FRDC
FISHERIES RESEARCH & DEVELOPMENT CORPORATION

Elucidating the nutritional requirements of farmed hybrid abalone

David S. Francis, Melissa M. Rocker, Kieren Searle, Abdul Lathiff Inamul Hassan, Erin E. Cumming, Timothy D. Clark, John A. Donald, Giovanni M. Turchini, Thomas S. Mock

December 2021

FRDC Project No **2017/113**

ISBN: 978-0-7300-0903-0

Elucidating the nutritional requirements of farmed hybrid abalone
2017/113

2021

Published by Deakin University 2021.

Researcher Contact Details

Name:	David S. Francis
Address:	Deakin University Queenscliff Marine Science Centre 2A Bellarine Highway, Queenscliff, VIC 3225
Phone:	03 5563 3083
Email:	d.francis@deakin.edu.au

FRDC Contact Details

Address:	25 Geils Court Deakin ACT 2600
Phone:	02 6122 2100
Email:	frdc@frdc.com.au
Web:	www.frdc.com.au

In submitting this report, the FRDC has agreed to Deakin University publishing the report in its edited form.

Contents

List of Tables

List of Figures

Acknowledgements

Funding for this project was provided by the Australian Abalone Growers Association, and the Fisheries Research and Development Corporation (FRDC) on behalf of the Australian Government. The support of the Australian Abalone Growers Association is gratefully acknowledged. The level of enthusiasm and encouragement made for a highly fruitful collaboration, adding significantly to the outcomes achieved. Significant in-kind support was 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. We thank Ridley AgriProducts for the supply of core dietary ingredients utilised for the manufacture of experimental diets.

Abbreviations

Abbreviation	Definition
AA	amino acid
AAGA	Australian Abalone Growers Association
ADC	apparent digestibility coefficient
ANOVA	analysis of variance
DHA	docosahexaenoic acid
DM	dry matter
E:S	enzyme to substrate ratio
ED	energy deposition
EER	energy efficiency ratio
EPA	eicosapentaenoic acid
FA	fatty acid
FCR	feed conversion ratio
FID	flame ionisation detector
FMOC	fluorenylmethyloxycarbonyl chloride
GIM	gastrointestinal model
HSD	honestly significant difference
HSP	heat shock protein
ICP-MS	inductivity-coupled plasma mass-spectrometry
K	condition factor
LC	long chain
MSEI	mass specific energy ingestion
MUFA	monounsaturated fatty acid
MWCO	molecular weight cut off
n-3	omega-3 fatty acid
n-3 LC PUFA	omega-3 long chain polyunsaturated fatty acid
n-3 PUFA	omega-3 polyunsaturated fatty acid
n-6	omega-6 fatty acid
n-6 LC PUFA	omega-6 long chain polyunsaturated fatty acid
n-6 PUFA	omega-6 polyunsaturated fatty acid
n-6/n-3	omega-6 to omega-3 ratio
NFE	nitrogen free extract
NMI	non-methylene interrupted
NuSea.Lab	The Nutrition and Seafood Laboratory
O:N	oxygen to nitrogen ratio
OPA	o-phthaldialdehyde
PC	principal component
PCA	principal component analysis
PD	protein deposition
PER	protein efficiency ratio
PUFA	polyunsaturated fatty acid
RP-HPLC	reverse phase high-performance liquid chromatography
SDA	specific dynamic action
SEM	standard error of the mean
SFA	saturated fatty acid
SGR	specific growth rate
SMR	standard metabolic rate
UV	ultraviolet

Executive Summary

The Australian abalone aquaculture industry continues to search for performance improvements in farmed hybrid abalone (*Haliotis laevigata* x *H. rubra*) as a means of satisfying the increasing consumer demand for this seafood delicacy. However, commercially relevant research is urgently required to optimise the composition of formulated feeds for hybrid abalone considering the industry's current dependence on formulations developed for other species. Importantly, the level of dietary protein still needs to be optimised specific to hybrid abalone life stage and environmental conditions. To this end, research led by Deakin University's Nutrition and Seafood Laboratory (NuSea.Lab) and the Australian Abalone Growers Association (AAGA), conducted a series of experiments which aimed to:

1) Characterise existing commercial feeds for hybrid abalone,

2) Identify an optimal dietary protein inclusion level for hybrid abalone,

3) Determine whether there is a metabolic cost to increasing the level of dietary protein, and

4) Investigate methodologies for rapid meal assessment.

This research project was the first in 25 years to comprehensively profile existing commercial feeds and found large differences between their nutritional composition, driven largely by geographical region and manufacturer. The crude proximate composition of existing diets broadly aligned with published requirements for Greenlip Abalone, but areas of improvement were identified with respect to their amino acid and fatty acid compositions. These results informed the experimental diets that were manufactured and trialled by Deakin University.

Growth trials on both sub-adult and juvenile hybrid abalone clearly showed that efficiencies in growth performance can be achieved by increasing the level of dietary protein from 35% to between 41-44%, with 41% providing arguably both the fastest and most efficient growth, thereby aligning with on-farm growth rate and weight gain data presented by (Stone et al., 2016). In the present experiments, the improved performance in abalone fed 41% dietary protein was supported by numerous performance parameters, including measurements of weight gain, growth rate, food conversion ratio, nutrient utilisation efficiency and nutrient digestibility. Also, it was shown that strategic increases in dietary protein inclusion was most effective during

periods of higher growth, where water temperatures ≥ 17 °C. Comparatively, at water temperatures of 12 °C, where abalone show extremely slow growth, a least cost approach to feeding is suggested. Analysis of fatty acid and amino acid compositions of abalone tissue showed that increasing the level of dietary protein had no negative effect on the nutritional composition of the abalone and therefore does not diminish the quality of the final commercial product, nor is it expected to affect consumer acceptance.

In depth metabolic assessments of both sub-adult and juvenile abalone overwhelmingly showed that increasing the level of dietary protein to ~40% does not increase oxygen consumption in hybrid abalone or reduce tolerance to oxidative stress caused by high water temperatures.

Rapid meal assessment using an *in vitro* gastro-intestinal method highlighted that such methods may not accurately reflect the slow and prolonged digestion that occurs in abalone. However, in conjunction with *in vivo* methods may aid in evaluating raw materials used in abalone feed formulations.

This research project provides clear evidence to Australian hybrid abalone producers and feed manufacturers that increases in growth performance can be achieved by changing the composition currently used diets, and that implementing these changes does not compromise the health status of the abalone during stressful summer growing conditions.

Keywords

Aquaculture, Australian hybrid abalone (*Haliotis laevigata* x *Haliotis rubra*), nutrition, growth performance, protein requirement, fatty acid, amino acid, metabolism, oxidative stress, in vitro gastrointestinal model, specific dynamic action

Introduction

Background

Globally, around 100 abalone species belonging to family haliotidae have been documented (Venter et al., 2018). As a renowned seafood delicacy, abalone fetches a high market price and is sold in different forms, including live, frozen and canned. However, declining wild abalone catch is a major industry concern (Cook, 2014 & 2016; Venter et al., 2018), where catches have declined from 15,000 mt in 1970 to 5,000 mt in 2016 due to overfishing, disease, poaching, ocean acidification, increased predation and habitat degradation (Cook, 2014 & 2016; Venter et al., 2018; FAO, 2018). Notably, during the same timeframe, abalone aquaculture production has grown substantially to reach 153,500 mt in 2016 from nearly zero production in 1970 (FAO, 2018). These trends reveal that the abalone aquaculture sector has been undergoing a period of continuous growth and proving to be a viable option to cater for growing global demand. Like other aquaculture sectors, China represents the largest producer and exporter of cultured abalone. Other major exporting countries include Australia and South Korea, while Hong Kong, Japan and Singapore are the leading abalone importers (FAO, 2018).

Australia is projected to increase its current abalone production of 757 tonnes (Mobsby et al., 2018; FAO, 2018) by three fold over the next decade (AAGA *pers. comm.*). Australian abalone aquaculture is dominated by two haliotid species, the naturally occurring Greenlip Abalone (*H. laevigata* Donovan) and an interspecies hybrid abalone of the Greenlip and Blacklip Abalone (*H. laevigata* Donovan x *H. rubra* Leach). The historical focus of both the wild-catch fishery and aquaculture industries has generated a significant body of literature relating to the Greenlip abalone. Conversely, relatively little published literature exists focusing on the Greenlip x Blacklip Ablone hybrid, or hybridism amongst haliotids in general. In Australia, abalone are cultured in flow through aquaculture systems and culture practices are well established. However, Australian abalone aquaculture is characterized by a prolonged culture period, slow and variable growth (Day & Fleming, 1992), and summer mortality (Vandepeer, 2006; Stone et al., 2014) which has the potential to lower the profit margin (Huchette et al., 2003).

Australian abalone grow-out aquaculture solely depends on formulated feeds to satisfy nutritional requirements. Thus, optimization of formulated feeds is crucial to achieving

economically viable growth. Feed is a major operational expense in abalone aquaculture and accounts for 30% of the total operational cost (Stone et al., 2014). Therefore, minor improvements in feed formulations have the potential to bring significant economic benefit to abalone farmers. This has been demonstrated previously via a successful series of in-depth nutritional studies focussing on Australian Greenlip and Blacklip abalone, that resulted in feed cost reductions and improved growth and health condition for these species (Fleming et al., 1996; Coote, 1998; Dunstan et al., 2001; Vandepeer, 2005 & 2006; Stone et al., 2013, 2014 & 2016), urging industry to optimize the feed formulation specific to Australian hybrid abalone.

Protein content of the formulated feed should be neither lower nor greater than an abalone's actual requirement. A lower protein level can limit growth and promote fatty tissue buildup (Britz & Hecht, 1997). Similarly, when protein is high, it may affect the nutrient balance of the feed by displacing other key nutrients which can then result in reduced abalone growth (Mercer et al. 1993; Coote et al., 2000). Further, it can result in the use of protein for energy fulfillment, reduced protein utilization (Britz, 1996; Britz and Hecht, 1997; Gómez-Montes et al., 2003), water quality deterioration and increased feed cost (Coote et al., 2000). Therefore, feed containing excess protein does not favor growth, but rather, increases the cost of production (Stone et al., 2013). These facts demand the need for optimizing dietary protein levels to minimize the negative consequences associated with low or excess dietary protein inclusion, whilst achieving maximum growth.

Abalone protein requirement is greatly influenced by species, size, environmental conditions (especially temperature), dietary protein source and digestibility, the protein to energy ratio of the feed, and feeding frequency (Coote et al., 2000; Tung and Alfaro, 2011). Likewise, requirements are also known to vary with sex and to a lesser extent, genotype (Coote et al., 2000). An analysis of abalone wild feed preference at various life stages revealed that they initially feed on microalgae containing 12-35% crude protein (Brown et al., 1997) and later, during the sub adult stage, they feed on macroalgae containing comparatively lower protein ranging 11-19% crude protein (dry matter basis) (Mai et al., 1994). However, higher soft tissue protein deposition was observed with increasing abalone size in different species (Knauer et al., 1994; King et al., 1996). The experimental findings relating to the abalone size dependent optimal protein requirement seems unclear. Stone et al. (2013) describes a slightly higher optimal protein requirement for smaller size abalone, suggesting 29% and 24% dietary protein

for one-year old and two-year old Greenlip Abalone, respectively, when reared at 14 °C. Yet differences in protein requirement were minimal at 22°C. In contrast, nutritional studies with South African Abalone (*Haliotis midae*) revealed higher optimal protein requirements (44%) for larger size abalone (7-14 g) over smaller cohorts (0.2-1 g, 34% dietary protein) (Britz & Hecht, 1997). Given these contrasting observations, investigating different size cohorts on similar dietary protein inclusion levels over lengthened experimental periods may disclose more conclusive evidence to support size specific protein requirement.

As poikilothermic aquatic animals, temperature is a key environmental factor for abalone, greatly influencing physiological functions related to feed intake, metabolism and growth (García-Esquivel et al., 2007; Kaushik, 1986). Improved abalone growth performance and survival in response to increasing temperature has been established previously in different abalone species, resulting in increased feed consumption and metabolism (Britz et al., 1997; Stone et al., 2013; Bansemer et al., 2015), altered digestive physiology and morphology (Schaefer et al., 2013), and increased gut enzyme activity (Edwards & Condon, 2001; Bansemer et al., 2016). However, increasing temperature impacts nutrient digestibility and absorption via a reduction in feed residence time in the gut (Bansemer et al., 2016; Currie et al., 2015; Kaushik, 1986). Further, when temperature increases above its optima, there is a reduction in feed intake and negative impacts on metabolic rate. This positive growth occurs only within an optimal temperature range which is species (Gilroy & Edwards, 1998) and size (Steinarsson & Imsland, 2003; Searle et al., 2006) specific. As the physiology and energetics of abalone change with temperature, it is reasonable to suggest that their nutritional requirements may change also (Green et al., 2011). Therefore, there is a need to optimize dietary protein levels to match temperature specific requirements. This is most applicable during extreme conditions (winter and summer) with a view of maximizing protein deposition in soft tissue, ultimately improving growth and reducing the lengthy duration of abalone culture.

Growths trial on juvenile Greenlip Abalone with varying protein levels (27, 30, 33 and 36%) at three different temperatures (14, 17 and 20 °C) revealed that growth significantly increased with temperature, but growth rate was not significantly different between dietary protein levels at each of the tested temperatures. However, at 20 °C feed consumption was reduced in dietary treatments containing higher protein levels. Subsequently, a dietary protein level of 35% protein was suggested at high temperatures and 29% at 14 and 17 °C in consideration of

improved feed conversion ratios (FCR) (Bansemer et al., 2015). Similarly, an increased dietary protein level was recommended for one-year (29 to 35% crude protein) and two-year old (24 to 34% crude protein) Greenlip Abalone when temperature increases from 14 to 22 °C considering increased protein deposition in both age groups (Stone et al., 2013). It is important to note that both studies recommended increasing dietary protein requirement with temperature despite finding significant temperature and protein interactions. As both studies were conducted over a relatively short duration (91 and 84 days in Bansemer et al., 2015 & Stone et al., 2013 respectively), the slow and variable growth nature of abalone resulted in insufficient growth to detect any significant dietary protein effects. Therefore, extending the culture duration to obtain sufficient growth may reveal significant temperature and dietary protein interactions.

The desired quantity of dietary protein in abalone formulated feed is achieved using range of protein sources of varying qualities, where the protein content, amino acid profile and subsequent digestibility is taken into consideration (Fleming et al., 1996). The commonly used protein sources in formulated diets for Australian abalone include fishmeal, casein and soybean meal (Vandepeer, 2005). However, the expensive nature, finite availability and limited local production of these ingredients (Vandepeer, 2005) have prompted the utilization of alternative protein sources including various soy and vegetable-based meal isolates.

The performance of hybrid abalone in culture is superior to that of Greenlip Abalone in relation to growth, meat yield and feed utilisation (Freeman, 2001; Guo, 2009; Mateos et al., 2010; Lafarga de la Cruz & Gallardo-esca, 2011). Further, comparatively higher feeding and nutrient intake rates have been observed in Australian hybrid abalone over Greenlip Abalone with formulated and macroalgae feed sources at different rearing temperatures (Currie et al. 2015). Notably, 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 (Stone et al., 2016). As such, there is scope for improvement of Australian hybrid abalone performance via species, temperature and size specific nutritional intervention. However, detailed information relating to the nutritional requirements of farmed Australian hybrid abalone is currently scarce to non-existent.

Poor nutrition will be reflected in physiological parameters such as respiration and excretion rates, as the animals require more oxygen and will produce more waste products from such synthesis processes. These processes can be directly measured by monitoring the specific dynamic action (SDA) associated with the digestion and assimilation of food (Jobling, 1981; McCue, 2006). SDA quantification involves measuring the rise in oxygen consumption that occurs post-feed ingestion and has been used extensively in fish (Fu et al., 2005; Jobling, 1981; LeGrow & Beamish, 1986) and invertebrates such as prawns (Du & Niu, 2002), crabs (McGaw & Reiber, 2000) and mussels (Gaffney & Diehl, 1986). Several studies have demonstrated an increase in oxygen consumption following feeding in abalone (Gaty & Wilson, 1986; Lyon 1996), while Barkai and Griffiths (1987) found no difference between freshly collected and starved animals. Few have attempted to quantify SDA of feeding in abalone. Excretion of nitrogenous wastes (principally ammonia in abalone) can be used to calculate energetic expenditure on excretion (Farías et al., 2003; Lopez & Tyler, 2006; Peck et al., 1987). More importantly, nitrogen excretion rates can be used in conjunction with oxygen consumption as an Oxygen to Nitrogen ratio (O:N) for monitoring the relative proportion of proteins and carbohydrates being catabolised. Using O:N, Romo et al., (2010); Vosloo and Vosloo (2010) demonstrated that protein catabolism dominates in abalone facing stress induced by rapid temperature or salinity fluctuations. This combination of SDA measurement and O:N ratio clearly has application is assessing the suitability of dietary protein inclusion as well as monitoring how the animals utilise protein when responding to stress.

Abalone possess several methods for coping with environmental stressors such as increased temperature. Initially, abalone can utilise a substantial capacity for anaerobic glycolysis (Donovan et al., 1999; Donovan & Taylor, 2008) when oxygen uptake across the gills is insufficient to meet increased metabolic demands. A longer-term response involves the synthesis of protein chaperones called heat shock proteins (HSPs) (Drew et al., 2001; Farcy et al., 2007). Both strategies are energetically costly. Anaerobic glycolysis is inherently inefficient and requires extended recovery from an oxygen debt (Donovan & Taylor, 2008) while synthesising heat shock proteins (HSP) is resource and energy intensive (Sokolova et al., 2012). According to dynamic energy budget theory, energy tied up in digestion related respiratory and excretory functions stemming from poor nutrition is energy that cannot be diverted to processes aimed at conserving homeostasis during stress (Kooijman, 2010; Sokolova, et al.

2012). Monitoring the distribution of energy to physiological processes may shed light on how diet composition can maximise efficiency of resource allocation. Furthermore, combining manipulation of nutritional inputs with the manipulation of stress factors may reveal new methods of dietary intervention to improve stress tolerance and improve periods of sub-optimal growth.

Need

The farming of quality Australian abalone is a profitable industry, producing an estimated total ~1,000 tonnes live weight with a value of $35 million in the financial year ending July 2017. Notably, over the next 10 years, abalone production is forecast to increase by > 300% to ~3,600 tonnes with an estimated total value of ~$120 million based on current day market prices. However, to facilitate this growth, detailed knowledge pertaining to the nutritional requirements of farmed abalone species is paramount. Currently, hybrid abalone culture is carried out via the provision of feeds developed specifically for Greenlip Abalone. While these feeds promote good growth and survival in both species, it is considered that the ideal protein requirements of abalone may vary in relation to temperature (season), age (stage of growth) and species (Greenlip vs. hybrid). The issue is further exacerbated by varying and site-specific environmental conditions; especially high summer temperatures, causing incidents of elevated mortality and low winter temperatures suppressing growth. As such, on-farm performance of hybrid abalone has significant scope for improvement via nutritional intervention. Carefully planned and targeted RD&E effort that builds on the nutritional knowledge amassed for Greenlip Abalone therefore has the capacity to make rapid steps in relation to the productivity of the hybrid abalone aquaculture industry. Those gains are in turn expected to be transferable back to Greenlip Aquaculture.

The Australian Abalone Growers Association (AAGA) identified ‘Nutrition’ as an RD&E investment priority in its 2015-2020 Strategic Plan, with a Strategic Goal to ‘Implement a Nutrition Program for Health, Survivorship and Meat Weight Gain’. This project will assist industry in achieving its projected growth within the timeframe of AAGA’s Strategic Plan by developing formulated feeds that are tailored to the major seasonal trends experienced by the abalone farming industry. Depending on the differences found in nutritional requirements

this may result in the development of age/size-specific, temperature/season-specific and/or species-specific diets.

Objectives

This project sets out to achieve four key objectives towards advancing our knowledge on the nutritional requirements of farmed hybrid abalone and improving on-farm performance during inclement environmental conditions:

1. Explore the key nutritional requirements of farmed hybrid abalone (*Haliotis laevigata* x *H. rubra*) with respect to stage of growth and environmental rearing temperature through a comprehensive nutritional profiling of available commercial abalone feeds (sourced domestically and internationally).

2. Profile the nutritional characteristics of commercially available abalone aquafeeds towards improved hybrid abalone feed formulations through two long-term growth trials at three relevant rearing temperatures (i.e., 12, 17 and 22°C) evaluating protein inclusion levels (32-44%) in sub-adult hybrid abalone (Task 2.1) and graded protein inclusion levels (35-47%) in juvenile hybrid abalone (Task 3.1).

3. Understand the impacts of nutritional conditioning on the survival of sub-adult (Task 2.2) and juvenile (Task 3.2) abalone in response to adverse temperature conditions.

4. Development of rapid screening assays for diet digestibility and on-farm performance assessment.

Task 1. Comprehensive nutritional profiling of available commercial abalone feeds

Introduction

The development of commercial feeds for abalone is relatively recent, with formal research commencing in Japan in the late 1980's (Fleming et al., 1996). Since then, rising consumer demand for abalone products and subsequent increased abalone production has coincided with global nutritional development. This has resulted in commercial diets better optimised for specific abalone species and growing conditions. Yet despite this, anecdotal evidence from farmers suggests the need and desire to further improve formulations to maximise growth and product quality (Mark Gervis, Southern Ocean Mariculture *pers. comm.*). Current commercial dietary formulations differ significantly dependent on production region and the species farmed, yet such differences are not necessarily related to the nutritional requirement of the farmed species. The availability, and relatedly, the cost of various raw materials in different regions influences commercially competitive least cost dietary formulations. For example, diets manufactured in China and Japan typically contain greater amounts of locally produced seaweed given its high availability and relative low cost (Mulvaney, 2016). Further, disparity between dietary formulations can arise due to the various approaches toward ingredient selection and manufacture method utilised by different research groups to develop artificial diets. It has now been 25 years since Fleming et al.,, (1996) conducted a thorough review of available commercial abalone diets, therefore, an updated assessment of the current status of commercial feeds used in abalone aquaculture is long overdue, especially given the strides made in determining the nutrient requirements of various abalone species in recent years.

A popular approach used to determine the optimal dietary nutritional profile for farmed abalone has previously involved matching the nutritional profile of the animals' soft tissue, particularly with respect to the amino acid composition (Fleming et al., 1996; Gorfine, 1991; King et al., 1996). Therefore, there is value in synthesising the nutritional profiles of farmed abalone in conjunction with the nutritional profiles of commercial diets. Typically, a close match between the dietary and soft tissue nutritional profile is considered an indication that the nutritional demand of the animal will be met. Synthesising and quantifying nutritional

data, from both diets and soft tissue, however, presents unique challenges given the varied approaches used by research groups to present nutritional data (Fleming et al., 1996; Mock et al., 2020). Consequently, there is a necessity to carefully select comparative methods, and data standardisation techniques (e.g., unit conversions) on the extracted data.

As a matter of due diligence, and in response to the needs of abalone farmers, a quantitative review of the analysed chemical composition of currently available commercial abalone diets from Australia and overseas, as well as published nutritional profiles of abalone diets and tissue published in the literature, was conducted. Taken together, this is expected to serve as an invaluable point of reference and platform for further nutritional optimisation/ refinement in subsequent feeding experiments.

Methods

Sample collection

Seventeen commercially compounded abalone feeds were collected from abalone farming regions globally and subjected to comprehensive nutritional profiling to characterise and benchmark the nutritional composition of commercial formulations. Diets were analysed for proximate composition (moisture, protein, lipid, ash and nitrogen free extract) alongside a detailed analysis of their fatty acid and amino acid profiles. Each of these parameters was determined in the dedicated nutrition laboratories of The Nutrition and Seafood Laboratory (NuSea.Lab), Deakin University, School of Life and Environmental Sciences, using standard published analytical procedures. Further, the nutritional profiles of both abalone tissue and diets were identified in the published literature using a combination of key words and search techniques including Web of Knowledge® and Google Scholar®. Data were only extracted and used in the subsequent statistical analysis if complete, or near complete, fatty acid and amino acid profiles were reported. Specifically, diet, wild abalone and farmed abalone soft tissue fatty acid data from Dunstan et al., (1996), farmed soft tissue fatty acid data from Guest et al., (2008) and amino acid data of both wild and farmed abalone from Fleming et al., (1996), Coote et al., (2000) and Daume et al., (2003) were extracted and used in the subsequent analysis.

Proximate and biochemical analyses

The proximate composition of all diets and abalone were determined according to standard published analytical procedures and methods of NuSea.Lab, Deakin University (detailed in (Mock et al., 2019; Rocker et al., 2021). Moisture content was calculated by drying samples in an oven at 80°C to constant weight. Crude protein was measured in an automated Kjeltec 2300 (Tecator, Sweden) as Kjeldahl nitrogen (N × 6.25). Lipid concentration was determined by dichloromethane:methanol (2:1) cold extraction (Folch et al., 1957). Ash content was determined by incinerating samples in a muffle furnace (S.E.M., Australia) at 550°C. Nitrogen free extract (NFE) was calculated by mass difference. Total energy was calculated on the basis of the established combustion enthalpies of 23.6 kJ g^{-1}, 39.5 kJ g^{-1}, and 17.2 kJ g^{-1} for protein, lipid, and carbohydrate, respectively.

Amino acid composition analysis

Amino acid composition was determined using reverse phase high-performance liquid chromatography (RP-HPLC) (1260 Agilent infinity II series systems, Agilent Technologies, Santa Clara, USA). Samples were initially acid hydrolysed using 6 M HCl for 22 hours, followed by derivatisation using o-phthaldialdehyde (OPA) and fluorenylmethyloxycarbonyl chloride (FMOC) as described in detail by Lewis et al., (2019). Amino acid peaks were identified relative to known external standards injected at five concentrations to facilitate an accurate quantification.

Fatty acid composition analysis

Following lipid extraction, total lipid extracts from the samples were reconstituted in 1.0 ml of dichloromethane:methanol (MeOH) (2:1) for saponification. Two mL of 5% potassium hydroxide (KOH) in 80/20 MeOH/H_2O water was added and samples were heated to 60 °C for 3 h. After cooling, 1.0 mL MilliQ water was added. Samples were purified three times with the addition of 1.8 mL 4:1 hexane:$CHCl_3$ (DCM), a 10 s vortex, centrifugation for 3 min at 1000 rpm, and collection of the aqueous MeOH/H_2O extract. The aqueous MeOH/H_2O extracts were methylated at 100 °C for 1 h with 2.0 mL acetyl chloride:methanol (1:10) after addition of 100 µL internal standard C23:0 (0.75 mg mL^{-1}). Two ml of potassium carbonate (1 M) and 1.7 mL hexane were added, and samples were vortexed for 10 s then centrifuged at 1000 rpm for 3

min at room temperature. Fatty acids (FA) were analysed with gas chromatography—FID (Agilent Technologies 7890A, USA) equipped with a DB23 capillary column (30 m, 0.25 mm internal diameter, 0.25 µm film thickness; SGE Analytical Science, Ringwood, Victoria, Australia), a flame ionisation detector (FID), an Agilent Technologies 7693 autosampler injector and a split injection system (split ratio 50:1) following Francis & Turchini (2017). Areas of resulting individual FA peaks were corrected by theoretical relative FID response factors Ackman (2002) and identified and quantified against known external standards (mixed and individual standards from Sigma-Aldrich, Inc., St. Louis, USA and NuChek Prep Inc., Elysian, USA) using GC ChemStation software (Agilent Technologies, USA). FA concentration was standardised to weight of total lipid concentration and expressed as mg FA g $diet^{-1}$ for quantitative comparisons. Individual FA concentrations, sums of FA classes, FA ratios, and percentage composition of FA were calculated.

Statistical analyses

All statistical analyses were performed using R (Version 3.5.3, R Core Team 2019). Proximate composition, amino acid composition and fatty acid composition were reported as g kg^{-1} of diet and mg g^{-1} diet, respectively. For literature data, percentage fatty acids were converted into mg g^{-1} lipid values using a diet conversion ratio of 0.8 and an abalone soft tissues conversion ratio of 0.6. Trends in relative amino acid and fatty acid percentage composition were characterised with principal component analyses (PCA). Score plots explore the trends of samples within the data set and loading plots reveal the respective loadings of amino acids or fatty acids influencing the samples. Samples with similar compositions are located in the same general areas on the score plots.

Results and Discussion

Seventeen commercially available abalone diets were analysed for their proximate (moisture, protein, lipid, ash and nitrogen free extract) composition and energy content, amino acid composition and fatty acid composition (**Table 1**). Thirteen of the diets originated internationally (AB1-AB13) (eleven from China, one from South Africa and one from Taiwan), whilst four were of domestic (Australia) origin (AB14-AB17). Notably, the Chinese diets were received in powdered form and the South African diet was delivered in characteristic leaf-shaped pellets.

In relation to crude protein concentration, two of the sampled diets (AB14 and AB17) were manufactured above the target set for Greenlip Abalone based on the results of previous nutritional investigations [35% crude protein; (Stone et al., 2014a)]. However, it is also noteworthy to highlight the low protein concentration apparent in AB15, which falls below the current recommendation for Greenlip Abalone, aligning more closely with the values adopted prior to the work of Stone et al., (2014a). In relation to the crude composition of the majority of international abalone feeds (AB1-AB13), the crude protein composition also falls around the 350 g kg^{-1} mark, despite these diets being manufactured for different abalone species. This potentially points towards similar requirements for crude protein for international species, or alternatively and more likely, a lack of information regarding the nutritional requirements for these species, necessitating the adoption of formulations designed and published for similar proxy species (e.g., *H. laevigata*). Of all the profiled diets, the largest differences were noted in the international feeds with regard to their ash concentrations. These varied from 250-288 g kg $diet^{-1}$ and were markedly higher than the values obtained for the domestic formulations (50-88 g kg $diet^{-1}$). These values quite likely relate to the dietary inclusion of dried seaweed, reported anecdotally (Nick Savva *pers. comm.*) and further confirmed by the aroma of the diet and the level of observed pigment of the solvent extracted lipid fraction.

Differences between the composition of commercial diets and abalone soft tissue are visualised using the fatty acid profiles of seventeen commercial diets and published accounts of both wild abalone species (*H. laevigata* and *H. rubra*) and farmed abalone (*H. laevigata* and *H. laevigata* x *H. rubra*) (Dunstan et al., 1996; Guest et al., 2008) (**Figure 1**). The distance

between the samples on the scatterplot (**Figure 1a**) indicates the degree of similarity. As such, tight clustering of points indicates a high degree of similarity. The clustering of the diets versus the tight clustering of the abalone tissue samples are highlighted by colour (yellow and purple, respectively). Statistically speaking, considerable distance separates diet and the animal samples, indicating differences in fatty acid profiles. The individual fatty acids driving the clustering of samples are visualised in the loading plot (**Figure 1b**). The colour and length of arrows indicate the strength of each fatty acid's influence on the clustering.

Our analyses indicate a disconnect in the composition of analysed feeds and abalone flesh profiles with respect to key fatty acids (as indicated by the discrete clustering of these groups) (**Figure 1**). As per expectation, all diets were dominated by triacylglycerols despite evidence suggesting that the lipid class profile of abalone muscle is rich in polar lipids (data not reported), pointing to the importance of the source of the dietary lipid. Considering the benefits imparted by fatty acids from both a growth and health promoting perspective, potential gains may be possible by paying greater attention to the dietary lipid profile in combination with the crude concentration of protein provided. In **Figure 1**, commercial diet samples are grouped on the negative side of the x-axis which explains 47.5% of the variation in the data and is largely influenced by the fatty acids on the negative side of the x-axis, specifically 18:3n-3, 18:2n-6, and 18:1n-9. These shorter chain fatty acids are recognised as being of terrestrial origin, in this case likely derived from the inclusion of protein sources such as soybean meal in formulations. Notably, the key fatty acid separating the AB12, AB10, AB9 and AB5 diets from the others (negative x-axis and positive y-axis) is DHA (22:6n-3, docosahexaenoic acid), widely recognised as a health promoting fatty acid of marine origin. Following recent discussions with the manufacturer of the AB12 product, this can be attributed to the large fishmeal component incorporated into the dietary formulation. The most prominent fatty acids separating out the abalone samples were 20:4n-6, 22:5n-3 and 22:2NMI. The former two FA are both documented to play important roles in immune response and easily sourced for incorporation into aquafeeds, while 22:2NMI (non-methylene-interrupted dienoic fatty acid) is known to be biosynthesised *de novo* by the animal itself and may be an alternative/complement to essential PUFA in the lipid membranes of molluscs (Barnathan, 2009; Pernet et al., 2007).

Commercial diets and published accounts of *Haliotis spp.* both farmed (*H. laevigata* and *H. rubra*) and wild (*H. iris*, *H. midae*, *H. rubra* and *H. rufescens*) (Coote et al., 2000; Daume et al., 2003; Fleming et al., 1996) are also different when visualised by amino acid composition (**Figure 2**). Our analyses indicate minimal differences in the amino acid composition of analysed feeds profiles with respect to the essential amino acids (as indicated by the two discrete clusters of international and domestic diets). The tight clustering of the abalone diets is visualised in yellow on the positive side of the x-axis with domestic diets along the negative y-axis and international diets along the positive y-axis. However, there is a high level of variation in the abalone flesh profiles indicated by the large spread of abalone samples on the negative side of the x-axis (**Figure 2a**). Statistically, there is considerable distance between the diets and the animal samples (on the negative side of the x-axis), showing that their amino acid profiles are different. Furthermore, alanine, threonine and glycine are driving the differentiation between the abalone animal samples and the diets (**Figure 2b**).

Taken together, the results of this analysis allude to several areas of potential improvement concerning the qualitative aspects of abalone diet composition. Whilst crude macronutrient concentrations (particularly protein and lipid) generally aligned with the documented requirements established for species such as Greenlip Abalone, alterations to ingredient profiles to better reflect those of wild abalone could provide benefit from growth and health angles. Notably, whilst the composition of individual amino acids aligned between the sampled diets and wild abalone from a presence absence perspective, further refinement of diets to better reflect wild abalone from a quantitative g kg^{-1} diet point of view is required. Likewise, similar efficiencies could be realised by placing a focus on considerations around the fatty acid composition, particularly with respect to the discrepancies between the concentrations of n-6 and n-3 LC PUFA which were notably lower on average in the sampled diets compared to the profiles evident in wild sampled animals.

However, at the request of AAGA, the need to adhere as close as possible to current-day commercial feed formulations was identified as a priority with a view of facilitating the comparison of results stemming from the subsequent growth experiments and those generated previously on-farm or in a research setting. As such, the findings and suggestions generated by **Task 1** and **Task 2** will form the basis of future investigations.

Table 1: Proximate composition, amino acids and fatty acids of commercial abalone diets.

	AB1	AB2	AB3	AB4	AB5	AB6	AB7	AB8	AB9	AB10	AB11	AB12	AB13	AB14	AB15	AB16	AB17
Proximate composition (g kg^{-1} diet)																	
Moisture	54.8	52.6	57.7	57.4	56.3	53.4	60.4	55.2	52.5	53.9	54.9	66.7	70.1	94.6	94.6	77.2	69.0
Protein	354.9	352.0	347.4	352.7	339.8	344.5	354.5	338.3	385.0	340.2	349.8	339.8	292.0	377.3	263.9	350.7	397.2
Lipid	42.8	32.5	27.9	26.1	29.9	29.5	27.5	26.1	32.9	37.5	30.4	21.6	46.8	25.1	29.9	42.0	43.0
Ash	262.6	272.8	246.5	250.2	288.4	260.5	255.0	257.7	250.3	258.5	263.3	69.8	88.3	50.6	57.2	55.9	73.4
NFE	284.8	290.1	320.5	313.6	285.6	312.1	302.6	322.7	279.2	309.9	301.5	502.0	502.8	452.4	554.4	474.3	417.4
Energy (MJ kg^{-1})	15.0	14.6	14.8	14.7	14.1	14.7	14.7	14.6	15.2	14.8	14.6	17.5	17.4	17.7	16.9	18.1	18.3
	AB1	**AB2**	**AB3**	**AB4**	**AB5**	**AB6**	**AB7**	**AB8**	**AB9**	**AB10**	**AB11**	**AB12**	**AB13**	**AB14**	**AB15**	**AB16**	**AB17**
Amino Acid composition (mg g^{-1} diet)																	
Histidine	6.8	6.9	6.9	7.1	7.2	7.0	7.1	6.8	8.2	8.3	7.2	7.9	7.5	9.6	6.8	9.5	8.6
Serine	14.4	14.3	14.3	14.9	14.9	14.7	15.1	14.8	18.1	17.3	15.2	14.5	13.9	17.7	13.0	17.9	18.2
Arginine	21.3	21.7	21.3	22.4	20.9	21.9	22.3	21.5	22.6	21.6	22.2	18.3	14.4	24.2	15.2	28.8	23.8
Glycine	17.8	18.2	17.8	18.5	19.3	18.3	18.5	18.8	19.0	17.8	19.0	17.7	12.8	16.1	12.6	16.6	15.5
Aspartic acid	32.5	32.5	32.2	32.9	33.8	35.1	32.9	32.6	38.0	36.5	35.2	31.3	21.4	32.6	24.3	35.5	32.9
Glutamic acid	52.3	52.9	52.4	53.3	52.1	54.7	52.7	50.9	59.2	56.5	55.4	49.4	70.7	78.9	59.3	71.1	82.4
Threonine	12.2	12.3	12.1	12.8	13.3	12.7	13.0	13.2	16.3	15.7	13.0	13.9	10.3	12.7	9.4	13.7	13.0
Alanine	16.9	17.0	16.9	17.3	17.7	18.0	17.7	18.9	21.3	20.7	18.0	19.4	12.2	14.8	10.9	14.4	13.4
Proline	15.4	15.6	15.5	15.9	16.5	15.6	15.8	15.6	18.5	17.3	16.2	16.2	19.1	22.6	15.4	18.6	21.3
Lysine	18.2	18.2	18.0	18.3	19.5	19.0	18.3	18.3	22.6	21.7	19.2	21.0	13.4	16.4	13.8	15.3	13.9
Tyrosine	8.9	9.3	8.9	9.2	9.1	9.2	9.3	9.0	11.4	10.5	8.9	6.6	7.0	8.6	4.7	7.7	9.1
Methionine	5.4	5.6	5.4	5.2	5.3	5.3	5.3	4.7	5.1	4.7	4.8	6.0	4.3	4.9	3.3	4.6	3.9
Valine	15.6	15.9	15.8	16.4	16.6	16.4	16.5	16.9	19.9	19.4	16.1	17.4	11.9	15.4	11.2	14.8	14.9
Isoleucine	14.3	14.4	14.4	14.8	14.7	14.7	14.8	14.6	17.4	16.9	14.5	15.3	13.7	18.1	13.2	18.2	18.2
Leucine	24.4	24.5	24.4	25.2	24.8	25.0	25.2	25.0	29.5	28.5	25.1	27.5	22.2	27.1	19.8	25.9	26.7
Phenylalanine	15.0	15.1	15.1	15.6	15.1	15.4	15.6	15.3	17.4	16.8	15.4	15.3	9.5	11.8	8.7	10.8	11.3
Total	291.3	294.5	291.4	299.8	300.8	302.9	300.0	297.1	344.7	330.1	305.4	297.8	264.5	331.4	241.5	323.5	326.9
	AB1	**AB2**	**AB3**	**AB4**	**AB5**	**AB6**	**AB7**	**AB8**	**AB9**	**AB10**	**AB11**	**AB12**	**AB13**	**AB14**	**AB15**	**AB16**	**AB17**
Fatty Acid composition (mg g^{-1} diet)																	
4:0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.0	0.0	0.0	0.0	0.0
6:0	1.8	2.1	2.1	2.1	2.0	2.1	1.9	2.1	2.1	2.1	2.1	2.1	0.0	0.0	0.0	0.0	0.0
10:0	0.9	1.0	1.1	1.0	1.0	1.0	1.0	1.1	1.1	1.1	1.1	1.1	0.0	0.0	0.0	0.0	0.0
14:0	0.4	0.4	0.3	0.3	0.7	0.3	0.4	0.3	0.6	0.7	0.4	0.8	0.4	0.4	0.2	0.3	0.3
15:0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1
16:0	4.7	4.4	3.3	3.3	4.4	3.3	3.5	3.3	5.2	5.2	3.8	2.7	5.8	3.1	3.9	5.5	5.4
16:1n-7	0.4	0.4	0.3	0.3	0.7	0.3	0.3	0.3	0.9	0.8	0.4	0.8	0.3	0.3	0.5	0.3	0.4
17:0	0.1	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.1

18:0	1.5	1.4	1.0	1.0	1.4	1.0	1.0	1.0	1.1	1.0	1.2	0.6	1.4	0.7	1.0	1.8	1.1
18:1n-9	5.7	5.3	4.3	4.4	3.0	4.3	4.1	3.9	2.8	2.6	4.8	1.7	7.3	4.3	7.1	10.1	13.3
18:1n-7	0.3	0.3	0.2	0.3	0.4	0.3	0.3	0.3	0.5	0.5	0.3	0.4	0.6	0.3	0.5	0.4	0.8
18:2n-6	6.4	5.9	5.1	5.3	4.1	5.0	5.0	4.8	4.7	4.7	5.8	1.4	17.6	6.8	7.0	12.0	7.2
18:3n-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18:3n-3	0.5	0.5	0.4	0.4	0.6	0.4	0.4	0.5	0.7	0.8	0.5	0.2	1.8	0.7	0.9	1.4	2.0
20:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.2	0.3
20:1n-11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1
20:1n-9	0.2	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.3	0.2	0.1	0.3	0.3	0.9
21:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
20:2n-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1
20:3n-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
20:4n-6	0.4	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.4	0.3	0.3	0.2	0.1	0.1	0.1	0.1	0.1
22:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.2	0.4	0.8
20:4n-3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3
22:1n-11	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.0
22:1n-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.0
20:5n-3	0.5	0.4	0.3	0.3	0.6	0.4	0.4	0.5	0.7	1.0	0.4	1.3	0.6	0.6	0.2	0.2	0.2
24:0	0.2	0.2	0.1	0.2	0.0	0.1	0.1	0.1	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.2
22:5n-6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2
24:1n-9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0
22:5n-3	0.2	0.2	0.0	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1
22:6n-3	0.8	0.8	0.6	0.7	0.9	0.6	0.7	0.7	0.7	1.1	0.8	1.4	0.7	0.6	0.4	0.8	1.3
Total	25.5	24.1	19.9	20.2	20.8	19.8	19.9	19.4	22.5	22.7	22.4	15.2	37.8	18.8	23.2	34.7	35.5
SFA	9.9	9.8	8.2	8.1	9.9	8.1	8.0	8.1	10.3	10.2	8.9	7.4	8.1	4.7	5.5	8.5	8.4
MUFA	6.9	6.2	5.0	5.1	4.3	5.0	4.9	4.6	4.4	4.1	5.7	3.2	8.8	5.3	8.7	11.4	15.5
n-3 PUFA	2.0	1.8	1.3	1.4	2.4	1.5	1.7	1.6	2.1	2.9	1.7	3.0	3.2	1.9	1.7	2.5	4.0
n-3 LC PUFA	1.4	1.4	1.0	1.0	1.8	1.0	1.2	1.2	1.4	2.1	1.2	2.7	1.4	1.2	0.7	1.1	2.0
n-6 PUFA	6.8	6.2	5.4	5.6	4.2	5.3	5.3	5.0	5.7	5.5	6.1	1.6	17.7	6.8	7.3	12.3	7.6
n-6 LC PUFA	0.4	0.3	0.3	0.3	0.1	0.3	0.3	0.3	0.4	0.3	0.3	0.2	0.1	0.1	0.2	0.3	0.3
n-6/n-3	3.5	3.4	4.0	4.0	1.8	3.7	3.1	3.1	2.7	1.9	3.6	0.5	5.5	3.5	4.4	4.9	1.9

Fatty acids < 0.1 mg g^{-1} sample are not included in this table. SFA, sum of saturated fatty acids; MUFA, sum of monounsaturated fatty acids; n-3/n-6 PUFA, sum of all omega-3/6 fatty acids with two or more double bonds; n-3/6 LC PUFA, sum of all omega-3/6 fatty acids ≥ 20 C and with two or more double bonds.

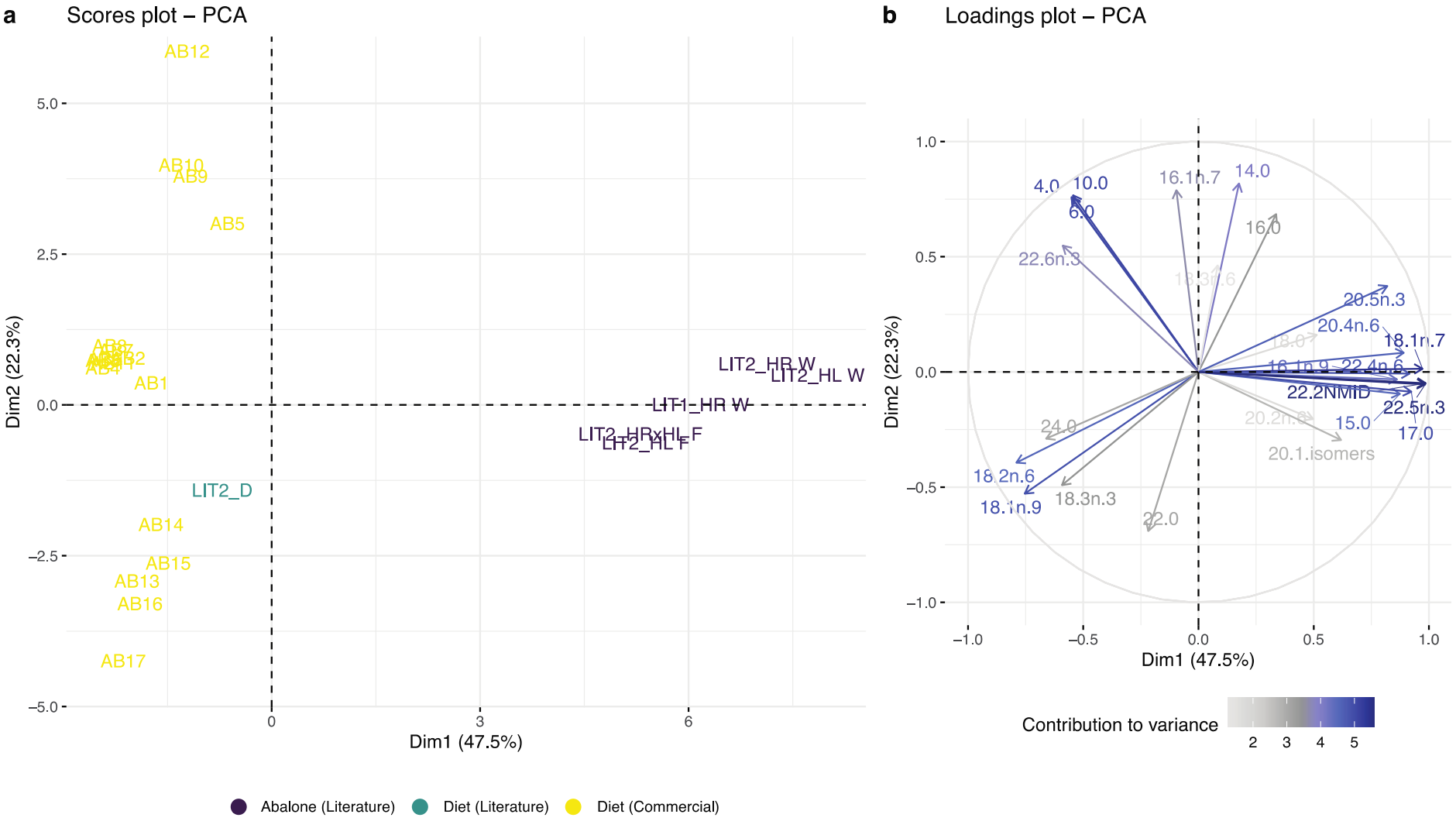

Figure 1: PCA of Commercial diets and abalone based on fatty acid composition

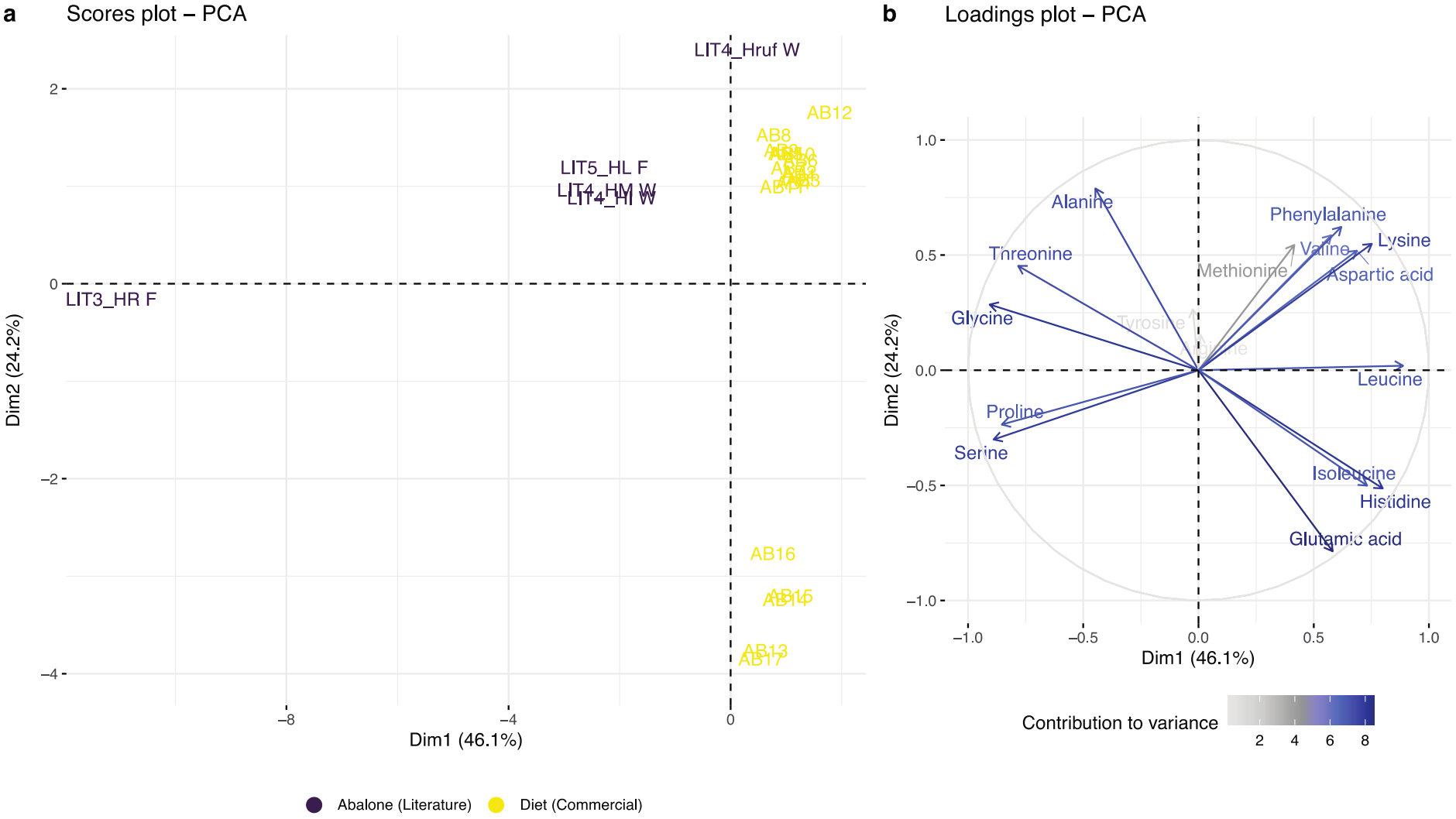

Figure 2: PCA of Commercial diets and abalone based on amino acid composition

Task 2.1 Sub-adult hybrid abalone growth trial

Introduction

As reflected in existing commercial feed formulations for farmed abalone, protein and carbohydrate are the predominant macronutrients required to satisfy the physiological requirements of the farmed species. Notably, however, while dietary energy levels are typically 16-17 MJ kg^{-1}, a wide range of protein (20-50%) and carbohydrate (30-60%) levels are implemented dependant on the manufacturer (see Task 1). Lipid remains a relatively minor dietary component (3-4%), owing to the low utilisation of lipids in abalone species and therefore low dietary requirement (Bansemer et al., 2016a; Fleming et al., 1996). However, the wide range of macronutrient inclusion rates in commercial feeds do not adequately reflect the importance of providing ideal nutrient requirements for abalone. Specifically, when protein is included below dietary requirement levels, growth declines and fatty tissue build up is promoted (Britz & Hecht, 1997). Conversely, when included above requirement levels, other nutrients may be displaced, negatively affecting the nutrient balance of the feed by promoting the utilization of dietary protein for energy, as opposed to tissue synthesis, ultimately leading to a reduction in growth performance, water quality deterioration and increased feed cost (Britz & Hecht, 1997; Britz et al., 1997; Coote et al., 2000; Gómez-Montes et al., 2003; Mercer et al., 1993; Stone et al., 2013). Although information on hybrid abalone is scarce, preliminary work from an on-farm growth trial found that a high protein feed (39.8%) in comparison to a standard protein feed (32.6%), produced better growth performance and increased economic returns (Stone et al. 2016).

Detailed information on the nutrient requirements of abalone at different life stages, however, is severely lacking. The utilisation of dietary nutrients and thus the nutritional requirements of both finfish and shellfish are dependent on life stage and size (Austreng et al., 1987; Bureau et al., 2000; Glencross, 2008; Handeland et al., 2008). Furthermore, the metabolism and growth rate of abalone is size dependent (Steinarsson & Imsland, 2003; Venter et al., 2018). As highlighted in Greenlip Abalone, differences in growth performance in response to dietary change, including an increase in dietary protein, are more pronounced in smaller abalone (Stone et al., 2016). Water temperature is another major determinant of the nutrient utilisation and growth rate of abalone (Bansemer et al., 2015a; Bansemer et al.,

2015b), and must be considered when assessing the efficacy of different dietary formulations given the flow-through culture systems typically used by abalone farmers in Australia are subject to seasonal changes in water temperature. Clearly, there is a need to optimise commercial feed formulations specific to Australian hybrid abalone under varying environmental conditions and specific to abalone size.

Considering the potentially dire consequences of a combination of poorly formulated feeds and challenging culture conditions, the present experiment aimed to establish the optimal protein requirements of Australian farmed sub-adult hybrid abalone reared at different water temperatures. 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.

Methods

The experimental system, animals, stocking and acclimation

The current growth trial was conducted using a flow-through seawater system in an air temperature (20 °C) and photoperiod-controlled laboratory at Deakin University, Queenscliff Marine Station, Queenscliff, Victoria, for 143 days. The photoperiod was held at 12 hours complete dark and 12 hours low-intensity light to mimic Australian commercial abalone farm conditions. The experimental system consisted of three identical experimental systems, each holding 15 tanks. The water temperature in the tanks for each of the three systems was set to 12, 17 and 22 °C, representing, winter, average annual and summer water temperatures, respectively. The individual culture units were 12.5 L blue plastic rectangular tanks (dimensions: 39.2 × 28.8 × 11.0 cm). Each tank was supplied with filtered (5 µm 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 escapees.

Australian hybrid abalone, at 20 months of age (sub-adult), were sourced from Jade Tiger Abalone (Craig Mostyn Group, Indented Head, Victoria) in September 2018. Abalone were lightly sedated using a commercially available anaesthetic approved by the Deakin University Animal Ethics Committee (Aqui-S, isoeugenol 540 mg L^{-1}) to minimise stress and transported to the Deakin University Queenscliff Marine Science Centre. Initially, abalone were acclimated to the experimental system at a water temperature reflective of on-farm temperature at the time of collection. Following acclimation, 20 abalone were individually weighed and assigned to each of the tanks. Care was taken to minimise variability among individual weights and total biomass per tank. Shell length was also recorded using a Vernier calliper. During the two-week acclimation period, the abalone were fed a diet consisting of 30% crude protein and the same raw materials as those used in the test diets. The abalone were fed every second day on a restricted ration to minimise the size variation between treatments. During the acclimation period, the water temperature, dependent on the assigned temperature treatment, was changed by 1 °C per day until the predetermined temperatures were reached. Water temperatures were maintained to within ± 1 °C of the pre-determined water temperature throughout the growth trial.

Experimental diets, feeding and faeces collection

Five experimental diets, fed to triplicate tanks of abalone within each temperature treatment, were formulated to contain graded dietary protein levels: 320, 350, 380, 410 and 440 g kg^{-1} and assigned the labels P32, P35, P38, P41 and P44, respectively (**Table 2**). The protein levels were achieved by altering the inclusion levels of the principal protein sources, namely, rice and pea protein isolate, at the expense of pregelatinized starch and an inert filler, diatomaceous earth. All other dietary ingredients remained identical and were included at similar levels across the experimental diets. Consistent with commercial formulations, the diets were formulated to contain 3-4% dietary lipid, using fish and canola oil. Diets were formulated to be isoenergetic (19 MJ kg^{-1}). The amino acid composition of the experimental diets was balanced to match the soft tissue composition of parent species (*Haliotis laevigata* and *Haliotis rubra*) due to the lack of amino acid composition data on Australian hybrid

abalone. All the dietary ingredients were analysed for proximate composition prior to diet formulation.

Experimental diets were cold extruded into flat pellets (diameter: 4mm) using a commercial benchtop pasta extruder. Prior to feeding, dry matter leaching was quantified to evaluate diet water stability at each of the experimental water temperatures, as described in Stone et al. (2013). Abalone were fed to satiation once per day at 1600 hrs to ensure growth was not limited by diet availability. Tanks were cleaned daily at 0900 hrs by siphoning out uneaten feed pellets and faeces. Feed consumption was determined by subtracting the equivalent mass of uneaten feed from the amount fed to each tank. Further, this was used to adjust feeding rate, where a 0.5 g day^{-1} increase in feed was implemented when the number of uneaten pellets was < 20 per tank. Faeces were collected once daily at 1400 hrs using a pipette, before being freeze-dried and frozen at -20 °C until subsequent analysis. All the experimental diets contained 0.1% of titanium dioxide (TiO_2) as an inert marker for subsequent digestibility analysis.

Water quality management

Water temperature and dissolved oxygen were measured daily using a handheld dissolved oxygen meter. Salinity and pH were measured weekly using a handheld refractometer and pH meter, respectively. Flow rates were checked weekly using a flowmeter and held at 500 mL min^{-1} throughout the growth trial. The cartridge filters (5 µm and 1 µm) were backwashed weekly to ensure adequate water flow.

Growth performance and nutrient digestibility

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 (PD%), energy deposition (ED%), soft body to shell ratio and condition factor (K) were calculated as described in detail by Britz et al., (1997) and Bansemer et al., (2015a).

Apparent digestibility coefficients for dry matter, protein, lipid, carbohydrate and energy were estimated using equations described in detail by Lewis et al., (2019) and Cho et al., (1982); with the exception that TiO_2 was used as the inert marker.

Biochemical analyses

Immediately prior to the commencement of the experiment, 30 abalone were sampled and stored at -20 °C until subsequent analysis. Similarly, at the end of the trial, seven abalone per tank were collected and stored at -20 °C until subsequent analysis. Moisture, ash, protein and 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 x 6.25) and dichloromethane: methanol (2:1) cold extraction of Folch et al., (1957), respectively as reported in detail by (Mock et al., 2019). Nitrogen free extract (NFE) was calculated by mass difference. The amino acid composition was determined using reverse-phase high-performance liquid chromatography (RP-HPLC) (1260 Agilent infinity II series systems, Agilent Technologies, Santa Clara, USA). Samples were initially acid hydrolysed using 6 M HCl for 22 hours, followed by derivatisation using o-phthaldialdehyde (OPA) and fluorenylmethyloxycarbonyl chloride (FMOC) as described in detail by Lewis et al., (2019).

The TiO_2 content of the experimental diets and faeces was analysed using wet-ash digestion followed by colourimetric determination as described in detail by Myers et al., (2004). Briefly, 0.5 g of faeces was digested in 13 mL of concentrated H_2SO_4 at 420 °C for 2 hours with the addition of 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. The total liquid weight was made up to 100 g by adding distilled water, and subsequently vacuum filtered through a Whatman No. 541 filter paper 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 to 10 mg of TiO_2. TiO_2 free faeces, was analysed to correct for background organic matter interference.

Statistical analyses

Statistical analyses were performed using R (Version 3.5.3, R Core Team 2019). All the data, with the exception of diets, were reported as mean ± standard error and replicate data were pooled for each treatment (n = 3). Upon confirmation of homogeneity of variance and normality, data were subjected to a one-way analysis of variance (ANOVA) to assess potential differences between dietary protein inclusion levels at each temperature separately. When necessary, data were $\log_{10}$-transformed to maintain homogeneity of variance and normality. Where significant differences were detected, data were then analysed post hoc using a Tukey's HSD (honestly significant difference) test. Similarly, polynomial regressions analyses were performed at each temperature against dietary protein levels. Significance was considered at $p < 0.05$ for all the statistical tests.

Results

General observations

Proximate analysis confirmed that the experimental feeds were in line with dietary formulations (**Table 3**). Accordingly, there was a step-wise increase in dietary protein concentration between P32 and P44. Total energy was constant across the feeds (19.5 MJ kg^{-1}). A small increase in dietary lipid was recorded with increasing dietary protein in the experimental diets due to a small, yet unavoidable, amount of lipid in the protein meals.

All abalone readily accepted the experimental feeds and appeared healthy throughout the experiment with only five mortalities recorded, all of which were due to an isolated mechanical issue in an individual tank, which was rectified the following day. Abalone shells were increasingly colonised with a calcareous tubeworm (*Spirobis sp*.) over the duration of the experiment; however, this did not appear to impair feeding behaviour or adversely affect health. Notably, the shells of abalone subjected to 22 °C appeared to have the highest coverage of the tubeworm.

Abalone growth performance

In general, growth performance indices, with the exception of total feed consumption and condition factor, revealed a consistent trend of increasing growth performance with increasing dietary protein inclusion levels (**Table 4** & **Figure 3)** However, this trend appeared more pronounced at the higher water temperatures, particularly at 22 °C.

Within each temperature, differences in growth performance between the different dietary protein levels were assessed using a one-way analysis of variance (ANOVA) and Tukey's post hoc analysis. At 12 °C, abalone in the P41 treatment attained the highest final weight, where they grew from an initial weight of 12.5 g to reach 18.15 g, however, this result was not statistically significant. The P41 treatment did, however, record a significantly lower total feed consumption, compared to the P35, P38 and P44 treatments. Related to enhanced growth and lower feed consumption, abalone in the P41 treatment recorded a significantly lower (superior) FCR (1.47) compared to P32, P35 and P38. P41 also recorded a superior SGR (0.26) compared to P32 (0.21), although differences were not significant.

At 17 °C, abalone were visibly larger compared to those subjected to 12 °C. Furthermore, statistical differences in growth performance parameters were more apparent, including for final weight, where abalone in the P44 treatment were significantly larger than those in P35 (21.7 g compared to 19.7 g, respectively). Again, growth performance tended to increase with increasing dietary protein inclusion where FCR ranged from 1.93 to 1.54 in P35 and P44, respectively. P44 also recorded a numerically superior SGR compared to the other treatments, with recorded values ranging from 0.33 to 0.39 in P32 and P44, respectively.

At 22 °C, abalone grew faster compared to the other experimental temperatures and as mentioned, differences in growth performance parameters between the protein inclusion levels were the most pronounced, with numerous statistically significant differences recorded. Abalone doubled their weight, with weight gain ranging between 110.4 and 146.4% in P35 and P44, respectively. SGR also increased with increasing protein inclusion, ranging from 0.53 to 0.65, in P35 and P44, respectively, while FCR decreased from 1.51 to 1.14 between P32 and P38, respectively.

Nutrient deposition and feed digestibility

The retention of dietary nutrients, namely, protein and total energy, fed to abalone in the present experiment was calculated in terms of both deposition % and deposition efficiency ratios (**Table 5** & **Figure 4**). In general, both dietary protein and dietary energy were more efficiently deposited in abalone reared at higher water temperatures, particularly at 22 °C, regardless of the level of dietary protein. However, when considering the effect of dietary protein inclusion level at each temperature, there were several significant differences between the dietary treatments. At 12 °C, and in-line with the superior feed conversion mentioned above, abalone in the P41 treatment exhibited superior PER and EER. In terms of PD%, however, there was a general decrease with increasing dietary protein, with values ranging between 35.4 and 16.8 in P32 and P44, respectively. At 17 °C, there were fewer significant differences, however, ED% was higher in P41 (14.4) compared to P32 (7.1). At 22 °C, EER was significantly affected by dietary treatment, ranging from 38.2 to 49.5 in P32 and P38, respectively. Interestingly, ED%, appeared to increase with increasing protein inclusion, ranging from 17.1 to 24.0 in P32 and P44, respectively.

Apparent digestibility coefficients (ADC %), revealed in most instances that dietary protein level had a limited effect on the digestibility of macronutrients. Specifically, dietary protein digestibility was unaffected across all experimental temperatures (**Table 6** & **Figure 5**). However, in general, P38 recorded slightly lower ADC values for most nutrients. Further, there was a slight, yet observable decrease in ADC values with increasing water temperatures, for example protein with values averaged across dietary treatments of approximately, 80, 78 and 76% in abalone subjected to 12, 17 and 22 °C, respectively. Within the 12 °C experiment, the digestibility of both dry matter (ranging from 70.2 to 73.6% in P38 and P35, respectively) and lipid (ranging from 69.3 to 77.6% in P38 and P41, respectively) were significantly affected by the level of dietary protein. At 17 °C, only the digestibility of lipid was significantly affected by the level of dietary protein (ranging from 65.3 to 74.4 in P38 and P41, respectively). Finally, at 22 °C, the digestibility of dry matter, lipid and energy were all significantly affected by the level of dietary protein with consistently lower values recorded in P38.

Abalone tissue proximate, amino acid and fatty acid composition

In general, there were very few differences in the proximate composition of abalone soft tissue between dietary treatments within each experimental temperature (**Table 7**). Soft tissue moisture being the exception, which ranged from 749.0 to 780.7 mg g^{-1} in P41 and P38, respectively. When comparing experimental temperatures, regardless of dietary treatment, there was a slight, yet observable, decrease in soft tissue protein in abalone subjected to 22 °C (~660 mg g^{-1}) compared to those reared at 12 °C (~690 mg g^{-1}).

The amino acid composition of abalone tissue showed few clear patterns with respect to increasing dietary protein levels (**Table 8** & **Figure 6**). In general, amino acids present in the highest concentrations in abalone tissue were glutamic acid (~90 mg g^{-1} dry tissue), glycine (~50 mg g^{-1} dry tissue) and arginine (~65 mg g^{-1} dry tissue). The only significant difference recorded was at 12 °C, where the concentration of glycine in abalone in the P44 treatment was higher compared to P32 (58.7 versus 51.9 mg g^{-1} dry tissue, respectively).

When reported as percentage of total amino acids, principal component analysis showed that amino acid composition of abalone soft tissues was generally clustered by rearing temperature, as opposed to dietary protein inclusion level (**Figure 6**). Abalone reared at the three temperatures separated along PC1 (principal component 1; the x-axis) by temperature, which explains 44.87% of the variation in AA composition. Generally, abalone reared at 17 °C clustered on the positive end of the x-axis and abalone reared at 12 and 22 °C clustered on the negative end of the x-axis. Additionally, PC2 (principal component 2; y-axis) explained 24.7% of the variation in AA composition and abalone reared at 12 and 22 °C separated along this axis with positive and negative values, respectively. The AA composition of abalone reared at 22 °C was characterised by higher percentages glycine (negative scores on PC1 and PC2), while AA composition of abalone reared at 12 °C was characterised by higher percentages of glutamic acid, isoleucine, valine, arginine, aspartic acid and histidine (negative PC1 scores and positive PC2 scores). Furthermore, AA composition of abalone reared at 17 °C was characterised by higher percentages of phenylalanine, leucine, alanine, proline, lysine, threonine, serine and tyrosine (positive PC1 scores).

In general, fatty acid compositions were dominated by a few individual fatty acids, namely, 16:0, 17:0, 18:1n-9 and 18:2n-6 (**Table 9**). However, also in relative abundance were n-3 LC PUFA, such as 20:5n-3, 22:5n-3 and 22:6n-3. The fatty acid composition of abalone tissue differed only slightly between dietary treatment groups within both the 12 °C and 17 °C experiments. However, numerous significant differences were apparent between treatment groups within the 22 °C experiment. Fatty acid compositions varied little between dietary treatments P32-P38, however, an increase in the concentration of numerous fatty acids, predominantly 16:0, 18:1n-9 and to a lesser extent 20:5n-3, was seen in the P41 treatment, with P44 representing a middle ground.

Despite this, principal component analysis showed that the fatty acid compositions of abalone tissues appeared to be dictated primarily by rearing temperature, as opposed to dietary protein level (**Figure 7**) as indicated by the clustering of abalone samples. Abalone reared at three temperatures separated along PC1 (x-axis), which explains 46.46% of the variation in FA composition. Abalone reared at 12 °C had the lowest PC1 scores, abalone reared at 17 °C had PC1 scores near 0 and abalone reared at 22 °C had the highest PC1 scores. PC2 (y-axis) explains 24.77% of the variation in FA composition. Abalone reared at 12 and 22 °C had positive PC2 scores and abalone reared at 17 °C had negative PC2 scores. The FA composition of abalone reared at 12 °C was characterised by higher percentages of long chain, polyunsaturated FA (20:2n-6, 20:3n-6, 22:2n-6, 24:5n-3). Conversely, the FA composition of abalone reared at 22 °C was characterised by higher percentages of monounsaturated FA (18:1n-9, 18:1n-9t, 20:1n-9, 20:1n-11, 22:1n-11). Interestingly, abalone reared at 17 °C were characterised by higher percentages of health-promoting FA (20:5n-3 and 22:6n-3) which separated abalone reared at 17 °C from abalone reared at 12 °C and 22 °C along PC2 (y-axis).

Table 2: Formulation of the experimental diets fed to sub-adult Australian hybrid abalone.

	P32	P35	P38	P41	P44
Nominal protein level (g kg^{-1})	320	350	380	410	440
Ingredient Composition (g 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	19	18	16.5	15
Fish meal	50	50	50	50	50
Gluten	50	50	50	50	50
Gelatin	50	50	50	50	50
Fish oil	15	15	15	15	15
Lecithin	10	10	10	10	10
Canola Oil	2.1	2.1	2.0	2.0	2.0
Celite	4	4	4	4	4
Titanium dioxide	1	1	1	1	1
Vitamin & mineral mix	7	7	7	7	7
Vitamin C	0.5	0.5	0.5	0.5	0.5
Choline	5	5	5	5	5
Vitamin E	1	1	1	1	1
Monosodium phosphate	7.5	7.5	7.5	7.5	7.5
Calcium sulphate	5	5	5	5	5
Agar	5	5	5	5	5
Sodium alginate	5	5	5	5	5
Methionine	5	5	5	5	5
Lysine	5	5	5	5	5
Arginine	2	2	2	2	2
Threonine	2	2	2	2	2

Table 3: Proximate, amino acid and fatty acid composition of the experimental diets fed to sub-adult Australian hybrid abalone.

	P32	P35	P38	P41	P44
Proximate composition (g kg^{-1} dry diet)					
Dry matter	914.2	913.2	915.6	917.8	916.0
Protein	321.0	350.1	380.2	413.3	436.6
Lipid	31.8	33.9	34.1	36.5	37.8
Ash	56.4	52.8	56.1	55.6	56.3
NFE	590.8	563.2	529.7	494.5	469.4
Energy (MJ kg^{-1})	19.0	19.3	19.4	19.7	19.7
Amino Acid composition (mg g^{-1} diet)					
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	23.3	24.6	25.7	26.6
Aspartic acid	24.4	26.6	29.0	31.2	34.1
Glutamic acid	54.3	57.6	62.0	65.7	70.4
Threonine	11.2	12.5	13.6	13.7	15.3
Alanine	15.9	17.0	18.2	19.2	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
Fatty acid composition (mg g^{-1} diet)					
11:0	0.00	0.00	0.05	0.04	0.00
13:0	3.07	3.10	3.00	2.85	3.36
14:0	0.69	0.70	0.70	0.73	0.78
15:0	0.09	0.09	0.09	0.10	0.10
16:0	4.98	5.42	5.45	5.78	6.30
16:1n-7	0.77	0.74	0.75	0.76	0.82
16:2n-4	0.17	0.20	0.22	0.20	0.24
16:3n-4	0.12	0.13	0.12	0.14	0.17
18:0	0.99	1.05	1.05	1.11	1.20
18:1n-9	5.20	5.65	5.60	5.96	6.49
18:1n-7	0.51	0.51	0.51	0.53	0.57
18:2n-6	5.29	6.06	5.92	6.32	6.99
18:3n-3	0.82	0.90	0.91	0.97	1.07
20:0	0.14	0.15	0.15	0.16	0.17
18:4n-3	0.16	0.16	0.15	0.16	0.17
20:1n-11	0.00	0.00	0.00	0.12	0.13
20:1n-9	0.64	0.65	0.66	0.57	0.62
20:4n-6	0.12	0.13	0.13	0.13	0.15
20:3n-3	0.00	0.00	0.00	0.03	0.00
20:4n-3	0.09	0.09	0.09	0.10	0.10
22:1n-11	0.24	0.25	0.24	0.25	0.27
22:1n-9	0.08	0.09	0.09	0.09	0.10
20:5n-3	1.00	1.00	0.97	0.97	1.11
22:2NMI	0.06	0.14	0.07	0.06	0.13
22:4n-6	0.03	0.00	0.00	0.10	0.03
24:0	0.07	0.08	0.10	0.35	0.11

22:5n-3	0.18	0.18	0.18	0.31	0.19
22:6n-3	1.39	1.39	1.36	1.40	1.54
Total	26.92	28.88	28.57	30.30	32.93
SFA	10.04	10.61	10.60	11.12	12.02
MUFA	7.45	7.89	7.85	8.29	9.01
n-3 PUFA	3.63	3.73	3.66	3.94	4.19
n-3 LC PUFA	2.65	2.67	2.60	2.81	2.95
n-6 PUFA	5.45	6.19	6.05	6.55	7.17
n-6 LC PUFA	0.15	0.13	0.13	0.23	0.18
n-6/n-3	1.50	1.66	1.65	1.67	1.71

Fatty acids < 0.01 mg g^{-1} sample for all dietary treatments are not included in this table. SFA, sum of saturated fatty acids; MUFA, sum of monounsaturated fatty acids; n-3/n-6 PUFA, sum of all omega-3/6 fatty acids with two or more double bonds; n-3/6 LC PUFA, sum of all omega-3/6 fatty acids ≥ 20 C and with two or more double bonds.

Table 4: Growth performance of sub-adult Australian hybrid abalone

	P32	P35	P38	P41	P44	Sig.
12°C						
Initial weight (g)	12.47 ± 0.02	12.52 ± 0.02	12.55 ± 0.00	12.57 ± 0.04	12.52 ± 0.03	
Final weight (g)	16.79 ± 0.32	17.38 ± 0.30	17.45 ± 0.34	18.15 ± 0.38	17.71 ± 0.41	
Initial shell length (mm)	43.4 ± 0.4	43.1 ± 0.3	43.2 ± 0.3	43.5 ± 0.3	43.5 ± 0.3	
Final shell length (mm)	49.4 ± 0.4	50.1 ± 0.4	50.0 ± 0.5	50.6 ± 0.4	50. 5 ± 0.4	
SGR	0.21 ± 0.01	0.23 ± 0.01	0.24 ± 0.01	0.26 ±0.02	0.24 ± 0.02	
Survival (%)	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	
Weight gain (%)	34.3 ± 2.5	39.0 ± 2.4	39.6 ± 2.7	45.2 ± 3.0	41.7 ± 3.3	
Biomass gain (g tank^{-1})	73.3 ± 4.3	90.7 ± 2.4	91.1 ± 8.7	104.9 ± 10.1	93.2 ± 1.2	
FCR	2.12 ± 0.14b	2.11 ± 0.09b	2.15 ± 0.17b	1.47 ± 0.12a	1.79 ± 0.02ab	**
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	
Soft tissue to shell ratio	1.37 ± 0.03	1.45 ± 0.05	1.41 ± 0.03	1.52 ± 0.07	1.42 ± 0.03	
K	0.78 ± 0.01	0.78 ± 0.01	0.80 ± 0.02	0.79 ± 0.01	0.77 ± 0.01	
Feed consumption (g tank^{-1})	154.2 ± 2.0ab	191.7 ± 7.5c	193.2± 4.0c	152.0 ± 3.7a	167.0 ± 0.9b	***
17°C						
Initial weight (g)	12.48 ± 0.03	12.52 ± 0.04	12.50 ± 0.00	12.52 ± 0.03	12.50 ± 0.03	
Final weight (g)	19.72 ± 0.36a	21.23 ± 0.39ab	20.88 ± 0.49ab	20.97 ± 0.51ab	21.72 ± 0.52b	**
Initial shell length (mm)	43.5 ± 0.4	43.7 ± 0.4	43.9 ± 0.4	43.7 ± 0.4	43.3 ± 0.4	
Final shell length (mm)	51.6 ± 0.4a	52.8 ± 0.4ab	51.0 ± 0.5ab	51.6 ± 0.5ab	52.9 ± 0.5b	*
SGR	0.33 ± 0.01	0.38 ± 0.01	0.36 ± 0.02	0.36 ± 0.02	0.39 ± 0.02	
Survival (%)	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	
Weight gain (%)	57.7 ± 2.9a	69.8 ±3.1ab	67.0 ± 3.9ab	67.7 ± 4.1ab	73.8 ± 4.1b	*
Biomass gain (g tank^{-1})	142.2 ±2.7	174.2 ± 8.3	166.8 ± 13.4	169.0 ± 13.3	184.5 ± 9.3	
FCR	1.93 ± 0.06a	1.76 ± 0.08a	1.87 ± 0.12a	1.61 ± 0.10a	1.54 ± 0.07a	* †
Shell growth rate (µm day^{-1})	58.4 ± 2.7a	68.0 ± 3.1ab	56.6 ± 3.5a	57.9 ± 3.5a	70.6 ± 3.5b	**
Soft tissue to shell ratio	1.39 ± 0.03	1.41 ± 0.03	1.40 ± 0.03	1.43 ± 0.03	1.44 ± 0.03	
K	0.78 ± 0.01	0.79 ± 0.01	0.80 ± 0.01	0.78 ± 0.01	0.79 ± 0.01	
Feed consumption (g tank^{-1})	274.1 ± 4.3a	305.4 ± 1.9b	308.0 ± 5.4b	269.0 ± 4.9a	282.2 ± 2.7a	***
22°C						
Initial weight (g)	12.47 ± 0.02	12.50 ± 0.03	12.55 ± 0.08	12.53 ± 0.02	12.50 ± 0.05	
Final weight (g)	26.30 ± 0.60a	27.67 ± 0.57ab	29.31 ± 0.59bc	29.17 ± 0.69bc	30.88 ± 0.68c	***
Initial shell length (mm)	43.6 ± 0.3	44.0 ± 0.4	43.8 ± 0.4	43.4 ± 0.3	43.3 ± 0.4	
Final shell length (mm)	57.3 ± 0.5a	58.8 ± 0.4ab	60.0 ± 0.5bc	59.1 ± 0.5bc	60.2 ± 0.5c	***
SGR	0.53 ± 0.02a	0.57 ± 0.01ab	0.61 ± 0.01bc	0.60 ± 0.01bc	0.65 ± 0.01c	***
Survival (%)	91.7 ± 8.3	100 ± 0	100 ± 0	100 ± 0	100 ± 0	
Weight gain (%)	110.4 ± 4.8a	120.8 ± 4.6ab	133.3 ± 4.7bc	132.2 ± 5.5bc	146.4 ± 5.4c	***
Biomass gain (g tank^{-1})	266.2 ±11.4a	303.4 ± 17.7ab	335.2 ± 2.4bc	334.2 ± 15.5bc	367.5 ± 15.3c	**
FCR	1.51 ± 0.06b	1.31 ± 0.09ab	1.14 ± 0.01a	1.25 ± 0.05ab	1.16 ± 0.05a	**
Shell growth rate (µm day^{-1})	99.8 ± 3.5a	108.0 ± 3.1ab	119.0 ± 3.3bc	114.3 ± 3.5bc	121.0 ± 3.7c	***
Soft tissue to shell ratio	1.33 ± 0.03a	1.38 ± 0.04ab	1.40 ± 0.03ab	1.51 ± 0.02b	1.43 ± 0.03ab	*
K	0.81 ± 0.01	0.79 ± 0.01	0.79 ± 0.01	0.82 ± 0.01	0.82 ± 0.01	
Feed consumption (g tank^{-1})	400.2 ± 1.6a	395.3 ± 2.3b	381.6 ± 3.3b	417.0 ± 3.1a	424.1 ± 3.3ab	**

SGR, specific growth rate; FCR, feed conversion ratio; K, condition factor. Data are expressed as mean ± SEM. Values in the same row with different superscripts are significantly different (one-way ANOVA and Tukey's post hoc test). *, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. †ANOVA may indicate significant differences, however significant differences were not detected in post-hoc.

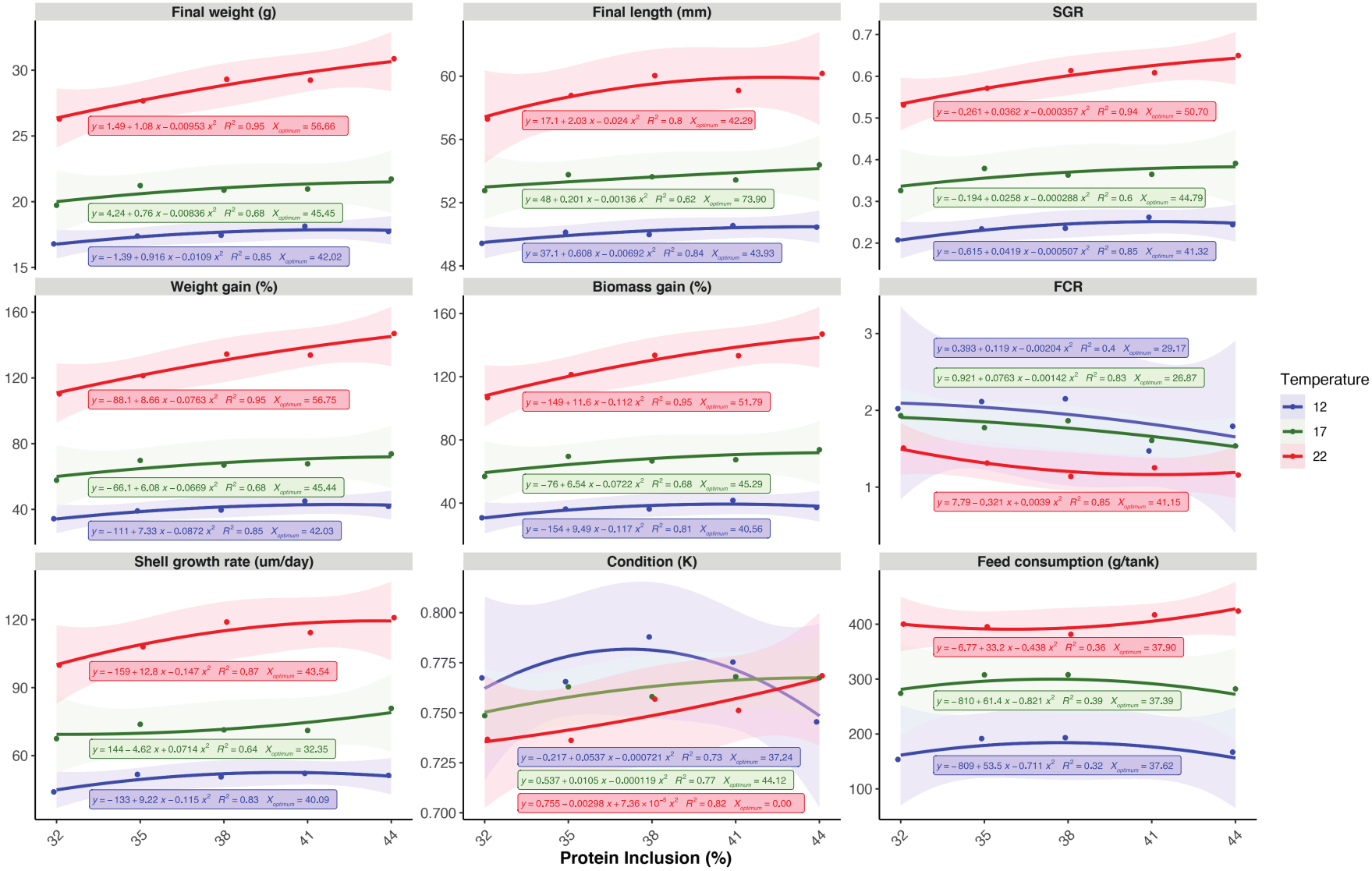

Figure 3: Regressions of growth performance of sub-adult Australian hybrid abalone.

Table 5: Nutrient retention efficiency of sub-adult Australian hybrid abalone

	P32	P35	P38	P41	P44	Sig.
12°C						
PER	1.64 ± 0.11ab	1.41 ± 0.06ab	1.33 ± 0.10a	1.79 ± 0.14b	1.36 ± 0.01a	*
PD%	35.4 ± 3.3b	24.5 ± 2.3ab	23.8 ± 1.4ab	24.5 ± 3.9ab	16.8 ± 4.1a	*
EER (g MJ^{-1})	28.7 ± 1.8a	26.7 ± 1.1a	26.5 ± 2.0a	37.9 ± 2.9b	30.5 ± 0.3ab	**
ED%	15.1 ± 2.2	13.3 ± 1.5	13.4 ± 0.5	13.8 ± 2.3	9.0 ± 3.6	
17°C						
PER	1.71 ± 0.06	1.68 ± 0.07	1.53 ± 0.10	1.63 ± 0.10	1.59 ± 0.07	
PD%	17.1 ± 2.8	25.0 ± 2.1	22.3 ± 0.2	24.6 ± 1.7	22.9 ± 1.0	
EER (g MJ^{-1})	29.9 ± 1.0	31.8 ± 1.4	30.4 ± 2.0	34.5 ± 2.1	35.7 ± 1.5	
ED%	7.1 ± 1.6a	12.6 ± 1.2b	12.9 ± 0.8b	14.4 ± 1.2b	14.0 ± 0.8b	**
22°C						
PER	2.19 ± 0.09	2.28 ± 0.14	2.48 ± 0.03	2.08 ± 0.08	2.11 ± 0.09	
PD%	32.9 ± 2.7	35.9 ± 2.1	37.4 ± 0.3	36.6 ± 1.3	35.7 ± 2.3	
EER (g MJ^{-1})	38.2 ± 1.5a	43.1 ± 2.7ab	49.5 ± 0.6b	44.1 ± 1.7ab	47.3 ± 1.9b	**
ED%	17.1 ± 0.9a	20.0 ± 0.9a	21.3 ± 0.2a	23.3 ± 0.7b	24.0 ± 1.1b	***

PER, protein efficiency ratio; PD, protein deposition; EER, energy efficiency ratio; ED, energy deposition. Data are expressed as mean ± SEM. Values in the same row with different superscripts are significantly different (one-way ANOVA and Tukey's post hoc test). *, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

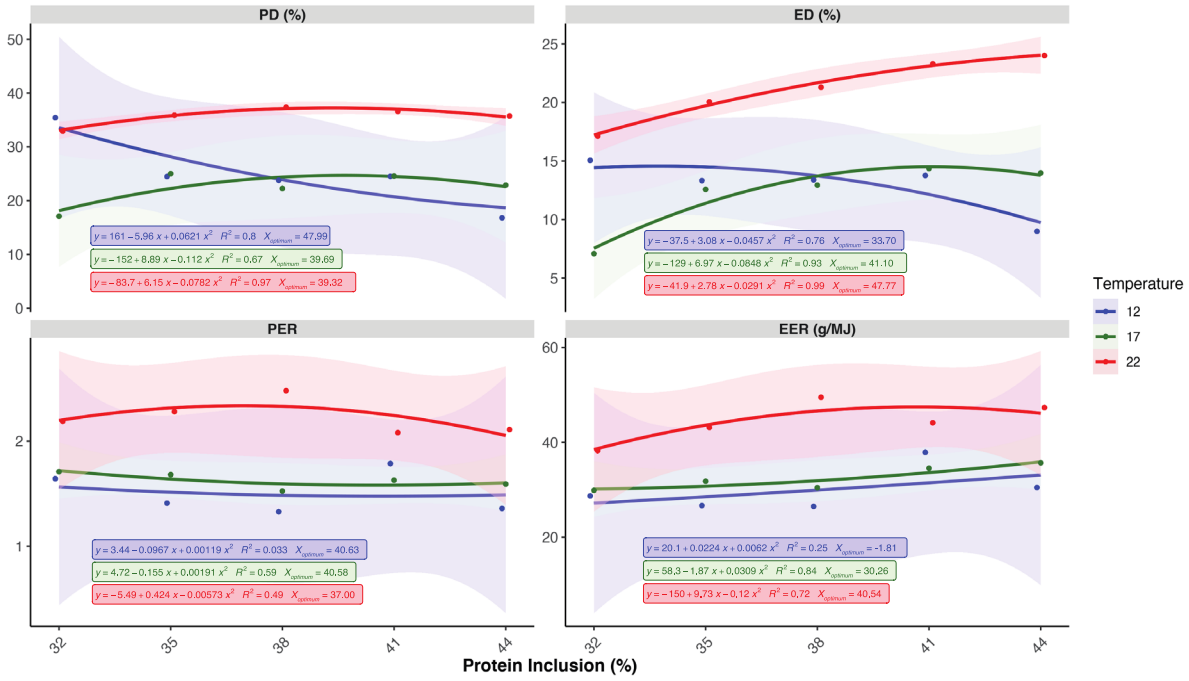

Figure 4: Regressions of nutrient efficiency of sub-adult Australian hybrid abalone.

Table 6: Apparent digestibility coefficient of experimental diets fed to sub-adult Australian hybrid abalone

	P32	P35	P38	P41	P44	Sig.
12°C						
DM	73.3 ± 0.2c	73.6 ± 0.2c	70.2 ± 0.3a	71.4 ± 0.6ab	72.9 ± 0.5bc	***
Protein	80.2 ± 0.4	81.9 ± 0.3	79.7 ± 0.6	79.7 ± 0.9	80.2 ± 0.9	
Lipid	72.5 ± 0.7b	77.3 ± 0.3c	69.3 ± 0.3a	77.6 ± 0.2c	76.2 ± 0.6c	***
NFE	89.1 ± 0.2	88.7 ± 0.2	88.3 ± 0.2	87.7 ± 0.7	88.2 ± 0.3	
Energy	84.7 ± 0.2	84.9 ± 0.2	83.1 ± 0.4	83.0 ± 0.7	83.4 ± 0.6	
17°C						
DM	72.1 ± 1.3	72.3 ± 0.2	69.5 ± 0.2	70.3 ± 0.5	71.8 ± 1.6	
Protein	78.0 ± 0.6	79.3 ± 0.3	78.8 ± 2.1	77.8 ± 0.6	78.1 ± 1.2	
Lipid	66.5 ± 2.9ab	74.0 ± 0.6b	65.3 ± 1.0a	74.4 ± 1.5b	71.7 ± 2.3ab	*
NFE	88.6 ± 0.4	88.2 ± 0.4	87.7 ± 0.1	87.2 ± 0.1	88.3 ± 0.9	
Energy	83.0 ± 0.6	83.2 ± 0.2	82.1 ± 1.0	81.5 ± 0.4	81.9 ± 0.9	
22°C						
DM	71.6 ± 0.2c	71.6 ± 0.3c	68.3 ± 0.4a	69.5 ± 0.3ab	70.9 ± 0.2bc	***
Protein	76.5 ± 0.5	77.0 ± 0.3	75.2 ± 0.7	75.5 ± 0.8	75.7 ± 0.4	
Lipid	65.2 ± 0.7b	70.2 ± 2.0b	58.3 ± 0.8a	69.4 ± 1.4b	65.7 ± 0.7b	***
NFE	88.6 ± 0.5	88.6 ± 0.5	88.2 ± 0.4	87.9 ± 0.1	89.0 ± 0.1	
Energy	82.3 ± 0.3c	82.0 ± 0.2bc	80.1 ± 0.6a	80.2 ± 0.4a	80.4 ± 0.3ab	**

ADC, apparent digestibility coefficient; DM, dry matter; NFE, nitrogen free extract (calculated). Data are expressed as mean ± SEM. Values in the same row with different letters are significantly different (one-way ANOVA and Tukey's post hoc test). *, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

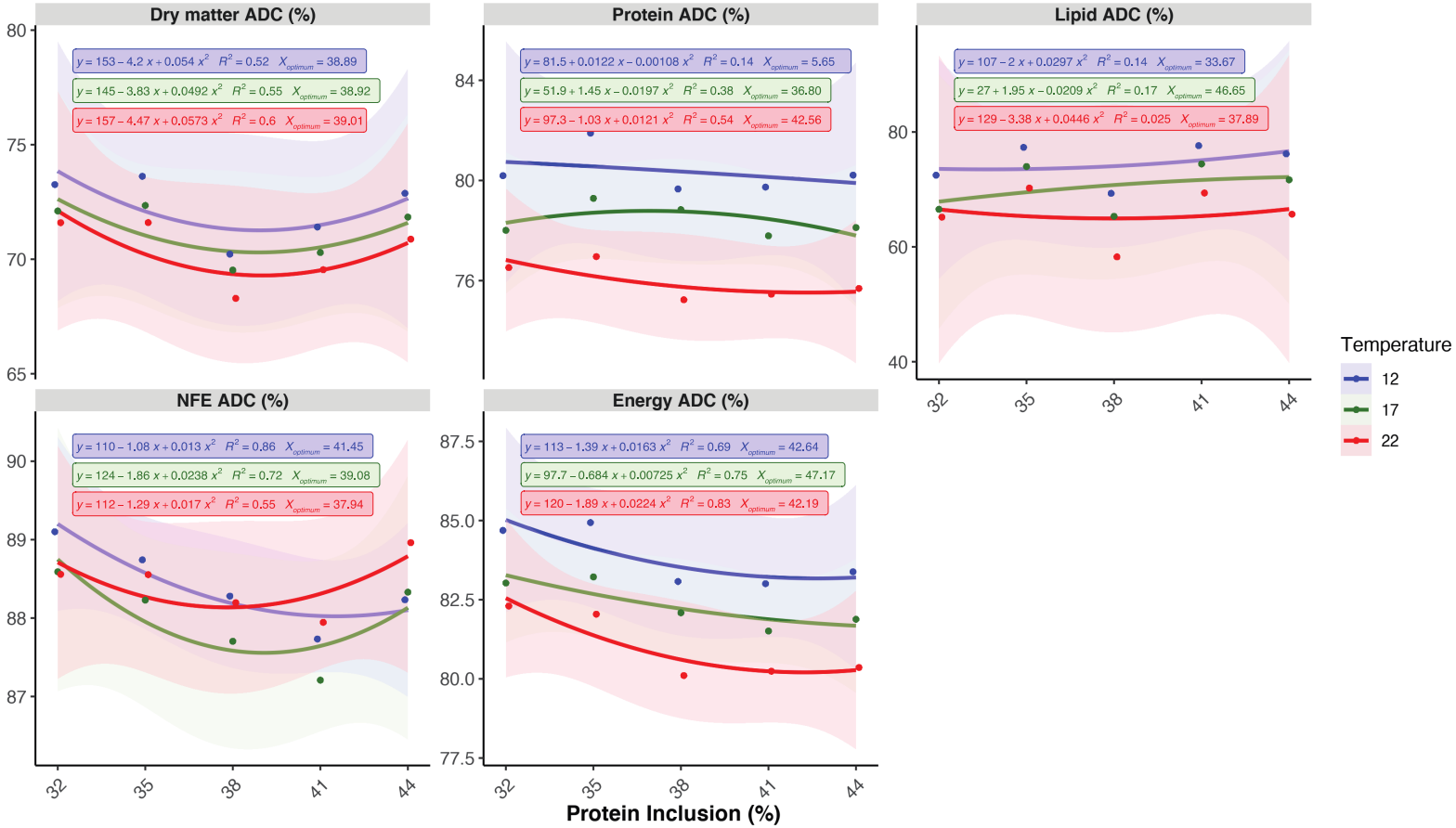

Figure 5: Regressions of apparent digestibility coefficients of sub-adult Australian hybrid abalone.

Table 7: Proximate composition and energy content of sub-adult Australian hybrid abalone tissue

	P32	P35	P38	P41	P44	Sig.
12°C						
Moisture	769.1 ± 5.2	777.8 ± 9.4	775.9 ± 5.0	791.9 ± 5.0	797.9 ± 10.2	
Protein	702.6 ± 7.7	690.9 ± 16.1	690.7 ± 15.9	688.8 ± 4.2	691.3 ± 11.6	
Lipid	61.6 ± 1.5	67.1 ± 1.8	66.2 ± 1.9	63.8 ± 1.8	62.3 ± 0.8	
Ash	108.1 ± 4.8	87.9 ± 3.1	86.6 ± 3.4	89.9 ± 3.3	94.2 ± 11.1	
NFE	127.7 ± 6.9	154.2 ± 20.8	156.5 ± 14.0	157.5 ± 3.3	152.2 ± 1.6	
Energy	21.2 ± 0.1	21.6 ± 0.1	21.6 ± 0.1	21.5 ± 0.1	21.4 ± 0.3	
17°C						
Moisture	811.1 ± 7.0	785.0 ± 13.0	788.2 ± 6.8	785.7 ± 6.6	784.5 ± 7.8	
Protein	684.2 ± 8.7	685.5 ± 12.3	689.5 ± 6.3	690.6 ± 8.5	673.7 ± 0.8	
Lipid	57.4 ± 1.6	59.5 ± 4.1	60.5 ± 0.9	61.3 ± 0.9	64.3 ± 1.6	
Ash	107.0 ± 4.8	92.5 ± 5.4	95.4 ± 2.6	93.3 ± 6.3	97.5 ± 3.1	
NFE	151.3 ± 9.3	162.5 ± 13.5	154.6 ± 7.6	154.8 ± 13.9	164.5 ± 3.1	
Energy	21.0 ± 0.1	21.3 ± 0.1	21.3 ± 0.0	21.4 ± 0.1	21.3 ± 0.1	
22°C						
Moisture	774.5 ± 6.6b	772.3 ± 1.8b	780.7 ± 2.1b	749.0 ± 1.7a	765.2 ± 3.0ab	***
Protein	657.3 ± 11.8	668.8 ± 16.4	677.9 ± 8.6	649.4 ± 4.1	680.4 ± 6.2	
Lipid	63.8 ± 1.7	64.1 ± 0.7	62.9 ± 1.8	66.7 ± 1.2	66.5 ± 0.9	
Ash	97.9 ± 8.0	102.6 ± 9.1	99.7 ± 7.2	91.8 ± 2.8	89.9 ± 5.2	
NFE	181.0 ± 15.2	164.5 ± 6.8	159.4 ± 0.6	192.1 ± 4.4	163.2 ±5.0	
Energy	21.1 ± 0.1	21.1 ± 0.3	21.2 ± 0.1	21.3 ± 0.1	21.5 ± 0.1	

NFE, nitrogen free extract (calculated). Data are expressed as mean ± SEM. Values in the same row with different letters are significantly different (one-way ANOVA and Tukey's post hoc test). *, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Table 8: Amino acid composition of sub-adult Australian hybrid abalone tissue

	P32	P35	P38	P41	P44	Sig.
12°C						
Aspartic acid	66.3 ± 1.4	67.0 ± 1.7	66.5 ± 1	65.7 ± 1	67.8 ± 0.7	
Glutamic acid	92.4 ± 1.8	93 ± 2.3	92.9 ± 1.5	91.8 ± 1.3	94.8 ± 1.2	
Serine	29.3 ± 1.3	30.3 ± 0.4	30.4 ± 1.2	30.4 ± 0.5	30.6 ± 0.7	
Histidine	9.8 ± 0.2	9.9 ± 0.0	9.7 ± 0.1	9.6 ± 0.1	9.8 ± 0.1	
Glycine	51.9 ± 0.7	55.7 ± 0.6	54.7 ± 1.0	54.9 ± 2.1	58.7 ± 1.9	
Threonine	24.4 ± 0.9	25.0 ± 0.3	24.8 ± 0.8	24.5 ± 0.4	25.0 ± 0.3	
Arginine	69.6 ± 1.1	68.4 ± 1.8	68.9 ± 1.3	68.9 ± 1.0	69.6 ± 0.9	
Alanine	33.0 ± 0.9	33.1 ± 0.6	33.2 ± 0.9	32.9 ± 0.6	33.9 ± 0.7	
Tyrosine	13.9 ± 0.9	15.1 ± 0.2	15.0 ± 0.5	15.1 ± 0.5	15.4 ± 0.3	
Valine	27.6 ± 0.7	27.2 ± 0.8	27.1 ± 0.2	26.8 ± 0.4	27.7 ± 0.2	
Methionine	15.4 ± 0.5	15.3 ± 0.5	15.3 ± 0.2	15.2 ± 0.3	15.6 ± 0.2	
Phenylalanine	21.8 ± 0.6	21.8 ± 0.5	21.8 ± 0.2	21.4 ± 0.3	22.1 ± 0.0	
Isoleucine	23.7 ± 0.6	23.4 ± 0.8	23.3 ± 0.3	23.0 ± 0.4	23.9 ± 0.0	
Leucine	42.5 ± 1.1	42.4 ± 1.0	42.4 ± 0.8	42.0 ± 0.7	42.9 ± 0.2	
Lysine	33.0 ± 0.2b	30.2 ± 0.6a	31.5 ± 0.8ab	30.3 ± 0.8ab	32.1 ± 0.1ab	*
Proline	25.2 ± 1.4	25.3 ± 0.6	24.9 ± 0.4	25.0 ± 0.3	26.1 ± 0.4	
Total	579.9 ± 12.5	583.0 ± 11.8	582.5 ± 10.0	577.4 ± 7.7	596.1 ± 6.2	
	P32	**P35**	**P38**	**P41**	**P44**	**Sig.**
17°C						
Aspartic acid	60.0 ± 2.3	64.8 ± 5.6	66.2 ± 1.9	67.4 ± 5.6	62.9 ± 2.9	
Glutamic acid	84.9 ± 3.1	92.9 ± 8.4	93.9 ± 2.4	96.2 ± 8.5	89.8 ± 4.6	
Serine	30.0 ± 1.2	31.8 ± 2	32.2 ± 1	33.6 ± 2.1	32.1 ± 1.5	
Histidine	8.7 ± 0.4	8.9 ± 1.1	9.2 ± 0.7	8.6 ± 1.0	8.4 ± 0.2	
Glycine	46.8 ± 2.3	53.6 ± 3.4	55.1 ± 3.9	55.0 ± 2.1	54.4 ± 3.6	
Threonine	24.1 ± 0.8	25.9 ± 2.3	26.3 ± 0.9	27.2 ± 2.4	25.5 ± 0.9	
Arginine	59.0 ± 3.0	65.0 ± 5.7	64.5 ± 1.4	68.7 ± 6.0	62.3 ± 3.1	
Alanine	31.0 ± 0.6	33.3 ± 2.3	34.0 ± 0.8	34.1 ± 2.2	32.9 ± 1.4	
Tyrosine	13.7 ± 0.5	14.6 ± 1.5	15.6 ± 0.4	16.7 ± 2.0	15.2 ± 0.5	
Valine	23.3 ± 0.7	26.1 ± 2.7	26.5 ± 1.0	26.7 ± 2.6	24.5 ± 0.8	
Methionine	14.1 ± 0.4	15.3 ± 1.5	15.4 ± 0.3	15.8 ± 1.4	14.8 ± 0.7	
Phenylalanine	20.1 ± 0.7	22.1 ± 2.2	22.5 ± 0.9	23.1 ± 2.3	21.2 ± 0.7	
Isoleucine	20.4 ± 0.5	23 ± 2.6	23.7 ± 0.6	23.7 ± 2.5	21.4 ± 0.8	
Leucine	39.7 ± 1.0	43.1 ± 4.2	43.5 ± 1.3	45.1 ± 4.3	41.7 ± 1.5	
Lysine	30.2 ± 0.6	32.0 ± 3.9	33.2 ± 1.6	35.1 ± 3.6	32.3 ± 1.4	
Proline	25.3 ± 1.6	27.9 ± 2.0	30.0 ± 1.5	30.0 ± 2.1	28.8 ± 1.8	
Total	531.4 ± 18.4	580.2 ± 51.1	591.8 ± 17.0	607.2 ± 50.6	568.2 ± 25.9	
	P32	**P35**	**P38**	**P41**	**P44**	**Sig.**
22°C						
Aspartic acid	60.8 ± 2.1	68.6 ± 0.5	64.1 ± 0.7	64.9 ± 4.4	64.6 ± 0.5	
Glutamic acid	83.8 ± 4	96.0 ± 1.2	89.2 ± 1.5	88.6 ± 5.7	90.1 ± 0.9	
Serine	29.5 ± 1.0	32.5 ± 1.6	31.0 ± 0.7	29.7 ± 1.7	30.4 ± 0.1	
Histidine	9.2 ± 0.2	10.5 ± 0.4	10.1 ± 0.8	9.6 ± 0.8	9.4 ± 0.1	
Glycine	50.7 ± 2.4	59.1 ± 2.9	54.7 ± 0.9	52.3 ± 3.3	56.6 ± 1.9	
Threonine	23.7 ± 0.7	26.2 ± 0.4	24.8 ± 0.4	24.9 ± 1.7	24.8 ± 0.2	
Arginine	60.3 ± 3.4	68.9 ± 2.5	64.7 ± 1.3	60.3 ± 3.9	63.9 ± 1.4	
Alanine	29.9 ± 1.2	34.3 ± 0.6	31.6 ± 0.5	31.8 ± 2.1	32.1 ± 0.1	
Tyrosine	13.8 ± 0.7	15.5 ± 0.4	15.0 ± 0.2	14.1 ± 0.7	14.7 ± 0.1	
Valine	23.6 ± 1.2	27.1 ± 0.6	25.1 ± 0.5	26 .0 ± 2.0	26.1 ± 0.2	
Methionine	13.7 ± 0.7	15.6 ± 0.1	14.6 ± 0.3	14.7 ± 1.0	14.6 ± 0.1	
Phenylalanine	19.6 ± 0.9	21.7 ± 0.2	20.7 ± 0.1	20.7 ± 1.5	20.7 ± 0.1	
Isoleucine	20.1 ± 1.0	23.3 ± 0.7	21.5 ± 0.5	22.5 ± 1.8	22.5 ± 0.2	
Leucine	38.1 ± 1.7	43.3 ± 0.2	40.7 ± 0.5	40.5 ± 2.8	41.0 ± 0.4	

Lysine	29.2 ± 1.1	31.5 ± 1.2	30.6 ± 0.5	29.8 ± 2.4	31.2 ± 0.7
Proline	22.3 ± 1.7	26.4 ± 0.4	23.6 ± 0.9	24.8 ± 1.4	23.7 ± 1.7
Total	528.3 ± 20.8	600.6 ± 9.4	562.0 ± 6.9	555.1 ± 37.0	566.1 ± 3.0

Data are expressed as mean ± SEM. Values in the same row with different letters are significantly different (one-way ANOVA and Tukey's post hoc test). *, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

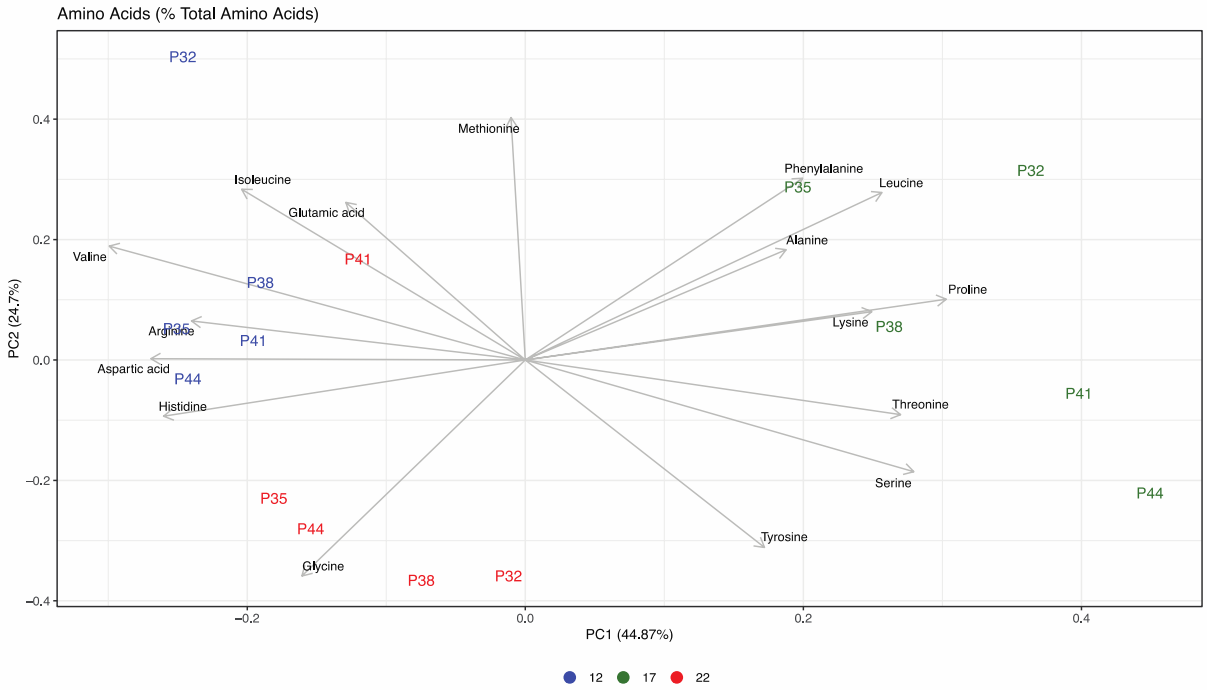

Figure 6: PCA of sub-adult Australian hybrid abalone based on amino acid composition

Table 9: Fatty acid composition of sub-adult Australian hybrid abalone tissue

	Initial	P32	P35	P38	P41	P44	Sig.
12°C							
10:0	0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
12:0	0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
13:0	2.6	3.0 ± 0.1	2.4 ± 0.2	2.5 ± 0.2	2.3 ± 0.2	2.1 ± 0.2	
14:0	0.4	0.4 ± 0.0	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	
15:0	0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
15:1n-5	0.1	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
16:0	5.3	6.2 ± 0.1	6.4 ± 0.2	6.2 ± 0.5	5.8 ± 0.1	5.4 ± 0.2	
16:1n-7	0.2	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	
17:0	2.8	3.1 ± 0.1	3.1 ± 0.1	3.0 ± 0.2	2.8 ± 0.3	2.6 ± 0.2	
16:2n-4	0.2	0.3 ± 0.0b	0.3 ± 0.0ab	0.3 ± 0ab	0.2 ± 0ab	0.2 ± 0.0a	*
18:0	1.4	1.6 ± 0.1	1.6 ± 0.0	1.6 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	
18:1n-9 t	0.2	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	
18:1n-9	2.6	2.4 ± 0.1	2.7 ± 0.3	2.6 ± 0.3	2.4 ± 0.1	2.2 ± 0.2	
18:1n-7	1.1	1.3 ± 0.0	1.6 ± 0.3	1.3 ± 0.2	1.3 ± 0.1	1.0 ± 0.0	
18:2n-6	6.0	3.4 ± 0.0	3.5 ± 0.3	3.8 ± 0.6	3.1 ± 0.2	3.3 ± 0.3	
18:3n-3	0.7	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	
18:4n-3	0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
20:1n-11	0.9	0.8 ± 0.0	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.0	0.6 ± 0.0	
20:1n-9	0.7	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.1	0.5 ± 0.0	0.5 ± 0.1	
21:0	0.6	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	
20:2n-6	1.0	0.7 ± 0.1	0.7 ± 0.0	0.7 ± 0.1	0.6 ± 0.0	0.6 ± 0.1	
20:3n-6	0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
Unknown	0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	
20:4n-6	0.8	0.9 ± 0.0	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	
22:1n-9	0.2	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
20:5n-3	1.5	2.5 ± 0.1	2.2 ± 0.1	2.3 ± 0.2	2.0 ± 0.2	1.9 ± 0.1	
22:2NMI	2.1	1.9 ± 0.0	1.8 ± 0.1	1.8 ± 0.2	1.7 ± 0.1	1.5 ± 0.1	
22:2n-6	0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	
22:4n-6	0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
24:0	0.0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0 ± 0.0	
22:5n-6	0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0 ± 0.0	
22:5n-3	1.4	1.7 ± 0.1	1.7 ± 0.0	1.6 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	
22:6n-3	0.7	1.0 ± 0.0	0.9 ± 0.0	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	
24:5n-3	0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
Total	34.3	34.9 ± 1.1	34.3 ± 0.7	34.0 ± 2.7	30.5 ± 1.1	28.9 ± 1.8	
SFA	13.2	15.2 ± 0.7	14.4 ± 0.3	14.4 ± 1.0	13.2 ± 0.6	12.4 ± 0.7	
MUFA	5.8	5.6 ± 0.1	6.3 ± 0.6	5.8 ± 0.6	5.4 ± 0.3	4.7 ± 0.3	
n-3 PUFA	4.6	6.0 ± 0.2	5.5 ± 0.1	5.5 ± 0.5	4.9 ± 0.4	4.7 ± 0.3	
n-3 LC PUFA	3.8	5.4 ± 0.2	5.0 ± 0.1	4.9 ± 0.4	4.4 ± 0.4	4.2 ± 0.3	
n-6 PUFA	8.2	5.4 ± 0.3	5.4 ± 0.3	5.7 ± 0.6	4.7 ± 0.3	4.9 ± 0.5	
n-6 LC PUFA	2.2	1.9 ± 0.2	1.9 ± 0.0	1.9 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	
n-6/n-3	1.78	0.9 ± 0.04	0.97 ± 0.04	1.03 ± 0.04	0.97 ± 0.03	1.03 ± 0.04	
		P32	**P35**	**P38**	**P41**	**P44**	**Sig.**
17°C							
10:0		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
12:0		0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
13:0		2.1 ± 0.0	2.3 ± 0.2	2.1 ± 0.4	1.2 ± 0.1	1.7 ± 0.4	
14:0		0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	
15:0		0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
15:1n-5		0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
16:0		4.8 ± 0.2	5.8 ± 0.9	5.6 ± 0.5	5.3 ± 0.2	5.9 ± 0.1	
16:1n-7		0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	
17:0		2.7 ± 0.2	2.8 ± 0.3	3.0 ± 0.2	2.8 ± 0.0	3.0 ± 0.1	

16:2n-4	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	
18:0	1.2 ± 0.1	1.4 ± 0.2	1.4 ± 0.1	1.3 ± 0.0	1.4 ± 0.0	
18:1n-9 t	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	
18:1n-9	2.0 ± 0.1	2.6 ± 0.5	2.6 ± 0.4	2.4 ± 0.1	2.7 ± 0.0	
18:1n-7	1.0 ± 0.1	1.2 ± 0.3	1.1 ± 0.1	1.0 ± 0.0	1.0 ± 0.0	
18:2n-6	2.6 ± 0.2	3.4 ± 0.5	3.4 ± 0.4	3.3 ± 0.2	3.8 ± 0.0	
18:3n-3	0.3 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	
18:4n-3	0.1 ± 0.0ab	0.1 ± 0ab	0.1 ± 0.0b	0.1 ± 0.0b	0.1 ± 0.0a	*
20:1n-11	0.7 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.0	0.8 ± 0.0	
20:1n-9	0.5 ± 0.0	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	
21:0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	
20:2n-6	0.4 ± 0.0	0.5 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	
20:3n-6	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
Unknown	0.8 ± 0.8	0.8 ± 0.8	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	
20:4n-6	0.7 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.7 ± 0.0	0.8 ± 0.0	
22:1n-11	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	
22:1n-9	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
20:5n-3	1.9 ± 0.1	2.1 ± 0.3	2.1 ± 0.2	2.1 ± 0.0	2.3 ± 0.1	
22:2NMI	1.5 ± 0.1	1.6 ± 0.2	1.7 ± 0.1	1.5 ± 0.0	1.7 ± 0.0	
22:4n-6	0.1 ± 0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
24:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	
22:5n-6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	
22:5n-3	1.5 ± 0.2	1.5 ± 0.2	1.7 ± 0.2	1.4 ± 0.0	1.5 ± 0.0	
22:6n-3	0.8 ± 0.0	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.0	
24:5n-3	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
Total	26.5 ± 1.7	30.8 ± 4.3	30.6 ± 3.2	27.3 ± 0.9	31.0 ± 0.8	
SFA	11.5 ± 0.6	13.1 ± 1.7	12.9 ± 1.3	11.2 ± 0.3	12.9 ± 0.6	
MUFA	4.5 ± 0.3	5.6 ± 1.1	5.3 ± 0.6	4.9 ± 0.2	5.4 ± 0.1	
n-3 PUFA	4.7 ± 0.3	5.1 ± 0.6	5.3 ± 0.5	4.8 ± 0.1	5.4 ± 0.1	
n-3 LC PUFA	4.3 ± 0.3	4.6 ± 0.6	4.8 ± 0.4	4.3 ± 0.1	4.8 ± 0.1	
n-6 PUFA	3.9 ± 0.3	4.9 ± 0.7	4.9 ± 0.6	4.7 ± 0.2	5.2 ± 0.0	
n-6 LC PUFA	1.3 ± 0.1	1.5 ± 0.2	1.5 ± 0.1	1.3 ± 0.0	1.4 ± 0.0	
n-6/n-3	0.84 ± 0.02	0.95 ± 0.06	0.92 ± 0.03	0.96 ± 0.01	0.97 ± 0.02	
	P32	**P35**	**P38**	**P41**	**P44**	**Sig.**
22°C						
13:0	2.3 ± 0.2	2.2 ± 0.1	2.3 ± 0.2	2.4 ± 0.0	2.4 ± 0.2	
14:0	0.4 ± 0ab	0.4 ± 0.0a	0.4 ± 0.0a	0.5 ± 0.0b	0.4 ± 0.0ab	**
15:0	0.2 ± 0.0bc	0.2 ± 0bc	0.2 ± 0.0a	0.2 ± 0.0c	0.2 ± 0.0ab	**
15:1n-5	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0b	0.1 ± 0.0ab	*
16:0	6.6 ± 0.1a	6.6 ± 0.2a	6.5 ± 0.1a	8.1 ± 0.2b	7.3 ± 0.4ab	**
16:1n-7	0.3 ± 0.0ab	0.3 ± 0.0ab	0.3 ± 0.0a	0.4 ± 0.0b	0.3 ± 0.0ab	*
17:0	3.1 ± 0.1ab	3.1 ± 0.2ab	3.0 ± 0.1a	3.5 ± 0.1b	2.9 ± 0.0ab	*
16:2n-4	0.3 ± 0.0ab	0.3 ± 0.0ab	0.3 ± 0.0a	0.4 ± 0.0b	0.3 ± 0.0ab	**
18:0	1.5 ± 0.0a	1.5 ± 0.0a	1.4 ± 0.0a	1.8 ± 0.1b	1.6 ± 0.1ab	**
18:1n-9 t	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.2 ± 0.0b	0.2 ± 0.0b	**
18:1n-9	3.4 ± 0.2a	3.3 ± 0.1a	3.2 ± 0.2a	4.6 ± 0.3b	3.9 ± 0.2ab	**
18:1n-7	1.3 ± 0.0ab	1.3 ± 0.1a	1.2 ± 0.0a	1.5 ± 0.1b	1.2 ± 0.0ab	*
18:2n-6	4.1 ± 0.3a	4.0 ± 0.1a	4.0 ± 0.1a	5.7 ± 0.2b	5.4 ± 0.4b	**
18:3n-3	0.5 ± 0.0ab	0.5 ± 0.0a	0.5 ± 0.0a	0.7 ± 0.0c	0.6 ± 0.0bc	***
18:4n-3	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	* †
20:1n-11	1.0 ± 0.0ab	1.0 ± 0.1a	0.9 ± 0.0a	1.2 ± 0.1b	1.0 ± 0.0ab	*
20:1n-9	0.6 ± 0.1ab	0.6 ± 0.0a	0.6 ± 0.0a	0.8 ± 0.0b	0.7 ± 0.0ab	*
21:0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	
20:2n-6	0.5 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.6 ± 0.1	0.5 ± 0.0	
20:3n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
20:4n-6	0.9 ± 0.0ab	0.9 ± 0.1ab	0.8 ± 0.0a	1.0 ± 0.0b	0.9 ± 0.0ab	*
20:4n-3	0.0 ± 0.0ab	0.0 ± 0.0a	0.0 ± 0ab	0.2 ± 0.0b	0.1 ± 0.0ab	*

22:1n-11	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
22:1n-9	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
20:5n-3	2.4 ± 0.1ab	2.2 ± 0.1ab	2.1 ± 0.1a	2.6 ± 0.1b	2.2 ± 0.1.0a	*
22:2NMI	1.7 ± 0.0a	1.8 ± 0.1ab	1.7 ± 0.0a	2.1 ± 0.1b	1.8 ± 0.0a	**
22:4n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
22:5n-3	1.5 ± 0.0	1.5 ± 0.1	1.4 ± 0.1	1.7 ± 0.1	1.4 ± 0.0	
22:6n-3	1.1 ± 0.1ab	1.0 ± 0.1a	1.0 ± 0.1a	1.3 ± 0.0b	1.1 ± 0.0ab	*
24:5n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	
Total	35.0 ± 1.0a	34.2 ± 1.2a	33.2 ± 0.6a	42.7 ± 1.3b	37.6 ± 1.7ab	**
SFA	14.3 ± 0.3a	14.3 ± 0.5a	14.0 ± 0.3a	16.8 ± 0.4b	15.2 ± 0.7ab	*
MUFA	6.9 ± 0.4a	6.6 ± 0.3a	6.3 ± 0.2a	8.8 ± 0.4b	7.5 ± 0.3ab	**
n-3 PUFA	5.8 ± 0.2ab	5.5 ± 0.2a	5.3 ± 0.2a	6.7 ± 0.0b	5.7 ± 0.2a	**
n-3 LC PUFA	5.2 ± 0.2ab	4.9 ± 0.2a	4.7 ± 0.2a	5.8 ± 0.0b	4.9 ± 0.2a	**
n-6 PUFA	5.7 ± 0.4ab	5.6 ± 0.2a	5.5 ± 0.1a	7.6 ± 0.3c	7.0 ± 0.4bc	**
n-6 LC PUFA	1.5 ± 0.0a	1.6 ± 0.0a	1.5 ± 0.0a	1.8 ± 0.1b	1.6 ± 0.1ab	**
n-6/n-3	0.98 ± 0.03a	1.01 ± 0.01ab	1.05 ± 0.02ab	1.15 ± 0.04bc	1.24 ± 0.03c	**

Fatty acids < 0.1 mg g^{-1} sample for all dietary treatments at all experimental temperatures are not included in this table. SFA, sum of saturated fatty acids; MUFA, sum of monounsaturated fatty acids; n-3/n-6 PUFA, sum of all omega-3/6 fatty acids with two or more double bonds; n-3/6 LC PUFA, sum of all omega-3/6 fatty acids ≥ 20 C and with two or more double bonds. Data are expressed as mean ± SEM. Values in the same row with different superscripts are significantly different (one-way ANOVA and Tukey's post hoc test). *, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. †ANOVA may indicate significant differences, however significant differences were not detected in post-hoc.

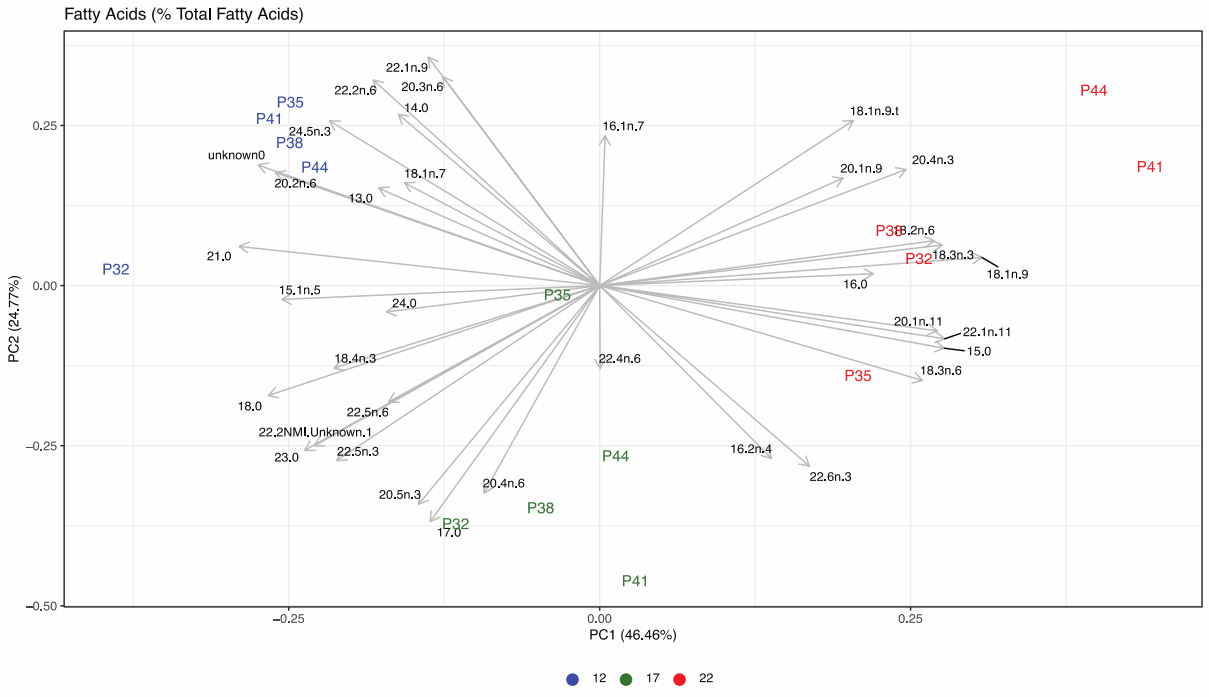

Figure 7: PCA of sub-adult Australian hybrid abalone based on fatty acid composition

Discussion

Slow and variable growth and high summer mortality limit the potential future growth of the Australian abalone aquaculture industry (Day & Fleming 1992; Stone et al., 2014b; Vandepeer 2006). While large seasonal fluctuations in water temperature, beyond the thermal optimal of abalone species, drive these undesirable outcomes, artificial temperature control in flow-through systems remains unviable. Previous research has identified the potential to enhance the growth of cultured abalone through dietary manipulation, including increasing the dietary protein level (Stone et al. 2016). However, this remains understudied and unverified across size cohorts and water temperatures in a controlled environment for hybrid abalone. To this end, the current study aimed to identify the optimal protein inclusion level in diets for cultured sub-adult Australian hybrid abalone at three distinct water temperatures representative of winter, spring/autumn and summer growing conditions.

A suite of measured performance parameters were calculated with a focus on growth performance and nutrient utilisation. As expected, weight gain was substantially higher at 22 °C compared to 12 °C, yet, a major finding was that growth performance, including, FCR, improved with increasing dietary protein at each of the experimental temperatures tested and weight gain improved at both 17 °C and 22 °C with increasing dietary protein. This trend showed little sign of abating within the tested range of dietary protein inclusion levels, suggesting that the optimal inclusion level may have been beyond the tested range (i.e., > 44% dietary protein). However, it should be noted that the sub-adult abalone used in the present experiment experience relatively slow growth rates compared to juvenile abalone (Mulvaney et al., 2013; Stone et al., 2013). Therefore, the trial duration may not have been sufficient to fully realise the potential maximum growth performance at the higher dietary protein inclusion levels and may have resulted in an inability to accurately identify a maximum dietary protein inclusion level. In the present study, the SGR of abalone reared at 22 °C was comparable with that of a previous experiment conducted under commercial conditions (~0.6) (Stone et al., 2016). Furthermore, a systematic review of SGR in abalone indicates that the SGRs obtained in the present experiment were comparable or exceeded those observed in previous published works (Kirkendale et al., 2010).

Nevertheless, the observed improvements in growth performance with increasing dietary protein levels were more pronounced at higher temperatures, particularly at 22 °C. Specifically, at 22 °C a 36% higher weight gain was observed when dietary protein was increased from 35% to 44% of the diet, with abalone fed the latter diet achieving a total weight gain of 146%. This is in agreement with Stone et al. (2016) who found improved growth performance of hybrid abalone at high water temperatures (20-22 °C) in hybrid abalone fed a relatively high dietary protein level (39%). The same increase in dietary protein at 12 °C, however, only resulted in an 8% increase in weight gain, concomitant with a much lower total weight gain (42%), which again, was somewhat expected given the very slow growth observed in similar abalone species reared at water temperatures of up to 14 °C (Stone et al. 2013). Interestingly, and in contrast to the present study, a reduction in growth performance (reduced SGR and increased FCR) was observed in Greenlip and Blacklip Abalone when fed diets in excess of 35% dietary protein (Coote et al. 2000). This is testament to the importance of conducting species-specific research (e.g., hybrid abalone) when determining optimal inclusion levels for macronutrients and individual dietary ingredients.

Clearly, enhancing the growth performance of cultured animals remains a major objective for aquaculture producers, however, the efficient use of dietary nutrients is vital to ensure industry expansion is both economically and environmentally sustainable (Glencross, 2020). An inefficient use of dietary nutrients, particularly dietary protein, by cultured fish and shellfish may result in an increased cost of production as well as negative environmental outcomes such as eutrophication of the surrounding environment due to an increase in discharged undigested nitrogen (Crab et al., 2007; Howarth & Marino, 2006). In the present study, an increase in dietary protein had no negative effect on the apparent digestibility of diet dry matter (70%), protein (75-80%) or lipid, regardless of rearing temperature. Although, it should be noted that while the apparent digestibility of dry matter was within the range expected for other abalone species (Fleming, 1995; Montaño-Vargas et al., 2002; Sales & Britz, 2001, 2002), the apparent digestibility of protein was on the lower end of the ranges reported for South African Abalone (*Haliotis middae*) (Sales & Britz 2001, 2002). In the latter studies, protein source selection was found to have a significant impact on protein digestibility, yet remains underexplored in hybrid abalone. Regardless, the lower protein digestibility observed in the present experiment may have limited their potential

performance, contributing to an inability to ascertain a maximum dietary protein inclusion level. Despite this, protein and energy deposition efficiency parameters, including protein and energy deposition % and energy efficiency ratios were not negatively affected by an increase in dietary protein. In fact, increased deposition efficiencies were typically observed at the higher dietary protein levels (38-44%). In summary, this research highlights that improvements in the growth performance of hybrid abalone owing to an increase in the level of dietary protein does not incur the cost of inefficient nutrient utilisation.

Abalone are prized by consumers for their taste, cultural significance and healthy nutritional profile (Mulvaney, 2016). Considering the relative healthiness of seafood is a major determinant of seafood consumption in Australia (Christenson et al., 2017), any change to the nutritional composition of commercial diets must consider the potential impact on the nutritional profile of the abalone tissue. Previous research has shown the potential to modulate the nutritional profile, including the amino acid and fatty acid profile of abalone through changes to the diet (Cochet et al., 2013; Mulvaney, 2016). Promisingly, in the present study, increasing the dietary protein level had no effect on the amino acid profile of abalone soft tissue, regardless of rearing temperature. While the same was true for the fatty acid profile of hybrid abalone reared at 12 °C and 17 °C, there appeared to be an effect of diet on the concentration of fatty acids in abalone reared at 22 °C. Most notably, increased tissue levels of 18:0, 18:1n-9 and to a lesser extent 20:5n-3 (EPA) were recorded. The exact reasons behind this apparent increase in the concentration of fatty acids, despite no increase in total lipid should be further explored with additional research. Importantly, an increase in the dietary protein level did not cause a decrease in the concentrations of human health beneficial fatty acids of the omega-3 series, 20:5n-3 or 22:6n-3 (DHA). Taken together, this suggests that a potential increase in the dietary protein level for hybrid abalone will have minimal effect on the nutritional quality of the final product delivered to the consumer.

Conclusions

The present experiment aimed to establish the optimal level of dietary protein in diets for farmed hybrid abalone. It was clearly demonstrated that increasing the dietary protein level from 32 to 44% resulted in stepwise improvements in growth performance with no negative effects on mortality, nutrient utilisation, or the final nutritional profile. Furthermore, this

effect was manifestly greater at higher water temperatures representative of summer growing conditions in Australia. This suggests that the development of seasonally tailored diets targeting improved growth during periods of faster growth (i.e., summer) may yield significant benefits to production. Notably, an optimal dietary protein inclusion level was difficult to ascertain in the present experiment. It is suggested that future work that employs an extended trial duration or utilises relatively faster growing size cohorts (i.e., juvenile abalone) may better elucidate a maximum protein inclusion level in hybrid abalone with respect to growth performance. Moreover, future trials could experiment with different sources of protein in diets, given that not all protein sources are equally digested and therefore have the potential to affect the performance of farmed abalone.

Task 3.1 Juvenile hybrid abalone growth trial

Introduction

In **Task 2.1** of the current report, the results of an experiment using hybrid abalone sub-adults provided strong evidence that significant gains in growth performance can be achieved by increasing the dietary protein inclusion rate. These improvements did not coincide with a deterioration in the nutrient utilisation efficiency or nutritional quality of the cultured animal. The previous experiment tested a range of dietary protein inclusion levels (32-44% of the diet) which were considered broad enough to elucidate the optimal inclusion level for hybrid abalone sub-adults. However, for numerous growth parameters, performance was superior at the higher or highest protein inclusion levels tested, limiting the ability to identify a maximum or optimal dietary protein inclusion level. This was, in-part, attributed to a relatively short culture period given the slow growth rates of sub-adult abalone and, potentially, an unexplored effect of dietary protein source, which saw protein digestibility values that were slightly lower than expected, which may have impacted abalone performance. Taken together, this suggests that the effect of a dietary protein inclusion > 44% should be examined in a size cohort of abalone with expectedly faster growth rates (e.g. juveniles). In the previous experiment, however, it was clear that an increase in the dietary protein inclusion level had the greatest benefit in abalone reared at higher water temperatures (22 °C), where overall growth rates were substantially faster compared to animals reared at 12 °C and 17 °C. It was therefore recommended that seasonally tailored diets, namely during the summer growing period, that utilise relatively high levels of dietary protein would enhance growth performance and ultimately improve the production of cultured hybrid abalone.

Beyond optimising macronutrient inclusion levels in diets for abalone, there is growing interest to investigate novel ingredients which may promote growth performance and improve the nutritional quality of cultured animals. Considering the diet of wild abalone consists primarily of macroalgae, their digestive system is well-suited to their inclusion in formulated feeds (Bansemer et al., 2016). Specifically, it has been demonstrated that the inclusion of *Ulva spp.* in formulated feeds for hybrid abalone provided good growth and improved tissue levels of n-3 LC PUFA compared to non-algae supplemented feeds

(Mulvaney, 2016). Therefore, the present experiment aimed to evaluate the efficacy of a diet containing *Ulva ohnoi* alongside a diet containing no added macroalgae.

Accordingly, the present experiment examined a different size class of abalone (juvenile) compared to **Task 2.1** (sub-adult) while also increasing the upper range of protein inclusion levels in the experimental diets. It is expected that taken together with the promising results of **Task 2.1**, a more comprehensive understanding of the nutritional requirements for Australian hybrid abalone will be achieved. Ultimately, leading to the development and adoption of optimised dietary formulations and feeding strategies for the Australian hybrid abalone aquaculture industry.

Methods

The experimental system, animals, stocking and acclimation

The design of the experimental system, stocking protocol and acclimation period used in the present experiment was identical to that presented in detail in **Task 2.1** in the present report. The exception being the size and age of the abalone used in the current experiment were ~10-months of age and each tank was stocked with 30 individuals with the trial lasting 150 days.

Experimental diets, feeding and faeces collection

Six experimental diets, fed to triplicate tanks of abalone within each temperature treatment, were formulated to contain graded dietary protein levels: 350, 380, 410, 440 and 470 g kg^{-1} and assigned the labels P35, P38, P41, P44 and P47, respectively (**Table 10**). An additional dietary treatment was formulated to contain 410 g kg^{-1} protein with the addition of green seaweed (*Ulva ohnoi*) at 150 g kg^{-1} and labelled P41U. The protein levels were achieved by altering the inclusion levels of the principal protein sources, namely, soybean meal, casein and lupin flour at the expense of pregelatinised starch and the inert filler, diatomaceous earth. All other dietary ingredients remained identical and were included at similar levels across the experimental diets. Consistent with commercial formulations, the diets were formulated to contain 3-4% dietary lipid, using fish and canola oil. Diets were formulated to

be isoenergetic; (18 MJ kg^{-1}). As in **Task 2.1**, the amino acid composition of the experimental diets was balanced to follow the soft tissue composition of the parent species (*Haliotis laevigata* and *Haliotis rubra*). All the dietary ingredients were analysed for proximate composition prior to diet formulation.

The pelletisation of the experimental diets, feeding regime and faeces collection was identical to the methods described in detail in **Task 2.1**. All the experimental diets contained 0.1% of yttrium oxide (Y_2O_3) as an inert marker for subsequent digestibility analysis.

Yttrium oxide analysis

The Y_2O_3 content of the experimental diets and faeces was determined by digesting 50 mg of homogenous sample in 4.5 mL 70% nitric acid at 90 °C in a water bath for 1 hour. This was repeated the following day. On the third day, 0.5 mL 30% hydrogen peroxide was added, and the digestion was conducted at 80 °C for 3 hours. Subsequently, 5 mL Milli-Q water was added to the samples and allowed to cool. Once cooled, samples were vortexed and centrifuged at 3,000 RCF for 3 minutes. Samples were diluted 1:20 in Milli-Q water and stored until subsequent analysis by inductivity-coupled plasma mass-spectroscopy (ICP-MS). ICP-MS was conducted at the Plant Chemistry Research Laboratory of the School of Life and Environmental Sciences, Deakin University, Burwood Campus, VIC, Australia.

All other materials and methods used in the current study, namely, water quality management, growth performance and digestibility calculations, biochemical and statistical analysis were conducted in accordance with those described in detail in **Task 1** and **Task 2.1**.

Results

General observations

Proximate analysis confirmed that the experimental feeds closely represented dietary formulations (**Table 11**). Accordingly, there was a stepwise increase in dietary protein concentration between P35 and P47. Total energy was similar between diets, ranging from 19-20 MJ kg^{-1} in P41U and P47, respectively.

Abalone readily accepted the experimental feeds and appeared healthy throughout the experiment with no mortalities recorded. Compared to the previous experiment, there was considerably less colonisation by calcareous tubeworm (*Spirobis sp*.), nevertheless no impact on health or feeding behaviour was observed.

Notably, the addition of *U. ohnoi* in P41U impacted pellet stability where a faster absorption and subsequent deterioration of the pellet was observed, particularly evident in the first two hours after immersion in water.

Abalone feed consumption and growth performance

In general, with respect to growth and feed conversion parameters, there was an observable increase in performance with increasing dietary protein inclusion until P41, after which, performance declined. This pattern was consistent across the three experimental temperatures. Feed consumption and condition factor, however, were an exception and did not follow this pattern (**Table 12** & **Figure 8**).

As with **Task 2.1**, within each experimental temperature, the differences in growth performance parameters between dietary treatments was assessed using a one-way analysis of variance (ANOVA) and Tukey's post hoc analysis. With respect to the 12 °C experiment, expectedly, growth performance and feed consumption was observably lower compared to both the 17 °C and 22 °C experiments. Here, abalone grew from an initial weight of 3.28 g to obtain final weights ranging from 6.49 and 7.04 g in P35 and P41, respectively. However, these differences were not significant. In fact, few significant differences between dietary treatments were detected between dietary treatments except for total feed consumption which was highest in P47.

Differences in growth performance and feed consumption parameters between dietary treatments were more apparent in the 17 °C experiment, where abalone grew noticeably faster compared to those reared at 12 °C. Multiple growth performance and feed efficiency parameters were superior in the P41 treatment and were significantly improved compared to P35 and P38. For example, final weight ranged from 11.13 to 13.55 g in P35 and P41, respectively and FCR ranged from 1.26 to 1.00 in P35 and P41, respectively. Total feed

consumption peaked in P44 and, with the exception of P47, was significantly higher compared to the other dietary treatments.

Similar to the 17 °C experiment, at 22 °C, the P41 treatment outperformed other dietary treatments, particularly P41U, with respect to multiple growth parameters. Final weights ranged from 12.33 to 16.96 g in P41U and P41, respectively despite a higher total feed consumption in P41. Relatedly, FCR was significantly lower (superior) in P41 (1.22) compared to P41U (1.93).

Nutrient deposition and feed digestibility

The retention of dietary nutrients, namely, protein and total energy, fed to abalone in the present experiment was calculated in terms of both deposition % and deposition efficiency ratios (**Table 13** & **Figure 9**). In general, regardless of dietary treatment, the deposition and retention of dietary protein and energy appeared to be highest in abalone reared at 17 °C and poorest in those reared at 12 °C. A few common trends appeared when comparing dietary treatments within each temperature experiment, for example, nutrient retention and deposition appeared more efficient in P38 and P41.

There were few differences recorded between dietary treatments when focussing on the 12 °C experiment, with the exception of EER which ranged from 24.8 to 29.3 in P47 and P38, respectively. At 17 °C, P41 recorded superior PER and EER compared to poorer performing treatments such as P44 and P47. Most notably, PER ranged from 1.9 in both P44 and P47, compared to 2.4 in P41. The 22 °C experiment proved to be a mid-point in energy and protein retention among all the experimental temperatures, with numerous significant differences recorded between dietary treatments. The P41U treatment was clearly detrimental to nutrient retention when compared with P41, with ED% ranging from 16.8 to 26.1% in P41U and P41, respectively.

Apparent digestibility coefficients (ADC%), calculated for both proximate composition and individual amino acids, revealed that dietary nutrients were, in general, well digested, however, the effect of dietary protein inclusion level was not uniform across the different experimental temperatures (**Table 14**, **Table 15** & **Figure 10**). Regardless of experimental

temperature or dietary treatment, several individual amino acids were highly digested, including, methionine, proline and lysine (~90%).

At 12 °C, where the digestibility of dietary nutrients was lower compared to the other experimental temperatures, there was no clear relationship with increasing dietary protein. Despite, relatively low dry matter digestibility (69%), the digestibility of protein was high and unaffected by dietary treatment, ranging from 83.4 to 87.3% in P41U and P44, respectively. At 17 °C, the effect of dietary protein level on the digestibility of nutrients was clearer, with numerous significant differences recorded. Most notably, digestibility values increased from P35 to P44, after which, values plateaued. For example, protein ADC % ranged from 84.1 to 90.4 % in P35 and P44, respectively and this was reflected in the values recorded for individual amino acids. While a similar trend of increasing ADC values between P35 and P44 was recorded in abalone reared at 22 °C, values declined at the highest protein inclusion level (P47). The digestibility of lipid was a notable example, ranging from 77.0 to 88.7% in P35 and P44, respectively. Despite differences in the digestibility of macronutrients between dietary treatments at 22 °C, there was no difference between treatments with respect to individual amino acids.

In general, the inclusion of *U. ohnoi* in the diet did not appear to affect the digestibility of dietary nutrients, when comparing P41 and P41U, regardless of experimental temperature.

Abalone tissue proximate, amino acid and fatty acid composition

The soft tissue proximate composition of abalone in the current experiment was dictated by dietary treatment (**Table 16**). Specifically, protein concentrations in soft tissue increased with increasing dietary protein in all experimental temperatures and ranged from 584.6 to 697.2 mg g^{-1} dry tissue in P35 and P47, respectively in abalone reared at 17 °C. This increase was inversely proportional to decreasing NFE concentrations. Lipid concentrations remained relatively constant across dietary treatments, however, P35 exhibited slightly, albeit significantly lower soft tissue lipid compared to both P41 and P41U. Differences in soft tissue amino acid concentrations between dietary treatments reflected the increasing tissue protein concentrations concomitant with increasing dietary protein inclusion levels (**Table 17**). However, differences were most noticeable at 12 °C and 17 °C. At all experimental

temperatures, the most prominent individual amino acids were glutamic acid, aspartic acid, arginine and glycine.

When reported as percentage of total amino acids, experimental temperature appeared to have some influence on amino acid composition (**Figure 11**). Generally, abalone soft tissue samples clustered by rearing temperature along PC1 (x-axis), which explains 36.63% of variation in AA composition. Abalone reared at 12 °C clustered on the negative end of PC1 (strongly driven by isoleucine and valine) and abalone reared at 17 and 22 °C clustered on the positive end of PC1 (strongly driven by serine). Additionally, PC2 (y-axis) explained 28.68% of the variation in AA composition. Generally, low protein diets (< 40% protein) had positive scores on PC2, while high protein diets (> 40% protein) had negative scores on PC2. Positive PC2 scores were driven by proline and alanine, while negative PC2 scores were driven by methionine, threonine, tyrosine and leucine.

On a mg g^{-1} dry basis, the fatty acid composition of abalone soft tissue was typified by relatively high levels of 13:0, 16:0, 18:1n-9 and 18:2n-6, regardless of experimental temperature or dietary treatment (**Table 18**). Despite relatively small numerical differences in individual fatty acid concentrations, there were several significant differences between dietary treatments, with each experimental temperature. This included 18:3n-3 which was present in significantly lower concentrations in P47 at both 12 °C and 17 °C.

When reported as percentage of total fatty acids, the resulting fatty acid compositions were clustered according to experimental temperature and protein inclusion level (**Figure 12**). Abalone reared at three temperatures separated along PC1 (x-axis), which explains 44.34% of the variation in FA composition. Abalone reared at 12 °C had the lowest PC1 scores, abalone reared at 17 °C had PC1 scores near 0 and abalone reared at 22 °C had the highest PC1 scores. The FA composition of abalone reared at 12 °C was characterised by higher percentages of short chain, saturated and monounsaturated FA (15:0, 16:0, 16:1n-7, 18:1n-7, 18:1n-9, 16:2n-4 and 18:2n-6). Conversely, the FA composition of abalone reared at 22 °C was characterised by higher percentages of long chain, omega-3 and omega-6 FA (20:3n-3, 20:5n-3, 22:5n-3, 22:6n-3, and 20:2n-6, 20:3n-6, 22:2n-6, 22:5n-6).

Abalone fed at six different diets separated along PC2 (y-axis), which explains 19.07% of the variation in FA composition. Abalone fed lower protein inclusion diets (P35, P38 and P41) had lower PC2 scores, while abalone fed higher protein diets (P44 and P47) and a diet with *Ulva spp.* Inclusion (P41U) had higher PC2 scores. The FA composition of abalone fed lower protein inclusion was characterised by higher percentages of 18:1n-9t, 20:1n-11 and 22:4n-6, compared to higher percentages of 18:4n-3 and 20:4n-6 in abalone tissues fed high protein and *Ulva spp.* supplemented diets.

Table 10: Formulation of the experimental diets fed to juvenile Australian hybrid abalone.

	P35	**P38**	**P41**	**P44**	**P47**	**P41 U**
Nominal protein level (g kg^{-1})	350	380	410	440	470	410
Ingredient Composition (g kg^{-1})						
Soy selecta	126.3	144.4	162.4	180.5	198.6	144.7
Lupin flour	63.2	72.2	81.2	90.3	99.3	72.4
Casein	126.3	144.4	162.4	180.5	198.6	144.7
Pregelatinised starch	429.1	385.9	342.7	299.4	256.2	268.2
Diatomaceous earth	24.3	23.0	21.8	20.5	19.2	0.0
Canola oil	5.3	4.6	4.0	3.3	2.7	4.5
Green seaweed (*Ulva ohnoi*)	0	0	0	0	0	150
Diatomaceous earth	10	10	10	10	10	0
Fishmeal	50	50	50	50	50	50
Gluten	50	50	50	50	50	50
Gelatin	50	50	50	50	50	50
Fish oil	5	5	5	5	5	5
Yttrium	0.5	0.5	0.5	0.5	0.5	0.5
Lecithin	3	3	3	3	3	3
Vit & min mix	4	4	4	4	4	4
Vit C (STAY-C) 35%	0.5	0.5	0.5	0.5	0.5	0.5
Choline	5	5	5	5	5	5
Vitamin E	1	1	1	1	1	1
Monosodium phosphate	7.5	7.5	7.5	7.5	7.5	7.5
Calcium sulphate	5	5	5	5	5	5
Agar	5	5	5	5	5	5
Sodium alginate	5	5	5	5	5	5
Methionine	6	6	6	6	6	6
Lysine	6	6	6	6	6	6
Arginine	6	6	6	6	6	6
Threonine	6	6	6	6	6	6

Table 11: Proximate, amino acid and fatty acid composition of the experimental diets fed to juvenile Australian hybrid abalone.

	P35	P38	P41	P44	P47	P41 U
Proximate composition (g kg^{-1} dry diet)						
Dry matter	971.3	960.8	970.3	969.9	972.4	972.5
Protein	369.5	385.7	425.7	466.2	491.0	416.8
Lipid	29.9	28.2	31.9	33.0	31.0	35.4
Ash	58.5	59.5	57.7	59.7	60.2	95.3
NFE	542.0	526.5	484.7	441.1	417.9	452.5
Energy (MJ kg^{-1})	19.2	19.3	19.6	19.9	20.0	19.0
Amino Acid composition (mg g^{-1} diet)						
Histidine	7.1	7.8	8.7	9.4	10.4	8.7
Serine	13.7	13.9	16.9	19.3	21.9	16.8
Arginine	21.8	23.6	25.6	27.3	30.2	25.6
Glycine	19.5	20.4	21.1	22.5	23.9	22.2
Aspartic acid	26.1	29.0	32.1	35.4	39.6	32.6
Glutamic acid	67.1	72.6	80.1	87.5	96.6	78.4
Threonine	15.5	16.2	18.1	20.2	21.9	18.3
Alanine	13.8	14.8	15.8	17.3	18.8	16.9
Proline	31.5	32.8	35.5	38.5	42.9	35.1
Lysine	22.9	23.1	25.5	23.6	29.4	22.7
Tyrosine	8.0	8.8	11.0	10.9	13.3	10.4
Methionine	10.0	10.6	11.6	11.9	12.7	11.3
Valine	15.9	17.6	18.9	21.0	22.8	19.4
Isoleucine	14.0	15.7	16.8	18.9	20.7	17.0
Leucine	23.5	25.5	28.5	31.4	35.0	28.2
Phenylalanine	13.7	15.0	16.7	18.4	20.5	16.7
Total	324.1	347.4	383.0	413.6	460.7	380.4
Fatty acid composition (mg g^{-1} diet)						
13:0	3.2	3.0	2.8	3.0	2.7	2.9
14:0	0.2	0.2	0.3	0.3	0.3	0.3
15:1n-5	0.0	0.0	0.0	0.0	0.0	0.0
16:0	1.9	2.5	2.6	2.9	2.7	2.7
16:1n-7	0.3	0.3	0.3	0.4	0.3	0.3
16:2n-4	0.0	0.1	0.1	0.1	0.1	0.1
18:0	0.7	0.9	0.9	1.0	1.0	0.9
18:1n-9	10.2	12.0	11.8	12.2	10.9	10.4
18:1n-7	0.6	0.7	0.7	0.7	0.7	0.7
18:2n-6	4.4	5.4	5.5	6.0	5.5	4.7
18:3n-3	1.5	1.7	1.7	1.8	1.6	1.4
20:0	0.2	0.2	0.2	0.2	0.2	0.2
18:4n-3	0.0	0.0	0.0	0.0	0.0	0.1
20:1n-9	0.3	0.4	0.4	0.4	0.4	0.4
20:4n-3	0.1	0.1	0.1	0.2	0.2	0.1
22:1n-11	0.0	0.1	0.1	0.1	0.1	0.1
20:5n-3	0.3	0.3	0.3	0.3	0.3	0.3
24:0	0.0	0.1	0.1	0.1	0.0	0.0
22:5n-3	0.0	0.0	0.0	0.1	0.0	0.0
22:6n-3	0.4	0.5	0.5	0.5	0.4	0.4
Total	24.3	28.7	28.3	30.1	27.5	26.0
SFA	6.2	6.9	6.8	7.4	6.9	6.9
MUFA	11.4	13.6	13.3	13.8	12.4	12.0
n-3 PUFA	2.3	2.7	2.6	2.8	2.6	2.4
n-3 LC PUFA	0.8	0.9	0.9	1.0	0.9	0.9
n-6 PUFA	4.4	5.4	5.5	6.0	5.5	4.7
n-6 LC PUFA	0.0	0.0	0.0	0.0	0.0	0.0
n-6/n-3	2.0	2.0	2.1	2.1	2.2	2.0

Fatty acids < 0.1 mg g^{-1} sample for all dietary treatments are not included in this table. SFA, sum of saturated fatty acids; MUFA, sum of monounsaturated fatty acids; n-3/n-6 PUFA, sum of all omega-3/6 fatty acids with two or more double bonds; n-3/6 LC PUFA, sum of all omega-3/6 fatty acids ≥ 20 C and with two or more double bonds.

Table 12: Growth performance of juvenile Australian hybrid abalone

	P35	P38	P41	P44	P47	P41 U	Sig.
12°C							
Initial weight (g)	3.28 ± 0.0	3.28 ± 0.0	3.28 ± 0.0	3.28 ± 0.0	3.28 ± 0.0	3.28 ± 0.0	
Final weight (g)	6.49 ± 0.12	6.69 ± 0.19	7.04 ± 0.19	6.91 ± 0.04	6.75 ± 0.16	6.67 ± 0.07	
Initial shell length (mm)	28.0 ± 0.0	28.0 ± 0.0	28.0 ± 0.0	28.0 ± 0.0	28.0 ± 0.0	28.0 ± 0.0	
Final shell length (mm)	35 ± 0.2	35.4 ± 0.3	36.2 ± 0.3	36.1 ± 0.3	35.8 ± 0.2	35.5 ± 0.3	
SGR (% day^{-1})	0.45 ± 0.01	0.47 ± 0.02	0.50 ± 0.02	0.49 ± 0.00	0.47 ± 0.02	0.47 ± 0.01	
Survival (%)	100	100	100	100	100	100	
Weight gain (%)	97.8 ± 3.7	103.8 ± 5.8	114.6 ± 5.8	110.7 ± 1.3	105.9 ± 4.9	103.2 ± 2.2	
Biomass gain (g $tank^{-1}$)	73.8 ± 3.4	80.2 ± 9.7	90.6 ± 5.8	86.8 ± 1.3	82 ± 4.8	77.1 ± 1.5	
FCR	2.15 ± 0.07	1.9 ± 0.24	1.87 ± 0.1	1.88 ± 0.02	2.10 ± 0.09	2.01 ± 0.05	
Shell growth rate (µm day^{-1})	46 ± 1.1	48.6 ± 1.9	54.2 ± 2	53.1 ± 1.7	51.4 ± 1.3	49.3 ± 2.1	
Condition factor	0.87 ± 0.01	0.87 ± 0.02	0.86 ± 0.01	0.85 ± 0.01	0.85 ± 0.01	0.86 ± 0.01	
Feed consumption (g $tank^{-1}$)	158.2 ± 1.9b	147.8 ± 0.8a	167.9 ± 3.0c	163.0 ± 0.7bc	171.3 ± 2.8c	154.7 ± 1.5ab	***
	P35	**P38**	**P41**	**P44**	**P47**	**P41 U**	**Sig.**
17°C							
Initial weight (g)	3.25 ± 0.00	3.25 ± 0.00	3.25 ± 0.00	3.25 ± 0.00	3.25 ± 0.00	3.25 ± 0.00	
Final weight (g)	11.13 ± 0.19a	11.64 ± 0.14ab	13.55 ± 0.18c	13.26 ± 0.44c	13.49 ± 0.42c	12.49 ± 0.05bc	***
Initial shell length (mm)	28.1 ± 0.0	28.1 ± 0.0	28.1 ± 0.0	28.1 ± 0.0	28.1 ± 0.0	28.1 ± 0.0	
Final shell length (mm)	41.3 ± 0.2a	41.7 ± 0.2ab	43.9 ± 0c	43.6 ± 0.6c	43.5 ± 0.3c	42.9 ± 0.1bc	***
SGR (% day^{-1})	0.8 ± 0.01a	0.82 ± 0.01ab	0.93 ± 0.01c	0.91 ± 0.02c	0.92 ± 0.02c	0.88 ± 0bc	***
Survival (%)	100	100	100	100	100	100	
Weight gain (%)	242.6 ± 5.8a	258.2 ± 4.4ab	317.0 ± 5.7c	308.0 ± 13.5c	315.1 ± 13.1c	284.4 ± 1.7bc	***
Biomass gain (g $tank^{-1}$)	213.6 ± 5.8a	228.8 ± 4.2ab	285.9 ± 5.4c	277.2 ± 13.4c	284.1 ± 12.8c	254.4 ± 1.5bc	***
FCR	1.26 ± 0.09b	1.14 ± 0.03ab	1.00 ± 0.03a	1.19 ± 0.05ab	1.13 ± 0.05ab	1.12 ± 0.00ab	*
Shell growth rate (µm day^{-1})	87.8 ± 1.3a	90.7 ± 1.4ab	105.1 ± 0.3c	103.6 ± 3.7c	102.6 ± 2.0c	99.0 ± 0.7bc	***
Condition factor	0.92 ± 0.01	0.93 ± 0.00	0.93 ± 0.01	0.92 ± 0.01	0.95 ± 0.02	0.91 ± 0.01	
Feed consumption (g $tank^{-1}$)	269.4 ± 14.3a	260.3 ± 2.4a	285.4 ± 3.1a	329.4 ± 1.5b	320.3 ± 1.8b	285.5 ± 0.7a	***
	P35	**P38**	**P41**	**P44**	**P47**	**P41 U**	**Sig.**
22°C							
Initial weight (g)	3.27 ± 0.00	3.27 ± 0.00	3.27 ± 0.00	3.27 ± 0.00	3.27 ± 0.00	3.27 ± 0.00	
Final weight (g)	14.86 ± 0.21ab	16.47 ± 0.97b	16.97 ± 1.24b	15.40 ± 0.34ab	15.65 ± 0.99ab	12.33 ± 0.85a	*
Initial shell length (mm)	28.1 ± 0.0	28.1 ± 0.0	28.1 ± 0.0	28.1 ± 0.0	28.1 ± 0.0	28.1 ± 0.0	
Final shell length (mm)	45.3 ± 0.3ab	46.8 ± 1.1b	47.0 ± 1.2b	45.5 ± 0.3ab	45.5 ± 0.9ab	42.8 ± 0.8a	*

SGR (% day^{-1})	0.99 ± 0.01ab	1.05 ± 0.04b	1.07 ± 0.05b	1.01 ± 0.01ab	1.01 ± 0.04ab	0.85 ± 0.05a	*
Survival (%)	100	100	100	100	100	100	
Weight gain (%)	354.4 ± 6.3ab	403.6 ± 29.7b	419.0 ± 37.9b	370.8 ± 10.3ab	378.7 ± 30.3ab	277 ± 25.9a	*
Biomass gain (g tank^{-1})	325.3 ± 6.2ab	373.6 ± 29.1ab	383.7 ± 41.5b	341.5 ± 10.1ab	349.2 ± 29.8ab	249.4 ± 25.4a	*
FCR	1.44 ± 0.03ab	1.23 ± 0.10a	1.22 ± 0.13a	1.50 ± 0.04ab	1.45 ± 0.13ab	1.93 ± 0.22b	*
Shell growth rate (µm day^{-1})	114.5 ± 1.8ab	125.0 ± 7.0b	125.9 ± 7.8b	116.2 ± 2.2ab	116.1 ± 5.9ab	97.8 ± 5.4a	*
Condition factor	0.93 ± 0.00	0.93 ± 0.01	0.95 ± 0.00	0.94 ± 0.01	0.96 ± 0.03	0.91 ± 0.03	
Feed consumption (g tank^{-1})	466.6 ± 1.8bc	454.1 ± 3.9a	455.7 ± 1.9ab	513.1 ± 2.7e	499.6 ± 1d	469.5 ± 1.5c	***

Data are expressed as mean ± SEM. Values in the same row with different letters are significantly different (one-way ANOVA and Tukey's post hoc test). *, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Table 13: Nutrient retention efficiency of juvenile Australian hybrid abalone

	P35	**P38**	**P41**	**P44**	**P47**	**P41 U**	**Sig.**
12°C							
PER	1.3 ± 0.0	1.5 ± 0.2	1.3 ± 0.1	1.2 ± 0.0	1.0 ± 0.0	1.2 ± 0.0	
PD%	18.0 ± 1.4	21.4 ± 3.7	19.3 ± 1.5	17.4 ± 1.6	16.1 ± 0.2	19.4 ± 0.8	
EER (g MJ^{-1})	25.3 ± 0.9ab	29.3 ± 3.7b	28.6 ± 1.4ab	27.8 ± 0.3ab	24.8 ± 1.1a	27.3 ± 0.7ab	*
ED%	14.6 ± 0.8	17.1 ± 2.5	15.5 ± 1.2	14.6 ± 1.1	12.9 ± 0.4	15.5 ± 0.6	
17°C							
PER	2.3 ± 0.2ab	2.4 ± 0.1b	2.5 ± 0.1b	1.9 ± 0.1a	1.9 ± 0.1a	2.2 ± 0ab	**
PD%	32.2 ± 3	35.8 ± 1.3	37.3 ± 1.2	32.5 ± 1.4	33.9 ± 1.4	38.1 ± 0.9	
EER (g MJ^{-1})	43.3 ± 3.1a	47.4 ± 1.2ab	53.2 ± 1.4b	43.9 ± 1.9a	46.0 ± 1.9ab	48.8 ± 0.2ab	*
ED%	24.7 ± 2.0	26.6 ± 0.6	28.9 ± 0.6	25.7 ± 1.4	26.0 ± 1.2	28.3 ± 0.6	
22°C							
PER	2.0 ± 0.0ac	2.2 ± 0.2c	2.1 ± 0.2bc	1.5 ± 0ab	1.5 ± 0.1ab	1.3 ± 0.1a	**
PD%	30.0 ± 1.6ac	34.6 ± 1.3c	34.1 ± 3.5bc	24.8 ± 0.5ab	26.6 ± 2.1ac	21.9 ± 1.8a	**
EER (g MJ^{-1})	37.8 ± 0.8ab	44.3 ± 3.6b	44.6 ± 4.8b	34.7 ± 0.8ab	36.2 ± 3.2ab	29.1 ± 3.0a	*
ED%	21.9 ± 0.9ab	25.7 ± 1.9b	26.1 ± 3.1b	19.7 ± 0.6ab	21.3 ± 1.9ab	16.8 ± 1.5a	*

PER, protein efficiency ratio; PD, protein deposition; EER, energy efficiency ratio; ED, energy deposition. Data are expressed as mean ± SEM. Values in the same row with different letters are significantly different (one-way ANOVA and Tukey's post hoc test). *, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

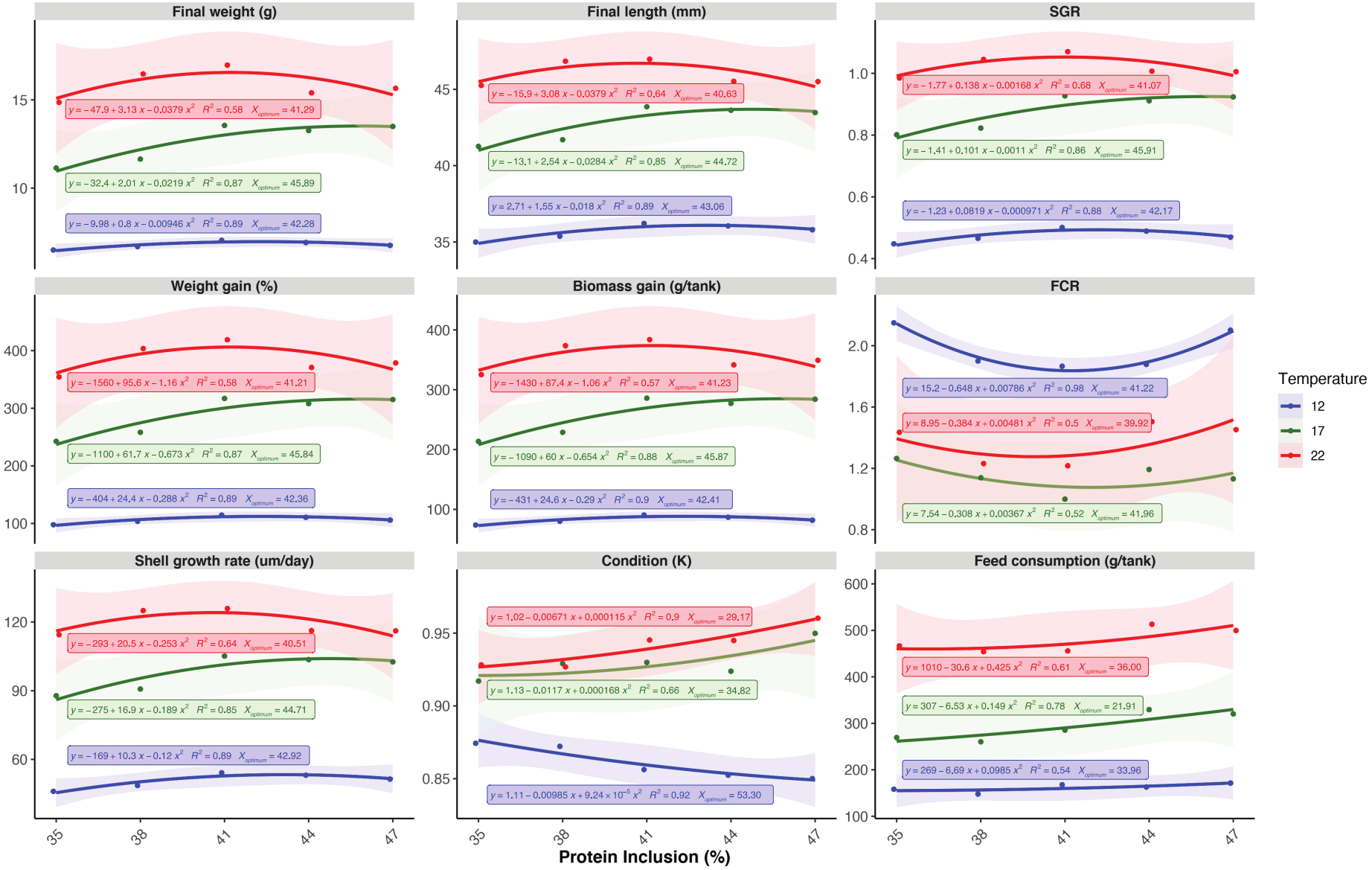

Figure 8: Regressions of growth performance of juvenile Australian hybrid abalone.

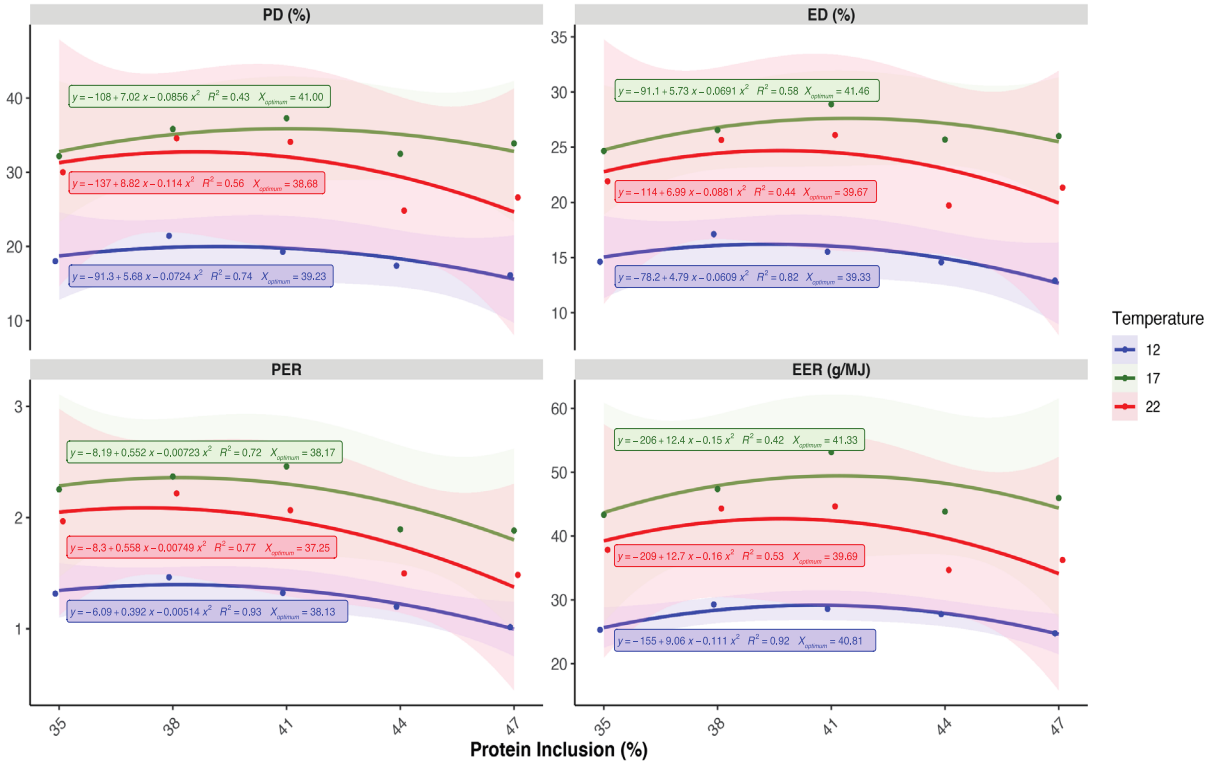

Figure 9: Regressions of nutrient efficiency of juvenile Australian hybrid abalone.

Table 14: Apparent digestibility coefficient of experimental diets fed to juvenile Australian hybrid abalone

	P35	P38	P41	P44	P47	P41 U	Sig
12°C							
DM	68.5 ± 2.7	68.4 ± 2.4	65.6 ± 1.2	71.3 ± 2.2	69.5 ± 0.8	69.3 ± 1.0	
Protein	85.1 ± 1.3a	83.9 ± 1.4a	83.7 ± 0.4a	86.9 ± 0.5a	87.3 ± 0.6a	83.4 ± 0.4a	* †
Lipid	69.9 ± 2.6a	75.3 ± 2.0ab	74.3 ± 1.7ab	81.4 ± 2.1b	80.4 ± 1.3b	74.7 ± 0.2ab	**
NFE	78.2 ± 1.8	77.5 ± 1.4	73.4 ± 0.9	76.6 ± 2.1	73.7 ± 0.3	75.8 ± 0.7	
Energy	79.0 ± 1.4	78.5 ± 1.5	76.6 ± 0.7	80.7 ± 1.4	80.0 ± 0.5	77.1 ± 0.5	
17°C							
DM	73.7 ± 1.1a	77.5 ± 0.6ab	80.6 ± 1.7bc	81.9 ± 0.3bc	81.6 ± 0.8bc	84.4 ± 0.4c	***
Protein	84.1 ± 1.2a	86.2 ± 0.8ab	88.4 ± 1.4bc	90.4 ± 0.2c	90.3 ± 0.4c	90.1 ± 0.1bc	**
Lipid	71.8 ± 2.2a	79.5 ± 0.6b	82.4 ± 1.5bc	85.5 ± 0.5c	87.1 ± 0.5c	83.7 ± 0.1bc	***
NFE	83.2 ± 0.9a	84.9 ± 0.2ab	87.4 ± 1.2bc	87.4 ± 0.3bc	86.7 ± 0.8ab	90.2 ± 0.5c	***
Energy	81.4 ± 1.2a	83.6 ± 0.5ab	86.3 ± 1.4bc	87.7 ± 0.0c	87.6 ± 0.6bc	88.3 ± 0.3c	***
22°C							
DM	77.3 ± 1.1ab	77.7 ± 0.6ab	80.1 ± 0.7b	79.8 ± 1.0ab	74.3 ± 2.1a	81.2 ± 0.9b	*
Protein	84.3 ± 1.1a	85.5 ± 0.5a	88.0 ± 0.3a	88.2 ± 0.8a	83.7 ± 1.7a	87.7 ± 0.9a	* †
Lipid	77.0 ± 0.9a	83.7 ± 0.5b	86.3 ± 0.9b	88.7 ± 0.7b	84.9 ± 2.4b	85.6 ± 0.6b	***
NFE	86.4 ± 0.9b	86.0 ± 0.6ab	86.8 ± 1.0b	86.1 ± 0.6ab	82.3 ± 1.1a	87.7 ± 0.7b	*
Energy	83.5 ± 1.1a	84.3 ± 0.5a	86.2 ± 0.7a	86.3 ± 0.6a	81.7 ± 1.7a	86.0 ± 0.8a	* †

ADC, apparent digestibility coefficient; DM, dry matter; NFE, nitrogen free extract (calculated). Data are expressed as mean ± SEM. Values in the same row with different letters are significantly different (one-way ANOVA and Tukey's post hoc test). *, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. †ANOVA may indicate significant differences, however significant differences were not detected in post-hoc.

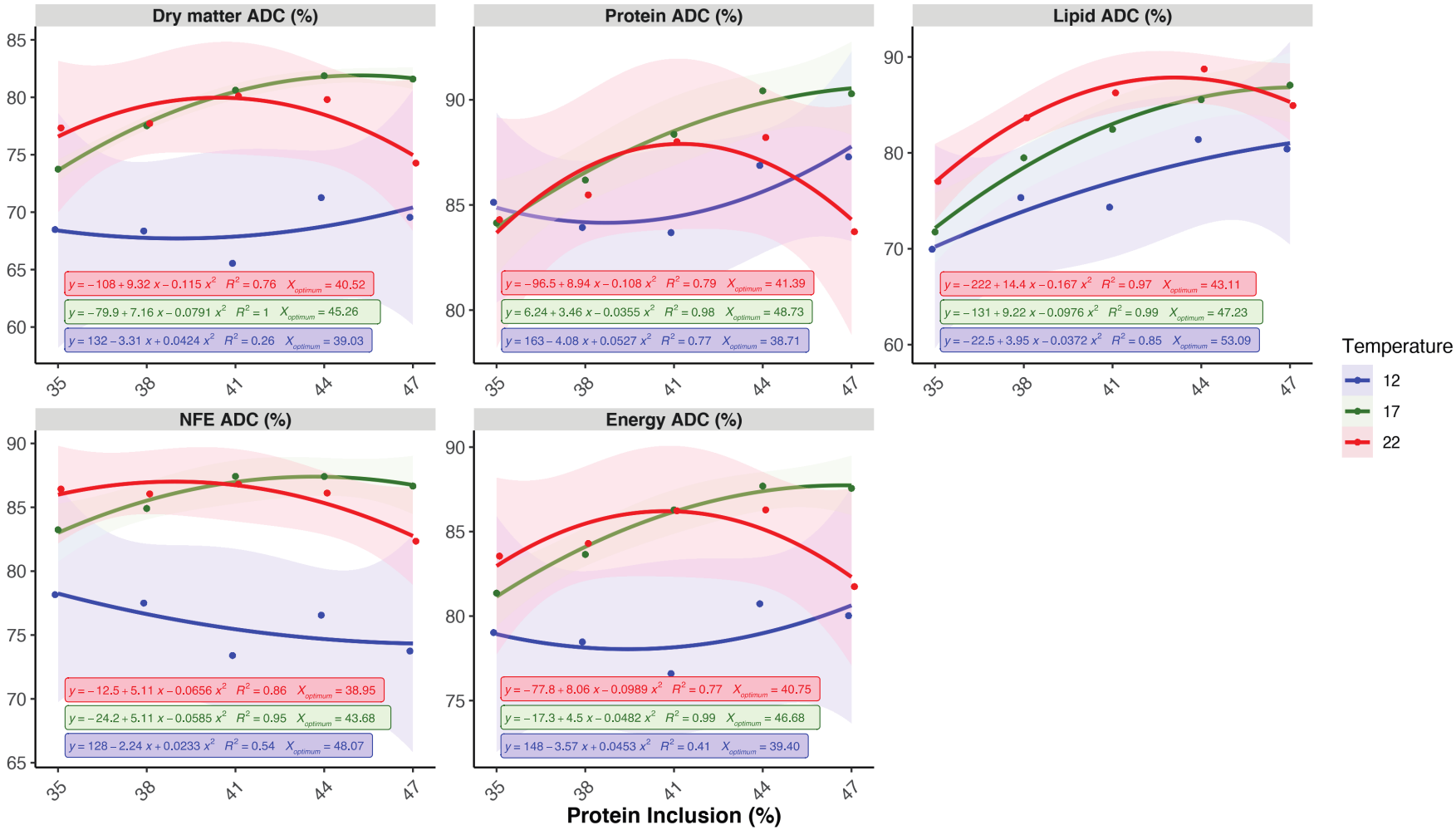

Figure 10: Regressions of apparent digestibility coefficients of juvenile Australian hybrid abalone.

Table 15: Apparent digestibility coefficient of amino acids of experimental diets fed to juvenile Australian hybrid abalone

	P35	P38	P41	P44	P47	P41 U	Sig.
12°C							
Aspartic acid	76.4 ± 2.2ab	74.6 ± 1.6a	75.9 ± 1.8ab	80.4 ± 1.9ab	82.8 ± 1.6b	76.1 ± 0.6ab	*
Glutamic acid	87.0 ± 1.3a	85.7 ± 0.9a	86.4 ± 1.0a	88.9 ± 1.0a	90.2 ± 0.9a	86.5 ± 0.3a	* †
Serine	81.6 ± 1.1ab	78.1 ± 2.1a	78.7 ± 1.4ab	84.9 ± 2.0ab	86.2 ± 1.3b	80.8 ± 1.5ab	*
Histidine	80.4 ± 1.7ab	79.8 ± 1.3a	80.4 ± 1.2ab	84.2 ± 1.3ab	86.0 ± 1.0b	81.3 ± 0.3ab	*
Glycine	84.6 ± 1.4	82.9 ± 1.0	82.9 ± 1.1	85.5 ± 1.6	86.3 ± 1.1	83.5 ± 0.5	
Threonine	84.6 ± 1.2	82.7 ± 1.2	82.6 ± 1.1	86.7 ± 1.7	87.5 ± 1.1	83.6 ± 0.8	
Arginine	85.6 ± 1.0a	83.9 ± 1.3a	83.7 ± 0.8a	87.2 ± 1.4a	88.7 ± 1.0a	85.0 ± 0.8a	* †
Alanine	78.8 ± 1.8	77.1 ± 1.6	76.8 ± 1.3	81.1 ± 2.1	82.8 ± 1.4	77.5 ± 0.9	
Tyrosine	82.2 ± 1.4ab	80.9 ± 1.5a	82.8 ± 1.3ab	85.4 ± 1.7ab	88.0 ± 1.1b	83.4 ± 1.0ab	*
Valine	81.1 ± 2.0	80.1 ± 1.2	81.4 ± 1.3	84.1 ± 1.6	85.7 ± 1.3	80.5 ± 0.6	
Methionine	91.3 ± 1.0	90.7 ± 0.5	91.4 ± 0.4	92.2 ± 0.8	92.9 ± 0.6	90.9 ± 0.3	
Phenylalanine	81.0 ± 1.8ab	79.3 ± 1.3a	80.5 ± 1.4ab	84.1 ± 1.4ab	85.9 ± 1.3b	80.0 ± 0.6ab	*
Isoleucine	80.7 ± 2.0a	79.6 ± 1.4a	80.9 ± 1.5a	84.1 ± 1.4a	85.8 ± 1.3a	79.9 ± 0.5a	* †
Leucine	82.4 ± 1.5ab	80.6 ± 1.2a	81.7 ± 1.4ab	85.1 ± 1.3ab	86.9 ± 1.2b	81.3 ± 0.5ab	*
Lysine	86.7 ± 0.8	86.7 ± 0.9	86.5 ± 0.8	87.7 ± 0.6	89.6 ± 0.3	86.6 ± 0.5	
Proline	90.2 ± 1.1	88.8 ± 0.7	89.3 ± 0.9	91.2 ± 0.9	92.1 ± 0.8	89.4 ± 0.4	
Total	84.2 ± 1.4a	82.7 ± 1.1a	83.3 ± 1.2a	86.3 ± 1.3a	87.8 ± 1.1a	83.5 ± 0.5a	* †
	P35	**P38**	**P41**	**P44**	**P47**	**P41 U**	**Sig.**
17°C							
Aspartic acid	75.8 ± 1.8a	79.6 ± 1.2ab	82.3 ± 2.4ac	86.0 ± 0.7bc	87.6 ± 1.0c	86.4 ± 0.3c	***
Glutamic acid	86.2 ± 1.0a	88.0 ± 0.8ab	89.7 ± 1.4ac	91.8 ± 0.4bc	92.8 ± 0.6c	92.4 ± 0.2c	***
Serine	83.6 ± 1.5a	83.7 ± 0.3a	87.6 ± 1.7ab	90.3 ± 0.3b	91.4 ± 0.6b	90.3 ± 0.7b	***
Histidine	80.3 ± 1.5a	82.9 ± 1.1a	85.8 ± 1.9ab	88.5 ± 0.5b	89.7 ± 0.7b	89.4 ± 0.2b	***
Glycine	85.1 ± 1.2a	86.8 ± 0.7ab	88.1 ± 1.5ab	90.3 ± 0.4b	90.9 ± 0.7b	90.7 ± 0.1b	**
Threonine	85.5 ± 1.2a	86.6 ± 0.5a	88.8 ± 1.5ab	91.1 ± 0.3b	91.7 ± 0.7b	91.0 ± 0.4b	***
Arginine	84.9 ± 1.2a	86.9 ± 0.6ab	88.4 ± 1.7ac	90.8 ± 0.5bc	91.9 ± 0.7c	91.7 ± 0.2c	***
Alanine	79.1 ± 1.6a	81.6 ± 0.9ab	83.7 ± 2.3ac	87.2 ± 0.5bc	88.2 ± 0.9c	86.9 ± 0.2bc	**
Tyrosine	81.1 ± 1.5a	83.1 ± 0.6ab	87.5 ± 1.6bc	89.0 ± 0.7c	91.1 ± 0.7c	90.0 ± 0.5c	***
Valine	80.3 ± 1.4a	83.4 ± 1.0ab	85.4 ± 2.0ac	88.5 ± 0.6bc	89.4 ± 0.9c	88.4 ± 0.2bc	***
Methionine	90.6 ± 0.7a	91.8 ± 0.5ab	93.0 ± 0.9ac	94.1 ± 0.3bc	94.5 ± 0.4c	94.0 ± 0.0bc	**
Phenylalanine	79.8 ± 1.4a	82.7 ± 1.1ab	85.2 ± 2.0ac	88.3 ± 0.6bc	89.6 ± 0.9c	88.1 ± 0.2bc	***
Isoleucine	79.1 ± 1.4a	82.7 ± 1.2ab	84.6 ± 2.2ac	88.1 ± 0.6bc	89.2 ± 0.9c	87.9 ± 0.2bc	***
Leucine	81.4 ± 1.3a	83.9 ± 1.0ab	86.3 ± 1.9ac	89.1 ± 0.6bc	90.4 ± 0.8c	89.1 ± 0.2bc	***
Lysine	87.5 ± 0.5a	89.4 ± 0.6ab	91.3 ± 1.1bc	91.7 ± 0.3bc	93.1 ± 0.3c	92.5 ± 0.2c	***
Proline	89.8 ± 0.8a	90.9 ± 0.6ab	92.3 ± 1.0ac	93.7 ± 0.3bc	94.5 ± 0.5c	94.2 ± 0.1c	***
Total	83.9 ± 1.2a	85.9 ± 0.8ab	87.9 ± 1.6ac	90.3 ± 0.5bc	91.3 ± 0.7c	90.6 ± 0.2c	***
	P35	**P38**	**P41**	**P44**	**P47**	**P41 U**	**Sig.**
22°C							
Aspartic acid	75.4 ± 1.7	78.9 ± 1.1	80.8 ± 2.2	83.2 ± 1.3	78.2 ± 2.3	82.6 ± 1.3	
Glutamic acid	86.2 ± 1.0	85.9 ± 1.3	87.2 ± 3.3	90.6 ± 0.7	87.5 ± 1.3	90.6 ± 0.8	
Serine	81.5 ± 1.4	81.2 ± 1.4	85.8 ± 2.5	87.5 ± 1.4	83.9 ± 1.0	86.7 ± 1.2	
Histidine	80.6 ± 1.5	82.5 ± 0.8	84.2 ± 2.4	86.7 ± 0.9	83.0 ± 1.7	87.0 ± 1.1	
Glycine	85.3 ± 0.9	83.0 ± 3.2	86.5 ± 1.8	88.5 ± 0.7	85.1 ± 1.5	88.3 ± 0.7	
Threonine	84.6 ± 1.1	85.5 ± 0.8	87.6 ± 1.5	89.0 ± 1.0	85.4 ± 1.2	88.1 ± 1.0	
Arginine	84.5 ± 1.6	84.1 ± 1.8	87.2 ± 2.4	88.8 ± 1.1	85.1 ± 1.5	89.6 ± 0.9	
Alanine	78.2 ± 1.8	79.0 ± 1.4	81.9 ± 2.2	84.2 ± 1.3	79.6 ± 2.2	82.9 ± 1.2	
Tyrosine	78.0 ± 2.4	82.3 ± 2.4	85.7 ± 2.0	85.8 ± 1.3	82.7 ± 1.4	86.2 ± 1.3	
Valine	80.2 ± 0.8	82.4 ± 0.2	83.7 ± 1.9	86.5 ± 0.8	82.9 ± 2.2	85.4 ± 1.1	
Methionine	90.9 ± 0.5	88.6 ± 2.9	91.8 ± 1.7	93.5 ± 0.3	91.5 ± 0.9	92.7 ± 0.5	
Phenylalanine	79.9 ± 1.3	81.2 ± 0.5	83.3 ± 2.5	86.2 ± 1.0	82.1 ± 2.0	85.3 ± 1.1	
Isoleucine	79.5 ± 1.2	82.3 ± 0.4	82.9 ± 2.2	86.3 ± 0.8	82.2 ± 2.3	85.3 ± 1.1	

Leucine	81.1 ± 1.3	82.3 ± 0.5	84.4 ± 2.3	87.0 ± 0.9	83.2 ± 1.8	86.4 ± 1.1
Lysine	86.3 ± 1.2	85.7 ± 0.8	87.7 ± 1.5	87.4 ± 1.1	87.2 ± 1.2	89.5 ± 0.8
Proline	90.4 ± 0.7	89.1 ± 1.7	90.9 ± 2.1	93.1 ± 0.6	91.3 ± 0.9	93.4 ± 0.5
Total	83.6 ± 1.2	83.9 ± 0.8	86.0 ± 2.3	88.3 ± 0.9	85.0 ± 1.5	88.1 ± 0.9

Data are expressed as mean ± SEM. Values in the same row with different letters are significantly different (one-way ANOVA and Tukey's post hoc test). *, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. †ANOVA may indicate significant differences, however significant differences were not detected in post-hoc.

Table 16: Proximate composition and energy content of juvenile Australian hybrid abalone tissue

	P35	**P38**	**P41**	**P44**	**P47**	**P41 U**	**Sig.**
12°C							
Moisture	750.6 ± 2.0	751.1 ± 3.3	758.3 ± 3.2	762.7 ± 6.3	763.7 ± 1.2	755.3 ± 3.4	
Protein	614.0 ± 4.6a	627.6 ± 2.9ab	643.9 ± 3.8bc	655.5 ± 3.6c	683.1 ± 7.4d	654.9 ± 1c	***
Lipid	74.6 ± 3.3	77.4 ± 1.9	76.9 ± 3.6	76.4 ± 1.2	70.2 ± 1.5	76.0 ± 1.8	
Ash	83.9 ± 1.2	81.3 ± 2.4	85.6 ± 1.7	85.8 ± 1.3	86.2 ± 0.8	82.1 ± 0.2	
NFE	227.5 ± 8.6d	213.7 ± 5.2cd	193.7 ± 1.8bc	182.4 ± 3.7ab	160.5 ± 7.4a	187.0 ± 2.4b	***
Energy	213.5 ± 0.9a	215.4 ± 0.8ab	215.6 ± 0.5ab	216.2 ± 0.7ab	216.5 ± 0.3ab	216.8 ± 0.5b	*
17°C							
Moisture	743.6 ± 1.7	745.6 ± 4.0	752.0 ± 0.4	743.3 ± 2.9	749.1 ± 2.8	743.9 ± 3.3	
Protein	584.6 ± 11.7a	610.7 ± 7.0ab	625.0 ± 10.3bc	658.4 ± 8.6c	697.2 ± 1.4d	658.3 ± 4.2c	***
Lipid	73.7 ± 0.3	73.6 ± 1.8	75.7 ± 2.1	76.7 ± 1.3	71.7 ± 0.8	76.3 ± 0.7	
Ash	76.5 ± 0.2ab	84.9 ± 2.2b	83.4 ± 1.6b	73.6 ± 0.6a	83.7 ± 3.8b	80.2 ± 1.3ab	**
NFE	265.1 ± 11.5d	230.8 ± 7.2cd	215.9 ± 11.7bc	191.3 ± 7.7bc	147.4 ± 4.7a	185.3 ± 4.2ab	***
Energy	212.7 ± 0.8a	212.9 ± 0.3a	214.5 ± 1.3ab	218.6 ± 0.3c	218.2 ± 0.7c	217.4 ± 0.4bc	***
22°C							
Moisture	736.8 ± 2.5	739.4 ± 0.9	740.2 ± 4.3	745.2 ± 0.6	741.7 ± 2.0	745.5 ± 1.3	
Protein	589.8 ± 7.7a	607.8 ± 21.0a	634.2 ± 15.3ab	648 ± 4ab	678.2 ± 9.1b	646.6 ± 12.2ab	**
Lipid	69.0 ± 2.0a	78.7 ± 2.9ab	81.7 ± 1.3b	75.4 ± 2.0ab	79.3 ± 2.8ab	83.7 ± 1.8b	**
Ash	74.4 ± 1.0	75.9 ± 2.2	78.7 ± 2.6	79.3 ± 1.0	78.5 ± 0.9	81.2 ± 2.1	
NFE	266.8 ± 8.6c	237.5 ± 17.3bc	205.3 ± 18.4ab	197.3 ± 2.5ab	164.1 ± 8.9a	188.5 ± 10.1ab	***
Energy	212.3 ± 1.1a	215.4 ± 1.1ab	217.3 ± 0.7bc	216.7 ± 0.4bc	219.6 ± 0.9c	218.1 ± 0.8bc	***

NFE, nitrogen free extract (calculated). Data are expressed as mean ± SEM. Values in the same row with different letters are significantly different (one-way ANOVA and Tukey's post hoc test). *, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Table 17: Amino acid composition of juvenile Australian hybrid abalone tissue

	P35	P38	P41	P44	P47	P41 U	Sig.
12°C							
Aspartic acid	56.4 ± 0.4ab	55.0 ± 0.5a	57.1 ± 0.6ac	58.4 ± 0.2bc	62.4 ± 0.7d	59.0 ± 0.2c	***
Glutamic acid	81.4 ± 1.3ab	79.3 ± 0.6a	81.3 ± 1.5ab	83.1 ± 0.3ab	88.8 ± 1.1c	84.5 ± 0.5bc	***
Serine	24.7 ± 0.6	26.6 ± 0.4	26.4 ± 1.2	24.9 ± 0.8	26.9 ± 0.5	27.2 ± 0.3	
Histidine	7.9 ± 0.2ab	7.5 ± 0.0a	8.0 ± 0.1b	8.1 ± 0.0b	8.8 ± 0.1c	8.3 ± 0.1bc	***
Glycine	42.7 ± 1.2a	42.9 ± 0.8a	43.0 ± 1.1a	45.4 ± 0.3ab	47.8 ± 1.1b	45.0 ± 0.9ab	*
Threonine	23.6 ± 0.1a	24.1 ± 0.2a	24.8 ± 0.4ab	24.5 ± 0.4ab	26.7 ± 0.4c	25.7 ± 0.2bc	***
Arginine	51.0 ± 0.8a	50.3 ± 0.7a	51.1 ± 0.7a	52.5 ± 0.1a	56.4 ± 0.8b	53.7 ± 1.0ab	**
Alanine	29.8 ± 0.4ab	29.2 ± 0.2a	29.6 ± 0.4ab	30.3 ± 0.2ab	32.0 ± 0.3c	30.8 ± 0.3bc	***
Tyrosine	13.1 ± 0.3a	13.8 ± 0.1a	14.3 ± 0.4ac	13.9 ± 0.4ab	15.3 ± 0.1c	15.1 ± 0.2bc	**
Valine	24.2 ± 0.4ab	23.5 ± 0.1a	24.6 ± 0.7ab	25.6 ± 0.2bc	26.8 ± 0.1c	25.4 ± 0.2bc	**
Methionine	11.6 ± 0.2ab	11.4 ± 0.2a	12.0 ± 0.3ac	12.2 ± 0.1bcd	13.0 ± 0.2d	12.7 ± 0.1cd	***
Phenylalanine	18.4 ± 0.2ab	17.8 ± 0.1a	18.6 ± 0.2bc	19.1 ± 0.0cd	20.3 ± 0.2e	19.3 ± 0.1d	***
Isoleucine	21.5 ± 0.4ab	20.2 ± 0.0a	21.1 ± 0.7ab	22.4 ± 0.2bc	24.1 ± 0.2c	22.1 ± 0.2b	***
Leucine	36.3 ± 0.3ab	35.4 ± 0.2a	36.8 ± 0.5ac	37.7 ± 0.3bc	40.5 ± 0.5d	38.0 ± 0.1c	***
Lysine	28.4 ± 0.7	27.1 ± 1.0	29.0 ± 1.2	28.7 ± 1.6	31.0 ± 2.2	27.0 ± 1.8	
Proline	31.3 ± 0.6	29.9 ± 0.2	31.2 ± 0.8	30.6 ± 0.2	32.0 ± 0.8	29.9 ± 0.5	
Total	502.3 ± 5.2ab	493.9 ± 2.7a	509.0 ± 6.6ab	517.5 ± 2.7b	552.7 ± 7.4c	523.9 ± 1.6b	***
	P35	**P38**	**P41**	**P44**	**P47**	**P41 U**	**Sig.**
17°C							
Aspartic acid	50.9 ± 1.6a	52.7 ± 2.0a	53.6 ± 1.2a	57.3 ± 1.8ab	62.6 ± 1.4b	56.9 ± 1.3ab	**
Glutamic acid	74.9 ± 2.1a	76.4 ± 2.5a	78.3 ± 1.8a	82.8 ± 2.4ab	89.9 ± 1.7b	82.4 ± 2.1ab	**
Serine	25.0 ± 0.9	25.3 ± 0.5	25.9 ± 0.6	25.8 ± 1.6	29.2 ± 1.1	26.9 ± 1.2	
Histidine	7.4 ± 0.3a	7.3 ± 0.3a	7.4 ± 0.2ab	7.9 ± 0.1ab	8.4 ± 0.2b	7.7 ± 0.1ab	*
Glycine	41.2 ± 1.5a	41.2 ± 1.0a	43.2 ± 1.6a	45.7 ± 2.1ab	51.6 ± 0.2b	46.3 ± 2.6ab	**
Threonine	22.0 ± 0.7a	22.6 ± 0.6a	23.5 ± 0.3a	24.3 ± 1.1ab	27.3 ± 0.9b	24.7 ± 0.6ab	**
Arginine	47.2 ± 1.2a	48.0 ± 1.8a	49.6 ± 0.8a	52.2 ± 1.7ab	57.2 ± 0.9b	52.7 ± 1.0ab	**
Alanine	27.8 ± 0.7a	28.2 ± 1.1a	28.4 ± 0.6a	30.3 ± 0.9ab	32.7 ± 0.7b	30.0 ± 0.8ab	**
Tyrosine	12.5 ± 0.7a	13.1 ± 0.8ab	13.6 ± 0.5ab	14.2 ± 0.8ab	16.5 ± 0.8b	14.7 ± 0.7ab	*
Valine	21.4 ± 0.6a	22.1 ± 0.9ab	22.7 ± 0.7ab	24.9 ± 0.5bc	26.6 ± 0.3c	24.1 ± 0.3ac	***
Methionine	10.4 ± 0.3a	10.8 ± 0.3ab	11.2 ± 0.4ab	12.4 ± 0.4bc	13.3 ± 0.2c	12.5 ± 0.1bc	***
Phenylalanine	16.3 ± 0.5a	17.0 ± 0.6ab	17.4 ± 0.3ab	18.6 ± 0.4bc	20.3 ± 0.3c	18.4 ± 0.3bc	***
Isoleucine	18.4 ± 0.5a	19.1 ± 0.9ab	19.5 ± 0.6ab	21.7 ± 0.5bc	23.3 ± 0.2	20.9 ± 0.4ac	**
Leucine	32.6 ± 0.9a	33.8 ± 1.2a	34.7 ± 0.8a	37.2 ± 1.2ab	40.6 ± 1.0b	36.8 ± 0.7ab	**
Lysine	25.5 ± 0.7	26.1 ± 1.7	27.1 ± 1.2	27.9 ± 2.0	30.7 ± 3.4	27.4 ± 2.1	
Proline	29.8 ± 0.2	30.6 ± 0.4	29.5 ± 1.4	31.7 ± 1.4	32.7 ± 0.8	29.3 ± 1.7	
Total	463.4 ± 12.0	474.3 ± 15.7a	485.7 ± 11.6a	514.9 ± 18.6ab	562.9 ± 13.6b	511.5 ± 15.2ab	**
	P35	**P38**	**P41**	**P44**	**P47**	**P41 U**	**Sig.**
22°C							
Aspartic acid	54.1 ± 1.4a	54.0 ± 1.9a	57.0 ± 3.4a	60.8 ± 1.0a	61.3 ± 0.8a	59.4 ± 0.5a	* †
Glutamic acid	79.2 ± 1.3	78.2 ± 2.9	83.7 ± 5.9	86.9 ± 1.7	88.0 ± 2.2	85.4 ± 0.8	
Serine	26.2 ± 1.1	26.1 ± 0.7	27.9 ± 0.8	28.8 ± 1.3	30.1 ± 0.7	28.4 ± 1.1	
Histidine	8.0 ± 0.3	7.7 ± 0.3	8.0 ± 0.5	8.0 ± 0.0	8.3 ± 0.2	8.2 ± 0.2	
Glycine	44.1 ± 0.6	42.5 ± 2.4	45.2 ± 3.5	48.5 ± 0.6	50.8 ± 0.9	47.4 ± 0.4	
Threonine	23.2 ± 0.7a	23.5 ± 0.5a	25.3 ± 1.3ab	26.6 ± 0.7ab	28.2 ± 0.3b	26.5 ± 0.7ab	**
Arginine	49.9 ± 0.8	50.1 ± 2.1	51.8 ± 4.1	54.7 ± 0.8	55.9 ± 1.4	54.0 ± 0.9	
Alanine	28.8 ± 0.5	28.2 ± 1.1	30.2 ± 2.0	31.3 ± 0.5	31.8 ± 0.7	30.9 ± 0.4	
Tyrosine	13.8 ± 0.3a	14.4 ± 0.4ab	15.5 ± 0.7ac	16.5 ± 0.3c	17.3 ± 0.2c	16.2 ± 0.4bc	***
Valine	22.5 ± 0.3	22.4 ± 1.2	23.7 ± 1.9	25.4 ± 0.3	25.4 ± 0.4	24.9 ± 0.5	
Methionine	11.2 ± 0.5a	11.3 ± 0.5ab	12.6 ± 0.7ac	12.6 ± 0.5ac	13.5 ± 0.2c	13.3 ± 0.1bc	**
Phenylalanine	17.2 ± 0.2a	17.5 ± 0.5a	18.5 ± 1.1a	19.4 ± 0.2a	19.7 ± 0.3a	19.3 ± 0.1a	* †
Isoleucine	19.3 ± 0.3	19.3 ± 1.1	20.5 ± 1.9	21.9 ± 0.3	21.8 ± 0.4	21.4 ± 0.5	
Leucine	34.4 ± 0.7a	34.6 ± 1.2a	37.0 ± 2.5a	39.0 ± 0.5a	39.8 ± 0.9a	38.3 ± 0.4a	* †
Lysine	24.0 ± 1.6	25.9 ± 1.6	26.8 ± 3.7	28.7 ± 0.6	30.4 ± 1.8	27.9 ± 0.8	
Proline	28.3 ± 1.1	27.9 ± 0.9	29.5 ± 1.7	30.8 ± 0.8	30.9 ± 1.0	28.6 ± 0.6	
Total	484.3 ± 9.9	483.4 ± 18	513.2 ± 35.4	540.0 ± 7.9	553 ± 11.6	530.3 ± 4.1	

Data are expressed as mean ± SEM. Values in the same row with different superscripts are significantly different (one-way ANOVA and Tukey's post hoc test). *, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. †ANOVA may indicate significant differences, however significant differences were not detected in post-hoc.

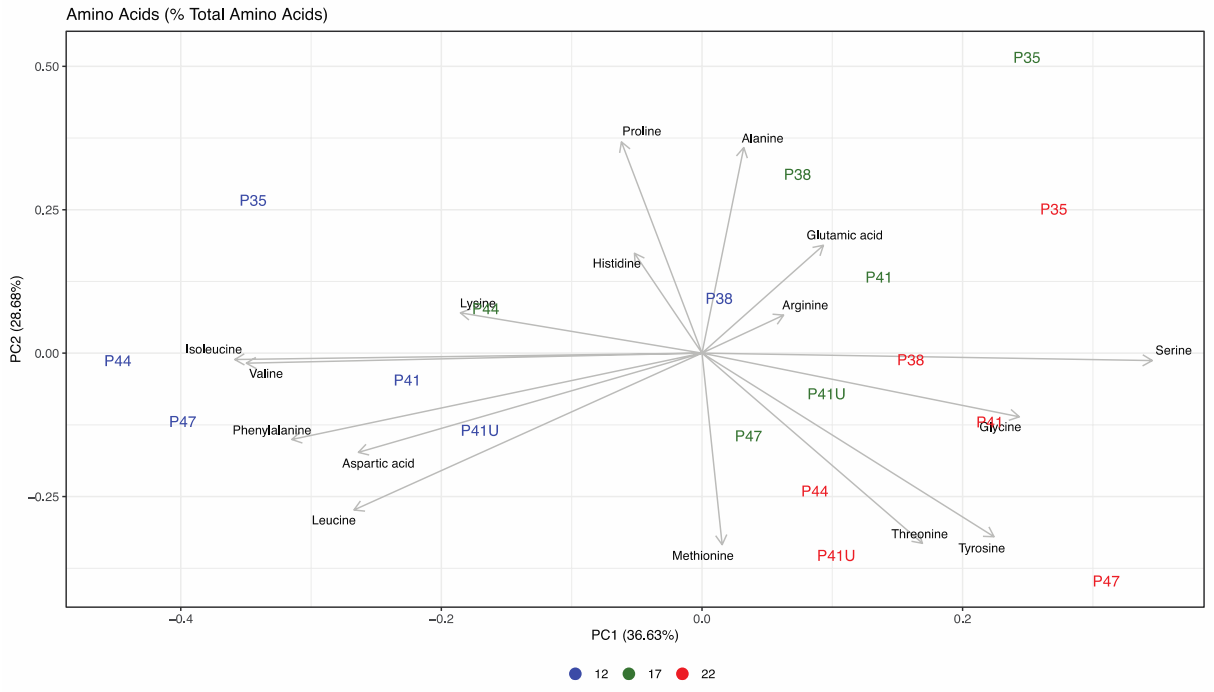

Figure 11: PCA of juvenile Australian hybrid abalone based on amino acid composition

Table 18: Fatty acid composition of juvenile Australian hybrid abalone tissue (mg g^{-1} dry weight basis).

	P35	P38	P41	P44	P47	P41 U	Sig.
12°C							
13:0	4.1 ± 0.1	4.6 ± 0.1	4.6 ± 0.2	4.2 ± 0.3	4.5 ± 0.2	4.7 ± 0.2	
14:0	0.7 ± 0.0b	0.7 ± 0.0b	0.7 ± 0.0b	0.5 ± 0.0ab	0.4 ± 0.0a	0.55 ± 0.0ab	**
15:0	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0b	***
15:1n-5	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	
16:0	6.6 ± 0.1	6.6 ± 0.1	6.7 ± 0.2	6.5 ± 0.1	6.2 ± 0.1	6.9 ± 0.1	
16:1n-7	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	
16:2n-4	0.5 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	
18:0	1.8 ± 0.0	1.7 ± 0.0	1.8 ± 0.1	1.7 ± 0.1	1.6 ± 0.0	1.7 ± 0.0	
18:1n-9 t	0.4 ± 0.2	0.2 ± 0.2	0.3 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	
18:1n-9	7.0 ± 0.4	7.6 ± 0.5	7.7 ± 0.7	7.3 ± 0.1	5.9 ± 0.4	7.3 ± 0.3	
18:1n-7	1.8 ± 0.1	1.8 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	1.9 ± 07	
18:2n-6	6.3 ± 0.1	6.7 ± 0.3	7.0 ± 0.49	6.7 ± 0.1	5.7 ± 0.3	6.7 ± 0.2	
18:3n-3	1.8 ± 0.0b	1.8 ± 0.1b	1.8 ± 0.2b	1.5 ± 0.1ab	1.2 ± 0.1a	1.6 ± 0.1ab	**
20:0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
18:4n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	
20:1n-11	0.9 ± 0.0c	0.8 ± 0a.0c	0.9 ± 0.0bc	0.8 ± 0.0ac	0.7 ± 0.0a	0.8 ± 0.0ab	**
20:1n-9	1.2 ± 0.0a	1.2 ± 0.1a	1.1 ± 0.1a	1.1 ± 0.1a	1.0 ± 0.0a	1.0 ± 0.0a	* †
21:0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	
20:2n-6	1.3 ± 0.0b	1.3 ± 0.1ab	1.2 ± 0.0ab	1.2 ± 0.1ab	1.1 ± 0.0a	1.1 ± 0.1ab	*
20:3n-6	0.1 ± 0.0b	0.1 ± 0.0ab	0.1 ± 0.0b	0.1 ± 0.0ab	0.1 ± 0.0ab	0.1 ± 0.0a	*
Unknown	0.5 ± 0.5	0.5 ± 0.5	0.5 ± 0.5	0.4 ± 0.4	0 ± 0.0	0.0 ± 0.0	
20:4n-6	0.7 ± 0.1	0.7 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	
20:3n-3	0.2 ± 0.0d	0.2 ± 0.0cd	0.2 ± 0.0bd	0.2 ± 0.0abc	0.1 ± 0.0a	0.2 ± 0.0ab	***
22:1n-9	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	
20:5n-3	1.6 ± 0.0	1.5 ± 0.0	1.6 ± 0.1	1.5 ± 0.0	1.5 ± 0.1	1.6 ± 0.1	
22:2NMI	2.2 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	2.1 ± 0.0	2.1 ± 0.1	
22:2n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
22:4n-6	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
22:5n-6	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	
22:5n-3	1.5 ± 0.0	1.5 ± 0.1	1.5 ± 0.0	1.4 ± 0.0	1.4 ± 0.0	1.7 ± 0.1	
22:6n-3	0.7 ± 0.0	0.7 ± 0.2	0.7 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	0.8 ± 0.0	
24:5n-3	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	
Total	43.8 ± 1.7	44.8 ± 1.2	45.7 ± 2.6	43.1 ± 0.6	39.2 ± 0.8	43.8 ± 1.1	
SFA	14.0 ± 0.3	14.5 ± 0.3	14.7 ± 0.3	13.9 ± 0.5	13.6 ± 0.3	14.7 ± 0.3	
MUFA	11.5 ± 0.6	12.1 ± 0.6	12.3 ± 0.9	11.5 ± 0.1	9.92 ± 0.5	11.6 ± 0.4	
n-3 PUFA	6.1 ± 0.1b	5.9 ± 0ab	6.1 ± 0.3b	5.5 ± 0.2ab	5.2 ± 0.1a	5.9 ± 0.2ab	*
n-3 LC PUFA	4.2 ± 0.1	4.0 ± 0.1	4.1 ± 0.1	3.9 ± 0.1	3.8 ± 0.1	4.1 ± 0.1	
n-6 PUFA	8.9 ± 0.2	9.3 ± 0.4	9.5 ± 0.5	9.2 ± 0.2	8.1 ± 0.2	9.0 ± 0.3	
n-6 LC PUFA	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.0	2.5 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	
n-6/n-3	1.5 ± 0.0	1.6 ± 0.1	1.6 ± 0.0	1.7 ± 0.1	1.6 ± 0.0	1.5 ± 0.0	
	P35	**P38**	**P41**	**P44**	**P47**	**P41 U**	**Sig.**
17°C							
13:0	4.0 ± 0.1	3.3 ± 0.4	3.5 ± 0.3	4.1 ± 0.2	4.1 ± 0.1	4.0 ± 0.3	
14:0	0.7 ± 0.0b	0.6 ± 0.1ab	0.6 ± 0.0ab	0.5 ± 0.0ab	0.5 ± 0.0a	0.6 ± 0.0ab	*
15:0	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.2 ± 0.0b	***
15:1n-5	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	
16:0	7.1 ± 0.2	6.8 ± 0.5	7.3 ± 0.2	7.3 ± 0.2	7.2 ± 0.1	7.9 ± 0.2	
16:1n-7	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	
16:2n-4	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	
18:0	1.7 ± 0.0	1.7 ± 0.1	1.7 ± 0.0	1.7 ± 0.1	1.7 ± 0.0	1.8 ± 0.0	
18:1n-9 t	0.4 ± 0.0a	0.4 ± 0.0a	0.4 ± 0.0a	0.3 ± 0.0a	0.3 ± 0.0a	0.3 ± 0.0a	* †
18:1n-9	7.8 ± 0.1	8.8 ± 0.6	8.9 ± 0.3	8.5 ± 0.6	8.3 ± 0.2	8.8 ± 0.2	
18:1n-7	1.9 ± 0.0	1.9 ± 0.1	2.0 ± 0.1	1.9 ± 0.1	1.8 ± 0.0	2.0 ± 0.0	

18:2n-6	7.3 ± 0.3	7.8 ± 0.4	7.7 ± 0.2	7.6 ± 0.5	7.3 ± 0.1	7.7 ± 0.2	
18:3n-3	1.7 ± 0.1c	1.7 ± 0.1bc	1.6 ± 0.1bc	1.5 ± 0.1ab	1.3 ± 0.0a	1.7 ± 0.0bc	**
20:0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
18:4n-3	0.1 ± 0.0ab	0.1 ± 0.0a	0.1 ± 0.0ab	0.1 ± 0.0ab	0.1 ± 0.0ab	0.1 ± 0.0b	*
20:1n-11	1.0 ± 0.0a	0.9 ± 0.1a	0.9 ± 0.0a	0.8 ± 0.1a	0.8 ± 0.0a	0.8 ± 0.1a	* †
20:1n-9	1.4 ± 0.1c	1.2 ± 0.1bc	1.0 ± 0.0ab	1.1 ± 0.1ab	0.9 ± 0.0a	1.1 ± 0.0ab	**
21:0	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	
20:2n-6	1.1 ± 0.1b	1.1 ± 0.0b	0.9 ± 0ab	1.0 ± 0ab	0.8 ± 0.0a	0.9 ± 0.0a	**
20:3n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
20:4n-6	0.7 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	0.9 ± 0.1	0.9 ± 0.0	0.8 ± 0.1	
20:3n-3	0.1 ± 0.0c	0.1 ± 0.0bc	0.1 ± 0.0ac	0.1 ± 0.0ab	0.1 ± 0.0a	0.1 ± 0.0ac	**
20:4n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
22:1n-9	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	
20:5n-3	1.6 ± 0.1	1.4 ± 0.1	1.5 ± 0.0	1.5 ± 0.1	1.5 ± 0.0	1.7 ± 0.1	
22:2NMI	2.5 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	
22:2n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
22:4n-6	0.2 ± 0.0b	0.1 ± 0.0ab	0.1 ± 0.0a	0.1 ± 0ab	0.1 ± 0ab	0.1 ± 0.0ab	*
24:0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
22:5n-6	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.0	
22:5n-3	1.4 ± 0.0	1.1 ± 0.0	1.4 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.4 ± 0.0	
22:6n-3	0.7 ± 0ab	0.7 ± 0ab	0.7 ± 0.0ab	0.7 ± 0.0ab	0.7 ± 0.0a	0.8 ± 0.0b	*
24:5n-3	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.1	0.2 ± 0.0	0.4 ± 0.2	
24:6n-3	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	
Total	46.1 ± 1.1	45.3 ± 2.7	46.1 ± 1.0	45.6 ± 1.9	44.2 ± 0.2	47.6 ± 0.8	
SFA	14.3 ± 0.3	13.1 ± 1.1	13.8 ± 0.6	14.4 ± 0.5	14.2 ± 0.1	15.0 ± 0.5	
MUFA	12.8 ± 0.3	13.5 ± 0.8	13.5 ± 0.4	12.9 ± 0.8	12.5 ± 0.2	13.5 ± 0.2	
n-3 PUFA	6.1 ± 0.3	5.8 ± 0.2	6.0 ± 0.3	5.5 ± 0.1	5.4 ± 0.2	6.3 ± 0.3	
n-3 LC PUFA	4.3 ± 0.2	3.9 ± 0.2	4.2 ± 0.3	4.0 ± 0.0	3.9 ± 0.2	4.5 ± 0.2	
n-6 PUFA	9.6 ± 0.3	10.0 ± 0.5	9.9 ± 0.2	9.9 ± 0.5	9.4 ± 0.1	10.0 ± 0.2	
n-6 LC PUFA	2.4 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	2.4 ± 0.1	2.1 ± 0.1	2.2 ± 0.0	
n-6/n-3	1.6 ± 0.1	1.7 ± 0.0	1.7 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	1.6 ± 0.0	
	P35	**P38**	**P41**	**P44**	**P47**	**P41 U**	**Sig.**
22°C							
13:0	3.5 ± 0.2	3.4 ± 0.1	3.5 ± 0.1	3.4 ± 0.2	3.7 ± 0.1	3.4 ± 0.1	
14:0	0.5 ± 0.0	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.0	0.5 ± 0.1	0.5 ± 0.2	
15:0	0.1 ± 0.0a	0.2 ± 0a	0.1 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0b	**
15:1n-5	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
16:0	6.6 ± 0.1a	7.4 ± 0.3ab	7.7 ± 0.2bc	7.1 ± 0.1ab	7.7 ± 0.2bc	8.4 ± 0.3c	**
16:1n-7	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.1	
16:2n-4	0.3 ± 0.0a	0.3 ± 0.0ab	0.3 ± 0.0ab	0.3 ± 0.0ab	0.3 ± 0.0ab	0.3 ± 0.0b	*
18:0	1.5 ± 0.0a	1.7 ± 0.0ac	1.6 ± 0.1ac	1.6 ± 0.0ab	1.8 ± 0.0bc	1.8 ± 0.0c	**
18:1n-9 t	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	
18:1n-9	8.0 ± 0.4a	9.9 ± 0.7ab	10.8 ± 0.8b	9.0 ± 0.3ab	9.6 ± 0.7ab	11 ± 0.5b	*
18:1n-7	1.9 ± 0.1a	2.1 ± 0.1ab	2.2 ± 0.1ab	2.0 ± 0.0ab	2.1 ± 0.1ab	2.4 ± 0.1b	*
18:2n-6	6.67 ± 0.3	7.9 ± 0.5	8.4 ± 0.5	7.3 ± 0.3	7.6 ± 0.5	8.3 ± 0.3	
18:3n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	
18:3n-4	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.1 ± 0.0a	* †
18:3n-3	1.3 ± 0.0	1.6 ± 0.1	1.6 ± 0.1	14.0 ± 0.1	1.4 ± 0.1	1.6 ± 0.1	
20:0	0.1 ± 0.0a	0.1 ± 0.0ac	0.1 ± 0bc	0.1 ± 0.0ab	0.1 ± 0.0ac	0.1 ± 0.0c	**
18:4n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
20:1n-11	0.9 ± 0.0	1.0 ± 0.1	1.0 ± 0.0	0.9 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	
20:1n-9	1.1 ± 0.0	1.1 ± 0.1	1.0 ± 0.0	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	
21:0	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	
20:2n-6	0.9 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.1	
20:3n-6	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
Unknown	0.9 ± 0.0	0.9 ± 0.1	1.0 ± 0.0	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.2	
20:4n-6	0.7 ± 0.0	0.8 ± 0.1	0.9 ± 0.0	0.8 ± 0.0	0.9 ± 0.1	0.9 ± 0.1	

20:3n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
22:1n-9	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	
20:5n-3	1.3 ± 0.0a	1.3 ± 0.0a	1.4 ± 0.1a	1.3 ± 0.0a	1.4 ± 0.1ab	1.6 ± 0.1b	**
22:2NMI	2.3 ± 0.0	2.4 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	2.4 ± 0.0	2.4 ± 0.1	
22:2n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
22:4n-6	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	
22:5n-6	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
22:5n-3	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.1	1.1 ± 0.0	1.1 ± 0.1	1.3 ± 0.1	
22:6n-3	0.6 ± 0.0a	0.7 ± 0.0a	0.7 ± 0.0a	0.6 ± 0.0a	0.7 ± 0.0a	0.8 ± 0.0b	**
24:5n-3	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	
Total	42.2 ± 1.1a	47.6 ± 2.2ab	49.3 ± 1.6ab	44.1 ± 1.3ab	47.0 ± 1.8ab	50.9 ± 2.0b	*
SFA	12.7 ± 0.3a	13.8 ± 0.4ab	14.0 ± 0.3ab	13.3 ± 0.4ab	14.4 ± 0.4ab	14.8 ± 0.5b	*
MUFA	12.5 ± 0.6a	14.8 ± 1.0ab	15.8 ± 0.9ab	13.5 ± 0.4ab	14.4 ± 0.9ab	16.2 ± 0.7b	*
n-3 PUFA	4.7 ± 0.0a	5.1 ± 0.2ab	5.2 ± 0.2ab	4.7 ± 0.2a	5.0 ± 0.1a	5.7 ± 0.2b	**
n-3 LC PUFA	3.3 ± 0.0a	3.4 ± 0.1ab	3.4 ± 0.2ab	3.3 ± 0.1a	3.4 ± 0.1ab	3.9 ± 0.2b	*
n-6 PUFA	8.7 ± 0.4	10.1 ± 0.6	10.5 ± 0.5	9.1 ± 0.4	9.5 ± 0.5	10.3 ± 0.4	
n-6 LC PUFA	1.9 ± 0.0	2.1 ± 0.1	2.0 ± 0.1	1.8 ± 0.1	1.9 ± 0.0	1.9 ± 0.1	
n-6/n-3	1.8 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	1.9 ± 0.0	1.9 ± 0.1	1.8 ± 0.1	

Individual fatty acids < 0.1 mg g^{-1} sample for all dietary treatments at all experimental temperatures are not included in this table. SFA, sum of saturated fatty acids; MUFA, sum of monounsaturated fatty acids; n-3/n-6 PUFA, sum of all omega-3/6 fatty acids with two or more double bonds; n-3/6 LC PUFA, sum of all omega-3/6 fatty acids ≥ 20 C and with two or more double bonds. Data are expressed as mean ± SEM. Values in the same row with different letters are significantly different (one-way ANOVA and Tukey's post hoc test). *, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. †ANOVA may indicate significant differences, however significant differences were not detected in post-hoc.

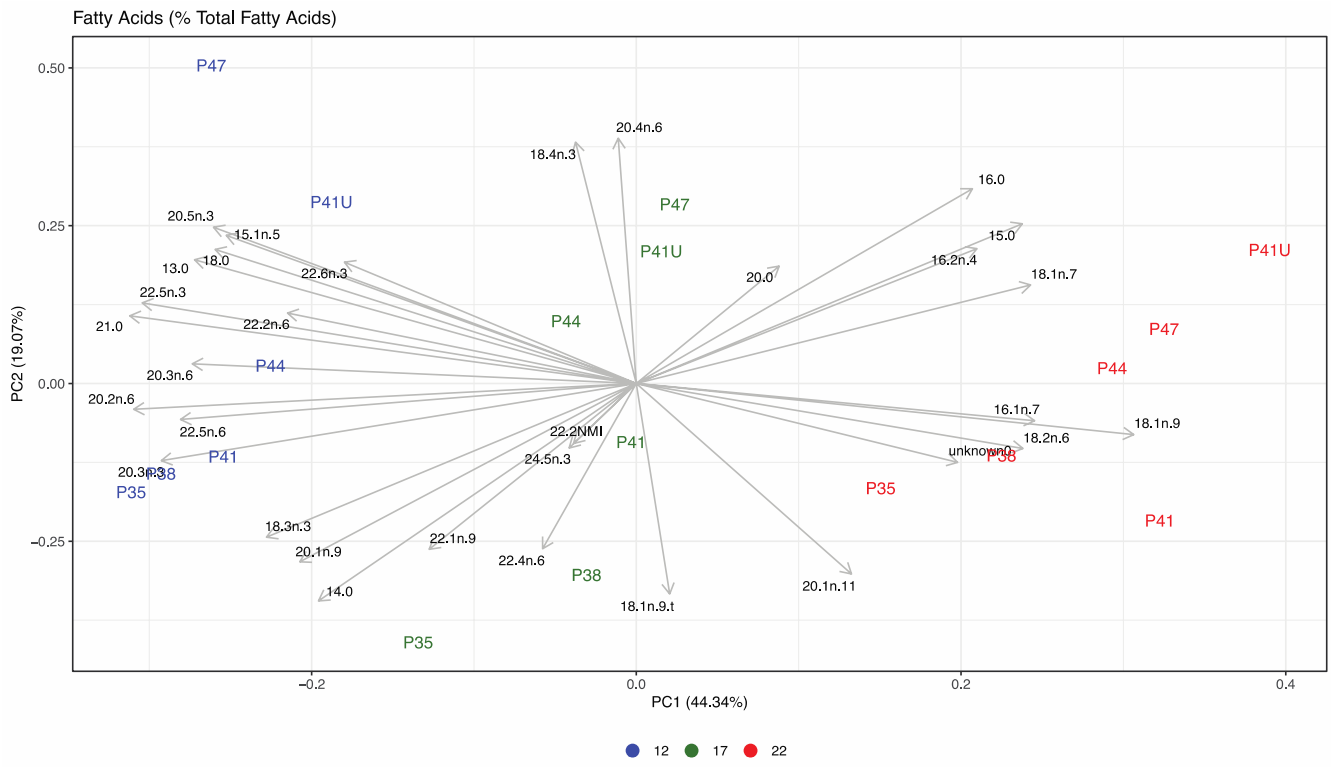

Figure 12: PCA of juvenile Australian hybrid abalone based on fatty acid composition

Discussion

With the same overarching objectives and rationale, the present experiment built on the results obtained and presented in **Task 2.1**. The aim, therefore, was to provide a more comprehensive understanding of the effect of altering the dietary protein inclusion level in formulated diets for Australian hybrid abalone. This was achieved by investigating a different size class of abalone (starting weight 3.3 g) and expanding the upper range of dietary protein inclusion levels compared to those assessed in **Task 2.1**. The ultimate goal being to provide nutritional solutions to production challenges faced by producers, such as slow and variable growth (Freeman, 2001).

In general, the juvenile abalone used in the present experiment exhibited faster growth rates compared to the sub-adult abalone used previously in **Task 2.1**, with SGRs ranging from 0.5 to 1.1 at 12 °C and 22 °C, respectively, which compares, or exceeds growth rates reported for similar sized Greenlip Abalone (Stone et al., 2013). Growth rate comparisons with other Australian hybrid abalone are hindered due to a lack of published information with comparable abalone sizes. With respect to the experimental diets, the results of the present experiment amplified those presented in **Task 2.1**, in terms of the positive effect on growth and feed conversion associated with an increase in the level of dietary protein. At both 17 °C and 22 °C, abalone fed a diet containing 41% dietary protein exhibited higher weight gain and improved feed conversion compared to those fed relatively low (35%) dietary protein. This supports data presented by Stone et al. (2016), where Australian hybrid abalone fed a diet containing 39% protein resulted in an increased SGR and higher total biomass gain compared to those fed a diet containing 32% protein over an 18 month culture period. However, the present study provides added detail with respect to culture temperature, nutrient digestibility and nutrient utilisation efficiency, whilst suggesting scope for further increased performance based on the higher inclusion levels trialled. Specifically, at 17 °C, a 74% improvement in weight gain percentage was observed in abalone fed a diet containing 41%, compared to 35%, dietary protein (317% vs 243%, respectively). This was reflected in the digestibility of dietary nutrients, which showed a positive relationship with increasing dietary protein up to 41-44% inclusion. In general, however, protein was highly digested in the present study (typically 85-90%), with an observable improvement on the previous experiment (**Task 2.1**), and values comparable to previous studies on similar abalone species (Sales & Britz, 2003; Sales & Britz

2001; Shipton & Britz, 2002). Importantly, the present study identified an upper limit with respect to dietary protein inclusion for juvenile abalone. In most cases, this limit appeared to be in the range of 41-44%, when considering growth, feed efficiency, nutrient deposition efficiency and nutrient digestibility parameters as the primary measures of performance. Beyond this, declines in these key performance measures were observed.

As with the previous experiment, abalone grew observably faster when reared at 22 °C compared to both 12 °C and 17 °C, although to lesser extent compared to the latter. Abalone grew to a final weight of 17 g when provided a diet containing 41% protein at 22 °C, compared to 13.6 g and 7 g at 17 °C and 12 °C, respectively when provided the same level of dietary protein. Notably, FCR was superior in abalone reared at 17 °C compared to 22 °C. In agreement with the previous experiment and previous published research, improvements in growth performance were observed in abalone fed increased levels of dietary protein (Stone et al. 2016). Importantly, this research attests to the importance considering season when aiming to enhance growth performance by altering the level of dietary protein. Clearly, improvements are magnified during periods where water temperatures are in excess of 17 °C.

The nutritional composition of the edible portion of farmed seafood products, including abalone, remains a major driver of demand from Australian consumers (Christenson et al., 2017). Expectedly, the total protein content of abalone tissue was proportional to the dietary protein inclusion level, with a concomitant increase in the concentration of individual amino acids. An increase in the level of protein in abalone tissue was associated with a decrease in tissue levels of NFE. As seafood is regarded as a reliable source of dietary protein to consumers, this is not considered a detriment to the quality of the final product. Despite being relatively low in total lipid, particular attention is paid to the fatty acid composition of abalone tissue, particularly, regarding human health beneficial n-3 LC PUFA. Specifically, tissue levels of 20:5n-3 (EPA) and 22:6n-3 (DHA) remain synonymous with seafood, including abalone, consumption (Bogard et al. 2019). In the present experiment, an increase in the dietary protein inclusion level did not adversely affect the concentration of n-3 LC PUFA in the tissue of abalone. Of interest, however, was the slightly increased tissue levels of n-3 LC PUFA in abalone fed a diet containing 15% *U. ohnoi*, despite no difference in the dietary level of n-3 LC PUFA compared to other diets. This potentially suggests an increase in the deposition or

retention of dietary provided n-3 LC PUFA in abalone fed formulated feed supplemented with added seaweed. However, this apparent improvement in retention efficiency may in fact be linked to the poor growth performance of abalone fed a diet containing 15% *U. ohnoi*. Most notably, a higher FCR was recorded during periods of increased growth in hybrid abalone fed a diet containing 15% *U. ohnoi* compared to a diet containing a similar protein inclusion level but no added macroalgae. In part, this can be attributed to the poorer pellet stability and resultant increase in dry matter leaching observed in the P41U diet. Given the naturally slow feeding behaviour of abalone, pellet stability remains a key factor in diet development (Sales & Janssens, 2004). While red macroalgae containing agar or carrageenan and brown algae containing alginates may improve pellet stability (Chao et al., 2010; Stone et al., 2013), the composition of dried green macroalgae (e.g. *Ulva* spp), makes it susceptible to pellet deterioration, which may negatively affect abalone performance and nutrient retention (Bansemer et al., 2016a). Future studies may explore methods to improve the stability of diets formulated with relatively high inclusions of green macroalgae such as increasing the amount of added binding agents or by investigating macroalgae species with naturally higher concentrations of polysaccharides (e.g. alginates, carrageenan or agar).

Conclusions

The present experiment aimed to evaluate the effect of dietary protein inclusion level on the growth performance, nutrient utilisation and nutritional quality of juvenile Australian hybrid abalone. The results of the present experiment built upon those presented previously **Task 2.1** by evaluating diets containing a higher level of dietary protein and by utilising a different life stage of abalone compared to the previous experiment. Overwhelmingly, it was shown that a dietary protein inclusion level in the range of 41-44% provided for optimal growth performance in juvenile abalone with no adverse effects on nutrient utilisation, retention, or nutritional quality. Importantly, the present Task showed that beyond this level, performance declined, suggesting the optimal protein inclusion level, at least for hybrid abalone was identified. In conjunction with the previous experiment, it was shown that improvements in growth performance resulting from an increase in dietary protein were maximised during periods of faster growth, namely where water temperatures were ≥ 17 °C. Such information should prove valuable to producers of Australian hybrid abalone to implement nutritional strategies that enhance production while not compromising efficiency or quality.

Tasks 2.2 and 3.2 Temperature stress events for sub-adult and juvenile Australian hybrid abalone

Introduction

Onshore aquaculture requires careful maintenance of culture conditions such as water quality, temperature, and feed provision. The interactions and feedbacks between these factors complicate the task of ensuring good culture conditions, particularly at the high animal densities often employed in onshore aquaculture.

Oxygen concentration is typically one of the first water quality parameters to decline with poor management. Mechanical aeration, water replacement or biological production by algae are common methods employed to replace oxygen. However, these methods are not always reliable or cost effective. Therefore, it is useful to understand the specific oxygen requirements of the species under culture and how it varies when culture conditions such as temperature and feed provision change. Aquaculture managers can use this information to inform operational practices in a proactive way by predicting periods of high oxygen demand.

Abalone farms rely on high animal densities in flow through aquaculture systems to maintain productivity in these slow growing species. These systems typically use large quantities of seawater, making temperature control uneconomical. Subsequently, farmed abalone are subject to seasonal fluctuations in local sea temperatures throughout the year. Abalone, like many marine ectotherms, display a non-linear increase in basal metabolic rate as water temperature increases (Díaz et al., 2000; Gaty & Wilson, 1986; Kang et al., 2019; Tripp-Valdez et al., 2017). A recent review of thermal preferences and thermal limits amongst abalone species and found large differences between species (Morash & Alter 2016). For example; thermal preferences and limits are generally in line with water temperatures typical of the species' natural geographic distribution. Recently developed interspecies hybrid abalone (*Haliotis laevigata* X *Haliotis rubra*) purportedly have larger thermal tolerance windows and greater growth rates compared to their parent species (Alter et al., 2017; Chen et al., 2016). As such, hybrid abalone are an attractive candidate for aquaculture for producers looking to reduce grow-out times and on-farm mortality rates related to adverse sea water temperatures (Alter et al., 2017; Lafarga-De la Cruz et al., 2013). Yet, the relationship between

water temperature and metabolic rate is underreported in hybrid abalone in the Australian context.

Diet formulations for abalone have undergone significant revision over the last two decades with the intent of increasing growth rates and decreasing feed costs for this slow growing species (Fleming et al., 1996; Sales & Janssens, 2004). Recent research has focussed on increasing the inclusion of crude protein in diets to levels well above what wild abalone would typically encounter in a natural habitat. While this has been successful in significantly improving growth rates and therefore the profitability of abalone farms, questions remain regarding the flow-on effects of maintaining high dietary protein levels in a naturally or nominally herbivorous animal. Supplying dietary protein, above the requirement for tissue synthesis, likely results in higher levels of protein catabolism to meet the metabolic energy requirement for maintaining homeostasis in both fish (Mock et al., 2019) and shellfish, including abalone (Gómez-Montes et al., 2003; Kreeger, 1993). Protein catabolism and the excretion of resultant metabolic waste products incurs additional energetic costs to the organism as well as increasing the amount of nitrogenous waste, mostly, in the form of ammonia discharged to the surrounding environment (Huchette et al., 2003). Energy budgets developed for abalone suggest a small energetic cost associated with nitrogenous excretion in abalone (Barkai & Griffiths, 1988), however, conceivably, this may be challenged if the level of protein catabolism increases due to a sub-optimal dietary protein: energy ratio.

The hybrid abalone and the closely related Greenlip Abalone are the most common abalone in the Australian abalone aquaculture industry. Few studies, however, have examined the effects of water temperature on oxygen consumption and none have examined the combined effects of meal ingestion and assimilation (referred to henceforth as specific dynamic action; SDA) on the pattern of oxygen consumption of these species. Furthermore, existing published data appears contradictory. For example, metabolic rate for fed European abalone (*Haliotis tuberculate*) increased approximately 30% compared to unfed animals (Gaty & Wilson 1986). Furthermore, inconsistent increases in oxygen consumption of fed versus unfed South African Abalone (*Haliotis midae)* (Lyon, 1995) and no differences in metabolic rates between recently fed and starved *H. midae* have been found (Barkai & Griffiths, 1987). Considering the growing popularity of cultured Australian hybrid abalone, a better understanding of the relationship between temperature, feeding status and metabolic rate is required.

Abalone present a unique challenge for measurement of specific dynamic action (SDA; **Figure 13**) due to an inherently slow feeding mechanism and variable meal sizes. Abalone feed over several hours under darkened conditions, yet, are sensitive to external stimulus (e.g., sound, light, vibrations) (Morikawa & Norman, 2003), which may interrupt normal feeding. In fish, forced feeding of meals has been used to assess specific dynamic action in difficult or easily startled species. Unfortunately, the feeding physiology and susceptibility to injury of abalone preclude force feeding as a viable method to control meal size. Alternatively, the method proposed in the present experiments detailed in **Task 2.2** and **3.2** used groups of cultured abalone immediately following a long-term growth/feeding trial (**Task 2.1** and **3.1**) to assess the average specific dynamic action. This is based on the notion that abalone subjected to a constant temperature and diet regime would result in a predictable response to feed provision.

The aim of the present study (presented herein as **Tasks 2.2** and **3.2**) was to establish the relationship between temperature and metabolic rate in the Australian hybrid abalone (*H. rubra x H. laevigata*). Additionally, the specific dynamic action (SDA) of the hybrid abalone were characterised at three relevant temperatures and two life stages (sub-adult and juvenile) for the first time. Finally, the effect of dietary crude protein content on the profile of the specific dynamic response was evaluated. These objectives were achieved by measuring the standard metabolic rate (SMR) of the abalone, meal size, peak metabolic rate attained post-feeding, time to return to SMR and total energy invested over the course of feeding and subsequent digestion. Such an investigation is considered fundamental to ensure any potential shift in feed formulation or management strategy on-farm does not jeopardise the physiological or health status of farmed hybrid abalone during seasonal fluctuations in water temperature.

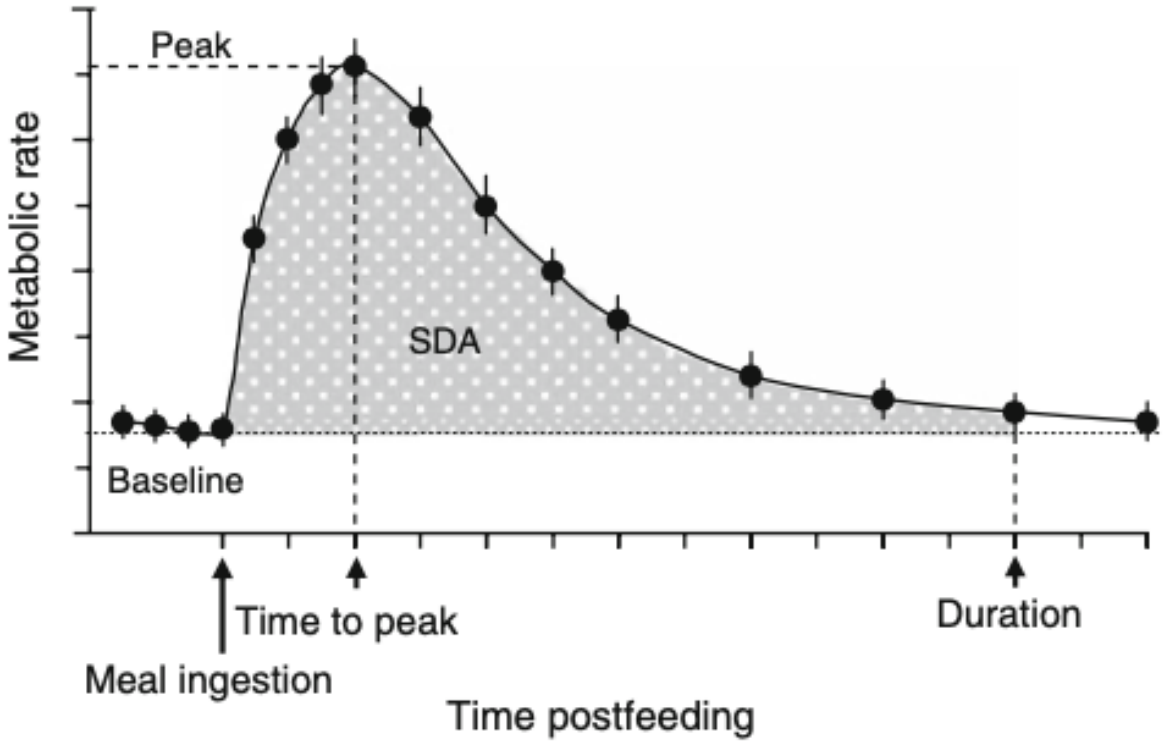

Figure 13: Typical specific dynamic action

Methods

All methods presented in detail below pertain to both **Tasks 2.2** and **3.2**, with any exceptions otherwise stated below.

Animals and holding conditions

Hybrid abalone (*Haliotis rubra x H. laevigata)* were obtained from the Jade Tiger Abalone Farm at Indented Head, Victoria, Australia, and were 20-months old (sub-adult; ~12.5 g) and 8-months old (juvenile ~3.3 g) at the commencement of the experiments presented in **Task 2.1** and **3.1**, respectively. The experiments took place at the Queenscliff Marine Station, Victoria, Australia. The maintenance of water quality and the experimental tank set up were identical to those presented in detail in **Task 2.1**. Briefly, laboratory conditions were controlled with air temperature at 20 °C and controlled photoperiod of 12:12 h light to dark and abalone were subjected to one of three culture temperatures (12 °C, 17 °C and 22 °C).

Diet preparation

For **Task 2.2** using sub-adult abalone, a subset of three diets from **Task 2.1** were used in determination of abalone SDA metrics and the temperature stress experiment. These diets have previously been identified as P32, P38 and P44 and represent a spread of 12% protein inclusion within the diets. See **Table 2** and **Table 3** in **Task 2.1**.

For **Task 2.2** and **3.2** using juvenile abalone, a subset of four diets from **Task 3.1** were used. These diets have previously been identified as P35, P41, P47 and P41+Ulva and represent a spread of 12% protein inclusion within the diets. See **Table 10** and **Table 11** in **Task 3.1**.

Oxygen consumption determination

After 120 and 150 days subjected to the experimental conditions for sub-adult and juvenile abalone, respectively, oxygen consumption rates (mO_2) were measured for groups of abalone within their holding tanks. Mean weights of the sub-adult animals during the respirometry measurement period were 16.8 g, 20.7 g and 28.8 g for 12 °C, 17 °C and 22 °C treatments, respectively. Mean weights of the juvenile animals during the respirometry measurement period were 6.8 g, 12.6 g, and 15.3 g for 12 °C, 17 °C and 22 °C treatments, respectively. All

abalone were fasted for three to five days before mO_2 measurements to ensure postprandial processes did not influence the measurements.

Tank-based respirometry measurements

In the tank-based respirometry analyses, mO_2 measurements were performed on three replicate tanks for each dietary treatment at each of the three culture temperatures (12 °C, 17 °C, 22 °C). Flow-through water to the tanks was stopped, and water circulation was instead provided by 300 L hr^{-1} aquarium pumps (Ehiem) located in separate sumps and operated by an automatic timer set to 24:8 min on to off. Pyroscience Firesting-02 4-channel optical oxygen meters and Pyroscience 3 mm robust optical oxygen probes were inserted into the circulation pump outlet and pO_2 was recorded every 5 s. Oxygen probes were calibrated at 0% and 100% saturation in seawater by bubbling nitrogen or atmospheric air through seawater taken from the appropriate temperature culture system. Tanks were sealed at the upper water/air interface by plastic bubble wrap, cut to tightly fit the inner dimensions of the tank. Efficacy of atmospheric oxygen exclusion was confirmed by the addition of de-oxygenated water to a single empty tank and monitored for two hours to check for changes in pO_2. Oxygen consumption measurements commenced at 1000 hrs (time -7) and feed was provided at 1700 hrs (time 0) (**Figure 14**). Uneaten feed pellets and faeces were siphoned from the tanks at 0800 hrs (time 15) the following day and the number of pellets remaining quantified to obtain total amount of feed consumed. Once abalone returned to standard metabolic rates, measurements of live weight, shell length and width were recorded. Background oxygen consumption for each tank was quantified while abalone were absent for biometry measurements. Live weight calculations were corrected for mortalities or sampled abalone.

Quantifying epibiont and microbial respiration

Oxygen consumption of epibionts (e.g., calcareous tubeworm; *Spirobis sp*.) and associated microbes were quantified by analysing the oxygen consumption of recently shucked abalone shells in the individual respirometry chambers. This was calculated as O_2 consumed min^{-1} mm^{2} $^{-1}$ and subtracted from the final respiration values for the total shell surface area in each group respiration tank.

Simulated summer temperature challenge

The effect of sudden high temperature fluctuation was examined to observe physiological interactions between temperature stress and high protein diets. A sub-set of abalone acclimated to 22 °C during **Task 2.1** (from dietary treatments P32, P38 and P44) and **Task 3.1** (from dietary treatments P35, P41, P47 and P41U) were maintained in their respective experimental tanks following the growth trial and group respirometry experiments. Thirteen sub-adult abalone and twenty-two juvenile abalone were present in each tank for **Tasks 2.2** and **3.2**, respectively. mO_2 measurements were continued from the group-based respirometry, however feed was provided daily at 1700 hrs followed by quantification at 0800 hrs the following day. Remaining feed and faeces were subsequently removed. For sub-adult abalone, an acute temperature stress event (over the course of 11 days) was simulated by raising the water temperature to 26 °C over the course of 3 days (~1.3 °C day^{-1}), maintaining 26 °C for 5 days, and reducing the temperature back to 22 °C over the course of 3 days (~1.3 °C day^{-1}). For juvenile abalone, an acute temperature stress event (over the course of 15 days) was simulated by raising the water temperature to 28 °C over the course of 5 days (~1.2 °C day^{-1}), maintaining 28 °C for 7 days, and reducing the temperature back to 22 °C over the course of 3 days (2 °C day^{-1}).

Feed was provided as the temperature was increased to 26 °C, however, feed provision was stopped once the temperature profile reached 26 °C in accordance with abalone on-farm practices. Briefly, feed was provided daily at 1.5% body weight day^{-1} until 26 °C was reached at which point feed provision ceased. Feed provision was restarted once the water temperature decreased below 26 °C. Abalone tanks were checked twice daily for mortalities by checking the flinching response of abalone to stimulus. Abalone that showed no flinching response, poor flinching response, or poor attachment (inability to remain attached to a vertical inner surface of the tank) were declared dead.

Data analysis

Oxygen consumption data are presented as mg O_2 g soft $tissue^{-1}$ min^{-1}. A significant proportion of abalone live weight is made up of non-biologically active shell. To account for this a live

weight to soft tissue weight regression was calculated by sacrificing 244 abalone and measuring live weight, soft tissue weight, shell weight and shell size.

A strong pattern of increased nocturnal activity was evident in the data. This was attributed to increased locomotory activity of the abalone as they browsed for food in accordance with the findings of (Buss et al., 2015). To prevent oxygen consumption associated with nocturnal activity interfering with the calculation of SDA variables, values generated from a sine equation relating time of day to oxygen consumption (for each treatment replicate) was subtracted from the data, similar to the method used by Roe et al., (2004). This equation was developed using >3 days of recorded data after abalone had returned to standard metabolic rate. The normalisation equation was minimised by setting the minimum to 0 to prevent reduction of oxygen consumption values associated with times of low activity. Standard metabolic rate statistics were found to be unaffected by the increased nocturnal oxygen consumption rates and were therefore calculated from un-modified data.

Data analysis for metabolic oxygen consumption was carried out on LabChart 7 software and R (Version 3.5.3, R Core Team 2019) following methods in Chabot et al., (2016) for the measurement of SDA. The SDA metrics calculated and used in further analysis were: (1) Standard metabolic rate (SMR), mO_2 in a post-absorptive resting state; (2) $mO_{2\ peak}$, the maximum mO_2 achieved after feeding; (3) t_{peak}, the time between food provision and $mO_{2\ peak}$; (4) duration, the time between food provision and return of mO_2 to SMR; (5) SDA energy, energy used (kJ) between feed provision and return of mO_2 to SMR; (6) factorial scope, $mO_{2\ peak}$ divided by SMR; and (7) SDA coefficient, the proportion of energy ingested that is expended in the measured SDA response. mO_2 values were converted to energy values using the coefficient 14.32 kJ O_2 g^{-1}.

Statistical analysis

All statistical analyses were performed using R (Version 3.5.3, R Core Team 2019). Data used for analysis of specific dynamic action metrics were log_{10} transformed to improve normality and homogeneity of variance. Numerical values presented in tables are not transformed. The means of SDA metrics were compared using one-way ANOVAs to test for differences between

protein inclusion levels within temperature treatments. Significance was considered at $p < 0.05$ for all the statistical tests.

Results

Sub-adult Australian hybrid abalone (Task 2.2)

Specific dynamic action response and SDA metrics

The average SDA response profiles of sub-adult Australian hybrid abalone by temperature treatments are presented in **Figure 14** and broken down into specific SDA metrics (**Table 19** & **Figure 15**), including the mass specific energy ingestion (MSEI) of fasted hybrid abalone reared at three temperatures and fed diets containing three different protein inclusion levels. Abalone maintained at 17 °C ingested approximately 19% more energy than those reared at 12 °C, while abalone reared at 22 °C ingested approximately 88% more energy than those reared at 12 °C. No trend of energy ingestion was apparent amongst the dietary protein levels within 12 °C and 17 °C temperature treatments, however, within the 22 °C treatment, energy ingestion was significantly lower at 32% dietary protein inclusion compared to 38% and 44% protein inclusion.

Standard metabolic rate (SMR) increased with each increase in temperature. Abalone maintained at 17 °C had a metabolic rate 28% higher than those at 12 °C, while for animals maintained at 22 °C metabolic rate was 65% higher than those at 12 °C. Temperature appears to be the stronger influence on SMR in abalone. The effect of dietary protein level on SMR was less clear. There appears to be a non-significant trend of increased SMR with increasing dietary protein within the 17 °C and 22 °C temperature treatments, however, this trend was not apparent in the 12 °C treatment with the lower SMR observed in the 32% protein diet.

Peak mO_2 ($mO_{2\ peak}$) represents the highest recorded metabolic rate observed in post feeding (digesting) hybrid abalone. Peak mO_2 of hybrid abalone appears to follow the same highly temperature sensitive trend as energy ingestion. Animals maintained at 17 °C reached a peak mO_2 12.7% higher than those at 12 °C while animals maintained at 22 °C reached a peak mO_2

108.1% higher than those at 12 °C. The effects of dietary protein levels on peak mO_2 were not significant.

Peak mO_2 time (t_{peak}) represents the time taken for the animals to reach the previously reported peak metabolic rate after feeding. Time taken to reach peak mO_2 also appears to be temperature dependent. Animals maintained at 17 °C reached peak mO_2 approximately 12.8 hours earlier than those at 12 °C while animals maintained at 22 °C reached a peak mO_2 approximately 20.9 hours earlier than those at 12 °C. Again, the effects of dietary protein levels on peak mO_2 were not clear.

The duration of the SDA response is the time taken from feed being provided to the animals to the animals returning to standard metabolic rate. Hybrid abalone maintained at 12 °C returned to SMR on average 91.5 hours after feeding began. Animals maintained at 17 °C returned to SMR 45.9 hours after feeding while those at 22 °C took an average of 90.7 hours. Dietary protein makes no observable difference on SDA duration. It can be observed that the animals maintained at 17 °C returned to SMR faster than the animals maintained at 12 °C or 22 °C. This is likely due to a combination of factors. The 17 °C animals exhibit higher initial metabolic rate and higher peak mO_2 than the 12 °C animals, however, the 17 °C animals only demonstrated a small increase in mass specific energy ingestion.

SDA expenditure, SDA co-efficient and factorial scope of hybrid abalone appears to follow a temperature sensitive trend in which animals maintained at 22 °C have higher values with more variability than those maintained at both 12 °C and 17 °C. The effects of dietary protein levels on these three SDA metrics were not significant.

Respirometry of sub-adult abalone during temperature stress event

Figure 16 presents a timeline of metabolic activity measured as mass-specific oxygen consumption for hybrid abalone exposed to a simulated summer stress event. Four distinct peaks can be observed during the temperature increase period, occurring between approximately 2300 h and 0200 h and ranging from 4.0-5.0 mg O_2 kg^{-1} min^{-1}. These peaks in activity are attributed to abalone locomotion and feeding behaviours. The troughs during this period rarely drop below 3.0 mg O_2 kg^{-1} min^{-1}. This represents a nearly 3-fold increase over the SMR observed for fasted abalone reared at 22 °C and is approximately the same as the

peak mO_2 for abalone reared at 22 °C. Once 26 °C was reached and feed provision stopped, the abalone ceased to show the distinct patterns of nocturnal locomotion present at lower temperatures. During this time mO_2 steadily declines and after 96 h (SDA duration of 22 °C abalone) is approximately 2.0 mg O_2 kg^{-1} min^{-1}. This represents a near 2-fold increase in SMR over 22 °C and 3-fold increase over 12 °C acclimated abalone. After 96 h the abalone mO_2 continues to decline at a slower rate, however, some separation between the protein treatments appears to develop. As the temperature declines, nocturnal activities and the recommencement of feeding return, although mO_2 never reaches the same levels observed during the temperature increase period. No mortalities were observed during this experiment.

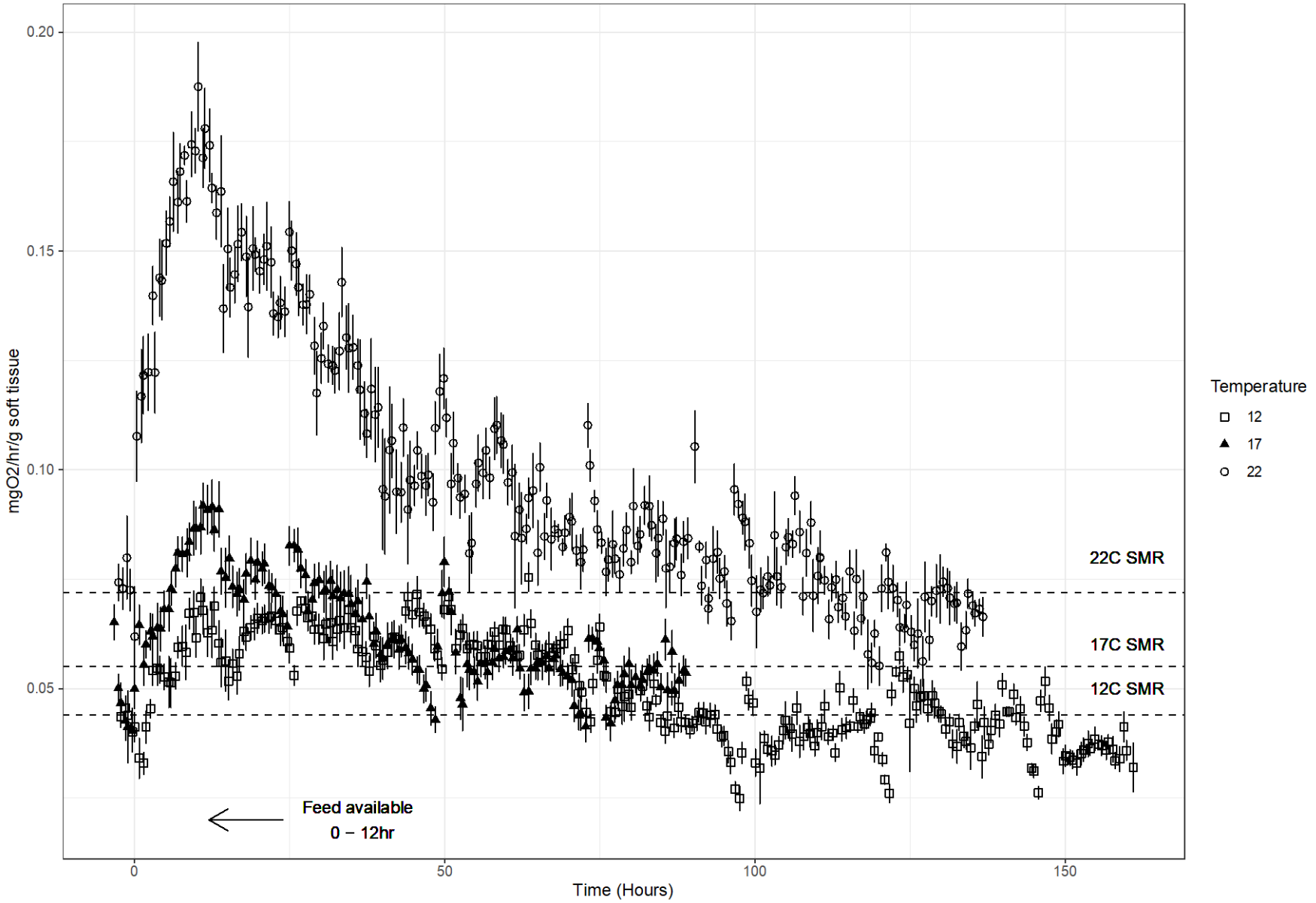

Figure 14: Average SDA mO_2 mass normalised of sub-adult Australian hybrid abalone.

Table 19: Respirometry of sub-adult Australian hybrid abalone

Temperature	12°C				17°				22°C			
Protein level	32%	38%	44%	Sig.	32%	38%	44%	Sig.	32%	38%	44%	Sig.
Tank sof tissue mass (*g*)	143.88 ± 1.73	150.34 ±3.44	150.90 ± 0.72		170.09 ± 1.13	180.03 ± 5.34	187.04 ± 3.89		204.87 ± 19.02a	247.35 ± 0.41b	259.78 ± 5.68b	**
Mass specific energy ingestion (kJ*/g soft tissue*)	234.23 ± 21.59	272.91 ± 4.17	248.84 ± 18.33		318.23 ± 31.80	309.94 ± 18.20	271.75 ± 16.67		549.54 ± 39.27a	438.90 ± 10.90b	433.47 ± 17.74b	*
Standard metabolic rate (SMR) (*mg O_2/hr/g soft tissue*)	0.040 ± 0.001a	0.047 ± 0.001b	0.043 ± 0.001b	**	0.053 ± 0.003	0.054 ± 0.009	0.059 ± 0.004		0.066 ± 0.005	0.072 ± 0.005	0.075 ± 0.003	
Peak mO_2 *(mg O_2/hr/g soft tissue)*	0.065 ± 0.001	0.071 ± 0.001	0.072 ± 0.003		0.084 ± 0.003	0.086 ± 0.011	0.101 ± 0.003		0.176 ± 0.013	0.169 ± 0.008	0.173 ± 0.002	
Peak net *(mg O_2/hr/g soft tissue)*	0.026 ± 0.001	0.025 ± 0.002	0.028 ± 0.004		0.031 ± 0.001a	0.033 ± 0.003ab	0.043 ± 0.003b	*	0.110 ± 0.009	0.097 ± 0.009	0.097 ± 0.003	
$Time_{peak}$ *(hr)*	31.20 ± 1.31	35.98 ± 3.30	28.39 ± 9.63		14.95 ± 5.38	11.52 ± 0.24	11.52 ± 0.68		9.65 ± 0.88	10.01 ± 0.86	9.90 ± 0.37	
Duration *(hr)*	75.31 ± 2.19	73.48 ± 0.96	77.50 ± 4.33		29.86 ± 4.94	29.01 ± 4.67	33.54 ± 5.01		63.48 ± 0.77	57.70 ± 10.32	60.29 ± 3.94	
SDA expenditure (*kJ*)	19.47 ± 1.83	16.04 ± 3.57	19.88 ± 3.53		8.68 ± 2.02	7.70 ± 1.44	11.41 ± 2.96		50.89 ± 7.56	47.24 ± 10.67	47.21 ± 4.01	
SDA co-efficient (*(SDA/MSEI)*100*)	8.44 ± 1.01	5.89 ± 1.34	7.92 ± 1.07		2.90 ± 0.89	2.47 ± 0.38	4.10 ± 0.88		9.31 ± 1.49	10.79 ± 2.49	11.00 ± 1.31	
Factorial Scope (*Peak mO_2/SMR*)	1.65 ± 0.05	1.53 ± 0.06	1.64 ± 0.09		1.59 ± 0.05	1.62 ± 0.07	1.73 ± 0.08		2.69 ± 0.09	2.36 ± 0.17	2.30 ± 0.08	

Data are expressed as mean ± SEM. Values within temperature treatments within the same row with different letters are significantly different (one-way ANOVA and Tukey's post hoc test). *, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

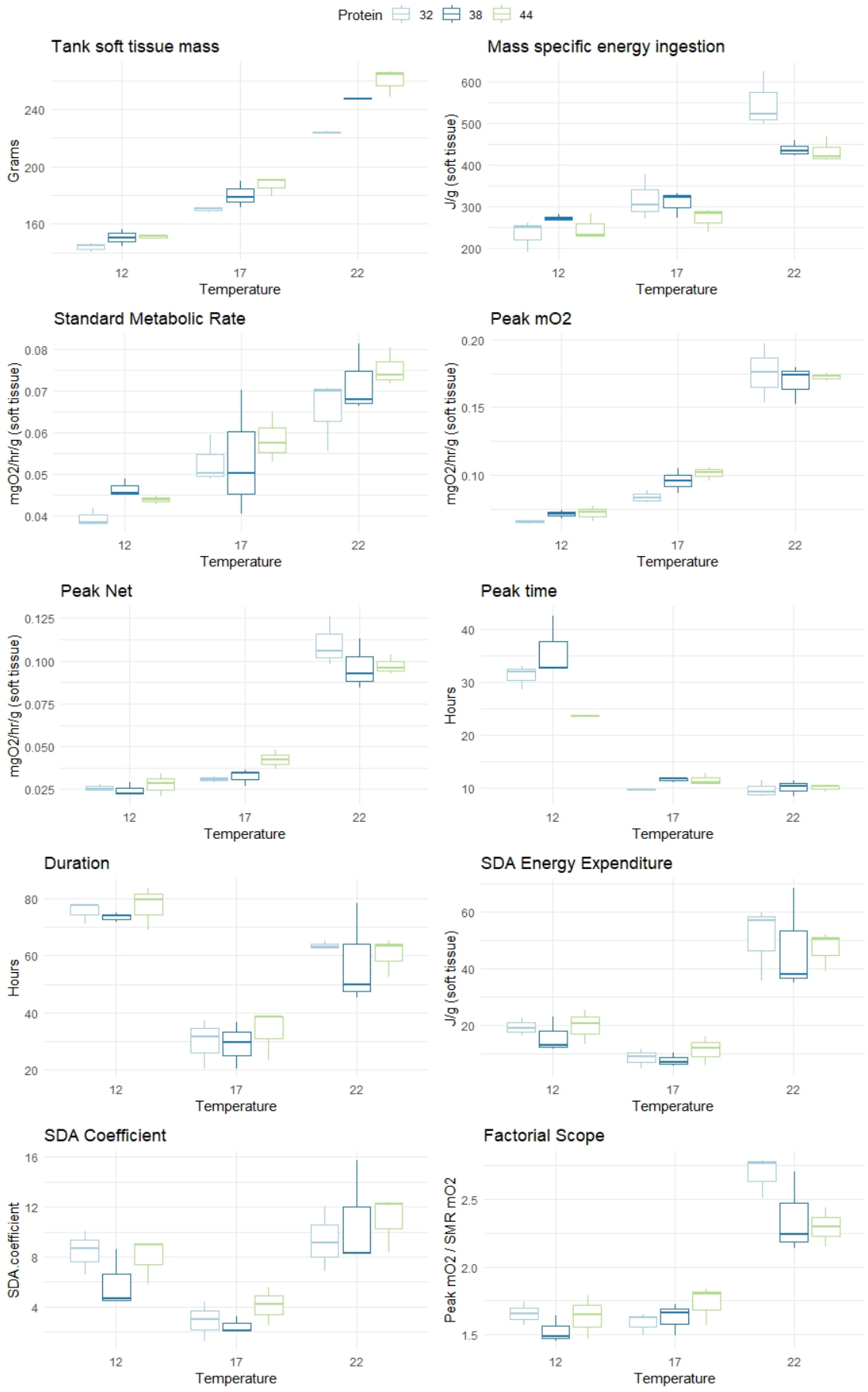

Figure 15: SDA metrics for respirometry of sub-adult Australian hybrid abalone

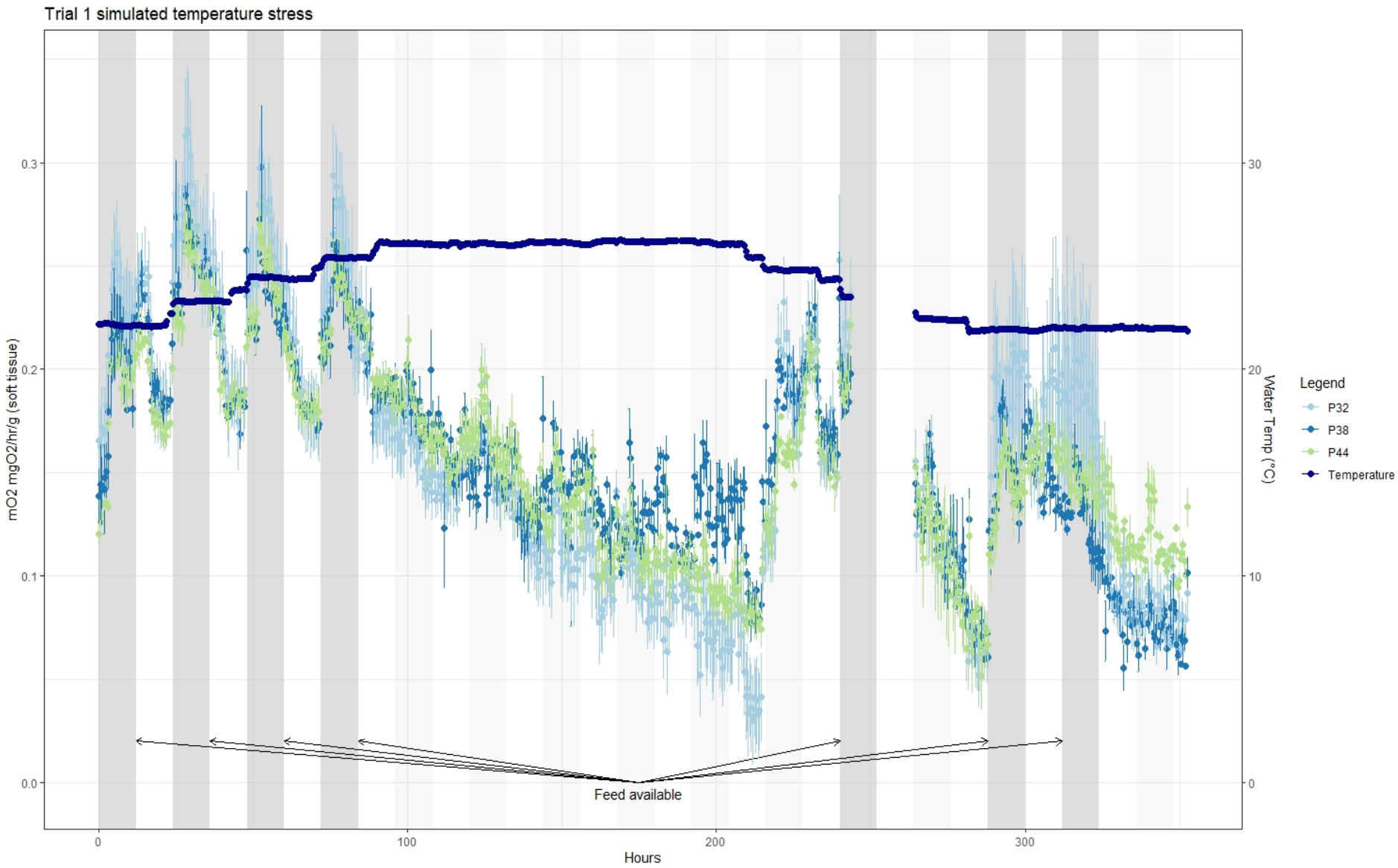

Figure 16: Respirometry of sub-adult Australian hybrid abalone during temperature stress event.

Juvenile Australian hybrid abalone (Task 3.2)

Specific dynamic action response and SDA metrics

The average SDA response profiles of juvenile Australian hybrid abalone by temperature treatments are presented in (**Figure 17**) and broken down into specific SDA metrics in **Table 20** and **Figure 18**, including the MSEI of fasted hybrid abalone reared at three temperatures and fed diets containing four different dietary protein inclusion levels. Abalone maintained at 17 °C ingested approximately 7% more energy than those reared at 12 °C while abalone reared at 22 °C ingested approximately 80% more energy than those reared at 12 °C. No trend of energy ingestion is apparent amongst the dietary protein levels within 12 °C, 17 °C and 22 °C temperature treatments.

Standard metabolic rate (SMR) increased with each increase in temperature. Abalone maintained at 17 °C had a metabolic rate 32% higher than those at 12 °C, while for animals maintained at 22 °C metabolic rate was 50% higher than those at 12 °C. The effect of dietary protein level on SMR, however, was less clear.

Abalone maintained at 17 °C reached an $mO_{2\ peak}$ that was 52% higher than those maintained at 12 °C while animals maintained at 22 °C reached an $mO_{2\ peak}$ that was 150% higher than those at 12 °C. The effects of dietary protein levels on $mO_{2\ peak}$ were not significant within the 12 °C and 17 °C treatments. However, within the 22 °C treatment, $mO_{2\ peak}$ was significantly higher in abalone fed P41U compared to P35, P41 and P44 dietary treatments.

As well as $mO_{2\ peak}$, the time taken to reach peak mO_2 ($time_{peak}$) also appears to be temperature dependent. Specifically, abalone maintained at 17 °C reached a peak mO_2 that was 52% higher than and approximately 37.3 hours earlier those at 12 °C while abalone maintained at 22 °C reached a peak mO_2 that was 150% higher than and approximately 37.3 hours earlier than those at 12 °C. The effects of dietary protein levels on peak mO_2 were not significant within the 12 °C and 17 °C treatments. However, within the 22 °C treatment, peak mO_2 was significantly higher at P41U compared to P35, P41 and P44 dietary treatments. Meanwhile the effect of dietary protein levels on $time_{peak}$ is not clear.

Hybrid abalone maintained at 12 °C returned to SMR on average 122 hours after feeding began. Animals maintained at 17 °C returned to SMR approximately 114 hours after feeding while those at 22 °C were returned to SMR notably faster (64 hours).

Mortality results

Results of the temperature stress trial do not show a significant effect of dietary protein level (**Figure 19**) or dried *Ulva spp.* Inclusion (**Figure 20**) on the number of mortalities in either of the comparisons tested. This indicates that the mortalities recorded during the high temperature stress are likely to induced through a mechanism not directly related to the digestive processes or processes unaffected by the moderate differences in protein concentration or used in this study.

Respirometry of juvenile abalone during temperature stress event

Figure 21 presents a timeline of metabolic activity measured as mass-specific oxygen consumption for juvenile hybrid abalone exposed to a simulated summer stress event. Two distinct peaks can be observed during the baseline period at 22 °C. As the temperature begins to increase, three peaks can be observed during the temperature increase period (from 22 °C to approximately 24.5 °C), occurring between approximately 2300 h and 0200 h and ranging from 0.15-0.2 mg O_2 kg^{-1} min^{-1}. These peaks in activity are attributed to abalone locomotion and feeding behaviours. The troughs during this period rarely drop below 0.05 mg O_2 kg^{-1} min^{-1}. Feed provision was stopped as per on-farm practices once 26 °C was reached. Maximum temperature (28 °C) was reached at approximately 192:00 h. At this time, the abalone ceased to show the distinct patterns of nocturnal locomotion present at lower temperatures. During this time mO_2 steadily declines and after 64 h (SDA duration of 22 °C juvenile abalone) is approximately 0.075 mg O_2 kg^{-1} min^{-1}. After 96 h the abalone mO_2 continues to decline at a slower rate. Interestingly, separation between the Ulva supplemented treatment and the other protein treatments develops during the temperature stress event. As the temperature declines, the recommencement of feeding return, although mO_2 never reaches the same levels observed during the temperature increase period.

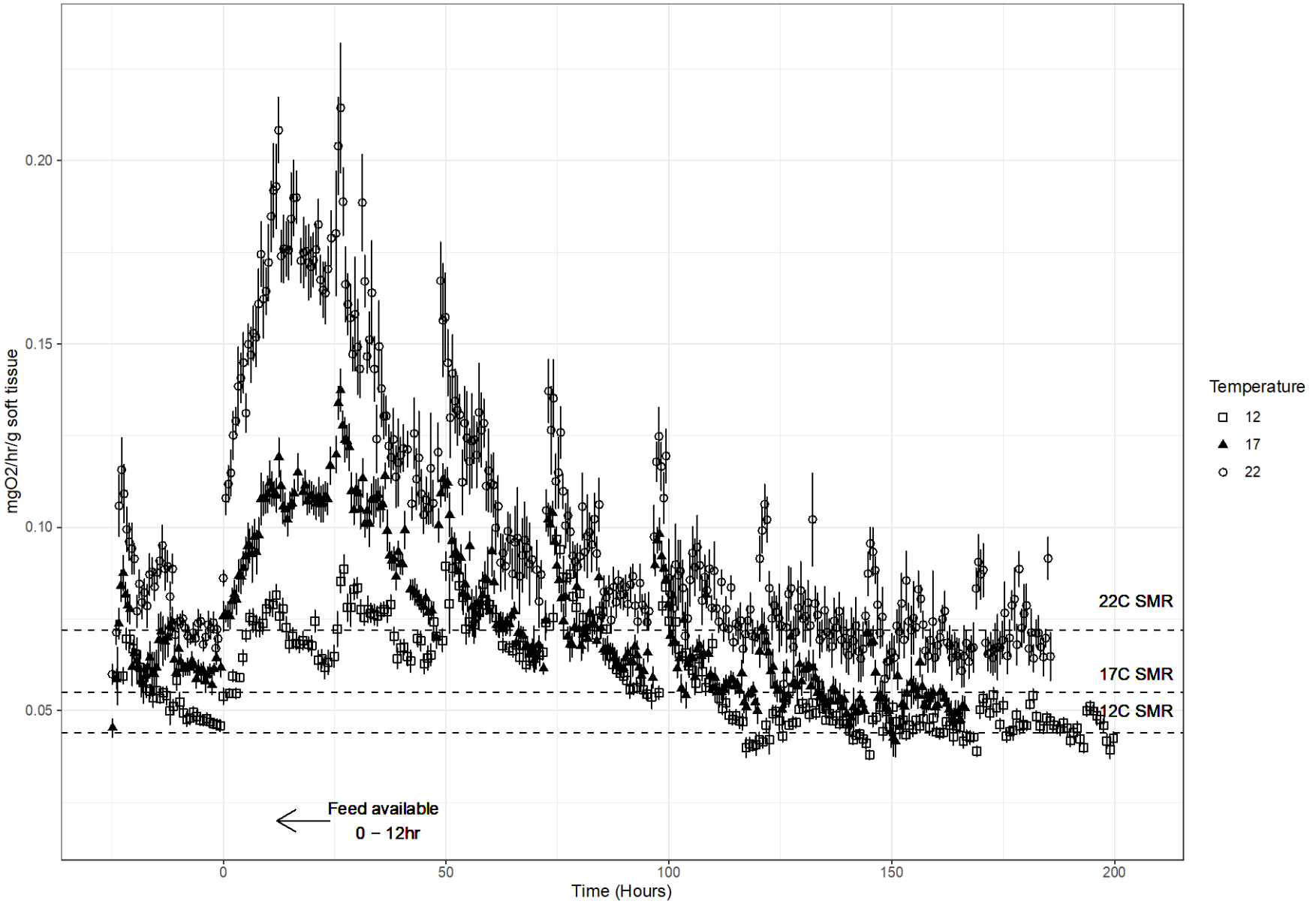

Figure 17: Average SDA mO_2 mass normalised of juvenile Australian hybrid abalone.

Table 20: Respirometry of juvenile Australian hybrid abalone

Temperature	12°C					17°					22°C				
Protein level	35%	41%	47%	41% + Ulva	Sig.	35%	41%	47%	41% + Ulva	Sig.	35%	41%	47%	41% + Ulva	Sig.
Tank soft tissue mass (*g*)	77.02 ± 2.11	85.81 ± 4.52	79.77 ± 2.52	77.58 ± 1.21		132.27 ± 4.54a	162.15 ± 2.73b	167.19 ± 3.70b	152.87 ± 1.57b	***	179.62 ± 3.00	197.46 ± 16.67	184.32 ± 11.49	143.89 ± 12.57	
Mass specific energy ingestion (k*J/g soft tissue*)	260.16 ± 31.34	341.04 ± 19.44	342.75 ± 7.91	302.24 ± 11.00		367.40 ± 23.36	312.92 ± 6.05	309.67 ± 14.39	344.32 ± 3.57		536.01 ± 2.53	488.26 ± 49.99	535.22 ± 35.69	683.13 ± 73.30	
Standard metabolic rate (SMR) (*mg O_2/hr/g soft tissue*)	0.044 ± 0.003	0.042 ± 0.003	0.040 ± 0.002	0.046 ± 0.002		0.064 ± 0.004	0.055 ± 0.002	0.053 ± 0.005	0.056 ± 0.005		0.064 ± 0.002	0.060 ± 0.004	0.065 ± 0.002	0.069 ± 0.003	
Peak mO_2 *(mg O_2/hr/g soft tissue)*	0.083 ± 0.005	0.077 ± 0.002	0.076 ± 0.006	0.077 ± 0.001		0.129 ± 0.005	0.117 ± 0.006	0.111 ± 0.008	0.118 ± 0.005		0.177 ± 0.004a	0.184 ± 0.008a	0.188 ± 0.009a	0.232 ± 0.011b	**
Peak net *(mg O_2/hr/g soft tissue)*	0.039 ± 0.005	0.035 ± 0.001	0.037 ± 0.004	0.030 ± 0.001		0.065 ± 0.002	0.058 ± 0.004	0.056 ± 0.003	0.069 ± 0.003		0.112 ± 0.004a	0.124 ± 0.006a	0.123 ± 0.008a	0.163 ± 0.012b	**
$Time_{peak}$ *(hr)*	69.51 ± 9.81ab	43.20 ± 4.90a	58.26 ± 7.43ab	74.06 ± 0.00b	*	24.71 ± 2.05	21.43 ± 4.35	22.03 ± 2.90	28.32 ± 0.57		26.37 ± 1.06	20.76 ± 3.64	15.41 ± 2.30	14.83 ± 2.99	
Duration *(hr)*	140.31 ± 24.13	116.05 ± 1.01	117.07 ± 1.95	116.82 ± 0.26		109.97 ± 1.08	111.00 ± 2.21	118.99 ± 8.68	118.17 ± 8.41		62.77 ± 1.89	60.80 ± 3.92	65.47 ± 1.56	69.90 ± 5.50	
SDA expenditure (*J/g soft tisse*)	49.83 ± 5.16	44.92 ± 0.69	48.63 ± 5.02	39.85 ± 1.07		48.36 ± 1.50	47.67 ± 4.63	47.54 ± 2.56	53.65 ± 6.58		64.48 ± 4.84a	66.00 ± 5.60a	69.49 ± 4.48a	93.55 ± 10.01b	*
SDA co-efficient (*(SDA expenditure/MSEI)*100*)	19.25 ± 0.96a	13.25 ± 0.77b	14.27 ± 1.79b	13.22 ± 0.52b	*	13.24 ± 0.64	15.30 ± 1.77	15.48 ± 1.47	15.59 ± 1.94		12.03 ± 0.87	13.59 ± 0.72	13.00 ± 0.37	14.19 ± 2.74	
Factorial Scope (*Peak mO_2/SMR*)	1.90 ± 0.14	1.85 ± 0.06	1.92 ± 0.06	1.65 ± 0.03		2.02 ± 0.05	2.05 ± 0.04	2.12 ± 0.06	2.50 ± 0.34		2.74 ± 0.08	3.07 ± 0.15	2.89 ± 0.11	3.36 ± 0.22	

Data are expressed as mean ± SEM. Values within temperature treatments within the same row with different letters are significantly different (one-way ANOVA and Tukey's post hoc test). *, ** and *** indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

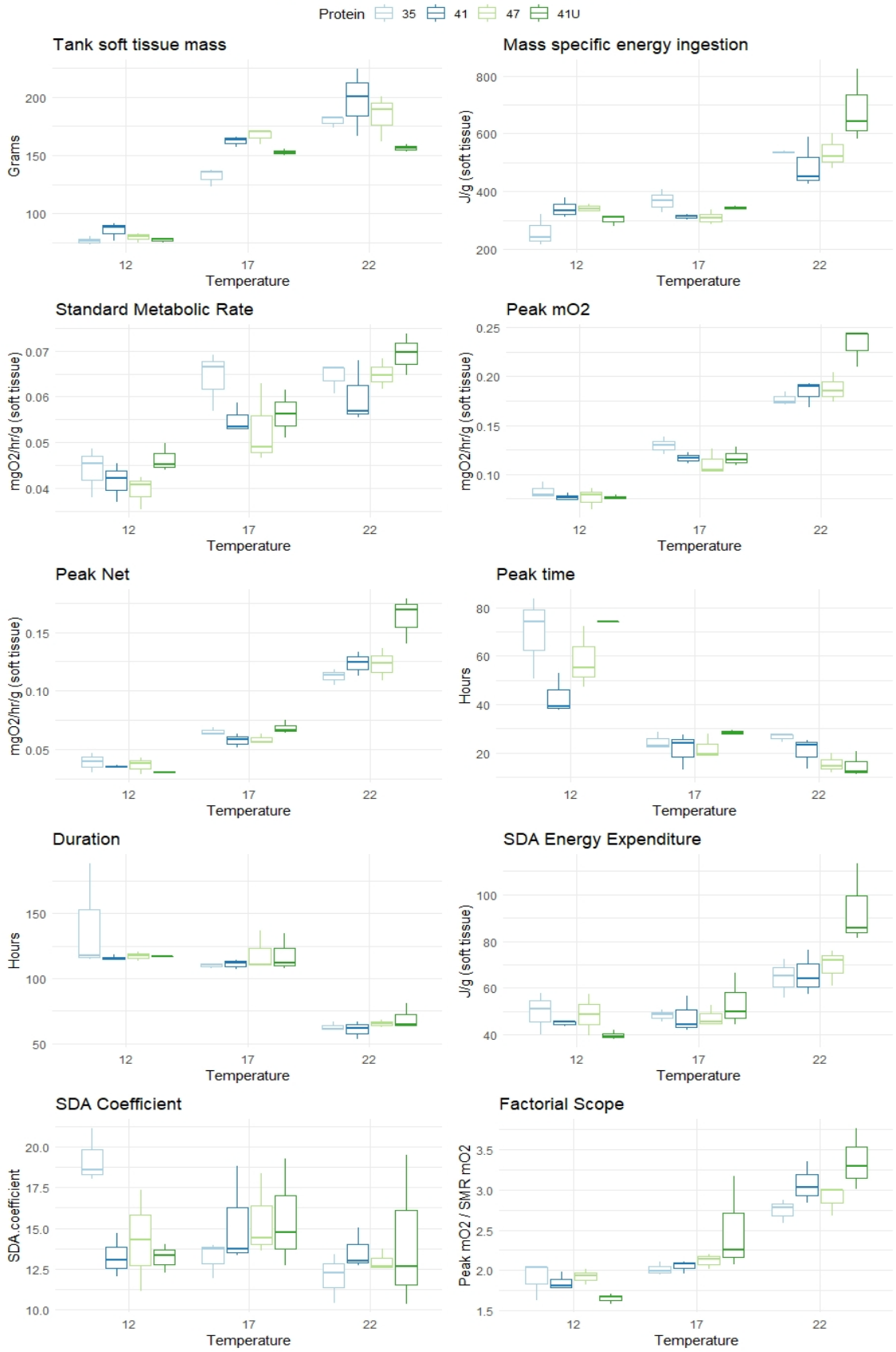

Figure 18: SDA metrics for respirometry of juvenile Australian hybrid abalone

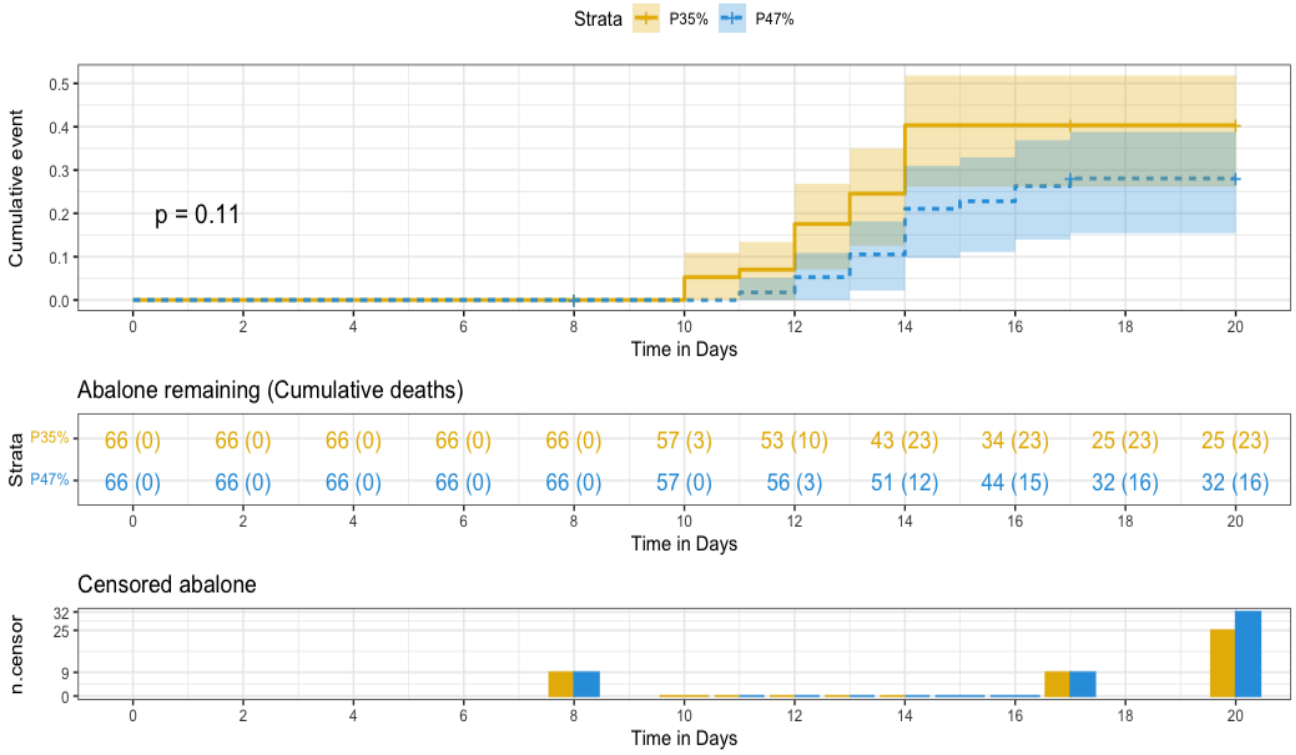

Figure 19: Mortality response of juvenile Australian hybrid abalone fed 35% and 47% protein inclusion diets during temperature stress event.

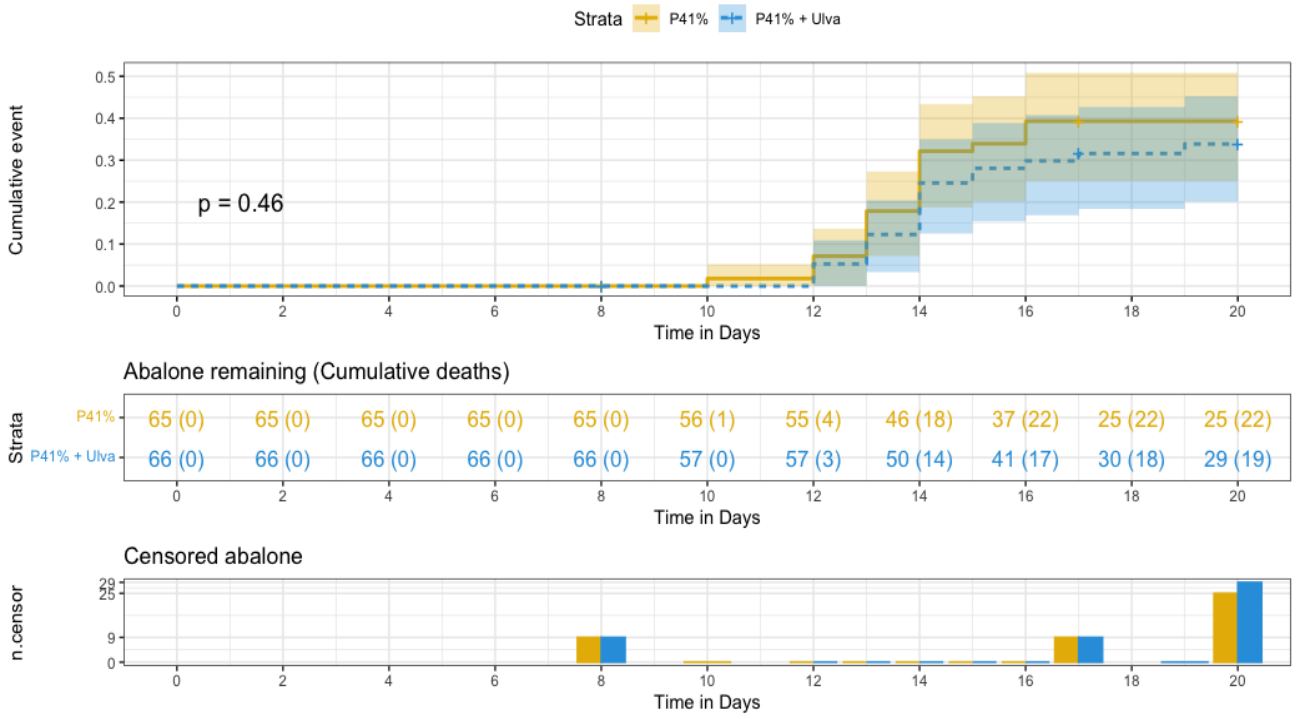

Figure 20: Mortality response of juvenile Australian hybrid abalone fed 41% and 41% + *Ulva* protein inclusion diets during temperature stress event.

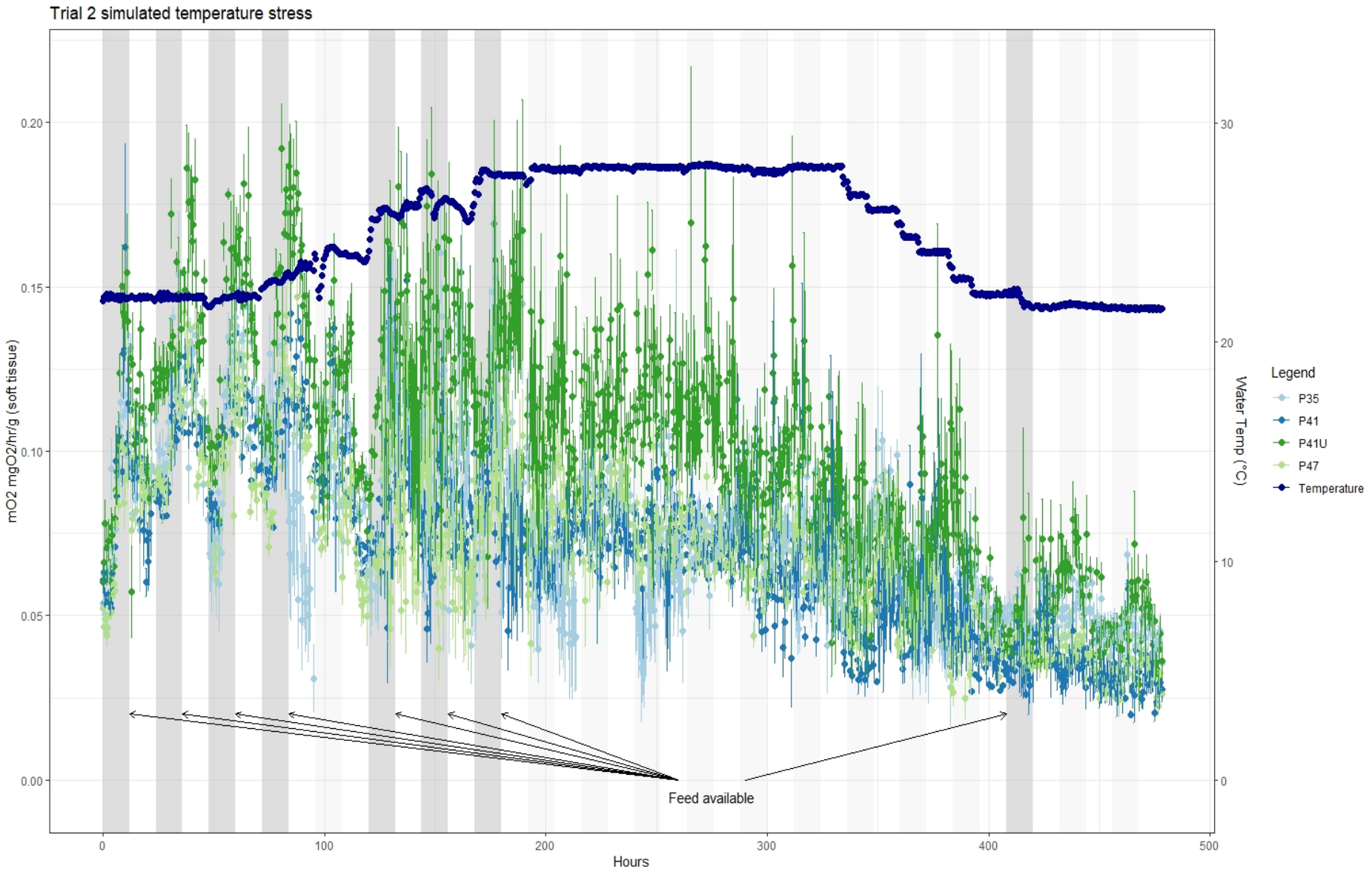

Figure 21: Respirometry of juvenile Australian hybrid abalone during temperature stress event.

Discussion

SDA response

The results from SDA on both the sub-adult and juvenile abalone (**Tasks 2.2** and **3.2**, respectively) indicate that digestive metabolic rates, regardless of life stage are significantly affected by temperature. Furthermore, these results indicate that the cost of feed digestion in abalone is largely unchanged in terms of O_2 consumed when the level of dietary protein is increased from 32% to 44% crude protein in sub-adult abalone and increased from 35% to 47% juvenile abalone, regardless of the *Ulva* supplementation. These findings are in line with findings regarding dietary protein level in other abalone species (Montano-Vargas et al. 2005). Taken together with the results detailed in **Task 2.1** and **Task 3.1**, it is clear that increasing the dietary protein inclusion level in diets for hybrid abalone to, at least, 41% would likely yield significant gains in terms of growth performance while having no adverse physiological consequences on the farmed abalone with regard to metabolic or oxidative stress.

This study of hybrid abalone metabolic physiology has confirmed the distinct non-linear trend of increasing metabolic activity with increasing temperature that is typical of ectothermic animals (Secor, 2009), and specifically abalone (Gaty & Wilson, 1986; Paul & Paul, 1998). The results of this study also suggest that not all features of metabolic physiology respond to temperature equally. The difference between SMR and peak mO_2 in the 12 °C and 22 °C treatment (regardless of life stage) suggests that baseline metabolic costs do not increase with temperature at the same rate as the total metabolic capacity. Specifically, the differences between SMR and peak mO_2 is magnified in abalone reared at higher water temperatures. Furthermore, peak net mO_2 includes metabolic costs associated with ingestion, somatic growth, digestion and excretion processes. Peak net mO_2 increased ~4-fold between 12 °C and 22 °C due to a relatively small SMR increase but a marked increase in peak mO_2. Peak net mO_2 was less than SMR in both the 12 °C and 17 °C treatments for sub-adult and juvenile abalone; however, at 22 °C peak net mO_2 was between 1.5 and 2 times greater than SMR. This shows that hybrid abalone have greater capacity for metabolic work (growth) at higher temperatures. The metabolic responses of hybrid abalone to the three rearing temperatures of these experiments largely agree with and support the growth, feed conversion and digestibility metrics presented earlier in this report in **Task 2.1** and **3.1** and to published

reports on similar species (Bansemer et al., 2016b; Paul & Paul, 1998). Simply, increased metabolic rates due to higher temperature drives higher energy (food) ingestion rates which manifests in other physiological responses, namely, increased growth performance.

A positive relationship was observed between SMR and dietary protein inclusion level within sub-adult abalone reared at higher water temperatures (17 and 22 °C). This accords with theoretical additional costs associated the catabolism of dietary provided protein as an energy substrate (Gómez-Montes et al., 2003; Kreeger, 1993; Mock et al., 2019). This trend would be expected to be also present (and likely accentuated) in the peak mO_2 results. Although, this was the case at 17 °C, peak mO_2 was similar across dietary treatments at 22 °C. Additionally, temperature significantly affected the peak time of the SDA response with the 17 °C treatment being similar to the 22 °C treatment (~10 h and ~20 h for the sub-adult and juvenile abalone, respectively) and the 12°C treatment being approximately 3-fold longer (~30 h and ~60 h for the sub-adult and juvenile abalone, respectively). Given that most abalone farms feed in the afternoon, this puts the peak of metabolic activity due to digestion and assimilation at approximately midday to early afternoon the next day when temperatures are high (22 °C). This peak of metabolic activity likely coincides with the peak of daily water temperature fluctuations experienced during summer. This may represent a serious source of stress for abalone as metabolic demands may easily outstrip the capacity of the animals. Peak time also takes longer to reach at 12 °C compared to 17 °C or 22 °C. This trend was evident in both the sub-adult and juvenile abalone respirometry data and is indicative of higher SMR in the 17 °C and 22 °C reared abalone coupled with an increased rate of response in the digestive processes.

A caveat exists regarding the animal size used within temperature treatments of the respirometry data. All abalone were from the same brood cohort, and therefore were all the same age and life history stage at the start of the growth trials (~12.5 g for **Task 2.1** and ~3.3 g for **Task 3.1**). Unavoidably, for respirometry and temperature stress experiments (**Tasks 2.2** and **3.2**), average animal sizes differed between temperature treatments due to the long temperature and diet acclimation time required by the preceding growth trials (143 days for sub-adult and 150 days for juvenile abalone). Due to the slow metabolic rate of abalone, and resultantly, long acclimation periods, differences in growth performance are inevitable. However, published allometric scaling constants show that large abalone use less O_2 g^{-1} of

soft tissue than smaller abalone (Farías et al., 2003). The effect of accounting for allometric scaling would be to amplify the differences in standard metabolic rate (SMR), Peak mO_2 and SDA expenditure between temperature treatments.

Temperature stress events

The lack of nocturnal activity attributed to locomotion during the peak of the temperature stress events appears to be highly unusual for a nocturnally browsing animal. SDA experiments conducted at 12, 17 and 22 °C exhibited noticeable nocturnal patterns that interfered with the calculation of SDA metrics, however, these distinct diurnal O_2 consumption patterns have been observed in other abalone species (Chacon et al., 2003; Kemp, 2018; Montano-Vargas et al., 2005). It was necessary to subtract the calculated increase in nocturnal activity based on time of day to normalise the mO_2 in these experiments. The complete absence of nocturnal activity during the peak of the stress events suggests that the abalone would/could not feed even if food had been provided. The same period saw an apparent continual decline of mO_2. No clear effect of dietary treatment could be discerned from the sub-adult experiment; however, within the juvenile experiment, abalone fed the *Ulva* supplemented diet appeared to consume slightly more O_2 /maintain slightly higher levels of metabolic activity than abalone fed the protein only diets. However, the same continual decline of mO_2 was still apparent in the *Ulva* supplemented diet treatment. It therefore appears likely that the stress experienced by abalone during summer mortality events is a result of excessively high temperatures and low dissolved oxygen and not from dietary formulations. It is unknown whether the mO_2 would continue to decline until death or would reach a lower bound around which it would stabilise. Since summer temperature stress events rarely last more than a few days the relevance of this speculation is questionable.

Task 4. Development of rapid screening assays

Introduction

Traditionally, raw material quality and digestibility in abalone feed formulations have been assessed using the *in vivo* faecal collection technique, where abalone are grown over a prolonged period to collect an adequate quantity of faeces for subsequent chemical analysis (Montaño-Vargas et al.,, 2002; Sales & Britz, 2001). Although it is still considered as the best and most accurate among the available methods, it is also well-known for associated logistical limitations and constraints in execution, particularly those surrounding time, effort and financial investment. Further, the emergence of a large number of alternative feed ingredients and the complexities associated with their varying nutrient quality has spurred industry and researchers to identify rapid and comparatively inexpensive ways of analysing feed ingredient quality and digestibility (Moyano et al., 2015; Shipton & Britz, 2002). In this context, several *in-vitro* digestibility methods have widely been tested on several terrestrial and aquatic species.

Among all the different tested methods, the gastrointestinal model (GIM) method has emerged as an ideal alternative as it more closely represents the physiological state of the digestive process in target organisms, with results comparable to the traditionally employed *in vivo* faecal collection method (Lewis et al., 2019; Moyano et al., 2015; Shipton & Britz, 2002). The GIM is a two-chambered bioreactor separated by a semi-permeable membrane. Crude enzymes extracted from the target species are added to the chamber along with the test substrate at a rate to maintain the same relative enzyme to substrate ratio (E:S) at the pH and temperature similar to the digestive tract of the target species. The use of a peristaltic pump further mimics the target organism's absorption process by immediately removing of end products of enzymatic reaction through the membrane. The GIM approach has been used previously to determine the *in vitro* protein digestibility in few aquatic species, including fish, shellfish, octopus and lobster (Hamdan et al., 2014; Lewis et al., 2019; Perera et al., 2010; Shipton & Britz, 2002).

As mentioned, a key performance parameter for fish and, by extension, aquafeed and its constituent ingredients are recorded digestibility values. *In vitro* GIM's been suggested as a

complimentary approach to *in vivo* apparent digestibility assessments (Morales & Moyano, 2010). GIM allows for assessment of the bioaccessibility (fraction which can be potentially hydrolysed from food matrix) and bioavailability (fraction which can be readily available for absorption and utilisation) of nutrients (Parada & Aguilera, 2007). Given consistent relationships can be established between *in vitro* GIM and *in vivo* digestibility values, GIM has the potential to provide aquaculture industries with a rapid and safe tool for digestibility assessments. Furthermore, considering limited live animals are required, there are fewer ethical, logistical, and financial restrictions (Morales & Moyano, 2010). GIM assessments may provide insight into reliable meal selections that could be rapidly implemented pre-, post-, or peri-temperature events to optimise performance and condition of the target species.

The development of methodology to screen new feed ingredients will provide a rapid assessment of suitability for incorporation into abalone feed formulations. The information generated here will subsequently facilitate the rapid establishment of ingredient and dietary digestibility values without the need for time consuming and expensive *in vivo* estimations, assisting in the development of more economical feed formulations without compromising growth performance. Therefore, to permit the rapid assessment of digestibility and subsequent quality, abalone diets and their constituent protein sources will be evaluated using *in vitro* digestion technologies. Briefly, crude enzymes will be extracted from abalone gut samples and assessed for their acid and alkaline protease activities. Following these processes, the acid and alkaline phases of digestive hydrolysis will be simulated in a two chambered bioreactor with a semi-permeable membrane over a 240 minute period. The concentrations of total nitrogen and amino acids in collected dialysates will then be quantified and correlated with the results of *in vivo* sampling efforts.

Methods

Gastrointestinal model assay

Preparation and characterisation of digestive enzyme extracts

Preparation of the crude enzyme extracts and determination of the activities of acid and alkaline proteases were performed as described previously (Morales and Moyano, 2010) with minor modifications to mimic abalone digestive process and physiological environment. Following the final sampling of the Australian hybrid abalone growth and temperature stress trial (**Task 2.1** and **Tasks 2.2** and **3.2**), remaining abalone (~3-year old, average weight – 40 g) were transferred to a large holding tank (22 °C) and reared on a commercial diet for two months prior to sampling. Twenty abalone were shucked, and their whole digestive tract were removed after 12 h of their last feeding as protease enzyme peak activity occurs at this point. The pH (Micro combination pH electrode, Thermo Scientific, USA) of the crop, stomach, and intestine were measured.

Subsequently, crude enzyme extracts were prepared by manual homogenisation of the digestive tract in distilled water (1:10, w/v) followed by centrifugation (12,000 rpm, 3 °C, 15 min). Supernatants were stored at -20 °C until used in the assays. Acid protease activity in stomach extracts was measured using the method of (Anson 1938) with minor modifications, using substrate haemoglobin and citric acid-sodium phosphate buffer (pH 5.6), reflecting the pH of the stomach and crop of abalone as most of the digestion occurs at these two sites. The measured enzyme activity was used to determine the enzyme to substrate ratio (E:S) to use in the gastrointestinal model (GIM).

Operational conditions of the assay

Hydrolysis in the GIM assay was simulated in a two-chambered bioreactor separated by a semi-permeable dialysis membrane with a molecular weight cut off (MWCO) of 3500 Da (Spectra Por 6, Spectrum Medical Industries, Los Angeles, CA, USA). *In vitro* digestibility was simulated by placing fishmeal (~250 mg protein) suspended in deionised water in the upper chamber of the bioreactor containing citric acid-sodium phosphate buffer at pH 5.6 and agitated with a magnetic stirrer. Abalone crude enzyme extracts were added to the upper chamber to match the estimated enzyme to substrate ratio (E:S). The amino acids and

peptides that passed through the semi-permeable membrane were continuously removed from the bioreactor by a peristaltic pump circulating 0.5 mL min^{-1} of citric acid-sodium phosphate buffer for 225 min. GIM temperature was maintained at 22 °C by placing inside an incubation oven as the abalone were grown at that temperature. Samples were separately collected every 45 min and subjected to amino acid analysis using high performance liquid chromatography. All samples were run in duplicate, with duplicate blanks where no enzyme extracts were added for the entirety of the assay.

Results and Discussion

Hybrid abalone crude protease enzyme activity was low and ranged between 0.08-0.12 U mg^{-1} soluble protein (mean ± SE – 0.093 ± 0.007 U mg^{-1} soluble protein) (U = enzyme catalytic activity unit). However, this phenomenon has been observed previously among other abalone species. Previous enzymatic studies on *Haliotis laevigata* revealed that trypsin activities were low and ranged between 0.11-0.83 U mg^{-1} soluble protein depending on the age of abalone and feed protein content (Bansemer et al., 2016b). Being herbivorous, abalone predominantly eat carbohydrate-rich macroalgae in the wild, which contains 11-19% of protein. Therefore, their enzymatic profile is mainly dominated by various carbohydrase enzymes. In contrast, very high protease enzyme activities (stomach crude protease enzyme activity – 256 U mg^{-1} soluble protein, and intestinal crude protease enzyme activity – 1460 U mg^{-1} soluble protein) were recorded with carnivore fish, Barramundi (Lewis et al., 2019).

In vitro protein digestibility of fish meal using hybrid abalone crude enzyme for 225 min was low and ranged between 0.88-1.05% (mean ± SE – 0.97 ± 0.04%). Similarly, the individual amino acid concentration of the *in vitro* digesta was also very low (**Table 21**). However, *in vivo* protein digestibility of fishmeal with Greenlip and Blacklip abalone were comparatively high, with ADC values of 46%, and 56%, respectively (Vandepeer & Barneveld, 2003). Therefore, the protein digestibility coefficients obtained in the current study was far lower than the values reported for its parental species in the traditional *in vivo* studies. However, studies on barramundi revealed that *in vitro* and *in vivo* protein digestibility were highly correlated (Spearman correlation – 0.96) and comparable, albeit considerably lower than the ADC values obtained using *in vivo* approaches (Lewis et al., 2019).

The lower *in vitro* protein digestibility value obtained for abalone in this study is likely related to the issues in simulating the reaction time similar to the digestion duration time. The reaction time in the current study was only 225 min, whereas the actual digestion time in abalone is more than 24 hours (Currie et al., 2015). Generally, reaction time in *in vitro* GIM studies was restricted to 4-6 hours only, as longer reaction times can lead to bacterial contamination, autohydrolysis of enzymes and saturation by reaction by-products resulting reduced enzyme reaction (Moyano et al., 2015). Therefore, simulating such a long digestion process of abalone in *in vitro* GIMs may prove difficult.

Although care was taken to mimic the relative enzyme to substrate ratio (E:S) in the GIM similar to the abalone digestive tract, the volume of the GIM chamber (12.5 mL) was far higher than the actual volume of the digestive tract. Hence, the enzymatic reaction in the GIMs occurred at a lower enzyme to substrate ratio. Further, enzymes were collected from the digestive tract of the abalone which were conditioned on a commercial diet. However, there is evidence suggesting abalone are capable of regulating enzymatic secretion considering the protein quantity and source (Bansemer et al. 2016b). Therefore, the employed enzyme to substrate ratio (E:S) may not be relevant to the current study as fishmeal was used as the substrate.

pH is a strong determinant of protease enzyme activity by influencing protein solubility. The current *in vitro* GIM was carried out at pH 5.6 by mimicking the actual pH in the abalone stomach and crop. However, in the current study, as the abalone stomach pH falls within the isoelectric point ranges of fishmeal (pH 4.8-6.2), the protein might be precipitated. Because the protein molecules become electrically neutral when it reaches its isoelectric point, hence no binding sites for water (Morales & Moyano 2010). As a result, there might be a reduced rate of reaction between enzyme and substrate. Moreover, one of the significant weaknesses of the *in vitro* GIM is that it doesn't simulate the microbial digestion occurring in the target animal. However, there is evidence suggesting the presence of various microorganisms in the abalone digestive tract and their significant role in the abalone digestion process (Harris et al., 1998).

Conclusions

It is essential to recognise that digestion is a myriad of complex processes; therefore, the GIM is designed to simulate, not reproduce, the digestive processes occurring in the gastrointestinal tract of the animal. The use of GIM *in vitro* digestibility assessment is a relatively new concept in fish nutrition which has been tested on very few aquatic species. However, strong correlations have been reported between *in vivo* and *in vitro* protein digestibility in numerous fish species (Fenerci & Şener, 2005; Lewis et al., 2019; Perera et al., 2010; Shipton & Britz, 2002) . Interestingly, all the studies were conducted on fish species which exhibit higher enzymatic activity and rapid digestion in two phases, strong acidic followed by alkaline (Moyano et al., 2015). For example, Barramundi show higher protease enzyme activity (stomach and intestinal protease enzyme activities are 256 and 1460 U mg^{-1} soluble protein, respectively), strong acidic digestion in the stomach (pH 3.4) followed by alkaline digestion in the intestine (pH 7.8) and faster digestion (~6 hrs) (Lewis et al., 2019). Whereas, abalone shows low protease enzyme activity (0.933 U mg^{-1} soluble protein), weak acidic digestion in the stomach and crop (pH 5.6) and the intestine (pH 6.4), and slow digestion (24 hrs). Therefore, it is possible that GIM assessments may not reflect the slow and prolonged digestion occurring in abalone. However, strong correlations may be possible when used in conjunction with targeted *in vivo* trials which aim to evaluate the efficacy of different raw materials used in abalone feed formulations. As such, there may be scope to investigate this topic further in future AAGA – Deakin University projects.

Table 21: Amino acids and protein digestibility based on *in vitro* GIM

	Replicate 1	Replicate 2
Aspartic acid	0.0069	0.0035
Glutamic acid	0.1291	0.1168
Serine	0.0465	0.0413
Histidine	0.3211	0.2835
Glycine	0.1603	0.1439
Threonine	0.0292	0.0202
Arginine	0.4466	0.3115
Alanine	0.6842	0.4829
Tyrosine	0.0398	0.0390
Valine	0.2036	0.1559
Methionine	0.0523	0.0469
Phenylalanine	0.0340	0.0308
Isoleucine	0.0339	0.0271
Leucine	0.1924	0.1689
Lysine	0.3421	0.3347
Proline	0.0579	0.0814
Total Amino acids	2.7800	2.2848
Protein digestibility	1.05	0.88

Implications

The outcomes of the present investigation have wide ranging impacts across the abalone and aquatic animal nutrition sectors. Prior to the execution of this project, the formulation of compounded feeds for farmed hybrid abalone was informed by results obtained from studies conducted using the Greenlip Abalone as a proxy. Anecdotal evidence suggested a departure in the requirements for protein in hybrid abalone, but there was no conclusive evidence available to support this. Resultantly, the growth potential of hybrid abalone was not fully realised leading to longer grow-out periods and lower profit returns.

Based on the data detailed herein, revised protein level recommendations can be presented to farmers and feed manufacturers, promoting improved growth in farmed hybrid abalone, with minimal to no impact on the physiological performance of animals when subjected to periods of summer heat stress. Likewise, changes to dietary formulations to incorporate higher protein inclusion levels had little impact on the nutritional composition and quality of the final farmed product, indicating that, if implemented, higher protein inclusion levels will not negatively impact consumer acceptance. However, consumer preference did not factor into the investigations of the current project and ultimately requires verification.

The approach to test each of the experimental diet formulations at three rearing temperatures revealed a series of noteworthy trends that have the potential to inform and benefit on-farm feed management protocols. In particular, these were associated with the regularity of on-farm feeding during the cold winter months, where growth performance in hybrid abalone was poor regardless of the level of protein offered. Noting the feasibility issues associated with heating water in a flow-through facility, adopting strategies that save on feed costs and farm labour, whilst maintaining similar levels of growth, should be investigated in a commercially relevant setting.

Recommendations

This project set out to evaluate the optimal protein requirement of farmed hybrid abalone in response to observations that on-farm growth performance was hindered due to the absence of a diet developed specific to the Australian hybrid abalone and relying on diets developed for Greenlip Abalone aquaculture. With a view of developing hybrid abalone specific feeds tailored to seasonal conditions, a series of experiments were conducted across three different water temperatures reflective of winter (12°C), summer (22°C), and average (17°C) rearing environments. Based on the results of this project, the following recommendations are put forward for consideration by AAGA and the broader abalone industry with respect to hybrid abalone aquaculture:

1. Dietary requirements for crude protein developed for Greenlip Abalone aquaculture are insufficient for hybrid abalone. The results of the current project suggest that significant performance gains can be realised by increasing the crude protein inclusion level beyond the current 35% to a level of 41%. The benefits of this higher inclusion level were particularly notable at 17°C and 22°C, but less evident at 12°C due to overall slow growth. As such, it is recommended that industry adopts a seasonal approach to feed formulations whereby a cost efficient diet containing a crude protein level of 35% (or potentially lower) is manufactured and fed during the slower growing winter months, with a switch to a 41% crude protein diet during the warmer months when feeding activity, animal metabolism and ultimately growth rates increase.

2. Considering the current project was conducted in an experimental setting it is important that the recommendations above are verified in a commercial setting across farms with adequate replication and controls to properly assess the benefits of a higher protein diet. Ideally, investigations would be carried out over the entire grow-out cycle, permitting the establishment of a commercially relevant cost-benefit analysis reflecting the increases associated with formulating diets containing higher levels of crude protein.

3. Prior to the implementation of on-farm trials, technical evaluations are required on behalf of the feed manufacturers to assess the viability of reaching the revised protein inclusion level whilst maintaining the prerequisite high level of pellet stability for commercial farming. Notably, the experimental formulations achieved in the present project may be challenging to achieve on a commercial scale.

Further development

Moving forward there are considerable opportunities to realise further gains for hybrid abalone aquaculture via continued R&D effort focussing on nutrition and the interaction of this discipline with health and genetics. Whilst these suggestions are by no means exhaustive, avenues for further investigation stemming from the current body of work include:

- Comprehensive evaluations of various protein sources, including maximum inclusion rates and their subsequent digestibility. This information is severely lacking in the public realm and recognised as a fundamental step for formulating optimised nutritional approaches.

- Investigations into the quantitative and qualitative aspects of meal inclusion to better align the amino acid composition of formulated feeds with that of both farmed and wild abalone species.

- Manipulation of dietary fatty acid composition, particularly with respect to omega-6 long chain polyunsaturated fatty acids and their documented role in modulating the immune response of aquatic animals.

- Evaluation of bioactive compounds that have the potential to increase resilience to summer temperature extremes.

Extension and Adoption

This project was executed with the direct involvement of the Australian Abalone Growers Association (AAGA). AAGA have been closely involved in all aspects of project planning and execution. Regular milestone reports have been submitted to the FRDC and AAGA, with key results being distributed electronically when available. Likewise, the project team have taken part in numerous AAGA executive meetings and presented project overviews and key findings at four AAGA annual general meetings. Due to COVID-19 restrictions, the project team has thus far been unable to present the final results in person. However, with restrictions easing, this will be scheduled for the next AGM (date to be confirmed). Likewise, COVID-19 has impeded the ability of the PhD students embedded in the project to attend international symposia to present the results of their research.

Given the early distribution of the results emanating from the two growth trials, the team has been informed anecdotally that higher protein inclusion level diets are being trialled independently on farm. Whilst the project team are yet to see the results of these investigations, it is envisaged that plans for further on-farm extension will be discussed in a future forum with AAGA and relevant feed manufacturers to validate the results of the current research activities.

Project coverage

To date, there hasn't been any media coverage or technical articles developed specific to this project with the exception of FRDC milestone reports and AAGA presentations. Following discussions with AAGA, we will gauge the need/ desire to disseminate the current research findings through mainstream media channels and relevant trade publications. It is expected that the findings of this work, alongside that of other AAGA IPA funded research activities, would provide an ideal topic for coverage in the FRDC's FISH magazine.

Project materials developed

No project materials have been developed to date. However, noting the involvement of two Deakin University PhD candidates, eight scientific articles are under preparation for submission to peer-reviewed journals in the coming months.

The following project overview was prepared and posted on the website of Deakin University's Nutrition and Seafood Laboratory.

https://lab.org.au/portfolio_page/elucidating-the-nutritional-requirements-of-farmed-hybrid-abalone/

References

Ackman, RG 2002, 'The gas chromatograph in practical analyses of common and uncommon fatty acids for the 21st century', *Analytica Chimica Acta*, vol. 465, no. 1-2, pp. 175-92.

Alter, K, Andrewartha, SJ, Morash, AJ, Clark, TD, Hellicar, AD, León, RI & Elliott, NG 2017, 'Hybrid abalone are more robust to multi-stressor environments than pure parental species', *Aquaculture*, vol. 478, pp. 25-34.

Anson, ML 1938, 'The estimation of pepsin, trypsin, papain, and cathepsin with hemoglobin', *The Journal of general physiology*, vol. 22, no. 1, p. 79.

Austreng, E, Storebakken, T & Åsgård, T 1987, 'Growth rate estimates for cultured Atlantic salmon and rainbow trout', *Aquaculture*, vol. 60, no. 2, pp. 157-60.

Bansemer, MS, Harris, JO, Qin, JG, Adams, LR, Duong, DN & Stone, DA 2015a, '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.

Bansemer, MS, Qin, JG, Currie, K-L & Stone, DA 2015b, '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. 885-92.

Bansemer, MS, Qin, JG, Harris, JO, Howarth, GS & Stone, DA 2016a, 'Nutritional requirements and use of macroalgae as ingredients in abalone feed', *Reviews in Aquaculture*, vol. 8, no. 2, pp. 121-35.

Bansemer, MS, Qin, JG, Harris, JO, Schaefer, EN, Wang, H, Mercer, GJ, Howarth, GS & Stone, DA 2016b, 'Age-dependent response of digestive enzyme activities to dietary protein level and water temperature in greenlip abalone (*Haliotis laevigata*)', *Aquaculture*, vol. 451, pp. 451-6.

Barkai, R & Griffiths, C 1988, 'An energy budget for the South African abalone *Haliotis midae* Linnaeus', *Journal of Molluscan studies*, vol. 54, no. 1, pp. 43-51.

Barkai, R & Griffiths, CL 1987, 'Consumption, absorption efficiency, respiration and excretion in the South African abalone *Haliotis midae*', *South African Journal of Marine Science*, vol. 5, no. 1, pp. 523-9.

Barnathan, G 2009, 'Non-methylene-interrupted fatty acids from marine invertebrates: Occurrence, characterization and biological properties', *Biochimie*, vol. 91, no. 6, pp. 671-8.

Bogard, JR, Farmery, AK, Baird, DL, Hendrie, GA & Zhou, SJN 2019, 'Linking Production and Consumption: The Role for Fish and Seafood in a Healthy and Sustainable Australian Diet', vol. 11, no. 8, p. 1766.

Britz, PJ & Hecht, T 1997, 'Effect of dietary protein and energy level on growth and body composition of South African abalone, *Haliotis midae*', *Aquaculture*, vol. 156, no. 3-4, pp. 195-210.

Britz, PJ, Hecht, T & Mangold, S 1997, '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.

Bureau, DP, Azevedo, PA, Tapia-Salazar, M & Cuzon, G 2000, 'Pattern and cost of growth and nutrient deposition in fish and shrimp: Potential implications and applications', *Avances en Nutrición Acuícola*.

Buss, J, Jones, D, Lumsden, A, Harris, J, Bansemer, M & Stone, D 2015, 'Restricting feed ration has more effect than diet type on the feeding behaviour of greenlip abalone *Haliotis laevigata*', *Marine and Freshwater Behaviour and Physiology*, vol. 48, no. 1, pp. 51-70.

Chabot, D, Steffensen, JF & Farrell, AP 2016, 'The determination of standard metabolic rate in fishes', vol. 88, no. 1, pp. 81-121.

Chacon, O, Viana, MT, Farias, A, Vazquez, C & Garcia-Esquivel, Z 2003, 'Circadian metabolic rate and short-term response of juvenile green abalone (*Haliotis fulgens* Philippi) to three anesthetics', *Journal of Shellfish Research*, vol. 22, no. 2, pp. 415-22.

Chao, W-R, Huang, C-Y & Sheen, S-S 2010, 'Development of formulated diet for post-larval abalone, *Haliotis diversicolor supertexta*', *Aquaculture*, vol. 307, no. 1-2, pp. 89-94.

Chen, N, Luo, X, Gu, Y, Han, G, Dong, Y, You, W & Ke, C 2016, 'Assessment of the thermal tolerance of abalone based on cardiac performance in *Haliotis discus hannai, H. gigantea* and their interspecific hybrid', *Aquaculture*, vol. 465, pp. 258-64.

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

Christenson, JK, O'Kane, GM, Farmery, AK & McManus, A 2017, 'The barriers and drivers of seafood consumption in Australia: A narrative literature review', *International Journal of Consumer Studies*, vol. 41, no. 3, pp. 299-311.

Cochet, M, Brown, M, Kube, P, Fluckiger, M, Elliott, N & Delahunty, C 2013, 'Sensory and physicochemical assessment of wild and aquacultured green and black lip abalone (*Haliotis laevigata* and *Haliotis rubra*)', *Journal of Shellfish Research*, vol. 32, no. 1, pp. 81-8.

Coote, T, Hone, P, Van Barneveld, R & Maguire, G 2000, 'Optimal protein level in a semipurified diet for juvenile greenlip abalone *Haliotis laevigata*', *Aquaculture Nutrition*, vol. 6, no. 4, pp. 213-20.

Crab, R, Avnimelech, Y, Defoirdt, T, Bossier, P & Verstraete, W 2007, 'Nitrogen removal techniques in aquaculture for a sustainable production', *Aquaculture*, vol. 270, no. 1, pp. 1-14.

Currie, K-L, Lange, B, Herbert, EW, Harris, JO & Stone, DA 2015, 'Gastrointestinal evacuation time, but not nutrient digestibility, of greenlip abalone, *Haliotis laevigata* Donovan, is affected by water temperature and age', *Aquaculture*, vol. 448, pp. 219-28.

Daume, S, Long, BM & Crouch, P 2003, 'Changes in amino acid content of an algal feed species (*Navicula* sp.) and their effect on growth and survival of juvenile abalone (*Haliotis rubra*)', *Journal of applied phycology*, vol. 15, no. 2, pp. 201-7.

Day, R & Fleming, A 1992, *The determinants and measurement of abalone growth. In 'Abalone of the World: Biology, Fisheries and Culture'.(Eds SA Shepherd, MJ Tegner and SA Guzman Del Proo.) pp. 141–168*, Blackwell Scientific: Oxford.

Díaz, F, del Río-Portílla, MA, Sierra, E, Aguilar, M & Re-Araujo, AD 2000, 'Preferred temperature and critical thermal maxima of red abalone *Haliotis rufescens*', *Journal of thermal biology*, vol. 25, no. 3, pp. 257-61.

Donovan, D, Baldwin, J & Carefoot, T 1999, 'The contribution of anaerobic energy to gastropod crawling and a re-estimation of minimum cost of transport in the abalone, *Haliotis kamtschatkana* (Jonas)', *Journal of Experimental Marine Biology and Ecology*, vol. 235, no. 2, pp. 273-84.

Donovan, DA & Taylor, HH 2008, 'Metabolic consequences of living in a wave-swept environment: Effects of simulated wave forces on oxygen consumption, heart rate, and activity of the shell adductor muscle of the abalone *Haliotis iris*', *Journal of Experimental Marine Biology and Ecology*, vol. 354, no. 2, pp. 231-40.

Drew, B, Miller, D, Toop, T & Hanna, P 2001, 'Identification of expressed HSPs in blacklip abalone (*Haliotis rubra* Leach) during heat and salinity stresses', *Journal of Shellfish Research*, vol. 20, no. 2, pp. 695-703.

Du, L & Niu, C-J 2002, 'Effects of Dietary Protein Level on Bioenergetics of the Giant Freshwater Prawn, *Macrobrachium rosenbergii* (De Man, 1879) (Decapoda, Natantia)', *Crustaceana*, vol. 75, no. 7, pp. 875-89.

Dunstan, GA, Baillie, HJ, Barrett, SM & Volkman, JK 1996, 'Effect of diet on the lipid composition of wild and cultured abalone', *Aquaculture*, vol. 140, no. 1-2, pp. 115-27.

Farcy, E, Serpentini, A, Fiévet, B & Lebel, J-M 2007, 'Identification of cDNAs encoding HSP70 and HSP90 in the abalone *Haliotis tuberculata*: Transcriptional induction in response to thermal stress in hemocyte primary culture', *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, vol. 146, no. 4, pp. 540-50.

Farías, A, García-Esquivel, Z & Viana, MT 2003, 'Physiological energetics of the green abalone, *Haliotis fulgens*, fed on a balanced diet', *Journal of Experimental Marine Biology and Ecology*, vol. 289, no. 2, pp. 263-76.

Fenerci, S & Şener, E 2005, '*In vivo* and *in vitro* protein digestibility of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1972) fed steam pressured or extruded feeds', *Turkish Journal of Fisheries and Aquatic Sciences*, vol. 5, no. 1.

Fleming, A 1995, 'Digestive efficiency of the Australian abalone *Haliotis rubra* in relation to growth and feed preference', *Aquaculture*, vol. 134, no. 3-4, pp. 279-93.

Fleming, AE, Van Barneveld, RJ & Hone, PW 1996, 'The development of artificial diets for abalone: a review and future directions', *Aquaculture*, vol. 140, no. 1-2, pp. 5-53.

Folch, J, Lees, M & Sloane Stanley, GHS 1957, 'A simple method for the isolation and purification of total lipides from animal tissues', *Biological Chemistry*, vol. 226, no. 1, pp. 497-509.

Francis, DS & Turchini, GM 2017, 'Retro-engineering the protein sparing effect to preserve n-3 LC-PUFA from catabolism and optimise fish oil utilisation: A preliminary case study on juvenile Atlantic salmon', *Aquaculture*, vol. 468, Part 1, pp. 184-92.

Freeman, KA 2001, 'Aquaculture and related biological attributes of abalone species in Australia. A review'.

Fu, S-J, Xie, X-J & Cao, Z-D 2005, 'Effect of dietary composition on specific dynamic action in southern catfish *Silurus meridionalis* Chen', *Aquaculture Research*, vol. 36, no. 14, pp. 1384-90.

Gaffney, PM & Diehl, WJ 1986, 'Growth, condition and specific dynamic action in the mussel *Mytilus edulis* recovering from starvation', *Marine Biology*, vol. 93, no. 3, pp. 401-9.

Gaty, G & Wilson, JH 1986, 'Effect of body size, starvation, temperature and oxygen tension on the oxygen consumption of hatchery-reared ormers *Haliotis tuberculata* L', *Aquaculture*, vol. 56, no. 3, pp. 229-37.

Glencross, BD 2008, 'A factorial growth and feed utilization model for barramundi, *Lates calcarifer* based on Australian production conditions', *Aquaculture Nutrition*, vol. 14, no. 4, pp. 360-73.

Glencross, BD 2020, 'A feed is still only as good as its ingredients: An update on the nutritional research strategies for the optimal evaluation of ingredients for aquaculture feeds', *Aquaculture Nutrition*.

Gómez-Montes, L, Garcıa-Esquivel, Z, D'Abramo, LR, Shimada, A, Vásquez-Peláez, C & Viana, MT 2003, 'Effect of dietary protein: energy ratio on intake, growth and metabolism of juvenile green abalone *Haliotis fulgens*', *Aquaculture*, vol. 220, no. 1-4, pp. 769-80.

Gorfine, H 1991, 'An artificial diet for hatchery-reared abalone *Haliotis rubra*', *Marine Science Laboratories, Internal Rep*, no. 190.

Guest, M, Nichols, P, Frusher, S & Hirst, A 2008, 'Evidence of abalone (*Haliotis rubra*) diet from combined fatty acid and stable isotope analyses', *Marine Biology*, vol. 153, no. 4, pp. 579-88.

Hamdan, M, Tomás-Vidal, A, Martínez, S, Cerezo-Valverde, J & Moyano, FJ 2014, 'Development of an *in vitro* model to assess protein bioavailability in diets for common octopus (*Octopus vulgaris*)', *Aquaculture Research*, vol. 45, no. 12, pp. 2048-56.

Handeland, SO, Imsland, AK & Stefansson, SO 2008, 'The effect of temperature and fish size on growth, feed intake, food conversion efficiency and stomach evacuation rate of Atlantic salmon post-smolts', *Aquaculture*, vol. 283, no. 1–4, pp. 36-42.

Harris, J, Burke, C & Maguire, G 1998, 'Characterization of the digestive tract of greenlip abalone, *Haliotis laevigata* Donovan. II. Microenvironment and bacterial flora', *Journal of Shellfish Research*, vol. 17, no. 4, pp. 989-94.

Howarth, RW & Marino, R 2006, 'Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: evolving views over three decades', *Limnology and Oceanography*, vol. 51, no. 1part2, pp. 364-76.

Huchette, SM, Koh, C & Day, RW 2003, 'Growth of juvenile blacklip abalone (*Haliotis rubra*) in aquaculture tanks: effects of density and ammonia', *Aquaculture*, vol. 219, no. 1-4, pp. 457-70.

Jobling, M 1981, 'The influences of feeding on the metabolic rate of fishes: a short review', *Journal of Fish Biology*, vol. 18, no. 4, pp. 385-400.

Kang, HY, Lee, Y-J, Song, W-Y, Kim, T-I, Lee, W-C, Kim, TY & Kang, C-K 2019, 'Physiological responses of the abalone *Haliotis discus hannai* to daily and seasonal temperature variations', *Scientific Reports*, vol. 9, no. 1, p. 8019.

Kemp, JOG 2018, 'Aspects of the nutritional physiology of the perlemoen *Haliotis midae* (L.) and red abalone *H. rufescens* (Swainson)'.

King, RH, Rayner, CJ, Kerr, M, Gorfine, HK & McShane, PE 1996, 'The composition and amino acid balance of abalone (*Haliotis rubra*) tissue', *Aquaculture*, vol. 140, no. 1-2, pp. 109-13.

Kirkendale, L, Robertson-Andersson, DV & Winberg, PC 2010, 'Review on the use and production of algae and manufactured diets as feed for sea-based abalone aquaculture in Victoria'.

Kooijman, SALM 2010, *Dynamic energy budget theory for metabolic organisation*, Cambridge university press.

Kreeger, D 1993, 'Seasonal patterns in utilization of dietary protein by the mussel *Mytilus trossulus*', *Marine Ecology-Progress Series*, vol. 95, pp. 215-.

Lafarga-De la Cruz, F, Núñez-Acuña, G & Gallardo-Escárate, C 2013, 'Hybridization between *Haliotis rufescens* and *Haliotis discus hannai*: evaluation of fertilization, larval development, growth and thermal tolerance', *Aquaculture Research*, vol. 44, no. 8, pp. 1206-20.

LeGrow, SM & Beamish, FWH 1986, 'Influence of Dietary Protein and Lipid on Apparent Heat Increment of Rainbow Trout, *Salmo gairdneri*', *Canadian Journal of Fisheries and Aquatic Sciences*, vol. 43, no. 1, pp. 19-25.

Lewis, MJ, Francis, DS, Blyth, D, Moyano, FJ, Smullen, RP, Turchini, GM & Booth, MA 2019, '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.

Lopez, LM & Tyler, P 2006, 'Energy budget of cultured female abalone *haliotis tuberculata* (L.)', *Journal of Shellfish Research*, vol. 25, no. 2, pp. 385-9.

Lyon, RG 1995, 'Aspects of the physiology of the South African abalone, *Haliotis midae* L., and implications for intensive abalone culture', Rhodes University.

Lyon, RG 1996, 'Aspects of the physiology of the South African abalone, *Haliotis Midae* L., and implications for intensive abalone culture', Rhodes University.

McCue, MD 2006, 'Specific dynamic action: A century of investigation', *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, vol. 144, no. 4, pp. 381-94.

McGaw, IJ & Reiber, CL 2000, 'Integrated physiological responses to feeding in the blue crab *Callinectes sapidus*', *Journal of Experimental Biology*, vol. 203, no. 2, p. 359.

Mercer, J, Mai, K-S & Donlon, J 1993, '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 & development*, vol. 23, no. 2-3, pp. 75-88.

Mock, TS, Francis, DS, Drumm, DW, Versace, VL, Glencross, BD, Smullen, RP, Jago, MK & Turchini, GM 2020, 'A systematic review and analysis of long-term growth trials on the effect of diet on omega-3 fatty acid levels in the fillet tissue of post-smolt Atlantic salmon', *Aquaculture*, vol. 516, p. 734643.

Mock, TS, Francis, DS, Jago, MK, Glencross, BD, Smullen, RP, Keast, RSJ & Turchini, GM 2019, '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.

Montaño-Vargas, J, Shimada, A, Vásquez, C & Viana, MaT 2002, 'Methods of measuring feed digestibility in the green abalone (*Haliotis fulgens*)', *Aquaculture*, vol. 213, no. 1, pp. 339-46.

Montano-Vargas, J, Viana, MT, D'Abramo, LR, Shimada, A & Vasquez-Pelaez, C 2005, 'Growth and energy utilization of juvenile pink abalone *Haliotis corrugata* fed diets containing different levels of protein and two starch: lipid ratios', *Journal of Shellfish Research*, vol. 24, no. 4, pp. 1179-85.

Morales, GA & Moyano, FJ 2010, 'Application of an in vitro gastrointestinal model to evaluate nitrogen and phosphorus bioaccessibility and bioavailability in fish feed ingredients', *Aquaculture*, vol. 306, no. 1-4, pp. 244-51.

Morash, AJ & Alter, K 2016, '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. 342-68.

Morikawa, Y & Norman, C 2003, 'Perception of light intensity by Haliotis discus discus based on locomotor activity patterns', *Fisheries Science*, vol. 69, no. 3, pp. 478-86.

Moyano, FJ, Saenz de Rodriganez, MA, Díaz, M & Tacon, AG 2015, 'Application of *in vitro* digestibility methods in aquaculture: constraints and perspectives', *Reviews in Aquaculture*, vol. 7, no. 4, pp. 223-42.

Mulvaney, WJ 2016, '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'.

Mulvaney, WJ, Winberg, PC & Adams, L 2013, 'Comparison of macroalgal (*Ulva* and *Grateloupia* spp.) and formulated terrestrial feed on the growth and condition of juvenile abalone', *Journal of applied phycology*, vol. 25, no. 3, pp. 815-24.

Myers, W, Ludden, P, Nayigihugu, V & Hess, B 2004, 'A procedure for the preparation and quantitative analysis of samples for titanium dioxide', *Journal of Animal Science*, vol. 82, no. 1, pp. 179-83.

Parada, J & Aguilera, J 2007, 'Food microstructure affects the bioavailability of several nutrients', *Journal of Food Science*, vol. 72, no. 2, pp. R21-R32.

Paul, A & Paul, J 1998, 'Respiration rate and thermal tolerances of pinto abalone *Haliotis kamtschatkana*', *Journal of Shellfish Research*, vol. 17, pp. 743-6.

Peck, LS, Culley, MB & Helm, MM 1987, 'A laboratory energy budget for the ormer *Haliotis tuberculata* L', *Journal of Experimental Marine Biology and Ecology*, vol. 106, no. 2, pp. 103-23.

Perera, E, Moyano, FJ, Rodriguez-Viera, L, Cervantes, A, Martínez-Rodríguez, G & Mancera, JM 2010, '*In vitro* digestion of protein sources by crude enzyme extracts of the spiny lobster *Panulirus argus* (Latreille, 1804) hepatopancreas with different trypsin isoenzyme patterns', *Aquaculture*, vol. 310, no. 1-2, pp. 178-85.

Pernet, F, Tremblay, R, Comeau, L & Guderley, H 2007, 'Temperature adaptation in two bivalve species from different thermal habitats: energetics and remodelling of membrane lipids', *Journal of Experimental Biology*, vol. 210, no. 17, pp. 2999-3014.

Rocker, M, Lewis, MJ, Mock, TS, Francis, DS, Bellagamba, F, Moretti, VM, Quinn, GP, Smullen, RP & Turchini, GM 2021, 'Poultry offal meal production conditions impact meal quality and digestibility in Atlantic salmon (*Salmo salar*)', *Aquaculture*, p. 736909.

Roe, JH, Hopkins, WA, Snodgrass, JW & Congdon, JD 2004, 'The influence of circadian rhythms on pre- and post-prandial metabolism in the snake *Lamprophis fuliginosus*', *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, vol. 139, no. 2, pp. 159-68.

Romo, ZM, Re, AD, Díaz, F & Mena, A 2010, 'Physiological responses of pink abalone *Haliotis corrugata* (Gray, 1828) exposed to different combinations of temperature and salinity', *Aquaculture Research*, vol. 41, no. 7, pp. 953-60.

Sales, J & Britz, P 2003, 'Apparent and true availability of amino acids from common feed ingredients for South African abalone (*Haliotis midae* L.)', *Aquaculture Nutrition*, vol. 9, no. 1, pp. 55-64.

Sales, J & Britz, PJ 2001, 'Evaluation of different markers to determine apparent nutrient digestibility coefficients of feed ingredients for South African abalone (*Haliotis midae* L.)', *Aquaculture*, vol. 202, no. 1, pp. 113-29.

Sales, J & Britz, PJ 2002, 'Evaluation of the reference diet substitution method for determination of apparent nutrient digestibility coefficients of feed ingredients for South African abalone (*Haliotis midae* L.)', *Aquaculture*, vol. 207, no. 1, pp. 113-23.

Sales, J & Janssens, G 2004, 'Use of feed ingredients in artificial diets for abalone: a brief update', in *Nutrition Abstracts and Reviews: Series B*, vol. 74, pp. 13N-21N.

Secor, SM 2009, 'Specific dynamic action: a review of the postprandial metabolic response', *Journal of Comparative Physiology B*, vol. 179, no. 1, pp. 1-56.

Shipton, T & Britz, P 2002, 'Evaluation of an in vitro digestibility technique for the prediction of protein digestibility in the South African abalone, *Haliotis midae* L', *Aquaculture Nutrition*, vol. 8, no. 1, pp. 15-21.

Sokolova, IM, Frederich, M, Bagwe, R, Lannig, G & Sukhotin, AA 2012, 'Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates', *Marine Environmental Research*, vol. 79, pp. 1-15.

Steinarsson, A & Imsland, AK 2003, 'Size dependent variation in optimum growth temperature of red abalone (*Haliotis rufescens*)', *Aquaculture*, vol. 224, no. 1-4, pp. 353-62.

Stone, DA, Bansemer, MS, Currie, K-L, Saunders, L & Harris, JO 2016, '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.

Stone, DA, Bansemer, MS & Harris, JO 2014a, *Development of formulated diets for cultured abalone*, Australian Seafood Cooperative Research Centre.

Stone, DA, Bansemer, MS, Lange, B, Schaefer, EN, Howarth, GS & Harris, JO 2014b, 'Dietary intervention improves the survival of cultured greenlip abalone (*Haliotis laevigata* Donovan) at high water temperature', *Aquaculture*, vol. 430, pp. 230-40.

Stone, DA, Harris, JO, Wang, H, Mercer, GJ, Schaefer, EN & Bansemer, MS 2013, 'Dietary protein level and water temperature interactions for greenlip abalone *Haliotis laevigata*', *Journal of Shellfish Research*, vol. 32, no. 1, pp. 119-30.

Tripp-Valdez, MA, Bock, C, Lucassen, M, Lluch-Cota, SE, Sicard, MT, Lannig, G & Pörtner, HO 2017, 'Metabolic response and thermal tolerance of green abalone juveniles (*Haliotis fulgens*: Gastropoda) under acute hypoxia and hypercapnia', *Journal of Experimental Marine Biology and Ecology*, vol. 497, pp. 11-8.

Vandepeer, M 2006, 'Preventing summer mortality of abalone in aquaculture systems by understanding interactions between nutrition and water temperature', *FRDC final report. SARDI Aquatic Sciences, Adelaide*.

Venter, L, Loots, DT, Vosloo, A, Jansen van Rensburg, P & Lindeque, JZ 2018, 'Abalone growth and associated aspects: now from a metabolic perspective', *Reviews in Aquaculture*, vol. 10, no. 2, pp. 451-73.

Vosloo, D & Vosloo, A 2010, 'Response of cold-acclimated, farmed South African abalone (*Haliotis midae*) to short-term and long-term changes in temperature', *Journal of thermal biology*, vol. 35, no. 7, pp. 317-23.