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ORIGINAL PAPER

Phenolic concentrations of brown seaweeds and relationships to nearshore environmental gradients in Western Australia

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Abstract Phenolic compounds are found in all brown macroalgae and function as cell wall structure, UV protection and as herbivore deterrents. The concentrations of phenolic compounds vary among taxa and between temperate and tropical ecosystems. Australasia has high concentrations of soluble phenolics compared to other regions. Presently, relationships between phenolic concentrations and environmental gradients are unclear. The purpose of this study was to determine the soluble phenolic concentrations of brown seaweeds along temperate and tropical ecosystems of the Western Australia coastline. We tested the hypothesis that phenolic concentrations are related to local and broad-scale abiotic environmental gradients. Strong environmental gradients of coastal Western Australia provided the opportunity to characterize phenolic compounds across one large gradient. Phenolic concentrations of brown seaweeds at seven study locations varied across latitude with higher concentrations found at higher latitudes and were comparable to seaweeds from similar latitudes in Australia. This trend coincided with a negative relationship between photosynthetically active radiation and phenolic compounds, and a positive relationship with salinity. We also found phenolic concentrations were positively related to salinity in tropical Shark Bay but this was dependent on species. Environmental conditions are important in regulating concentrations of phenolic compounds. Multiple factors influence the concentrations of macroalgal phenolic compounds creating unique distributions among geographical regions. This study highlighted the importance of considering multiple factors when studying phenolic ecology and suggests photosynthetically active radiation and salinity as important drivers of phenolic compound distribution in Western Australia.

Keywords Latitudinal gradient · Macroalgae · Photosynthetically active radiation · Polyphenolics · Salinity

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Introduction

Phenolic compounds are chemically diverse primary and secondary metabolites found in terrestrial and aquatic primary producers that provide cellular and ecological functions through their oxidative capacity (Appel 1993; Appel et al. 2001). In marine macroalgae, phenolic compounds are most common in brown seaweeds, where they are composed of polymers of phloroglucinol, and often are referred to as phlorotannins. Globally, phenolic concentrations of brown seaweeds range from negligible to 20% of tissue dry mass (Ragan and Glombitza 1986) with higher concentrations generally found at higher latitudes (Steinberg and Paul 1990; Steinberg et al. 1991). This pattern stands in contrast to other chemical compounds used in marine allelopathic interactions that are generally more abundant in tropical environments (Hay 1996). While globally structured by

latitude, phenolic compounds can occur regionally in high concentrations in low latitude areas such as the Caribbean (Targett et al. 1992). Studies of phenolic compounds on regional scales provide conflicting relationships to latitude and associated environmental conditions (Steinberg 1989; Van Alstyne et al. 1999; Le Lann et al. 2012; Tanniou et al. 2014). Phenolic concentrations differ across spatial scales ranging from a few meters to 1000's of kilometers (Steinberg 1989; Pavia and Åberg 1996; Van Alstyne et al. 1999; Le Lann et al. 2012) as well as among closely (Van Alstyne et al. 1999; Connan et al. 2004) and distantly related taxa (Steinberg 1989).

Although ubiquitous in brown seaweeds, the distribution of, and factors that relate to, phenolic compounds are not well understood at regional and global scales. Phenolic compounds primarily strengthen cell walls (Schoenwaelder and Clayton 1999) and function in wound healing and repair (Lüder and Clayton 2004). They are also known to protect against abiotic stressors like ultraviolet radiation (Swanson and Druehl 2002; Jormalainen and Honkanen 2004) and salinity by acting as oxidant scavenging molecules (Pedersen 1984; Ragan and Glombitza 1986). In addition to cellular functions, phenolic compounds act as allelopathic compounds and defend seaweeds against some herbivores (Amsler and Fairhead 2006) as well as fungal and bacterial infections (Plouguerné et al. 2012; Tanniou et al. 2014). Phenolic concentrations in brown seaweeds are generally higher in Australasia than in the northern hemisphere (Steinberg 1989; Van Altena and Steinberg 1992; Van Alstyne et al. 1999; Yates and Peckol 1993). This difference is possibly a result of differences in herbivory pressures between the two regions (Estes and Steinberg 1988; Steinberg et al. 1995). Because of this, we examined seaweeds of Western Australia to see if they follow the typical trend of higher concentrations at higher latitudes and higher concentrations than the northern hemisphere.

Western Australia is a global hotspot for temperate marine macroalgal diversity (Wernberg et al. 2011; Bennett et al. 2016) but the distribution of phenolic compounds in this region is currently unknown. The Leeuwin Current is the dominant ocean current along nearshore Western Australia. It originates in the Indonesian through-flow and continues around the southern coast of Australia across the Great Australian Bight (Wernberg et al. 2013) carrying warm tropical waters south to higher latitudes. This creates strong abiotic gradients against which many ecological gradients have been studied (McGowran et al. 1997; Kendrick et al. 2009; Wernberg et al. 2013). Along these gradients, herbivore activity is similar to global patterns (Vermeij 1978; Gaines and Lubchenco 1982), with higher grazing rates at lower latitudes (Vanderklift and Kendrick 2004; Poore et al. 2012). Additionally, localized abiotic gradients exist. For example, a salinity gradient in tropical Shark Bay (Fig. 1) ranges from marine to salinities higher than 60 psu at the southern terminus (Walker 1985). Additionally, tidal exchanges within tropical Cygnet Bay can be as large as 10 m resulting in an extremely variable intertidal environment. Local gradients such as these, along with the geographic scope of Western Australia, represent a unique opportunity to advance the understanding of marine phenolic compound distribution on continental scales.

In this study, we quantified phenolic compounds of dominant brown seaweeds in Western Australia and investigated potential relationships of phenolic concentrations to environmental conditions among taxa across broad and fine spatial scales throughout coastal Western Australia. We specifically aimed to answer the following questions: (1) What variability of soluble phenolic concentrations exists among macroalgal taxa at different spatial scales? (2) How do phenolic concentrations of brown seaweeds from nearshore Western Australia compare to other regions? (3) Do phenolic concentrations of seaweeds correlate with local environmental gradients? These questions were investigated by sampling brown seaweeds from seven geographically distinct nearshore study locations of Western Australia and comparing those findings to environmental conditions gathered both in situ and from satellite imagery.

Methods

Study locations

Brown marine seaweeds (Class Phaeophyceae) were collected from high intertidal to 12-m depth at seven nearshore study locations in Western Australia (Fig. 1) from March to May 2015. One to seven sites were sampled (Table 1) within each location to cover known within-location gradients. The study locations represent temperate (three locations) and tropical (four locations) systems in Western Australia and span 17 degrees of latitude (Table 1). Thirty-one species of seaweed were haphazardly collected during this study. Species continuity of collections was maintained when possible, but this was infrequent and mostly different algal species were collected at study locations separated by 150–1000 km (Fig. 1). We collected *Hormophysa cuneiformis* at three locations, *Padina* sp. at three locations, and *Sargassum ligulatum* and *Sirophysalis trinodis* at five locations (Table 2). Seaweeds collected during this study represented the dominant habitat-forming brown taxa present at each study location. The taxonomic families collected were: Dictyotaceae, Lessoniaceae, Sargassaceae, and Sporochnaceae. Both Dictyotaceae and Sargassaceae were distributed throughout sampling locations, comprising 94% of species collections, whereas Lessoniaceae and

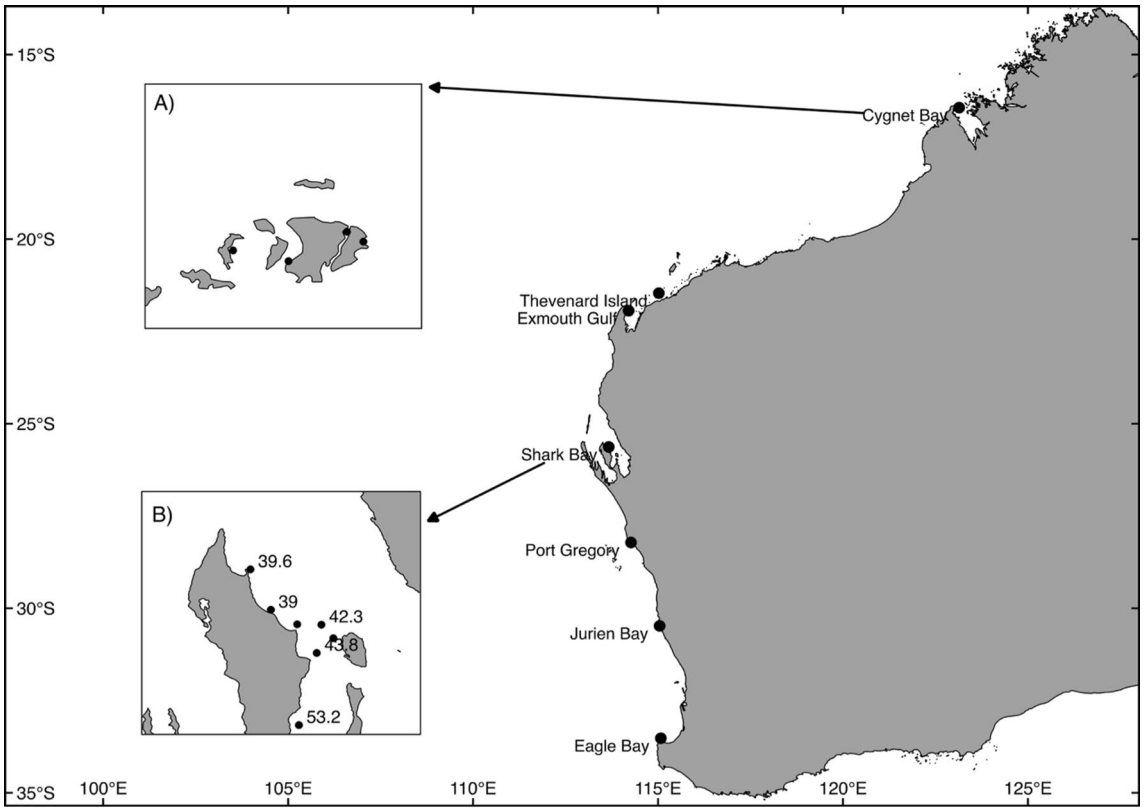

Fig. 1 Map of study locations along coastal Western Australia. The *inset* map of Cygnet Bay **a** shows the locations of sample sites around Sunday Island. *Inset map* of Shark Bay **b** shows sampling sites along with the associated salinity of each site

Table 1 Physical characteristics of seven study locations in Western Australia and the macroalgal sampling summary of each location

Location	Longitude (E)	Latitude (S)	Region	Exposure	Substrate/habitat	Envir. gradients sampled	Depth (m)	No. of sites	No. species collected
Eagle Bay (EB)	115.1	−33.56	Temperate	North-facing, shallow	Sandy reef flat	None	2	1	6
Jurien Bay (JB)	115.09	−30.49	Temperate	West-facing, exposed	Complex coral/limestone	Depth	6.5–10	3	8
Port Gregory (PG)	114.22	−28.18	Temperate	Lagoon and exposed reef	Sandstone boulders	Depth	1.5–11	3	6
Shark Bay (SB)	113.22	−25.62	Tropical	North-facing; shallow bay	Flat sand, seagrass	Salinity	1-2.5	7	5
Exmouth Gulf (EG)	114.2	−21.94	Tropical	North-facing	Flat sand/reef	None	1.5	1	6
Thevenard Island (TI)	115	−21.46	Tropical	Offshore Island; east side	Flat sand with coral heads	None	2	1	8
Cygnet Bay (CB)	122.88	−16.56	Tropical	East-facing; tidal	Intertidal reef flat	None	Intertidal	3	7

Seaweeds at each location were collected at different numbers of sites. Species collected indicates the number of species collected at each location. Sampling sites crossed known environmental gradients

Table 2 Phenolic concentrations (% DM, means +/− SE) of brown seaweeds collected at seven study locations along the coast of Western Australia

Order	Family	Species	Location	*N*	Total phenolics (%DM)
Fucales	Dictyotaceae	*Dictyopteris muelleri*	JB	5	0.48 ± 0.03
		Dictyota australis	TI	5	1.63 ± 0.34
		Dictyota ciliolata	PG	3	0.95 ± 0.45
		Dictyota ceylanica	TI	4	0.35 ± 0.03
			EG	5	0.35 ± 0.03
		Dictyota naevosa	PG	3	1.06 ± 0.43
		Dictyota sp.	PG	2	0.43 ± 0.03
		Dilophus sp.	JB	3	1.11 ± 0.14
		Distromium sp.	PG	12	2.24 ± 0.38
			JB	4	4.40 ± 0.72
		Lobophora variegata	EG	10	0.31 ± 0.01
			EB	5	8.73 ± 1.05
		Padina sp.	CB	6	2.18 ± 0.17
			TI	4	1.73 ± 0.03
			EG	4	1.95 ± 0.11
		Spatoglossum macrodontum	JB	3	1.81 ± 0.35
		Zonaria turneriana	JB	5	4.78 ± 0.39
	Sargassaceae	*Cystophora #1*	EB	4	9.36 ± 0.68
		Cystophora grevillei	EB	3	10.99 ± 2.63
		Hormophysa cuneiformis	CB	12	2.59 ± 0.19
			EG	5	0.72 ± 0.10
			SB	24	0.76 ± 0.12
		Myriodesma serrulata	JB	5	1.17 ± 0.15
		Sargassopsis decurrens	TI	6	0.51 ± 0.07
			SB	20	0.99 ± 0.12
		Sargassum ligulatum	CB	8	1.13 ± 0.14
			TI	5	0.25 ± 0.03
			EG	6	0.33 ± 0.04
		Sargassum linearifolium	EB	6	4.16 ± 0.75
		Sargassum marginatum	TI	6	0.70 ± 0.11
		Sargassum olygocystin	PG	5	0.28 ± 0.11
		Sargassum paradoxum	PG	19	1.28 ± 0.10
			JB	5	2.94 ± 0.27
		Sargassum polycystum	CB	8	1.42 ± 0.14
		Sargassum polyphyllum	EG	5	0.63 ± 0.08
		Sargassum rasta	CB	6	0.81 ± 0.05
		Sargassum unknown #4	TI	5	0.31 ± 0.07
		Scaberia agardhii	EB	5	4.73 ± 0.65
		Sirophysalis trinodis	CB	6	1.14 ± 0.15
			TI	3	0.87 ± 0.19
			EG	5	0.55 ± 0.12
			SB	13	1.05 ± 0.34
			EB	3	3.56 ± 0.92
		Turbinaria gracilis	CB	15	2.96 ± 0.28
Laminariales	Lessionaceae	*Ecklonia radiata*	JB	13	3.37 ± 0.28
Sporochnales	Sporochnaceae	*Sporochnus moorei*	SB	4	1.15 ± 0.32

N is the number of tissue samples analyzed. Study locations are abbreviated as follows: *CB* Cygnet Bay, *JB* Jurien Bay, *PG* Port Gregory, *SB* Shark Bay, *EG* Exmouth Gulf, *TI* Thevenard Island, *CB* Cygnet Bay

Sporochnaceae were represented by one species and location each.

Seaweeds were collected across local environmental gradients of depth and salinity (Table 1; Fig. 1). In tropical Shark Bay, three species of macroalgae; *Hormophysa cuneiformis, Sargassopsis decurrens* and *S. trinodis* were collected at seven sites encompassing salinities from 39.0 to 53.5 (Walker 1985) (Fig. 1; Table 1). *Sargassum paradoxum* and the subtidal kelp *Ecklonia radiata* were collected at three sites within temperate Jurien Bay at depths ranging from 5.0 to 10.0 m. *Sargassum paradoxum* was also collected at three sites within temperate Port Gregory ranging from 1.5 to 11.0 m depth (Table 1). At the intertidal study location of tropical Cygnet Bay, the tidal range is up to 10 m creating a large and dynamic intertidal zone. *Hormophysa cuneiformis* and *Turbinaria gracilis* were collected at four high intertidal sites within Cygnet Bay (Table 1).

Environmental parameters

Abiotic environmental conditions for each of the seven study locations across nearshore Western Australia were gathered from MODIS Aqua at 4 km resolution from seasonal composites averaged over the sampling period (March–May 2015) (Table 3). Using QGIS we extracted data for diffuse light attenuation (at 490 nm using the KD2 algorithm), daytime sea surface temperature (SST), photosynthetically available radiation (PAR) and chlorophyll a concentration. Mean salinity values for each study site were taken from the CSIRO Atlas of Regional Seas (CARS) as a 5-year average from 2003 to 2008 (Ridgway 2009).

Tissue collection and chemical analysis

Meristem tissue samples of healthy-looking brown seaweeds individuals were haphazardly collected at each study location ($n = 3$–24 different plants per species) and kept cool in an icebox in the field. Seaweeds were identified to the lowest taxonomic level (Womersley 1987; Huisman 2000, 2015). A representative of each species was lodged at the University of Western Australia Herbarium. Seaweeds were cleaned of epiphytes and debris and frozen at −20 °C while in the field. Frozen seaweeds were transported to the University of Western Australia no later than 1 week after collection where, upon arrival, samples were stored at −80 °C. Tissue samples were later freeze-dried prior to chemical analysis.

We analyzed soluble phenolic compounds using a modified Folin–Ciocalteu method from Van Alstyne (1995). This assay measures the redox activity of a methanolic extract by reacting with the available hydroxyl groups accessible to the reagents as well as non-polar hydroxylated aromatic compounds that comprise <5% of all Folin–Ciocalteu compounds (Van Alstyne 1995). Freeze-dried seaweed samples were ground to a fine powder using a mixer/mill. Ten milligrams of ground tissue from each individual sample were extracted in 1.0 ml 80% methanol for 24 h in the dark. Extracts were either analyzed the following day or stored at −80 °C until analysis. Forty microliters of Folin–Ciocalteu reagent were added to 100 µl of extract (diluted 1:20 with Type-1 reagent grade water). The solution was then made alkaline by the addition of 100 µl Na_2CO_3 after 5 min. Absorbance was read at 765 nm using a FLUOStar microplate reader after a 30-min incubation at 50 °C. Three replicate extracts per sample were analyzed with phloroglucinol (Sigma 79330_FLUKA) as a standard (Van Alstyne 1995) and converted to phenolic concentrations as a percent of dry mass.

Statistical analysis

All statistical analyses were run using R Studio (R Core Team 2013). Average phenolic concentration of the macroalgae at each study location was calculated as the mean of all species collected at each location. Phenolic concentration data were fourth-root transformed to pass the assumptions of normality and homogeneity of variance with a Cochran's Test prior to analysis. To test for differences in phenolic concentrations of the study location

Table 3 Values of environmental variables extracted from MODIS satellite data for the seven nearshore study locations of Western Australia

Factor	Eagle Bay	Jurien Bay	Port Gregory	Shark Bay	Exmouth Gulf	Thevenard Island	Cygnet Bay
Chlorophyll a (mg m^{-3})	0.43	1.69	1.15	3.29	3.06	4.25	1.11
Diffuse attenuation (490 nm m^{-1})	0.06	0.15	0.11	0.24	0.22	0.29	0.11
Photosynthetically active radiation (Einstein m^{-2} day^{-1})	26.75	29.65	30.98	32.01	35.78	34.41	43.48
Salinity	35.76	35.66	35.66	39–53.5	35.05	35.15	35.07
Sea surface temperature (°C)	26.30	22.70	22.00	25.40	26.30	26.60	28.51

Values represent seasonal composites for the austral fall of 2015. Chlorophyll a values are reported in mg m^{-3}, diffuse attenuation at 490 nm m^{-1}, photosynthetically active radiation in Einstein m^{-2} day^{-1}, salinity, sea surface temperature in °C.

Table 4 Results of ANOVAs comparing phenolic concentrations of study locations and sample sites to the mean phenolic concentration of (a) the study location and of (b) the mean concentrations of each family

Taxa	Factor	DF	MS	*F*	*P*
(a)					
Assemblage	Location	6	0.29	16.77	<0.001
	Location (Site)	16	0.28	0.62	0.85
	Error	45	0.13		
(b)					
Family	Family	3	0.02	2.86	0.04
	Location	6	0.27	16.63	<0.001
	Location (site)	16	0.03	0.68	0.79
	Error	42	0.12		

Tests were performed on fourth-root transformed data. All seven study locations were used in each statistical test

averages, we used a two-way nested analysis of variance (ANOVA) with collection site nested within study location followed by Tukey's HSD post hoc tests (Table 4). Differences in phenolic concentrations among algal families and locations were tested with a two-way ANOVA with species nested within location and family (Table 4). Differences in phenolic concentrations for individual species collected at multiple locations were tested with one-way ANOVAs with location as the fixed factor (Table 5).

We investigated relationships between environmental conditions and phenolic concentrations with a multiple linear regression analysis. We considered Cygnet Bay, the only high intertidal lagoon study location, ecologically different from the other six study locations for the purpose of this analysis and excluded it from the regression models. We assessed the importance of predictor environmental conditions on phenolic concentration with a multiple regression analysis that included chlorophyll a, diffuse attenuation, PAR, salinity and SST. The contribution of each factor to the regression model was reported with partial eta-squared (η^2) values (Table 6). We removed chlorophyll a from the model because of collinearity with attenuation (cutoff r^2 value = 0.95). We also assessed the relationship between the multiple regression model and individual species distributed across multiple study locations. Phenolic concentrations of *H. cuneiformis, S. decurrens*, and *S. trinodis* were compared to salinity in tropical Shark Bay. Phenolic concentrations of *E. radiata* and *S. paradoxum* in temperate Jurien Bay and *S. paradoxum* were compared to depth.

Table 5 ANOVA results comparing the phenolic concentrations of (a) brown seaweed species across multiple study locations and (b) species between multiple sites within a given study location in Western Australia

Species	Factor	DF	MS	*F*	*P*	Location
(a)						
Hormophysa cuneiformis	Location	2	0.56	33.7	<0.001	SB, EG, CB
	Error	38	0.02			
Sargassum ligulatum	Location	2	0.19	30.75	<0.001	EG, TI, CB
	Error	16	0.01			
Sirophysalis trinodis	Location	4	0.14	4.44	0.008	EB, SB, EG, TI, CB
	Error	25	0.03			
(b)						
Ecklonia radiata	Site	2	0.001	0.01	0.98	JB
	Error	10	0.01			
Sargassum paradoxum	Site	3	0.09	9.99	<0.001	PG
	Error	20	0.01			
Hormophysa cuneiformis	Site	4	0.06	4.1	0.01	SB
	Error	19	0.02			
Sargassopsis decurrens	Site	2	0.03	2.65	0.11	SB
	Error	12	0.01			
Sirophysalis trinoids	Site	2	0.22	12.96	0.002	SB
	Error	10	0.02			
Hormophysa cuneiformis	Site	1	0.03	6.99	0.02	CB
	Error	10	0.005			
Turbinaria gracilis	Site	2	0.049	4.97	0.03	CB
	Error	12	0.001			

Tests were performed on fourth-root transformed data. The study locations used in each variance test are noted under "Location"

Table 6 Results from multiple regression analyses comparing the relationships of abiotic conditions to brown seaweed phenolic concentrations of (a) study locations and (b) individual families and species. Regressions comparing phenolic concentrations of individual species across local environmental gradients within sites are shown in (c)

Taxa	Regression	Factor	*P*	r^2	Partial η^2	Relationship	Location(s)
(a)							
All species	Single	Latitude	< 0.001	0.49			EB, JB, PG, SB, EG, TI
All species	Multiple		< 0.001	0.57			EB, JB, PG, SB, EG, TI
		Attenuation			0.003	Negative	
		PAR			0.18	Negative	
		Salinity			0.15	Positive	
		SST			0.0001	Negative	
(b)							
Dictyotaceae	Multiple		< 0.001	0.49			EB, JB, PG, SB, EG, TI
		Attenuation			0.036	Negative	
		PAR			0.109	Negative	
		Salinity			0.14	Positive	
		SST			0.05	Negative	
Sargassaceae	Multiple		< 0.001	0.65			EB, JB, PG, SB, EG, TI
		Attenuation			0.03	Negative	
		PAR			0.27	Negative	
		Salinity			0.21	Positive	
		SST			0.009	Negative	
Hormophysa cuneiformis	Multiple		< 0.001	0.64			CB, EG, SB
		Attenuation			-1.33^{-14}	Negative	
		PAR			0	–	
		Salinity			0	–	
		SST			$-5.26\ e^{-16}$	Positive	
Sargassum ligulatum	Multiple		< 0.001	0.79			CB, EG, TI
		Attenuation			$-4.6\ e^{-15}$	Negative	
		PAR			0	–	
		Salinity			0	–	
		SST			$-4.18\ e^{-15}$	Positive	
Sirophysalis trinodis	Multiple		0.007	0.42			CB, EB, EG, SB, TI
		Attenuation			0.067	Negative	
		PAR			0.093	Negative	
		Salinity			0.095	Positive	
		SST			0.1	Negative	
(c)							
Hormophysa cuneiformis	Single	Salinity	0.18	0.08		Pos	SB
Sargassopsis decurrens	Single	Salinity	0.03	0.3		Pos	SB
Sirophysalis trinodis	Single	Salinity	0.02	0.29		Pos	SB
Ecklonia radiata	Single	Depth	0.88	0.02		–	JB
Sargassum paradoxum	Single	Depth	0.08	0.13		Neg	JB
Sargassum paradoxum	Single	Depth	0.23	0.16		Neg	PG

The multiple regression model compared phenolic concentration to diffuse attenuation, PAR, salinity and sea surface temperature. Each regression used either all or a subset of the study locations as indicated

Results

Phenolic concentrations of seaweeds in this study ranged from $0.25 \pm 0.03\%$ to $10.99 \pm 2.63\%$ of dry weight (Table 2). Of the 46 species collected, 32 were low phenolic species (<2% DM, Steinberg 1989) and 14 were high phenolic species (>2% DM, Steinberg 1989). The majority of high phenolic species were found in the temperate locations. Only three species with phenolic concentrations higher than 2% were present in tropical study locations;

Hormophysa cuneiformis, Padina sp. and *Turbinaria gracilis* collected in Cygnet Bay (Table 2).

Average phenolic concentrations of brown seaweeds at each study location were significantly different among locations (ANOVA, $F(6,45)=16.77$, $P<0.001$,) (Table 4). Lower phenolic concentrations were generally found in tropical latitudes north of Shark Bay. The highest phenolic concentration of a study location, found at the highest latitude, was 11 times greater than the lowest average location concentration found at tropical Thevenard Island (Fig. 2a) and was higher than all other study locations (Tukey's HSD, $P<0.001$). Phenolic concentrations in tropical Cygnet Bay and temperate Jurien Bay were higher than those found at study locations between Port Gregory and Thevenard (Tukey's HSD, $P<0.01$).

Phenolic concentrations were significantly different among macroalgal families (ANOVA, $F(3,42)=2.86$, $P=0.04$) (Fig. 3; Table 4). Mean phenolic concentrations of the Lessoniaceae were significantly higher than the Sargassaceae and Dictyotaceae (Tukey's HSD, $P=0.01$) (Fig. 3). Mean phenolic concentrations of macroalgal families were also significantly different among study locations (ANOVA, $F(3,298)=6.89$, $P<0.001$) (Table 4). The phenolic concentration of the Dictyotaceae was up to eight times higher in temperate Eagle Bay than tropical Exmouth Gulf while the concentration of the Sargassaceae was 12 times higher in Eagle Bay than Exmouth Gulf (Fig. 4).

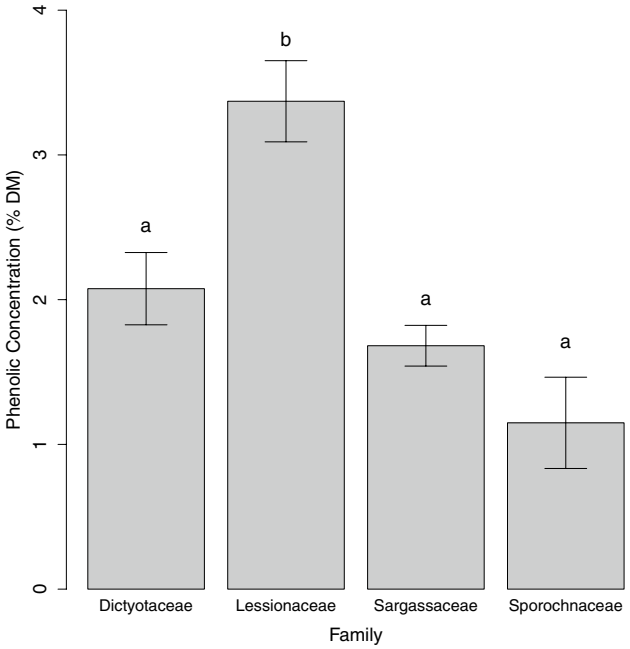

Fig. 3 Phenolic concentrations (as % dry mass) of brown seaweed families collected at seven study locations across nearshore Western Australia. *Each bar* represents the mean phenolic concentration averaged over the seven locations. Values are means ± SE. *Letters* indicate significant differences between families (from Tukey's HSD)

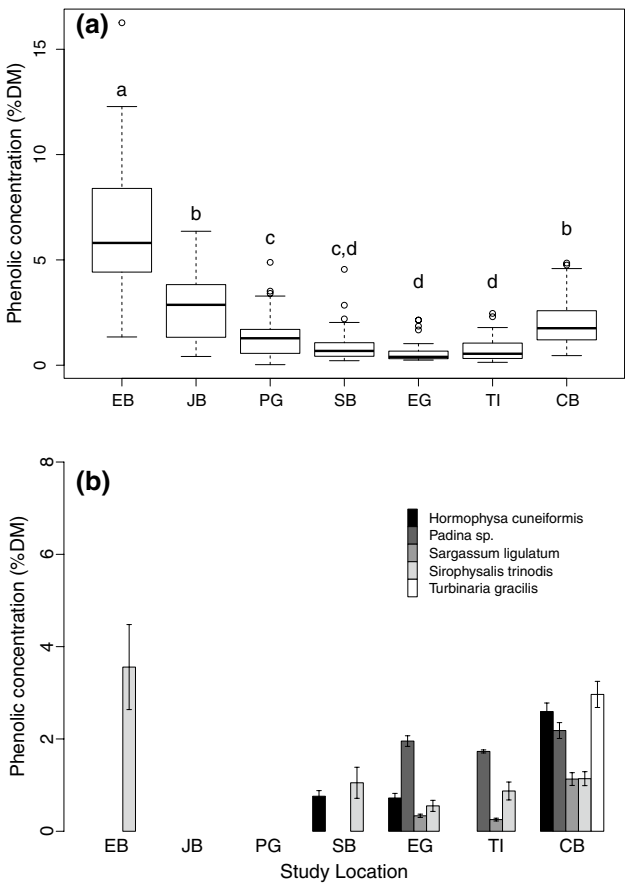

Fig. 2 Mean phenolic concentration (as % dry mass) of **a** the dominant brown seaweeds (as a composite of all species collected) and **b** individual species found across study locations along nearshore Western Australia. Values shown are means ± SE. Collection locations are ordered by high to low latitude from *left* to *right*. *Letters* indicate significant differences between study locations (from Tukey's HSD)

Phenolic concentration of individual species varied among study locations. In general, the highest phenolic concentrations of species were found either in tropical Cygnet Bay or temperate Eagle Bay (Fig. 2b). Phenolic concentrations of *Sirophysalis trinodis* collected at five locations (Table 2), varied up to 6.5 times between locations (ANOVA, $F(4,25)=4.44$, $P=0.008$) (Table 5) with the highest concentrations found at the highest latitude location. The phenolic concentration of the tropical *Sargassum ligulatum* was 3.4 times higher in tropical Cygnet Bay than the tropical locations Exmouth Gulf or Thevenard Island (ANOVA, $F(2,16)=30.75$, $P<0.001$). The phenolic concentration of *Lobophora variegata* was 28 times higher in temperate Eagle Bay than in tropical Exmouth Gulf (ANOVA, $F(1,13)=690.1$, $P<0.001$). The highest phenolic concentration of *H. cuneiformis*, also found in Cygnet Bay (ANOVA, $F(2,38)=33.7$, $P<0.001$) was 3.5 times higher than Eagle Bay or tropical Shark Bay.

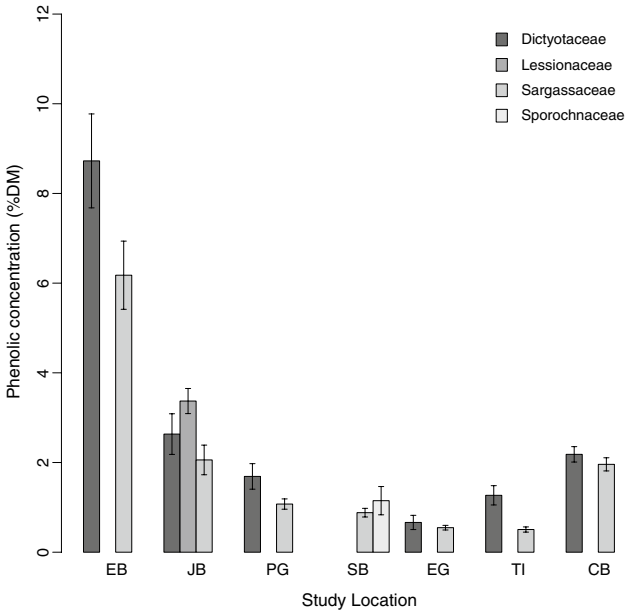

Fig. 4 Phenolic concentrations (as % dry mass) of macroalgal families collected at seven nearshore study locations along Western Australia. Study locations are organized highest to lowest latitude, *left* to *right*. Values shown are means ± SE

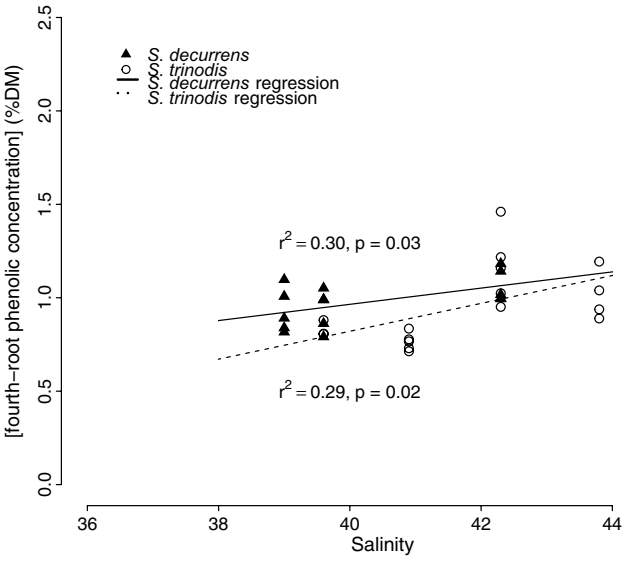

Fig. 5 Linear regressions comparing fourth-root transformed phenolic concentrations of *Sargassopsis decurrens* (*triangles*) and *Sirophysalis trinodis* (*circles*) to salinity in Shark Bay, Western Australia. *Regression lines* indicate the relationship between salinity and phenolics for *S. decurrens* (*solid*) and *S. trinodis* (*dashed*)

Additionally, phenolic concentrations of some macroalgal species varied significantly among sample sites within study locations. Phenolic concentrations of *H. cuneiformis* (ANOVA, $F(4,19)=4.1$, $P=0.01$) and *S. trinodis* (ANOVA, $F(2,10)=12.96$, $P=0.002$) (Table 5) varied up to six times among sample sites within tropical Shark Bay while phenolic concentrations of *S. decurrens* were similar among sites (Table 2). Phenolic concentrations of *H. cuneiformis* (ANOVA, $F(1,10)=6.99$, $P=0.02$) and *T. gracilis* (ANOVA, $F(2,12)=4.97$, $P=0.03$) in tropical Cygnet Bay varied by 1.4 times and 1.8 times between sample sites, respectively (Table 2). Phenolic concentrations of *E. radiata* and *S. paradoxum* did not differ significantly among collection sites in temperate Jurien Bay.

Environmental conditions during the sampling period varied across the latitudinal gradient (Table 3). Photosynthetically active radiation generally decreased with latitude and ranged from 26.8 Einstein m^2 day^{-1} in Eagle Bay to 43.5 Einstein m^2 day^{-1} in tropical Cygnet Bay. Average sea surface temperatures were highest at low latitudes and ranged from 19.9 °C in Eagle Bay to 28.5 °C in Cygnet Bay. There was no clear latitudinal pattern in diffuse attenuation coefficients, which ranged from 0.06 m^{-1} in temperate Eagle Bay to 0.29 m^{-1} at tropical Thevenard Island or in salinity, which ranged from 35.1 in tropical Exmouth Gulf to 49.5 (mean value) in Shark Bay (Table 3). The salinity range among sample sites in Shark Bay, however, was 35.0–53.2.

The multiple regression model found relationships between phenolic concentration averages of study locations (excluding Cygnet Bay) as well as individual species phenolic concentrations. A strong relationship was found between average phenolic concentrations and environmental variation across study locations (multiple regression; $r^2=0.57$, $P<0.001$) (Table 6a). Mean phenolic concentrations of macroalgae across all study locations were positively correlated to salinity ($\eta^2 = 0.15$) and negatively correlated to PAR ($\eta^2 = 0.18$). Similarly, phenolic concentrations of the Dictyotaceae and Sargassaceae were positively correlated to salinity ($\eta^2 = 0.14$ and $\eta^2 = 0.21$, respectively) and negatively with PAR ($\eta^2 = 0.11$ and $\eta^2 = 0.27$, respectively) (Table 6b). While phenolic concentrations of *S. trinodis* were negatively related to PAR ($\eta^2 = 0.09$) and SST ($\eta^2 = 0.10$) (Table 6), phenolic concentrations of *H. cuneiformis* and *Sargassum ligulatum* were negatively related to diffuse attenuation ($\eta^2 < 0.001$ and $\eta^2 < 0.001$, respectively) and positively related to SST ($\eta^2 < 0.001$ and $\eta^2 < 0.001$, respectively) (Table 6c).

There were also strong relationships between phenolic concentrations of individual species and local abiotic gradients at individual study locations. Phenolic concentrations of *S. decurrens* ($r^2=0.30$, $F(1,13)=5.61$, $P=0.03$) and *S. trinodis* ($r^2=0.29$, $F(1,15)=6.22$, $P=0.02$) in tropical Shark Bay were positively related to salinity (Fig. 5) but not in *H. cuneiformis* (Table 6c). Phenolic concentration differences among sites were not related to depth in temperate Jurien Bay or Port Gregory. We found no relationship

between phenolic concentration and depth in *E. radiata* in Jurien Bay or *S. paradoxum* in Port Gregory (1.5–11.5 m depths) and Jurien Bay (3–10 m depths) (Table 6).

Discussion

In this study, we investigated latitudinal and environmental relationships to soluble phenolic concentrations in a range of brown seaweeds along the coast of Western Australia spanning 17 degrees of latitude. Macroalgal phenolic concentrations in Western Australia were variable across multiple spatial scales and among taxa (Table 2). In this study, macroalgal phenolic compounds were found in higher concentrations at higher latitudes, and in some groups were also correlated to associated gradients in salinity and PAR. Seaweeds of Western Australia generally had lower phenolic concentrations than seaweeds of other global areas, but displayed similar spatial variability to regions of comparable size (e.g., Steinberg 1989; Van Alstyne et al. 1999).

Phenolic concentrations of seaweeds were highest in the temperate latitudes of Western Australia. The average phenolic concentration of temperate Eagle Bay was similar to sites at comparable latitudes in eastern and southern Australia (Steinberg 1989) (Table 2). In general, tropical seaweeds have low concentrations of phenolics (Steinberg 1986; Hay and Fenical 1988; Van Alstyne and Paul 1990) and seaweeds of Western Australia follow this trend (Table 2). We found phenolic concentrations were three times lower in tropical study locations than temperate ones, with the exception of tropical Cygnet Bay, which had phenolic concentrations comparable to those from temperate Jurien Bay (Fig. 2a). This is similar to seaweeds from the Caribbean Sea (Targett et al. 1992) which also had anomalously high levels of phenolics for a tropical region. The largest within-family differences in phenolic concentrations between locations occurred in the Dictyotaceae and Sargasscaeae, which were almost an order of magnitude higher in temperate Eagle Bay than tropical Exmouth Gulf (Fig. 4). Interestingly, *Sirophysalis trinodis* contained phenolic concentrations that classified it as a high phenolic species in temperate Eagle Bay and a low phenolic species in tropical locations (Table 2). Species found across temperate and tropical regions, like *S. trinodis*, must adapt to a broader range of environmental conditions and pressures by increasing the physiological importance of phenolics to stress in temperate regions and decreasing it in tropical ones.

The multiple regression models highlighted patterns of environmental factors likely to be important influences on phenolic compound distribution in brown seaweeds. There were significant positive relationships between seaweed phenolic concentrations and salinity across study locations of Western Australia (Table 6a) as well as a subset of individual species (Table 6b). High salinity can cause oxidative stress in seaweeds and phenolic compounds act as antioxidative compounds to combat the elevated production of reactive oxygen species (Pedersen 1984; Ragan and Glombitza 1986; Zubia et al. 2007). Phenolic concentrations were also found in higher concentrations at high salinities in *Ascophyllum nodosum* (Pedersen 1984). While salinity influences phenolic concentrations, species salinity tolerances may also play a role. Phenolic concentrations of some individual species were positively related to salinity in Shark Bay (Table 6c). Seaweeds in tropical Shark Bay are tolerant to a wide a range of salinity, which would explain why only *S. trinodis* and *S. decurrens* showed higher phenolic concentrations at higher salinities (Fig. 5) and not *Hormophysa cuneiformis*. With a potentially broader salinity tolerance, a species like *H. cuneiformis* would not need to increase cellular antioxidant activity through the increased production of phenolic compounds.

We aimed to quantify differences phenolic concentrations of seaweeds throughout coastal Western Australia. The coastline of Western Australia stretches almost 20,000 km (including offshore islands) and encompasses a variety of temperate and tropical macroalgal assemblages (Kendrick et al. 1990; Huisman and Borowitzka 2003; Wernberg et al. 2012; Huisman 2015). This resulted in sampling of different macroalgal species at each study location with little species continuity (Table 2). A few macroalgal species (*H. cuneiformis* and *S. trinodis*) were found at multiple locations while all but two species were restricted to either temperate or tropical study locations. Despite limited species continuity, the geographic scope of this project highlighted the differences in distribution of phenolic compounds across temperate and tropical ecosystems unlike other studies that encompassed only temperate (Steinberg 1989; Tanniou et al. 2014; Van Alstyne 1999) or tropical (Fleury et al. 1994; Pavia and ÅBerg 1996; Steinberg 1986; Stiger et al. 2004; Targett et al. 1992) latitudes.

It is interesting that we found a similar distribution of phenolic compounds across temperate and tropical latitudes in the distantly related taxa of the Dictyotaceae and Sargassaceae (Fig. 4). Previously, no latitudinal patterns in phenolic concentration were found in multiple seaweed taxa across 15 degrees of latitude from the northeast Pacific region (Van Alstyne et al. 1999). Phenolic concentration variability was often as high among geographically close sites as far ones. Additionally, variation in phenolic concentration of *Ascophyllum nodosum* was small at large spatial scales but large between at distances of a few meters (Pavia and ÅBerg 1996). Our findings indicate strong environmental gradients may be present in nearshore Western Australia that overcome the inherent variability in phenolic compounds between taxa as well as geographically

close study locations. Because of the range of temperate and tropical conditions found among study locations (Table 1), it is difficult to determine which of those conditions are most influential in situ, as responses of phenolic compounds to environmental conditions are known to be species specific (Ragan and Jensen 1978; Steinberg 1994; Tala et al. 2016). Nevertheless, regardless of which factors ultimately drive phenolic concentrations, the differences in concentrations we found are greater than the effects of study location variability.

We found no relationship between phenolic concentrations of seaweeds and depth in individual species collected at temperate Jurien Bay across a depth gradient of 6.5–10.0 m or Port Gregory across 1.5–11.0 m depth (Table 6c). Many environmental gradients are associated with increasing depth, including decreasing temperatures and light levels (Nybakken 1993). Phenolic compounds are known to function as UV protection and UV-B radiation has been highlighted as a driver of phenolic concentrations (Hay 1996; Pavia et al. 1997; Pavia and Brock 2000; Mannino et al. 2014). The sampling depths of this study (0–12 m) may experience similar UV-B and temperature conditions in the clear warm waters of the Leeuwin Current (Kendrick et al. 2009) without and impact to the phenolic concentrations of subtidal seaweeds. However, we found very high phenolic concentrations in *Turbinaria gracilis* and *H. cuneiformis* from tropical Cygnet Bay, which was the only intertidal site sampled. Phenolic concentrations of *H. cuneiformis* were 3.5 times higher in Cygnet Bay than the other three tropical locations sampled. Also, *T. gracilis* in Cygnet Bay (Table 2) had two to four times more phenolic compounds than reported elsewhere in the Indo-Pacific for *Turbinaria sp*. (Steinberg 1986; Van Alstyne and Paul 1990; Targett et al. 1992; Stiger et al. 2004). Within Cygnet Bay, 10.0 m tidal swings leave seaweeds in high intertidal lagoons exposed to high temperatures and elevated UV-B radiation. Phenolic compounds absorb UV-B radiation within the range of 195–265 nm commonly associated with photooxidation (Pavia et al. 1997; Henry and Van Alstyne 2004). The ability of phenolic compounds to protect seaweeds from photooxidation likely resulted in high concentrations of phenolics of tropical intertidal seaweeds like those in Cygnet Bay compared to the tropical subtidal study locations.

Herbivory is a known driver of both constitutive and inducible chemical defense traits in marine seaweeds (Cronin and Hay 1996; Peckol et al. 1996; Pavia and Toth 2000; Haavisto 2016). Herbivory pressures on seaweeds, as a result of more abundant large-bodied herbivores in the tropics, are generally higher at lower latitudes in Western Australia (Vanderklift and Kendrick 2004; Poore et al. 2012) and globally (Vermeij 1978; Gaines and Lubchenco 1982). While marine chemical defenses are generally globally more abundant at lower latitudes (Hay and Fenical 1988), low phenolic concentrations in tropical regions of Western Australia are paradoxical, considering the suggested defensive role of phenolic compounds in tropical brown seaweeds (Steinberg 1986, 1989; Targett and Boettcher 1995). Temperate herbivores are often deterred by high phenolic concentrations (Iken et al. 2009; McCarty and Sotka 2013) but tropical herbivores readily consume phenol-rich algae (>2% dry mass phenolics) (Steinberg and Paul 1990; Steinberg et al. 1991) indicating a lack of deterrence by phenolic compounds. Herbivore deterrence from other compounds such as non-polar metabolites (Steinberg and Paul 1990) or other bioactive compounds (Amsler and Fairhead 2006) are possibly more important in this role for tropical seaweeds. Phenolic compounds in tropical seaweeds of Western Australia may have other primary functions such as light protection. Low palatability resulting in herbivore deterrence is likely a secondary characteristic of these compounds.

While we do not know the specific phenolic species found in the taxa examined in this study, the phenolic concentrations provided here should be a relative measure of the reductive potential of the compounds, and thus of their bioactivity. Phenolic compounds are diverse group of multifunctional molecules, which among higher-order taxa, differ in quantity, sizes, steric configurations, and numbers of hydroxyl groups (Ragan and Glombitza 1986; Amsler and Fairhead 2006). The Folin–Ciocalteu method measures extractable, non-bound phenolics by quantifying the reducing ability of accessible hydroxyl groups in the compounds and thus can be used to compare reactivities among broader taxonomic groups. Because the types and structures of phenolic compounds within species are likely similar, the Folin–Ciocalteu method can be used for intraspecific comparisons of phenolic concentrations (Appel et al. 2001).

In conclusion, we found concentrations of phenolic compounds in brown seaweeds were variable across multiple taxonomic and spatial scales. This study spanned both temperate and tropical ecosystems and identified similar distribution patterns of phenolic compounds among seaweed families across latitude. Our multiple regression models related phenolic concentration to salinity and PAR, suggesting common evolutionary mechanisms across taxa. Lower tropical phenolic concentrations found here and in other studies (Steinberg 1986; Steinberg and Paul 1990) indicate a lack of deterrence by phenolic compounds in tropical areas with high grazing pressures in Western Australia. Additionally, the higher phenolic concentrations in Cygnet Bay indicate phenolic compounds likely mediate elevated stresses associated with the intertidal environment. Field experiments measuring the phenolic response of seaweeds to environmental gradients will help identify the mechanistic drivers behind the gradient correlations

presented in this study. Expanding on models such as those presented here will increase the understanding of the evolutionary pressures that shape regional distributions of phenolic compounds.

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Compliance with ethical standards

Conflict of interest Daniel H van Hees declares that he has no conflict of interest. Ylva S Olsen declares that she has no conflict of interest. Thomas Wernberg declares that he has no conflict of interest. Kathryn L Van Alstyne declares that she has no conflict of interest. Gary A Kendrick declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with animals performed by any of the authors.

Author contributions D.H.vH and G.A.K conceived of the ideas; D.H.vH collected the data; D.H.vH and K.V.A ran the laboratory analyses; D.H.vH, K.L.V.A, Y.O, T.W and G.A.K analysed the data; D.H.vH led the writing of the manuscript with contributions from G.A.K, K.L.V.A., T.W., and Y.S.O.

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