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Growth and feed utilisation of greenlip abalone (*Haliotis laevigata*) fed nutrient enriched macroalgae

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ABSTRACT

Wild greenlip abalone predominantly consume macroalgae, but are fed formulated diets under culture conditions. This study aims to (i) investigate the effect of nutrient enrichment (non-enriched and enriched) and fresh macroalgae type (*Ulva* sp., *Gracilaria cliftonii*, and an equal combination of *Ulva* sp. and *G. cliftonii*) on the growth and feed utilisation of greenlip abalone and (ii) compare the performance of abalone fed fresh macroalgae to those separately fed either one of the three commercial formulated diets. Abalone were fed to excess at 16:00 h daily, and uneaten feed was collected the following morning. Nutrient enrichment increased the protein level for *Ulva* sp. and *G. cliftonii* from 5.3 to 27.7% and 12.9 to 38.1%, respectively. The growth of abalone fed *G. cliftonii* was superior to animals fed *Ulva* sp. The effect of enrichment was macroalgae species-dependent. While abalone fed enriched *Ulva* sp. exhibited superior growth to those fed non-enriched *Ulva* sp., animals fed non-enriched and enriched *G. cliftonii* exhibited similar growth. However, abalone fed non-enriched *G. cliftonii* had superior apparent protein deposition and protein efficiency ratio, compared to animals fed enriched *G. cliftonii*. Feeding an equal mix of *Ulva* sp. and *G. cliftonii* had a positive synergistic effect on abalone growth, compared to animals fed mono-specific algae. Abalone fed each commercial diet exhibited improved growth and feed utilisation compared to animals fed fresh macroalgae. The results of the current study suggest that the use of fresh *G. cliftonii* as a source of carbohydrates may spare protein when feeding formulated diets to abalone. When formulated diets are unavailable or are inappropriate to feed abalone, a mix of enriched *Ulva* sp. and *G. cliftonii* may be used. However, feeding fresh macroalgae alone to cultured greenlip abalone should be avoided, if growth is the parameter of interest and we recommend that commercial formulated diets be fed to cultured greenlip abalone.

Statement of relevance: Abalone diet development with fresh macroalgae.

1. Introduction

Greenlip abalone (*Haliotis laevigata*) are cultured throughout southern Australia in land-based systems and fed formulated diets until market size (Fleming and Hone, 1996; Stone et al., 2013; Bansemer et al., 2014). However, the predominant diet of wild greenlip abalone is macroalgae (Shepherd, 1973). Macroalgae are also utilised as feed for cultured abalone in numerous countries including China, Korea and Chile (Kirkendale et al., 2010). Macroalgae improve abalone health and marketability and may also stimulate abalone feeding activity, which may improve growth, compared to those fed formulated diets (Allen et al., 2006; Brown et al., 2008; Lange et al., 2014; Stone et al., 2014; Buss et al., 2015). In Australia, feeding macroalgae to greenlip abalone was previously limited due to the prohibition of wild macroalgae collection on mainland Australia. Recently however, there has been increasing research to aid the development of an Australian macroalgae aquaculture industry, which would be capable of supplying high quality feed for farmed abalone (Lorbeer et al., 2013).

Numerous macroalgae species are cultured globally and abalone accept a variety of macroalgae species. Two macroalgae genera, the red algae *Gracilaria* spp. and the green algae *Ulva* spp. have been identified as excellent candidates for abalone feed (Naidoo et al., 2006; Viera et al., 2011). Dietary protein is the first growth limiting macronutrient for abalone, and the optimal dietary protein level for abalone ranges from 24% to 47% (Bansemer et al., 2014). However, non-enriched macroalgae are generally low in protein (11–19% dry) (Viera et al., 2011). Two management options to overcome protein and nutrient limitations when feeding fresh macroalgae to abalone are recommended: (i) prior to

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feeding, macroalgae should be cultured in a nutrient enriching medium; and (ii) macroalgae should be fed as mixed macroalgae diets (Shpigel et al., 1999; Viera et al., 2011). Some macroalgae genera, including *Gracilaria* spp. and *Ulva* spp., are able to assimilate exogenous inorganic nitrogen for amino acid and protein synthesis (Hernández et al., 2002; Taylor et al., 2006). Culturing macroalgae in a nutrient enriching medium can increase the protein level to >30% (Viera et al., 2011). Abalone fed nitrogen/protein-enriched macroalgae exhibited superior growth compared to those fed non-enriched macroalgae (Shpigel et al., 1999; Viera et al., 2011). In addition to nutrient enrichment, feeding mixed macroalgae to abalone is recommended as it provides a superior balance of essential nutrients, such as amino acids, compared to monospecific macroalgal diets (Viera et al., 2011). Although nutrient enrichment and mixed macroalgae diets have been tested for a range of other abalone species, macroalgae preference and nutritional requirements of abalone are species-specific. For example, while most abalone species generally prefer brown macroalgae species (e.g. *Macrocystis* spp., *Ecklonia* spp. and *Laminaria* spp.), Australian abalone generally prefer red algae and *Ulva* spp., and avoid brown algae (Fleming, 1995; Flores-Aguilar et al., 2007; Cornwall et al., 2009). There is a need to investigate the growth and feed utilisation of greenlip abalone fed nutrient-enriched algae, before fresh macroalgae is incorporated into commercial on-farm feeding practices for greenlip abalone.

It is important to note that if macroalgae form part of the feeding regime for cultured abalone, growth should not be compromised compared to current formulated diets. The current literature relating to the growth of abalone fed fresh macroalgae and formulated diets is controversial and conflicting (Naidoo et al., 2006; Garcìa-Esquivel and Felbeck, 2009; Hernández et al., 2009; Mulvaney et al., 2013a). For example, red abalone (*Haliotis rufescens*) fed a formulated diet exhibited superior growth to abalone fed *Macrocystis pyrifera* (Garcìa-Esquivel and Felbeck, 2009). In contrast, Mulvaney et al. (2013a) reported significantly lower growth rates for hybrid abalone (*H. laevigata* × *Haliotis rubra*) fed a formulated diet, compared to abalone fed fresh macroalgae (*Ulva* spp. and *Grateloupia* spp.). Further research is required to investigate the growth and feed utilisation of abalone fed fresh macroalgae and commercial diets. In this study, we aimed to: (i) investigate the effect of nutrient enrichment (non-enriched and enriched) and macroalgae type (*Ulva* sp., *Gracilaria cliftonii* and an equal mix of *Ulva* sp. and *G. cliftonii*) on growth performance and feed utilisation of greenlip abalone; and (ii) compare the growth performance and feed utilisation of abalone fed fresh macroalgae to those separately fed either one of the three commercial formulated diets.

2. Methods

2.1. Experimental animals and system

Greenlip abalone (0.80 ± 0.01 g; shell length 17.94 ± 0.03 mm) were purchased from Kangaroo Island Abalone Pty Ltd. (Smith Bay, SA, Australia). Prior to stocking, abalone were held in a flow-through seawater system at South Australia Research and Development Institute Aquatic Sciences (West Beach, SA, Australia), and fed a commercial formulated diet ad libitum ("Abgrow premium" 5 mm chip; Eyre Peninsula Aquafeed Pty Ltd., Lonsdale, SA, Australia).

The experiment was conducted in a photoperiod and temperature controlled laboratory described in Stone et al. (2013). Briefly, abalone were housed in one of 36 12.5 L blue plastic culture units (bottom surface area of 1129 cm^2), and were supplied with sand filtered, UV treated, flow-through seawater at a rate of 300 mL min^{-1}. Water depth was held at 2.5 cm (effective tank water volume of 2.8 L) using a standpipe with a mesh screen (0.8 mm) on the outlet to retain uneaten food. Water temperature was held at 22 °C by using a 3 kW immersion heater (240 V, JQ20; Austin & Cridland, Carlton, NSW, Australia) in the system sump.

2.2. Stocking

Abalone were gently prised from the holding tank using a spatula. Fifteen animals were weighed, measured and stocked into four replicate culture units per dietary treatment. Abalone were acclimated to the system for two weeks and were fed a commercial formulated diet ("Abgrow premium" 5 mm chip). After seven days the water temperature was slowly raised from 19 °C to the final temperature of 22 °C. Dead abalone were recorded, measured, weighed and replaced with abalone of a similar weight, which had been fed their respective diet at 22 °C.

2.3. Diets and feeding

In this 93 day study, we utilised a 3 × 2 factorial design, three macroalgae types (*Ulva* sp., *G. cliftonii*, and a mixed algae diet consisting of an equal mixture of both species) were fed as either non-enriched or nutrient/protein enriched treatments. In addition, abalone were separately fed either one of the three commercial formulated diets, which acted as controls to compare with animals fed fresh macroalgae.

Fresh *Ulva* sp. and *G. cliftonii* were collected from intertidal sand-flats at Outer Harbor (Gulf St. Vincent, SA, Australia), and cultured in four 4000 L tanks, under ambient sunlight. Non-enriched *Ulva* sp. and *G. cliftonii* were supplied with fresh seawater at a rate of 8 L min^{-1}. Enriched *Ulva* sp. and *G. cliftonii* were held in static seawater and enriched fortnightly with 8 L of modified F2 nutrient media (Guillard and Ryther, 1962; Lange et al., 2014). Tanks were provided with aeration through a bottom-central pipeline to keep macroalgae in motion. The *G. cliftonii* were covered with shade cloth (80% nominal shade) to reduce epiphytic growth, while *Ulva* sp. was exposed to direct sunlight. During the experimental period, macroalgae were sub-sampled weekly, and samples within a tank were pooled for proximate composition analyses. Three formulated diets (3–5 mm chip) were supplied by different feed companies (Eyre Peninsula Aquafeed; Aquafeeds Australia [formally Adam and Amos], Mount Barker, SA, Australia; and Skretting Australia, Cambridge, TAS, Australia) and were stored at −20 °C prior to feeding. Proximate composition of macroalgae and commercial formulated diets is presented in Table 1.

Prior to feeding, macroalgae were spun dry in a salad spinner (Woolworths, Baulkham Hills NSW, Australia), weighed into individual feeding containers for each tank and topped up with seawater. Commercial formulated diets were fed as supplied. Abalone were fed to excess daily at 16:00 h. Feed rates were maintained at 14% and 4.5% abalone biomass day^{-1} for macroalgae and commercial diet treatments, respectively, and were adjusted throughout the experiment based on monthly bulk weight checks.

Tanks were cleaned and uneaten feed was collected by sieving the entire tank contents through a fine mesh at 08:30 h daily. Uneaten macroalgae were spun dry in a salad spinner and weighed. Uneaten formulated diets were collected, stored at −20 °C and were later dried at 105 °C for 16 h. Daily feed consumption was calculated by the difference between feed offered and uneaten feed in dry weight. To account for macroalgae growth, *Ulva* sp. and *G. cliftonii* were added to tanks without animals present at 22 °C at 16:00 h daily, collected at 08:30 h, spun dry in a salad spinner and weighed. To account for the leaching loss of formulated diets, diets were added to tanks without animals present at 22 °C. The weight difference of feed measured between 16:00 h and 08:30 h was used as a correction factor to calculate the apparent feed consumption rate.

2.4. Biochemical and water quality analyses

The proximate composition analyses of diets and whole body tissue were conducted according to methods in the British Pharmacopoeia Commission (2004) or German Institute for Standardization (DIN) (2000). At the commencement of the experiment, the soft tissue of 40 animals ($n = 4$ replicates) were collected, shucked and stored at −20

Table 1
Nutrient composition of non-enriched, nutrient-enriched and mixed fresh macroalgae diets and commercial formulated diets (dry g 100 g^{-1}).

	Non-enriched macroalgae			Enriched macroalgae			Commercial formulated diets		
	Ulva sp.	*G. cliftonii*	Mixed[a]	*Ulva* sp.	*G. cliftonii*	Mixed[a]	Diet A	Diet B	Diet C
Proximate composition									
Moisture	79.3	84.5	81.9	80.8	85.6	83.2	7.9	10.0	8.9
Crude protein	5.3	12.9	9.1	27.7	38.1	32.9	36.9	34.0	36.7
Lipid	1.6	1.8	1.7	1.8	1.6	1.7	5.2	5.0	6.7
Gross energy (MJ kg^{-1})	14.17	16.19	15.18	16.91	16.23	16.57	16.83	16.89	17.01
Ash	27.7	27.7	27.7	24.3	28.9	26.6	7.3	6.9	8.3
Carbohydrate[b]	65.4	57.6	61.5	46.2	31.4	38.8	50.6	54.1	48.3
Amino acids									
Arginine	0.20	0.53	0.37	2.06	3.25	2.66	1.77	1.83	1.98
Histidine	0.08	0.16	0.12	0.30	0.31	0.31	0.73	0.73	0.80
Isoleucine	0.17	0.52	0.35	0.74	0.91	0.83	1.29	1.26	1.26
Leucine	0.29	0.76	0.53	1.28	1.33	1.31	2.23	2.13	2.20
Lysine	0.17	0.51	0.34	1.03	0.95	0.99	1.99	1.96	1.75
Methionine	0.08	0.09	0.09	0.28	0.23	0.26	0.39	0.31	0.35
Phenylalanine	0.21	0.57	0.39	0.93	0.92	0.93	1.56	1.42	1.45
Threonine	0.18	0.54	0.36	0.84	0.94	0.89	1.14	1.08	1.11
Valine	0.26	0.63	0.45	1.12	1.06	1.09	1.39	1.39	1.44
Total amino acids	3.47	9.14	6.31	18.58	19.47	19.03	29.23	26.99	27.54

[a] Composition of mixed macroalgae diet is calculated based on feeding an equal mix (1:1 by wet weight) of *Ulva* sp. and *Gracilaria cliftonii*.
[b] Carbohydrate = 100% − (protein % + lipid % + ash %).

°C to analyse the initial soft tissue protein and energy composition. At the end of the experiment, five abalone from each tank were collected, shucked and stored at −20 °C. Abalone were later pooled for each tank for the analysis of soft tissue protein and energy composition.

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 the feed values were based on an as fed and dry values:

Biomass gain (g $tank^{-1}$) = (final weight + ∑mortality weight) − (initial weight + ∑replacement weight)
Specific growth rate (SGR, % day^{-1}) = ([ln final weight − ln initial weight] / days) × 100
Shell growth rate (μm day^{-1}) = (final shell length − initial shell length) / days
Apparent feed consumption = (feed offered − uneaten feed collected − ([total feed offered × % leaching loss without animals] + [uneaten feed collected / % retained without animals × % leaching loss without animals]) / 2) / tank biomass (Stone et al., 2013)
Apparent feed conversion ratio (FCR) = feed consumed/abalone weight gain
Apparent protein efficiency ratio (PER) = abalone weight gain/protein consumed
Apparent energy efficiency ratio (EER) = abalone weight gain/energy consumed
Apparent protein deposition = ([final soft body protein − initial soft body protein] / protein intake) × 100
Apparent energy deposition = ([final soft body energy − initial soft body energy] / energy intake) × 100.

Water quality parameters were monitored daily. Water temperature was measured using an alcohol filled thermometer. Dissolved oxygen (mg L^{-1} and % saturation) was measured using a dissolved oxygen meter (OxyGuard International A/S, Birkerød, Denmark). The pH was measured using a meter (Oakton pHtestr 20; Oakton Instruments, Vernon Hills, IL, USA). Salinity (g L^{-1}) was measured using a portable salinity refractometer (model RF20, Extech Instruments, Nashua, NH, USA).

2.5. Statistical analyses

IBM SPSS (Version 22 for Windows; IBM SPSS Inc., Chicago, IL, 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 Shapiro–Wilk test, respectively. All percentage data were arcsine transformed before analyses. Initial weight, initial shell length and mortality were compared between all treatments and were analysed using a one-factor ANOVA. To assess the effect of nutrient enrichment (non-enriched and enriched) and macroalgae types (*Ulva* sp., *G. cliftonii* and mixed diet) on abalone performance, data were analysed using two-factor (2 × 3) ANOVA. When significant main effects were observed, Fisher's least significant difference post-hoc test was used to detect significant differences between treatment means. When significant interactions between macroalgae types and nutrient enrichment were observed, pairwise comparisons were used to determine significant differences between treatment combinations (Fisher's least significant difference). To correct for the experimentwise error rate for pairwise comparisons, a significance level of $P < 0.01$ was used. There were no significant differences in performance between abalone separately fed either one of the three commercial diets (one-factor ANOVA). Data for abalone fed the three commercial diets were pooled ($n = 12$), and used as a control to compare to each fresh macroalgae treatment ($n = 4$ replicates $treatment^{-1}$; one-factor ANOVA; Dunnett's post-hoc test). A significance level of $P < 0.05$ was used for all statistical tests. All values are presented as means ± standard error of the mean, unless otherwise stated. If the SE was <0.01 it is reported as "0.01".

3. Results

3.1. General observations

The average initial weight and shell length of abalone were 0.80 ± 0.01 g and 17.94 ± 0.03 mm, respectively, and were not significantly different between diets (one-factor ANOVA; $P = 0.329$ and 0.819, respectively). Throughout the study, water quality parameters were maintained at levels appropriate for greenlip abalone: water temperature (21.9 ± 0.4, 20.8–23.0 °C [mean ± standard deviation, range]), dissolved oxygen (97 ± 4, 88–107% saturation; 7.0 ± 0.5, 6.0–8.7 mg L^{-1}), pH (8.2 ± 0.1, 7.6–8.6) and salinity (35 ± 1, 34–36).

Animals exhibited normal signs of feeding behaviour and fed actively on all diets during the study. No gross signs of disease were observed in abalone. The overall mortality rate of abalone during the study was 0.76%, and was not affected by diet (one-factor ANOVA; $P = 0.845$). Nutrient enrichment of *Ulva* sp. and *G. cliftonii* increased dietary protein level (dry) from 5.3 to 27.7% and 12.9 to 38.1%, respectively (Table 1).

Table 2
Growth performance, feed efficiency and nutrient retention of greenlip abalone fed non-enriched and nutrient-enriched mono- and mixed-macroalgae diets and commercial diets.[1]

Enrichment		Non-enriched macroalgae			Enriched macroalgae			Dunnet's test (*P* value)[2]	2 factor ANOVA (*P* value)[3]		
Macroalgae	Control commercial diets	*Ulva* sp.	*G. cliftonii*	Mixed	*Ulva* sp.	*G. cliftonii*	Mixed		Macroalgae (A)	Enrichment (B)	A × B
Growth performance											
Biomass gain (g tank^{-1})	70.24 ± 2.07^{a}	4.29 ± 0.11^{b}	38.06 ± 1.69^{b}	29.85 ± 2.85^{b}	23.58 ± 2.80^{b}	37.14 ± 6.86^{b}	45.31 ± 2.27^{b}	<0.001	<0.001	<0.001	0.020
SGR (% day^{-1})	2.07 ± 0.03^{a}	0.33 ± 0.01^{b}	1.55 ± 0.03^{b}	1.36 ± 0.07^{b}	1.10 ± 0.05^{b}	1.35 ± 0.02^{b}	1.70 ± 0.04^{b}	<0.001	<0.001	<0.001	<0.001
Shell growth rate (μm day^{-1})	181.49 ± 3.34^{a}	23.56 ± 0.96^{b}	128.85 ± 4.09^{b}	104.65 ± 7.15^{b}	75.85 ± 1.64^{b}	109.63 ± 1.98^{b}	143.05 ± 3.04^{b}	<0.001	<0.001	<0.001	<0.001
Feed utilisation											
Feed consumption rate (g as fed kg abalone^{-1} day^{-1})	10.61 ± 0.45^{b}	25.42 ± 1.27^{a}	62.19 ± 0.80^{a}	51.72 ± 0.10^{a}	38.54 ± 0.59^{a}	61.38 ± 0.25^{a}	48.12 ± 1.22^{a}	<0.001	<0.001	<0.001	<0.001
Apparent FCR (as fed)	0.67 ± 0.03^{b}	7.78 ± 0.49^{a}	4.68 ± 0.13^{a}	4.35 ± 0.22^{a}	3.51 ± 0.37^{a}	4.48 ± 0.62^{a}	3.39 ± 0.12^{a}	<0.001	0.001	<0.001	<0.001
Nutrient retention											
Apparent PER	5.20 ± 0.22^{b}	3.41 ± 0.09^{c}	9.77 ± 0.20^{a}	10.93 ± 0.57^{a}	4.78 ± 0.35^{b}	3.66 ± 0.04^{c}	5.54 ± 0.19^{b}	<0.001	<0.001	<0.001	<0.001
Apparent PD	28.86 ± 1.56^{b}	6.37 ± 2.26^{c}	73.62 ± 3.47^{a}	74.68 ± 3.92^{a}	32.35 ± 0.88^{b}	27.15 ± 1.47^{b}	38.69 ± 2.04^{a}	<0.001	<0.001	<0.001	<0.001
Apparent EER	11.73 ± 0.45^{a}	5.23 ± 0.32^{b}	10.06 ± 0.27^{a}	9.95 ± 0.48^{a}	10.74 ± 1.24^{a}	12.23 ± 2.31^{a}	12.53 ± 0.42^{a}	<0.001	0.014 (U < G = M)	0.001 (NE < E)	0.287
Apparent ED	3.41 ± 0.17^{a}	0.90 ± 0.12^{b}	3.03 ± 0.14^{a}	2.90 ± 0.10^{a}	2.86 ± 0.19^{a}	3.08 ± 0.13^{a}	3.54 ± 0.13^{a}	<0.001	<0.001	<0.001	<0.001
Proximate composition											
Moisture (%)	77.24 ± 0.37	77.93 ± 0.09	77.15 ± 0.57	77.50 ± 0.27	76.96 ± 0.59	77.23 ± 0.53	78.12 ± 0.57	0.690	0.440	0.829	0.264
Protein (% dry)	38.99 ± 0.71^{b}	48.23 ± 1.70^{a}	50.32 ± 2.37^{a}	48.57 ± 2.96^{a}	47.10 ± 2.28^{a}	50.25 ± 2.45^{a}	49.41 ± 2.29^{a}	<0.001	0.505	0.949	0.917
Energy (MJ kg^{-1} dry)	19.18 ± 0.33	19.38 ± 0.64	19.65 ± 0.26	19.72 ± 0.23	19.21 ± 0.14	20.41 ± 0.14	19.58 ± 0.16	0.337	0.091	0.578	0.268

SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio; PD, protein deposition; EER, energy efficiency ratio; ED, energy deposition. U, *Ulva* sp.; G, *Gracilaria cliftonii*; M, mixed macroalgae diets; NE, non-enriched; E, enriched. Initial soft tissue content: moisture (76.92%) protein (67.37% dry) and energy (20.06 MJ kg^{-1} dry).

[1] (mean ± SE; $n = 4$). SE less than 0.01 is reported as "0.01". A significance level of $P < 0.05$ was used for all statistical tests.

[2] Abalone fed the three commercial diets were pooled ($n = 12$), and used as a control and compared to abalone fed fresh macroalgae ($n = 4$ treatment^{-1}; one-factor ANOVA; Dunnett's post-hoc test). ^{a,b,c} values without a common superscript compared to the control are significantly different (^{a} indicates the highest value; $P < 0.05$).

[3] 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 [$P < 0.01$]).

3.2. Interactive effects between macroalgae type and enrichment

3.2.1. Growth performance

Biomass gain, SGR and shell growth rate of abalone were significantly affected by the interaction between enrichment (non-enriched and enriched) and macroalgae type (*Ulva* sp., *G. cliftonii* and mixed diet) (two-factor ANOVA; $P < 0.001$; Table 2). While abalone fed *G. cliftonii* exhibited superior growth to animals fed *Ulva* sp., abalone fed enriched mixed macroalgae exhibited significantly superior growth to those fed other macroalgae treatments. The interaction between enrichment and macroalgae type was due to the significant superior growth performance for abalone fed enriched *Ulva* sp. and mixed macroalgae, while abalone fed enriched *G. cliftonii* exhibited inferior growth, compared to abalone fed respective non-enriched treatments ($P < 0.001$; Table 2).

3.2.2. Feed use

Feed consumption rate (g as fed kg abalone^{-1} day^{-1}) was significantly influenced by the interaction between enrichment and macroalgae type ($P < 0.001$; Table 2). The significant interaction was due to the significant increase in feed consumption rate of abalone fed enriched *Ulva* sp. compared to abalone fed non-enriched *Ulva* sp., while abalone fed enriched *G. cliftonii* and mixed macroalgae diets had similar or significantly lower feed consumption rates compared to non-enriched treatments, respectively. Abalone fed *G. cliftonii* (non-enriched and enriched) had significantly higher feed consumption rates than abalone fed mixed macroalgae treatments, which were significantly higher than those fed *Ulva* sp. ($P < 0.001$; Table 2).

The apparent FCR (as fed) was significantly affected by the interaction between enrichment and macroalgae type ($P < 0.001$; Table 2). The interaction was due to the significantly superior FCR for abalone fed enriched *Ulva* sp. compared to abalone fed non-enriched *Ulva* sp., while feeding abalone enriched *G. cliftonii* and mixed macroalgae diets did not significantly influence FCR.

3.2.3. Soft tissue composition and nutrient use

The soft tissue composition (moisture, protein and energy) of abalone was not significantly influenced by macroalgae type, enrichment, and the interaction between the two factors ($P > 0.05$; Table 2).

Apparent protein efficiency ratio (PER) and apparent protein deposition of abalone were significantly influenced by the interaction between macroalgae type and enrichment ($P < 0.001$; Table 2). The interaction between enrichment and macroalgae type was similar for apparent PER and apparent protein deposition. The interaction was due to the significant increase in PER and protein deposition for abalone fed enriched *Ulva* sp. compared to non-enriched *Ulva* sp., while the apparent PER and protein deposition for abalone fed non-enriched *G. cliftonii* and mixed macroalgae diets were significantly higher than those fed enriched equivalent treatments. Abalone fed non-enriched *G. cliftonii* and non-enriched mixed macroalgae had significantly higher apparent PER than abalone fed other diets.

The apparent energy efficiency ratio (EER) was significantly affected by macroalgae type ($P = 0.014$; *Ulva* sp. < *G. cliftonii* = mixed) and enrichment ($P = 0.001$; non-enriched < enriched), but not by the interaction between these two factors ($P = 0.287$; Table 2). Apparent energy deposition of abalone was significant influenced by the interaction between enrichment and macroalgae type ($P < 0.001$). Energy deposition increased for abalone fed enriched macroalgae, although the effect was more pronounced for abalone fed *Ulva* sp., compared to abalone fed *G. cliftonii* and mixed macroalgae diets. Moreover, the increase of energy deposition was also more pronounced for abalone fed enriched mixed macroalgae diets, compared to enriched *G. cliftonii.*

3.3. Comparison between commercial diets and macroalgae

3.3.1. Growth performance

The performance of abalone separately fed either one of the three commercial diets was analysed using one-factor ANOVA. There were no significant differences between abalone fed the three commercial diets. Data from abalone fed the three commercial diets were pooled ($n = 12$), and used as a control to compare against the abalone fed fresh macroalgae ($n = 4$ per treatment; one-factor ANOVA; Dunnett's post-hoc test). Abalone fed commercial diets exhibited significantly superior biomass gain, SGR and shell growth rate than animals fed any of the six macroalgae diets (one-factor ANOVA; Dunnett's post-hoc test; $P < 0.001$; Table 2).

3.3.2. Feed use

Abalone fed commercial diets had significantly lower feed consumption rates (g as fed kg abalone^{-1} day^{-1}) than those fed fresh macroalgae ($P < 0.001$; Table 2). On a dry basis however, the feed consumption rates of abalone fed commercial diets were similar to animals fed non-enriched and enriched *G. cliftonii* and mixed macroalgae treatments ($P > 0.05$). The feed consumption rates of abalone fed commercial diets were significantly higher than those fed non-enriched and enriched *Ulva* sp. ($P < 0.05$; Table 2).

Apparent FCR (as fed) for abalone fed commercial diets was significantly lower than for those fed all macroalgae diets ($P < 0.001$). On a dry basis however, the apparent FCR for abalone fed commercial diets was similar to abalone fed all macroalgae treatments, except for non-enriched *Ulva* sp. Abalone fed non-enriched *Ulva* sp. had significantly higher apparent FCR (dry) than abalone fed commercial diets ($P < 0.05$; Table 2).

3.3.3. Soft tissue composition and nutrient use

The soft tissue moisture and energy content of abalone fed commercial diets were not significantly different from abalone fed macroalgae treatments ($P > 0.05$; Table 2). In contrast, the soft tissue protein content of abalone fed commercial diets was significantly lower than abalone fed macroalgae ($P < 0.001$; Table 2).

Apparent PER of abalone fed commercial diets was significantly lower than abalone fed non-enriched *G. cliftonii* and non-enriched mixed macroalgae, statistically similar to abalone fed enriched *Ulva* sp. and enriched mixed macroalgae, and significantly superior to abalone fed non-enriched *Ulva* sp. and enriched *G. cliftonii* ($P < 0.001$; Table 2). Apparent protein deposition for abalone was significantly influenced by diet, and the effect was similar to apparent PER, but differed from abalone fed enriched *G. cliftonii* and enriched mixed macroalgae. Abalone fed enriched *G. cliftonii* and enriched mixed macroalgae had statistically similar and significantly superior protein deposition compared to abalone fed commercial diets, respectively (Table 2).

Apparent EER and apparent energy deposition for abalone fed commercial diets were similar to abalone fed all macroalgae treatments, except for non-enriched *Ulva* sp. Abalone fed non-enriched *Ulva* sp. had significantly higher EER and energy deposition than abalone fed commercial diets ($P > 0.05$; Table 2).

4. Discussion

Greenlip abalone (1-year old; 1.8 g) require ~35% dietary protein to achieve optimal growth at 22 °C (Stone et al., 2013). However, the protein level of non-enriched macroalgae is ~11–19% (dry) (Viera et al., 2011). In the current study, nutrient enrichment was successful, and increased protein levels of *Ulva* sp. and *G. cliftonii* from 5.3 to 27.7%, and from 12.9 to 38.1%, respectively, which is comparable to previous studies that utilised nutrient/protein enriched macroalgae as feed for abalone (Shpigel et al., 1999; Viera et al., 2011; Mulvaney et al., 2013a). Furthermore, protein levels of enriched *Ulva* sp. and *G. cliftonii* approached or exceeded the optimal protein level for greenlip abalone

at 22 °C (~35%; Stone et al., 2013). Abalone fed *G. cliftonii* outperformed those fed *Ulva* sp., which is likely related to the higher preference and intake for red macroalgae by wild greenlip abalone. However, the benefit of enrichment on abalone growth and feed utilisation was macroalgae species-dependent.

In the current study, abalone fed non-enriched *Ulva* sp. exhibited inferior growth, compared to those fed enriched *Ulva* sp. Previous studies reported greenlip abalone readily accepted and consumed non-enriched *Ulva* sp. (Stone et al., 2014; Bansemer et al., 2015b). In the current study however, abalone fed non-enriched *Ulva* sp. had depressed feed intake compared to those fed other diets. In addition to depressed feed intake, non-enriched *Ulva* sp. was low in protein (5.3%) and energy (14.17 MJ kg^{-1}). Nutrient enrichment of *Ulva* sp. improved protein (27.7%) and energy (16.91 MJ kg^{-1}) levels and also improved the feed intake. The improved nutritional profile, particularly the protein level and amino acid composition, and feed intake likely caused superior growth for abalone fed enriched *Ulva* sp., compared to animals fed non-enriched *Ulva* sp. The growth benefit of feeding enriched *Ulva* sp. to abalone is consistent with previous abalone growth studies (Shpigel et al., 1999; Viera et al., 2011). In contrast to the current study, an equal mix of non-enriched *Ulva australis* and *Ulva laetevirens* promoted similar or superior growth for a closely related hybrid abalone (*H. laevigata* × *H. rubra*), compared to those separately fed either one of the commercial diets, or enriched *U. laetevirens* and *Grateloupia turuturu* combinations (Mulvaney et al., 2013a). It should be noted that the authors collected *U. australis* and *U. laetevirens* from abalone nursery tanks and outflow drains, which were likely exposed to higher inorganic nitrogen levels, evident by the higher protein level (>20% dry; Mulvaney et al., 2013a), than non-enriched *Ulva* sp. utilised in the current study.

In contrast to *Ulva* sp., abalone fed non-enriched *G. cliftonii* exhibited similar growth to animals fed enriched *G. cliftonii*. Although dietary protein is the first limiting macronutrient for abalone growth (Fleming and Hone, 1996; Britz and Hecht, 1997), other nutritional factors in *G. cliftonii* may be of equal importance for abalone growth. In the current study, nutrient enrichment increased the dietary protein level of *G. cliftonii*, but this was at the expense of carbohydrates. Red macroalgae species contain unique carbohydrates that are specific to the group, including agar, carrageenan and floridean starch. Greenlip abalone are anatomically and biochemically adapted to digest and utilise these unique carbohydrates, which may also be important to abalone growth (Shepherd, 1973; Harris et al., 1998). This notion is further supported by superior protein utilisation (apparent protein deposition and PER) observed in abalone fed non-enriched *G. cliftonii* to animals fed enriched *G. cliftonii*. While numerous studies have focused on optimising protein levels in abalone diets (Mai et al., 1995; Britz, 1996; Dunstan, 2010; Stone et al., 2013), abalone fed high protein diets (>35% crude protein) deaminate protein to supply energy for metabolism, rather than protein deposition and tissue growth (Bansemer et al., 2015a). To overcome this problem, carbohydrates in *G. cliftonii* may be an available energy source to spare protein and improve protein utilisation when abalone are fed high protein diets. However, the growth performance of abalone fed *G. cliftonii* was inferior to animals fed formulated diets. To improve growth and protein utilisation, feeding formulated diets in conjunction with fresh macroalgae (*M. pyrifera*, *Lessonia berteroana* or *Lessonia spicata*) also improved the PER of red abalone (*H. rufescens*; Kemp et al., 2015). Moreover, abalone (*Haliotis discus hannai*) exhibited superior feed efficiency when fed a formulated diet with dietary inclusions of dried macroalgae meal (combination of *Laminaria digitata*, *Palmaria palmate* and *Ulva lactuca*) compared to animals fed fresh *L. digitata* alone (O'Mahoney et al., 2014). Based on results from the current study, we recommend further research to investigate the use of fresh *G. cliftonii* and formulated diet combinations, dried *G. cliftonii* meal inclusions or inclusions of carbohydrate extracts from *G. cliftonii*, which may ultimately improve greenlip abalone growth and nutrient utilisation.

Compared to abalone fed *G. cliftonii* and *Ulva* sp. separately, feeding enriched mixed macroalgae (*G. cliftonii* and *Ulva* sp.) had a synergistic effect on abalone growth and FCR. Abalone fed enriched mixed macroalgae diets were supplied with higher protein level and amino acid levels, particularly lysine, the first limiting amino acid, compared to animals fed enriched *Ulva* sp. or *G. cliftonii*, respectively (Fleming et al., 1996). These results are consistent with previous studies that have utilised mono- and mixed-macroalgae diets. For example, Viera et al. (2011) reported superior growth performance for *Haliotis tuberculata coccinea* fed a diet consisting of mixed macroalgae species. The authors suggested that abalone fed mixed macroalgae were supplied with a superior balance of essential nutrients and amino acid profile to animals fed mono-specific macroalgal diets, and concluded that fresh macroalgae can be fed to abalone until market size (Viera et al., 2011).

However, formulated diets are currently fed to cultured greenlip abalone in Australia. If macroalgae are to be used as part of the feeding regime for cultured abalone, it is important that the growth and feed utilisation of animals are comparable to currently used commercially available feeds. In the current study, although fresh macroalgae supported excellent growth, abalone fed formulated diets exhibited superior growth to those fed fresh macroalgae. Commercial formulated diets contain highly palatable and digestible dietary ingredients, which include fish meal, cereal grains, oilseeds and pulses, which are carefully formulated to optimise dietary energy, lipid, protein and amino acid levels, and essential vitamins and minerals for growth (Stone et al., 2013; Bansemer et al., 2014). While the protein level of enriched macroalgae was similar to commercial diets, commercial diets had superior amino acid profiles compared to fresh macroalgae, which likely influenced abalone growth. Although numerous studies have focused on comparing the growth of abalone fed fresh macroalgae and formulated diets, results are conflicting. Formulated diets promoted superior growth rates for red abalone (*H. rufescens*; Garcìa-Esquivel and Felbeck, 2009). However, South African abalone (*Haliotis midae*), *H. rufescens* and *H. laevigata* × *H. rubra* fed fresh macroalgae outperformed those fed formulated diets (Naidoo et al., 2006; Hernández et al., 2009; Mulvaney et al., 2013a). In addition, Hernández et al. (2009) and Mulvaney et al. (2013a) used pre-weaned animals, and these results may differ if animals had been weaned on to formulated diets prior to the commencement of the study. This is supported by extremely poor growth for *H. laevigata* × *H. rubra* fed a commercial diet (0.39% day^{-1}; Mulvaney et al., 2013a), compared to the growth for greenlip abalone fed commercial diets in the current study (2.07% day^{-1}). However, further differences in feeding regimes and feed availability between studies may have also influenced results. This study highlights the importance of species-specific data, for both macroalgae species and abalone species, and not applying general information from one abalone or macroalgae species to another.

In conclusion, based on results from the current study, we recommend the use of formulated diets for cultured greenlip abalone, as they support excellent growth and feed utilisation. We recommend that greenlip abalone should not be fed fresh macroalgae alone, as this practice may lead to sub-optimal growth. Australian abalone farms are not currently set up to feed fresh macroalgae, which would require further additional infrastructure. Recently, there has been increased interest to culture Australian abalone in offshore sea–cage systems, where formulated diets may be inappropriate as abalone feed, due to diet stability problems (Mulvaney et al., 2013b). Under these conditions, it may be beneficial to feed enriched-mixed macroalgae to greenlip abalone, although this would likely result in a longer grow-out period.

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