Restricting feed ration has more effect than diet type on the feeding behaviour of greenlip abalone Haliotis laevigata

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Restricting feed ration has more effect than diet type on the feeding behaviour of greenlip abalone *Haliotis laevigata*

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Abalone farmed in Australia are predominantly fed formulated feeds, while in the wild, their diet consists of a mix of macroalgae. Here we investigated the feeding behaviour of juvenile greenlip abalone, *Haliotis laevigata;* fed live macroalgae and formulated diets at different rations; and observed their feeding behaviour using a novel ventral video monitoring technique. Four different diets (commercial chip, experimental flake, *Ulva* sp. and *Gracilaria cliftonii*) at two feeding rations (excess vs. restricted) were tested. Diet type had no effect on abalone movement, but macroalgal diets resulted in higher feed intakes. Restricting feed rations induced greater movement. Abalone moved little during the light period and moved mostly during darkness, except for animals on the restricted feed ration where feeding commenced during the light period. On farms, this phenomenon may be a useful behavioural indicator for identifying underfed abalone. Despite their lower intake, formulated diets promoted higher energy and nutritional intake, indicating that quantity of feed consumed is not solely indicative of nutritional gain. From a research perspective, the novel ventral monitoring method has created opportunities for further behavioural studies in molluscs.

Keywords: greenlip abalone; *Haliotis laevigata*; aquaculture; formulated diet; macroalgae; feed ration; feeding behaviour; novel video monitoring

Introduction

In the wild, juvenile abalone feed on diatoms before transferring to a range of different macroalgae species as they mature to adults (Tutschulte & Connell 1988; Stepto & Cook 1993; Naidoo et al. 2006). Abalone show feeding preferences for specific macroalgae species, with some red algae being highly favoured by a variety of abalone species, particularly Australian temperate species (Shepherd 1975; Tutschulte & Connell 1988; Shepherd & Steinberg 1992). The green algae, *Ulva australis*, was shown to be of second preference or sometimes avoided in the wild by the same abalone species (Shepherd 1975; Fleming 1995). Despite the preference for specific types of macroalgae, formulated feed has been used exclusively in Australian land-based abalone

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aquaculture since 1996 because it is more cost-effective in terms of the protein and energy supplied (Fleming et al. 1996; Stone et al. 2013).

Formulated feeds have a significant nutrient leaching problem for farmers in spite of their economic efficiency (Fleming et al. 1996). Between 26 and 54% of the amino acids leach out over a 24-h period, which compromises nutritional quality and, potentially, water quality (Coote 1998). Formulated feed is typically made with binding agents to delay leaching and allow chips to stay intact for approximately 48 h following immersion (Fleming et al. 1996). Adverse microbial and protozoan growth may occur after this time (Bissett et al. 1998). Despite minimising nutrient leaching loss, the binding agents also increase chip hardness, which has been shown to discourage feed consumption by abalone (Gorfine 1991; Fleming et al. 1996). Understanding how feeding regimes can therefore be manipulated by abalone farmers to optimise feed intake, growth and production efficiency is important. Studies of feeding behaviour to gain this understanding should be conducted with multiple diets, particularly to compare common formulated feed used in Australian abalone aquaculture with the natural, wild diets of abalone.

Previous studies investigating abalone diets compared their effect on growth rates (Naidoo et al. 2006; Dang et al. 2011; Stone et al. 2013; Bansemer et al. 2014), nutritional and health benefits (Shpigel et al. 1999; Schaefer et al. 2013; Stone et al. 2014; Lange et al. 2014), foraging behaviour in the wild (Tutschulte & Connell 1988), feeding preferences (McShane et al. 1994) and feeding stimuli (Allen et al. 2006). Little is known about the effect of feed availability and diet type on the feeding behaviour of abalone.

Allen et al. (2006), previously filming from a dorsal view in a laboratory setting, assessed the feeding behaviour of two-year-old New Zealand blackfoot abalone (paua) (*Haliotis iris*) fed a formulated diet and found that summer ingestion rates were higher than those in winter. Despite reporting two peaks where maximum locomotion occurred during darkness (2030–2330 and 0100–0530 h), the authors did not report peak feeding activity. Tutschulte and Connell (1988), also observing from a dorsal view, analysed the feeding behaviour of adult and juvenile pink (*Haliotis corrugata*) and green (*Haliotis fulgens*) abalone, in the wild in the Pacific Ocean off Santa Catalina Island, California, USA. Using dorsal view time-lapse cinematography, Tutschulte and Connell (1988) reported that green and pink adult abalone fed on macroalgae equally day and night, whereas juvenile abalone fed exclusively at night. Homing behaviour was also reported for pink abalone between one and two years of age under laboratory conditions and in the wild. Tutschulte and Connell (1988) also concluded that one- and two-year-old pink abalone were most active at night. The findings were supported by Day and Branch (2002), who observed juvenile South African abalone (*Haliotis midae*) behaviour from a dorsal view in the wild in Atlantic Ocean waters off the Cape Peninsula, South Africa.

Formulated feeds produce superior growth rates to macroalgal diets for abalone of up to 30 mm in shell length (Bansemer et al. 2014). The growth rates decrease for larger abalone across all diets (Kirkendale et al. 2010). The time of feed introduction and light intensity have also been shown to affect abalone feeding behaviour, with darkness stimulating both higher grazing and growth rates compared to light exposure for post-larvae, six-day-old red abalone (*Haliotis rufescens*) in static conditions (Searcy-Bernal & Gorrostieta-Hurtado 2007). Feed intake and growth rates have been reported to increase by 24 and 260%, respectively, for red abalone juveniles (40 mm) when cultivated in complete darkness (Ebert & Houk 1984). No feeding activity was observed during daylight hours in ass's ear abalone (*Haliotis asinina*). The highest feeding

activity occurred during darkness between 1800 and 0200 h. and ceased entirely before sunrise (Tahil & Juino-Menez 1999).

The aim of this study was to compare feeding behaviour of juvenile greenlip abalone presented with formulated compounded diets or live macroalgal diets at different feeding rations using video monitoring from a ventral view.

Materials and methods

Experimental design

The effect of diet type (commercial chip, experimental flake, live *Ulva* sp. and live *Gracilaria cliftonii*) and feed ration (excess vs. restricted ration) on the feeding behaviour of the greenlip abalone was investigated in a factorial experiment comprised of eight treatment combinations (Table 1). The commercial formulated diet (Abgrow Premium, $5 \times 5 \times 2$ mm chip) was provided by Eyre Peninsula Aquafeeds (EPA) Pty Ltd (Lonsdale, S.A, Australia). The ingredient formulation of the experimental flake diet was based on a commercial greenlip abalone grow-out diet (Aqua Feeds Australia Pty Ltd, Mt Barker, S.A, Australia), but was designed to have a physical structure (large thin flakes, 1 mm thick) that mimicked the thalloid structure of *Ulva* sp. when submersed in water.

Experimental animals

Two-year-old greenlip abalone were sourced from South Australian Mariculture Pty. Ltd (Port Lincoln, S.A., Australia) and delivered to the South Australian Research and Development Institute (SARDI), Aquatic Sciences Centre (ASC) at West Beach, South Australia. One month prior to the commencement of the experiment, abalone were held in a 200-L tank provided with sand-filtered, UV-treated, temperature-controlled (22 ± 1 °C) flow-through seawater and fed the 5 mm Abgrow Premium commercial feed chip *ad libitum* daily.

Experimental stocking and system

Five abalone (initial mean weight, 12.57 ± 0.01 g; shell length, 45.57 ± 0.01 mm, $n = 40$) were randomly harvested from the 200-L holding tank without anaesthetic,

Table 1. Experimental treatment combinations used to assess the feeding behaviour of greenlip abalone (*H. laevigata*).

Treatment	Diet type	Feed ration (% body weight day^{-1})	
		Excess	Restricted
1 & 2	Commercial diet	2.84	0.44
3 & 4	Experimental flake	2.84	0.44
5 & 6	Live *Ulva* sp.	4.73	1.17
7 & 8	Live *Gracilaria cliftonii*	8.00[a]	1.17

[a]*G. cliftonii* was fed to excess at 4.73% bw day^{-1} for the first five days and then at 8.00% body weight day^{-1} thereafter.

weighed, measured, tagged with different coloured glue-on FPN shellfish tags (Hallprint Pty. Ltd, Hindmarsh Valley, S.A. Australia) and systematically interspersed into each of the eight 12 L clear glass aquaria (30 cm × 20 cm × 20 cm deep; $n = 1$ per treatment). Each aquarium was supplied with sand-filtered, UV-treated, temperature-controlled (22 ± 1 °C) flow-through seawater at a flow rate of 0.22 L min^{-1}. The water level in each aquarium was set at 6 cm (water volume, 3.6 L) using a screened (nominal pore size 0.8 mm) standpipe at the outlet. The photoperiod was controlled at 12 h of low-intensity fluorescent lighting at 3.4 lux (0700–1900 h) and 12 h of darkness (1900–0700 h) throughout the experiment. Animals were acclimatised to their respective aquarium for 13 days before filming of feeding behaviour commenced.

Feeding

Over the course of the experiment (13 days of acclimatisation and three days of film-ing), abalone were fed their respective diets and feed rations (Table 1) daily at 1600 h. All uneaten feed was collected at 0840 h the following morning when the aquaria were cleaned. Feed rations were based on feed intake rates for similar-sized greenlip abalone previously determined during a preliminary study in the same experimental system. In brief, greenlip abalone (21.1 g) were held in aquaria at 22 °C and fed to excess with either the *Ulva* sp. (4.73% body weight (bw) day^{-1}) or the commercial formulated diet (2.84% bw day^{-1}). Uneaten feed was collected and dried, and after correcting for leach-ing loss and moisture content, abalone consumed 1.17% bw day^{-1}of *Ulva* sp. and 0.44% bw day^{-1} of the commercial diet. Smaller greenlip abalone consume feed at a higher proportion of their body weight than larger abalone (Stone et al. 2013). Applying the predetermined feed rates to the smaller abalone in the current study therefore results in a restricted feed ration. As the feed intake rates of *G. cliftonii* and the experimental flake diet were not predetermined in the preliminary study, the restricted feed rates for *Ulva* sp. and the commercial diet were used for *G. cliftonii* and the experimental flake diets, respectively. However, greenlip abalone fed ravenously on *G. cliftonii* during the first five days of the acclimation period and consumed most of the feed ration offered, when we attempted to feed to excess. Consequently, on day six of the acclimation per-iod, the feed rate of *G. cliftonii* was increased to 8% bw day^{-1} to ensure feeding was to excess, and remained at this rate thereafter.

Calculation of apparent feed consumption

Allowing for a 10-day feed acclimation period, the daily feed intake for greenlip aba-lone was recorded over the final six days of the experiment. Collected uneaten feed samples from each aquaria were weighed wet, then oven dried at 60 °C for 48 h to a constant dry weight. Dry matter feed leaching losses (formulated diet) or gain (live macroalgae growth) was determined according to the methods of Stone et al. (2013). This value was used as a correction factor to calculate the apparent feed consumption. The calculations for the apparent feed consumption were based on as-fed values for feed intake and wet values for abalone weight.

Video monitoring

The video monitoring system comprised a flat top table with four recessed rectangular holes; one for each aquarium. Four video cameras (HD Pro Webcam C920; Logitech,

Newark, CA, USA) were located under the table top, one for each aquarium. During the 12-h dark phase, the underside of each aquarium was illuminated (6.0 lx) with red light (12 V Nelson Mini Spot Pond Lights, HPM Industries Pty. Ltd Preston, NSW, Australia). White fluorescent lighting provided illumination (3.4 lx) during the 12-h light phase. Video footage of the animals' movements and feeding behaviour was captured ventrally using a desktop computer and the 'Security Monitor Pro' software package (v5 4-Camera, Desk-Share, Plainview, NY, USA). The eight treatments were distributed over the eight aquaria using a randomised block design. Four aquaria (two diets fed the excess and restricted feed rations) were filmed at any one time. Replicate 24-h feeding events were filmed over three consecutive days for each treatment combination.

Measurement of activity and feeding behaviour

Video footage was observed and scored for the first 10 min of every 30-min period for the three 24-h replicate periods, using a video software program (VLC Media Player, Version 2.0.5 Twoflower, Paris, France). Observed activity was assigned to one of four categories (Table 2). Depending upon the level of activity, video footage was reviewed at varying speed of up to $16 \times$ play speed. The mean percentage of time that each of the activities was exhibited within each treatment was determined from the average of all abalone activity in each tank over the 10-min observation period. This process was repeated for each 10-min interval for each of the three 24-h replicate periods.

Measurement of distance and velocity travelled

To accurately measure distance and velocity travelled by each abalone, the perimeter size of the aquarium was scaled to match the size of the computer screen. Movement over time was tracked and recorded and multiplied by the correction factor between the aquarium size and screen size. The mean distance travelled and velocity within each treatment was determined over the 10-min observation period. This process was repeated for each 10-min interval for each of the three 24-h replicate periods. In order to investigate the effect of feed ration on velocity in more detail, each replicate 24-h period was divided into five nominal periods: the first dark period (1900–2159 h), the second dark period (2200–0059 h), the third dark period (0100–0359 h), the fourth dark period (0400–0659 h) and the light period (0700–1859 h).

Table 2. The scoring system used to measure the feeding behaviour of greenlip abalone (*H. laevigata*).[a]

Behaviour	Description
Quiescent	Shell held tightly to aquarium surface, cephalic and mantle tentacles retracted, no movement
Alert	Shell raised off aquarium surface, tentacles extended, extension from foot, rotation
Moving	Moving an appreciable distance in any direction
Feeding	Actively ingesting food items

[a]Modified from Allen et al. (2006).

Homing behaviour

After a three-day acclimation period, the homing behaviour of greenlip abalone was determined over a period of 13 days. This was achieved by photographing tagged abalone in each aquarium from above at 0840 h daily when the abalone were quiescent. Daily photographs were evaluated by comparing similarities or differences in the location of abalone at the same time of each day. The coloured FPN glue-on shellfish tags enabled tracking of each individual. The homing behaviour of each individual abalone was assigned to one of three categories: (1) homing (returned to same location); (2) moved (different location); or (3) returned (adjacent to home, but unable to return to exact homing location due to the presence of another animal). Data for each tank over the 13-day period was then converted to a proportion (%) of homing and returned divided by all three categories.

Biochemical analyses

The moisture, ash, crude lipid, gross energy and crude protein contents of the commercial diet, experimental flake diet and *Ulva* sp. were analysed according to the methods of AOAC International (1995). Moisture content was determined by oven drying to a constant weight at 105 °C for 16 h and 60 °C for 48 h, respectively. Crude protein ($N \times 6.25$) was determined by the Kjeldahl method. Crude lipid was analysed using a Soxtherm rapid extraction system (Gerhardt GmbH & Co. KG, Königswinter, Germany) with petroleum liquid (BP 100 °C) as the extracting solvent. Ash was determined using a muffle furnace at 550 °C for 16 h. The gross energy contents of the commercial diet and the experimental flake diet were determined using a bomb calorimeter calibrated with benzoic acid. The gross energy content of the *Ulva* sp. and *G. cliftonii* were calculated using the values of 17.2, 23.6 and 39.5 MJ kg^{-1} for carbohydrate, protein and lipid, respectively (NRC 2011) (Table 3). The crude protein, crude lipid, ash and NFE values (dry basis) of the *G. cliftonii* were sourced from Kumar et al. (2010), whereas moisture was determined as above.

Table 3. The biochemical composition of diets fed to greenlip abalone (*H. laevigata*).

Component (as fed)	Diet			
	Commercial diet	Experimental flake	*Gracilaria cliftonii*[a]	*Ulva* sp.
Moisture (g kg^{-1})	100.0	100.0	834.0	841.0
Crude protein (g kg^{-1})	306.0	420.0	18.4	18.0
Crude lipid (g kg^{-1})	45.0	48.0	0.7	3.4
Ash (g kg^{-1})	62.0	67.0	51.8	38.7
NFE (g kg^{-1})[1]	587.0	465.0	95.1	98.9
Gross energy (MJ kg^{-1})[b,c]	15.2	17.4	2.1	2.3

[a]The crude protein, crude lipid, ash and NFE values of the *G. cliftonii* were sourced from Kumar et al. (2010).
[b]NFE = nitrogen-free extract was calculated by difference = 100% − (crude protein% + total fat % + ash%).
[c]The gross energy content of *Ulva* sp. and *G. cliftonii* were calculated using the values of 17.2, 23.6 and 39.5 MJ kg^{-1} for carbohydrate, protein and lipid, respectively (NRC 2011).

Statistical analysis

All statistical analyses were done using IBM SPSS, Version 20 for Windows (IBM SPSS Inc., Chicago, IL, USA). The normality of data was assessed using the Shapiro–Wilk test. Homogeneity of variances among means was assessed using Levene's test for equality of variance errors. ANOVA was used to assess the effects of diet type and feed ration on dependent variables. Where significant interactions were observed, the data were analysed using Student Newman–Keuls (SNK) multiple range test. The significance level was set at $p < 0.05$ for all tests unless otherwise stated. All values are presented as means ± standard error of the mean.

Results

Abalone were observed to feed actively on all diets. No visual signs of disease or mortalities were observed in experimental animals. The average proportion of time greenlip abalone exhibited quiescent or moving behaviours, in any 24-h period, was not significantly affected by diet type, feed ration or the interaction between the two factors ($p > 0.05$; Table 4).

There was a significant effect of feed ration on the average proportion of time greenlip abalone exhibited alert behaviour ($p = 0.021$). There was, however, no significant effect for feed type ($p > 0.05$; Table 4), and there was no significant interaction between feed ration and type ($p > 0.05$; Table 4) for this behaviour. Abalone fed the restricted feed ration spent a significantly greater proportion of time exhibiting alert behaviour ($46.4 \pm 2.65\%$) compared to abalone fed the excess feed ration ($37.8 \pm 2.66\%$; Table 4).

After the addition of feed to the aquaria, feed ration had a significant effect on the time abalone commenced feeding ($p = 0.005$), but there were no significant effects of diet type or an interaction between diet type and feed ration for this behaviour ($p > 0.05$; Table 4). Abalone fed the restricted feed ration began to feed earlier (1.92 ± 0.68 h) in the light period (Figures 1(b), 2(b), 3(b) and 4(b)), than abalone fed to excess (3.54 ± 0.67 h) (Table 4), which commenced feeding directly before or just after darkness (Figures 1(a), 2(a), 3(a), and 4(a)).

Feed ration significantly affected the average proportion of time greenlip abalone spent feeding ($p < 0.001$). In contrast, neither diet type nor the interaction between diet type and feeding ration significantly affected the average proportion of time spent feeding for all dark and light photoperiods ($p > 0.05$; Tables 4 and 5). Restricted feed ration resulted in significantly less time feeding ($0.76 \pm 0.08\%$) compared to the excess feed ration ($3.66 \pm 0.25\%$; Table 4).

Similarly, feed ration significantly affected the average velocity of greenlip abalone over a 24-h period ($p = 0.002$), with neither diet type nor the interaction between diet type and feed ration significantly affecting the average velocity ($p > 0.05$). Abalone on the restricted feed ration moved at a higher average velocity (2.65 ± 0.08 cm min^{-1} or 0.59 body lengths min^{-1}) compared to abalone fed the excess feed ration (1.85 ± 0.24 cm min^{-1} or 0.41 body lengths min^{-1}) (Figure 5; Table 5).

For the five nominal periods, the average velocity of abalone was not significantly affected by feed ration in the first and fourth dark periods, however, it was significantly affected in the second ($p = 0.034$) and third dark periods ($p \leq 0.001$). Abalone on the restricted feed ration moved at a higher average velocity (second dark period, 5.61 ± 0.66 cm min^{-1} or 1.23 body lengths min^{-1}; third dark period, 6.13 ± 0.67 cm min^{-1} or 1.35 body lengths min^{-1}) than the abalone fed the excess feed ration (second dark

Table 4. The average proportion of time greenlip abalone (*H. laevigata*) exhibited each feeding behaviour in response to diet type and feed ration over a 24-h period.[a]

Diet type	Commercial diet		Experimental flake		*Ulva* sp.		*Gracilaria cliftonii*		Two-factor ANOVA *p* values[b,c]		
									Diet type (a)	Feed ration (b)	Interaction (a × b)
Feed rate (as fed)	Excess	Restricted	Excess	Restricted	Excess	Restricted	Excess	Restricted			
Feeding behaviour											
Quiescent (%)	49.37 ± 0.53	46.58 ± 6.89	45.70 ± 4.98	37.26 ± 4.08	59.74 ± 4.45	52.83 ± 7.79	52.69 ± 8.02	44.89 ± 2.44	0.084	0.087	0.954
Alert (%)	41.71 ± 0.24	45.44 ± 5.84	42.10 ± 5.58	52.97 ± 4.69	30.81 ± 4.83	40.10 ± 6.39	36.38 ± 7.52	47.03 ± 1.98	0.123	(>) 0.021	0.895
Moving (%)	5.43 ± 0.19	7.30 ± 1.29	9.19 ± 1.14	8.95 ± 1.29	5.48 ± 2.15	6.13 ± 1.41	6.33 ± 0.75	7.47 ± 0.46	0.052	0.308	0.854
Feeding (%)	3.50 ± 0.25	0.68 ± 0.26	3.02 ± 0.64	0.82 ± 0.43	3.96 ± 0.79	0.94 ± 0.01	4.14 ± 0.30	0.61 ± 0.04	0.563	(>) <0.001	0.489
Time to commence feeding (h)	5.50 ± 0.58	2.00 ± 1.00	2.67 ± 0.44	1.83 ± 0.83	3.00 ± 0.29	2.83 ± 0.93	3.00 ± 0.29	1.00 ± 0.00	0.100	(>) 0.005	0.086

[a]Mean ± se; *n* = 3 replicates from separate 24-h observation periods.
[b]There were no significant interactions and there was no significant effect of diet type on any feeding behaviour (Two-factor ANOVA; SNK; *p* > 0.05).
[c]For feed ration, the symbol (>) indicates that the response to the restricted feed ration was significantly greater than the response to the excess feed ration (*p* < 0.05).
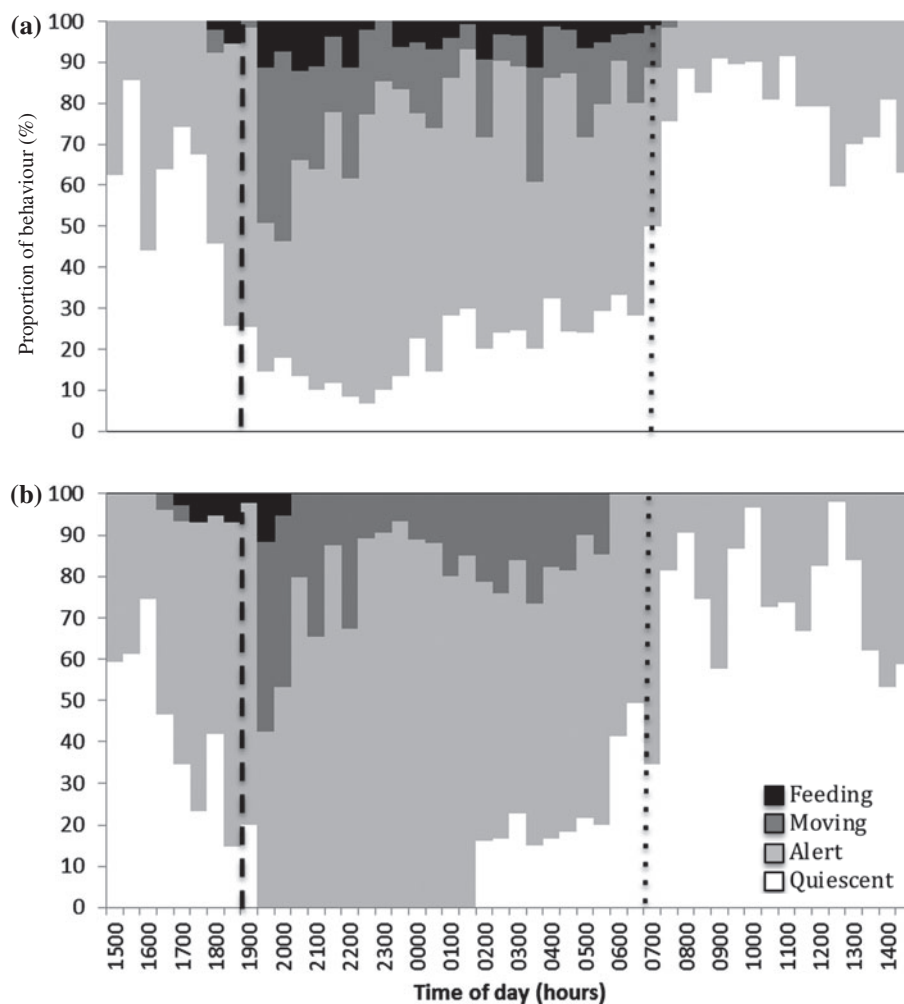
Figure 1. The proportion of time greenlip abalone (*H. laevigata*) exhibited each behaviour over a 24-h period when fed an excess feed ration (1a) or a restricted feed ration (1b) of the experimental flake diet (Means; $n = 3$ replicate observations of 10 min at every 30 min interval over three consecutive 24 h periods). Feed introduced to the system at 1600 h, lights off (dashed line) at 1900 h, lights on (dotted line) again at 0700 h the following day and uneaten feed collected and aquaria cleaned at 0840 h the following day.

period, 3.89 ± 0.39 cm min^{-1} or 0.85 body lengths min^{-1}; third dark period, 2.69 ± 0.25 cm min^{-1} or 0.59 body lengths min^{-1}) (Table 5). As abalone exhibited very little to no movement during the light period (0700–1859 h), the average velocities during this period could not be statistically analysed. Despite this, the main trends still showed that the abalone on the restricted feed ration moved at higher average velocities (Table 5).

When feeding was restricted, abalone stopped feeding early in the dark period (latest at 2200 h; Figures 1(b), 2(b), 3(b) and 4). When fed to excess, abalone continued to feed throughout the night until 0700 h the next morning (Figures 1(a), 2(a), 3(a), and 4(a)). However, in the hours leading up to 0700 h, quiescent behaviour increased in
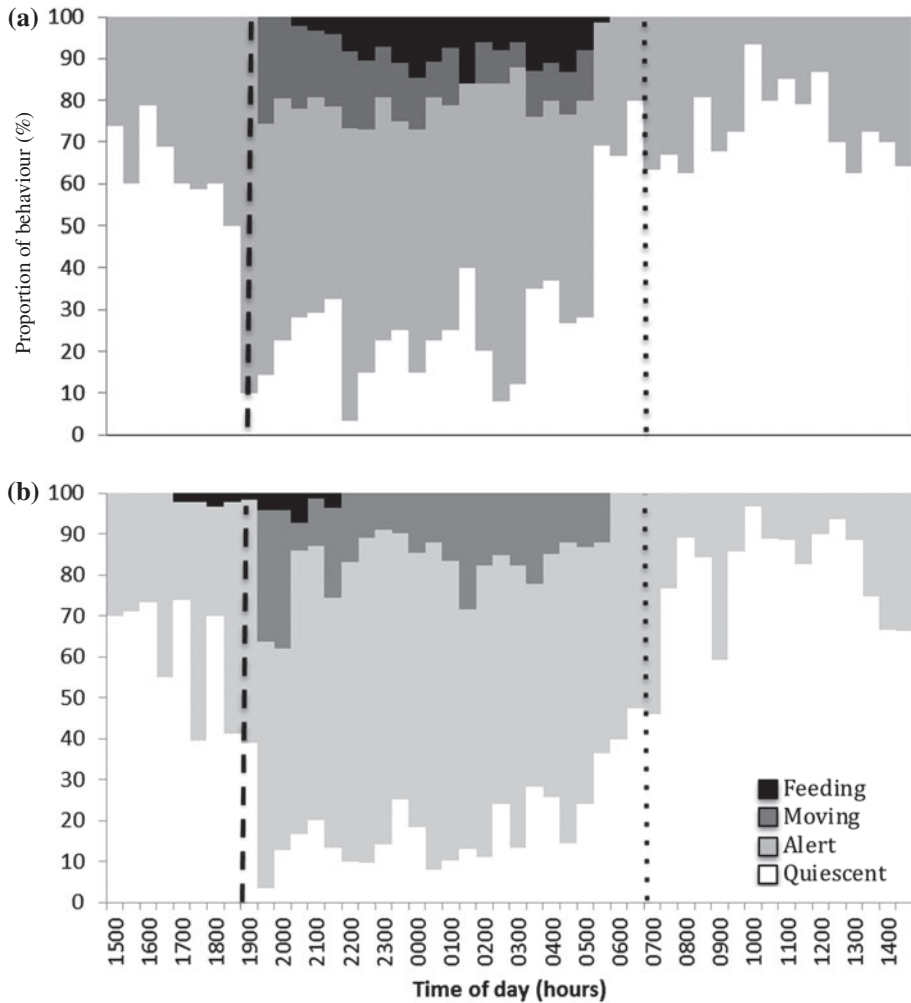
Figure 2. The proportion of time greenlip abalone (*H. laevigata*) exhibited each behaviour over a 24-h period when fed an excess feed ration (2a) or a restricted feed ration (2b) of the commercial diet (Means; *n* = 3 replicate observations of 10 min at every 30 min interval over three consecutive 24 h periods). Feed introduced to the system at 1600 h, lights off (dashed line) at 1900 h, lights on (dotted line) again at 0700 h the following day and uneaten feed collected and aquaria cleaned at 0840 h the following day.

all treatments, and was the dominant behaviour during the light period (0700–1900 h; Figures 1–4).

There was no significant effect of diet type or feed ration on the homing behaviour of abalone and there was no significant interaction between the two factors ($p > 0.05$). Abalone exhibited homing behaviour $62.1 \pm 1.9\%$ of the time.

No statistical comparison of feed and nutrient intake rates for abalone fed the restricted feed ration could be done because abalone ate all feed supplied. The analysis of feed and nutrient intake rate was restricted to diet type alone in the excess feed ration treatments (Table 6), where significant effects were detected ($p < 0.001$). Across the four diet types, feed intake rate was highest for *G. cliftonii*, followed by the
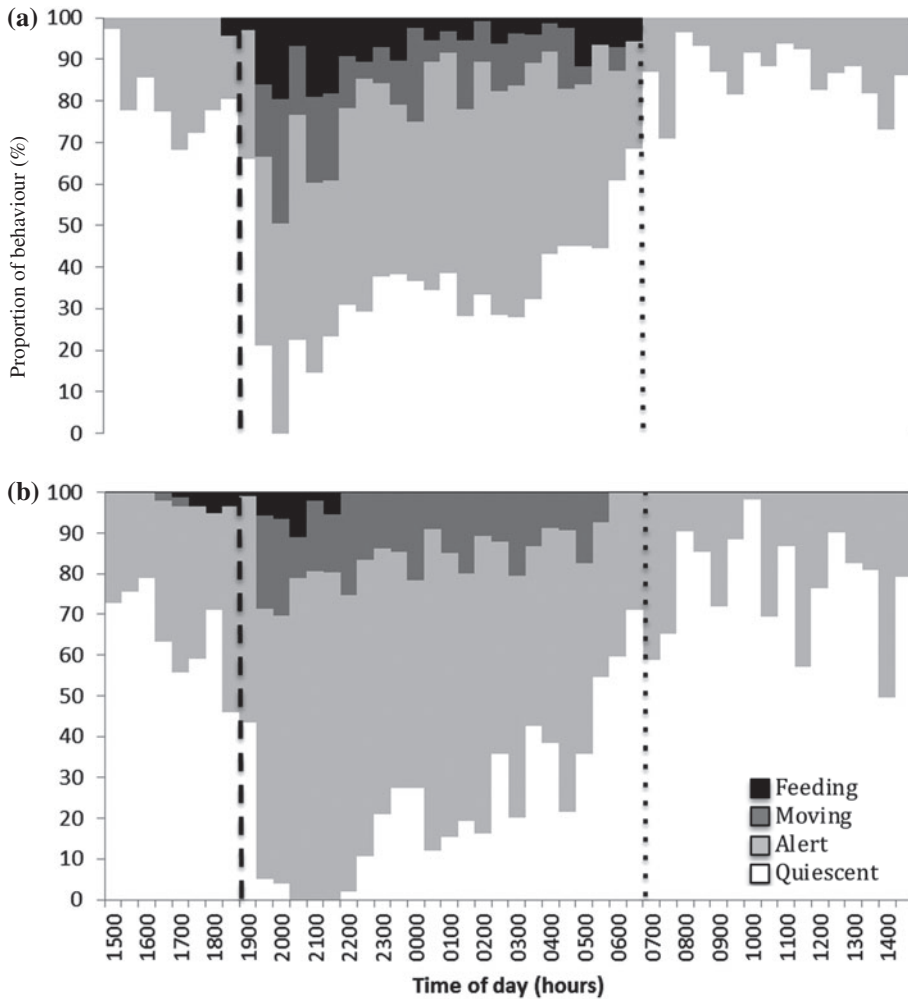
Figure 3. The proportion of time greenlip abalone (*H. laevigata*) exhibited each behaviour over a 24-h period when fed an excess feed ration (3a) or a restricted feed ration (3b) of *Ulva* sp. (Means; *n* = 3 replicate observations of 10 min at every 30 min interval over three consecutive 24 h periods). Feed introduced to the system at 1600 h, lights off (dashed line) at 1900 h, lights on (dotted line) again at 0700 h the following day and uneaten feed collected and aquaria cleaned at 0840 h the following day.

experimental flake diet, with the commercial diet and *Ulva* sp. lowest. As a result, protein, carbohydrate and energy intake rates were significantly lower for the two macroalgal diet treatments compared to the two formulated diet treatments (*Ulva* sp. < *G. cliftonii* < commercial diet < experimental flake). Lipid intake rates also differed between diet types (*Ulva* sp. = *G. cliftonii* < commercial diet < experimental flake).

Discussion

This study is the first to accurately distinguish the amount of time abalone spend feeding compared to other behaviours. In the current study, greenlip abalone spent a shorter
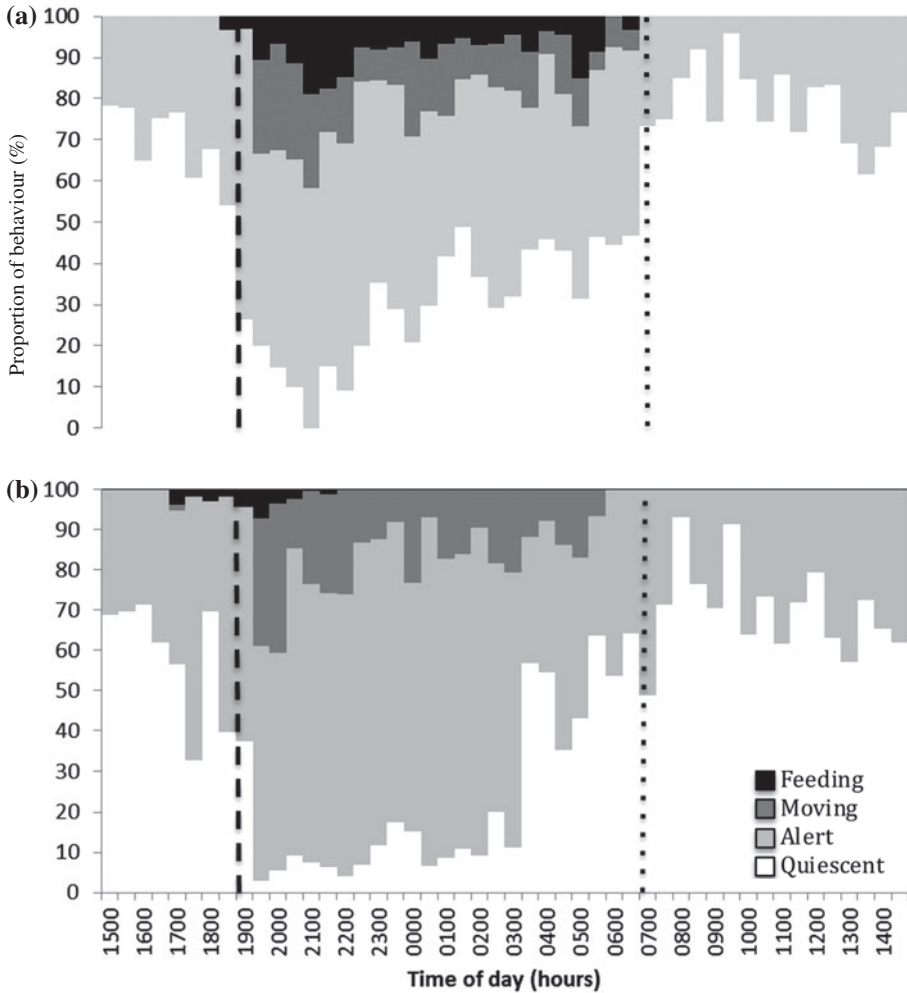
Figure 4. The proportion of time greenlip abalone (*H. laevigata*) exhibited each behaviour over a 24-h period when fed an excess feed ration (4a) or a restricted feed ration (4b) of *G. cliftonii* (Means; $n = 3$ replicate observations of 10 min at every 30 min interval over three consecutive 24 h periods). Feed introduced to the system at 1600 h, lights off (dashed line) at 1900 h, lights on (dotted line) again at 0700 h the following day and uneaten feed collected and aquaria cleaned at 0840 h the following day.

proportion of time feeding (Figures 1–4) than cultured northern abalone, *Haliotis kamtschatkana*, continuously fed from 1600 to 0700 h (Lloyd & Bates 2008). However, the determination of the feeding activity of these northern abalone was conducted from a dorsal view, where the behavioural responses of feeding, moving or being alert could not be discerned (Lloyd & Bates 2008). In the current study, the method of ventral monitoring enabled us to distinguish between the different behaviours, and accurately determine how greenlip abalone responded to variations in feed types and feed rations.

Greenlip abalone in the current study fed predominantly in the dark period. Numerous studies have supported both wild and cultured *Haliotis* spp. being nocturnal feeders (Ino 1952; Tutschulte 1967; Momma & Sato 1969; Momma & Sato 1970; Poore 1972;

Table 5. The average velocity of greenlip abalone (*H. laevigata*) in response to diet type and feed ration over a 24-h period.

Diet type	Commercial diet		Experimental flake		*Ulva* sp.		*Gracilaria cliftonii*		Two-factor ANOVA p values[b,c]		
Feed rate	Excess	Restricted	Excess	Restricted	Excess	Restricted	Excess	Restricted	Diet type (a)	Feed ration (b)	Interaction (a × b)
Rate of movement (cm min⁻¹)											
24 h period 1530–1529 h[a]	1.60 ± 0.01	2.84 ± 0.33	2.55 ± 0.25	2.52 ± 0.21	1.45 ± 0.61	2.54 ± 0.02	1.78 ± 0.24	2.69 ± 0.07	0.394	(>) 0.002	0.157
Dark period 1900–2159 h	3.61 ± 0.08	6.23 ± 1.88	6.95 ± 0.95	8.11 ± 0.79	4.40 ± 2.55	4.35 ± 1.88	4.40 ± 0.61	7.27 ± 1.53	0.204	0.138	0.743
Dark period 2200–0059 h	3.95 ± 0.08	5.63 ± 1.63	4.52 ± 1.05	4.12 ± 0.07	2.79 ± 0.87	7.31 ± 1.59	4.30 ± 1.08	5.37 ± 0.74	0.914*	(>) 0.034*	0.165*
Dark period 0100–0359 h	2.40 ± 0.08	8.14 ± 2.79	3.37 ± 0.14	5.31 ± 1.24	2.23 ± 0.90	5.49 ± 0.09	2.76 ± 0.44	5.57 ± 1.54	0.685	(>) 0.001	0.451
Dark period 0400–0659 h	2.14 ± 0.00	2.15 ± 0.14	4.15 ± 1.40	2.27 ± 0.39	0.88 ± 0.44	2.14 ± 0.16	2.12 ± 0.42	2.49 ± 0.28	0.061*	0.886*	0.081*
Light period 0700–1859 h	0.00 ± 0.00	0.01 ± 0.01	0.10 ± 0.10	0.03 ± 0.02	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.23 ± 0.02	na	na	na

Note: na = not statistically analysed due to lack of movement during daylight hours.
*Normality was not reached, despite transformation.
[a]Mean ± se; n = 3 replicate 24-h observation periods.
[b]There were no significant interactions and there was no significant effect of diet type on any feeding behaviour (Two-factor ANOVA; SNK; *p* > 0.05).
[c]For feed rate treatments, the symbol (>) indicates that the response to the restricted feed ration was significantly greater than the response to the excess feed ration (*p* < 0.05).
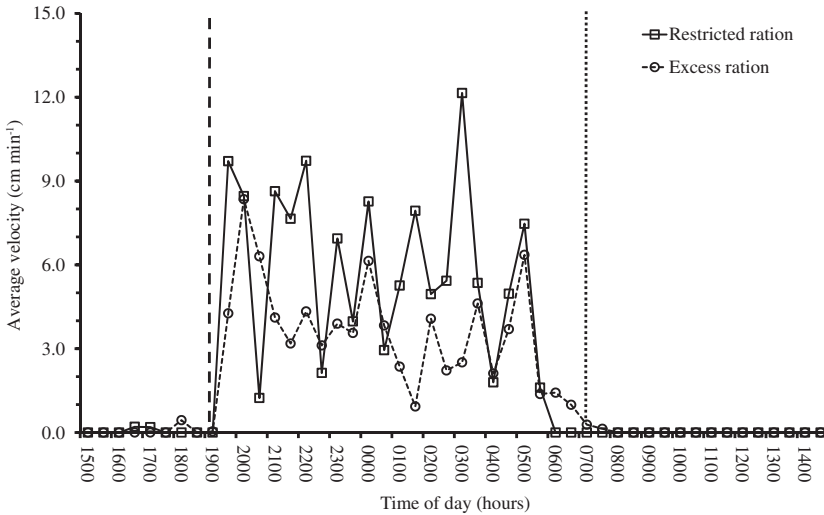
Figure 5. The average velocity of greenlip abalone (*H. laevigata*) over a 24-h period when fed excess or restricted feed rations ($n = 12$ observations of 10 min at every 30 min interval over three consecutive 24 h periods for each data point). Feed introduced to the system at 1600 h, lights off (dashed vertical line) at 1900 h, lights on (dotted vertical line) again at 0700 h the following day and uneaten feed collected and aquaria cleaned at 0840 h the following day.

Shepherd 1973a; Ebert & Houk 1984; Tutschulte & Connell 1988; Hahn 1989; Tahil & Juino-Menez 1999; Garcia-Esquivel et al. 2007; Pereira et al. 2007; Searcy-Bernal & Gorrostieta-Hurtado 2007; Lloyd & Bates 2008). The nocturnal feeding behaviour of *Haliotis* spp. is considered to be an evolved response to reduce exposure to predation (Hahn 1989). Foraging by cultured three-year-old northern abalone on live kelp (*Nereocystis luetkeana*) was reported to begin approximately two hours after sunset, regardless of feed ration or stocking density (Lloyd & Bates 2008).

   In the current study, greenlip abalone fed a restricted feed ration commenced feeding in the light period, irrespective of diet type (Figures 1–4; Table 4). Wild abalone are known to prolong foraging activity when food resources are limited (Poore 1972; Shepherd 1973b; Sloan & Breen 1988; Lloyd & Bates 2008), as similarly demonstrated in the current study through velocity measurements (Figure 5). However, in most reported instances, the increase in foraging does not extend into the daylight hours (Hahn 1989; Garcia-Esquivel et al. 2007; Lloyd & Bates 2008). This highlights that feeding during the daylight, as observed in greenlip abalone during the current study, is typically uncommon for *Haliotis* spp. and is likely to be an adaptive behavioural response to hunger. This behavioural trait, although unusual for *Haliotis* spp., may be used to identify underfeeding and may complement other feed management tools. A similar increase in foraging behaviour to restricted feed ration was also observed in cultured northern abalone (Lloyd & Bates 2008). This may adversely affect growth rates, as movement is energetically taxing due to an increase in metabolic activity and mucus excretion rates (Shepherd 1973b; Donovan & Carefoot 1998). Shepherd (1973b) reported that in the wild, when drift algae become limited, growth stunting occurred in several *Haliotis* spp.

   Greenlip abalone are generally sedentary for most of their lives, occupying rocky zones in rough water environments, opportunistically feeding on a range of drifting

Table 6. The average feed and nutrient intake rates (as fed) for greenlip abalone (*H. laevigata*) in response to diet type and feed ration.[a–d]

Diet type	Commercial diet		Experimental flake		*Ulva* sp.		*Gracilaria cliftonii*	
Feed ration	Excess	Restricted	Excess	Restricted	Excess	Restricted	Excess	Restricted
Feed intake (g kg bw^{-1} d^{-1})	10.56 ± 0.62[c]	4.29	20.91 ± 0.67[b]	4.23	6.42 ± 0.78[c]	11.47	47.63 ± 6.07[a]	11.55
Protein (g kg bw^{-1} d^{-1})	3.23 ± 0.19[b]	1.31	8.78 ± 3.59[a]	1.78	0.12 ± 0.01[d]	0.21	0.88 ± 0.11[c]	0.11
Carbohydrate (g kg bw^{-1} d^{-1})	6.20 ± 0.36[b]	2.52	9.72 ± 0.31[a]	1.97	0.64 ± 0.08[d]	1.13	4.53 ± 0.58[c]	1.11
Lipid (g kg bw^{-1} d^{-1})	0.48 ± 0.03[b]	0.20	1.00 ± 0.03[a]	0.20	0.02 ± 0.00[c]	0.04	0.03 ± 0.00[c]	0.01
Energy (MJ kg bw^{-1} d^{-1})	0.16 ± 0.01[b]	0.07	0.36 ± 0.01[a]	0.07	0.01 ± 0.00[d]	0.03	0.10 ± 0.01[c]	0.02

[a]Mean ± se; n = 6 replicates from separate 24-h observation periods.
[b]As abalone ate all of the restricted feed rations at each meal time, there were no variances for the restricted feeding treatments mean value for each variable, therefore, statistical analysis was only done for the variables for the treatments fed to excess.
[c]Means within each row with different superscripts are significantly different (One-factor ANOVA; SNK; $p < 0.001$).
[d]When reported standard errors are 0.00, the actual values were < 0.001.

macroalgae species (Shepherd 1973b; Shepherd 1975; Tutschulte & Connell 1988; Stepto & Cook 1993; Naidoo et al. 2006). Shepherd (1973b) also found that the type of diet of greenlip abalone changed over the seasons and in relation to location. In addition, they have been observed to show homing behaviour and reside on the same rock for months at a time when food is available, only moving to alternate substrates when food is scarce (Shepherd 1973b). Homing behaviour was also reported for pink abalone between one and two years of age under laboratory conditions and in the wild (Tutschulte & Connell 1988). Juvenile greenlip abalone in the current study were also observed to show homing behaviour in the absence of sufficient feed, regardless of diet type. This further supports the way diet type affects the feeding behaviour of abalone to a much lesser extent than food availability.

The feeding behaviour of greenlip abalone changed throughout the night in response to feed availability and time, but not diet type. Feed ration and diet type had no significant effect on the average velocity of greenlip abalone during the first period of darkness, between 1900 and 2159 h, when food was not limiting for either factor. However, as previously discussed, abalone are recognised as nocturnal feeders, and as feed was introduced three hours prior to darkness, the velocity of abalone during foraging in response to diet type and feed ration would be expected to remain the same in the presence of sufficient food during this period (Figures 1–4). Several authors have reported restricted feed availability increasing the proportion of movement and foraging time of abalone (Poore 1972; Shepherd 1973b; Sloan & Breen 1988; Lloyd & Bates 2008). Our analysis of separate periods within the dark period provided more resolution to interpret feeding behaviour, and this is demonstrated in our finding that feeding behaviour differed in more pronounced ways as night progressed. By 2200 h, all restrictively fed greenlip abalone had consumed all available food (Figures 1(b), 2(b), 3(b) and 4(b)). Following this, more movement was observed in abalone up until 0359 h (Table 5; Figure 5). This was likely a result of the abalone searching for food to satisfy their appetites.

Quiescent behaviour was dominant in all greenlip abalone from 0400 h until the following evening (Table 5). The cessation of movement during this period has previously been noted for other *Haliotis* spp. (Shepherd 1973b; Tutschulte & Connell 1988; Tahil & Juino-Menez 1999; Pereira et al. 2007; Lloyd & Bates 2008), demonstrating that as daytime approaches, the presence or absence of food has minimal effect on abalone movement. Juvenile abalone, in particular, have been reported to follow this trend, displaying quiescent behaviour during daylight, actively feeding during darkness and resuming quiescent behaviour before dawn (Tutschulte & Connell 1988; Pereira et al. 2007). The high proportion of quiescent behaviour displayed by greenlip abalone during the light period in this study may be an evolutionary response to increased vulnerability to predation on active wild abalone during daylight hours (Shepherd 1973b; Hahn 1989; Jenkins 2004).

In this study, greenlip abalone, when fed either of the macroalgal diets or the experimental flake diet, were observed to eat the same thallus or diet fragment until it was fully consumed. The physical structure of the experimental flake diet may have better imitated the thalloid structure of macroalgae, possibly influencing the higher feed intake compared to commercial diet (Table 6). However, differences in ingredient formulation and texture may have also contributed. Abalone fed the commercial diet were observed to graze briefly on one chip before quickly moving on to the next. This indicates that abalone may be preferentially grazing on the water-softened surface layer of the chip and rejecting the harder core. This suggests that chip hardness may be an influencing

feeding behaviour and intake, which has been previously reported in the literature (Gorfine 1991; Fleming et al. 1996; Allen et al. 2006). Further research is required in this area.

In the current study, there were vast differences in the nutrient density of the diets (Table 3). The moisture content had the greatest influence on nutrient density between the diets with moisture levels being considerably higher in the macroalgal diets. As a result, dietary protein, lipid, nitrogen free extract and energy levels were highest in the formulated diets compared to the macroalgal diets, while ash levels were in closer agreement (Table 3). The commercial diet was formulated to meet the nutrient requirements of greenlip abalone and is considered to be highly palatable compared to *Ulva* sp. (Stone et al. 2014). The experimental flake diet was based on a commercial diet formulation currently used with greenlip abalone, but differed in physical structure. It was designed to mimic the thalloid structure of macroalgae and was expected to induce a higher feed intake rate than the commercial diet chip treatment. Macroalgae may be expected to result in higher feed intake rates than formulated feeds as this is the natural food source for wild abalone species (Tutschulte & Connell 1988; Stepto & Cook 1993; Naidoo et al. 2006). However, red algal species, such as *Gracilaria* spp., are considered to be primary preferred feed sources for greenlip abalone in the wild and are also a preferred food source in the laboratory, whereas *Ulva* spp. are considered to be a secondary feed choice or are actively avoided (Shepherd 1975; McShane et al. 1994; Fleming 1995). For abalone to obtain comparable nutrient intake levels, it could be expected that diet intake rates would have to increase concomitantly as dietary moisture content increased. However, the obvious lack of intake of *Ulva* sp. by abalone in this study suggests that this species of macroalgae lacks attractiveness for greenlip abalone.

It has been reported that abalone show a preference for certain dietary components which may result in greater feed consumption (Harada 1992; Shepherd & Steinberg 1992), but results from this study indicate that greater feed consumption does not necessarily equate to greater nutrient or energy acquisition as this depends on a range of factors. While red macroalgae, such as *G. cliftonii*, may be a large component of the natural diet of greenlip abalone, greater nutritional value was derived from consuming quantitatively less of the experimental flake or commercial diets (Table 6). This may have consequences for growth. Greenlip abalone grew significantly faster and exhibited superior feed efficiency when fed the commercial formulated diet or the experimental flake diet fed in the chip form (Aqua Feeds Australia Pty Ltd, Mt Barker, SA, Australia) compared to when fed either *G. cliftonii* or *Ulva* sp., although *G. cliftonii* was a superior diet compared to *Ulva* sp. (Bansemer et al. 2014). Additionally, other *Haliotis* spp. are known to grow faster when fed formulated diets than when fed macroalgae (Nie et al. 1986; Hahn 1989).

Conclusion

The ventral filming method used in this study enabled feeding behaviour to be more accurately discerned than in previous studies. Feed ration and photoperiod had far greater impacts on the feeding behaviour of abalone than diet type. In regards to photoperiod, abalone exhibited the most movement and feeding behaviour during darkness, supporting the notion that nocturnal feeding is preferred. Abalone nevertheless began feeding in daylight in response to restrictive feed rations. This behavioural trait may be used as a tool for on-farm feed management to identify changing feeding requirements in abalone. Restricted feed rations also induced greater movement which may have

consequences for energy expenditure. Nutrient dense formulated diets provided higher total nutritional intake, a finding supporting their benefits over non-enriched macroalgae options.

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