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Mollusc epifaunal assemblages are simplified due to habitat shifts under ocean acidification

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ABSTRACT

Ocean acidification can have profound effects on marine organisms, particularly those that rely on calcium carbonate for shell and skeleton formation, resulting in structural changes to marine ecosystems. Here, we contrast the structure of marine mollusc communities (epifauna) associated with an abundant shallow-water macroalga, *Halopteris scoparia*, in an area with seawater carbonated by natural CO₂ seeps and three reference sites, off the Azores archipelago. Epifaunal mollusc abundance and diversity were significantly lower at the CO₂ seep compared to reference sites whilst species accumulation curves and Jaccard multivariate analyses showed that the mollusc assemblage was consistently less diverse at the CO₂ seep. Most of the abundant epifaunal species that were present at the CO₂ seep were also found at reference sites, but less common or rare species were generally absent from the former. We conclude that while some molluscs are likely to cope with ocean acidification, the overall biodiversity of epifaunal molluscs will be simplified under these conditions in a future ocean.

1. Introduction

Many macroalgal species have a diverse and abundant assemblage of small motile invertebrates (Sánchez-Moyano et al., 2000; Costa and Ávila, 2001; Kelaher et al., 2001; Kelaher and Castilla, 2005; Berthelsen et al., 2015; Navarro-Barranco et al., 2018), which make an important contribution to secondary production (Taylor, 1998) and play an important role as food source for higher trophic levels (e.g. fish) (Wellenreuther and Connell, 2002). Such epifaunal communities contribute to nutrient cycling, energy transfer, and overall ecosystem health (Brodie et al., 2014). Invertebrate assemblages vary among host macroalgal species (Bruno et al., 2005; Navarro-Barranco et al., 2018) and respond to spatial variations in environmental conditions (Sánchez-Moyano et al., 2000; Matias, 2013; Berthelsen et al., 2015). Macroalgal associated communities are useful for investigating the effects of ecological processes such as species-area relationships (Matias et al., 2011), the impact of species invasions (Vásquez-Luis et al., 2012) and biodiversity and ecosystem function relationships (Bruno et al.,

2005).

Increasing atmospheric CO₂ concentrations are decreasing surface ocean pH (Jiang et al., 2023), a process named ocean acidification. This change in pH can have profound effects on marine organisms, particularly those that rely on calcium carbonate for shell and skeleton formation, by affecting their viability (Shirayama and Thornton, 2005; Martins et al., 2021), which can, in turn, result in cascading structural changes in marine ecosystems (Doney et al., 2020) and the services they provide (Hall-Spencer and Harvey, 2019).

Organisms with calcareous structures (e.g. coralline algae, molluscs) are expected to be more vulnerable to ocean acidification via interference of acidified conditions on calcification and dissolution processes (Kroeker et al., 2011; Rodolfo-Metalpa et al., 2011; Ricevuto et al., 2012). For instance, Couto et al. (2012) reported abnormal shells of gastropod limpets (*Patella* spp.) living near shallow-water hydrothermal vents, Garilli et al. (2015) found dwarfing (the so-called 'Lilliput effect') in two gastropod species as a response to carbonated seawater, whilst Martins et al. (2021) found that, despite high settlement rates of a

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numerically dominant bivalve under acidified conditions, the population failed to grow with few individuals reaching adult size. In contrast, non-calcareous vegetation (macroalgae and seagrasses) may be expected to thrive under more acidified ocean conditions by taking advantage of the increased CO₂ availability (e.g., Hall-Spencer et al., 2008) although there is still debate regarding the suitability of venting sites for these photosynthetic organisms (see Olivé et al., 2017; Foo et al., 2018).

Marine shallow-water CO₂ seeps are natural sources of carbon dioxide that emerge from the seafloor, creating areas of elevated pCO₂ (Cardigos et al., 2005; Aiuppa et al., 2021). These seeps can be used as analogues for the effects of chronic ocean acidification at scales that are difficult to mimic in mesocosms (Hall-Spencer et al., 2008). Provided that care is taken to account for potential confounding effects (e.g. presence of metals and H₂S, Vizzini et al., 2013; Mishra et al., 2020), the use of natural CO₂ seeps can show how ocean acidification leads to ecosystem simplification and the consequent restructuring of marine communities, offering a glimpse into the future of our ocean under increased atmospheric CO₂ levels (Ávila et al., 2007; Hall-Spencer et al., 2008; Fabricius et al., 2015; Agostini et al., 2018, 2021).

In this study, we use an asymmetrical sampling design to contrast the structure of the marine molluscs (gastropods and bivalves) associated with an abundant shallow-water macroalga *Halopteris scoparia* (Linnaeus) Sauvageau, 1904 in areas with naturally carbonated seawater (associated with the presence of sublittoral CO₂ seeps) and reference conditions, off the Azores Archipelago. Considering the known effects of acidified seawater on species with calcareous structures, we hypothesize that macroalgae would support an impoverished assemblage of shelled molluscs (in terms of density and richness), in areas influenced by CO₂ seeps.

2. Materials and methods

2.1. Study sites

In the Azores, volcanic CO₂ seeps occur around several islands and seamounts (Ávila et al., 2004; Cardigos et al., 2005; Couto et al., 2015; Viveiros et al., 2016; Wallenstein et al., 2009, 2013). At São Miguel Island, Ponta da Lobeira (Ribeira Quente) is one such site, where a shallow-water (2–7 m depth), accessible and strong degassing CO₂ seep is located (Fig. 1).

In this area, carbon dioxide accounts for >99 mol% of gas composition, with low concentration of other gases (helium, hydrogen sulfide, hydrogen and methane) (Martins et al., 2021). Seawater temperature and salinity are not locally affected (Martins et al., 2021).

The seep and our three selected reference sites (Fig. 1) are all located off the southern shore of São Miguel Island and characterized by medium-to large-sized boulders, which are replaced by sandy bottoms at around 5 m depth. All sites are exposed to similar environmental

conditions (e.g. wave action, currents, light, salinity, temperature) except for the presence of a CO₂ seep at Ribeira Quente.

Halopteris scoparia is a conspicuous marine brown macroalga in shallow-water habitats around the Azores (e.g. Neto, 2001; Martins et al., 2013; Sangil et al., 2018) and was abundant at all the sampled sites. Previous studies have shown that *Halopteris scoparia* can sustain diverse invertebrate assemblages (Sánchez-Moyano et al., 2000; Costa and Ávila, 2001; Vázquez-Luis et al., 2012; Navarro-Barranco et al., 2018; Ávila et al., 2024).

2.2. Sampling design

We modified a Before-After Control-Impact (Underwood, 1991, 1992, 1993, 1994) study design to assess whether there were consistent differences the CO₂ seep and several reference sites following Glasby (1997) to avoid spatially confounding ‘pseudo-replication’ (Hurlbert, 1984). In our situation only post-impact data existed (an After-Control-Impact design; see Glasby, 1997). Although other degassing seeps occur in the archipelago, the one at Ribeira Quente is the most extensive shallow-water degassing site occurring in the Azores. Other degassing seeps in the region are either small in area (e.g. Porto Formoso), much deeper (e.g. Ponta da Espalamarca, Faial at 40 m depth, Martins et al., 2021) or in areas with substantial urbanisation, human presence and influenced by changes in temperature (e.g. hot baths at Ferraria). As such, Ribeira Quente offers a unique opportunity in the entire archipelago for such studies by providing stable conditions over a large enough area which is amenable to sampling and experimental work in shallow-waters that are easily accessible by road. The uniqueness of these conditions makes it difficult, if not impossible, to find seeps in the region without major differences in factors such as light, depth, substratum and temperature. Hence our choice to use an asymmetrical sampling design. Moreover, a gradient sampling design, although commonly used in pollution studies where there is often a distinct source of pollution (e.g., sewage outfall), was probably not the best option in this region, since degassing is diffuse across a stretch of the coast, making it practically impossible to determine a number of sampling sites over a gradient, whilst keeping all the other variables (e.g., substratum, depth).

Here, we compared a CO₂ seep (off Ribeira Quente) and three reference sites (R1: São Roque, R2: Caloura and R3: Ponta Garça; Fig. 1). Sampling was conducted during July 2020 across the four sites. At each site, 10 replicates of *Halopteris scoparia* individuals were collected by SCUBA diving by carefully placing 200 mL plastic jars on top of individual algae, which was removed by sliding a spatula underneath and sealing the lid. Sampled individuals of each alga were haphazardly selected at least 3 m apart from each other at depths of 3–5 m. Additionally, five water samples (250 ml) were collected in the vicinity of the first five seaweed samples. This was performed prior to the removal of individuals to avoid the suspension of particles.

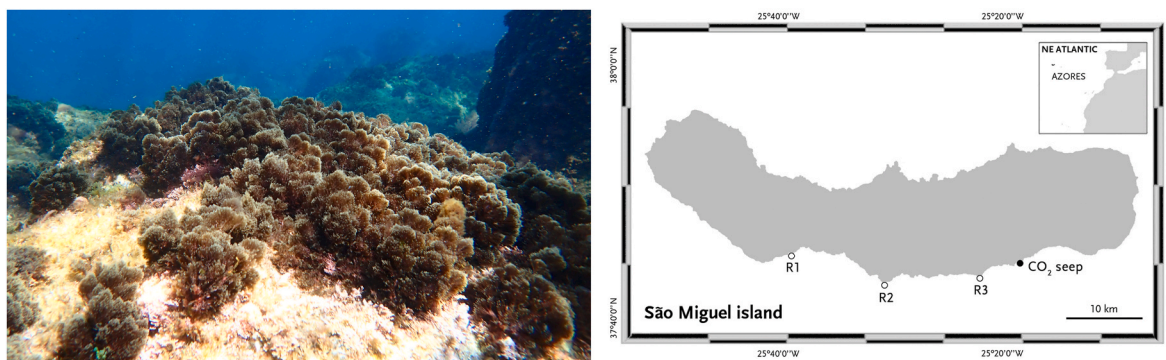


Fig. 1. A typical stand of the macroalga *Halopteris scoparia* off Sao Miguel Island, in the Azores and (right) sampling sites (R1: São Roque; R2: Caloura; R3: Ponta Garça; CO₂ seep: Ribeira Quente).

Water samples were kept in dark conditions, brought to the laboratory and their carbonate chemistry measured immediately. For each sample, a bench meter (HI-2002 Edge® pH Meter by Hanna Instruments) was used to record pH, conductivity and temperature set using a TRIS seawater buffer, supplied by A. Dickson. Additionally, samples for total alkalinity (TA) (~80 ml) were filtered through a 0.22 µm membrane and measured within 48h by potentiometric titration, following Dickson et al. (2003), and using a Metrohm Titrino Plus 848 equipped with a 869 Compact Sample Changer, calibrated with certified reference material supplied by A. Dickson. Furthermore, about 60 ml of water sample was filtered through a Minisart® syringe filter membrane (0.22 µm) and stored at -20 °C until the analysis of the dissolved inorganic nutrients. Concentrations of nitrate, nitrite, silicate and phosphate were measured following Hansen and Koroleff (1999), using a spectrophotometer (Cary 60 Probe, Varian). The carbonate chemistry parameters were calculated from temperature, salinity, silicate, phosphate, total pH and TA using the program CO₂sys (Lewis and Wallace, 1998), with the equilibrium constants determined by Mehrbach et al. (1973) as refitted by Dickson and Millero (1987).

Biological samples were gently washed on a 500 µm mesh, preserved in 70 % ETOH and stored until analyses. Upon inspection, the alga was removed, and the retained invertebrates were washed into a Petri dish and examined under a dissection microscope. Individuals were rapidly sorted into large taxonomical groups (e.g. amphipods, gastropods, bivalves). The malacofauna (gastropods and bivalves), for which there was taxonomical expertise among the authors, were identified to species level and counted. The remaining assemblage was preserved in 70 % ETOH and stored. The alga was oven-dried for 48 h at 60 °C, after which it was weighed.

2.3. Data analysis

A 1-way asymmetrical Analysis of Co-Variance (ANCOVA) was used to compare the structure of the malacofaunal assemblage at the CO₂ seep and the three reference sites. A general model of the analysis as well as the calculation of the expected mean squares and the consequent determination of denominators for each test was adapted from Terlizzi et al. (2005) and is presented in Table S1 (see supplementary online material). Generally, the factor 'site' was partitioned into two components: one contrasting the CO₂ seep and reference sites (RQ vs. R's) and the other contrasting reference sites among each other (R's). This was also true for the residuals associated with variability among quadrats, respectively within RQ and R's (see Terlizzi et al., 2005 for a detailed description of these procedures). Since there was significant variation in algal biomass among sites (ANOVA $F_{3,36} = 4.66, P = 0,008$), we included algal biomass as a covariate for standardization in the above analyses. Prior to analysis, Cochran's test was used to check for problems of heteroscedasticity and data were transformed where appropriate (Underwood, 1997). In addition, heterogeneity of slopes was tested by crossing both covariates with the other factor(s) and checking for significant interactions (Quinn and Keough, 2002). The inclusion of algal biomass as a covariate in the analysis increases its power when the relationship between the variable and the covariate is significant (Quinn and Keough, 2002), but not otherwise. As such, where the covariate algal biomass was not significant, the same general asymmetrical design was tested without the covariate, with an ordinary asymmetrical analysis of variance (ANOVA). This was used to compare the richness (number of species per sample), total numbers of individuals as well as the abundances of the most common species individually (those with >10 individuals).

Similarly, the same design was applied to a Permutational Analysis of Variance (PERMANOVA) to test for differences in the assemblage as a whole. This analysis was run twice: once on the untransformed Bray-Curtis dissimilarity matrix, and again on the Jaccard dissimilarity matrix; the rationale being that the former (Bray-Curtis) is more influenced by changes in abundances of species, whereas the latter (Jaccard)

completely discards abundance data and is therefore influenced only by changes in species composition.

Since a substantially lower number of individuals were generally observed in samples from the CO₂ seep, which may impact estimates of sample richness (Gotelli and Colwell, 2001), we used the software EstimateS (Colwell, 2013) to compute species accumulation curves based on the individual-based rarefaction curves calculated for each sample.

3. Results

3.1. Water chemistry

Water samples collected at the CO₂ seep had a much greater concentration of pCO₂, and a lower pH_t than the reference sites (Table 1). These values are in line with those measured by Martins et al. (2021) indicating that seawater carbonate chemistry conditions at the CO₂ seep are persistent. Dissolved oxygen was rather similar among all the sampled sites, with the venting site showing values within the range of those found in reference sites (Table 1).

3.2. Biological assemblages

A total of 3515 mollusc individuals were counted and identified to 17 species: 13 gastropods and 4 bivalves (see Table S2, supplementary online material). Overall, the most numerous species were the gastropods *Setia subvaricosa* (Gofas, 1990) (67 % of the total number of individuals), *Tricolia azorica* (Dautzenberg, 1889) (17 % of the total number of individuals) and *Skeneopsis planorbis* (O. Fabricius, 1780) (9 % of the total number of individuals). The richness of the mollusc assemblage associated with *H. scoparia* s in the CO₂ seep was significantly lower than that in reference sites (Fig. 2a, Table 2), with mean number of species of 2.7 ± 0.4 (mean \pm SE) per sample compared to 5.6 ± 0.2 at the reference sites. The number of individuals per sample was also significantly lower at the CO₂ seep (36.3 ± 7.7 , Reference sites: 105.1 ± 11.9) (Fig. 2b–Table 2). In both cases, there was significant and positive variation associated with the covariate (sample's algal biomass). Species accumulation curves showed that a clear asymptote was not attained in most sites (Fig. 3). In addition, the richness of the mollusc assemblage at the CO₂ seep was smaller than that found in the three reference sites across most of the range of number of individuals (Fig. 3).

When considering the individual abundances of the most common species, there was often significant and positive variation in their abundances with algal biomass, except in the cases of *P. vroomi* and *R. ermelindoi*. For these, ANOVA detected a significant greater numbers of *R. ermelindoi* in reference sites (Fig. 4, Table 3), which is not surprising since it was absent from the CO₂ seep samples. No significant variation

Table 1

Carbonate chemistry for each sampling site, assuming 2 µmol kg⁻¹ of silicate and 0.5 µmol kg⁻¹ phosphate. Data refer to the average and corresponding standard deviation (in brackets) of 5 replicates per site sampled off Sao Miguel Island, Azores. TA: total alkalinity; DIC: dissolved inorganic content; DO: Dissolved oxygen.

Site	TA (µmol kg ⁻¹)	DIC (µmol kg ⁻¹)	pCO ₂ (µatm)	pH _t	DO (mg L ⁻¹)
R1	2429.4 (± 4.3)	2171.7 (± 4.0)	464.6 (± 2.6)	8.01 (+/- < 0.01)	8.9 (± 0.7)
R2	2422.3 (± 5.0)	2168.9 (± 4.7)	476.6 (± 2.9)	8.00 (± 0.01)	8.5 (± 0.5)
R3	2434.8 (± 6.2)	2157.0 (± 7.3)	435.9 (± 4.5)	8.03 (+/- < 0.01)	9.6 (± 0.4)
CO ₂ seep	2450.1 (± 6.7)	2231.7 (± 8.1)	604.7 (± 16.9)	7.91 (± 0.01)	8.5 (± 0.2)

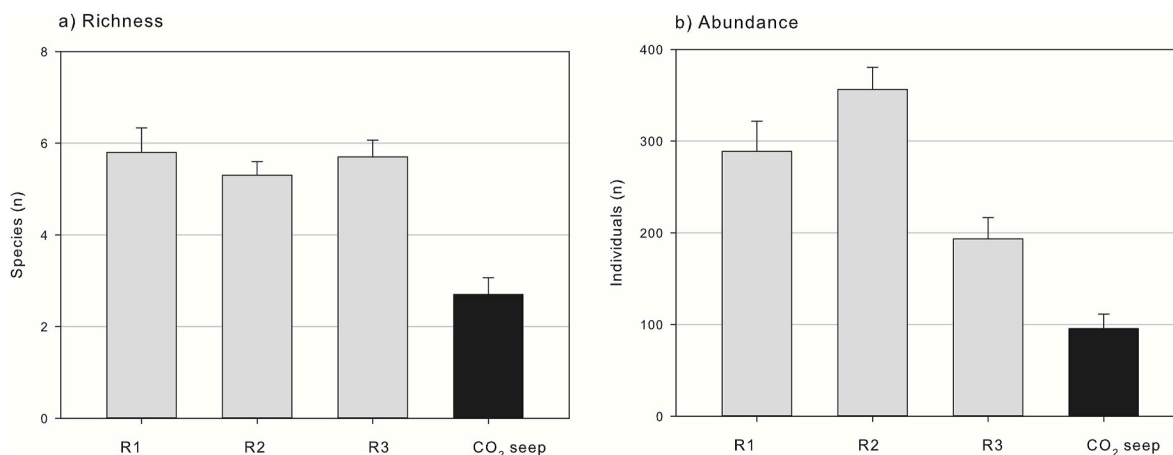


Fig. 2. Mean (\pm SE, $n = 10$) richness (a) and abundance of individuals (b) for mollusc assemblages associated with macroalga *Halopteris scoparia* samples collected using 200 ml jars across reference sites (R1, R2 and R3) and a CO₂ seep off Sao Miguel island, the Azores.

Table 2

Asymmetrical analysis of covariance comparing the richness and numbers of molluscs per sample inhabiting *Halopteris scoparia* at a CO₂ seep and reference (R's) sites. Algal biomass was used as covariate.

Source	df	Richness		No. individuals	
		MS	F	MS	F
Covariate	1	21.96	50.05***	256.80	154.17***
Site	3	21.98		47.50	
CO ₂ vs. R's	1	60.73	23.27	110.30	6.85*
R's	2	2.61	2.10**	16.10	6.39**
Covariate x Site	3	0.44	0.42	1.67	0.79
Covariate x CO ₂ vs. R's	1	1.16	2.84	2.34	2.69
Covariate x R's	2	0.08	†	1.33	†
Residual	32	1.04		2.11	
Res CO ₂	8	0.41		0.87	
Res R's	24	1.25		2.52	
Transformation		None		sq-rt	
Cochran's test		C = 0.44		C = 0.40	

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, † eliminated from the model ($p > 0.25$, Underwood, 1997).

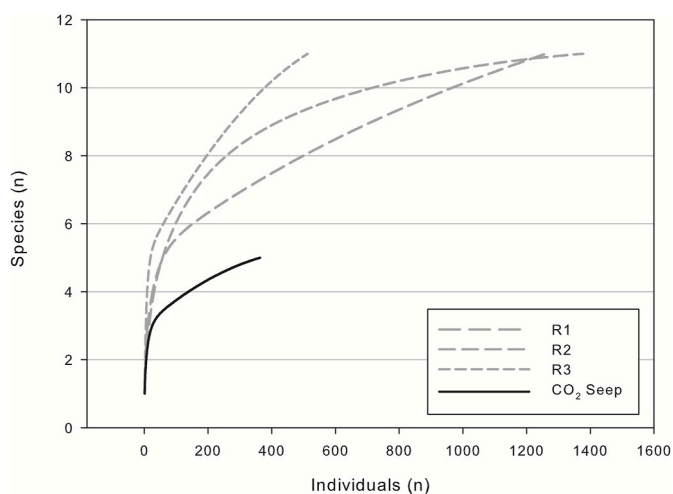


Fig. 3. Species accumulation curves based on the individual-based rarefaction curves calculated for each reference site (R1, R2 and R3) and CO₂ seep.

was found in the numbers of *P. vroomi* between the CO₂ seep and reference sites (Fig. 4, Table 3). For most of the other common species (*S. subvaricosa*, *T. pullus azorica*, *B. nanum*, *E. fulgida* and *S. cingulata*),

there was significant variation with algal biomass, but ANCOVA failed to detect significant variation in their abundances between the CO₂ seep and reference sites (Fig. 4, Table 3), even though their numbers tended to be lower or absent in the CO₂ seep (Fig. 4). In the case of *S. planorbis*, there was a significant interaction between algal biomass and the CO₂ vs R's comparison (Table 3) indicating heterogeneity of slopes between its abundance and algal biomass between the CO₂ seep and reference sites. In addition, there was a significant CO₂ vs R's effect indicating that its abundance was significantly greater in the reference sites (10.00 ± 1.90) compared to the CO₂ seep (0.20 ± 0.13) (Fig. 4, Table 3).

No significant differences were found between the CO₂ seep and reference sites when multivariate analyses were dominated by abundance data (Bray-Curtis), but a significant difference was found when analyses were based on species incidences (Jaccard) (Fig. 5, Table 4), over and above a significant interaction with the covariate. As above, in both cases, there was significant variation explained by the covariate.

4. Discussion

Marine shallow water CO₂ seeps provide invaluable insights into the potential impacts of current climate change and associated ocean acidification on marine ecosystems (Agostini et al., 2021). The effects of elevated pCO₂ on marine community interactions underscore the intricate relationships and dependencies within marine ecosystems (Bibby et al., 2007; Sunday et al., 2017). As the threats of ocean acidification grow, knowledge gained from studying these natural laboratories becomes increasingly needed to inform adaptation and mitigation responses to declining ocean health and resilience (Fabricius et al., 2011; Laffoley et al., 2022; Hudson et al., 2023).

Research at temperate and tropical CO₂ seeps using artificial substrata has shown that calcifying mollusc abundance and diversity falls with increasing acidification (Allen et al., 2017). Raising pH and seawater carbonate saturation levels through photosynthesis may alleviate acidification and benefit calcifying molluscs to such an extent that kelp cultivation may help oyster cultivation (Xiao et al., 2021). Our results show that bivalves and gastropods associated with the macroalga *H. scoparia* collected from a CO₂ seep in the Azores were substantially impoverished compared to reference sites (Fig. 2). Samples from the CO₂ seep tended to have a reduced number of rare species and lower abundances of the dominant ones. Previous studies in the Azores reached similar conclusions (Costa and Ávila, 2001; Ávila et al., 2004, 2007, 2024). Thus, cumulatively, these studies convincingly demonstrate that mollusc assemblages associated with macroalgae at CO₂ seeps support fewer species and reduced abundances of common species. This is consistent with the view that hard-shelled organisms, such as gastropods and bivalves, are negatively affected by the lower carbonate saturation

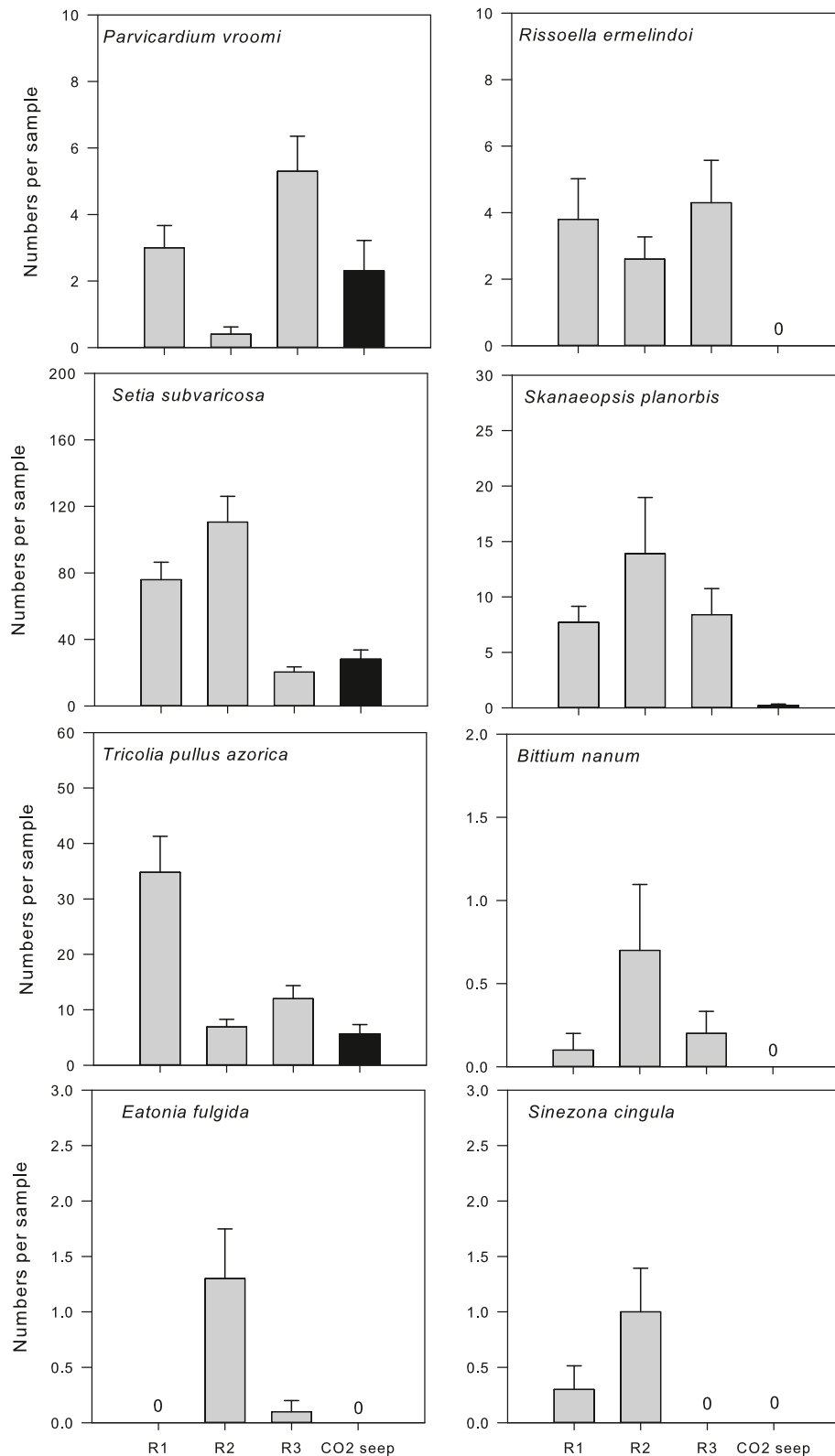


Fig. 4. Mean (\pm SE) abundances of most common species per 200 ml jar seaweed sample in each reference site (R1, R2 and R3) and CO₂ seep. Note scaling differences on y-axis.

conditions found at CO₂ seeps (Shirayama and Thornton, 2005; Couto et al., 2012; Martins et al., 2021; Hall-Spencer et al., 2022).

This reduced diversity found in the CO₂ seep was not just the result of the fewer numbers of individuals counted. When using individual-based rarefaction curves, there was evidence that for a similar number of

individuals, the CO₂ seep still supported fewer species (Fig. 3). This suggests that there is a strong signal of environmental filtering at CO₂ seeps, which is most likely related to the less alkaline (or more acidified) conditions that impacts the assemblage by limiting the numbers of hard-shelled species that can thrive under these conditions. In particular, as

Table 3

Asymmetrical analysis of covariance (or variance) comparing the abundances (per sample) of the most common molluscan species inhabiting *Halopteris scoparia* at CO₂ seep and reference (R's) sites. Algal biomass was used as covariate, where significant. df = degrees of freedom. *p < 0.05, **p < 0.01, ***p < 0.001, † eliminated from the model (p > 0.25, Underwood, 1997).

Source	df	<i>Parvicardium vroomi</i>		<i>Rissoella ermelindoi</i>		<i>Setia subvaricosa</i>		<i>Skeneopsis planorbis</i>	
		MS	F	MS	F	MS	F	MS	F
Covariate	1	–	–	–	–	213.37	51.43***	824.08	12.97**
Site	3	40.97	–	36.89	–	33.04	–	236.88	–
CO ₂ vs. R's	1	2.70	0.04	95.41	12.50**	41.64	1.45	630.37	4502.64***
R's	2	60.10	11.23***	7.63	0.64	28.75	16.21***	40.14	†
Covariate x Site	3	–	–	–	–	4.15	2.83	63.52	†
Covariate x CO ₂ vs. R's	1	–	–	–	–	2.98	0.63	102.54	732.43
Covariate x R's	2	–	–	–	–	4.73	2.67	44.01	†
Residual	32 (36)	6.13	–	8.89	–	1.47	–	69.71	–
Res CO ₂	8 (9)	8.46	–	0.00	–	0.55	–	0.14	–
Res R's	24 (27)	5.35	–	11.86	–	1.77	–	92.90	–
Transformation		none	–	none	–	sqrt(x)	–	none	–
Cochran's		C = 0.45	–	C = 0.45	–	C = 0.44	–	C = 0.77*	–

Source	df	<i>Tricolia pullus azorica</i>		<i>Bittium nanum</i>		<i>Eatonia fulgida</i>		<i>Sinezona cingulata</i>	
		MS	F	MS	F	MS	F	MS	F
Covariate	1	17.21	6.82*	0.14	10.60**	8.50	9.13**	8.98	11.31**
Site	3	34.81	–	0.96	–	2.07	–	0.63	–
CO ₂ vs. R'	1	30.84	0.84	0.79	0.76	1.87	0.86	1.41	1.77
R's	2	36.79	39.63***	1.04	1.52	2.17	3.83*	0.25	†
Covariate x Site	3	2.52	3.10*	0.01	0.03	0.93	2.19	0.79	2.21
Covariate x CO ₂ vs. R's	1	3.07	1.37	0.02	no test	0.53	0.47	0.85	1.12
Covariate x R's	2	2.25	2.42	0.01	†	1.13	2.00	0.76	1.60
Residual	32	0.81	–	0.51	–	0.42	–	0.36	–
Res CO ₂	8	0.47	–	0.00	–	0.00	–	0.00	–
Res R's	24	0.93	–	0.69	–	0.57	–	0.48	–
Transformation		sqrt(x)	–	none	–	none	–	none	–
Cochran's		C = 0.40	–	C = 0.85*	–	C = 0.89*	–	C = 0.70*	–

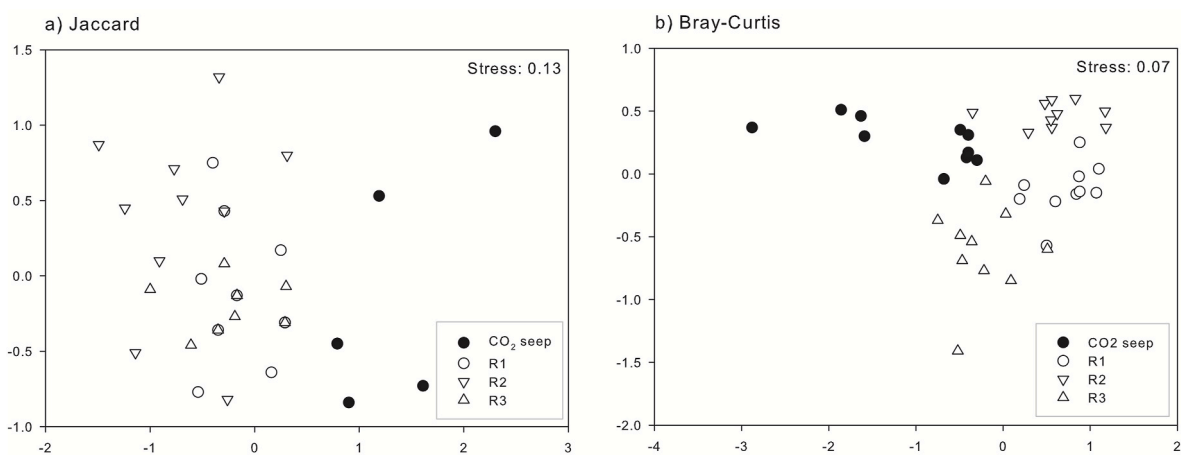


Fig. 5. Non-metric multidimensional scaling of (a) Jaccard and (b) Bray-Curtis similarity matrices for mollusc assemblages across sites.

Table 4

Asymmetrical analysis of permutational Covariance (PERMANCOVA) comparing the structure of mollusc assemblages between CO₂ seep and reference (R's) sites, based on the Bray-Curtis and Jaccard dissimilarity matrices. Algal biomass was used as covariate.

Source	df	Bray-Curtis					Jaccard				
		MS	F	P	Perm	P(MC)	MS	F	P	Perm	P(MC)
Covariate	1	16996.00	41.07	0.001	999	0.001	4032.00	5.25	0.002	999	0.001
Site	3	6182.67	–	–	–	–	6283.67	–	–	–	–
CO ₂ seep vs. R's	1	8548.10	1.70	0.300	24	0.295	13485.00	5.03	0.234	24	0.024
R's	2	4999.95	11.89	0.001	998	0.001	2683.15	3.49	0.001	999	0.001
Covariate x Site	3	3400.67	8.22	0.001	999	0.001	1520.33	1.98	0.017	999	0.001
Covariate x CO ₂ seep vs. R's	1	8275.20	19.60	0.001	999	0.001	3877.20	5.04	0.001	999	0.002
Covariate x R's	2	963.40	2.28	0.010	998	0.021	341.87	0.44	0.933	997	0.923
Residual	32	413.84	–	–	–	–	768.19	–	–	–	–
Res CO ₂ seep	8	388.75	–	–	–	–	766.88	–	–	–	–
Res R's	24	422.21	–	–	–	–	768.63	–	–	–	–

shown by the much lower number of species that was overall found in samples originating from the CO₂ seep (Kroeker et al., 2011; Ricevuto et al., 2012), CO₂ seep sites appear to prevent the occurrence of mollusc species, e.g., *Alvania angioyi*, *Crisilla postrema*, that are common in sites with normal environmental conditions. Moreover, while it has been found that few species can adapt to such conditions, i.e. via the production of thicker shells (Leung et al., 2019) acidified conditions tend to cause reduced shell thickness due to dissolution (Couto et al., 2012; Harvey et al., 2018) and thus limit the ability of species to grow or survive under such conditions (Zhao et al., 2023).

Unlike the study of Allen et al. (2021), who found that the effects of elevated CO₂ on biofilm communities were mostly driven by species turnover (species replacements), in our dataset, all the species present at the CO₂ seep were also found in reference sites, a result that is similar to that of Harvey et al. (2020) looking at the effects of elevated CO₂ on biofilm communities on marine plastic. This suggests that the relative importance of species turnover and nestedness for community assembly vary under elevated CO₂ conditions depending on other local factors.

Our study focused on bivalves and gastropods as these were the groups for which there was taxonomic expertise available within the team. Bringing experts with taxonomic knowledge in other groups (e.g. amphipods, bryozoans) could help assess how general our findings are across a more diverse range of taxa, as done by Hall-Spencer et al. (2022) in the Mediterranean Sea.

CRedit authorship contribution statement

Gustavo M. Martins: Writing – original draft, Visualization, Formal analysis, Conceptualization, Writing – review & editing, Methodology, Investigation. **Javier Herrero:** Writing – review & editing, Investigation, Data curation. **Cristina Canella:** Writing – review & editing, Investigation, Data curation. **Sérgio P. Ávila:** Writing – review & editing, Methodology, Investigation, Data curation. **Afonso Prestes:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Joana Barcelos e Ramos:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Jason M. Hall-Spencer:** Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **João Faria:** Writing – original draft, Visualization, Conceptualization, Methodology, Investigation, Data curation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Gustavo M. Martins reports financial support was provided by Foundation for Science and Technology. Gustavo M. Martins reports financial support was provided by Programa Operacional Açores (2020). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecss.2025.109422>.

Data availability

Data will be made available on request.

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