Received: 15 January 2020 | Revised: 22 July 2020 | Accepted: 3 August 2020

DOI: 10.1111/are.14832

ORIGINAL ARTICLE

Aquaculture Research WILEY

'Energy budgets for greenlip abalone (*Haliotis laevigata* Donovan) fed live macroalgae compared to commercial formulated diets'

Duong N. Duong[1] | David A.J. Stone[1,2,3,4] | Jian G. Qin[1] | Matthew S. Bansemer[1,2] | James O. Harris[1,3]

[1]College of Science and Engineering, Flinders University, Adelaide, SA, Australia

[2]SARDI Aquatic Sciences, Henley Beach, SA, Australia

[3]Marine Innovation Southern Australia, Adelaide, SA, Australia

[4]School of Animal and Veterinary Sciences, The University of Adelaide, Roseworthy, SA, Australia

Correspondence
James O. Harris, College of Science and Engineering, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia.
Email: James.Harris@flinders.edu.au

FUNDING INFORMATION
This work was supported by the South Australian Government Primary Industries and Regions South Australia Agribusiness Accelerator Program; Flinders University; the Australian Abalone Growers' Association; and the AusAID Australian Development Scholarship for Duong Ngoc Duong.

Abstract

Energy budgets were developed for greenlip abalone (*Haliotis laevigata*) under different feeding scenarios to predict growth, metabolic and waste outputs. The energy budgets of abalone fed live macroalgae (*Ulva* sp. or *Gracilaria cliftonii*) and an equal mix of those two macroalgae species with and without nutrient enrichment, or one of three control formulated commercial diets, at 22°C for 93 days were investigated. Among non-enriched algal treatments, abalone fed *G. cliftonii* and mixed diet treatments had significantly higher ingested feed energy and absorbed energy than those fed *Ulva* sp. Abalone fed non-enriched *G. cliftonii* invested significantly more energy into somatic growth than those fed *Ulva* sp. and the mixed diet treatment. For diets with nutrient enrichment, ingested feed energy and absorbed energy rate of abalone fed *G. cliftonii* and mixed diet treatments were significantly higher than those fed *Ulva* sp. Nutrient enrichment increased crude protein in live macroalgae, and ammonia excretion energy rate was higher for abalone fed enriched macroalgae compared to non-enriched. Abalone fed the control commercial diets spent more energy in all components compared to those fed the live macroalgae treatments. The major component of the energy budgets in abalone fed the commercial diets and live macroalgae diets was somatic growth energy, ranging from 25.5% to 37.7% of ingested feed energy, except for abalone fed live *Ulva* sp. where the major component was respiration energy (38.5%). Overall, abalone fed the formulated diets or live non-enriched *G. cliftonii* could increase ingested feed energy, absorbed energy and somatic growth energy.

KEYWORDS
abalone, energy budgets, enrichment, macroalgae, nutrition

1 | INTRODUCTION

Abalone aquaculture has developed rapidly in many countries due to increasing global demand. The high value of abalone has stimulated considerable effort into the development and optimization of intensive abalone culture (Cook & Gordon, 2010). The successful culture of this species relies on understanding and managing many aspects of production, including reproduction (Freeman et al., 2006), nutrition (Britz, 1996a, 1996b; Mai, Mercer, & Donlon, 1994, 1995a, 1995b), dietary manipulation (Bansemer et al., 2016a; Bansemer

et al., 2016b; Stone, Bansemer, & Harris, 2014; Stone et al., 2014), ecology and habitat (Shepherd, 1973; Shepherd & Turner, 1985), and environmental requirements (Freeman, 2001).

Abalone are herbivorous gastropods, consuming mainly macroalgae in the wild (Mottet, 1978). In aquaculture, live macroalgae diets are widely used for culturing abalone in many countries including China, Korea, South Africa and Chile (Kirkendale, Robertson-Andersson, & Winberg, 2010). Feeding live macroalgae to abalone results in a number of advantages including improved marketability, less nutrient leaching compared to formulated feeds and most importantly, very low mortality (Bansemer et al., 2016c; Kirkendale et al., 2010). However, live macroalgae are relatively low in nutrient density and quality, particularly protein, which can affect greenlip abalone (*Haliotis laevigata*) growth (Bansemer et al., 2016a; Bansemer et al., 2016b; Bansemer et al., 2016c). Previous studies showed that the culture of macroalgae in nutrient-rich waters enhances their nutritional profile, specifically via increased protein content. Feeding nutrient-enriched macroalgae improved growth rates of the green ormer (*Haliotis tuberculata coccinea*) (Viera et al., 2011), the Pacific abalone (*Haliotis discus hannai*) (Shpigel, Ragg, Lupatsch, & Neori, 1999), the South African abalone (*Haliotis midae*) (Naidoo, Maneveldt, Ruck, & Bolton, 2006), Roe's abalone (*Haliotis roei*) (Boarder & Shpigel, 2001) and greenlip abalone (Bansemer et al., 2016a), compared to non-enriched equivalent treatments. However, in several countries, such as Australia, formulated diets are preferred for abalone culture as they promote much faster growth than macroalgae diets (Bansemer et al., 2016a).

The determination of energy budgets is an efficient method for studying energy flow, transformation and losses of energy consumed (Jobling, 1993). Energy budgets provide a framework for the evaluation of various ways in which energy derived from dietary nutrients is allocated and utilized (Lawrence & Lane, 1982). The energy budget is derived using a range of variable including ingested feed energy, egested faecal energy, somatic growth energy, shell growth energy, reproduction energy, respiration energy, ammonia excretion energy and mucus production energy (Barkai & Griffiths, 1988; Peck, Culley & Helm, 1987). An understanding of the balance between energy intake, energy expenditure and energy losses is not only useful for the evaluation of species for aquaculture, but also for the prediction of growth patterns, reproductive strategies, waste production, mortality and population dynamics in cultured species (Donovan & Carefoot, 1998; Peck et al., 1987). The measurement of physiological processes, such as respiration, excretion and mucus production, may allow feeding regimes, production rates and efficiencies of culture systems to be assessed when costs of feed and product returns are known (Peck et al., 1987).

Greenlip abalone is currently one of the main species of abalone cultured in Australia (Freeman et al., 2006). Although live macroalgae are a feed source of wild greenlip abalone, land-based cultured post-juvenile abalone in Australia are fed almost exclusively with commercial diets. Some previous studies have been carried out to investigate the survival, health and growth of greenlip abalone fed live or dried macroalgae or commercial formulated diets (Bansemer et al., 2016a; Bansemer et al., 2016b; Dang, Li, Speck, & Benkendorff, 2011; Lange, Currie, Howarth, & Stone, 2014; Stone et al., 2014), while energy budgets have been developed for haliotids such as *Haliotis tuberculata* (Lopez & Tyler, 2006; Peck et al., 1987), *H. midae* (Barkai & Griffiths, 1987, 1988), the northern abalone (*Haliotis kamtschatkana*) (Donovan & Carefoot, 1998), the green abalone (*Haliotis fulgens*) (Farías, García-Esquivel, & Viana, 2003; Gómez-Montes et al., 2003) and the Thai or Ass's-ear abalone (*Haliotis asinina*) (Ganmanee, Sirirustananun, & Jarayabhand, 2010). However, there are no energy budgets of greenlip abalone fed either live macroalgae (with or without nutrient enrichment) or formulated diet available.

In this study, we aimed to (a) establish energy budgets for greenlip abalone fed either live macroalgae (*Ulva* sp. and *Gracilaria cliftonii*) with and without nutrient enrichment or commercial diets currently used in the culture of this species; and (b) examine whether components of the energy budget for juvenile greenlip abalone are affected by diet type (live non-enriched or nutrient-enriched macroalgae, or commercial diets).

2 | MATERIALS AND METHODS

The energetic study reported in this manuscript followed on from a 93-day growth trial with greenlip abalone reported in Bansemer et al. (2016a). The energetics study involved three separate experimental phases: (a) collecting and analysing initial and final abalone tissue samples, and feed samples from the growth study; (b) determination of energy allocation values using respirometry following the completion of the growth trial; and (c) mucus production evaluation following the completion of the growth trial.

2.1 | Experimental animals

One-year-old greenlip abalone were obtained from Kangaroo Island Abalone Pty Ltd and prior to the commencement of the experiments were held in 1000-L tanks supplied with flow-through ambient temperature sand-filtered seawater at South Australian Research and Development Institute (SARDI) Aquatic Science Centre (ASC) at West Beach, South Australia. During this period, abalone were fed a commercial formulated diet (5 mm chip; Abgrow diet, Eyre Peninsula Aquafeeds Pty Ltd, Lonsdale, SA, Australia) at a rate of ~3% body weight per day.

2.2 | Experimental diets

In this study, we utilized a 3 × 2 factorial design, with three live macroalgae types (live *Ulva* sp., live *G. cliftonii* or an equal mix of these two live macroalgae species) were fed as either non-enriched or nutrient-/protein-enriched treatments. In addition, greenlip abalone were separately fed either one of the three commercial formulated diets (Eyre Peninsula Aquafeeds, Aquafeeds Australia and Skretting

Australia), which acted as controls for comparisons with the response of animals fed live macroalgae treatments.

Information on the actual commercial diet ingredient formulations was limited due to confidentiality. However, proximate dietary analysis of the nutrients and energy was completed for all diets (Table 1). Two species of live macroalgae (*Ulva* sp. and *G. cliftonii*) were collected from the intertidal sand flats at Outer Harbor, Gulf St Vincent, S.A., Australia, and then cultured in seawater parabolic tanks at SARDI ASC. The culture water of live *Ulva* sp. and *G. cliftonii* was enriched fortnightly with 8 L of modified F2 nutrient media (Bansemer et al., 2016a; Guillard & Ryther, 1962).

2.3 | Growth trial phase

The 93-day growth experiment was conducted at the SARDI ASC, and the experimental system and design was previously described in Bansemer et al. (2016a). In brief, 36 culture tanks (12.5 L rectangular blue plastic tanks; 39.2 long × 28.8 wide × 11.0 cm deep; Nally IH305, Viscount Plastics Pty Ltd) were used in this study. Each culture tank had a screened standpipes on the tank outlets to maintain a water depth of 3 cm and an effective water volume of 3.4 L. All experimental tanks were provided with single-pass flow-through water from the reservoir by gravity at 300 ml/min, and water temperature was maintained at 22 ± 1°C. The light was provided using fluorescent lighting (low light intensity 3.4 lx), and the photoperiod during the experimental period was set as 12-hr low light and 12-hr dark.

At stocking, the experiment was conducted in a completely randomized manner where dietary treatments were assigned randomly to the 36 experimental tanks (9 treatments; $n = 4$). Fifteen greenlip abalone (initial weight, 0.80 ± 0.01 g and shell length [SL], 17.94 ± 0.03 mm; $n = 540$) were stocked into one of four replicate culture units per dietary treatment. Experimental animals were acclimated in the experimental system at 18°C for 1 week and given their respective experimental diets. After 1 week, water temperature was slowly increased, ~1°C/day to 22°C. This temperature was maintained for the remainder of the 93-day trial. Mortalities during the acclimation period and the experimental period were removed, weighed and replaced with animals of a similar weight that had been fed with the corresponding diet and held in the same tanks, provided with the same water under the same temperatures.

Abalone were fed at 16:00 hr daily with a feeding rate of 14% body weight per day for live *Ulva* sp. and *G. cliftonii* and 4% body weight per day for the commercial formulated diets. Feeding time and amount were established using information from Buss et al. (2015) and Stone et al. (2013). Macroalgae diet treatments were prepared prior to feeding. The live *Ulva* sp. and *G. cliftonii*

TABLE 1 Nutrient composition of live non-enriched and enriched macroalgal diets and formulated commercial diets as g 100 g^{-1} dry basis (reported by Bansemer et al., 2016a)

	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)	14.2	16.2	15.2	16.9	16.2	16.6	16.8	16.9	17.0
Ash (%)	27.7	27.7	27.7	24.3	28.9	26.6	7.3	6.9	8.3
Carbohydrate (%; calculated)[b]	65.4	57.6	61.5	46.2	31.4	38.8	50.6	54.1	48.3
Amino acids (g 100 g^{-1} diet as fed)									
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[c]	3.47	9.14	6.31	18.58	19.47	19.03	29.23	26.99	27.54

[a]Proximate composition of mixed macroalgae diet is calculated based on feeding an equal mix of *Ulva* sp. and *G. cliftonii*.

[b]Carbohydrate = 100%−(protein % + lipid % + ash %).

[c]Total amino acids were measured using different assay to individual amino acids.

were collected from the culture tanks and spun several times until completely de-watered using a salad spinner (Woolworths, Baulkham Hills NSW, Australia). The live *Ulva* sp. and *G. cliftonii* then were weighed and placed into 100-ml plastic feeding containers and topped up with seawater and stored indoors at ambient temperature until fed.

Uneaten feed was collected at 08:30 hr in the morning of the following day by pouring the entire tank contents through a fine mesh (500 μm). The uneaten formulated diets were collected, stored frozen at −20°C and subsequently dried in an oven at 105°C for 16 hr, while uneaten live *Ulva* sp. or live *G. cliftonii* were spun in a salad spinner and weighed again.

Feed intake was corrected for macroalgal growth and also for commercial diet leaching and collection losses. For calculating the growth of macroalgae at 22°C, *Ulva* sp. and *G. cliftonii* were weighed out into 100-ml feed containers at 13:00 hr and then introduced to tanks without abalone at 16:00 hr and collected and weighed at 08:30 hr following day. The increased weight of macroalgae was determined after being de-watered in a spinner. We also determined the proportion of uneaten formulated feed that was lost through leaching and through the collection net without animals in the tank, and then, we used this correction factor to calculate the corrected apparent feed intake per tank as per Stone et al. (2013). The amount of feed consumed for the macroalgae diet treatments was calculated by subtracting the amount of uneaten feed collected from the tank from the amount of feed offered and then correcting by adding the amount of growth of macroalgae. The amount of feed consumed for the commercial diet treatments was calculated by subtracting the amount of uneaten feed collected from the tank from the amount of feed offered and then correcting by subtracting the leaching loss.

With regard to sample collections, at stocking, 40 abalone (initial samples) and subsamples of each of the experimental diets were collected and stored at −80°C. At the completion of the growth phase, five abalone per tank were collected and also stored −80°C for the analysis of tissue and shell energy content.

Water quality was maintained at appropriate level for greenlip abalone (Harris, Maguire, Edwards, & Hindrum, 1999; Harris, Maguire, Edwards, & Johns, 1999; Stone et al., 2013). The water quality components included water temperature (21.9 ± 0.4°C), dissolved oxygen (97 ± 4% saturation or 7.0 ± 0.5 mg/L), pH (8.2 ± 0.1) and salinity (35 ± 1 ppt) (Bansemer et al., 2016a).

2.4 | Respirometry phase

Oxygen consumption and ammonia excretion were measured in individual respiration chambers following the growth phase. The metabolism of abalone is composed of standard metabolism, active metabolism and specific dynamic action (Jobling, 1994). However, only the standard metabolic rate was measured in this study as it allowed to evaluate the effects of different diets on oxygen consumption in abalone during the 93-day trial, while the measurement of other metabolic components such as specific dynamic action was eliminated due to fasting abalone.

For the successful operation of the respiration chamber system, the following factor had to be considered. To avoid hypoxia depressing their metabolic rates of abalone during incubation, the oxygen levels in the respiration chambers needed to be maintained above 70% oxygen saturation level (Harris et al., 1999). To ensure the oxygen saturation of the respiration chambers remained above 70% during the incubation period, a preliminary experiment was conducted.

In the preliminary experiment, five greenlip abalone (~6.94 g per abalone), previously fed the commercial diet, were stocked into each of four respiration chambers. Each chamber was supplied with aerated (~99% oxygen saturation) and temperature-controlled seawater (22 ± 1°C). The abalone were allowed to acclimate to the chambers for three days. During this period, they were fed to excess for two days, and on the fourth day, commencing at 09:00 hr the oxygen concentration (% saturation) in the chambers was measured at 09:00 hr, after 30, 60 and 90 min of incubation. Results indicated oxygen saturation levels in each chamber remained above the 70% (~85%) for up to 60 min and declined to ~69% saturation after 90 min (Figure 1). Based on these results, it was decided to run the incubation periods in the main respiration experiment for 60 min.

For the main respiration experiment, following final harvest of the growth phase, five abalone of each treatment (1.09–5.48 g per abalone) from each tank were randomly selected and introduced into a 1-L respiration chamber (n = 4 per treatment) for oxygen consumption and ammonia excretion measurements. Each chamber was supplied with aerated (~98% oxygen saturation) and temperature-controlled (22 ± 1°C) seawater for three days to reduce the effects of handling on oxygen consumption. For the first 2 days, abalone were fed (4% bw per day for the control commercial treatments and 14% bw per day for live macroalgae treatments) and then starved on the third day. At 09:00 hr on the fourth day, initial dissolved oxygen levels (mg/L and % saturation) in each respiration chamber were measured using a dissolved oxygen meter (OxyGuard International A/S, Birkerød, Denmark). Subsequently, the water supply was cut off for 1 hr of incubation. After an hour, the chambers were partly opened one by one, with just enough space to allow the head of oxygen meter (probes) into the water. This step was performed as quickly as possible to limit the diffusion of oxygen in the air into the water. In addition, there were four control chambers, which were run and sampled under the same condition as the other experimental chambers, but with no animals inside to account for the biological oxygen demand present in the water. The actual oxygen consumption value was adjusted based on these control chambers.

Water samples for the determination of ammonia excretion were also collected from each chamber, using a 5-ml syringe, immediately following the initial and 1-hr oxygen sampling times. Water samples were stored at −20°C. Ammonia was analysed using the salicylate hypochlorite method of Bower & Holm-Hansen (1980) and a

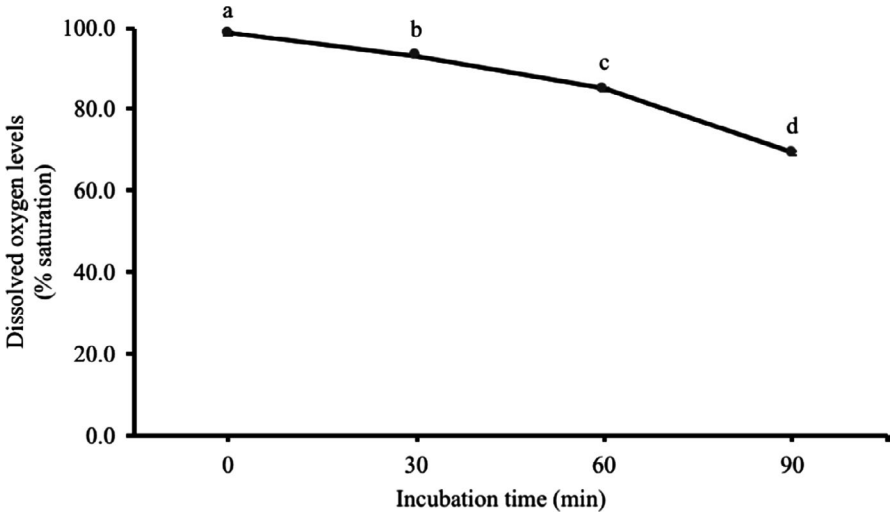

FIGURE 1 Optimization of the incubation period for respiration sampling based on the changes in dissolved oxygen levels (% saturation) in the 1-L respiration chambers containing five greenlip abalone (6.94 g abalone^{-1}) following 90-min incubation at 22°C (mean ± SE; $n = 4$; means which share the same superscript are not significantly different; $p > 0.05$; one-factor ANOVA; Tukey's HSD test)

FLUOstar Omega spectrophotometer at 640 nm (Omega software version 1.02–BMG Labtech, Germany).

2.5 | Pedal mucus production

Although fifteen abalone (0.80 g) were stocked into each tank, only five abalone per tank were available for energy budget study at the end of the experiment (10 abalone went to other studies and abalone size was generally small [1.09–5.48 g]). Thus, we were unable to collect a sufficient amount of mucus from each individual treatment to analyse energy content. In order to achieve the required quantity of mucus, the total number of 80 abalone (similar size and same batch which were used in this experiment) from the holding tank was placed onto four previously weighed 250-mm-diameter crystal plates and then immersed in a tank supplied with fresh seawater. Abalone were carefully removed after 10 min, and the plates were rinsed using distilled water to remove faeces and seawater. The plates were then dried at 70°C for 1 hr and reweighed, and mucus production was calculated by subtraction. The dried mucus was carefully scraped from the plate, and its energy content was analysed (Davies, 1993; Donovan & Carefoot, 1998).

2.6 | Determination of the components of the energy budget

Energy budgets were calculated for greenlip abalone by measuring each component of the energy budget in the equation described by Peck et al. (1987) and Lopez and Tyler (2006), with one modification (the component for shell growth energy [S] was added):

$$I - E = Pg + Pr + R + U + M + S$$

where I = ingested feed energy; E = egested faecal energy; Pg = somatic growth energy; Pr = reproduction energy (as gonad tissue is laid down when abalone are ~ three years old (Wells & Mulvay, 1995), Pr of one-year abalone was not investigated in this study); R = respiration energy; U = ammonia excretion energy; *M* = pedal mucus production energy; and S = shell growth energy.

Partitioning of each energy component (somatic growth, respiration, ammonia excretion, shell and pedal mucus production energy) was calculated as a percentage of feed ingestion energy, while the egested faecal energy was obtained from 100% ingested feed energy minus absorbed energy (Ab) (Ganmanee et al., 2010).

All energy allocation components were determined using samples of abalone, feed or faecal material that had been freeze-dried for 48 hr to constant mass. The energy content of each component was determined by bomb calorimetry using a micro-bomb calorimeter and the methods described below.

2.6.1 | Ingested feed energy (I)

Ingested feed energy rate (J g abalone^{-1} hr^{-1}) = [(amount of consumed feed × energy content of feed)/(initial weight + final weight)/2)]/time.

2.6.2 | Somatic growth energy (Pg)

Somatic growth energy rate (J g abalone^{-1} hr^{-1}) = [(final tissue energy–initial tissue energy)/[(initial weight + final weight)/2)]/time.

2.6.3 | Respiration energy (R)

Initially, the oxygen uptake rate was calculated as follows: oxygen uptake rate (mg g abalone^{-1} hr^{-1}) = [(initial levels of oxygen in the chamber–final levels of oxygen in the chamber–oxygen consumed in blank chamber) × volume of the chamber]/(biomass × time). Then, the rate of oxygen consumption was converted to energy equivalents (J g abalone^{-1} hr^{-1}) by multiplying by 14.77 J mg O_2^{-1} for

carbohydrate respiration, 13.72 J mg O_2^{-1} for lipid respiration and 13.39 J mg O_2^{-1} for protein respiration (Elliott & Davison, 1975).

2.6.4 | Ammonia excretion energy

Ammonia excretion rate (mg g abalone^{-1} hr^{-1}) = [(final concentration of ammonia in the chamber−initial concentration of ammonia in the chamber−concentration of ammonia in blank chamber) × volume of the chamber]/(biomass × time).

To account for energy loss through ammonia excretion, the conversion factor of 24.85 J mg NH_3^{-1} was used to convert the amount of ammonia production into energy value (J g abalone^{-1} hr^{-1}) (Elliott & Davison, 1975).

2.6.5 | Apparent energy digestibility, absorbed energy (Ab) and egested faecal energy (E)

Throughout the 93 days of the experiment, faecal material from each tank was picked up with a plastic 10-ml pipette and placed into a fine mesh to drain water out three times daily (11:00, 14:00 and 17:00 hr). Faecal samples were then transferred to a 50-ml container and stored in a −80°C freezer. At the end of experiment, samples were freeze-dried for 48 hr to constant mass and then determined energy content. The apparent digestibility coefficient (ADC) was also determined from the freeze-dried faecal material by using the ash insoluble acid (AIA) method of Van Keulen and Young (1977), modified by Montaño-Vargas, Shimada, Vásquez, and Viana (2002). Values for nutrient ADCs are reported in Bansemer et al. (2016a).

The apparent digestible energy coefficient (%) = 100 × [1−(F/D × D_{AIA}/F_{AIA})], where F is the per cent of nutrient or energy in faeces, D is the per cent of nutrient or energy in diet, D_{AIA} is the per cent of AIA in diet, and F_{AIA} is the per cent of AIA in faeces (Cho & Kaushik, 1990).

The amount of absorbed energy taken up by greenlip abalone was calculated using the following formula: absorbed energy (J g abalone^{-1} hr^{-1}) = (ingested feed (g) × energy content of the feed (J/g) × apparent digestible energy coefficient (%)/weight abalone (g)/time (hr).

The amount of egested faecal energy from greenlip abalone was calculated using the following formula: egested faecal energy rate (J g abalone^{-1} hr^{-1}) = Ingested feed energy (J g abalone^{-1} hr^{-1})−absorbed energy (J g abalone^{-1} hr^{-1}).

2.6.6 | Pedal mucus production energy (M) and shell growth energy (S)

Pedal mucus production energy rate (J g abalone^{-1} hr^{-1}) = (Mucus production × energy content of mucus)/(biomass × time).

Shell growth energy rate (J g abalone^{-1} hr^{-1}) = [(final shell energy content−initial energy content of shell)/(initial weight + final weight)/2]/time.

2.7 | Biochemical analysis

The biochemical compositions of the diets and test ingredients were analysed following the methods of the AOAC (1995). Crude protein (N × 6.25) was determined by the Kjeldahl method. Crude lipid was analysed with a Soxtherm rapid extraction system (Gerhardt GmbH and Co. KG, Konigswinter, Germany) with petroleum liquid (BP 100°C) as the extracting solvent. Ash was determined using a muffle furnace at 550°C for 16 hr. Carbohydrate was calculated by difference between 100% and total percentage of protein, lipid and ash (Table 1).

2.8 | Statistical analysis

The statistical program IBM SPSS (version 22 for Windows; IBM SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. In order to ensure normal distribution, the survival data were transformed, when Levene's test for equality of variance was not satisfied. To assess the effects of nutrient enrichment (non-enriched vs. enriched) and macroalgae treatment (*Ulva* sp., *G. cliftonii* and mixed diet) on the energy budget of greenlip abalone, the data were analysed using a two-factor ANOVA. When significant main effects were observed, Tukey's HSD post hoc test was used to detect significant differences between treatment means. When a significant interaction between macroalgae treatment and nutrient enrichment was observed, Tukey's HSD test was performed for macroalgae treatment within non-enriched or enriched diets. As there was no significant difference between different indices for the commercial diets, data for abalone fed the three commercial diets were pooled ($n = 12$) and used as a control to compare each of the six live macroalgae treatments ($n = 4$ replicates per treatment; 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.

3 | RESULTS

3.1 | General observation

The overall survival rate was 99.24%, and experimental abalone were healthy, showed no gross signs of disease and exhibited normal feeding behaviour over period of study.

3.2 | Ingested feed energy (I)

Each component of the energy budgets (J g abalone^{-1} hr^{-1}) for greenlip abalone fed the live macroalgae treatments, with and without nutrient enrichment, is displayed in Table 2. The ingested feed energy rate was significantly affected by the macroalgae treatment ($p < 0.001$; two-factor ANOVA; Table 2), nutrient enrichment ($p = 0.047$) and the interaction between the two factors ($p < 0.001$). Abalone fed *G. cliftonii* (4.56 J g abalone^{-1} hr^{-1}) and

TABLE 2 Energy budget components (J g abalone^{-1} hr^{-1}) of greenlip abalone fed control commercial diet, non-enriched and enriched mono- and mixed macroalgae diets[†]

Enrichment		Non-enriched macroalgae (NE)			Enriched macroalgae (E)			2-factor ANOVA (p-value)[‡]			Dunnett's test[§]
Diet	Control commercial diets[§]	*Ulva* sp.	*G. cliftonii.*	Mixed	*Ulva* sp.	*G. cliftonii*	Mixed	Macroalgae treatment (A)	Enrichment (B)	A × B	(p-value)
I	5.98 ± 0.18	2.44 ± 0.12^{a,*}	4.77 ± 0.06^{b*}	4.48 ± 0.01^{b,*}	3.43 ± 0.17^{a,*}	4.35 ± 0.02^{b,*}	4.39 ± 0.06^{b,*}	***(U < G=M)	(NE < E)	***	***
Ab	5.17 ± 0.15	1.83 ± 0.09^{a,*}	4.28 ± 0.06^{b,*}	3.90 ± 0.06^{b,*}	3.03 ± 0.15^{a,*}	3.81 ± 0.09^{b}	3.87 ± 0.06^{b,*}	*** (U < G=M)	* (NE < E)	***	***
Pg	1.75 ± 0.06	0.27 ± 0.06^{a,*}	1.72 ± 0.10^{c}	1.37 ± 0.05^{b,*}	1.27 ± 0.12*	1.56 ± 0.18	1.55 ± 0.05	*** (U < G=M)	*** (NE < E)	***	***
R	1.63 ± 0.07	0.95 ± 0.25*	1.03 ± 0.05*	1.01 ± 0.07*	0.72 ± 0.03*	0.75 ± 0.13*	0.94 ± 0.06*	NS	NS	NS	***
U	0.19 ± 0.03	0.07 ± 0.03*	0.09 ± 0.02	0.06 ± 0.01*	0.10 ± 0.03	0.15 ± 0.01	0.15 ± 0.01	NS	** (NE < E)	NS	***
E	0.81 ± 0.06	0.61 ± 0.03	0.49 ± 0.11*	0.58 ± 0.06	0.40 ± 0.05*	0.54 ± 0.08*	0.53 ± 0.10*	NS	NS	NS	**
M	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	NA	NA	NA	NA
S	0.04 ± 0.01	0.01 ± 0.01*	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01*	0.03 ± 0.01	0.04 ± 0.01	*** (U < G=M)	* (NE < E)	NS	***

Note: Values without a common superscript compared to the control are significantly different, *significant at $p < 0.05$; **significant at $p < 0.005$; ***significant at $p < 0.001$. NS: no significant interaction ($p > 0.05$).

Abbreviations: I, ingested feed energy; Ab, absorbed energy; Pg, somatic growth energy; Pr, reproduction energy; R, respiration energy; U, ammonia excretion energy; E, egested faecal energy; M, pedal mucus production energy; and S, shell growth energy (respiration energy, ammonia excretion energy and mucus production energy were calculated at the end of experiment, while others were measured using final and initial data); NA, not applicable.

[†]Data presented as mean ± *SE*; n, 4. SE <0.01 is reported as '0.01'.

[‡]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 (Tukey's HSD test; $p < 0.05$). [a–c]For parameters with a significant interaction, differences in type of macroalgae are compared within non-enriched or enriched diets (one-factor ANOVA, Tukey's HSD test); values without a common superscript are different ($p < 0.05$). U: *Ulva* sp.; G: *G. cliftonii*; M: mixed macroalgae diets; NE: non-enriched macroalgae; E: enriched macroalgae.

[§]Abalone fed the three commercial diets were pooled (due to no significant differences in performance between abalone separately fed either one of the three commercial diets (one-factor ANOVA), $n = 12$) and used as a control and compared to abalone fed fresh macroalgae ($n = 4$ per treatment; one-factor ANOVA; Dunnett's post hoc test).

mixed diets (4.44 J g abalone^{-1} hr^{-1}) had significantly higher ingested feed energy rates than those fed the *Ulva* sp. treatments (2.93 J g abalone^{-1} hr^{-1}) ($p < 0.001$). The ingested feed energy rate was significantly higher in abalone fed the nutrient enrichment diet treatments (4.06 J g abalone^{-1} hr^{-1}) compared to non-nutrient-enriched treatments (3.90 J g abalone^{-1} hr^{-1}) ($p = 0.047$). The interaction was due to a significant increase in ingested feed energy rate for abalone fed the live enriched *Ulva* sp. compared to those fed the corresponding non-enriched live *Ulva* sp. However, there was a significant reduction in ingested feed energy rate of abalone fed the live enriched *G. cliftonii* compared to those fed live non-enriched *G. cliftonii*. In abalone fed the non-enriched treatments, the highest ingested feed energy rate was found in live *G. cliftonii* which was significantly different from the live *Ulva* sp. ($p < 0.001$; one-factor ANOVA; Tukey's HSD test) but not from the mixed diet ($p = 0.070$). For enriched diets, abalone fed the live *Ulva* sp. had a significantly lower ingested feed energy rate than those fed the other live macroalgae diets ($p < 0.001$). The ingested feed energy rate of abalone fed the control commercial diets (pooled mean value) was significantly higher than those fed live macroalgae diets ($p < 0.001$; Dunnett's post hoc test; Table 2).

3.3 | Absorbed energy (Ab)

The absorbed energy rate was significantly influenced by macroalgae treatment ($p < 0.001$; two-factor ANOVA; Table 2), nutrient enrichment ($p = 0.021$) and the interaction between the two factors ($p < 0.001$). Absorbed energy rate of abalone fed *G. cliftonii* (4.04 J g abalone^{-1} hr^{-1}) and mixed diets (3.88 J g abalone^{-1} hr^{-1}) was significantly higher than in those fed *Ulva* sp. (2.43 J g abalone^{-1} hr^{-1}) ($p < 0.001$). Nutrient enrichment (3.57 J g abalone^{-1} hr^{-1}) improved absorbed energy of abalone compared to non-nutrient enrichment (3.34 J g abalone^{-1} hr^{-1}). The significant interaction was due to a significant increase in absorbed energy rate for abalone fed live enriched *Ulva* sp. compared to those fed the same diets without enrichment, whereas there was significant reduction in the absorbed energy rate for abalone fed live enriched *G. cliftonii* compared to those fed live non-enriched *G. cliftonii*. For non-enriched diets, abalone fed the live *Ulva* sp. had a significantly lower absorbed energy rate than those fed live *G. cliftonii* and the mixed diets ($p < 0.001$; one-factor ANOVA; Tukey's HSD test). Similarly, for diets with nutrient enrichment, the absorbed energy rate for abalone was significantly lower for those fed the live *Ulva* sp. than the mixed diet ($p = 0.001$) and the live *G. cliftonii* ($p = 0.002$). Abalone fed the control commercial diets (pooled mean value) had a significantly higher absorbed energy rate than those fed live macroalgae diets ($p < 0.001$; Dunnett's test; Table 2).

3.4 | Somatic growth energy (Pg)

Somatic growth energy rate was significantly affected by macroalgae treatment ($p < 0.001$; two-factor ANOVA; Table 2), nutrient enrichment ($p = 0.001$) and the interaction between the two factors ($p < 0.001$). Abalone fed live *G. cliftonii* (1.64 J g abalone^{-1} hr^{-1}) and the mixed diet treatments (1.46 J g abalone^{-1} hr^{-1}) invested significantly more energy in somatic growth than those fed the live *Ulva* sp. (0.77 J g abalone^{-1} hr^{-1}) ($p < 0.001$). Nutrient enrichment improved somatic growth energy rate of abalone (1.46 J g abalone^{-1} hr^{-1}) compared to non-nutrient enrichment (1.12 J g abalone^{-1} hr^{-1}). The interaction was due to a significantly higher somatic growth energy rate for abalone fed the live enriched *Ulva* sp. than those fed live non-enriched *Ulva* sp., while the somatic growth energy rate was significantly lower in abalone fed live enriched *G. cliftonii* than those fed the same diets without enrichment. Abalone fed live non-enriched *G. cliftonii* invested the highest energy for somatic growth and were significantly different to those fed the other macroalgal diets (live non-enriched *Ulva* sp., $p < 0.001$; non-mixed diet, $p = 0.007$; one-factor ANOVA; Tukey's HSD test). For enriched macroalgae, there was no significant difference in the somatic growth energy rate of abalone ($p > 0.05$). Abalone fed the control commercial diets (pooled mean value) had a higher somatic growth energy rate than those fed live non-enriched *Ulva* sp. ($p < 0.001$), non-enriched mixed diet ($p = 0.02$) and the live enriched *Ulva* sp. ($p = 0.003$; Dunnett's test; Table 2). However, no significant difference was found in somatic growth energy rate of abalone fed the control commercial diets (pooled mean value) compared to those fed the live non-enriched *G. cliftonii*, live enriched *G. cliftonii* and the enriched mixed diets ($p > 0.05$).

3.5 | Respiration energy (R)

The respiration energy rate of greenlip abalone was not affected by macroalgae treatment ($p = 0.331$), nutrient enrichment ($p = 0.070$) or the interaction between the two factors ($p = 0.626$; two-factor ANOVA; Table 2). Abalone fed the control commercial diets (pooled mean value) had a significantly higher respiration energy rate than those fed live macroalgae treatments ($p < 0.001$; Dunnett's test; Table 2).

3.6 | Ammonia excretion energy (U)

Nutrient enrichment significantly affected ammonia excretion energy rate ($p = 0.002$; two-factor ANOVA; Table 2), while macroalgae treatment ($p = 0.293$) and the interaction between the two factors ($p = 0.431$) did not. Abalone fed enriched diets (0.13 J g abalone^{-1} hr^{-1}) had higher ammonia excretion energy rates than those fed the corresponding non-enriched diets (0.08 J g abalone^{-1} hr^{-1}) ($p = 0.002$). Ammonia excretion energy rate for abalone fed the control commercial diets (pooled mean value) was significantly higher than those fed live non-enriched *Ulva* sp. ($p = 0.019$; Dunnett's test; Table 2) and non-enriched mixed diets ($p = 0.012$), but was not significantly different to the remaining dietary treatments ($p > 0.05$).

3.7 | Egested faecal energy (E)

The egested faecal energy rate was not significantly affected by macroalgae treatment ($p = 0.794$), nutrient enrichment ($p = 0.283$) or the interaction between the two factors ($p = 0.250$; two-factor ANOVA; Table 2). Egested faecal energy rate of abalone fed the control commercial diets (pooled mean value) was significantly higher than those fed live *G. cliftonii* and the mixed diets with or without nutrient enrichment ($p = 0.003$), but not from those fed the live enriched and non-enriched *Ulva* sp. diets ($p > 0.05$; Dunnett's test; Table 2).

3.8 | Mucus production energy (M) and shell growth energy (S)

The mucus production energy rate was 0.05 J g abalone^{-1} hr^{-1} for all treatments in this study.

The shell growth energy rate was significantly affected by macroalgae treatment ($p = 0.001$; two-factor ANOVA; Table 2) and nutrient enrichment ($p = 0.035$), but was not influenced by an interaction between the two factors ($p = 0.084$). Shell growth energy rate was significantly lower for abalone fed live *Ulva* sp. (0.018 J g abalone^{-1} hr^{-1}) compared to those fed live *G. cliftonii* (0.029 J g abalone^{-1} hr^{-1}) ($p = 0.021$) and the mixed diets (0.036 J g abalone^{-1} hr^{-1}) ($p < 0.001$). Abalone fed live *G. cliftonii* and the mixed diet had similar shell growth energy rates ($p = 0.090$). Abalone fed diets with nutrient enrichment (0.031 J g abalone^{-1} hr^{-1}) had significantly higher shell growth energy rates than those fed the same diets without nutrient enrichment (0.024 J g abalone^{-1} hr^{-1}). Shell growth energy rate in abalone fed the control commercial diets (pooled mean value) was significantly higher than those fed live *Ulva* sp. with or without nutrient enrichment ($p < 0.001$), but not from those fed the other diets ($p > 0.05$; Dunnett's test; Table 2).

3.9 | Energy budgets

The energy budgets for abalone fed live macroalgae or the control commercial diets (pooled mean value) are displayed in Table 3. The major components of the energy budget varied depending on the diets. The absorbed energy (%) was the lowest in abalone fed the live non-enriched *Ulva* sp. The major components of the energy budget for abalone fed the live non-enriched *Ulva* sp. were respiration energy (38.5%) and egested faecal energy (25.0%). With the exception of live non-enriched *Ulva* sp., abalone fed the other diets investigated in the current study allocated most energy to somatic growth, ranging from 25.5% for abalone fed the control commercial diets (pooled mean value) to 37.7% for abalone fed the live enriched *Ulva* sp. Ammonia excretion energy formed a smaller proportion of energy budgets (from 1.43% in non-enriched mixed diet to 3.35% in the live enriched *G. cliftonii* and the enriched mixed diet). Mucus production and shell growth energy accounted for the smallest proportion of the energy budgets, ranging from 0.81% to 1.97% and from 0.42% to 0.87% respectively.

4 | DISCUSSION

The somatic growth energy values obtained in the current study demonstrate that abalone fed live *G. cliftonii* invested more energy in somatic growth than those fed live *Ulva* sp. The differences in the somatic growth energy of abalone fed live *G. cliftonii* and *Ulva* sp. may be attributed to feeding preference, digestibility and utilization of the macroalgae. This species' known preference for red macroalgae (Bansemer et al., 2016b; Fleming, 1995; Shepherd, 1973; Stuart & Brown, 1994) resulted in higher ingested feed energy in the current study. Feed preference of abalone may reflect more efficiency in the ability to digest preferred algae species. Greenlip abalone are anatomically and biochemically adapted to digest and utilize unique carbohydrates found in live *G. cliftonii* such as agar and floridean starch, which are energy sources available for metabolism and growth (Bansemer et al., 2016b; Stuart & Brown, 1994). According to Stuart & Brown (1994), blackfoot abalone, *Haliotis iris*, fed a diet containing *Gracilaria chilensis* assimilated protein, lipid and carbohydrates more efficiently than those fed sea lettuce, *Ulva lactuca*. Similarly, greenlip abalone fed live *G. cliftonii* had higher absorbed energy than those fed live *Ulva* sp. in the current study. Lower absorbed energy of abalone fed live *Ulva* sp. reflects the low nutritional value of this macroalgae, particularly in protein content and amino acid profiles. Additionally, *Ulva* spp. are known to contain some anti-nutrients such as saponins, tannins and phytic acid that may inhibit digestion (Azaza et al., 2008).

Nutrient enrichment of live macroalgal feed is an important economic consideration in the culture of abalone as it can markedly influence their growth rate due to increased protein content and energy density of the feed (Bansemer et al., 2016a; Shpigel et al., 1999; Viera et al., 2011). For example, the protein contents of *Ulva rigida* and *Gracilaria cornea* cultured using waste water effluents in ponds were increased from 16.6% to 33.8% and 11.3% to 29.4% respectively (Viera et al., 2011). Similarly, Bansemer et al. (2016a) reported that live *Ulva* sp. and *G. cliftonii* cultured in a nitrogen-/protein-enriching medium increased protein levels from 5.3% to 27.7% and 12.9% to 38.1% respectively. Typically, abalone fed nutrient-enriched macroalgae displayed higher growth rates compared with those fed non-enriched algae (Bansemer et al., 2016a; Shpigel et al., 1999; Viera et al., 2011). In the current study, nutrient enrichment significantly affected ingested feed energy rate, somatic growth energy rate and ammonia excretion energy rate of greenlip abalone. However, the advantage of nutrient enrichment on somatic growth energy rate of greenlip abalone was dependent on the macroalgae treatment. Greenlip abalone fed enriched live *Ulva* sp. and the enriched mixed diet treatments expended more energy on somatic growth than those fed without enrichment, but no effect of nutrient enrichment was found in the enriched *G. cliftonii* treatment. Ogino & Kato (1964) were among the first to suggest that the

TABLE 3 The components of the energy budgets (%) of greenlip abalone (*Haliotis laevigata*) fed the control commercial diets or live enriched and non-enriched macroalgae[a,b]

Diet	Control commercial diets	Non-enriched macroalgae			Enriched macroalgae		
		Ulva sp.	*G. cliftonii*	Mixed	*Ulva* sp.	*G. cliftonii*	Mixed
I	100	100	100	100	100	100	100
Ab	86.50 ± 0.77	75.00 ± 0.01	89.80 ± 2.54	87.00 ± 1.31	88.40 ± 1.21	87.50 ± 1.92	88.10 ± 2.09
Pg	25.50 ± 0.46	10.80 ± 2.39	36.00 ± 1.81	30.50 ± 1.07	37.70 ± 5.20	36.00 ± 4.25	30.50 ± 1.07
R	27.40 ± 1.21	38.50 ± 9.98	21.60 ± 1.31	22.60 ± 1.58	21.00 ± 0.78	17.30 ± 2.95	21.40 ± 1.63
U	3.11 ± 0.39	3.03 ± 1.34	1.89 ± 0.35	1.43 ± 0.33	3.05 ± 0.76	3.35 ± 0.20	3.35 ± 0.20
E	13.50 ± 0.77	25.00 ± 0.01	10.20 ± 2.55	13.00 ± 0.31	11.60 ± 1.21	12.50 ± 1.92	11.90 ± 2.09
M	0.81 ± 0.02	1.97 ± 0.10	1.00 ± 0.01	1.07 ± 0.01	1.41 ± 0.07	1.10 ± 0.01	1.09 ± 0.01
S	0.67 ± 0.04	0.42 ± 0.09	0.62 ± 0.10	0.74 ± 0.05	0.78 ± 0.13	0.66 ± 0.10	0.87 ± 0.08
Unexplained energy	29.00	20.30	28.70	30.70	24.50	29.10	30.90

Note: Abalone fed the three commercial diets were pooled ($n = 12$) and live macroalgae diets ($n = 4$ treatment^{-1}).

Abbreviations: I, ingested feed energy; Ab, absorbed energy; Pg, somatic growth energy; Pr, reproduction energy; R, respiration energy; U, ammonia excretion energy; E, egested faecal energy; M, pedal mucus production energy; and S, shell growth energy.

Absorbed energy (Ab) and other energy components (Pg, R, U, E, M and S) were calculated as a percentage of ingested feed energy. Egested faecal energy obtained from 100% ingested feed energy minus absorbed energy. Unexplained was calculated as I (100%)–(Pg + Pr + R + U + M + S + E).

[a]Unavailable data for Pr as abalone showed not visible signs on gonad development. Respiration energy, ammonia excretion energy and mucus production energy were calculated at the end of experiment, while others were measured using final and initial data.

[b]Data presented as mean ± SE; $n = 4$.

growth of abalone is influenced by the content of protein in the diet. Dietary protein deficiency may result in a reduction in growth rates, whereas those in excess may cause extra feed costs to the producer and negative effects to water quality (Coote, Hone, Van Barneveld, & Maguire, 2000). Similarly, Mai, Mercer, and Donlon (1995b) and Britz (1996b) reported that the availability of suitable quantity and quality of dietary protein is considered to be a prime factor that affects the growth of abalone fed macroalgae diets. Thus, if *Ulva* sp. is to be used as a feed, it will be necessary to enrich it for culturing abalone. However, in some cases, energy of diets is an issue when the growth of abalone is independent of dietary protein levels.

The growth of some abalone species was significantly affected by dietary digestible energy as these animals eat to satisfy their energy requirements (Green, Jones, & Britz, 2011; Stone et al., 2013). In the present study, abalone fed live non-enriched and enriched *G. cliftonii* exhibited similar somatic growth energy even though the protein content was increased by a factor of over five in the enriched diet compared to the diet without enrichment. According to Stone et al. (2013), the growth rate of greenlip abalone was not affected by dietary protein levels when the dietary digestible energy (12.5 MJ/kg) was constant between diets. It is possible that in the current study, the somatic growth energy rate of abalone fed live non-enriched and enriched *G. cliftonii* was not significantly different due to the similarity of dietary digestible energy levels.

In the present study, the somatic growth energy rate of abalone fed the control commercial diets was similar to those fed live enriched or non-enriched *G. cliftonii*, even though dietary crude protein was different. It is again possible that dietary digestible energy was similar between the commercial diet and *G. cliftonii* diets. Therefore, once the growth of abalone was protein-independent, the energy or protein:energy ratio may be the next important component that affects somatic growth energy of abalone fed the commercial diet or *G. cliftonii* diets. Further study is required to clarify the role of somatic growth energy of greenlip abalone.

In this study, the components of the energy budgets for greenlip abalone varied greatly, and were dependent on macroalgae treatments and nutrient enrichment. One-year-old greenlip abalone fed live non-enriched *Ulva* sp. expended most of their energy into respiration or egested faeces, while those fed other diets channelled the major component of the ingested feed energy into somatic growth. Lopez & Tyler (2006) used female *H. tuberculata* (~0.96 g) fed macroalgae (66% *Palmaria palmata* and 34% *U. lactuca*) at 22°C to determine the energy budget and reported that 37.3% of ingested feed energy was invested into somatic growth. Peck et al. (1987) also reported that somatic growth energy accounted for the major proportion of the energy budget for the Ormer fed *U. lactuca* at 15°C (37.5% of ingested feed energy) in ~0.04 g abalone. The possible reason for higher egested faecal energy in abalone fed non-enriched *Ulva* sp. in the current study may be due to poorly digestible green macroalgae components such as anti-nutritional factors including saponins, tannins and phytic acid, lower β-galactosidase and suboptimal amino acid profiles (Azaza et al., 2008; Wahbeh, 1997).

In the current study, ammonia excretion energy of greenlip abalone fed the control commercial diets and live macroalgae diet treatments only accounted for a small proportion of the energy budgets, ranging from 1.43% to 3.35% of ingested feed energy. Previous studies also reported minimal energy losses due to ammonia excretion in the South African abalone (<1%) (Barkai & Griffiths, 1988), ass's-ear abalone (~1.33%) (Ganmanee et al., 2010) and green ormer

(~1.53%) (Lopez & Tyler, 2006). Additionally, ammonia excretion energy was significantly influenced by nutrient enrichment in this study. Ammonia is the major nitrogenous excretory product in abalone, and excretion rates may be impacted by the quality and quantity of dietary protein (Bayne & Newell, 1983). Ammonia excretion was reported to increase with increasing protein and decreasing carbohydrate content in the diet in some abalone species (Rychly, 1980; Yang, Liou, & Liu, 2002). This is consistent with observations in the current study of the nutrient-enriched macroalgal treatments. Thus, the level of ammonia in the water should be considered in a farming situation, especially when enriched macroalgae is given to abalone as enriched diets may foul the water more quickly.

Previous studies showed that mucus production was affected by stress or fluctuations in water temperature. For example, large energy loss via mucus production energy (23.3%–29.1% of ingested feed energy) of *H. tuberculata* fed *U. lactuca* was found to be due to stress (Peck et al., 1987), while it increased from 4.0% in the winter to 16.0% in summer of ingested feed energy in the northern abalone, *H. kamtschatkana* (Donovan & Carefoot, 1998). Other research has shown that mucus production energy formed small portions of the energy budget and was independent of the diet. For example, Lopez and Tyler (2006) and Montaño-Vargas, Viana, D'Abramo, Shimada, and Vasquez - Pelaez (2005) reported that energy losses in the form of mucus production were not affected by diet and accounted for only 0.99% of the ingested feed energy in the ormer, fed both seaweed and formulated diets, or 0.73%–1.23% in the pink abalone (*Haliotis corrugata* Wood) fed formulated diets containing different protein content and starch/lipid ratios. It was also <1% of ingested feed energy in the South African abalone (Barkai & Griffiths, 1988). In the present study, we were not able to measure the mucus production energy corresponding to dietary treatments at the end of the experiment due to small size of animals. Thus, the absolute value of pedal mucus production energy (from 80 animals) was calculated and accounted for <2% of ingested feed energy. This lower mucus production energy may contribute to the high unexplained energy in this study (range of 20.30%–30.90%) since mucus is used for many processes in molluscs such as epithelial protection, egestion of faeces and cleaning the gills (Davies & Alison, 1998), but only pedal mucus production was measured in this energy budget. In addition, the amount of mucus washed away by water should also be considered. The methods used for the estimation of mucus production need further refinement in future studies.

In conclusion, somatic growth energy and respiration accounted for the largest proportion of the energy budgets, while the contribution from the other components was relatively small. The control commercial diets or live *G. cliftonii* or mixed macroalgae diets improved absorbed energy and somatic growth energy rate. While, in some cases, nutrient enrichment (protein) led to improvements in the energy budgets, however, improvements were dependent on macroalgae species. Overall, nutrient enrichment was more beneficial for enhancing the value of *Ulva* sp. The results of this study will assist in predicting feed consumption, respiration, somatic growth, ammonia excretion and nutrient effluent levels of greenlip abalone production at the optimal growth temperature (22°C) for live enriched and non-enriched *Ulva* sp. and *G. cliftonii*. Further research to understand changes to the components of the energy budget under sub-optimal conditions and at different abalone sizes is recommended.

CONFLICT OF INTEREST STATEMENT

The authors have declared that there is no conflict of interests.

ETHICS STATEMENT

Greenlip abalone are not covered in the current Australian Code for the Care and Use of Animals for Scientific Purposes, and as such, this work required no approval from an ethics committee. Nevertheless, we always extend the practices embedded in this Code to the animals in our care.

ACKNOWLEDGMENTS

The authors also wish to thank Joel Scanlon (Aquafeeds Australia) and Dr Tom Coote and Kym Heidenreich (Eyre Peninsula Aquafeeds) for their technical and financial contributions to the supply and manufacture of the diets. The authors also wish to acknowledge Ms. Krishna-Lee Currie and Dr. Hoang Hai Thanh of Flinders University and Dr. Sasi Nayar and Mr. Kristen Bott of SARDI Aquatic Sciences for technical assistance.

AUTHOR CONTRIBUTION

Duong Ngoc Duong made contributions to experimental design, running the experiment, collection and analysis of samples and interpretation of data, manuscript writing and revision. David A Stone provided the funding and experimental animals, laboratory facilities and contributed to the concept and experimental design, interpretation of data, and manuscript writing and revision. Matthew S Bansemer was involved in the concept, experimental design, running the experiment, sample preparation and analysis and provided inputs into the manuscript and revision. James O Harris contributed to concept, experimental design and manuscript preparation and revision, while Jian Guang Qin contributed to concept, experimental design and manuscript preparation and revision.

DATA AVAILABILITY STATEMENT

The authors have declared that this original energy budget research of greenlip abalone was done with the absence of shared data or data sets.

ORCID

Duong N. Duong https://orcid.org/0000-0002-2242-2808
Jian G. Qin https://orcid.org/0000-0003-2448-8058
James O. Harris https://orcid.org/0000-0002-0997-3015

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How to cite this article: Duong DN, Stone DAJ, Qin JG, Bansemer MS, Harris JO. 'Energy budgets for greenlip abalone (*Haliotis laevigata* Donovan) fed live macroalgae compared to commercial formulated diets'. *Aquac Res*. 2020;00:1–13. https://doi.org/10.1111/are.14832