2083.24	2082.01	2084.14
($\pm$S.E)	22.81	23.24	15.37	24.45	19.41	29.37	14.01	18.38	17.97	15.4	16.9	14.41
CO_3^{2-} ($\mu mol\ kg^{-1}$)[a]	200.29	208.46	204.97	197.13	200.19	195.89	88.76	89.36	90.39	84.27	85.51	85.47
($\pm$S.E)	7.74	8.98	6.59	8.92	8.51	12.21	3.73	5.82	5.92	5.22	5.13	5.26
Ω_{cal}[a]	4.81	5	4.92	4.83	4.81	4.8	2.13	2.15	2.17	2.02	2.05	2.05
($\pm$S.E)	0.19	0.22	0.16	0.21	0.21	0.29	0.09	0.14	0.14	0.13	0.12	0.13
Ω_{ara}[a]	3.13	3.16	3.21	3.08	3.13	3.06	1.39	1.4	1.41	1.35	1.34	1.34
($\pm$S.E)	0.12	0.14	0.1	0.14	0.13	0.19	0.06	0.09	0.09	0.08	0.08	0.08

592

593   **Table 1.** Physico-chemical parameters of kreisels used to assign ocean acidification (OA) treatments (mean $\pm$ SE): temperature (°C),
594   pH (NIST scale), salinity, total alkalinity (TA $\mu Eq\ kg^{-1}$), dissolved inorganic carbon (DIC $\mu mol\ kg^{-1}$), carbon dioxide partial pressure
595   ($pCO_2\ \mu atm$), bicarbonate concentration ($HCO_3^-\ \mu mol\ kg^{-1}$), carbonate concentration ($CO_3^{2-}\ \mu mol\ kg^{-1}$), calcite ($\Omega_{cal}$) and aragonite
596   saturation (Ω_{ara}). Header tanks 1 and 2 supplied sea water at current oceanic conditions, header tanks 3 and 4 represent OA conditions.
597   All ionic concentrations were calculated in CO2SYS (Pierrot *et al.*, 2006), using CO_2 disassociation constants K_1 and K_2 from
598   Mehrbach et al. (1973) refit by Dickson and Millero (1987), and KSO_4 from Dickson et al. (2007)

25

Figure Legends

600 **Fig. 1.** Illustration of the filters that determine the composition of a local community. The

601 dispersal filter is not included as it did not form part of the current experiment. The abundances

602 of species present in the regional pool represent the potential community. The members of the

603 regional pool that are able to cope with ocean acidification and a cubzoan predator in

604 combination determine the final community composition. Changes in OA may alter final

605 community composition by directly affecting the strength of the abiotic filter, or by increasing

606 susceptibility to predation, thereby increasing the strength of the biotic filter. After Lawton

607 (2000).

608 **Fig. 2.** Multidimensional scaling plot illustrating how OA and the presence/absence of predators

609 alters community composition. Each point represents the community contained within a single

610 plankton kreisel at the end of the experiment. Distances between points are proportional to

611 similarities in community composition, meaning nearby points represent similar communities.

612 **Fig. 3.** Consequences of (ocean acidification) OA and predator presence/absence on the Log_{10}

613 abundances ($\pm$ 1 standard error) of zooplankton within the community. **a)** Calanoida copepods,

614 **b)** Pleocyemata, **c)** Nauplii of all copepods, **d)** Dendrobranchiata, **e)** Amphipoda, **f)** Mysida, **g)**

615 Cyclopoida copepods. Different letters above bars indicate treatments that are significantly

616 different from each other. In each panel, the black "null model" bar represents the predicted

617 additive effects of OA and scyphozoan predators if these two factors acted independently, and

618 asterisks indicate synergistic interaction
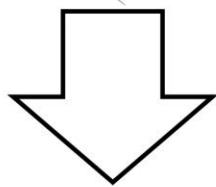
26

619   Figure 1
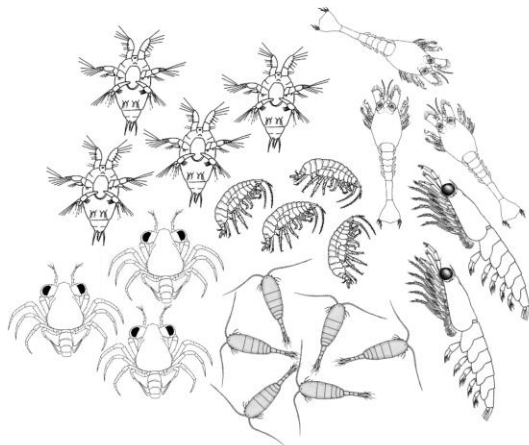
Potential community
composition, determined
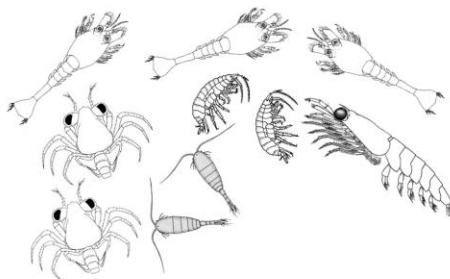by regional pool
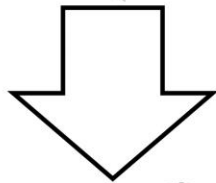
Abiotic filter (altered pH)
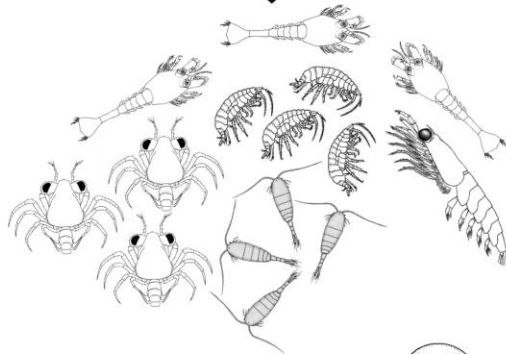
Community able to
overcome abiotic
conditions

Biotic filter (cubozoan
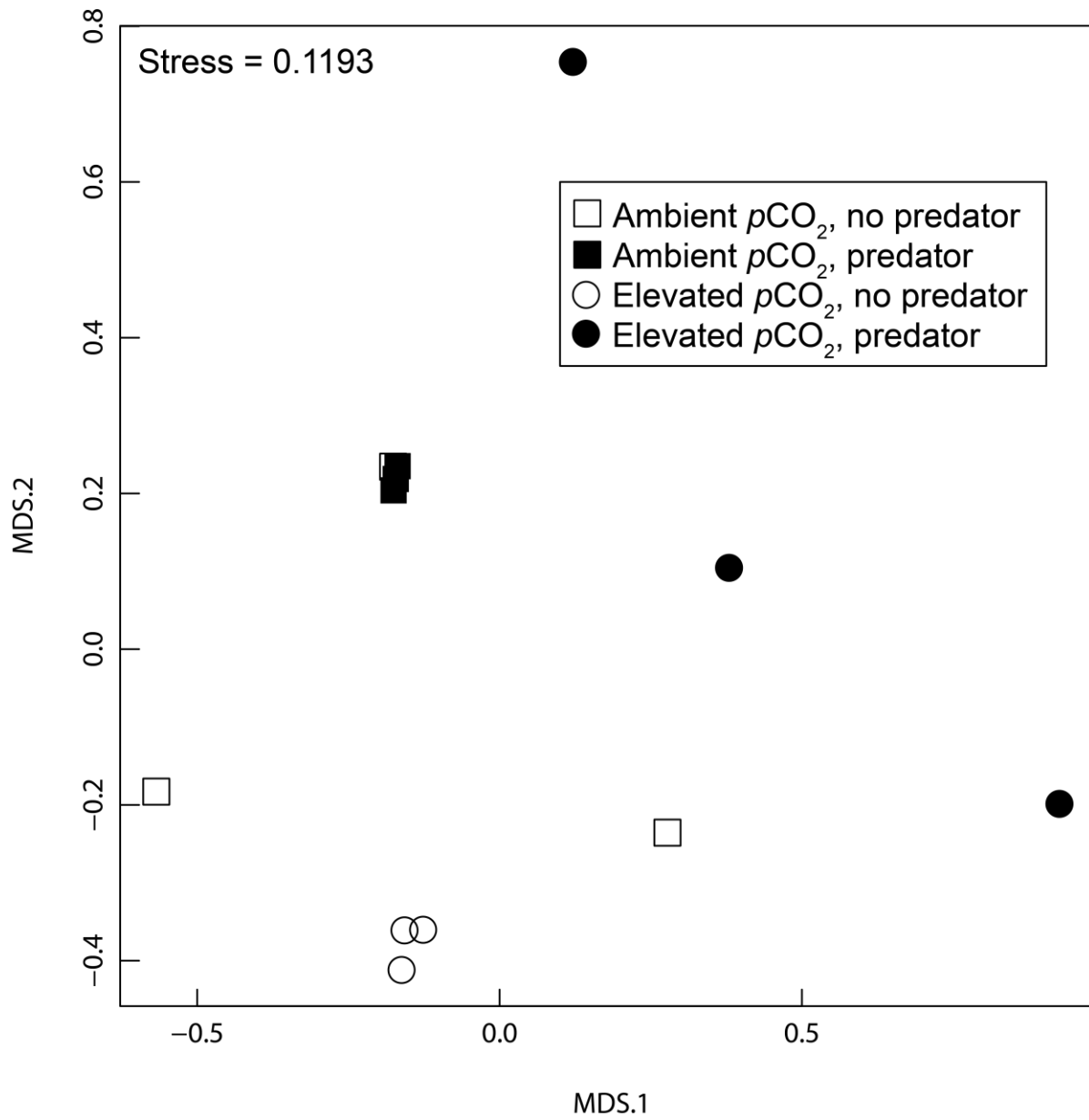predator)

Community able to
overcome abiotic and
biotic conditions

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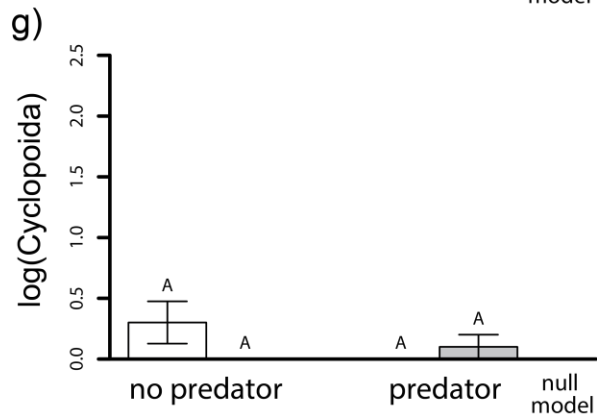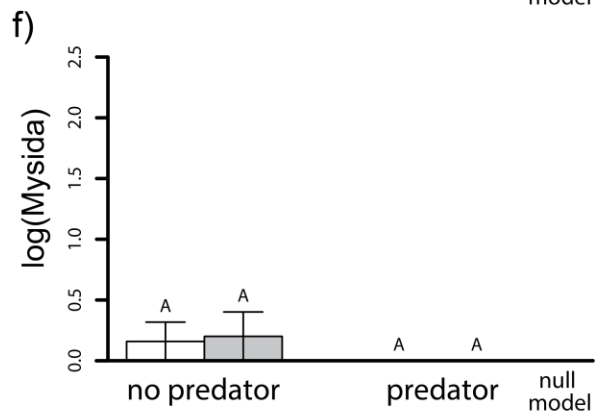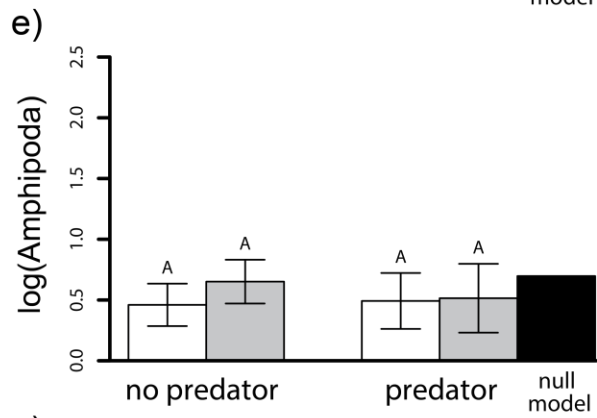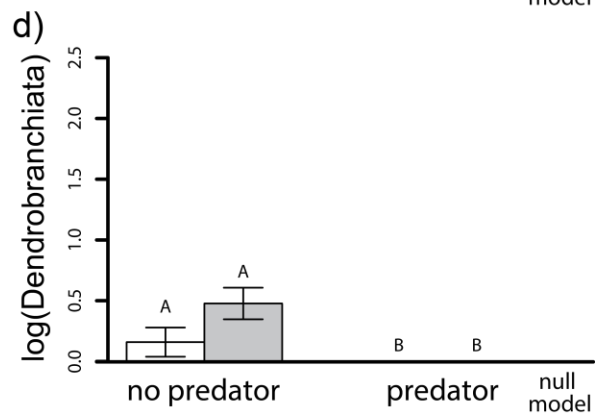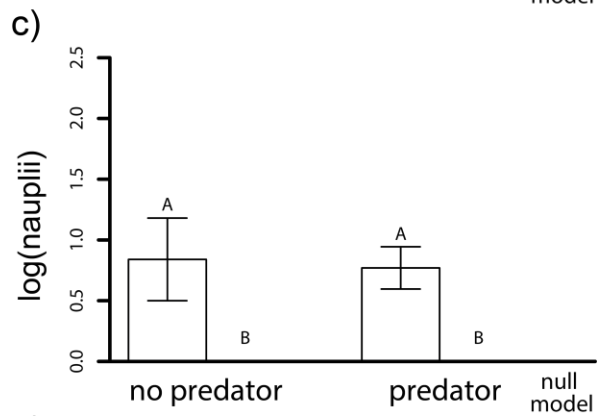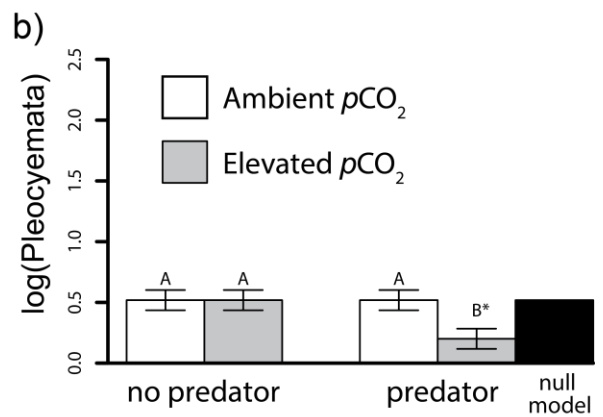622

623   Figure 2

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632   Figure 3

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