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Research Article

Nutritional and Metabolomic Changes of Juvenile Farmed Abalone (*Haliotis iris*) in New Zealand

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Seasonal variations play a crucial role in the physiology, immune responses, and nutritional profile of aquatic animals. Unpredictable water temperature fluctuations, especially those caused by climate change, may negatively affect feed consumption and growth of cultured organisms, such as abalone. In addition, metabolic and nutritional changes across different seasons may have significant effects on aquaculture production. This study aimed to investigate biochemical and metabolic alterations in healthy abalone (*Haliotis iris*) during 1 year of grow out in a land-based farm in New Zealand. Proximate analyses were used to identify nutritional variations in whole animal tissues, and a gas chromatography–mass spectrometry-based metabolomics approach was used to identify metabolic changes in adductor muscle of abalone during different seasons in the 1-year sampling period. Results showed that protein content was higher in warmer months compared with colder months, whereas lipid, ash, and carbohydrate contents remained generally constant throughout the year. Metabolic profile fluctuations indicated higher amounts of glutamic acid, glutathione, methionine, lysine, serine, tyrosine, and glycine in January and March compared with October and July, indicating possible amino acid breakdown and collagen degradation due to warmer temperatures. Although the proximate analyses findings revealed no signs of nutritional deficiencies in abalone among seasons, the metabolic profiles suggested possible thermal stress during summer months. This study provides a foundation for further nutritional studies to optimise seasonal diets for farmed *Haliotis iris* and highlights the need to monitor thermal stress effects, especially during summer and/or heatwave events.

1. Introduction

The abalone industry forms part of a dynamic primary production sector that has been continuously growing due to global demands for seafood. The high nutritional quality and meat flavour of abalone make this seafood especially sought after in Asia, where it is considered as a delicacy. Global abalone production has dramatically increased from 20,370 Mt in 1970s to 73,206 Mt in 2010 and to 174,162 Mt in 2016/17 [1, 2]. From the total abalone production in 2015, abalone aquaculture contributed 95%, whereas fisheries contributed only 5% [1]. This proportion shift from fisheries to aquaculture reflects a growing demand for reliable and sustainable products, which necessitate responsible farming practices. To this end, a significant number of innovations have been developed to enhance abalone culturing systems, allowing farms to improve production, especially in those countries where abalone farming is just starting to develop. However, one of the most significant bottlenecks of land-based abalone aquaculture is the high cost of the feed, which has been documented to be up to 50% of the production cost [3]. Thus, there has been a growing interest in feed innovation and technology to optimise abalone nutrition according to the requirements of the species and the availability and supply of formulated and natural diets. Another challenge for the abalone aquaculture industry is the length of time required to rear individuals, which is normally 4–5 years to attain market size [4]. To overcome this limitation, new feed technologies have been developed to optimise nutrition and growth. These technologies use different approaches, such as feeding a combination of formulated and natural diets [5], increasing protein content in artificial feeds [6], and adding specific amino

acids [7] or fatty acids [5, 8] to enhance the nutritional profile.

In New Zealand, the abalone industry has grown to 700 t per year in 2019 [9]. This production is based on one species, the New Zealand black-footed abalone (*Haliotis iris*), which is mainly exported to China, Japan, Hong Kong, Australia, Taiwan, Cambodia, Singapore, and Malaysia [10], generating export revenues of almost NZD 20 million in 2021 [11, 12]. Although abalone land-based operations have appeared and disappeared over the years, one major farm (Moana New Zealand Ltd) has persisted and contributes most of the production of cocktail-sized (60–94 mm) farmed abalone worth NZD 2.4 million in 2021 as well as wild caught abalone sales of up to NZD 22.6 million [12].

Currently, farmed *Haliotis iris* are fed artificial diets from early juveniles to adults. However, the artificial diets are not species or season specific and are not optimised to supply the exact nutritional requirements for different seasons. To date, only few studies have investigated the changes in nutritional profile of abalone meat as a function of the type of feed and seasonal changes. Some studies have focused on the development of formulated feeds using alternative ingredients (e.g., insect- and plant-based proteins) to enhance growth and nutritional composition. For example, Bautista-Teruel and Millamena [13] reported that the growth of *Haliotis asinina* was faster on artificial feeds compared with natural feeds due to the high levels of protein and more balanced amino acid profile. Other studies have also shown that growth is enhanced when the amino acid profile in the feed formulation resembles the amino acid profile of animal's tissues [14]. Mai et al. [15] determined that the presence and amount of some essential amino acids, such as arginine, methionine, and threonine, in diets directly affected the nutritional profile of *Haliotis tuberculata* and *Haliotis discus hannai*. In terms of lipid composition, Bautista-Teruel et al. [16] reported that the lipid content of *Haliotis asinina* increased when animals received higher amounts of dietary lipids. Specifically, the supplementation of linoleic acid (LA) (18 : 2n−6), alpha LA (18 : 3n−3), and n−3 highly unsaturated fatty acids produced animals with higher contents of these fatty acids in the animals, which also grew faster, probably due to the contribution of these fatty acids on biomembrane composition [17]. Britz and Hecht [6] found that the protein composition in the soft bodies of *Haliotis midae* was directly affected by the level of protein and lipids in their diets, resulting in better growth when 44% protein and 6% lipid contents were given. However, a diet containing 10% or more in lipids impaired growth [6]. These studies highlight the need for a deep understating of the nutritional profile of abalone species when wanting to provide the optimal feed to maximise productivity.

The environmental conditions for cultivation are also important factors to consider when optimising nutritional value and growth of abalone. For example, oxygen levels, temperature, and water chemistry may affect animals in different ways during different seasons and may also alter the composition and quality of the feed. These effects are reflected in the animal's nutritional composition, immunity [18], and growth [19]. For example, abnormally high ocean water temperatures, which have been reported in the past few years due to climate change, have been associated with increased mortality in different *Haliotis* species [20–23]. Warmer seasons have also been found to slightly increase protein levels and decrease moisture in *Haliotis discus* [24], whereas glycogen, an indicator of palatability in abalone meat, has been found to be higher in summer than in winter in *Haliotis discus* [25]. Indeed, abalone with low levels of glycogen in their soft tissues have been characterised as watery and lacking taste. In terms of amino acid profiles, the total amount of free amino acids (FAAs) also varies from season to season. In *Haliotis discus*, FAAs, such as glutamine, arginine, glycine, glutamic acid, alanine, and serine tend to be higher in animals right after summer compared with other months. Coincidentally, glutamic acid and glycine are taste-active components that are associated with *umami* taste, a reason for harvesting abalone in summer months in Japanese traditions [24]. Other nutrients, such as polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), tend to be found in higher concentrations in *Haliotis rubra* and *Haliotis laevigata* harvested in winter and spring than in summer. Conversely, saturated fatty acids are significantly higher in summer than other seasons [26].

From a sustainability point of view, information on the nutritional profile of commercial aquatic species over different seasons is relevant to the development of a more sustainable aquaculture industry. This knowledge is particularly important during the juvenile phase, when animals experience rapid growth and start to build resources for reproduction. Thus, farmers aiming to improve and optimise production practices may adjust the inclusion of certain ingredients or bioactives in artificial feeds accordingly. Furthermore, the effect of specific feed ingredients on the performance of abalone may be of interest when testing alternative sources of proteins replacing fishmeal for more sustainable aquafeeds.

Previous nutritional studies have evaluated the benefits of macronutrients, such as lipids, carbohydrates, and proteins as well as amino acids, fatty acids, vitamins, and minerals in molluscan species, such as mussels [27–29] and clams [30]. For abalone, nutritional profiles have been well documented for *Haliotis diversicolor* [31], *Haliotis discus* [24], *Haliotis laevigata* × *Haliotis rubra* [32], *Haliotis rubra*, and *Haliotis laevigata* [26], but limited information is available on the nutritional profile of *Haliotis iris*.

The nutritional status of a species can be investigated with a range of analytical techniques, such as proximate analysis and metabolomics. Proximate analysis provides a general overview on macronutrient levels within broad types of compounds (e.g., lipids, proteins, and carbohydrates), whereas metabolic profiling provides a snapshot of endogenous metabolites, such as amino acids, fatty acids, organic acids, and other biomarkers involved in biological processes [21]. Previous studies have successfully demonstrated the effectiveness of using metabolic profiling techniques to investigate the nutritional state of abalone stocks within different cultured conditions, such as diet quality [7, 33], water temperature [21], and handling [34, 35]. Such studies provide valuable

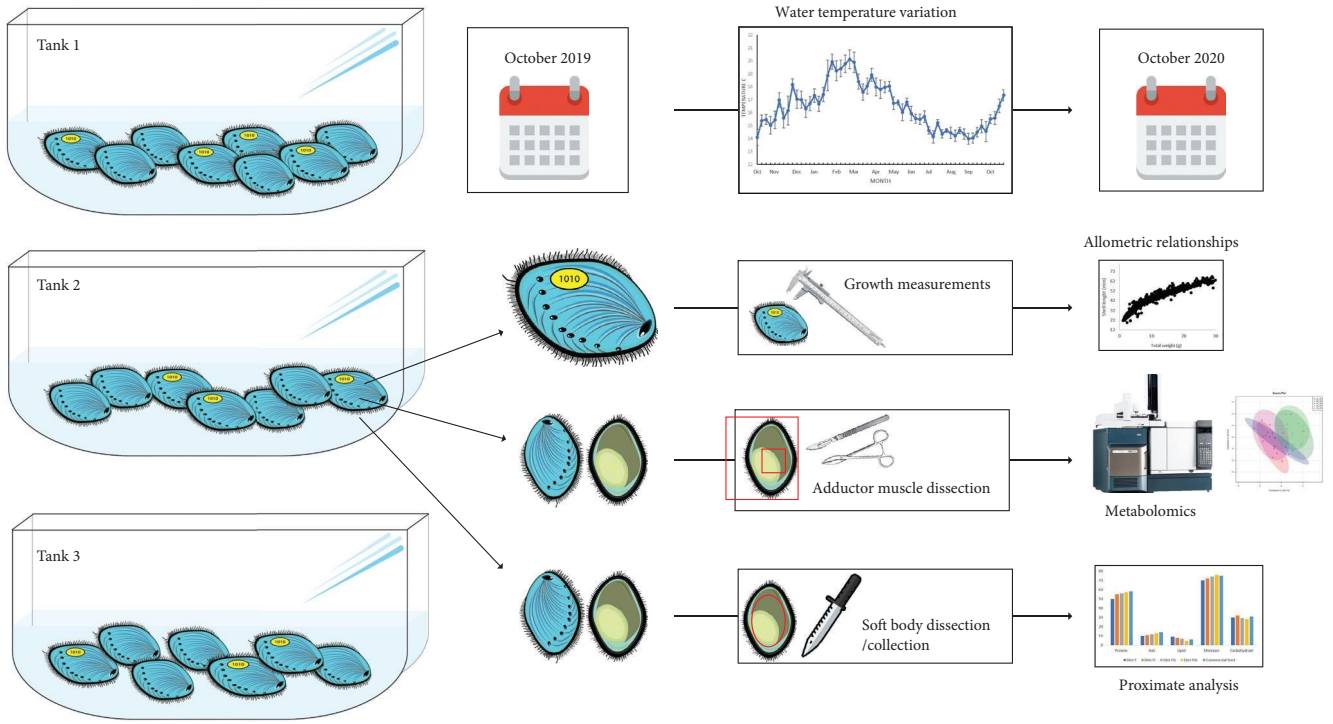

FIGURE 1: Summary of the experimental workflow.

information about the capacity to optimise nutritional state and profitability in aquaculture settings using these techniques. Thus, the aim of this study is to evaluate the nutritional status and metabolic profiles in juvenile New Zealand black-footed abalone (*Haliotis iris*) within farm conditions across seasons. To this end, we applied traditional proximate analyses and novel metabolomics techniques to identify animal growth and meat quality parameters and their variations over different seasons.

2. Materials and Methods

2.1. Experimental Setup. This study was conducted within a commercial abalone (*Haliotis iris*) farm at Moana New Zealand Limited, Ruakaka, Northland, New Zealand. Healthy juvenile abalone (1-year old) were randomly selected from the farm stock for this experiment. The animals ranged in size from 21 to 38 mm in shell length and had wet weights of 1.2–5.9 g. About 500 animals were placed within each of three experimental cylindrical tanks (85 cm radius × 85 cm height × 7 cm of water coverage), which was consistent with the culturing densities at the farm. A total of 50 animals from each tank were tagged with a special tag designed in the farm (vinyl tags with a spring). Tags were adhered and embedded into the shell with the aid of forceps. The tagging process was performed over 1 day and the water temperature was 15.7°C.

The tanks were all linked to the farm's recirculating water system with 100% water exchange every 4 hr with filtered seawater (60 μm filter). The water flow through the containers was at a rate of 10 L/min, which equates to a total water exchange of 12 times per hour. The tanks were drained and cleaned typically once a month. Briefly, the stopper at the bottom of the tank was pulled off from the tank, and with the tipping action of the water supply faeces, debris, and uneaten food residues were flashed out. Animals remained in the tank while cleaning was performed. Water temperature was measured according to the farm procedures with an electronic device that controlled water temperature every 10 min per day for the whole farm. Water pH and dissolved oxygen were measured with a multiparameter device every day and the values were maintained within 7.9–8.1 and >99%, respectively.

At the beginning of the 12 months of sampling (October 2019), abalone started being fed with a commercial diet (Marifeed® S34) according to their body weight of 1.11%–1.25% per day. The feeding rate was adjusted according to the higher temperature, especially during December, January, and February being increased by 10%. Abalone were fed once every other day in the afternoon.

2.2. Sampling of Animals. Every 4 weeks (except for April, May, August, and September 2020 due to COVID-19 lockdowns), removal of tagged abalone from tanks was performed with the aid of a blunt knife by carefully lifting the foot off the surface of the tank. Then, the animals were dried with paper towels, and their maximum shell lengths and widths (mm) and total animal wet weights (g) were recorded. Lengths were measured with a vernier calliper (Mitutoyo 0–125 mm, Warwickshire, UK) to the nearest 0.1 mm, and weights were measured with a digital balance to the nearest 0.1 g.

At each sampling point, 20 animals per tank were collected for proximate analysis and seven animals in total for metabolomic analyses (Figure 1). For proximate analysis, the whole soft body was collected and placed in dry ice (−80°C) until further processing. For metabolomic analyses, the

adductor muscle was dissected, placed into a 2 mL cryovial (Biostor™), quenched in liquid nitrogen for 10 min, and then stored in dry ice (−80°C) for transportation (2 hr drive) to the −80°C freezer located at the Auckland University of Technology (Auckland, New Zealand), where samples were stored until further analysis.

Diagram illustrating the sampling design and data collection from October 2019 to October 2020.

2.3. Growth Parameters. Tagged animals were used to provide information about growth with the following equations:

$$\text{Weight gain} = \frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times 100, \quad (1)$$

$$\text{Shell gain} = \frac{\text{final shell length} - \text{initial shell length}}{\text{initial shell weight}} \times 100. \quad (2)$$

Specific growth rates were calculated to determine the effectiveness of animal growth using the following equation:

$$\text{SGR(specific growth rate)}\left(\frac{\%}{\text{day}}\right) = 100 \times \frac{\text{Ln average final weight} - \text{Ln average initial weight}}{\text{number of days}}. \quad (3)$$

Daily growth rate was calculated to describe the longitude of growth as:

$$\text{DGR(daily growth rate)}\left(\frac{\mu\text{m}}{\text{day}}\right) = 1000 \times \frac{\text{Final shell length} - \text{initial shell length}}{\text{number of days}}. \quad (4)$$

Muscle yield (%):

$$(\text{muscle weight} \div \text{total body weight}) \times 100\%. \quad (5)$$

Condition factor (CF) is an index to evaluate the relationship between the weight of the abalone per unit shell length [36]. It was calculated following Britz [37].

$$\text{CF} = [5.575 \times (\text{final weight} \div \text{shell length}^{2.99})]. \quad (6)$$

2.4. Proximate Composition Analyses. Proximate analyses were conducted on the commercial feed and animal soft tissues to obtain crude protein, crude lipid, ash, and moisture contents following AOAC (1995). For moisture determination, three replicates of frozen tissue from each tank were freeze-dried (Christ alpha series freeze dryer, Osterode am Harz, Germany) for 48 hr. Then, the dried tissues were ground up to powder using a grinder (IKA A11 model analytical mill, Germany) with the addition of liquid nitrogen to avoid denaturation/oxidation of metabolites. The Kjeldahl method was used for crude protein determination, and the Bligh and Dyer method was used [38] for crude lipid quantification, adapted for small samples. For crude protein extractions, 0.2 ± 0.02 g of dried abalone samples was used with a factor of 6.25 for nitrogen conversion. Briefly, the samples were digested in Velp tubes with 7 g of catalyst (9 : 1 w/w mixture of potassium sulphate and cupric sulphate) and 10 mL of concentrated sulphuric acid. The tubes with samples and blanks (no sample material) were placed in a digestor (Velp Scientifica Ltd. Usmate, Italy). First, all samples were boiled at 420°C for 1 hr and once cooled, diluted with 40 mL distilled water. The samples were distilled in a Kjeldahl system with NaOH 35% and boric acid as indicator. The distilled solution was collected in a 250 mL conical flask where the indicator was placed, and the distilled ammonia was received until the solution was 150 mL. The change of colour from red to green indicated that the collection of ammonia was successful. Titration was performed with standardised 0.1 M HCl solution. The percentage of crude protein was calculated with the formula:

$$\text{Crude protein}\left(\frac{\text{g}}{\text{kg}}\right) = 6.25 \times 100\% \times \frac{(V_s - V_b) \times \text{Conc.HCl standard}(0.1\,M) \times \text{NaOH molar}(14.01)}{\text{sample(g)}}, \quad (7)$$

where V_b is the amount of standard 0.1 M HCl solution used in the blank titration, V_s is the amount of standard 0.1 M HCl solution used in the sample, S is the sample weight (g), and 6.25 is the conversion factor to determine protein percentage in meat.

A lipid extraction method was modified for small samples [38]. Dried abalone soft tissue (0.1 g) samples were hydrated in 0.8 mL distilled Millipore water and 3 mL mixture of methanol (2 mL) and chloroform (1 mL) in 15 mL screw-top glass tubes. A vortex was used for 2 min to enhance the extraction process. After this time, 1 mL chloroform was added and vortexed for 30 s. One millilitre of Millipore distilled water was added after this time and mixed again for 30 s. The final solution was centrifuged at 3,000 rpm for 5 min. The lower organic solvent layer was collected into a preweighted 8 mL glass tube. A nitrogen stream was used until all solvent was vaporised. Finally, the net weight of the lipids in the sample was recorded.

The ash content was obtained by complete combustion in a furnace at 550°C for 6 hr. Carbohydrate content in dried samples was calculated as follows:

$$\%\text{carbohydrate (in dry tissue)} = 100\% - (\text{protein}\% + \text{ash}\% + \text{lipid}\%). \quad (7)$$

2.5. Metabolomic Analysis. Seven abalone at each sampling point were analysed. Samples of abalone adductor muscle were freeze-dried for 48 hr (Christ alpha series freeze dryer,

Osterode am Harz, Germany) and ground up using a mortar and pestle into fine powder before analysis. Metabolite extractions were derivatised via methyl chloroformate alkylation, following Nguyen et al. [39]. Briefly, 7 mg of powdered tissues were slowly thawed on ice and mixed with 20 μL of d_4-alanine (10 mM) as an internal standard. Extractions were performed using 400 μL of cold (−20°C) 50% MeOH:H_2O solution. The mixture was vortexed vigorously for 2 min using a tissue homogeniser, frozen in dry ice, and then thawed again. Extracts were cold (−6°C) centrifuged at 2,500 rpm for 5 min at 4°C (Hermle laboratories, Model Z216MK, Germany), and the supernatants from the extractions were collected in 1.5 mL Eppendorf plastic vials placed on dry ice. Similarly, the second extraction was performed with 400 μL of cold (−20°C) 80% MeOH:H_2O. The supernatant was collected and mixed with the previous supernatant from the first extraction on ice.

Derivatised samples were transferred into 2 mL amber gas chromatography–mass spectrometry (GC–MS) glass vials fitted with 300 μL inserts with bottom springs (Sigma- Aldrich, St. Louis, MO, USA) and then analysed on an Agilent 7890B GC coupled to an Agilent MSD5977A mass spectrometer detector (Agilent Technologies, CA, USA), with an electron ionisation source operated at 70 eV. The system was equipped with a ZB-1701GC capillary column (30 m × 250 μm internal diameter × 0.15 μm film thickness with a 5 m guard column) (Phenomenex, Torrance, CA, USA). The instrument parameters were set according to Smart et al. [40]. The detailed protocol is accessible through Nguyen et al. [34].

Different types of quality controls (QCs) were used to guarantee reproducibility of GC–MS measurements, such as d_4-alanine, blank samples, and pooled biological QC samples from all samples after extraction. Blank samples contained only 20 μL of 10 mM d_4-alanine. Blank samples and pooled QC samples were extracted and derivatised with the other samples. For QC purposes, chloroform solvent and nonderivatised n-alkanes (C10–C40) were injected at the beginning of the analysis. This was followed by pooled QC samples and blank. The samples were injected in a random fashion after QCs. Injections of pooled QCs were repeated after every five samples. On the final day of the analysis, all pooled QC samples were run again to compare with the previous days.

2.6. Statistical Analyses. For size measurements, simple linear regression was used. For proximate composition, data followed a normal distribution (Kolmogorov–Smirnov test, $p < 0.05$) and were statistically treated by one-way ANOVA and Tukey's test was applied for multiple comparison of means at $p < 0.05$ on the statistical package XLSTAT (Addinsoft, Version 2022.3.1). For ANOVA purposes, month of sampling was considered as a fixed factor and tank as a random factor.

For metabolomic profiling, statistical analyses were performed using the integrated web-based platform MetaboAnalyst 5.0 (metaboanalyst.ca). Data were normalised by autoscaling (mean centered and divided by the standard deviation of each variable). A one-way ANOVA ($p < 0.05$) was used to assess effects of months on abalone metabolite profiles. Chemometric analysis via partial least-squares-discriminant analysis (PLS-DA) was performed to facilitate visualisation of the major trends. A heatmap of detected metabolites in adductor muscle was generated to visualise differences.

Table 1: Monthly water temperatures during the study period.

Month	Min °C	Max °C	Mean ± SD
October 2019	10.6	16.3	15 ± 0.7
November 2019	14	19	16.6 ± 1.3
December 2019	15.2	19	16.9 ± 0.8
January 2020	15.7	21.9	17.9 ± 1.3
February 2020	17.4	21.6	19.6 ± 0.8
March 2020	16	21.4	18.6 ± 1.1
April 2020	16	19.7	18.1 ± 0.8
May 2020	14.6	18.7	16.8 ± 0.8
June 2020	14.1	17.2	15.7 ± 0.5
July 2020	12.9	15.8	14.6 ± 0.5
August 2020	12.9	15.2	14.4 ± 0.4
September 2020	13	16.2	14.3 ± 0.6
October 2020	13.3	18.1	15.9 ± 0.9

3. Results

3.1. Water Temperatures. The water temperatures showed weekly and seasonal variations, with a minimum of 10.6°C in October 2019 and a maximum of 21.9°C in January 2020 (Table 1 and Figure 2). Temperatures fluctuated from 10.6 to19°C in spring 2019 (October, November, and December), 15.7–21.4°C in summer 2020 (January, February, and March), 14.1–19.7°C in autumn 2020 (April, May, and June), 12.9–16.2°C in winter 2020 (July, August, and September), and 13.3–18.1°C in Spring 2020 (October 20).

3.2. Survival. Abalone survival was relatively high during the 1-year grow-out period, with an overall average of 86% from the beginning to the end of the study (Figure 3).

3.3. Growth Parameters. The size relationships showed expected results based on normal growth behaviour of molluscs (Figure 4). Most of the relationships showed that while animals mature, the variability of sizes and weight increased. Relationships such as shell length–total weight and shell width–total weight showed a low r^2 indicating a wide range of weights in animals with the same shell length or width. In addition, in the shell weight–shell length relationship ($r^2 = 0.9216$), shell weight was more variable as the shell increased in size. The relationship shell weight – total weight ($r^2 = 0.9835$) indicated a reliable regression model. Similarly, the relationship soft body weight–total weight ($r^2 = 0.9967$) showed that soft body weight is a good predictor variable that explains 99.7% the total weight. Animals showed an overall specific growth rate (SGR)% of 0.82 ± 0.08, an overall shell length gain (%) of 93.33 ± 28.51, and a total weight gain (%) of 665.48 ± 234.13 (Table 2). The highest SGR% per day was in November 2019 (0.72% ± 0.27%) and December 2019 (0.74% ± 0.25%) compared with the lowest value in July 2020 (0.42% ± 0.23%). The highest weight gain

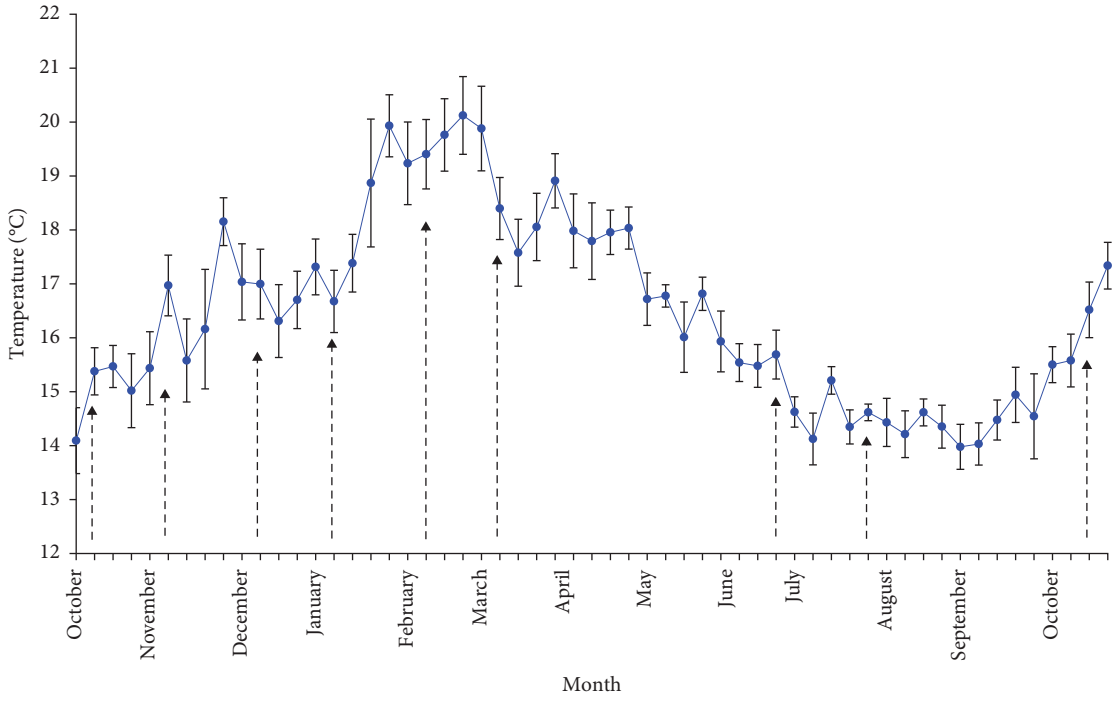

FIGURE 2: Average weekly water temperatures in the recirculating system from October 2019 to October 2020. Error bars denote the minimum and maximum weekly temperatures. Dotted arrows indicate the sampling events.

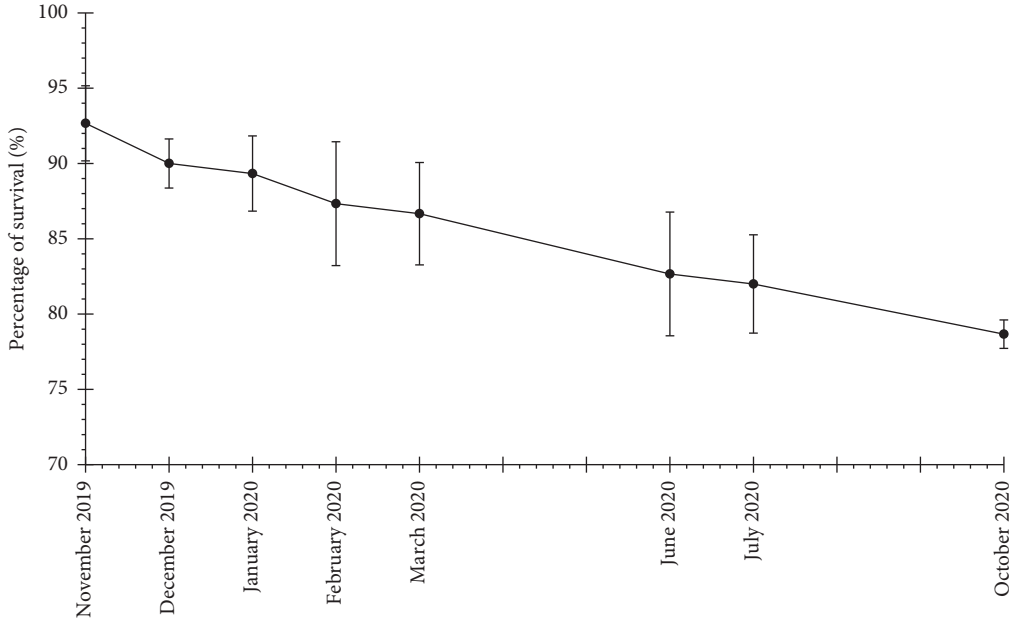

FIGURE 3: Survival percentages for farmed abalone during 1 year of grow out. Data represent means and bars standard deviation ($n = 150$).

occurred in November 2019 and the lowest in July 2020. In addition, animals showed an overall shell length gain of $70.44 \pm 14.00\,\mu$m/day. The highest shell length gain was observed in January 2020 with $8.87\% \pm 3.94\%$ followed by November 2019 with $7.48\% \pm 3.86\%$, and the lowest gain was observed in July 2020 with $3.29\% \pm 1.69\%$. Daily growth measurements revealed that animals grew at the highest rate of $101.68 \pm 44.42\,\mu$m/day during January 2020 and at a lowest rate of $54.52 \pm 27.34\,\mu$m/day during July 2020.

The soft body: shell ratio (SB/S) ranged from 3.72 ± 1.27 in Oct 19 to 2.27 ± 0.17 in October 2020, theCF ranged from 0.64 ± 0.04 in October 2019 to 0.67 ± 0.06 in October 2020, and the muscle yield ranged from 77.15 ± 4.04 in October 2019 to 69.35 ± 1.64 in October 2020 (Table 3). SB/S and CF

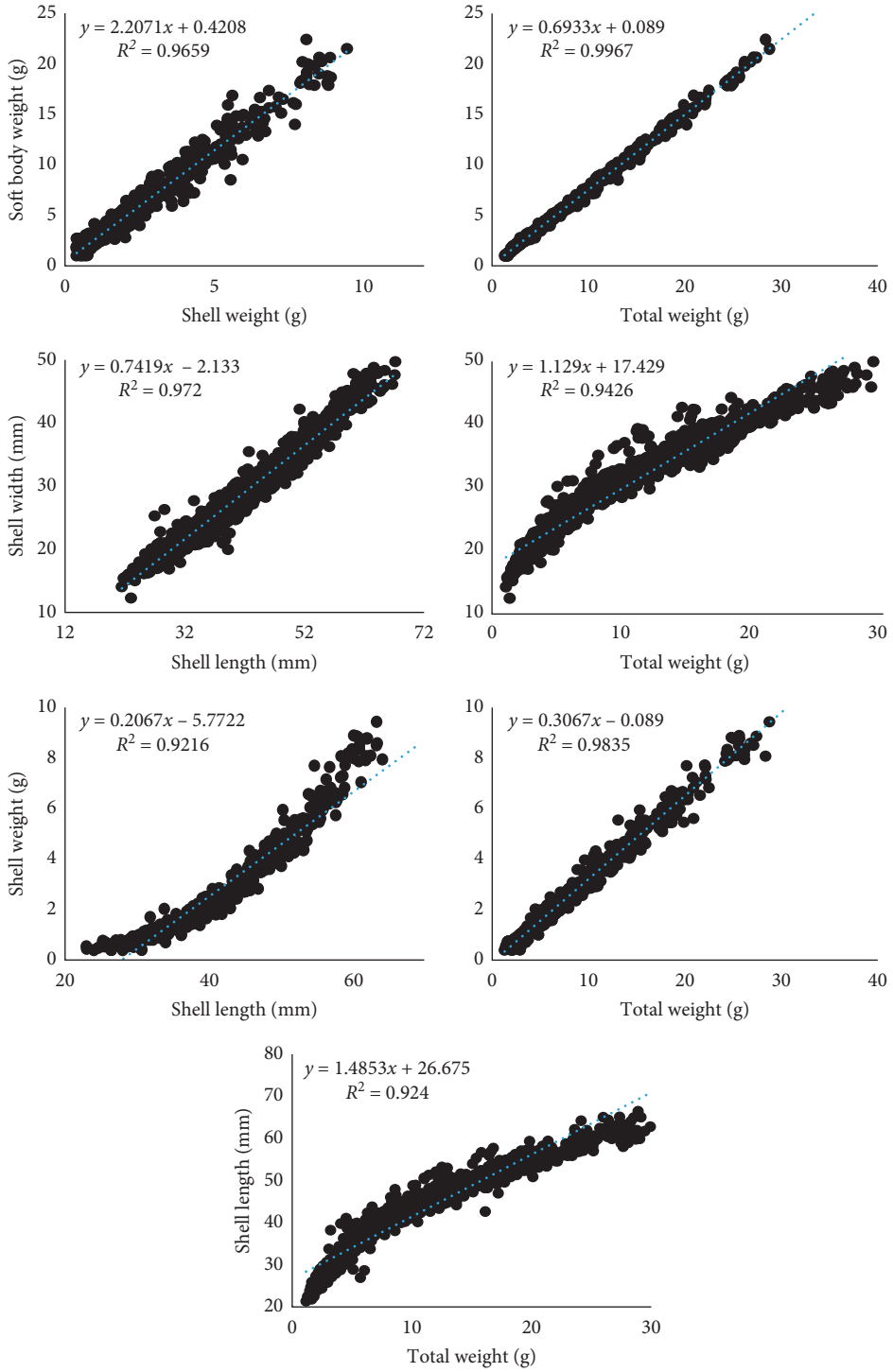

Figure 4: Size relationships for farmed abalone from October 2019 to October 2020.

showed that animals had heavier soft bodies in the first months (October 2019, November 2019, December 2019, and January 2020) compared with the last months (February 2020, March 2020, June 2020, July 2020, and October 2020).

3.4. Proximate Composition Analyses. The proximate composition of the commercial feed used during the study was 34.7% protein, 5.2% lipid, and 42.6% carbohydrate.

Differences among carbohydrate, protein, lipid, and ash content in wet and dry weight of abalone meat are presented in Table 4. Both are presented, but only wet weight-based results are discussed. The protein levels (wet weight) in the soft tissues of abalone were significantly higher in February 2020 (14.79 ± 0.48) and March 2020 (14.55 ± 1.03) compared with colder months October 2019 (12.38 ± 0.07) and November 2019 (12.37 ± 0.76). There were no significant

Table 2: Growth measurements of *Haliotis iris* during 1 year of grow out.

	November –2019	December –2019	January –2020	February –2020	March –2020	July –2020	Total (October–2019 to October –2020)
Initial weight (g)	2.70 ± 0.73	3.47 ± 0.96	4.19 ± 1.16	5.01 ± 1.40	6.14 ± 1.80	12.25 ± 3.62	2.70 ± 0.73
Final weight (g)	3.47 ± 0.96	4.19 ± 1.16	5.01 ± 1.40	6.14 ± 1.80	7.14 ± 2.13	13.88 ± 3.95	19.93 ± 5.63
Weight gain per day (%)	2.22 ± 1.07	2.84 ± 1.28	2.78 ± 1.99	3.40 ± 1.75	3.45 ± 2.07	5.43 ± 3.22	658.94 ± 243.54
Weight gain (%)	29.40 ± 12.26	23.36 ± 8.45	17.97 ± 9.96	23.71 ± 10.28	15.79 ± 8.82	12.72 ± 7.52	665.48 ± 234.13
Initial length (mm)	28.35 ± 2.78	30.35 ± 2.76	32.15 ± 3.07	34.88 ± 3.63	37.07 ± 3.92	47.16 ± 4.97	28.35 ± 2.78
Final length (mm)	30.35 ± 2.76	32.15 ± 3.07	34.88 ± 3.63	37.07 ± 3.92	39.45 ± 4.11	48.02 ± 4.76	55.00 ± 5.78
Shell length gain per day (μm)	59.33 ± 29.39	87.20 ± 14.87	101.68 ± 44.42	61.35 ± 33.99	89.90 ± 41.17	54.52 ± 27.34	70.44 ± 14.00
Shell length gain (%)	7.48 ± 3.86	6.32 ± 3.32	8.87 ± 3.94	6.20 ± 3.58	6.89 ± 3.42	3.29 ± 1.69	93.33 ± 28.51
SGR (%) day	0.72 ± 0.27	0.74 ± 0.25	0.58 ± 0.30	0.60 ± 0.24	0.51 ± 0.27	0.42 ± 0.23	0.82 ± 0.08

Abbreviations: SGR, specific growth rate (% body weight per day). Data represents means and standard deviations ($n = 150$).

Table 3: Shell weight, shell length, shell width, soft body weight, soft body: shell ratio, and condition factor of *Haliotis iris* collected in different months October 2019 to October 2020.

	(Baseline) October2019	November–2019	December–2019	January 2020	February –2020	March–2020	June 2020	July 2020	October–2020
Wet shell weight (mg)	0.61 ± 0.16	0.93 ± 0.25	1.17 ± 0.31	1.58 ± 0.40	2.23 ± 0.44	2.67 ± 0.62	4.85 ± 1.02	5.39 ± 1.05	7.84 ± 0.86
Wet soft body weight (mg)	2.16 ± 0.66	2.60 ± 0.79	3.06 ± 0.81	4.06 ± 1.09	5.02 ± 1.19	6.15 ± 1.61	11.03 ± 2.56	12.34 ± 2.53	17.80 ± 2.21
Soft body: shell ratio SBS/S	3.72 ± 1.27	2.80 ± 0.52	2.62 ± 0.32	2.58 ± 0.27	2.25 ± 0.26	2.30 ± 0.23	2.27 ± 0.27	2.30 ± 0.26	2.27 ± 0.17
Muscle yield (%)	77.15 ± 4.04	73.14 ± 4.14	72.21 ± 2.27	71.87 ± 2.10	68.99 ± 2.55	69.53 ± 2.14	69.24 ± 2.62	69.47 ± 2.49	69.35 ± 1.64
Condition factor	0.64 ± 0.04	0.63 ± 0.07	0.63 ± 0.06	0.6 ± 0.05	0.61 ± 0.07	0.63 ± 0.04	0.66 ± 0.04	0.67 ± 0.05	0.67 ± 0.06

Data represent means and standard deviation ($n = 150$).

differences among the lipid levels (wet weight) from different months and they ranged between 1.22% and 1.32%. The ash content was significantly higher in October 2019 (2.49 ± 0.01) compared with colder months June 2020 (2.07 ± 0.11) and July 2020 (2.24 ± 0.28). The moisture content varied from 76.57% to 81.03% with significantly higher values in warmer months October 2019 (79.21 ± 0.17), November 2019 (81.03 ± 1.11), and October 2020 (79.65 ± 0.90) compared with other months. The carbohydrate content varied from 2.99% to 5.45%. Significantly lower values were observed in November 2019 (2.99 ± 0.34) and October 2020 (3.27 ± 0.56) compared with other months.

3.5. Metabolomics. Metabolite identification yielded 100 annotated compounds from spectra of abalone muscle tissues. Most of these compounds consisted of amino acids, organic acids, and fatty acids. The multivariate data analysis via PLS-DA score plot revealed some clear separations between sampling times through the year (Figure 5(a)). Overall, samples in October (years 2019 and 2020) were clearly distinct from other months (January 2020, March 2020, and July 2020). However, there was no good separation between October samples in 2019 and 2020. Similarly, there were some overlaps in distribution of samples in January 2020, March 2020, and July 2020. The first two components contributed to 49.7% of the total variation. The PLS-DA model cross validation *via* LOOCV showed accuracy of 0.6, R^2 of 0.83, and Q^2 of 0.61, indicating a good prediction model. Furthermore, PLS-DA analysis also identified 27 metabolites with variable importance in projection (VIP) scores greater than 1 which are important classifiers (Figure 5(b)).

The univariate data analysis *via* one-way ANOVA identified 31 metabolites that were significantly different between sampling times. A heatmap of these metabolites was generated to visualise the detail differences (Figure 6), which divided the metabolites into 3 main clusters (A, B & C). Metabolites in cluster A are fatty acids, whereas most of amino acids are in cluster C. Cluster B includes some amino acids, fatty acids, and organic acids. As the PLS-DA score plot, the heatmap also grouped sampling times into two main groups: The first group with October samplings (2019 and 2020) and the remaining sampling times (January 2020, March 2020, and July 2020) in the second group. The first sampling group (Octobers) shared a similar abundance of

Table 4: Proximate composition in *Haliotis iris* based on dry weight and wet weight (%).

		October–2019	November–2019	December–2019	January–2020	February–2020	March–2020	Jun–2020	July–2020	October–2020
Carbohydrate (%)	Dry weight	22.3 ± 0.03^{abc}	15.76 ± 1.69^{d}	22.15 ± 2.36^{abc}	20.65 ± 2.76^{abc}	19.56 ± 1.43^{cd}	20.01 ± 2.19^{bcd}	24.37 ± 6.36^{ab}	24.61 ± 2.70^{a}	16.06 ± 2.42^{d}
	Wet weight	4.64 ± 0.05^{a}	2.99 ± 0.34^{b}	5.21 ± 0.81^{a}	4.64 ± 0.74^{a}	4.49 ± 0.41^{a}	4.54 ± 0.42^{a}	5.44 ± 1.59^{a}	5.45 ± 0.64^{a}	3.27 ± 0.56^{b}
Protein (%)	Dry weight	59.57 ± 0.17^{cd}	65.19 ± 1.14^{a}	62.78 ± 1.92^{abcd}	62.53 ± 2.86^{abcd}	64.45 ± 1.52^{ab}	63.82 ± 2.05^{abc}	60.85 ± 6.36^{bcd}	59.48 ± 2.29^{d}	66.65 ± 2.01^{a}
	Wet weight	12.38 ± 0.07^{cd}	12.37 ± 0.76^{d}	14.69 ± 0.86^{ab}	13.98 ± 0.52^{abc}	14.79 ± 0.48^{a}	14.55 ± 1.03^{ab}	13.51 ± 1.37^{bcd}	13.21 ± 1.19^{cd}	13.56 ± 0.71^{bcd}
Lipid (%)	Dry weight	6.13 ± 0.16^{ab}	6.85 ± 1.19^{a}	5.53 ± 0.37^{b}	5.89 ± 0.65^{b}	5.52 ± 0.49^{b}	5.67 ± 0.49^{b}	5.49 ± 0.88^{b}	5.86 ± 0.78^{b}	5.30 ± 0.78^{b}
	Wet weight	1.28 ± 0.05	1.30 ± 0.18	1.29 ± 0.14	1.32 ± 0.17	1.29 ± 0.14	1.29 ± 0.15	1.22 ± 0.2	1.3 ± 0.21	1.08 ± 0.16
Ash (%)	In dry weight	12 ± 0.33^{a}	12.20 ± 0.29^{a}	9.54 ± 0.65^{d}	10.92 ± 0.54^{b}	10.47 ± 0.47^{bc}	10.50 ± 0.30^{bc}	9.29 ± 0.34^{d}	10.05 ± 0.70^{c}	11.99 ± 0.52^{a}
	In wet weight	2.49 ± 0.01^{a}	2.32 ± 0.18^{ab}	2.24 ± 0.13^{bc}	2.44 ± 0.07^{ab}	2.4 ± 0.07^{ab}	2.39 ± 0.13^{ab}	2.07 ± 0.11^{c}	2.24 ± 0.28^{bc}	2.44 ± 0.13^{ab}
Moisture (wet weight)	–	79.21 ± 0.17^{ab}	81.03 ± 1.11^{a}	76.57 ± 1.66^{b}	77.62 ± 0.89^{b}	77.05 ± 0.70^{b}	77.22 ± 1.17^{b}	77.76 ± 0.93^{b}	77.79 ± 1.68^{b}	79.65 ± 0.90^{a}
Protein: Lipid ratio	–	$9.72^{b} \pm 0.30$	$9.63^{b} \pm 1.02$	$11.42^{ab} \pm 0.87$	$10.79^{ab} \pm 1.73$	$11.87^{ab} \pm 1.09$	$11.34^{ab} \pm 0.98$	$11.34^{ab} \pm 2.03$	$10.33^{b} \pm 1.45$	$12.87^{a} \pm 1.70$

Data represent means and standard deviation of three technical replicates ($n = 15$). Means with different superscripts (a, b, c) in each column are significantly different from Tukey post hoc tests ($p < 0.05$).

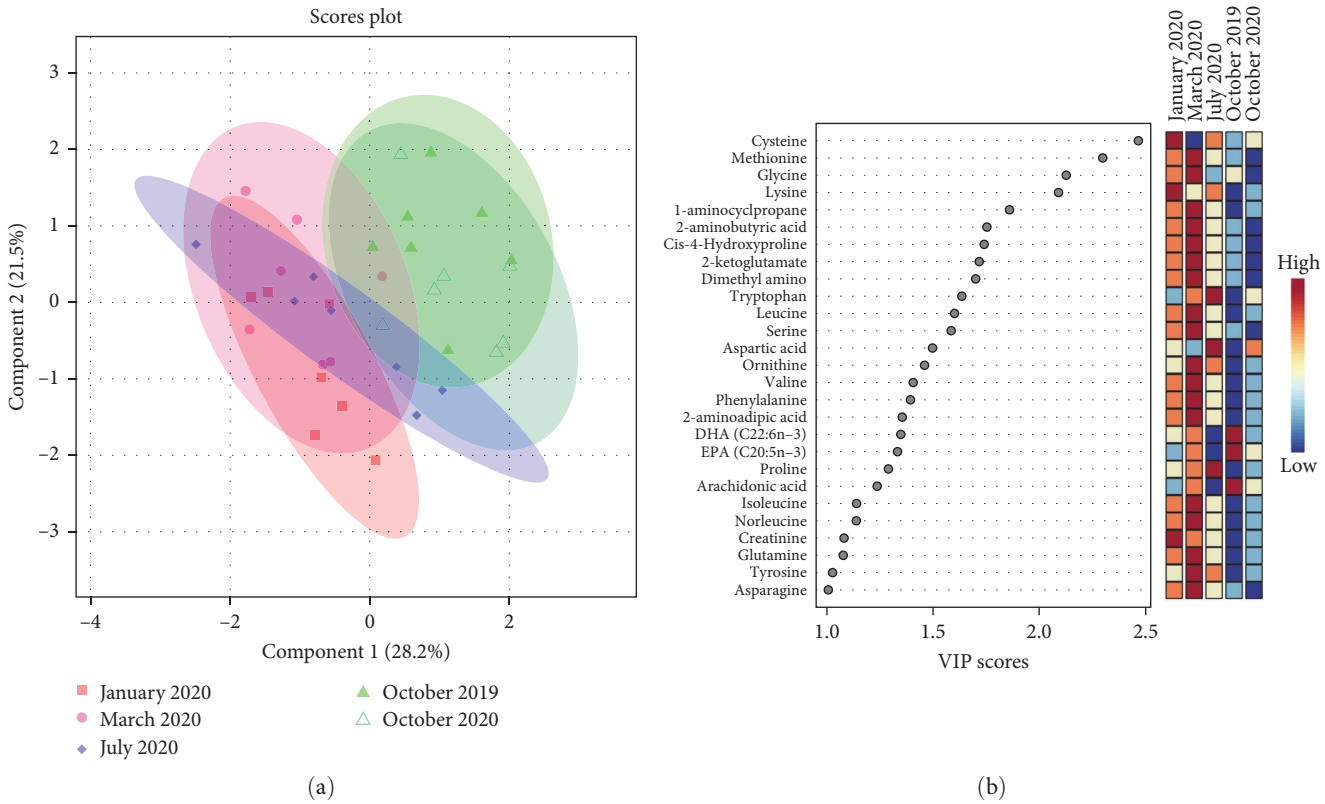

FIGURE 5: PLS-DA analysis of abalone metabolite profiles at different sampling times. (a) PLS-DA score plot. (b) List of 27 metabolites with PLS-DA VIP scores greater than 1.

metabolites in cluster C but had some differences with clusters A and B, respectively. Similarly, most metabolites in cluster C of the second sampling group (January 2020, March 2020, and July 2020) were similar between three sampling times, whereas metabolites in clusters A and B of March 2020 sampling were higher than those of January and July 2020 samplings. The January and July 2020 samplings shared similar levels of metabolites identified by one-way ANOVA. In contrast, the most obvious difference among sampling times was observed between October 2020 and July 2020, where most of metabolites were higher in July 2020 than in October 2020, with the exception of cysteine and aspartic acid.

4. Discussion

Growth variations revealed similar trends for *Haliotis iris* as other *Haliotis* species with better growth in warmer water temperatures compared with colder temperatures. Our study reported an overall SGR of 0.82% per day in 12-month trial with an SGR of 0.42% in winter and 0.74% in the beginning of summer. These values indicate that *Haliotis iris* in the North Island of New Zealand grow faster in summer compared with winter, following the trend documented for other abalone species [41]. However, previous studies differ which season produces the most rapid growth in abalone as the variation might be related mainly to microscopic food supply coming from ocean waters [42]. The SGR values in this study are higher than the ones reported by Allen et al. [5] in the same species with an overall SGR of 0.45%–0.51% and the SGR of 0.24% and 0.52% in winter and summer, respectively. This discrepancy in SGR between the two studies is expected as growth is a result of a multiplicity of factors including species, environment, and intrinsic characteristics of the animals.

Apart from determining the growth performance of *Haliotis iris* in a monthly basis, another objective of this project was to identify the proximate composition variation of juvenile *Haliotis iris* during 1 year of grow out. In general, many marine species show proximate composition variation relative to the season. These variations are influenced by factors such as availability of food, temperature of the water, differences in diet, and physical–chemical changes brought about by the maturity of the animal [43]. The results of this study indicate that proximate composition of *Haliotis iris* varies along the year with protein levels within the expected range for the species higher in summer than winter ($p < 0.05$), and ash, carbohydrate, and moisture levels without much variation among months. As protein levels are directly related to muscle building, their fluctuations affect the final product weight and monetary value, therefore its understanding becomes relevant to the abalone industry [3]. Significant differences in protein levels have only been reported in wild abalone [24] and farmed abalone [44, 45] when exposed to different water temperatures but not to different months of the year. The protein level differences presented here might be explained by better feed intake

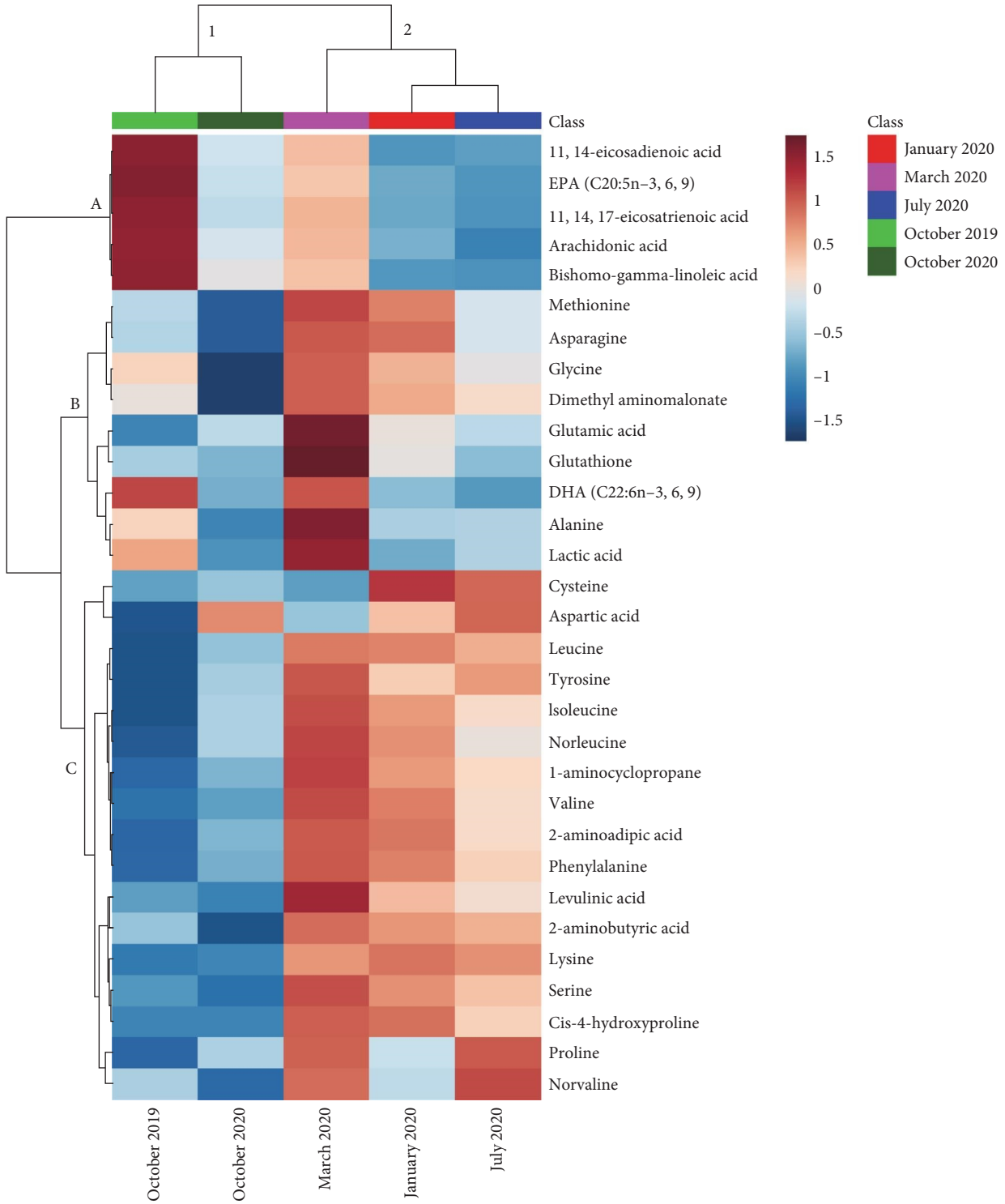

FIGURE 6: Heatmap of 31 metabolites of abalone muscle tissue that were different between five sampling times (one-way ANOVA, $p < 0.05$).

during warmer seasons compared with winter that leads to increase protein consumption and protein animal composition. This is possible as temperature is one of the limiting factors in feed intake [46]. For example, it has been observed that when temperature exceeds 26°C, *Haliotis laevigata* feed intake is substantially reduced and abalone severely stressed [47]. In addition, protein levels in the soft body of abalone is ascribed to be directly related to the dietary protein content when temperature remains the same [46]. Because only one commercial feed was used during the whole trial, this may suggest that the differences are attributable to water temperature, reproductive status, and age of abalone. Further studies to evaluate the influence of these factors on the nutritional composition of abalone are recommended.

The protein values found in this study mirror those of previous studies that have examined the effect of different feeds in proximate composition. Similar to our results, Tung and Alfaro [48] and Allen et al. [5] determined *Haliotis iris* protein levels between 10.6%–12.9% and 18.6%–19.3%, respectively. Hatae et al. [24] reported protein levels between 14.2% and18.4% in *Haliotis discus*.

While protein was the most significant nutrient varying during the year; moisture, ash, lipid, and carbohydrate showed differences without much variation among months. Seasonal variation of moisture might indicate that *Haliotis iris* possess higher values of moisture right before summer compared to other months. This trend has also been reported for other aquatic species, such as Atlantic Mackerel [43] and blue mussel [49]. Moisture is an important attribute for juiciness and determines marketability of abalone; therefore, the harvest of abalone before and during summer would be correlated with better sensory attributes. Significant moisture variations have been reported in abalone exposed to 20°C and 25°C in a laboratory setup showing higher levels in the latter [19] compared with lower temperatures; however, there are no studies outside the laboratory.

The ash content varied from 2.1% to 2.5% (wet weight) and was significantly lower in December 2019, June 2020, and July 2020 compared with other months. These results differ from previous studies documenting ash values of 1.4% [50], 1.1%–1.9% [24], and 1.8%–2.8% [31]. A possible explanation of the different mineral contents along the year might be the salinity fluctuations of coastal waters. Mineral content, most importantly sodium and potassium, increases when water salinity increases [51].

The lipid content variations were not significant across months (wet weight). These results are similar to those reported by Hatae et al. [24]. It has been documented that lipid levels significantly change in abalone meat when lipid or carbohydrates are modified in the diet [52, 53] or when temperatures are significantly low [54], but no reports have documented significant seasonal lipid variation [24].

4.1. Metabolomic Profile. Another objective of our study was to identify significant metabolic variations during the year of grow out. To our knowledge, previous studies have reported abalone metabolite profiles facing different stressors, such as heat and hypoxia [55], heat stress [56], and harvesting and transportation [35] but not seasonal variation in *Haliotis iris*. In this study, metabolomic profiling showed that only 31 metabolites were significantly different at four different sampling times (October 2019, January 2020, March 2020, July 2020, and October 2020). From these metabolites, proteinogenic and nonproteinogenic amino acids, fatty acids, and organic acids were identified.

FAAs in *Haliotis iris* have been mostly reported using non-GC–MS techniques such as liquid chromatography–mass spectrometry. So far, this is the first study evaluating the metabolomic profile of *Haliotis iris* in different seasons. Our study shows that many amino acids such as methionine, asparagine, glycine, cysteine, aspartic acid, leucine, tyrosine, isoleucine, norleucine, valine, phenylalanine, lysine, serine, and proline were higher in warmer months January 2020 and March 2020 compared with other months, whereas glutamic acid, glutathione, and alanine were higher in March 2020 and lower in the rest of the months. Cysteine, glutamic acid, glutathione, glycine, lysine, methionine, and serine belong to the glutathione metabolism pathway and are related to flavour and taste, which have been documented to increase when abalone is exposed to mild stressors, such as transportation [57]. Possibly, higher water temperatures might stress abalone's metabolism favouring the increased levels of flavour-related free amino acids which are an indicator of the best time for harvesting. From all metabolites contributing to flavour, glutamic acid is considered as the strongest, whereas glycine provides a fresh sweetness flavour. Both metabolites contribute to the *umami* flavour and sourness [58], and their levels have been documented to increase during summer in *Haliotis discus* [24], *Haliotis diversicolor* [31] and other mollusc species such as oysters [59].

This study documents the higher levels of valine, serine, and proline in summer compared with winter, which is similar to *Haliotis discus* [24]. Although more data introducing other variables are needed to establish a clear link between the season and the levels of free amino acids, warmer months produced higher fluctuations of amino acids related to flavour, tenderness, and taste. Decreased levels of amino acids during colder months might reflect amino acid breakdown and their usage for energy supply [7] due to high energy demands in winter [57]. Valine is an essential amino acid and its accumulation is associated with altered protein turnover, thermal stress [60]. Proline is a nonessential amino acid that accumulates in response to temperature variations and osmotic stress. In contrast, its reduced levels are seen when protein catabolism takes place for energy production [7]. In addition, proline, hydroxyproline, and glycine are the main degradation products of collagen and their increment during summer months January 2020 and March 2020 might indicate more tenderness of the meat. Based on a previous work by Hatae et al. [24] on *Haliotis discus*, collagen degradation products were higher in summer compared with winter, leading to a more tender carcass.

Other amino acids such as leucine, isoleucine, aspartic acid, tyrosine, and phenylalanine were found to increase in warmer months compared with colder. These compounds, which are mostly linked to osmoregulation, have been found to be elevated in thermal stress and hypoxia [34, 61, 62], transportation [57], and muscle damage [63], but to our knowledge no elevation has been reported in different seasons. From these amino acids, aspartic acid has been suggested to be an important biomarker of stress and health status in *Haliotis iris* [57]. However, its levels have not been found elevated in summer in our study suggesting that thermal stress did not occur during this period. To elaborate further in this premise, further research should be done to investigate the impact of seasons on abalone nutritional composition in studies longer than 1 year. Significant elevated free amino acids such as arginine, glycine, and a lesser quantity of methionine, glutamic acid, and alanine account normally for 81%–94% of the total free amino acids in abalone muscle [31]. Their main role is osmoregulation;

therefore, their levels fluctuate according to season [24], developmental stage [64], feed [13, 15], and culture setup [65, 66].

In our study, some key fatty acids such as EPA, DHA, and arachidonic acid (ARA) showed high levels in October 2019 but low levels in October 2020. The decreasing levels of these metabolites along the months are unclear and might not be directly related to the season. These changes can also be associated with many other factors such as developmental age of the animal [67], farm conditions (aquaculture setup), and muscle disposition preparing for maturity which were not controlled in this study. In terms of seasonality, previous studies have reported different results; for example, Su et al. [26] concluded that abalone had higher levels of n−3 PUFAs in winter compared with summer. However, a previous study performed by the same research group concluded no significant differences in these metabolites in Australian abalone during different seasons. The available evidence on fatty acid composition in abalone does not appear to be uniform as their levels might be linked not only to temperature fluctuations but also to diet composition [16], culture system [66], and life stage. Our findings agree with other studies concluding EPA, ARA, and DHA are the most relevant PUFAs in this species. In fact, abalone is a rich source of n−3 PUFAs, such as EPA, DHA, and docosapentaenoic acid (DPA) [26] and n−6 PUFAs, such as LA and ARA [68].

EPA and ARA, relevant n−3 PUFAs, for maintaining the membrane fluidity under lower temperatures in aquatic animals [69] were reduced during winter (July 2020) and after winter (October 2020). The decreased levels of EPA and ARA in our study might be related to the higher expenditure of these metabolites for abalone metabolism and its reduction on muscle concentration. As poikilotherm, abalone do not possess a temperature regulation system and therefore must rely on membrane protection for temperature adaptation [54]. ARA is the major PUFA found in *Haliotis discus* [68, 70] and *Haliotis rubra x Haliotis laevigata* [32], and it has been found to be higher close to winter and summer in the latter species [26]. These findings match with our results showing that ARA levels in *Halitis iris* are higher after summer (March 2020) and winter (October 2019) compared with the following months. Apart from temperature regulation, ARA plays a vital role in muscle metabolism opening up ATP-sensitive potassium channels to hyperpolarise and activate muscle contraction in abalone. The decreasing levels of EPA and ARA along the year might also suggest a better capability of biomembranes to cope with temperature fluctuations as animals mature. In addition, DHA levels decrease as animals age. DHA is commonly found in the lowest proportion in abalone [70], and this might be attributed to an adaptation to a low lipid diet such as seaweed, characteristic of aquatic herbivores [71]. DHA has found to be in low levels as abalone have low conversion rate from DPA to DHA [62].

5. Conclusions

In conclusion, this study provides evidence of significant variations of *Haliotis iris* in terms of growth performance, nutritional profile, and specific metabolites during summer and winter months. Protein composition was the most relevant macronutrient affected during the year of grow out, whereas carbohydrate, ash, and moisture showed less variation but significant. Lipid level variation was not significant across months. Significant metabolites, such as some amino acids related to flavour and tenderness, fatty acids related to membrane temperature regulation, and organic acids showed strong seasonal changes with higher levels mostly in summer compared with winter. Our findings suggest that abalone pass through metabolic and nutritional changes along 1 year of grow out and those variations are not exclusively associated with seasonal changes, as they are also affected by other factors that were not considered in this study such as temperature, feed availability, and developmental stage. The data obtained from this study provide a baseline information for further studies that envisage to evaluate longer effects of seasons on the nutritional status of *Haliotis iris*.

Data Availability

Data will be made available upon request.

Conflicts of Interest

The authors have no relevant financial or nonfinancial interests to disclose.

Authors' Contributions

Natalia Bullon played a supporting role in conceptualization and a lead role in data curation, formal analysis, investigation, methodology, project administration, validation, writing–original draft, and writing–review and editing. Seyedehsara Masoomi Dezfooli played a supporting role in sample processing. Tim Young played a supporting role in data curation. Ali Seyfoddin played a lead role in conceptualization, funding acquisition, and resources; a supporting role in supervision; and an equal role in writing–review and editing. Andrea C. Alfaro played an equal role in funding acquisition and writing–review and editing, a supporting role in resources, and a lead role in supervision.

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