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## 8 Changes in fish communities due to benthic habitat shifts under 9 ocean acidification conditions

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## 22 **Abstract**

23 Ocean acidification will likely change the structure and function of coastal marine ecosystems over  
24 coming decades. Volcanic carbon dioxide seeps generate dissolved CO<sub>2</sub> and pH gradients that provide  
25 realistic insights into the direction and magnitude of these changes. Here, we used fish and benthic  
26 community surveys to assess the spatio-temporal dynamics of fish community properties off CO<sub>2</sub> seeps  
27 in Japan. Adding to previous evidence from ocean acidification ecosystem studies conducted elsewhere,  
28 our findings documented shifts from calcified to non-calcified habitats with reduced benthic complexity.  
29 In addition, we found that such habitat transition led to decreased diversity of associated fish and to  
30 selection of those fish species better adapted to simplified ecosystems dominated by algae. Our data  
31 suggest that near-future projected ocean acidification levels will oppose the ongoing range expansion of  
32 coral reef-associated fish due to global warming.

33 **Keywords:** carbon dioxide, biogenic habitat complexity, scleractinian coral cover, reef-associated fish

34

35

## 36 **1. Introduction**

37

38 Shifts in marine biogenic habitats in response to anthropogenic activities and a range of stressors have  
39 been documented since the 1960s (Hughes 1994). In tropical coral reef ecosystems examples include  
40 studies of the impacts of overfishing (Jackson et al. 2001), outbreaks of coral-eating predators (De'ath et  
41 al. 2012), diseases (Hughes 1994), pollution (McCulloch et al. 2003), hurricanes (Hughes 1994), and  
42 extreme temperatures (Hoegh-Guldberg 1999). Ocean warming is changing coastal marine communities,  
43 for example due to (1) warm-water species (e.g. corals and tropical fish) moving poleward following  
44 their thermal physiological niche, (2) changes in the strength of interspecific interactions (e.g. increase  
45 of herbivory from warm-water fish leading to the loss of kelp forests at their low latitude limits), and (3)  
46 a decrease in biogenic habitat complexity (e.g. Vergès et al. 2014, 2016; Hall-Spencer & Harvey 2019).  
47 Ocean Acidification (OA), the alteration of seawater carbonate chemistry due to rising atmospheric CO<sub>2</sub>  
48 concentrations, adds an extra set of stressors to those caused by warming. Meta-analyses show that

49 decreased seawater pH due to OA may impair calcification and accelerate dissolution for many calcifying  
50 habitat-formers, while rising  $p\text{CO}_2$  levels may enhance the primary production and carbon fixation rates  
51 of non-calcifying autotrophs (Falkenberg et al. 2013; Harvey et al. 2013; Kroeker et al. 2013; Wittmann  
52 and Pörtner 2013). As a result, there will be losers and winners under OA conditions, with effects on  
53 ecosystems documented along gradients in seawater pH at  $\text{CO}_2$  seeps around the world (e.g., Hall-  
54 Spencer et al. 2008; Fabricius et al. 2011) including reductions in habitat complexity, shifts in  
55 competitive interactions and changes in species dominance.

56 While  $\text{CO}_2$  seeps are not perfect analogues for ocean acidification (e.g., Fabricius et al. 2017), they  
57 nevertheless comprise one of the very few field-based tools available to assess OA effects on ecosystems  
58 and communities-(Hall-Spencer and Harvey 2019). To date, our knowledge about the ecosystem effects  
59 of OA is advancing rapidly with increasing evidence from temperate (Hall-Spencer et al. 2008;  
60 Nagelkerken et al. 2015; Milazzo et al. 2019), subtropical (Agostini et al. 2018) and tropical (Fabricius  
61 et al. 2011, 2014; Inoue et al. 2013; Enochs et al. 2015)  $\text{CO}_2$  seeps. The responses of biogenic habitats  
62 to OA differ regionally. Shifts from diverse to depauperate scleractinian species assemblages and from  
63 hard to soft coral communities have been observed in Papua New Guinea (Fabricius et al. 2011), Palau  
64 (Barkley et al. 2015) and in Southern Japan (Inoue et al. 2013). As carbon dioxide levels increase, there  
65 is a shift in community dominance from corals to seaweeds in the Northern Mariana Islands (Enochs et  
66 al. 2015) and at a subtropical-temperate transition zone in Japan (Agostini et al. 2018). How such OA-  
67 induced habitat shifts affect fish requires further study as most work at  $\text{CO}_2$  seeps to date at has focused  
68 on bacteria, algae and invertebrates (Hall-Spencer et al. 2008; Fabricius et al. 2014; Sunday et al. 2017;  
69 Milazzo et al. 2019).

70 Very few attempts have been made to estimate the effects of OA-induced habitat simplification on fish  
71 communities (Munday et al. 2014; Nagelkerken et al. 2015, 2017). A study at three  $\text{CO}_2$  seeps in Papua  
72 New Guinea reported reduced coral reef complexity at elevated  $\text{CO}_2$  respect to control sites. However,

73 there was no difference in fish species richness between seep and control sites, and no difference in fish  
74 community structure at two out of the three seep-control groups examined (Munday et al. 2014). The  
75 only slight differences detected in fish community structure were mostly driven by small-bodied fish  
76 species showing strong habitat preferences (Munday et al. 2014). In another paper, Nagelkerken et al.  
77 (2015) documented shifts from kelp/macroalgae and seagrass to low-relief turf-algae at temperate CO<sub>2</sub>  
78 seeps that seemed to cause a loss of fish predators and predatory release of prey fish species, even though  
79 their antipredator responses were compromised.

80 At present, how ocean warming and acidification will affect ecosystem properties and functioning is still  
81 under debate. Some modelling attempts suggest that declines in aragonite saturation state ( $\Omega_{\text{arag}}$ ) will  
82 limit the poleward expansion of tropical coral reefs that is underway due to ongoing warming (Yara et  
83 al. 2012; van Hooijdonk et al. 2014), as will insufficient light in winter for coral algal symbiont  
84 photosynthesis (Muir et al. 2015).

85 Here, we evaluate the temporal consistency of changes in fish communities in response to biogenic  
86 habitat shifts off CO<sub>2</sub> seeps located in the NW Pacific (Japan), in a region that has naturally low levels  
87 of  $p\text{CO}_2$ , high carbonate saturation levels and elevated local seawater temperatures (Midorikawa et al.  
88 2005). These conditions allow the coexistence of both canopy-forming macroalgae and scleractinian  
89 coral communities at ambient CO<sub>2</sub> conditions, while a transition to low-relief algal turf habitats occurs  
90 at elevated CO<sub>2</sub> levels (Agostini et al. 2018; Harvey et al. 2019). To assess how fish community  
91 properties changed spatially along the  $p\text{CO}_2$  gradient, we coupled fish and benthic community  
92 assessments (habitat complexity, canopy height, and % cover), along a CO<sub>2</sub> gradient and at control sites  
93 off Shikine Island (Eastern Japan). The study was carried out over two time periods (June and September)  
94 to investigate whether the effects of OA-mediated habitat shifts on fish community composition and  
95 structure are temporally consistent, specifically when acute seasonal typhoons (usually from July to  
96 September), may affect benthic community structure and habitat complexity. In addition, we carried out

97 fish trait comparisons between the different CO<sub>2</sub> sites, to assess changes in taxonomic richness and  
98 abundance of fish subdivided by geographic distribution (Tropical, Subtropical and Temperate), trophic  
99 guilds (Carnivore, Omnivore, Herbivore and Planktivore) and affinity to coral reef habitats.  
100 We expected that fish communities would change in composition and structure as a result of decreasing  
101 habitat complexity along a spatial CO<sub>2</sub> gradient and that these changes would be consistent over time.  
102 Since it has been suggested that OA may decrease overall habitat complexity (e.g. from complex corals  
103 and canopy-forming algae to low-profile algae and turfs; Sunday et al. 2017), we expected that fish  
104 community species richness would decrease with increasing levels of CO<sub>2</sub>. As CO<sub>2</sub> enrichment acts both  
105 as a stressor for scleractinian corals and as a substrate for primary producers, we also expected that the  
106 number of species of fish from tropical and subtropical origins would decrease, while the diversity of  
107 herbivorous fish would increase in elevated CO<sub>2</sub> conditions.

108

## 109 **2. Materials and Methods**

110

### 111 *2.1 Study sites and carbonate chemistry*

112

113 Shikine is a volcanic island east of the Izu peninsula in Japan (34° 19' 9" N, 139° 12' 18" E) with many  
114 CO<sub>2</sub> seeps in shallow waters. Based on previous geochemical investigations (Agostini et al. 2015), our  
115 survey locations were selected to avoid potentially confounding geochemical factors (e.g. high sulfides,  
116 negative redox potential, altered total alkalinity and elevated temperature). One location (Elevated-CO<sub>2</sub>)  
117 was within Mikawa Bay and a second location (Control) characterised by ambient CO<sub>2</sub> conditions was  
118 positioned in an adjacent bay with similar depths (3-12 m), and exposure to wind and currents (Fig. 1).  
119 To document spatial variation in the carbonate chemistry, a WQC24 multi-parameter logger (DKK-TOA  
120 Corporation, Tokyo, Japan) and a HydroC® CO<sub>2</sub> II sensor (Contros System & Solutions GmbH,  
121 Germany) were deployed between 9:00 am and 3:00 pm by scuba divers along four and five 100-m

122 transects in the Elevated- and Control CO<sub>2</sub> locations, respectively (Fig. 1). Every 10 meters the seawater  
123 pH (NBS scale), temperature (T, °C), salinity, and depth (m) were recorded for 5 minutes with the DKK-  
124 TOA, whilst the HydroC® CO<sub>2</sub> II sensor recorded measures of *p*CO<sub>2</sub> (µatm) every 5 seconds. The CO<sub>2</sub>  
125 sensor detects dissolved CO<sub>2</sub> molecules that diffuse through a thin film composite membrane into an  
126 internal gas circuit containing a detector chamber where the *p*CO<sub>2</sub> is determined by means of an IR  
127 absorption spectrometer. Both loggers were positioned at 1 meter from the sea-bottom at an average  
128 depth range of 3-12 meters, and were attached to a floating buoy equipped with a GPS (eTrex30x,  
129 Garmin) to record the exact position of each measurement. Total alkalinity (TA) was measured from  
130 seawater samples collected underwater at each location (N = 24 in June; N = 25 in September). Water  
131 samples were immediately filtered at 0.45 µm using disposable cellulose acetate filters (Dismic,  
132 Advantech, Japan) and stored at room temperature in the dark (for no more than one week) until  
133 measurement. TA was measured by titration (TiTouch i915, Metrohm) with HCl at 0.1 mol l<sup>-1</sup>, and  
134 calculated from the Gran function between pH 4.2 and 3.0. The titrations were cross-validated using a  
135 working standard (SD: ± 9 µmol kg<sup>-1</sup>) and against certified reference material purchased from the A.G.  
136 Dickson laboratory (Batch 152). The CO<sub>2</sub>SYS software (Pierrot et al. 2006) was used to calculate *p*CO<sub>2</sub>  
137 (Table 1) from T, pH, salinity and TA values, and to control *in situ* continuous measurements of *p*CO<sub>2</sub>  
138 recorded by the HydroC® CO<sub>2</sub> II sensor. The dissociation constants from Mehrbach (1973), as adjusted  
139 by Dickson and Millero (1987), HSO<sub>4</sub> using Dickson (1990), and total borate concentrations from  
140 Uppström (1974) were used for carbonate chemistry calculations (Table 1). The HydroC® CO<sub>2</sub> II sensor  
141 was not employed in the September survey due to logistic constraints.

142 The carbonate chemistry measurements along the nine 100-m transects were used to identify five  
143 sampling CO<sub>2</sub> sites in the rocky subtidal zone between 3 and 12 m depth in Mikawa bay and the Control  
144 bay: one ‘High-CO<sub>2</sub>’ (High), one ‘Mid-CO<sub>2</sub>’ (Mid) and one ‘Low-CO<sub>2</sub>’ (Low), and two ‘Ambient-CO<sub>2</sub>’

145 (Ref 1 and Ref 2; Fig. 1; Table 1). In each CO<sub>2</sub> site, both the benthic habitats and the fish communities  
 146 were characterised as detailed below.

147

148 *Table 1 - Seawater chemistry of the subtidal sampling sites off Shikine Island. Values from June and September*  
 149 *surveys are reported as mean (±SD). Minimum (Min) and maximum (Max) pCO<sub>2</sub> values are also reported.*  
 150 *pCO<sub>2</sub> calc.= pCO<sub>2</sub> levels calculated with CO2SYS. pCO<sub>2</sub> meas.= pCO<sub>2</sub> levels measured with the HydroC®*  
 151 *CO<sub>2</sub> II logger.*

### a) June 2016

CO <sub>2</sub> location	CO <sub>2</sub> site	Salinity	T °C	pH nbs	n (pH)	TA (μmol kg <sup>-1</sup> )	pCO <sub>2</sub> calc. (μatm)	pCO <sub>2</sub> meas. (μatm)	n (pCO <sub>2</sub> meas.)
Elevated	High	34.5 (0.05)	19.9 (0.5)	7.87 (0.15)	11	2249.9	971.7 (434.0) Min:591.8 Max:2062.1	952.8 (450.8) Min:567.7 Max:2360.4	417
Elevated	Mid	34.6 (0.07)	19.4 (0.5)	8.09 (0.05)	17	2253.4	497.2 (72.6) Min:373.8 Max:645.8	552.1 (188.2) Min:368.2 Max:1552.1	843
Elevated	Low	34.7 (0.05)	19.5 (0.7)	8.16 (0.05)	16	2270.5	404.9 (54.8) Min:361.2 Max:523.2	402.1 (53.4) Min:358.0 Max:591.1	555
Control	Ref 1	34.7 (0.05)	18.9 (0.3)	8.21 (0.01)	28	2253.1	348.2 (10.6) Min:322.0 Max:367.6	347.9 (13.9) Min:307.2 Max:373.8	994
Control	Ref 2	34.8 (0.05)	19.5 (0.2)	8.25 (0.03)	27	2250.8	311.5 (29.3) Min:255.4 Max:356.7	311.8 (29.1) Min:251.9 Max:357.8	913

### b) September 2016

CO <sub>2</sub> location	CO <sub>2</sub> site	Salinity	T °C	pH nbs	n (pH)	TA (μmol kg <sup>-1</sup> )	pCO <sub>2</sub> calc. (μatm)
Elevated	High	33.9 (0.1)	26.0 (0.1)	7.65 (0.09)	4	2267.8	1646.4 (397.3) Min:1220.9 Max:2026.5
Elevated	Mid	33.9 (0.2)	25.1 (1.0)	7.91 (0.12)	6	2257.3	849.7 (291.0) Min:524.4 Max:1372.0
Elevated	Low	33.9 (0.0)	25.6 (0.1)	8.13 (0.01)	4	2269.3	459.3 (12.7) Min:442.7 Max:473.1
Control	Ref 1	33.9 (0.2)	26.3 (1.4)	8.18 (0.01)	4	2249.9	395.4 (11.9) Min:379.3 Max:407.8
Control	Ref 2	33.9 (0.2)	26.2 (1.7)	8.16 (0.01)	4	2249.5	420.8 (13.6) Min:409.2 Max:439.8

152



153

## 154 2.2 Benthic habitat characterisation

155

156 Average canopy height and percentage cover (% cover) of benthic taxa were recorded in the June and  
157 September surveys along 25 m strip transects positioned in the five CO<sub>2</sub> sites (High, Mid, Low, Ref1 and  
158 Ref2). Specifically, four transects were deployed in the ‘High-CO<sub>2</sub>’ site (High), six transects within the  
159 ‘Mid-CO<sub>2</sub>’ (Mid) site, six transects within the ‘Low-CO<sub>2</sub>’ (Low) site, and nine transects each in the two  
160 ‘Ambient CO<sub>2</sub>’ sites (Ref1 and Ref2; Fig. 1). Within each 25 m benthic transect, the canopy height was  
161 measured every meter using the point-intercept method, and a Biotic Habitat Profile (BHP) ratio was  
162 estimated as a proxy of biotic complexity. BHP, conceptually similar to the well-established chain  
163 method, was calculated *a posteriori* by dividing the contoured distance following the measured canopy  
164 profile by the linear distance (i.e. 25 meters).

165 The % cover of benthic taxa was assessed in ten photoquadrats, positioned at ca. 5 meters apart along  
166 each 25 meter transect. An Olympus Stylus Tough TG3 with a PT056 camera housing was mounted on  
167 a 1 x 1 m frame. The % cover was estimated using the open-access software Image-J  
168 (<http://rsb.info.nih.gov/ij/>; Schneider et al. 2012) by tracing the 2-dimensional outline of each benthic  
169 morphological taxon. For each photoquadrat the % cover of the following benthic groups was recorded:  
170 Turf algae, crustose coralline algae (CCA), Non-Canopy-forming fleshy algae (<5 cm canopy height),  
171 Canopy-forming algae ( $\geq$  5 cm canopy height), *Caulerpa chemnitzia* var. *peltata*, Table corals, Soft  
172 Corals, Encrusting Corals, Massive Corals (i.e. boulder corals with massive growth forms), Anemones  
173 and Sponges.

174

## 175 2.3 Fish surveys

176 Species composition and relative abundance of fish were visually censused within standard linear 25x5  
177 m transects (Harmelin-Vivien et al. 1985). A total of 73 and 37 transects were carried out on June and

178 September 2016, respectively. Fish transects were located haphazardly within each sampling CO<sub>2</sub> site at  
179 4-10 m depth, and were conducted between 09.00 am and 03.00 pm by a scuba diver leaving behind a  
180 25 m measure tape, while counting and identifying all the fish encountered 2.5 m either side of the tape  
181 (125 m<sup>2</sup>; Harmelin-Vivien et al. 1985). Water visibility exceeded 15 m for all counts. Each sampling day,  
182 only two spatially separated transects (at >20 m distance each other) were gathered at each CO<sub>2</sub> site to  
183 avoid temporal dependence of data (Stewart-Oaten et al. 1986).

184 To make trait-mediated comparisons among different CO<sub>2</sub> conditions, the different fish species were also  
185 subdivided by geographical origin (Tropical, Subtropical and Temperate), by trophic guild (Carnivore,  
186 Omnivore, Herbivore and Planktivore), and by their association with coral reefs (i.e. coral reef associated  
187 and non-associated species) following Nakamura et al. (2013) and FishBase (<http://www.fishbase.org/>).

#### 188 *2.4 Data analyses*

189

190 Changes in benthic habitat composition among CO<sub>2</sub> sites were analysed using non-metric  
191 multidimensional scaling technique (nMDS) and tested with Permutational Multivariate Analysis of  
192 Variance (PERMANOVA; Anderson and Braak 2003) using the software PRIMER 6 and  
193 PERMANOVA+ β3 package (Clarke and Gorley 2006). The analysis was performed on Bray-Curtis  
194 measures in a multivariate context of untransformed % cover data, using 9999 permutations of the  
195 appropriate units. Two fixed factors were considered: “CO<sub>2</sub> site” with 5 levels (High, Mid, Low, Ref 1  
196 and Ref 2) and “Season” with two 2 levels (June and September).

197 To identify the relevant variables characterising the benthic habitat which were responsible for the  
198 variation in fish community structure and composition, we used a Multivariate Distance Based Linear  
199 Model [DISTLM, Anderson et al. 2008]. Both the fish community and the benthic datasets were square  
200 root transformed and the step-wise model selection method based on the AIC criterion was performed to  
201 assess the benthic variables related with Bray-Curtis resemblance matrix of the fish dataset. To visualise

202 the fish community data as a biplot, the Distance-based redundancy analysis (dbRDA; Legendre and  
203 Anderson 1999; McArdle and Anderson 2001) using the benthic variables identified by the DISTLM  
204 routine was used.

205 To investigate the potential relationship between  $p\text{CO}_2$  and habitat complexity (as BHP index, log  
206 transformed) we used an Additive Mixed Model (AMM) with a Gaussian distribution and the identity  
207 link. AMM was used because it allows for the modelling of the non-linear effects of continuous  
208 explanatory variables by incorporating smooth functions (Wood 2011). Specifically, the cubic regression  
209 spline was used as a one-dimensional non-parametric smoothing function and the number of knots  $k$  was  
210 set to 6, to prevent the models from producing complex non-linear relationships of little biological  
211 significance (Wood 2006). To account for the lack of independence of the BHP data of the same Site per  
212 Season we used Site  $\times$  Season as random intercept, which is assumed to be normally distributed with  
213 mean 0 and variance  $\sigma^2$ . Data for both sampling campaigns (June and September) were combined into a  
214 single data set and Season was modelled as a factor. The model was fitted using maximum likelihood  
215 (ML) parameter estimation. The analysis was performed using the R package 'mgcv' (Wood 2011).

216 We also used a method derived from meta-analysis to assess the effects of the different  $\text{CO}_2$  conditions  
217 on the number ( $S$ ) and abundance ( $N$ ) of fish species, also considering their classification by geographical  
218 distribution ( $S_{\text{origin}}$ ), trophic guild ( $N_{\text{trophic}}$ ) and coral reef association ( $S_{\text{coral}}$ ). To this aim, we calculated  
219 the effect size (a metric that quantifies the difference between the control and experimental groups) as  
220 the response ratio, i.e. the natural logarithm of the ratio between the averaged response variable values  
221 ( $S$ ,  $N$ ,  $S_{\text{origin}}$ ,  $N_{\text{trophic}}$ ,  $S_{\text{coral}}$ ) recorded at the Elevated  $\text{CO}_2$  (High, Mid and Low  $\text{CO}_2$  sites) and Control  
222 (Ref1 and Ref2 sites) locations. These analyses were performed using the R (R Core Team 2018) package  
223 'metafor' (Viechtbauer 2010).

224

### 3. Results and Discussion

225  
226

227 Benthic communities changed from rocky habitats dominated by scleractinian corals and canopy-forming  
228 macroalgae to rocky reefs covered in low-profile and turf algae from Control (Ref1 and Ref2) to Elevated  
229 CO<sub>2</sub> sites (Low, Mid and High) and this observation was consistent in both of the considered time periods  
230 (Fig. 2; PERMANOVA: CO<sub>2</sub> site x Season, Pseudo-F= 7781.2; P(perm)=0.0001). Composition and  
231 structure of benthic communities did not differ between sites within ambient CO<sub>2</sub> condition both in June  
232 (i.e., Ref1 = Ref 2; Pair-wise T test, t= 1.52; P(perm)=0.08) and September (Pair-wise T test, t= 1.20;  
233 P(perm)=0.28) surveys, whilst they did significantly differ in most of the pair-wise comparisons of sites  
234 within the elevated CO<sub>2</sub> sites (Table S1).

235 Table, massive and encrusting corals were common at control sites in both sampling periods. They are  
236 able to survive at this high latitude (34° N) in the NW Pacific due to the warm northward flow of the  
237 Kuroshio Current (Veron and Minchin 1992). Hard corals were absent along transects taken at elevated  
238 CO<sub>2</sub> conditions. In contrast to some other CO<sub>2</sub> seep systems (Suggett et al. 2012; Inoue et al. 2013), soft  
239 corals were rare and were absent in our elevated CO<sub>2</sub> transects. Instead, dense mats of *Caulerpa*  
240 *chemnitzia* var. *peltata* and the diatom *Biddulphia biddulphiana* covered most of the rocky substrata at  
241 the elevated CO<sub>2</sub> sites in June. These species were not seen in our transects in September, often revealing  
242 a covering of crustose coralline algae or low profile turf algae on the rocks (Fig. 2). As recently suggested,  
243 this was likely due to strong wave energy during typhoon activity that occurs in summer and early autumn  
244 (from late July to early October each year) on Shikine Island (Harvey et al. 2019). This major seasonal  
245 habitat shift resulted in an overall loss of canopy height due to the increase in the abundance of a few  
246 low profile algal and turf species which may outcompete large and slow-growing species under ocean  
247 acidification conditions (Harley et al. 2012; O'Brien & Scheibling 2018; Harvey et al. 2019). The ability  
248 of a few opportunistic species to withstand OA effects, benefit from CO<sub>2</sub> enrichment and displace

249 dominant habitat-forming species (such as canopy-forming algae or coral early stages), has been  
250 previously documented (e.g. Connell et al. 2018; Agostini et al. 2018) and can be attributed to stunted  
251 successional development (Gaylord et al. 2015).

252 Previous CO<sub>2</sub> seeps studies revealed both detrimental and no effects of elevated CO<sub>2</sub> levels on the eco-  
253 physiological and behavioral performances of fish (e.g. Munday et al. 2014; Nagelkerken et al. 2015;  
254 Milazzo et al. 2016; Cattano et al. 2017; Di Franco et al. 2019). Here we focus on fish responses at the  
255 community level.

256 Fish assemblages changed significantly between sites along the CO<sub>2</sub> gradient, and between elevated CO<sub>2</sub>  
257 and reference sites. The DISTLM procedure revealed differences in fish assemblage composition and  
258 structure, and identified five variables that best explained such patterns: the table and massive corals, the  
259 turf, the non-canopy forming algae and the CCA. The dbRDA ordination of the dataset constrained by  
260 these variables showed that the difference in the fish communities along the CO<sub>2</sub> gradient was best  
261 explained by turf, massive and non-canopy algae, while the differences between June and September  
262 were best explained by table corals and CCA (Fig. 3). Thus, the clear change of fish communities from  
263 ambient to elevated CO<sub>2</sub> conditions was associated with a biogenic habitat shift under OA conditions.

264 To date, few studies have documented the effects of OA-induced habitat changes on the structure and  
265 composition of fish communities. Munday et al. (2014) found that fish communities differed little  
266 between CO<sub>2</sub> seeps and nearby control reefs in Papua New Guinea, suggesting that such similarities were  
267 due to the contribution of highly mobile fish species, which are able to move in and out of small CO<sub>2</sub>  
268 seep areas. The few observed differences in the abundance of certain fish species were related to coral  
269 community changes (from branched to massive corals) between CO<sub>2</sub> exposed and un-exposed reefs (see  
270 Fabricius et al. 2014) rather than by the direct effects of high CO<sub>2</sub> on fish. Nagelkerken et al. (2015)  
271 described habitat characteristics and fish species composition at two different CO<sub>2</sub> seeps, documenting  
272 predator reductions and habitat shifts at elevated CO<sub>2</sub> conditions, which together led to an increase of a

273 few territorial fish species. A more recent study carried out off the White Island CO<sub>2</sub> seeps in New  
274 Zealand documented loss of fish diversity and homogenisation of fish communities under OA conditions,  
275 suggesting that elevated CO<sub>2</sub> indirectly boosted the abundance of a single species thus altering the  
276 competitive relationships among species and suppressing the abundance of the competitive subordinates  
277 (Nagelkerken et al. 2017). Contrary to these previous studies, where fish community differences between  
278 CO<sub>2</sub> exposed and unexposed reefs were evaluated focusing on a few species with narrow home ranges,  
279 here we compared the structure and the composition of entire fish communities finding significant  
280 differences among the four CO<sub>2</sub> conditions considered.

281 The effects of OA on fish communities may depend on how elevated CO<sub>2</sub> affects the different species  
282 directly and indirectly. By altering acid-base balance and the processing of sensory information, elevated  
283 CO<sub>2</sub> concentrations affect physiological and behavioural performance in fish, although such effects are  
284 species- and trait-specific (reviewed in Cattano et al. 2018). In addition, by promoting shifts in biogenic  
285 habitat (Milazzo et al. 2019), habitat simplification (Sunday et al. 2017) and food web simplification  
286 (Fabricius et al. 2014; Vizzini et al. 2017), high CO<sub>2</sub> conditions may indirectly affect some fish species,  
287 especially those with highly specialised habitat and resource use. Conversely, generalist species could  
288 cope better with the predicted effects of OA on habitat and resources (e.g. Wilson et al. 2008a). This  
289 inter-specific variability in the extent to which fish may respond to OA-driven habitat modifications  
290 could play a role in shaping the direction of community shift and the composition of novel fish  
291 communities under elevated CO<sub>2</sub> conditions (Nagelkerken et al. 2017).

292 Our findings support the prediction that OA simplifies habitat composition and reduces habitat  
293 complexity (Sunday et al. 2017). Biogenic habitat complexity (BHP) decreased with increasing  $p\text{CO}_2$   
294 levels, in a non-linear relationship (Fig. 4a). When looking at model residuals including both seasons,  
295 habitat complexity was high at  $p\text{CO}_2$  values below ~500  $\mu\text{atm}$ , whilst this relationship was significantly  
296 negative for values up to ~ 1000  $\mu\text{atm}$  (i.e. decreased complexity with increasing  $p\text{CO}_2$ ), after which no

297 significant effects were detected probably due to the low sample size (Fig.4b). Overall, this trend was  
298 consistent in the two sampling campaigns (Fig. 4c). The minimum index values were reached between  
299 ~500 and ~1000  $\mu\text{atm}$ , where table corals disappeared being replaced mainly by turf, non-canopy algae  
300 and *Caulerpa chemnitzia* var. *peltata*, and where the only calcifying groups were represented by CCA or  
301 a few encrusting corals. The consistent complexity reduction in the elevated  $\text{CO}_2$  sites compared to  
302 control conditions matches the habitat shift from corals and canopy-forming macroalgae to turf  
303 dominated reefs, which indeed provides fewer structure and habitat functions than corals (Filbee-Dexter  
304 and Wernberg, 2018). Such transition to less complex habitats may lead to the loss of a suite of resources  
305 for fish (e.g. food and space availability) and to the alteration of important ecological processes, such as  
306 foraging, settlement and predation avoidance. OA research in  $\text{CO}_2$  seeps has documented altered  
307 properties and non-linear responses for invertebrate communities along  $\text{CO}_2$  gradients (e.g. Fabricius et  
308 al. 2014; Milazzo et al. 2019), whilst no effects were detected in fish communities despite lower habitat  
309 complexity under elevated  $\text{CO}_2$  conditions (Munday et al. 2014).

310 Here for the first time, a consistent temporal response of the fish fauna to the OA-mediated habitat shift  
311 was documented. Overall, the number of species declined by 35% and 57% when comparing fish species  
312 richness at Control vs High  $\text{CO}_2$  sites in June and September, respectively (see Table S3). In addition,  
313 average species richness and abundance significantly decreased from Control to elevated  $\text{CO}_2$  conditions,  
314 with the lowest values recorded at the High and Mid  $\text{CO}_2$  sites (Fig. 5a; Table S3). Such a pattern was  
315 consistent in both June and September (Fig. 5a; Table S3) and confirms previous evidence that shifts  
316 toward less complex habitat causes decreased fish diversity and abundance (e.g. Wilson et al. 2008b), as  
317 well as simplified and homogenised fish communities under OA conditions (Nagelkerken et al. 2017).  
318 Trait-based approaches are increasingly being used to characterise ecological changes in disturbed  
319 ecosystems (Mouillot et al. 2013). Environmental alterations may not only lead to biodiversity loss, but  
320 also to changes in community function, through the selection of species with traits well-adapted to the

321 new environmental conditions. Since functional groups may show specific habitat requirements and  
322 preferences, the occurrence and abundance of certain species can be strongly influenced by habitat  
323 composition and characteristics. As expected, trait-based comparisons of fish communities along the CO<sub>2</sub>  
324 gradient reveal a change in composition towards fish species of less tropical and sub-tropical origin (Fig.  
325 5b), which is very likely related to the observed loss of scleractinian corals at >400 ppm CO<sub>2</sub> and to the  
326 disappearance of the more specialised associated fish fauna they support. Therefore, the observed  
327 transitions from corals/high-profile algae to low-profile/turf algae support the available models  
328 suggesting that OA will hold temperature-induced poleward expansion of coral habitats (Yara et al.  
329 2012), and this will cascade on the composition of the associated fish fauna with a decreasing pool of  
330 tropical, sub-tropical and coral reef associated species under elevated CO<sub>2</sub> conditions. In this regard,  
331 Nakamura et al. (2013) documented an increase of warm-water tropical fish along the Japan high-latitude  
332 waters. Our findings rather suggest that if ongoing OA will act as a supplementary game-changer in the  
333 near future, this could not be the case.

334 When looking at fish trophic guilds we found that, relative to ambient CO<sub>2</sub> conditions, the abundance of  
335 herbivorous fish was significantly higher at the High CO<sub>2</sub> site in June, but not in September when  
336 seasonal typhoons caused the removal of macroalgal and turf cover particularly from the high CO<sub>2</sub> area  
337 (Fig. 5c). Conversely, omnivorous species showed the opposite pattern being more abundant after the  
338 typhoon season. These results support previous evidence showing that an increased abundance of  
339 herbivore species at elevated CO<sub>2</sub> conditions can be driven by the greater biomass of primary producers  
340 associated with enhanced nutritional quality (Vizzini et al. 2017). When turf and algae are removed by  
341 wave action, the availability and type of food changes, with repercussions on trophic guild composition  
342 (i.e. less herbivorous and more omnivorous species). In addition to this, results from recent experiments  
343 suggest that by increasing their macrophyte consumption and defecation rates, herbivore population  
344 growth could further modify trophic processes in benthic systems under OA conditions, hence amplifying



345 detritus biomass production and potentially altering energy pathways and ecosystem functioning (Zarco-  
346 Perello et al. 2019).

347 By contrast, a consistent decrease in the abundance of carnivore species was observed along the CO<sub>2</sub>  
348 gradient in both the sampling seasons (Fig. 5c). Such switches in the composition of functional groups  
349 (i.e. increase of herbivore and reduction in carnivores) appears to be a common community property in  
350 elevated CO<sub>2</sub> systems and has been described by other ecosystem studies carried out in temperate natural  
351 CO<sub>2</sub> vent systems (Vizzini et al. 2017; Nagelkerken et al. 2015; Milazzo et al., 2019). In this regard, we  
352 speculate that piscivorous species may be attracted to more complex habitat characterised by higher prey  
353 abundance, while herbivores may prefer less complex areas where primary production is higher and this  
354 resource can be accessed more easily. We also found a consistent decline in the abundance of  
355 planktivorous fish and in the number of coral reef associated species from ambient to elevated CO<sub>2</sub>  
356 conditions (Fig. 5c and 5d). Again, this seems to be related to the loss of coral species at the more elevated  
357 CO<sub>2</sub> sites, which provide structural complexity and represent the preferred habitat for many coral fish  
358 species and their prey (Coker et al. 2014; Smith et al. 2016). Our findings add to previous evidence from  
359 ocean warming studies showing fish diversity declines following extensive coral loss due to bleaching  
360 events (e.g. Pratchett et al. 2011). In this regard, we suggest that OA may act as an additional bottleneck  
361 for fish community resilience under predicted ocean change scenarios.

362 Natural analogues have many benefits for furthering knowledge about the responses of fish to OA  
363 conditions, but they are not perfect analogues for acidifying oceans. The seep areas are localised and so  
364 fish can move in and out, complicating dose-response assessments (Hurlbert 1984; Munday et al. 2014;  
365 Cornwall and Hurd 2015). Moreover, carbonate chemistry can be highly variable at CO<sub>2</sub> seeps (Cattano  
366 et al. 2016; Cattano et al. 2017; Agostini et al., 2018) and toxic areas around volcanic vents need to be  
367 avoided in studies of the effects of ocean acidification (Vizzini et al. 2013). Nonetheless, the seeps allow  
368 studies of the ecosystem effects of OA and over time, using multiple CO<sub>2</sub> seep locations, a pattern is

369 beginning to emerge about the effects of OA on fish communities. An additional caveat with volcanic  
370 CO<sub>2</sub> seeps is that ocean acidification is occurring concurrently with warming, thus preventing their use  
371 as analogues for future oceans (Rastrick et al. 2018). This issue can be addressed by assessing ecosystem  
372 responses at CO<sub>2</sub> seep in different biogeographic regions, or by manipulating temperature along CO<sub>2</sub>  
373 gradients (Alessi et al. 2019), or by exploiting marine heat waves to assess the combined stress of rising  
374 temperature and elevated CO<sub>2</sub> (Rodolfo-Metalpa et al. 2011). In this context, recent evidence combining  
375 laboratory, mesocosm and meta-analyses of CO<sub>2</sub> seep research suggests that OA affects the outcome of  
376 ocean warming (Goldenberg et al 2018).

377 In summary, our data provide direct evidence that shifts in biogenic habitat and complexity reduction  
378 underpin a major loss (35-57%) of marine fish diversity as levels of carbon dioxide in seawater increase  
379 in coastal waters off Japan. Ocean acidification is expected to limit the poleward range expansion of  
380 coral reef-associated fish that is now occurring due to warming in Japan (Yamano et al. 2011; Agostini  
381 et al 2018; Kumagai et al. 2018) and elsewhere (Baird et al. 2012; Serrano et al. 2013; Denis et al. 2015;  
382 Tuckett et al. 2017). By enhancing the competitive strength of algae at the expense of structurally  
383 complex calcifying organisms, such as corals, our surveys indicate that ocean acidification will change  
384 fish community function with a greater abundance of herbivorous fish species but fewer carnivores and  
385 planktivores. Such changes in fish communities would cause major changes in trophic processes and  
386 energy pathways, as well as affecting fisheries. We conclude that the indirect effects of rising CO<sub>2</sub> levels  
387 on coastal ecosystems will have a profound impact on reef fish communities.

388

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390

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#### 400 **Author contributions**

401 CC and MM conceived the experiment and wrote the first draft of the manuscript. CC, SA, BPH,  
402 SW, KI, JH-S and MM carried out the sampling surveys. FQ and GT performed the statistical  
403 analyses. All authors contributed to the latest version of the manuscript.

#### 404 **Competing financial interests**

405

406 The authors declare no competing financial interests.

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409 **References**

410

- 411 Agostini S, Wada S, Kon K, Omori A, Kohtsuka H, Fujimura H et al. (2015) Geochemistry of two  
 412 shallow CO<sub>2</sub> seeps in Shikine Island (Japan) and their potential for ocean acidification research.  
 413 *Regional Studies in Marine Science*, **2**, 45–53.
- 414 Agostini S, Harvey BP, Wada S, Kon K, Milazzo M, Inaba K, Hall-Spencer JM (2018) Ocean  
 415 acidification drives community shifts towards simplified non-calcified habitats in a  
 416 subtropical–temperate transition zone. *Scientific Reports*, **8**:11354.
- 417 Alessi C, Giomi F, Furnari F, Sarà G, Chemello R, Milazzo M (2019) Ocean acidification and  
 418 elevated temperature negatively affect recruitment, oxygen consumption and calcification of the reef-  
 419 building *Dendropoma cristatum* early life stages: Evidence from a manipulative field study. *Science*  
 420 *of The Total Environment*, **693**, 133476.
- 421 Anderson M, Braak CT (2003) Permutation tests for multi-factorial analysis of variance. *Journal of*  
 422 *statistical computation and simulation*, **73**(2), 85–113.
- 423 Anderson M, Gorley RNRN, Clarke K, Anderson MJ, Gorley RN, Clarke KR, Anderson M, Gorley  
 424 R, Anderson MJ (2008) PERMANOVA+ for PRIMER. Guide to software and statistical methods.
- 425 Baird AH, Sommer B, Madin JS (2012) Pole-ward range expansion of *Acropora* spp. along the east  
 426 coast of Australia. *Coral Reefs*, **31**(4), 1063–1063.
- 427 Barkley HC, Cohen AL, Golbuu Y, Starczak VR, De Carlo TM, Shamberger KEF (2015) Changes  
 428 in coral reef communities across a natural gradient in seawater pH. *Science Advances*, **1**, e1500328.
- 429 Cattano C, Giomi F, Milazzo M (2016) Effects of ocean acidification on embryonic respiration and  
 430 development of a temperate wrasse living along a natural CO<sub>2</sub> gradient. *Conservation physiology*,  
 431 **4**(1), cov073.
- 432 Cattano C, Calò A, Di Franco A, Firmamento R, Quattrocchi F, Sdiri K et al. (2017) Ocean  
 433 acidification does not impair predator recognition but increases juvenile growth in a temperate wrasse  
 434 off CO<sub>2</sub> seeps. *Marine environmental research*, **132**, 33–40.
- 435 Cattano C, Claudet J, Domenici P, Milazzo M (2018) Living in a high CO<sub>2</sub> world: A global meta-  
 436 analysis shows multiple trait-mediated fish responses to ocean acidification. *Ecological monographs*,  
 437 **88**(3), 320–335.
- 438 Clarke KR, Gorley RN (2006) User manual/tutorial. Primer-E Ltd., Plymouth, 93.
- 439 Coker DJ, Wilson SK, Pratchett MS (2014) Importance of live coral habitat for reef fishes. *Reviews*  
 440 *in Fish Biology and Fisheries*, **24**(1), 89–126.
- 441 Connell SD, Doubleday ZA, Foster NR, Hamlyn SB, Harley CD, Helmuth B et al (2018) The duality  
 442 of ocean acidification as a resource and a stressor. *Ecology*, **99**(5), 1005–1010.
- 443 Cornwall CE, Hurd CL (2015) Experimental design in ocean acidification research: problems and  
 444 solutions. *ICES Journal of Marine Science*, **73**(3), 572–581.

445 De'ath G, Fabricius KE, Sweatman H, Puotinen M (2012) The 27-year decline of coral cover on the  
446 Great Barrier Reef and its causes. *Proceedings of the National Academy of Sciences U.S.A.*, **109**,  
447 17995–17999.

448 Denis V, De Palmas S, Benzoni F, Chen CA (2015). Extension of the known distribution and depth  
449 range of the scleractinian coral *Psammocora stellata*: first record from a Taiwanese mesophotic reef.  
450 *Marine Biodiversity*, **45**(4), 619–620.

451 Di Franco A, Calò A, Sdiri K, Cattano C, Milazzo M, Guidetti P (2019) Ocean acidification affects  
452 somatic and otolith growth relationship in fish: evidence from an in situ study. *Biology letters*, **15**(2),  
453 20180662.

454 Dickson AG (1990) Thermodynamics of the dissociation of boric acid in synthetic seawater from  
455 273.15 to 318.15 K. *Deep Sea Research Part A. Oceanographic Research Papers*, **37**(5), 755–766.

456 Dickson AG, Millero FJ (1987) A comparison of the equilibrium constants for the dissociation of  
457 carbonic acid in seawater media. *Deep Sea Research Part A. Oceanographic Research Papers*,  
458 **34**(10), 1733–1743.

459 Enochs IC, Manzello DP, Donham EM, Kolodziej G, Okano R, Johnston L et al. (2015) Shift from  
460 coral to macroalgae dominance on a volcanically acidified reef. *Nature Climate Change*, **5**, 1083–  
461 1088.

462 Falkenberg LJ, Russell BD, Connell SD (2013) Contrasting resource limitations of marine primary  
463 producers: implications for competitive interactions under enriched CO<sub>2</sub> and nutrient regimes.  
464 *Oecologia* **172**, 575–583 (2013).

465 Fabricius KE, Langdon C, Uthicke S, Humphrey C, Noonan S, De'ath G et al. (2011) Losers and  
466 winners in coral reefs acclimatized to elevated carbon dioxide concentrations. *Nature Climate*  
467 *Change*, **1**, 165–169

468 Fabricius KE, De'ath, G, Noonan S, Uthicke S (2014) Ecological effects of ocean acidification and  
469 habitat complexity on reef-associated macroinvertebrate communities. *Proceeding of the Royal*  
470 *Society B: Biological Sciences*, **281**, 20132479

471 Filbee-Dexter K, Wernberg T (2018). Rise of turfs: A new battlefield for globally declining kelp  
472 forests. *BioScience*, **68**(2), 64–76.

473 Gaylord B, Kroeker KJ, Sunday JM, Anderson KM, Barry JP, Brown NE et al. (2015) Ocean  
474 acidification through the lens of ecological theory. *Ecology*, **96**(1), 3–15

475 Goldenberg SU, Nagelkerken I, Marangon E, Bonnet A, Ferreira CM, Connell SD (2018) Ecological  
476 complexity buffers the impacts of future climate on marine consumers. *Nature Climate Change*, **8**,  
477 229–233. Hall-Spencer JM, Rodolfo-Metalpa R, Martin S, Ransome E, Fine M, Turner SM et al.  
478 (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature*, **454**,  
479 96–99.

480 Hall-Spencer JM, Harvey BP (2019) Ocean acidification impacts on coastal ecosystem services due  
481 to habitat degradation. *Emerging Topics in Life Sciences*, **3**(2), 197–206.

482 Harley CD, Anderson KM, Demes KW, Jorve JP, Kordas RL, Coyle TA, Graham MH (2012) Effects  
483 of climate change on global seaweed communities. *Journal of Phycology*, **48**(5), 1064–1078.

484 Harmelin-Vivien ML, Harmelin JG, Chauvet C, Duval C, Galzin R, Lejeune P et al. (1985)  
485 Evaluation visuelle des peuplements et populations de poissons méthodes et problèmes.

486 Harvey BP, Gwynn-Jones D, Moore PJ (2013) Meta-analysis reveals complex marine biological  
487 responses to the interactive effects of ocean acidification and warming. *Ecology and evolution*, **3**(4),  
488 1016–1030.

489 Harvey BP, Agostini S, Kon K, Wada S, Hall-Spencer JM (2019) Diatoms Dominate and Alter  
490 Marine Food-Webs When CO<sub>2</sub> Rises. *Diversity*, **11**(12), 242.

491 Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's coral reefs.  
492 *Marine and freshwater research*, **50**(8), 839-866.

493 van Hooidonk R, Maynard JA, Manzello D, Planes S (2014). Opposite latitudinal gradients in  
494 projected ocean acidification and bleaching impacts on coral reefs. *Global Change Biology*, **20**, 103–  
495 112.

496 Hughes TP (1994) Catastrophes, phase-shifts, and large scale degradation of a Caribbean coral reef.  
497 *Science*, **265**, 1547–1551.

498 Hurlbert SH (1984) Pseudoreplication and the design of ecological field experiments. *Ecological*  
499 *monographs*, **54**(2), 187–211.

500 Inoue S, Kayanne H, Yamamoto S, Kurihara H (2013) Spatial community shift from hard to soft  
501 corals in acidified water. *Nature Climate Change*, **3**, 683–687.

502 Jackson JBC, Kirby MX, Berger WH et al. (2001) Historical Overfishing and the Recent Collapse of  
503 Coastal Ecosystems. *Science*, **293**, 629–637.

504 Kroeker KJ, Kordas RL, Crim R, Hendriks IE, Ramajo L, Singh GS, Duarte CM, Gattuso JP (2013)  
505 Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with  
506 warming. *Global Change Biology*, **19**(6), 1884–1896.

507 Kumagai NH, Molinos JG, Yamano H, Takao S, Fujii M, Yamanaka Y (2018) Ocean currents and  
508 herbivory drive macroalgae-to-coral community shift under climate warming. *Proceedings of the*  
509 *National Academy of Sciences*, **115**(36), 8990–8995.

510 Legendre P, Anderson MJ (1999) Distance-based redundancy analysis: testing multispecies  
511 responses in multifactorial ecological experiments. *Ecological monographs*, **69**(1), 1–24.

512 McArdle BH, Anderson MJ (2001) Fitting multivariate models to community data: a comment on  
513 distance-based redundancy analysis. *Ecology*, **82**(1), 290–297.

514 McCulloch M, Fallon S, Wyndham T, Hendy E, Lough J, Barnes D (2003). Coral record of increased  
515 sediment flux to the inner Great Barrier Reef since European settlement. *Nature*, **421**(6924), 727–  
516 730.

517 Mehrbach C (1973) Measurement of the apparent dissociation constants of carbonic acid in seawater  
518 at atmospheric pressure.

519 Midorikawa T, Nemoto K, Kamiya H, Ishii M, Inoue H Y (2005) Persistently strong oceanic CO<sub>2</sub>  
520 sink in the western subtropical North Pacific. *Geophysical Research Letters*, **32**, L05612.

521 Milazzo M, Cattano C, Alonzo SH, Foggo A, Gristina M, Rodolfo-Metalpa R et al. (2016) Ocean  
522 acidification affects fish spawning but not paternity at CO<sub>2</sub> seeps. *Proceedings of the Royal Society*  
523 *B: Biological Sciences*, **283**(1835), 20161021.

524 Milazzo M, Alessi C, Quattrocchi F, Chemello R, D'Agostaro R, Gil J et al (2019) Biogenic habitat  
525 shifts under long-term ocean acidification show nonlinear community responses and unbalanced  
526 functions of associated invertebrates. *Science of The Total Environment*, **667**, 41–48.

527 Mouillot D, Graham NA, Villéger S, Mason NW, Bellwood DR (2013) A functional approach reveals  
528 community responses to disturbances. *Trends in ecology & evolution*, **28**(3), 167–177.

529 Muir PR, Wallace CC, Done T, Aguirre JD (2015) Limited scope for latitudinal extension of reef  
530 corals. *Science*, **348**, 1135–1138.

531 Munday PL, Cheal AJ, Dixson DL, Rummer JL, Fabricius KE (2014) Behavioural impairment in  
532 reef fishes caused by ocean acidification at CO<sub>2</sub> seeps. *Nature Climate Change*, **4**, 1–6.

533 Nagelkerken I, Russell BD, Gillanders BM, Connell SD (2015) Ocean acidification alters fish  
534 populations indirectly through habitat modification. *Nature Climate Change*, **6**, 89 – 95.

535 Nagelkerken I, Goldenberg SU, Ferreira CM, Russell BD, Connell SD (2017) Species interactions  
536 drive fish biodiversity loss in a high-CO<sub>2</sub> world. *Current Biology*, **27**(14), 2177–2184.

537 Nakamura Y, Feary DA, Kanda M, Yamaoka K (2013) Tropical fishes dominate temperate reef fish  
538 communities within western Japan. *PLoS ONE*, **8**, e81107.

539 O'Brien JM Scheibling RE (2018) Turf wars: competition between foundation and turf-forming  
540 species on temperate and tropical reefs and its role in regime shifts. *Marine Ecology Progress Series*,  
541 **590**, 1–17.

542 Pierrot D, Lewis E, Wallace DWR (2006) MS Excel Program Developed for CO<sub>2</sub> System  
543 Calculations. ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center, Oak Ridge  
544 National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee. doi:  
545 10.3334/CDIAC/otg.CO2SYS\_XLS\_CDIAC105a.

546 Pratchett MS, Hoey AS, Wilson SK, Messmer V, Graham NA (2011) Changes in biodiversity and  
547 functioning of reef fish assemblages following coral bleaching and coral loss. *Diversity*, **3**(3), 424-  
548 452.

549 R Core Team 2018 R: A Language and Environment for Statistical Computing. R Foundation for  
550 Statistical Computing, Vienna, Austria.

551 Rastrick SS, Graham H, Azetsu-Scott K, Calosi P, Chierici M, Fransson A et al (2018) Using natural  
552 analogues to investigate the effects of climate change and ocean acidification on Northern  
553 ecosystems. *ICES Journal of Marine Science*, **75**(7), 2299–2311.

554 Rodolfo-Metalpa R, Houlbrèque F, Tambutté E, Boisson F, Baggini C, Patti FP, Jeffree R et al.  
555 (2011) Coral and mollusc resistance to ocean acidification adversely affected by warming. *Nature*  
556 *Climate Change*, **1**, 308–312.

557 Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis.  
558 *Nature methods*, **9**(7), 671.

- 559 Serrano E, Coma R, Ribes M, Weitzmann B, García M, Ballesteros E (2013) Rapid northward spread  
560 of a zooxanthellate coral enhanced by artificial structures and sea warming in the western  
561 Mediterranean. *PLoS One*, **8**(1), e52739.
- 562 Smith JN, De'ath G, Richter C, Cornils A, Hall-Spencer JM, Fabricius KE (2016) Ocean acidification  
563 reduces demersal zooplankton that reside in tropical coral reefs. *Nature Climate Change*, **6**, 1124-  
564 1129
- 565 Stewart-Oaten A, Murdoch WW, Parker KR (1986) Environmental impact assessment:  
566 "pseudoreplication" in time? *Ecology*, **67**, 929–940
- 567 Suggett DJ, Hall-Spencer JM, Rodolfo-Metalpa R, Boatman TG, Payton R, Tye Pettay D et al. (2012)  
568 Sea anemones may thrive in a high CO<sub>2</sub> world. *Global Chang Biology*, **18**, 3015–3025
- 569 Sunday JM, Fabricius KE, Kroeker KJ, Anderson KM, Brown NE, Barry JP et al (2017) Ocean  
570 acidification can mediate biodiversity shifts by changing biogenic habitat. *Nature Climate Change*,  
571 **7**(1), 81.
- 572 Tuckett CA, de Bettignies T, Fromont J, Wernberg T (2017) Expansion of corals on temperate reefs:  
573 direct and indirect effects of marine heatwaves. *Coral Reefs*, **36**(3), 947–956.
- 574 Uppström L R (1974) The boron/chlorinity ratio of deep-sea water from the Pacific Ocean. *Deep-Sea*  
575 *Research and Oceanographic Abstracts*, **21**, 161–162.
- 576 Vergés A, Steinberg PD, Hay ME, Poore AGB, Campbell AH, Ballesteros E et al. (2014) The  
577 tropicalization of temperate marine ecosystems: climate-mediated changes in herbivory and  
578 community phase shifts. *Proceeding of the Royal Society B: Biological Sciences*, **281**, 20140846
- 579 Vergés A, Doropoulos C, Malcolm HA, Skye M, Garcia-Pizá M, Marzinelli EM et al. (2016) Long-  
580 term empirical evidence of ocean warming leading to tropicalization of fish communities, increased  
581 herbivory, and loss of kelp. *Proceedings of the National Academy of Sciences U.S.A.*, 201610725.
- 582 Veron JEN, Minchin PR (1992) Correlations between sea surface temperature, circulation patterns  
583 and the distribution of hermatypic corals of Japan. *Continental Shelf Research*, **12**(7-8), 835–857.
- 584 Viechtbauer W (2010) Conducting meta-analyses in R with the metafor package. *Journal of*  
585 *Statistical Software*, **36**(3), 1–48.
- 586 Vizzini S, Di Leonardo R, Costa V, Tramati CD, Luzzu F, Mazzola A (2013) Trace element bias in  
587 the use of CO<sub>2</sub> vents as analogues for low pH environments: Implications for contamination levels in  
588 acidified oceans. *Estuarine, Coastal and Shelf Science*, **134**, 19-30.
- 589 Vizzini S, Martínez-Crego B, Andolina C, Massa-Gallucci A, Connell SD, Gambi MC (2017) Ocean  
590 acidification as a driver of community simplification via the collapse of higher-order and rise of  
591 lower-order consumers. *Scientific reports*, **7**(1), 4018.
- 592 Wilson SK, Burgess SC, Cheal AJ, Emslie M, Fisher R, Miller I et al (2008a) Habitat utilization by  
593 coral reef fish: implications for specialists vs. generalists in a changing environment. *Journal of*  
594 *Animal Ecology*, **77**(2), 220–228.



595 Wilson SK, Fisher R, Pratchett MS, Graham NAJ, Dulvy NK, Turner RA et al (2008b) Exploitation  
596 and habitat degradation as agents of change within coral reef fish communities. *Global Change*  
597 *Biology*, **14**, 2796–2809

598 Wittmann AC, Pörtner HO (2013) Sensitivities of extant animal taxa to ocean acidification. *Nature*  
599 *Climate Change*, **3**(11), 995.

600 Wood SN (2006) Low-rank scale-invariant tensor product smooths for generalized additive mixed  
601 models. *Biometrics*, **62**(4), 1025–1036.

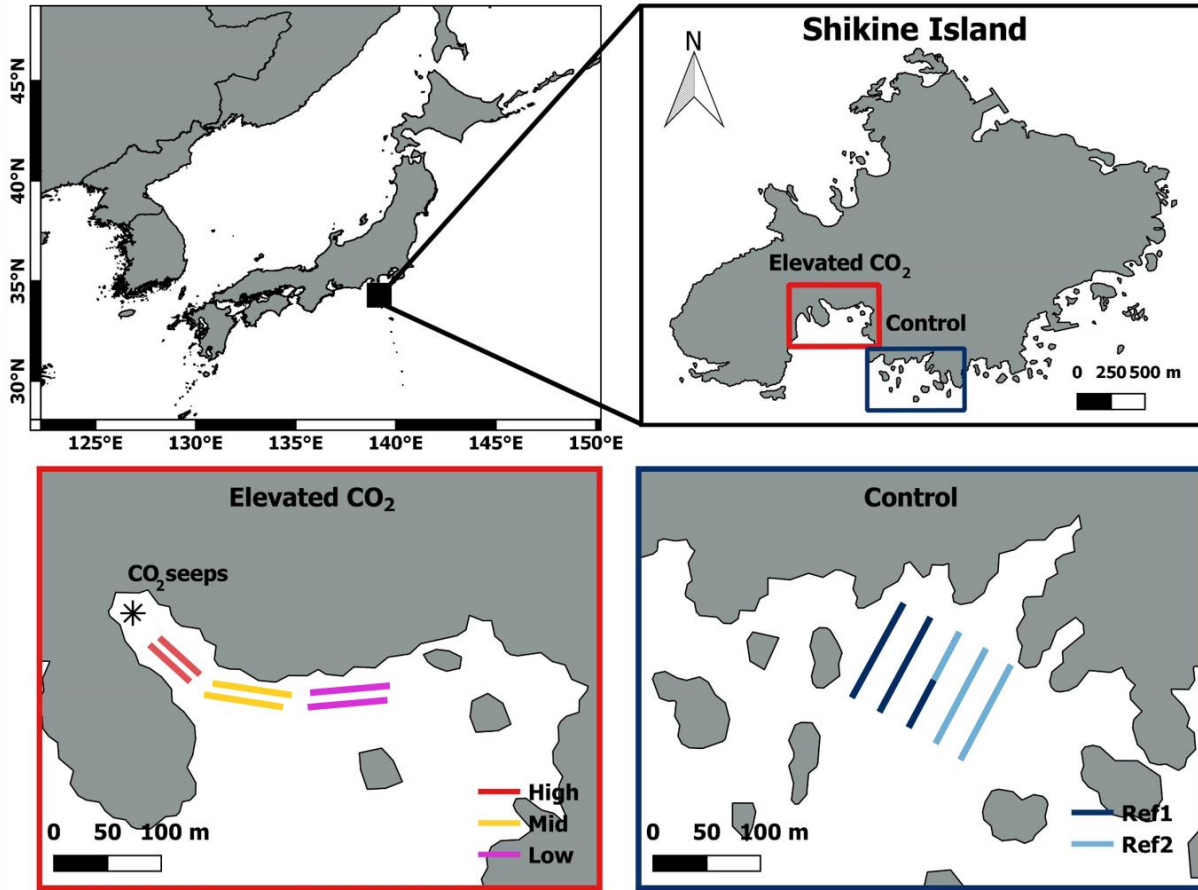
602 Wood SN (2011) Fast stable restricted maximum likelihood and marginal likelihood estimation of  
603 semiparametric generalized linear models. *Journal of the Royal Statistical Society: Series B*  
604 (*Statistical Methodology*), **73**(1), 3–36.

605 Yamano H, Sugihara K, Nomura K (2011) Rapid poleward range expansion of tropical reef corals in  
606 response to rising sea surface temperatures. *Geophysical Research Letters*, **38**(4).

607 Yara, Y, Vogt M, Fujii M, Yamano H, Hauri C, Steinacher M et al. (2012) Ocean acidification limits  
608 temperature-induced poleward expansion of coral habitats around Japan. *Biogeosciences*, **9**, 4955–  
609 4968.

610 Zarco-Perello S, Langlois TJ, Holmes T, Vanderklift MA, Wernberg T (2019) Overwintering tropical  
611 herbivores accelerate detritus production on temperate reefs. *Proceedings of the Royal Society B*,  
612 **286**(1915), 20192046.

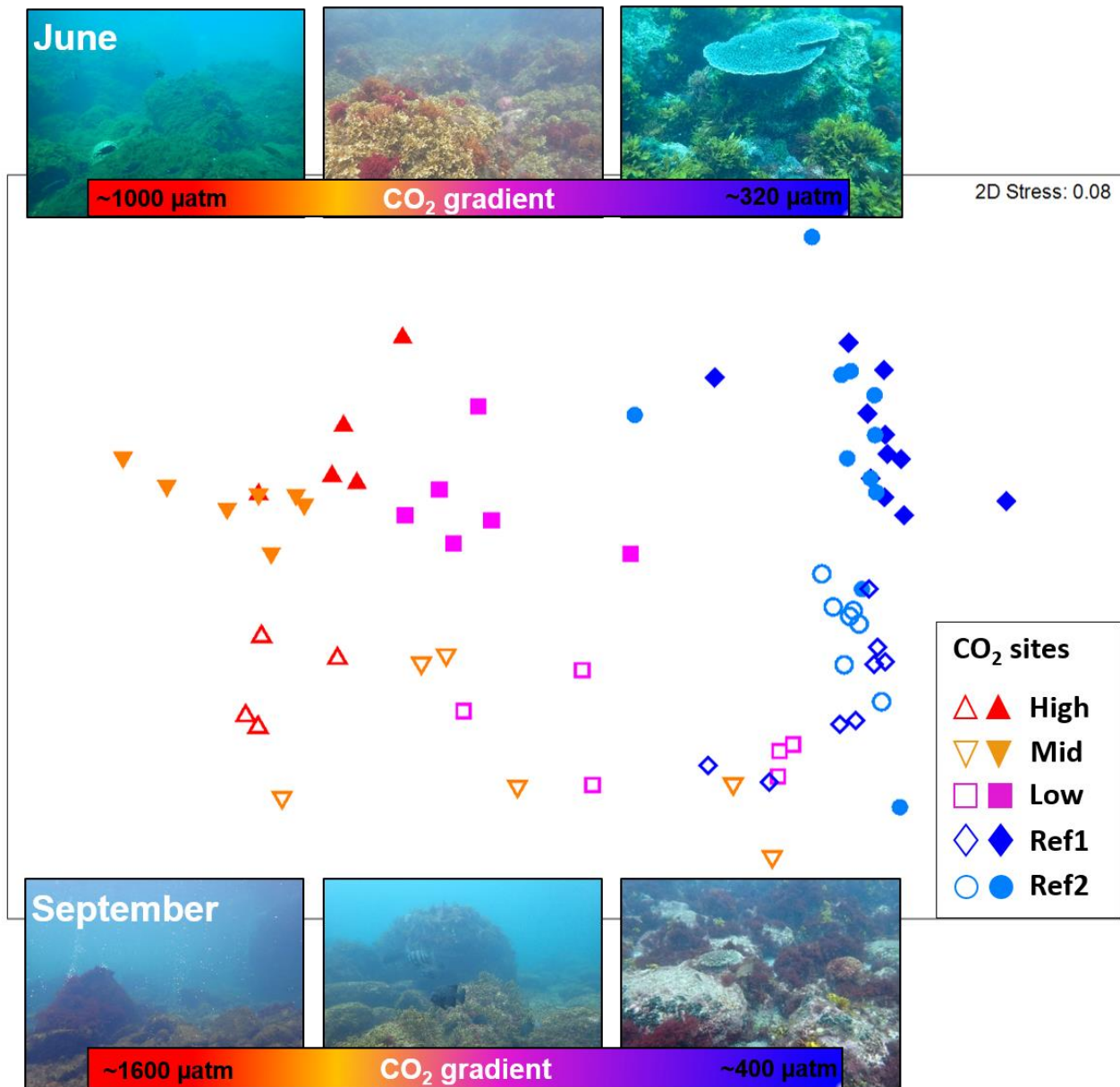
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615 *Figure 1 – Map of the study showing Shikine Island, the two CO<sub>2</sub> locations (Elevated and Control) and*  
 616 *the five CO<sub>2</sub> sites (High, Mid, Low, Ref1 and Ref2) where the benthic and fish 25m-transects were*  
 617 *positioned.*

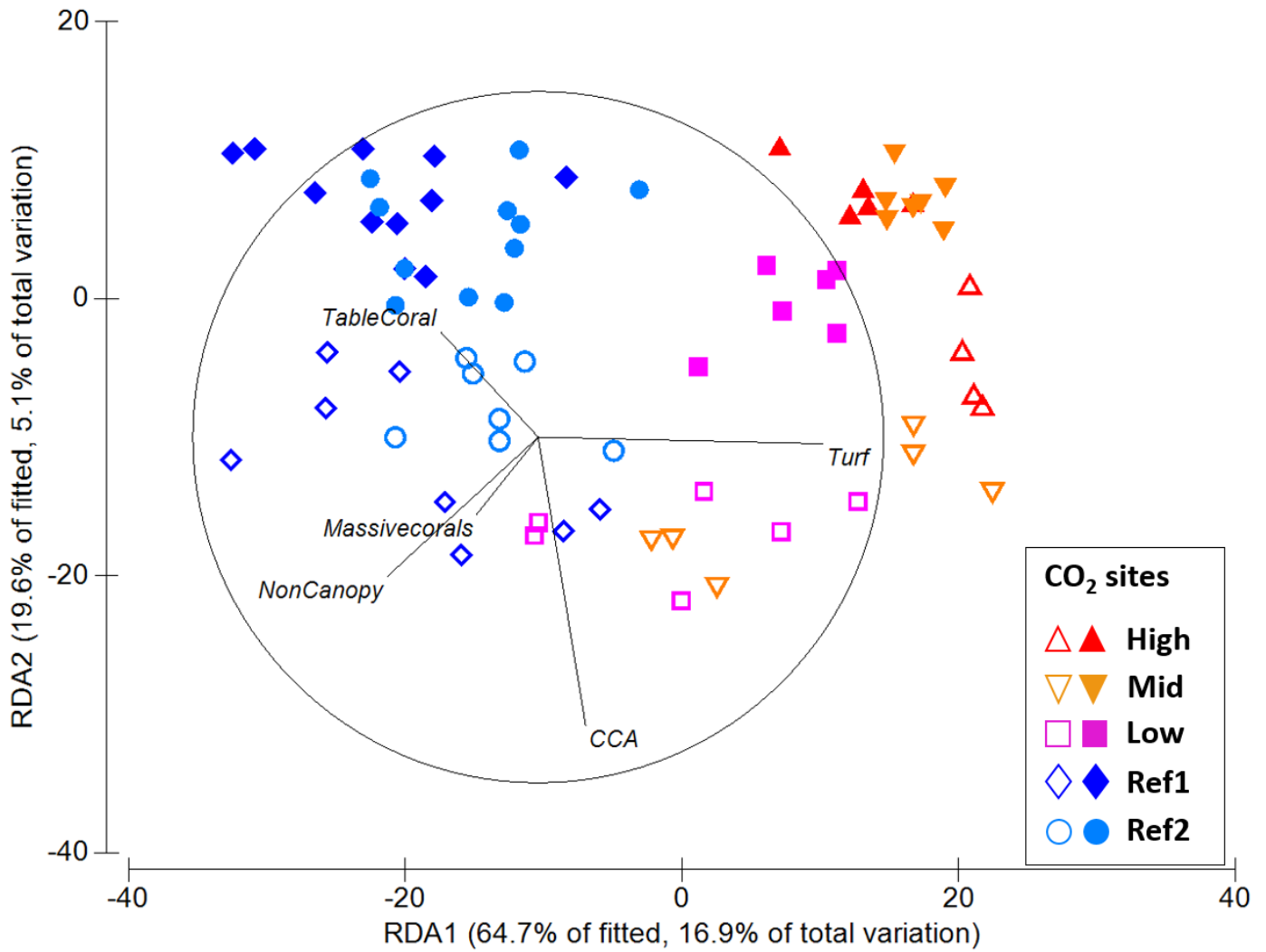
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620 *Figure 2 – Non-metric multidimensional scaling (nMDS) on benthic habitats at the five CO<sub>2</sub> sampling*  
 621 *sites (High, Mid, Low, Ref1 and Ref2) in June (filled symbols) and September (empty symbols).*  
 622 *Photomosaics of benthic community shifts observed in June and September along the CO<sub>2</sub> gradient are*  
 623 *also reported. The figure shows a clear community shifts in both seasons from zooxanthellate*  
 624 *scleractinian corals and canopy-forming macroalgae at Control CO<sub>2</sub> location (Ref1 and Ref2) to*  
 625 *macroalgae at the Low and Mid CO<sub>2</sub> sites and turf algae at the High CO<sub>2</sub> site.*

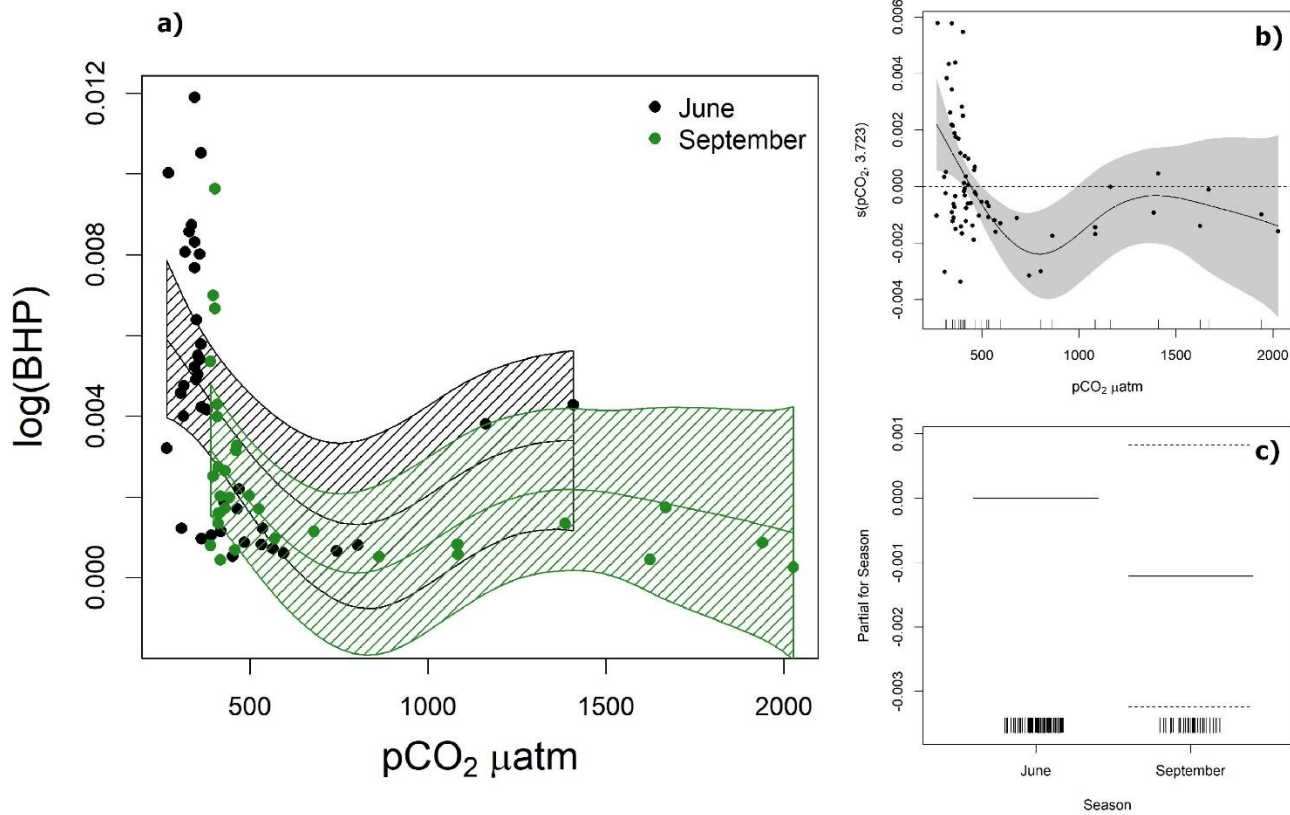
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628 *Figure 3 – dbRDA ordination of fish community data from five sampling sites (High, Mid, Low, Ref1 and*  
 629 *Ref2) constrained by five environmental variables (CCA, Massive corals, table corals, Turf and non*  
 630 *canopy algae) identified by DISTLM as significant in explaining 84,3% of fitted and 22% of total*  
 631 *variation. Vectors represent strength of variables in the model. Their length in relation to the circle*  
 632 *radius (radius = 1.0) and their direction indicate the strength and the sign, respectively, of the*  
 633 *relationship between the variable and the axes. Filled and empty symbols represent data from June and*  
 634 *September, respectively.*

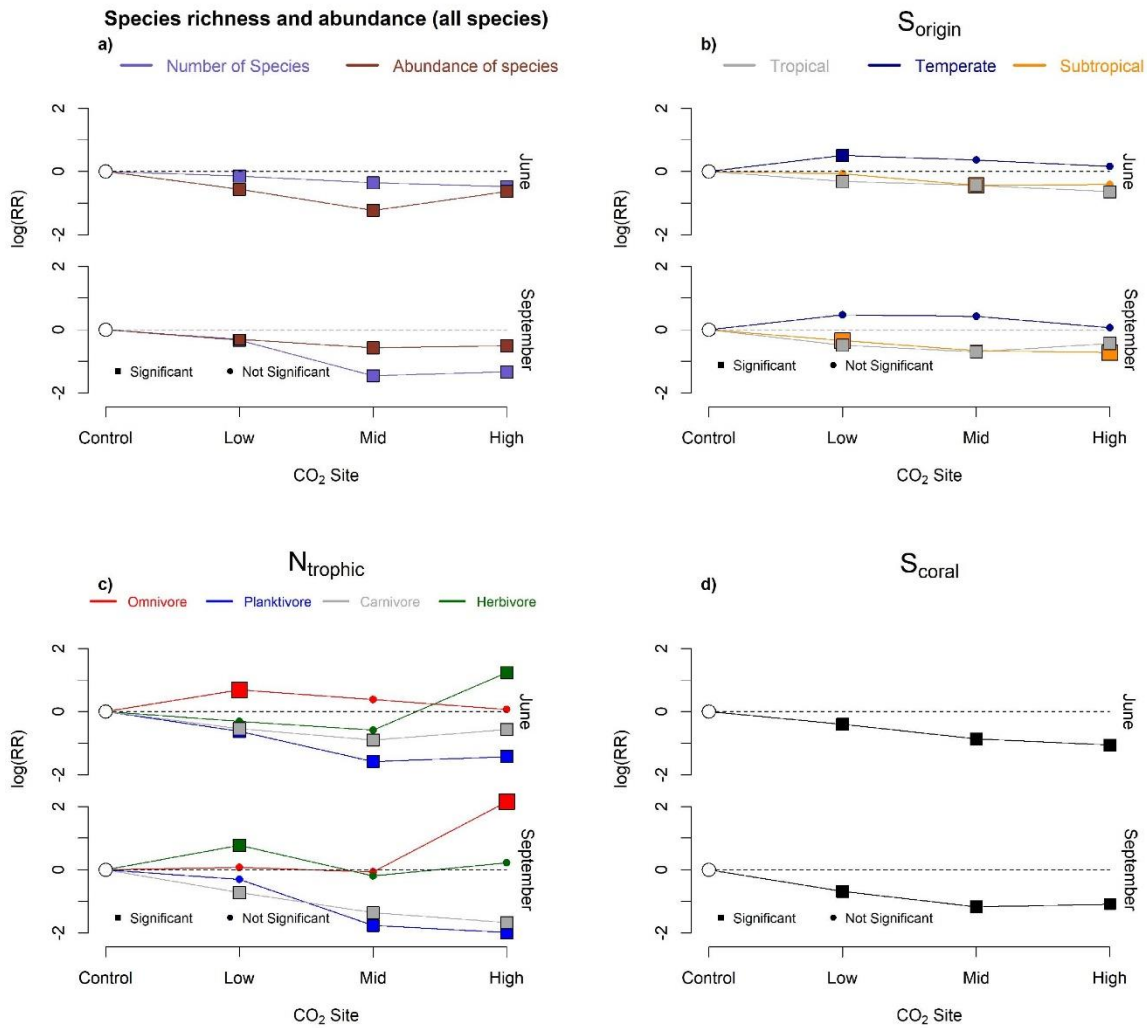
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637 *Figure 4 –AMM (Additive Mixed Model) showing the relationship between  $p\text{CO}_2$  values and habitat*  
 638 *complexity (BHP). a) Fitted values ( $\pm 95\%$  confidence intervals) of  $p\text{CO}_2$  values and habitat complexity*  
 639 *(log-transformed BHP index) for June (black dots) and September (green dots); b) model residuals*  
 640 *(black dots) and estimated smoothing curve (solid line with  $\pm 95\%$  confidence interval) showing the  $p\text{CO}_2$*   
 641 *effects on BHP. The effect is significant (i.e. positive or negative) when the CI (grey area) does not*  
 642 *overlap the zero (dashed line); c) partial effect (solid lines) of the factor Season on the BHP index*  
 643 *(dashed lines indicate the  $\pm 95\%$  confidence interval for September). The vertical lines reported on the*  
 644 *x-axis of panels b and c represent the observations from the different  $p\text{CO}_2$  values and from the two*  
 645 *seasons, respectively.*

646



647

648 *Figure 5 - Meta-analysis-derived approach to assess overall and trait-based changes of fish community*  
 649 *at the different elevated CO<sub>2</sub> sites, both in June and September. Each point represents the log response*  
 650 *ratio (LnRR) of the average value for the different fish community variables recorded at Low, Mid and*  
 651 *High CO<sub>2</sub> sites relative to the ambient CO<sub>2</sub> condition (i.e. Control location: Ref1 and Ref2 together),*  
 652 *indicating the sign and the strength of change. a) Number and abundance of fish species (all the censused*  
 653 *species); b) S<sub>origin</sub>: number of species by their geographic distribution (Tropical, Sub-tropical or*  
 654 *Temperate); c) N<sub>trophic</sub>: abundance of species by their trophic guild (Omnivore, Planktivore, Carnivore*  
 655 *or Herbivore); d) S<sub>coral</sub>: number of coral-reef associated fish species. Squares indicate significant effects*  
 656 *per p < 0.05. Small circles indicate no significant differences.*

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