Diffusion Boundary Layers Ameliorate the Negative Effects of Ocean Acidification on the Temperate Coralline Macroalga *Arthrocardia corymbosa*

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Abstract

Anthropogenically-modulated reductions in pH, termed ocean acidification, could pose a major threat to the physiological performance, stocks, and biodiversity of calcifiers and may devalue their ecosystem services. Recent debate has focussed on the need to develop approaches to arrest the potential negative impacts of ocean acidification on ecosystems dominated by calcareous organisms. In this study, we demonstrate the role of a discrete (i.e. diffusion) boundary layer (DBL), formed at the surface of some calcifying species under slow flows, in buffering them from the corrosive effects of low pH seawater. The coralline macroalga *Arthrocardia corymbosa* was grown in a multifactorial experiment with two mean pH levels (8.05 ‘ambient’ and 7.65 a worst case ‘ocean acidification’ scenario projected for 2100), each with two levels of seawater flow (fast and slow, i.e. DBL thin or thick). Coralline algae grown under slow flows with thick DBLs (i.e., unstarved with regular replenishment of seawater to their surface) maintained net growth and calcification at pH 7.65 whereas those in higher flows with thin DBLs had net dissolution. Growth under ambient seawater pH (8.05) was not significantly different in thin and thick DBL treatments. No other measured diagnostic (recruit sizes and numbers, photosynthetic metrics, %C, %N, %MgCO₃) responded to the effects of reduced seawater pH. Thus, flow conditions that promote the formation of thick DBLs, may enhance the subsistence of calcifiers by creating localised hydrodynamic conditions where metabolic activity ameliorates the negative impacts of ocean acidification.

Citation: Cornwall CE, Boyd PW, McGraw CM, Hepburn CD, Pilditch CA, et al. (2014) Diffusion Boundary Layers Ameliorate the Negative Effects of Ocean Acidification on the Temperate Coralline Macroalga *Arthrocardia corymbosa*. PLoS ONE 9(5): e97235. doi:10.1371/journal.pone.0097235

Editor: Gretchen E. Hofmann, University of California- Santa Barbara, United States of America

Received: October 1, 2013; Accepted: April 16, 2014; Published: May 13, 2014

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Funding: C.E.C was supported by a University of Otago Doctoral Scholarship and a University of Otago Postgraduate Publishing Bursary. C.L.H. was funded by a Performance Based Research Fund from the University of Botany, University of Otago, and a University of Otago Research Grant. P.W.B. was funded by a New Zealand MPI grant to Coasts and oceans. C.M.M was funded by a New Zealand Foundation for Research Science & Technology (Grant U00X0802). C.L.H and P.W.B. were funded by a Royal Society of New Zealand Marsden grant (UOO0914). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

The uptake of a large proportion of anthropogenic CO₂ emissions is currently reducing surface seawater pH (a process termed ocean acidification, OA), and is projected to decrease pH by approximately 0.35 units by 2100 [1–3]. Perturbation experiments simulating OA reveal that many calcifying organisms experience lower net calcification rates, resulting in considerably reduced net calcification [4,5]. Other detrimental physiological and ecological effects include altered behavioural traits, and reduced reproduction and overall survival for some species [6–8]. Together, these detrimental effects may fundamentally change the structure and function of ecosystems dominated by calcareous organisms, resulting in changes in their productivity, biodiversity and stability [9–11]. Cold temperate coastal ecosystems may be particularly under threat as calcifying organisms here straddle multiple trophic levels and also deliver essential ecosystem services [12,13]. For example, coralline algae are calcifying primary producers that cover large areas of temperate rocky shores beneath kelp canopies [14,15], provide settlement cues for invertebrate larvae [16–18], and are pivotal to the functioning of these ecosystems [19]. Little is known about the cumulative effects of OA on such potentially vulnerable temperate ecosystems, or on what type of ecosystem(s) might replace them over large expanses of coralline-dominated coastline.

There has been recent discussion on the ability of photosynthetic calcifying organisms to biologically modify their local pH environment, thereby ameliorating the negative effects of ocean acidification [20–26]. However, this hypothesis remains untested by long-term perturbation experiments. In addition, discussion of the effects of climate change on coastal biota [27] has so far included little mention of the important role that organism-seawater flow interactions (ecomechanics)[28] can play in modifying the effects of OA, particularly under slow water flows. Recent
studies suggest that OA could be less detrimental to calcifying species that photosynthesize, such as coralline algae, growing in slow-flow compared to fast-flow habitats. This has been suggested because the diffusion boundary layer (hereafter 'DBL') provides a thin but significant biologically-controlled buffer between the calcifying organism’s external structure and the outer bulk seawater, potentially reducing rates of dissolution [20,21].

The DBL is a μm to cm thick region where the movement of solutes to and from metabolically active surfaces is dominated by molecular diffusion [29]. Within the DBL, biological processes, such as photosynthesis, greatly alter the chemical micro-environment [20,21]. In some cases, DBL formation may reduce the uptake of dissolved inorganic carbon and nitrogen, and the rate of removal of O2 released by photosynthesis [30–35] but their presence is critically important to other processes such as the timing of gamete and spore release [36,37], the deployment of photosynthetic organisms as an example, pH and metabolic processes can result in conditions at the organism’s extracellular enzymes [38] and maintaining steep concentration gradients of bioactive chemicals [39]. When the DBL is thick, metabolic processes can result in conditions at the organism’s surface being very different to those in the overlying bulk seawater. Using photosynthetic organisms as an example, pH and O2 concentrations at the organism’s surface are higher than in the bulk seawater in the light due to photosynthesis, and lower in the dark due to respiration [40–43].

Our study organism, the small (<10 cm) geniculate coralline alga Arthrocardia corymbosa (Fig. 1), was selected as it belongs to a genus found in temperate regions worldwide [44] and has well-characterised effects on local pH (DBL scale) and seawater flow [20]. Velocities within Arthrocardia canopies are 80–99% slower than the bulk seawater flow, which even under fast flows results in thick (up to 18 mm) DBLs at its surface in which the pH is modified by metabolic activity [20]. It is well known that pH at the surface of calcareous species varies due to photosynthesis/respiration [20,21,40]. Based on this knowledge, Hurd et al (2011) hypothesised that a thick diffusion boundary layer will protect coralline algae from OA. Here we test this hypothesis by growing A. corymbosa in two pH treatments, one with today’s seawater (ambient pH 8.05) and the second using that projected for 2100 (7.65) [3]. If the DBL acts as a protective mechanism at low pH, the greatest chance of detecting this effect would be at very slow flows were a thick DBL forms. We therefore used two velocity treatments that would promote the formation of relatively thick and thin DBLs around the coralline alga. For each pH treatment, there were two seawater velocity/DBL thickness levels, a fast flow, ‘thin DBL’ treatment with instantaneous velocities up to 5 cm s⁻¹ provided by vigorous stirring in the culture tank and a slow flow, ‘thick DBL’ treatment characterised by no mechanical stirring but complete exchange of the seawater every 4.4 h to provide environmental mimicry of the periodic erosion of DBLs evident in nature [45]. Our results support the hypothesis that slow seawater flows ameliorate the negative effects of high CO2/low pH on coralline algae, and we suggest that habitats in which thick DBLs can form will offer a refuge for some calcareous species from OA.

Materials and Methods

Collection of seaweed

Thirty assemblages of the articulate coralline alga Arthrocardia corymbosa (Fig. 1) were collected from a Macrocystis pyrifera-dominated kelp forest near Karitane, South Island, New Zealand (45°38′20″S, 170°40′15″E) on 22nd September 2011 using SCUBA. Field collections took place using permits provided by the New Zealand Ministry of Fisheries to the University of Otago, on publically owned land. No endangered organisms were used during this study. For a detailed description of the seaweed community composition and the light environment at the site, see Hepburn et al. [46]. Each assemblage contained 10 individual A. corymbosa anchored to a base of crustose coralline algae (Mesophyllum sp.). Assemblages were removed from the rock using hammers and chisels without touching the A. corymbosa individuals. Assemblages were then placed into zip-lock bags underwater and transferred to an insulated bin, in the dark, for the 30-min transport back to the laboratory. The average height of the assemblages was 5 cm. Six of these assemblages were used to assess the amount of CaCO₃ in the seaweed tissue before the experiment began, using destructive techniques.

Experimental design

The remaining 24 A. corymbosa assemblages were each placed into one of four experimental treatments for 40 days. Treatment levels were multifactorial with the two mean pH values (8.05 and 7.63 measured on the total scale) crossed with the two flow treatments (i.e. thin or thick DBL). Each combination pH/flow was replicated in 6 individual culture tanks. Assemblages were individually tied with nylon thread to circular perspex plates (60 mm diameter), that was perforated with six 1 cm diameter holes to allow seawater to flow around the seaweed and placed into one of 24 650-ml culture chambers.

pH levels within each culture chamber were maintained using a modified version of the automated culture system described by McGraw et al.[47] that uses spectrophotometric pH measurements to measure and maintain the experimental seawater pH. The culture system was housed in a walk-in growth room at 10.5°C, under a mean photon flux density (PFD) of 18 μmol photons m⁻² s⁻¹ with a 12 h:12 h light:dark cycle. Seawater (salinity ≈ 34 S₀) was collected from Portobello Marine Laboratory, Otago Harbour, New Zealand (45°52.51’S, 170°30.9’E), filtered to 0.5 μm (Filter Pure® polypropylene spun melt) and ultraviolet sterilized (Aquastep 25 watt Ultraviolet Sterilizer). Twice a day, this water was used to fill a 150 L storage tank that fed into a mixing tank, and then into 24, independently replicated, 1 L header tanks.

Each of the 24 A. corymbosa assemblages was grown in an individual 630 mL Perspex flow-through culture chamber, which was attached to the outflow of a 1 L perspex header tank. Each 1 L header tank was automatically refilled from a single 1 L mixing tank. Target pH levels were achieved in each mixing tank by adding equal amounts of 0.2 M HCl and NaHCO₃ to 1 L of seawater that was pumped from the 150 L storage tank. This method of chemical manipulation of the seawater carbonate system is chemically identical to that achieved using CO₂ [1,48]. Before the newly-mixed seawater was transferred to the appro-
assemblages were attached. The plates were photographed using a
juveniles was measured on the perspex plates to which the seaweed
were used to calculate DIC. Using these values, [CO2], [CO3
products from the macroalgal surface.
slow-flow treatment the stirrer bar was stationary and water motion was
placement in the culture chamber and then the velocity measured for
in the culture chamber were measured using a Nortek Ventrino micro-
the seawater in the mixing chamber was replenished. Therefore, although the slow-flow treatment was
new seawater was replenished in each of the 24 culture chambers was
header tanks approximately every 4.4 h. The order in which the
performed every 4.4 h as the seawater within each culture chamber
system delivered new seawater at the target pH to each of the 24
treatment was fully replenished more than 5 times per day,
and 400 mL samples were taken to determine total alkalinity
(100

Growth, photosynthesis and elemental analysis

The relative growth rate (RGR) of A. corymbosa was measured by

RGR(%day−1) = (ln(W2/W1)/t2 − t1) × 100 (1)

where t1 = day 1, t2 = day 40, W1 is wet weight on day 1 and W2 is
A. corymbosa reproduced during the experiment. Recruitment of juveniles was measured on the perspex plates to which the seaweed assemblages were attached. The plates were photographed using a Cannon Powershot A620 digital camera attached to a Zeiss Axioskop 2 microscope at 20 times magnification. For each perspex plate, the number of individual recruits was recorded on a random 10 × 10 mm sub-sample, using the software ImageJ 1.42q [53]. The mean surface area of the recruits for each replicate was estimated from 10 randomly-selected recruits from each plate.
The Fv/Fm of one individual from each replicate assemblage was measured at the end of the experiment, using a Pulse Amplitude Modulated (PAM) chlorophyll fluorescence meter (Diving PAM, Walz, Germany). This model has a red light-emitting diode, and both gain and dampening were set to 2. On all occasions, Fv was > 130 before measurements were made. Each assemblage was dark-adapted for 30 min prior to measurements [54]. Relative electron transport rates (rETRmax) for each replicate assemblage were determined using the rapid light curve function. A. corymbosa individuals were exposed to 9 light steps of 20 s duration with the irradiance increasing from 0 to 1234 μmol photons m−2 s−1. Equations using least-squares non-linear regression were then used to determine rETRmax, a (light-use efficiency), b (photoinhibition) and I50 (light saturation point) [55].

RGR (% day−1) = \left( \frac{\ln(W_2/W_1)}{t_2 - t_1} \right) \times 100

\begin{align*}
\text{where} \quad t_1 &= \text{day 1}, \quad t_2 = \text{day 40}, \quad W_1 \text{ is wet weight on day 1 and } W_2 \text{ is wet weight on day 40.}
A. corymbosa \text{ reproduced during the experiment. Recruitment of juveniles was measured on the perspex plates to which the seaweed assemblages were attached. The plates were photographed using a Cannon Powershot A620 digital camera attached to a Zeiss Axioskop 2 microscope at 20 times magnification. For each perspex plate, the number of individual recruits was recorded on a random } 10 \times 10 \text{ mm sub-sample, using the software ImageJ 1.42q [53]. The mean surface area of the recruits for each replicate was estimated from 10 randomly-selected recruits from each plate.}
\text{The } F_v/F_{m_0} \text{ of one individual from each replicate assemblage was measured at the end of the experiment, using a Pulse Amplitude Modulated (PAM) chlorophyll fluorescence meter (Diving PAM, Walz, Germany). This model has a red light-emitting diode, and both gain and dampening were set to 2. On all occasions, } F_v \text{ was } > 130 \text{ before measurements were made. Each assemblage was dark-adapted for 30 min prior to measurements [54]. Relative electron transport rates (rETR}_{max} \text{ for each replicate assemblage were determined using the rapid light curve function. A. corymbosa individuals were exposed to 9 light steps of 20 s duration with the irradiance increasing from 0 to 1234 } \mu\text{mol photons m}^{-2} \text{s}^{-1}. \text{ Equations using least-squares non-linear regression were then used to determine } rETR_{max}, a \text{ (light-use efficiency), } b \text{ (photoinhibition) and } I_{50} \text{ (light saturation point) [55].}
\end{align*}
using *A. corymbosa* at pH 8.00 and 7.65 [20]. These results demonstrated a strong relationship between H\(^+\) and O\(_2\) concentrations within the DBL (R\(^2\) = 0.92; due to the uptake and release of CO\(_2\) and O\(_2\) during photosynthesis and respiration) and showed that the DBL thickness measured using changes in H\(^+\) and O\(_2\) are of equal size. O\(_2\) concentration can therefore be confidently used as a proxy for [H\(^+\)]. We first converted O\(_2\) concentration to [H\(^+\)] using separate relationships in the dark and in the light at both pH 7.65 and pH 8.05. Then, the calculated [H\(^+\)] deviations at the surface of *A. corymbosa* were multiplied by deviations in the bulk seawater measured during the current study (see above section). Finally, [H\(^+\)] was used to calculate \(\Omega_C\) based on total alkalinity measurements made in the bulk seawater.

**Estimation of net calcification**

The net calcification rate of one *A. corymbosa* individual from each replicate assemblage was estimated from the amount of inorganic tissue gained over 40 days relative to the starting weight of inorganic tissue measured in individuals sacrificed at day 0. Formula (1) for RGR was used to calculate net calcification, except the coefficient \(W\) was replaced with \(WC\) (the weight of CaCO\(_3\)). \(WC\) is determined by the following formula:

\[
WC = W \times (1 - O)
\]

where \(W\) = g dry weight and \(O\) = the proportion of organic tissue (g\(^{-1}\) of dry weight). For \(WC\), \(O\) of the initial sacrificed *A. corymbosa* sample was used, all other parameters were obtained from the experimental *A. corymbosa* on day 0 and 40.

The proportion of organic tissue \(O\) was determined by taking one whole *A. corymbosa* thallus from each replicate (0.06±0.01 g of dried seaweed), then dissolving the sample in 10% HCl and re-weighing it. The ratio of wetdry weight for day 0 replicates was calculated by measuring the wet weight of 20 *A. corymbosa* samples collected from the field at day 0, then dividing by their weight after drying at 80°C for 24 h. This ratio had an R\(^2\) of 0.94.

\(\Omega\) (saturation states of minerals) has been used in many past studies to show the relationship between net calcification and carbonate chemistry where, as \(\Omega\) declines, net calcification also declines for many organisms [62]. \(\Omega_C\), (saturation state for calcite), which is proportional to [CO\(_2\)\(^{-}\)] in the seawater, may have no direct physiological role in controlling net calcification [63], although related carbonate parameters (namely H\(^+\), HCO\(_3\)\(^{-}\) and Ca\(^{2+}\)) likely do. We refer to \(\Omega_C\) throughout the text but do not infer that it controls any aspect of calcification directly.

**Statistical analyses**

RGR, net calcification rates, recruit numbers and sizes, all pigments, the \(\%\) MgCO\(_3\), \(\%\) CaCO\(_3\), C:N, \(\delta^{13}\)C, \(\delta^{15}\)N, \(\tau\)ETR\(_{max}\), \(\zeta\), \(\beta\), \(I_0\), and H\(^+\) deviations in the bulk seawater and H\(^+\) concentrations at the surface were analysed using an analysis of variance (ANOVA) with the pH and the level of seawater flow/DBL thickness (diffusion boundary layer) as the random factor and pH and flow/DBL thickness as the fixed factors. All statistical analyses were performed in R version 2.7.0 [64].

**Results**

The DBL in the fast flow treatment was thinner than those in the slow flow treatments (mean 0.03 and 14.77 mm respectively, see Table S1). The calcite saturation state at the surface of the seaweed at pH 7.65 was very different for the thin compared to thick DBL treatment. \(\Omega_C\) at the surface of the seaweed was estimated at the start (initial) and end of the 4.4 h cycle in both the light and dark (Fig. 2). At pH 8.05, \(\Omega_C\) at the surface of the seaweed increased in the light and decreased in the dark (relative to the initial value), although the range in \(\Omega_C\) was less in the thin compared to the thick DBL treatment (Fig. 2, Table S1). For the pH 7.65/thick DBL treatment, \(\Omega_C\) was also higher in the light, but at night \(\Omega_C\) was comparable to the initial \(\Omega_C\). In the pH 7.65/thin DBL treatment, \(\Omega_C\) in the dark also remained similar to the initial value, however \(\Omega_C\) in the light was significantly lower than in any other treatment (Table S2, P<0.01; Fig. 2). Therefore, in the pH 7.65/thin DBL treatment, the seaweed encounter a lower mean value of \(\Omega_C\) over the day/night cycle compared to the thick DBL/pH 7.65 treatment.

There was a significant effect of pH treatment on growth rates (P=0.01), with higher growth at pH 8.05 than 7.65, no significant effect of flow/DBL thickness (P=0.67) but and a near-significant interaction term (P=0.08 Table S2). Trends in net calcification mirrored those for growth rates (Fig. 1b), with negative rates of net calcification observed in the thin DBL/pH 7.65 treatment. The only difference between the two diagnostics being that the interaction between DBL thickness and pH treatments was significant for net calcification (P<0.01). The significant interaction term was due differences in the effect of DBL thickness on calcification at pH 8.05 and 7.65. This significant interaction term was due to the effect of DBL thickness being opposite at pH 8.05 compared to 7.65, where the mean calcification rates were highest in thin DBL treatments at pH 8.05 and lowest in thin DBL treatments at pH 7.65 (Fig. 3a). The magnitude of the change in rates of growth (Fig. 3a) and calcification (Fig. 3b) across the treatments were relatively comparable.

![Figure 2. Carbonate saturation state (\(\Omega_C\)) of seawater at the surface of the coralline alga *Arthrocardia corymbosa*. Computed values of the carbonate saturation state (\(\Omega_C\)) of seawater at the surface of the coralline alga *Arthrocardia corymbosa*, in the light and dark, under four combinations of pH and DBL (diffusion boundary layer) thickness (see Supplementary Information). The \(\Omega_C\) initial value is that of new seawater that has been introduced into the culture chamber. \(\Omega_C\) day is the calculated \(\Omega_C\) at the seaweed surface after 4.4 h incubation in the light, and \(\Omega_C\) night is that after 4.4 h in the dark. The bars represent the mean ± standard error of 6 individual replicates, and in some the error bars are too small to be seen. doi:10.1371/journal.pone.0097235.g002](https://www.plosone.org).
Discussion

Our study revealed contrasting findings - differences in the rates of growth and net calcification between treatments, yet no evidence from the other metrics that physiological or reproductive performance was altered by pH and/or water motion/DBL thickness. The most compelling explanation to reconcile these seemingly disparate findings is that the regular exposure to lower pH in the thin DBL/pH 7.65 treatment resulted in a lower mean value of δ13C at the seaweed surface hence increasing the chemically-induced dissolution of calcium carbonate structures at the surface of the organism, rather than reducing biologically-mediated changes to gross calcification (as net growth of adults was negative in this treatment. Importantly, our results support our initial hypotheses, and those of Hurd et al. [21] who suggested that OA should have a lesser effect on the calcification rates of photosynthetic species growing in slow-flows than those in fast-flows. The present study confirms that in slow-flow conditions, where the DBL is thick, carbonate chemistry at the seaweed surface is conducive to higher net calcification (hence influencing non-significant trends in growth, as the two are intertwined in coralline algae [65]). This occurs through a combination of metabolic processes and boundary-layer dynamics. Conversely, in fast-flows the calcifying seaweed surface is constantly in contact with low pH seawater, due to erosion of the DBL, most likely leading to increased dissolution.

The interplay of bulk seawater pH, organisinal modification of pH, and water motion are summarised in Fig. 5. These factors interact to set up a pronounced diurnal cycle of vertical pH gradients from the calcified surface of the organism to the outermost extent of the DBL. Thus, an eco-mechanical [28] view of environmental heterogeneity reveals that coralline algae can encounter a wide range of conditions with respect to diurnal changes in pH from almost constant conditions in fast flows, to a wide range of pH and δ13C over the diurnal cycle in slow flows. Slow flows will likely provide periods of respite from constantly low pH (or δ13C) in a future ocean influenced by OA, allowing increased net calcification. Such slow-flow conditions may act as a stop-gap, enabling calcifiers to adapt or acclimate to lower pH conditions projected for the coming decades [21].

Fast flow rates did not significantly increase the growth or photosynthesis of *Arthrocardia corymbosa* here, nor was there an effect of flow on pH or photosynthetic physiology. There are three possible reasons why fast flow did not enhance growth at ambient pH. First, the high variability in seaweed physiological responses here masked any significant trends. If we had used higher numbers of culture tanks here (n>6), it may have resulted in significant differences as a result of increased power during the analysis. Second, *A. corymbosa*’s photosynthetic rates were saturated for CO2 at the irradiances levels used here at ambient pH. This suggestion is supported by the observation that the δ13C data indicate no change in the proportion of diffusive CO2 used by *A. corymbosa* grown under fast and slow flows [66]. A third explanation is that the replenishment of the DBL every 4.4 hours provided sufficient inorganic nitrogen to *A. corymbosa*. While the DBL was thick enough to reduce the dissolution of calcareous structures in the pH 7.65/thick DBL treatment, its formation did not result in inorganic nitrogen or DIC limitation because the organic carbon and nitrogen content (C:N ratio) of *A. corymbosa* did not vary significantly. This is in line with recent findings that the DBL only acts to limit inorganic nitrogen or carbon under conditions where the concentration in the bulk seawater is already relatively low for some species [67,68].

Our suggestion that the DBL acts to protect coralline algae from OA is in line with a growing body of evidence that DBLs can be beneficial to seaweeds. For example, seaweeds sense seawater flow via changes in the carbonate system within the DBL and thereby control the timing of gamete and spore release [36,37]; extracellular enzymes are deployed at the seaweed surface within the DBL [30] and they help maintain steep concentration gradients of anti-fouling chemicals [39]. Although they can limit nutrient uptake in some circumstances, velocities required for maximal nutrient uptake are dependent on seaweed morphology, and are usually between 0.5 and 2 cm s⁻¹ [35,69–71]. The effects of OA *in situ* for calcareous photosynthetic species, and the epibionts that grow on their surface, will likely be directly related

Figure 3. Effect of DBL thickness and pH on the relative growth rate and net calcification rate. The effect of DBL thickness/seawater flow and pH on the A) relative growth rate and B) net calcification rate of the coraline alga *Arthrocardia corymbosa*. Thick DBL (diffusion boundary layer) = no mechanical stirring with regular replenishment of the seawater media and fast flow = 5 cm s⁻¹. The bars represent the mean ± standard error of 6 individual replicates. doi:10.1371/journal.pone.0097235.g003

Although there were clear effects of pH and DBL thickness on rates of growth and net calcification (Fig. 3), little change was observed for other diagnostics of seaweed health. No significant differences between treatments were detected for photosynthetic competence (Fₚ/Fₘₑ), chlorophyll a content and the C:N ratio (Fig. 4a–c; P>0.3 on all occasions). Similarly, other measures of seaweed health that included accessory pigment composition, photosynthetic capacity and elemental composition did not differ among treatment (P>0.21 on all occasions, Tables S2–S3, Figures S1–S4). The seaweeds reproduced during the 40 d experiment, and the number and size of the recruits were also comparable for all treatments (P>0.3 on all occasions, Table S2, Fig. 4d).
to the micro-pH environment encountered by individual organisms [21,72,73].

The extent of the impacts of OA for some marine organisms will therefore be dependent on their locality and factors involved in modifying the DBL thickness. Species with complex morphologies that trap or slow flow around them will have thicker DBLs [20,21,74], potentially resulting in more robust responses to OA. Likewise, species with faster metabolic rates, or with semi-enclosed spaces where calcification (or DIC uptake for calcification) and photosynthesis co-occurs, will encounter greater biological modification of pH at the site of calcification [75–77], also resulting in more robust responses to the effects of OA compared to species with slow metabolic rates and/or external calcification sites [78]. Importantly, organisms in areas with high wave energy or fast currents will likely show the typical species-specific responses to OA (usually in the form of reductions in net calcification rates and, subsequently, in growth rates and the survival of organisms) [79]. In contrast, the impacts of OA may be reduced for organisms living within areas of slow flow, such as in embayments, wide estuaries or within the canopies of larger seaweed that can reduce flow rates [34,80,81] and alter pH conditions [23,82,83]. Therefore, the positive effect of a thick DBL shown here at

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**Figure 4. Diagnostics of the health of the coralline alga *Arthrocardia corymbosa*.** Physiological and reproductive metrics used as diagnostics of the health of the coralline alga *Arthrocardia corymbosa* grown under four combinations of DBL (diffusion boundary layer) thickness and pH. (a) photosynthetic competence \(F_v/F_m\), (b) chlorophyll \(a\) content, (c) elemental stoichiometry (C:N molar ratio) and (d) reproductive output (number and size of recruits). The bars and points represent the mean ± standard error of 6 individual replicates. doi:10.1371/journal.pone.0097235.g004

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**Figure 5. Modification of seawater pH at the surface of the coralline algae.** Conceptual scheme of the interplay between seawater carbonate chemistry (i.e. pH), organismal modification of the local chemical environment during a day/night cycle and flow regime illustrating the potential feedbacks between these inter-related processes. Slow flow denotes the slow flow conditions used in our study. doi:10.1371/journal.pone.0097235.g005
pH 7.65 indicates that the prevailing view that the DBL negatively impacts primary producers needs to be re-evaluated [34,34].

There were no statistical differences in the size or numbers of recruits after 40 days in our experiment. This may mean that either 1) different processes control the growth and development of micro-stages of calcareous seaweed, as with non-calcareous seaweed and coral species [85-87], or that 2) the growth and net calcification of micro-stage coralline algae is very slow, meaning that if the experiment was longer then the full effects of OA and flow may have been observed on recruit sizes. The growth rates of juvenile *A. corymbosa* in a previous experiment did not resemble to those of the adults for the first 40 days [82], and only after 22 weeks did they mirror those of the adults (M.Y. Roleca et al., in prep). Because recruits in specific culture tanks may have germinated at different times, it is possible that after a longer duration they may have mirrored the adult growth and net calcification rates. Recruit numbers were also unaffected by pH and flow, though there was a non-significant tendency for recruit numbers to be higher in treatments with faster adult net calcification rates, suggesting a trade-off between energetic demands induced by faster dissolution rates and the energetic demands of reproduction. However, the fact that recruit sizes and numbers were not significantly altered by OA confirms previous research [82] that indicates that the effects of OA on coralline recruitment may be species specific.

In addition to the physiological insights gained from this study, our work has important implications on how OA culture experiments are conducted. Flow conditions within experiments are rarely detailed, making it very difficult to predict the carbonate chemistry at the surface of organisms, which therefore makes comparisons between studies difficult. Vigorous water motion via mixing (bubbling or stirring or shaking) is often recommended [48,88] to reduce the formation of diffusion boundary layers around marine primary producers, thereby maximizing nutrient uptake; however this mixing will exacerbate the negative effects of OA as the protective diffusion boundary layers will not form. Conversely, conducting experiments with no/slow-flow will result in markedly different carbonate chemistry conditions at the surface of the cultured organism, which will depend largely on the metabolic activity of the organism [21]. Therefore, if experiments are conducted on the same study species but under different flow regimes, the biological responses could vary, making it difficult to compare, and extrapolate, the responses of organisms to OA. We provide the first examination of how the DBL could influence the growth of calcareous species under conditions simulating OA. However, the source of this flow used here does not exactly mimic the source of flow in the field. Vortex flow here was produced from beneath the seaweed, whereas in the field flow will usually either be unidirectional or oscillatory. Long-term measurements of flow at a scale relevant to understorey species is needed before conclusions can be drawn about the role of the DBL in situ. That notwithstanding, we recommend that seawater velocity is monitored and reported for all OA research, along with the other important environmental factors (e.g. photon flux density, photoperiod, nutrients, rate of seawater exchange), in order to better facilitate the interpretation of results.

Seaweed beds could provide regions of slow flow/higher pH where calcifying organisms could subside in future low pH seawater. The physical and chemical environment within a seaweed canopy is very different to that of the bulk seawater – i.e., the seawater flow is much reduced [89] and the metabolism of seaweed also results in higher pH values within areas influenced by their photosynthesis during the day [90,91]. However, further research is required to fully examine the role of kelp canopies on understorey organisms. For example, the flow rates in our slow flow treatment were zero for over four hours, and while velocity can reach zero within seaweed canopies [89], the temporal dynamics will be a function of seaweed density, size and bulk seawater velocity, which is usually greater than zero [20,89,92,93].

Future research should examine what protective role the DBL could play at a greater range of seawater velocities, and for a greater range of species, than those used here. In addition, kelp canopies alter other environmentally important variables, such as light intensity [14,94,95] or nutrient concentrations [96], which could interact with OA or other environmental stressors to influence calcification, photosynthetic or growth rates of coralline algae [97,98].

The experimental findings, in conjunction with previously published flow measurements beneath seaweed canopies [20,89], provide the first insights into how the localised chemical and hydrodynamic processes that occur at the surface of the understorey species could interact with global-scale processes such as ocean acidification. Together, they reveal that regions of slow flow may help to conserve important biodiversity of calcifiers in selected regions. Rau et al. [99] discuss the need for novel conservation strategies to offset the effects of OA, and put forward several options including boosting the resilience of calcifiers to OA through breeding programmes. Rau et al. [99] also advocated the potential management of local carbonate chemistry using geochemical modification of seawater, but pointed out that such an approach would be time-consuming, and expensive even to modify a hundreds of m² of the seafloor/reefs. If our results are transferable to the field, we propose that such temporal and logistical hurdles could be overcome by maintaining seaweed beds, characterised by localised biological modification of pH and seawater flow, as potential refugia (km²) from OA.

**Supporting Information**

**Figure S1** The proportion of total organic tissue, CaCO₃ and MgCO₃ in *A. corymbosa*. The proportion of total organic tissue, CaCO₃ and MgCO₃ in *A. corymbosa* after 40 days in thick and thin DBL treatments at pH 7.65 and 8.05. (EPS)

**Figures S2** Chlorophyll fluorescence parameters of *A. corymbosa*. (a) Relative maximum electron transport (βETR_max), (b) degree of photoinhibition (β), (c) light use efficiency (χ) and (d) irradiance saturation point (I_s) of *A. corymbosa* measured using chlorophyll fluorescence at day 40 in each treatment. (EPS)

**Figure S3** Pigment content of *A. corymbosa*. (A) phycoerythrin and (B) phyocyanin content of *A. corymbosa* grown for 40 days with thick and thin DBLs at pH 7.65 and 8.05. (EPS)

**Figure S4** δ¹³C and δ¹⁵N of *A. corymbosa*. Organic tissue δ¹³C and δ¹⁵N of *A. corymbosa* grown for 40 days with thick and thin DBLs at pH 7.65 and 8.05. (EPS)

**Table S1** Physical conditions during the experiment and the seawater carbonate chemistry parameters. (DOCX)

**Table S2** Analysis of variance results of biotic (non-elemental and pigment) responses of *A. corymbosa* to the experimental treatments. (DOCX)
Table S3  Analysis of variance results of elemental and pigment bioic responses of A. corymbosa to the experimental treatments.  

( DOX)  

Acknowledgments  
We thank: Stewart Bell for field assistance, Kim Currie for the use of laboratory equipment, Christine Davies for laboratory assistance, Julia Mullarney for ADV measurements, Lisa Buckle for the preparation of Fig. 4, and Peter Dillingham for statistical advice. We acknowledge the support of the management committee of the East Otago Taiapa (customary fishing reserve) within which the field collections took place.

Author Contributions  
Conceived and designed the experiments: CEC CLH CDH. Performed the experiments: CEC JMN. Analyzed the data: CEC CMM. Contributed reagents/materials/analysis tools: CMM CLH CAP AMS. Wrote the paper: CEC PWB CLH. Editing the manuscript: CEC PWB CMM CDH CAP JMN AMS CLH.

References  
1. Gattuso JP, Gao K, Lee K, Rost B, Schulz KG (2010) Approaches and tools to manipulate the carbonate chemistry. In: Ribesell U, Fabry VJ, Hansson L, Gattuso JP, editors. Guide to best practices for ocean acidification research and data reporting. Luxembourg: Publications Office of the European Union. pp. 41–51.
2. Raven J, Caldeira K, Elderfield H, Hoegh-Guldberg O, Lai P, et al. (2005) Ocean acidification due to increasing atmospheric carbon dioxide. The Royal Society. 3. Caldeira K, Wickett ME (2003) Anthropogenic carbon and ocean pH. Nature 425: 365. 4. Findlay HS, Kendall MA, Spicer JI, Widdicombe S (2009) Future high CO2 in the intertidal may compromise adult barnacle Chthamalus stellatus survival and embryonic development stage. Mar Ecol Prog Ser 389: 193-202. 5. Ferrari MCO, Manassra RP, Dixon DL, Munday PL, McCormick MI, et al. (2012) Effects of ocean acidification on learning in coral reef fishes. PLoS ONE 7: e31478. 6. Kroeker KJ, Kordas RL, Crim RN, Hendriks IE, Ramajo L, et al. (2013) Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Glob Change Biol 19: 1684-1696. 7. Fabry VJ, Feely RA, Perry RA, Orr JC (2008) Impacts of ocean acidification on calcifying organisms in marine ecosystems: an organism-to-ecosystem perspective. Annu Rev Ecol Syst 41: 127-147.
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Sardene V, Wahl M (2013) Differential responses of calcifying and non-calcifying algal cells to ocean acidification: implications for ocean diversity. PLoS ONE 8: e370455.