Title: Fish assemblages cope with ocean acidification in a shallow volcanic CO_2 vent benefiting from an adjacent recovery area

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Abstract

Shallow CO_2 vents are used to test ecological hypotheses about the effects of ocean acidification (OA). Here, we study fish assemblages associated to *Cymodocea nodosa* meadows, long-term exposed to high pCO_2/low pH conditions. Using underwater visual census, we assessed fish community structure and biodiversity in a low pH site, a close control site and a far control site, hypothesising a decline in biodiversity and a homogenisation of fish assemblages under OA conditions. Our findings revealed that fish diversity did not show a univocal spatial pattern, or even significant relationships with pH, but correlated with seagrass leaf canopy. Among-site similarity was found in juveniles' abundance, contrary to the forecasted impacts of OA on early life stages. Despite this, pH was an important driver in structuring fish assemblages, which however showed high similarity between the low pH site and the close control. This unexpected pattern may represent a combined response of fish mobility, enhanced food resources in the acidified site, and a 'recovery area' effect of the adjacent control site.

Keywords: Mediterranean Sea, CO_2 seeps, pH, Fish, Seagrass, *Cymodocea nodosa*, Biodiversity, Community structure, Underwater visual census, Juveniles

Introduction

The increase in anthropogenic CO_2 absorbed by the ocean and the consequent pH reduction, known as ocean acidification (OA), are causing changes in the carbon chemistry balance with direct and indirect effects on marine ecosystems and biodiversity (Barry et al. 2011, Cheung et al. 2009, Fabricius et al. 2011, Foo et al., 2018, Nagelkerken & Connell, 2015). OA can exert negative direct effects on organisms, especially calcifying species (i.e. algae, corals, mollusks and echinoderms) impairing survival, calcification, growth, development, reproduction and abundance (Kroeker et al. 2013). More difficult is to disentangle the indirect effects of OA, which are as important as direct ones to forecast the overall ecosystem responses (Riebesell & Gattuso, 2015). Indeed, OA may affect indirectly the marine realm by reducing habitat complexity through the decline in habitat-forming species that support high biodiversity (Barry et al. 2011). A recent study gathering data from naturally acidified sites (i.e., CO_2 vents) projected indirect negative effects of OA on biodiversity due to the reduction in structural complexity of biogenic habitat (i.e. coral reefs, mussel beds and some macroalgal habitats) (Sunday et al. 2017). Nevertheless, there is a lack of evidence that biodiversity will increase in systems where habitat-forming species could benefit from OA (i.e., seagrasses) (Sunday et al. 2017).

Direct and indirect effects of OA have been documented on fish, although they are considered able to cope with low pH thanks to their capacity of acid-base regulation (Ishimatsu et al. 2008). For instance, OA can directly affect olfactory and auditory functions (Dixson et al. 2010, Simpson et al. 2011), behaviour and neurosensory functions (Munday et al. 2014, Nilsson et al. 2012), reproduction (Miller et al. 2013), as well as calcareous structures (Bignami et al. 2013, Mirasole et al. 2017, Di Franco et al. 2019). On the other hand, indirect effects can be driven by habitat alteration and interactions with other species (Nagelkerken et al. 2015, 2017). Moreover, early life stages of fish are considered particularly vulnerable to OA, with consequences on fish population replenishment (Baumann et al. 2011, Munday et al. 2010, Rossi et al. 2016). Accordingly, it has been hypothesized

69 that fish biodiversity will be negatively affected by OA in a near future (Cheung et al. 2009) and
70 decline of catches worldwide is expected (Cheung, 2018).
71 CO_2 vents represent a great opportunity to test the effects of OA at high levels of the biological
72 hierarchy (i.e., community and ecosystem), as they are naturally acidified environments and are
73 widely used to fill the gap between laboratory experiments and the real world (Riebesell & Gattuso,
74 2015). Nevertheless, so far, most studies in CO_2 vents mainly focused on calcareous species and
75 ecosystem shifts driven by a decline not only in species richness at low pH conditions (Hall-Spencer
76 et al. 2008, Fabricius et al. 2011, Kroeker et al. 2012, Foo et al. 2018), but also a loss of functional
77 diversity (Teixido et al. 2018). Only recently, a few studies carried out in CO_2 vents addressed the
78 response of fish to OA at species (Nagelkerken et al. 2015, Cattano et al. 2017) and community levels
79 (Munday et al. 2014, Nagelkerken et al. 2017). The latter studies showed contrasting results on
80 changes in community structure and biodiversity, probably influenced by the different habitat use of
81 the fish studied. Indeed, although both studies found fish behavioural abnormalities, Nagelkerken et
82 al. (2017) found a loss in biodiversity of highly resident benthic fish, while Munday et al. (2014)
83 found only minor differences in the community structure of more mobile necto-benthic fish.
84 This study was conducted in the shallow CO_2 vent located in Vulcano Island (Aeolian Archipelago,
85 Italy) where the seagrass *Cymodocea nodosa* (Ucria) Ascherson, 1870, forms large meadows. In this
86 area, previous studies highlighted lower *C. nodosa* density and biomass close to the CO_2 vent area
87 compared with control sites, although overall characterized by a more intense metabolism and
88 photosynthetic activity (Apostolaki et al. 2014). *C. nodosa* has a crucial role in terms of primary
89 production, biodiversity and food web complexity (Cancemi et al. 2002) and is one of the most
90 important habitat-forming species for coastal fish, providing shelter from predators, nursery ground
91 and food (Guidetti & Bussotti, 2000).
92 The main aim of this study was to assess the effect of high pCO_2/low pH on fish assemblages in a
93 coastal area characterised by seagrass meadows. We compared fish assemblages long-term exposed
94 to high pCO_2/low pH conditions (low pH site) to those in normal pH conditions from two controls in

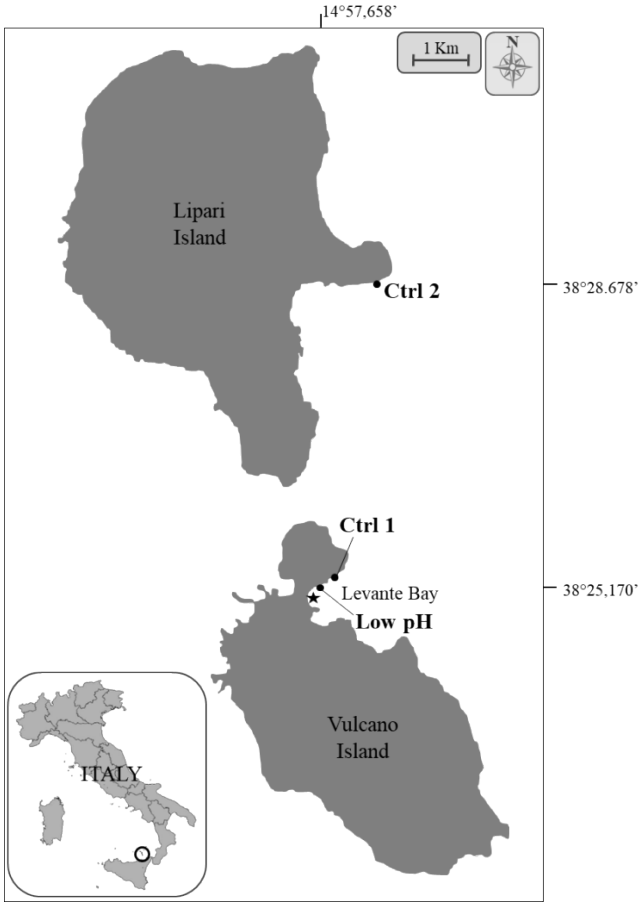
two periods of the year. In all sites and periods, we assessed species composition, fish biodiversity, community and size-class structure, as well as *C. nodosa* meadow complexity in terms of shoot density and leaf canopy height. We hypothesised a decline in fish biodiversity and a homogenization of fish assemblage (over simplification of the community structure through the numerical dominance of only a few species, *sensu* Nagelkerken et al. 2017) due to the combination of the stress effect of low pH and the expected reduction in seagrass structural complexity. We also expect that the detrimental effects are more severe on juveniles due to their higher vulnerability (Dixson et al. 2010, Munday et al. 2010, 2014).

Materials and methods

Study area and fieldwork

The shallow venting system of Vulcano Island (Aeolian Archipelago, 24 km off the NE coast of Sicily, Italy) is one of the most active and studied vent in the whole Mediterranean Sea. The main venting area occurs at about 1 m depth on the south-western part of Levante Bay, on the eastern side of the Island (Fig. 1) (Boatta et al. 2013). Overall, gas composition is dominated by CO_2 (97-99% vol.), which generates a pH gradient (from 5.5. to 8.1) along the north shore of the bay (Boatta et al. 2013, Italiano 2009). The area is characterized by acidic and reducing conditions, causing changes in major and trace element geochemical fluxes at the sediment-seawater interface (Vizzini et al. 2013). Emissions include also small quantities of H_2S (< 2.2%), which rapidly decrease with distance from the vent (Boatta et al. 2013). Water composition in terms of major elements and compounds (Cl, SO_4, Na, K, Ca and Mg) is similar to that of Mediterranean surface waters, while greater variability has been recorded for dissolved Fe concentrations, which showed high values close to the vents (Boatta et al. 2013). Seawater carbonate chemistry parameters in the Levante Bay range between 2.78 and 3.17 mmol kg^{-1} for total alkalinity and 0.02 and 3.64 for aragonite saturation state (Boatta et al. 2013). Fish assemblage was studied through non-destructive underwater visual censuses (UVCs) (Harmelin-Vivien et al. 1985) along 10 m long × 5 m wide strip transects (total area 50 m^2), randomly placed

121 within *Cymodocea nodosa* meadows, at a depth between 2 and 5 m in three sites: a low pH site
122 (hereafter low pH) about 250 m far from the primary CO_2 vent in Levante Bay, and two Control sites:
123 Control 1 (Ctrl 1) about 500 m far from the primary vent, and Control 2 (Ctrl 2) in Lipari Island,
124 about 6.5 km far from the primary vent (Fig. 1). Controls were chosen for the same orientation (South-
125 East), depth (2-5 m) and vegetal coverage (*C. nodosa* meadow) as the low pH site. Visual census was
126 carried out in two periods of the year (Period I: from September to November 2014; Period II: from
127 May to July 2015), between 11.00 AM and 3.00 PM (18 replicates in total per site and period). Cryptic
128 fish (i.e. Blenniidae, Gobiidae, Scorpaenidae) were kept out from the surveys to avoid
129 underestimation (Willis 2001). Fish were identified to species level and abundance was estimated by
130 using seven pre-established abundance classes (1, 2-5, 6-10, 11–30, 31–50, 51–100, >100 number of
131 individuals) according to Harmelin-Vivien et al. (1985). Abundance was calculated by taking into
132 account the mid-point of each abundance class and expressed as mean fish abundance (ind. 50 m^{-2}).
133 Fish were also assigned to three size-classes: small (S), medium (M) and large (L), corresponding
134 each to one-third of the maximum total length reported in the literature, according to FishBase online
135 database (Froese & Pauly, 2016), and grouped into trophic groups following the classification by
136 Guidetti & Sala (2007). In agreement with literature (Guidetti & Bussotti, 2000; Harmelin-Vivien et
137 al. 1985), we considered small, medium and large size corresponding to different fish life stages,
138 analogues of juveniles, sub-adults and adults.
139 Seawater temperature, salinity, dissolved oxygen (DO), pH and redox potential (Eh) were measured
140 in each site during the entire sampling period using a multiparameter probe (Hanna Instrument,
141 HI98194). Additionally, seagrass (*C. nodosa*) shoot density and leaf canopy height were measured.
142 *C. nodosa* shoot density was estimated by counting shoots (three replicates per transect) within a 400
143 cm^2 surface through a 20 x 20 cm frame and then converted to square meter. Leaf canopy height was
144 measured by recording the maximum length of the highest leaf in each shoot analysed (five shoots
145 per square, five replicates per transect).



146

147 **Fig. 1** - Map of the study area showing the sampling sites in Vulcano (Low pH and Ctrl 1) and Lipari Islands (Ctrl 2)
148 (Aeolian Archipelago, Italy). The primary vent is located in Levante Bay (Vulcano) and is indicated by the star.

149

150 Data analysis and statistics

151 Univariate permutational analysis of variance (ANOVA) was used to test for differences among sites
152 (fixed factor with 3 levels: low pH, Ctrl 1 and Ctrl 2) and between periods (fixed factor and orthogonal
153 to Site, with 2 levels: PI and PII) for *Cymodocea nodosa* features (shoot density and leaf canopy
154 height), fish diversity (species richness S, total abundance N, and size-class abundance N: N_{small},
155 N_{medium}, N_{large}). Data were previously transformed using the log (x + 1) notation and resembled using
156 Bray Curtis similarity matrices (Primer 6/PERMANOVA+ software, Plymouth Marine Laboratory).
157 Linear regression analysis was run to test the relationship between fish diversity (S, N, N_{small}, N_{medium},
158 N_{large}) and both *C. nodosa* features (shoot density and leaf canopy height) and pH values, by using
159 Statistica software (Stat Soft V. 10).

160 Permutational ANOVA was used also to test for differences among sites and between periods in
161 abundance of the most abundant species (N> 2% in at least one sampling site) taken individually
162 (univariate analysis), all together and grouped in size-class (N_{small}, N_{medium}, N_{large}) (multivariate
163 analysis). The analysis was carried out after transformation using the log (x + 1) notation and
164 resemblance using Bray Curtis similarity matrices (Primer 6/PERMANOVA+ software, Plymouth
165 Marine Laboratory). Only in the analysis of size-class abundance, a dummy variable = 1 was added
166 to improve interpretability of displays and significance of tests when denuded samples were found
167 (Clarke et al. 2006). To visualize the differences in fish abundance (total and size-class abundance)
168 among sites and between periods, a constrained ordination (with the interaction "site x period" as
169 constrained factor) was performed using canonical analysis of principal coordinates (CAP, Anderson
170 & Willis 2003). A similarity percentage procedure (SIMPER) was used also to identify fish species
171 mostly contributing to dissimilarities among sites.
172
173 **Results**
174 Environmental features
175 Temperature did not show large among-site wide variability, but increased from Period I (mean ± s.d.
176 22.3 ± 2.4 °C; range: 20.1-25.5 °C) to Period II (23.9 ± 3.0 °C; 19.0-29.5 °C). Salinity (38.7 ± 0.4
177 psu; 38.2-39.2 psu) and DO (7.3 ± 0.3 mg l^{-1}; 6.2-8.6 mg l^{-1}) varied little among sites and between
178 periods. On the other hand, pH showed among-site variability, being on average 7.80 ± 0.09 in the
179 Low pH site, 8.19 ± 0.03 in Ctrl 1 and 8.22 ± 0.02 in Ctrl 2. Consistently, mean Eh was 79.3 ± 5.6
180 mV in the Low pH site, 85.6 ± 8.3 mV in Ctrl 1 and 111.6 ± 13.7 mV in Ctrl 2.
181 *C. nodosa* shoot density showed significant differences for the interaction 'site x period' (Pseudo-F
182 $_{(2, 318)} = 25.76$, $p < 0.001$). Pair-wise tests highlighted significantly lower densities in the Low pH site
183 than in both Controls in Period I ($p < 0.001$), and also in Period I than in Period II at all sites (Fig.
184 2a). No among-site and between-period differences in *C. nodosa* leaf canopy height were detected
185 (Fig. 2b).

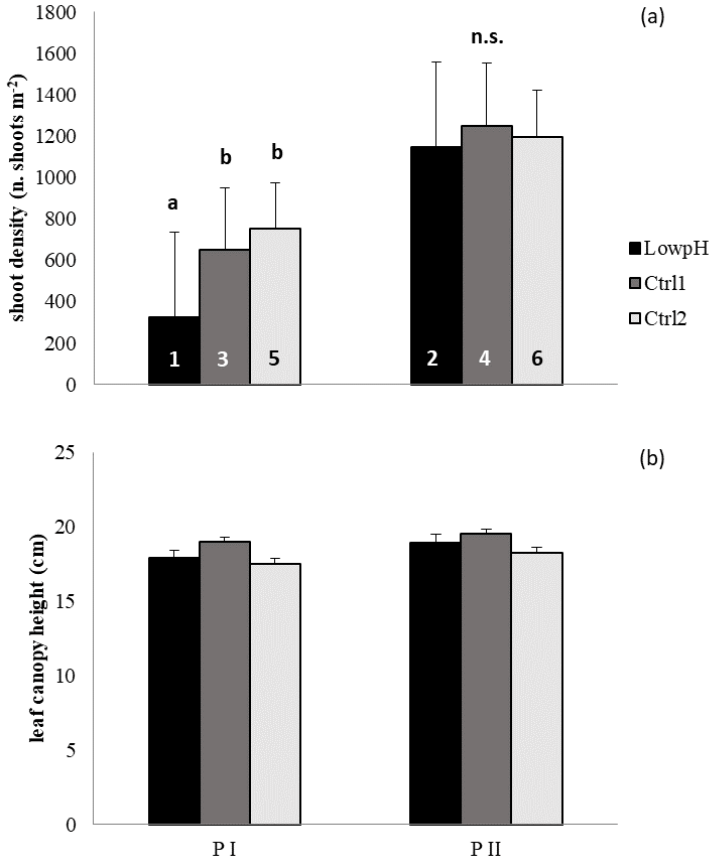


Fig. 2 - Mean value (±SE) of a) *Cymodocea nodosa* shoot density (n. shoots m^{-2}) and b) leaf canopy height (cm) in the three sites (Low pH, Ctrl 1 and Ctrl 2) and the two periods (PI and PII). Letters and numbers indicate homogeneous groups within sites and periods, respectively. n.s. indicates not significant differences among sites.

Fish assemblages

Overall, nineteen fish species belonging to six different families were recorded. Assemblages were dominated by Labridae (10 species) and Sparidae (5 species), followed by Mullidae, Mugilidae, Pomacentridae and Serranidae (Annex I). Most species belonged to the invertivorous trophic group with the exception of one herbivore (*Sarpa salpa*), one detritivore (*Mugil cephalus*), one piscivore (*Serranus scriba*) and two planktivores (*Chromis chromis* and *Oblada melanura*). In general, the most abundant species belonged to the families Labridae (i.e. *Coris julis* and *Symphodus ocellatus*), Pomacentridae (*C. chromis*) and Sparidae (i.e. *Diplodus vulgaris* and *S. salpa*).

The most abundant fish species (N> 2% in at least one site) showed significant differences among the three sites, except for *M. surmuletus* (Table 1). In particular, three species showed comparable

abundances in Low pH and Ctrl 1 sites, and lower (*D. vulgaris*) or higher (*C. chromis* and *C. julis*) than in Ctrl 2. Three species (*S. ocellatus, S. salpa* and *S. tinca*) showed similar abundances in Low pH and Ctrl 2, while only one species showed lower abundance in Low pH than both Controls (*O. melanura*), and another species differed among the three sites with a marked increase from Low pH to Ctrl 1 and Ctrl 2 (*T. pavo*). By comparing periods, only three species (*C. julis*, *S. tinca* and *T. pavo*) showed a significantly higher abundance in Period I than in Period II, while one species (*S. salpa*) showed the opposite trend.

Source of variation		***C. chromis***				***C. julis***				***D. vulgaris***			
	df	**MS**	**Pseudo-F**	**p**	**Pair-wise**	**MS**	**Pseudo-F**	**p**	**Pair-wise**	**MS**	**Pseudo-F**	**p**	**Pair-wise**
Site	2	6895.3	13.0	**	C2 < Low = C1	1622.8	8.8	***	C2 < Low = C1	5783.3	19.2	***	C1 = Low < C2
Period	1	1318.9	2.5	n.s.		4858.9	26.4	***	PII < PI	494.3	1.6	n.s.	
Site*Period	2	493.7	0.9	n.s.		411.1	2.2	n.s.		701.0	2.3	n.s.	
Residuals	102	528.9				183.8				300.8			

Source		***M. surmuletus***				***O. melanura***				***S. ocellatus***			
	df	**MS**	**Pseudo-F**	**p**	**Pair-wise**	**MS**	**Pseudo-F**	**p**	**Pair-wise**	**MS**	**Pseudo-F**	**p**	**Pair-wise**
Site	2	120.0	0.4	n.s.		2714.9	8.0	**	Low < C2 = C1	1516.6	3.0	*	C2 = Low < C1
Period	1	187.9	0.6	n.s.		93.5	0.3	n.s.		252.6	0.5	n.s.	
Site*Period	2	657.3	2.3	n.s.		395.8	1.2	n.s.		758.4	1.5	n.s.	
Residuals	102	285.2				338.6				498.7			

Source		***S. salpa***				***S. tinca***				***T. pavo***			
	df	**MS**	**Pseudo-F**	**p**	**Pair-wise**	**MS**	**Pseudo-F**	**p**	**Pair-wise**	**MS**	**Pseudo-F**	**p**	**Pair-wise**
Site	2	2175.1	4.0	*	C1 < C2 = Low	1799.1	5.4	**	C2 = Low < C1	4387.1	16.8	***	Low < C1 < C2
Period	1	3040.8	5.6	*	PI < PII	2061.3	6.2	*	PII < PI	1011.4	3.9	*	PII < PI
Site*Period	2	840.9	1.5	n.s.		387.3	1.2	n.s.		587.8	2.2	n.s.	
Residuals	102	542.8				332.5				260.7			

Table 1 - Results of univariate permutational ANOVA testing for differences among Sites and between Periods in abundance of the most abundant species (> 2%). Probability levels: n.s. = not significant- $p > 0.05$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

At multivariate level, fish assemblage showed significant differences for the interaction 'site x period' (Pseudo-$F_{(2,102)} = 1.87$, $p < 0.05$). Pair-wise tests revealed that the three sites differed each other in both sampling periods, and, in turns, the two periods were significantly different in all sites. Nevertheless, site seems the main factor discriminating the three groups in the CAP ordination (Fig. 3). In particular, Low pH is located on the down-right side of the graph, Ctrl 2 on the up-left side and Ctrl 1 interspersed between the other two sites, with no evident separation between periods within each site. Vectors superimposed onto the graph, showed that the main species contributing to the ordination were *C. chromis, C. julis* and *S. tinca* for samples from Low pH and Ctrl 1, and *D. vulgaris* and *T. pavo* mainly for samples from Ctrl 2. Furthermore, SIMPER analysis highlighted the lowest dissimilarity between Low pH and Ctrl 1, and the highest dissimilarity between Low pH and Ctrl 2, due to the species *C. chromis*, *S. salpa*, *D. vulgaris* and *T. pavo*. On the other hand, the dissimilarity between the two controls was due mainly to *C. chromis*, *D. vulgaris* and *S. ocellatus* (Table 2).

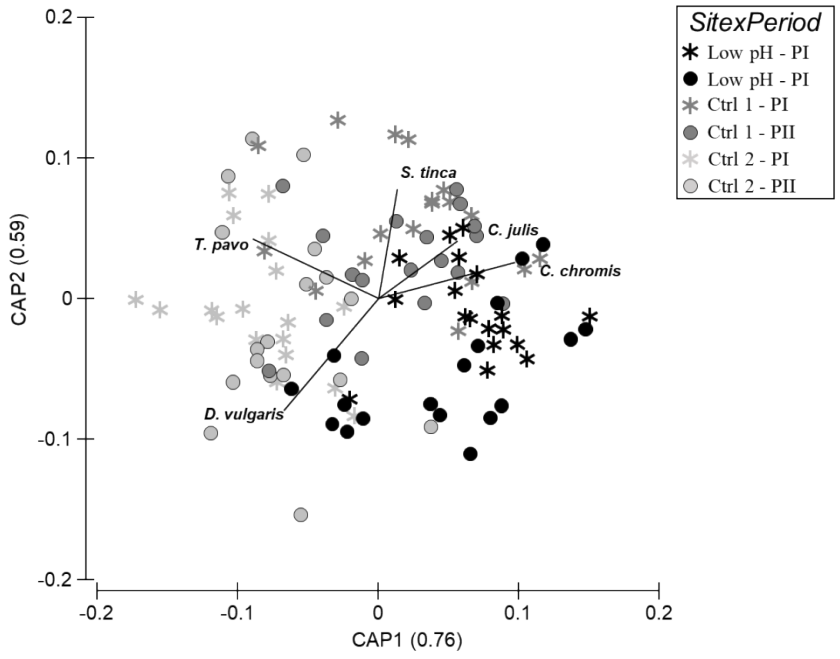


Fig. 3 - Canonical analysis of principal coordinates (CAP) of abundance of the most abundant species (> 2%) in the three sites (Low pH, Ctrl 1 and Ctrl 2) and the two periods (PI and PII). Vectors of the species contributing most to the ordination (Pearson correlation > 0.4) are superimposed. See the annex I for the whole scientific name of the species. The correlation of each axis with the resulted ordination is also indicated.

Species	Contr. %	Av. Ab.		Contr. %	Av. Ab.		Contr. %	Av. Ab.	
		Low pH	Ctrl 1		Low pH	Ctrl 2		Ctrl 1	Ctrl 2
C. chromis	17.32	1.35	1.33	15.79	1.35	0.27	14.8	1.33	0.27
C. julis	8.53	1.27	1.25	9.07	1.27	0.79	9.29	1.25	0.79
D. vulgaris	7.21	0.49	0.30	13.38	0.49	1.19	12.95	0.30	1.19
M. surmuletus	6.76	0.39	0.32	6.68	0.39	0.37	6.07	0.32	0.37
O. melanura	8.20	0.02	0.71	5.92	0.02	0.50	9.84	0.71	0.50
S. ocellatus	12.23	0.47	0.92	8.01	0.47	0.40	12.00	0.92	0.40
S. salpa	12.77	1.08	0.27	14.00	1.08	0.77	9.69	0.27	0.77
S. tinca	10.70	0.58	1.03	8.78	0.58	0.57	9.69	1.03	0.57
T. pavo	7.30	0.18	0.56	11.02	0.18	0.98	9.13	0.56	0.98
	Av. Dis. = 61.59			**Av. Dis. = 67.41**			**Av. Dis. = 66.42**		

234 **Table 2** - SIMPER analysis showing fish species contributing most to the dissimilarity among sites and average
235 abundance at each site (Low pH, Ctrl 1 and Ctrl 2). Av. Ab.: Average abundances; Contr.%: dissimilarity contribution;
236 Av. Dis.: Average dissimilarity.
237

238 Fish grouped in size-classes differed among sites (N_{small}: Pseudo-$F_{(2,102)} = 3.20$, $p < 0.01$; N_{medium}:
239 Pseudo-$F_{(2,102)} = 4.58$, $p < 0.001$; N_{large}: Pseudo-$F_{(2,102)} = 5.20$, $p < 0.001$) and between periods (N_{small}:
240 Pseudo-$F_{(1,102)} = 7.88$, $p < 0.001$; N_{medium}: Pseudo-$F_{(1,102)} = 2.61$, $p < 0.05$; N_{large}: Pseudo-$F_{(1,102)} =$
241 2.50, $p < 0.05$). Pair wise tests showed that each class differed among the three sites, in both periods.
242 Likewise CAP ordination of the whole assemblage, CAP of size-class abundance did not highlight
243 any temporal separation, but partial spatial separations. In more detail, small and medium-sized fish
244 from Low pH and Ctrl 1 showed a partial overlap in the centre-right side of the graphs, while Ctrl 2
245 laid in the centre-left side (Fig. 4a, b). Large fish from the three sites were even more distinct along
246 the first axis (Fig. 4c). Moreover, the main species contributing to discriminate the sites were *C. julis*
247 and *D. vulgaris* for small-sized fish, *D. vulgaris* and *S. ocellatus* for medium-sized fish, and *C.*
248 *chromis* and *C. julis* for large-sized fish.
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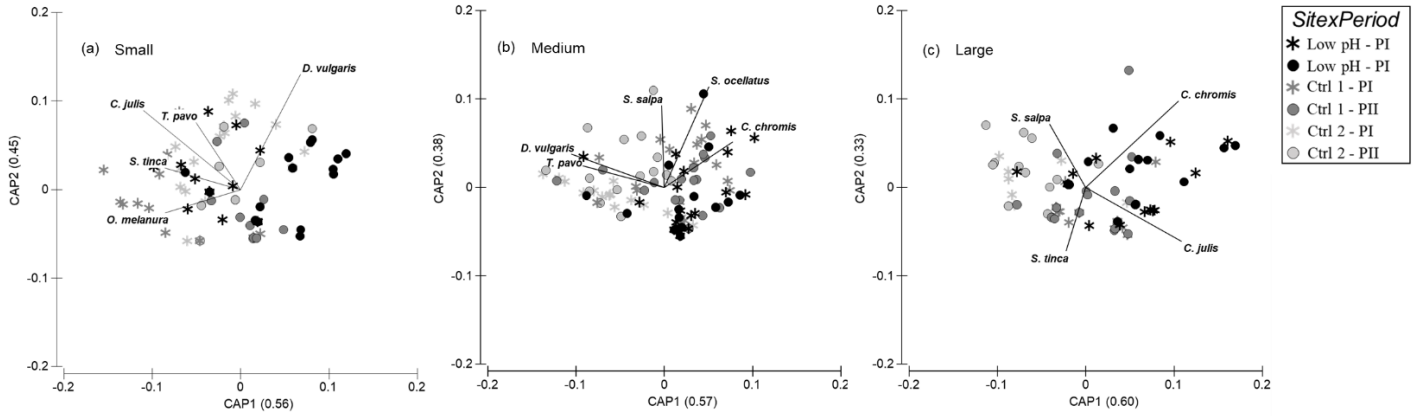


Fig. 4 - Canonical analysis of principal coordinates (CAP) of density of the most abundant species (> 2%) grouped per size-class: a) N_{small}, b) N_{medium} and c) N_{large} in the three sites (Low pH, Ctrl 1 and Ctrl 2) and the two periods (PI and PII). Vectors of the species contributing most to the ordination (Pearson correlation > 0.4) are superimposed. See the annex I for the whole scientific name of the species. The correlation of each axis with the resulted ordination is also indicated.

Fish biodiversity, in terms of species richness (S) and total abundance (N), showed different patterns when comparing sites and periods. Overall, S differed among sites (Pseudo-$F_{(2,102)} = 4.71$, $p < 0.05$) and periods (Pseudo-$F_{(1,102)} = 5.66$, $p < 0.05$), but not for their interaction. In more detail, significantly higher S values were observed in Ctrl 1 than in the other sites, and in Period I than in Period II (Fig. 5a). Despite the high spatial variability of N in Period I, any significant difference was detected, nor clear spatial or temporal patterns (Fig. 5b). Results of the regression analysis between fish diversity (S and N) and environmental variables (*C. nodosa* shoot density, leaf canopy height, pH) revealed a significant positive relationship only between both S and N and leaf canopy height (S: $n = 108$, $r = 0.22$, $p < 0.05$; N: $n = 108$, $r = 0.33$, $p < 0.01$).

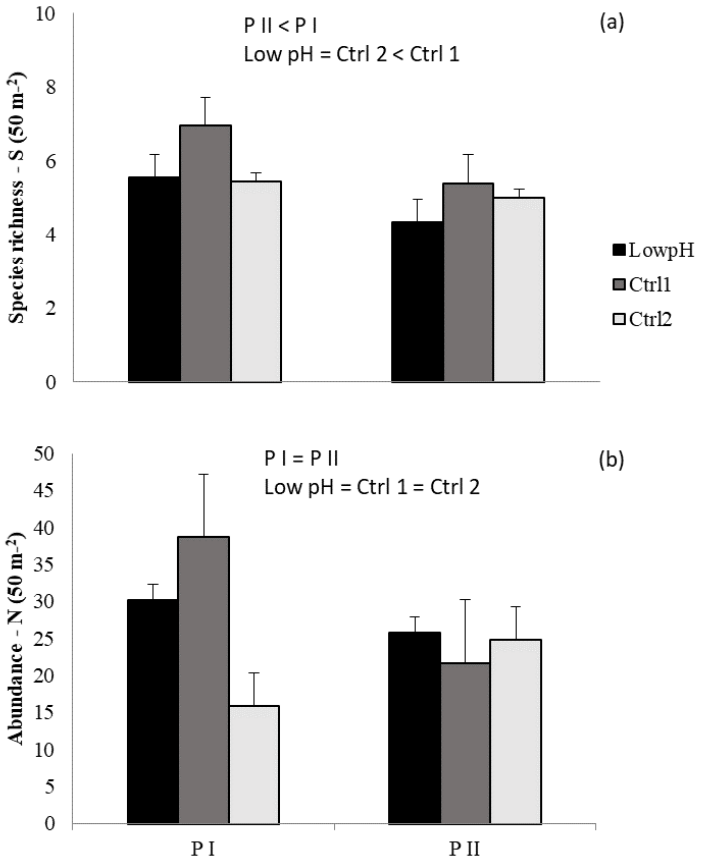
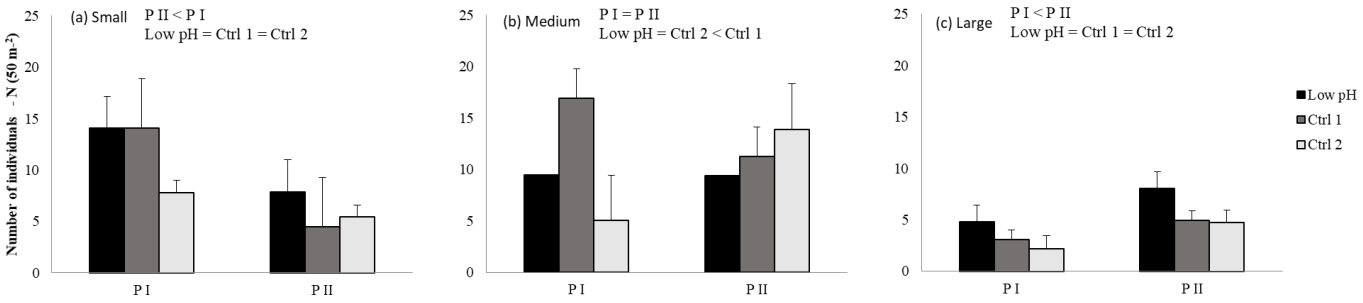


Fig. 5 - Mean value (±SE) of a) species richness (S) and b) abundance (N) of fish in the three sites (Low pH, Ctrl 1 and Ctrl 2) and the two periods (PI and PII). Results of univariate permutational ANOVA pair-wise tests are also showed.

As regards size-class abundance (N_{small}, N_{medium}, N_{large}), small- and large-sized individuals showed contrasting temporal trends (Fig. 6a, c): N_{small} decreased significantly from Period I to Period II (Pseudo-$F_{(1,102)} = 4.88$; $p < 0.05$), when N_{large} increased (Pseudo-$F_{(1,102)} = 5.07$; $p < 0.05$). Abundance of medium-sized fish, N_{medium}, did not differ between periods, but it was higher in Ctrl 1 than in the other sites (Pseudo-$F_{(2,102)} = 5.43$; $p < 0.01$) (Fig. 6b). Moreover, likewise total abundance N, N_{small} was positively correlated with *C. nodosa* leaf canopy height (n= 108, r = 0.37; $p < 0.001$)



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278 **Fig. 6** – Size-class abundance (N) (±SE) of a) small (N_{small}), b) medium (N_{medium}) and c) large (N_{large}) fish in the three sites
279 (Low pH, Ctrl 1 and Ctrl 2) and the two periods (PI and PII). Results of univariate permutational ANOVA pair-wise tests
280 are also showed.
281

282

283 **Discussion**

284 The forecasted decrease in ocean pH over the next decades (IPCC, 2014) is predicted to negatively
285 affect marine ecosystems and biodiversity through direct and indirect effects (Cheung et al. 2009,
286 Sunday et al. 2017). Even though calcareous organisms are considered more susceptible (Doney et
287 al. 2009), also fish are expected to be directly and/or indirectly affected by ocean acidification (OA),
288 with consequences on their distribution and abundance (Perry et al. 2005, Cattano et al. 2018).
289 In the present study, we investigated fish communities in a shallow volcanic CO_2 vent to test the
290 hypothesis of a decline in fish biodiversity and the homogenization of fish assemblage in the low pH
291 site due to the combination of the stress effect of acidic conditions and the expected reduction in
292 seagrass structural complexity. We also hypothesised that juveniles are more affected by low pH due
293 to their higher vulnerability to OA (Dixson et al. 2010, Munday et al. 2010, 2014).
294 Fish diversity, in terms of species richness and abundance, did not show a univocal spatial pattern
295 and significant relationships with pH. However, fish assemblages changed among sites with *Chromis*
296 *chromis* and *Coris julis* higher in both the acidified site and the close control than in the far control,
297 while *Diplodus vulgaris* and *Thalassoma pavo* showed an opposite trend. Moreover, *C. chromis*
298 dominated the assemblages in both low pH site and the adjacent control, together with *Sarpa salpa*
299 in the former site, and *Symphodus ocellatus* in the latter. In contrast, no species clearly dominated the
300 fish assemblage in the far control, except for *S. salpa* in only one of the two investigated periods.

These results suggest that pH played a role in shaping fish assemblage, with a certain species overlap between the low pH site and the close control, although the latter was characterised by boosted species richness.

S. salpa, which was one of the most influent species in discriminating sites (above all low pH *vs*. the two controls) is one of the most important herbivorous fish in the Mediterranean Sea. The minor content of phenolic substances, which are deterrent to herbivory (Arnold et al. 2012) and boost the palatability of *Cymodocea nodosa* long-term exposed to high pCO_2 (Apostolaki et al. 2014), may have led to a higher attractiveness of the acidified site for the high mobile *S. salpa*, which indeed was more abundant than in the close control. This result opens interesting scenarios about the role that herbivores may have in future acidified oceans, because of their control on primary producers through top-down forces (Poore et al. 2012). Nowadays, new insights are emerging about the role of herbivores and their compensatory effect under high CO_2 conditions to maintain communities' resistance to disturbance (Ghedini et al. 2015). Indeed, compensatory dynamics are important stabilising mechanisms through which communities respond to environmental changes (Ghedini et al. 2015).

Similarly to *S. salpa*, the spatial patterns observed for the damselfish *C. chromis,* which showed a clear dominance and a significantly higher abundance in both the low pH and close control, than in the further control site, suggest its high tolerance to OA. Indeed, although a high species-specific sensitivity to elevated CO_2 was observed in other damselfish species (Ferrari et al. 2011), more recent studies highlighted a high resistance of damselfish to OA (Kwan et al. 2017), as well as an improved aerobic performance when exposed to high pCO_2 (Rummer et al. 2013) and an unaffected gregarious behavior (group cohesion) under high pCO_2 levels (Cattano et al. 2019).

On the other hand, among the species that mainly contributed to discriminate sites, particularly interesting is also the case of the two sympatric Labridae, the rainbow wrasse *C. julis* and the ornate wrasse *T. pavo*, which are widespread in the Mediterranean Sea. Here, their different spatial distribution was striking: *C. julis* abundance was similar in the Low pH and the adjacent control site,

and higher than in the far control, while *T. pavo* showed the highest abundance in the far control and the lowest in the acidified site. These findings let hypothesize that *C. julis* is more tolerant to acidified conditions compared to the sympatric species and open a new question regarding the combined effects of warming and acidification. Indeed, recent studies showed that the interaction of these species is potentially exacerbated by seawater warming and that their distribution differs according to water temperature (Milazzo et al. 2013, 2016).

Looking at the whole assemblage, the close control site had a fish community structure partially overlapping both the low pH site and the far control, and showed the lowest dissimilarity with the low pH site. This result, together with the among-site similarity in fish total abundance, provides evidence that fish did not avoid the low pH site, and this is possibly because the boosted food resources (low-order invertebrates; Vizzini et al. 2017) counteract the low pH-induced stress. At the same time, fish may take advantage from their mobility to escape chronic exposure to high pCO_2/low pH values and take shelter in the close control, which may represent an adjacent 'recovery area'. This hypothesis is supported also by the high percentage of invertivorous fish (~ 75 %) in the low pH site and the similar isotopic ($\delta^{13}C$, $\delta^{15}N$) composition and niches of fish (Mirasole, unpublished data) between the low pH site and the close control site. The high cost for the acid-base regulation under acidified conditions may be indeed compensated by the higher prey availability associated to macrophytes in the acidified area (Vizzini et al. 2017), corroborating the results found by Nagelkerken et al. (2015; 2017) where the increase in fish density was driven by higher resource abundances.

When explaining the overall similarity of fish assemblage between the low pH and adjacent control site, natural fluctuations of pH must be also taken into account. Due to the geo-morphological settings of the Levante Bay, acidified water masses mostly run parallel to the northern shoreline of the bay, when predominant winds belong to north-western sectors (Boatta et al. 2013). However, the pH gradient created by the primary vent along the northern cost of the bay is reduced or deleted when wind direction is from south-east (Sirocco wind). As suggested by Kroeker et al. (2012), it is possible

353 that natural fluctuations in carbonate chemistry in vent areas allow organisms to better tolerate, or
354 even adapt to, these particular conditions. Indeed, laboratory studies found negative effects on
355 organisms mainly when there is a sharp and constant reduction in pH, without time of adaptation
356 (Ishimatsu et al. 2008). Evolutionary mechanisms may help organisms to adapt from one generation
357 to another, but this mechanism implies genetic variation (Crozier & Hutchings, 2014).
358 Transgenerational acclimation occurs when the environment experienced by parents influences the
359 performance of offspring (Allan et al. 2014, Sunday et al. 2014, Cattano et al. 2016) showing that
360 adaptive plasticity mechanisms can have a crucial role for fish, improving their resilience to
361 environmental change (Munday 2014, Fox et al. 2019). However, on the other hand, it has been
362 already suggested that population replenishment may occur from "non-acidified populations"
363 (Kroeker et al. 2012, Munday et al. 2014). Indeed, most fish species analysed in the present study
364 have a long pelagic larval stage ranging between 10 and 30 days (Raventos & MacPherson, 2001). In
365 this case, plastic responses in physiology, morphology, or behaviour may help maintaining fitness in
366 a new stressful environment and take less time (days to months, or within a life stage). Moreover, a
367 meta-analysis on different taxonomic groups highlighted that highly mobile organisms (i.e. fish or
368 crustaceans) with developed intracellular/extracellular pH regulatory mechanisms may be more
369 resilient to OA (Kroeker et al. 2013).
370 Considering fish size-class, only sub-adults showed among-site differences, with significantly higher
371 abundance in the close control site than in the acidified and in the far control site. In contrast, the
372 similar abundance of juveniles across sites rules out the expected negative effects of low pH values
373 on fish early life stages, as previously found both in laboratory experiments (Baumann et al. 2011)
374 and in field studies (Rossi et al. 2016). The contrasting results with literature may be due to a species-
375 specific vulnerability (Ferrari et al. 2011) and/or constrained experimental conditions, such as limited
376 exposure time and high pCO_2 levels (Ishimatsu et al. 2008), and suggest that, contrarily to what
377 expected, juveniles may cope with OA natural conditions. Recent studies conducted in the same area

of Vulcano island, highlighted bigger sizes (Cattano et al. 2017) and larger otoliths (Di Franco et al. 2019) in *S. ocellatus* juveniles, probably resulting in higher survival rates.

As regards the relation between fish assemblage and seagrass meadows, species richness, total and juvenile abundances correlated positively only with *C. nodosa* leaf canopy height, which however did not change between low pH site and controls. This pattern confirms the role of seagrass meadow structure in supporting fish diversity, and, in particular, juveniles (Guidetti & Bussotti, 2000, 2002, Bell & Westoby, 1986, Hori et al. 2009), regardless of pH conditions. On the other hand, consistently with Apostolaki at al. (2014), *C. nodosa* shoot density was overall lower in the Low pH site, and in the first sampling period (i.e. autumn) when fish species richness reached higher values. These results may indicate a stronger effect of low pH conditions on *C. nodosa* density in autumn/winter when the seagrass is featured by low structural complexity (Guidetti & Bussotti, 2000; Cancemi et al. 2002), and suggests a lower effect of density than leaf canopy height in shaping fish diversity, accordingly with the literature (Bell & Westoby, 1986, Hori et al. 2009).

Overall, the patterns in species distribution and fish diversity observed in this study were not consistent with those found in another shallow CO_2 vent in the southern hemisphere (New Zealand), where species richness dropped in the acidified site compared with the control site, due to a strong homogenization of community structure (Nagelkerken et al. 2017). Nagelkerken et al. (2017) found a reduction of predatory pressure coupled with an increase of behaviourally dominant fish and a loss of local biodiversity. This pattern was also attributed to the OA-mediated enhancement of food resources, which balanced the stressful conditions at the vent site. Here we do not find a loss in species richness, nor a decrease of fish abundance, although a few species were prevalent in the low pH site. The dissimilar results may be due to the different habitat use of the fish assemblages. Indeed, the different mobility and habitat use of the fish addressed, highly territorial benthic fish in Nagelkerken et al. (2017) *vs*. nekto-benthic fish in this study, may explain the contrasting results. On the other hand, in another CO_2 vent (Papua New Guinea), consistently with the present study, diversity and community structure of the reef nekton-benthic fish differed little between acidified and control sites

(Munday et al. 2014). If Munday et al. (2014) attributed this pattern to a different habitat complexity and composition in corals, rather than to a direct effect of high CO_2, here pH was an important driver of fish assemblage, as its spatial and temporal structure was independent on that of seagrass meadows. In conclusion, our findings suggest that near-future OA predictions (pH = 7.8 in the year 2100, IPCC 2014) may influence fish assemblages. In general, the nekto-benthic fish studied were able to cope with OA, form well-structured fish assemblages, despite dominated by high-tolerant species, and seem to benefit from an adjacent 'recovery area'. Therefore, because of their behavioural and physiological features (mobile habitus, capacity of acid-base regulation) and indirect effects of high pCO_2/low pH in CO_2 vents (i.e. greater food availability), fish can balance the higher energetic cost to live under high pCO_2 environments without major changes in community structure. Being habitat use crucial in influencing fish response to environmental stressors, further investigations are needed to assess if this pattern is exacerbated in highly territorial benthic fish exposed to high pCO_2/low pH conditions. Also, further data are needed to forecast the species-specific responses of other seagrass systems and the resulting impact on associated fauna. Finally, this study confirms the need to use *in situ* experiments and naturally acidified environments to evaluate the responses of whole communities long-term exposed to low pH conditions.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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602 **Annex I** - List of all fish species censused in the three sites (Low pH, Ctrl 1 and Ctrl 2). Trophic group and total relative
603 abundance (%) of each species in the two periods (PI and PII) are indicated. Trophic groups: Invertivore (INV);
604 Detritivore (DET); Planktivore (PLK); Small piscivore (PISC); Herbivore (HER).

Family	Species	Trophic Group	Low pH		Ctrl 1		Ctrl 2	
			PI	PII	PI	PII	PI	PII
Labridae	*Coris julis*	INV	7.7	3.5	6.8	4.5	6.3	1.9
	Labrus viridis	INV	<2	<2	<2	<2	<2	<2
	Symphodus cinereus	INV	<2	<2	<2	<2	<2	<2
	Symphodus doderleini	INV	<2	<2	<2	<2	<2	<2
	Symphodus ocellatus	INV	7.2	<2	15.6	2.5	<2	7.2
	Symphodus mediterraneus	INV	<2	<2	<2	<2	<2	<2
	Symphodus roissali	INV	<2	<2	<2	<2	<2	<2
	Symphodus rostratus	INV	<2	<2	<2	<2	<2	<2
	Symphodus tinca	INV	3.1	<2	6.1	2.7	3.5	2.3
	Thalassoma pavo	INV	<2	<2	2.2	<2	8.4	4.0
Mugilidae	*Mugil cephalus*	DET	<2	<2	<2	<2	<2	<2
Mullidae	*Mullus surmuletus*	INV	<2	4.5	<2	<2	2.3	<2
Pomacentridae	*Chromis chromis*	PLK	12.1	9.5	12.0	12.2	<2	4.7
Serranidae	*Serranus scriba*	PISC	<2	<2	<2	<2	<2	<2
Sparidae	*Oblada melanura*	PLK	<2	<2	9.6	<2	3.6	3.6
	Diplodus annularis	INV	<2	<2	<2	<2	<2	<2
	Diplodus sargus	INV	<2	<2	<2	<2	<2	<2
	Diplodus vulgaris	INV	<2	3.1	<2	<2	8.0	8.0
	Sarpa salpa	HER	16.4	21.4	<2	4.7	<2	26.2

605