



RESEARCH

Differential thermal tolerances in geographically distinct lineages of a dominant reef building alga

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Abstract How organisms respond to increasing temperatures could be attributed to existing thermal tolerances or that certain populations are living well below their thermal limits. To address these ideas, we exposed geographically distinct (1144–2332 km apart) lineages of the dominant reef-building crustose coralline alga, *Porolithon* cf. *onkodes*, from the Australian Great Barrier Reef and Lord Howe Island to an increasing temperature (1 °C h⁻¹) experiment, where individual average oxygen production was measured continuously. Molecular phylogenetic analysis revealed the existence of hidden lineages within this alga, but individuals are morpho-anatomically identical. The tropical, low latitude lineage supported the climate variability hypothesis, in which some populations existing in already warmer and more stable thermal environments may be living at or near their thermal thresholds. On average, there was a ~92% decrease in O₂ produced after a 1 °C increase in the tropical, low latitude lineage. However, the high latitude lineage did not support this hypothesis, as individuals continuously decreased the amount of O₂ produced with increasing temperature. The central lineage responded uniquely,

maintaining a stable level of O₂ for almost 5 °C above their acclimation temperature. Our results indicate that the climate variability hypothesis only partially explains the thermal tolerance in this alga, and we suggest local oceanographic processes, latitudinal effects, and importantly, cryptic speciation influences the responses of different lineages of the critically important reef-building alga *P. cf. onkodes* to rising temperatures.

Resumen La forma en que los organismos responden al aumento de las temperaturas puede atribuirse a sus tolerancias térmicas existentes o a que ciertas poblaciones viven muy por debajo de sus límites térmicos. Para abordar estas ideas, expusimos linajes geográficamente distintos (separados entre 1 144 y 2 332 km) de la principal alga coralina incrustante constructora de arrecifes, *Porolithon* cf. *onkodes*, procedentes de la Gran Barrera de Coral australiana y de la isla Lord Howe, a un experimento de incremento de temperatura (1 °C h⁻¹), midiendo continuamente la producción media de oxígeno de cada individuo. El análisis filogenético molecular reveló la existencia de linajes crípticos dentro de esta alga, aunque los individuos son morfoanatómicamente idénticos. El linaje tropical de baja latitud apoyó la hipótesis de la variabilidad climática, según la cual algunas poblaciones que ya habitan en entornos térmicos más cálidos y estables podrían encontrarse en o cerca de sus umbrales térmicos. En promedio, la producción de O₂ disminuyó alrededor de un 92 % tras un aumento de 1 °C en este linaje tropical. Sin embargo, el linaje de alta latitud no respaldó esta hipótesis, ya que los individuos redujeron continuamente la producción de O₂ a medida que aumentaba la temperatura. El linaje central respondió de manera única, manteniendo un nivel estable de O₂ hasta casi 5 °C por encima de su temperatura de aclimatación. Nuestros resultados indican que la hipótesis de la variabilidad climática solo explica parcial-

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mente la tolerancia térmica en esta alga, y sugerimos que los procesos oceanográficos locales, los efectos latitudinales y, de forma importante, la especiación críptica influyen en las respuestas de los diferentes linajes de la alga constructora de arrecifes *P. cf. onkodes* frente al aumento de temperaturas.

Keywords Thermal tolerance · Crustose coralline algae · Thermal limits · Cryptic species · Heat-stress · Coral reefs

Introduction

Current ocean warming, caused by increased CO₂ emissions to the atmosphere from human activities, is a matter of great concern due to its effects on individuals, populations, and communities (Parmesan 1996; Walther et al. 2002). Ocean warming, for example, causes mass coral bleaching and widespread coral mortality in coral reefs (Hughes et al. 2017) and has been responsible for regional mortality of kelps along a variety of temperate and cold-water regions (Wernberg et al. 2016). Ocean warming and rapid increases in seawater temperature may exceed the fundamental thermal niche of species and surpass the physiological capacity of individuals, potentially leading to species and population die offs (Hoegh-Guldberg and Bruno 2010; Comte and Olden 2017; Frölicher et al. 2018). Increased seawater temperature has also induced shifts in the geographic distribution of many species, particularly along latitudinal gradients, including poleward migrations in the leading edge of populations and potential contractions in the trailing (warm) edge of populations (Heron et al. 2016). The “climate variability hypothesis” proposes that with increased latitude, there is a positive relationship between thermal tolerance and the level of climatic variability experienced by individuals and their populations (Stevens 1989; Bozinovic et al. 2011). That is, populations living at higher latitudes will be more resistant to warming owing to their broader thermal niches compared with tropical populations that might already be living near their optimal temperature (Bennett et al. 2018). Based on current ideas, species currently living at or near their thermal limits could be pushed over their existing thermal tolerance limits with increasing temperature (Hochachka and Somero 2002; Miller and Stillman 2012). Alternatively, species may be able to compensate for increases in temperature through acclimation or widening their thermal performance curve to incorporate higher temperatures (Hochachka and Somero 2002; Miller and Stillman 2012). The plasticity to environmental stressors (e.g., shifting thermal regimes) may arise from (i) tolerances species develop in their current or historical habitats (Sandoval-Castillo et al. 2020), or (ii) because certain populations are living well below their thermal limits and may be able to accommodate further increases in temperature without surpassing their thermal limits (Stillman

2003; Miller and Stillman 2012). It is important to understand the thermal tolerances of populations along latitudinal geographical distribution gradients in order to predict (and mitigate) the impacts of climate change on marine ecosystems. This is particularly relevant for ecosystems that are sensitive to thermal stress, such as highly diverse coral reef ecosystems.

Coral reefs are hotspots for biodiversity, supporting many marine taxa and people (Moberg and Folke 1999). Coral reefs along the eastern coast of Australia, including the Great Barrier Reef (GBR) and Lord Howe Island, provide a unique opportunity to study species variation in geographic range and physiological responses, such as thermal tolerance limits. Reefs along the eastern coast of Australia extend for more than 3000 km along a latitudinal gradient, from the tropics to the subtropics, where average seawater temperature during the summer ranges from around 29–24 °C, respectively. Marine habitats along this coast are also experiencing rapid warming at a rate of ~0.20 °C/decade (Heron et al. 2016), with the occurrence of extreme climatic and anomalous temperature events, like heatwaves, driving mass coral bleaching events (Kim et al. 2019; Kamenos and Hennige 2024). The climate variability hypothesis has found support in a number of studies in terrestrial and marine organisms (Bozinovic et al. 2011; Mota et al. 2018; Clark et al. 2020). However, studies on reef-building corals suggest absence of a correlation between latitude and temperature optima for photosynthesis (Oliver & Palumbi 2011; Bennett et al. 2015; Schoepf et al. 2015; Jurriaans & Hoogenboom 2019). How latitudinal gradients impact the thermal capacity of other crucial reef building species, such as crustose coralline algae (CCA), is unknown. CCA are critically important for coral reef ecology, including stabilization of loose coral rubble and construction of reef framework (Adey 1998), and induction of settlement and metamorphosis of coral larvae contributing to reef resilience (Harrington et al. 2004; Ritson-Williams et al. 2009; Abdul Wahab et al. 2023). Populations of the CCA *Porolithon onkodes*, a primary reef building species (Littler 1973; Adey et al. 1982) and one of the most important calcareous alga for reef cementation (e.g., Dechnik et al. (2017)), span along latitudinal gradients and it is possible high latitude populations are potentially more resistant to warming than their more tropical counterparts. This information is valuable to understand the potential impacts of ocean warming on reef stabilization, cementation, and growth.

In the current study, we aimed to investigate the thermal physiology (photosynthetic response) of individuals from three populations of the reef-building alga *Porolithon cf. onkodes* along a latitudinal gradient on the Eastern Australian coast. We sampled from reefs surrounding Lizard Island, Heron Island, and Lord Howe Island. Due to the potential reproductive isolation of these populations (geographic

separation > 1000 km) and the difficulties in morphological identification of coralline algae, the possibility that populations of *P. cf. onkodes* represent hidden lineages (or cryptic species) was considered and investigated through molecular phylogenetic analysis. In the context of the climate variability hypothesis, we predicted the tropical, low latitude (trailing edge, more stable) population would be less tolerant to further increases in temperature because this tropical population is living near their thermal threshold. On the other hand, we predicted that the high latitude (leading edge, more variable) population, which we hypothesized is living furthest from its thermal limit, would perform the best under increasing temperatures, while the central population would perform somewhere between the tropical, low latitude, and high latitude populations in terms of response. By examining the variability in thermal physiology of these three geographically distinct populations, we can further explore if populations are adapted to their current environments, which would be seen in a similar response across locations. Alternatively, thermal tolerance could be at a population (or lineage or cryptic species) level, which would be seen as differences in response to increasing temperature dependent on location.

Material and methods

Collections and identification

Fragments of the widespread and abundant crustose coralline algae (CCA) species, *Porolithon cf. onkodes*, of the orange color morph, were collected from three

geographically distinct locations along the Eastern Coast of Australia (Fig. 1) through the Austral summer months of 2018 and 2019. The tropical, low latitude, and central sites, Lizard Island (14.6645 S, 145.4651 E) and Heron Island (23.4423 S, 151.9148 E), are located in the Great Barrier Reef, while the high latitude site, Lord Howe Island (31.5553 S, 159.0821 E), is the southernmost true coral reef in the world (Fig. 1). The approximate annual seawater temperature ranges for 2018–2019 were 24 to 29 °C at Lizard Island, 21 to 27 °C at Heron Island, and 19 to 25 °C at Lord Howe Island. Temperature data were sourced from Australian Institute of Marine Science publicly available sea temperature observing system (Australian Institute of Marine Science) and seatemperature.info for Lord Howe Island. Fragments, ~3 cm², of *P. cf. onkodes* were collected using hammer and chisel from reefs surrounding Lizard Island, Heron Island, and Lord Howe Island between 1 and 4 m depth, all from similar well exposed shallow reef environments. After collection, fragments were brought back to respective research stations, Lizard Island Research Station (LIRS), Heron Island Research Station (HIRS), Lord Howe Island Research Station (LHIRS), and thoroughly cleaned of epiphytes. Any exposed, non-living calcium carbonate skeleton was sealed using Coral Glue® (Ecotech Marine). Algae were allowed to acclimatize in flow through environments at respective collection temperatures (27.0 °C, 26.5 °C, and 26.3 °C for Lizard Island, Heron Island, and Lord Howe Island, respectively) and light levels (~240–250 μmol photons m⁻² s⁻¹ PAR) for 3 days after collection prior to incubations. Algal fragments were examined morphologically (smooth crusts and presence of well-defined trichocyte fields) and anatomically (presence of cell fusions, absence

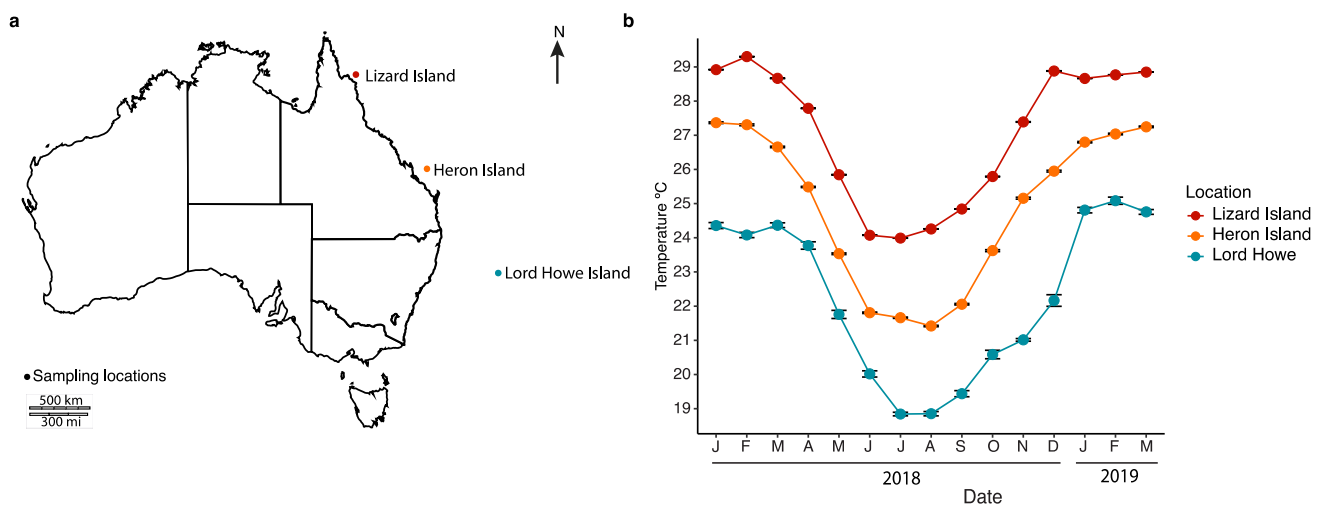


Fig. 1 **A** Map of Australia with points at sampling and experimental sites along the East Coast of Australia. The distance between Lizard Island and Heron Island is 1190 km and between Heron Island and Lord Howe Island is 1144 km. **B** Monthly average seawater tempera-

tures from January 2018–March 2019. Error bars are ± standard error. Data sourced from Australian Institute of Marine Science publicly available sea temperature observing system (Australian Institute of Marine Science) and seatemperature.info for Lord Howe Island

of secondary pit connections, and presence of uniporate reproductive chambers or conceptacles) using light microscopy. All individuals used fit the taxonomic description of *P. onkodes* (Heydrich) Foslie (Adey et al. 1982; Maneveldt and Keats 2014). To further investigate their taxonomic status and the potential existence of cryptic species (i.e., taxa that are morphologically indistinguishable but are on different evolutionary trajectories, e.g., Struck et al. (2018)), we sequenced the ribulose biphosphate carboxylase large chain (*rbcL*) gene from our samples (following the metabolic incubations described further below) and compared the phylogenetic affinities among individuals of the three populations. We further compared our sequences from the current study with sequences of existing herbarium type material (see Molecular analyses section below). Fragments used for morpho-anatomical and genetic analyses from each location were vouchered in the G.D-P. herbarium at Griffith University, Brisbane, Australia.

Photosynthetic response

To test the effects of increased seawater temperature on algal metabolism, through conducting an acute increasing temperature experiment, 18–20 fragments of *P. cf. onkodes* were used for photosynthetic incubations at each location. Fragments of algae were placed into 150 mL acrylic incubation chambers filled with ambient seawater. Each chamber contained a magnetic stir bar, a temperature sensor (Pt100), and a PreSens dipping oxygen optode (DP-PSt3). Chambers were placed within a water bath with an immersion circulating heater (CORIO CD, Julabo) that controlled temperature within ± 0.01 °C. The water bath was positioned on top of a magnetic stir plate and under an LED light (MarsAqua 300 W LED, Full Spectrum) set to light levels mimicking the average, at depth light level at each collection location (240–250 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ PAR). The oxygen sensors were calibrated daily prior to each incubation run to 100% O_2 , done by bubbling seawater with air, and 0% O_2 , achieved through addition of sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$) to seawater. Before each incubation run, algae were held at the temperature measured at time and location of collection (27.0 °C, 26.5 °C, and 26.3 °C for Lizard Island, Heron Island, and Lord Howe Island, respectively) for 30 min and allowed to acclimatize. After acclimatization, the internal temperature of the incubation chambers was increased by $1 \text{ }^\circ\text{C h}^{-1}$ (a rate of about $0.017 \text{ }^\circ\text{C min}^{-1}$), simulating an acute increasing temperature experiment. Starting temperatures for the incubations were based on the temperatures at the collection site. The recorded temperature at Lizard Island at the beginning of our experiment (December) was $1 \text{ }^\circ\text{C}$ lower than the average record provided by AIMS (Australian Institute of Marine Science), and this difference may be due to differences in specific locality of the sampling site

compared to AIMS monitoring site within the Lizard Island reef. At the time of collection, Lord Howe Island was experiencing a minor heatwave which brought the temperature over $1 \text{ }^\circ\text{C}$ it's normal sea water temperature average for the month of March. Dissolved O_2 and temperature were continuously measured and recorded every minute throughout the entirety of the incubations. Incubations were continued until $\text{O}_2\%$ in the incubation chambers plateaued and began to decrease (or, on average, algae began to respire or use O_2 faster than producing it) and did not recover or temperature reached $+4.5 \text{ }^\circ\text{C}$ above collection temperature (Fig. 2). Hyperoxic conditions in the experiment can be ruled out as O_2 generally decreased or stayed constant during the incubations. $\text{O}_2\%$ was converted to $\mu\text{g O}_2 \text{ h}^{-1} \text{ cm}^{-2}$ after incubations to obtain net photosynthesis/ O_2 production, and was normalized to controls (incubation chambers without algae) that were run with each incubation, and to the surface area of each algal fragment (determined through the aluminum foil technique (Marsh Jr 1970)). Color change of the surficial pigmented tissue was monitored throughout the incubations as an indication of bleaching/health of algae.

Molecular analyses

Following the net photosynthesis incubations, all algal fragments were placed in silica gel for DNA sequencing. Once in the laboratory, algal tissue was collected by scraping the thallus surface with a razor blade. DNA extractions followed the protocol described in Jeong et al. (2019), and *rbcL* gene sequences were used in the current study. Detailed methods can be found in Supplementary Materials, Methods, and Molecular analyses. To explore whether individuals from the three locations belong to the same species or represent cryptic taxa, we estimated *rbcL* sequence divergence values between populations (Supplementary Table S2). To further understand the phylogenetic relationships between individuals collected from the three locations in our study, and more broadly with other species of the genus *Porolithon*, representative coralline *rbcL* sequences of all described species of the genus *Porolithon* were downloaded from GenBank (Supplementary Table S1) and phylogenetic trees were constructed using *Adeylithon boseneci* and *Hydrolithon* sp. as outgroups. Further methods on alignment and tree construction can be found in Supplementary Materials, Methods, and Molecular analyses.

Statistical analysis

Statistical analyses were performed in R Studio in the statistical software R v3.6.1 (www.rstudio.com). Oxygen production ($\mu\text{mol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$) was calculated for each individual at every $0.5 \text{ }^\circ\text{C}$ increment in ramp temperature. To evaluate how thermal responses differed among populations of *P. cf.*

onkodes, we fit a generalized linear mixed-effects model (GLMM) using the *glmmTMB* package with a Tweedie distribution and log link (McGillcuddy et al. 2025), which allowed modeling of non-linear decline and accommodated the presence of negative and near-zero O_2 values. Fixed effects included ramp temperature, population (location of acclimation), and their interaction; individual identity was included as a random intercept to account for repeated measures.

To meet the requirements of the log link, oxygen production values were shifted by adding the absolute minimum value plus a small constant to ensure all values were positive. Model fit was assessed by comparing Akaike Information Criterion (AIC) values with a linear mixed-effects model (LMM), and residual diagnostics were performed using the *DHARMa* package (Hartig et al. 2024). The Tweedie model showed substantially improved fit ($\Delta AIC > 68$) and reduced residual deviation and was therefore used for inference.

To compare thermal sensitivities among populations, we used the *emtrends()* function from the *emmeans* package (Lenth et al. 2025) to estimate the slope of log-transformed O_2 production versus ramp temperature for each population. Pairwise comparisons of slopes were adjusted using Tukey's method. Welch's t-tests (Welch 1947) were used for targeted within-population comparisons between specific ramp temperatures.

Results

Populations of *P. cf. onkodes* responded differently to increasing temperature during the acute temperature experiment (Fig. 2). Both ramp temperature ($^{\circ}C$) and acclimation temperature (i.e., location of population) significantly influenced O_2 production ($p < 0.0001$, Table 1), and a significant

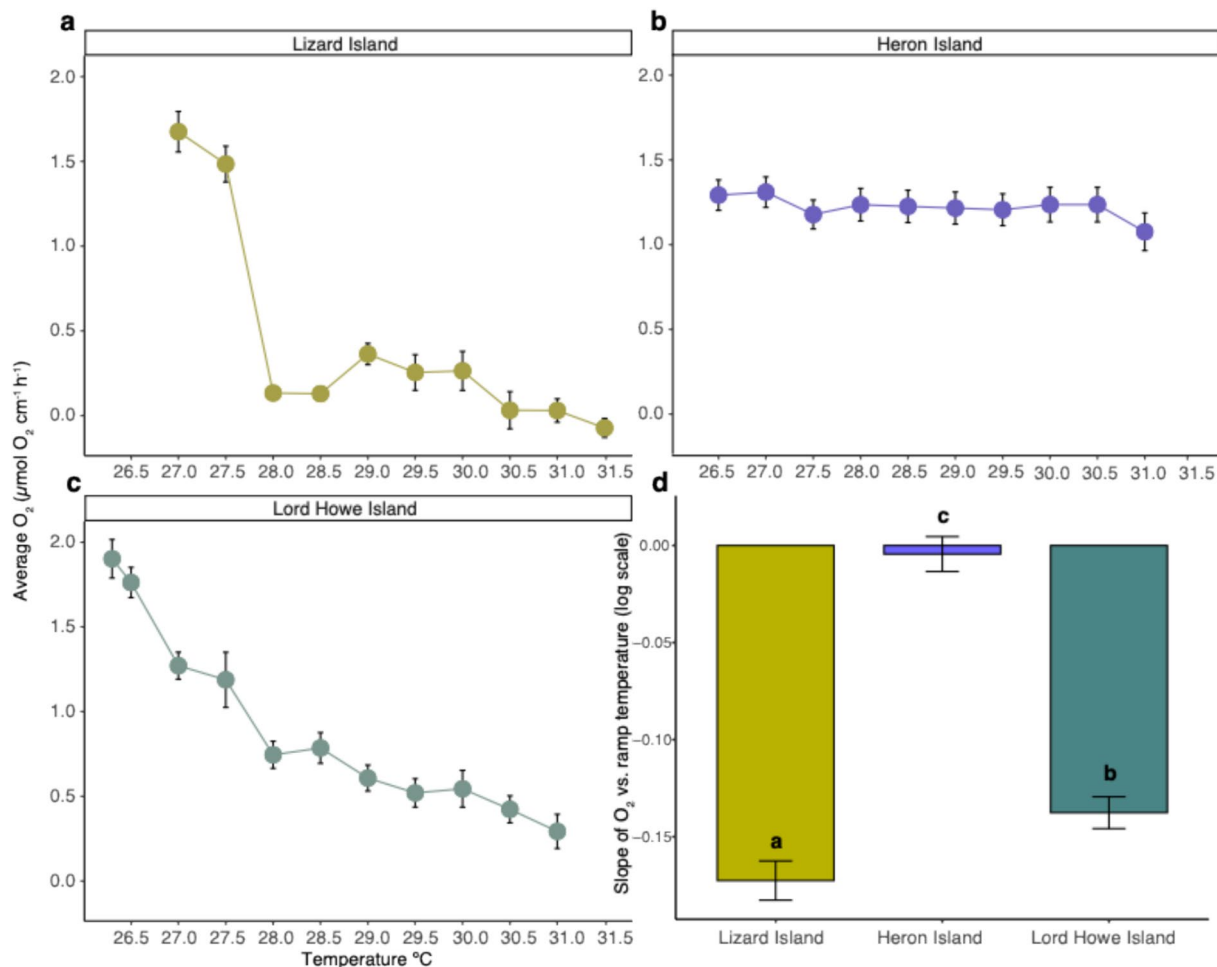


Fig. 2 Photosynthetic response of three populations of the crustose coralline alga *Porolithon cf. onkodes* across a latitudinal gradient: Lizard Island (tropical, low latitude), Heron Island (central reef), and Lord Howe Island (high latitude). Panels a–c show the change in average O_2 production ($\mu mol\ O_2\ cm^{-2}\ h^{-1} \pm SE$) at each 0.5 $^{\circ}C$ incre-

ment in ramp temperature. Panel d shows the estimated slopes ($\pm SE$) of log-transformed O_2 production versus ramp temperature, derived from a generalized linear mixed model (Tweedie distribution with log link). Lowercase letters indicate statistically significant differences among populations

Table 1 Summary of fixed effects and slope estimates from a generalized linear mixed-effects model (GLMM) of oxygen production in *Porolithon* cf. *onkodes* using a Tweedie distribution with log link. Fixed effects include ramp temperature, population (location), and their interaction. Estimated marginal trends (slopes) were calculated

Effect	Estimate	SE	z-value	p-value	Comparison
Intercept (Lizard Island)	18.572	1.093	16.989	< 0.0001	
Ramp temperature (RampT)	−0.677	0.039	−17.541	< 0.0001	
Heron Island	−18.119	1.286	−14.088	< 0.0001	
Lord Howe Island	−8.522	1.281	−6.655	< 0.0001	
RampT×Heron Island	0.668	0.045	14.768	< 0.0001	
RampT×Lord Howe Island	0.316	0.046	6.946	< 0.0001	
Estimated Slope–Lizard Island	−0.677	0.0135			a
Estimated Slope–Heron Island	−0.509	0.0122			c
Estimated Slope–Lord Howe	−0.642	0.0130			b
Lizard–Heron	−0.168	0.0135	−12.441	< 0.0001	Lizard < Heron
Lizard–Lord Howe	−0.035	0.0130	−2.690	0.0196	Lizard < Lord Howe
Heron–Lord Howe	0.133	0.0122	10.946	< 0.0001	Heron > Lord Howe

interaction between these factors ($p < 0.0001$) indicated that the relationship between ramp temperature and O_2 production varied by population (Fig. 2a–c).

Model comparison showed that a Tweedie generalized linear mixed-effects model with a log link fit the data substantially better than a linear model ($\Delta AIC = 68.3$), capturing the nonlinear decline observed in the tropical and high-latitude populations more effectively. Slope estimates from this model revealed that thermal sensitivity differed significantly among populations ($p < 0.0001$; Table 1). Lizard Island exhibited the steepest decline in O_2 production with increasing temperature, followed by Lord Howe Island, while Heron Island showed the most thermally stable response (Fig. 2d). All pairwise differences in slopes were statistically significant (Tukey-adjusted $p < 0.05$), confirming that the rate of decline in O_2 production was significantly greater in Lizard Island and Lord Howe Island populations than in Heron Island, and also significantly greater in Lizard Island than in Lord Howe Island.

Across all ramp temperatures, individuals from Heron Island consistently produced more O_2 than those from Lizard and Lord Howe Islands. Although Heron Island O_2 production began to decline beyond 30.5 °C, this decline was not statistically significant (Welch's t -test, $p = 0.30$; Fig. 2b). In contrast, the Lizard Island and Lord Howe Island populations showed steady, significant declines across nearly the entire temperature range (Fig. 2a, c).

The phylogenetic analysis using *rbcL* gene sequences of the individuals confirmed that all our samples belong to the genus *Porolithon*; however, they formed three distinct clades representing each of the three geographical localities (Fig. 3). The pairwise divergences (0–1.9%) among individuals within the same localities were relatively low; however,

using *emtrends()* to assess how the rate of change in O_2 production differed across locations. Lowercase letters indicate significant differences between slope estimates based on Tukey-adjusted pairwise comparisons. Significance codes are indicated in the comparison column

the divergence value among the clades ranged between 3.4 and 8.0% (Lizard Island vs. Heron Island: 3.4–3.8%, Lizard Island vs. Lord Howe Island: 6.5–6.9%, and Heron Island vs. Lord Howe Island: 7.3–8.0%, Supplementary Table S2). This suggests the existence of cryptic species (or lineages) under the name *Porolithon* cf. *onkodes*. Based on our light microscopy examinations, we were unable to detect morpho-anatomical differences between individuals from the three localities.

Discussion

Ocean warming is driving latitudinal range shifts in marine populations and pushing species past their thermal tolerance limits. In the context of macrophysiology, the climate variability hypothesis proposes populations in higher latitudes will have a greater thermal tolerance breadth than those closer to the equator (Stevens 1989; Bennett et al. 2019; Clark et al. 2020). In our study, we examined if populations of the important coral reef-building alga, *Porolithon* cf. *onkodes*, vary along a latitudinal gradient off the eastern coast of Australia. Our study provides only partial support for this hypothesis, as the algal population closest to the equator (tropical, low latitude, trailing edge, Lizard Island 14.7° S) was more sensitive to thermal stress than the central population in Heron Island (23.4° S); however, the population located in the highest latitude reef (leading edge, Lord Howe Island, 31.6° S) was as sensitive to temperature stress as the tropical, low latitude (trailing edge) population from Lizard Island. The central population along the latitudinal gradient (Heron Island) exhibited the most thermally stable response, with a significantly shallower rate of decline in O_2

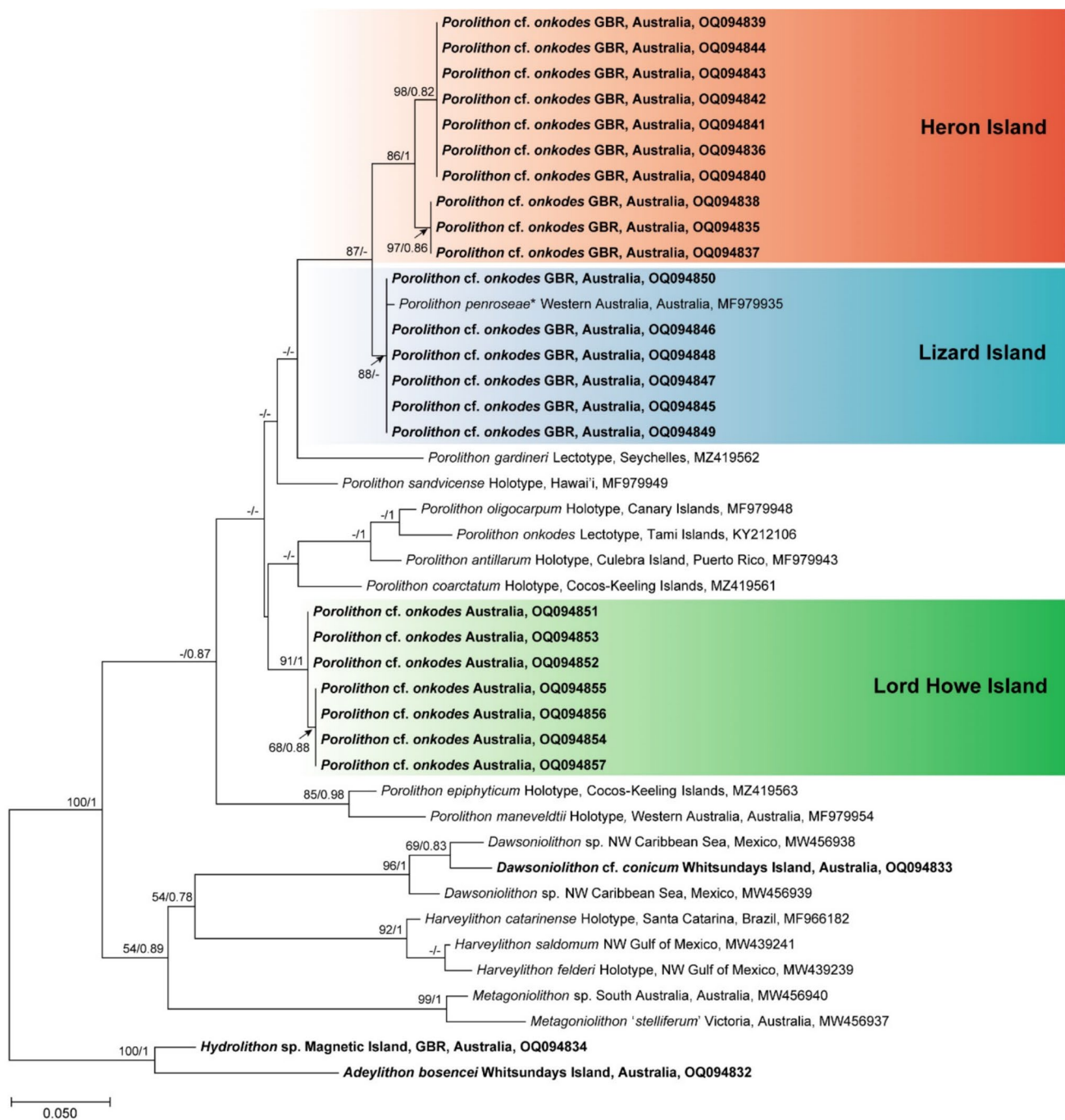


Fig. 3 Phylogenetic tree based on maximum likelihood (ML) of *rbcL* sequences. GenBank accession numbers and country/island are provided. Values above branches denote maximum likelihood bootstrap values (BP) in % > 50/Bayesian posterior probability (BPP) > 0.75,

respectively. BP values < 50% and BPP values < 0.75 are indicated by hyphens (-). New sequences are in bold. **Porolithon penroseae* was recently described and this species might have been misidentified as *Porolithon* cf. *onkodes* elsewhere in Australia (Huisman 2018)

production with increasing temperature. Our data suggest that local variability in climatic and oceanographic factors (e.g., currents) can be more important than the latitudinal gradient, supporting previous studies (Bennett et al. 2019; Clark et al. 2020). This is important for understanding the impacts of warming on marine populations as it provides evidence

of varying thermal tolerances in closely related populations and/or lineages that are morphologically identical but are on different evolutionary trajectories (i.e., cryptic species). The current study showed both tropical, low latitude and high latitude populations/lineages being more sensitive than central populations/lineages along a latitudinal gradient, the central

population potentially able to acclimate physiologically and adapt to future thermal challenges and potentially continue to contribute to reef cementation and growth.

The responses of the Lizard Island (tropical, low latitude) and Heron Island (subtropical, central) populations to thermal stress support the climate variability hypothesis because the tropical, low latitude (trailing edge) population/cryptic species is at the edge of their thermal tolerance, while the central (subtropical) population/cryptic species is more tolerant to warming. Previous studies of thermal stress on *P. onkodes* population from Lizard Island found elevated seawater temperature in combination with reduced pH lowered the thermal tolerance of *P. onkodes* to additional, acute increases in temperature (Page et al. 2021). The high sensitivity of the tropical, low latitude (trailing edge) population in our study is consistent with previous studies on coralline algae from the Northern Hemisphere (Kolzenburg et al. 2023, 2021), where thermally distinct populations of the articulated alga *Corallina officinalis* were compared and it was found that the Southern (trailing edge) population was already living closer to their thermal limits. Studies in fleshy brown macroalgae (Pearson et al. 2009; Clark et al. 2020), coral algal symbionts (Jurriaans and Hoogenboom 2019), seagrasses (Mota et al. 2018), and a number of animal species (e.g., copepods (Pereira et al. 2017), crabs (Stillman 2003)), have also found higher sensitivity in low latitude (trailing edge) populations along latitudinal gradients. High sensitivity to thermal stress in trailing edge (warm) populations/cryptic species suggests that these individuals are overall more vulnerable to ocean warming than central populations/cryptic species along the latitudinal gradient, as predicted by the climate variability hypothesis.

On the other hand, the comparison of thermal tolerances among all examined populations (low latitude, central, and high latitude) does not support our original hypothesis (or the climate variability hypothesis) that the Lord Howe Island leading edge (higher latitude) *P. cf. onkodes* population would have a wider thermal breadth to handle increases in temperature. The Lord Howe Island population showed a negative photosynthetic response to the thermal stress experiment similar to that of the tropical, low latitude population. With every increase in temperature, their O_2 production decreased. The central *P. cf. onkodes* population (Heron Island) maintained a relatively stable rate of O_2 production across the temperature ramp, with a significantly shallower slope of decline in log-transformed O_2 production, distinguishing it from both the low and high latitude populations, also showing the least sensitivity to acute warming in this population. The tropical, low latitude, and high latitude populations of *P. cf. onkodes* both consistently decreased their rate of photosynthesis with increasing temperature. There is evidence in reef corals that thermal tolerance does not always align with temperature at local environments or

latitudinal gradient, for example, two coral species from the GBR showed differences in thermal tolerance across populations; however, these differences did not directly relate to temperature of the collection environment (Jurriaans and Hoogenboom 2019). The response of the central population of *P. cf. onkodes* could be indicative of acclimation or plasticity to increasing temperatures (Stitt et al. 2014; Sandoval-Castillo et al. 2020), with our data showing the central population to be least susceptible to ocean warming. The differences in thermal tolerance and variation in plasticity to increasing temperature found across populations could be indicative of genetic variation (Via 1993; Pereira et al. 2017), and this view is supported by the *rbcL* gene divergence values between lineages of *Porolithon* which results in unique plastic responses to thermal stress.

The partial support of the climate variability hypothesis, provided by our data, suggests that species' thermal tolerance breadth is influenced not only by the variability in temperature along the latitudinal gradient, but also by local climatic factors. Bennett et al. (2015, 2019) and Clark et al. (2020) argue that although temperature variability (along a latitudinal gradient) may place a strong selection pressure on the thermal niche of marine species, local oceanographic factors (e.g., currents, upwelling, water quality, etc.) may play an even more important role than the latitudinal gradient. Clark et al. (2020) also suggest that local scale topography and local environmental conditions may be more important in driving physiology and species' distributions than larger regional effects of temperature variation among latitudes in the brown macroalga *Hormosira banksii*. In our case, for instance, the Capricorn Bunker group, where Heron Island is located, is influenced by a unique oceanographic eddy that brings cooler, nutrient-enriched oceanic subsurface water to the reef zone (Weeks et al. 2010). Further, recent marine heat waves along the Eastern Australian coast (including the GBR and Lord Howe Island) (2016, 2017, 2020 e.g. Hughes et al. (2017)) have triggered mass coral bleaching events which have varied in extent and intensity each year affecting the ecology of the different regions differently. During the 2019, summer season when the present study was conducted, Lord Howe Island experienced sustained heat stress, where sea surface temperatures exceeded monthly average values by as much as 2 °C causing a coral bleaching event in this region (Moriarty et al. 2019), in March, when the experiment was conducted, temperatures were 1 °C above monthly average. The central and northern GBR reefs, during this time frame, were not affected, and this local marine heatwave, although minor, could have influenced the sensitivity to thermal stress we documented in the Lord Howe Island population (Marzonie et al. 2023). The influence of these type of local climatic and oceanographic phenomena most likely contribute to the variability in CCA thermal stress responses and may overwhelm the influence of thermal variability along latitudinal gradients.

Another contributing factor to differences found in the response to thermal stress across these populations, besides local oceanographic and latitudinal influences, is cryptic speciation. Genetic variation in CCA assemblages and populations is widespread (De Jode et al. 2019; Zhan et al. 2022), and can be driven by depth, geographic distance, sedimentation, and substrate type among others (De Jode et al. 2019; Zhan et al. 2022; Martin et al. 2024). From phylogenetic analysis of the samples used within our study, we found genetic divergence values between the populations that suggest existence of cryptic species (or lineages) in *Porolithon* cf. *onkodes*. The taxonomy of the genus *Porolithon*, and more broadly the CCA, is complex and unresolved (Gabrielson et al. 2018; Pezzolesi et al. 2019; Twist et al. 2019; Jeong et al. 2023). Cryptic species of CCA (De Jode et al. 2019; Martin et al. 2024) and other red macroalgae (Muangmai et al. 2015) have been found to have physiological differences, similarly to the individuals sampled during the present study. For example, Martin et al. (2024) found differences in photosynthetic performance among cryptic species of *Lithophyllum stictiforme* growing at same depths, however, did not address latitudinal variation. In our study, the distinct slopes of photosynthetic response to warming between populations suggest that underlying genetic differences may be influencing their physiological plasticity and thermal sensitivity. The findings from the current study suggest that one hypothesis (e.g., climate variability hypothesis) does not necessarily explain the differences in response of populations of *P. cf. onkodes*, but instead we should consider not only local oceanographic influences and latitudinal effects, but also the contribution of cryptic speciation.

Extreme climatic events are becoming more frequent and severe, including the occurrence of both prolonged and sudden acute temperature events. The latter of these, the sudden acute temperature events, are only recently being found to be pivotal in shaping marine ecosystems (Smale et al. 2019). Here, we provide evidence suggesting that acute temperature events can impact the physiological process of photosynthesis across different populations (or lineages or cryptic species) of the crucial, reef building species of CCA, *P. cf. onkodes*. If the process of photosynthesis is impaired in the critically important calcifying alga *P. cf. onkodes* (e.g. Dechnik et al. (2017)), this could directly affect their reef building function by negatively impacting their growth, calcification, and therefore the structure of reefs. The Heron Island lineage of *P. cf. onkodes*, located in the center of the population distribution along the eastern Australian coast, exhibited the shallowest slope of decline in O_2 production with increasing temperatures, suggesting a greater capacity to withstand acute thermal stress. The Heron Island lineage therefore is likely, (i) living furthest from its thermal limit, and (ii) will be tolerant to sudden, acute increases in temperature. It is worth considering, however, that the effects

of ocean warming will not act independently of those from ocean acidification (elevated pCO_2 /declined seawater pH) and it is likely that the effects of increased seawater temperature on CCA physiology will be exacerbated by ocean acidification influences (Martin and Gattuso 2009; Diaz-Pulido et al. 2012; Cornwall et al. 2019; Page et al. 2021). The tropical, low latitude (Lizard Island), and high latitude reef, Lord Howe Island, populations/lineages will be largely impacted by sudden, acute increases in temperature, suggesting these populations are already living close to or at their thermal limits and their potential for adaptation to future climates are uncertain. Impacts on algal metabolism could lead to changes in species composition along the latitudinal/thermal gradient as the ocean continues to warm and acidify. In light of the findings from this study, conservation and management strategies must consider the differential responses of hidden lineages in crustose coralline algae to thermal stress to best protect species, particularly those that play fundamental roles in coral reef building and resilience.

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Author contributions Conceptualization was contributed by Tessa M Page and Guillermo Diaz-Pulido; Methodology was involved by Tessa M Page; Formal analysis, investigation, writing—original draft preparation, and writing—review and editing were performed by Tessa M Page, So Young Jeong, and Guillermo Diaz-Pulido; Funding acquisition and supervision were done Guillermo Diaz-Pulido.

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Data availability Data, sequence alignments, and code are available from Figshare <https://doi.org/10.6084/m9.figshare.c.6351587> [53].

Declarations

Conflict of interest The authors declare no financial or non-financial competing or conflicting interests to declare that are relevant to the content of this article.

Ethical approval Crustose coralline algae fragments were collected under Great Barrier Reef Marine Park Authority permit num-

ber G18/41291.1 and under Department of Primary Industries Marine Parks permit LHIMP/R/18011/12092018.

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