Tropical CO_2 seeps reveal the impact of ocean acidification on coral reef invertebrate recruitment

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ABSTRACT

Rising atmospheric CO_2 concentrations are causing ocean acidification by reducing seawater pH and carbonate saturation levels. Laboratory studies have demonstrated that many larval and juvenile marine invertebrates are vulnerable to these changes in surface ocean chemistry, but challenges remain in predicting effects at community and ecosystem levels. We investigated the effect of ocean acidification on invertebrate recruitment at two coral reef CO_2 seeps in Papua New Guinea. Invertebrate communities differed significantly between 'reference' (median pH 7.97, 8.00), 'high CO_2' (median pH 7.77, 7.79), and 'extreme CO_2' (median pH 7.32, 7.68) conditions at each reef. There were also significant reductions in calcifying taxa, copepods and amphipods as CO_2 levels increased. The observed shifts in recruitment were comparable to those previously described in the Mediterranean, revealing an ecological mechanism by which shallow coastal systems are affected by near-future levels of ocean acidification.

1. Introduction

Atmospheric carbon dioxide concentrations increased from preindustrial levels of 280 ppm to 400 ppm in 2016, increasing oceanic uptake of CO_2 and lowering both the pH and the carbonate saturation level of surface waters (Gattuso et al., 2015). Concentrations of corrosive H^+ ions are now 30% higher than in the preindustrial age and are expected to be 150% higher by 2100 (Williamson and Turley, 2012). Seawater saturation states of aragonite and calcite are falling rapidly, making it more difficult for some organisms to maintain shells and exoskeletons and causing the expansion of areas that are corrosive to carbonates (Rodolfo-Metalpa et al., 2011; Chan and Connolly, 2013; Jackson et al., 2014).

Laboratory experiments have shown that the early life-history stages of a wide range of marine invertebrates are acutely vulnerable to elevated pCO_2 (Kurihara, 2008; Kroeker et al., 2013; Parker et al., 2013). Furthermore, key settlement processes of larval attachment and metamorphosis are affected to elevated pCO_2 (Ko et al., 2014; Dineshram et al., 2016). This has raised concerns that benthic recruitment from the meroplankton may be severely affected (Ross et al., 2011; Byrne, 2012; Weatherdon et al., 2015). To assess the likely effects of ongoing ocean acidification on larval success we need to factor in altered larval-substratum interactions (Doropoulos et al., 2012; Uthicke et al., 2013) and changes in organism interactions (Gaylord et al., 2015).

To date, the effects of ocean acidification on marine invertebrate recruitment remain poorly documented, although work at upwelling areas and at volcanic CO_2 seeps show that recruitment success at high seawater pCO_2 depends on a range of environmental factors (Thomsen et al., 2010). These acidified environments can be useful for investigations into the ecosystem effects of ocean acidification since they have gradients in seawater pCO_2 and carbonate saturation that provide 'space for time' analogs in environmentally realistic settings (Wernberg et al., 2012; Andersson et al., 2015). Coral reefs in waters with low carbonate saturation erode easily (Jackson et al., 2014) and surveys of reefs affected by CO_2 seeps have major declines in macrobenthic species richness in acidified waters (Fabricius et al., 2011, 2014; Enochs et al., 2015). We do not yet know how ocean acidification might affect invertebrate recruitment to coral reefs.

Here, we assessed the effects of elevated CO_2 on invertebrate recruitment and community development using artificial recruitment substrata to standardize habitat characteristics at CO_2 seeps on two coral reefs in Papua New Guinea. We compare our findings to data collected using similar methods at CO_2 seeps in Italy (Cigliano et al., 2010) to assess the

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likely effects of ocean acidification on benthic invertebrate recruitment. Based on previous laboratory and field data on the effects of ocean acidification on early life-history stages and key settlement processes in marine invertebrates, we hypothesize that elevated CO_2 conditions will affect species composition and community structure, with the strongest effects observed in the recruitment of calcifying invertebrates.

2. Materials and methods

2.1. Study area and seawater chemistry

Invertebrate recruitment was investigated at 2–4 m depth on two coral reefs in Papua New Guinea (Fig. 1) where gas comprising 99% CO_2 was bubbling up through the sea bed into the water column (see Fabricius et al., 2015 for further details). The seeps have been active for at least 70 years and may be significantly older (Fabricius et al., 2011). Adjacent reference sites had ambient levels of CO_2 but were otherwise similar in terms of topography, geomorphology, wave exposure, temperature, alkalinity and salinity.

Two water chemistry data sets were collected. The first was pH logging data (from SBE 18 pH Sensors (Sea-Bird Scientific, Halifax) and SeaFET (Satlantic) sensors) deployed at reference, high CO_2, and extreme CO_2 stations at both reefs between April 2012 and November 2014. The second data set was from 863 individual seawater samples collected at reference, high CO_2, and extreme CO_2 stations at both reefs between 2011 and 2013. Each seawater sample from reference and high CO_2 stations at each reef was collected next to nylon scouring pads that we deployed as standard recruitment substrata (see below). Individual seawater samples collected from extreme CO_2 stations did not correspond to specific scouring pads. We took pH measurements of each seawater sample at the time of collection using a Mettler Toledo InLab Expert Pro pH electrode and a SG78 pH/Temperature meter (see Fabricius et al., 2014). A subset of 541 seawater samples from Dobu (reference: 64, high CO_2: 96, extreme CO_2: 22) and Upa Upasina (reference: 184, high CO_2: 148, extreme CO_2: 99) reefs were preserved in mercuric chloride for subsequent laboratory processing (see Fabricius et al., 2015). Total alkalinity (TA) and dissolved inorganic carbon (DIC) for 509 samples were determined with a Vindta 3C meter (Marianda, Kiel). In the remaining 32 samples total alkalinity was determined using a Metrohm 855 automated open cell potentiometric titrator (Metrohm AG, Herisau, Fangue et al., 2010). Using established chemical relationships, variables describing seawater carbonate chemistry (pCO_2, $\Omega_{aragonite}$ and $\Omega_{calcite}$) were calculated using the Seacarb v2.4.8 package in R (Lavigne and Gattuso, 2013).

2.2. Invertebrate sampling

We collected invertebrates using the same type of standardized recruitment substrata as Cigliano et al. (2010), i.e. 8 cm diameter scouring pads made of tightly rolled 0.25 cm^2 nylon mesh. The scouring pads were fixed at haphazard locations ~2 cm above the benthos at 2–4 m water depth along 50 m stretches of reef slope (Dobu reference: 11, high CO_2: 24, extreme CO_2: 11, Upa Upasina reference: 12, high CO_2: 23, extreme CO_2: 11) in January 2013. After 116–127 days they were removed and preserved. In the laboratory, the nylon netting was unrolled and rinsed on a 500 μm sieve. Material retained by the sieve was stored in 70% industrial methylated spirit before examination under a ×40 stereomicroscope. A total of 75,889 invertebrates were removed and sorted into 30 taxa. Identification to a greater taxonomic resolution was not possible as the region is exceptionally species-rich with many species undescribed, and comprehensive identification keys were unavailable. A subsample of 4644 invertebrates from 15 randomly selected artificial recruitment substrata from Dobu reef (5 per station) were
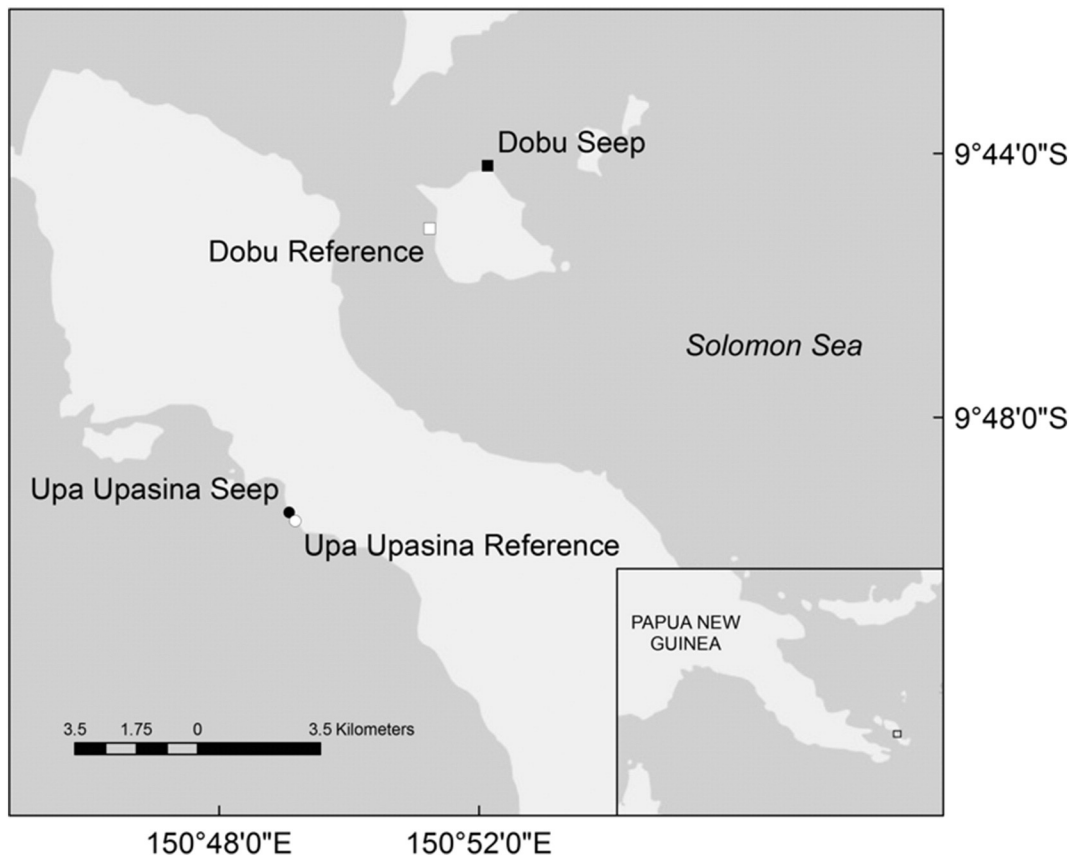

Fig. 1. Milne Bay Province in eastern Papua New Guinea showing two sets of reference sites (white filled) plus high CO_2 and extreme CO_2 stations (black filled).
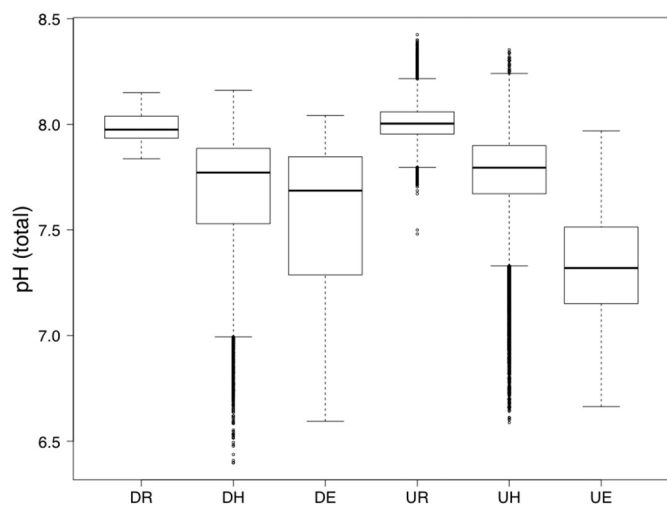
Fig. 2. Seawater pH (total scale) recorded from Seabird and SeaFET loggers at reference, high, and extreme CO_2 stations at Dobu and Upa Upasina reefs, between April 2012 and November 2014. Horizontal black lines represent median values. Boxes represent 25th and 75th percentiles. Whiskers represent maximum and minimum values, excluding outliers, which are marked with circles.

separated into 73 operational taxonomic units (OTUs) based on morphology, to calculate community diversity and evenness.

2.3. Univariate analyses

Univariate analyses were conducted using Minitab version 16. The effect of elevated CO_2 on the most abundant taxa was tested using generalized linear models (GLM) with CO_2 level and reef as fixed factors. Where necessary, abundance data were transformed to reduce structure in analytical residuals and homogenize variances. Shannon-Wiener Diversity (H′) and Pielou's Evenness (J′) were calculated based on the OTU data from the subsample of 15 artificial recruitment substrata from Dobu reef. Diversity and evenness were then analyzed using ANOVA with CO_2 level as a fixed factor. Where significant main effects were found, Tukey's HSD tests were used to test pair-wise differences between reference, high CO_2, and extreme CO_2 stations.

2.4. Multivariate analyses

Multivariate analyses were conducted using PRIMER v.6 (Primer-E Ltd., Plymouth; Clarke and Gorley, 2006) with the PERMANOVA add-on (Anderson et al., 2008). To test the effect of elevated CO_2 on invertebrate communities, abundance data were log transformed and a Bray-Curtis similarity matrix was calculated. We then used Type III SS PERMANOVA with CO_2 level (reference, high CO_2, and extreme CO_2) and reef (Dobu, Upa Upasina) as fixed factors. Possible confounding due to non-homogeneity of dispersions for fixed factors in PERMANOVA was investigated using PERMDISP tests (Anderson, 2004). Pairwise tests in PERMANOVA were used to compare community structure between stations at each reef independently due to a significant reef × CO_2

level interaction. SIMPER analysis using log-transformed abundance data was conducted to assess which taxa contributed most to dissimilarity between stations. A non-metric multidimensional scaling plot (NMDS) was constructed based on a Bray-Curtis similarity matrix to visualize sample overlap and centroid separation. Taxa vectors, based on log transformed abundance data, were then fitted to the NMDS plot using the envfit function of the R-package 'vegan' (Oksanen et al., 2016).

3. Results

3.1. Seawater chemistry

Logger data showed a gradient of mean seawater pH, ranging from pH 8.00 at reference stations outside seep areas, to pH 7.32 at extreme CO_2 stations within areas of intense bubbling. Variability in pH was greater at elevated CO_2 stations than at the more stable reference stations (Fig. 2, Table 1). Individual seawater samples showed that median aragonite and calcite saturation (Ω) ranged from 4.13 and 6.22 at the reference stations, to 1.33 and 1.85 at extreme CO_2 stations, respectively. Dissolved inorganic carbon ranged from 1924.50 µmol kg^{-1} at reference stations to 2248.92 µmol kg^{-1} at extreme CO_2 stations.

3.2. Univariate results

In the data set including all samples, calcified Foraminifera, Gastropoda, and Bivalvia, were significantly less abundant at both high and extreme CO_2 stations than at reference sites (Fig. 3). Significant abundance differences were also apparent in several crustacean groups (Fig. 3); for example, Amphipoda and Copepoda were significantly less abundant at high and extreme CO_2 stations compared to the reference stations. Tanaidacea meanwhile appeared resilient to ocean acidification, and were significantly more abundant at elevated CO_2 stations driven by very high abundance at extreme CO_2 stations at Dobu reef. Polychaeta were significantly less abundant at extreme CO_2 stations, and calcifying tube dwelling species were only found at reference stations.

In the Dobu reef subsamples, diversity (H′) and evenness (J′) were significantly lower at elevated CO_2 stations (One-way ANOVA; Table 2). Tukey's post-hoc tests for both diversity and evenness revealed no significant differences between reference and high CO_2 stations, but significant differences between extreme, and both high and reference CO_2 stations (Table 2).

3.3. Multivariate results

Invertebrate communities changed significantly with CO_2 at the two reefs (reef × CO_2 level interaction: p < 0.001, Table 3). Samples from Upa Upasina reef had significant non-homogeneity of dispersions ($F_{2,43}$ = 14.431; p < 0.001) but inspection of the MDS plot indicated that this did not confound the significant CO_2 level effect, with clear centroid separation being evident. Pairwise tests revealed significant differences between invertebrate communities in all combinations of CO_2 levels at both sites (all combinations p < 0.001, Table 3). SIMPER analysis of log-transformed abundance data showed that heavily calcified taxa (Foraminifera, Gastropoda, Bivalvia) and abundant crustaceans

Table 1

Seawater carbonate chemistry derived from individual seawater samples at reference, high and extreme CO_2 stations (median pH$_T$, TA, pCO$_2$, DIC, $\Omega_{aragonite}$, $\Omega_{calcite}$), and Seabird/SeaFET logger data (median pH$_T$ in parentheses) for Dobu and Upa Upasina reefs.

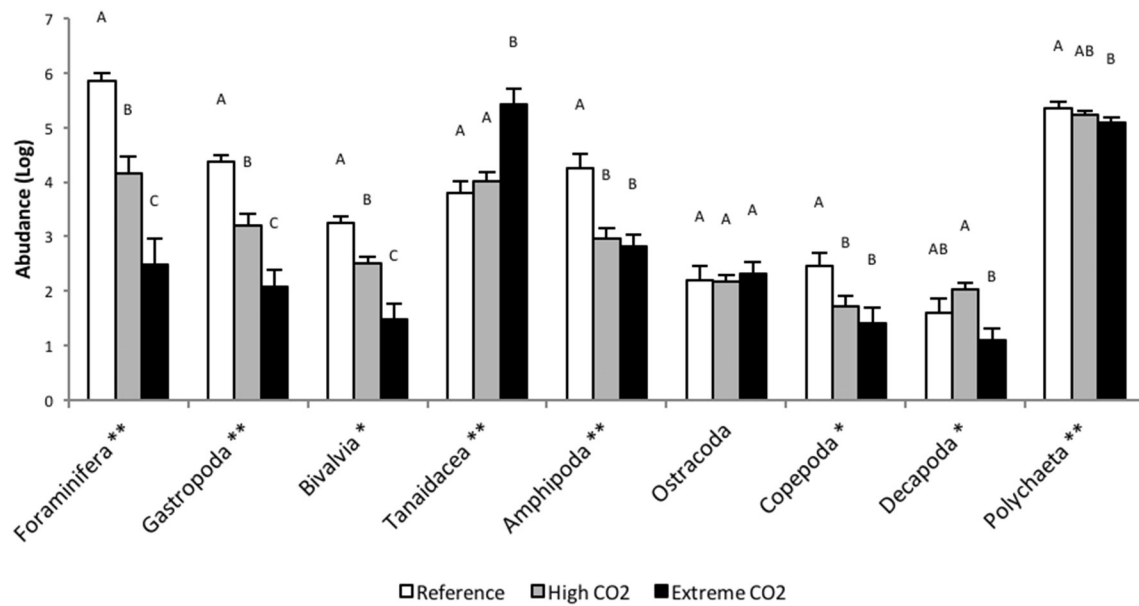| Reef	Station	pH$_T$ (total scale)	TA (µmol kg^{-1})	pCO$_2$ (µatm)	DIC (µmol kg^{-1})	$\Omega_{aragonite}$	$\Omega_{calcite}$
Dobu	Reference	7.99 (7.97)	2235.00	442.40	1926.60	3.56	5.31
	High	7.69 (7.77)	2289.98	995.99	2129.73	2.10	3.15
	Extreme	7.51 (7.69)	2306.99	1990.13	2233.39	1.33	1.96
Upa Upasina	Reference	7.99 (8.00)	2269.82	341.89	1924.50	4.13	6.22
	High	7.84 (7.79)	2302.60	604.50	2047.30	2.87	4.31
	Extreme	7.67 (7.32)	2354.96	1150.10	2248.92	1.85	2.76

Fig. 3. Mean (+ standard error) log-transformed abundance of the numerically dominant taxa at reference, high CO_2, and extreme CO_2 stations on Dobu and Upa Upasina reefs combined. *: overall significant effect of CO_2 level ($p < 0.05$); **: significant CO_2 level and CO_2 level x Reef interaction ($p < 0.05$). Superscripts indicate significant between-station differences (pair-wise Tukey's post-hoc tests ($p < 0.05$)).

(Tanaidacea, Amphipoda, Ostracoda) contributed most to dissimilarity of communities between CO_2 levels (Table 4). The NMDS plot of invertebrate community composition (Fig. 4) shows distinct clustering of invertebrate communities from each CO_2 level at Dobu and Upa Upasina reefs, respectively.

4. Discussion

Carbon dioxide seeps provide useful analogs for ocean acidification as they reveal direct and indirect ecological effects in natural settings (Hall-Spencer et al., 2008; Andersson et al., 2015). Surveys of these seep systems have consistently shown that rising carbon dioxide causes species diversity to fall, and they reveal the mechanisms that drive changes in ecosystem function (Sunday et al., 2016). Elevated CO_2 can have direct effects, for example through carbonate dissolution (Rodolfo-Metalpa et al., 2011), benefits to photosynthesis (Cornwall et al., submitted), increased metabolic costs (Garilli et al., 2015) and effects on fish behavior (Milazzo et al., 2016). They can also have indirect effects that drive shifts in coastal systems, such as decreased habitat complexity (Fabricius et al., 2015), altered underwater soundscapes (Rossi

et al., 2016) and changes in inter- and intraspecific interactions of every kind (Gaylord et al., 2015; Smith et al., 2016).

Experimental laboratory work indicates that the larval and juvenile stages of marine invertebrates are especially vulnerable to ocean acidification (Kroeker et al., 2013; Parker et al., 2013) although field research has established that ample food supplies can confer resilience to settling invertebrates when faced with elevated CO_2. In the nutrient rich waters of the Baltic, serpulids, mussels and barnacles recruit well to settlement plates even in water that has 2000 ppm pCO_2 and is undersaturated with carbonate (Thomsen et al., 2010). Our analyses of standardized substrata on tropical coral reefs have shown that ocean acidification can adversely affect calcareous invertebrate recruitment, with major shifts in invertebrate community structure. Our results are strikingly similar to those seen in oligotrophic conditions in the Mediterranean, where calcified organisms also struggle to settle and grow at >800 ppm pCO_2 (Cigliano et al., 2010). This is presumably because organisms with less food are unable to meet the increased metabolic costs of living in acidified seawater (Wood et al., 2008; Garilli et al., 2015), and or the cues from substrata such as crustose coralline algae

Table 2

Results of combined One-way ANOVA analyses of Shannon-Weiner Diversity (H′) and Pielou's Evenness (J′) scores from reference, high CO_2, and extreme CO_2 stations at Dobu Reef.

	df	MS	F	p
Diversity (H′)				
CO_2 level	2	0.9562	20.72	<0.001
Error	12	0.0462		
Total	14			
High CO_2, reference				0.133
High CO_2, extreme CO_2				0.030
Reference, extreme CO_2				<0.001
Evenness (J′)				
CO_2 level	2	0.01941	8.54	0.005
Error	12	0.00227		
Total	14			
High CO_2, reference				0.783
High CO_2, extreme CO_2				0.020
Reference, extreme CO_2				0.006

Table 3

Results of Type III SS PERMANOVA analysis of invertebrate recruitment community data from reference, high CO_2, and extreme CO_2 stations at Dobu and Upa Upasina reefs. Pairwise tests are presented for each combination of stations at each reef.

	df	MS	Pseudo-F	p
CO_2 level	2	3927.9	12.704	<0.001
Reef	1	11,780	38.102	<0.001
CO_2 level × reef	2	2124.7	6.8722	<0.001
Error	86	309.18		
Total	91			

			T	p
Upa Upasina				
High CO_2, reference			2.193	<0.001
High CO_2, extreme CO_2			2.572	<0.001
Reference, extreme CO_2			3.056	<0.001
Dobu				
High CO_2, reference			3.016	<0.001
High CO_2, extreme CO_2			2.907	<0.001
Reference, extreme CO_2			4.202	<0.001

Table 4

Results of log transformed SIMPER analyses based on abundance data from reference, high CO_2, and extreme CO_2 stations. For each pair of stations, the five taxa contributing the greatest percentage of dissimilarity between communities are shown.

	Percentage dissimilarity contributed
Reference vs high CO_2	
Foraminifera	9.24%
Amphipoda	8.32%
Tanaidacea	7.02%
Gastropoda	6.57%
Ostracoda	5.91%
Reference vs extreme CO_2	
Foraminifera	13.00%
Gastropoda	8.70%
Tanaidacea	8.54%
Bivalvia	7.06%
Amphipoda	6.61%
High CO_2 vs extreme CO_2	
Foraminifera	10.06%
Tanaidacea	8.92%
Gastropoda	7.51%
Bivalvia	6.93%
Amphipoda	6.53%

which induce settlement in many reef organisms are missing (Fabricius et al., 2015).

The most dramatic effects on invertebrate recruitment occurred in our 'extreme CO_2' treatments. This was not surprising since areas with intense bubbling experience such rapid variations in carbonate chemistry that they are unrealistic analogs for the global effects of ocean acidification as very few taxa can survive (Kerrison et al., 2011; Small et al., 2015). Such extreme areas are usually avoided in studies of the effects of ocean acidification on coral reef ecology (Fabricius et al., 2011, 2014; Enochs et al., 2015), but they can be useful in showing which organisms are extremely resilient to elevated CO_2. Tanaid abundance was much higher under these extreme conditions in Papua New Guinea (Fig. 3, Table 4) which is interesting since tanaids were also tolerant of very

high CO_2 levels in the Mediterranean (Cigliano et al., 2010). Perhaps they may benefit from reduced competition and/or increased food availability (Johnson et al., 2015), they also brood their young and so may have been able to adapt through multigenerational exposure (Sunday et al., 2014).

Of particular interest were areas where seawater chemistry was comparable to 2100 acidification scenarios under RCP 8.5 (our 'high CO_2' stations; Hoegh-Guldberg et al., 2014). Here, we found differences in the invertebrate taxa that settled on the two reefs systems, but in each case there were significant shifts in community composition between the reference sites and the 'high CO_2' treatments (Fig. 2, Fig. 4). Heavily calcified taxa were adversely affected, in line with many laboratory studies showing that elevated seawater CO_2 can increase the energetic cost of producing calcareous structures (Ries et al., 2009), and can cause growth abnormalities that disrupt settlement resulting in high levels of mortality (Kurihara, 2008; Parker et al., 2013; Waldbusser et al., 2014; Jansson et al., 2015). The abundances of calcified polychaetes, foraminiferans and juvenile molluscs were significantly lower at elevated CO_2 (Fig. 3) which corroborates previous work at volcanic seeps in Papua New Guinea and the Mediterranean (Cigliano et al., 2010; Fabricius et al., 2011; Pettit et al., 2015).

Some crustaceans are known to be resilient to elevated CO_2 (Wittmann and Pörtner, 2013; Hall-Spencer and Allen, 2015) and we found that juvenile decapod recruitment was unaffected in 'high CO_2' treatments. Decapod abundance was significantly lower in established communities on these reefs, so we suspect that predation may drive this difference given that reduced habitat complexity under elevated CO_2 conditions provides less shelter for adult decapods (Fabricius et al., 2014). Amphipods and copepods were significantly less abundant in recruitment communities developed under near-future elevated CO_2 conditions, in line with work at CO_2 seeps in the Mediterranean (Cigliano et al., 2010). Low recruitment of these small crustaceans would be of particular importance in coral reef habitats, as these groups are key food sources for many corals and reef fish.

Our findings support the notion that early life-history stages of calcifying taxa are particularly vulnerable to ocean acidification and may act
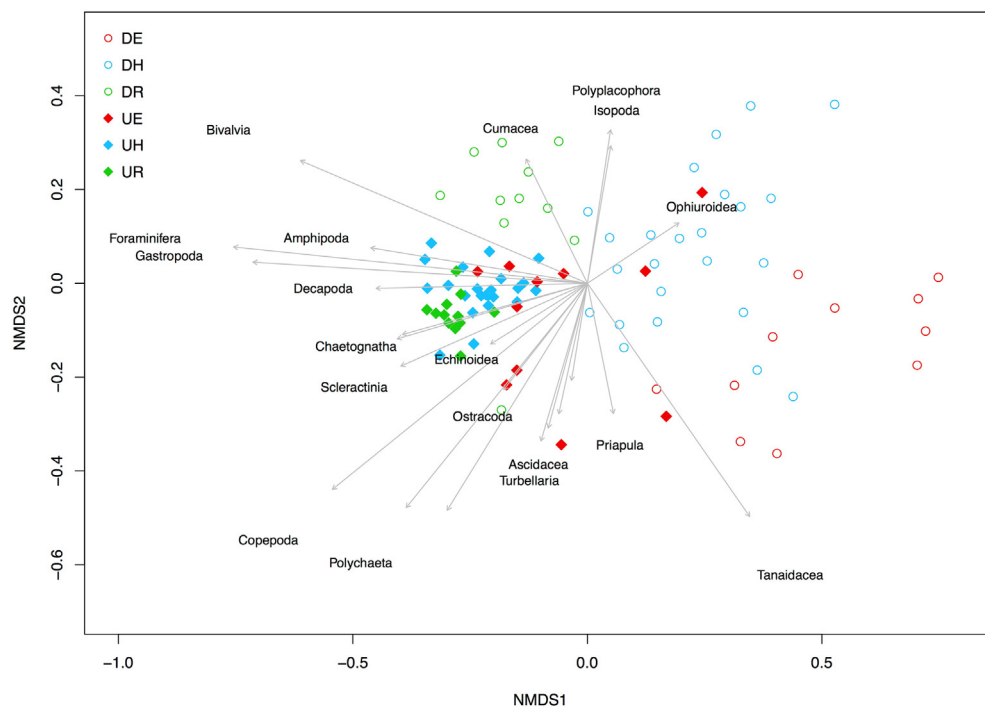

Fig. 4. Non-metric multidimensional scaling (NMDS) plot of invertebrate community composition for reference (green), high CO_2 (blue), and extreme CO_2 (red) stations at Upa Upasina (closed diamonds) and Dobu (open circles) reefs. Arrows indicate linear vectors of log transformed taxa abundance ($p < 0.05$), fitted using the envfit function of the R-package 'vegan'. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

as bottlenecks for species survival (Weatherdon et al., 2015). There is hope that our experimental approach over-estimates the effects of ocean acidification on invertebrate recruitment, since plankton drifting into experimental seep sites experience a rapid jump in pCO_2 levels (Ziveri et al., 2014). Exposure to gradually increasing CO_2 levels over multiple generations may improve the chances of vulnerable taxa though adaptation and transgenerational acclimation as ocean acidification progresses (Sunday et al., 2014). Work in the laboratory and in the wild has shown that molluscs and polychaetes with limited larval dispersal can adapt to ocean acidification (Calosi et al., 2013; Garilli et al., 2015; Rodríguez-Romero et al., 2015). We found that non-calcifying polychaetes were remarkably resilient to ocean acidification, in line with findings other studies at CO_2 seep sites (Cigliano et al., 2010; Fabricius et al., 2014).

In conclusion, levels of seawater CO_2 expected by 2100 profoundly altered invertebrate recruitment on two coral reefs in Papua New Guinea. Calcifying taxa (e.g. foraminiferans, bivalves and gastropods) and certain crustaceans (copepods and amphipods) were highly vulnerable to elevated CO_2 causing significant shifts in invertebrate recruitment dynamics. The effects were very similar to those observed in oligotrophic conditions in the Mediterranean (Cigliano et al., 2010) but unlike those observed in eutrophic conditions in the Baltic where recruitment was not impaired since the settling larvae are able to obtain enough food to meet the metabolic costs of dealing with ocean acidification (Thomsen et al., 2013). This highlights the vulnerability of invertebrate recruitment processes to elevated CO_2 conditions on oligotrophic tropical coral reefs.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.marpolbul.2016.12.031.

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