Faculty of Science and Engineering

School of Biological and Marine Sciences

2020-07

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http://hdl.handle.net/10026.1/15586

10.1016/j.scitotenv.2020.138501 Science of The Total Environment Elsevier BV

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- 2 This is the author's accepted manuscript. The final published version of this work (the version of
- 3 record) is published by Elsevier in *Science of the Total Environment*. The manuscript was made
- 4 available online on the 5 April 2020 at
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# <sup>8</sup> Changes in fish communities due to benthic habitat shifts under <sup>9</sup> ocean acidification conditions

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#### 22 Abstract

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

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

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#### 36 **1. Introduction**

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

decreased seawater pH due to OA may impair calcification and accelerate dissolution for many calcifying habitat-formers, while rising  $pCO_2$  levels may enhance the primary production and carbon fixation rates of non-calcifying autotrophs (Falkenberg et al. 2013; Harvey et al. 2013; Kroeker et al. 2013; Wittmann and Pörtner 2013). As a result, there will be losers and winners under OA conditions, with effects on ecosystems documented along gradients in seawater pH at  $CO_2$  seeps around the world (e.g., Hall-Spencer et al. 2008; Fabricius et al. 2011) including reductions in habitat complexity, shifts in competitive interactions and changes in species dominance.

While CO<sub>2</sub> seeps are not perfect analogues for ocean acidification (e.g., Fabricius et al. 2017), they 56 nevertheless comprise one of the very few field-based tools available to assess OA effects on ecosystems 57 58 and communities-(Hall-Spencer and Harvey 2019). To date, our knowledge about the ecosystem effects of OA is advancing rapidly with increasing evidence from temperate (Hall-Spencer et al. 2008; 59 Nagelkerken et al. 2015; Milazzo et al. 2019), subtropical (Agostini et al. 2018) and tropical (Fabricius 60 61 et al. 2011, 2014; Inoue et al. 2013; Enochs et al. 2015) CO<sub>2</sub> seeps. The responses of biogenic habitats to OA differ regionally. Shifts from diverse to depauperate scleractinian species assemblages and from 62 hard to soft coral communities have been observed in Papua New Guinea (Fabricius et al. 2011), Palau 63 (Barkley et al. 2015) and in Southern Japan (Inoue et al. 2013). As carbon dioxide levels increase, there 64 is a shift in community dominance from corals to seaweeds in the Northern Mariana Islands (Enochs et 65 66 al. 2015) and at a subtropical-temperate transition zone in Japan (Agostini et al. 2018). How such OAinduced habitat shifts affect fish requires further study as most work at CO<sub>2</sub> seeps to date at has focused 67 68 on bacteria, algae and invertebrates (Hall-Spencer et al. 2008; Fabricius et al. 2014; Sunday et al. 2017; 69 Milazzo et al. 2019).

Very few attempts have been made to estimate the effects of OA-induced habitat simplification on fish
communities (Munday et al. 2014; Nagelkerken et al. 2015, 2017). A study at three CO<sub>2</sub> seeps in Papua
New Guinea reported reduced coral reef complexity at elevated CO<sub>2</sub> respect to control sites. However,

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

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

Here, we evaluate the temporal consistency of changes in fish communities in response to biogenic 85 habitat shifts off CO<sub>2</sub> seeps located in the NW Pacific (Japan), in a region that has naturally low levels 86 87 of  $pCO_2$ , high carbonate saturation levels and elevated local seawater temperatures (Midorikawa et al. 2005). These conditions allow the coexistence of both canopy-forming macroalgae and scleractinian 88 coral communities at ambient CO<sub>2</sub> conditions, while a transition to low-relief algal turf habitats occurs 89 90 at elevated  $CO_2$  levels (Agostini et al. 2018; Harvey et al. 2019). To assess how fish community properties changed spatially along the  $pCO_2$  gradient, we coupled fish and benthic community 91 assessments (habitat complexity, canopy height, and % cover), along a CO<sub>2</sub> gradient and at control sites 92 93 off Shikine Island (Eastern Japan). The study was carried out over two time periods (June and September) to investigate whether the effects of OA-mediated habitat shifts on fish community composition and 94 structure are temporally consistent, specifically when acute seasonal typhoons (usually from July to 95 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.

We expected that fish communities would change in composition and structure as a result of decreasing 100 101 habitat complexity along a spatial CO<sub>2</sub> gradient and that these changes would be consistent over time. Since it has been suggested that OA may decrease overall habitat complexity (e.g. from complex corals 102 103 and canopy-forming algae to low-profile algae and turfs; Sunday et al. 2017), we expected that fish community species richness would decrease with increasing levels of CO<sub>2</sub>. As CO<sub>2</sub> enrichment acts both 104 105 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 herbivorous fish would increase in elevated CO<sub>2</sub> conditions. 107

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- **2. Materials and Methods**
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#### 111 2.1 Study sites and carbonate chemistry

Shikine is a volcanic island east of the Izu peninsula in Japan (34° 19' 9" N, 139° 12' 18" E) with many 113 CO<sub>2</sub> seeps in shallow waters. Based on previous geochemical investigations (Agostini et al. 2015), our 114 survey locations were selected to avoid potentially confounding geochemical factors (e.g. high sulfides, 115 116 negative redox potential, altered total alkalinity and elevated temperature). One location (Elevated-CO<sub>2</sub>) was within Mikawa Bay and a second location (Control) characterised by ambient CO<sub>2</sub> conditions was 117 118 positioned in an adjacent bay with similar depths (3-12 m), and exposure to wind and currents (Fig. 1). To document spatial variation in the carbonate chemistry, a WQC24 multi-parameter logger (DKK-TOA 119 Corporation, Tokyo, Japan) and a HydroC® CO<sub>2</sub> II sensor (Contros System & Solutions GmbH, 120 121 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 pH (NBS scale), temperature (T, °C), salinity, and depth (m) were recorded for 5 minutes with the DKK-123 TOA, whilst the HydroC<sup>®</sup> CO<sub>2</sub> II sensor recorded measures of pCO<sub>2</sub> (µatm) every 5 seconds. The CO<sub>2</sub> 124 sensor detects dissolved CO<sub>2</sub> molecules that diffuse through a thin film composite membrane into an 125 126 internal gas circuit containing a detector chamber where the  $pCO_2$  is determined by means of an IR absorption spectrometer. Both loggers were positioned at 1 meter from the sea-bottom at an average 127 depth range of 3-12 meters, and were attached to a floating buoy equipped with a GPS (eTrex30x, 128 Garmin) to record the exact position of each measurement. Total alkalinity (TA) was measured from 129 seawater samples collected underwater at each location (N = 24 in June; N = 25 in September). Water 130 samples were immediately filtered at 0.45 µm using disposable cellulose acetate filters (Dismic, 131 Advantech, Japan) and stored at room temperature in the dark (for no more than one week) until 132 measurement. TA was measured by titration (TiTouch i915, Metrohm) with HCl at 0.1 mol 1<sup>-1</sup>, and 133 calculated from the Gran function between pH 4.2 and 3.0. The titrations were cross-validated using a 134 working standard (SD:  $\pm$  9 µmol kg<sup>-1</sup>) and against certified reference material purchased from the A.G. 135 136 Dickson laboratory (Batch 152). The CO<sub>2</sub>SYS software (Pierrot et al. 2006) was used to calculate  $pCO_2$ (Table 1) from T, pH, salinity and TA values, and to control *in situ* continuous measurements of pCO<sub>2</sub> 137 recorded by the HydroC<sup>®</sup> CO<sub>2</sub> II sensor. The disassociation constants from Mehrbach (1973), as adjusted 138 by Dickson and Millero (1987), HSO<sub>4</sub> using Dickson (1990), and total borate concentrations from 139 Uppström (1974) were used for carbonate chemistry calculations (Table 1). The HydroC<sup>®</sup> CO<sub>2</sub> II sensor 140 was not employed in the September survey due to logistic constrains. 141

The carbonate chemistry measurements along the nine 100-m transects were used to identify five sampling CO<sub>2</sub> sites in the rocky subtidal zone between 3 and 12 m depth in Mikawa bay and the Control 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

148Table 1 - Seawater chemistry of the subtidal sampling sites off Shikine Island. Values from June and September149surveys are reported as mean ( $\pm$ SD). Minimum (Min) and maximum (Max) pCO<sub>2</sub> values are also reported.150pCO<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®151CO<sub>2</sub> II logger.

CO <sub>2</sub> location	CO <sub>2</sub> site	Salinity	T°C	pH nbs	n (pH)	TA (µmol kg <sup>-1</sup> )	pCO2 calc. (µatm)	pCO2 meas. (µatm)	n (pCO <sub>2</sub> meas.)
Elevated	High	34.5	19.9	7.87	11	2249.9	971.7 (434.0)	952.8 (450.8)	417
		(0.05)	(0.5)	(0.15)			Min:591.8	Min:567.7	
							Max:2062.1	Max:2360.4	
Elevated	Mid	34.6	19.4	8.09	17	2253.4	497.2 (72.6)	552.1 (188.2)	843
		(0.07)	(0.5)	(0.05)			Min:373.8	Min:368.2	
							Max:645.8	Max:1552.1	
Elevated	Low	34.7	19.5	8.16	16	2270.5	404.9 (54.8)	402.1 (53.4)	555
		(0.05)	(0.7)	(0.05)			Min:361.2	Min:358.0	
							Max:523.2	Max:591.1	
Control	Ref 1	34.7	18.9	8.21	28	2253.1	348.2 (10.6)	347.9 (13.9)	994
		(0.05)	(0.3)	(0.01)			Min:322.0	Min:307.2	
							Max:367.6	Max:373.8	
Control	Ref 2	34.8 19.5 8	0.25		2250.8	311.5 (29.3)	311.8 (29.1)		
			8.25	27		Min:255.4	Min:251.9	913	
		(0.05)	(0.2)	(0.03)			Max:356.7	Max:357.8	

#### a) June 2016

#### 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	Uigh	33.9	26.0	7.65	4	2267.8	1646 4 (207 2) Min: 1220 0 May: 2026 5
	nigii	(0.1)	(0.1)	(0.09)			1040.4 ( <i>377.3)</i> Will. 1220.9 Max. 2020.3
Elevated	Mid	33.9	25.1	7.91	6	2257.3	849.7 (291.0) Min:524.4 Max:1372.0
	Milu	(0.2)	(1.0)	(0.12)	0		
Elevated	Low	33.9	25.6	8.13	4	2269.3	459.3 (12.7) Min:442.7 Max:473.1
	LOW	(0.0)	(0.1)	(0.01)			
Control	Dof 1	33.9	26.3	8.18	4	2249.9	395.4 (11.9) Min:379.3 Max:407.8
	Kel I	(0.2)	(1.4)	(0.01)			
Control	D-f 2	33.9	26.2	8.16	4	2249.5	420.8 (13.6) Min:409.2 Max:439.8
	Kel Z	(0.2)	(1.7)	(0.01)			

#### 154 2.2 Benthic habitat characterisation

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

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

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175 *2.3 Fish surveys* 

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

September 2016, respectively. Fish transects were located haphazardly within each sampling  $CO_2$  site at 4-10 m depth, and were conducted between 09.00 am and 03.00 pm by a scuba diver leaving behind a 25 m measure tape, while counting and identifying all the fish encountered 2.5 m either side of the tape (125 m<sup>2</sup>; Harmelin-Vivien et al. 1985). Water visibility exceeded 15 m for all counts. Each sampling day, only two spatially separated transects (at >20 m distance each other) were gathered at each  $CO_2$  site to avoid temporal dependence of data (Stewart-Oaten et al. 1986).

To make trait-mediated comparisons among different CO<sub>2</sub> conditions, the different fish species were also subdivided by geographical origin (Tropical, Subtropical and Temperate), by trophic guild (Carnivore, Omnivore, Herbivore and Planktivore), and by their association with coral reefs (i.e. coral reef associated 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  $CO_2$  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+  $\beta$ 3 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).

To identify the relevant variables characterising the benthic habitat which were responsible for the variation in fish community structure and composition, we used a Multivariate Distance Based Linear Model [DISTLM, Anderson et al. 2008]. Both the fish community and the benthic datasets were square root transformed and the step-wise model selection method based on the AIC criterion was performed to assess the benthic variables related with Bray-Curtis resemblance matrix of the fish dataset. To visualise the fish community data as a biplot, the Distance-based redundancy analysis (dbRDA; Legendre and
Anderson 1999; McArdle and Anderson 2001) using the benthic variables identified by the DISTLM
routine was used.

To investigate the potential relationship between  $pCO_2$  and habitat complexity (as BHP index, log 205 206 transformed) we used an Additive Mixed Model (AMM) with a Gaussian distribution and the identity link. AMM was used because it allows for the modelling of the non-linear effects of continuous 207 explanatory variables by incorporating smooth functions (Wood 2011). Specifically, the cubic regression 208 spline was used as a one-dimensional non-parametric smoothing function and the number of knots k was 209 set to 6, to prevent the models from producing complex non-linear relationships of little biological 210 211 significance (Wood 2006). To account for the lack of independence of the BHP data of the same Site per Season we used Site x Season as random intercept, which is assumed to be normally distributed with 212 mean 0 and variance  $\sigma^2$ . Data for both sampling campaigns (June and September) were combined into a 213 214 single data set and Season was modelled as a factor. The model was fitted using maximum likelihood (ML) parameter estimation. The analysis was performed using the R package 'mgcv' (Wood 2011). 215

We also used a method derived from meta-analysis to assess the effects of the different CO<sub>2</sub> conditions 216 on the number (S) and abundance (N) of fish species, also considering their classification by geographical 217 distribution (S<sub>origin</sub>), trophic guild (N<sub>trophic</sub>) and coral reef association (S<sub>coral</sub>). To this aim, we calculated 218 the effect size (a metric that quantifies the difference between the control and experimental groups) as 219 the response ratio, i.e. the natural logarithm of the ratio between the averaged response variable values 220 (S, N, Sorigin, Ntrophic, Scoral) recorded at the Elevated CO<sub>2</sub> (High, Mid and Low CO<sub>2</sub> sites) and Control 221 222 (Ref1 and Ref2 sites) locations. These analyses were performed using the R (R Core Team 2018) package 'metafor' (Viechtbauer 2010). 223

#### **3. Results and Discussion**

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Benthic communities changed from rocky habitats dominated by scleractinian corals and canopy-forming 227 macroalgae to rocky reefs covered in low-profile and turf algae from Control (Ref1 and Ref2) to Elevated 228 229 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 structure of benthic communities did not differ between sites within ambient CO<sub>2</sub> condition both in June 231 (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; 232 233 P(perm)=0.28) surveys, whilst they did significantly differ in most of the pair-wise comparisons of sites within the elevated CO<sub>2</sub> sites (Table S1). 234 235 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 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 238 corals were rare and were absent in our elevated CO<sub>2</sub> transects. Instead, dense mats of Caulerpa 239 chemnitzia var. peltata and the diatom Biddulphia biddulphiana covered most of the rocky substrata at 240

(from late July to early October each year) on Shikine Island (Harvey et al. 2019). This major seasonal
habitat shift resulted in an overall loss of canopy height due to the increase in the abundance of a few
low profile algal and turf species which may outcompete large and slow-growing species under ocean
acidification conditions (Harley et al. 2012; O'Brien & Scheibling 2018; Harvey et al. 2019). The ability
of a few opportunistic species to withstand OA effects, benefit from CO<sub>2</sub> enrichment and displace

the elevated  $CO_2$  sites in June. These species were not seen in our transects in September, often revealing

a covering of crustose coralline algae or low profile turf algae on the rocks (Fig. 2). As recently suggested,

this was likely due to strong wave energy during typhoon activity that occurs in summer and early autumn

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

Milazzo et al. 2016; Cattano et al. 2017; Di Franco et al. 2019). Here we focus on fish responses at the community level.

Fish assemblages changed significantly between sites along the CO<sub>2</sub> gradient, and between elevated CO<sub>2</sub> 256 257 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 turf, the non-canopy forming algae and the CCA. The dbRDA ordination of the dataset constrained by 259 these variables showed that the difference in the fish communities along the  $CO_2$  gradient was best 260 261 explained by turf, massive and non-canopy algae, while the differences between June and September were best explained by table corals and CCA (Fig. 3). Thus, the clear change of fish communities from 262 263 ambient to elevated CO<sub>2</sub> conditions was associated with a biogenic habitat shift under OA conditions.

To date, few studies have documented the effects of OA-induced habitat changes on the structure and 264 composition of fish communities. Munday et al. (2014) found that fish communities differed little 265 266 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  $CO_2$ seep areas. The few observed differences in the abundance of certain fish species were related to coral 268 269 community changes (from branched to massive corals) between CO<sub>2</sub> exposed and un-exposed reefs (see Fabricius et al. 2014) rather than by the direct effects of high  $CO_2$  on fish. Nagelkerken et al. (2015) 270 described habitat characteristics and fish species composition at two different CO<sub>2</sub> seeps, documenting 271 predator reductions and habitat shifts at elevated CO<sub>2</sub> conditions, which together led to an increase of a 272

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, suggesting that elevated  $CO_2$  indirectly boosted the abundance of a single species thus altering the 275 competitive relationships among species and suppressing the abundance of the competitive subordinates 276 277 (Nagelkerken et al. 2017). Contrary to these previous studies, where fish community differences between CO<sub>2</sub> exposed and unexposed reefs were evaluated focusing on a few species with narrow home ranges, 278 279 here we compared the structure and the composition of entire fish communities finding significant differences among the four CO<sub>2</sub> conditions considered. 280

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

Our findings support the prediction that OA simplifies habitat composition and reduces habitat complexity (Sunday et al. 2017). Biogenic habitat complexity (BHP) decreased with increasing  $pCO_2$ levels, in a non-linear relationship (Fig. 4a). When looking at model residuals including both seasons, habitat complexity was high at  $pCO_2$  values below ~500 µatm, whilst this relationship was significantly negative for values up to ~ 1000 µatm (i.e. decreased complexity with increasing  $pCO_2$ ), after which no

significant effects were detected probably due to the low sample size (Fig.4b). Overall, this trend was 297 consistent in the two sampling campaigns (Fig. 4c). The minimum index values were reached between 298 ~500 and ~1000 µatm, where table corals disappeared being replaced mainly by turf, non-canopy algae 299 and *Caulerpa chemnitzia* var. *peltata*, and where the only calcifying groups were represented by CCA or 300 301 a few encrusting corals. The consistent complexity reduction in the elevated  $CO_2$  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 and Wernberg, 2018). Such transition to less complex habitats may lead to the loss of a suite of resources 304 305 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 CO<sub>2</sub> seeps has documented altered properties and non-linear responses for invertebrate communities along CO<sub>2</sub> gradients (e.g. Fabricius et 307 al. 2014; Milazzo et al. 2019), whilst no effects were detected in fish communities despite lower habitat 308 complexity under elevated CO<sub>2</sub> conditions (Munday et al. 2014). 309

Here for the first time, a consistent temporal response of the fish fauna to the OA-mediated habitat shift 310 was documented. Overall, the number of species declined by 35% and 57% when comparing fish species 311 richness at Control vs High CO<sub>2</sub> sites in June and September, respectively (see Table S3). In addition, 312 average species richness and abundance significantly decreased from Control to elevated CO<sub>2</sub> conditions, 313 314 with the lowest values recorded at the High and Mid  $CO_2$  sites (Fig. 5a; Table S3). Such a pattern was consistent in both June and September (Fig. 5a; Table S3) and confirms previous evidence that shifts 315 316 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).

Trait-based approaches are increasingly being used to characterise ecological changes in disturbed ecosystems (Mouillot et al. 2013). Environmental alterations may not only lead to biodiversity loss, but 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 preferences, the occurrence and abundance of certain species can be strongly influenced by habitat 322 composition and characteristics. As expected, trait-based comparisons of fish communities along the CO<sub>2</sub> 323 gradient reveal a change in composition towards fish species of less tropical and sub-tropical origin (Fig. 324 325 5b), which is very likely related to the observed loss of scleractinian corals at >400 ppm  $CO_2$  and to the disappearance of the more specialised associated fish fauna they support. Therefore, the observed 326 327 transitions from corals/high-profile algae to low-profile/turf algae support the available models suggesting that OA will hold temperature-induced poleward expansion of coral habitats (Yara et al. 328 2012), and this will cascade on the composition of the associated fish fauna with a decreasing pool of 329 tropical, sub-tropical and coral reef associated species under elevated CO<sub>2</sub> conditions. In this regard, 330 Nakamura et al. (2013) documented an increase of warm-water tropical fish along the Japan high-latitude 331 waters. Our findings rather suggest that if ongoing OA will act as a supplementary game-changer in the 332 near future, this could not be the case. 333

334 When looking at fish trophic guilds we found that, relative to ambient  $CO_2$  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 seasonal typhoons caused the removal of macroalgal and turf cover particularly from the high CO<sub>2</sub> area 336 (Fig. 5c). Conversely, omnivorous species showed the opposite pattern being more abundant after the 337 338 typhoon season. These results support previous evidence showing that an increased abundance of herbivore species at elevated  $CO_2$  conditions can be driven by the greater biomass of primary producers 339 associated with enhanced nutritional quality (Vizzini et al. 2017). When turf and algae are removed by 340 wave action, the availability and type of food changes, with repercussions on trophic guild composition 341 342 (i.e. less herbivorous and more omnivorous species). In addition to this, results from recent experiments suggest that by increasing their macrophyte consumption and defecation rates, herbivore population 343 growth could further modify trophic processes in benthic systems under OA conditions, hence amplifying 344

detritus biomass production and potentially altering energy pathways and ecosystem functioning (ZarcoPerello et al. 2019).

By contrast, a consistent decrease in the abundance of carnivore species was observed along the CO<sub>2</sub> 347 gradient in both the sampling seasons (Fig. 5c). Such switches in the composition of functional groups 348 (i.e. increase of herbivore and reduction in carnivores) appears to be a common community property in 349 elevated CO<sub>2</sub> systems and has been described by other ecosystem studies carried out in temperate natural 350 351 CO<sub>2</sub> vent systems (Vizzini et al. 2017; Nagelkerken et al. 2015; Milazzo et al., 2019). In this regard, we speculate that piscivorous species may be attracted to more complex habitat characterised by higher prey 352 353 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 planktivorous fish and in the number of coral reef associated species from ambient to elevated  $CO_2$ 355 conditions (Fig. 5c and 5d). Again, this seems to be related to the loss of coral species at the more elevated 356 CO<sub>2</sub> sites, which provide structural complexity and represent the preferred habitat for many coral fish 357 species and their prey (Coker et al. 2014; Smith et al. 2016). Our findings add to previous evidence from 358 ocean warming studies showing fish diversity declines following extensive coral loss due to bleaching 359 events (e.g. Pratchett et al. 2011). In this regard, we suggest that OA may act as an additional bottleneck 360 for fish community resilience under predicted ocean change scenarios. 361

Natural analogues have many benefits for furthering knowledge about the responses of fish to OA conditions, but they are not perfect analogues for acidifying oceans. The seep areas are localised and so fish can move in and out, complicating dose-response assessments (Hurlbert 1984; Munday et al. 2014; Cornwall and Hurd 2015). Moreover, carbonate chemistry can be highly variable at CO<sub>2</sub> seeps (Cattano et al. 2016; Cattano et al. 2017; Agostini et al., 2018) and toxic areas around volcanic vents need to be avoided in studies of the effects of ocean acidification (Vizzini et al. 2013). Nonetheless, the seeps allow 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 CO2 seep research suggests that OA affects the outcome of
376	ocean warming (Goldenberg et al 2018).

In summary, our data provide direct evidence that shifts in biogenic habitat and complexity reduction 377 underpin a major loss (35-57%) of marine fish diversity as levels of carbon dioxide in seawater increase 378 379 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 planktivores. Such changes in fish communities would cause major changes in trophic processes and 385 386 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

#### 389 Acknowledgements

390

We would like to thank the technical staff of Shimoda Marine Research Center (University of Tsukuba)
for field assistance and the use of RV *Tsukuba*, and Prof. Alessandro Aiuppa and Dr Rossella Di Napoli

- 393 (University of Palermo, Italy) for providing the HydroC Carbon Dioxide Sensor. We are grateful to Prof.
- 394 Nakamura for help with fish identification. This study was supported by the 'International Education and
- Research Laboratory Program', University of Tsukuba. Travel costs were funded by the University of
- 396 Palermo for C.C. and by a Japan Society for the Promotion of Science Short Term Invitation Fellowship
- 397 (Grant Number: S16073) and by JAMBIO for M.M.
- 398 Images used within the graphical abstract are courtesy of the Integration and Application Network,
- 399 University of Maryland Center for Environmental Science (ian.umces.edu/symbols/).

#### 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.
- 407

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**615** Figure 1 - Map of the study showing Shikine Island, the two  $CO_2$  locations (Elevated and Control) and **616** the five  $CO_2$  sites (High, Mid, Low, Ref1 and Ref2) where the benthic and fish 25m-transects were **617** positioned.



Figure 2 – Non-metric multidimensional scaling (nMDS) on benthic habitats at the five  $CO_2$  sampling sites (High, Mid, Low, Ref1 and Ref2) in June (filled symbols) and September (empty symbols). Photomosaics of benthic community shifts observed in June and September along the  $CO_2$  gradient are also reported. The figure shows a clear community shifts in both seasons from zooxanthellate scleractinian corals and canopy-forming macroalgae at Control  $CO_2$  location (Ref1 and Ref2) to macroalgae at the Low and Mid  $CO_2$  sites and turf algae at the High  $CO_2$  site.



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



Figure 4 –AMM (Additive Mixed Model) showing the relationship between pCO<sub>2</sub> values and habitat 637 complexity (BHP). a) Fitted values ( $\pm 95\%$  confidence intervals) of pCO<sub>2</sub> values and habitat complexity 638 (log-transformed BHP index) for June (black dots) and September (green dots); b) model residuals 639 (black dots) and estimated smoothing curve (solid line with  $\pm 95\%$  confidence interval) showing the pCO<sub>2</sub> 640 effects on BHP. The effect is significant (i.e. positive or negative) when the CI (grey area) does not 641 overlap the zero (dashed line); c) partial effect (solid lines) of the factor Season on the BHP index 642 (dashed lines indicate the ±95% confidence interval for September). The vertical lines reported on the 643 x-axis of panels b and c represent the observations from the different  $pCO_2$  values and from the two 644 645 seasons, respectively.



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