Hindawi
Aquaculture Research
Volume 2023, Article ID 1676340, 13 pages
https://doi.org/10.1155/2023/1676340

Research Article

Optimal Dietary Protein Requirement of Subadult Australian Hybrid Abalone (*Haliotis rubra* × *Haliotis laevigata*) at Different Rearing Temperatures

Abdul Lathiff Inamul Hassan,[1] Thomas S. Mock,[1] Kieren Searle,[1] Melissa M. Rocker,[1] Giovanni M. Turchini,[12] and David S. Francis[1]

[1]*Nutrition and Seafood Laboratory, School of Life and Environmental Sciences, Deakin University, Geelong, Victoria, Australia*
[2]*School of Agriculture and Food, The University of Melbourne, Melbourne, Victoria, Australia*

Correspondence should be addressed to David S. Francis; d.francis@deakin.edu.au

Received 6 October 2022; Revised 20 November 2022; Accepted 12 January 2023; Published 4 February 2023

Academic Editor: Femi Fawole

Australian abalone aquaculture is characterised by a prolonged culture period and slow and variable growth, and abalone is cultured in fluctuating water temperatures ranging between 10 and 25°C with distinct seasons. Temperature is a crucial environmental factor influencing abalone's physiology and energetics, leading to a change in nutritional requirements. However, feeds are generally formulated to match the nutritional requirements at their optimal temperature. Hence, there is a need to optimise dietary protein levels to match temperature-specific requirements during extreme conditions (winter and summer). Given this, a growth trial evaluating five experimental feeds consisting of graded protein inclusion levels (320, 350, 380, 410, and 440 g·kg^{-1}) was conducted on subadult hybrid abalone (*Haliotis rubra* × *H. laevigata*) at three different temperatures reflecting winter (12°C), summer (22°C), and the annual average water temperature (17°C) for 143 days. At lower water temperature (12°C), there was a marginal improvement in growth performance as dietary protein levels increased from 320 to 440 g·kg^{-1}. However, at higher water temperatures (when the culture water temperature is above 17°C), there was a significant improvement in growth performance as dietary protein levels increased from 320 to 440 g·kg^{-1} as evidenced by an improved weight gain, specific growth rate, and feed conversion ratio. Furthermore, increasing dietary protein levels did not compromise the nutritional quality of the abalone tissue at all three tested temperatures. Therefore, during periods of higher water temperatures, feeding Australian hybrid abalone with a relatively high dietary protein level (410 g·kg^{-1}) is expected to result in improved growth, shorter culture duration, and profit maximisation.

1. Introduction

Globally, there are around 100 abalone species documented as belonging to the family Haliotidae [1]. Of these species, several are renowned as seafood delicacies, commanding a high market price for live, frozen, and processed/canned products. However, a declining wild abalone catch is a major industry concern [1–3]. Catches declined from 15,000 to 5,000 mt between 1970 and 2016 due to overfishing, disease, poaching, ocean acidification, increased predation, and habitat degradation [1–4]. Notably, during a similar timeframe, abalone aquaculture production has grown from its infancy to reach 153,500 mt in 2020 [4]. As such, the abalone aquaculture sector represents the only viable means of catering to growing global demand.

Although small on a global scale, Australia's farmed abalone production (~1400 tonnes, in 2021) is projected to undergo a three-fold increase over the next decade, with the vast majority of products being exported internationally [5, 6]. In Australia, three major species drive this production: blacklip (*Haliotis rubra*), greenlip (*Haliotis laevigata*), and their hybrid (*Haliotis rubra* × *Haliotis laevigata*) [7]. These species are typically cultured in flow-through aquaculture systems where culture practices are well established.

Australian abalone aquaculture is characterised by a prolonged culture period, slow and variable growth [8], and high summer mortality [9, 10]; all of these threaten the economic potential of the industry [11]. Artificially controlling water temperature in flow-through systems is considered economically unviable, resulting in significant seasonal fluctuations throughout the typical three-year culture period, ranging from 10°C in the winter to 25°C in the summer in popular growing locations [12, 13]. These seasonal extremes are substantially disparate from the reported thermal optima of 17.0 and 18.3°C, for blacklip and greenlip abalone, respectively [14]. As temperature control is not a viable option, alternative solutions are required to optimise the health and growth of cultured abalone and ultimately ensure the continued expansion of the abalone aquaculture industry.

The grow-out stage of abalone culture in Australia is reliant on formulated feeds to satisfy nutritional requirements. Thus, nutritional manipulation may offer a solution to, or at least mitigate, the negative effects on the growth and health status of abalone imposed by suboptimal growing conditions. As with all cultured species, feed is a major operational expense in abalone aquaculture, accounting for approximately 30% of the total operating cost [15]. Therefore, minor improvements in abalone feed would likely bring significant economic benefits to abalone farmers. In fact, the optimisation of formulated feeds has played a major role in ensuring the economically and environmentally sustainable growth of numerous commercially important aquaculture industries. As an example, formulated feeds tailored for both life stages and seasons are now commonplace in salmonid aquaculture; however, the same degree of nutritional progress has not yet been achieved for many shellfish species, including abalone [12, 16]. Nevertheless, a series of in-depth nutritional studies have demonstrated the potential to reduce feed costs and improve the growth and health condition of both Australian greenlip and blacklip abalone [9, 10, 12, 13, 17–20].

Notably, several on-farm observations have revealed that Australian hybrid abalone exhibited superior growth, meat yield, and feed utilisation compared to greenlip abalone [21–24]. Furthermore, these observations have been consistent across a range of diet types and water temperatures [25]. Yet, further improvements in growth performance for Australian hybrid abalone are achievable. In a preliminary farm growth trial, Australian hybrid abalone fed a high protein feed (39.8% crude protein) in comparison to a standard protein feed (32.6% crude protein) demonstrated better growth performance and economic returns [13]. Such observations have urged the industry to optimise the dietary protein levels specific to Australian hybrid abalone with a view of obtaining similar species-specific improvements recorded recently with Australian greenlip abalone [9, 12, 13].

As poikilothermic aquatic animals, the temperature is a key environmental factor for abalone, greatly influencing physiological functions related to feed intake, metabolism, and growth [26, 27]. Improved abalone growth performance and survival in response to increasing temperature has been established previously in different abalone species [12, 16, 28]. However, when temperature increases above its optimal, there is a simultaneous reduction in feed intake and metabolic rate. As such, this positive growth occurs only within an optimal temperature range, which is species- [29] and [30, 31] size-specific. As the physiology and energetics of abalone change with temperature, it is reasonable to suggest that their nutritional requirements may change as well [32]. As such, there is a need to optimise dietary protein levels to match temperature-specific requirements.

The objective of the current study was to establish the optimal protein requirements of Australian farmed hybrid abalone with respect to rearing water temperature (season). The outcomes of this study will significantly contribute towards the development of season-specific feeds for hybrid abalone and, therefore, facilitate the projected growth of the Australian abalone aquaculture industry.

2. Materials and Methods

The present article presents the results of a trial that formed a part of a larger investigation of the nutritional requirements of farmed hybrid abalone; as such, some of the information presented herein also appears in a technical report prepared for the Fisheries Research and Development Corporation (FRDC) [33].

2.1. Experimental System and Animals. The feeding trial was conducted using a photoperiod-controlled flow-through seawater system at Deakin University, Queenscliff Marine Science Centre, Queenscliff, Victoria, over a 143-day period. The photoperiod was held at 12 hours of complete darkness and 12 hours of low-intensity light to mimic Australian commercial abalone farming conditions where, during daylight hours, the infiltration of external light into the experimental system was kept to a minimum. The experimental system consisted of three identical tables, each holding 15 tanks. The water temperature in the tanks for each of the three tables was set to 12, 17, and 22°C, representing the winter, average annual, and summer water temperatures, respectively. Water temperature was controlled by heatpump units (Aquahort Ltd., Auckland, New Zealand). The individual tanks consisted of 12.5 L blue plastic rectangular tanks, (dimensions of $39.2 \times 28.8 \times 11.0$ cm). Within each temperature, five experimental diets (P32, P35, P38, P41, and P44) were examined in triplicate. Each tank was supplied with UV-treated, filtered (5 and 1 μm cartridge), and temperature-controlled seawater at a flow rate of 500 mL·min^{-1}. Water depth was maintained at 8.5 cm to give a practical water volume of 9.6 L, and the water was aerated using air stones to maintain dissolved oxygen levels near saturation. A hide consisting of three ceramic tiles (26.2×8.6 cm) attached to PVC celuka board strips was placed in each tank to increase the available surface area for attachment. Additionally, a 2 cm strip of synthetic grass was fastened around the inner perimeter of the tank, on the high-water level, to prevent abalone from escaping.

Twenty-month-old Australian hybrid abalone were obtained from Jade Tiger Abalone (Craig Mostyn Group, Intended Head, Victoria) in September 2018. Abalone were lightly sedated (Aqui-S, isoeugenol 40 mg·L^{-1}) to minimise stress, and transported to the Deakin Queenscliff Marine Science Centre. Initially, the abalone were acclimated to the experimental system at a water temperature reflective of source location. Following acclimation, twenty abalone were individually weighed, measured, and assigned to each of the tanks and randomly allocated to one of five experimental treatments in triplicate. The initial weight and shell length of the abalone were not significantly different across different treatments. The average initial shell length and weight were 43.57 ± 0.05 mm and 12.51 ± 0.01 g, respectively. Water temperatures were held within ±1°C of the nominated temperature throughout the growth trial. The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. As invertebrates were used for this experiment, no ethical approval was required. Nevertheless, all possible steps towards minimizing animal suffering were taken.

2.2. Experimental Diets, Feeding, and Faeces Collection. Five isoenergetic experimental diets (17-18 MJ·kg^{-1}) were formulated to contain graded dietary protein levels of 320 (P32), 350 (P35), 380 (P38), 410 (P41), and 440 (P44) g·kg^{-1} by increasing the levels of principal protein sources, namely, rice and pea protein isolates, and decreasing the levels of pregelatinised starch (Table 1). All other dietary ingredients remained identical and were maintained at similar inclusion levels across the experimental diets. Fish oil and canola oil were used as the principal lipid sources, and the dietary lipid level was formulated at 3-4%, consistent with commercial dietary formulations. The amino acid composition of the experimental diets was balanced to match the soft tissue composition of the parent species (*Haliotis laevigata* and *Haliotis rubra*) due to the lack of amino acid composition data on Australian hybrid abalone. Detailed ingredient and proximate composition of the experimental diets is provided in Tables 1 and 2. All dietary ingredients were analysed for proximate composition prior to diet formulation (data not shown).

Experimental diets were cold extruded into flat pellets (diameter~4 mm) using a commercial benchtop pasta extruder, then dried at 35°C for 48 hours in a purpose built drying room with air extraction. Prior to feeding, dry matter leaching was quantified to evaluate diet water stability at 12, 17, and 22°C, respectively, as described in Stone et al. [12]. Abalone were fed their respective diets to satiation daily between 1600 and 1700 hours to ensure growth was not limited by diet availability. Tanks were cleaned daily between 0800 and 1000 hrs by siphoning out uneaten feed pellets and faeces. Feed consumption was quantified by subtracting uneaten feed from feed fed by counting the number of uneaten pellets in each tank and multiplying by the average weight of a feed pellet on an as-fed basis.

All experimental diets contained 0.1% of titanium dioxide (TiO_2) as an inert marker for subsequent digestibility analysis. Faeces were collected once daily at 1400 hrs using a pipette, freeze-dried, and frozen at −20°C until subsequent analysis.

2.3. Water Quality Management. Water temperature and dissolved oxygen were measured daily using a handheld dissolved oxygen meter (OxyGuard® Handy Polaris 2 Dissolved Oxygen Meter). Salinity and pH were measured weekly using a refractometer (Atago® S/Mill hand refractometer) and pH meter (Apera Instruments® PH20 pH tester), respectively. Flow rates were checked weekly and held at 500 mL·min^{-1} throughout the growth trial. The cartridge filters (5 and 1 μm) were backwashed weekly to ensure adequate water flow.

2.4. Growth Performance and Nutrient Digestibility. Abalone and feed weight measurements were recorded on a wet basis, and shell lengths were measured across the longest axis. Growth performance indices, including specific growth rate (SGR), shell growth rate, biomass gain, feed conversion ratio (FCR), protein efficiency ratio (PER), energy efficiency ratio (EER), protein deposition, energy deposition, soft body to shell ratio, and condition factor, were calculated as described in detail by Britz et al. [28] and Bansemer et al. [16].

Apparent digestibility coefficients (ADC%) for dry matter, protein, lipid, nitrogen-free extract (NFE), and energy were estimated following equations described in detail by Lewis et al. [34] and Cho et al. [35] using titanium oxide (TiO_2) as the internal marker, where

$$\text{ADC diet}(\%) = 100\,x\left[1-\left(\frac{\text{Nutrient in faeces}(\%)}{\text{Nutrient in diet}(\%)}\,x\,\frac{\text{Mmarker in diet}(\%)}{\text{Mmarker in faeces}(\%)}\right)\right]. \quad (1)$$

2.5. Biochemical Analyses. At the beginning of the experiment, 30 abalone were sampled and stored immediately at −20°C until subsequent analysis. Similarly, at the end of the trial, seven abalone per tank were collected and directly stored at −20°C until subsequent analysis. Moisture, ash, crude protein, and crude lipid contents were determined using oven drying at 80°C to a constant weight, incinerating in a muffle furnace at 550°C, automated Kjeltech 2300 (nitrogen × 6.25) and dichloromethane: methanol (2 : 1) cold extraction of Folch et al. [36]; respectively as reported in detail in Mock et al. [37] Nitrogen-free extract (NFE) was calculated by subtracting crude protein, crude lipid, and ash

from 100%. The amino acid composition was determined using reverse-phase high-performance liquid chromatography (1260 Agilent infinity II series systems, Agilent Technologies, Santa Clara, USA). Samples were initially subjected to acid hydrolysis using 6 M HCl for 22 hours, followed by derivatisation with o-phthaldialdehyde (OPA) and fluorenylmethyloxycarbonyl chloride (FMOC), as described in detail by Lewis et al. [34].

The TiO_2 content of the experimental diets and faeces was analysed using wet-ash digestion followed by colourimetric estimation as described in detail by Myers et al. [38]. Briefly, 0.5 g of faeces sample was digested at 420°C for 2 hours along with 13 mL of concentrated H_2SO_4 and a reaction catalyst containing 3.5 g of K_2SO_4 and 0.4 g of $CuSO_4$. Upon digestion, samples were allowed to cool for 30 minutes, and 10 mL of 30% H_2O_2 was added. Then the total liquid weight was brought up to 100 g by adding distilled water, and the sample was vacuum filtered through Whatman No. 541 to remove any particles. Finally, the absorbance was measured at 410 nm using a microplate reader (Varioskan LUX Multimode Microplate Reader, Thermofisher Scientific). A standard curve was developed using working standards ranging from 0, 2, 4, 6, 8, and 10 mg of TiO_2. In addition, the absorbance of TiO_2-free faeces, was analysed for background correction to nullify the organic matter interference.

2.6. Statistical Analyses. All the data, except ingredient and proximate composition of experimental feeds, were reported as mean ± standard error and replicate data were pooled for each treatment ($n = 3$). Upon confirmation of homogeneity of variance and normality using Levene's test and the Shapiro–Wilk test, respectively, the data were subjected to a two-way ANOVA. Where there was a significant interaction between the two independent factors (dietary protein level, $n = 5$, and water temperature, $n = 3$), one-way ANOVA with Tukey's post hoc test of multiple comparisons was performed for the response variable across all treatment groups ($n = 15$). Where no significant interaction was recorded, a one-way ANOVA and Tukey's post hoc test of multiple comparisons were performed between the dietary protein levels within each experimental temperature separately. Regression analyses (second-order polynomial regression) were performed separately at each temperature against the dietary protein level for key performance parameters. Significance was considered at $P < 0.05$ for all the statistical tests. Statistical analyses were performed using R (Version 3.6.3, R Core Team 2020).

3. Results

3.1. General Observations. Proximate composition analysis confirmed that all the experimental feeds were in line with their respective formulations (Table 2). The dietary protein level of the experimental feeds increased in a sequential manner, ranging from 303.8 to 410.7 g·kg^{-1} diet (wet weight basis). Similarly, the concentrations of individual amino acids increased with increasing dietary protein levels. Total lipid and energy were constant across the feeds (~35 g·kg^{-1} and ~17.8 MJ·kg^{-1}, respectively). Water temperature and dissolved oxygen stayed within ±1°C of the nominated temperature throughout the duration of the experiment, and average values are reported in Table 3. With the progression of the experiment, abalone shells were increasingly colonised by a calcareous tube worm, identified as belonging to the family *Spirorbidae*; however, there were no signs of unhealthy or impaired feeding behaviour. Notably, the shells of abalone subjected to 22°C appeared to have a higher coverage of tubeworm compared to those at 17°C and 12°C.

3.2. Abalone Growth Performance. All experimental feeds were readily accepted by abalone at all three tested temperatures (Table 4 and Figure 1). In general, growth performance parameters improved in a stepwise manner with increasing temperature, and there was a general tendency for improvement with increasing dietary protein level (Table 4). There was a significant temperature-protein interaction for final weight, weight gain, and feed consumption. Both final weight and feed consumption were higher in all treatment groups at 22°C compared to those at both 12 and 17°C and tended to be higher with increasing dietary protein level; however, feed consumption was numerically higher at the mid-range of the tested protein levels (P38). Furthermore, at 22°C abalone more than doubled their initial weight, with weight gain ranging between 110.4 and 146.4% in P32 and P44 (Table 4), respectively.

As mentioned, for the growth performance parameters where there was no significant interaction between dietary protein level and water temperature, a one-way ANOVA and Tukey's post hoc analysis were performed to determine the effect of dietary protein level separately at each temperature. A significant effect of dietary protein level on FCR was recorded at each experimental temperature, where FCR lowered (improved) with an increasing dietary protein level. At 17°C, FCR ranged from 1.93 to 1.54 between P32 and P44, respectively, and at 22°C FCR ranged between 1.51 and 1.14 in P32 and P38, respectively. Both temperature and dietary protein level significantly affected SGR, yet significant differences between dietary protein levels were only recorded at 22°C, where SGR ranged from 0.53 to 0.65 in P32 and P44, respectively.

Trends of improving growth performance at higher water temperatures and increased dietary protein levels were demonstrated by regression analysis (Figure 1), where a clear improvement in both weight gain % and SGR are seen at 22°C compared to both 17 and 12°C. Differences between dietary protein levels are most pronounced at 22°C, where a clear improvement in both of these growth performance parameters can be observed with increasing dietary protein levels (where x = dietary protein level, weight gain $\% = -88.1 + 8.66x - 0.0763x^2$, $R^2 = 0.95$, and $\text{SGR} = 0.261 + 0.0362x - 0.000357x^2$, $R^2 = 0.94$). Over the entire experimental duration, there were five dead abalone in a single tank at 22°C on the same

Table 1: Ingredient composition of the experimental diets fed to Australian hybrid abalone at three water temperatures.

	Experimental diets*				
	P32	P35	P38	P41	P44
Ingredient composition ($g \cdot kg^{-1}$)					
Rice protein isolate	107.8	125.8	143.5	161.1	179.2
Pea protein isolate	107.8	125.8	143.5	161.1	179.2
Pregelatinised starch	531.2	497.3	462.9	429.4	394.7
Diatomaceous earth	21.0	19.0	18.0	16.5	15.0
Fish meal	50.0	50.0	50.0	50.0	50.0
Gluten	50.0	50.0	50.0	50.0	50.0
Gelatin	50.0	50.0	50.0	50.0	50.0
Fish oil	15.0	15.0	15.0	15.0	15.0
Lecithin	10.0	10.0	10.0	10.0	10.0
Canola oil	2.1	2.1	2.0	2.0	2.0
Celite[1]	4.0	4.0	4.0	4.0	4.0
Titanium dioxide	1.0	1.0	1.0	1.0	1.0
Vitamin and mineral mix	7.0	7.0	7.0	7.0	7.0
Vitamin C	0.5	0.5	0.5	0.5	0.5
Choline	5.0	5.0	5.0	5.0	5.0
Vitamin E	1.0	1.0	1.0	1.0	1.0
Monosodium phosphate	7.5	7.5	7.5	7.5	7.5
Calcium sulphate	5.0	5.0	5.0	5.0	5.0
Agar	5.0	5.0	5.0	5.0	5.0
Sodium alginate	5.0	5.0	5.0	5.0	5.0
Methionine	5.0	5.0	5.0	5.0	5.0
Lysine	5.0	5.0	5.0	5.0	5.0
Arginine	2.0	2.0	2.0	2.0	2.0
Threonine	2.0	2.0	2.0	2.0	2.0

*Experimental diets: P32 = 320 $g \cdot kg^{-1}$ protein, P35 = 350 $g \cdot kg^{-1}$ protein, P38 = 380 $g \cdot kg^{-1}$ protein, P41 = 410 $g \cdot kg^{-1}$ protein, and P44 = 440 $g \cdot kg^{-1}$ protein.

day due to a blockage in the incoming water to that tank, which occurred late in the growth trial and did not negatively impact feed intake on a per animal basis.

3.3. Nutrient Retention Efficiency. Significant interactions between dietary protein level and temperature were recorded for PER, PD, EER, and ED, which, in general, were higher at 22°C, indicating both dietary protein and dietary energy were more efficiently deposited in abalone reared at higher water temperatures (Table 5). Both ED and EER showed a general trend toward increased values as dietary protein levels increased, and this was most evident at the higher water temperatures. At 22°C, where differences were most obvious, PER, PD, and EER were all numerically higher at P38 compared to the other dietary protein levels.

3.4. Nutrient Digestibility. Both dietary protein level and temperature have a significant effect on the apparent digestibility coefficients (ADC%) of macronutrients in the present experiment, with the exception of NFE, which was unaffected by temperature, and, interestingly, protein digestibility was not significantly affected by dietary protein level at any of the three experimental water temperatures. In general, the digestibility of dry matter, protein, lipid, and energy were higher at 12°C and appeared to decrease with increasing water temperature. At 12°C, dietary protein level

Table 2: Proximate and amino acid composition ($mg \cdot g^{-1}$ diet as fed) of the experimental diets fed to Australian hybrid abalone at three water temperatures.

	Experimental diets[1]				
	P32	P35	P38	P41	P44
Proximate composition					
Moisture	85.8	86.8	84.4	82.2	84.1
Protein	303.8	336.6	354.4	385.0	410.7
Lipid	30.1	36.3	31.5	37.6	37.6
Ash	55.3	50.4	55.7	54.5	52.8
NFE[2]	525.0	489.9	474.0	440.8	415.0
Energy ($MJ \cdot kg^{-1}$)[3]	17.4	17.8	17.8	18.2	18.3
Amino acids					
Histidine	6.1	6.7	7.3	7.8	8.6
Serine	13.7	14.9	16.3	17.3	18.6
Arginine	22.5	24.8	26.4	28.7	31.2
Glycine	22.9	23.4	24.6	25.8	26.6
Aspartic acid	24.4	26.6	29.0	31.2	34.1
Glutamic acid	54.3	57.6	62.0	65.8	70.4
Threonine	11.2	12.5	13.6	13.7	15.3
Alanine	16.0	17.0	18.2	19.3	20.6
Proline	21.3	22.0	23.3	24.4	25.3
Lysine	18.1	19.3	20.5	21.6	23.3
Tyrosine	6.5	8.3	8.4	9.8	11.4
Methionine	9.0	10.4	8.9	10.1	12.7
Valine	14.6	16.0	17.3	18.7	20.6
Isoleucine	12.0	13.2	14.3	15.4	17.0
Leucine	22.3	24.5	26.5	28.3	30.9
Phenylalanine	14.1	15.5	16.8	18.1	19.7
Total	288.9	312.6	333.2	355.7	386.2

[1]See Table 1 for detailed experimental feed information. [2]Nitrogen-free extract (NFE) = (100 − (crude protein + crude lipid + ash)). [3]Energy was calculated using the values of 17.2, 23.6, and 39.5 $MJ \cdot kg^{-1}$ for NFE, protein, and lipid, respectively. Information presented in the table herein also appears in the technical report by [33].

Table 3: Water temperature and dissolved oxygen (average values of daily measurements) recorded throughout the 12, 17, and 22°C abalone growth experiments.

	12°C	17°C	22°C
Temperature (°C)	12.2	16.9	21.9
Dissolved oxygen (mg L^{-1})	8.6	7.6	6.7
Dissolved oxygen (% saturation)	99.9	96.5	96.1

significantly affected dry matter and lipid digestibility, which were both lower in P38 (70.2 and 69.3%, respectively). Similarly, at 17°C, lipid digestibility was also lowest in P38 (65.3%). Similar trends were also apparent at 22°C, where dry matter, lipid, and energy digestibility were lowest in abalone fed P38.

3.5. Abalone Soft Tissue Proximate and Amino Acid Composition. There was no significant effect of dietary protein level on any of the tissue nutrient levels analysed in any of the three experimental temperatures, except tissue moisture levels, where there was a significant interaction of dietary protein level and temperature (Table 5). Temperature had a significant effect on tissue levels of protein, lipid, and

Table 4: Growth performance of Australian hybrid abalone fed five experimental diets with increasing protein concentrations at three water temperatures.

	12°C					17°C					22°C					Temperature (A)	Protein level (B)	A × B interaction
	P32	P35	P38	P41	P44	P32	P35	P38	P41	P44	P32	P35	P38	P41	P44			
Initial weight (g)	12.47 ± 0.02	12.52 ± 0.02	12.55 ± 0.00	12.57 ± 0.04	12.52 ± 0.03	12.48 ± 0.03	12.52 ± 0.04	12.50 ± 0.00	12.52 ± 0.03	12.50 ± 0.03	12.47 ± 0.02	12.50 ± 0.03	12.55 ± 0.08	12.53 ± 0.02	12.50 ± 0.05	ns	ns	ns
Initial length (mm)	43.4 ± 0.40	43.1 ± 0.31	43.2 ± 0.34	43.5 ± 0.34	43.5 ± 0.33	43.5 ± 0.35	43.7 ± 0.35	43.9 ± 0.35	43.7 ± 0.35	43.3 ± 0.36	43.6 ± 0.32	44.0 ± 0.35	43.8 ± 0.36	43.4 ± 0.31	43.3 ± 0.36	ns	ns	ns
Final weight (g)	16.79 ± 0.33^{f}	17.38 ± 0.14^{ef}	17.45 ± 0.47^{ef}	18.13 ± 0.26^{ef}	17.73 ± 0.35^{ef}	19.72 ± 0.23^{de}	21.23 ± 0.38^{d}	20.88 ± 0.64^{d}	20.97 ± 0.7^{d}	21.72 ± 0.49^{d}	26.29 ± 0.09^{c}	27.67 ± 0.91^{bc}	29.31 ± 0.05^{ab}	29.24 ± 0.76^{ab}	30.88 ± 0.71^{a}			*
SGR[1]	0.21 ± 0.01	0.23 ± 0.01	0.24 ± 0.01	0.26 ± 0.02	0.24 ± 0.02	0.33 ± 0.01	0.38 ± 0.01	0.36 ± 0.02	0.36 ± 0.02	0.39 ± 0.02	0.53 ± 0.02^{a}	0.57 ± 0.01^{ab}	0.61 ± 0.01^{bc}	0.60 ± 0.01^{bc}	0.65 ± 0.01^{c}	***	***	ns
Survival (%)	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	91.7 ± 8.3	100 ± 0	100 ± 0	100 ± 0	100 ± 0	ns	ns	ns
Weight gain (g)	34.3 ± 2.67^{f}	39.07 ± 1.1^{ef}	39.59 ± 3.75^{ef}	45.04 ± 2.09^{ef}	41.85 ± 2.82^{ef}	57.79 ± 1.82^{de}	69.8 ± 3.04^{d}	67.03 ± 5.15^{d}	67.72 ± 5.5^{d}	73.79 ± 3.91^{d}	110.34 ± 0.72^{c}	121.37 ± 7.27^{bc}	134.49 ± 0.42^{ab}	133.93 ± 6.11^{ab}	147 ± 5.72^{a}			*
Weight gain (%)	30.69 ± 1.71^{f}	36.24 ± 0.97^{ef}	36.31 ± 3.45^{ef}	41.7 ± 3.9^{ef}	37.23 ± 0.36^{ef}	56.94 ± 1.06^{de}	69.59 ± 3.51^{d}	66.73 ± 5.38^{d}	67.47 ± 5.15^{d}	73.77 ± 3.58^{d}	106.77 ± 4.54^{c}	121.34 ± 6.82^{bc}	133.58 ± 1.73^{ab}	133.32 ± 6.28^{ab}	147.05 ± 6.68^{a}			*
Condition factor (K)	0.78 ± 0.01	0.78 ± 0.01	0.80 ± 0.02	0.79 ± 0.01	0.77 ± 0.01	0.78 ± 0.01	0.79 ± 0.01	0.80 ± 0.01	0.78 ± 0.01	0.79 ± 0.01	0.81 ± 0.01	0.79 ± 0.01	0.79 ± 0.01	0.82 ± 0.01	0.82 ± 0.01	*		ns
Shell growth rate (μm·day^{-1})	44.0 ± 3.2	51.7 ± 2.6	50.6 ± 2.9	52.2 ± 2.8	51.3 ± 3.1	58.4 ± 2.7^{a}	68.0 ± 3.1^{ab}	56.6 ± 3.5^{a}	57.9 ± 3.5^{a}	70.6 ± 3.5^{b}	99.8 ± 3.5^{a}	108.0 ± 3.1^{ab}	119.0 ± 3.3^{bc}	114.3 ± 3.5^{bc}	121.0 ± 3.7^{c}	***	***	ns
Final length (mm)	49.4 ± 0.43	50.1 ± 0.36	50.0 ± 0.45	50.6 ± 0.39	50.5 ± 0.43	51.6 ± 0.38^{a}	52.8 ± 0.42^{ab}	51.0 ± 0.51^{ab}	51.6 ± 0.50^{ab}	52.9 ± 0.51^{b}	57.3 ± 0.48^{a}	58.8 ± 0.42^{ab}	60.0 ± 0.46^{bc}	59.1 ± 0.48^{bc}	60.2 ± 0.49^{c}	***	***	ns
Feed consumption (g fish^{-1})	7.38 ± 0.08^{f}	9.16 ± 0.39^{e}	9.31 ± 0.18^{e}	7.12 ± 0.17^{f}	7.98 ± 0.04^{ef}	13.36 ± 0.21^{d}	15.31 ± 0.08^{bc}	16.6 ± 0.87^{b}	12.99 ± 0.21^{d}	13.7 ± 0.14^{cd}	26.59 ± 0.53^{a}	26.99 ± 0.19^{a}	27.22 ± 0.28^{a}	25.83 ± 0.24^{a}	26.3 ± 0.25^{a}			*
FCR[2]	1.73 ± 0.14^{ab}	1.89 ± 0.12^{a}	1.93 ± 0.16^{a}	1.28 ± 0.04^{b}	1.54 ± 0.09^{ab}	1.85 ± 0.07^{ab}	1.77 ± 0.08^{ab}	1.99 ± 0.08^{a}	1.55 ± 0.1^{b}	1.49 ± 0.06^{b}	1.92 ± 0.05^{a}	1.79 ± 0.12^{ab}	1.62 ± 0.02^{abc}	1.55 ± 0.06^{bc}	1.44 ± 0.06^{c}		***	

[1]SGR: specific growth rate (on weight) and [2]FCR: feed conversion ratio. Data are expressed as mean ± SEM values in the same row with different superscripts that are significantly different (two-way ANOVA and Tukey's post hoc analysis, using temperature (A) and dietary protein level (B) as factors). Where there was no significant interaction between A and B, one-way ANOVA and Tukey's post hoc analysis were performed comparing the dietary protein level within temperature, ns = nonsignificant. *, **, and *** indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

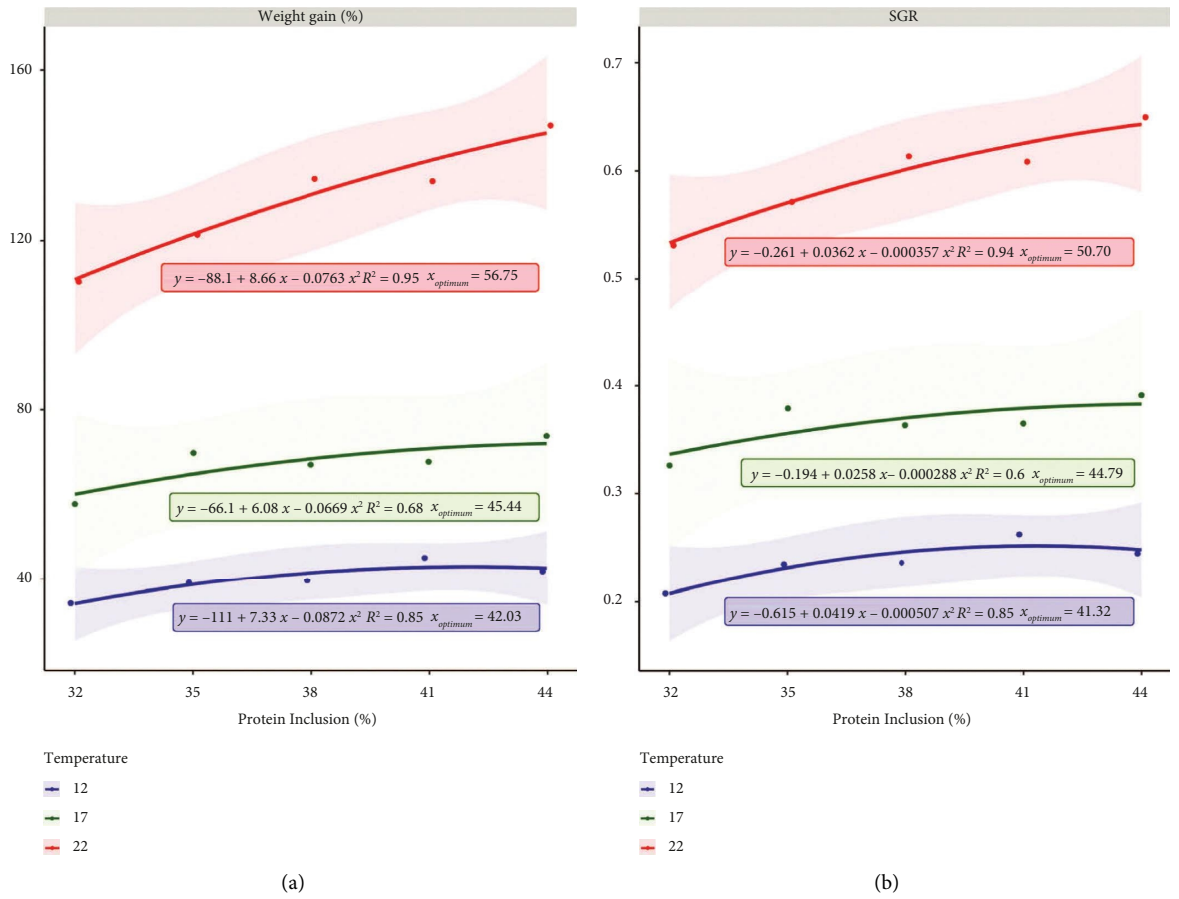

FIGURE 1: Second-order polynomial regression of weight gain percentage and SGR of Australian hybrid abalone fed five experimental diets with increasing protein concentrations at three water temperatures. Values at each dietary protein level for each temperature represent treatment means ($n = 3$) with confidence intervals (shaded area).

NFE, where tissue protein concentration appeared to decrease with increasing water temperature. In general, the individual amino acids glutamic acid, glycine, and arginine were present in high concentrations in abalone tissue irrespective of experimental temperature or dietary protein level (Table 6). Histidine, tyrosine, and methionine were the least abundant amino acids. The only significant difference in tissue concentration of amino acids was recorded at 12°C, where the concentration of glycine in abalone in the P44 treatment was higher than that in the P32 treatment (58.7 versus 51.9 $mg{\cdot}g^{-1}$ dry tissue, respectively).

4. Discussion

The primary objective of the present study was to identify the optimal dietary protein inclusion level for Australian hybrid abalone with respect to rearing temperature. Identifying the season-specific nutritional requirements of Australian hybrid abalone has the potential to enhance growth performance and, in turn, reduce the culture duration and maximise the production efficiency of the industry. High growth rates, survival rates, and positive feeding responses demonstrated that the animals used in the present study were healthy and uncompromised. Furthermore, hybrid abalone growth rates in the current trial were comparable to those observed on-farm (Jade Tiger Abalone™, pers. comm.) and similar aged greenlip abalone [12]. Furthermore, a systematic review of SGR in abalone indicates that the SGR obtained in the present experiment was comparable to or exceeded that observed in previously published works [39].

The physiological functions of abalone, a poikilothermic aquatic animal, relative to feeding, metabolism, and growth are heavily influenced by environmental factors, especially water temperature [12, 16, 26, 28]. In the present experiment, numerous growth performance parameters, including SGR, final length, and shell growth rate, were substantially higher at 22°C compared to 17°C, whereas there was a comparatively smaller difference between 12°C and 17°C. These temperature-related growth patterns are consistent with those observed in other abalone species [12, 28, 32, 40]. Therefore, the improved abalone growth performance with increasing culture temperature may be attributed to related effects of an increased feed consumption and a higher metabolic rate.

Growth performance differences between dietary protein levels were more pronounced at higher temperatures,

Table 5: Nutrient retention efficiency, digestibility, and tissue proximate composition of Australian hybrid abalone fed five experimental diets with increasing protein concentrations at three water temperatures.

	12°C					17°C					22°C					Temperature (A)	Protein level (B)	A × B interaction
	P32	P35	P38	P41	P44	P32	P35	P38	P41	P44	P32	P35	P38	P41	P44			
Nutrient efficiency																		
PER	1.6 ± 0.1^{de}	1.4 ± 0.1^{e}	1.3 ± 0.1^{e}	1.8 ± 0.1^{bcde}	1.4 ± 0^{e}	1.7 ± 0.1^{cde}	1.7 ± 0.1^{cde}	1.5 ± 0.1^{e}	1.6 ± 0.1^{de}	1.6 ± 0.1^{e}	2.2 ± 0.1^{ab}	2.3 ± 0.1^{a}	2.5 ± 0^{a}	2.1 ± 0.1^{abcd}	2.1 ± 0.1^{abc}			**
PD (%)	35.4 ± 3.3^{abc}	24.5 ± 2.3^{bcde}	23.8 ± 1.4^{cde}	24.5 ± 3.9^{bcde}	16.8 ± 4.1^{e}	17.1 ± 2.8^{e}	25 ± 2.1^{abcde}	22.3 ± 0.2^{de}	24.6 ± 1.7^{bcde}	22.9 ± 1^{de}	32.9 ± 2.7^{abcd}	35.9 ± 2.1^{abc}	37.4 ± 0.3^{a}	36.6 ± 1.3^{ab}	35.7 ± 2.3^{abc}			**
EER ($g \cdot MJ^{-1}$)	28.7 ± 1.8^{fg}	26.7 ± 1.1^{g}	26.5 ± 2^{g}	37.9 ± 2.9^{cdef}	30.5 ± 0.3^{efg}	29.9 ± 1^{efg}	31.8 ± 1.4^{efg}	30.4 ± 2^{efg}	34.5 ± 2.1^{defg}	35.7 ± 1.5^{cdefg}	38.2 ± 1.5^{bcde}	43.1 ± 2.7^{abcd}	49.5 ± 0.6^{a}	44.1 ± 1.7^{abc}	47.3 ± 1.9^{ab}			**
ED (%)	15.1 ± 2.2^{bcd}	13.3 ± 1.5^{bcde}	13.4 ± 0.5^{bcde}	13.8 ± 2.3^{bcde}	9 ± 3.6^{de}	7.1 ± 1.6^{e}	12.6 ± 1.2^{cde}	12.9 ± 0.8^{cde}	14.4 ± 1.2^{bcde}	14 ± 0.8^{bcde}	17.1 ± 0.9^{abc}	20 ± 0.9^{abc}	21.3 ± 0.2^{ab}	23.3 ± 0.7^{a}	24 ± 1.1^{a}			**
ADC%																		
Dry matter	73.3 ± 0.2^{c}	73.6 ± 0.2^{c}	70.2 ± 0.3^{a}	71.4 ± 0.6^{ab}	72.9 ± 0.5^{bc}	72.1 ± 1.3	72.3 ± 0.2	69.5 ± 0.2	70.3 ± 0.5	71.8 ± 1.6	71.6 ± 0.2^{c}	71.6 ± 0.3^{c}	68.3 ± 0.4^{a}	69.5 ± 0.3^{ab}	70.9 ± 0.2^{bc}	***	***	ns
Protein	80.2 ± 0.4	81.9 ± 0.3	79.7 ± 0.6	79.7 ± 0.9	80.2 ± 0.9	78.0 ± 0.6	79.3 ± 0.3	78.8 ± 2.1	77.8 ± 0.6	78.1 ± 1.2	76.5 ± 0.5	77.0 ± 0.3	75.2 ± 0.7	75.5 ± 0.8	75.7 ± 0.4	***	ns	ns
Lipid	72.5 ± 0.7^{b}	77.3 ± 0.3^{c}	69.3 ± 0.3^{a}	77.6 ± 0.2^{c}	76.2 ± 0.6^{c}	66.5 ± 2.9^{ab}	74.0 ± 0.6^{b}	65.3 ± 1.0^{a}	74.4 ± 1.5^{b}	71.7 ± 2.3^{ab}	65.2 ± 0.7^{b}	70.2 ± 2.0^{b}	58.3 ± 0.8^{a}	69.4 ± 1.4^{b}	65.7 ± 0.7^{b}	***	***	ns
NFE	89.1 ± 0.2	88.7 ± 0.2	88.3 ± 0.2	87.7 ± 0.7	88.2 ± 0.3	88.6 ± 0.4	88.2 ± 0.4	87.7 ± 0.1	87.2 ± 0.1	88.3 ± 0.9	88.6 ± 0.5	88.6 ± 0.5	88.2 ± 0.4	87.9 ± 0.1	89.0 ± 0.1	ns	*	ns
Energy	84.7 ± 0.2	84.9 ± 0.2	83.1 ± 0.4	83.0 ± 0.7	83.4 ± 0.6	83.0 ± 0.6	83.2 ± 0.2	82.1 ± 1.0	81.5 ± 0.4	81.9 ± 0.9	82.3 ± 0.3^{c}	82.0 ± 0.2^{bc}	80.1 ± 0.6^{a}	80.2 ± 0.4^{a}	80.4 ± 0.3^{ab}	***	***	ns
Tissue proximate composition ($mg \cdot g^{-1}$ dry basis)																		
Moisture	769.1 ± 9^{bc}	777.8 ± 16.2^{abc}	775.9 ± 8.6^{abc}	791.9 ± 8.7^{ab}	797.9 ± 17.6^{ab}	811.1 ± 12.1^{a}	785 ± 22.4^{ab}	788.2 ± 11.8^{ab}	785.7 ± 11.5^{ab}	784.5 ± 13.5^{abc}	774.5 ± 11.4^{bc}	772.3 ± 3.1^{bc}	780.7 ± 3.6^{abc}	749 ± 2.9^{c}	765.2 ± 5.2^{bc}			**
Protein	702.6 ± 7.7	690.9 ± 16.1	690.7 ± 15.9	688.8 ± 4.2	691.3 ± 11.6	684.2 ± 8.7	685.5 ± 12.3	689.5 ± 6.3	690.6 ± 8.5	673.7 ± 0.8	657.3 ± 11.8	668.8 ± 16.4	677.9 ± 8.6	649.4 ± 4.1	680.4 ± 6.2	***	ns	
Lipid	61.6 ± 1.5	67.1 ± 1.8	66.2 ± 1.9	63.8 ± 1.8	62.3 ± 0.8	57.4 ± 1.6	59.5 ± 4.1	60.5 ± 0.9	61.3 ± 0.9	64.3 ± 1.6	63.8 ± 1.7	64.1 ± 0.7	62.9 ± 1.8	66.7 ± 1.2	66.5 ± 0.9	***	ns	
Ash	108.1 ± 4.8	87.9 ± 3.1	86.6 ± 3.4	89.9 ± 3.3	94.2 ± 11.1	107.0 ± 4.8	92.5 ± 5.4	95.4 ± 2.6	93.3 ± 6.3	97.5 ± 3.1	97.9 ± 8.0	102.6 ± 9.1	99.7 ± 7.2	91.8 ± 2.8	89.9 ± 5.2	ns	ns	
NFE	127.7 ± 6.9	154.2 ± 20.8	156.5 ± 14.0	157.5 ± 3.3	152.2 ± 1.6	151.3 ± 9.3	162.5 ± 13.5	154.6 ± 7.6	154.8 ± 13.9	164.5 ± 3.1	181.0 ± 15.2	164.5 ± 6.8	159.4 ± 0.6	192.1 ± 4.4	163.2 ± 5.0	**	ns	
Energy ($MJ \cdot kg^{-1}$)	21.2 ± 0.1	21.6 ± 0.1	21.6 ± 0.1	21.5 ± 0.1	21.4 ± 0.3	21.0 ± 0.1	21.3 ± 0.1	21.3 ± 0.0	21.4 ± 0.1	21.3 ± 0.1	21.1 ± 0.1	21.1 ± 0.3	21.2 ± 0.1	21.3 ± 0.1	21.5 ± 0.1	ns	ns	

PER: protein efficiency ratio; PD: protein deposition; EER: energy efficiency ratio; ED: energy deposition; ADC: apparent digestibility coefficient; NFE: nitrogen-free extract (calculated). Data are expressed as mean ± SEM values in the same row with different superscripts that are significantly different (two-way ANOVA and Tukey's post hoc analysis, using temperature (A) and the dietary protein level (B) as factors. Where there was no significant interaction between A and B, a one-way ANOVA with Tukey's post hoc analysis was performed comparing the dietary protein level within temperature. ns = nonsignificant. *, **, and *** indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. Information presented in the table herein also appears in the technical report by [33].

Table 6: Amino acid composition (mg·g^{-1} dry basis) of Australian hybrid abalone fed five experimental diets with increasing protein concentrations at three water temperatures.

	12°C						17°C						22°C					
	P32	P35	P38	P41	P44	*P* value	P32	P35	P38	P41	P44	*P* value	P32	P35	P38	P41	P44	*P* value
Aspartic acid	66.3 ± 1.4	67.0 ± 1.7	66.5 ± 1	65.7 ± 1	67.8 ± 0.7	ns	60.0 ± 2.3	64.8 ± 5.6	66.2 ± 1.9	67.4 ± 5.6	62.9 ± 2.9	ns	60.8 ± 2.1	68.6 ± 0.5	64.1 ± 0.7	64.9 ± 4.4	64.6 ± 0.5	ns
Glutamic acid	92.4 ± 1.8	93 ± 2.3	92.9 ± 1.5	91.8 ± 1.3	94.8 ± 1.2	ns	84.9 ± 3.1	92.9 ± 8.4	93.9 ± 2.4	96.2 ± 8.5	89.8 ± 4.6	ns	83.8 ± 4	96.0 ± 1.2	89.2 ± 1.5	88.6 ± 5.7	90.1 ± 0.9	ns
Serine	29.3 ± 1.3	30.3 ± 0.4	30.4 ± 1.2	30.4 ± 0.5	30.6 ± 0.7	ns	30.0 ± 1.2	31.8 ± 2	32.2 ± 1	33.6 ± 2.1	32.1 ± 1.5	ns	29.5 ± 1.0	32.5 ± 1.6	31.0 ± 0.7	29.7 ± 1.7	30.4 ± 0.1	ns
Histidine	9.8 ± 0.2	9.9 ± 0.0	9.7 ± 0.1	9.6 ± 0.1	9.8 ± 0.1	ns	8.7 ± 0.4	8.9 ± 1.1	9.2 ± 0.7	8.6 ± 1.0	8.4 ± 0.2	ns	9.2 ± 0.2	10.5 ± 0.4	10.1 ± 0.8	9.6 ± 0.8	9.4 ± 0.1	ns
Glycine	51.9 ± 0.7	55.7 ± 0.6	54.7 ± 1.0	54.9 ± 2.1	58.7 ± 1.9	ns	46.8 ± 2.3	53.6 ± 3.4	55.1 ± 3.9	55.0 ± 2.1	54.4 ± 3.6	ns	50.7 ± 2.4	59.1 ± 2.9	54.7 ± 0.9	52.3 ± 3.3	56.6 ± 1.9	ns
Threonine	24.4 ± 0.9	25.0 ± 0.3	24.8 ± 0.8	24.5 ± 0.4	25.0 ± 0.3	ns	24.1 ± 0.8	25.9 ± 2.3	26.3 ± 0.9	27.2 ± 2.4	25.5 ± 0.9	ns	23.7 ± 0.7	26.2 ± 0.4	24.8 ± 0.4	24.9 ± 1.7	24.8 ± 0.2	ns
Arginine	69.6 ± 1.1	68.4 ± 1.8	68.9 ± 1.3	68.9 ± 1.0	69.6 ± 0.9	ns	59.0 ± 3.0	65.0 ± 5.7	64.5 ± 1.4	68.7 ± 6.0	62.3 ± 3.1	ns	60.3 ± 3.4	68.9 ± 2.5	64.7 ± 1.3	60.3 ± 3.9	63.9 ± 1.4	ns
Alanine	33.0 ± 0.9	33.1 ± 0.6	33.2 ± 0.9	32.9 ± 0.6	33.9 ± 0.7	ns	31.0 ± 0.6	33.3 ± 2.3	34.0 ± 0.8	34.1 ± 2.2	32.9 ± 1.4	ns	29.9 ± 1.2	34.3 ± 0.6	31.6 ± 0.5	31.8 ± 2.1	32.1 ± 0.1	ns
Tyrosine	13.9 ± 0.9	15.1 ± 0.2	15.0 ± 0.5	15.1 ± 0.5	15.4 ± 0.3	ns	13.7 ± 0.5	14.6 ± 1.5	15.6 ± 0.4	16.7 ± 2.0	15.2 ± 0.5	ns	13.8 ± 0.7	15.5 ± 0.4	15.0 ± 0.2	14.1 ± 0.7	14.7 ± 0.1	ns
Valine	27.6 ± 0.7	27.2 ± 0.8	27.1 ± 0.2	26.8 ± 0.4	27.7 ± 0.2	ns	23.3 ± 0.7	26.1 ± 2.7	26.5 ± 1.0	26.7 ± 2.6	24.5 ± 0.8	ns	23.6 ± 1.2	27.1 ± 0.6	25.1 ± 0.5	26.0 ± 2.0	26.1 ± 0.2	ns
Methionine	15.4 ± 0.5	15.3 ± 0.5	15.3 ± 0.2	15.2 ± 0.3	15.6 ± 0.2	ns	14.1 ± 0.4	15.3 ± 1.5	15.4 ± 0.3	15.8 ± 1.4	14.8 ± 0.7	ns	13.7 ± 0.7	15.6 ± 0.1	14.6 ± 0.3	14.7 ± 1.0	14.6 ± 0.1	ns
Phenylalanine	21.8 ± 0.6	21.8 ± 0.5	21.8 ± 0.2	21.4 ± 0.3	22.1 ± 0.0	ns	20.1 ± 0.7	22.1 ± 2.2	22.5 ± 0.9	23.1 ± 2.3	21.2 ± 0.7	ns	19.6 ± 0.9	21.7 ± 0.2	20.7 ± 0.1	20.7 ± 1.5	20.7 ± 0.1	ns
Isoleucine	23.7 ± 0.6	23.4 ± 0.8	23.3 ± 0.3	23.0 ± 0.4	23.9 ± 0.0	ns	20.4 ± 0.5	23 ± 2.6	23.7 ± 0.6	23.7 ± 2.5	21.4 ± 0.8	ns	20.1 ± 1.0	23.3 ± 0.7	21.5 ± 0.5	22.5 ± 1.8	22.5 ± 0.2	ns
Leucine	42.5 ± 1.1	42.4 ± 1.0	42.4 ± 0.8	42.0 ± 0.7	42.9 ± 0.2	ns	39.7 ± 1.0	43.1 ± 4.2	43.5 ± 1.3	45.1 ± 4.3	41.7 ± 1.5	ns	38.1 ± 1.7	43.3 ± 0.2	40.7 ± 0.5	40.5 ± 2.8	41.0 ± 0.4	ns
Lysine	33.0 ± 0.2^{b}	30.2 ± 0.6^{a}	31.5 ± 0.8^{ab}	30.3 ± 0.8^{ab}	32.1 ± 0.1^{ab}	*	30.2 ± 0.6	32.0 ± 3.9	33.2 ± 1.6	35.1 ± 3.6	32.3 ± 1.4	ns	29.2 ± 1.1	31.5 ± 1.2	30.6 ± 0.5	29.8 ± 2.4	31.2 ± 0.7	ns
Proline	25.2 ± 1.4	25.3 ± 0.6	24.9 ± 0.4	25.0 ± 0.3	26.1 ± 0.4	ns	25.3 ± 1.6	27.9 ± 2.0	30.0 ± 1.5	30.0 ± 2.1	28.8 ± 1.8	ns	22.3 ± 1.7	26.4 ± 0.4	23.6 ± 0.9	24.8 ± 1.4	23.7 ± 1.7	ns
Total	579.9 ± 12.5	583.0 ± 11.8	582.5 ± 10.0	577.4 ± 7.7	596.1 ± 6.2	ns	531.4 ± 18.4	580.2 ± 51.1	591.8 ± 17.0	607.2 ± 50.6	568.2 ± 25.9	ns	528.3 ± 20.8	600.6 ± 9.4	562.0 ± 6.9	555.1 ± 37.0	566.1 ± 3.0	ns

Data are expressed as mean ± SEM. Data were subjected to one-way ANOVA and Tukey's post hoc test. ns = nonsignificant. *, **, and *** indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. Information presented in the table herein also appears in the technical report by [33].

especially at 17 and 22°C. At 22°C, in particular, there was a clear positive relationship between both weight gain percentage and SGR and increasing dietary protein level. Nevertheless, it was interesting to note an improvement in FCR with increasing dietary protein levels at each of the experimental temperatures, which were all lowest between 38 and 44% dietary protein. This clearly suggests that protein utilisation for tissue synthesis is not negatively impacted by increasing the dietary protein levels. Similar trends in FCR were also observed in studies with greenlip abalone [12, 16].

Taken together, some of the recorded growth performance parameters may suggest that the optimal dietary protein inclusion level may have been at or even beyond the tested range (i.e., >44% dietary protein). However, it should be noted that abalone generally exhibit slow growth; hence, shorter experimental trials may be insufficient to fully realise maximum growth performance, resulting in an inability to identify the optimal protein inclusion level [12]. However, the present experiment, conducted over 143 days, was considerably longer in comparison to other studies on similar-sized abalone by Bansemer et al. [16] and Stone et al. [12], who conducted 75- and 84-day growth trials, respectively. Nevertheless, even in the present study, it could have been that the duration was still not sufficient to clearly identify the optimal protein level. Therefore, further extending the abalone culture duration in future studies is critical to accurately detecting key differences in growth parameters to identify the optimal dietary protein inclusion level.

Further exploration of the optimal dietary protein inclusion level in Australian hybrid abalone may avert the potential negative consequences when the dietary protein level exceeds the animal's physiological requirement. Among others, this may include a displacement of other nutrients, a negative effect on the nutrient balance of the feed, which in turn promotes the utilisation of dietary protein for energy as opposed to tissue synthesis, increased nitrogen waste discharge and water quality deterioration, and increased feed cost [41–44]. Therefore, it is essential to consider the effect of increasing dietary protein levels on feed utilisation efficiency, namely growth conversion and nutrient deposition while emphasising the impact on growth performance.

In the present experiment, there was an interaction between dietary protein level and water temperature on feed intake. In general, however, feed intake was slightly higher in abalone-fed diets containing 38% protein, particularly at 12 and 17°C; however, it was clear that water temperature had a much larger effect on feed intake. Similar trends in feed consumption were also reported in several previous studies, in line with the well-held notion that abalone feed consumption is primarily determined by culture temperature [9, 12, 28, 40, 45–47]. However, this can also be influenced by species [40], size or life stage, and the diet type (formulated vs. natural) [9, 12, 28, 45, 46]. Moreover, the dietary protein level [12], protein to energy ratio [32, 48], and dissolved oxygen concentration of the culture water [49] may also affect feed consumption. As the abalone in the present experiment were fed isoenergetic diets in excess, they were able to consume sufficient food to fulfil their energy requirements, which is a commonly observed strategy for aquatic animals [50]. The metabolic rate and concomitant energy expenditure of abalone are intrinsically linked to water temperature. Therefore, the increased feed consumption observed in abalone in the present experiment in response to increasing water temperature can likely be attributed to an increase in energy requirement, which in turn is associated with higher metabolic activity levels [32]. Furthermore, abalone exhibit a faster gut evacuation rate at higher water temperatures, resulting in a faster return to appetite and therefore an increase in feed consumption [45].

The digestibility of macronutrients appeared to be negatively impacted by increasing temperatures; whereas digestive enzyme activity is known to be higher at higher temperatures, the rapid gastric evacuation reduces the contact between feed and enzymes, leading to poor digestibility [27, 51, 52]. Conversely, digestive enzyme activity typically decreases with decreasing water temperatures, which may increase the time in which ingested feed is exposed to digestive enzymes due to a reduced gastric evacuation rate. Contrastingly, digestibility studies with *H. midae* revealed significantly higher digestibility coefficients at 18°C compared to 14°C or 22°C [53]. However, in a study on greenlip abalone, where a direct total faecal collection method was utilised to estimate digestibility, it was revealed that gastrointestinal evacuation time, but not nutrient digestibility, was affected by temperature [45].

Furthermore, higher dietary protein inclusion levels often deteriorate the nutritional quality of abalone tissue [54, 55]. Promisingly, in the present study, increasing the dietary protein level did not affect the nutrient composition and amino acid profile of abalone soft tissue, regardless of rearing temperature. Furthermore, an interactive effect of dietary protein level and temperature was recorded for nutrient utilisation indicators for both energy (ED and EER) and protein (PER and PED). Although, in general, the results suggested that both protein and energy were better deposited in abalone fed between 38 and 41% dietary protein and in abalone reared at 22°C. Despite the need to further uncouple the interactive effect of dietary protein level and rearing temperature, it is evident that the use of a higher dietary protein feed during periods of elevated water temperature can improve the growth performance of abalone. Importantly, this can occur without compromising the efficient utilisation of dietary nutrients or the nutritional quality of the abalone tissue.

In conclusion, the trends in Australian hybrid abalone growth performance and nutrient utilisation observed in the current growth trial conducted using subadult Australian hybrid abalone have clearly suggested that significant growth benefits could be realised via the implementation of temperature-specific (seasonal) optimisation of dietary protein levels in formulated feeds for abalone. The degree to which the growth performance benefits observed in the present lab-based experiment would translate to an on-farm scenario may depend on the influence of inherent differences

in culture conditions between the two, namely differences in water filtration and daily water temperature fluctuations. Nevertheless, currently, commercial diets for Australian abalone contain 35% protein and are fed throughout the entire grow-out culture period. However, considering the growth performance, feed utilisation, feed conversion, and nutrient utilisation and deposition observed, the present study strongly suggests that an improvement in growth performance can be achieved by increasing the dietary protein level beyond 35%. Furthermore, those benefits will be best realised during periods of elevated water temperatures (~22°C). Conversely, during periods of relatively low water temperature (~12°C), where differences in performance between dietary protein levels are minimal, a cost-effective approach, either by implementing a reduced feeding frequency or a least-cost diet formulation, may enhance the production efficiency of Australian hybrid abalone.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This project was financially supported by the Australian Abalone Growers' Association and the Fisheries Research and Development Corporation on behalf of the Australian Government (FRDC Project No. 2017/113). They acknowledge the in-kind support provided by Jade Tiger Abalone, Yumbah, AbTas, Southern Ocean Mariculture, and 888 Abalone in the form of experimental animals, feeds, experimental system design, farm data and industry know-how. The authors thank Ridley AgriProducts for the supply of core dietary ingredients utilised for the manufacture of experimental diets. Open access publishing facilitated by Deakin University, as part of the Wiley-Deakin University agreement via the Council of Australian University Librarians.

References

[1] L. Venter, D. T. Loots, A. Vosloo, P. Jansen van Rensburg, and J. Z. Lindeque, "Abalone growth and associated aspects: now from a metabolic perspective," *Reviews in Aquaculture*, vol. 10, no. 2, pp. 451–473, 2018.

[2] P. A. Cook, "The worldwide abalone industry," *Modern Economy*, vol. 5, no. 13, pp. 1181–1186, 2014.

[3] P. A. Cook, "Recent trends in worldwide abalone production," *Journal of Shellfish Research*, vol. 35, no. 3, pp. 581–583, 2016.

[4] Fao, *Fishery and Aquaculture Statistics (FishStatJ) (Fisheries and Aquaculture Division, 2020)*, Food and Agriculture Orginization of the United Nations, Rome, Italy, 2020.

[5] Austrade, *A Culinary Treasure of the Sea Australian Abalone, Australian Trade and Investment Commission*, Australian Government, 2021.

[6] D. Mobsby, A. Bath, and R. Curtotti, "Fisheries: outlook to 2022-23," *Agricultural Commodities*, vol. 8, no. 1, pp. 128–137, 2018.

[7] A. J. Morash and K. Alter, "Effects of environmental and farm stress on abalone physiology: perspectives for abalone aquaculture in the face of global climate change," *Reviews in Aquaculture*, vol. 8, no. 4, pp. 342–368, 2016.

[8] R. Day and A. Fleming, "The determinants and measurement of abalone growth," in *Abalone of the World: Biology, Fisheries and Culture*, S. A. Shepherd and M. J. Tegner, Eds., pp. 141–168, Blackwell Scientific, Oxford, UK, 1992.

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

[10] M. Vandepeer, "Preventing summer mortality of abalone in aquaculture systems by understanding interactions between nutrition and water temperature," *Fisheries Research and Development Corporation*, 2006.

[11] S. M. Huchette, C. Koh, and R. W. Day, "Growth of juvenile blacklip abalone (Haliotis rubra) in aquaculture tanks: effects of density and ammonia," *Aquaculture*, vol. 219, no. 1-4, pp. 457–470, 2003.

[12] D. A. Stone, J. O. Harris, H. Wang, G. J. Mercer, E. N. Schaefer, and M. S. Bansemer, "Dietary protein level and water temperature interactions for greenlip AbaloneHaliotis laevigata," *Journal of Shellfish Research*, vol. 32, no. 1, pp. 119–130, 2013.

[13] D. A. Stone, M. S. Bansemer, K.-L. Currie, L. Saunders, and J. O. Harris, "Increased dietary protein improves the commercial production of hybrid abalone (Haliotis laevigata × Haliotis rubra)," *Journal of Shellfish Research*, vol. 35, no. 3, pp. 695–701, 2016.

[14] S. Edwards, "Thermal preferences and tolerances of Greenlip and Blacklip abalone," in *Proceedings of the 3rd Annual Abalone Aquaculture Workshop*, P. Hone, Ed., pp. 60–62, South Australian Research and Development Institute, Adelaide, Australia, 1996.

[15] D. A. Stone, M. S. Bansemer, and J. O. Harris, "Development of formulated diets for cultured abalone," *Australian Seafood Cooperative Research Centre*, 2014.

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

[17] T. Coote, *'Optimising the nutrient specifications of manufactured feeds for farmed juvenile greenlip abalone (Haliotis laevigata Donovan)*, Ph.D thesis, University of Tasmania, Hobart, Australia, 1999.

[18] G. A. Dunstan, J. K. Volkman, and G. B. Maguire, "Optimisation of essential lipids in artificial feeds for Australian abalone," *Fisheries Research and Development Corporation*, 2000.

[19] A. E. Fleming, R. J. Van Barneveld, and P. W. Hone, "The development of artificial diets for abalone: a review and future directions," *Aquaculture*, vol. 140, no. 1-2, pp. 5–53, 1996.

[20] M. Vandepeer, "Abalone aquaculture subprogram: manufactured diet development," *Fisheries Research and Development Corporation*, 2005.

[21] K. A. Freeman, "Aquaculture and related biological attributes of abalone species in Australia," *A review, Fisheries Western Australia*, 2001.

[22] X. Guo, "Use and exchange of genetic resources in molluscan aquaculture," *Reviews in Aquaculture*, vol. 1, no. 3-4, pp. 251–259, 2009.

[23] F. Lafarga de la Cruz and C. Gallardo-Escárate, "Intraspecies and interspecies hybrids in Haliotis: natural and experimental evidence and its impact on abalone aquaculture," *Reviews in Aquaculture*, vol. 3, no. 2, pp. 74–99, 2011.

[24] H. Mateos, P. Lewandowski, and X. Su, "Seasonal variations of total lipid and fatty acid contents in muscle, gonad and digestive glands of farmed Jade Tiger hybrid abalone in Australia," *Food Chemistry*, vol. 123, no. 2, pp. 436–441, 2010.

[25] K.-L. Currie, H. Davidson, M. S. Bansemer, J. O. Harris, and D. A. Stone, "Ventral videographic assessment of the feeding behavior of juvenile greenlip [Haliotis laevigata(donovan, 1808)] and hybrid (H. laevigata×Haliotis rubra) abalone in response to dietary and temperature manipulation," *Journal of Shellfish Research*, vol. 35, no. 3, pp. 641–651, 2016.

[26] Z. García-Esquivel, S. Montes-Magallón, and M. A. González-Gómez, "Effect of temperature and photoperiod on the growth, feed consumption, and biochemical content of juvenile green abalone, *Haliotis fulgens*, fed on a balanced diet," *Aquaculture*, vol. 262, no. 1, pp. 129–141, 2007.

[27] S. J. Kaushik, "Environmental effects on feed utilization," *Fish Physiology and Biochemistry*, vol. 2, no. 1, pp. 131–140, 1986.

[28] P. J. Britz, T. Hecht, and S. Mangold, "Effect of temperature on growth, feed consumption and nutritional indices of Haliotis midae fed a formulated diet," *Aquaculture*, vol. 152, no. 1-4, pp. 191–203, 1997.

[29] A. Gilroy and S. Edwards, "Optimum temperature for growth of Australian abalone: preferred temperature and critical thermal maximum for blacklip abalone, Haliotis rubra (Leach), and greenlip abalone, Haliotis laevigata (Leach)," *Aquaculture Research*, vol. 29, no. 7, pp. 481–485, 1998.

[30] T. Searle, R. D. Roberts, and P. M. Lokman, "Effects of temperature on growth of juvenile Blackfoot abalone, Haliotis iris Gmelin," *Aquaculture Research*, vol. 37, no. 14, pp. 1441–1449, 2006.

[31] A. Steinarsson and A. K. Imsland, "Size dependent variation in optimum growth temperature of red abalone (*Haliotis rufescens*)," *Aquaculture*, vol. 224, no. 1-4, pp. 353–362, 2003.

[32] A. J. Green, C. L. Jones, and P. J. Britz, "The protein and energy requirements of farmed South African abalone Haliotis midae L. cultured at optimal and elevated water temperatures," *Aquaculture Research*, vol. 42, no. 11, pp. 1653–1663, 2011.

[33] D. S. Francis, M. M. Rocker, K. Searle et al., *Elucidating the Nutritional Requirements of Farmed Hybrid Abalone*, Deakin University, Geelong, Australia, 2021.

[34] M. J. Lewis, D. S. Francis, D. Blyth et al., "A comparison of in-vivo and in-vitro methods for assessing the digestibility of poultry by-product meals using barramundi (*Lates calcarifer*); impacts of cooking temperature and raw material freshness," *Aquaculture*, vol. 498, pp. 187–200, 2019.

[35] C. Cho, S. Slinger, and H. Bayley, "Bioenergetics of salmonid fishes: energy intake, expenditure and productivity," *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, vol. 73, no. 1, pp. 25–41, 1982.

[36] J. Folch, M. Lees, and G. Stanley, "A simple method for the isolation and purification of total lipides from animal tissues," *Journal of Biological Chemistry*, vol. 226, no. 1, pp. 497–509, 1957.

[37] T. S. Mock, D. S. Francis, M. K. Jago et al., "The impact of dietary protein: lipid ratio on growth performance, fatty acid metabolism, product quality and waste output in Atlantic salmon (*Salmo salar*)," *Aquaculture*, vol. 501, pp. 191–201, 2019.

[38] W. Myers, P. Ludden, V. Nayigihugu, and B. Hess, "Technical Note: a procedure for the preparation and quantitative analysis of samples for titanium dioxide1," *Journal of Animal Science*, vol. 82, no. 1, pp. 179–183, 2004.

[39] L. Kirkendale, D. V. Robertson-Andersson, and P. C. Winberg, "Review on the use and production of algae and manufactured diets as feed for sea-based abalone aquaculture in Victoria," *Shoalhaven Marine and Freshwater Centre*, vol. 7, 2010.

[40] M. S. Bansemer, J. G. Qin, K.-L. Currie, and D. A. Stone, "Temperature-dependent feed consumption patterns for greenlip (Haliotis laevigata) and hybrid (H. Laevigata × Haliotis rubra) abalone fed fresh macroalgae or a formulated diet," *Journal of Shellfish Research*, vol. 34, no. 3, pp. 885–892, 2015.

[41] P. J. Britz and T. Hecht, "Effect of dietary protein and energy level on growth and body composition of South African abalone," *Aquaculture*, vol. 156, no. 3-4, pp. 195–210, 1997.

[42] T. Coote, P. Hone, R. Van Barneveld, and G. Maguire, "Optimal protein level in a semipurified diet for juvenile greenlip abalone Haliotis laevigata," *Aquaculture Nutrition*, vol. 6, no. 4, pp. 213–220, 2000.

[43] L. Gómez-Montes, Z. Garcıa-Esquivel, L. R. D'Abramo, A. Shimada, C. Vásquez-Peláez, and M. T. Viana, "Effect of dietary protein: energy ratio on intake, growth and metabolism of juvenile green abalone *Haliotis fulgens*," *Aquaculture*, vol. 220, no. 1-4, pp. 769–780, 2003.

[44] J. Mercer, K.-S. Mai, and J. Donlon, "Comparative studies on the nutrition of two species of abalone, Haliotis tuberculata Linnaeus and Haliotis discus hannai Ino I. Effects of algal diets on growth and biochemical composition," *Invertebrate Reproduction and Development*, vol. 23, no. 2-3, pp. 75–88, 1993.

[45] K.-L. Currie, B. Lange, E. W. Herbert, J. O. Harris, and D. A. Stone, "Gastrointestinal evacuation time, but not nutrient digestibility, of greenlip abalone, Haliotis laevigata Donovan, is affected by water temperature and age," *Aquaculture*, vol. 448, pp. 219–228, 2015.

[46] K. Hahn, "Nutrition and growth of abalone," *Handbook of Culture of Abalone and Other marine Gastropods*, CRC Press, Boca Raton, FL, USA, 1st edition, 1989.

[47] N. Uki, "Feeding behavior of experimental populations of the abalone, Haliotis discus hannai," *Bulletin of Tohoku Regional Fisheries Research Laboratory*, vol. 43, pp. 53–58, 1981.

[48] M. N. Bautista-Teruel, A. C. Fermin, and S. S. Koshio, "Diet development and evaluation for juvenile abalone, Haliotis asinina: animal and plant protein sources," *Aquaculture*, vol. 219, no. 1-4, pp. 645–653, 2003.

[49] J. O. Harris, G. B. Maguire, S. J. Edwards, and D. R. Johns, "Low dissolved oxygen reduces growth rate and oxygen consumption rate of juvenile greenlip abalone," *Aquaculture*, vol. 174, no. 3-4, pp. 265–278, 1999.

[50] S. S. De Silva and T. A. Anderson, *Fish Nutrition in Aquaculture*, Vol. 1, Springer Science and Business Media, , Berlin, Germany, 1994.

[51] J. Brett and D. Higgs, "Effect of temperature on the rate of gastric digestion in finger ling sockeye salmon, *Oncorhynchus nerka*," *Journal of the Fisheries Research Board of Canada*, vol. 27, no. 10, pp. 1767–1779, 1970.

[52] I. J. McGaw and N. M. Whiteley, "Effects of acclimation and acute temperature change on specific dynamic action and gastric processing in the green shore crab, *Carcinus maenas*," *Journal of Thermal Biology*, vol. 37, no. 8, pp. 570–578, 2012.

[53] M. G. Dixon, *'The effect of temperature and photoperiod on the digestive physiology of the South African abalone Haliotis midae*, Ph.D thesis, Rhodes University South Africa, Grahamstown, South Africa, 1992.
[54] M. Cochet, M. Brown, P. Kube, M. Fluckiger, N. Elliott, and C. Delahunty, "Sensory and physicochemical assessment of wild and aquacultured green and black lip abalone (Haliotis laevigataandHaliotis rubra)," *Journal of Shellfish Research*, vol. 32, no. 1, pp. 81–88, 2013.
[55] W. J. Mulvaney, *Effect of various seaweed diets on the growth performance and fatty acid profiles of abalone (Haliotis laevigata x H. rubra) in a trial offshore culture system in South-Eastern Australia*, Ph.D thesis, University of Wollongong, Wollongong, Australia, 2016.