Ocean acidification reverses the positive effects of seawater pH fluctuations on growth and photosynthesis of the habitat-forming kelp, *Ecklonia radiata*

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Ocean acidification (OA) is the reduction in seawater pH due to the absorption of human-released CO₂ by the world’s oceans. The average surface oceanic pH is predicted to decline by 0.4 units by 2100. However, kelp metabolically modifies seawater pH via photosynthesis and respiration in some temperate coastal systems, resulting in daily pH fluctuations of up to ±0.45 units. It is unknown how these fluctuations in pH influence the growth and physiology of the kelp, or how this might change with OA. In laboratory experiments that mimicked the most extreme pH fluctuations measured within beds of the canopy-forming kelp *Ecklonia radiata* in Tasmania, the growth and photosynthetic rates of juvenile *E. radiata* were greater under fluctuating pH (8.4 in the day, 7.8 at night) than in static pH treatments (8.4, 8.1, 7.8). However, pH fluctuations had no effect on growth rates and a negative effect on photosynthesis when the mean pH of each treatment was reduced by 0.3 units. Currently, pH fluctuations have a positive effect on *E. radiata* but this effect could be reversed in the future under OA, which is likely to impact the future ecological dynamics and productivity of habitats dominated by *E. radiata*.

Ocean acidification (OA) is a decline in surface seawater pH caused by the sustained absorption of anthropogenically-derived atmospheric CO₂. OA is predicted to cause widespread change in many marine ecosystems, primarily through its potential to reduce the net calcification rates of calcareous species, and to alter the behaviour of invertebrates and fishes. However, some species of non-calcareous macroalgae may benefit from OA, including canopy-forming kelps. Canopy-forming kelps are species of large, brown macroalgae of the orders Laminariales (true kelps) and Fucales (functional equivalents) that form extensive forests or beds between latitudes of ~35–65°. Kelps play a pivotal role in providing habitat, food, and nursery areas for numerous species, and they strongly influence the structure of understorey invertebrate and macroalgal assemblages, primarily through the alteration of light and water motion. The majority of research into the resilience and stability of kelp forests has been concerned with external processes such as over-fishing, increased temperature, or terrestrial inputs that act to disturb or maintain the system. However, internal processes may also be important in maintaining kelp forests through feedback mechanisms. One internal mechanism could be the capacity of kelp to elevate seawater pH via their photosynthetic activity. The diel range in seawater pH within some kelp beds can be almost 1 pH unit (i.e. ±0.45) due to the uptake of dissolved inorganic carbon (DIC) via photosynthesis during the day that causes pH to increase, and respiration which produces CO₂ during the night, thus lowering seawater pH. The largest daily pH fluctuations of 0.90 units (7.96–8.86) have been recorded in giant kelp forests (*Macrocystis pyrifera*) leading to suggestions that this process may buffer these systems from the effects of OA.

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It is unclear how OA will influence kelp physiology and ecology\(^6,30,31\), and it is unknown how kelps respond to fluctuations in pH caused by their own metabolic activity. For non-calcareous (i.e. fleshly and foliose) macroalgae, the effects of OA are variable, increasing the growth and photosynthetic rates of certain species\(^6,30,32\), decreasing the growth rates of others\(^33,35\), or having no detectable effect\(^30,31,36\). No study to date has examined the response of non-calcareous macroalgae to fluctuations in pH (although the recruitment of a range of fleshly macroalgae was reported in a study examining the effects of pH fluctuations on juvenile coralline algae\(^37\)). For coralline algae, pH fluctuations negatively affect growth rates, under both ambient mean pH levels and under mean levels expected for the future as a result of OA\(^22,37,38\). For kelp, however, reductions in seawater pH may have little effect on photosynthesis and growth, because they actively uptake HCO\(_3^−\) (the most abundant form of DIC in seawater) using carbon concentrating mechanisms (CCMs)\(^8,31,39\). Alternatively, the projected 200% increase in seawater CO\(_2\) concentration could benefit kelp by increasing the diffusive uptake of CO\(_2\), which might result in the down-regulation of the CCM\(^8\). Increased uptake of CO\(_2\) could therefore result in elevated rates of growth and photosynthesis for kelp\(^8\). However, in kelp beds the high (metabolically-induced) daytime pH might have an opposing effect because CO\(_2\) concentrations are reduced as pH increases, and so in this case growth and photosynthetic rates might be reduced.

*Ecklonia radiata* is a dominant canopy-forming member of the order Laminariales in the Southern Hemisphere, ranging from South Africa, to southern Australia and New Zealand\(^30\). Despite its importance as an ecosystem engineer, creating habitat and food for thousands of species\(^40\), we know almost nothing about the pH environment that it experiences daily, nor its responses to pH fluctuations, both now and those predicted to occur in the future. To address this knowledge gap, we took measurements of pH within several *E. radiata* beds in south eastern Tasmania, Australia, to assess the daily pH fluctuations. Using these field values to provide an environmental context for laboratory experiments, in one experiment we grew juvenile *E. radiata* under fluctuating (8.1 ± 0.3 units in the day and −0.3 units in the night) and constant pH regimes (pH 8.4, 8.1, and 7.8) over 21 days in the laboratory, and in a second similar experiment at pH −0.3 units in all treatments (hereafter “ambient” and “OA” respectively). We hypothesized that: 1) Seawater pH fluctuates on a diel cycle within *E. radiata* beds due to their photosynthetic activity, reducing at night and increasing during the day; 2) simulated fluctuations in pH will reduce growth (measured as a change in thallus length) and photosynthetic rates (O\(_2\) evolution) of *E. radiata* compared to static pH treatments with the same mean pH possibly as a result of up-regulation of the CCM; 3) pH conditions simulating OA (0.3 pH\(_{\text{NBS}}\) units lower than present day) will cause an increase in *E. radiata* growth and photosynthetic rates relative to treatments with present day (8.1) and high pH (8.4); and 4) RNA:DNA ratios will increase in treatments where growth increases, reflecting increased protein synthesis and hence increased total RNA content. The carbon isotope composition of seaweed tissue can be used to infer changes in the relative use of CO\(_2\) and HCO\(_3^−\) \(^{22,33,38}\), because CO\(_2\) is more depleted in \(^{13}\)C (i.e. its \(^{81}\)C is lower) compared to HCO\(_3^−\) \(^{41}\). Thus, we also hypothesized that 5) *E. radiata* \(^{13}\)C (i.e. the difference between tissue and source seawater DIC in each treatment) would increase with declining pH (i.e. increasing CO\(_2\)) as a result of increased use of diffusive CO\(_2\) over active uptake of HCO\(_3^−\) \(^{42}\).

**Results**

**Field Measurements.** Seawater pH showed clear diel cycles at all 3 sites where pH was measured. The range in pH on the total scale (pH\(_5\)); all subsequent field measurements are referred to on the total scale) was larger (0.40 units) within the more sheltered, shallower sites (where pH was measured only during daylight hours over 3 days) than at deeper, more wave-exposed sites (0.05–0.09 units; over 21 days). Seawater pH within the sheltered, shallow (1.5 m depth) *E. radiata*/Phyllospora comosa bed at Darlington, Maria Island, displayed a clear increase over the course of the day, with a minimum of pH 7.97 ± 0.06 at 08:00 on day 2 and maximum of 8.37 ± 0.01 at 14:00 on day 3 (Table 1). pH was tightly correlated with oxygen concentration ($r = 0.89 ± 0.06, P < 0.001$). Seawater pH in Fortescue Bay in late autumn and mid-winter showed a similar diel pattern, but it had a smaller range than at Maria Island; pH fluctuated 0.08 pH units (8.01 to 8.09) at 12.5 m depth over 3 days. At the mouth of Fortescue Bay in early spring, pH varied by 0.09 pH units (8.06 to 8.15) over 21 days at 7 m, and 0.05 units (8.06 to 8.11) over 21 days at 25 m (Table 1). At this site pH was tightly correlated with oxygen concentration at 7 m ($r = 0.93$, \(p < 0.001\).
P < 0.01), but only weakly correlated with oxygen concentration at 25 m (r = 0.37, P < 0.01). pH within the E. radiata canopy, determined from Niskin sampling in late spring, ranged from 8.13 to 8.19 over daylight hours with the lowest values occurring at 06:45 and the highest values occurring at 16:45, while pH measurements made over the same time period above the adjacent soft sediment benthos ranged between 8.15 and 8.16 pH units with no indication of diel fluctuations.

pH within sealed bags containing E. radiata sporophytes in the shallow E. radiata/Phyllospora comosa bed increased significantly more (from 8.00 ± 0.02 to 8.72 ± 0.05) over the course of the day than control bags without E. radiata (from 8.00 ± 0.02 to 8.14 ± 0.03; ANOVA, F_{1,37} = 142.31, P < 0.001). Oxygen concentrations also increased significantly more (84.43%) within the bags containing E. radiata compared to controls (ANOVA, F_{1,37} = 36.86, P < 0.01). Oxygen concentration was positively correlated with pH, both in bags with and without E. radiata (r = 0.94 ± 0.02).

**Laboratory experimental conditions.** pH treatments within both of the experiments were maintained within 0.05 units of the treatments target throughout all experiments, and the standard error of pH in each tank for each treatment was smaller than the measurement error (<0.01). The pH_{f} of treatments (with treatment pH_{NBS} labels in parentheses) was 7.78 (7.8), 8.09 (8.1) and 8.35 (8.4) for growth experiment 1 and 7.54 (7.5), 7.80 (7.8) and 8.06 (8.1) for growth experiment 2. Alkalinity (A_{K}) did not vary substantially across treatments within experiments, being 2294.57 ± 10.79 μmol kg^{-1} (mean ± s.e.) in the first experiment and 2352.66 ± 16.40 μmol kg^{-1} in the second. DIC measured in each treatment was inversely related to the pH of that treatment (Table S1). Salinity was 35.00 ± 0.01 in each treatment.

**Growth experiment 1: ambient seawater, mean pH 8.1.** Relative growth rates of blades were on average 47% greater in the fluctuating pH treatment than in all other treatments (Table 2, Fig. 1a). Net photosynthetic rates were higher (on average 253% higher) in the fluctuating pH treatment compared to the static pH 7.8 and pH 8.1 treatments (Table 2, Fig. 2a). RNA:DNA ratios were significantly higher in the fluctuating and static pH 8.4 treatments than at constant pH 8.1 and pH 7.8 (mean 101% higher, Tukey’s Honestly Significant Difference (THSD): P < 0.01). RNA:DNA ratios also increased significantly more (84.43%) within the bags containing E. radiata compared to controls (ANOVA, F_{1,37} = 36.86, P < 0.01). Oxygen concentration was positively correlated with pH, both in bags with and without E. radiata (r = 0.94 ± 0.02).

**Growth experiment 2: OA conditions, mean pH 7.8.** Relative growth rates were similar between treatments (Table 2, Fig. 1b). However, net photosynthesis was 49% lower in the fluctuating pH treatment relative to the static pH 7.8 treatment (Table 2, THSD: P = 0.03, Fig. 2b). There were no significant differences between treatments in RNA:DNA ratios (Table 2, Fig. 3b), total RNA, and total DNA content (Table 2, Fig. S1). There was no effect of experimental treatment on ETR_{max} (Table 2, Fig. S4b). Although F_{v}/F_{m} was significantly lower in the fluctuating pH treatment (0.69 ± 0.01, mean ± s.e.) than the pH 7.8 (0.72 ± 0.01) and 8.1 (0.73 ± 0.01) treatments (Table 2, Fig. S2b), again these values indicate normal functionality of PSII. Similar to Experiment 1, Δ^{13}C values increased with decreasing pH during the day, and were significantly higher in the pH 7.5 and 7.8 treatments compared to the fluctuating pH treatment (Table 2, THSD: P = 0.02 and 0.03, Fig. 4b). C:N was similar for all treatments (Table 2, Fig. S3).

**Discussion**

We measured diel changes in pH within E. radiata beds of up to 0.4 units, and found that when these fluctuations are simulated in laboratory experiments, rates of blade growth and photosynthesis (measured as O_{2} evolution)

| Table 2. Analysis of variance (ANOVA) table displaying F-values, P-values, and Tukey Honestly Significantly Different (THSD) differences between treatments for all measured responses during the ambient and ocean acidification (OA) experiments. Degrees of Freedom = 3 for Treatments and 21 to 17 for residuals. α = 0.05. For THSD, F = Fluctuating treatment, all other treatments named by their mean pH_{NBS}. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Response        | Ambient experiment | OA experiment | F-value | P-value | F-value | P-value | THSD |
| Blade length RGR | 5.434 (0.007) F > 8.4 = 8.1 = 7.8 | 2.356 (0.100) F > 8.1 = 7.8 |  |  |  |  |  |
| Photosynthetic rates | 14.72 (<0.001) F > 8.1 = 7.8 | 3.840 (<0.001) F > 7.8 F |  |  |  |  |  |
| Δ^{13}C          | 4.385 (0.020) F < 8.1 = 7.8; 8.4 < 7.8 | 4.782 (0.014) F < 7.5 F |  |  |  |  |  |
| Fv/Fm            | 1.435 (0.264) | 2.680 (0.080) F |  |  |  |  |  |
| RNA:DNA         | 4.551 (0.015) 8.4 > 7.8 | 5.500 (0.008) F < 8.1 = 7.8 |  |  |  |  |  |
| Total RNA       | 23.170 (<0.001) F = 8.4 > 8.1 = 7.5 | 0.950 (0.438) F |  |  |  |  |  |
| C:N             | 9.530 (<0.002) F = 8.4 > 8.1 = 7.5 | 5.500 (0.008) F < 8.1 = 7.8 |  |  |  |  |  |
| Total RNA       | 8.23 (0.001) F = 8.4 > 8.1 = 7.8 | 1.150 (0.358) F |  |  |  |  |  |
Figure 1. Relative growth rates (RGR) of juvenile *Ecklonia radiata* over 21 days, measured as linear extension of the blade under (a) ambient pH conditions (fluctuating pHNBS [8.4 during the day, 7.8 at night], constant pHNBS at 8.4, 8.1 and 7.8); and (b) OA conditions (the same treatments as in (a) but with pHNBS reduced by 0.3 units in each treatment). Data are displayed as means ± standard error, *n* = 4–6. * denotes significantly different treatments, as revealed by Tukey’s Honestly Significant Difference tests (*α* = 0.05).

Figure 2. Net photosynthetic rates of juvenile *Ecklonia radiata* (μmol O₂ h⁻¹ mm⁻²) after 21 days under treatments of: ambient pH conditions (fluctuating pHNBS [8.4 during the day, 7.8 at night], constant pHNBS at 8.4, 8.1 and 7.8); and (b) OA conditions (the same treatments as in (a) but with pHNBS reduced by 0.3 units in each treatment). Data are displayed as means ± standard error, *n* = 4–6. Bars sharing a letter within panels are not significantly different, as revealed by Tukey’s Honestly Significant Difference tests (*α* = 0.05).
of juvenile *E. radiata* increased compared to static pH treatments. However, this positive effect of fluctuating pH on growth and photosynthetic rates was not apparent when the experimental pH was reduced in all treatments.
by 0.3 units, simulating future OA. Moreover, pH fluctuations under simulated OA had a negative impact on net photosynthesis relative to static pH treatments with the same mean pH.

These findings are important in the context of predicting the responses of kelp-based communities to OA. Past research has considered that metabolically-induced pH fluctuations in habitats dominated by photosynthetic species (macroalgae, seagrasses, corals) could act as a refuge from OA for calcifying species because higher pH during the day might facilitate calcification. However, this refuge may be less effective than previously considered in a future, reduced pH ocean, if these pH fluctuations no longer benefit the kelp that are responsible for the pH changes. Increased variability in pH has a negative impact on coralline algae under ambient mean seawater pH, the effects of which are enhanced by OA. However, in the case of coralline algae, reduced pH in general is known to slow growth and net calcification, possibly through elevated dissolution rates. The mechanisms responsible for the negative impacts (relative to fluctuating pH under current conditions) of fluctuating pH under OA conditions on the non-calcareous E. radiata are unclear (see below). Further research is required to determine whether other wide-spread and abundant habitat-forming macroalgal species will respond to pH fluctuations predicted for the future in a similar way to E. radiata, or whether this is a species-specific response.

Polarization by macrophyte habitats, further research is required that combines physical modelling with long-term, spatially-extensive measurements of pH, water motion, depth and biotic characteristics that are replicated through space and time. Once these data are obtained, the role that macrophyte habitats could play in modifying the effects of OA on resident organisms can be more thoroughly understood.

Contrary to our initial hypotheses, the net photosynthetic and growth rates of Ecklonia radiata were not elevated in treatments with reduced daytime pH (and more CO₂) relative to present ocean conditions. These supports past research indicating that increased concentrations of DIC, particularly CO₂, associated with OA may not benefit this species. The Δ13C of E. radiata displayed a clear trend of a greater reliance on diffusive CO₂ as a carbon source for photosynthesis in treatments with lower daytime pH, as hypothesized initially. However, we saw no evidence of increased growth rates as might be expected if CCMs are down-regulated, despite the increasing reliance on diffusive CO₂ at low pH; this is similar to the growth responses of Ulva rigida and Macrocystis pyrifera to elevated CO₂.

Rather than increased growth at higher DIC concentrations (i.e. lower pH), we found that the rates of E. radiata growth and photosynthesis were maximal under the experimental treatment that most closely simulated the pH fluctuations that it currently encounters in the field (daytime pH 8.4, night-time pH 7.8), suggesting that it is physiologically adapted to these pH/DIC conditions. We suggest that the specific combination of a daytime pH 8.4 and night-time pH 7.8 provides a seawater carbonate chemistry that is conducive to growth, photosynthesis, and RNA synthesis by Ecklonia. The static pH 8.4 treatment itself appeared to be slightly beneficial to Ecklonia because RNA synthesis was increased, along with evidence of increased O₂ evolution, although there was no evidence of increased growth. The explanation for this finding of stimulated metabolism when the daytime pH was 8.4 may be related to enhanced bicarbonate uptake at higher pH, which has been previously observed at very high pH (~9.0) for green seaweeds, but not brown seaweeds. However, we know relatively little of the diversity of bicarbonate uptake mechanisms in kelps, although this knowledge is required to further understand how they will respond to OA (see references within 31,39).

This is the first documentation of seaweed metabolism being enhanced by diel fluctuations in seawater carbonate chemistry. The mechanisms are unknown but are likely to involve metabolic processes that are enhanced by higher H⁺ and/or DIC at night, along with decreased H⁺ and/or DIC during the day. Further studies are needed including those using enzyme inhibitors and gene expression, to elucidate why this specific combination of pH/DIC during the day and night stimulated growth of E. radiata. The next step in quantifying how seawater carbonate chemistry influences growth and photosynthesis of macroalgae should investigate the activity of different CCMs in macroalgae grown for long periods of time under pH treatments similar to ours. This can be done by combining direct assessments of specific CCMs with gene expression data.

This study is the first to examine the effects of fluctuations in pH on a non-calcareous macroalgae, and our findings build on those of earlier studies which highlight the importance of acknowledging pH fluctuations when assessing how ecologically important calcifying and non-calcifying primary producers might be affected by ocean acidification in dynamic coastal environments. The finding that the positive effects of fluctuating pH were not apparent under OA has implications for the abilities of kelps to act as refugia for calcifying organisms in the future (cf 27,28,43). This study raises many questions, but it now seems clear that it is important to ascertain how the magnitude of macrophyte-induced diel pH fluctuations in shallow environments influence the physiology and ecology of marine species now and in a future lower pH ocean. How different magnitudes of fluctuations combine with changes in mean pH to influence organisms now needs to be addressed for a range of species before we can begin to predict how long-term changes in near-shore pH due to OA could impact these ecosystems.
Methods

Field measurements. To determine whether pH within *Ecklonia radiata* beds follows a daily cycle similar to those occurring in other ecosystems22,24, seawater pH (and where possible dissolved oxygen) was measured on five different sampling occasions at two general locations, *viz.* Darlington (Maria Island), and Fortescue Bay, Tasmania:

(1) In a shallow (1.5 m), sheltered *E. radiata*/Phyllopora comosa bed, an environment likely to have extreme pH fluctuations (Darlington), at regular intervals between 07:30 and 17:30 on each day between April 19-21 2014. At this location pH and dissolved oxygen (DO) were measured using a pH meter (Thermo Scientific Orion Star A216 pH/RDO/DO meter), pH electrode (Thermo Scientific Orion 8107 BNUMD Ross Ultra pH/ATC Triode) and DO probe (Thermo Scientific Orion 087100MF Field RDO probe). Seawater was collected in situ using bottles (100 ml plastic sealed bottles) because surge prohibited the use of sensors in the shallow water;

(2) At 7 m and 25 m depth on an exposed coast (Fortescue Bay) where pH fluctuations are likely to be minimal, between 5–26 September 2014. *In situ* SeaPHOX loggers with SeaFET pH sensors were used to measure pH and DO hourly at these sites;

(3) In a moderately exposed site at 12.5 m (Fortescue Bay), most representative of *E. radiata* beds on the east coast of Tasmania and where pH change is likely to be intermediate between the situations described in 1) and 2), between 20-22 May 2014. An ENVOC pHTempion combined pH and temperature logger was used to measure pH at 5 min intervals as we did not have access to the primary sensors (the seaPHOX) at this time;

(4) Within sealed bags containing either seawater only, or seawater and an adult sporophyte of *E. radiata*, to determine the extent to which *E. radiata* metabolism can change pH in the field, and to separate the changes caused from photosynthesis and respiration of phytoplankton from that caused by *E. radiata*, at Darlington, on each of 18, 19 and 21 April 2014 using the pH meter, probe and DO probe described in 1) and

(5) Within the shallow (7 m) exposed *E. radiata* bed (described in 2) above) and on the adjacent soft-sediment substratum (Fortescue Bay, 20 m depth), to determine whether pH changes are localised within beds or evident over larger spatial scales, using a Niskin sampler and the pH meter, probe and DO probe described in 1) and 4), between 06:45 and 16:45 on 27 November 2014.

These five sampling procedures were used to capture a range of examples of pH change that could occur in *E. radiata* beds. Electrodes were calibrated initially using pH 7 and pH 9 NBS buffers on site, then pH on the total scale (pHT) was calculated afterwards using Tris and amp buffers at 14 °C. All pH measurements are given on the total scale, unless otherwise noted. The DO probe was calibrated by measuring the oxygen concentration of seawater that had been bubbled with air for 10 min (100% saturation) and by measuring the DO content of seawater total scale, unless otherwise noted. The DO probe was calibrated by measuring the oxygen concentration of seawater at deployment, a mid-point and collection. See supplementary methods (SI 1) for a full description and rationale of the methods of water sampling and pH measurement.

Laboratory growth experiment 1: ambient seawater pH. Approximately 50 juvenile *Ecklonia radiata* individuals of blade length 40–100 mm were collected from Fortescue Bay (43.123079° S, 147.974848° E) on August 22nd 2014 using SCUBA. Individuals were collected at 8–12 m, placed into black plastic bags containing seawater, and on the surface placed in an insulated container filled with seawater for transport (2 h) to the laboratory. Individuals were acclimated to laboratory conditions in 0.5 μm filtered (Whatman® GF/C filters; GE Healthcare UK Limited, Little Chalfont, UK) and UV sterilised (Emperor Aquatics Smart HO UV steriliser, Star A216 pH/RDO/DO meter), pH electrode (Thermo Scientific Orion 8107 BNUMD Ross Ultra pH/ATC Triode) and DO probe (Thermo Scientific Orion 087100MF Field RDO probe). Seawater was collected into the experimental tanks over time. The fluctuating pH treatment was achieved by changing bags over at 08:00, 11:00, 14:00, 17:00, and 20:00, with bags lined with a metallic film to keep pH constant for short term storage. Seawater then drained from the bags into the experimental tanks with constant water motion (using magnetic stirrers) to break down the diffusion boundary layer. See22,37,32 for a description of the chambers. Trials were conducted to test the extent that juvenile *E. radiata*...
Ecklonia radiata could alter pH in the culture tanks under the experimental light conditions (i.e. at 0 and 28 μmol photons m⁻² s⁻¹), and revealed that tanks required exchange of seawater every 3 hours during the day and every 12 hours at night to ensure that pH remained within 0.05 units of the target.

**Growth experiment 2: OA conditions.** Approximately 50 E. radiata sporelings were collected from the site at Fortescue Bay (as above) on August 20th 2014. All experimental conditions and methods were the same as the growth experiment 1 described above, except that the target pH of each treatment was reduced by 0.3 pH units across all treatments to simulate conditions expected to occur in the future as a result of OA. Three individual sporophytes (two replicates subject to constant pH 7.5 and one replicate under constant pH 7.8) became necrotic and were discarded during the experiment.

**Biotic responses.** Linear extension of the blade length (tip of blade to blade-stipe junction) of each sporophyte was calculated from photographs of individuals placed on a grid on days 1 and 21, using the software program ImageJ. Relative growth rate was calculated relative to the initial size.

Photosynthetic rates were determined on day 21 in each culture tank at experimental light levels at 11:00 after 3 hours in the light, using an Orion RDO probe (ORI087100MDW). Oxygen production was standardised to surface area of the thallus (in mm²) over an hour.

The performance of PSII (Fv/Fm) and maximum relative electron transport rates (rETRmax) were measured to assess any changes in photo-physiology during the experiments. Fv/Fm and rETRmax were measured using a Pulse Amplitude Modulation (PAM) chlorophyll fluorescence meter (Diving PAM, Walz, Germany) on day 21. Fv/Fm measurements were made on E. radiata individuals that had been dark adapted for 15 minutes. The diving PAM had a blue-light-emitting diode, and dampening and gain were set to 1 and 2 respectively. Fv was above 120 on all occasions.

Tissue samples from the meristem were taken at the end of the experiment on day 21 from each individual for determination of δ¹³C, C:N ratios, and RNA:DNA ratios. C:N was measured to indicate whether nutrient limitation occurred, and along with rETRmax and Fv/Fm, was used only as an indication of the physiological state of the algae. δ¹³C and C:N ratios were determined using the methods outlined in using a NA1500 elemental analyser coupled to a Thermo Scientific Delta V Plus via a ConFlo IV. Combustion and reduction were achieved at 1020°C and 650°C respectively. Values were normalised to the VPDB scale via a 3 point calibration using certified reference material. Both precision and accuracy were dasticity), and passed all tests, except for δ¹³C in the ambient pH experiment; 2 outliers were removed from the differences between treatments. Data were checked for violations of ANOVA assumptions (normality and homoscedasticity), and revealed that tanks required exchange of seawater every 3 hours during the day and every 12 hours at night to ensure that pH remained within 0.05 units of the target.

**Statistical analysis.** All statistical analyses were conducted using the statistical software R v. 3.1.1. Correlations between pH and oxygen concentrations during field measurements were calculated using Pearson’s correlation coefficient. Two-way ANOVAs were used to assess whether there were differences between pH and oxygen concentration changes in bags placed in the field, with a fixed factor of ‘Treatment’ (2 levels: chambers containing Ecklonia radiata and control bags with no kelp) and a random factor ‘Day’ (3 levels: April 18th, 19th and 21st). In the laboratory-based experiments, one-way ANOVAs were used to estimate whether there were significant differences between pH treatments for RGR, oxygen evolution, Fv/Fm, rETRmax, C:N ratios, Δ¹³C, RNA:DNA ratios, and RNA and DNA content. When main effects in one-way ANOVAs were significant (at α = 0.05), Tukey’s Honest Significant Difference (THSD) post-hoc tests were used to determine the nature of differences between treatments. Data were checked for violations of ANOVA assumptions (normality and homoscedasticity), and passed all tests, except for Δ¹³C in the ambient pH experiment; 2 outliers were removed from the pH 8.1 treatment as they returned values that were substantially different to other replicates in the treatment.

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**Acknowledgements**

Cayne Layton, Matthew Cameron, and Masa Tatsumi provided invaluable assistance in the field. Bronte Tilbrook, Eric Van Oijen, and Kristina Patterson enabled the collection of vital field data. This study was funded by an ARC Discovery grant to CRJ and Jeffrey Wright.

**Author Contributions**

D.B. carried out the laboratory experiments. D.B. and C.E.C. collected field data. D.B. and C.E.C. analysed the data. D.B., C.E.C., C.L.H. and C.R.J. designed the study. D.B. and C.E.C. wrote the original draft, with subsequent contributions by other authors. A.T.R. collected vital laboratory data and contributed materials.

**Additional Information**

Supplementary information accompanies this paper at http://www.nature.com/srep

**Competing financial interests:** The authors declare no competing financial interests.

**How to cite this article:** Britton, D. *et al.* Ocean acidification reverses the positive effects of seawater pH fluctuations on growth and photosynthesis of the habitat-forming kelp, *Ecklonia radiata*. *Sci. Rep.* **6**, 26036; doi: 10.1038/srep26036 (2016).

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