2021-01-10

Ocean acidification locks algal communities in a speciespoor early successional stage

Harvey, BP

<http://hdl.handle.net/10026.1/16821>

10.1111/gcb.15455

Global Change Biology

Wiley

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.

This is the author's accepted manuscript. The final published version of this work (the version of record) was published by Wiley in *Global Change Biology* online on 10 January 2021 at <https://onlinelibrary.wiley.com/doi/10.1111/gcb.15455>

This work is made available online in accordance with the publisher's policies. Please refer to any applicable terms of use of the publisher.

Ocean acidification locks algal communities in a species-poor early successional stage

Ben P. Harvey¹, Koetsu Kon¹, Sylvain Agostini¹, Shigeki Wada¹, Jason M. Hall-Spencer^{1,2}

¹ Shimoda Marine Research Center, University of Tsukuba, 5-10-1 Shimoda, Shizuoka 415-0025, Japan

² Marine Biology and Ecology Research Centre, University of Plymouth, Plymouth PL4 8AA, UK.

Correspondence: Ben P Harvey; Email: ben.harvey@shimoda.tsukuba.ac.jp

Keywords: Global change ecology, CO₂ seeps, Ecosystem function, Community dynamics, Inhibition, Competition, Turf algae

ABSTRACT

Long-term exposure to CO₂-enriched waters can considerably alter marine biological community development, often resulting in simplified systems dominated by turf algae that possess reduced biodiversity and low ecological complexity. Current understanding of the underlying processes by which ocean acidification alters biological community development and stability remains limited, making the management of such shifts problematic. Here, we deployed recruitment tiles in reference (pH_T 8.137 ± 0.056 SD) and CO₂-enriched conditions (pH_T 7.788 ± 0.105 SD) at a volcanic CO₂ seep in Japan in order to assess the underlying processes and patterns of algal community development. We assessed (i) algal community

succession over six months in two different seasons (Cooler months: January–July, and warmer months: July–January), (ii) the effects of initial community composition on subsequent community succession (by reciprocally transplanting pre-established communities for a further six months), and (iii) the community net production of the resulting communities, in order to assess how any changes in community composition will alter their functioning (following 12 months recruitment). Settlement tiles became dominated by turf algae under CO₂-enrichment and had a lower biomass, diversity and complexity than tiles from the reference conditions. The enriched-CO₂ conditions enabled opportunistic *r*-selected species to competitively exclude others and lock the community in a species-poor early successional stage. This pattern was consistent across seasons, and when considering both new- and pre-established algal communities. In terms of community functioning, the elevated *p*CO₂ community exhibited greater net community production, and yet this apparent boost to the community did not result in benefits for the ecosystem such as increased community cover, biomass, biodiversity or structural complexity. Taken together, this shows that both new and established communities become simplified with rising CO₂ levels. Our transplant of pre-established communities from enriched-CO₂ to reference conditions showed the high resilience of the algal communities, with these transplanted communities indistinguishable from communities maintained entirely in reference conditions. This shows that meaningful reductions in *p*CO₂ will enable the recovery of algal communities. By understanding the ecological processes responsible for driving shifts in community composition, we can better assess how communities are likely to be altered by ocean acidification.

OA locks algal succession to early stage

Community development and succession

1 INTRODUCTION

The oceanic uptake of anthropogenic carbon dioxide emissions is a global environmental issue termed ocean acidification. The effects of ocean acidification are detrimental to a wide range of marine organisms (Harvey et al., 2013; Kroeker, Kordas, et al., 2013), and this affects ecosystem functioning and the goods and services that people derive from marine resources (Gattuso et al., 2015; Hall-Spencer & Harvey, 2019). To better understand the effects of ocean acidification, there has been an effort in recent years to move beyond aquarium-based experiments on single species towards *in-situ* experiments (e.g. Albright et al., 2016, 2018; Brown et al., 2016), long-term mesocosm observations (e.g. Algueró-Muñiz et al., 2017; Moulin et al., 2015), and studies using natural CO₂ seeps (e.g. Agostini et al., 2018; Fabricius et al., 2011; Hall-Spencer et al., 2008; Milazzo et al., 2014). These approaches have shown that long-term exposure to ocean acidification conditions projected for the end of the century fundamentally alters the composition of marine biological communities, usually resulting in simplified systems with reduced biodiversity and less ecological complexity (Agostini et al., 2018; Sunday et al., 2017; Vizzini et al., 2017). Many of these studies have been observation-based, and so an understanding of the underlying processes responsible for driving these patterns in community development remains limited. To help assess the future effects of ocean acidification, it would be useful to better understand how community development processes are affected by rising levels of seawater CO₂ (Gaylord et al., 2015), and how such changes will influence their associated ecosystem functioning.

Ecological theory suggests that the successional trajectories of ‘disturbed’ marine subtidal communities will be primarily driven by physical stresses, competition for resources through the mechanisms of ‘facilitation’ and ‘inhibition’ (J. H. Connell & Slatyer, 1977), and the strength of associated bottom-up and top-down interactions. One of the difficulties in predicting how community development will be affected by ocean acidification is that the

changes in carbonate chemistry can simultaneously act as both resource and stressor (S. D. Connell et al., 2013, 2018; Milazzo et al., 2019). It provides a bottom-up resource to primary producers by enhancing the availability of bicarbonate and CO₂ (S. D. Connell et al., 2013; Koch et al., 2013), but also acts as a physical stressor to many organisms (including calcified primary producers) via negative effects on their physiology (Harvey et al., 2013; Kroeker, Kordas, et al., 2013). Subsequently, marine communities are expected to be re-organised by the effects of ocean acidification. Ocean acidification alters the initial successional trajectories of algal communities, which lead to dominance by fleshy algae over calcified algae in acidified conditions projected for the end of the century in both temperate and tropical settings (Crook et al., 2016; Kroeker et al., 2012). Enriched CO₂ alters competitive interactions, acting as a physical stressor to calcified macroalgae whereas turf algae can use the additional carbon to boost growth, which allows turf algae to attain dominance (also see S. D. Connell et al., 2018). Fast-growing opportunistic (*r*-selected) turf algal species are usually suppressed beneath macroalgal canopies on temperate reefs (C. R. Johnson & Mann, 1988) and by top-down control of grazers in coral reefs (Hughes et al., 2007). In the absence of strong competition or compensatory processes (e.g. S. D. Connell et al., 2018; Ghedini et al., 2015; Ghedini & Connell, 2016), turf species can become dominant thereby changing the ecosystem state.

Under present-day conditions, it has been suggested that despite bottom-up control of primary production being pervasive, top-down control by consumers has a stronger influence on the trajectories of algal community succession (Gruner et al., 2008; Hillebrand et al., 2007). For example, intense grazing by sea urchins and herbivorous fish can prevent kelp forest growth resulting in ‘urchin barrens’ dominated by crustose coralline algae (Ling et al. 2015; Kelly et al., 2016). Ocean acidification is expected to reduce bottom-up control on those species which are carbon-limited, as long as sufficient nutrients are available (Celis-Plá et al., 2015; Gordillo et al., 2003; Li et al., 2012). Top-down control by benthic invertebrates in acidified conditions

may also diminish, given that at CO₂ seeps the abundance and size of many marine fauna are reduced (Garilli et al., 2015; Harvey et al., 2016, 2018), with such examples as the observed number of sea urchin feeding halos being reduced in a CO₂ seep (Kroeker, Gambi, et al., 2013). Fish communities include a greater proportion of herbivorous fish within acidified conditions (during the period of peak macroalgae biomass; Cattano et al., 2020), and so it may be possible in some systems for fish to maintain top-down control (Baggini et al., 2015). Taken together, any strong reductions in bottom-up and/or top-down control are likely to alter community successional trajectories and allow *r*-selected opportunistic species to outcompete other species and dominate under ocean acidification.

Seasonality is an important aspect of shallow-water ecosystems, and yet the consequences of seasonally-induced environmental fluctuations have rarely been considered in ocean acidification studies (Baggini et al., 2014; Godbold & Solan, 2013). Algal communities in temperate and warm temperate ecosystems experience large seasonal changes in environmental conditions (Fig. 1), which result in considerable temporal shifts with a period of high recruitment and peak biomass typically occurring in late spring. Thus the responses of algal communities to ocean acidification will likely be strongly influenced by seasonality (Baggini et al., 2014). In the Northern Pacific Ocean, this is further complicated by the occurrence of typhoons, which typically occur between July and October in Japan. Typhoons act as a substantial physical disturbance that affects benthic community structure and habitat complexity, such as the removal of corals (Done, 1992), macroalgae (Cattano et al., 2020) and seagrass cover (Wilson et al., 2020), and can indirectly change the community function of associated species (e.g. fish; Cattano et al., 2020).

Observations at natural CO₂ seeps worldwide provide a good understanding of how long-term ocean acidification simplifies the composition of climax communities (Foo et al., 2018;

González-Delgado & Hernández, 2018; Hall-Spencer & Harvey, 2019), yet it remains unclear whether these simplified communities develop due to altered successional trajectory, stunted community development (*via* successional inhibition), or driven by reduced bottom-up and/or top-down control. To address these gaps, we deployed recruitment tiles in reference (~350 $\mu\text{atm } p\text{CO}_2$) and acidified (~900 $\mu\text{atm } p\text{CO}_2$) conditions (using a natural CO_2 seep area) to assess the early to mid-successional trajectories of algal communities in two different seasons (cooler months - January to July, and warmer months - July to January) to end of the century $p\text{CO}_2$ conditions (the RCP 8.5 scenario, 851 to 1370 μatm ; IPCC, 2013). The study was carried out over these two time periods to investigate whether the effects of ocean acidification on community development are temporally consistent. Following this we carried out a reciprocal transplant of some of those established communities, in order to assess the effects of initial community composition on subsequent community succession in the reference and acidified conditions. Finally, we assessed the community production of these reciprocally transplanted communities (including the associated sessile invertebrate communities which contribute in terms of respiration), in order to assess how any changes in community composition will alter their ecosystem functioning.

2 MATERIALS AND METHODS

2.1 *Experimental Design*

To investigate our core question of how ocean acidification influences early community succession of algal communities, experiments using recruitment tiles were carried out using an acidified area of the Shikine Island CO_2 seep, Japan (34°19'9" N, 139° 12'18" E), and a nearby reference $p\text{CO}_2$ area in an adjacent bay (~ 600 m away by the shortest route). Both the reference and acidified locations (hereafter '350 μatm ' and '900 μatm ', respectively) have had their carbonate chemistry and biology well characterised previously (Agostini et al., 2015, 2018; Cattano et al., 2020; Harvey et al., 2018, 2019; Kerfahi et al., 2020; Witkowski et al., 2019),

and we present two-months of additional original pH_T (Fig. S1) and temperature data collected with a Durafet sensor (SeaFET, Sea-Bird Scientific, Halifax, Canada) using the same approach as Agostini et al. (2018). Salinity was measured concurrently using Hobo conductivity loggers (U24-002-C, Onset, MA, USA), and discrete samples for total alkalinity were collected throughout the study period, with total alkalinity measured using an auto-titrator (916 Ti-Touch, Metrohm, Switzerland). In summary, the ‘350 μatm ’ site had a mean pH_T of 8.137 ± 0.056 (SD) and the ‘900 μatm ’ site had a mean pH_T of 7.781 ± 0.105 (SD), and the mean carbonate chemistry of the two locations is presented in Table 1. Long-term temperature data were recorded over a one-year period by deploying a temperature logger (HOBO Pendant Temperature/Light 64K Data Logger, Onset, MA, USA) at ~6 m depth in each site. The ‘900 μatm ’ elevated $p\text{CO}_2$ location represents an end-of-the-century projection for reductions in pH (the RCP 8.5 scenario; IPCC, 2013), and was not confounded by differences in temperature, salinity, dissolved oxygen, total alkalinity, nutrients or depth relative to reference sites used for comparison (Agostini et al., 2015, 2018; Harvey et al., 2019). Our basalt recruitment tiles were 130 x 130 x 15 mm and were secured using individual anchor bolts (8.5 mm width, 70 mm length) drilled into rock by SCUBA divers at ~6 m depth (Nemo Underwater Drill, Nemo Power Tools, CA, USA). The tiles at each site were deployed haphazardly across a *ca.* 400 m^2 area (with at least 5 m between individual tiles), fixed to upward-facing substrata.

Table 1. Summary of the carbonate chemistry for the 350 μatm and 900 μatm locations. The pH_T (350 μatm , $n = 1964$; 900 μatm , $n = 10,818$), salinity (350 μatm , $n = 1964$; 900 μatm , $n = 10,818$), and total alkalinity (A_T ; 350 μatm , $n = 56$; 900 μatm , $n = 47$) are measured values. All other values were calculated using the carbonate chemistry system analysis program CO2SYS: Seawater $p\text{CO}_2$, dissolved inorganic carbon (DIC), bicarbonate (HCO_3^-), carbonate (CO_3^{2-}), carbon dioxide (CO_2), saturation states for calcite (Ω_calcite), and aragonite ($\Omega_\text{aragonite}$). Values are presented as mean, with standard deviation below. NOTE: ‘350 μatm ’ carbonate chemistry data is sourced from Agostini et al. 2018, ‘900 μatm ’ carbonate chemistry data is averaged across Agostini et al. 2018 and original data collected between 2017/05/31 and 2017/08/08.

Location	pH_T	Salinity (psu)	A_T ($\mu\text{mol kg}^{-1}$)	$p\text{CO}_2$ (μatm)	DIC ($\mu\text{mol kg}^{-1}$)	HCO_3^- ($\mu\text{mol kg}^{-1}$)	CO_3^{2-} ($\mu\text{mol kg}^{-1}$)	Ω_calcite	$\Omega_\text{aragonite}$
‘350 μatm ’	8.137	34.504	2264.29	316.057	1962.694	1740.629	211.979	5.087	3.301
	0.056	0.427	15.34	47.466	34.376	55.084	22.221	0.534	0.348
‘900 μatm ’	7.788	34.351	2268.33	841.148	2125.785	1984.889	115.150	2.771	1.805
	0.106	0.484	19.45	291.762	39.381	52.510	21.308	0.512	0.336

2.1.1 Seasonal experiment

For the first experiment, five recruitment tiles were individually deployed in each location (350 and 900 μatm) during the cooler months of January 2017 – July 2017 (hereafter termed ‘Cold Period’), with eight recruitment tiles deployed in each location (350 and 900 μatm) during the warmer months of July 2017 – January 2018 (hereafter termed ‘Warm Period’). Mean seawater temperature ($\pm$ SD) during the ‘Cold Period’ was 18.14 ± 1.81 °C at 350 μatm and 18.07 ± 1.63 °C at 900 μatm , and during the ‘Warm Period’ was 22.86 ± 2.97 °C at 350 μatm and 22.67 ± 2.83 °C at 900 μatm . See Fig. 1A for a conceptual overview of the experimental design.

2.1.2 Reciprocal experiment

For the second experiment, tiles from the ‘Warm Period’ of the seasonal experiment were used (each tile had an algal community following 6-months recruitment). Sixteen tiles from the seasonal experiment were reciprocally transplanted into the 350 μatm and 900 μatm locations for a further six months to assess the effects of initial community composition on subsequent community succession in reference and acidified conditions (four tiles in each combination, see Fig. 1B).

OA locks algal succession to early stage

Figure 1. Conceptual representation of the recruitment tile treatments. A) Tiles were deployed for six-months during the 'Cold Period' or 'Warm Period' in either reference $p\text{CO}_2$ (350 μatm ; black line) or acidified conditions (900 μatm ; grey line). **B)** Tiles from the 'Warm Period' were then used as part of a reciprocal transplant either being transplanted into 350 μatm or 900 μatm conditions for a further six-months.

2.2 *Community analysis*

For both the seasonal experiment and the reciprocal experiment, following the six-months experimental period each tile was brought into the laboratory and photographed (Nikon D7200, Nikon, Japan). For each twelve-month tile, two photos were taken to image the upper story community, and (after removal of the upper-story community by hand) the under-story community. These data were then combined for analysis. Community composition was assessed using ImageJ (Abràmoff et al., 2004) by overlaying 64 points on a grid, and recording the abundance of each algal functional group underlying that point. Functional groups used followed Steneck & Dethier (1994), with algal grouping being based on their morphology, thallus size and complexity (microalgae, filamentous algae, foliose algae, corticated foliose algae, corticated macrophytes, leathery macrophytes, articulated calcareous algae and crustose algae). The habitat complexity of each tile was determined by combining the abundance of each algal functional group with a rank (0 to 5) based on the biogenic habitat complexity provided by that functional group. The ranking was based on categories assigned by Steneck & Dethier (1994): microalgae = 1, filamentous algae = 2, foliose algae = 3, corticated foliose algae = 3.5, corticated macrophytes = 4, leathery macrophytes = 5, articulated calcareous algae = 4 and crustose algae = 3. The habitat complexity score was then normalised to between 0 and 1.

2.3 *Community production*

Community production and respiration of individual tiles was assessed by measuring, with an Orion 4-Star pH and dissolved oxygen meter (Thermo Scientific, USA), the changes in dissolved oxygen concentrations during an incubation within a 2.5 L seawater container (15 cm wide x 20 cm length x 10 cm height) in a temperature-controlled water bath. Magnetic stirrers (M-1 Controller and MS101A Stirrer, AS-One, Japan) were used to continuously mix the seawater within each container throughout measurements. Seawater for each treatment

($\text{pH}_{\text{NBS}} 8.05 \pm 0.01 \text{ SD}$ vs. $\text{pH}_{\text{NBS}} 7.83 \pm 0.01 \text{ SD}$) was collected from the same location off-shore (oceanic $\text{pH}_{\text{NBS}} 8.05$) and treatments of $\text{pH}_{\text{NBS}} 7.83 \pm 0.01 \text{ SD}$ were acquired by bubbling with pure CO_2 (Fukurow pH Controller; Aqua Geek, Kawaguchi, Japan). Community production and respiration were measured over a 150-minute period; first determining oxygen production (60-minute light period, *ca.* $200 \mu\text{mol m}^{-2} \text{ s}^{-1}$), and after a 30-minute dark period, oxygen consumption (60-minute dark period). Net community production and community respiration are measured during the light and dark periods, respectively, with gross primary production calculated as the net community production minus community respiration. All three measurements are presented as $\text{mol O}_2 \text{ hr}^{-1} \text{ m}^{-2}$. To assess whether any changes in respiration rates were driven by an altered sessile invertebrate community for the 12-month communities, the community composition and total cover of the sessile fauna (located on the underside of the tile) was assessed, following the same procedure as the community analysis (based on the percent cover of the sessile faunal species).

2.4 Statistical analysis

Statistical analyses were conducted using R (version 3.6.0; Team & R Development Core Team, 2019), with the ‘vegan’ (Oksanen et al., 2019), ‘mvabund’ (Wang et al., 2012) and base ‘stats’ package used for statistical analysis, and the ‘ggplot2’ (Wickham, 2016) and ‘ggpubr’ (Kassambara, 2019) packages used for figure production. For each of the analyses performed, the package and specific function used in R are listed below as ‘package::function’.

For the seasonal experiment, differences in community composition (based on percentage cover) between $p\text{CO}_2$ (two levels: $350 \mu\text{atm}$ and $900 \mu\text{atm}$) and Season (two levels: ‘Cold Period’ and ‘Warm Period’) were assessed using an nMDS (vegan::metaMDS) and a permutational analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarity (vegan::vegdist and vegan::adonis). To test for differences in the percentage cover of the

specific algal functional groups, we employed a two-way GLM (Family: Binomial (Link = Logit)), with $p\text{CO}_2$ (two levels: 350 μatm and 900 μatm) and Season (two levels: ‘Cold Period’ and ‘Warm Period’) as fixed factors (stats::glm).

For the reciprocal transplant experiment, differences in community composition (based on percentage cover) between Community Origin (two levels: 350 μatm and 900 μatm), Community Destination (two levels: 350 μatm and 900 μatm) and Time (two levels: 6-months and 12-months) as fixed factors were assessed using an nMDS (vegan::metaMDS) and a PERMANOVA based on Bray-Curtis dissimilarity (vegan::vegdist and vegan::adonis). As the starting communities at 6-months could influence the resulting communities at 12-months on a particular tile, we accounted for this repeated measure in the PERMANOVA by using the ‘strata’ argument within vegan::adonis. To test for differences in the percentage cover of the specific algal functional groups at 12-months, we employed a two-way GLM (Family: Binomial (Link = Logit)), with Community Origin (two levels: 350 μatm and 900 μatm) and Community Destination (two levels: 350 μatm and 900 μatm) as fixed factors (stats::glm).

Differences in community production and respiration of the 12-month communities were tested using a two-way GLM (Family: Gaussian (Link = Identity)), with Community Origin (two levels: 350 μatm and 900 μatm) and Community Destination (two levels: 350 μatm and 900 μatm) as fixed factors (stats::glm). To assess whether changes in respiration were driven by an altered sessile invertebrate community for the 12-month communities, we assessed for differences in the community composition (based on percentage cover) and total percentage cover of the sessile fauna. Differences in the community composition of the sessile fauna, with Community Origin (two levels: 350 μatm and 900 μatm), and Community Destination (two levels: 350 μatm and 900 μatm) as fixed factors, were assessed using a PERMANOVA based on Bray-Curtis dissimilarity (vegan::vegdist and vegan::adonis). Differences in the total

percentage cover of the sessile fauna were tested using a two-way GLM (Family: Gaussian (Link = Identity)), with Community Origin (two levels: 350 μ atm and 900 μ atm) and Community Destination (two levels: 350 μ atm and 900 μ atm) as fixed factors (stats::glm).

Post-hoc comparisons for all PERMANOVAs were achieved using a Bonferroni-corrected pairwise PERMANOVA. For the reciprocal transplant, only comparisons chosen *a priori* were tested; these were between Time (two levels: 6-months and 12-months) for each of the four combinations of Community Origin (two levels: 350 μ atm and 900 μ atm) and Community Destination (two levels: 350 μ atm and 900 μ atm). The assumptions of the generalised linear models (GLMs) were met, with the response variable independent, the mean-variance relationship suitable (assessed by plotting the residuals vs. fits; mvabund::manyglm), and dispersion parameters not being under- or over-dispersed (assessed using quasibinomial error distributions in R; stats::glm).

3 RESULTS

3.1 Effects of $p\text{CO}_2$ and Season on Community Composition

Overall community composition following six months settlement were highly separated by nMDS, being significantly affected by both ' $p\text{CO}_2$ ' and 'Season' (PERMANOVA: $p\text{CO}_2$ * Season, $F_{1,25} = 4.351$, $p = 0.012$; Fig. 2A; Table 2). Community composition was similar between seasons at 350 μ atm (*post-hoc* Bonferroni-adjusted, $p = 0.102$), but greatly differed between seasons at 900 μ atm (*post-hoc* Bonferroni-adjusted, $p = 0.018$). The typical newly settled community at 350 μ atm during the 'Warm Period' largely comprised of a low coverage (< 10 %) of microalgae and turf algae (Fig. 2B) as well as corticated foliose algae, and a high coverage (~ 75 %) of both corticated macrophytes (Fig. 2C, including *Chondracanthus tenellus* (Harvey) Hommersand, 1993) and crustose coralline algae (Fig. 2D, including *Lithophyllum*

288 *sp.* Philippi, 1837). The community at 350 μatm during the ‘Cold Period’ was similar, the only
 289 difference being a significantly lower cover (approximately half) of corticated macrophytes
 290 relative to the ‘Warm Period’ (Fig. 2C).

291

Figure 2 – (A) nMDS of community composition based on algal functional groups. Treatments are displayed by $p\text{CO}_2$ (‘350 μatm ’ – blue; ‘900 μatm ’ - red) and season (‘Warm Period’ – triangles; ‘Cold Period’ – crosses). (B-D) Percentage cover (%) of (B) microalgae and turf algae, (C) corticated macrophytes, and (D) crustose coralline algae following 6-months settlement at either 350 μatm $p\text{CO}_2$ (‘Warm Period’ – darker blue, ‘Cold Period’ – lighter blue) or 900 μatm $p\text{CO}_2$ (‘Warm Period’ – darker red, ‘Cold Period’ – lighter red). Two-way GLM ($p\text{CO}_2$ * Season) results are presented in the top-right of B-D. See Table S1 for more detailed statistics.

Table 2. PERMANOVA summary of $p\text{CO}_2$ (350 vs. 900 μatm) and Season (‘Cold Period’ vs. ‘Warm Period’) for the algal communities.

Term	Df	Sum Sq.	Mean Sq.	F	p	
$p\text{CO}_2$	1	1.995	1.995	32.58	0.001	***
Season	1	0.378	0.378	6.18	0.003	**
$p\text{CO}_2$ * Season	1	0.266	0.266	4.35	0.014	*
Residuals	22	1.347	0.061			
Total	25	3.986				

Relative to 350 μatm CO_2 , at 900 μatm the newly settled community during the ‘Warm Period’ showed increased coverage by microalgae and turf (from almost zero to ~30 % coverage; Fig. 2B), as well as increased corticated foliose algae (ten-fold increase; including the species *Zonaria diesingiana* J.Agardh, 1841), but a three-fold decrease in corticated macrophytes (Fig. 2C) and a four-fold decrease in crustose coralline algae (Fig. 2D). This resulted in a community with roughly similar coverage of the microalgae and turf, corticated macrophytes and crustose coralline algae. During the ‘Cold Period’, the 900 μatm communities showed a high coverage of microalgae and turf algae (~72 %; Fig. 2B), but an absence of corticated foliose algae. Both the corticated macrophytes (Fig. 2C) and crustose coralline algae (Fig. 2D) were similar in coverage between the ‘Warm Period’ and ‘Cold Period’ at 900 μatm CO_2 .

Table 3. PERMANOVA summary of Community Origin (‘Origin’: 350 vs. 900 μatm), Community Destination (‘Destination’: 350 vs. 900 μatm), and Time (‘6-months’ vs. ‘12-months’) for algal communities grown on settlement tiles off Shikine Island, Japan.

Term	<i>Df</i>	Sum Sq.	Mean Sq.	<i>F</i>	<i>p</i>	
Origin	1	0.910	0.910	13.05	0.001	***
Destination	1	0.669	0.669	9.59	0.002	**
Time	1	1.267	1.267	18.17	0.001	***
Origin * Destination	1	0.141	0.141	2.03	0.085	
Origin * Time	1	0.490	0.490	7.03	0.003	**
Destination * Time	1	1.16	1.16	16.66	0.001	***
Origin * Destination * Time	1	0.074	0.074	1.06	0.356	
Residuals	23	1.604	0.070			
Total	30	6.136				

303

304 3.2 Effects of early-stage composition and $p\text{CO}_2$ on subsequent community succession

305 After performing a reciprocal transplant of the established six-month communities for a further
 306 six-months, it was found that regardless of the conditions under which the communities were
 307 established (i.e. ‘Community Origin’), the communities converged to form similar
 308 communities based on the $p\text{CO}_2$ conditions they were currently residing in (i.e. ‘Community
 309 Destination’) (Fig. 3; Table 3).

310 At 12 months the 350 μatm CO_2 recruitment tiles typically had a corticated macrophyte
 311 upperstory (Fig. 4B, including *Codium coactum* Okamura, 1930 and *Chondracanthus*
 312 *tenellus*), with an understory of microalgae and turf algae (Fig. 4A), as well as crustose

Figure 3 – nMDS of community composition based on algal functional groups. Communities are grouped as those exposed to 350 μatm throughout (darker blue), transplanted from 900 μatm to 350 μatm (lighter blue), transplanted from 350 μatm to 900 μatm (lighter red), and exposed to 900 μatm throughout (darker red). The initial starting 6-month communities are displayed with open symbols and dashed lines, and the 12-month communities following the transplant are displayed with solid symbols and lines.

coralline algae (Fig. 4C, including *Lithophyllum* sp.). Both the ‘350 to 350 μ atm’ and the ‘900 to 350 μ atm’ communities had a higher total cover than at 6-months (significant increase by ~40 % cover for both). This was due to an increased cover of understory microalgae and turf algae in the ‘350 to 350 μ atm’ community (Fig. 4A), and increased corticated macrophytes (Fig. 4B; both as understory and canopy) for both communities. Overall the ‘350 to 350 μ atm’ community was most complex, followed by the ‘900 to 350 μ atm’ community (Fig. 4D).

At 900 μ atm CO₂, a typical community after 12 months lacked canopy algae and was predominantly comprised of turf algae (Fig. 4A, including *Biddulphia biddulphiana*) with some corticated foliose algae (including *Zonaria diesingiana*), and minimal cover of corticated macrophytes (Fig. 4B) and crustose coralline algae (Fig. 4C). The community that was transplanted into 900 μ atm (‘350 to 900 μ atm’) showed a decline in total cover (significant decrease by ~45% cover), and a large reduction in complexity (Fig. 4D) due to the loss of corticated macrophytes and crustose coralline algae (Figs 4B and 4C). The community that was consistently maintained at 900 μ atm (‘900 to 900 μ atm’) showed an increased coverage by turf and microalgae (Fig. 4A), but overall displayed similar levels of total cover (~100 % cover at 6-months and ~120 % cover at 12-months) and complexity (Fig. 4D) relative to the initial 6-month community.

OA locks algal succession to early stage

Figure 4. Percentage cover (%) of functional groups following a further 6-months settlement: (A) microalgae and turf algae, (B) corticated macrophytes, (C) and crustose coralline algae. (D) structural complexity of the communities. Communities are grouped as those exposed to 350 μatm throughout (darker blue), transplanted from 900 to 350 μatm (lighter blue) or 350 to 900 μatm (lighter red), and exposed to 900 μatm throughout (darker red). Two-way GLM results are presented in the top-right of each. See Table S2 for more detailed statistics.

3.3 Effects of early-stage composition and $p\text{CO}_2$ on community production

Net community production was reduced for the 350 μatm communities compared to the 900 μatm communities (Fig. 5A). This is due to greatly increased community respiration rates for those communities at 350 μatm (Fig. 5B), which resulted in the ‘350 – 350 μatm ’ communities showing no net community production, and the ‘900 – 350 μatm ’ communities having a negative net community production (Fig. 5A). Overall, the resulting communities had similar levels of gross community production (Fig. 5C).

OA locks algal succession to early stage

Figure 5. (A) Net community production (mol O₂ hr⁻¹ m⁻²), (B) Community respiration (mol O₂ hr⁻¹ m⁻²), and (C) Gross community production (mol O₂ hr⁻¹ m⁻²). Communities are grouped as those exposed to 350 μatm throughout (darker blue), transplanted between locations (900 to 350 μatm, lighter blue; and 350 to 900 μatm, lighter red), and exposed to 900 μatm throughout (darker red). See Table S3 for more detailed statistics.

337

338 3.4 Effects of early-stage composition and pCO₂ on sessile invertebrate community

339 To assess whether increased respiration was driven by an altered sessile invertebrate
 340 community for the 12-month communities, the community composition and total cover of the
 341 sessile fauna was assessed. The sessile invertebrate community composition of the 12-month
 342 communities was altered by both ‘Community Origin’ and ‘Community Destination’
 343 (PERMANOVA: $F_{1,14} = 15.07$, $p < 0.001$ and $F_{1,14} = 3.68$, $p = 0.042$, respectively; Fig. S2),
 344 with a greater coverage of ascidians (Didemnidae) and hydrozoans (Leptomedusae) in the

elevated $p\text{CO}_2$ conditions ('350 – 900 μatm ' and '900 – 900 μatm ') and a greater coverage of polychaetes (Serpulidae) in the reference $p\text{CO}_2$ conditions ('350 – 350 μatm ' and '900 – 350 μatm ') (see Fig. S3). However, the overall coverage of sessile fauna did not significantly differ between the treatments (GLM: 'Community Origin' $t = 1.17$, $p = 0.27$ and 'Community Destination' $t = -0.18$, $p = 0.86$), with no interaction (GLM: 'Community Origin' * 'Community Destination' $t = -0.07$, $p = 0.95$).

4 DISCUSSION

Ocean acidification alters the competitive abilities of algae and so is expected to change the structure, composition and functioning of both coastal and open ocean marine habitats (Cornwall et al., 2017; Hall-Spencer & Harvey, 2019). Observations at volcanic CO_2 seeps in the photic zone have shown profound ecosystem shifts towards simplified non-calcareous communities that are often algal dominated with lower biodiversity and reduced ecological complexity (Agostini et al., 2018; S. D. Connell et al., 2018; Foo et al., 2018; González-Delgado & Hernández, 2018; Kroeker, Gambi, et al., 2013). The underlying processes by which ocean acidification affects the structure of shallow-water marine communities are not clearly established and require additional investigation, although significant progress has been made in recent years (see Kroeker et al., 2011, 2012; Kroeker, Gambi, et al., 2013; Porzio et al., 2013; Teixidó et al., 2018; Vizzini et al., 2017). We found that an enriched CO_2 environment had a positive effect on r -selected, fast-growing microalgae and turf algal species in the early stages of community development. This species-poor, low complexity, early-successional stage was then locked-in as it inhibited the settlement and growth of corticated macrophytes. We highlight the potential ecological processes responsible for this change in a temperate rocky reef community exposed to enriched CO_2 conditions.

Ocean acidification altered competitive dominance after 6 months with substrata in the acidified conditions being dominated by microalgae and turf algae, rather than the corticated macrophytes and crustose coralline algae that dominated in reference $p\text{CO}_2$ conditions. This pattern was temporally consistent, showing that regardless of the growing season ocean acidification truncates the normal successional trajectories of communities (Baggini et al., 2014; Kroeker et al., 2012). The benefits of seawater acidification to opportunistic species, such as turf algae, over others (including calcareous species) is well established (S. D. Connell et al., 2013, 2018; Cornwall et al., 2017). Lowered carbonate saturation is a stressor to calcified macroalgae (Brinkman & Smith, 2015; Enochs et al., 2015; Fabricius et al., 2011; Kamenos et al., 2016) whilst turf algae and other fast-growing opportunistic species can use the additional carbon from ocean acidification to grow and compete for resources (Harvey et al., 2019; Kroeker et al., 2012; Porzio et al., 2013), thereby attaining dominance (S. D. Connell et al., 2018). The shift from typical coastal habitat-forming species (such as corals and kelp forests) to turf algal dominance causes a loss of structural complexity and associated ecosystem services (O'Brien & Scheibling, 2018; Rogers et al., 2014).

Before turf algae overgrew the recruitment tiles in acidified conditions (between 6 and 12 months, see Fig. 3B), both crustose coralline algae and corticated macrophytes recruited onto the substrata. This suggests that the divergence in community composition was not due to limited recruitment or a physiological intolerance to the acidified conditions, but was driven by altered competitive interactions (Crook et al., 2016; Kroeker et al., 2012) and/or loss of compensatory processes (S. D. Connell et al., 2018; Ghedini et al., 2015). Although bottom-up control helps stimulate algal growth on coral and rocky reefs, grazing pressure determines whether turf algae dominate (Mumby et al., 2006). The grazing pressure of large benthic invertebrates in acidified sites is thought to be lowered due to physiological impacts (Calosi et al., 2013; O'Donnell et al., 2010) which cause their size and abundance to be reduced compared

to the reference $p\text{CO}_2$ areas (Harvey et al. unpublished, this site and Vulcano, Italy; Connell et al. 2018, White Island, New Zealand). Similarly, the observed number of sea urchin feeding halos has also previously been found to be reduced in the Ischia CO_2 seeps (Kroeker, Gambi, et al., 2013). Fish communities also play a key role in top-down control and, in our site, communities included more herbivorous fishes than the surrounding non-acidified areas (Cattano et al., 2020). Clearly, the increased turf algae supported a greater herbivorous fish population than in the reference conditions, but those same herbivores alone are not able to control the increased growth of the boosted turf algae (also see Baggini et al., 2015; S. D. Connell et al., 2018). Although outside the scope of this study, this may support the notion that the fish are preferentially consuming different algae other than the turfs and further reinforcing the ecological shift.

After 12 months, assemblages in reference $p\text{CO}_2$ conditions continued to gain species through time and had developed more structurally complex communities with clearly defined understory and canopy species. The assemblages in the elevated $p\text{CO}_2$ became arrested in terms of their successional development due to competition for space by the turf algae. A similar overgrowth and dominance by turf algae was observed on recruitment tiles in the Ischia CO_2 seep (Kroeker et al., 2012; Porzio et al., 2013). This community development in our study resulted in a similar community composition at 6 months and 12 months with only the abundance of the turf algae being increased. At 12 months, communities on the tiles were visually indistinguishable from the surrounding rocky substrata (Fig. S3). Similar declines in macroalgal diversity with increasing $p\text{CO}_2$ have also been demonstrated in Methana, Greece (Baggini et al., 2014). The simplification of marine ecosystems has been observed across CO_2 seeps (Agostini et al., 2018; Brown et al., 2018; Cigliano et al., 2010; Fabricius et al., 2011; Kroeker, Gambi, et al., 2013; Vizzini et al., 2017), with such changes leading to a functional-biodiversity loss in the system (Teixidó et al., 2018). It is likely that such simple systems are

maintained by reinforcing feedback loops (sediment trapping, changes in physicochemical environment, and recruitment inhibition) that facilitate turf algal dominance. Turf algae can inhibit successional development through reducing primary substratum availability (Airoidi, 1998; S. D. Connell & Russell, 2010) and by trapping sediment which alters settlement surface chemistry and reduces the survival of other recruits (Airoidi, 2003; Gorman & Connell, 2009). Such dominance by short-lived species, which then locks the system in place, can lead to decreased stability in the system (Stachowicz et al., 2007), with implications for the functioning of the system under future ocean acidification (Teixidó et al., 2018).

In terms of community dynamics, both the reference and elevated $p\text{CO}_2$ conditions appeared to overwhelm any ecological resistance that would have otherwise resisted ecosystem change. This was demonstrated by the established algal communities that were transplanted from reference to elevated $p\text{CO}_2$ conditions converging (in terms of community composition) to almost match the community formed under elevated $p\text{CO}_2$ conditions (and vice-versa). This suggests that acidification-driven ecological shifts to simplified turf algae communities will occur regardless of the state that the community is in, and means that the community successional trajectory is not fixed from the initial bare substratum during primary or secondary succession. The prevention of such shifts by ecosystem management will require resilience building in order to mitigate the future degradation of ecosystems (Billé et al., 2013; Falkenberg et al., 2013). In contrast, the convergence of the communities transplanted from elevated $p\text{CO}_2$ conditions to the reference $p\text{CO}_2$ conditions could mean that recovery from a degraded state is possible. This would likely be due to sufficient compensatory processes at our reference $p\text{CO}_2$ location, and/or the turf algae losing its competitive edge in the absence of elevated $p\text{CO}_2$. Therefore, a combination of conservation strategy and meaningful reductions in atmospheric CO_2 emissions could achieve substantial recovery of the abundance, structure and function of shallow coastal marine ecosystems (Duarte et al., 2020).

Despite possessing highly divergent communities, gross oxygen production was similar between all of the transplanted tiles. Net oxygen production, however, was positive in the acidified conditions, but balanced between productivity and respiration for the reference $p\text{CO}_2$ communities due to elevated respiration. Ecosystems that are more developed and stable will tend towards rates of oxygen production and respiration being equal, tending to not accumulate further biomass. Early stage ecosystems will tend to have a higher productivity per biomass, but will be lacking in terms of biomass and species diversity (Cooke, 1967). This further supports the concept that the algal community developing under elevated $p\text{CO}_2$ is arrested into a typical early stage community dominated by *r*-selected species. Previous studies in CO_2 seeps have generally focussed on the primary production or photophysiology of individual species of algae (e.g. Celis-Plá et al., 2015; Porzio et al., 2018, 2020) with the aim of assessing their physiological response to ocean acidification, rather than the effects on overall community net production (making comparisons difficult). The sessile invertebrate communities differed in community composition between the reference and elevated $p\text{CO}_2$ sites, but not in percentage cover, suggesting that they were not a sizeable contributor toward such large changes in net oxygen production. Instead, the decreased net oxygen was likely driven by the greater algal biomass (as well as low surface to volume ratio) of the more highly structurally complex reference community compared to the high surface to biomass ratio of the turf algae. Taken together, this suggests that the greater net production stimulated by ocean acidification does not translate into enhanced ecosystem benefits, such as increased community cover, biomass, biodiversity or structural complexity, as well as an altered sessile invertebrate community.

Natural analogues provide a number of benefits for advancing our understanding about the responses of shallow water marine communities to ocean acidification conditions, but they are not perfect analogues. Carbonate chemistry at some CO_2 seeps can be highly variable (S. P. S. Rastrick et al., 2018), and areas in close proximity to CO_2 vents can be enriched in some metals

and toxins (Vizzini et al., 2013; Zitoun et al., 2020). It is possible to reduce such confounding factors by avoiding toxic areas and only selecting sites a suitable distance away, since contamination from hydrothermal fluids can be quickly diluted by mixing with seawater (Agostini et al., 2015; Pichler et al., 2019). The gas being released at our study site is $98 \pm 3 \%$ CO_2 , and although concentrations of hydrogen sulfide are detected at the main vent, they are below detection limits ~ 50 m away from the main vents (Agostini et al., 2015) and the study site used in this study is more than 300 m away from the main vent. An additional consideration for CO_2 seeps is that they demonstrate the consequences of future ocean acidification but in the absence of concurrent ocean warming (Hughes et al., 2017), and temperatures will mediate the response of organisms and communities to future ocean acidification. Such an issue can be addressed by comparing CO_2 seep systems under different thermal regimes and assess the consistency of responses (V. R. Johnson et al., 2012), or by manipulating temperature along CO_2 gradients (Alessi et al., 2019). Despite these caveats, the use of CO_2 seeps is still invaluable for providing a window into the future state of organisms, communities and ecosystems to future ocean acidification (S. P. S. Rastrick et al., 2018).

In conclusion, ocean acidification can set the course of successional development in algal communities that benefit turf algae, causing reduced algal biomass, diversity and complexity. Altered carbonate chemistry can enable opportunistic *r*-selected species to competitively exclude other species and lock the community in a species-poor early successional stage. The ecological process responsible for this shift in community composition was not simply altering community trajectory during primary succession, as the same shift occurred in pre-established communities. This highlights that without reducing atmospheric CO_2 emissions we may increasingly observe the loss of large algal habitats and the spread of fast-growing, small opportunistic species that can utilise additional inorganic carbon. By understanding the ecological processes responsible for driving shifts in community composition, we can begin to

494 better assess how communities are likely to be altered by ocean acidification. Finally, our
495 results show that the recovery of shallow water marine communities is possible if meaningful
496 reductions in CO₂ emissions are implemented, as encouraged by the Paris Agreement.

497

ACKNOWLEDGEMENTS

We thank the technical staff at ‘Shimoda Marine Research Center, University of Tsukuba’ for their assistance aboard RV *Tsukuba II* and at the study site, and the Japan Fisheries agencies of Nijima/Shikine Island (Tokyo prefecture) for their support. This project was heavily supported by the ‘International Education and Research Laboratory Program’, University of Tsukuba. This work was also supported by JSPS KAKENHI Grant Number 17K17622, and we acknowledge funding support from the Ministry of Environment, Government of Japan (Suishinhi: 4RF-1701).

Some of the images used within the graphical abstract are courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/).

COMPETING INTERESTS

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

BH conceived the idea, designed the methodology, analysed the data, and led the writing of the manuscript. BH and KK carried out the image analysis. BH and SA performed the oxygen production measurements. All authors assisted with field work, contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY

[Upon acceptance] Raw data used to create Figs 2-5 and Tables 1-3 will be supplied and stored in the Pangaea repository (<http://pangaea.de>).

REFERENCES

Abràmoff, M. D., Magalhães, P. J., & Ram, S. J. (2004). Image processing with ImageJ. *Biophotonics International*, 11(7), 36–42.

Agostini, S., Harvey, B. P., Wada, S., Kon, K., Milazzo, M., Inaba, K., & Hall-Spencer, J. M. (2018). Ocean acidification drives community shifts towards simplified non-calcified habitats in a subtropical-temperate transition zone. *Scientific Reports*, 8, 11354. <https://doi.org/10.1038/s41598-018-29251-7>

Agostini, S., Wada, S., Kon, K., Omori, A., Kohtsuka, H., Fujimura, H., Tsuchiya, Y., Sato, T., Shinagawa, H., Yamada, Y., & Inaba, K. (2015). Geochemistry of two shallow CO₂ seeps in Shikine Island (Japan) and their potential for ocean acidification research. *Regional Studies in Marine Science*, 2, Supplement, 45–53.

Airoidi, L. (1998). Roles of disturbance, sediment stress, and substratum retention on spatial dominance in algal turf. *Ecology*, 79(8), 2759–2770. [https://doi.org/10.1890/0012-9658\(1998\)079\[2759:RODSSA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1998)079[2759:RODSSA]2.0.CO;2)

Airoidi, L. (2003). The effects of sedimentation on rocky coast assemblages. *Oceanography and Marine Biology, An Annual Review*, 41, 169–171.

Albright, R., Caldeira, L., Hosfelt, J., Kwiatkowski, L., Maclaren, J. K., Mason, B. M., Nebuchina, Y., Ninokawa, A., Pongratz, J., Ricke, K. L., Rivlin, T., Schneider, K.,

- 542 Sesboüé, M., Shamberger, K., Silverman, J., Wolfe, K., Zhu, K., & Caldeira, K. (2016).
 543 Reversal of ocean acidification enhances net coral reef calcification. *Nature*,
 544 *531*(7594), 362–365.
- 545 Albright, R., Takeshita, Y., Koweeck, D. A., Ninokawa, A., Wolfe, K., Rivlin, T., Nebuchina,
 546 Y., Young, J., & Caldeira, K. (2018). Carbon dioxide addition to coral reef waters
 547 suppresses net community calcification. *Nature*, *555*, 516.
 548 <https://doi.org/10.1038/nature25968>
- 549 Alessi, C., Giomi, F., Furnari, F., Sarà, G., Chemello, R., & Milazzo, M. (2019). Ocean
 550 acidification and elevated temperature negatively affect recruitment, oxygen
 551 consumption and calcification of the reef-building *Dendropoma cristatum* early life
 552 stages: Evidence from a manipulative field study. *Science of The Total Environment*,
 553 *693*, 133476.
- 554 Algueró-Muñiz, M., Alvarez-Fernandez, S., Thor, P., Bach, L. T., Esposito, M., Horn, H. G.,
 555 Ecker, U., Langer, J. A. F., Taucher, J., Malzahn, A. M., Riebesell, U., & Boersma, M.
 556 (2017). Ocean acidification effects on mesozooplankton community development:
 557 Results from a long-term mesocosm experiment. *PLOS ONE*, *12*(4), e0175851.
 558 <https://doi.org/10.1371/journal.pone.0175851>
- 559 Baggini, C., Issaris, Y., Salomidi, M., & Hall-Spencer, J. (2015). Herbivore diversity improves
 560 benthic community resilience to ocean acidification. *Journal of Experimental Marine*
 561 *Biology and Ecology*, *469*(0), 98–104. <https://doi.org/10.1016/j.jembe.2015.04.019>
- 562 Baggini, C., Salomidi, M., Voutsinas, E., Bray, L., Krasakopoulou, E., & Hall-Spencer, J. M.
 563 (2014). Seasonality affects macroalgal community response to increases in $p\text{CO}_2$. *PLoS*
 564 *One*, *9*(9), e106520.

- 565 Billé, R., Kelly, R., Biastoch, A., Harrould-Kolieb, E., Herr, D., Joos, F., Kroeker, K., Laffoley,
566 D., Oschlies, A., & Gattuso, J.-P. (2013). Taking Action Against Ocean Acidification:
567 A Review of Management and Policy Options. *Environmental Management*, 52(4),
568 761–779. <https://doi.org/10.1007/s00267-013-0132-7>
- 569 Brinkman, T. J., & Smith, A. M. (2015). Effect of climate change on crustose coralline algae
570 at a temperate vent site, White Island, New Zealand. *Mar. Freshwater Res.*, 66(4), 360–
571 370.
- 572 Brown, N. E. M., Milazzo, M., Rastrick, S. P. S., Hall-Spencer, J. M., Therriault, T. W., &
573 Harley, C. D. G. (2018). Natural acidification changes the timing and rate of succession,
574 alters community structure, and increases homogeneity in marine biofouling
575 communities. *Global Change Biology*, 24(1), e112–e127.
576 <https://doi.org/10.1111/gcb.13856>
- 577 Brown, N. E. M., Therriault, T. W., & Harley, C. D. G. (2016). Field-based experimental
578 acidification alters fouling community structure and reduces diversity. *Journal of*
579 *Animal Ecology*, 85(5), 1328–1339. <https://doi.org/10.1111/1365-2656.12557>
- 580 Calosi, P., Rastrick, S. P. S., Graziano, M., Thomas, S. C., Baggini, C., Carter, H. A., Hall-
581 Spencer, J. M., Milazzo, M., & Spicer, J. I. (2013). Distribution of sea urchins living
582 near shallow water CO₂ vents is dependent upon species acid-base and ion-regulatory
583 abilities. *Mar. Pollut. Bull.*, 73(2), 470–484.
- 584 Cattano, C., Agostini, S., Harvey, B. P., Wada, S., Quattrocchi, F., Turco, G., Inaba, K., Hall-
585 Spencer, J. M., & Milazzo, M. (2020). Changes in fish communities due to benthic
586 habitat shifts under ocean acidification conditions. *Science of The Total Environment*,
587 725, 138501. <https://doi.org/10.1016/j.scitotenv.2020.138501>

- 588 Celis-Plá, P. S. M., Hall-Spencer, J. M., Horta, P. A., Milazzo, M., Korbee, N., Cornwall, C.
589 E., & Figueroa, F. L. (2015). Macroalgal responses to ocean acidification depend on
590 nutrient and light levels. *Frontiers in Marine Science*, 2, 26.
591 <https://doi.org/10.3389/fmars.2015.00026>
- 592 Cigliano, M., Gambi, M. C., Rodolfo-Metalpa, R., Patti, F. P., & Hall-Spencer, J. M. (2010).
593 Effects of ocean acidification on invertebrate settlement at volcanic CO₂ vents. *Marine*
594 *Biology*, 157(11), 2489–2502. <https://doi.org/10.1007/s00227-010-1513-6>
- 595 Connell, J. H., & Slatyer, R. O. (1977). Mechanisms of succession in natural communities and
596 their role in community stability and organization. *The American Naturalist*, 111(982),
597 1119–1144. <https://doi.org/10.1086/283241>
- 598 Connell, S. D., Doubleday, Z. A., Foster, N. R., Hamlyn, S. B., Harley, C. D., Helmuth, B.,
599 Kelaher, B. P., Nagelkerken, I., Rodgers, K. L., Sarà, G., & Russell, B. D. (2018). The
600 duality of ocean acidification as a resource and a stressor. *Ecology*, 99(5), 1005–1010.
601 <https://doi.org/10.1002/ecy.2209>
- 602 Connell, S. D., Kroeker, K. J., Fabricius, K. E., Kline, D. I., & Russell, B. D. (2013). The other
603 ocean acidification problem: CO₂ as a resource among competitors for ecosystem
604 dominance. *Philosophical Transactions of the Royal Society B: Biological Sciences*,
605 368(1627), 20120442. <https://doi.org/10.1098/rstb.2012.0442>
- 606 Connell, S. D., & Russell, B. D. (2010). The direct effects of increasing CO₂ and temperature
607 on non-calcifying organisms: Increasing the potential for phase shifts in kelp forests.
608 *Proc. Biol. Sci.*, 277(1686), 1409–1415.
- 609 Cooke, G. D. (1967). The pattern of autotrophic succession in laboratory microcosms.
610 *BioScience*, 17(10), 717–721. <https://doi.org/10.2307/1294089>

- 611 Cornwall, C. E., Revill, A. T., Hall-Spencer, J. M., Milazzo, M., Raven, J. A., & Hurd, C. L.
 612 (2017). Inorganic carbon physiology underpins macroalgal responses to elevated CO₂.
 613 *Scientific Reports*, 7, 46297. <https://doi.org/10.1038/srep46297>
- 614 Crook, E. D., Kroeker, K. J., Potts, D. C., Rebolledo-Vieyra, M., Hernandez-Terrones, L. M.,
 615 & Paytan, A. (2016). Recruitment and succession in a tropical benthic community in
 616 response to in-situ ocean acidification. *PLOS ONE*, 11(1), e0146707.
 617 <https://doi.org/10.1371/journal.pone.0146707>
- 618 Done, T. (1992). Effects of tropical cyclone waves on ecological and geomorphological
 619 structures on the Great Barrier Reef. *Continental Shelf Research*, 12(7–8), 859–872.
- 620 Duarte, C. M., Agusti, S., Barbier, E., Britten, G. L., Castilla, J. C., Gattuso, J.-P., Fulweiler,
 621 R. W., Hughes, T. P., Knowlton, N., Lovelock, C. E., Lotze, H. K., Predragovic, M.,
 622 Poloczanska, E., Roberts, C., & Worm, B. (2020). Rebuilding marine life. *Nature*,
 623 580(7801), 39–51. <https://doi.org/10.1038/s41586-020-2146-7>
- 624 Enochs, I. C., Manzello, D. P., Donham, E. M., Kolodziej, G., Okano, R., Johnston, L., Young,
 625 C., Iguel, J., Edwards, C. B., Fox, M. D., Valentino, L., Johnson, S., Benavente, D.,
 626 Clark, S. J., Carlton, R., Burton, T., Eynaud, Y., & Price, N. N. (2015). Shift from coral
 627 to macroalgae dominance on a volcanically acidified reef. *Nature Climate Change*,
 628 5(12), 1083–1088. <https://doi.org/10.1038/nclimate2758>
- 629 Fabricius, K. E., Langdon, C., Uthicke, S., Humphrey, C., Noonan, S., De'ath, G., Okazaki,
 630 R., Muehllehner, N., Glas, M. S., & Lough, J. M. (2011). Losers and winners in coral
 631 reefs acclimatized to elevated carbon dioxide concentrations. *Nature Climate Change*,
 632 1(3), 165–169. <https://doi.org/10.1038/nclimate1122>

- 633 Falkenberg, L. J., Connell, S. D., & Russell, B. D. (2013). Disrupting the effects of synergies
634 between stressors: Improved water quality dampens the effects of future CO₂ on a
635 marine habitat. *Journal of Applied Ecology*, 50(1), 51–58.
636 <https://doi.org/10.1111/1365-2664.12019>
- 637 Foo, S. A., Byrne, M., Ricevuto, E., & Gambi, M. C. (2018). The carbon dioxide vents of
638 Ischia, Italy, a natural system to assess impacts of ocean acidification on marine
639 ecosystems: An overview of research and comparisons with other vent systems.
640 *Oceanography and Marine Biology: An Annual Review*, 56, 237–310.
641 <https://doi.org/10.1201/9780429454455-9>
- 642 Garilli, V., Rodolfo-Metalpa, R., Scuderi, D., Brusca, L., Parrinello, D., Rastrick, S. P. S.,
643 Foggo, A., Twitchett, R. J., Hall-Spencer, J. M., & Milazzo, M. (2015). Physiological
644 advantages of dwarfing in surviving extinctions in high-CO₂ oceans. *Nature Climate*
645 *Change*, 5(7), 678–682. <https://doi.org/10.1038/nclimate2616>
- 646 Gattuso, J.-P., Magnan, A., Billé, R., Cheung, W. W. L., Howes, E. L., Joos, F., Allemand, D.,
647 Bopp, L., Cooley, S. R., Eakin, C. M., Hoegh-Guldberg, O., Kelly, R. P., Pörtner, H.-
648 O., Rogers, A. D., Baxter, J. M., Laffoley, D., Osborn, D., Rankovic, A., Rochette, J.,
649 ... Turley, C. (2015). Contrasting futures for ocean and society from different
650 anthropogenic CO₂ emissions scenarios. *Science*, 349(6243), aac4722.
651 <https://doi.org/10.1126/science.aac4722>
- 652 Gaylord, B., Kroeker, K. J., Sunday, J. M., Anderson, K. M., Barry, J. P., Brown, N. E.,
653 Connell, S. D., Dupont, S., Fabricius, K. E., Hall-Spencer, J. M., Klinger, T., Milazzo,
654 M., Munday, P. L., Russell, B. D., Sanford, E., Schreiber, S. J., Thiyagarajan, V.,
655 Vaughan, M. L. H., Widdicombe, S., & Harley, C. D. G. (2015). Ocean acidification
656 through the lens of ecological theory. *Ecology*, 96(1), 3–15.

- 657 Ghedini, G., & Connell, S. D. (2016). Organismal homeostasis buffers the effects of abiotic
658 change on community dynamics. *Ecology*, 97(10), 2671–2679.
659 <https://doi.org/10.1002/ecy.1488>
- 660 Ghedini, G., Russell, B. D., & Connell, S. D. (2015). Trophic compensation reinforces
661 resistance: Herbivory absorbs the increasing effects of multiple disturbances. *Ecology*
662 *Letters*, 18(2), 182–187. <https://doi.org/10.1111/ele.12405>
- 663 Godbold, J. A., & Solan, M. (2013). Long-term effects of warming and ocean acidification are
664 modified by seasonal variation in species responses and environmental conditions.
665 *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368(1627),
666 20130186.
- 667 González-Delgado, S., & Hernández, J. C. (2018). The importance of natural acidified systems
668 in the study of ocean acidification: What have we learned? *Advances in Marine Biology*,
669 80, 57–99. <https://doi.org/10.1016/bs.amb.2018.08.001>
- 670 Gordillo, F. J., Figueroa, F. L., & Niell, F. X. (2003). Photon- and carbon-use efficiency in *Ulva*
671 *rigida* at different CO₂ and N levels. *Planta*, 218(2), 315–322.
- 672 Gorman, D., & Connell, S. D. (2009). Recovering subtidal forests in human-dominated
673 landscapes. *Journal of Applied Ecology*, 46(6), 1258–1265.
674 <https://doi.org/10.1111/j.1365-2664.2009.01711.x>
- 675 Gruner, D. S., Smith, J. E., Seabloom, E. W., Sandin, S. A., Ngai, J. T., Hillebrand, H., Harpole,
676 W. S., Elser, J. J., Cleland, E. E., Bracken, M. E. S., Borer, E. T., & Bolker, B. M.
677 (2008). A cross-system synthesis of consumer and nutrient resource control on producer
678 biomass. *Ecol. Lett.*, 11(7), 740–755.

- 679 Hall-Spencer, J. M., & Harvey, B. P. (2019). Ocean acidification impacts on coastal ecosystem
680 services due to habitat degradation. *Emerging Topics in Life Sciences*.
681 <https://doi.org/10.1042/ETLS20180117>
- 682 Hall-Spencer, J. M., Rodolfo-Metalpa, R., Martin, S., Ransome, E., Fine, M., Turner, S. M.,
683 Rowley, S. J., Tedesco, D., & Buia, M.-C. (2008). Volcanic carbon dioxide vents show
684 ecosystem effects of ocean acidification. *Nature*, 454(7200), 96–99.
685 <https://doi.org/10.1038/nature07051>
- 686 Harvey, B. P., Agostini, S., Kon, K., Wada, S., & Hall-Spencer, J. M. (2019). Diatoms
687 dominate and alter marine food-webs when CO₂ rises. *Diversity*, 11(12).
688 <https://doi.org/10.3390/d11120242>
- 689 Harvey, B. P., Agostini, S., Wada, S., Inaba, K., & Hall-Spencer, J. M. (2018). Dissolution:
690 The Achilles’ heel of the triton shell in an acidifying ocean. *Frontiers in Marine*
691 *Science*, 5, 371. <https://doi.org/10.3389/fmars.2018.00371>
- 692 Harvey, B. P., Gwynn-Jones, D., & Moore, P. J. (2013). Meta-analysis reveals complex marine
693 biological responses to the interactive effects of ocean acidification and warming.
694 *Ecology and Evolution*, 3(4), 1016–1030. <https://doi.org/10.1002/ece3.516>
- 695 Harvey, B. P., McKeown, N. J., Rastrick, S. P. S., Bertolini, C., Foggo, A., Graham, H., Hall-
696 Spencer, J. M., Milazzo, M., Shaw, P. W., Small, D. P., & Moore, P. J. (2016).
697 Individual and population-level responses to ocean acidification. *Scientific Reports*, 6,
698 20194. <https://doi.org/10.1038/srep20194>
- 699 Hillebrand, H., Gruner, D. S., Borer, E. T., Bracken, M. E. S., Cleland, E. E., Elser, J. J.,
700 Harpole, W. S., Ngai, J. T., Seabloom, E. W., Shurin, J. B., & Smith, J. E. (2007).
701 Consumer versus resource control of producer diversity depends on ecosystem type and

- 702 producer community structure. *Proceedings of the National Academy of Sciences*,
 703 104(26), 10904. <https://doi.org/10.1073/pnas.0701918104>
- 704 Hughes, T. P., Barnes, M. L., Bellwood, D. R., Cinner, J. E., Cumming, G. S., Jackson, J. B.
 705 C., Kleypas, J., van de Leemput, I. A., Lough, J. M., Morrison, T. H., Palumbi, S. R.,
 706 van Nes, E. H., & Scheffer, M. (2017). Coral reefs in the Anthropocene. *Nature*,
 707 546(7656), 82–90. <https://doi.org/10.1038/nature22901>
- 708 Hughes, T. P., Rodrigues, M. J., Bellwood, D. R., Ceccarelli, D., Hoegh-Guldberg, O.,
 709 McCook, L., Moltschaniwskyj, N., Pratchett, M. S., Steneck, R. S., & Willis, B. (2007).
 710 Phase shifts, herbivory, and the resilience of coral reefs to climate change. *Current*
 711 *Biology*, 17(4), 360–365. <https://doi.org/10.1016/j.cub.2006.12.049>
- 712 IPCC. (2013). *Climate Change 2013—The Physical Science Basis: Working Group I*
 713 *Contribution to the Fifth Assessment Report of the IPCC* (No. 0521880092; p. 1535).
 714 Cambridge University Press.
- 715 Johnson, C. R., & Mann, K. H. (1988). Diversity, patterns of adaptation, and stability of Nova
 716 Scotian kelp beds. *Ecological Monographs*, 58(2), 129–154.
 717 <https://doi.org/10.2307/1942464>
- 718 Johnson, V. R., Russell, B. D., Fabricius, K. E., Brownlee, C., & Hall-Spencer, J. M. (2012).
 719 Temperate and tropical brown macroalgae thrive, despite decalcification, along natural
 720 CO₂ gradients. *Glob. Chang. Biol.*, 18(9), 2792–2803.
- 721 Kamenos, N. A., Perna, G., Gambi, M. C., Micheli, F., & Kroeker, K. J. (2016). Coralline algae
 722 in a naturally acidified ecosystem persist by maintaining control of skeletal mineralogy
 723 and size. *Proceedings of the Royal Society B: Biological Sciences*, 283(1840),
 724 20161159. <https://doi.org/10.1098/rspb.2016.1159>

- 725 Kassambara, A. (2019). Ggpubr:“ggplot2” based publication ready plots. *R Package Version*
 726 *0.2.4*. <https://CRAN.R-project.org/package=ggpubr>
- 727 Kerfahi, D., Harvey, B. P., Agostini, S., Kon, K., Huang, R., Adams, J. M., & Hall-Spencer, J.
 728 M. (2020). Responses of intertidal bacterial biofilm communities to increasing $p\text{CO}_2$.
 729 *Marine Biotechnology*. <https://doi.org/10.1007/s10126-020-09958-3>
- 730 Koch, M., Bowes, G., Ross, C., & Zhang, X.-H. (2013). Climate change and ocean acidification
 731 effects on seagrasses and marine macroalgae. *Global Change Biology*, *19*(1), 103–132.
 732 <https://doi.org/10.1111/j.1365-2486.2012.02791.x>
- 733 Kroeker, K. J., Gambi, M. C., & Micheli, F. (2013). Community dynamics and ecosystem
 734 simplification in a high- CO_2 ocean. *Proceedings of the National Academy of Sciences*
 735 *of the United States of America*, *110*(31), 12721–12726.
 736 <https://doi.org/10.1073/pnas.1216464110>
- 737 Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C.
 738 M., & Gattuso, J.-P. (2013). Impacts of ocean acidification on marine organisms:
 739 Quantifying sensitivities and interaction with warming. *Global Change Biology*, *19*(6),
 740 1884–1896. <https://doi.org/10.1111/gcb.12179>
- 741 Kroeker, K. J., Micheli, F., & Gambi, M. C. (2012). Ocean acidification causes ecosystem
 742 shifts via altered competitive interactions. *Nature Climate Change*, *3*(2), 156–159.
- 743 Kroeker, K. J., Micheli, F., Gambi, M. C., & Martz, T. R. (2011). Divergent ecosystem
 744 responses within a benthic marine community to ocean acidification. *Proceedings of*
 745 *the National Academy of Sciences of the United States of America*, *108*(35), 14515–
 746 14520. <https://doi.org/10.1073/pnas.1107789108>

- 747 Li, W., Gao, K., & Beardall, J. (2012). Interactive effects of ocean acidification and nitrogen-
748 limitation on the diatom *Phaeodactylum tricornutum*. *PloS One*, 7(12), e51590–e51590.
749 PubMed. <https://doi.org/10.1371/journal.pone.0051590>
- 750 Milazzo, M., Alessi, C., Quattrocchi, F., Chemello, R., D’Agostaro, R., Gil, J., Vaccaro, A.
751 M., Mirto, S., Gristina, M., & Badalamenti, F. (2019). Biogenic habitat shifts under
752 long-term ocean acidification show nonlinear community responses and unbalanced
753 functions of associated invertebrates. *Science of The Total Environment*, 667, 41–48.
754 <https://doi.org/10.1016/j.scitotenv.2019.02.391>
- 755 Milazzo, M., Rodolfo-Metalpa, R., Chan, V. B. S., Fine, M., Alessi, C., Thiyagarajan, V., Hall-
756 Spencer, J. M., & Chemello, R. (2014). Ocean acidification impairs vermetid reef
757 recruitment. *Scientific Reports*, 4, 4189. <https://doi.org/10.1038/srep04189>
- 758 Moulin, L., Grosjean, P., Leblud, J., Batigny, A., Collard, M., & Dubois, P. (2015). Long-term
759 mesocosms study of the effects of ocean acidification on growth and physiology of the
760 sea urchin *Echinometra mathaei*. *Marine Environmental Research*, 103, 103–114.
761 <https://doi.org/10.1016/j.marenvres.2014.11.009>
- 762 Mumby, P. J., Dahlgren, C. P., Harborne, A. R., Kappel, C. V., Micheli, F., Brumbaugh, D. R.,
763 Holmes, K. E., Mendes, J. M., Broad, K., & Sanchirico, J. N. (2006). Fishing, trophic
764 cascades, and the process of grazing on coral reefs. *Science*, 311(5757), 98–101.
- 765 O’Brien, J., & Scheibling, R. (2018). Turf wars: Competition between foundation and turf-
766 forming species on temperate and tropical reefs and its role in regime shifts. *Marine*
767 *Ecology Progress Series*, 590, 1–17.

- 768 O'Donnell, M. J., Todgham, A. E., Sewell, M. A., Hammond, L. M., Ruggiero, K., Fangué, N.
769 A., Zippay, M. L., & Hofmann, G. E. (2010). Ocean acidification alters skeletogenesis
770 and gene expression in larval sea urchins. *Mar. Ecol. Prog. Ser.*, 398, 157–171.
- 771 Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.
772 R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., &
773 Wagner, H. (2019). The vegan package. *Vegan: Community Ecology Package. R*
774 *Package Version 2.5-5* [[Https://Cran.r-Project.Org/Package=vegan](https://Cran.r-Project.Org/Package=vegan)].
- 775 Pichler, T., Biscéré, T., Kinch, J., Zampighi, M., Houlbrèque, F., & Rodolfo-Metalpa, R.
776 (2019). Suitability of the shallow water hydrothermal system at Ambitle Island (Papua
777 New Guinea) to study the effect of high $p\text{CO}_2$ on coral reefs. *Marine Pollution Bulletin*,
778 138, 148–158. <https://doi.org/10.1016/j.marpolbul.2018.11.003>
- 779 Porzio, L., Arena, C., Lorenti, M., De Maio, A., & Buia, M. C. (2020). Long-term response of
780 *Dictyota dichotoma* var. *Intricata* (C. Agardh) Greville (Phaeophyceae) to ocean
781 acidification: Insights from high $p\text{CO}_2$ vents. *Science of The Total Environment*, 731,
782 138896. <https://doi.org/10.1016/j.scitotenv.2020.138896>
- 783 Porzio, L., Buia, M. C., Ferretti, V., Lorenti, M., Rossi, M., Trifuoggi, M., Vergara, A., &
784 Arena, C. (2018). Photosynthesis and mineralogy of *Jania rubens* at low pH/high $p\text{CO}_2$:
785 A future perspective. *Science of The Total Environment*, 628–629, 375–383.
786 <https://doi.org/10.1016/j.scitotenv.2018.02.065>
- 787 Porzio, L., Garrard, S. L., & Buia, M. C. (2013). The effect of ocean acidification on early algal
788 colonization stages at natural CO_2 vents. *Marine Biology*, 160(8), 2247–2259.
789 <https://doi.org/10.1007/s00227-013-2251-3>

- 790 Rastrick, S. P. S., Graham, H., Azetsu-Scott, K., Calosi, P., Chierici, M., Fransson, A., Hop,
791 H., Hall-Spencer, J., Milazzo, M., Thor, P., Kutti, T., & Handling editor: C. Brock
792 Woodson. (2018). Using natural analogues to investigate the effects of climate change
793 and ocean acidification on Northern ecosystems. *ICES Journal of Marine Science*,
794 75(7), 2299–2311. <https://doi.org/10.1093/icesjms/fsy128>
- 795 Rogers, A., Blanchard, J. L., & Mumby, P. J. (2014). Vulnerability of Coral Reef Fisheries to
796 a Loss of Structural Complexity. *Current Biology*, 24(9), 1000–1005.
797 <https://doi.org/10.1016/j.cub.2014.03.026>
- 798 Stachowicz, J. J., Bruno, J. F., & Duffy, J. E. (2007). Understanding the effects of marine
799 biodiversity on communities and ecosystems. *Annu. Rev. Ecol. Evol. Syst.*, 38, 739–
800 766.
- 801 Steneck, R. S., & Dethier, M. N. (1994). A functional group approach to the structure of algal-
802 dominated communities. *Oikos*, 69(3), 476–498.
- 803 Sunday, J. M., Fabricius, K. E., Kroeker, K. J., Anderson, K. M., E, B. N., Barry, J. P., Connell,
804 S. D., Dupont, S., Gaylord, B., Hall-Spencer, J. M., Klinger, T., Milazzo, M., Munday,
805 P. L., Russell, B. D., Sanford, E., Thiyagarajan, V., Vaughan, M. L. H., Widdicombe,
806 S., & Harley, C. D. G. (2017). Ocean acidification can mediate biodiversity shifts by
807 changing biogenic habitat. *Nature Climate Change*, 7, 81–85.
808 <https://doi.org/10.1038/nclimate3161>
- 809 Team, R. D. C., & R Development Core Team, R. (2019). *R: A Language and Environment*
810 *for Statistical Computing*.

- 811 Teixidó, N., Gambi, M. C., Parravacini, V., Kroeker, K., Micheli, F., Villéger, S., &
 812 Ballesteros, E. (2018). Functional biodiversity loss along natural CO₂ gradients. *Nature*
 813 *Communications*, 9(1), 5149. <https://doi.org/10.1038/s41467-018-07592-1>
- 814 Vizzini, S., Di Leonardo, R., Costa, V., Tramati, C. D., Luzzu, F., & Mazzola, A. (2013). Trace
 815 element bias in the use of CO₂ vents as analogues for low pH environments:
 816 Implications for contamination levels in acidified oceans. *Estuar. Coast. Shelf Sci.*, 134,
 817 19–30.
- 818 Vizzini, S., Martínez-Crego, B., Andolina, C., Massa-Gallucci, A., Connell, S. D., & Gambi,
 819 M. C. (2017). Ocean acidification as a driver of community simplification *via* the
 820 collapse of higher-order and rise of lower-order consumers. *Scientific Reports*, 7(1),
 821 4018. <https://doi.org/10.1038/s41598-017-03802-w>
- 822 Wang, Y., Naumann, U., Wright, S. T., & Warton, D. I. (2012). Mvabund—an R package for
 823 model-based analysis of multivariate abundance data. *Methods in Ecology and*
 824 *Evolution*, 3(3), 471–474.
- 825 Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag.
- 826 Wilson, S. S., Furman, B. T., Hall, M. O., & Fourqurean, J. W. (2020). Assessment of
 827 Hurricane Irma Impacts on South Florida Seagrass Communities Using Long-Term
 828 Monitoring Programs. *Estuaries and Coasts*, 43(5), 1119–1132.
 829 <https://doi.org/10.1007/s12237-019-00623-0>
- 830 Witkowski, C. R., Agostini, S., Harvey, B. P., van der Meer, M. T. J., Sinninghe Damsté, J. S.,
 831 & Schouten, S. (2019). Validation of carbon isotope fractionation in algal lipids as a
 832 $\delta^{13}\text{C}_{\text{CO}_2}$ proxy using a natural CO₂ seep (Shikine Island, Japan). *Biogeosciences*,
 833 16(22), 4451–4461. <https://doi.org/10.5194/bg-16-4451-2019>

834 Zitoun, R., Connell, S. D., Cornwall, C. E., Currie, K. I., Fabricius, K., Hoffmann, L. J.,
835 Lamare, M. D., Murdoch, J., Noonan, S., Sander, S. G., Sewell, M. A., Shears, N. T.,
836 van den Berg, C. M. G., & Smith, A. M. (2020). A unique temperate rocky coastal
837 hydrothermal vent system (Whakaari–White Island, Bay of Plenty, New Zealand):
838 Constraints for ocean acidification studies. *Marine and Freshwater Research*, 71(3),
839 321–344.

840