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DIETARY PROTEIN LEVEL AND WATER TEMPERATURE INTERACTIONS FOR GREENLIP ABALONE *HALIOTIS LAEVIGATA*

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ABSTRACT Land-based grow-out of greenlip abalone (*Haliotis laevigata*) in Australia is predominantly practiced using a single-diet feeding strategy, despite geographical and seasonal differences in water temperature that influence feed intake and growth. This 12-wk study investigated the interactions between 2 abalone year classes (1 y old, 1.8 g; 2 y old, 22.9 g), 3 water temperatures (14°C, 18°C, and 22°C), and 4 dietary protein levels (1 y old: 27%, 30%, 33%, and 36% crude protein; 2 y old: 24%, 27%, 30%, and 33% crude protein) to evaluate the potential to use diets to suit specific water temperatures and aged animals. Diets were formulated to be isoenergetic (~12.5 MJ/kg digestible energy), and contain fat levels of ~3.6% and digestible protein levels ranging from 17.99%–28.57%. Feed was fed to excess daily and uneaten food was collected. Temperature significantly affected the specific growth rate of both year classes of abalone. There was no significant effect of dietary protein level on specific growth rate; however, abalone compensated for a reduction of dietary protein by consuming more feed. This observation was evident with significant increases in feed conversion ratios as dietary protein levels decreased for both year classes. With the exception of abalone grown at 14°C, when protein deposition decreased with increasing dietary protein level, protein deposition increased with an increase in dietary protein for both year classes. For 1-y-old abalone, as temperature increased from 14–22°C, the optimum crude dietary protein levels increased from ~29%–~35%. For 2-y-old abalone, the optimum crude protein level appeared to be less, and increased from 24% at 14°C to 34% at 22°C. There is scope to use multidiet feeding strategies in the production of greenlip abalone.

KEY WORDS: greenlip abalone, nutrition, protein, temperature, age, *Haliotis laevigata*

INTRODUCTION

The aquaculture production of Australian greenlip abalone (*Haliotis laevigata*) occurs predominantly in land-based systems, which experience large seasonal fluctuations in water temperatures, and are reliant on formulated feeds. Typically, when juvenile greenlip abalone are weaned off microalgae they are on-grown using a single-diet feeding strategy with crude protein (CP) levels in the range of 25%–30%. This range is based on previous research conducted by Coote et al. (2000) on 1 size class of juvenile greenlip abalone (0.5–2.5 g) at 20°C. Wild abalone have a distinct shift in dietary preference during their life cycle, initially feeding on microalgae, then as subadults they shift to a predominately macroalgae diet (Shepherd & Turner 1985, Won et al. 2010). The reported protein levels (dry matter basis) of microalgae and macroalgae used for cultured abalone range from 12%–35% (Brown et al. 1997) and 11%–19% (Mai et al. 1994), respectively. This shift suggests that their nutritional requirements may change over time with respect to protein. There is significant scope to capitalize on this shift in feeding behavior and, in combination with investigations into protein requirements at fluctuating seasonal water temperatures, develop and implement multidiet feeding strategies aimed at improving the productivity of the greenlip abalone culture industry.

Temperature is an important environmental factor and impacts the growth of organisms significantly (Hochachka & Somero 2002). Water temperatures experienced during the grow-out of greenlip abalone oscillate seasonally between 10°C and 25°C. The optimum temperature for growth, and the 50% critical thermal maxima, of greenlip abalone (shell length (SL), 82 mm) were reported to be 18.3°C and 27.5°C, respectively (Gilroy & Edwards 1998). Steinarsson and Imsland (2003) reported size-dependent variation in the optimum growth temperature of red abalone (*Haliotis rufescens*), with temperature optimum for growth having a symmetrical, concave relationship with SL from 16 mm to maturity, reaching a peak of 17.8°C at 44 mm in SL. Cho and Kim (2012) investigated the effects temperature (20–26°C) on the growth performance of juvenile (4.7 g) *Haliotis discus hannai* and reported an optimum of 20°C for their culture. Green et al. (2011) reported a significant reduction in the growth rate of *Haliotis midae* as temperature increased from 18–24°C.

The reported optimal dietary protein levels for a range of abalone species are variable (Fleming et al. 1996). Uki et al. (1986) and Taylor (1992), using casein as the main protein source, reported optimal CP levels of 38% for *Haliotis discus hannai* and at least 30% for *Haltiotis kamtschatkana*, respectively. Sales et al. (2003), also using casein as the main dietary protein source, reported an optimum protein level of 36% (dry matter basis) for 5-g *Haliotis midae* cultured at 16°C, and reported depressed growth at a dietary protein level of 28%. These findings differ from the lower optimum CP level of 27% reported for juvenile greenlip abalone (0.6–2.5 g) cultured at 20°C (Coote et al. 2000). Similarly, Bautista-Teruel and Millamena (1999) reported an optimum CP level of 27% for juvenile *Haliotis asinina* (0.6–3.0 g) at 27–31°C. Some initial research has been carried out to investigate the interactions of animal size and dietary protein level with a view to develop multidiet feeding strategies in related abalone species. Britz and Hecht (1997) reported that maximum growth of *H. midae* at 18°C occurred in large (7.8 ± 0.25 g) abalone fed a crude dietary

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protein level of 44%, whereas the maximum growth occurred in small (0.2 ± 0.01 g) abalone at 34%. Shipton and Britz (2001) also demonstrated differences in the protein requirements of juvenile (initial SL, 10–20 mm; weight, 0.5 g) and young adult (initial SL, 40–50 mm; weight, 20 g) *H. midae*.

When reviewing the results from these studies, it becomes apparent that variables such as species, animal size, water temperature, and nutrient digestibility may play a pivotal role in the differences observed. All the previous studies used high-quality protein sources, and although some studies gave consideration to the "ideal protein ratio" concept, none were formulated on a digestible protein basis. Protein is an expensive, yet essential, dietary component for abalone and it is crucial that it is palatable and digestible, and has an adequate amino acid profile to support maximum protein deposition and growth (Britz & Hecht 1997, Bautista-Teruel et al. 2003).

This study aimed to investigate the interactions of dietary protein level and water temperature on the growth performance and feed use of 1- and 2-y-old size classes of greenlip abalone. In this study, test diets were formulated to ensure a gradation of digestible protein levels to cover the range considered to be commercially applicable to land-based production of abalone in southeastern Australia. We selected a range of highly palatable and digestible ingredients, formulated at realistic inclusion levels, using protein and energy digestibility data reported for greenlip abalone by Fleming et al. (1998) and Vandepeer (2005). With respect to amino acid composition, diets were formulated using the ideal protein ratio concept using soft tissue amino acid values for similar-size greenlip abalone reported by Coote et al. (2000). All diets contained ~3.5% lipid, as recommended for greenlip abalone by Van Barneveld et al. (1998) and Dunstan et al. (2000), and contained crude and digestible energy levels of ~17.4 MJ/kg and ~12.5 MJ/kg, respectively. The diets were tested at a range of seasonal temperatures (14°C, 18°C, and 22°C) representative of those experienced by growing abalone in land-based facilities in southeastern Australia.

MATERIALS AND METHODS

Experimental Animals

Greenlip abalone of 2 distinct year-class cohorts (1-y-old and 2-y-old were spawned in September 2010 and 2009, respectively) were purchased from South Australian Mariculture at Boston Point, Port Lincoln, South Australia, in September 2011. Abalone of each year class were fed a commercial diet (Eyre Peninsula Aquafeed Pty Ltd, Lonsdale, South Australia, Australia) prior to the trial and had not been used previously for any other experiments. On arrival at the SARDI Aquatic Science Center, West Beach, Adelaide, the abalone were placed in a flow-through seawater system overnight and then stocked into the experimental system at ambient water temperature (15°C) during the following 2 days.

Experimental System

The room temperature (20 ± 1°C) and photoperiod [12 h low intensity, fluorescent lighting at 3.4 Lux (equates to dark limit of civil twilight under a clear sky):12 h dark] were controlled. The experimental facility consisted of 3 identical temperature-controlled (14°C, 18°C, and 22°C) saltwater systems supplied with flow-though UV-treated seawater (model 025120–2, 120 W; Emperor Aquatics, Pottstown, PA). Each system was comprised of a 780-L sump, 780-L intermediate tank, and 780-L header tank (Solid Nally MegaBins, MS7800; Viscount Plastics Pty Ltd., Hawthorn East, Vic, Australia), and 36 12.5-L blue plastic culture units (Nally IH305, Viscount Plastics Pty Ltd.; length, 39.2 cm; width, 28.8 cm; depth, 11.0 cm; bottom surface area, 1,129 cm^2). Water temperature control was achieved by using either chillers (3 hp, 240V, 50 Hz; Daeil Cooler Co., Ltd., Busan, Korea) or 3-kW immersion heaters (240V, 3 kW; JQ20, Austin & Cridland, Carlton, NSW, Australia). The culture units were each provided with temperature-controlled flow-through water from the reservoir by gravity feed at a rate of 300 mL/min. Water level was set at 2.5 cm in each culture unit using a standpipe with a mesh screen (nominal mesh size, 0.8 mm) on the outlet to retain uneaten food.

Stocking

Abalone were anesthetized using 1 mL (1 y old, $n = 960$) or 2 mL (2 y old, $n = 480$) ethyl p-amino benzoate (Sigma Chemicals, Balcatta, WA, Australia)/L of seawater in a 40-L plastic container with 30 L oxygenated, ambient temperature (15°C) seawater. Twenty 1-y-old and ten 2-y-old animals were screened from larger populations, weighed, measured, and stocked, using systematic interspersion, into 4 replicate culture units per treatment combination. A sample of abalone from each year class was also collected, shucked, and stored frozen at –20°C for proximate analysis. Because of the low ambient water temperature (15°C) at stocking, a 1-wk acclimation period was used to raise or lower the water temperature slowly (~1°C/day) to the desired 14°C, 18°C, or 22°C. The water temperature was then maintained at the desired treatment temperatures (±1.0°C) throughout the remainder of the 84-day experiment. Dead abalone were recorded, measured, weighed, and replaced with abalone of a similar weight and size that had been held at the same treatment water temperature and fed a commercial diet.

Water Quality

Throughout the study, water quality was maintained at levels appropriate for good growth of greenlip abalone (Table 1). Water temperature and dissolved oxygen (measured in milligrams per liter and percent saturation) were measured daily using an OxyGuard Handygamma dissolved oxygen meter (OxyGuard International A/S, Birkerød, Denmark). The pH was measured daily using a meter (Oakton pHtestr 20; Oakton Instruments, Vernon Hills, IL). Salinity (measured in grams per liter) was determined weekly using a portable salinity refractometer (model RF20; Extech Instruments, Nashua, NH). Light intensity was measured using a LI-COR 1400 Quantum light meter (LI-COR Environmental, Lincoln, NE).

Diets and Feeding

The proximate composition of the ingredients was analyzed. The diets were then formulated, mixed, and extruded into flat pellets using a commercial pasta machine (La Prestigiosa medium; IPA, Vicenza, Italy) and cold-pressing technology, and contained nominal protein levels of 27%, 30%, 33%, and 36% CP for 1-y-old abalone and 24%, 27%, 30%, and 33% CP

TABLE 1.

Summary of water quality for each temperature system.

Temperature system	Temperature (°C) ($n = 83$)	Dissolved oxygen (mg/L) ($n = 83$)	Dissolved oxygen (% saturation) ($n = 83$)	pH ($n = 83$)	Salinity (ppt) ($n = 12$)
14°C	14.1 ± 0.20	8.0 ± 0.19	99.1 ± 1.64	8.3 ± 0.05	35.0 ± 0.62
	(13.2–14.7)	(6.8–8.7)	(88.4–104.2)	(8.1–8.4)	(34.0–36.0)
18°C	18.1 ± 0.22	7.3 ± 0.26	95.7 ± 2.96	8.3 ± 0.04	35.0 ± 0.62
	(17.6–19.0)	(6.2–8.1)	(79.2–102.7)	(8.2–8.4)	(34.0–36.0)
22°C	22.0 ± 0.41	6.7 ± 0.41	92.5 ± 4.45	8.3 ± 0.05	35.0 ± 0.62
	(21.0–23.0)	(5.3–8.1)	(73.6–101.6)	(8.2–8.4)	(34.0–36.0)

Values are mean ± SD. Values in parentheses represent the range of values. Data for dissolved oxygen, pH, and salinity are for entire experiment, whereas the data for water temperature is from the end of the temperature acclimation period.

for 2-y-old abalone. The ingredient composition and chemical composition of the diets are displayed in Table 2. All diets were formulated to be isoenergetic on a digestible basis. The diets were also formulated, using book values, so the ratio of each essential amino acid to lysine was equal to or greater than that analyzed in the soft body tissue of *Haliotis laevigata* reported by Coote et al. (2000). The different diet series were chosen according to the general understanding of the specific requirements of proteins for the small and large animals. Feed rates were maintained in the range of 4.00%–5.25% (1 y old) and 1.00%–1.75% (2 y old) total biomass of abalone per tank per day. These rations were in excess of the animals' daily requirements. The rations were adjusted based on the biomass at stocking and from monthly weight checks for all treatments. Feeding was carried out at 4:00 PM daily. Cleaning and collection of food waste took place daily at 8:30 AM and was done by sieving the entire tank contents through a fine mesh. The wet, uneaten feed was collected and stored frozen at –20°C. The wet uneaten feed was dried in an oven at 105°C for 16 h. The amount of dry feed consumed was calculated by subtracting the amount of dry uneaten feed from the amount of dry feed offered. We then determined the proportion of uneaten feed that was lost through the collection net without animals in the tank, and then we used this correction factor to calculate the corrected apparent feed intake per tank.

At the end of the experiment, feeding was stopped 24 h prior to harvest to ensure digestive tracts were empty before harvesting. Eight 1-y-old abalone and four 2-y-old abalone from each tank were collected, shucked, and stored frozen at –20°C, and were later pooled for each tank to analyze whole tissue proximate composition.

The dry matter leaching loss of each diet was determined in triplicate after a period of 1 h, 2 h, 4 h, 8 h, and 16 h of immersion at 14°C, 18°C, and 22°C. This was achieved by placing 1 g of each diet into 25 mL saltwater at the required temperature and then extracting the supernatant, by syringe, and then oven drying the remaining pellets at 105°C for 16 h.

Performance Indices

All data reported for individual animal performance were based on the individual data recorded from each tank; biomass gain and feed conversion ratio (FCR) take the weight of mortality into account. Performance indices for weight gain, specific growth rate (SGR), and apparent nutrient efficiency and deposition ratios were calculated as described by Stone et al. (2003). The condition factor (CF) was calculated using the equation derived from *Haliotis midae* by Britz and Hecht (1997):

$$\mathrm{CF} = 5{,}575 \times \frac{\text{Weight (in grams)}}{\text{Length (in millimeters)}^{2.99}}$$

The optimum protein levels for each year class at each water temperature were estimated using the average of the X_{max} values of SGR and protein deposition. The X_{max} and Y_{max} values for 1-y-old animals were determined directly from the equation for the second-order polynomial relationship, whereas, the X_{max} and Y_{max} values for 2-y-old animals were determined directly off the graph.

Biochemical Analyses

The proximate composition analyses of ingredients, diets, and whole body tissue were conducted according to methods in the British Pharmocopoeia Commission (2004) or German Institute for Standardization (DIN) Standards (2000). Abalone collected per tank for whole body proximate analyses were pooled, freeze-dried, then ground. Tissue samples were freeze-dried to a constant weight at –50°C to determine moisture content. Diet samples were oven-dried to a constant weight at 105°C for 16 h to determine moisture content. Crude protein ($N \times 6.25$) was determined by the Kjeldahl method (BP A219 H Determination of Nitrogen). Crude lipid was analyzed using a Soxtherm rapid extraction system (Gerhardt GmbH & Co. KG, Königswinter, Germany) with petroleum liquid (BP 100°C) as the extracting solvent. Ash was determined by a muffle furnace at 550°C for 16 h. Gross energy content was determined using a bomb calorimeter (Method 51900-1, DIN Standards 2000) calibrated with benzoic acid.

Statistical Analyses

Homogeneity of variance among mean values was assessed using Levene's test for equality of variance errors. Data from each year class was analyzed separately using 2-factor analysis of variance (ANOVA), with water temperature as the first factor and dietary protein level as the second factor. When significant interactions were observed, the data from all treatment combinations for the given variable were analyzed using 1-factor ANOVA. The Student Newman-Keuls (SNK) test was used to identify significant differences among multiple treatment means. Regressions were also applied to SGR, FCR, and protein deposition data in an attempt to determine the optimum

TABLE 2.

Ingredient and nutrient composition of experimental diets.

Ingredient composition (g/100 g diet as fed)	Nominal CP level (%)				
	24	27	30	33	36
Salmon fish meal	4.00	4.00	4.00	4.00	4.00
Solvent-extracted soybean meal	16.40	18.90	21.40	23.90	26.47
Lupins (dehulled)	17.95	20.80	23.60	26.40	29.14
Waxy maize starch	30.40	30.67	29.07	27.59	19.96
Pregelatinized waxy maize starch	15.40	10.00	5.62	1.15	0.00
Wheat gluten meal	5.00	5.00	5.00	5.00	5.00
Casein	4.43	5.48	6.53	7.59	8.63
Diatomaceous earth	2.61	1.76	1.79	1.77	4.60
Fish oil	1.61	1.22	0.84	0.46	0.10
EPA vitamin/mineral premix	0.20	0.20	0.20	0.20	0.20
Sodium alginate	0.30	0.30	0.30	0.30	0.30
Vitamin E	0.01	0.01	0.01	0.01	0.01
Calcium sulfate	0.50	0.43	0.36	0.30	0.22
Monosodium phosphate	0.75	0.72	0.68	0.65	0.61
Arginine	0.27	0.31	0.37	0.41	0.46
Threonine	0.17	0.20	0.23	0.27	0.30
Ingredient composition *(g/100 g diet as fed), analyzed and (calculated)*					
Moisture	10.10	10.35	10.48	10.61	10.30
CP	24.20	27.00	31.10	34.30	37.30
Digestible protein (calculated)	17.99	20.27	23.54	26.13	28.57
Lipid	3.60	3.60	3.60	3.70	3.50
GE (MJ/kg)	17.46	17.00	17.25	17.64	17.27
Digestible energy (MJ/Kg) (calculated)	12.72	12.24	12.35	12.57	12.53
Ash	5.68	5.02	5.31	5.24	8.17
NFE (calculated)	66.52	64.38	59.99	56.76	51.03
Digestible CP:GE (g/MJ)	14.14	16.57	19.06	20.79	22.80
Calculated amino acids (g/100 g diet)					
Arginine	1.95	2.22	2.50	2.77	3.04
Histidine	0.64	0.72	0.80	0.89	0.97
Isoleucine	1.13	1.28	1.44	1.59	1.74
Leucine	1.86	2.10	2.35	2.59	2.83
Lysine	1.34	1.52	1.71	1.90	2.09
Methionine	0.41	0.46	0.51	0.57	0.62
Phenylalanine	1.16	1.31	1.46	1.61	1.76
Threonine	1.07	1.22	1.37	1.53	1.67
Tryptophan	0.27	0.30	0.34	0.37	0.41
Valine	1.26	1.42	1.59	1.75	1.92

CP, crude protein; GE, gross energy; NFE, nitrogen-free extract, = 100% – (Protein % + Lipid % + Ash %); EPA, Eyre Peninsula Aquafeed Pty Ltd.

protein level for growth of each year class. A significance level of $P < 0.05$ was used for all statistical tests. All statistical analyses were done using IBM SPSS, version 19 for Windows (IBM SPSS Inc., Chicago, IL). All values are presented as mean ± SE of the mean.

RESULTS

General Observations

There were no significant differences in the initial weight, tank biomass, and SL among treatments for 1-y-old or 2-y-old abalone at the commencement of the experiment ($P > 0.05$). The overall mean value for the abalone of each year class were initial weight, 1.75 ± 0.01 g and 22.93 ± 0.09 g; initial tank biomass, 34.96 ± 0.09 g/tank and 229.29 ± 0.88 g/tank; and initial SL, 23.31 ± 0.03 mm and 56.64 ± 0.08 mm for 1- and 2-y-old abalone, respectively. Animals exhibited normal signs of feeding behavior throughout the study and no gross symptoms of disease were observed. Overall, the mortality rate for the experiment was 2.92% and the majority of the mortalities occurred within the first 2–3 wk after stocking. Interestingly, there was a significant effect of water temperature on mortality rate of 1-y-old abalone (2-factor ANOVA, $P < 0.001$). A higher mortality rate was observed at 14°C (6.60 ± 1.09%) compared with either 18°C (1.25 ± 0.56%) or 22°C (1.25 ± 0.72%). There was no significant effect of protein level on mortality rate ($P = 0.421$), and there was no significant interaction between water temperature and protein level ($P = 0.054$). The mortality rate (2.71 ± 0.83%) of 2-y-old abalone was not significantly affected by water temperature ($P = 0.676$), protein level ($P = 0.421$), or the interaction between these 2 factors ($P = 0.877$).

The analyzed protein contents of the diets were slightly higher than the formulated nominal values (Table 2). Diet dry matter leaching loss was significantly affected ($P < 0.001$) by diet type, water temperature, and immersion time in water. There were no significant interactions among these factors ($P > 0.05$). The differences in percent leaching losses among diets types were significant; however, they were relatively small (diet 1, 5.83% > diet 2, 3.73% = diet 3, 3.68% = diet 4, 4.05% = diet 5, 3.86%; 3-factor ANOVA; pooled SE, 0.316%; SNK). Leaching loss increased with increasing water temperature (Fig. 1A) and with increasing submersion time until 8 h (Fig. 1B).

Growth Performance

There were significant effects ($P < 0.001$) of water temperature on final individual weight (Table 3) and SGR for 1-y-old abalone (Fig. 2A). There were significant increases in final individual weight and SGR observed as the temperature increased from 14–22°C. There was no significant effect of protein level on final individual weight ($P = 0.449$) or SGR ($P = 0.200$). There were no significant interactions between water temperature and protein level for final individual weight or SGR ($P = 0.439$ and 0.237, respectively). However, there were definite trends for an increase in final weight and SGR of 1-y-old abalone at 22°C as the protein level increased from 27% up to 33% (Table 3, Fig. 2A). Although, in comparison, a relatively neutral and negative response was observed to increasing protein level for weight gain and SGR of 1-y-old abalone cultured at 18°C and 14°C, respectively. There were also significant effects ($P < 0.001$) of water temperature on the final individual weight and SGR of 2-y-old abalone. There was a significant increase in final individual weight (Table 4) and SGR (Fig. 2B) observed for 2-y-old abalone as the temperature increased from 14–18°C and 22°C. There were no significant differences for final individual weight ($P = 0.424$) or SGR ($P = 0.796$) between 18C and 22C. There was no significant effect of protein level on final individual weight ($P = 0.692$) or SGR ($P = 0.465$). There were no significant interactions between water temperature and protein level for final individual weight or SGR ($P = 0.811$ and 0.936, respectively).

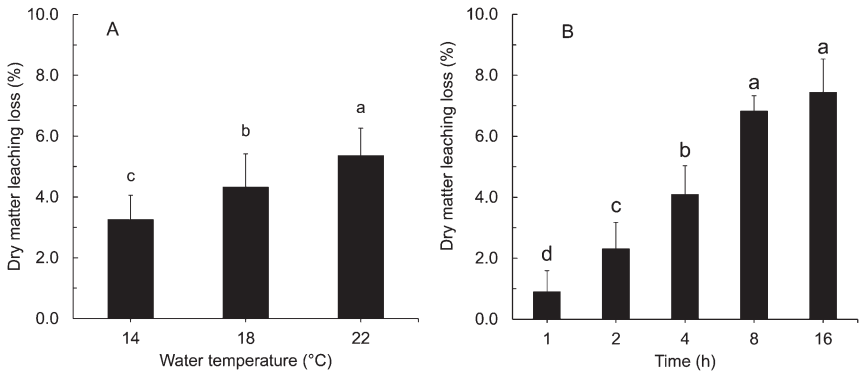

Figure 1. (A, B) Dry matter leaching loss of experimental abalone diets at 1 h, 2 h, 4 h, 8 h, and 16 h after submersion at 3 water temperatures (14, 18, and 22°C). Diet type (diet 1, 5.8% > diet 2, 3.7% = diet 3, 3.7% = diet 4, 4.1% = diet 5, 3.9%; pooled SE, 0.32%) water temperature and submersion time significantly affected leaching loss ($P < 0.001$). The interaction was not significant ($P > 0.05$). Three-factor ANOVA, SNK, $n = 15$ for temperature and $n = 9$ for time. Mean ± SE. Values that share the same superscript are not significantly different ($P > 0.05$).

There was a significant effect of water temperature on biomass gain for 1-y-old abalone (Table 3; $P < 0.001$). A progressive significant increase in biomass gain was recorded as the temperature increased from 14–22°C. There was also a significant effect of water temperature on biomass gain for 2-y-old abalone (Table 4; $P < 0.001$). The biomass gain was significantly lower at 14°C compared with either 18°C or 22°C. There was no significant effect of protein level on biomass gain for 1-y-old ($P = 0.342$) or 2-y-old ($P = 0.631$) abalone, and there was no significant interaction between the 2 factors for either 1-y-old ($P = 0.414$) or 2-y-old ($P = 0.900$) abalone.

There were significant effects of water temperature on final SL, shell growth rate, and CF for 1-y-old abalone (Table 3; $P < 0.001$). Significant increases in final SL and shell growth rate were observed as the temperature increased from 14–22°C. In contrast, there was a significant decrease in CF observed as the temperature increased from 14–22°C. There was no significant effect of protein level on final SL ($P = 0.623$), shell growth rate ($P = 0.527$), or CF ($P = 0.572$). However, for shell growth rate, similar trends were observed in response to increasing protein level at each temperature when compared with weight gain (Table 3) and SGR (Fig. 2A) of abalone of the same year class. There were no significant interactions between water temperature and protein level for final SL ($P = 0.424$), shell growth rate ($P = 0.427$), and CF ($P = 0.924$) for 1-y-old abalone. For 2-y-old abalone, there were significant increases in final SL and shell growth rate observed as the temperature increased from 14–22°C (Table 4; $P < 0.001$). In contrast, the CF of 2-y-old abalone was significantly lower at 22°C (Table 4; $P = 0.005$) compared with 14°C and 18°C. There was no significant effect of protein level on final SL ($P = 0.995$), shell growth rate ($P = 0.893$), and condition factor ($P = 0.697$). However, there was a trend for shell growth rate to increase in response to increasing protein level at 22°C for 2-y-old abalone; however, this did not equate to an increase in SGR (Fig. 2B). There were no significant interactions between water temperature and protein level for final SL ($P = 0.692$), shell growth rate ($P = 0.424$), and CF ($P = 0.496$) for 2-y-old abalone.

Feed Use

There were significant effects of water temperature ($P < 0.001$) and protein level ($P < 0.001$) on feed consumption rate (Table 3) and apparent economic FCR (Fig. 3A) for 1-y-old abalone.

There were also significant interactions observed between these variables for feed use ($P < 0.001$). The interaction for feed consumption rate may be explained by the neutral response of feed intake to additional protein at 14°C compared with the significant reductions in feed intake that began at the 33% protein level at 18°C, and at the 30% protein level at 22°C (Table 3; 1-factor ANOVA, SNK). The interaction for apparent FCR may be explained by the significant positive relationship between FCR and protein level at 14°C, compared with the significant negative relationships observed between FCR and protein level at both 18°C and 22°C (Fig. 3A; 1-factor ANOVA, SNK). There were significant effects of water temperature on feed consumption rate for 2-y-old abalone (Table 4; $P < 0.001$). There were also significant effects of protein level on feed consumption rate ($P < 0.001$). There was also a significant interaction between water temperature and protein level for feed consumption rate ($P < 0.001$). The interaction for feed consumption rate may be explained by the significantly negative relationship between this variable and protein level at 22°C, compared with the positive relationships at both 14°C and 18°C up to the 30% protein level (Table 4; 1-factor ANOVA, SNK).

There were significant effects of water temperature on FCR for 2-y-old abalone (Fig. 3B; $P < 0.001$). The FCR at 14°C was significantly higher than at either 18°C or 22°C. There were also significant effects of protein level on FCR. The FCRs of 27% and 30% protein were significantly higher than 33% protein, whereas 24% and 33% protein were not significantly different ($P = 0.082$). There was no significant interaction between water temperature and protein level for FCR ($P = 0.338$).

Soft Tissue Composition

For 1-y-old greenlip abalone there was a significant effect of water temperature on soft tissue moisture composition ($P < 0.001$). Moisture content was inversely related to increasing water temperature (Table 3). There was no significant effect of protein level ($P = 0.091$) and the interaction between the 2 factors ($P = 0.373$). There was no significant effect of water temperature ($P = 0.686$), protein level ($P = 0.129$), or interaction between the 2 factors ($P = 0.104$) on soft tissue moisture composition of 2-y-old greenlip abalone (Table 4).

Final dry tissue protein content of 1-y-old abalone was significantly affected by water temperature ($P < 0.001$) and

TABLE 3.

Growth performance and soft tissue composition of 1-y-old greenlip (mean ± SE, $n = 4$).

Temperature (°C)	14				18				22					ANOVA							
														Temp (°C) (A)*			Protein level (%) (B)†				
Nominal protein (%)	27	30	33	36	27	30	33	36	27	30	33	36	SE	14	18	22	27	30	33	36	A × B‡
Growth performance																					
Final weight (g/fish)	3.09	3.05	3.16	2.84	4.57	4.75	4.65	4.66	5.58	5.90	6.00	6.03	0.15	Z	Y	X	NS	NS	NS	NS	NS
Biomass gain (g/tank)§	23.50	26.61	27.67	25.11	56.01	59.12	60.17	57.62	80.32	83.42	84.48	81.92	2.08	Z	Y	X	NS	NS	NS	NS	NS
Final shell length (mm)	28.91	28.67	29.05	28.04	34.76	34.93	34.73	34.89	37.54	38.10	38.36	38.18	0.36	Z	Y	X	NS	NS	NS	NS	NS
Shell growth rate (μm/day)	66.53	63.63	68.93	57.73	136.73	138.63	135.43	137.63	167.73	176.48	180.00	175.73	4.31	Z	Y	X	NS	NS	NS	NS	NS
Condition factor	0.74	0.75	0.74	0.74	0.63	0.65	0.64	0.63	0.61	0.62	0.61	0.63	0.01	X	Y	Z	NS	NS	NS	NS	NS
Feed consumption rate (mg/individual/day)	62.17^{f}	62.48^{f}	55.80^{f}	59.24^{f}	136.73^{c}	138.27^{c}	109.16^{e}	118.24^{de}	168.30^{a}	158.38^{b}	123.25^{d}	123.05^{d}	3.31	*	*	*	*	*	*	*	*
Soft tissue composition																					
Moisture (%)	81.75	82.24	82.55	82.35	79.49	80.11	79.89	79.66	77.29	78.37	78.38	79.81	0.52	X	Y	Z	NS	NS	NS	NS	NS
Protein (% dry)	66.45^{ab}	66.30^{ab}	63.86^{abc}	67.47^{a}	60.70^{cd}	62.70^{bc}	64.74^{abc}	65.50^{abc}	58.57^{d}	62.06^{bc}	64.26^{abc}	63.85^{abc}	1.01	*	*	*	*	*	*	*	*
Fat (% dry)	6.59	7.28	6.18	6.37	6.61	6.27	5.87	5.83	6.91	7.09	6.23	5.98	0.30	NS	NS	NS	x	x	Y	y	NS
Ash (% dry)	11.36	10.15	11.49	11.13	10.73	10.49	10.75	10.75	9.71	9.65	9.92	10.86	0.60	NS	NS	NS	NS	NS	NS	ns	NS
NFE (% dry)	16.58	16.27	18.48	15.04	21.96	20.55	18.64	17.93	24.81	21.20	19.60	19.32	1.32	Y	X	X	x	xy	xy	y	NS
Energy (MJ/kg dry)	20.18	20.00	19.85	21.00	20.30	19.68	19.98	20.15	20.18	20.30	20.10	19.98	0.23	NS	NS	NS	NS	NS	NS	NS	NS

*X, Y, Z: For variables with a significant effect of temperature and no interaction, values without a common uppercase letter are significantly different (X 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 lowercase letter are significantly different (w indicates the highest value; $P < 0.05$). ‡A × B = 2-factor ANOVA interactions. §Biomass gain = (Weight of final animals + Weight of mortalities) – (Weight of stocked animals + Weight of replacement animals). ¶Significant interaction ($P < 0.05$). [a, b, c, d]: For variables with a significant interaction, differences in all protein levels are compared across all temperatures (1-factor ANOVA, SNK test). Values without a common superscript are significantly different ([a] indicates the highest value; $P < 0.05$). One-y-old initial soft tissue content of protein (66.41% dry), lipid (6.01% dry), ash (12.08% dry), NFE (15.50% dry), and energy (19.95 MJ/kg dry). NFE, nitrogen-free extract, = 100% – (Protein % + Fat % + Ash %). NS, nonsignificant at $P > 0.05$.

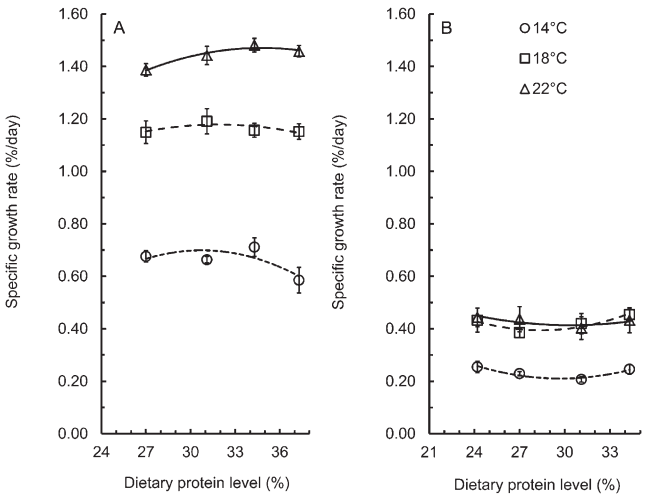

Figure 2. (A, B) Specific growth rate of 1-y-old (A) and 2-y-old (B) abalone fed a range of dietary protein levels (1-y-old, 27%, 30%, 33%, and 36%; 2-y-old, 24%, 27%, 30%, and 33%) at 3 different water temperature for 84 days ($n = 4$ tanks, mean ± SE. One-y-old at 14°C: $y = -0.0023x^2 + 0.14x - 1.48$, $R^2 = 0.62$, $X_{max} = 30.8$, $Y_{max} = 0.70$; at 18°C: $y = -0.0011x^2 + 0.070x + 0.069$, $R^2 = 0.60$, $X_{max} = 31.6$, $Y_{max} = 1.17$; and at 22°C: $y = -0.0014x^2 + 0.099x - 0.25$, $R^2 = 0.95$, $X_{max} = 35.3$, $Y_{max} = 1.50$. Two-y-old at 14°C: $y = 0.0016x^2 - 0.093x + 1.59$, $R^2 = 0.9172$, $X_{max} = 24.2$, $Y_{max} = 0.26$; at 18°C: $y = 0.0018x^2 - 0.11x + 1.88$, $R^2 = 0.86$, $X_{max} = 24.2$, $Y_{max} = 0.43$; and at 22°C: $y = 0.0009x^2 - 0.057x + 1.28$, $R^2 = 0.66$, $X_{max} = 24.2$, $Y_{max} = 0.44$. The values for X_{max} and Y_{max} for 1-y-old abalone are determined from the second-order polynomial equation, whereas, the X_{max} and Y_{max} values for 2-y-old abalone are determined directly off the graph.

protein content ($P < 0.001$; Table 3). There was also a significant interaction for tissue protein content ($P = 0.034$). The interaction is explained by the significant reduction in tissue protein for the 1-y-old abalone fed the 27% crude protein diet at 22°C, whereas tissue protein was not significantly affected in the 2 other water temperature groups (Table 3, 1-factor ANOVA, SNK). There were significant effects of water temperature on final dry tissue protein content for 2-y-old abalone (Table 4; $P = 0.003$). Tissue protein was significantly higher at 14°C. Tissue protein levels were significantly ($P < 0.001$) and positively related to increasing dietary protein levels. There was no significant interaction between water temperature and protein level for final tissue protein content ($P = 0.758$).

There were no significant effects of water temperature on soft tissue fat content for 1-y-old abalone ($P = 0.075$; Table 3). However, there was a significant effect of dietary protein level ($P = 0.003$), with abalone fed diets containing 27% and 30% CP having slightly, although significantly, higher fat levels compared with those fed 33% or 36% CP. There were no significant interactions between the 2 factors ($P = 0.699$). For 2-y-old abalone, the fat content at 14°C was significantly higher than at either 18°C or 22°C (Table 4; $P = 0.001$). There were no significant effects of protein level on fat content ($P = 0.065$), and there was no significant interaction between water temperature and protein level ($P = 0.733$).

There was no significant effect of water temperature ($P = 0.073$; 0.638) or dietary protein level on the dry soft tissue ash content of 1-y-old ($P = 0.404$) or 2-y-old ($P = 0.654$) abalone, and there were no significant interactions between the 2 factors ($P = 0.830$, $P = 0.404$; Tables 3 and 4), respectively. There was no significant effect of water temperature ($P = 0.372$, $P = 0.217$) or dietary protein level ($P = 0.118$, $P = 0.352$) on the dry soft tissue energy content of 1- or 2-y-old abalone and there were no significant interactions ($P = 0.054$, $P = 0.298$; Tables 3 and 4), respectively.

There was a significant effect of water temperature on soft tissue nitrogen-free extract (NFE) content for 1- and 2-y-old abalone (Tables 3 and 4; $P < 0.001$). For 1- and 2-y-old abalone, the NFE contents were significantly lower at 14°C compared with either 18°C or 22°C. There was a significant effect of protein level on NFE content on 1-y-old ($P = 0.015$) and 2-y-old ($P < 0.001$) abalone. For 1-y-old abalone, there was a significant progressive reduction in NFE content as the dietary protein content increased from 27%–36% (Table 3). The NFE content of 2-y-old abalone fed the 30% and 33% CP diets were significantly lower than those of the abalone fed diets containing 24% and 27% CP protein (Table 4). There was no significant interaction between the 2 factors for either year class ($P = 0.245$, $P = 0.894$).

Nutrient Use

There was a significant effect of water temperature (Fig. 4A; $P < 0.001$) and protein level on protein deposition ($P = 0.015$) for 1-y-old abalone. There was also a significant interaction between water temperature and protein level for protein deposition ($P = 0.018$). The interaction for protein deposition for 1-y-old abalone may be explained by the significant positive response to increasing protein level occurring at the 33% protein level at 22°C compared with the nonsignificant neutral and significant negative responses observed at 18°C and 14°C, respectively (Fig. 4A; 1-factor ANOVA, SNK). There was a significant effect of water temperature ($P = 0.041$) and protein level on protein deposition for 2-y-old abalone (Fig. 4B; $P = 0.048$), and there was a significant interaction between water temperature and protein level for protein deposition ($P = 0.008$). The interaction may be explained by the significant increase in protein deposition observed at the 33% protein level at 22°C compared with the nonsignificant responses to increasing protein level recorded at 18°C and 14°C (Fig. 4A; 1-factor ANOVA, SNK).

DISCUSSION

Our overarching goal is to optimize abalone growth by formulating specific levels of dietary protein for seasonal water temperatures throughout the production cycle. The information obtained from this study is essential to achieving this goal and will be used to formulate diets in future experiments on optimum dietary protein levels in commercial situations.

Animals fed actively on the experimental diets with no apparent gross symptoms of disease. The mortality rate observed in this study was low and compared favorably with mortality rates observed at commercial facilities during routine tank harvesting/stocking procedures. The growth rates recorded in this study were favorable when compared with other laboratory-based studies by Coote et al. (2000) and Vandepeer (2005), and were also comparable with those observed in commercial facilities. Coote et al. (2000) reported an SGR of 1.03%/day for juvenile greenlip abalone stocked at ~1 g (SL, 19 mm) and grown at 20°C for 85 days. Vandepeer

TABLE 4.

Growth performance and soft tissue composition of 2-y-old greenlip abalone (mean ± SE, $n = 4$).

Temperature (°C)	14				18				22					ANOVA							
														Temp (°C) (A)*			Protein level (%) (B)†				
Nominal protein (%)	24	27	30	33	24	27	30	33	24	27	30	33	SE	14	18	22	24	27	30	33	A × B‡
Growth performance																					
Final weight (g/fish)	28.72	27.78	27.58	28.22	32.25	31.49	32.28	33.84	33.09	33.72	32.35	33.02	1.03	Y	X	X	NS	NS	NS	NS	NS
Biomass gain (g/tank)§	54.45	48.60	46.50	53.45	98.63	87.11	97.44	110.07	103.35	104.94	97.16	102.49	9.38	Y	X	X	NS	NS	NS	NS	NS
Final shell length (mm)	60.19	59.38	59.65	59.48	62.79	62.25	62.33	62.62	63.42	64.54	64.37	64.47	0.60	Z	Y	X	NS	NS	NS	NS	NS
Shell growth rate (µm/day)	40.23	35.33	36.15	29.60	75.40	65.93	67.88	73.78	82.65	89.83	88.85	97.45	5.83	Z	Y	X	NS	NS	NS	NS	NS
Condition factor	0.77	0.77	0.76	0.78	0.76	0.76	0.78	0.80	0.76	0.73	0.71	0.72	0.02	X	X	Y	NS	NS	NS	NS	NS
Feed consumption rate (mg/fish/day)	136.20^{e}	155.78^{d}	168.71^{cd}	131.37^{e}	199.06^{b}	209.55^{b}	208.45^{b}	169.43^{cd}	228.58^{a}	228.03^{a}	208.68^{b}	174.38^{c}	4.74	*	*	*	*	*	*	*	*
Soft tissue composition																					
Moisture (%)	80.66	80.35	80.68	82.43	80.76	80.71	81.08	82.92	80.18	82.81	80.18	80.42	0.79	NS	NS	NS	NS	NS	NS	NS	NS
Protein (% dry)	60.90	63.30	65.19	65.78	59.03	60.44	60.47	64.36	57.72	58.68	62.18	64.00	1.32	X	Y	Y	y	xy	wx	w	NS
Fat (% dry)	6.96	6.17	7.27	6.52	6.52	5.74	6.20	6.41	5.59	5.43	6.23	5.44	0.36	X	X	Y	NS	NS	NS	NS	NS
Ash (% dry)	9.32	8.67	9.08	9.60	8.90	8.90	9.62	8.94	8.39	9.26	8.70	9.23	0.42	NS	NS	NS	NS	NS	NS	NS	NS
NFE (% dry)	22.82	21.87	18.46	18.10	25.56	24.94	23.71	20.29	28.31	26.63	22.83	21.33	1.49	Y	X	X	x	x	y	y	NS
Energy (MJ/kg dry)	20.18	20.08	20.00	20.08	19.85	20.25	19.83	20.23	19.85	19.48	19.85	20.20	0.20	NS	NS	NS	NS	NS	NS	NS	NS

*X, Y, Z: For variables with a significant effect of temperature and no interaction, values without a common uppercase letter are significantly different (X 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 lowercase letter are significantly different (w indicates the highest value; $P < 0.05$). ‡A × B = 2-factor ANOVA interactions. §Biomass gain = (Weight of final animals + Weight of mortalities) – (Weight of stocked animals + Weight of replacement animals). ¶Significant interaction ($P < 0.05$). [a, b, c, d]: For variables with a significant interaction, differences in all protein levels are compared across all temperatures (1-factor ANOVA, SNK test). Values without a common superscript are significantly different ([a] indicates the highest value; $P < 0.05$). Two-y-old initial soft tissue content of protein (64.54% dry), lipid (5.68% dry), ash (10.67% dry), NFE (19.11% dry), and energy (20.22 MJ/kg dry). NFE, nitrogen-free extract, = 100% – (Protein % + Fat % + Ash %). NS, nonsignificant at $P > 0.05$.

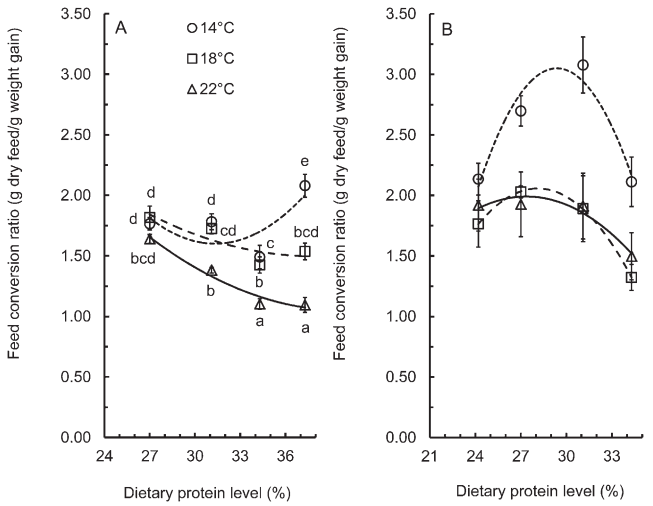

Figure 3. (A, B) Apparent economic feed conversion ratio of 1-y-old (A) and 2-y-old (B) abalone fed a range of dietary protein levels (1-y-old, 27%, 30%, 33%, and 36%; 2-y-old, 24%, 27%, 30%, and 33%) at 3 different water temperature for 84 days (n = 4 tanks, mean ± SE). One-y-old at 14°C: $y = 0.011x^2 - 0.71x + 12.69$, $R^2 = 0.51$, $X_{max} = 31.3$, $Y_{max} = 1.61$; at 18°C: $y = 0.0029x^2 - 0.22x + 5.68$, $R^2 = 0.76$, $X_{max} = 37.3$, $Y_{max} = 1.48$; and at 22°C: $y = 0.0036x^2 - 0.29x + 6.82$, $R^2 = 0.97$, $X_{max} = 37.3$, $Y_{max} = 1.05$. Two-y-old at 14°C: $y = -0.037x^2 + 2.15x - 28.47$, $R^2 = 0.93$, $X_{max} = 24.2$, $Y_{max} = 2.14$; at 18°C: $y = -0.019x^2 + 1.079x - 13.11$, $R^2 = 1.00$, $X_{max} = 34.3$, $Y_{max} = 1.32$; and at 22°C: $y = -0.0098x^2 + 0.54x - 5.36$, $R^2 = 0.94$, $X_{max} = 34.3$, $Y_{max} = 1.50$. The values for X_{max} and Y_{max} for 1-y-old abalone are determined from the second-order polynomial equation, whereas the X_{max} and Y_{max} values for 2-y-old abalone are determined directly off the graph.

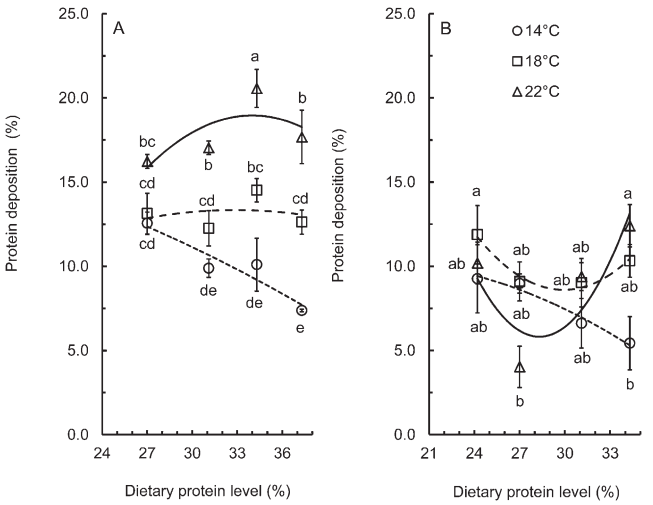

Figure 4. (A, B) Protein deposition of 1-y-old (A) and 2-y-old (B) abalone fed a range of dietary protein levels (1-y-old 27%, 30%, 33%, and 36%; 2-y-old: 24%, 27%, 30%, and 33%) at 3 different water temperature for 84 days (n = 4 tanks, mean ± SE). One-y-old at 14°C: $y = -0.0062x^2 - 0.051x + 18.27$, $R^2 = 0.88$, $X_{max} = 27.1$, $Y_{max} = 12.33$; at 18°C: $y = -0.014x^2 + 0.92x - 1.75$, $R^2 = 0.047$, $X_{max} = 32.8$, $Y_{max} = 13.31$; and at 22°C: $y = -0.063x^2 + 4.30x - 54.13$, $R^2 = 0.53$, $X_{max} = 34.0$, $Y_{max} = 19.00$. Two-y-old at 14°C: $y = -0.016x^2 + 0.53x + 5.93$, $R^2 = 0.97$, $X_{max} = 24.2$, $Y_{max} = 9.26$; at 18°C: $y = 0.096x^2 - 5.74x + 94.47$, $R^2 = 0.96$, $X_{max} = 34.3$, $Y_{max} = 10.33$; and at 22°C: $y = 0.2049x^2 - 11.599x + 170.01$, $R^2 = 0.7386$, $X_{max} = 34.3$, $Y_{max} = 12.41$). The values for X_{max} and Y_{max} for 1-y-old abalone are determined from the second-order polynomial equation, whereas, the X_{max} and Y_{max} values for 2-y-old abalone are determined directly off the graph.

(2005) also reported an SGR of 1.05 for greenlip abalone (2.25 g; SL, 25 mm) grown for 50 days at 18°C.

In this study, animals were fed to excess, and feed was not a limiting factor. When feed is not limiting, temperature is the most important environmental factor influencing the growth rate of an animal (Britz et al. 1997). The optimal water temperature for greenlip abalone is 18.3°C (Gilroy & Edwards 1998); below this, a reduction in growth may occur because the metabolic rate of a poikilothermic organism is temperature dependent (Quartararo et al. 1998, Freeman 2001). In this study, the SGR of 1-y-old abalone was more responsive to increases in water temperatures than 2-y-old abalone. Size-dependent optimal water temperature for growth has also been observed in red abalone (*Haliotis rufescens*), with a symmetrical, concave relationship peaking at an SL of 44 mm at 17.8°C (Steinarsson & Imsland 2003). The optimal water temperature for juvenile *Haliotis discus hannai* (4.7 g) growth was at 20°C (Cho & Kim 2012). An optimal water temperature of 20°C was similarly observed in *Haliotis midae* (Britz et al. 1997). Britz et al. (1997) fed a commercial diet to *H. midae* (1 g; SL, 18 mm) for 84 days and observed a significant increase in growth rate as temperature increased from 12–20°C, with mortality rates between 1% and 4.4%. However, at water temperatures warmer than 20°C, the growth rate was significantly reduced and mortalities increased drastically from 12% at 22°C to 43% at 24°C (Britz et al. 1997). Green et al. (2011) also reported significant reductions in the growth rate of *H. midae* as temperature increased from 18°C to 22°C to 24°C. In contrast, as water temperatures increased from 14–22°C, 1-y-old abalone were observed to have significant increases in SGR and shell growth rate with relatively low mortalities. However, 2-y-old abalone differed in their response, and maximum growth was attained at 18°C and remained constant at 22°C with no apparent increase in mortality. Greenlip abalone have been observed previously to have a critical thermal maxima, defined as the point at which abalone can no longer hold on to the substrate, which is considered equivalent to death, at a water temperature of 27.5°C (Gilroy & Edwards 1998). Abalone in this study clearly had not reached their thermal tolerance level.

The growth parameters of 1-y-old abalone were more responsive to both temperature and protein level than 2-y-old abalone. Although care must be exercised when comparing results from different studies, differences in the growth rate between juvenile and subadult greenlip abalone were similarly observed between other studies by Coote et al. (2000) and Vandepeer (2005). Similarly, Britz and Hecht (1997) observed higher growth rates in *Haliotis midae*, ranging from 1.5%–2.2% body weight/day for 0.2–1-g abalone compared with 0.1%–0.5% body weight/day for 7–14-g abalone. Basal metabolic rate and maintenance requirements are proportional to body weight and are greater in larger animals; as such, SGR has a tendency to be higher in smaller animals. In addition, subsequent histological examination showed that eggs were present in 2-y-old abalone prior to the commencement of the experiment; therefore, abalone might have partitioned some energy for reproductive maturation instead of for somatic growth.

Growth rates alone do not appear to be a sensitive measure of the effects of dietary protein inclusion for abalone, when fed

to excess, in short-term experiments such as this one. No significant effect of protein level on final individual weight gain, SGR, or shell growth rate was observed for either year class. For 1-y-old abalone held at either 18°C or 22°C, there was a general trend for growth parameters to increase as protein level increased, and reached an optimum between 32.2% and 34.7% CP, respectively. In comparison, the growth rates of 2-y-old abalone were lower. As mentioned previously, the animals in the current study were fed to excess. The protein availability initially limits protein accumulation by an animal. If protein is not limiting, protein accumulation is limited by the dietary energy content (Coote et al. 2000). Animals eat to satisfy their energy requirements. Digestible dietary energy (~12.5 MJ/kg) was constant between diets in the current study, whereas the protein-to-energy ratio increased from 14.14–22.80 with increasing protein level. Bautista-Teruel and Millamena (1999) determined, by regression analysis, the optimal dietary protein level for *Haliotis asinina* (0.6 ± 0.03 g) at 27–31°C to be 27% with an estimated metabolizable energy level of 12.98 MJ/kg. In the current study, we observed that abalone fed low-protein diets increased their food consumption rate as an offset to increase protein intake resulting in no significant difference in growth rates between different protein levels in the diet.

Two-y-old animals in our study exhibited slow and highly variable growth rates. Highly variable growth rates have been observed in protein requirement studies in a number of other abalone studies (Britz 1996a, Britz 1996b, Coote et al. 2000). Britz (1996a) and Coote et al. (2000) concluded that variable growth rates reduced statistical power and resulted in an inability to detect significant treatment effects, other than between the most extreme dietary treatments. Britz (1996a) concluded that an increase in the duration of the experiment might have led to significant dietary effects of economic importance. Alternatively, it may be desirable to use a restrictive feeding method to halt compensatory nutrient intake in future nutrient requirement studies for greenlip abalone.

Considering the inability to estimate optimal dietary protein levels by significant differences in growth rates alone, an estimate was attained by investigating the combined responses of SGR, feed intake, FCR, and protein deposition. Significant differences were observed in feed intake, FCR, and protein deposition in relation to increasing dietary protein level for both 1- and 2-y-old abalone. In this study, feed intake was significantly reduced as dietary protein levels increased and, as a result, 1-y-old abalone at 18°C and 22°C had significant improvements in FCR as dietary protein increased. One-y-old abalone at 22°C had significantly higher protein deposition at 34.3% protein, whereas a nonsignificant increase was observed at 18°C as dietary protein increased. The improved FCR of 1-y-old abalone, based on lower feed intakes, coupled with a numerically higher SGR and improved protein deposition at higher dietary protein levels suggest that the optimal CP level at 12.5 MJ/kg digestible energy at 18°C and 22°C is 32.2% (24.4% dietary protein) and 34.7% (26.7% dietary protein), respectively.

Significant reductions in feed intake with increasing dietary protein level were also observed at 18°C and 22°C for 2-y-old abalone. There were also strong negative second-order polynomial relationships between protein level and FCR for 2-y-old abalone at these temperatures (Fig. 3B), indicating that FCR improved at the 34.3% CP level. In contrast to 1-y-old abalone, 2-y-old abalone did not show an improved SGR or protein deposition at higher dietary protein levels. These results suggest that 2-y-old animals should be fed with 34% CP (25.9% dietary protein) at 18°C or 22°C. However, more research is need to determine the optimal level of protein for these 2-y-old abalone, which are larger and have a slower growth rate.

Regarding the results for SGR, FCR, and protein deposition at 14°C, we recommend an optimal level of 29.0% CP (21.9% dietary protein) and 24% CP (17.8% dietary protein) for 1- and 2-y-old abalone, respectively.

The optimal dietary protein levels for greenlip abalone recommended from this study are higher than previously reported by Coote et al. (2000). They are similar to those reported by Taylor (1992) for *Haliotis kamtschatkana* (≥30% CP). However, they are considerably lower than the level of 38% CP recommended by Uki et al. (1986) for *Haliotis discus hannai*, or by Britz (1996a), who recommended a much higher value of 47% CP for *Haliotis midae*. Assuming that abalone in the aforementioned studies were unable to increase their feed intake when fed lower protein diets, Coote et al. (2000) hypothesized that the difference in their study occurred because of suboptimal essential amino acid profiles in the experimental diets. In addition to the hypothesis of Coote et al. (2000), we also hypothesize that the greater differences may have also been a result of the unavailability of nutrients resulting from diets not being formulated on a digestible basis, using reliable apparent digestibility coefficients, and developed specifically for the species tested. The higher protein level recommended by Uki et al. (1986) and Britz (1996a) may have also occurred because of species-specific protein requirements.

Interestingly, and although inconclusive, the results suggest similar or slightly lower optimum protein levels for larger 2-y-old versus 1-y-old greenlip abalone. In contrast, Britz and Hecht (1997), using fishmeal as the major protein source, reported maximum growth of *Haliotis midae* at 18°C occurred in large (age, 20 mo; initial SL, 36 mm; weight, 7.8 ± 0.25 g) abalone fed a crude dietary protein level of 44%, whereas the maximum growth occurred in small (age, 6 mo; initial SL, 11 mm; weight, 0.2 ± 0.01 g) abalone at 34%. Higher protein deposition occurred in larger abalone fed a protein level of 34% or 44%. In contrast, small abalone had higher protein deposition when fed a protein level of 24% or 34% (Britz & Hecht 1997). This may indicate species differences, so further research is needed.

In our study, significantly higher CFs for 1- and 2-y-old abalone were observed as water temperature decreased. Britz et al. (1997) also observed this response in *Haliotis midae* and hypothesized the higher CF occurred as a result of a decrease in energy requirements and a partitioning of energy into glycogen reserves at colder water temperatures. However, the results in our study conflict with this hypothesis, because NFE and fat levels were observed to increase with increasing water temperature, and increasing dietary protein levels, in both age classes. This result indicates species differences in relation to energy use, and suggests further research is required. It is also possible that the reduced CF observed in our study was a result of an increased proportion of new shell being put forward by the faster growing abalone at the warmer water temperatures. Meat and shell growth have been reported to be independent in related mollusc species (Palmer 1981).

In conclusion, there were marked differences between the growth performance, feed use, and nutrient deposition of 1- and 2-y-old greenlip abalone in relation to increasing water temperatures and dietary protein levels. In southeastern Australia, commercial diets for greenlip abalone currently contain CP levels in the range of 25%–30%, and are used during the entire grow-out period of the production cycle. The results from the current study suggest the dietary protein levels for commercial greenlip abalone should be altered to ensure that higher protein diets are used during periods of rapid growth, which may be attained at and above the optimal water temperature of 18°C. Lower protein diets may be used for the larger, slower growing 2-y-old abalone during periods of coldwater temperatures. Further research is needed to clarify the change, over time, for optimum dietary protein levels between 1- and 2-y-old greenlip abalone. In addition, the optimum dietary protein levels for larger 3-y-old+ greenlip abalone also need to be determined. With regard to improved growth at increasing water temperatures, it may be a viable option to heat nursery systems to improve growth and productivity, and, ultimately, to reduce the length of the production cycle for greenlip abalone. Based on the information obtained from the current study, we note that there is great potential to introduce multidiet feeding strategies that provide optimum protein levels for different life stages, and at different seasonal water temperatures, experienced throughout the production cycle of greenlip abalone.

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