

# Seasonal growth and calcification of a reef-building crustose coralline alga on the Great Barrier Reef

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**ABSTRACT:** Crustose coralline algae (CCA) play a central role in tropical reef ecology, helping to build and stabilise the reef framework and, due to their high Mg-calcite skeleton, are potential indicators for assessing the impacts of ocean acidification. However, basic information on CCA seasonal growth and calcification is relatively unknown on the Great Barrier Reef (GBR). This study provides seasonal baseline rates of vertical growth, marginal (horizontal) growth and calcification of the abundant CCA species *Porolithon onkodes*, together with the effect of reduced irradiance on these metrics to better understand the natural variability between CCA living in exposed and shaded areas. Seasonal variation was detected in each of the metrics, with maximum vertical growth and calcification observed in spring and marginal extension in autumn. Annual vertical growth rate was 1.45 mm yr<sup>-1</sup>, absolute marginal growth rate (surface area) was 11.12 cm<sup>2</sup> yr<sup>-1</sup>, and absolute marginal extension rate (diameter) was 24.66 mm yr<sup>-1</sup>. Net calcification, determined using the buoyant weight method (includes secondary calcification deposits) was approximately 2.72 to 3.40 g CaCO<sub>3</sub> cm<sup>-2</sup> yr<sup>-1</sup>, while gross calcification determined using growth metrics (just newly deposited CaCO<sub>3</sub>) was 0.43 to 0.59 g CaCO<sub>3</sub> cm<sup>-2</sup> yr<sup>-1</sup>. Shaded treatments yielded higher growth and gross calcification rates compared to exposed in all seasons except summer. This data provides empirical information necessary to monitor the impacts of future climate change on the GBR and to better understand the response of CCA to manipulative experiments on ocean acidification and warming.

**KEY WORDS:** Coralline algae · Growth · Calcification · Seasonal · Great Barrier Reef

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## INTRODUCTION

Coralline algae play crucial roles in the ecology of tropical and temperate reefs worldwide and are among the most abundant marine organisms living on hard substrate in the euphotic zone (Steneck 1986). As well as stabilising the reef framework (Littler & Littler 1984), coralline algae make significant carbonate sediment contributions (Chave & Wheeler 1965), provide hard substrate and settlement cues for corals and commercially important species (Diaz-Pulido et al. 2010), are an important food source and habitat (Littler & Littler 1984, Foster 2001) and transfer carbon from the biological to the geological cycle by way of calcification (Steneck 1986). With a soluble high Mg-calcite skeleton, corallines have also been

identified as one of the most sensitive marine calcifying organisms to ocean acidification (Anthony et al. 2008, Jokiel et al. 2008, Martin & Gattuso 2009), and consequently, a potential indicator species for future climate change. However, despite their ecological importance, little information is available on coralline algae autoecology, and baseline growth and calcification rates *in situ* in tropical environments such as the Great Barrier Reef (GBR) are largely unknown.

While polar and temperate corallines have received attention due to their capacity as palaeoenvironmental recorders (Halfar et al. 2000, Kamenos et al. 2008), tropical corallines have been examined little (Figueiredo 1997). Currently, available information on crustose coralline algae (CCA) growth and calcification in warmer climates is restricted to a

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handful of studies on tropical reefs scattered worldwide. Studies that have been conducted in tropical environments include growth measurements in the Caribbean (Adey & Vassar 1975, Steneck & Adey 1976, Stearn et al. 1977), Brazil (Figueiredo 1997, Villas Bôas et al. 2005), Japan (Matsuda 1989), and the GBR (Ringeltaube 2001). Research on calcification rates in the GBR is limited to 2 studies (Chisholm et al. 1990, Chisholm 2000) that estimated calcification using seawater carbonate chemistry measurements. The scarcity of baseline information on basic physiological and ecological processes of CCA in tropical environments such as the GBR needs to be addressed given the important roles these species play on reefs and the rapidly changing environmental conditions.

There are a number of environmental factors that can influence the growth and calcification of corallines in the natural environment (Edyvean & Ford 1987, Fabricius & De'ath 2001). Temperature and irradiance are 2 key factors that regulate coralline growth and calcification rates, both independently (Adey 1970, Steneck 1986, Leukart 1994, Figueiredo 1997, Halfar et al. 2011, Short et al. 2015) and interactively (Leukart 1994, Macchiavello et al. 1998). In temperate and polar environments, the highest growth and calcification rates (of various species and morphologies) are reported over the warmer months of spring and summer, when these 2 factors (irradiance and seawater temperature) are at their peak (Adey & McKibbin 1970, Potin 1990, Freiwald & Henrich 1994, Foster 2001, Steller et al. 2007, Halfar et al. 2008, Martin et al. 2013, Egilsdottir et al. 2015). Of the limited studies conducted on tropical CCA growth and calcification, few have investigated seasonality (with the exception of Figueiredo 1997, Short et al. 2015). Aside from temperature and light, other variables such as seawater chemistry (Jokiel et al. 2008, Kuffner et al. 2008, Martin & Gattuso 2009, Gao & Zheng 2010, Diaz-Pulido et al. 2012), nutrients (Ichiki et al. 2000, Langdon & Atkinson 2005, Russell et al. 2009), herbivory (Adey & Vassar 1975, Matsuda 1989, O'Leary & McClanahan 2010), water movement (Agegian 1981, Larkum et al. 2003), space competition (Matsuda 1989, Paine 1990, Steneck et al. 1991) and reproductive cycle (Edyvean & Ford 1987, Figueiredo 1997) can also influence growth and calcification. While these factors may prove to be just as important in governing rates of growth in tropical CCA, quantifying the effects of irradiance and temperature on growth was given priority in this study. This was to ascertain the influence of these 2 key factors in tropical shallow reef environments of the GBR where sea-

sonal changes in temperature and light are not as pronounced as they are in colder climes.

*Porolithon onkodes* (Heydrich) Foslie is a smooth, non-geniculate coralline alga that can grow to several centimetres thick (Farr et al. 2009, R. Steneck et al. unpubl. data). It is one of the most widespread tropical to subtropical non-geniculate coralline algae, and has been reported to be the single-most important ecological species because of its wide distribution (Maneveltdt & Keats 2014) and importance to reef development (Doty & Morrison 1954, Littler 1972). *P. onkodes* is also the most abundant species of CCA on the GBR (Dean et al. 2015, R. Steneck et al. unpubl. data), due to its ability to withstand desiccation to some extent (Ringeltaube 2001, R. Steneck et al. unpubl. data) and survive intense grazing (R. Steneck et al. unpubl. data). Information on how key reef-building CCA species such as *P. onkodes* respond to current environmental conditions and seasonal variation is vital if we are to effectively monitor the health of the GBR (and other Pacific and Indo-Pacific reefs in general) and accurately assess the natural and anthropogenic impacts associated with climate change and ocean acidification. Once baseline seasonal variations in growth and calcification rates are established, this information can be utilised for a multitude of conservation and management practices such as assessing the impacts of local disturbances and recovery times, refining carbon cycle modeling and better understanding the response of CCA in manipulative experiments concerning ocean acidification and warming.

The focus of this study was to address the lack of critical knowledge on baseline CCA growth and calcification rates in the GBR. To achieve this, we aimed to establish *in situ* seasonal baseline vertical growth, marginal growth and calcification (both net and gross) rates of the abundant and important reef-building CCA species *P. onkodes* on Heron Island (GBR). Additionally, we explored the effects of irradiance on these growth and calcification rates. We hypothesised that the maximum seasonal growth rates would occur over the summer season, and that the samples in the exposed treatments would have higher growth rates than those in the shaded treatment.

## MATERIALS AND METHODS

### Study site and environmental parameters

Seasonal growth and calcification rates of the encrusting coralline alga *Porolithon cf. onkodes* (Heydrich) Foslie were compared under 2 different irra-

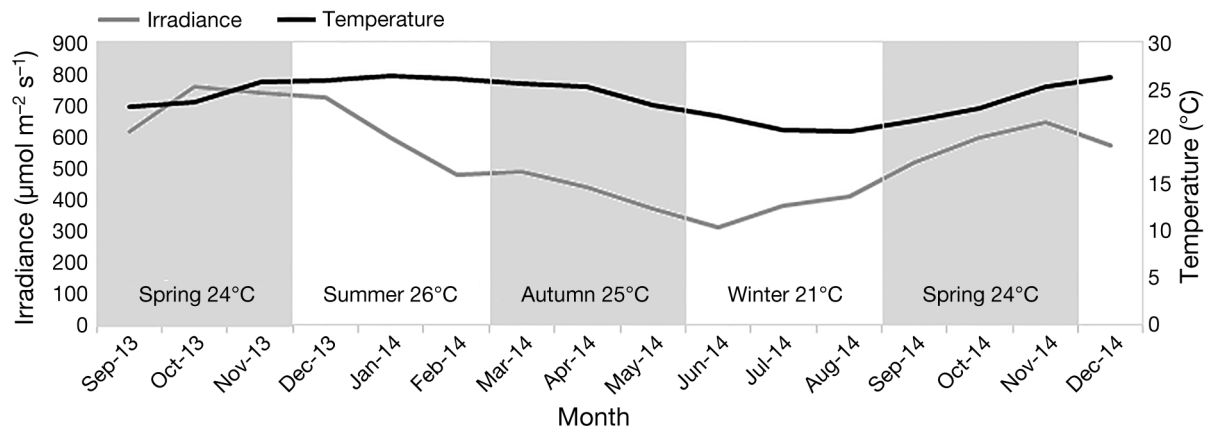


Fig. 1. Average seasonal variance in seawater irradiance and temperature levels at Heron Island on the Great Barrier Reef from spring 2013 to spring 2014 (AIMS 2016)

diance treatment levels during 5 separate 3 mo sampling periods, representing the different climatic seasons from spring 2013 to summer 2014. This was an *in situ* experiment based on the Tenements 1 site ( $23^{\circ}26'0.4''\text{S}$ ,  $151^{\circ}55'41.3''\text{E}$ ) of Heron Island, on the southern GBR. Heron Island has a sub-tropical climate, with the majority of rain falling over summer and autumn, and a relatively dry winter and spring. The climatic seasons were defined as spring (September to December), summer (December to March), autumn (March to June) and winter (June to September). The highest average seasonal temperatures and irradiance during this study (as displayed in Fig. 1) occurred over the summer season at  $26.4^{\circ}\text{C}$  and  $635 \mu\text{mol m}^{-2} \text{s}^{-1}$  (AIMS 2016), while the lowest were recorded in winter at  $21.2^{\circ}\text{C}$  and  $382 \mu\text{mol m}^{-2} \text{s}^{-1}$  (AIMS 2016). These average values were calculated using the daily values for the 3 mo period of each season. Seasonal changes in carbonate chemistry in the southern GBR have been reported by Shaw et al. (2012) and Shaw & McNeil (2014) in both offshore reef and reef flat sites. These studies reported ranges (minimum–maximum) of pH levels of 8.04–8.10 and 7.59–8.56, total alkalinity of 2289–2316 and 1922–2538  $\mu\text{mol kg}^{-1}$ , and  $p\text{CO}_2$  of 343–404 and 70–1325  $\mu\text{atm}$  for the offshore reef and reef flat sites respectively.

### Sample collection and staining

At the beginning of each climatic season (commencing with spring in September 2013), specimens were carefully collected using hammer and chisel from the nearby reef crest and upper reef slope (<6 m) of Tenements 1 and transported back to the

outdoor flow-through tank facilities on Heron Island Research Station (HIRS) where seawater was supplied directly from the reef lagoon. Samples were cleaned by hand to remove epiphytes, invertebrates and loose material, and cut to size ( $3 \times 3 \text{ cm}$  chips). After cutting, samples were placed in 5 l of fresh seawater and stained using the Alizarin Red stain at  $0.25 \text{ g l}^{-1}$  for 24 h (Blake & Maggs 2003). During staining, each container had a small pump for circulation and was placed in a temperature-controlled room to maintain the seasonal *in situ* water temperature and prevent overheating. After staining, samples were rinsed with fresh seawater to remove any residual stain and set into Selleys Aqua Knead It<sup>TM</sup> epoxy to secure the sample and minimise potential dissolution of the underside exposed skeleton. The epoxy and CCA were placed into a small PVC ring (40 mm diameter  $\times$  10 mm height) set inside a previously prepared 90 mm PVC ring filled with cement and covered with a thin layer of epoxy to keep a uniform surface (of the same substrate). The purpose of the rings (in addition to minimising dissolution) was to provide a flat, uniform surface for surficial marginal growth to occur (Fig. 2). Holes were drilled into the outer cement ring, allowing cable ties to attach the samples to racks; each ring was labelled using alphabet beads set into the epoxy (visible from the back) (Fig. 2). Photographs of each ring with the CCA sample embedded in epoxy were taken prior to the experiments using a Canon G12 camera with a ruler to calibrate distance for marginal growth measurements. Once prepared (approx. 4 to 6 d), samples were transported to the reef site in fresh seawater and attached to galvanised racks (2 rack<sup>-1</sup>) secured to the reef slope at approximately 5 to 6 m depth (high tide).

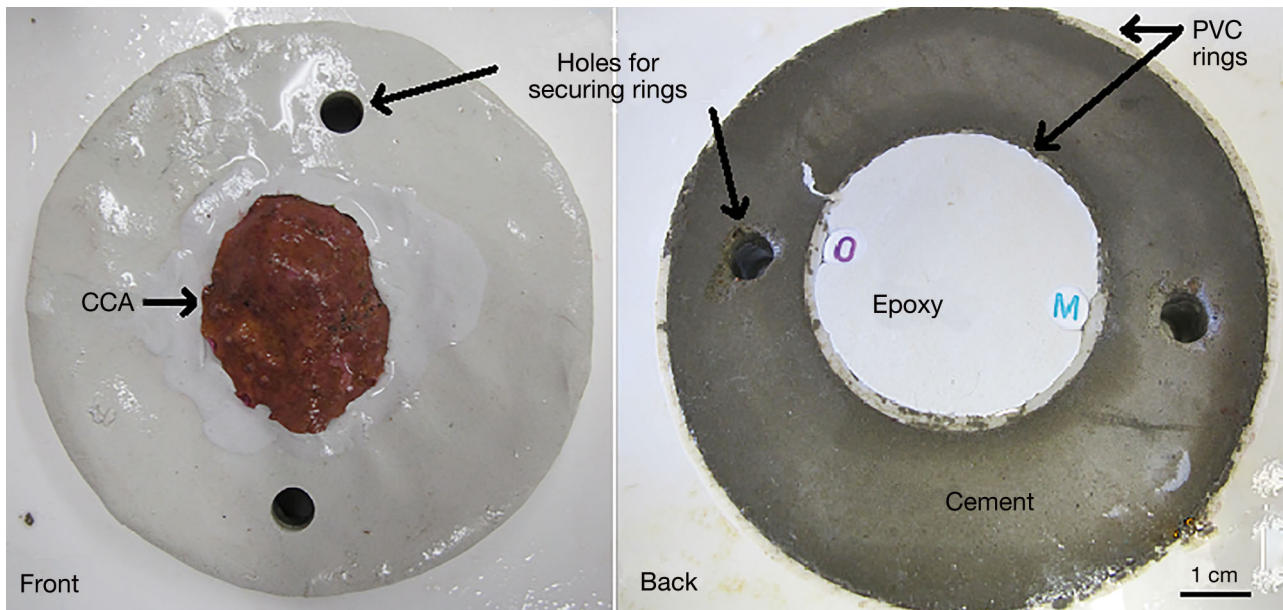


Fig. 2. Epoxy rings, comprised of 40 mm PVC pipe filled in with Selleys Aqua Knead It™ epoxy, set inside a previously prepared 90 mm PVC ring filled with cement and covered with a thin layer of epoxy to keep a uniform surface over which marginal growth of *Porolithon onkodes* could be measured

The irradiance treatment consisted of 2 levels: (1) ambient: between approximately 382 and 635  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$  depending on season (Fig. 1) and (2) shaded: samples protected from direct sunlight during the highest irradiance hours of the day (Fig. 3), receiving 35 to 60  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$  during these midday hours. Shade was created using 70% UV-protection shade cloth fixed to racks so that the cloth was 20 cm above the CCA. *In situ* irradiance and water temperature were measured during de-

ployment and collection of samples using a Walz diving pulse-amplitude modulated (PAM) fluorometer. Further daily irradiance levels and water temperature were taken from the Australian Institute of Marine Science (AIMS) weather stations located on Heron Island reef (AIMS 2016). There were 20 samples per irradiance level: 10 racks containing 2 samples each were exposed to ambient irradiance, and the other 10 racks were shaded. Following a 3 mo period, all samples were collected

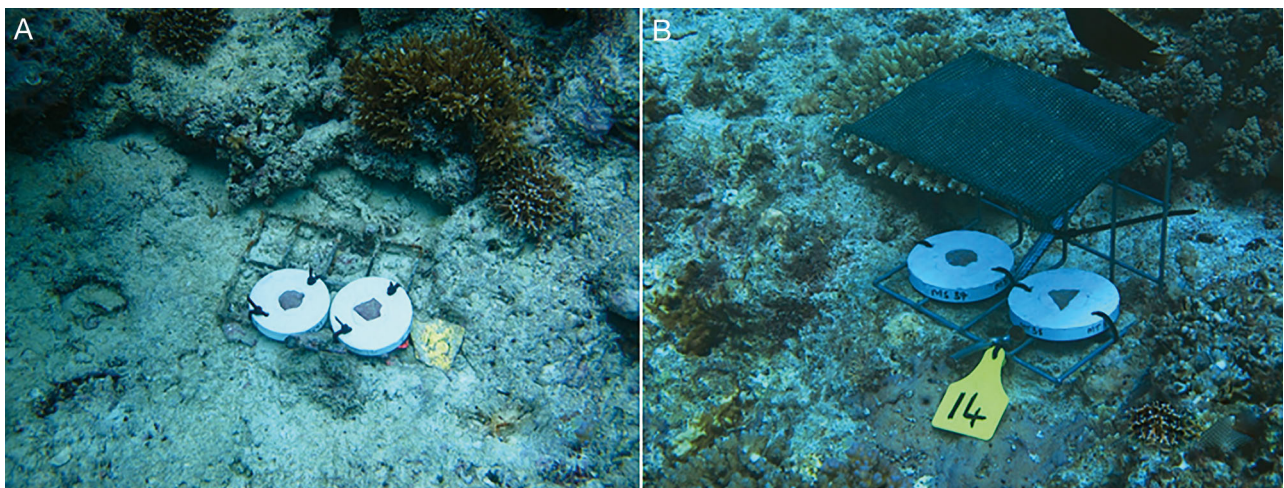


Fig. 3. PVC rings with embedded *Porolithon onkodes* fragments attached to the reef slope on either (A) exposed or (B) shaded racks at 5 to 6 m depth (high tide)

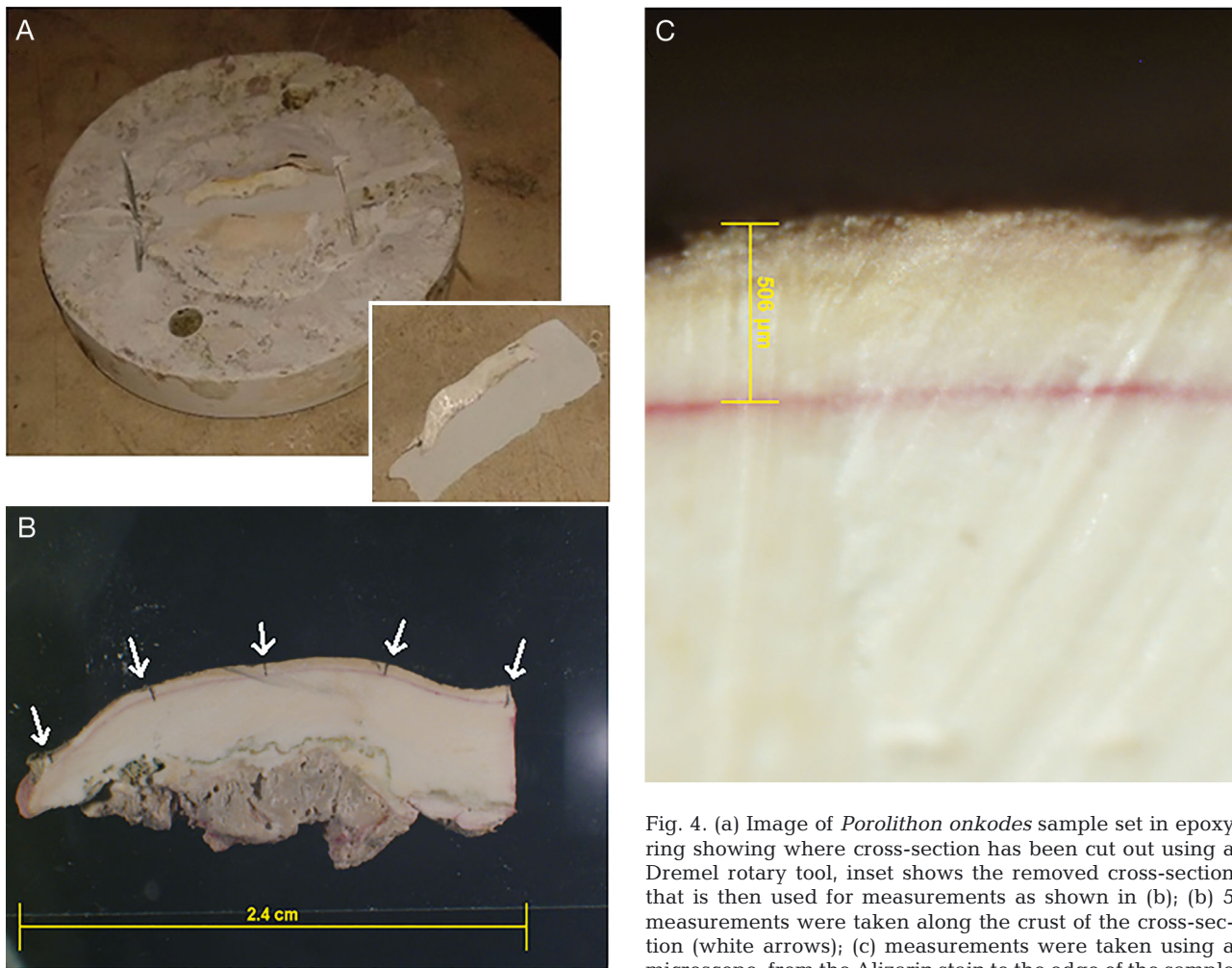


Fig. 4. (a) Image of *Porolithon onkodes* sample set in epoxy ring showing where cross-section has been cut out using a Dremel rotary tool, inset shows the removed cross-section that is then used for measurements as shown in (b); (b) 5 measurements were taken along the crust of the cross-section (white arrows); (c) measurements were taken using a microscope, from the Alizarin stain to the edge of the sample

and replaced with new CCA samples (prepared as described above) for the following season. All shade cloths were scrubbed at the beginning of each season to remove turf algae. Collected samples were oven-dried at 60°C for 24 h.

Dead (bleached) and unhealthy (discoloured or declining tissue) samples were excluded from the following measurements.

### Growth measurements

In this study, 'vertical' growth rate refers to the vertical extension of the crust which increases crust thickness, whereas 'marginal' growth rate refers to the lateral extension of the crust which increases surficial area. To determine vertical growth rate, a ~3 mm thick cross-section was sliced from the middle of the sample (Fig. 4a) using a Dremel rotary tool with a diamond cut wheel. Vertical growth ( $\mu\text{m}$ )

was then obtained by measuring the distance from the alizarin mark to the growing edge of the crust under a compound microscope (Olympus BX53) using a 4 $\times$  objective (Fig. 4c). A total of 5 measurements at equal distance apart were taken along the cross-section of each crust and averaged to obtain the growth measurement. The annual vertical extension growth rate was calculated by summing the average growth in each of the 4 seasons (normalised to 90 d season<sup>-1</sup>).

To determine marginal growth over the 3 mo period, the surface area of each sample was photographed before deployment and after collection. Absolute marginal growth (useful for comparisons with other studies) was calculated by subtracting final from initial surface area ( $\text{mm}^2$ ) using the ImageJ software program (<https://imagej.nih.gov/ij/>) (Fig. 5).

Changes in diameter (derived in ImageJ by tracing around the edge of the crust) were also calculated using the final and initial perimeter measurements

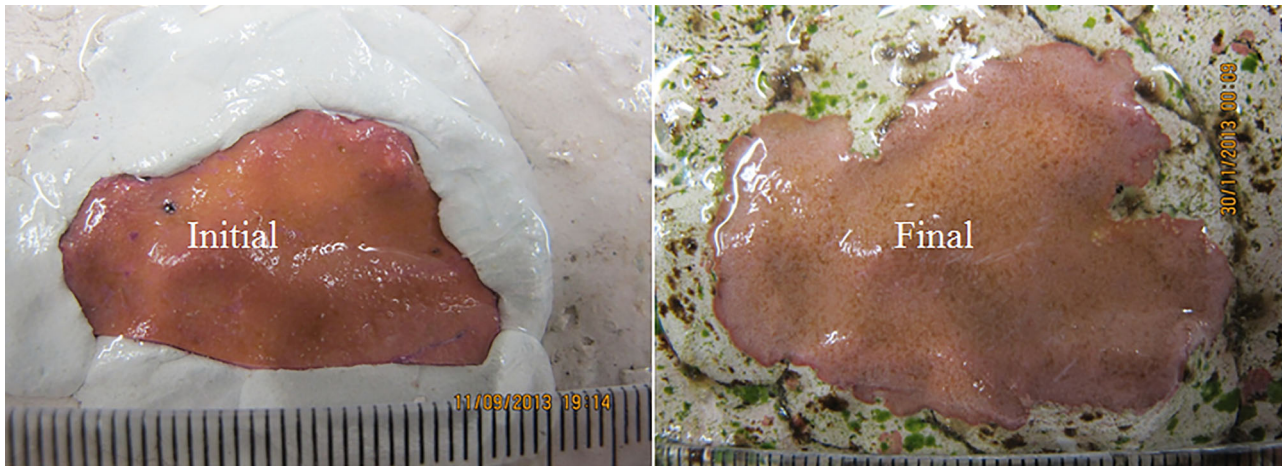


Fig. 5. Marginal growth of *Porolithon onkodes*, determined using the ImageJ program by tracing around the edge of the crust and calculating the difference between final and initial measurements

(mm). For statistical analyses, percentage change in surface area was used to normalise marginal extension to crust starting size. Annual marginal extension rate was calculated by summing the average daily absolute growth in each of the 4 seasons (normalised to 90 d season<sup>-1</sup>).

### Calcification measurements

Seasonal net and gross calcification rates of *P. onkodes* were determined independently. Net calcification rates, determined by the buoyant weight method (Davies 1989), include secondary calcification processes such as infilling of the older skeleton and skeletal dissolution, whereas gross calcification, based on geometry techniques using volume derived from the seasonal growth metrics, excludes these processes, giving the rate of newly deposited CaCO<sub>3</sub> only.

For net calcification, each fragment (set inside the rings) was buoyantly weighed (to the nearest 0.01g) at the start of each season directly before deployment, and then again upon collection (after careful removal of fouling organisms). Net deposition of CaCO<sub>3</sub> was then estimated as the difference between these initial and final weights ( $W_0$  and  $W_1$ , converted to dry weight based on the measured density of the seawater and CaCO<sub>3</sub>), normalised to the surface area (initial and final surface area:  $A_0$  and  $A_1$ ) of each fragment and divided by the number of days ( $d$ ) in that particular season. This was then multiplied by 365 and expressed in units of grams per unit area per year (g CaCO<sub>3</sub> cm<sup>-2</sup> yr<sup>-1</sup>):

$$\text{Buoyant weight net calcification} = \left( \frac{W_1 - W_0}{(A_0 + A_1)/2} \right) / d \quad (1)$$

Gross calcification rates were estimated using the fragments' marginal ( $A_0$  and  $A_1$ ) and vertical extension (centre of the fragment,  $V_0$  and edge of the fragment,  $V_1$ ) measurements together with the skeletal density ( $sd$ ) of *P. onkodes* from Heron Island (2.58 g cm<sup>-3</sup>, as determined by (Reyes-Nivia 2013):

$$\text{Mass of fragment centre } (M_0) = \left( \frac{V_0}{A_0} \right) \times sd \quad (2)$$

$$\begin{aligned} \text{Mass of fragment edge } (M_1) \\ = \sum [(A_1 - A_0) \times V_1] \times sd \end{aligned} \quad (3)$$

$$\text{Total mass of fragment new growth } (M_3) = M_0 + M_1 \quad (4)$$

$$\begin{aligned} \text{Net calcification rate (g CaCO}_3 \text{ cm}^{-2} \text{ yr}^{-1}) \\ = \frac{M_3}{d} \times \frac{365}{A_1} \end{aligned} \quad (5)$$

### Statistical analysis

To determine any significant differences in the rates of vertical growth, marginal growth and calcification rates between seasons and irradiance levels, general linear model 2-way ANOVAs were used with season and light treatment as fixed factors, and CCA fragments as replicates. Data normality and homogeneity of variance were tested using Kolmogorov-Smirnov and Levene's tests, respectively. Data for vertical and marginal growth were also log<sub>10</sub> transformed to meet requirements prior to analysis. Tukey's test was used for post hoc comparisons.

## RESULTS

## Vertical growth measurements

Vertical growth rates of *Porolithon onkodes* on the reef slope at Heron Island varied significantly over the climatic seasons (2-way ANOVA,  $F_{4,128} = 22.462$ ,  $p < 0.001$ ; Table 1, Fig. 6a). A significant increase in growth was observed during spring (both 2013 and 2014), with daily growth rates up to 45% higher than in the other 3 seasons. The highest mean growth for the exposed irradiance treatment,  $4.97 \pm 1.06 \mu\text{m d}^{-1}$ , was reported in spring 2013, while the lowest occurred in autumn at  $3.39 \pm 0.61 \mu\text{m d}^{-1}$  (Table 2, Fig. 6a). Annual vertical extension (calculated using seasonal means) for the exposed irradiance treatment was  $1.45 \text{ mm yr}^{-1}$  ( $3.98 \mu\text{m d}^{-1}$ ), and  $1.68 \text{ mm yr}^{-1}$

Table 1. ANOVA results for vertical and marginal growth and calcification rates of *Porolithon onkodes*. ns: not significant

Source of variation	df	F	p	Conclusion: Tukey's test
<b>Vertical growth</b>				
Season	4	22.46	<0.001	Spring > Summer, Winter, Autumn
Irradiance	1	8.854	0.003	Shaded > Exposed
Season $\times$ irradiance	4	2.141	0.079	ns
Error	128			
<b>Marginal growth (daily percentage increase)</b>				
Season	4	5.754	<0.001	Autumn > Winter & Spring 2014
Irradiance	1	7.955	0.006	Shaded > Exposed
Season $\times$ irradiance	4	1.658	0.165	ns
Error	114			
<b>Net calcification (buoyant weight method)</b>				
Season	4	2.556	0.043	Spring 2014 > Summer
Irradiance	1	2.020	0.158	ns
Season $\times$ treatment	4	1.828	0.129	ns
Error	103			
<b>Gross calcification (growth metrics method)</b>				
Season	4	17.311	<0.001	Spring 2013 > Autumn & Winter
Irradiance	1	22.163	<0.001	Shaded > Exposed
Season $\times$ treatment	4	3.715	0.007	
Error	111			

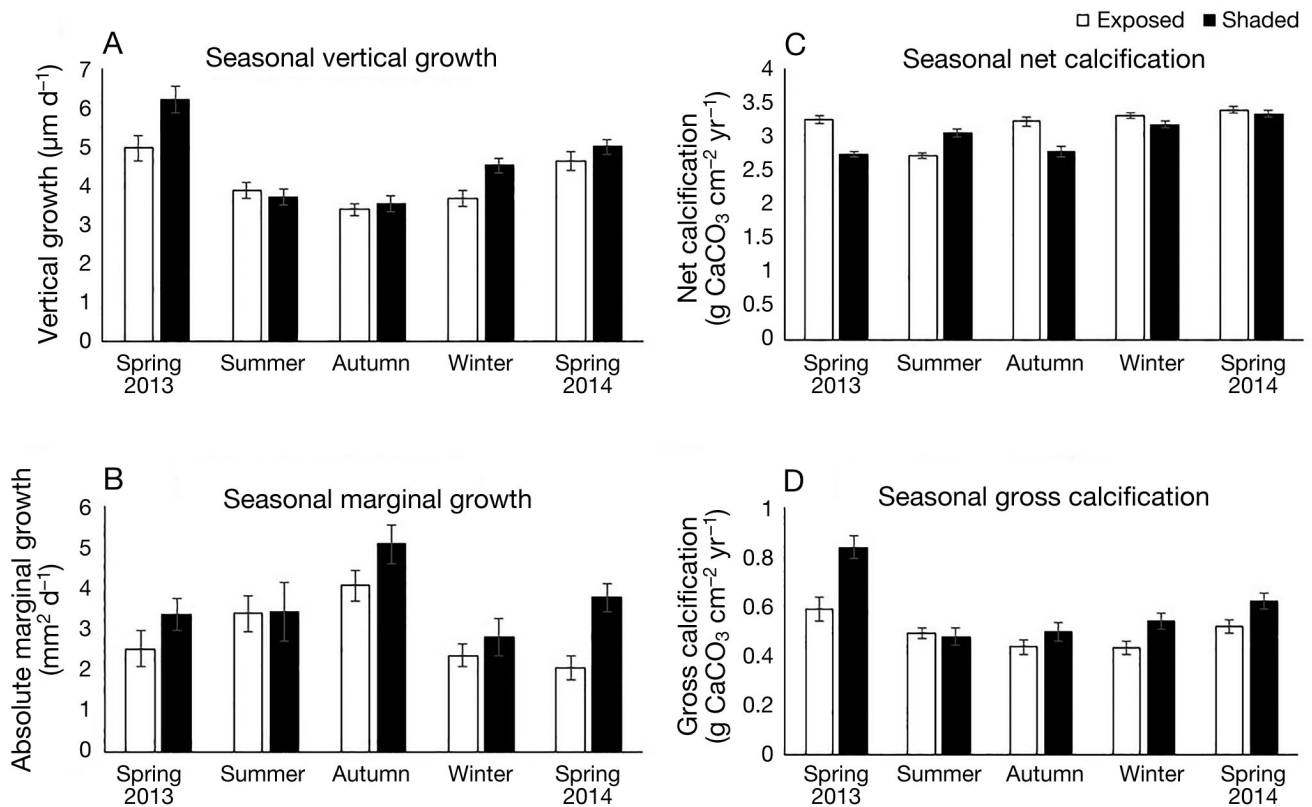


Fig. 6. Seasonal growth and calcification rates (mean  $\pm$  SE) of *Porolithon onkodes* in exposed and shaded irradiance treatments. (A) Seasonal vertical growth rate, (B) seasonal absolute marginal growth, (C) seasonal net calcification and (D) seasonal gross calcification

Table 2. Seasonal vertical and marginal growth and calcification rates of *Porolithon onkodes*

	Exposed				Shaded			
	n	Max.	Mean (SD)	Min.	n	Max.	Mean (SD)	Min.
<b>Vertical growth (<math>\mu\text{m d}^{-1}</math>)</b>								
Spring 2013	11	6.38	4.97 (1.06)	3.48	17	8.69	6.23 (1.45)	3.51
Summer	15	5.93	3.89 (0.80)	3.05	10	4.69	3.70 (0.66)	3.06
Autumn	15	4.53	3.39 (0.61)	2.26	12	4.91	3.54 (0.73)	2.43
Winter	19	5.60	3.67 (0.85)	2.10	10	5.53	4.52 (0.73)	3.28
Spring 2014	15	6.46	4.62 (0.92)	3.18	12	6.29	5.01 (0.66)	3.92
<b>Absolute marginal growth in area (<math>\text{mm}^2 \text{d}^{-1}</math>)</b>								
Spring 2013	11	6.13	2.53 (1.4)	1.01	14	6.75	3.35 (1.49)	1.15
Summer	15	6.08	3.38 (1.72)	0.50	9	7.15	3.41 (2.17)	0.62
Autumn	12	5.49	4.07 (1.28)	1.50	9	6.44	5.07 (1.43)	2.61
Winter	18	5.73	2.37 (1.24)	0.80	12	6.19	2.81 (1.55)	1.02
Spring 2014	12	4.08	2.06 (1.03)	0.91	12	6.05	3.77 (1.20)	1.84
<b>Absolute marginal growth in diameter (<math>\text{mm d}^{-1}</math>)</b>								
Spring 2013	11	0.15	0.061 (0.032)	0.027	14	0.14	0.077 (0.031)	0.028
Summer	15	0.15	0.078 (0.038)	0.014	9	0.16	0.076 (0.043)	0.019
Autumn	12	0.12	0.084 (0.026)	0.023	9	0.13	0.10 (0.026)	0.060
Winter	18	0.10	0.051 (0.022)	0.019	12	0.12	0.061 (0.030)	0.024
Spring 2014	12	0.08	0.041 (0.018)	0.022	12	0.11	0.075 (0.021)	0.038
<b>Net calcification (<math>\text{g CaCO}_3 \text{cm}^{-2} \text{yr}^{-1}</math>)</b>								
Spring 2013	10	4.07	3.27 (0.52)	2.54	13	3.48	2.75 (0.63)	1.41
Summer	15	3.33	2.72 (0.39)	2.05	9	4.77	3.06 (0.72)	2.35
Autumn	8	4.51	3.23 (0.64)	2.67	8	3.32	2.78 (0.39)	2.21
Winter	17	4.14	3.32 (0.51)	2.23	8	4.35	3.19 (0.75)	2.44
Spring 2014	11	4.66	3.40 (0.66)	2.71	11	4.24	3.35 (0.66)	2.37
<b>Gross calcification (<math>\text{g CaCO}_3 \text{cm}^{-2} \text{yr}^{-1}</math>)</b>								
Spring 2013	10	0.883	0.588 (0.160)	0.434	14	1.07	0.840 (0.170)	0.511
Summer	15	0.624	0.491 (0.087)	0.309	8	0.618	0.477 (0.100)	0.349
Autumn	12	0.622	0.436 (0.103)	0.271	10	0.715	0.497 (0.012)	0.386
Winter	18	0.665	0.432 (0.114)	0.256	11	0.741	0.541 (0.111)	0.350
Spring 2014	12	0.722	0.516 (0.092)	0.383	11	0.819	0.621 (0.109)	0.477

( $4.60 \mu\text{m d}^{-1}$ ) for the shaded treatment. A significant difference was found between irradiance treatments ( $F_{1,128} = 8.854$ ,  $p = 0.003$ ) with samples under the shaded treatment producing significantly higher vertical growth rates (up to 18%) than samples in the exposed treatment over spring 2013 and winter seasons.

### Marginal growth measurements

Marginal extension of *P. onkodes* also varied seasonally on Heron Island (2-way ANOVA on daily percentage increase,  $F_{4,114} = 5.754$ ,  $p < 0.001$ ; Fig. 6b). Post hoc results showed these significant seasonal variations were from autumn compared to winter ( $p < 0.001$ ), and autumn compared to spring 2014 ( $p = 0.002$ ) (Table 1). The highest absolute daily increase in surface area occurred in autumn, and this was consistent at both irradiance levels (ambient:  $4.07 \pm$

$1.28 \text{ mm}^2 \text{d}^{-1}$ , shaded:  $5.07 \pm 1.43 \text{ mm}^2 \text{d}^{-1}$ ; Table 2). Seasonal averages (spring 2013, summer, autumn and winter) gave an annual absolute marginal growth rate (surface area) of  $11.12 \pm 0.71 \text{ cm}^2 \text{yr}^{-1}$  for the exposed irradiance treatment, and  $13.53 \pm 0.82$  for the shaded treatment. The absolute marginal extension rate (diameter) for the exposed treatment was  $24.66 \pm 1.37 \text{ mm yr}^{-1}$ , and for the shaded treatment was  $28.38 \pm 1.54 \text{ mm yr}^{-1}$ .

The irradiance treatment affected the rate of marginal growth ( $F_{1,114} = 7.955$ ,  $p = 0.006$ ), with all seasons (aside from summer) reporting a higher daily percentage and absolute daily increase in growth under the shaded treatments. However, 1-way ANOVA revealed that this difference in marginal growth between exposed and shaded treatments was only significant in the spring 2014 season ( $p < 0.001$ ). The shaded samples in spring 2014 had an 82% higher absolute daily growth rate than the exposed samples.



### Net calcification

Seasonal variation was observed in net calcification rates of *P. onkodes* (2-way ANOVA,  $F_{4,103} = 2.556$ ,  $p = 0.043$ ; Table 1) with net calcification in the spring 2014 and winter seasons 25 and 22% higher than summer respectively (Table 2, Fig. 6c). No significant difference was found in the net calcification rate between irradiance levels or the interaction between season and irradiance.

### Gross calcification

A 2-way ANOVA using season and irradiance as factors on gross calcification revealed a significant difference, both interactively ( $F_{4,111} = 3.715$ ,  $p = 0.007$ ; Table 1) and independently (season:  $F_{4,111} = 17.311$ ,  $p < 0.001$ ; irradiance:  $F_{1,111} = 22.163$ ,  $p < 0.001$ ; Table 1). Further analysis using the post hoc Tukey's test revealed these seasonal differences to be between spring 2013 and autumn ( $p = 0.017$ ), and spring 2013 and winter ( $p = 0.006$ ), with spring 2013 gross calcification higher than autumn and winter by 36 and 35% respectively (Fig. 6d). Irradiance treatments produced differences, with the shaded treatment yielding significantly higher gross calcification rates than the exposed treatment in winter ( $p = 0.018$ ) and both spring 2013 and spring 2014 seasons ( $p = 0.001$  and  $0.022$  respectively). The biggest difference in gross calcification rate was found during the spring 2013 season with a 43% increase under the shaded treatment.

## DISCUSSION

This is the first study providing empirical data of seasonal *in situ* growth rates, and contributes significantly to the limited information available on calcification for coralline algae on the GBR. Results from this study demonstrated strong seasonal variability in all measured growth and calcification metrics and highlighted the effects of irradiance levels on these metrics in the tropical environment. Importantly, the growth and calcification metrics used in this study each displayed different trends, highlighting the varying responses of each metric to changing environmental conditions. The information collected in this study contributes substantially to our understanding of the ecology of a key calcifying component of the GBR, and provides a potentially useful foundation for exploring CCA responses to future environmental change on the GBR.

### Vertical growth

The vertical growth rate of  $1.4 \text{ mm yr}^{-1}$  recorded in our study was consistent with the mean annual vertical growth rate of  $1.4 \text{ mm yr}^{-1}$  reported for *Porolithon onkodes* by Matsuda (1989) in a shallow fore-reef environment on the Ryukyu Islands (Table 3). Adey & Vassar (1975) also reported similar vertical extension rates of 1 to  $5.2 \text{ mm yr}^{-1}$  for various encrusting corallines on the tropical reef St. Croix of the US Virgin Islands (Table 3). Although there was a significant difference between spring 2013 and spring 2014 ( $p = 0.033$ ), both seasons still recorded higher growth than the other 3, and this slight variation may be attributed to the 2014 season running 26 d longer into the warmer summer months, in which the rate of vertical growth was declining.

### Marginal growth

The marginal extension rates of  $25 \text{ mm yr}^{-1}$  ( $1.53 - 2.52 \text{ mm mo}^{-1}$ ) recorded for *P. onkodes* in our study were also comparable to various CCA species in similar tropical reef environments (including *P. onkodes*) as displayed in Table 3. These include rates of  $0.8 \pm 0.9 \text{ mm mo}^{-1}$  by Matsuda (1989), 0.97 to  $5.28 \text{ mm mo}^{-1}$  by Short et al. (2015), 0.6 to  $3.6 \text{ mm mo}^{-1}$  by Figueiredo (1997) and 0.3 to  $1.5 \text{ mm mo}^{-1}$  by Villas Bôas et al. (2005). Marginal extension rates reported by Ringeltaube (2001) during an 8 wk experiment at Heron Island were slightly higher ( $2.8$  to  $3.2 \text{ mm mo}^{-1}$ ) than those found in our study but this may be due to the measurements being taken from newly settled recruits, and from 3 different species of CCA (including *P. onkodes*).

### Net calcification

Net calcification rates of *P. onkodes* from Heron Island were calculated to be approximately  $2.72$  to  $3.40 \text{ g CaCO}_3 \text{ cm}^{-2} \text{ yr}^{-1}$ . This exceeds rates reported by Chisholm (2000) for *P. onkodes* using carbonate chemistry measurements on the northern GBR (Table 3). The use of 2 different methods, locations, experimental depths and seasons may account for the difference in calcification rates between these 2 studies. Other investigations using different methods to calculate calcification have reported even lower rates of *P. onkodes*, such as Short et al. (2015) using the buoyant weight method (Table 3). Although the calcification rate in the study by Short et al. (2015) is

Table 3. Vertical and marginal growth and calcification rates of coralline algae worldwide. CCA: crustose coralline algae

Taxon	Morphology	Region	Metric	Growth or calcification rate	Reference
<i>Bossiella gardneri</i>	Geniculate	California, USA	Vertical	3.3 mm yr <sup>-1</sup>	Smith (1972)
<i>Bossiella plumose</i>	Geniculate	British Columbia, Canada	Vertical	24.6 mm yr <sup>-1</sup>	Fisher & Martone (2014)
<i>Calliarthron cheilosporioides</i>	Geniculate	California, USA	Calcification	0.18–1.14 g CaCO <sub>3</sub> cm <sup>-2</sup> yr <sup>-1</sup>	Martone (2010)
		California, USA	Vertical	39.6 mm yr <sup>-1</sup>	Martone (2010)
<i>Calliarthron tuberculosum</i>	Geniculate	British Columbia, Canada	Vertical	20.4 mm yr <sup>-1</sup>	Fisher & Martone (2014)
		California, USA	Vertical	20.4 mm yr <sup>-1</sup>	Johansen & Austin (1970)
		California, USA	Vertical	4.2 mm yr <sup>-1</sup>	Smith (1972)
<i>Calliarthron</i> spp.	Geniculate	California, USA	Marginal	0.24–1.21 mm yr <sup>-1</sup>	Goldberg & Foster (2002)
		California, USA	Vertical	1–8 mm yr <sup>-1</sup>	Goldberg & Foster (2002)
<i>Corallina officinalis</i>	Geniculate	Massachusetts, USA	Vertical	16.8 mm yr <sup>-1</sup>	Andrake & Johansen (1980)
		Northern Ireland	Vertical	2.5 mm yr <sup>-1</sup>	Blake & Maggs (2003)
		Massachusetts, USA	Vertical	1.4–25 mm yr <sup>-1</sup>	Colthart & Johansen (1973)
<i>Corallina vancouveriensis</i>	Geniculate	British Columbia, Canada	Vertical	18 mm yr <sup>-1</sup>	Fisher & Martone (2014)
		California, USA	Vertical	4.2 mm yr <sup>-1</sup>	Smith (1972)
<i>Hydrolithon boergesenii</i>	Encrusting	St. Croix, Caribbean	Marginal	12 mm mo <sup>-1</sup>	Adey & Vassar (1975)
<i>Hydrolithon reinboldii</i>	Rhodolith	French Polynesia	Calcification	0.002 g CaCO <sub>3</sub> cm <sup>-2</sup> yr <sup>-1</sup>	Payri (1997)
		French Polynesia	Vertical	0.37 mm yr <sup>-1</sup>	Payri (1997)
<i>Lithophyllum congestum</i>	Encrusting	St. Croix, Caribbean	Marginal	0.9 mm mo <sup>-1</sup>	Steneck & Adey (1976)
		St. Croix, Caribbean	Vertical	8 mm yr <sup>-1</sup>	Steneck & Adey (1976)
<i>Lithophyllum incrustans</i>	Rhodolith	South West Wales	Calcification	0.04 g CaCO <sub>3</sub> cm <sup>-2</sup> yr <sup>-1</sup>	Edyvane & Ford (1987)
		South West Wales	Marginal	0.19–0.26 mm mo <sup>-1</sup>	Edyvane & Ford (1987)
		South West Wales	Vertical	0.27–0.3 mm yr <sup>-1</sup>	Edyvane & Ford (1987)
<i>Lithophyllum insipidum</i>	Encrusting	Ishigaki Island, Japan	Marginal	0.7 mm mo <sup>-1</sup>	Matsuda (1989)
		Ishigaki Island, Japan	Vertical	1.2 mm yr <sup>-1</sup>	Matsuda (1989)
		Northern Ireland	Vertical	0.45–0.65 mm yr <sup>-1</sup>	Blake & Maggs (2003)
<i>Lithothamnion glaciale</i>	Rhodolith	Troms, Norway	Calcification	420–1432 g CaCO <sub>3</sub> m <sup>-2</sup> yr <sup>-1</sup>	Freiwald & Henrich (1994)
		Troms, Norway	Vertical	0.6–1 mm yr <sup>-1</sup>	Freiwald & Henrich (1994)
<i>Lithothamnion muelleri</i>	Rhodolith	California, USA	Vertical	0.6 mm yr <sup>-1</sup>	Rivera et al. (2004)
<i>Neogoniolithon fosliei</i>	Encrusting	Ishigaki Island, Japan	Marginal	0.7 mm mo <sup>-1</sup>	Matsuda (1989)
<i>Neogoniolithon imbricatatum</i>	Encrusting	St. Croix, Caribbean	Marginal	2.1 mm mo <sup>-1</sup>	Adey & Vassar (1975)
<i>Neogoniolithon megacarpum</i>	Encrusting	St. Croix, Caribbean	Marginal	2.3 mm mo <sup>-1</sup>	Adey & Vassar (1975)
<i>Paragoniolithon conicum</i>	Encrusting	St. Croix, Caribbean	Marginal	1.9 mm mo <sup>-1</sup>	Adey & Vassar (1975)
		Ishigaki Island, Japan	Marginal	0.9 mm mo <sup>-1</sup>	Matsuda (1989)
		Ishigaki Island, Japan	Vertical	0.5 mm yr <sup>-1</sup>	Matsuda (1989)
<i>Phymatolithon calcareum</i>	Rhodolith	Northern Ireland	Vertical	0.9 mm yr <sup>-1</sup>	Blake & Maggs (2003)
<i>Porolithon gardeneri</i>	Branching	Hawaii, USA	Vertical	7–20 mm yr <sup>-1</sup>	Agegian (1981)
<i>Porolithon onkodes</i>	Encrusting	Ishigaki Island, Japan	Marginal	0.8 mm mo <sup>-1</sup>	Matsuda (1989)
		Ishigaki Island, Japan	Vertical	1.4 mm yr <sup>-1</sup>	Matsuda (1989)
		Marmion Lagoon, Western Australia	Calcification	0.14 g CaCO <sub>3</sub> cm <sup>-2</sup> yr <sup>-1</sup>	Short et al. (2015)
		Marmion Lagoon, Western Australia	Marginal	0.97–5.28 mm mo <sup>-1</sup>	Short et al. (2015)
<i>Porolithon pachydermum</i>	Encrusting	Great Barrier Reef, Australia	Marginal	2.8–3.2 mm mo <sup>-1</sup>	Ringeltaube (2001)
		Sueste & St. Barbara, Brazil	Marginal	0.6–3.6 mm mo <sup>-1</sup>	Figueiredo (1997)
		Rocas Atoll, Brazil	Marginal	0.3–1.5 mm mo <sup>-1</sup>	Villas Boas et al. (2005)
<i>Sporolithon durum</i>	Rhodolith	New Caledonia	Vertical	0.6 mm yr <sup>-1</sup>	Darrenougue et al. (2013)
Four spp. of CCA	Encrusting	Great Barrier Reef	Calcification	0.15–1.03 g CaCO <sub>3</sub> cm <sup>-2</sup> yr <sup>-1</sup>	Chisholm (2000)

within the same order of magnitude as rates reported by Chisholm (2000), the differences can be attributed to methodical, spatial or seasonal variances.

### Gross calcification

Gross calcification of *P. onkodes* from Heron Island reported an annual average of 0.432 to 0.588 g CaCO<sub>3</sub> cm<sup>-2</sup> yr<sup>-1</sup>. This annual average of gross calcification is markedly smaller than the net calcification rate. However, unlike net calcification measured using the buoyant weight method, gross calcification using the geometric method includes only the newly deposited CaCO<sub>3</sub>, excluding weight added by secondary calcification processes such as infilling of the older skeleton (e.g. by other minerals such as aragonite or dolomite) (Nash et al. 2012, Diaz-Pulido et al. 2014, Kraysky-Self et al. 2016). Further examination of the secondary calcification and infilling process is needed to better determine the rate and scale at which this infilling occurs.

### Seasonality

Seasonality had a major influence on both vertical and marginal growth of *P. onkodes*. Vertical growth of *P. onkodes* peaked during the spring season, while absolute marginal growth was highest over the autumn season. The maximum vertical and marginal growth rates of *P. onkodes* did not occur in summer in correlation with the highest temperature and irradiance (Figs. 1 & 6) as reported in studies from polar and temperate regions (Adey & McKibbin 1970, Halfar et al. 2008, Egilsdottir et al. 2015). The reasons for this deviation in expected summer growth are unknown, but may be because (1) temperature fluctuations in tropical reef environments are not as marked as in polar and temperate regions, therefore the algae are less exposed to changes in temperature variability. For example, a study by Freiwald & Henrich (1994) reported a 12°C difference between summer and winter temperatures, while in our study average temperature varied by only 5°C between winter and summer. (2) The steep incline in average temperature and irradiance at the end of winter (Fig. 1) is enough to trigger spring vertical growth. Although average temperatures and irradiance only varied by 5°C annually, there is a large increase of 3°C and 200 μmol m<sup>-2</sup> s<sup>-1</sup> respectively from winter to spring. (3) Elevated temperatures during summer may cause heat stress. CCA living in shallow tropical reef environments are already at the upper limits of

their temperature tolerance, and slight increases in maximum temperature may inhibit growth and calcification (Agegian 1985, Martin & Gattuso 2009). (4) Various ecological and physiological processes such as reproduction (Edyvean & Ford 1987), space competition (Matsuda 1989, Rogers 1997), nutrients (Bjork et al. 1995) or herbivory (Steneck & Adey 1976) may cause stress on CCA during summer. Although these factors each have the potential to individually or interactively influence the growth of CCA during the summer season, the effects that these have on seasonal changes in coralline growth and calcification of *P. onkodes* from Heron Island were not quantified in our baseline growth and calcification study. Until such research is carried out, the reasons behind the seasonal variation in the growth of *P. onkodes* on Heron reef remain speculative.

### Irradiance

This study demonstrates that irradiance is an important factor affecting the rates of growth of CCA on Heron Island. Samples attached under the shaded racks tended to grow faster (both vertically and marginally) than those attached to the exposed racks with full access to sunlight (as shown in Fig. 3). This result is to some extent unexpected, as it is generally accepted that a decrease in light availability results in decreased growth and calcification rates of CCA. It is likely, however, that under high light intensity, as found in shallow tropical reef environments such as Heron Island, CCA may be susceptible to light-induced stress (Payri & Maritorea 2001). This may occur particularly during the summer months when temperature is higher, potentially causing photoinhibition (e.g. Vásquez-Elizondo & Enríquez 2016). Although *P. onkodes* is typically a shallow water species that grows abundantly in shallow reef crests where irradiance can reach levels of approximately 2000 μmol m<sup>-2</sup> s<sup>-1</sup> (Payri & Maritorea 2001), there is an optimal light level at which growth is maximised. Diurnal cycles in photosynthetic capacity have been observed in various marine macroalgae species (Belshe et al. 2007, Edwards & Kim 2010), including red algae (Hanelt et al. 1993). In general, this cycle involves a high photosynthetic efficiency occurring in the morning and decreasing with increasing light intensity until maximal photoinhibition occurs around midday or early afternoon (Hanelt et al. 1993). In our study, the shaded samples were protected from high midday irradiance under the shade cloth. Therefore, during the midday hours of high

irradiance the exposed samples may have experienced reduced levels in photosynthetic activity, while the shaded samples may not have been affected by these photo-protection or inhibition processes. Without further physiological experiments, it is not possible to discern the mechanisms driving the patterns observed, however, this study does demonstrate that irradiance is an important factor affecting the growth of CCA in tropical reef environments.

Interestingly, rates of net calcification were unaffected by reduction of light intensity. This lack of response in calcification rates to changing irradiance levels was also unexpected as, along with growth, increased light is generally accepted to result in increased calcification (Potin 1990, Chisholm 2000, Martin et al. 2006, 2013). The fact that no changes were observed in net calcification between light treatments suggests that the buoyant weight method may not have been sensitive enough to detect subtle differences in calcification. Further, as this method represents the balance between new skeletal deposition and dissolution processes, the relationship between vertical and marginal growth with net calcification is not necessarily positive. Gross calcification, on the other hand, did respond to changes in irradiance levels with the shaded treatment producing higher rates of calcification than the exposed in all seasons except summer. Light has been shown to stimulate coralline algae calcification (Borowitzka 1987) with increasing light levels increasing calcification until saturation (Chisholm 2000, Martin et al. 2006). The lack of response in calcification rates to changes in light treatment (as found in net calcification) or the increased calcification rate under decreased irradiance (as reported in gross calcification) in this study may also indicate irradiance saturation. A study by (Payri & Maritorena 2001) in a similar coral reef environment found saturating irradiance for *P. onkodes* varied from 200 to 600  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . At these levels the exposed treatments may have been irradiance-saturated, resulting in no benefit from higher irradiance levels in the exposed over shaded treatments (net calcification) or even a reduction in calcification under the exposed treatment (gross calcification).

#### Comparison between metrics

The difference in the behaviour of the metrics used to document growth rate responses to seasonal changes in this study reveals a very interesting aspect of the life history of *P. onkodes* that, to our knowledge, had not been previously documented in the litera-

ture. While vertical growth rate was lowest during autumn, absolute marginal extension rate was highest during the same season. Similarly, when high rates of vertical growth occurred, rates of marginal growth were lower. This finding suggests a trade-off between energy expenditure in CCA biological traits such as vertical and marginal growth. These trade-offs may be in response to environmental, ecological and physiological processes such as reproductive cycles, space competition or herbivory (Adey & Vasar 1975, Edyvean & Ford 1987, Matsuda 1989). The cause of this decoupling in vertical and marginal growth direction is outside of scope of this study; however, it does draw attention to how little we know about CCA growth and calcification rates and the need for ongoing research into these topics if we are to accurately assess and monitor growth and calcification rates under future climate change scenarios.

#### CONCLUSIONS

We found significant variability in key parameters of the life history of the important reef-building coralline alga *Porolithon onkodes* in response to climatic seasons and light intensity on the GBR. Maximum rates of *in situ* growth and calcification varied seasonally and separately, with each measured metric achieving maximum rates in different seasons. Establishing the presence of seasonal variation in growth and gross calcification rates further refines existing baseline data and shows that seasonality needs to be taken into consideration in instances such as extrapolating growth and calcification rates for predictive modelling from short-term studies. This is particularly relevant in the context of ocean acidification and warming and the sensitivity of CCA to these stressors.

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