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

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Abstract

Ocean acidification will likely change the structure and function of coastal marine ecosystems over coming decades. Volcanic carbon dioxide seeps generate dissolved CO₂ and pH gradients that provide realistic insights into the direction and magnitude of these changes. Here, we used fish and benthic community surveys to assess the spatio-temporal dynamics of fish community properties off CO₂ seeps 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. 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 suggest that near-future projected ocean acidification levels will oppose the ongoing range expansion of coral reef-associated fish due to global warming.

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

1. Introduction

Shifts in marine biogenic habitats in response to anthropogenic activities and a range of stressors have been documented since the 1960s (Hughes 1994). In tropical coral reef ecosystems examples include studies of the impacts of overfishing (Jackson et al. 2001), outbreaks of coral-eating predators (De'ath et al. 2012), diseases (Hughes 1994), pollution (McCulloch et al. 2003), hurricanes (Hughes 1994), and extreme temperatures (Hoegh-Guldberg 1999). Ocean warming is changing coastal marine communities, for example due to (1) warm-water species (e.g. corals and tropical fish) moving poleward following their thermal physiological niche, (2) changes in the strength of interspecific interactions (e.g. increase of herbivory from warm-water fish leading to the loss of kelp forests at their low latitude limits), and (3) 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 CO₂ 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 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 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) 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 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 CO_2 seeps in Papua
72 New Guinea reported reduced coral reef complexity at elevated CO_2 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 CO₂ 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 (Ω_{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 habitat shifts off CO₂ seeps located in the NW Pacific (Japan), in a region that has naturally low levels of $p\text{CO}_2$, 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 coral communities at ambient CO₂ conditions, while a transition to low-relief algal turf habitats occurs at elevated CO₂ levels (Agostini et al. 2018; Harvey et al. 2019). To assess how fish community properties changed spatially along the $p\text{CO}_2$ gradient, we coupled fish and benthic community assessments (habitat complexity, canopy height, and % cover), along a CO₂ gradient and at control sites 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 structure are temporally consistent, specifically when acute seasonal typhoons (usually from July to September), may affect benthic community structure and habitat complexity. In addition, we carried out

fish trait comparisons between the different CO₂ sites, to assess changes in taxonomic richness and abundance of fish subdivided by geographic distribution (Tropical, Subtropical and Temperate), trophic 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 habitat complexity along a spatial CO₂ 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 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 CO₂. As CO₂ enrichment acts both as a stressor for scleractinian corals and as a substrate for primary producers, we also expected that the number of species of fish from tropical and subtropical origins would decrease, while the diversity of herbivorous fish would increase in elevated CO₂ conditions.

2. Materials and Methods

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 CO₂ seeps in shallow waters. Based on previous geochemical investigations (Agostini et al. 2015), our survey locations were selected to avoid potentially confounding geochemical factors (e.g. high sulfides, negative redox potential, altered total alkalinity and elevated temperature). One location (Elevated-CO₂) was within Mikawa Bay and a second location (Control) characterised by ambient CO₂ conditions was 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 Corporation, Tokyo, Japan) and a HydroC® CO₂ II sensor (Contros System & Solutions GmbH, 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 CO₂ 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® CO₂ II sensor recorded measures of *p*CO₂ (µatm) every 5 seconds. The CO₂
125 sensor detects dissolved CO₂ molecules that diffuse through a thin film composite membrane into an
126 internal gas circuit containing a detector chamber where the *p*CO₂ 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⁻¹, 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⁻¹) and against certified reference material purchased from the A.G.
136 Dickson laboratory (Batch 152). The CO₂SYS software (Pierrot et al. 2006) was used to calculate *p*CO₂
137 (Table 1) from T, pH, salinity and TA values, and to control *in situ* continuous measurements of *p*CO₂
138 recorded by the HydroC® CO₂ II sensor. The disassociation constants from Mehrbach (1973), as adjusted
139 by Dickson and Millero (1987), HSO₄ using Dickson (1990), and total borate concentrations from
140 Uppström (1974) were used for carbonate chemistry calculations (Table 1). The HydroC® CO₂ II sensor
141 was not employed in the September survey due to logistic constrains.

142 The carbonate chemistry measurements along the nine 100-m transects were used to identify five
143 sampling CO₂ sites in the rocky subtidal zone between 3 and 12 m depth in Mikawa bay and the Control
144 bay: one ‘High-CO₂’ (High), one ‘Mid-CO₂’ (Mid) and one ‘Low-CO₂’ (Low), and two ‘Ambient-CO₂’

145 (Ref 1 and Ref 2; Fig. 1; Table 1). In each CO₂ 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) pCO₂ values are also reported.*
 150 *pCO₂ calc.= pCO₂ levels calculated with CO2SYS. pCO₂ meas.= pCO₂ levels measured with the HydroC®*
 151 *CO₂ II logger.*

a) June 2016

CO ₂ location	CO ₂ site	Salinity	T °C	pH nbs	n (pH)	TA (μmol kg ⁻¹)	pCO ₂ calc. (μatm)	pCO ₂ meas. (μatm)	n (pCO ₂ 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 ₂ location	CO ₂ site	Salinity	T °C	pH nbs	n (pH)	TA (μmol kg ⁻¹)	pCO ₂ 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 CO₂ sites (High, Mid, Low, Ref1 and
158 Ref2). Specifically, four transects were deployed in the ‘High-CO₂’ site (High), six transects within the
159 ‘Mid-CO₂’ (Mid) site, six transects within the ‘Low-CO₂’ (Low) site, and nine transects each in the two
160 ‘Ambient CO₂’ 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 CO₂ 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 m²; 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 CO₂ site to
183 avoid temporal dependence of data (Stewart-Oaten et al. 1986).

184 To make trait-mediated comparisons among different CO₂ 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 CO₂ 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: “CO₂ 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 x Season as random intercept, which is assumed to be normally distributed with
 213 mean 0 and variance σ^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 CO_2 conditions
 217 on the number (S) and abundance (N) of fish species, also considering their classification by geographical
 218 distribution (S_{origin}), trophic guild (N_{trophic}) and coral reef association (S_{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_{origin} , N_{trophic} , S_{coral}) recorded at the Elevated CO_2 (High, Mid and Low 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

Benthic communities changed from rocky habitats dominated by scleractinian corals and canopy-forming macroalgae to rocky reefs covered in low-profile and turf algae from Control (Ref1 and Ref2) to Elevated CO₂ sites (Low, Mid and High) and this observation was consistent in both of the considered time periods (Fig. 2; PERMANOVA: CO₂ site x Season, Pseudo-F= 7781.2; P(perm)=0.0001). Composition and structure of benthic communities did not differ between sites within ambient CO₂ condition both in June (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; P(perm)=0.28) surveys, whilst they did significantly differ in most of the pair-wise comparisons of sites within the elevated CO₂ sites (Table S1).

Table, massive and encrusting corals were common at control sites in both sampling periods. They are able to survive at this high latitude (34° N) in the NW Pacific due to the warm northward flow of the Kuroshio Current (Veron and Minchin 1992). Hard corals were absent along transects taken at elevated CO₂ conditions. In contrast to some other CO₂ seep systems (Suggett et al. 2012; Inoue et al. 2013), soft corals were rare and were absent in our elevated CO₂ transects. Instead, dense mats of *Caulerpa chemnitzia* var. *peltata* and the diatom *Biddulphia biddulphiana* covered most of the rocky substrata at the elevated CO₂ 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 (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 CO₂ 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 CO₂ seeps studies revealed both detrimental and no effects of elevated CO₂ 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 CO₂ gradient, and between elevated CO₂
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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂
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 CO₂ exposed and un-exposed reefs (see
270 Fabricius et al. 2014) rather than by the direct effects of high CO₂ on fish. Nagelkerken et al. (2015)
271 described habitat characteristics and fish species composition at two different CO₂ seeps, documenting
272 predator reductions and habitat shifts at elevated CO₂ conditions, which together led to an increase of a

few territorial fish species. A more recent study carried out off the White Island CO₂ seeps in New Zealand documented loss of fish diversity and homogenisation of fish communities under OA conditions, suggesting that elevated CO₂ indirectly boosted the abundance of a single species thus altering the competitive relationships among species and suppressing the abundance of the competitive subordinates (Nagelkerken et al. 2017). Contrary to these previous studies, where fish community differences between CO₂ exposed and unexposed reefs were evaluated focusing on a few species with narrow home ranges, here we compared the structure and the composition of entire fish communities finding significant differences among the four CO₂ conditions considered.

The effects of OA on fish communities may depend on how elevated CO₂ affects the different species directly and indirectly. By altering acid-base balance and the processing of sensory information, elevated CO₂ concentrations affect physiological and behavioural performance in fish, although such effects are species- and trait-specific (reviewed in Cattano et al. 2018). In addition, by promoting shifts in biogenic habitat (Milazzo et al. 2019), habitat simplification (Sunday et al. 2017) and food web simplification (Fabricius et al. 2014; Vizzini et al. 2017), high CO₂ conditions may indirectly affect some fish species, especially those with highly specialised habitat and resource use. Conversely, generalist species could cope better with the predicted effects of OA on habitat and resources (e.g. Wilson et al. 2008a). This inter-specific variability in the extent to which fish may respond to OA-driven habitat modifications could play a role in shaping the direction of community shift and the composition of novel fish communities under elevated CO₂ 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 $p\text{CO}_2$ levels, in a non-linear relationship (Fig. 4a). When looking at model residuals including both seasons, habitat complexity was high at $p\text{CO}_2$ values below ~500 μatm , whilst this relationship was significantly negative for values up to ~1000 μatm (i.e. decreased complexity with increasing $p\text{CO}_2$), after which no

significant effects were detected probably due to the low sample size (Fig.4b). Overall, this trend was consistent in the two sampling campaigns (Fig. 4c). The minimum index values were reached between ~500 and ~1000 μatm , where table corals disappeared being replaced mainly by turf, non-canopy algae and *Caulerpa chemnitzia* var. *peltata*, and where the only calcifying groups were represented by CCA or a few encrusting corals. The consistent complexity reduction in the elevated CO_2 sites compared to control conditions matches the habitat shift from corals and canopy-forming macroalgae to turf 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 for fish (e.g. food and space availability) and to the alteration of important ecological processes, such as foraging, settlement and predation avoidance. OA research in CO_2 seeps has documented altered properties and non-linear responses for invertebrate communities along CO_2 gradients (e.g. Fabricius et al. 2014; Milazzo et al. 2019), whilst no effects were detected in fish communities despite lower habitat complexity under elevated CO_2 conditions (Munday et al. 2014).

Here for the first time, a consistent temporal response of the fish fauna to the OA-mediated habitat shift was documented. Overall, the number of species declined by 35% and 57% when comparing fish species richness at Control vs High CO_2 sites in June and September, respectively (see Table S3). In addition, average species richness and abundance significantly decreased from Control to elevated CO_2 conditions, 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 toward less complex habitat causes decreased fish diversity and abundance (e.g. Wilson et al. 2008b), as 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
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 CO₂
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 CO₂ 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 CO₂ 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 CO₂ conditions, the abundance of
335 herbivorous fish was significantly higher at the High CO₂ site in June, but not in September when
336 seasonal typhoons caused the removal of macroalgal and turf cover particularly from the high CO₂ 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 CO₂ 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 CO₂
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 CO₂ systems and has been described by other ecosystem studies carried out in temperate natural
351 CO₂ 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 CO₂
356 conditions (Fig. 5c and 5d). Again, this seems to be related to the loss of coral species at the more elevated
357 CO₂ 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 CO₂ 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 CO₂ seep locations, a pattern is

369 beginning to emerge about the effects of OA on fish communities. An additional caveat with volcanic
370 CO₂ 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 CO₂ seep in different biogeographic regions, or by manipulating temperature along CO₂
373 gradients (Alessi et al. 2019), or by exploiting marine heat waves to assess the combined stress of rising
374 temperature and elevated CO₂ (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).

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 CO₂ 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**

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614

615 *Figure 1 – Map of the study showing Shikine Island, the two CO₂ locations (Elevated and Control) and*
 616 *the five CO₂ sites (High, Mid, Low, Ref1 and Ref2) where the benthic and fish 25m-transects were*
 617 *positioned.*

618

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.

627

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.*

635

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

Figure 5 - Meta-analysis-derived approach to assess overall and trait-based changes of fish community at the different elevated CO₂ sites, both in June and September. Each point represents the log response ratio (LnRR) of the average value for the different fish community variables recorded at Low, Mid and High CO₂ sites relative to the ambient CO₂ 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 species); b) S_{origin}: number of species by their geographic distribution (Tropical, Sub-tropical or Temperate); c) N_{trophic}: abundance of species by their trophic guild (Omnivore, Planktivore, Carnivore or Herbivore); d) S_{coral}: number of coral-reef associated fish species. Squares indicate significant effects per $p < 0.05$. Small circles indicate no significant differences.