

VENTRAL VIDEOGRAPHIC ASSESSMENT OF THE FEEDING BEHAVIOR OF JUVENILE GREENLIP [*HALIOTIS LAEVIGATA* (DONOVAN, 1808)] AND HYBRID (*H. LAEVIGATA*×*HALIOTIS RUBRA*) ABALONE IN RESPONSE TO DIETARY AND TEMPERATURE MANIPULATION

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ABSTRACT This multifactorial study used a newly developed ventral videography technique to investigate the feeding behavior of 18-mo-old juvenile greenlip abalone (21.1 g, 55.6 mm), and hybrid abalone (23.4 g, 57.6 mm) fed either a formulated commercial diet or live *Ulva* sp. at 18°C and 22°C. Feeding behavior was scored in terms of the following activities: quiescence, alertness, moving, feeding, distance traveled, velocity, and homing. There was a significant effect of diet type and temperature on feeding behavior of abalone. Both types of abalone were more active and exhibited the highest velocity when fed the formulated diet at 22°C. Greenlip abalone spent a larger proportion of time feeding on *Ulva* sp. than on the formulated diet, regardless of the water temperature. In contrast, no significant difference was observed in the proportion of time feeding between diet types for hybrid abalone. Both types of abalone rapidly located and consumed *Ulva* sp. when feed was introduced into the aquaria at 1600 h (light phase). In contrast, abalone predominantly commenced feeding on the formulated diet in the dark phase, indicating the potential of *Ulva* sp. as feed attractant in abalone diet, to promote feeding. The mode in which abalone consumed feed also differed between diets. Abalone fed the formulated diet nibbled intermittently on random chips, whereas when fed *Ulva* sp., abalone engulfed entire individual fronds before moving on to the next available frond. Greenlip and hybrid abalone exhibited homing behavior at the completion of the dark phase, which was more pronounced in abalone fed *Ulva* sp. This new information may assist in refining feed design and feeding practices for the culture of these two types of abalone.

KEY WORDS: *Haliotis laevigata*, *H. laevigata*×*H. rubra*, feeding behavior, diet, temperature

INTRODUCTION

The abalone industry has developed significantly in Australia over the past decade. The stimulus for the industries' growth has been primarily due to an increased consumer demand, whereas global wild stocks have declined due to over fishing and the low survival of juveniles in rehabilitation programs (Kirkendale et al. 2010). Feed and feeding accounts for approximately 30% of the cost of abalone aquaculture production and, therefore, any research that can lead to an improvement in these areas may also lead to a decrease in the cost of production (Fleming et al. 1996, Stone et al. 2013). This is inclusive of understanding the feeding behavior of abalone in regard to different types of feed and environmental factors such as water temperature (Stone et al. 2013, Buss et al. 2015, Bansemer et al. 2015a).

Research thus far has demonstrated that abalone exhibit nocturnal feeding behavior. Fleming (1995) reported that the movement of greenlip abalone (*Haliotis laevigata*) was at its maximum during the early part of the dark period with movement predominately occurring between 2000 and 2400 h. Similarly, Allen et al. (2006) observed the feeding activity of 2-y-old blackfoot abalone (*Haliotis iris*) fed an artificial diet was maximum in darkness between 1900 and 2200 h. Wild and

laboratory-reared juvenile South African abalone (*Haliotis midae*) of different sizes have also been observed to be only active at night (Day & Branch 2002). Temperature has also been shown to have an effect on the behavior of abalone. In the wild, northern abalone (*Haliotis kamtschatkana*) exhibited seasonal variations in activity with 20% of all individuals observed crawling during summer compared with less than 5% during winter for observations made 0900–1200 h (Donovan & Carefoot 1998). In the laboratory, northern abalone were also observed to be more active in summer, but interestingly exhibited greater feeding activity in winter (Donovan & Carefoot 1998). Diet type has also been reported to influence the feeding behavior of abalone. Tutschulte and Connell (1988) reported that in the wild, adult (SL > 25 mm) pink (*Haliotis corrugata*) and green abalone (*Haliotis fulgens*) fed equally on macroalgae during both light and dark periods. In contrast, juvenile abalone (SL < 25 mm) of the same species fed exclusively at night, indicating that the diurnal feeding behavior may be influenced by size and age. In addition, movement and feeding behavior have been found to be influenced by feed ration. Buss et al. (2015) found that greenlip abalone fed on a restricted ration of either a macroalgae or formulated diet moved significantly more and started to exhibit feeding behavior earlier (during the light period) than when feed to excess. Homing behavior has also been observed in abalone. Tutschulte and Connell (1988) observed homing behavior for 1- to 2-y-old pink abalone, both in the laboratory and in the wild. Buss et al.

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(2015) also observed a moderate level of homing behavior in cultured greenlip abalone when fed live macroalgae or formulated diets.

In regard to previous abalone behavior studies, feeding behavior was predominately measured using dorsal viewing methods using either direct visual observations (Fleming 1995), or continuous (Allen et al. 2006) or time lapse videography (Tutschulte & Connell 1988). More recently, Buss et al. (2015) used a more accurate method of ventral videography to assess greenlip abalone behavior. The benefit of using this method over the dorsal methods is that the approach enables mouth movement and actual feeding activity of abalone to be accurately assessed so the observer can actually discern when abalone are eating, which was not possible to distinguish from merely being over fed when observed dorsally.

In Australia, as the harvesting of live attached natural macroalgae is prohibited, formulated diets are predominately used in land-based abalone aquaculture. This may be advantageous as formulated diets are significantly cheaper per unit protein and energy basis, compared with cultured live macroalgae (Kirkendale et al. 2010, Bansemmer et al. 2014). Taking into consideration that the natural diet of abalone is macroalgae (Shepherd & Steinberg 1992, Allen et al. 2006, Naidoo et al. 2006), greater consideration should be given as to how the change to formulated diets affects feeding behavior, in particular, examining how diet affects the ability of abalone to locate food.

Abalone rely on chemosensory and tactile cues to detect feed (Allen et al. 2006). It has been reported that the change from a natural diet to a formulated diet may alter chemosensory cues perceived by the variously colored abalone [*Haliotis diversicolor supertexta* (Reeve, 1846)] (Jan et al. 1981). Jan et al. (1981) also observed enhanced feeding behavior in variously colored abalone when exposed to an *Ulva* sp. extract. *Gracilaria* sp. have also been demonstrated to act as an effective feeding stimulant compared with a commercial formulated diet when offered to blackfoot abalone (Allen et al. 2006). When attempting to catch drift macroalgae, abalone often extend their cephalic tentacles and forefoot to detect and capture macroalgae fragments (Shepherd 1973, Jan et al. 1981, Allen et al. 2006). The abalone's ability to detect available food may be impaired in the absence of chemosensory and tactile stimulation provided by macroalgae. Abalone fed formulated diets that may lack chemosensory and tactile stimulus may lead to a reduced feeding response and feed ingestion rates (Allen et al. 2006). Formulated feed chips also leach nutrients rapidly when immersed in seawater (Fleming et al. 1996); a slower feeding response may also result in abalone consuming a nutritionally unbalanced diet. There is great interest in using dried macroalgae in compounded formulated diets for cultured abalone in Australia (Kirkendale et al. 2010, Bansemmer et al. 2014, Bansemmer et al. 2016) as they may work as attractants, aid in reducing nutrient leaching losses, and also provide health benefits for abalone (Dang et al. 2011, Bansemmer et al. 2014, Lange et al. 2014, Stone et al. 2014b).

The aim of his study was to measure the feeding behavior of juvenile (18 mo old) greenlip and hybrid abalone (*Haliotis laevigata* × *Haliotis rubra*) in relation to diet type (formulated diet versus live *Ulva* sp.) and water temperature (18°C versus 22°C). The observation method used in this study was the same as used by Buss et al. (2015) and differed from other studies by utilizing ventral videography through the transparent base of

aquaria, rather than dorsal viewing used by previous researchers (Fleming 1995, Allen et al. 2006). Improvements in the understanding of the feeding behavior of greenlip and hybrid abalone may assist in refining feed design and feeding practices for the culture of these types of abalone.

MATERIALS AND METHODS

Experimental Design

A factorial experiment, with eight treatment combinations (Table 1), was designed to investigate the effects of diet type (formulated commercial diet [Abgrow premium, 5 × 5 × 2-mm chip; Eyre Peninsula Aquafeed, Lonsdale, SA, Australia] versus live *Ulva* sp.) and water temperature (18°C versus 22°C) on the feeding behavior (Table 2) of greenlip and hybrid abalone. Feeding behavior was examined using ventral videography.

Experimental Animals

Eighteen-month-old juvenile greenlip and hybrid abalone, previously sourced from South Australian Mariculture (Boston Bay, SA, Australia), were held at the South Australian Research and Development Institute, Aquatic Sciences Center (SARDI ASC) at ambient water temperature (14–17°C) in two separate 5,000-l tanks prior to the experiment. Holding tanks were supplied with flow-through, sand-filtered, UV-treated seawater. Both types of abalone were fed the formulated diet *ad libitum* prior to the commencement of the experiment.

Experimental Culture System and Ventral Videography System

The experiment was housed in a photoperiod of 12 h of low-intensity fluorescent lighting at 3.4 lx (0700–1900 h) and 12 h of darkness (1900–0700 h) and air temperature-controlled (20°C) laboratory at the SARDI ASC. The experiment was run in a flow-through culture system composed of two separate temperature-controlled header tank systems, described in Stone et al. (2013). Each header tank system supplied four separate 12-l transparent acrylic aquaria (300 × 160 × 250 mm deep) with sand-filtered, UV-treated, temperature-controlled seawater at either 18°C or 22°C, respectively, at a flow rate of 0.22 l/min,

TABLE 1.

Experimental treatment combinations used to assess the feeding behavior of juvenile greenlip and hybrid abalone.*†

| Treatment | Species | Diet type | Temperature (°C) |
|-----------|----------|-----------------|------------------|
| 1 | Greenlip | Formulated diet | 18 |
| 2 | Greenlip | Formulated diet | 22 |
| 3 | Greenlip | <i>Ulva</i> sp. | 18 |
| 4 | Greenlip | <i>Ulva</i> sp. | 22 |
| 5 | Hybrid | Formulated diet | 18 |
| 6 | Hybrid | Formulated diet | 22 |
| 7 | Hybrid | <i>Ulva</i> sp. | 18 |
| 8 | Hybrid | <i>Ulva</i> sp. | 22 |

* The formulated diet was Abgrow premium feed (5-mm chip) supplied by Eyre Peninsula Aquafeed, Lonsdale, SA, Australia.

† *Ulva* sp. was batch cultured in aerated parabolic tanks provided with direct sunlight at SARDI ASC (Bansemmer et al. 2016).

TABLE 2.
Behavior category definitions used for analyzing the feeding behavior of greenlip and hybrid abalone.*

| Behavior | Description |
|-----------|--|
| Quiescent | No movement, shell held tightly to the aquarium surface, cephalic and mantle tentacles retracted |
| Alert | Shell raised off aquarium surface, cephalic and mantle tentacles extended, extension from foot, torso rotation |
| Moving | Moving an appreciable distance in any direction |
| Feeding | Actively ingesting food items |

* From Buss et al. (2015) and modified from Allen et al. (2006).

which is comparable to flow rates used in slab tanks used by the Australian abalone industry (Wassnig et al. 2010, Stone et al. 2014a). The water level in each aquarium was set at 60 mm (water volume, 2.9 l) using a screened (nominal pore size = 0.8 mm) standpipe at the outlet. Aquaria were located on a bench top during the initial 7-day acclimation period and were relocated, while maintaining the same water supply, to a specially designed videography system for a further acclimation and 3 days of video recording.

The videography system, designed to enable ventral monitoring of abalone feeding behavior, housed four aquaria at a time and was composed of a flat top table with four recessed rectangular holes (one for each aquarium). Four separate video cameras (HD Pro Webcam C920; Logitech, Newark, CA) were located 60 cm beneath each aquarium. During the 12-h dark phase, the underside of each aquarium was illuminated (6.0 l×) with red light (12 V Nelson Mini Spot Pond Lights, HPM Industries Pty. Ltd., Preston, New South Wales, Australia). Red light has been demonstrated to not inhibit the normal nocturnal feeding behavior of greenlip abalone (Buss et al. 2015, Currie et al. 2015). White fluorescent lighting provided illumination (3.4 l×) during the 12-h light phase. Video footage of abalone feeding behavior was captured ventrally through the transparent base of each aquarium using a desktop computer and the “Security Monitor Pro” software package (Version 5 4-Camera; Desk-Share, Plainview, NY). Four aquaria (one type of abalone fed the two diets at 18°C and 22°C; Table 1) were filmed at any one time. Replicate 24-h feeding events were recorded over three consecutive days for each treatment combination.

Experimental Stocking and Feeding

Greenlip abalone (21.1 g, 55.6-mm SL) or hybrid abalone (23.4 g, 57.6-mm SL) were randomly harvested from the 5,000-l holding tanks (ambient temperature 17°C) without anesthetic, weighed (g), and measured (mm) and five abalone were systematically interspersed into each of the eight aquaria using a randomized block design ($n = 1/\text{aquaria treatment}$; $n = 4$ aquaria for each type of abalone). This resulted in a stocking densities (2.2 kg/m³) comparable to those used for slab tanks by the Australian abalone producers (Wassnig et al. 2010, Stone et al. 2014a). Stocking of each type of abalone was staggered to allow access to the videography system. After stocking, water temperatures in each aquarium were slowly increased (~1°C/day) until they had reached 18°C or 22°C. These temperatures were then maintained (±1°C). Feeding occurred daily at 1600 h and at 0900 h the following day, aquaria were cleaned and

uneaten feed from each aquarium was collected, pooled, and stored at -20°C. Abalone were fed to excess for the entire experiment at a rate of 2.7% and 4.5% body weight (bw)/day for the formulated diet and *Ulva* sp., respectively. Feed rates were based on previously determined feed intake rates for similar-sized greenlip abalone cultured at 22°C in the same experimental facility (Stone et al. 2014a, Bansemmer et al. 2015b) and were chosen as restricted rations that affect the feeding behavior of greenlip abalone (Buss et al. 2015). The experiment ran for 17 days, during which time animals were acclimated to their respective feeds and aquarium for 14 days and then ventrally filmed for 3 days. Water quality was monitored daily and maintained at levels appropriate for cultured abalone throughout the experiment (Hutchinson & Vandeppeer 2004).

Calculation of Apparent Feed Consumption and Nutrient Intake

Allowing for an acclimation period to allow feeding rhythms to stabilize, feed intake for each treatment was recorded over the final 5 days of the experiment. Pooled frozen uneaten feed samples from each aquaria were 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) were determined according to the methods of Stone et al. (2013). These values were used as a correction factor to calculate the apparent feed consumption rate based on as-fed values for feed intake and wet values for abalone weight on a daily basis for each treatment. Nutrient intake was calculated by multiplying the daily feed intake by the proportion of each nutrient or energy in the diet (Table 3).

Measurement of Activity and Feeding Behavior

Video footage was observed and scored for the first 10 min of every 30-min period for the three 24-h replicate monitoring periods, using a video software program (VLC Media Player, Version 2.0.5; Twinflower, France). Observed feeding activities were designated to one of four categories based on the method described by Buss et al. (2015), which were adapted from Allen et al. (2006) (Table 2). 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 aquarium over the 10-min observation period. This process was repeated for each 30-min interval for each of the three 24-h replicate periods. To remove sampling bias, two people independently viewed and scored the video footage for each time, day, and treatment and the result of the mean scores is reported.

Measurement of Distance Traveled and Velocity

To measure distance traveled and velocity of each abalone, the area of the aquarium base was scaled to match the area of the computer screen and a correction factor was used to adjust and measure the movement over time. The mean distance traveled and velocity within each treatment was determined over the 10-min observation period. This process was repeated for each 30-min interval for each of the three 24-h replicate periods. Then each replicate 24-h period was further divided into six periods to gain a better understanding of the daily movement of abalone: 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), the light period (0700–1859 h), and the total distance traveled over 24 h.

TABLE 3.
The daily feed and nutrient intake rates for greenlip and hybrid abalone in response to diet type and water temperature.

| Intake rate (as fed) | Formulated diet | | <i>Ulva</i> sp. | |
|----------------------------|-----------------|------|-----------------|-------|
| | 18°C | 22°C | 18°C | 22°C |
| Greenlip abalone | | | | |
| Feed intake (g kg/bw/day) | 3.75 | 4.38 | 9.83 | 11.65 |
| Protein (g kg/bw/day) | 1.14 | 1.34 | 0.29 | 0.34 |
| Carbohydrate (g kg/bw/day) | 1.95 | 2.27 | 0.40 | 0.47 |
| Lipid (g kg/bw/day) | 0.11 | 0.13 | 0.02 | 0.02 |
| Energy (MJ kg/bw/day) | 0.57 | 0.67 | 0.14 | 0.17 |
| Hybrid abalone | | | | |
| Feed intake (g kg/bw/day) | 5.32 | 5.22 | 13.75 | 14.03 |
| Protein (g kg/bw/day) | 1.62 | 1.59 | 0.40 | 0.41 |
| Carbohydrate (g kg/bw/day) | 2.76 | 2.71 | 0.55 | 0.57 |
| Lipid (g kg/bw/day) | 0.16 | 0.15 | 0.02 | 0.02 |
| Energy (MJ kg/bw/day) | 0.81 | 0.79 | 0.20 | 0.20 |

The dry matter (DM), crude protein (CP), crude lipid (CL), carbohydrate (CHO) and gross energy (GE) contents (as fed) of the formulated diet were 893.4 g DM/kg, 304.8 g CP/kg, 29.4 g CL/kg, 518.7 g CHO/kg, and 15.2 MJ GE/kg, and *Ulva* sp. were 158.5 g DM/kg, 29.1 g CP/kg, 1.6 g CL/kg, 40.3 g CHO/kg, and 1.4 MJ GE/kg, respectively.

Measurement of Homing Behavior

The homing behavior of greenlip abalone was also determined during the 3-day video monitoring period. Images captured from the video footage from each aquaria were evaluated by comparing the location of abalone at the same time of each morning (0800 h) after feeding had ceased. The homing behavior of each individual abalone in each tank for each day was assigned a score according to one of three categories (Cat.): (Cat. 1) homing = 1 (returned to same location); (Cat. 2) returned = 1 (adjacent to home, but unable to return to exact homing location because of the presence of another animal); or (Cat. 3) moved = 0 (different location). Scores were then converted to a proportion of homing behavior (%) by the following equation: $(\text{Cat. 1} + \text{Cat. 2})/5 \times 100$.

Biochemical Analysis

The moisture, crude protein, crude lipid, ash, and gross energy levels of the formulated diet and *Ulva* sp. were analyzed according to the methods of AOAC International (1995). Moisture was determined by oven-drying to a constant weight at 60°C for 48 h. Crude protein ($N \times 6.25$) was determined by the Kjeldahl method. Crude lipid was analyzed using a Soxhtherm rapid extraction system (Gerhardt GmbH & Co. KG; Königswinter, Germany). Total carbohydrate was determined by the Molisch's test and a glucose standard curve. The gross energy contents of the formulated diet were determined by bomb calorimetry. 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.

Statistical Analysis

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. Apart from the proportion of homing behavior [abalone type \times diet type \times temperature, three-factor analysis of variance (ANOVA)], statistical comparisons between types of abalone (greenlip versus hybrid) were not made due to statistical interactions. Two-factor ANOVA was used to assess the effects of diet type (formulated diet versus *Ulva* sp.) and water temperature (18°C versus 22°C) on dependent variables. Where significant interactions were observed, the data were analyzed using Student Newman–Keuls (SNK) multiple range test. The significance level was set at $P < 0.05$. Values are presented as means \pm SE. Statistical analyses were done using IBM SPSS, Version 22 for Windows (IBM SPSS Inc., Chicago, IL).

RESULTS

General Observations

During the study, abalone appeared to exhibit normal behavior and no visual signs of disease or mortalities were observed. Once the water temperatures had been established for each treatment, water temperature, dissolved oxygen, salinity, and pH levels in aquaria ranged from 17.5°C to 18.6°C, 6.33 to 8.08 mg/l, 35.0 to 35.6, and 8.06 to 8.15, and from 21.3°C to 22.5°C, 6.54 to 7.53 mg/l, 35.0 to 36, and 8.04 to 8.17 for the 18°C and 22°C treatments, respectively. Feeding activity did not appear to be affected under the red lighting system. Abalone commenced moving almost immediately once the white lights were switched off and the red lights were switched on. Both types of abalone appeared to accept both diets readily. The mode in which both types of abalone consumed feed differed between diet types. Abalone fed the formulated diet grazed randomly and intermittently and nibbled on multiple chips, while either mobile or stationary. In contrast, when fed the *Ulva* sp., abalone tended to remain stationary and grazed continually on a single frond for up to 30 min, often engulfing the entire frond.

Feed and Nutrient Intake

The feed-intake rates of both types of abalone were considerably higher for *Ulva* sp. than the formulated diet (Table 3). Increasing water temperature had a positive impact on the feed intake rates of both the formulated diet and *Ulva* sp. for greenlip abalone, whereas the feed intake rate of hybrid abalone did not appear to be influenced by temperature alterations (Table 3). Overall, hybrid abalone tended to have higher feed intake rates than greenlip abalone. Hybrid abalone also had slightly higher nutrient intake rates than greenlip abalone at both water temperature when fed either diet (Table 3). The nutrient intake rates of both types of abalone were influenced by the nutrient density of the diet, than by feed intake rate (Table 3). The formulated diet was more nutrient dense than the *Ulva* sp. (Table 3). As a result, intake rates for protein, carbohydrate, lipid, and energy were far superior for abalone fed the formulated diet.

Abalone Behavior

Results for abalone feeding behavior are presented in Figures 1A, B; 2A, B; 3A, B; 4A, B and Table 4.

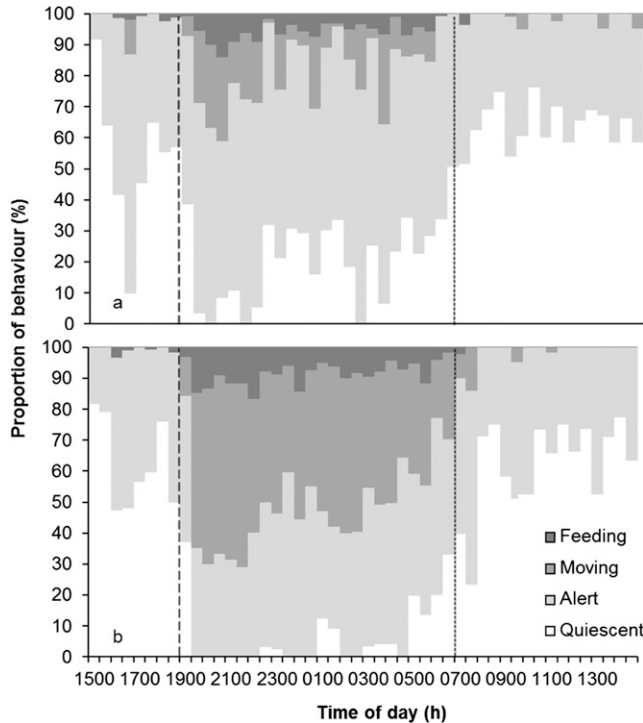


Figure 1. The proportion of time each type of feeding behavior was observed in greenlip abalone over a 24-h period ($n = 3$) in response to feeding the formulated diet at (A) 18°C and (B) 22°C. Feed introduced to aquaria at 1600 h and removed at 0900 h the following day. Photoperiod: 12 h light [white light on at 0700 h (dotted line) and off at 1900 h (dashed line)] and 12 h dark (red light provided during dark phase).

Quiescent Behavior

Greenlip abalone exhibited between 34.14% and 47.74% of time quiescent over a 24-h period (Table 4, Figs. 1A, B and 2A, B). There was a significant effect of diet type on the quiescent behavior of greenlip abalone ($P = 0.004$, formulated diet < *Ulva* sp., two-factor ANOVA, Table 4), whereas there was no significant effect of water temperature ($P = 0.178$) or significant interaction between the two factors ($P = 0.335$). Greenlip abalone exhibited the majority of quiescent behavior during the light phase (0700–1700 h, Fig. 1A, B).

The proportion of time hybrid abalone exhibited quiescent behavior over a 24-h period (Figs. 3A, B and 4A, B) appeared to be slightly higher than observed for greenlip abalone (Figs. 1A, B and 2A, B) and ranged from 47.92% to 56.09% (Table 4). There was no significant effect of diet type ($P = 0.447$) or water temperature ($P = 0.155$) on the quiescent behavior of hybrid abalone (Table 4), and there was no significant interaction between the two factors ($P = 0.126$). Hybrid abalone also exhibited the majority of quiescent behavior during the light phase (0700–1700 h, Figs. 3A, B and 4A, B).

Alert Behavior

Alert behavior was exhibited for 39.84%–50.23% of the time over the 24-h period by greenlip abalone (Figs. 1A, B and 2A, B) and was not significantly affected by diet type ($P = 0.351$), water temperature ($P = 0.140$), or by the interaction between the two factors ($P = 0.122$, two-factor ANOVA, Table 4).

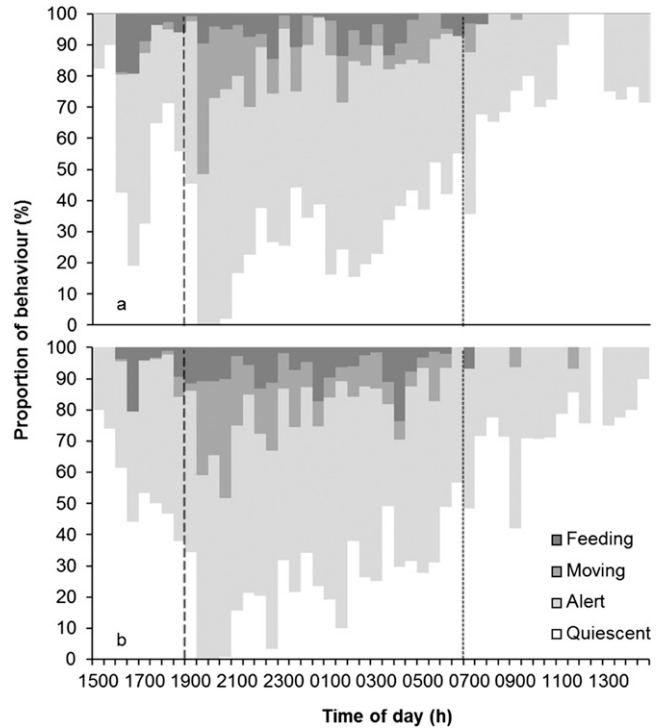


Figure 2. The proportion of time each type of feeding behavior was observed in greenlip abalone over a 24-h period ($n = 3$) in response to feeding the *Ulva* sp. at (A) 18°C and (B) 22°C. Feed introduced to aquaria at 1600 h and removed at 0900 h the following day. Photoperiod: 12 h light [white light on at 0700 h (dotted line) and off at 1900 h (dashed line)] and 12 h dark (red light provided during dark phase).

Overall, hybrid abalone exhibited slightly lower proportions of alert behavior over a 24-h period (31.05%–42.14%, Table 4, Figs. 2A, B and 3A, B) compared with greenlip abalone. For hybrid abalone, alert behavior was not significantly affected by diet type ($P = 0.327$), but was significantly affected by water temperature ($P = 0.002$, 22°C > 18°C) and there was no significant interaction between the two factors ($P = 0.540$, two-factor ANOVA, Table 4).

Moving Behavior

Greenlip abalone spent between 6.13% and 21.55% of their time moving during a 24-h period (Fig. 1A, B, Table 4). There was a significant effect of diet type ($P < 0.001$) and water temperature ($P < 0.001$) on the percentage of time greenlip abalone spent moving over the 24-h period (Table 4) and there was also a significant interaction between the two factors ($P < 0.001$, two-factor ANOVA, Table 4). Greenlip abalone spent a significantly higher proportion of time moving when fed the formulated diet at 22°C ($P < 0.001$, one-factor ANOVA, Table 4, Fig. 1B) than when fed the formulated diet at 18°C (Fig. 1A) or the *Ulva* sp. at either water temperature (Fig. 2A, B).

Overall, hybrid abalone spent a slightly lower proportion of time moving during a 24-h period (5.57% and 14.47%, Table 4, Figs. 3A, B and 4A, B) than greenlip abalone. Movement was significantly affected by water temperature ($P = 0.040$, two-factor ANOVA) with hybrid abalone moving significantly more at 22°C than at 18°C (Table 4). There were no significant effects of diet type ($P = 0.060$) or an interaction between the two

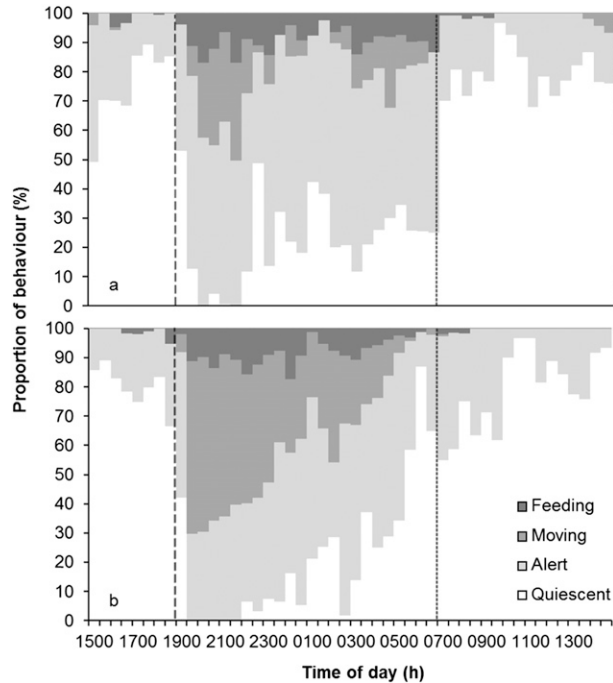


Figure 3. The proportion of time each type of feeding behavior was observed in hybrid abalone over a 24-h period ($n = 3$) in response to feeding the formulated diet at (A) 18°C or (B) 22°C. Feed introduced to aquaria at 1600 h and removed at 0900 h the following day. Photoperiod: 12 h light [white light on at 0700 h (dotted line) and off at 1900 h (dashed line)] and 12 h dark (red light provided during dark phase).

factors ($P = 0.098$) on the proportion of hybrid abalone movement over a 24-h period.

Feeding Behavior

Greenlip abalone spent a relatively small proportion of time feeding (2.81%–4.81%, Figs. 1A, B and 2A, B, Table 4), and feeding activity was significantly affected by water temperature ($P = 0.033$, 18°C < 22°C) over a 24-h period, but not by diet type ($P = 0.062$) or the interaction between the two factors ($P = 0.214$, two-factor ANOVA, Table 4). The proportion of feeding activity observed for hybrid abalone (Figs. 3A, B and 4A, B) was slightly higher than for greenlip abalone over a 24-h period and ranged from 4.16% to 5.36% (Table 4). The proportion of feeding activity was not significantly affected by diet type ($P = 0.730$), water temperature ($P = 0.906$), or the interaction between the two factors over a 24-h period ($P = 0.342$, two-factor ANOVA, Table 4).

There were no significant effects of diet type ($P = 0.112$) or water temperature ($P = 0.068$) or the interaction between the two factors ($P = 0.182$, two-factor ANOVA, Table 4) on the time taken to commence feeding following the introduction of feed to aquaria at 1600 h for greenlip abalone. The time taken for hybrid abalone to commence feeding following the introduction of feed to aquaria at 1600 h was similar to that of greenlip abalone (Table 4). Water temperature significantly reduced the time taken for hybrid abalone to commence feeding at 18°C compared with 22°C ($P = 0.041$, two-factor ANOVA, Table 4). There were no significant effects of diet type ($P = 0.327$), or the interaction between diet type and water temperature for the time to commence feeding for

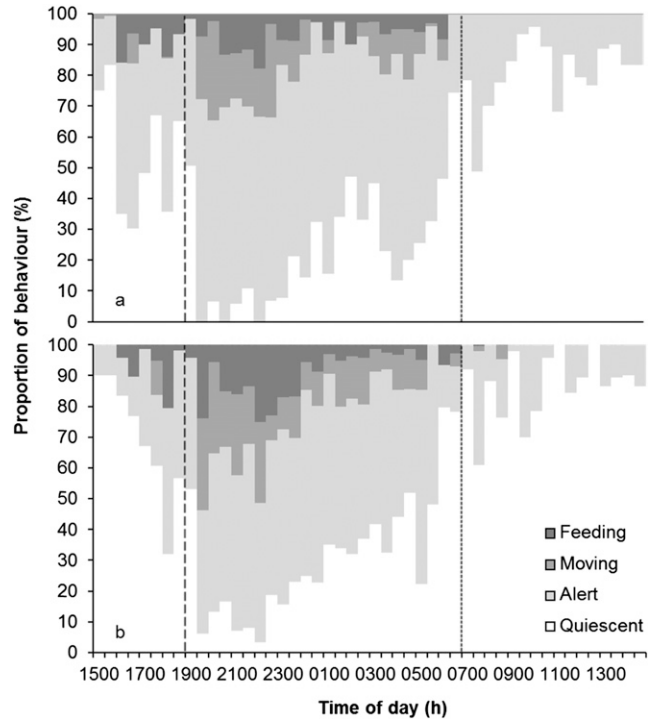


Figure 4. The proportion of time each type of feeding behavior was observed in hybrid abalone over a 24-h period ($n = 3$) in response to feeding the live *Ulva* sp. at (A) 18°C or (B) 22°C. Feed introduced to aquaria at 1600 h and removed at 0900 h the following day. Photoperiod: 12 h light [white light on at 0700 h (dotted line) and off at 1900 h (dashed line)] and 12 h dark (red light provided during dark phase).

hybrid abalone ($P = 0.506$). Both types of abalone were observed to commence feeding immediately on the introduction of *Ulva* sp. to the aquaria at 18°C (Table 4).

Greenlip ($P = 0.009$, two-factor ANOVA) and hybrid abalone ($P = 0.016$) spent a greater proportion of time feeding on *Ulva* sp. than the formulated diet between 1600 and 1900 h in the light phase (Table 4). This behavior for both types of abalone was not significantly affected by water temperature ($P > 0.05$) or the interaction between the two factors ($P > 0.05$, two-factor ANOVA, Table 4).

Velocity

The velocities of greenlip and hybrid abalone fed either the formulated diet or the *Ulva* sp. are displayed in Figure 5A, B, respectively, and in Table 5. Slight increases in velocity were observed for greenlip abalone at 18°C following the introduction of either diet to the aquaria at 1600 h (Fig. 5A). The majority of distance traversed by abalone fed either diet at either temperature occurred during the dark phase (1700–0700 h). Abalone of both types fed both diets typically exhibited a marked increase in velocity within 0–3 min of the commencement of the dark phase (1900 h) at both temperatures (Fig. 5A, B).

The maximum average velocity of abalone from each treatment was observed early in the first dark phase between 1900 and 2159 h (Table 5, Fig. 5A, B). There were interactive effects of diet type and water temperature ($P < 0.001$) observed for greenlip abalone during this period (1900–2159 h, Table 5). In contrast, no significant effects were observed for diet type

TABLE 4.
The average proportion of time greenlip or hybrid abalone exhibited each feeding behavior in response to diet type and water temperature over 24 h.*

| Feeding behavior | Formulated diet | | <i>Ulva</i> sp. | | Two-factor ANOVA*†‡§ | | |
|---|--------------------------|---------------------------|--------------------------|--------------------------|------------------------|---------------------|---------------------|
| | 18°C | 22°C | 18°C | 22°C | Diet type (a) | Temperature (b) | Interaction (a × b) |
| Greenlip abalone | | | | | | | |
| Quiescent (%) | 40.18 ± 4.60 | 34.14 ± 0.82 | 47.74 ± 1.19 | 46.53 ± 0.95 | 0.004 (FD < <i>U</i>) | 0.178 | 0.335 |
| Alert (%) | 50.23 ± 4.69 | 39.84 ± 3.05 | 41.84 ± 1.99 | 42.12 ± 1.70 | 0.351 | 0.140 | 0.122 |
| Moving (%) | 6.78 ± 0.41 ^b | 21.55 ± 2.41 ^a | 6.13 ± 0.61 ^b | 6.55 ± 0.59 ^b | <0.001 | <0.001 | <0.001§ |
| Feeding (%) | 2.81 ± 0.31 | 4.46 ± 0.41 | 4.29 ± 0.37 | 4.81 ± 0.55 | 0.062 | 0.033 (18°C < 22°C) | 0.214 |
| Time to start feeding after feed introduced at 1600 h (h) | 0.17 ± 0.17 | 2.00 ± 1.00 | 0.00 ± 0.00 | 0.33 ± 0.17 | 0.112 | 0.068 | 0.182 |
| Proportion of time feeding during the afternoon light phase (1600–1900 h) (%) | 1.19 ± 0.36 | 1.11 ± 1.11 | 8.47 ± 2.77 | 7.01 ± 2.44 | 0.009 (FD < <i>U</i>) | 0.701 | 0.731 |
| Hybrid abalone | | | | | | | |
| Quiescent (%) | 50.18 ± 0.87 | 50.33 ± 1.92 | 47.92 ± 3.84 | 56.09 ± 1.71 | 0.477 | 0.155 | 0.126 |
| Alert (%) | 38.70 ± 0.88 | 31.05 ± 3.13 | 42.14 ± 1.91 | 31.87 ± 1.56 | 0.327 | 0.002 (18°C > 22°C) | 0.540 |
| Moving (%) | 6.18 ± 1.47 | 14.47 ± 0.92 | 5.57 ± 1.23 | 6.67 ± 3.20 | 0.060 | 0.040 (18°C < 22°C) | 0.098 |
| Feeding (%) | 4.94 ± 0.50 | 4.16 ± 0.33 | 4.37 ± 1.23 | 5.36 ± 1.11 | 0.730 | 0.906 | 0.342 |
| Time to start feeding after feed introduced at 1600 h (h) | 0.17 ± 0.17 | 1.67 ± 0.73 | 0.00 ± 0.00 | 0.83 ± 0.60 | 0.327 | 0.041 (18°C < 22°C) | 0.506 |
| Proportion of time feeding during the afternoon light phase (1600–1900 h) (%) | 1.44 ± 0.31 | 3.97 ± 1.48 | 9.58 ± 3.76 | 7.28 ± 2.22 | 0.016 (FD < <i>U</i>) | 0.652 | 0.217 |

FD, formulated diet.

* Mean ± SE; $n = 3$ replicate 24-h observation periods.

† Where there was a significant effect of diet type ($n = 6$), details in parentheses indicate whether the FD was greater or less than *Ulva* sp. (*U*).

‡ Where there was a significant effect of temperature ($n = 6$), details in parentheses indicate whether 18°C was greater or less than 22°C.

§ Where a significant interaction occurred for a variable, mean values ($n = 3$) for diet type and temperature which share the same superscript are not significantly different ($P > 0.05$, one-factor ANOVA, SNK test).

($P = 0.073$), water temperature ($P = 0.102$), or the interactive effects ($P = 0.135$) on the velocity of hybrid abalone during the corresponding period. Even though significant effects were not observed for hybrid abalone, similar patterns in velocity were evident between types of abalone (Table 5). The average maximum velocities of greenlip (6.8 cm/min) and hybrid abalone (7.2 cm/min) when fed the formulated diet at 22°C were similar during 1900–2159 h (Fig. 5A, B). The velocity of both types of abalone fed the formulated diet at 22°C was higher than all other treatment combinations during 1900–2159 h (Table 5, Fig. 5A, B).

The velocity of abalone of both types from all treatments tended to progressively decrease from 2200 h onwards until the light phase commenced at 0700 h (Fig. 5A, B, Table 5). Abalone of both types remained practically dormant during the light phase (0700–1859 h). There were no significant effects of diet type, water temperature, or interactions between the two factors, on the velocity of either type of abalone during 0700–1859 h ($P > 0.05$, two-factor ANOVA, Table 5).

Large significant differences were observed in the total distance traveled by both types of abalone over the course of a 24-h period (Table 5). There were significant ($P < 0.001$) interactive effects of diet type and water temperature on the total distance traveled by greenlip abalone. Over a 24-h period, greenlip abalone fed the

formulated diet at 22°C traveled significantly further (33.07 m/day, $P < 0.001$) than when fed the *Ulva* sp. at 22°C or either of the diets at 18°C ($P < 0.05$, one factor ANOVA, SNK Table 5). In comparison, hybrid abalone followed a similar pattern to greenlip abalone over the same period, but the total distance traveled was effected by diet type ($P = 0.031$, formulated diet > *Ulva* sp.) and water temperature ($P = 0.045$, 18°C < 22°C), and there was no significant interaction between the two factors ($P = 0.070$, two-factor ANOVA, Table 5).

Homing Behavior

A high proportion of homing behavior was observed for both types of abalone. There was a significant effect of diet type on the proportion of homing behavior exhibited by abalone ($P = 0.028$, three-factor ANOVA). Abalone fed the *Ulva* sp. exhibited higher levels of homing behavior (90.0% ± 3.89%, $n = 12$) than abalone fed the formulated diet (74.4% ± 5.37%, $n = 12$). In contrast, there were no significant effect of the type of abalone ($P = 0.302$, three-factor ANOVA, $n = 12$, greenlip, 78.3% ± 3.86% versus hybrid, 86.1% ± 6.11%), water temperature ($P = 0.881$, $n = 12$, 18°C, 81.7 ± 3.86 versus 22°C, 82.8% ± 6.32%) or interactions between the three factors ($P > 0.05$) on the proportion of homing behavior.

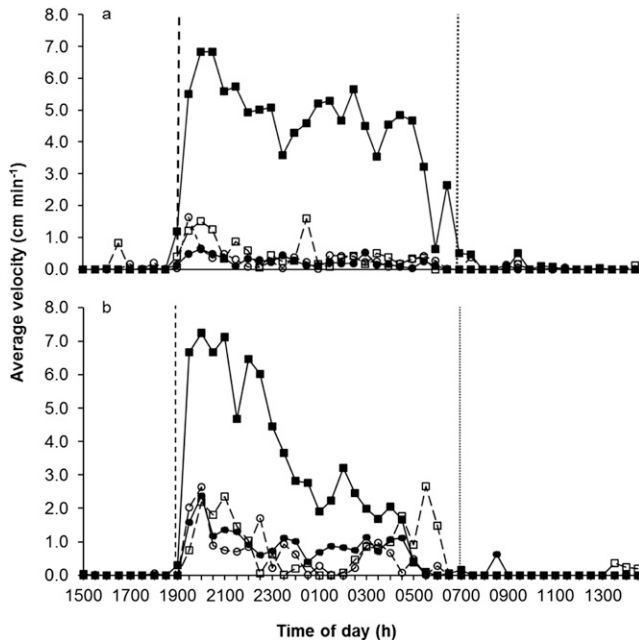


Figure 5. The average velocity (cm/min) of (A) greenlip and (B) hybrid abalone measured over a 24-h period at 18°C (□, dashed line) and 22°C (■, solid line) or *Ulva* sp. at 18°C (○, dashed line) and 22°C (●, solid line) throughout the night ($n = 3$ /replicate tanks treatment). Feed introduced to aquaria at 1600 h and removed at 0900 h the following day. Photoperiod: 12 h light [white light on at 0700 h (dotted line) and off at 1900 h (dashed line)] and 12 h dark (red light provided during dark phase).

DISCUSSION

Ventral videography used in this study enabled accurate differentiation between actual feeding activity and other behavioral activities of greenlip and hybrid abalone fed either a formulated diet or *Ulva* sp. at different water temperatures. The experimental aquaria system in this study was designed to use the same flow rates and stocking densities as used in Australian commercial slab tanks (Wassnig et al. 2010, Stone et al. 2014a). Hence, the present results may be comparable to the feeding behavior exhibited by abalone in commercial land-based systems in Australia. The feeding behavior recorded in the present system may deviate from those exhibited by abalone in the wild, as the density, aggregation, and food availability in wild abalone populations would vary between sites (Shepherd 1973).

Stereotypy may be observed in animals housed in confined spaces and may affect the results observed in behavioral studies (Fish et al. 2007). Stereotyped movement is a repetitive, non goal-directed action that is carried out in a uniform way (Fish et al. 2007). A stereotypy may be a simple movement for a stereotyped or recurrent utterance (Fish et al. 2007). Domenici and Kapoor (2010) considered the coefficient of variation ($CV = SD/mean$) as determinant of stereotypic behavior in fish behavioral studies. Coefficient of variation was considered inversely related to stereotypy, that is, behaviors with a high CV show low stereotypy. On the basis of CV for the different feeding behaviors observed in this study, which were variable (Table 4), there was no evidence of stereotypic behavior. Similarly, Buss et al. (2015) also observed that the behavior of greenlip abalone, in the same experimental system as used in this study, was not consistent or repetitive.

In this study, abalone were observed to be predominately nocturnal feeders, with comparatively little activity occurring during the daylight periods (Tables 4 and 5, Figs. 1–5). A major finding of this study was the difference in daylight feeding activity and behavior in both types of abalone, which was observed to be stimulated by diet type. During the light phase, both types of abalone fed more actively on *Ulva* sp. than the formulated diet (Table 4). Not only did greenlip and hybrid abalone spend approximately three and seven times longer, respectively, feeding on the *Ulva* sp. during the light phase, they also commenced feeding sooner on the administration of feed to the tanks compared with abalone fed the formulated diet. Interestingly, temperature also had a slight effect on this behavior, with both types of abalone tending to commence feeding sooner following the administration of feed to the tanks at 18°C than at 22°C. With regard to diet type, increased feeding activity may have been due to the *Ulva* sp. providing enhanced chemosensory and tactile stimulation. The various colored abalone has been observed to display enhanced feeding behavior when exposed to an *Ulva* sp. extract (Jan et al. 1981). In addition, Allen et al. (2006) reported higher feed intake in blackfoot abalone fed a formulated diet in the presence of dried *Gracilaria* sp. particles suspended in the culture water, compared with abalone fed without the suspended particles. Enhanced feeding activity has also been previously observed in greenlip abalone fed live *Ulva* sp. and *Gracilaria cliftonii* (Buss et al. 2015, Bansemmer et al. 2016). Buss et al. (2015) also reported improved daytime feeding activity in greenlip abalone fed a formulated diet that was designed and processed to have a physical structure that mimicked the thalloid structure of *Ulva* sp.; further suggesting the provision of tactile stimulation is important. This also highlights the advantages of the use of a natural ingredient that provides a stimulant that may enable abalone to locate feed easier. This could be particularly beneficial for the production of less motile, larger abalone. This recommendation is further supported by results from previous research where greenlip abalone fed formulated diets containing dried *Gracilaria* sp. or *Ulva* sp. at levels ranging from 5% to 20% were observed to display an immediate increase in locomotory activity, feeding behavior, and feed intake when diets were presented, whereas greenlip abalone remained quiescent when fed the control feed, containing no dried macroalgae, suggesting that the diets provided a chemosensory stimulus (Bansemmer 2015).

There were marked differences in the mode of feeding activity displayed by both types of abalone fed the *Ulva* sp. compared with the formulated diet. Shepherd (1973) described the physical feeding action of abalone on macroalgae involved shearing parts of the macroalgal frond off before ingestion. This feeding technique was not observed in this study. Macroalgal species differences may account for variations in feeding strategies. In this study, when fed the *Ulva* sp., abalone drew individual fronds directly into their mouth; this action was likened to sucking in a piece of spaghetti. Conversely, when fed the formulated diet, feeding occurred sporadically with abalone grazing on one chip briefly before moving on to another. Similarly, Buss et al. (2015) also reported the same difference in feeding behavior for greenlip abalone when fed formulated or live macroalgae diets. This indicates that abalone may be preferentially grazing on the water-softened surface layer of the chip while rejecting the harder core, and suggests abalone may require the surface of the feed chips to soften before the

TABLE 5.
The average velocity of greenlip and hybrid abalone during different periods of the day in response to diet type and water temperature.*

| | Formulated diet | | <i>Ulva</i> sp. | | Two-factor ANOVA†‡‡§ | | |
|---------------------------|--------------------------|---------------------------|---------------------------|--------------------------|----------------------|------------------------|---------------------|
| | 18°C | 22°C | 18°C | 22°C | Diet type (a) | Temperature (b) | Interaction (a × b) |
| Greenlip abalone | | | | | | | |
| | Velocity (cm/min) | | | | | | |
| 24-h period 1530–1529 h | 0.27 ± 0.01 ^b | 2.30 ± 0.34 ^a | 0.17 ± 0.03 ^b | 0.13 ± 0.02 ^b | <0.001 | <0.001 | <0.001§ |
| Dark period 1900–2159 h | 0.93 ± 0.12 ^b | 5.29 ± 0.48 ^a | 0.58 ± 0.01 ^{bc} | 0.36 ± 0.01 ^c | <0.001 | <0.001 | <0.001§ |
| Dark period 2200–0059 h | 0.53 ± 0.06 ^b | 4.58 ± 1.12 ^a | 0.17 ± 0.03 ^b | 0.28 ± 0.03 ^b | <0.001 | 0.001 | 0.001§ |
| Dark period 0100–0359 h | 0.27 ± 0.04 ^b | 4.81 ± 0.62 ^a | 0.28 ± 0.06 ^b | 0.23 ± 0.15 ^b | <0.001 | <0.001 | <0.001§ |
| Dark period 0400–0659 h | 0.20 ± 0.05 ^b | 3.42 ± 1.04 ^a | 0.21 ± 0.12 ^b | 0.10 ± 0.07 ^b | 0.001 | 0.005 | 0.002§ |
| Light period 0700–1859 h | 0.05 ± 0.01 | 0.07 ± 0.04 | 0.04 ± 0.02 | 0.01 ± 0.00 | 0.204 | 0.509 | 0.475 |
| Distance traveled (m/day) | 3.83 ± 0.18 ^b | 33.07 ± 4.87 ^b | 2.49 ± 0.41 ^b | 1.81 ± 0.33 ^b | <0.001 | <0.001 | <0.001§ |
| Hybrid abalone | | | | | | | |
| | Velocity (cm/min) | | | | | | |
| 24-h period 1530–1529 h | 0.43 ± 0.16 | 1.60 ± 0.32 | 0.32 ± 0.10 | 0.43 ± 0.28 | 0.031 (FD > U) | 0.045 (18°C < 22°C) | 0.070 |
| Dark period 1900–2159 h | 1.43 ± 0.25 | 5.45 ± 2.13 | 1.17 ± 0.29 | 1.30 ± 0.27 | 0.073 | 0.102 | 0.135 |
| Dark period 2200–0059 h | 0.38 ± 0.19 ^b | 4.38 ± 0.69 ^a | 0.72 ± 0.16 ^b | 0.79 ± 0.66 ^b | 0.054 | 0.017 | 0.008§ |
| Dark period 0100–0359 h | 0.37 ± 0.27 | 2.25 ± 0.45 | 0.40 ± 0.30 | 0.83 ± 0.83 | 0.164 | 0.166 | 0.205 |
| Dark period 0400–0659 h | 1.09 ± 1.08 | 0.71 ± 0.33 | 0.25 ± 0.12 | 0.44 ± 0.37 | 0.509 | 0.805 | 0.934 |
| Light period 0700–1859 h | 0.03 ± 0.03 | 0.01 ± 0.01 | 0.00 ± 0.00 | 0.03 ± 0.03 | 0.930 | 0.930 | 0.487 |
| Distance traveled (m/day) | 6.13 ± 2.36 | 23.07 ± 4.55 | 4.60 ± 1.39 | 6.23 ± 3.97 | 0.031 (FD > U) | 0.045 (18°C < 22°C) | 0.070 |

* Mean ± SE; $n = 3$ replicate 24-h observation periods.

† Where there was a significant effect of diet type ($n = 6$) the details in parentheses indicates whether the formulated diet (FD) was greater or less than *Ulva* sp. (U).

‡ Where there was a significant effect of temperature ($n = 6$) the details in parentheses indicates whether 18°C was greater or less than 22°C.

§ Where significant interactions occurred for a variable, mean values ($n = 3$) for diet type and temperature which share the same superscript are not significantly different ($P > 0.05$, one-factor ANOVA, SNK test).

radula is able to effectively rasp and convey feed to the mouth for ingestion. This behavior may be indicative of the requirement for feed of a certain level of hardness for abalone and also suggests that diet hardness may be influencing feeding behavior and potentially feed intake (McShane et al. 1994, Fleming et al. 1996, Allen et al. 2006). Increasing levels of live macroalgae and formulated pellet “hardness,” measured using a penetrometer, have been demonstrated to negatively influence feed intake in blacklip abalone (*Haliotis rubra*) (McShane et al. 1994). Conversely, making diets too soft leads to water stability problems. Further research is required in this area.

Differences in water temperature also had an influence on the movement and feeding behavior of both types of abalone (Table 4); however, results were variable. Overall, the average velocity and feeding activity of greenlip abalone increased with increasing water temperature (18°C < 22°C) but the effect was more pronounced when greenlip abalone were fed the formulated diet. Whereas, for hybrid abalone, while feeding activity remained relatively constant regardless of the water temperature or diet type, average velocity also increased when fed the formulated diet at 22°C. The increased movement and feeding activity at the higher water temperature may have been due to several factors that include (1) higher energy requirements, as metabolic rate is positively correlated with increased tissue synthesis and energy requirements (Jobling 1981, Stone et al. 2013); the act of searching for feed is energetically costly due to an increase in muscle activity and the secretion of mucus during

locomotion (Donovan & Carefoot 1998); or (2) differences in the mode of feeding, as previously discussed.

Locomotion was significantly affected by water temperature and diet for both types of abalone. Abalone held at 22°C and fed a commercial diet, moved more frequently and with a faster velocity than all other treatments. Over a 24-h period, greenlip and hybrid abalone fed the formulated diet and held at 22°C spent approximately 22% and 15% of their time moving, respectively; in contrast, other treatments spent approximately 6%–7% of their time moving (Table 4). Movement and velocity of both types of abalone were observed to peak during first phase of dark period (1900–2159 h, Fig. 5, Table 4), irrespective of diet and water temperature, and appeared to be correlated with increased feeding activity. The lower level of movement exhibited by both types of abalone fed the *Ulva* sp. at 22°C may have been due to a lower requirement to move to locate food because of superior tactile and chemosensory cues’ presence in the macroalgae (Jan et al. 1981, Allen et al. 2006).

On average, greenlip (0.41 body length/min) and hybrid abalone (0.28 body length/min) fed the formulated diet in this study moved at similar velocities when compared with greenlip abalone fed the same diet at a similar rate in the study of Buss et al. (2015), whereas, large differences were observed between studies when abalone were fed live *Ulva* sp. Buss et al. (2015) reported that greenlip abalone, fed to excess with either a formulated diet or *Ulva* sp. at 22°C, moved at a rate of 0.35 or 0.32 body length/min, respectively. Conversely, greenlip and

hybrid abalone in this study moved at the reduced rates of 0.02 and 0.07 body lengths/min, respectively, when fed the *Ulva* sp. at 22°C. In addition, the smaller greenlip (45.6 mm) abalone in the study reported by Buss et al. (2015) exhibited a slightly higher peak in maximum velocity (10 cm/min) during first phase of darkness (1900–2159 h), which also declined thereafter as the dark phase progressed. The observed difference in the maximum velocities of greenlip abalone between studies may have been due to reduced locomotory capacity of the larger abalone with increasing size (Tutschulte & Connell 1988).

In this study, both types of abalone consumed more *Ulva* sp. compared with the commercial formulated diet on an as-fed basis, regardless of water temperature. Even so, nutrient intake was lower in these treatments, due to the higher moisture content and lower nutrient density of the live macroalgae. Similarly, Bansemmer et al. (2016) and Buss et al. (2015) observed that greenlip abalone more readily accepted live macroalgae diets than formulated diets which resulted in higher feed intake rates but lower nutrient ingestion rates. Bansemmer et al. (2016) also reported reduced feed efficiency and growth in greenlip abalone when fed a mixed diet of live enriched *Ulva* sp. and *Gracilaria cliftonii* compared with commercial formulated diets. This demonstrates that although abalone may more readily consume live macroalgae, the inherent low dry matter content, nutrient density, and poor nutritional profile of the live product is problematic and may present practical nutrient delivery limitations (Bansemmer et al. 2014).

Abalone that show homing behavior, by returning to the same location after their moving period, produce a characteristic change to the substrate, termed a scar. A high proportion of homing behavior was observed for both types of abalone in this study; while water temperature had no effect on homing behavior, diet type did. Abalone fed *Ulva* sp. exhibited the highest levels of homing behavior (*Ulva* sp., 90% versus formulated diet, 74%). The differences in homing behavior between diets may be attributed to the reduced movement away from the scar that *Ulva* sp. fed abalone exhibited throughout the course of the dark phase in comparison with those fed the commercial diet, particularly at 22°C. Other studies have reported that abalone may exhibit a degree of homing behavior. Tutschulte and Connell (1988) observed homing behavior in pink and white abalone (*Haliotis sorenseni*) between 1 and 2 y of age under laboratory conditions and in the wild. Buss et al. (2015) reported a slightly lower level of homing behavior (62%) in smaller greenlip abalone (12.6 g, 45.6-mm SL) under laboratory conditions, which was not affected by diet type. Differences in homing results between this study and the study of Buss et al. (2015) may be attributed to differences in animal size. Tutschulte and Connell (1988) reported reduced movement away from home scars for abalone of increasing size. Homing behavior has also been recorded for Pacific or disc abalone [*Haliotis discus hannai*, (Ino 1953)] (Momma & Sato 1969) and blackfoot abalone (Allen et al. 2006). In contrast, there have

been several studies that have not found homing behavior in other species of abalone (Poore 1972), although the lack of homing behavior in the two aforementioned studies may have been due to improper assessment techniques, or the size of the abalone. The understanding of abalone homing behavior may be important to understand the potential ability of the abalone to remain within a certain area, particularly in terms of potential candidate species for sea ranching.

CONCLUSION

This study used a newly developed ventral videography technique to assess the feeding behavior of abalone. This technique was an improvement over previous studies as it eliminated any ambiguity that the abalone are eating and also allows for a better determination of the mode in which abalone consume different diets. Diet type, photoperiod, and water temperature were found to affect some aspect of abalone behavior. In regard to photoperiod, abalone exhibited the most movement and feeding activity during darkness, supporting the notion that nocturnal feeding is preferred. With the exception of when fed *Ulva* sp., abalone displayed more feeding activity during the daylight period than when fed a formulated diet, suggesting that chemosensory and tactile cues are involved in abalone's ability to detect feed. Abalone held at 22°C moved considerably more when fed a formulated diet than when held at 18°C or when fed *Ulva* sp. at either temperature. This can be attributed to several factors such as (1) water temperature affecting the metabolic rate of abalone, (2) diet affecting the abalone needs to actively search for food, and (3) feed ingested providing adequate nutrients for motility. The results from this study indicate that feeding abalone diets supplemented with dried macroalgae may be beneficial in stimulating feeding, which in turn may translate into improved growth.

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