Temperature-Dependent Feed Consumption Patterns for Greenlip (*Haliotis laevigata*) and Hybrid (*H. laevigata* × *Haliotis rubra*) Abalone Fed Fresh Macroalgae or a Formulated Diet

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TEMPERATURE-DEPENDENT FEED CONSUMPTION PATTERNS FOR GREENLIP (*HALIOTIS LAEVIGATA*) AND HYBRID (*H. LAEVIGATA* × *HALIOTIS RUBRA*) ABALONE FED FRESH MACROALGAE OR A FORMULATED DIET

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ABSTRACT Because of the nocturnal and slow feeding activity of abalone, farmed abalone are typically provided with feed throughout the night. Understanding the nocturnal feeding patterns of abalone is fundamental to feed management and productive abalone farming. In this study, the apparent feed consumption for greenlip abalone (*Haliotis laevigata*) and hybrid abalone (*H. laevigata* × *Haliotis rubra*) fed fresh *Ulva* sp. or a commercial formulated diet at 18°C or 22°C were investigated at night. Abalone were exposed to low light intensity (3.4 Lux) from 7:00 AM to 7:00 PM and darkness from 7:00 PM to 7:00 AM. Abalone were fed to excess daily at 4:00 PM and feed intake was determined at 7:00 PM, 10:00 PM, 1:00 AM, 4:00 AM, and 8:00 AM. When *Ulva* sp. was added to the tank, greenlip and hybrid abalone immediately displayed a feeding response, which was not observed in abalone fed the formulated diet. Abalone consumed *Ulva* sp. at a linear rate from 4:00 PM to 8:00 AM. In contrast, the apparent feed consumption rate of abalone fed the formulated diet was minimal from 4:00 PM to 7:00 PM, and was highest between 7:00 PM and 1:00 AM. Apparent feed consumption rate of abalone significantly increased as water temperature increased from 18°C to 22°C, but the effect was greater for hybrid abalone compared with greenlip abalone. The total apparent feed intake of both greenlip and hybrid abalone fed *Ulva* sp. was significantly greater than for both types of abalone fed the formulated diet. The total apparent feed intake on dry basis, and nutrient intake for abalone fed *Ulva* sp. was significantly lower than for abalone fed the formulated diet. This study indicates that the upper temperature range for feed intake in hybrid abalone is higher than in greenlip abalone. *Ulva* sp. can stimulate abalone feeding, though the high moisture content in algae can reduce nutrient intake.

KEY WORDS: greenlip abalone, hybrid abalone, feed consumption, nutrition, water temperature, *Haliotis laevigata*, *Haliotis rubra*

INTRODUCTION

Macroalgae are the predominate diet of wild abalone, and are also fed to farmed abalone (Shepherd 1973, Kirkendale et al. 2010). The nutrient intake and growth of abalone fed fresh macroalgae may be limited due to the high moisture content (~80%) and low nutrient density (Kirkendale et al. 2010, Bansemer et al. 2014a). In contrast, nutrient dense formulated diets are crucial to the success of land-based abalone culture as these diets contain an optimal nutritional profile for growth (Fleming & Hone 1996, Bansemer et al. 2014a).

Abalone rely on chemical and tactile stimuli to detect macroalgae, which are typically limited in commercial formulated diets (Allen et al. 2006). For example, greenlip abalone (*Haliotis laevigata*) graze briefly on a formulated diet chip before moving on to graze on the next, but abalone fed macroalgae (*Ulva* sp. or *Gracilaria cliftonii*) consume the whole fragment before recommencing foraging (Buss et al. 2015). Moreover, blackfoot abalone (*Haliotis iris*) spend most of the time sedentary when fed a formulated diet, but when dried and mulched *Gracilaria* spp. particles (300–500 μm) are suspended in the culture system abalone spend greater than 80% of their time feeding (Allen et al. 2006).

Because of the nocturnal and slow feeding activity of abalone, feed is typically provided to cultured abalone at night. Nutrient leaching loss from commercial formulated diets during extended periods of immersion is a major concern to the abalone industry (Fleming et al. 1996, Ruff et al. 2014). Binding agents such as agar, carrageenan, or alginate are typically used in formulated diets to retard nutrient leaching (Bautista-Teruel et al. 2013). If no binders are used in a formulated diet, the dry matter leaching loss is ~80% after 24 h (Bautista-Teruel et al. 2013). Therefore, the reduction of nutrient leaching from the diet has important implications on the nutrient intake and growth of abalone.

Understanding the nocturnal feeding patterns is fundamental to the development of successful feed management practices for abalone farming (Fleming et al. 1996). Various methods have been used to investigate the peak feeding activity of abalone including the observation of gut fullness (Britz et al. 1996), feed intake rate (Tahil & Juino-Menez 1999), and video monitoring (Allen et al. 2006, Buss et al. 2015). Discrepancies between studies are apparent, likely due to a number of factors including methodological differences, species-specific responses, water temperature variations, and differences in physical and biochemical characteristics of diets (Britz et al. 1996, Tahil & Juino-Menez 1999, Buss et al. 2015).

Two abalone types are primarily cultured in land-based systems throughout southern Australia, the greenlip abalone and hybrid abalone (*Haliotis laevigata* × *Haliotis rubra*). Greenlip abalone are more extensively farmed than hybrid abalone, but culturing hybrid abalone may be advantageous due to their superior growth rate, market appeal, and disease resistance

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compared with parental species (Fotedar & Phillips 2011). Australian abalone farms experience water temperature variations from 10°C to 24°C (Stone et al. 2013), which influences almost every aspect of abalone production including growth and feed consumption (Britz et al. 1997, Bansemer et al. 2015). A number of factors including species, genetic strain, and size influence the optimal water temperature of abalone (Britz et al. 1997, Gilroy & Edwards 1998, Stone et al. 2013, Bansemer et al. 2015).

To improve our understanding of the differences in the nocturnal feed consumption patterns between the most commonly cultured abalone in Australia, our aim was to understand the impact of diet type and water temperature on the nocturnal feed consumption patterns for greenlip and hybrid abalone. The knowledge on abalone feeding and feed consumption would provide insight into the improvement of management strategies in terms of time of feeding and the use of macroalgae for feeding stimulation.

MATERIALS AND METHODS

Experimental Animals

Greenlip abalone (weight: 21.35 ± 0.30 g, shell length: 55.50 ± 0.51 mm; $n = 100$) and hybrid abalone (weight: 25.50 ± 0.60 g, shell length: 58.87 ± 0.42 mm; $n = 100$) were purchased from South Australian Mariculture Pty. Ltd. (Port Lincoln, South Australia, Australia). Prior to the study, abalone were held at ambient water temperature in a 5000 l holding tank supplied with sand-filtered, ultraviolet (UV)-treated, flow-through seawater at the South Australian Research and Development Institute (SARDI) Aquatic Science Center (ASC) and fed a commercial formulated diet *ad libitum* ["Abgrow premium" 5 mm chip; Eyre Peninsula Aquafeeds (EPA), Lonsdale, South Australia].

Experimental System

The experiment was conducted in a temperature-controlled system previous described by Stone et al. (2013). Briefly, two identical systems were utilized, each system composed of twenty 12.5-l rectangular blue plastic tanks (Nally IH305, Viscount Plastics Pty. Ltd.). Each tank was supplied with temperature-controlled, sand-filtered, UV-treated, flow-through seawater at a rate of 300 ml/min. Water temperature was held at 18°C or 22°C (±1°C) by 3 kW immersion heaters (240 V, 3 kW, JQ20; Austin & Cridland, Carlton, NSW, Australia). A mesh screen (0.8-mm mesh size) on the outlet of each tank was used to retain uneaten feed. The photoperiod was held constant at 12-h low-intensity fluorescent lighting (3.4 Lux; 7:00 AM–7:00 PM): 12 h dark (7:00 PM–7:00 AM).

Experimental Design, Stocking and Animal Acclimation

The experiment used a factorial design with abalone type (greenlip or hybrid abalone), diet type [EPA "Abgrow premium" formulated diet (5mm chip) or fresh *Ulva* sp.], and water temperature (18°C or 22°C), resulting in a total of eight treatment combinations. The commercial formulated diet is routinely fed on-farm, whereas *Ulva* spp. has previously been reported to be excellent feed for abalone (Viera et al. 2011, Stone et al. 2014a). The water temperatures investigated in this study, represent on-farm temperatures experienced during the period of high feed consumption for both types of abalone (Stone et al. 2013, Lange et al. 2014, Stone et al. 2014b).

At the commencement of the experiment, abalone were removed from the holding tank (water temperature 14°C), weighed (±0.01 g), and measured (±0.01 mm), and five animals were systematically interspersed in to each experimental tank ($n = 5$ tanks/treatment). Animals were acclimatized to the experimental system for seven days, and the water temperature was slowly raised (1°C/day) to the desired temperature (18°C or 22°C). During the acclimation period, abalones were fed their respective diet to excess at 4:00 PM (fresh *Ulva* sp. 4.3%, formulated diet 2.6% of the abalone biomass/day). Tanks were cleaned at 8:00 AM the following day. No feed was left in the tanks between 8:00 AM and 4:00 PM.

Diets, Feeding and Sampling

During the experimental period, abalones were also fed to excess at 4:00 PM, at the same rate as used in the acclimation phase. During the experiment, each tank was systematically sampled five times, once at each sampling time (7:00 PM, 10:00 PM, 1:00 AM, 4:00 AM, and 8:00 AM), over five consecutive days. Sampling involved collecting uneaten feed, by sieving the entire tank contents through a fine mesh screen (500 μm). To ensure that feed intake was not limited after the removal of feed at each sampling period, tanks were immediately refed to excess and were recleaned at 8:00 AM the following morning. Abalones were not provided with feed between 8:00 AM and 4:00 PM each day.

Calculation of Cumulative Feed Consumption and Total Feed Intake

Collected uneaten feed samples were stored at –20°C, and were oven dried to a constant weight to obtain dry weights. To account for feed leaching losses (formulated diet) or growth (*Ulva* sp.), diets were added to tanks without animals present, and collected at each sampling period at 18°C and 22°C. This value was used as a correction factor to calculate the apparent feed consumption at each sampling period. The calculation for the apparent feed consumption for each sample time was based on dry values for feed intake and wet values for abalone weight. The apparent feed consumption for each collection period is expressed as:

$$\begin{aligned}&\text{Apparent feed consumption }(\text{g/kg abalone})\\&=\frac{\begin{aligned}&\text{feed offered} - \text{uneaten food collected }[(\text{total feed offered}\\&\quad\times\ \%\ \text{leaching loss without animals})\\&\quad+(\text{uneaten food collected}/\ \%\ \text{retained without animals}\\&\quad\times\ \%\ \text{leaching loss without animals})]/2\end{aligned}}{\text{tank biomass}}\end{aligned}$$

(Stone et al. 2013).

The total feed and nutrient intake was calculated using the apparent feed consumption formula (above) at 8:00 AM (16 h postfeeding). The total feed and nutrient intake were based on wet or dry values for the feed consumption, and wet weight of abalone. The calculations for the total feed intake and nutrient intake are expressed as g/kg abalone/day.

Biochemical and Water QualityAanalyses

The proximate composition of the diets was analyzed according to the methods of the AOAC International (1995) and are presented in Table 1. Moisture content was determined by oven drying samples to a constant weight. Crude protein (N × 6.25) was determined by the Kjeldahl method. Crude lipid was analyzed using a Soxtherm rapid extraction system (Gerhardt GmbH & Co. KG, Königswinter, Germany) with petroleum liquid (BP 100°C) as the extracting solvent. Total carbohydrate was determined by the Molisch's test and a glucose standard curve. Gross energy of the formulated diet was determined using a bomb calorimeter calibrated with benzoic acid. The gross energy content of the *Ulva* sp. was calculated using the values of 17.2, 23.6, and 39.5 MJ/kg for carbohydrate, protein, and lipid, respectively (NRC 2011).

Water quality parameters are presented in Table 2. Salinity was measured at the start of the five-day experimental period (35 g/L). The water temperature, dissolved oxygen (mg/l and % saturation) and pH were measured daily and maintained at levels appropriate for abalone throughout the study (Hutchinson & Vandepeer 2004).

Statistical Analyses

IBM SPSS (version 20 for Windows, IBM SPSS Inc., Chicago, IL) was used for all statistical analyses. Homogeneity of variances and normality among mean values were assessed using Levene's test for equality of variance errors and the standardized residuals against the predicted mean plot, respectively. Apparent total feed intake (as fed and dry basis) and nutrient intake were analyzed using three-factor analysis of variance (ANOVA) [Factors: abalone type (greenlip and hybrid abalone), diet type (fresh *Ulva* sp. and formulated diet), and water temperature (18°C and 22°C)]. When significant interactions were observed, pairwise comparisons were used to determine significant differences between treatment combinations. Pearson's linear and second order polynomial regression analyses were also applied between time and apparent cumulative feed consumption. To determine differences between relationships, apparent cumulative feed consumption was analyzed using three-factor analysis of covariance (ANCOVA) [(Factors: abalone type, water temperature, and diet type), with time included as a covariate (7:00 PM, 10:00 PM, 1:00 AM, 4:00 AM, and 8:00 AM)]. Data were log transformed to satisfy the assumption of ANCOVA. A significant level of $P < 0.05$ was used for all statistical tests. All values are presented as means ± SE of the mean ($n = 5$), unless otherwise stated. If the SE was less than 0.01 it is reported as "0.00."

TABLE 1.

Analyzed nutrient composition of test diets (g/100 g dry basis).

Item	Commercial formulated diet*	Fresh *Ulva* sp.
Moisture	10.66	84.15
Crude protein	34.12	18.36
Crude carbohydrate	58.06	25.42
Crude lipid	3.29	1.01
Gross energy (MJ/kg)	17.01	9.10†

* Commercial formulated diet: Eyre Peninsula Aquafeeds; Lonsdale, South Australia.

†Gross energy was calculated using the values of 23.60, 17.20, and 39.50 MJ/kg for protein, carbohydrate, and lipid, respectively (NRC 2011).

RESULTS

Apparent Cumulative Feed Consumption

Abalone fed actively on both diets throughout the study and immediately displayed an active feeding response post sample collection and refeeding. No visual signs of disease were observed in experimental animals.

For greenlip abalone, regression analyses showed significant positive second-order polynomial relationships between time and apparent cumulative feed consumption for abalone fed the formulated diet at 18°C ($R^2 = 0.841$; $P < 0.001$; Fig. 1) and 22°C ($R^2 = 0.915$; $P < 0.001$; Fig. 1). Due to the low x^2 value when a second-order polynomial relationship was fitted to the data, the apparent cumulative feed consumption for abalone fed *Ulva* sp. was analyzed using Pearson's linear regression analyses. There were significant positive linear relationships between time and cumulative feed consumption for greenlip abalone fed *Ulva* sp. at 18°C ($R^2 = 0.730$, $P < 0.001$; Fig. 1) and 22°C ($R^2 = 0.612$; $P < 0.001$; Fig. 1).

For hybrid abalone, regression analyses showed significant positive second-order polynomial relationships between time postfeeding and cumulative feed consumption for abalone fed the formulated diet at 18°C ($R^2 = 0.566$; $P < 0.001$; Fig. 2) and 22°C ($R^2 = 0.776$; $P < 0.001$; Fig. 2). Because of the reasons previously stated for greenlip abalone fed *Ulva* sp., the relationship between time and apparent cumulative feed consumption for hybrid abalone fed *Ulva* sp. was analyzed using Pearson's linear regression analyses. There were significant positive linear relationships between time and cumulative feed consumption for hybrid abalone fed *Ulva* sp. at 18°C ($R^2 = 0.440$; $P < 0.001$; Fig. 2) and 22°C ($R^2 = 0.883$; $P < 0.001$; Fig. 2).

To determine treatment effects on the apparent cumulative feed consumption, data were analyzed using ANCOVA, with log transformed time as a covariate to satisfy the linear assumption of ANCOVA. The apparent cumulative feed consumption was significant influenced by the covariate, time ($P <$ 0.001), and diet type (formulated diet greater than *Ulva* sp.; ANCOVA; Figs. 1 and 2). Apparent cumulative feed consumption was not significantly influenced by abalone type ($P = 0.468$), but was significantly affected by water temperature ($P <$ 0.001), and the interaction between water temperature and abalone type ($P = 0.009$; ACOVA; Figs. 1 and 2). Apparent cumulative feed consumption significantly increased as water temperature increased from 18°C to 22°C, this response was significantly higher for hybrid abalone. The apparent cumulative feed consumption was not significantly affected by the interaction between diet type and abalone type ($P = 0.111$), diet type and water temperature ($P = 0.990$), or abalone type, water temperature, and diet type ($P = 0.740$).

Apparent Total Feed Intake

The apparent total feed intake was significantly influenced by the interaction between abalone type, water temperature and diet type ($P = 0.039$; Table 3). The significant interaction was due to the significantly greater increase in apparent total feed

TABLE 2.

Summary of water quality for each temperature-controlled treatment.

Temperature system	Temperature (°C)	Dissolved oxygen (mg/l)	Dissolved oxygen (% saturation)	pH
18°C	18.2 ± 0.3	7.78 ± 0.25	101.1 ± 3.4	8.11 ± 0.03
	(17.5–18.6)	(7.33–8.08)	(95.4–109.6)	(8.06–8.15)
22°C	21.8 ± 0.4	7.10 ± 0.23	98.6 ± 3.1	8.1 ± 0.03
	(21.3–22.5)	(6.54–7.53)	(90.5–103.6)	(8.04–8.17)

Values are mean ± SD. Values in parentheses represent the range of values over the five-day experimental period.

intake for hybrid abalone as water temperature increased from 18°C to 22°C, compared with the response for greenlip abalone. Moreover, compared with greenlip abalone, a significantly greater increase in apparent total feed intake was observed for hybrid abalone fed *Ulva* sp. compared with hybrid abalone fed a formulated diet when water temperature increased from 18°C to 22°C.

The apparent total feed intake (dry basis) was significantly influenced by diet type (formulated diet greater than *Ulva* sp.; $P < 0.001$; Table 3). The apparent total dry mass feed intake was not significantly affected by abalone type ($P = 0.118$), but was significantly influenced by water temperature ($P < 0.001$) and the interaction between abalone type and water temperature ($P = 0.003$). The significant interaction between abalone type and water temperature for apparent total dry mass feed intake was due to the significantly greater increase in feed intake for hybrid abalone as water temperature increased from 18°C to 22°C, compared with greenlip abalone.

Apparent protein, carbohydrate, lipid, and energy intake of abalone was significantly influenced by diet type (formulated diet greater than *Ulva* sp.; $P < 0.001$; Table 3). The apparent protein, carbohydrate, lipid, and energy intake was not significantly affected by abalone type ($P = 0.118$), but was significantly influenced by water temperature ($P < 0.001$) and the interaction between abalone type and water temperature ($P = 0.003$). The significant interaction between abalone type and water temperature for apparent protein, carbohydrate, lipid, and energy intake occurred due to the significantly greater increase in feed intake for hybrid abalone as water temperature increased from 18°C to 22°C, compared with greenlip abalone.

DISCUSSION

Australian abalone farms typically use one to two layers of shade cloth to reduce light in the culture units during the day (Stone et al. 2014b). This low level of daytime light intensity was mimicked in this study by using low fluorescent lighting (7:00 AM to 7:00 PM; 3.4 Lux; comparable to the dark limit of civil twilight under a clear sky). The apparent feed consumption for greenlip and hybrid abalone at either 18°C or 22°C was minimal between the introduction of feed (4:00 PM) and the end of the light period (7:00 PM). On farm, abalone are typically fed before 5:00 PM, but during summer in southern Australia, sunset is not until ~8:30 PM, and total darkness may not occur until ~9:30 PM (Geoscience Australia 2014, Stone et al. 2014b). The dry matter leaching loss from commercial formulated diets is relativity low (~15% after 24 h; Ruff et al. 2014), but highly water soluble

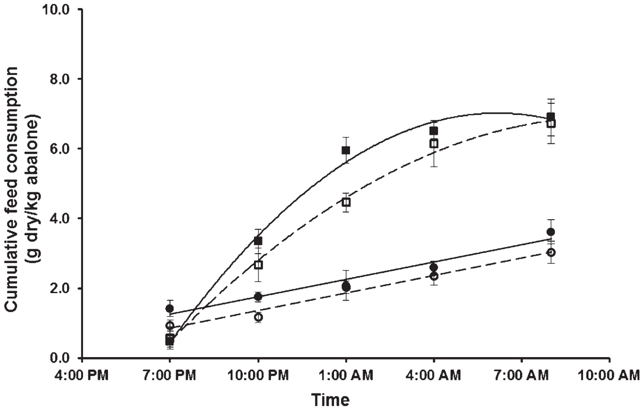

Figure 1. Cumulative feed consumption (dry basis, y) for greenlip abalone (*Haliotis laevigata*) fed the commercial formulated diet at 18°C (□, dashed line) and 22°C (■, solid line) or *Ulva* sp. at 18°C (○, dashed line) and 22°C (●, solid line) throughout the night ($n = 5$ replicate tanks/treatment; mean ± SE, x). Second order polynomial relationships: commercial formulated diet at 18°C, $y = –0.29x^2 –1.030x – 2.356$, $R^2 = 0.841$, $P < 0.001$; and at 22°C, $y = –0.53x^2 – 1.494x – 3.558$, $R^2 = 0.915$, $P < 0.001$. Linear relationships: *Ulva* sp. at 18°C, $y = 0.168x – 0.350$, $R^2 = 0.730$, $P < 0.001$; and at 22°C, $y = 0.165x – 0.771$, $R^2 = 0.612$, $P < 0.001$.

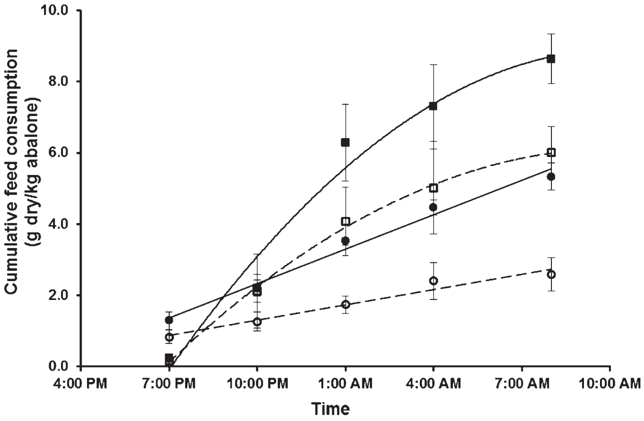

Figure 2. Cumulative feed consumption (dry basis, y) for hybrid abalone (*Haliotis laevigata* × *Haliotis rubra*) fed the commercial formulated diet at 18°C (□, dashed line) and 22°C (■, solid line) or *Ulva* sp. at 18°C (○, dashed line) and 22°C (●, solid line) throughout the night ($n = 5$ replicate tanks/treatment; mean ± SE, x). Second order polynomial relationships: commercial formulated diet at 18°C, $y = –0.026x^2 +0.939x – 2.468$, $R^2 = 0.566$, $P < 0.001$; and at 22°C, $y = –0.038x^2 + 1.405x – 3.957$, $R^2 = 0.776$, $P < 0.001$. Linear relationships: *Ulva* sp. at 18°C, $y = 0.144x – 0.445$, $R^2 = 0.440$, $P < 0.001$; and at 22°C, $y = 0.321x + 0.410$, $R^2 = 0.883$, $P < 0.001$.

TABLE 3.

Apparent total feed and nutrient intake for greenlip abalone (*Haliotis laevigata*) and hybrid abalone (*H. laevigata* × *Haliotis rubra*).

Abalone type	**Greenlip abalone**				**Hybrid abalone**										
Diet type	**Formulated diet**		**Fresh *Ulva* sp.**		**Formulated diet**		**Fresh *Ulva* sp.**		**ANOVA ($P =$)**						
Water temperature	**18°C**	**22°C**	**18°C**	**22°C**	**18°C**	**22°C**	**18°C**	**22°C**	**Species (A)**	**Diet (B)**	**Temperture (C)**	**A × B**	**A × C**	**B × C**	**A × B × C**
Feed consumption (g as fed/kg abalone/day)	7.52 ± 0.65	7.73 ± 0.59	19.11 ± 1.99	22.82 ± 2.18	6.72 ± 0.82	9.67 ± 0.78	16.33 ± 2.99	33.64 ± 2.37	0.078	<0.001	<0.001	0.179	0.003	0.001	0.039
Feed consumption (g dry/kg abalone/day)	6.72 ± 0.58	6.91 ± 0.53	3.03 ± 0.32	3.62 ± 0.35	6.00 ± 0.73	8.63 ± 0.70	2.59 ± 0.47	5.33 ± 0.38	0.118	<0.001 (>)	<0.001	0.858	0.003	0.722	0.846
Crude protein (g/kg abalone/day)	2.29 ± 0.20	2.36 ± 0.18	0.56 ± 0.06	0.66 ± 0.06	2.05 ± 0.25	2.95 ± 0.24	0.48 ± 0.09	0.98 ± 0.07	0.204	<0.001 (>)	0.001	0.813	0.010	0.445	0.346
Crude carbohydrate (g/kg abalone/day)	3.90 ± 0.34	4.01 ± 0.31	0.77 ± 0.08	0.92 ± 0.09	3.49 ± 0.42	5.01 ± 0.40	0.66 ± 0.12	1.36 ± 0.10	0.069	<0.001 (>)	0.006	0.344	0.035	0.608	0.529
Crude lipid (g/kg abalone/day)	0.22 ± 0.02	0.23 ± 0.02	0.03 ± 0.00	0.04 ± 0.00	0.20 ± 0.02	0.28 ± 0.02	0.03 ± 0.00	0.05 ± 0.00	0.284	<0.001 (>)	0.006	0.660	0.024	0.118	0.182
Gross energy* (MJ/kg abalone/day)	0.11 ± 0.01	0.12 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.10 ± 0.01	0.15 ± 0.01	0.02 ± 0.00	0.05 ± 0.00	0.235	<0.001 (>)	0.002	0.754	0.008	0.338	0.400

Values are mean ± SE; $n = 5$; SE < 0.01 is reported as "0.00."

A significance level of $P < 0.05$ was used for all statistical tests, for variables with a significant effect of diet ($P < 0.05$) and no interaction, < or > denotes that the variable for abalone fed the formulated diet was significantly less than or greater than abalone fed *Ulva* sp. For variables with a significant interaction, differences between treatments were analyzed using pairwise comparisons.

* Gross energy intake for abalone fed fresh *Ulva* sp. was calculated using the values of 23.60, 17.20, and 39.50 MJ/kg for protein, carbohydrate, and lipid, respectively (NRC 2011).

nutrients, such as vitamins, minerals, and free amino acids, may be leached between feeding and total darkness, before abalone have commenced feeding (Gadient & Schai 1994, Fleming et al. 1996, Coote et al. 2000). It may be beneficial to feed cultured abalone closer to darkness, but this may not be economically viable due to the high cost of labor in Australia. Currently, some hybrid abalone farms culture animals in total darkness during the day (Stone et al. 2014b), and it may also be beneficial to culture greenlip abalone under the same conditions; however, this practice may not be feasible due to logistical problems associated with occupational health and safety and the practicality of employees working in darkness.

It would be favorable to entice abalone to feed during the light period. In this study, greenlip and hybrid abalone immediately displayed a feeding response when *Ulva* sp. was added to the tank during the light period, and continued to consume *Ulva* sp. throughout the light period. This response was in contrast to abalone fed the formulated diet. *Ulva* sp. may act as an effective feeding stimulant to both greenlip and hybrid abalone. Previous studies have reported that a combination of tactile and chemosensory cues in macroalgae, that are not present in formulated diets, can stimulate a feeding response, and increase the feed intake and growth of abalone (Jan et al. 1981, Allen et al. 2006). For example, Jan et al. (1981) reported an immediate feeding response by abalone (*Haliotis diversicolor supertexta*) when a water soluble extract of *Ulva* spp. (25 g of dried *Ulva* spp. steeped in 1 l of seawater for 1 h, then filtered) was administrated to the tank, despite the absence of feed in the system. Moreover, numerically higher feed intake and significantly higher shell growth rates (15%) were reported for *Haliotis iris* fed a formulated diet with dried, mulched *Gracilaria* spp. particles (300–500 μm) suspended in the system compared with abalone fed a control formulated diet alone (Allen et al. 2006). Understanding the mechanism how fresh *Ulva* sp. stimulates the feeding activity of abalone has the potential to improve feeding efficiency in abalone farming. It would be beneficial in future studies to investigate whether the presence of cues from *Ulva* sp. also stimulates greenlip abalone to consume a formulated diet, particular during the light period.

In this study, the apparent cumulative feed consumption rate of abalone was dependent on diet type, abalone type, and water temperature. The cumulative feed consumption rate for greenlip abalone fed the formulated diet at 22°C was highest between 7:00 PM and 1:00 AM, but abalone ceased feeding after 1:00 AM. A similar peak in feed consumption rate was reported between 6:00 PM and 12:00 AM for *Haliotis midae* fed a formulated diet at 20°C (Britz et al. 1996). In contrast, the peak feeding activity, determined by ventral video monitoring, for greenlip abalone fed the same formulated diet as used in this study was between 10:00 PM and 6:00 AM at 22°C (Buss et al. 2015). Differences may have occurred due to size differences or methodology difference used by Buss et al. (2015) and to those used in this study. Moreover, in this study, feed consumption was prolonged, the apparent feed consumption rate for greenlip abalone at 18°C and hybrid abalone at 18°C and 22°C was highest between 7:00 PM and 4:00 AM, but abalone continued to feed at a lower rate until 8:00 AM. Nutrient leaching loss from commercial formulated diets is a major concern to the abalone industry (Fleming et al. 1996, Ruff et al. 2014). On the basis of the results from this study, we recommend that diets should ideally retard nutrient leaching loss from the addition of feed until morning (greater than16 h). Currently, commercial abalone feed companies exceed this recommendation and use binders and manufacturing processes to improve formulated diet stability and aim to minimize nutrient leaching loss for 24 h (Ruff et al. 2014).

The peak feed consumption rate for the ass's-ear abalone (*Haliotis asinina*) fed a mixed macroalgae diet (*Acanthophora specifera*, *Haliotis valentiae,* and *Laurencia papillosa*) occurred between 6:00 PM and 2:00 AM (Tahil & Juino-Menez 1999), which is similar to the aforementioned studies where abalone were fed a formulated diet (Britz et al. 1996, Buss et al. 2015). In contrast, greenlip and hybrid abalone in this study consumed *Ulva* sp. at a linear rate throughout the feeding period. Although the feed intake for abalone fed *Ulva* sp. was significantly higher (~300%) than abalone fed the formulated diet, the energy and nutrient intake of abalone fed *Ulva* sp. were significantly lower. Abalone regulate feed intake to achieve energy satiation (Fleming et al. 1996). In this study, the inherent high moisture content of *Ulva* sp. may have increased gut-fullness and inhibited feed intake upregulation to achieve energy satiation (Fleming et al. 1996, Alcantara & Noro 2005). It should be noted that in this study, *Ulva* sp. was cultured in unenriched seawater. *Ulva* spp. are able to utilize inorganic nitrogen and synthesise amino acids and protein when cultured in a nutrient-enriching medium (Viera et al. 2011, Nielsen et al. 2012). Abalone fed nutrient enriched *Ulva* spp. have higher feed intake and growth rates than those fed unenriched *Ulva* spp. (Shpigel et al. 1999, Viera et al. 2011, Bansemer et al. 2014b). The feeding patterns of greenlip and hybrid abalone in this study might have differed if abalone had been fed nutrient-enriched *Ulva* sp. that had higher protein and energy content. The feeding response of abalone fed nutrient-enriched macroalgae warrants further investigation.

Water temperature affects abalone growth, metabolic activity, and feed consumption (Britz et al. 1997, Bansemer et al. 2015). In this study, regardless of diet, the effect of water temperature on the cumulative feed consumption rate and feed intake was dependent on abalone type. The negligible increase in cumulative feed consumption rate and feed intake for greenlip abalone as water temperature increased from 18°C to 22°C is supported by a previous study by Stone et al. (2013). Stone et al. (2013) used greenlip abalone of a similar size [57mm shell length (SL)] and strain (South Australian Mariculture Pty Ltd.) to those used in this study, and found that raising water temperature from 18°C to 22°C had no significant effect on growth and feed intake when fed a formulated diet (31%–34% crude protein level). The optimal water temperature for hybrid abalone growth is unknown, but are generally cultured in cooler water compared with greenlip abalone (Stone et al. 2014b). It should be noted that Gilroy and Edwards (1998) reported that the optimal water temperature for a strain of Tasmanian greenlip abalone (82-mm SL) was 18.3°C, whereas the optima for blacklip abalone collected from a similar geographical location was 17.0°C. The notion that hybrid abalone have a lower water temperature optima than greenlip abalone was not supported in this study. The high survival (100%) and feed intake for hybrid abalone at 22°C suggest that the upper physiological range for hybrid abalone may be greater than or equal to 22°C. This was a short-term study, and the effect of chronic exposure to water temperature greater than or equal to 22°C is unknown. Moreover, cultured hybrid abalone may be exposed to suboptimal conditions, such as high stocking densities, low flow rates and low dissolved oxygen levels, which may also influence the physiological temperature range and ultimately survival for hybrid abalone (Wassnig et al. 2010). Further research on the optimal and physiological water temperature range for hybrid abalone would be beneficial to the abalone industry.

In conclusion, *Ulva* sp. induced an immediate feeding response of hybrid and greenlip abalone during the light period. The feed consumption of abalone fed a formulated diet was minimal during the light period. The high moisture content of *Ulva* sp. may restrict the energy and nutrient intake by greenlip and hybrid abalone and may ultimately compromise growth. To increase the feed intake of abalone fed a formulated diet during the light period on-farm, the cues present in fresh *Ulva* sp. that induced abalone feeding deserves further investigation. Compared with greenlip abalone and blacklip abalone, their hybrid can tolerate a higher temperature (greater than or equal to 22°C). This study is a step stone for further investigation on the use of live macroalgae and dried macroalgae meal in abalone diets.

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LITERATURE CITED

Alcantara, L. B. & T. Noro. 2005. Effect of macroalgae type and water temperature on macroalgae consumption rates of the abalone *Haliotis diversicolor* Reeve. *J. Shellfish Res*. 24:1169–1177.

Allen, V. J., I. D. Marsden, N. L. C. Ragg & S. Gieseg. 2006. The effects of tactile stimulants on feeding, growth, behaviour, and meat quality of cultured Blackfoot abalone, *Haliotis iris*. *Aquaculture* 257:294–308.

AOAC International. 1995. Official methods of analysis of AOAC International. 2 vols. 16th edition. Arlington, VA: Association of Analytical Communities.

Bansemer, M. S., J. G. Qin, J. O. Harris, G. S. Howarth & D. A. J. Stone. 2014a. Nutritional requirements and use of macroalgae as ingredients in abalone feed. *Rev. Aquaculture*. 5:1–15. doi: 10.1111/raq.12085.

Bansemer, M. S., J. G. Qin, J. O. Harris, G. S. Howarth & D. A. J. Stone. 2014b. Nutrient utilization and growth of greenlip abalone (*Haliotis laevigata*) fed nitrogen enriched macroalgae. Abstract presented (oral presentation), World Aquaculture Conference, Adelaide, Australia, 7–11 June 2014.

Bansemer, M. S., J. O. Harris, J. G. Qin, L. R. Adams, D. N. Duong & D. A. J. Stone. 2015. Growth and feed utilization of juvenile greenlip abalone (*Haliotis laevigata*) in response to water temperatures and increasing dietary protein levels. *Aquaculture* 436:13–20.

Bautista-Teruel, M. N., M. R. De La Pena & A. J. Asutilla. 2013. Evaluation of agar-bound microparticulate diet as alternative food in abalone hatchery: effects of agar concentrations and feeding frequencies. *J. Shellfish Res*. 32:9–15.

Britz, P. J., T. Hecht & J. Knauer. 1996. Gastric evacuation time and digestive enzyme activity in abalone *Halotis midae* fed a formulated diet. *S. Afr. J. Sci*. 17:297–303.

Britz, P. J., T. Hecht & S. Mangold. 1997. Effect of temperature on growth, feed consumption and nutritional indices of *Haliotis midae* fed a formulated diet. *Aquaculture* 152:191–203.

Buss, J. J., D. A. Jones, A. Lumsden, J. O. Harris, M. S. Bansemer & D. A. J. Stone. 2015. Restricting feed rate has more effect than diet type on the feeding behaviour of greenlip abalone *Haliotis laevigata*. *Mar. Freshwat. Behav. Physiol*. 48:51–70.

Coote, T. A., P. W. Hone, R. J. Van Barneveld & G. B. Maguire. 2000. Optimal protein level in a semipurified diet for juvenile greenlip abalone *Haliotis laevigata*. *Aquacult. Nutr*. 6:213–220.

Fleming, A. E. & P. W. Hone. 1996. Abalone aquaculture. *Aquaculture* 140:1–4.

Fleming, A. E., R. J. Van Barneveld & P. W. Hone. 1996. The development of artificial diets for abalone: a review and future directions. *Aquaculture* 140:5–53.

Fotedar, R. & B. Phillips. 2011. Recent advances and new species in aquaculture. West Sussex, UK: John Wiley & Sons.

Gadient, M. & E. Schai. 1994. Leaching of various vitamins from shrimp feed. *Aquaculture* 124:201–205.

Geoscience Australia. 2014. Australian Government Geoscience Australia [ONLINE]. Accessed October 21, 2014. Available at: http://www.ga.gov.au/geodesy/astro/sunrise.jsp.

Gilroy, A. & S. J. Edwards. 1998. Optimum temperature for growth of Australian abalone: preferred temperature and critical thermal maximum for blacklip abalone, *Haliotis rubra* (Leach), and greenlip abalone, *Haliotis laevigata* (Leach). *Aquacult. Res*. 29: 481–485.

Hutchinson, W. & M. Vandepeer. 2004. Water quality: effects and management on abalone farms. Adelaide: South Australian Research and Development Institute (Aquatic Sciences). 55 pp.

Jan, R. Q., K. T. Shao & K. H. Chang. 1981. Extract of sea lettuce *Ulva* reduces oxygen consumption of abalone (*Haliotis diversicolor supertexta* Lischke). *Bull. Inst. Zool. Acad. Sin*. 20:83–85.

Kirkendale, L., D. V. Robertson-Andersson & P. C. Winberg. 2010. Review on the use and production of algae and manufactured diets as feed for sea-based abalone aquaculture in Victoria. University of Wollongong and Shoalhaven Marine and Freshwater Centre, Department of Primary Industries, Fisheries Victoria, Victoria, Australia. 198 pp.

Lange, B., K. L. Currie, G. S. Howarth & D. A. J. Stone. 2014. Grape seed extract and dried macroalgae, *Ulva lactuca* Linnaeus, improve survival of greenlip abalone, *Haliotis laevigata* Donovan, at high water temperature. *Aquaculture* 433:348–360.

Nielsen, M. M., A. Bruhn, M. B. Rasmussen, B. Olesen, M. M. Larsen & H. B. Møller. 2012. Cultivation of *Ulva lactuca* with manure for simultaneous bioremediation and biomass production. *J. Appl. Phycol*. 24:449–458.

NRC. 2011. Nutrient Requirements of Fish and Shrimp. Washington, DC: National Academies Press.

Ruff, N., M. Bransden, B. Wybourne & R. Hauler. 2014. Optimized performance and efficiency in abalone feed. Abstract presented (oral presentation), World Aquaculture Conference, Adelaide, Australia, 7–11 June 2014.

Shepherd, S. A. 1973. Studies on southern Australian abalone (Genus *Halitosis*). Ecology of five sympatric species. *Aust. J. Mar. Freshwater Res.* 24:217–257.

Shpigel, M., N. L. Ragg, I. Lupatsch & A. Neori. 1999. Protein content determines the nutritional value of the seaweed *Ulva lactuca* L. for the abalone *Haliotis tuberculata* L. and *H. discus hannai* Ino. *J. Shellfish Res.* 18:227–233.

Stone, D. A. J., J. O. Harris, H. Wang, G. J. Mercer, E. N. Schaefer & M. S. Bansemer. 2013. Dietary protein level and water temperature interactions for greenlip abalone, *Haliotis laevigata*. *J. Shellfish Res.* 32:119–130.

Stone, D. A. J., M. S. Bansemer, B. Lange, E. N. Schaefer, G. S. Howarth & J. O. Harris. 2014a. Dietary intervention improves the survival of cultured greenlip abalone (*Haliotis laevigata* Donovan) at high water temperature. *Aquaculture* 430:230–240.

Stone, D. A. J., M. S. Bansemer & J. O. Harris. 2014b. Final Report. Development of formulated diets for cultured abalone. Prepared by the South Australian Research and Development Institute (Aquatic Sciences), Adelaide. CRC Project No. 2010/736. 131 pp.

Tahil, A. S. & M. A. Juino-Menez. 1999. Natural diet, feeding periodicity and functional response to food density of the abalone, *Haliotis asinina* L., (Gastropoda). *Aquacult. Res.* 30:95–107.

Viera, M. P., G. Courtois de Vicose, J. L. Gómez-Pinchetti, A. Bilbao, H. Fernandez-Palacios & M. S. Izquierdo. 2011. Comparative performances of juvenile abalone (*Haliotis tuberculata coccinea* Reeve) fed enriched vs non-enriched macroalgae: effect on growth and body composition. *Aquaculture* 319:423–429.

Wassnig, M., R. D. Roberts, A. Krsinich & R. W. Day. 2010. Effects of water flow rate on growth rate, mortality, and biomass return of abalone in slab tanks. *Aquacult. Res.* 41:839–846.