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Dietary inclusions of dried macroalgae meal in formulated diets improve the growth of greenlip abalone (*Haliotis laevigata*)

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Abstract Wild greenlip abalone predominantly consumes macroalgae, but under culture conditions in Australia, they are fed formulated diets. Dried macroalgae meals are promising ingredients for abalone diets. In this 92-day study, the growth, feed utilisation and digestive enzyme activities of greenlip abalone (*Haliotis laevigata*; 2.89 g) fed dried macroalgae meals (*Ulva* sp. meal or *Gracilaria cliftonii* meal) in formulated diets were investigated. Seven experimental formulated diets, a basal diet (0 % diet) and three inclusion levels of *Ulva* sp. meal (5, 10 and 20 % inclusions) and *Gracilaria* sp. meal (5, 10 and 20 % inclusions) were used. Diets were formulated to contain 35 % crude protein, 5 % crude lipid and 17.5 MJ kg^{-1} gross energy. A commercial diet was also fed to abalone and compared with the 0 % diet. Growth and feed conversion ratio (FCR) of abalone fed the 0 % diet and commercial diet were similar. Abalone fed 5 % *Gracilaria* sp. meal or *Ulva* sp. meal exhibited superior growth to abalone fed 0 %. However, increasing dietary *Gracilaria* sp. meal inclusions (>10 %) led to further growth improvements but impaired protein and energy retentions. In contrast, abalone fed >10 % *Ulva* sp. meal inclusions exhibited similar growth to those fed 0 and 5 % *Ulva* sp. Although *Ulva* sp. and *Gracilaria* sp. meals are currently not commercially viable, this study clearly demonstrates the potential to develop abalone feeds with inclusions of dried macroalgae meal. We recommend a dietary inclusion of 10 % *Gracilaria* sp. meal or 5 % *Ulva* sp. meal to improve abalone growth.

Keywords *Haliotis laevigata* · *Ulva* sp. meal · *Gracilaria* sp. meal · Digestive enzymes · Nutrition

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Introduction

In the wild, macroalgae forms a predominant dietary component of abalone and is also utilised as feed under culture conditions in a number of countries such as China, South Korea and Chile (Shepherd 1973; Kirkendale et al. 2010; Bansemer et al. 2014). In contrast, greenlip abalone (*Haliotis laevigata*) are primarily grown in land-based systems throughout southern Australia and are fed formulated diets until market size (Stone et al. 2013). Early Australian abalone farmers used information from these countries and utilised kelp as abalone feed (Stone et al. 2014). However, due to nutritional problems, kelp was quickly identified to be inappropriate and uneconomical as feed for Australian abalone (Stone et al. 2014). Recent research has primary focused on optimising the nutritional profile of formulated diets for greenlip abalone (Coote et al. 2000; Stone et al. 2013; Bansemer et al. 2015a).

Commercial formulated diets typically contain a range of palatable, digestible, nutritionally balanced and cost-effective ingredients, including fish meal, cereal grains, oilseeds and pulses (Stone et al. 2013). However, there are potential ecological, economical or nutritional problems associated with these ingredients. Although fish meal-based diets support excellent abalone growth, the aquaculture industry is tending to

reduce its reliance on this commodity due to ecological and economic implications (Britz 1996; Hardy and Tacon 2002). Plant protein sources, including de-hulled lupin and solvent-extract soybean meals, are commonly used sustainable and cost-effective ingredients (Stone et al. 2013). However, both of these ingredients contain a number of antinutritional factors that have been shown to be detrimental to fish health and may also impair abalone health (van den Ingh et al. 1991; Baeverfjord and Krogdahl 1996; Francis et al. 2001).

In contrast to commercial formulated diets, there are numerous benefits of feeding macroalgae to abalone, including feeding stimulation, health improvements and improved marketability (reviewed by Bansemer et al. 2014). For example, abalone (*Haliotis iris*) fed a diet with *Gracilaria* spp. particles, animals spent >80 % of their time feeding and exhibited a 15 % improved growth rate compared with those fed without an algae stimulant, which remained sedentary (Allen et al. 2006). In addition, macroalgae contain a number of biologically active compounds, including polysaccharides, proteins, pigments and polyphenols, which exhibit strong prebiotics, antimicrobial, antiviral, anti-infection and antioxidant activities (Chojnacka et al. 2012). These compounds are important during biotic and abiotic stress but may also promote growth under optimal conditions (Chojnacka et al. 2012).

In a recent review paper, Bansemer et al. (2014) summarised the nutritional requirement and rationalised the reasons for using macroalgae as ingredients in abalone feed. However, some abalone species exhibit sub-optimal growth when feeding fresh macroalgae, compared with those fed formulated diets due to high moisture content or low nutrient density in fresh algae (Viera et al. 2011; Bansemer et al. 2015b; Bansemer et al. 2016b). To overcome this problem, fresh macroalgae may be dried and milled into a product referred to as dried macroalgae meal. Two previous studies investigated the growth performance of abalone fed dietary inclusions of dried macroalgae meal in formulated diets (O'Mahoney et al. 2014; Viera et al. 2015). Based on results from these studies, macroalgae meal appears to be promising ingredients for abalone-formulated diets. Abalone (*Haliotis discus hannai*) fed a formulated diet with dietary inclusions of dried macroalgae meal (combination of *Laminaria digitata*, *Palmaria palmata* and *Ulva lactuca*) exhibited similar growth to those fed fresh *L. digitata* (O'Mahoney et al. 2014). However, the feed efficiency of *H. discus hannai* fed a formulated diet with dietary inclusions of dried macroalgae meal was superior to *H. discus hannai* fed fresh *L. digitata* (O'Mahoney et al. 2014).

In addition to the feeding stimulation, health improvements and improved marketability of abalone fed macroalgae, abalone are evolved to digest and utilise their wild macroalgae-based diet (Shepherd 1973; Erasmus et al. 1997; Harris et al. 1998). The nutritional composition of macroalgae, including carbohydrate, lipid and minerals, differs from formulated diet ingredients (Viola et al. 2001; Nelson et al. 2002; Yu et al. 2002). For example, the reserve carbohydrates of macroalgae and terrestrial ingredient are glucose polymers, but differ in chain length and branching degree (Viola et al. 2001; Yu et al. 2002). The primarily reserve carbohydrate in *Gracilaria* spp., floridean starch, lacks amylose and has a shorter glucose polymer chain length and a higher branching frequency than starch from terrestrial plants and reserve carbohydrate in *Ulva* sp. (Viola et al. 2001; Yu et al. 2002). Diet composition significantly influences the type and activity of digestive enzyme, and in turn the digestive capacity of abalone (Knauer et al. 1996; Erasmus et al. 1997; Bansemer et al. 2016a). While these studies have investigated digestive enzyme activity regulation in abalone fed fresh macroalgae or formulated diets separately, the digestive enzymes activity in abalone fed dried macroalgae meal inclusions is not clearly understood. Further research is required in this area to improve our understanding of the digestive physiology of greenlip abalone.

Although macroalgae meal inclusions in formulated diets for other abalone species were promising, the effect of dietary macroalgae meal inclusions in formulated diets for greenlip abalone is unknown. Based on previous studies, the effect of macroalgae meal inclusions appears to be species dependent but is also influenced by macroalgae species and inclusion level. In the current study, the effect of dietary inclusions of *Ulva* sp. meal and *Gracilaria cliftonii* meal (referred to as *Gracilaria* sp. meal) at graded levels (5, 10 and 20 %) on the growth performance, feed utilisation and digestive enzyme activities of greenlip abalone were investigated. These two macroalgae species were utilised in the current study due to their ease of culture and excellent nutritional profiles (Hernández et al. 2002; Martínez-Aragón et al. 2002; Naidoo et al. 2006; Viera et al. 2011). Furthermore, inclusion levels were selected in the current study based on the improved feeding stimulation at low inclusion levels (Allen et al. 2006) and improved growth, feed utilisation and health at higher inclusions levels (Lange et al. 2014; O'Mahoney et al. 2014).

Methods

Experimental animals and system

Greenlip abalone (weight, 2.89 ± 0.01 g; shell length, 22.41 ± 0.06 mm; $n = 480$) were purchased from South Australian Mariculture (Port Lincoln, SA, Australia). Prior to stocking, abalone were held in a flow-through seawater system at South Australia Research and Development Institute Aquatic Sciences (SARDI AS; West Beach, SA, Australia) and fed a

commercial diet ad libitum ("Abgrow premium" 5 mm chip; Eyre Peninsula Aquafeed Pty Ltd., Lonsdale, SA, Australia).

The experiment was conducted in a temperature-controlled system previously described in Stone et al. (2013). In brief, thirty-two 12.5 L rectangular blue plastic tanks (Nally IH305, Viscount Plastics Pty Ltd) were supplied with sand filtered, UV treated, flow-through seawater at a rate of 300 mL min^{-1}. Water level was set at 2.5 cm using a standpipe with a mesh screen (0.8 mm nominal mesh size) on the outlet to retain uneaten food. Water temperature was held at 22 °C by using 3 kW immersion heaters (240 V, A3122-1; Hotco, Australia).

Stocking

Abalone were gently prised from the substrate using a spatula. Fifteen animals were weighed, measured and stocked into one of four replicate culture units per dietary treatment. Abalone were stocked into the experimental system at 18 °C, acclimated to the experimental system for 1 week and fed their respective diets. After 1 week, water temperature was slowly raised (1 °C day^{-1}) to the final temperature of 22 °C. Dead abalone were measured, weighed, recorded and replaced with abalone of a similar weight, fed their respective diet at 22 °C.

Diets and feeding

Seven experimental formulated diets were investigated in the current study, a basal diet (0 % control diet), and three inclusion levels of *Ulva* sp. meal (5, 10 and 20 %) and *Gracilaria* sp. meal (5, 10 and 20 %). In addition, the performance of abalone fed the 0 % control diet was compared with those fed a commercially available formulated diet ("Abgrow premium" 5 mm chip).

Dried *Ulva* sp. meal (particle size <300 μm) was provided by Venus Shell Systems (Narrawallee, NSW, Australia). Dried *Gracilaria* sp. meal was produced at SARDI AS. Live *G. cliftonii* was collected from Outer Harbor (SA, Australia) and cultured in a 4000-L tank under ambient sunlight. One week prior to harvest, *G. cliftonii* was enriched with 8 L of F2 nutrient medium to increase dietary protein level (Guillard and Ryther 1962). Live *G. cliftonii* was then harvested, sun-dried for ~4 h, oven-dried at 45 °C for ~72 h and milled (particle size <300 μm).

Proximate composition of ingredients was analysed prior to diet formulation. Macroalgae meals (*Ulva* sp. or *Gracilaria* sp.) were included into a basal 0 % diet, formulated at SARDI AS at 5, 10 and 20 % inclusion levels, by reducing solvent extracted soybean meal, wheat flour and de-hulled lupin levels (Table 1). Diets were formulated to contain a 35 % crude protein level, 5 % crude lipid level and a gross energy content of 17.5 MJ kg^{-1}, based on the nutritional requirements reported for greenlip abalone (Stone et al. 2013; Bansemer et al. 2015a). The proximate composition, amino acid profile, fatty acid profile and mineral composition of test ingredients and experimental diets are displayed in Table 2.

Experimental diets were prepared by weighing the required dry ingredients, which were then mixed in a Hobart mixer (Hobart Corp., Troy, OH, USA) for 5 min. Water (~30 % of the total ingredient weight), fish oil, sodium alginate and calcium sulphate were then added to the dry ingredient mix and mixed for a further 5 min. The diets were manufactured using a TR110 pasta machine (Macchine Per Pasta SRL, Molina Di Malo, VI, Italy) and dried at 45 °C for 48 h, to produce a flat, sinking pellet (4 × 3 × 2 mm).

Abalone were fed to excess of their daily requirements based on the total tank biomass (4 % biomass day^{-1}) at 16:00 hours. Feed rates were based on the stocking biomass and adjusted from monthly weight checks. Tanks were cleaned the following day at 08:30 hours, and uneaten feed was collected by sieving the entire tank contents through a fine mesh. Collected feed was stored at −20 °C and was later dried at 105 °C for 16 h. Daily feed consumption was estimated by the difference between feed offered (dry), uneaten feed in dry weight and corrected for feed leaching loss. Feed consumption was corrected for leaching loss by calculating the feed lost between 08:30 and 16:00 hours by immersing diets in water at 22 °C in experimental tanks for 16.5 h without animals and sieved through a fine mesh net (500 μm) and dried to a constant weight. This value was also used as the diet stability.

Biochemical and water quality analyses

At the commencement of the experiment, the soft tissue of 20 animals ($n = 4$ replicates) were collected, shucked and stored at −20 °C to analyse the initial soft tissue proximate composition. At the conclusion of the experiment, five abalone from each tank were collected, shucked and stored at −20 °C. Abalone soft tissue was pooled for each tank, and proximate composition was analysed. Proximate composition analyses of diets and soft tissue composition were conducted according to methods in the British Pharmacopoeia Commission (2004) or German Institute for Standardization (2000). The gastrointestinal region (combined tissue and mucus) from four

Table 1 Ingredient composition of experimental diets

Ingredient composition (g (100 g)$^{-1}$ diet as fed)	Macroalgae species						
	NA	*Ulva* sp.			*Gracilaria cliftonii*		
	Macroalgal inclusion level (%)						
	0	5	10	20	5	10	20
Ulva sp. meal	0.00	5.00	10.00	20.00	0.00	0.00	0.00
Gracilaria cliftonii meal	0.00	0.00	0.00	0.00	5.00	10.00	20.00
Salmon fish meal	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Soy protein concentrate	8.00	8.00	8.00	8.00	8.00	8.00	8.00
Solvent extracted soybean meal	30.50	28.44	27.54	24.88	29.80	28.45	26.57
Wheat flour	29.20	27.82	26.92	24.78	25.90	22.31	15.82
Lupins (de-hulled)	23.96	22.40	19.20	14.00	22.76	22.50	20.66
Salmon fish oil	1.00	1.00	1.00	1.00	1.20	1.40	1.61
EPA vitamin/mineral premix	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin E	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Monosodium phosphate	0.61	0.61	0.61	0.61	0.61	0.61	0.61
Sodium alginate	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Calcium sulphate	0.22	0.22	0.22	0.22	0.22	0.22	0.22
SUM	100	100	100	100	100	100	100

NA no algae

abalone per tank were also collected at the conclusion of the experiment. Gastrointestinal samples were snap frozen in liquid nitrogen and stored at −80 °C prior to the analysis of digestive enzyme activity.

All data reported for animal performance were based on the pooled data from each tank. All calculations using abalone weight were based on wet values, while feed use values were based on dry values:

$$\text{Biomass gain}\left(\text{g tank}^{-1}\right) = \left(\text{final weight} + \sum\text{mortality weight}\right) - \left(\text{initial weight} + \sum\text{replacement weight}\right)$$

$$\text{Specific growth rate}\left(\text{SGR};\ \%\ \text{day}^{-1}\right) = (\left[\ln \text{final weight} - \ln \text{initial weight}\right] / \text{days}) \times$$

$$100\text{Shell growth rate}\left(\mu\text{m day}^{-1}\right) = (\text{final shell length} - \text{initial shell length}) / \text{days}$$

$$\text{Apparent feed consumption} = (\text{feed offered} - \text{uneaten feed collected} - (\text{uneaten feed collected} / (1 - \%\ \text{leaching loss without animals}) \times \%\ \text{leaching loss without animals})) / \text{tank biomass}$$

$$\text{Apparent feed conversion ratio (FCR)} = \text{feed consumed} / \text{abalone weight gain}$$

$$\text{Apparent protein efficiency ratio (PER)} = \text{abalone weight gain} / \text{protein consumed}$$

$$\text{Apparent energy efficiency ratio (EER)} = \text{abalone weight gain} / \text{energy consumed}$$

$$\text{Apparent protein deposition} = (\left[\text{final soft body protein} - \text{initial soft body protein}\right] / \text{protein intake}) \times 100$$

$$\text{Apparent energy deposition} = (\left[\text{final soft body energy} - \text{initial soft body energy}\right] / \text{energy intake}) \times 100$$

Water quality parameters were monitored daily. Water temperature was measured using a thermometer. Dissolved oxygen (mg L^{-1} and % saturation) was measured using a dissolved oxygen meter (OxyGuard International Denmark). The pH was measured using a pH meter (Oakton pHtestr 20; Oakton Instruments,

Table 2 Nutrient composition of test ingredients and experimental diets

	Ingredients (meals)		Diet							
	Macroalgal species									
	Ulva sp.	*Gracilaria* sp.	NA	*Ulva*				*Gracilaria*		
			Inclusion level (%)							
			EPA	0	5	10	20	5	10	20
Proximate composition (g 100 g^{-1} diet as fed)										
Moisture	10.6	3.8	12.2	12.7	11.9	12.0	11.9	12.1	11.8	11.3
Crude protein	33.6	25.2	29.7	34.6	35.0	34.8	34.7	35.1	35.0	35.2
Lipid	4.7	1.1	4.2	5.1	5.4	5.2	5.1	5.4	5.4	5.5
Gross energy (MJ kg^{-1})	15.67	13.09	16.94	17.54	17.58	17.34	17.00	17.45	17.24	16.86
Ash	16.9	30.9	5.8	4.8	5.9	6.9	8.8	6.5	8.0	10.9
Carbohydrate[a]	34.2	39.0	48.1	42.8	41.8	41.1	39.5	40.9	39.8	37.1
Amino acids (g 100 g^{-1} diet as fed)										
Alanine	2.55	1.14	1.10	1.38	1.45	1.56	1.69	1.47	1.42	1.56
Aspartic acid	4.19	1.95	2.66	3.36	3.46	3.75	3.78	3.62	3.42	3.46
Arginine	1.73	1.44	1.66	2.54	2.62	2.65	2.74	2.59	2.56	2.73
Glutamic acid	3.74	1.65	5.10	5.83	5.89	5.76	5.93	5.94	5.70	5.55
Glycine	1.68	1.00	1.20	1.60	1.65	1.63	1.73	1.65	1.63	1.65
Histidine	0.22	0.03	0.56	0.85	0.83	0.81	0.81	0.84	0.83	0.85
Isoleucine	1.11	0.63	1.03	1.46	1.48	1.40	1.41	1.50	1.47	1.47
Leucine	1.99	0.53	1.95	2.51	2.53	2.58	2.57	2.60	2.51	2.53
Lysine	1.50	1.19	1.80	1.97	1.99	2.09	2.19	2.18	1.99	1.89
Methionine	0.46	0.34	0.37	0.55	0.63	0.46	0.49	0.44	0.56	0.46
Phenylalanine	1.56	0.76	1.28	1.57	1.58	1.66	1.67	1.71	1.57	1.70
Proline	1.38	0.30	2.43	2.35	2.35	2.02	1.93	1.98	2.25	1.91
Serine	1.57	0.85	1.14	1.63	1.64	1.53	1.57	1.53	1.67	1.65
Threonine	1.43	0.81	0.90	1.36	1.39	1.24	1.30	1.25	1.39	1.33
Tyrosine	0.75	0.54	0.85	1.10	1.29	1.15	1.13	1.20	1.09	1.23
Valine	2.03	0.79	1.36	1.65	1.68	1.85	1.89	1.84	1.68	1.68
Fatty acids (mg 100 g^{-1} diet as fed)										
14:0	95	57	60	40	47	53	57	52	58	72
16:0	960	370	810	840	930	1020	970	970	920	960
18:0	480	<10	190	280	290	300	240	280	290	290
10:1	56	<10	<10	<10	<10	<10	<10	<10	<10	<10
14:1	10	<10	<10	<10	<10	<10	<10	<10	<10	<10
15:1	70	<10	<10	<10	<10	<10	<10	<10	<10	<10
16:1	58	<10	120	75	86	84	99	92	98	110
17:1	23	<10	<10	<10	<10	<10	<10	<10	<10	<10
18:1*n*–7	280	30	87	85	100	110	130	110	100	110
18:1*n*–9	24	46	1040	1440	1490	1340	1260	1440	1580	1620
18:2*n*–6	210	<10	1240	1710	1720	1430	1430	1660	1640	1520
20:4*n*–6	29	420	20	11	13	11	18	15	62	110
18:3*n*–3	680	<10	140	200	210	190	250	230	200	190
18:4*n*–3	550	<10	14	<10	17	16	37	26	<10	<10
20:4*n*–3	39	<10	<10	<10	<10	<10	<10	<10	<10	10
20:5*n*–3	85	<10	66	30	33	27	38	35	36	39
22:5*n*–3	79	<10	24	30	12	10	16	14	14	16

Table 2 (continued)

	Ingredients (meals)	Diet							
	Macroalgal species								
	Ulva sp.	*Gracilaria* sp.	NA	*Ulva*				*Gracilaria*		
			Inclusion level (%)							
			EPA	0	5	10	20	5	10	20
22:6*n*–3	<10	<10	140	98	98	68	97	98	110	110
Minerals (mg kg^{-1}as fed)										
Calcium	17,000	9300	4700	6100	6900	6900	8700	6200	7100	7700
Chromium	3.5	2.1	–	–	–	–	–	–	–	–
Cobalt	0.29	1.40	–	–	–	–	–	–	–	–
Copper	110.0	6.3	–	–	–	–	–	–	–	–
Iodine	59	200	–	–	–	–	–	–	–	–
Iron	360	1300	–	–	–	–	–	–	–	–
Magnesium	18,000	7300	–	–	–	–	–	–	–	–
Manganese	290	390	–	–	–	–	–	–	–	–
Molybdenum	0.60	0.48	–	–	–	–	–	–	–	–
Nickel	4.5	6.4	–	–	–	–	–	–	–	–
Phosphorus	14,000	4500	6200	8200	8600	8700	9800	8200	8500	8400
Potassium	16,000	72,000	–	–	–	–	–	–	–	–
Selenium	0.110	0.089	–	–	–	–	–	–	–	–
Sodium	29,000	44,000	–	–	–	–	–	–	–	–
Zinc	280	51	–	–	–	–	–	–	–	–
Diet stability (%)[b]	–	–	80.65	79.75	78.75	77.56	75.62	55.03	46.34	33.61

EPA Eyre Peninsula Aquafeed Pty Ltd, *NA* no algae, "–" variables not analysed

[a] Calculated by difference, carbohydrate% = 100 % − (moisture % + protein % + lipid % + ash %)

[b] Diet stability calculated by immersing diets in water at 22 °C in experimental tanks for 16.5 h and sieved through a fine mesh net (500 μm) and dried to a constant weight

USA). Salinity (g L^{-1}) was measured using a portable salinity refractometer (model RF20, Extech Instruments, USA).

Preparation of gut extracts and digestive enzymatic assays

Gastrointestinal samples from four abalone ($n = 4$) per tank replicate were partially thawed, weighed, pooled and homogenised in four volumes of distilled water (*W*/*V*) using a Dounce homogeniser. As each kit had set pH levels, the homogenate was resuspended in four volumes of the buffer supplied with each kit (*v*/*v*). The suspensions were centrifuged at 17,530×*g* for 20 min at 4 °C. The resulting supernatants were analysed in triplicate for α-amylase, β-glucosidase, β-galactosidase, trypsin and lipase activities at 22 °C using spectrophotometric techniques and commercial enzyme test kits.

Colourimetric analyses were used to determine α-amylase (EC 3.2.1.1) activity by reading the absorbance of samples at 405 nm at 10 and 20 min (cat. no. K711-100; Biovision, Inc., USA). Colourimetric analyses were used to determine β-glucosidase (EC 3.2.1.21) activity by reading the absorbance of samples at 405 nm at 0 and 20 min (cat. no. MAK129; Sigma-Aldrich, USA). Colourimetric analyses were used to determine β-galactosidase (EC 3.2.1.23) activity by reading the absorbance of samples at 405 nm at 0 and 30 min (cat. no. 75707; Thermo Scientific Inc., USA). Colourimetric analyses were used to determine trypsin (EC 3.4.21.4) activity by reading the absorbance of samples at 405 nm at 0 and 1 h (cat. no. K771-100; Biovision). Fluorometric analyses were used to determine lipase (EC 3.1.1.) activity by reading $E_x/E_m = 529/600$ nm at 0 and 40 min (cat. no. K724-100; Biovision). Total protein was determined using a bicinchoninic acid (BCA) protein assay kit with bovine serum albumin solution as the standard (Biovision, cat. no. K813-2500). Aside from specific β-galactosidase activity, which was defined as the ΔA405 nm h^{-1} mg^{-1} soluble protein, specific enzyme activities were defined as the amount of enzyme that catalysed the conversion of 1 μmol of substrate min^{-1} mg^{-1} of protein (i.e. U mg^{-1} soluble protein) at 22 °C.

Statistical analyses

IBM SPSS (version 22 for Windows; IBM SPSS Inc., USA) was used for all statistical analyses. Homogeneity of variances and normality among mean values were assessed using Levene's test for equality of variance errors and the standardised residuals against the predicted mean plot, respectively. All percentage data were arcsine transformed before analyses. Two-tailed *t* tests were used to determine differences between abalone fed the 0 % diet and commercial diet. Data were analysed using two-factor ANOVA to determine interactive effects between macroalgae meal species (*Ulva* sp. and *Gracilaria* sp.) and inclusion level (0, 5, 10 and 20 %). When significant interactions were observed, pairwise comparisons were used to determine significant differences between treatment combinations (Fisher's least significant difference). A significance level of $P < 0.05$ was used for all statistical tests. All values are presented as means ± standard error (SE) of the mean unless otherwise stated.

Results

General observations

There were no significant differences between diets for abalone initial weight and shell length ($P > 0.05$). The average initial weight and shell length were 2.89 ± 0.00 g and 22.41 ± 0.07 mm, respectively (Table 3). Water quality parameters were monitored daily and maintained at levels appropriate for greenlip abalone (mean ± standard deviation, range): water temperature (21.9 ± 0.3, 21.0–22.8 °C), dissolved oxygen (94 ± 4, 85–104 % saturation; 6.8 ± 0.3, 6.0–7.5 mg L^{-1}), pH (8.15 ± 0.05, 8.03–8.31) and salinity (36 ± 1, 34–37 ‰). Throughout the study, abalone exhibited normal signs of feeding and fed actively on all diets. No visual signs of disease were observed in experimental animals and abalone mortality during the study was low (5.63 %). Mortalities primarily occurred during the first 2 weeks due to handling and were not affected by diet ($P > 0.05$; Table 3).

Growth performance

Abalone fed the commercial diet and 0 % diet had similar final weight (12.45 and 11.49 g), biomass gain (143.29 and 129.16 g $tank^{-1}$), SGR (1.58 and 1.49 % day^{-1}), final shell length (45.89 and 44.51 mm) and shell growth rate (256.55 and 239.47 μm day^{-1}), respectively ($P > 0.05$; two-tailed *t* test).

Final weight, biomass gain, SGR, final shell length and shell growth rate were significantly influenced by macroalgae meal species (*Ulva* sp. and *Gracilaria* sp.), inclusion level (0, 5, 10 and 20 %) and the interaction between these two factors ($P < 0.05$; two-factor ANOVA; Table 3). The interaction between macroalgae species and inclusion level affected growth parameters similarly. Abalone fed 5 % *Ulva* sp. meal or 5 % *Gracilaria* sp. meal grew significantly more rapidly than abalone fed 0 %. Abalone fed 10 and 20 % *Gracilaria* sp. exhibited significantly superior growth, compared with those fed 0 and 5 % *Gracilaria* sp. (Table 3). In contrast, the growth performance of abalone fed 10 and 20 % *Ulva* sp. was similar to abalone fed 0 and 5 % *Ulva* sp.

Feed use

Feed consumption rate for abalone fed the commercial diet (10.21 g as fed kg $abalone^{-1}$ day^{-1}) was significantly higher than those fed the 0 % diet (9.81 g as fed kg $abalone^{-1}$ day^{-1}; $P = 0.004$; *t* test). However, feed conversion ratio was not significantly different between abalone fed the commercial diet (0.76) and the 0 % diet (0.73; $P = 0.107$; *t* test).

Feed consumption rate (g as fed kg $abalone^{-1}$ day^{-1}) of abalone was significantly influenced by macroalgae meal species, inclusion level and the interaction between macroalgae meal species and inclusion level ($P < 0.001$; two-factor ANOVA; Table 3). The significant interaction was due to a significant increase in feed consumption for abalone fed 5 and 10 % *Gracilaria* sp. compared with 0 %, while the feed consumption of abalone fed 5 and 10 % *Ulva* sp. inclusions were similar to those fed 0 % ($P > 0.05$). There was also a significantly greater increase in feed consumption for abalone fed 20 % *Gracilaria* sp. than 20 % *Ulva* sp., compared with abalone fed 0 %.

Macroalgae meal species, inclusion level and the interaction between these two factors had a significant effect on the apparent FCR of abalone ($P < 0.001$; two-factor ANOVA; Table 3). The significant interaction was due to significantly higher apparent FCR for abalone fed 5 and 10 % *Gracilaria* sp. than abalone fed 0 %. In contrast, abalone fed 5 and 10 % *Ulva* sp. had similar apparent FCRs to those fed 0 %. As the dietary *Gracilaria* sp. inclusion level increased from 5 to 20 %, apparent FCR for abalone significantly decreased, while abalone fed 20 % *Ulva* sp. had significantly higher apparent FCR than abalone fed 0, 5 and 10 % *Ulva* sp.

Soft tissue composition

Abalone fed the 0 % diet had significantly higher soft tissue moisture content (75.02 %), compared with the commercial diet (73.33 %; $P < 0.001$; *t* test). Soft tissue protein, lipid and energy contents were similar for abalone fed the 0 % diet (65.4 and 6.1 % and 19.72 MJ kg^{-1}, respectively) and the commercial diet (64.2 and 5.6 % and 20.03 MJ kg^{-1}, respectively; $P > 0.05$).

Soft tissue moisture, protein, energy or ash content of abalone was not influenced by macroalgae meal species,

Table 3 Growth performance, feed efficiency, nutrient retention and soft tissue composition of greenlip abalone fed dried macroalgae meal inclusions

	Macroalgal species							ANOVA (*P*)		
	Ulva sp. meal				*Gracilaria* sp. meal					
	Macroalgal inclusion level (%)									
	0	5	10	20	5	10	20	Species (A)	Inclusion level (%) (B)	A × B
Growth performance and mortality										
Initial weight (g)	2.90 ± 0.01	2.88 ± 0.01	2.89 ± 0.01	2.89 ± 0.01	2.89 ± 0.01	2.90 ± 0.01	2.88 ± 0.01	0.821	0.443	0.746
Final weight (g)	11.49 ± 0.35	11.84 ± 0.34	11.63 ± 0.11	11.73 ± 0.08	12.38 ± 0.23	13.48 ± 0.16	13.33 ± 0.33	<0.001	0.002	0.006
Biomass gain (g tank^{-1})	129.16 ± 5.28	134.25 ± 5.02	131.00 ± 1.78	132.08 ± 1.60	142.01 ± 3.37	158.77 ± 2.50	156.35 ± 4.63	<0.001	0.002	0.006
SGR (% day^{-1})	1.49 ± 0.03	1.53 ± 0.03	1.51 ± 0.01	1.52 ± 0.01	1.58 ± 0.02	1.67 ± 0.01	1.66 ± 0.03	<0.001	<0.001	0.003
Mortality (%)	5.00 ± 5.00	3.33 ± 1.92	8.33 ± 6.31	5.00 ± 3.19	13.33 ± 2.72	0.00 ± 0.00	5.00 ± 5.00	0.891	0.763	0.204
Somatic growth parameters										
Initial shell length (mm)	22.47 ± 0.21	22.28 ± 0.03	22.65 ± 0.28	22.27 ± 0.10	22.56 ± 0.28	22.47 ± 0.13	22.28 ± 0.05	0.832	0.444	0.668
Final shell length (mm)	44.51 ± 0.48	45.33 ± 0.48	45.25 ± 0.10	45.82 ± 0.15	46.27 ± 0.29	47.49 ± 0.28	47.76 ± 0.42	<0.001	<0.001	0.020
Shell growth rate (μm day^{-1})	239.47 ± 4.78	250.58 ± 5.39	245.63 ± 2.20	256.00 ± 1.21	257.76 ± 1.88	271.98 ± 2.58	276.99 ± 4.70	<0.001	<0.001	0.006
Feed utilisation										
Feed consumption rate (g as fed kg^{-1} abalone day^{-1})	9.42 ± 0.14	10.11 ± 0.29	9.84 ± 0.12	11.50 ± 0.23	13.91 ± 0.18	13.91 ± 0.28	13.03 ± 0.14	<0.001	<0.001	<0.001
Apparent FCR	0.73 ± 0.01	0.77 ± 0.03	0.75 ± 0.01	0.88 ± 0.02	1.03 ± 0.02	0.99 ± 0.03	0.93 ± 0.01	<0.001	<0.001	<0.001
Nutrient retention										
Apparent PER	3.48 ± 0.07	3.30 ± 0.11	3.36 ± 0.05	2.90 ± 0.06	2.43 ± 0.05	2.55 ± 0.07	2.70 ± 0.03	<0.001	<0.001	<0.001
Apparent PD	40.98 ± 0.82	40.24 ± 2.77	37.11 ± 1.44	34.72 ± 1.03	28.88 ± 0.54	29.84 ± 0.85	33.55 ± 1.71	<0.001	<0.001	0.001
Apparent EER	6.87 ± 0.13	6.57 ± 0.22	6.75 ± 0.10	5.91 ± 0.13	4.88 ± 0.10	5.17 ± 0.14	5.64 ± 0.07	<0.001	<0.001	<0.001
Apparent ED	25.38 ± 0.40	26.09 ± 1.52	23.81 ± 0.94	22.38 ± 1.05	18.25 ± 0.33	19.10 ± 0.49	21.92 ± 0.76	<0.001	<0.001	<0.001
Proximate composition										
Moisture (%)	75.02 ± 0.15	73.76 ± 0.68	75.85 ± 0.62	74.45 ± 0.44	75.28 ± 0.38	75.42 ± 0.09	74.71 ± 0.61	0.316	0.090	0.190
Protein (% dry)	65.4 ± 1.2	64.7 ± 0.9	64.4 ± 0.7	64.7 ± 0.6	66.1 ± 0.9	65.7 ± 0.6	66.5 ± 1.0	0.066	0.943	0.767
Lipid (% dry)	6.1 ± 0.1	6.1 ± 0.2	6.1 ± 0.2	5.7 ± 0.2	5.8 ± 0.2	6.6 ± 0.1	6.5 ± 0.2	0.085	0.186	0.030
Ash (% dry)	11.06 ± 0.42	9.19 ± 0.78	11.38 ± 0.49	11.36 ± 0.51	10.86 ± 0.59	10.74 ± 0.58	10.32 ± 0.56	0.993	0.255	0.101
Energy (MJ kg^{-1} dry)	19.72 ± 0.19	20.17 ± 0.16	19.67 ± 0.11	19.60 ± 0.21	20.00 ± 0.10	20.01 ± 0.13	20.19 ± 0.11	0.128	0.198	0.088

Initial soft tissue composition of greenlip abalone (dry): moisture (80.06 %), protein (73.8 %), lipid (4.3 %), ash (13.2 %) and energy (18.93 MJ kg^{-1}). Mean ± standard error, $n = 4$. A significance level of $P < 0.05$ was used for all statistical tests. Where significant main effects were detected, post hoc tests were used to determine differences between means (Fisher's least significant differences; $P < 0.05$). For variables with a significant interaction between macroalgae type and enrichment, differences between treatments were analysed using pairwise comparisons and are explained in text (Fisher's least significant differences)

SGR specific growth rate, *FCR* feed conversion ratio, *PER* protein efficiency ratio, *PD* protein deposition, *EER* energy efficiency ratio, *ED* energy deposition

inclusion level or the interaction between these two factors ($P > 0.05$; two-factor ANOVA; Table 3). Soft tissue lipid content of abalone was not influenced by macroalgae meal species ($P = 0.085$) or inclusion level ($P = 0.186$) but was significantly affected by the interaction between these two factors ($P = 0.030$; Table 3). The significant interaction was due to significantly higher lipid levels for abalone fed 20 % *Gracilaria* sp. than 20 % *Ulva* sp. meal, relative to abalone fed 5 % *Gracilaria* sp. and *Ulva* sp., respectively. Soft tissue lipid content was not influenced by other interactions between macroalgae meal species and inclusion level.

Nutrient use

Abalone fed the commercial diet had significantly higher apparent PER and apparent protein deposition (3.92 and 48.31, respectively) than abalone fed the 0 % diet (3.48 and 40.98, respectively) ($P = 0.002$ and $P < 0.001$, respectively; *t* test). Energy efficiency ratio was similar for abalone fed the commercial diet (6.88) and the 0 % diet (6.87; $P = 0.976$; *t* test). Apparent energy deposition for abalone fed the commercial diet was significantly higher than those fed the 0 % diet (27.58 and 25.38, respectively; $P = 0.017$; *t* test).

Macroalgae meal species, inclusion level and the interaction between macroalgae meal species and inclusion level significantly affected the apparent PER and EER of abalone ($P < 0.001$; two-factor ANOVA; Table 3). The interaction effects between macroalgae meal species and inclusion level was similar for both the apparent PER and EER of abalone. The PER and EER for abalone fed 5 and 10 % *Gracilaria* sp. meal was significantly lower than abalone fed 0 %. In contrast, PER and EER of abalone fed 5 and 10 % *Ulva* sp. were similar to those fed 0 %. In addition, PER and EER for abalone fed 20 % *Ulva* sp. and 20 % *Gracilaria* sp. were significantly lower and higher than animals fed 5 % *Ulva* sp. and 5 % *Gracilaria* sp., respectively.

Apparent protein deposition and apparent energy deposition of abalone was significantly affected by macroalgae meal species, inclusion level and the interaction between these two factors ($P < 0.001$; two-factor ANOVA; Table 3). The significant interaction was due to the significant lower apparent protein and energy depositions for abalone fed 5 and 10 % *Gracilaria* sp. meal relative to abalone fed 0 %, while protein and energy depositions for abalone fed 5 and 10 % *Ulva* sp. meal were similar to abalone fed 0 %. Additionally, abalone fed 20 % *Gracilaria* sp. or *Ulva* sp. meal diets had significantly reduced protein and energy depositions compared with abalone fed 0 %, but this response did not depend on macroalgae meal species.

Digestive enzymes

The α-amylase activity for abalone fed the 0 % diet was significantly higher than abalone fed the commercial diet (92.33 and 44.08 U mg^{-1} soluble protein, respectively; $P = 0.016$; *t* test). Abalone fed the 0 % diet and commercial diet had similar trypsin (0.38 and 0.30 U mg^{-1} soluble protein, respectively; $P = 0.435$), lipase (19.98 and 18.67 U mg^{-1} soluble protein, respectively; $P = 0.751$), β-glucosidase (3.18 and 3.42 U mg^{-1} soluble protein, respectively; $P = 0.875$) and β-galactosidase activities (0.35 and 0.33 ΔA405 nm h^{-1} mg^{-1} soluble protein, respectively; $P = 0.875$).

Trypsin activity of abalone was significantly affected by macroalgae species ($P = 0.003$), inclusion level ($P = 0.041$), and the interaction between these two factors ($P = 0.020$; two-factor ANOVA; Table 4). Abalone fed *Ulva* sp. had significantly higher trypsin activity than abalone fed corresponding *Gracilaria* sp. The significant interaction was primarily due to a more pronounced trypsin up-regulation for abalone fed 5 % *Ulva* sp. than 5 % *Gracilaria* sp., compared with abalone fed 0 % ($P < 0.05$; two-factor ANOVA; Table 4).

Abalone fed *Gracilaria* sp. had significantly higher β-galactosidase activity than those fed *Ulva* sp. ($P = 0.037$; two-factor ANOVA; Table 4). Inclusion level and the interaction between macroalgae species and inclusion level did not influence β-galactosidase activity ($P > 0.05$; Table 4). Lipase, α-amylase, β-glucosidase activities were not significantly influenced by macroalgae meal species, inclusion level, and the interaction between macroalgae meal species and inclusion level ($P > 0.05$; two-factor ANOVA; Table 4).

Discussion

Further development of a macroalgae industry in Australia is limited by cost-effective "farm to market" value chains. In order to develop cost-effective "farm to market" value chains and to maximise resource utilisation, it is important to develop a range of high-value macroalgae products that require complex downstream processing (e.g. extracted, desulfated/oversulfated or depolymerised) to low-value products that require less refinement and offer high yields (e.g. live/fresh or dried/milled) (Lorbeer et al. 2013). Therefore, our aim in the current study was to utilise macroalgae meal to improve abalone growth and also build linkage between the macroalgae and abalone industry. To achieve this aim, the effects of dietary inclusion of dried macroalgae meal (*Ulva* sp. and *Gracilaria* sp.) on the growth performance and feed utilisation of greenlip abalone was investigated in the current study. Specific growth grate of abalone ranged from 1.49 to 1.67 % day^{-1}, which compares favourably to other laboratory and commercial growth studies on greenlip abalone (Vandepeer 2005; Stone et al. 2013; Bansemer et al. 2015a).

Table 4 Trypsin, lipase, α-amylase, β-glucosidase and β-galactosidase activities in the gastrointestinal region of greenlip abalone fed dried macroalgae meal inclusions

	Macroalgal species							ANOVA (*P*)		
	Ulva				*Gracilaria*					
	Macroalgal inclusion level (%)									
	0	5	10	20	5	10	20	Species (A)	Inclusion level (%) (B)	A × B
Trypsin activity[a]	0.38 ± 0.04	1.08 ± 0.27	0.51 ± 0.08	0.66 ± 0.08	0.35 ± 0.07	0.39 ± 0.06	0.44 ± 0.07	0.003	0.041	0.020
Lipase activity[a]	19.98 ± 2.99	20.15 ± 2.94	18.61 ± 2.00	17.00 ± 3.59	21.62 ± 3.27	21.50 ± 2.95	18.65 ± 3.10	0.449	0.739	0.972
α-amylase activity[a]	92.33 ± 11.53	101.85 ± 7.49	103.06 ± 12.13	82.02 ± 11.24	99.44 ± 16.12	104.54 ± 23.92	112.42 ± 6.58	0.422	0.844	0.592
β-glucosidase activity[a]	3.18 ± 1.10	4.39 ± 0.37	4.72 ± 0.91	4.29 ± 1.24	4.40 ± 0.69	4.46 ± 0.98 t	3.46 ± 0.50	0.664	0.366	0.962
β-galactosidase activity[b]	0.35 ± 0.03	0.33 ± 0.01	0.31 ± 0.02	0.35 ± 0.02	0.37 ± 0.01	0.38 ± 0.02	0.36 ± 0.04	0.037 (U<G)	0.976	0.298

Mean ± standard error, $n = 4$, four pooled abalone per replicate. A significance level of $P < 0.05$ was used for all statistical tests. Where significant main effects were detected, post hoc tests were used to determine differences between means (Fisher's least significant differences; $P < 0.05$). For variables with a significant interaction between macroalgae type and enrichment, differences between treatments were analysed using pairwise comparisons and are explained in text (Fisher's least significant differences)

U Ulva sp. meal, *G Gracilaria* sp. meal

[a] U mg^{-1} soluble protein

[b] ΔA405 nm h^{-1} mg^{-1} soluble protein

The growth and feed conversion ratio of abalone fed the commercial diet and 0 % diet were similar, which gives confidence in interpreting results for experimental diets in the current study.

Abalone fed *Gracilaria* sp. or *Ulva* sp. meal inclusions immediately displayed active feeding behaviours when feed was added to the tank during the light phase. This behavioural response was not observed in abalone fed diets without macroalgae meal (0 % diet or commercial diet). Feeding stimulation by supplying macroalgae was reported in greenlip abalone fed live *Ulva* sp. (Bansemer et al. 2015b) and *Gracilaria* sp. (Buss et al. 2015). However, abalone fed live macroalgae exhibited inferior growth to those fed formulated diets (Bansemer et al. 2016b). In the current study, increased feeding stimulation for abalone fed 5 % *Gracilaria* sp. may have resulted in significantly higher feed intake, and in turn superior growth, to those fed the 0 % diet. These results are consistent with a previous study by Allen et al. (2006), which reported numerically higher feed intake and significantly higher shell growth rates (15 %) for *H. iris* fed a formulated diet with dried, mulched *Gracilaria* spp. particles (300–500 μm) suspended in the system, compared with a formulated diet alone (Allen et al. 2006). Feed attractants, supplied in the form of dried macroalgae inclusions (5 % *Ecklonia maxima*) in formulated diets, are currently utilised by a South African feed company for *Haliotis midae* (personal communication, Kurt Mätschke, Marifeed, Western Cape, South Africa). Results from the current study indicate that it would also be beneficial to formulate greenlip abalone diets with macroalgae meal (*Gracilaria* sp.) to stimulate feeding and also improve growth.

In the current study, abalone fed 10 and 20 % *Gracilaria* sp. inclusions exhibited superior growth performance to those fed other diets. All diets were formulated using highly palatable and digestible ingredients at realistic inclusion levels (Fleming et al. 1998; Vandepeer 2005; Stone et al. 2013). However, ingredients can be highly digested, but may be poorly utilised (Stone et al. 2003). The reserve carbohydrates in terrestrial plants and *Gracilaria* sp. are primarily glucose polymers with α-(1,4) glycosidic linkages, which are hydrolysed by α-amylase (Viola et al. 2001; Yu et al. 2002). However, floridean starch, the primarily reserve carbohydrate in *Gracilaria* spp., lacks amylose, has a shorter glucose polymer chain length and a higher branching frequency than starch from terrestrial plants and reserve carbohydrate in *Ulva* sp. (Viola et al. 2001; Yu et al. 2002). Structural carbohydrates of *Gracilaria* spp. also differ from other ingredients used in the current study. The primary structural carbohydrate in terrestrial plants is cellulose, while *Ulva* sp. also contains cellulose, xylans, ulvan and mannans are also present (McCandless 1981; Evans 1989; Lahaye and Robic 2007). In contrast, the most abundant structural carbohydrate in *Gracilaria* spp. is agar, which is composed of galactose and glucose repeating units with β-glycosidic linkages (McCandless 1981; Evans 1989; Lahaye and Robic 2007). As the digestive system of abalone is adapted to digest and utilise macroalgae, composition and structural carbohydrate differences may affect carbohydrate digestion and utilisation.

The digestive capacity of abalone is dependent on the type and activities of digestive enzymes. Digestive enzyme activities in abalone are significantly influenced by diet (Knauer et al. 1996; Erasmus et al. 1997; García-Carreño et al. 2003). For example, higher alginate lyase, carboxymethylcellulase and laminarinase activities were reported in abalone (*H. midae*) fed *Ecklonia maxima* than those fed *Gracilaria verrucosa* (Erasmus et al. 1997). In contrast, higher agarase and carrageenase activities were reported in abalone fed *G. verrucosa* than those fed *E. maxima*. The authors suggested that the regulation of carbohydrase activity in abalone was associated with carbohydrate differences between macroalgae species (Erasmus et al. 1997). In the current study, diet also affected abalone digestive enzyme activities. Agar is hydrolysed by agarose degrading enzymes, including β-galactosidase (Michel et al. 2006; Lee et al. 2014). Abalone significantly up-regulated β-galactosidase activities when fed *Gracilaria* sp. meal compared with those fed *Ulva* sp. meal. There was also tendency for α-amylase activities to increase with increased dietary inclusions of *Gracilaria* sp. The β-galactosidase activities and α-amylase activity up-regulation may increase carbohydrate utilisation for energy, and spare protein for growth. However, protein utilisation (apparent PER and protein deposition) of abalone fed a 10 and 20 % *Gracilaria* sp. meal inclusions was significantly lower than those fed the 0 % diet. Although abalone may have been supplied with carbohydrates, they efficiently digest and utilise, due to their increased energy requirements during periods of fast growth (Duong et al. 2014), abalone may have also deaminated protein for energy metabolism. This hypothesis is supported by a significantly lower energy deposition in abalone fed *Gracilaria* sp. meal, indicating that during periods of rapid growth abalone fed *Gracilaria* sp. meal may have a different energy budget to those fed diets without *Gracilaria* sp. meal. It would be beneficial in future studies to investigate greenlip abalone energy budgets when fed dried macroalgae meal inclusions to further improve the nutritional knowledge on greenlip abalone.

Superior growth performance for abalone fed *Gracilaria* sp. meal inclusions may also be related to fatty acid profile differences between *Gracilaria* sp. and *Ulva* sp. Abalone have low lipid requirements, but some fatty acids are essential for abalone growth (Nelson et al. 2002; Dunstan et al. 2000). The C20 long chain polyunsaturated fatty acids (LC PUFA) levels and higher arachidonic acid (ARA; 20:4*n*–6) to eicosapentaenoic acid (EPA; 20:5*n*–3) ratios can promote superior growth for abalone (*Haliotis fulgens*; Nelson et al. 2002). Eicosapentaenoic acid, a LC *n*–3 PUFA, is important

for cellular membrane structure and function, and controlling and regulating cellular metabolism (Dunstan et al. 2000; Bautista-Teruel et al. 2011). Additionally, ARA, a LC *n*–6 PUFA, is required for cell membrane function, combating infection, blood coagulation and as an anti-inflammatory (Nelson et al. 2002). In the current study, the EPA levels of experimental diets were relatively similar, but ARA levels in *Gracilaria* sp. diets increased due to high ARA levels in *Gracilaria* sp. meal. As a result, as dietary inclusions of *Gracilaria* sp. increased, diets contained higher C20 LC PUFA levels and ARA to EPA ratio, which may have also influenced abalone growth in the currents study. Recent research by Viera et al. (2015) however, suggested that abalone (*Haliotis tuberculata coccinea*) have the capacity to desaturate and chain elongate linoleic acid (18:2*n*–6, LA) to ARA. In the current study, *Ulva* sp. was low in ARA, but contained high levels of LA, which may have also supplemented low ARA levels. While the LC-PUFA biosynthesis in fish is well understood, few studies have focused on this area for abalone. Further research into fatty acid metabolism and gene expression of greenlip abalone, with particular focus on the desaturation and chain elongation of LA to ARA, is required before further conclusions can be made.

In addition to dietary macronutrients, micronutrients in *Gracilaria* sp. meal may also improve abalone growth. Dietary minerals are required for normal metabolic function. The optimal inclusion level for some minerals, including calcium, phosphorus, copper, iron, selenium and zinc, are established for abalone (Coote et al. 1996; Tan and Mai 2001; Wang et al. 2009; Wang et al. 2012). However, other dietary vitamin and mineral requirements for abalone are typically based on fish requirements (Sales and Janssens 2004). In the current study, *Gracilaria* sp. meal contained some minerals, including cobalt, iodine, iron and manganese, at levels considerably higher than *Ulva* sp. meal. These minerals may be required for optimal abalone growth. However, this area has not been thoroughly explored in previous studies, and further research to understand the mineral requirements and optimal level for abalone diets is required. The mineral composition of *Gracilaria* sp. meal may provide a useful benchmark to explore this area.

Abalone fed 5 % *Ulva* sp. meal inclusions also exhibited superior growth to those fed 0 %. However, in contrast to abalone fed 5 % *Gracilaria* sp., the apparent feed consumption and FCR of animals fed 5 % *Ulva* sp. were similar to those fed 0 %. Superior growth of abalone fed 5 % *Ulva* sp. may be related to digestive enzyme activity regulation. Trypsin activity was significantly up-regulated (184 %) in abalone fed 5 % *Ulva* sp. compared with those fed 0 %. Trypsin is important in protein digestion and is a useful indicator for fish growth (Lemieux et al. 1999; Rungruangsak-Torrissen et al. 2006). In the current study, the significant trypsin up-regulation by feeding 5 % *Ulva* sp. likely increased dietary protein utilisation and subsequent growth. Trypsin and other protease activities are typically influenced by dietary protein levels (Knauer et al. 1996). However, diets in the current study were isonitrogenous (~35 % crude protein). Resident bacteria in the gastrointestinal tract of abalone are associated with nutrient digestion (Erasmus et al. 1997; Harris et al. 1998). Dietary inclusions of *Ulva* sp. may alter the luminal environment and influence microbiota composition, which may have resulted in a proliferation of trypsin-secreting bacteria in the current study. This would ultimately increase the protein digestion and growth of abalone. However, further research on the complex interaction between dietary macroalgae meal inclusions, luminal environment and microbiota composition is required to support this hypothesis.

In Australia, there are currently no commercial producers of *Gracilaria* sp. However, results from the current study are positive and provide considerable scope to develop and grow a *Gracilaria* sp. industry in Australia, which will be capable of supplying *Gracilaria* sp. meal for abalone formulated diets to improve abalone production. In contrast, Venus Shell Systems in Australia already produce a high quality *Ulva* sp. product that was used in diets in the current trial. At present, *Ulva* sp. meal produced by Venus Shell Systems is primarily for human applications, and is sold for >AUS$ 20 kg^{-1}. At this price, dietary inclusions of *Ulva* sp. meal are not economically viable. However, this price is based on a 3 tonne/annum production capacity, and in the near future, Venus Shell Systems envisage a short term future price of AUS$ 10 kg^{-1} and an order of magnitude lower again once production exceeds 100 tonnes/annum (personal communication, Dr. Pia Winberg, Venus Shell Systems Pty. Ltd., Bomaderry, NSW, Australia). In addition to the two macroalgae species investigated in the current study, Australia has a diverse and endemic macroalgae species, and there are likely numerous other macroalgae species that may be beneficial to incorporate into formulated diets for greenlip abalone. For example, two red alga species, *Gelidium australe* and *Solieria robusta*, were identified as the best candidates for aquaculture in integrated multi-trophic aquaculture (IMTA), which have additional ecological benefits of removing nutrient wastes from other aquaculture species (Lorbeer et al. 2013; Wiltshire et al. 2014). Further research focused on incorporating different dried macroalgae meal species into formulated diet for greenlip abalone would also likely benefit both the Australian abalone and macroalgae industries.

In conclusion, when considering the growth and feed utilisation of greenlip abalone fed *Gracilaria* sp. meal inclusions, we recommend 10 % dietary inclusions of *Gracilaria* sp. meal for abalone diets. Although there are currently no commercial producers, results from the current study suggest the need to develop and grow a *Gracilaria* sp. industry to supply a high quality ingredient for abalone diets. With regard to *Ulva* sp. meal, once production is economically viable for

inclusion into abalone feeds, we recommend a dietary inclusion of 5 % *Ulva* sp. meal to stimulate digestive enzyme activity and improve abalone growth. Furthermore, up to 20 % inclusion of *Ulva* sp. meal did not compromise growth indicating *Ulva* sp. meal may be successfully used to replace solvent extracted soybean meal, de-hulled lupin meal and wheat meal in abalone formulated diets. Results from the current study will contribute to further improvements of formulated diets for abalone, which may ultimately lead to abalone growth improvements. Furthermore, this study built a linkage between the macroalgae and abalone industries and also provides a momentum to grow and diversify an Australian macroalgae industry.

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