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Growth and feed utilisation of juvenile greenlip abalone (*Haliotis laevigata*) in response to water temperatures and increasing dietary protein levels

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ABSTRACT

In this 91-day study, the interaction between four dietary crude protein (CP) levels (27, 30, 33 and 36% CP) and three water temperatures (14, 17 and 20 °C) on the growth and feed utilisation of 6-month old greenlip abalone (*Haliotis laevigata*) (0.91 g) were investigated. Diets were formulated to be isoenergetic (12.5 MJ kg^{-1} digestible energy), containing a lipid level of ~3.6% and digestible protein from 17.99 to 28.57%. Abalone were fed to excess at 16:00 h daily, and uneaten feed was collected the following day. The specific growth rate (SGR) of abalone improved significantly as water temperatures increased from 14 to 17 to 20 °C. In addition, apparent protein deposition was significantly higher in abalone at 17 and 20 °C compared to abalone at 14 °C. There was no significant effect of dietary protein level on SGR, but faster growing abalone at 20 °C compensated by consuming more feed when fed low dietary protein levels. In contrast, a significant positive relationship was observed between dietary protein level and feed consumption rate in slower growing abalone at 14 and 17 °C. A non-significant tendency for the apparent feed conversion ratio (FCR) to improve was observed in abalone fed high protein diets at 20 °C, while at 14 °C, abalone had a significantly poorer FCR, especially when fed high dietary protein levels. Based on results from the current study, it is plausible to heat land-based nursery systems in order to gain accelerated growth of juveniles before transfer to grow-out systems. Additionally, no benefits were apparent by feeding abalone high protein diets at 14 or 17 °C, and we therefore recommend a dietary protein level of 29% CP at 14 and 17 °C. While the SGR of abalone at 20 °C was not significantly influenced by dietary protein, the feed consumption rate decreased and there was a tendency for FCR to improve as dietary protein level increased. Therefore, it may be beneficial for abalone to be switched to a diet containing ~35% CP at water temperatures >20 °C.

1. Introduction

Greenlip abalone (*Haliotis laevigata*) are primarily grown in land-based systems throughout southern Australia. The water temperature during grow-out affects almost every aspect of on-farm production (Britz et al., 1997), and can range from below 10 °C in Tasmania during winter to above 24 °C in South Australia during summer. The optimal water temperature for growth for a Tasmanian greenlip abalone strain (82 mm shell length [SL]) was 18.3 °C (Gilroy and Edwards, 1998), while the optimal water temperature for growth for a South Australian greenlip abalone strain (23 mm SL) was 22 °C (Stone et al., 2013). The temperature dependent response in abalone growth may be attributed to genetics or animal size differences, the latter of which has also previously been reported in red abalone (*Haliotis rufescens*) (Steinarsson and Imsland, 2003).

Once juvenile greenlip abalone are weaned off a microalgae diet, they are fed a formulated diet for approximately three years until they reach market size. Dietary protein plays a major role in the nutritional value of formulated diets, as optimal growth is dependent on maximising protein deposition, which is limited by dietary protein availability (Britz and Hecht, 1997; Fleming and Hone, 1996; Shipton and Britz, 2001). The optimal dietary protein level is dependent on a number of factors including the abalone species, abalone size, water temperature, ingredient digestibility and dietary energy level

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(Bansemer et al., 2014; Bautista-Teruel and Millamena, 1999; Stone et al., 2013). The abalone industry is relatively new compared to other aquaculture sectors such as fin fish, which have successfully developed pre-starter, starter, grower and finisher diets for different grow-out stages (Ng and Romano, 2013; Sarker et al., 2013). There is an increased demand to introduce multi-diet feeding strategies for greenlip abalone by optimising the dietary protein level for each age class and water temperature throughout the production cycle. However, the optimal dietary protein level for greenlip abalone throughout their whole production cycle is not clear. Prior to 2013, Australian abalone diets were formulated to contain ~27% crude protein (CP) based on a growth trial for juvenile greenlip abalone (0.55–0.94 g) at 20 °C (Coote et al., 2000). Currently however, Australian abalone feed contains 30 to 35% CP as suggested by recent research for greenlip abalone (1.75 g) at 22 °C (Stone et al., 2013), but the authors also reported the optimal dietary protein level is dependent on both age (1 and 2-year old abalone) and water temperature (14, 18 and 22 °C). Further research, focused on the nutritional requirements of greenlip abalone soon after weaning (~6-month old abalone), is required to improve our understanding on feed formulation for juvenile abalone.

In this study, our aim was to identify the optimal dietary CP level for post-weaned greenlip abalone (6-month old) at 14, 17 and 20 °C. On-farm, in land-based facilities throughout southern Australia, the water temperature fluctuates throughout the grow-out period; the water temperatures selected in the current study represent the temperature range typically occurring from autumn, through winter, to early summer experienced by post-weaned juvenile greenlip abalone. The nominal dietary CP levels used in this study were 27, 30, 33 and 36%. These levels are considered to be commercially applicable to land-based abalone production in southern Australia. Diets used in this study were formulated on a digestible protein basis and contained highly palatable and digestible ingredients at realistic inclusion levels, using protein and energy digestibility data reported for greenlip abalone (Fleming et al., 1998; Vandepeer, 2005). Diets were formulated using the "ideal protein concept," such that the ratio of each essential amino acid to lysine was equal to, or greater than, the soft tissue amino acid values for greenlip abalone (Coote et al., 2000). Diets contained ~3.6% lipid (Dunstan et al., 2000; Van Barneveld et al., 1998), and ~17.4 MJ kg^{-1} crude and ~12.5 MJ kg^{-1} digestible energy levels. The results of this study will contribute towards the development of diets suitable for early weaned abalone at different water temperatures and the formulation of appropriate abalone diets for each grow-out stage throughout the abalone production cycle.

2. Methods

2.1. Experimental animals and system

Greenlip abalone (weight 0.91 ± 0.00 g; shell length 19.46 ± 0.02 mm; n = 864) were purchased from South Australian Mariculture (Port Lincoln, South Australia, Australia) in April 2013. Prior to stocking, abalone were held in a flow through seawater system at South Australia Research and Development Institute Aquatic Science Centre (SARDI ASC) (West Beach, South Australia, Australia) for two weeks and fed a commercial diet (~30% CP; Eyre Peninsula Aquafeed Pty Ltd., Lonsdale, SA, Australia).

The experiment was conducted in a photoperiod and temperature controlled laboratory described in Stone et al. (2013). The photoperiod was 12 h low intensity fluorescent lighting at 3.4 lx: 12 h dark. The air temperature was adjusted based on the incoming water temperature and ranged from 16.0 to 19.3 °C. Three identical culture systems (14, 17 or 20 °C) were supplied with 30 μm sand-filtered, UV treated seawater (Model 025120-2, 120w, Emperor Aquatics, Pottstown, PA, USA). Sixteen 12.5-L blue plastic culture units (Nally IH305, Viscount Plastics Pty Ltd.; 39.2 × 28.8 × 11.0 cm) per system were each supplied with flow-through seawater (300 mL min^{-1}). Water depth was held at 2.5 cm using a standpipe with a mesh screen (0.8 mm) on the outlet to retain uneaten feed. Water temperature was held at 14, 17 or 20 °C (±1 °C) throughout the experiment through the use of either immersion heaters (240 V, 3 kw, JQ20; Austin and Cridland, Carlton, NSW, Australia) or chillers (3 hp, 240 V, 50 Hz: Daeil Cooler Co., Ltd., Busan, Korea).

2.2. Stocking

Abalone were gently pried from the substrate using a spatula. Eighteen animals were weighed, measured and stocked into one of four replicate culture units per treatment combination. Animals were acclimated to the system for 16 days and fed their respective diets. After seven days the water temperature was either lowered or raised slowly (1 °C day^{-1}) to the desired water temperatures (14, 17 or 20 °C) and was maintained at these levels (±1 °C) throughout the remainder of the 75 day experiment. Dead abalone during the experiment were measured, weighed, recorded, and replaced with abalone of a similar weight and size that had been held at each respective water temperature and fed the commercial formulated diet.

2.3. Diets and feeding

At each temperature, animals were fed with one of four dietary protein levels (27, 30, 33 and 36% CP, Table 2). The proximate composition of the ingredients was analysed prior to diet formulation. Diets were formulated on a digestible protein and isoenergetic basis, based on data reported for greenlip abalone (Fleming et al., 1998; Vandepeer, 2005). Solvent-extracted soybean meal, de-hulled lupins, casein and fish meal were used as the main dietary protein source, while fish oil and de-hulled lupins were used as the main dietary lipids source. Diets were also formulated, using book values, so that the ratio of each essential amino acid to lysine was equal to, or greater than that analysed for the soft body tissue of greenlip abalone (Coote et al., 2000). Due to the difficulty in determining the amino acid requirement of abalone (Shipton et al., 2002), applying the "ideal protein concept" was concluded to be an acceptable alternative (Fleming et al., 1996). Diets were cold-pressed into flat pellets (4 × 3 × 2 mm thick) using a commercial pasta machine (La Prestigiosa medium; IPA. Vicenza. Italy). The dry matter leaching loss for each diet was determined in triplicate by submerging the diet (1 g) in seawater (25 mL) at 14, 17 and 20 °C for 16 h. After 16 h, the supernatant was removed, by syringe, and the remaining pellets were dried at 105 °C for 16 h. The dry matter leaching loss for all diets was highest at 20 °C, but was less than 8% dry weight. Abalone were fed to excess of their daily requirements (4% of the abalone biomass day^{-1}) at 16:00 h. Feed rates were maintained at these levels throughout the study based on monthly weight checks. Tanks were cleaned and uneaten feed was collected by sieving the entire tank contents through a fine mesh at 08:30 h and stored at −20 °C, and was later dried at 105 °C for 16 h. Daily feed consumption was estimated by the difference between feed offered and uneaten feed in dry weight. The proportion of uneaten feed lost between 08:30 to 16:00 h, from leaching and by sieving the entire tank contents through a fine mesh without animals in the tank, at the respective water temperatures, was used as a correction factor to calculate the apparent feed consumption rate.

2.4. Biochemical and water quality analysis

At the commencement of the experiment, the soft tissue of 50 animals (n = 4 replicates) were collected, shucked and stored at −20 °C to analyse the initial soft tissue proximate composition. At the conclusion of the experiment, 10 abalone from each tank were collected, shucked and stored at −20 °C. The abalone were later pooled for each tank for the analysis of soft tissue proximate composition. The proximate composition analyses of ingredients, diets, and whole body tissue

were conducted according to methods in the British Pharmacopoeia Commission (2004) or German Institute for Standardization (2000).

All data reported for animal performance were based on the pooled data from each tank. All calculations using abalone weight were based on wet values, while feed use values were based on dry values:

Biomass gain (g $tank^{-1}$)
(final weight + ∑mortality weight) − (initial weight + ∑replacement weight)
Specific growth rate (SGR, % day^{-1})
([ln final weight − ln intial weight]/days) × 100
Shell growth rate (μm day^{-1})
(final shell length − initial shell length)/days
Condition factor 5575 × (weight [g]/length [mm]$^{2.99}$) (Britz and Hecht, 1997)
Apparent feed consumption feed offered − uneaten feed collected − ([total feed offered × % leaching loss without animals] + [uneaten feed collected/% retained without animals × % leaching loss without animals])/2 (Stone et al., 2013)
Apparent feed conversion ratio (FCR) feed consumed/abalone weight gain
Apparent protein efficiency ratio (PER) abalone weight gain/protein consumed
Apparent energy efficiency ratio (EER) abalone weight gain/energy consumed
Apparent protein deposition ([final soft body protein-initial soft body protein]/protein intake) × 100
Apparent energy deposition ([final soft body energy-initial soft body energy]/energy intake) × 100.

Water quality parameters were measured daily and were maintained throughout the study at appropriate levels for the growth of abalone (Table 1). Water temperature was measured using a thermometer. Dissolved oxygen (mg L^{-1} and % saturation) was measured using a dissolved oxygen meter (OxyGuard International A/S, Birkerød, Denmark). The pH was measured using a meter (Oakton pHtestr 20; Oakton Instruments, Vernon Hills, IL, USA). Salinity (g L^{-1}) was measured using a portable salinity refractometer (model RF20, Extech Instruments, Nashua, NH, USA). Light intensity was measured using a LI-COR 1400 Quantum light meter (LI-COR Environmental, Lincoln, NE, USA).

2.5. Statistical analyses

IBM SPSS, Version 20 for Windows (IBM SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Homogeneity of variances and normality among mean values were assessed using Levene's test for equality of variance errors and the standardised residuals against the predicted mean plot, respectively. All percentage data was arcsine transformed before analyses. All variables were analysed using two-factor ANOVA, with water temperature as the first factor and dietary protein level as the second factor. When significant interactions were observed, post-hoc tests were used to detect significant differences between all treatment combinations (Student Newman–Keuls). Linear and second order polynomial regression analyses were also applied to SGR, feed consumption rate (mg individual^{-1} day^{-1}) and FCR. A significance level of $P < 0.05$ was used for all statistical tests. All values are presented as means ± standard error (SE) of the mean unless otherwise stated.

3. Results

3.1. General observations

The analysed protein content of the diets was slightly higher than the formulated nominal values (Table 2). There were no significant differences in the initial weight and shell length between treatments ($P > 0.05$). The average initial weight and shell length were 0.91 ± 0.00 g and 19.46 ± 0.02 mm, respectively. The overall mortality for the study was 4.05%, but was significantly higher at 14 °C (7.64%) compared to 17 °C (2.09%) and 20 °C (2.43%) ($P = 0.006$). Mortalities were not significantly influenced by dietary protein level ($P = 0.592$) or interaction between water temperature and dietary protein level ($P = 0.309$).

3.2. Growth performance

Water temperature had a significant effect on the final weight and shell length of greenlip abalone ($P < 0.001$; 14 < 17 < 20 °C; Table 3). Final individual weight and shell length were not significantly affected by dietary protein level ($P = 0.801$ and $P = 0.965$, respectively) or by the interaction of these two factors ($P = 0.924$ and $P = 0.965$, respectively).

Biomass gain, SGR and shell growth rate were also significantly affected by water temperature ($P < 0.001$; 14 < 17 < 20 °C; Table 3). Dietary protein level had no significant effect on SGR ($P = 0.772$), biomass gain ($P = 0.799$) or shell growth rate ($P = 0.840$) and there were no significant interactive effects between water temperature and dietary protein level on biomass gain ($P = 0.958$), SGR ($P = 0.927$) or shell growth rate ($P = 0.989$). In addition, there was no significant linear or second order polynomial relationship between dietary protein level and SGR for abalone at 14, 17 or 20 °C ($P > 0.05$). Condition factor was significantly affected by water temperature ($P < 0.001$; 14 > 17 > 20 °C; Table 3), but not significantly affected by dietary protein level ($P = 0.472$), or the interactive effects between these two factors ($P = 0.732$).

3.3. Feed use

Water temperature had a significant effect on feed consumption rate (mg abalone^{-1} day^{-1}) ($P < 0.001$), while dietary protein level did not ($P = 0.184$). However, feed consumption rate was significantly affected

Table 1
Summary of water quality for each water temperature system[a,b].

Nominal temperature	Actual temperature (°C)[c]	Dissolved oxygen (mg L^{-1})[d]	Dissolved oxygen (% saturation)[d]	pH[d]	Salinity (ppt)[d]
14 °C	14.0 ± 0.1 (13.8–14.1)	8.0 ± 0.3 (7.1–8.5)	99.3 ± 1.4 (95.8–103.0)	8.14 ± 0.05 (7.95–8.26)	35.7 ± 0.57 (34.0–38.0)
17 °C	17.0 ± 0.3 (16.1–17.9)	7.6 ± 0.2 (7.0–8.0)	98.2 ± 1.2 (94.5–101.8)	8.15 ± 0.04 (7.99–8.26)	35.7 ± 0.57 (34.0–38.0)
20 °C	19.9 ± 0.3 (19.0–20.9)	7.3 ± 0.2 (6.9–7.7)	97.2 ± 1.6 (91.3–102.0)	8.15 ± 0.04 (8.02–8.26)	35.7 ± 0.57 (34.0–38.0)

[a] Values means ± standard deviation, values in parentheses represent the range of values.
[b] Data for DO, pH and salinity for entire experiment, while the data for water temperature is from end of temperature acclimation period.
[c] n = 75.
[d] n = 91.

Table 2
Ingredient and nutrient composition of experimental diets.

	Nominal crude protein level (%)			
	27	30	33	36
Ingredients (g 100 g^{-1} diet as fed)				
Salmon fish meal	4.00	4.00	4.00	4.00
Solvent extracted soybean meal	18.90	21.40	23.90	26.47
Lupins (de-hulled)	20.80	23.60	26.40	29.14
Waxy maize starch	30.67	29.07	27.59	19.96
Pregelatinised waxy maize starch	10.00	5.62	1.15	0.00
Wheat gluten meal	5.00	5.00	5.00	5.00
Casein	5.48	6.53	7.59	8.63
Diatomaceous earth	1.76	1.79	1.77	4.60
Salmon fish oil	1.22	0.84	0.46	0.10
EPA vitamin/mineral premix	0.20	0.20	0.20	0.20
Sodium alginate	0.30	0.30	0.30	0.30
Vitamin E	0.01	0.01	0.01	0.01
Calcium sulphate	0.43	0.36	0.30	0.22
Monosodium phosphate	0.72	0.68	0.65	0.61
Arginine	0.31	0.37	0.41	0.46
Threonine	0.20	0.23	0.27	0.30
Ingredient composition (g 100 g^{-1} diet as fed), analysed and (calculated)				
Moisture	10.35	10.48	10.61	10.30
Crude protein	27.00	31.10	34.30	37.30
Digestible protein (calculated)[a]	20.27	23.54	26.13	28.57
Lipid	3.60	3.60	3.70	3.50
Gross energy (MJ kg^{-1})	17.00	17.25	17.64	17.27
Digestible energy (MJ kg^{-1})[a] (calculated)	12.24	12.35	12.57	12.53
Ash	5.02	5.31	5.24	8.17
NFE (calculated)	64.38	59.99	56.76	51.03
Digestible CP:GE (g MJ^{-1})[a]	16.57	19.06	20.79	22.80
Calculated amino acids (g 100 g^{-1})				
Arginine	2.22	2.50	2.77	3.04
Histidine	0.72	0.80	0.89	0.97
Isoleucine	1.28	1.44	1.59	1.74
Leucine	2.10	2.35	2.59	2.83
Lysine	1.52	1.71	1.90	2.09
Methionine	0.46	0.51	0.57	0.62
Phenylalanine	1.31	1.46	1.61	1.76
Threonine	1.22	1.37	1.53	1.67
Tryptophan	0.30	0.34	0.37	0.41
Valine	1.42	1.59	1.75	1.92

[a] Digestible protein and energy values based on data reported by Fleming et al. (1998) and Vandepeer (2005); NFE = Nitrogen free extract = 100% − (protein % + lipid % + ash %); EPA. Eyre Peninsula Aquafeed Pty Ltd.

by the interaction between water temperature and dietary protein level ($P = 0.002$; Table 3). When compared to abalone fed other dietary protein levels at their respective water temperatures, the feed consumption rate by abalone fed 36% dietary CP level at 17 °C was significantly higher, while the feed consumption rate by abalone fed 36% dietary CP level at 20 °C was significantly lower. The feed consumption rates of abalone at 14 and 17 °C were similar when fed the same dietary protein level. The feed consumption rate of abalone at 20 °C was significantly higher than abalone at 14 and 17 °C (Table 3). In addition, regression analyses indicated that there were significant moderate positive second order polynomial and linear relationships between dietary protein level and feed consumption rate for abalone at 14 °C ($R^2 = 0.536$, $P = 0.007$) and 17 °C ($R^2 = 0.501$, $P = 0.002$), respectively. In contrast, regression analyses indicated that there was a significant moderate negative linear relationship between dietary protein level and feed consumption rate for abalone at 20 °C ($R^2 = 0.501$, $P = 0.002$).

The apparent FCR was significantly affected by water temperature ($P < 0.001$; $14 > 17 = 20$ °C; Table 3), while dietary protein level had no significant influence on FCR ($P = 0.111$) and there was no significant interaction between water temperature and dietary protein level ($P = 0.137$). However, regression analyses indicated that there was a significant moderate positive second order polynomial relationship between dietary protein level and FCR for abalone at 14 °C ($R^2 = 0.406$, $P = 0.034$). While there were no significant relationships between dietary protein level and FCR for abalone at 17 or 20 °C ($P > 0.05$), there were positive and negative tendencies, respectively.

3.4. Soft tissue composition

Dietary protein level had a significant effect on the soft tissue moisture content ($P = 0.018$). The soft tissue moisture content was significantly higher in abalone fed 36% CP compared to abalone fed 27% CP (Table 3). There were no significant differences between abalone fed other diets. Water temperature had no significant effect on soft tissue moisture content ($P = 0.364$), and there were no significant interactions between water temperature and dietary protein level ($P = 0.441$). Soft tissue protein content in greenlip abalone was not significantly affected by water temperature ($P = 0.556$) or dietary protein level ($P = 0.637$), and there were no significant interactions between these two factors ($P = 0.606$). Soft tissue lipid content was significantly influenced by water temperature ($P < 0.001$; $14 = 17 > 20$ °C), but was not significantly affected by dietary protein level ($P = 0.073$), and there were no significant interactions between water temperature and dietary protein level ($P = 0.595$). Soft tissue ash content was not significantly affected by water temperature ($P = 0.962$) and dietary protein level ($P = 0.252$), and there were no significant interactions between these two factors ($P = 0.211$). Soft tissue energy was not significantly affected by water temperature ($P = 0.231$) and dietary protein level ($P = 0.317$), and there were no significant interactions between these two factors ($P = 0.519$; Table 3).

3.5. Nutrient use

The apparent PER was significantly affected by water temperature ($P < 0.001$; $14 < 17 = 20$ °C) and dietary protein level ($P < 0.001$). The PER of abalone fed a diet containing 27% CP compared to all other diets was significantly superior to that in all other treatments, while the PER of abalone fed a diet containing 36% CP was significantly inferior to that in all other treatments. The PER of abalone fed diets containing 30 and 33% CP was not significantly different ($P > 0.05$). There was no significant interaction between water temperature and dietary protein level for PER ($P = 0.771$).

Water temperature had a significant effect on apparent protein deposition ($P < 0.001$; $14 < 17 = 20$ °C). Protein deposition was significantly influenced by dietary protein level ($P = 0.002$), and was significantly superior in abalone fed a diet containing 27% CP compared to abalone fed other CP levels. The protein deposition of fed 30, 33 or 36% CP was not significantly different ($P > 0.05$). There was no significant interaction between water temperature and dietary protein level ($P = 0.567$).

The apparent EER was significantly influenced by water temperature ($P < 0.001$; $14 < 17 < 20$ °C). The EER was not significantly affected by dietary protein level ($P = 0.268$) or the interaction between water temperature and dietary protein level ($P = 0.429$). The apparent energy deposition was significantly affected by water temperature ($P < 0.001$; $14 < 17 = 20$ °C; Table 3) and dietary protein level ($P = 0.013$). Abalone fed 27% CP had a significant superior energy deposition compared to abalone fed 36% CP (27 = 30 = 33% CP; 30 = 33 = 36% CP). There was no significant interaction between these two factors ($P = 0.451$).

4. Discussion

The experimental animals fed actively on diets throughout the study and growth rates were comparable to those observed in commercial facilities and other laboratory-based studies (Coote et al., 2000; Stone et al., 2013; Vandepeer, 2005). For example, Coote et al. (2000) reported a SGR for greenlip abalone (~1 g) of 1.03% day^{-1} at 20 °C for 85 days (Coote et al., 2000), Vandepeer (2005) reported a SGR for greenlip abalone (2.3 g) of 1.05% day^{-1} at 18 °C for 50 days, while Stone et al.

Table 3
Growth performance, feed efficiency and nutrient retention of greenlip abalone at three water temperature fed four dietary protein levels (mean ± SE; n = 4).

Temperature (°C)	14				17				20				SE	ANOVA							
Nominal protein (%)	27	30	33	36	27	30	33	36	27	30	33	36		Temp (°C) (A)			Protein level (%) (B)				A × B
														14	17	20	27	30	33	36	
Growth performance and mortality																					
Initial weight (g)	0.91	0.91	0.92	0.92	0.91	0.91	0.92	0.91	0.91	0.91	0.92	0.91	0.01	NS			NS				NS
Final weight (g)	1.59	1.47	1.53	1.49	1.92	1.97	1.95	1.96	2.58	2.50	2.57	2.47	0.06	X	Y	Z	NS				NS
Biomass gain ($g\ tank^{-1}$)	11.74	10.19	10.49	10.20	18.18	18.97	19.02	18.80	29.97	28.40	29.81	28.03	1.16	X	Y	Z	NS				NS
SGR (% day^{-1})	0.62	0.53	0.56	0.54	0.82	0.86	0.82	0.84	1.15	1.11	1.14	1.10	0.04	X	Y	Z	NS				NS
Mortality (%)	5.56	2.78	9.72	12.50	2.78	1.39	2.78	1.39	4.17	2.78	1.39	1.39	0.82	Z	Y	Y	NS				NS
Somatic growth parameters																					
Initial shell length (mm)	19.40	19.46	19.55	19.53	19.36	19.51	19.48	19.48	19.37	19.41	19.51	19.45	0.02	NS			NS				NS
Final shell length (mm)	22.11	21.71	22.04	21.96	24.14	24.34	24.23	24.17	26.67	26.45	27.02	26.61	0.30	X	Y	Z	NS				NS
Shell growth rate (μm day^{-1})	29.98	24.96	27.57	26.60	52.65	53.17	52.61	55.89	80.27	78.21	83.41	79.12	3.34	X	Y	Z	NS				NS
Condition factor	0.85	0.83	0.82	0.81	0.78	0.79	0.79	0.80	0.78	0.78	0.75	0.76	0.01	Z	Y	X	NS				NS
Feed utilisation																					
Feed consumption rate (mg $individual^{-1}\ day^{-1}$)	15.66^{de}	14.60^{e}	16.16^{de}	18.44^{cd}	16.96^{de}	18.31^{cd}	18.60^{cd}	20.46^{c}	28.56^{a}	25.91^{ab}	25.84^{ab}	24.61^{b}	0.69	*	*	*	*	*	*	*	*
Apparent FCR	2.17	2.35	2.55	2.95	1.55	1.58	1.65	1.79	1.55	1.51	1.42	1.43	0.08	Z	Y	Y	NS				NS
Nutrient retention																					
Apparent PER	1.55	1.24	1.06	0.83	2.21	1.83	1.63	1.35	2.15	1.94	1.87	1.69	0.07	Y	Z	Z	z	y	y	x	NS
Apparent PD	17.90	14.55	12.40	10.25	24.56	16.41	16.77	12.41	23.90	18.58	20.97	15.29	0.84	Y	Z	Z	z	y	y	y	NS
Apparent EER	2.47	2.23	2.06	1.79	3.51	3.31	3.17	2.91	3.42	3.49	3.63	3.65	0.11	X	Y	Z	NS				NS
Apparent ED	10.69	8.52	9.08	7.33	15.02	12.79	11.40	10.55	13.71	13.63	14.22	13.03	0.43	Y	Z	Z	z	zy	zy	y	NS
Proximate composition																					
Moisture (%)	75.15	75.80	75.12	75.60	74.18	74.90	75.44	76.27	74.62	74.70	74.81	75.72	0.14	NS			y	zy	zy	z	NS
Protein (% dry)	50.95	54.77	51.51	54.51	52.03	46.77	53.44	49.58	53.35	48.65	55.20	49.12	0.92	NS			NS				NS
Lipid (% dry)	5.49	5.06	5.17	5.40	5.29	5.08	5.18	4.91	4.69	4.95	4.51	4.20	0.07	Z	Z	Y	NS				NS
Ash (% dry)	10.27	10.57	9.92	10.34	9.84	10.28	10.50	10.76	10.35	10.29	9.85	11.05	0.10	NS			NS				NS
Energy (MJ kg^{-1} dry)	19.92	20.02	19.94	20.11	20.02	19.56	19.97	19.62	19.88	19.65	20.04	19.38	0.07	NS			NS				NS

SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio; PD, protein deposition; EER, energy efficiency ratio; ED, energy deposition; Standard errors, SE.
Initial soft tissue content of greenlip abalone (dry): protein (46.79%), lipid (4.07%), ash (13.92%), and energy (18.71 MJ/kg dry).
X, Y, Z: For variables with a significant effect of temperature and no interaction, values without a common upper case letter are different (Z indicates the highest value; $P < 0.05$); w, x, y, z: For variables with a significant effect of protein level and no interaction, values without a common lower case letter are significantly different (z indicates the highest value; $P < 0.05$); * denotes parameters with a significant interaction (A × B; $P < 0.05$), difference in protein level are compared across all water temperatures (one-factor ANOVA, SNK test), $^{a, b, c, d, e}$: values without a common superscript are significantly different (^{a} indicates the highest value; $P < 0.05$); NS: denotes non significant differences ($P > 0.05$).

(2013) reported a calculated peak SGR of 1.48% day^{-1} for greenlip abalone (1.8 g) over 84 days at their optimal water temperature of 22 °C.

Water temperature is a key environmental variable that affects the survival, growth, feed consumption, nutritional requirements and digestive physiology of abalone (Britz et al., 1997; Edwards and Condon, 2001; Schaefer et al., 2013; Stone et al., 2013, 2014; Vandepeer, 2006). In the current study, the SGR, biomass gain, apparent protein deposition, PER, shell growth and feed consumption rate of greenlip abalone all significantly increased with water temperature from 14 to 20 °C. Significantly improved SGR with increasing water temperature was previously reported for 1-year old greenlip abalone up to 22 °C (Stone et al., 2013), and similarly in South African abalone (*Haliotis midae*) up to 20 °C (Britz et al., 1997). In the current study, the improved growth and protein deposition of abalone as water temperature increased may have occurred due to an increased efficiency at utilising dietary components, particularly protein, due to temperature dependent feed intake and digestive enzyme activity (Britz et al., 1997; Edwards and Condon, 2001; Hochachka and Somero, 2002). Edwards and Condon (2001) reported significantly higher (75%) protease activity as temperature increased from 9 to 24 °C in blacklip abalone (*Haliotis rubra*) and suggested that this would contribute to improved growth rates up to this species' optimal water temperature of 17 °C (Gilroy and Edwards, 1998).

The optimal water temperature for the growth of greenlip abalone is controversial. Gilroy and Edwards (1998) reported a calculated optimal of 18.3 °C for Tasmania stock, while more recently Stone et al. (2013) reported an optimal of 22 °C for South Australian stock. Although 22 °C was not used in the current study, the SGR of abalone was significantly superior at 20 °C compared to 17 or 14 °C, providing further support for Stone et al. (2013). The possible discrepancy may have been due to genetic differences as Gilroy and Edwards (1998) worked with a Tasmanian strain, while in the current study and Stone et al. (2013), a more heat tolerant South Australian strain was used. Additionally, Gilroy and Edwards (1998) used larger greenlip abalone (82 mm SL) compared to smaller abalone (19 mm SL) in the current study. In addition, water temperature optima may also decline with size, previous research showed age-dependent differences when water temperature was raised from 18 to 22 °C; the growth rates of 1-year old greenlip abalone (23 mm SL) significantly increased, whereas the growth rate of 2-year old abalone (57 mm SL) did not (Stone et al., 2013). Steinarsson and Imsland (2003) reported similar size dependent optimal water temperature for *H. rufescens*, which peaked at 17.8 °C for 44 mm SL abalone, and declined to 14.5 °C for 98 mm SL abalone. Lastly, the optimal water temperature reported by Gilroy and Edwards (1998) was not determined from growth studies, but was estimated from behavioural studies on temperature preference. These discrepancies highlight the importance of species, strain and size-specific data from experiments concerning the variable of interest, and to not rely on models generated from other variables. The superior growth rate at high water temperatures observed in the current study further supports the benefits of heating nursery systems during periods of low temperatures to improve growth. A cost-benefit analysis of implementing temperature controlled nursery systems is currently being undertaken on abalone farms throughout southern Australia. Further research investigating the optimal water temperature for different sized greenlip abalone, similar to Steinarsson and Imsland (2003), may be beneficial to increase on-farm production, especially with regard to implementing temperature controlled systems for other year classes of abalone.

To improve production without additional infrastructure, diet manipulation, particularly dietary protein levels, also leads to growth improvements (Britz, 1996; Britz and Hecht, 1997; Coote et al., 2000; Stone et al., 2013). Protein is an expensive dietary component and plays a major role in abalone nutrition. The optimal dietary protein level has been the focus of numerous studies for a range of abalone species, including *H. midae* (Britz, 1996; Britz and Hecht, 1997), *H. rubra* (Dunstan, 2010), green ormer (*Haliotis tuberculata*) (Mai et al., 1995), Pacific abalone (*Haliotis discus hannai*) (Mai et al., 1995) and greenlip abalone (Coote et al., 2000; Stone et al., 2013). The aims of these studies have been to reduce on-farm expense and increase production (Britz, 1996; Britz and Hecht, 1997; Fleming and Hone, 1996; Shipton and Britz, 2001). The protein requirements of greenlip abalone have been the focus of two previous studies, and both studies formulated diets using the "ideal protein concept" (Coote et al., 2000; Stone et al., 2013). Coote et al. (2000) used highly digestible protein sources (casein and semolina) and reported an optimal dietary CP level of 27% for juvenile greenlip abalone (0.55–0.94 g) at 20 °C. A recent investigation by Stone et al. (2013) used highly digestible protein sources (solvent-extracted soybean meal, de-hulled lupins, casein and fish meal) and reported water temperature- and size-dependent optimal dietary protein level for greenlip abalone. The optimal dietary CP level increased from ~29.0 to 32.2 to 34.7% CP for 1-year old abalone and from 24 to 34 and 34% CP for 2-year old abalone as water temperature increased from 14 to 18 to 22 °C, respectively (Stone et al., 2013). Due to space limitations in Stone et al. (2013), the protein requirements of the younger age class of greenlip abalone (~6 month old) investigated in the current study could not be determined at the same time as 1- and 2-year old . Although caution should be exercised when comparing between studies, the aim of the current study was to provide further information on the optimal dietary protein level for this age class of greenlip abalone. In the current study, the SGR of abalone was not significantly affected by dietary protein level. However, the benefit of high protein diets to SGR is masked by differences in feed consumption that also affected the FCR of abalone. As abalone were fed to satiation throughout the trial, the significant negative relationship between dietary protein level and feed consumption rate for abalone at 20 °C indicates that these faster growing abalone up regulated feed intake when fed low protein diets to increase protein intake and achieve near-maximum growth potential. In contrast, a significant positive relationship between feed consumption rate and dietary protein levels occurred in slow growing abalone at 14 and 17 °C. The positive relationship between dietary protein and feed consumption rate resulted in a significant positive relationship between dietary protein level and FCR for abalone at 14 °C. There were no significant relationships between dietary protein level and FCR for abalone at 17 °C and 20 °C, but a slight positive and negative tendency between dietary protein level and FCR, respectively. These results suggest that the interactive effects of water temperature and dietary protein on feed consumption rate and FCR may be influenced by increased digestive enzyme activity at warmer water temperatures (Edwards and Condon, 2001), differences in the energetics of abalone at different water temperatures (Duong et al., 2014) or alterations to the gastrointestinal tract morphology (Schaefer et al., 2013).

Feeding abalone high levels of dietary protein, up to 36%, did not necessarily translate to increased soft tissue protein deposition. In contrast, superior protein deposition was previously reported in 1-year old greenlip abalone fed increasing dietary protein levels at 22 °C (Stone et al., 2013). At sub-optimal water temperatures, below 22 °C, abalone may deaminate excess protein to supply energy for metabolism rather than protein deposition and tissue growth, subsequently resulting in increased feed costs and ammonia excretion (Chaitanawisuti et al., 2011). A recent finding by Duong et al. (2014), from samples collected from the same animals used in the current study, indicated that ammonia excretion was significantly higher when abalone were fed diets containing 36% CP compared to abalone fed 27% CP, further supporting this hypothesis. While dietary ingredients used in the current study were selected due to their relatively high protein and energy digestible coefficient (Fleming et al., 1998; Vandepeer, 2005), to reduce feed costs the energy requirements of abalone should ideally be satisfied by dietary carbohydrates (Dunstan, 2010). Increasing the dietary digestible energy supplied by carbohydrates as dietary protein level increased may have reduced the use protein for energy and in turn, increased the utilisation of dietary protein for protein deposition and growth. However, in

practical diet formulations, which were also utilised in the present study, there is limited room to economically manipulate the formulation to increase digestible carbohydrate levels. Lipid inclusion levels used in the current study are optimal for greenlip abalone (Dunstan et al., 2000), as such, are also a limited source of energy. It would be beneficial in future studies to investigate novel dietary ingredients, such as dried macroalgae, with particular consideration to the carbohydrate content, composition and carbohydrate digestibility, to achieve a protein sparing effect (Dunstan, 2010). This may also lead to further improvements to growth and reduction to feed cost.

Test diets were formulated with input from all of the Australian commercial abalone feed producers, and were designed to be isoenergentic, while maintaining the optimal crude lipid level of ~3.6% for greenlip abalone (Dunstan et al., 2000). Due to the inherent lipid content of the other dietary protein sources, fish oil levels decreased as dietary protein level increased. Fish oil, and the fish oil in fish meal, contains essential long chain n-3 polyunsaturated fatty acids (LC n-3 PUFAs): eicosapentaenoic acid (20:5n-3, EPA), docosapentaenoic acid (22:5n-3, DPA) and docosahexaenoic acid (22:6n-3, DHA) (Bautista-Teruel et al., 2011). LC n-3 PUFAs are important for cellular membrane structure and function, controlling and regulating cellular metabolism, and many other aspects of animal physiology (Bautista-Teruel et al., 2011). The LC n-3 PUFA fatty acid profiles of the salmon fish meal and salmon oil used in the current study were not analysed. However, using the analysed crude lipid level of salmon fish meal and salmon fish oil (16 and 100%, respectively; Table 2), and the fatty acid levels of Atlantic salmon by-products (Nichols et al., 2002), the EPA, DPA, DHA and $\sum$LC n-3 PUFA levels were calculated to be lowest in the 36% CP diet, 0.071, 0.023, 0.108 and 0.202%, respectively. There are conflicting reports in the literature pertaining to the LC n-3 PUFA requirement of abalone. Dunstan et al. (2000) suggested the EPA and DHA requirement of greenlip abalone was 0.3% of the diet, especially at cooler water temperatures, which would suggest that the 36% CP diet in the current study may have been LC n-3 PUFA deficient. However, Dunstan et al. (2000) also concluded that maximum growth would be achieved, with no addition of fish oil, when the dietary fish meal inclusion was greater than 8% (fish meal lipid level of 8%; EPA = 0.014; DPA = 0.002; DHA = 0.011; $\sum$LC n-3 PUFA = 0.026%), which was far exceeded in all diets used in the current study. In addition, the gene expression of Δ-6 desaturase and elongase 2 and the bioaccumulation of LC n-3 PUFA demonstrated in hybrid abalone (*H. laevigata* × *H. rubra*) suggest that this closely related hybrid are able to desaturate and chain elongate the precursor α-linolenic acid (18:3n-3, ALA) to EPA and DHA (Mateos et al., 2011). In the current study, the lipid fractions of de-hulled lupin meal, and to a lesser extent solvent extracted soybean meal, contained moderate levels of the precursor ALA (Chiofalo et al., 2012; Monteiro et al., 2012), and likely supplemented dietary LC n-3 PUFA. Moreover, Hernández et al. (2013) reported that if ALA is present in the diet, growth of abalone (*H. tuberculata*) is not compromised after 200 days compared to control animals fed a fish oil diet.

Throughout the study, no apparent gross disease symptoms, but a low number of mortalities were observed, which compared favourably with commercial facilities during routine tank harvesting of stocking procedures. However, significantly higher mortalities at 14 °C (7.64%) compared to 17 °C (2.09%) and 20 °C (2.43%) were observed. Stone et al. (2013) similarly observed significantly higher mortalities at 14 °C (6.60%), than 18 °C (1.25%) or 22 °C (1.25%). In contrast, summer mortality is the major concern to the abalone farmers in southern Australia during periods of high summer water temperatures (>22 °C) where mortality rates can be up to 50% (Vandepeer, 2006). The moderate, yet significantly higher mortalities at low water temperatures observed in the current study might be easily overlooked on-farm. A higher amount of "walk-outs" during the dark period, particularly after monthly weight checks, occurred at 14 °C compared to 17 and 20 °C. These animals were typically found alive, and returned to their respective tanks immediately. "Walk-outs" were also observed in *H. midae* when held at 12–20 °C, but they do not seem to be water temperature related (Britz et al., 1997). The reason for "walk-outs" and the higher mortalities at 14 °C deserves further investigation as it may be currently overlooked on-farm.

In conclusion, this study adds to the knowledge of the nutritional requirement of greenlip abalone throughout their production cycle, by investigating the optimal dietary protein level for 6-month old, post-weaned greenlip abalone at a more precise water temperature range. When considering SGR, feed consumption rate, FCR and protein deposition, there were no apparent benefits to feed 6-month old greenlip abalone high protein diets at 14 or 17 °C. Therefore, we recommend a dietary protein level of 29% CP (21.9% digestible protein) with a digestible energy level of 12.5 MJ kg^{-1} at 14 and 17 °C, which was the minimum recommendation for 1-year old greenlip abalone by Stone et al. (2013). Although care must be taken when comparing between studies, as different sized animals and water temperatures were used Stone et al. (2013) previously recommended a dietary protein level of 34.7% CP (26.7% digestible protein) for slightly larger 1-year old greenlip abalone at 22 °C. In the current study, although dietary protein had no significant effect on SGR of abalone at 20 °C, abalone consumed less feed and there was a tendency for an improved FCR, but also a significantly lower PER, as animals were fed increased dietary protein level, therefore, we suggest that it may beneficial for greenlip abalone to be switched to a diet containing 34.7% CP (26.7% digestible protein) once the water temperature reaches 20 °C. The dietary protein recommendations presented in this study were derived using greenlip abalone that have been selected for growth and survival at higher summer water temperatures, such as those experienced in South Australian waters and Port Phillip Bay, Victoria. It may be beneficial to switch to higher protein diets at lower temperatures in areas where abalone have been selected to grow at lower water temperatures, such as Tasmania and coastal Victoria. Further research to determine the appropriate water temperature to switch to higher protein diets in Tasmania and coastal Victoria may be required to fine tune on-farm feeding practices. The current study and Stone et al. (2013) provide a much clearer understanding of the protein requirements for 6-month, 1-year and 2-year old greenlip abalone at a range of relevant water temperatures.

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