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Does the dietary inclusion of *Ulva* meal improve the survival of abalone during summer on two Australian abalone farms?

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Contents

Tables

Figures

Plates

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Executive Summary

Background

A research priority identified by the Australian Abalone Growers' Association (AAGA) in 2019, prior to the commencement of the project, was to improve the survival of commercially cultured abalone during periods of high summer water temperatures (> 22°C). This research aimed to address this need. This project was developed by SARDI in collaboration with members of the Australian Abalone Growers' Association (AAGA), Aquafeeds Pty Ltd and researchers from Flinders University and The University of Adelaide. The primary aim of the project was focused on evaluating the effects of feeding a diet containing 10% dried *Ulva* sp. meal on improving the survival of Australian Greenlip (*H. laevigata*) and Tiger Abalone (*H. laevigata × H. rubra*) cultured throughout summer to autumn in two separate farm trials. Histopathological alterations of selected tissues of the digestive tract and gills of abalone were also assessed for each trial during this period. The secondary aim of the project evaluated the growth, feed utilisation and production cost of abalone for each trial. This project built on research that reported that dietary intervention, utilising dried *Ulva* sp. meal in a practical commercial formulated feed, reduced mortality in cultured Greenlip Abalone (*Haliotis laevigata*) associated with elevated summer water temperatures (26°C) under laboratory conditions (Lange et al., 2014).

Abalone cultured in southern Australia spend a large period of the summer in water temperatures above their preferred optimum (19-22°C, depending on stock genetics). When water temperatures exceed 22°C, increased health problems and mortality may occur (Vandepeer, 2006; Hooper et al., 2011; Hooper et al., 2014a, b; Lange et al., 2014; Stone et al., 2014a). This mortality is referred to as "summer mortality". Discussions with AAGA members based on historical records indicate that summer mortality events are typically sporadic between commercial culture units within a farm and may range from 15 to 50% each summer. Larger and more valuable 2 to 3-year-old stock is affected, however, in Tasmania small abalone are also affected (Nicholas Savva, Executive Officer, AAGA, Pers. Comm.). Similar size dependent mortality patterns in Greenlip Abalone exposed to heat stress have been reported repeatedly under laboratory conditions (Lange et al., 2014; Stone et al., 2014a; Duong et al., 2016; Bates et al., 2017; Buss et al., 2017; Shiel et al., 2017; Thomson et al., 2018).

Australian abalone cultured in land-based systems are fed formulated diets composed predominantly of terrestrial plant ingredients (Stone et al., 2013; Stone et al, 2014a). In contrast, in their natural environment, larger abalone have a distinct dietary preference for red (e.g., *Gracilaria* spp.) and green (e.g., *Ulva* spp.) macroalgae (Shepherd and Steinberg, 1992; Bansemer et al., 2016a). Macroalgae possess a range of attributes beneficial to the organisms consuming them, including bioactive compounds that exhibit antimicrobial and antioxidant properties, which when consumed, enhance the immune response of the abalone (Dang et al., 2011; Lange et al., 2013; Stone et al., 2014a; Bansemer et al., 2016a). Feeding live *Ulva* sp. to Greenlip Abalone exposed to heat stress (26°C) improved survival in the laboratory (Lange et al., 2014; Stone et al., 2014a). While feeding a commercial diet containing 30% dried *Ulva* sp. meal also improved survival as well as feed intake of Greenlip Abalone exposed to heat stress (26°C) under laboratory conditions (Lange et al., 2014). Feeding Greenlip Abalone diets containing either dried enriched *Ulva* sp. meal or *Ulva* sp. protein extract meal supported comparable growth to that obtained from feeding a commercial abalone diet (Bansemer et al., 2016b, Bates et al., 2017).

Aims/objectives

The project's main objective was to investigate if dietary intervention, using a diet containing 10% dried *Ulva* sp. meal, could be used to improve survival of Greenlip Abalone (*Haliotis laevigata*) and Tiger Abalone (*H. laevigata × H. rubra)* exposed to summer water temperatures on-farm under commercial conditions.

The secondary project objective was to determine if feeding a diet containing 10% dried *Ulva* sp. meal would improve growth performance, feed utilisation and ultimately production cost across a summer to autumn grow-out period in two separate farm trials for Greenlip and Tiger Abalone grown under commercial conditions.

Methods

Two separate on-farm commercial trials were run at Jade Tiger Abalone (Avalon, Victoria) [JTA Avalon] and Yumbah Narrawong (Allestree, Victoria). The trials were both run on a commercial basis using routine farming procedures used by each company. The trials were designed and run to investigate the potential to improve survival and growth by feeding diet containing 10% dried *Ulva* meal sp. compared to a commercial diet within farms. Given the differences in geographical locations, climatic conditions, stock genetics and culture methods employed at each farm, comparison between farm trials was not an objective of the study. The same two diets were used in each trial: Diet 1. Commercial (control) diet (Go2 diet, Aquafeeds Pty Ltd, Mt Barker, SA); and Diet 2. A practical commercial diet formulation containing 10% dried *Ulva* sp. meal ingredient (referred to as the *Ulva* diet, from here on). Within each separate farm trial abalone were fed either the commercial diet or the *Ulva* diet using commercial practices throughout the entire period. The trials conducted over summer commenced in December 2019 and ran for 112 days (d) (; 19/12/19 - 9/4/20) and 166 d (5/12/19 - 19/5/20) at JTA Avalon and Yumbah Narrawong, respectively. Based on discussions with participating AAGA members, investigations of the literature, and the geographical locations of the farms in Victoria, the theoretical temperature threshold level of > 22°C was considered applicable for the specific farm trials in this study to potentially experience summer mortality.

The commercial diet and the *Ulva* diet were formulated to contain ~36% crude protein, ≤4% crude lipid and ~18 MJ kg^{-1} gross energy (as fed) and were manufactured by Aquafeeds Pty Ltd using cooking extrusion technology (~4 mm diameter disc pellets). The *Ulva* diet was formulated by A/Prof David Stone (SARDI) and Mr Joel Scanlon (Aquafeeds Pty Ltd) as a practical commercial diet (for rapid industry adoption if successful) and contained 10% dried *Ulva* sp. meal (replaced plant-based ingredients) and other readily available and commonly used dietary ingredients. The JTA Avalon trial used 14 round concrete tanks (seven replicate round tanks per treatment; 10 m diameter and 0.8 m deep, water depth ~0.4 m) for the trial. The Yumbah Narrawong trial used eight concrete slab tanks (four replicate slab tanks per treatment; 16 m long and 2.5 m wide, water depth 3.0 cm). Survival, growth performance, feed and nutrient utilisation, and histopathology of selected gill and digestive tract tissues were measured during both farm trials. An economic analysis reported as the difference in basic sale revenue of cultured abalone fed each diet from the Yumbah Narrawong farm trial were calculated. The differences in basic sales revenue were calculated based on feed input costs (excluding freight costs) and live biomass yields for abalone grown using the commercial diet versus the *Ulva* diet. Calculations assumed an abalone farm gate value of \$35 kg^{-1} (Nicholas Savva, Executive Officer, AAGA, Pers. Comm.) and a cost premium of \$950 tonne $diet^{-1}$ for the *Ulva* diet compared to the commercial diet. Differences in basic sales revenue were reported on the cost difference between treatments for a slab tank of abalone over the 166 d trial period.

Results/key findings

Abalone in both farm trials experienced mild summers and water temperatures. However, the JTA Avalon trial experienced several instances where water temperatures exceeded 22°C during January and February. The maximum water temperature experienced during the Yumbah Narrawong trial was 22.5°C in early January and remained relatively low compared to those recorded at JTA Avalon. Summer mortality was observed during the farm trial at JTA Avalon, whereas water temperatures were too low during the Yumbah Narrawong trial to induce summer mortality. The dietary inclusion of 10% dried *Ulva* sp. meal did not result in any significant improvement in survival during either farm trial. In fact, survival appeared to be lower during both farm trials when 10% dried *Ulva* sp. meal was included in the diets (JTA Avalon survival: commercial diet survival 96.4% vs. 93.4% for the *Ulva* diet; Yumbah Narrawong survival: commercial diet 98.1% vs 97.5 % for the *Ulva* diet).

The dietary inclusion of 10% dried *Ulva* sp. meal did not appear to lead to any significant alteration in digestive tract or gill structure. Heat stress, in combination with commercial culture stressors, appeared to be a factor associated with histopathological alterations observed in abalone during the study, which were chiefly noted in the gill leaflet tips of Tiger Abalone. Gill leaflet tip damage scores appeared to be useful indicators of damage associated with the combination of culture stressors and heat stress in abalone.

In the Yumbah Narrawong trial, which was carried out during mild summer growing conditions, the dietary inclusion of 10% dried *Ulva* sp. meal improved growth (SGR increased by 9.6% combined with economically irrelevant differences in survival), feed and nutrient utilisation and live biomass yield for a mixed population of Tiger and Greenlip Abalone and compared to the commercial diet ultimately led to a $729 or 8.4% increase in basic sales revenue per slab tank for the 166-d trial period. Unfortunately, due to logistical constraints due to COVID-19, growth performance data were not available from JTA Avalon trial.

Implications

Overall, the dietary inclusion of 10% dried *Ulva* sp. meal did not lead to improvements in survival of Tiger or Greenlip Abalone cultured during summer. Based on the data obtained from the Yumbah Narrawong trial, improvements in growth performance (~9.6% improvement in SGR), feed and nutrient efficiency and basic sales revenue were obtained when abalone were cultured under mild summer growing conditions and fed the *Ulva* diet compared to the commercial control diet.

Data produced by this project support the concept that diets for improved abalone production should not be formulated on a least cost basis, but rather on an ingredient quality, and abalone and economic performance basis. Feeds formulated on this basis may support improved profitability.

There are numerous commercial benefits of producing larger abalone more quickly and cheaply. If the improved growth performance trends observed in the Yumbah Narrawong trial were maintained over an entire production cycle, the duration of a typical production cycle for Tiger or Greenlip Abalone in Australia may be shortened by up to ~10% (~3.5 months from 36 months to 32-33 months) by feeding the *Ulva* diet. This conclusion requires further validation, but the inclusion of 10% dried *Ulva* meal in the diet may enable farmers to harvest abalone sooner, which may reduce the exposure of the larger, more valuable stock to one less summer period. This factor alone would result in substantial improvements in productivity and decreased costs, and when combined with savings made with increased growth rates and feed efficiency, further improvements in productivity across the entire grow-out period for abalone may be achieved by feeding the *Ulva* diet.

The importation of large quantities of dried macroalgae meals into Australia for use in aquafeeds is currently restricted because of biosecurity concerns. Domestically produced macroalgae meals provide feed manufacturers and abalone producers with access to a range of new and improved ingredients which will reduce reliance on imported feed ingredients and enhance feed security on a national level. The improvements in growth and the economics of production observed in this study demonstrate that domestically produced dried macroalgae meal may be a viable alternative to imported macroalgae meals.

Results from the study demonstrate the importance of the inclusion of histopathological examination of the gills to assess abalone health in response to seasonal water temperature changes in future trials. In the current study gills appeared to be more sensitive to histopathological alterations than the digestive tract. For example, Tiger abalone exposed to a combination of commercial culture stressors and higher summer water temperatures during the JTA Avalon trial exhibited increasingly higher leaflet tip damage scores over the course of the trial while relatively small alterations were observed in the digestive tract.

Recommendations

Summer mortality was observed during the farm trial at JTA Avalon, but water temperatures were too low during the Yumbah Narrawong trial to induce summer mortality. We recommend well replicated farm trials that are designed to study summer mortality should be implemented on farms that experience high summer water temperatures. When adequate quantities of suitable quality dried macroalgal meal are available, trials should be run utilising dietary inclusion levels of up to 30% macroalgae.

The increase in basic sales revenue at Yumbah Narrawong was a positive finding in this study. Further research to assess the effects on growth of the inclusion of a range of dried macro algae meals into diets on the productivity for farmed abalone is recommended.

Given the sensitivity of the gills in response to the combined effects of culture stressors and heat stress, as exemplified by alterations to gill leaflet tip structure and function, it is recommended that future

summer mortality studies with abalone are designed to include the examination of histopathological alterations to the gills.

The lack of histopathological alterations observed in the digestive tract of abalone in this study suggested no adverse effects of the dietary inclusion of 10% dried *Ulva* meal. But the study was relatively short-term and histopathological alterations may have become apparent if the study had been run longer.

The sensitivity of the selected histopathological parameters differed between organs and also in response to summer and autumn water temperature fluctuations (gills were more sensitive than the digestive tract). To improve the sensitivity of future histopathological assessments of organs in abalone in response to heat stress it is recommended that the investigation of the application of more holistic approaches of histopathological scoring techniques, such as the gill alteration indices and leaflet prevalence scoring methods are undertaken. This will enable researchers to better serve the industries need to better understand the effects of heat stress on abalone health and survival.

Keywords

Greenlip Abalone, *Haliotis laevigata*; Tiger Abalone, *Haliotis laevigata x H. rubra*; Dietary macroalgae inclusion; Summer mortality; Survival, growth performance; feed utilisation; economic analysis.

Introduction

This project was developed in collaboration with the Australian Abalone Growers' Association (AAGA) and Aquafeed Australia to meet an industry need to improve the survival of commercially cultured Australian Greenlip Abalone (*H. laevigata*) and Tiger Abalone (*H. laevigata × H. rubra*) during periods of high summer water temperatures (> 22°C).

Abalone cultured in Australia spend a large period of the summer in water temperatures above their optimum (~19-22°C, depending on species and stock genetics; Gilroy and Edwards, 1998; Stone et al., 2013). When water temperatures exceed 22°C, increased health problems and mortality occur on abalone farms in Australia (Vandepeer 2006; Hooper et al., 2011; Hooper et al., 2014a, 2014b; Lange et al., 2014; Stone et al., 2014a). In Australia, this mortality is referred to as summer mortality (Vandepeer 2006; Hooper et al., 2011; Hooper et al., 2014a, 2014b; Shiel et al., 2018). Larger (2 to 3-year-old) more valuable stock are primarily affected, however, in Tasmania, younger one-year-old abalone are also affected (Nicholas Savva, Executive Officer, AAGA, Pers. Comm.). Summer mortality events are typically sporadic between commercial culture units and mortality rates may range from 15 to 50% over the summer growing period. Discussions with abalone farm managers participating in the current project indicated that these percentages are an accurate representation of the mortality patterns each summer (Joshua McIntyre, Farm Manager, JTA Avalon, Pers. Comm.; Luke Thorpe, Farm Manager; Yumbah Narrawong, Pers. Comm.). Similar mortality patterns have been demonstrated in Greenlip Abalone exposed to heat stress under laboratory conditions (Lange et al., 2014; Stone et al., 2014a; Duong et al., 2016; Bates et al., 2017; Buss et al., 2017; Shiel et al., 2017; Thomson et al., 2018).

Summer mortality is considered to be caused by a number of factors when abalone are exposed to heat stress above their optimal water temperature range (Vandepeer 2006; Hooper et al., 2011; Hooper et al., 2014a, 2014b; Shiel et al., 2018). Factors that may contribute to summer mortality in abalone include reduced water quality (elevated ammonia, lower dissolved oxygen, and increased bacteria levels) which may impact abalone immune status and overall health (Vandepeer. 2006). Handling stress, maturation and spawning stress may also be involved (Hooper et al., 2011; Cardinaud et al., 2014; Hooper et al., 2014b). Compromised gill epithelia cells due to exposure to handling damage and heat stress may act as portals of entry for bacteria (Hooper et al., 2014a, b). Nutritional supplementation may improve the health status of abalone and reduce the impacts of summer mortality (Lange et al., 2014; Stone et al., 2014a) and is likely to offer the most practical opportunity to reduce this problem for established operators.

In Australia, abalone cultured in land-based systems are currently fed formulated compounded diets composed predominantly of terrestrial plant, and to a lesser extent, marine ingredients (Stone et al., 2013; Stone et al, 2014a). In their natural environment, larger Australian abalone have a distinct dietary preference for red (e.g., *Gracilaria* spp.) and green (e.g., *Ulva* spp.) macroalgae (Shepherd and Steinberg, 1992; Bansemer et al., 2016a). Macroalgae possess a range of attributes that are beneficial to the organisms consuming them. Macroalgae contain a range of bioactive compounds that exhibit antimicrobial and antioxidant properties, that when consumed, may enhance the immune response of the abalone (Dang et al., 2011; Lange et al., 2013; Stone et al., 2014a; Bansemer et al., 2016a). Lange et al., (2014) reported that live *Ulva* sp. and dried enriched *Ulva* sp. meal had ferric-reducing antioxidant potentials of 0.040 and 1.72 µmol Fe^{2+} equivalent g^{-1}, respectively. Feeding live *Ulva* sp. has been reported to improve the survival of Greenlip Abalone exposed to heat stress (> 25°C) in the laboratory (Lange et al., 2014; Stone et al., 2014a). Dietary intervention, using a commercial diet formulated to contain up to 30% dried *Ulva* sp. meal, improved feeding, immune response, and survival of Greenlip Abalone exposed to heat stress (26°C) under laboratory conditions (Lange et al., 2014). Feeding formulated diets containing dried enriched *Ulva* sp. meal (Bansemer et al., 2016b) or *Ulva* sp. algal protein extracts (Bates et al., 2017) supports good growth and feed efficiency in Greenlip Abalone.

Heat stress may affect the structure and function of the digestive tract and gills in abalone and other animals (Hooper et al., 2014a; Vesco et al., 2020; Pedler et al. accepted; Thomson unpublished data). This in turn would impact nutrient uptake, health, growth and ultimately farm productivity. Hooper et al. (2014a) held Tiger Abalone at either 16°C or 26°C for seven days and reported an increase in haemocyte infiltration in the digestive gland at the higher temperature, indicating immuno-suppression and organ damage. Vesco et al. (2020) reported heat stress damaged the structural integrity of the intestinal epithelium of broiler chicken which impacted nutrient uptake and growth. Unfortunately, there is limited published data available on the effects of heat stress on the structural integrity of the intestinal epithelium of abalone. The thickness of the epithelial layer of the stomach and intestine of commercially cultured Tiger Abalone were reduced when cultured at moderate summer water temperatures (max 18.5°C) compared to winter water temperatures (min 10.5°C) (Thomson unpublished data). In the laboratory, the thickness of the epithelial layer of the stomach of juvenile and sub-adult Greenlip Abalone fed a formulated diet was also reduced when cultured for three months at 22°C compared to 14°C (Schafer et al. 2013). The two aforementioned examples suggest the potential for even more extreme alterations to the epithelial layer of the stomach and intestine when abalone are exposed to heat stress during summer.

The gills are important organs for the uptake of oxygen from seawater by abalone. They are also involved in transport of haemolymph and the exchange of other gases and substances vital for metabolism (Bevelander, 1988; Taylor and Ragg, 2005). The gills are sensitive to heat stress, particularly the leaflet structure (Hooper et al., 2014a; Pedler et al., accepted). A range of histopathological alterations to the structure of the gill leaflets due to heat stress have been observed in abalone including increased damage in the form of lifting of the epithelial layer (Pedler et al., accepted; Thomson unpublished data), epithelial cell loss (Hooper, et al. 2014a) and hemolymph channel enlargement (Pedler et al., accepted). An increase in gill damage will result in a reduction in gill function and growth, and also renders abalone more susceptible to risks of secondary infection from pathogens, including *Vibrio* spp. (Hooper et al., 2014a). All factors combined would also negatively impact on farm productivity.

Changes to diet formulations may also alter the structure and function of the digestive tract of abalone leading to potential impacts on health and growth. There is a lack of published information on this topic in relation to abalone. Increasing the dietary protein level in a formulated diet from 24% to 33% led to a decrease in crop epithelial layer thickness with no negative impact on growth in sub-adult Greenlip Abalone cultured at 14°C and 22°C (Schaefer et al. 2013). While there is a lack of published information on the effects of dietary ingredients on the structure and function of the digestive tract in abalone, published information available for marine vertebrate species has shown that altering diets can lead to negative morphological alterations to the digestive tract that may impact on an animal's health and growth. For example, replacing 20% of the dietary protein with soybean meal resulted in soybean meal induced enteritis in Common Carp and reduced growth (*Cyprinus carpio L*.) (Uran et al., 2008). While the addition of 30% dried *Ulva* sp. meal improved the survival of Greenlip Abalone when exposed to 26°C (Lange et al., 2014) there is a need to better understand the potential histopathological effects, whether negative or positive, of the inclusion of this dietary ingredient on the structure and function of the digestive tract and gills of abalone, particularly during period of heat stress.

Objectives

The primary objective of this project was to investigate if dietary intervention, using a diet containing 10% dried *Ulva* sp. meal, could be used to improve survival of Greenlip Abalone (*Haliotis laevigata*) and Tiger Abalone (*H. laevigata × H. rubra)* exposed to summer water temperatures on-farm under commercial conditions in two separate farm trials.

The secondary objective of the project was to determine if feeding a diet containing 10% dried *Ulva* sp. meal would improve growth performance, feed utilisation and ultimately production cost across a summer to autumn grow-out period in two separate farm trials for Greenlip and Tiger Abalone grown under commercial conditions.

Methods

2.1 Experimental design and treatments

Two separate trials, one at each farm, were run in order to increase the chance of experiencing high summer water temperatures on at least one commercial farm. Given the differences in geographical locations, climatic conditions, stock genetics and culture methods employed at each farm, comparison between farm trials was not an objective of the study. The two trials were run to evaluate the on-farm survival, health, growth and the basic economic benefits of feeding a practical diet (formulated using common commercial dietary ingredients for immediate commercial adoption) containing 10% dried *Ulva* sp. meal (which will be referred to as *Ulva* diet for the remainder of report) compared to a commercial diet, to both Greenlip Abalone (*Haliotis laevigata*) and Tiger Abalone (*H. laevigata × H. rubra*) to ameliorate mortality during the summer production cycle. The trials were run on a commercial basis using routine farming procedures used by each company.

The two abalone companies that participated in the project were:

1. Jade Tiger Abalone Avalon (JTA Avalon; Avalon, VIC); and
2. Yumbah Narrawong (Portland, VIC).

Based on discussions with staff at the participating AAGA farms, investigations of the literature (Vandepeer, 2006; Hooper et al., 2011; Hooper et al., 2014a, 2014b; Lange et al., 2014; Stone et al., 2014a), and the geographical locations of the farms in Victoria, the theoretical temperature threshold level of > 22°C was considered applicable to potentially induce abalone deaths related summer mortality for the farm trials reported in this study.

Additionally, please note, as the main objective of this project concentrated on survival and health, the growth and feed utilisation data used in this report has been derived from data provided by each farm and was not the primary focus of the report.

2.2 *Ulva* sp. meal ingredient, diet formulation and production

The dried *Ulva* sp. meal ingredient was commercially grown in Queensland and sourced by Aquafeeds Pty Ltd, and its biochemical composition is displayed in Table 1. Two diets were tested at two farms in this study. The commercial diet (Go2 diet, Aquafeeds Pty Ltd, Mt Barker, SA) and the *Ulva* diet were formulated to contain ~36% crude protein, ≤4% crude lipid and ~18 MJ kg^{-1} gross energy as fed. The biochemical composition of the diets is provided in Table 1. Diets were manufactured by Aquafeeds Pty Ltd using cooking extrusion technology (~4 mm diameter disc pellets). The *Ulva* diet was formulated by A/Prof David Stone (SARDI) and Mr Joel Scanlon (Aquafeeds Pty Ltd) to contain 10% dried *Ulva* sp. meal (replaced plant-based ingredients). As agreed with AAGA during the development of the project, apart from the algal inclusion content, dried *Ulva* sp. meal nutrient composition and diet proximate nutrient compositions, the ingredient formulations and the remaining nutrient composition of the commercial diet and the *Ulva* diet remain confidential to A/Prof David Stone and Mr Joel Scanlon. The water stability of both diets was assessed by Aquafeeds Pty Ltd, using in-house methods, and the stability of the *Ulva* diet was determined to be equivalent to, or slightly higher, compared to that of the commercial diet following 24 h of immersion at room temperature (~25°C; Joel Scanlon, Owner Manager, Aquafeeds Pty Ltd).

Table 1. The analysed biochemical composition of the dried *Ulva* sp. meal ingredient, commercial diet and *Ulva* diet used in the on-farm trials.

Item (as fed)	Dried *Ulva* sp. meal ingredient	Commercial diet[3]	*Ulva* diet[3]
Moisture (g 100 g^{-1})	10.84	5.29	3.94
Crude protein (g 100 g^{-1})	30.99	35.37	37.04
Lipid (g 100 g^{-1})	1.04	3.75	2.76
Ash (g 100 g^{-1})	30.56	6.71	9.20
Nitrogen free extract (g 100 g^{-1})[1]	26.57	48.88	47.06
Gross energy (MJ kg^{-1})[2]	12.22	18.32	18.09
Amino acids (g 100 g^{-1})			
Alanine	1.33	-	-
Arginine	2.64	-	-
Aspartic Acid	3.57	-	-
Glutamic Acid	4.74	-	-
Glycine	0.76	-	-
Histidine	2.43	-	-
Isoleucine	1.13	-	-
Leucine	2.36	-	-
Lysine	1.97	-	-
Methionine	0.45	-	-
Phenylalanine	0.83	-	-
Proline	2.19	-	-
Serine	0.42	-	-
Threonine	1.68	-	-
Tyrosine	0.76	-	-
Valine	1.28	-	-
Fatty acids (% total FAME)[4]			
Saturated fatty acids			
C12:0 Lauric	0.15	-	-
C14:0 Myristic	2.62	-	-
C15:0 Pentadecanoic	0.37	-	-
C16:0 Palmitic	19.84	-	-
C17:0 Margaric	0.34	-	-
C18:0 Stearic	4.65	-	-
C22:0 Behenic	0.47	-	-
Total saturated	*28.44*	-	-
Mono-unsaturated fatty acids			
C16:1 Palmitoleic	6.48	-	-
C18:1 Oleic	15.09	-	-
C20:1 Eicosenic	5.28	-	-
C22:1 Cetoleic	0.79	-	-
Total mono-unsaturated	*27.64*	-	-
Polyunsaturated fatty acids			
C18:2n6 Linoleic	32.72	-	-
C18:3n3 alpha-Linolenic	3.95	-	-
C20:5n3 Eicosapentaenoic	2.56	-	-
C22:5n3 Docosapentaenoic	0.34	-	-
C22:6n3 Docosahexaenoic	4.36	-	-
Total Poly-unsaturated	*43.93*	-	-
Omega 6 Fatty Acids	32.72	-	-
Omega 3 Fatty Acids	11.21	-	-
Total LC n-3 PUFA[5]	7.26	-	-
Total FAME (g 100 g^{-1})	*1.11*	-	-

[1] Nitrogen free extract (%) = 100% - [moisture (%) + protein (%) + lipid (%) + ash (%)].

[2] Dietary gross energy content (MJ kg^{-1}) calculated using dietary values (g kg^{-1}) for crude protein, lipid and nitrogen free extract multiplied by the following gross energy values for protein, 23.6 MJ kg^{-1}; lipid, 39.5 MJ kg^{-1}; and carbohydrate, 17.2 MJ kg^{-1} (NRC, 1993).

[3] Amino acid and fatty acid data for diets not provided, as agreed in the project development phase with AAGA.

[4] FAME = fatty acid methyl ester.

[5] Total LC n-3 PUFA = Sum of long chain omega 3 fatty acids = C20:5n3, Eicosapentaenoic + C22:5n3, Docosapentaenoic + C22:6n3, Docosahexaenoic

2.3 On-farm culture systems

The culture systems at each farm used for each trial represented typical culture systems and conditions utilised in the commercial production of abalone in Australia.

JTA Avalon committed 14 round concrete tanks to the trial (seven replicate round tanks per dietary treatment; Plate 1). The tanks were 10 m diameter and 0.8 m deep, with a working water depth of ~0.4 m. Tanks had a central drain-pipe. Tanks were supplied with ambient temperature seawater at a flow rate of 220 L min^{-1} throughout the trial. Tanks were cleaned regularly by draining and hosing to remove excess seaweed build up and waste feed and faecal material.

Plate 1. Ten metre diameter culture tanks used during the Jade Tiger Abalone Avalon farm trial.

Yumbah Narrawong committed eight concrete slab tanks to the trial (four replicate slab tanks per dietary treatment; Plate 2). The slab tanks were housed under a shade cloth roof and were 16 m long and 2.5 m wide, with a laminar flow water depth of 3.0 cm. Tanks were supplied with ambient temperature seawater at a flow rate of 170 L min^{-1}. Tanks were cleaned regularly using tipper flushers.

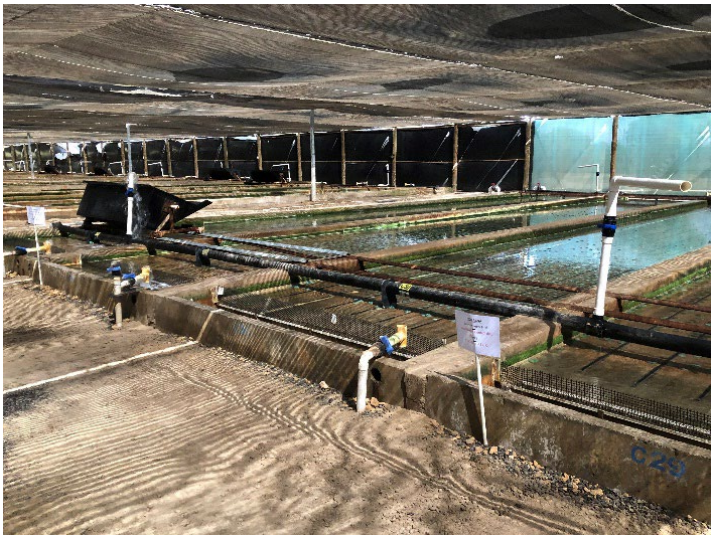


Plate 2. Slab culture tanks used during the Yumbah Narrawong farm trial.

2.4 History of abalone prior to the commencement of the on-farm trials

Prior to the commencement of each trial, abalone used at each farm were cultured using normal commercial methods. JTA Avalon used Tiger Abalone bred at their Indented Head site and held in the same outdoor tanks under the same conditions as used in the trial. Yumbah Narrawong used Greenlip Abalone and Tiger Abalone bred in-house and cultured in the same slab tanks under the same conditions as used in the trial. Prior to the commencement of both trials, abalone were fed a Skretting Halo diet (4 mm).

2.5 Stocking, running, and sampling of the on-farm trials

The on-farm trials were planned to start in November/December 2019, run for approximately four months and cover one summer growing period. Overall, the trials ran for 112 d (~3.7 months; 19/12/19 - 9/4/20) and 166 d (5.5 months; 5/12/19 – 19/5/20) at JTA Avalon and Yumbah Narrawong, respectively.

JTA Avalon

At JTA Avalon, the trial was stocked with Tiger Abalone (initial weight [mean ± stdev] 39.8 ± 5.46 g) at an average of 594 kg abalone per tank (~14,420 abalone tank^{-1}) with tansk randomly allocated to the two treatments on day 0. Abalone were fed daily, to slight excess, using normal farm practices with the Skretting Halo diet (Skretting Australia, Cambridge, Tasmania) from initial stocking until day 11 (30/12/19). Then daily feeding of the test diets using normal farm practices commenced on day 12 (31/12/19) and was concluded on day 112 (9/4/2020; total duration of feeding test diets was 101 d). Feed input and mortalities were recorded daily. Water temperature was measured manually at the inflow pipe of a tank located within the centre of the array of experimental tanks twice daily (between 7:00 and 8:00 am and between 3:00 and 4:00 pm) using an OxyGuard Handy Polaris 2 portable dissolved oxygen meter (OxyGuard International A/S, Farum, Denmark). The daily maximum air temperature (derived from daily air temperatures reported by the Bureau of Meteorology, Avalon airport, located adjacent to the JTA Avalon farm site) was also reported. At the conclusion of the trial, all tanks for each dietary treatment were randomly subsampled (3 abalone tank^{-1}) for histopathological evaluation and weighed ($\pm$1.00 g) and shell length measured ($\pm$1.0 mm). At this point JTA Avalon chose to on-grow abalone from this trial which resulted in each tank being partially harvested on different dates up to 18/8/20. Therefore, bulk performance data for growth was not available for this trial. However, data provided by JTA Avalon (initial weight, tank biomass and feed offered to each tank during the trial; n = 7 per treatment) and final weights and length data based on subsamples of 3 abalone per tank^{-1} from all tanks for each dietary treatment at the completion of survival monitoring trial, have been summarised in Table 2.

Over the course of the trial at JTA Avalon, Tiger Abalone were subsampled four times (initial stocking, day 11 (19/12/19); day 34 (22/1/20); day 69 (26/2/20); and harvest on day 112 (9/4/20) to assess proximate whole soft tissue composition and gill and digestive tract health (histology). At each subsampling event, three (n = 3) Tiger Abalone from each tank were randomly collected (21 abalone per dietary treatment) for histopathological evaluation; abalone were fixed whole in 10% formalin solution in seawater at room temperature until further processing of gill and digestive tract tissues took place at the University of Adelaide, School of Animal and Veterinary Sciences (Roseworthy, SA; refer to sections 2.7 -2.9 for histopathological processing and analysis details). A further three abalone per tank were randomly collected at each subsampling event and stored frozen for planned proximate composition analysis. Please note, due to COVID-19 disruptions it was not possible to transport the samples for proximate analysis back to SARDI West Beach. The samples remain frozen at JTA Avalon, therefore, proximate composition of abalone for this farm trial will not be reported in this report.

Yumbah Narrawong

At Yumbah Narrawong, the slab tanks in the trial (four tanks treatment^{-1}) were stocked and randomly allocated to treatments from day 0 (5/12/10) to day 4 (9/12/19). Each slab tank for each treatment was stocked with a 50:50 mix (~200 kg of each species) of Tiger Abalone (initial weight [mean ± stdev] 51.4 ± 5.07 g) and Greenlip Abalone (initial weight [mean ± stdev] 31.7 ± 2.37 g). This resulted in initial tank mean biomasses of 402.3 ± 1.83 kg tank^{-1} (n = ~10,268 abalone tank^{-1}) and 403.1 ± 3.28 kg tank^{-1} (~10, 279 total abalone tank^{-1}) for the commercial diet and *Ulva* diet treatments, respectively.

At Yumbah Narrawong, abalone were fed either the commercial or the *Ulva* diets daily, to slight excess, using normal farm practices from initial stocking to the split harvests. The split harvests resulted in two tanks from each treatment being harvested on day 141 (24/4/20) and day 166 (19/5/20). The total duration of feeding test diets was 166 d. At harvest, all abalone from each tank for each dietary treatment were bulk weighed for tank biomass. Feed input was measured for the 166 d duration while mortalities were recorded daily from stocking until the day 118 (1/4/20). At this point no further mortalities were recorded as it was deemed by Yumbah Narrawong farm management (Luke Thorpe, Farm Manager; Yumbah Narrawong, Pers. Comm.) and A/Prof David Stone that the water temperature challenge component of the experiment had finished. Additionally, during the recording of mortalities, farm staff did not discriminate between the number Tiger Abalone and Greenlip Abalone deaths, therefore, the mortality data reported in this report for this farm trial is based on the total treatment mortality over a 118 d period. Water temperature was measured throughout the trial in the inflow sump of a tank located within the array of experimental tanks using an automatic data logger.

Over the course of the trial at Yumbah Narrawong, abalone in all tanks were subsampled and weight checked by farm staff on a regular basis (days 0, 36 [10/1/20], 67 [10/2/20], 103 [17/3/20]) and final harvest (day 139; 22/4/20). Tiger Abalone and Greenlip Abalone were also subsampled 3 times throughout the trial (days 49 [23/1/20], 84 [27/2/20] and final harvest on day 139 [22/4/20]) to gill and digestive tract health (histology). At each subsampling event three Tiger Abalone and three Greenlip Abalone from each tank were randomly collected (12 of each type of abalone per dietary treatment) for histopathological evaluation; abalone were fixed whole in 10% formalin solution in seawater at room temperature until further processing and mounting of gill and digestive tract tissues took place at the University of Adelaide, School of Animal and Veterinary Sciences (Roseworthy, SA; refer to sections 2.7 -2.9 for histopathological processing and analysis details).

2.6 Growth performance and feed utilisation variables measured

All calculations using abalone weight and feed weights were based on wet values:

- Apparent biomass gain (kg tank^{-1}) = (final live bulk weight of abalone harvested from tank) - (initial live bulk weight of abalone stocked into tank)
- Specific growth rate (SGR, % d^{-1}) = (ln final weight - ln initial weight)/ time (d) × 100
- Apparent feed conversion ratio (FCR) = feed consumed / biomass gain
- Apparent protein efficiency ratio (PER) = abalone weight gain / protein consumed
- Condition factor = 5575 × (weight [g] / length [mm]$^{2.99}$) (Britz and Hecht, 1997)

A basic economic analysis, reported in terms of differences in basic sale revenue, of the cost of production of abalone from the Yumbah Narrawong on-farm trial was calculated. The basic sales revenue was based on the differences in feed input costs (excluding freight costs) and live biomass yields for abalone grown using the commercial diet versus the *Ulva* diet (Stone et al., 2016).

The analysis assumed an abalone farm gate value of \$35 kg^{-1} (AAGA, Pers. Comm.), a cost premium of ~\$950 $tonne^{-1}$ for the *Ulva* diet and was reported on a slab tank per experiment (166 d) basis. All other production costs were assumed to be equal for tanks from each treatment. Please note, due to insufficient data pertaining to final biomass, the basic economic analysis was not done for JTA Avalon trial.

2.7 Sample collection for histopathological evaluation of digestive tract and gill tissues

As previously mentioned, samples of gill and digestive tract tissue were collected from Tiger Abalone from JTA Avalon on four occasions (days 0 (initial), 11, 34, 69 and 112) for histopathological evaluation. At the initial sampling event, three abalone were randomly collected from each of seven tanks (n = 21 abalone). On each occasion three abalone were randomly collected from 14 tanks, seven tanks per dietary treatment (commercial diet and *Ulva* diet) providing 42 Tiger abalone in total for each individual sampling date. In total 147 Tiger abalone were collected from JTA Avalon for histopathological analysis.

For the Yumbah Narrawong trial samples of gill and digestive tract tissue were collected for histopathological evaluation from both Greenlip Abalone and Tiger Abalone on three occasions (Day 49, 84 and 139). Please note due to project contract delays, initial gill and digestive tract tissue samples were not collected at the commencement of this trial in December. On each sampling occasion six abalone (three Greenlip Abalone and three Tiger Abalone) were randomly collected from four tanks per dietary treatment (commercial diet and *Ulva* diet), providing 48 abalone in total for each sampling event. In total 144 abalone (72 Greenlip Abalone and 72 Tiger Abalone) were collected from Yumbah Narrawong for histopathological analysis.

2.8 Histological processing, staining, and imaging of digestive tract and gill tissues

All samples of gill and digestive tract tissue collected from JTA Avalon and Yumbah Narrawong were processed using the same methods. Please note, only tissue samples from live abalone were assessed during this project; therefore, results may represent characteristics either associated with survival or progression towards mortality. Abalone were fixed whole in 10% seawater buffered formalin. Three regions of tissue were collected from each individual abalone (Figure 1).

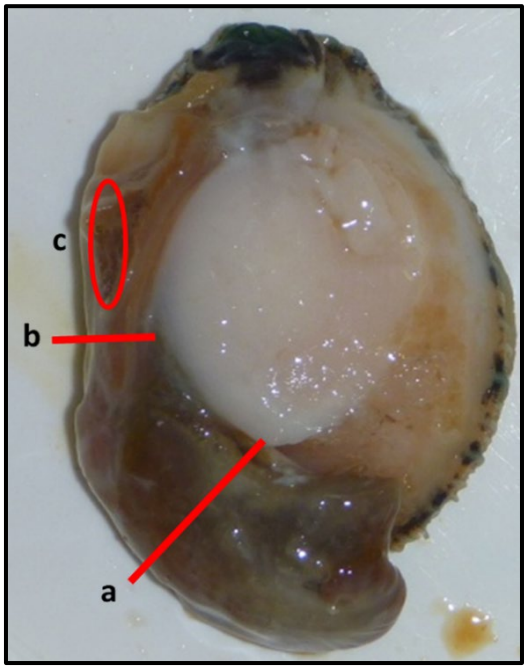
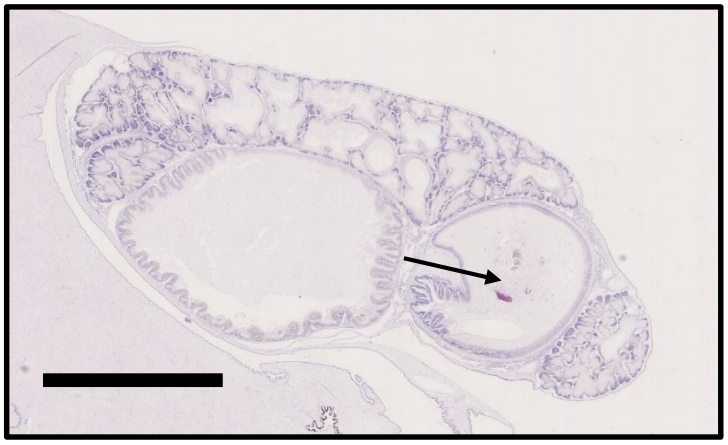


Figure 1. Location of digestive tract and gill tissue samples collected from Tiger Abalone (*H. laevigata* × *H. rubra*) at Jade Tiger Abalone Avalon and Greenlip Abalone (*H. laevigata*) and Tiger Abalone at Yumbah Narrawong to obtain the stomach (a), intestine (b) and left gill (c) sections.

In brief, incisions were made to obtain a cross-section containing the stomach, a second cross-section containing the intestines and separately, a portion of the left gill resulting in three tissues collected per abalone (Figure 2). Tissue samples were placed in separate histology cassettes resulting in three samples per animal. Cassettes were stored in 70% ethanol solution at room temperature. Fixed tissue samples were dehydrated through an ethanol series to histolene, embedded in paraffin wax and sectioned at 5 µm using a Leica RM 2235 rotary microtome (Leica Microsystems GmbH, Wetzlar, Germany). Sections were then floated onto Objekttra ger microscope slides (90-degree ground edges, twin frosted, ProSciTech Pty Ltd, Kirwan, QLD, Australia.) and dried before staining.

Sections containing stomach, intestine and gill samples were stained using Haematoxylin and Eosin (H&E) to show morphology of tissues and the presence of phagocytes (Figure 2A & C). Intestine sections were stained with Periodic acid Schiff and Alcian blue pH 2.5 (PAS/AB) stain to differentiate mucous cells that produce acidic (blue) or neutral (pink) mucins (Figure 2B). Whole slides were imaged using a Hamamatsu Nanozoomer 2.0HT and individual images captured using NDP.view2 (Nanozoomer Digital Pathology software, Hamamatsu Photonics K.K., Iwata City, Japan). Images were analysed, and measurements obtained using a mixture of NDP.view2 and Video Pro 32 (Colour Image Analysis System, version 6.210, Leading Edge Pty Ltd, Adelaide, Australia).



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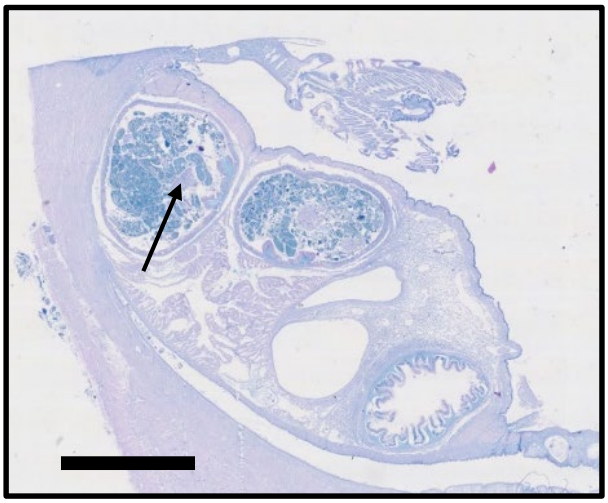

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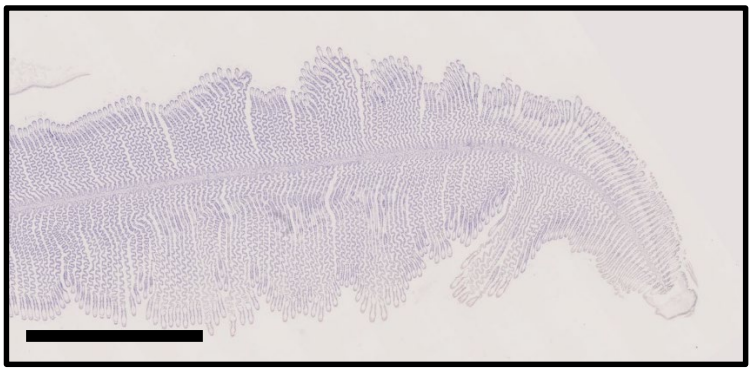


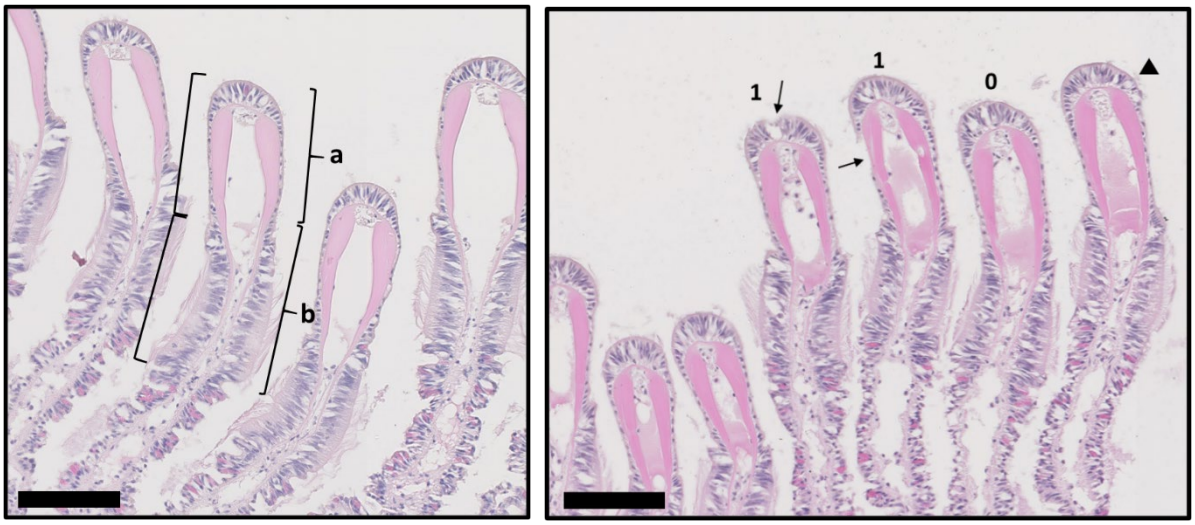
2C)

Figure 2. Photomicrographs of digestive tract and gill tissues collected from Greenlip Abalone (H. laevigata) and Tiger Abalone (H. laevigata × H. rubra). 2A is a cross section of the stomach stained with hematoxylin and eosin. Scale bar = 2.5 mm, magnification x 12.5. 2B is a cross section of the intestine stained with Periodic Acid Schiff/Alcian Blue 2.5. Scale bar = 2.5 mm, magnification x 12.5. 2C is a section of the left gill stained with hematoxylin and eosin. Scale bar = 2.5 mm, magnification x 12.5. Arrows (→) indicate target tissue sites (stomach [A] and intestine [B]) used for the measurement of histopathological parameters.

2.9 Histopathological measurements of digestive tract and gill tissues

Histopathological analysis of the gill and digestive tract utilised the same measurements for samples from JTA Avalon and Yumbah Narrawong. Two regions of the digestive tract were measured in each abalone: the stomach and intestine. Epithelial thickness measurements were taken for both the stomach and intestine over a 1000 µm section of the epithelium. The measured section was selected in the same location of the cross-section for each abalone to reduce bias. This section was measured at ~50 µm intervals from the basal lamina to the apical membrane of the epithelial cell resulting in 20 measurements. The mean of these 20 measurements was taken as the value for that individual abalone. The number of mucous cells was recorded in the same 1000 µm section of the intestine that was used to calculate epithelial thickness. Means for each individual tank were calculated and used as the replicate unit for data analysis.

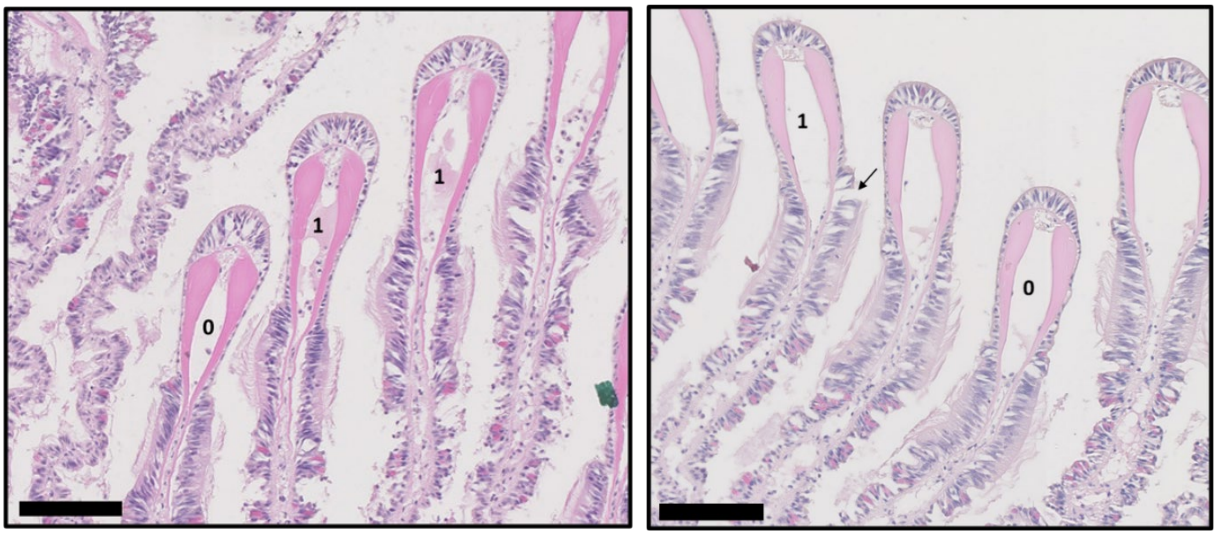
There were two sections of gill leaflets analysed in each abalone: (1) the leaflet tip; and (2) the columnar section, an area of columnar epithelial cells located below the leaflet tip (Figure 3A). For each animal 30 gill leaflets were analysed. The number of goblet cells in the leaflet tip was recorded (Figure 3), the mean of these 30 measurements was taken as the value for that individual abalone. Gill leaflet tips were scored for the absence (0) or presence (1) of damage in the form of an absence of epithelial cells (Figure 3B).



3A 3B)

Figure 2. Examples of gill sections stained with hematoxylin and eosin used for histopathological measurements of goblet cells and epithelial damage for Greenlip Abalone (*H. laevigata*) and Tiger Abalone (*H. laevigata × H. rubra*). 3A) Brackets indicate the two leaflet sections used for measurements of the leaflet tip (a) and columnar epithelial cells (b). Scale bar = 100 µm, magnification X 200. 3B) Gill leaflets showing goblet cells (arrowhead ▲) and leaflet tip damage scores of 0 (no damage) and 1 (damage), arrows (→) indicate damage location. Scale bars = 100 µm.

Gill leaflet tips were also scored for the presence (1) or absence (0) of fluid in the gill sinus (Figure 4A). The columnar section below the leaflet tip was scored for the presence (1) or absence (0) of damage in the form of absent epithelial cells and separation of epithelial cells from the basement membrane (Figure 4B).



4A) 4B)

Figure 3. Photomicrographs of gill leaflet tissues stained with hematoxylin and eosin showing the location of histopathological measurements for sinus fluid and columnar damage scoring for Greenlip Abalone (*H. laevigata*) and Tiger Abalone (*H. laevigata × H. rubra*). 4A) Gill leaflets showing examples of the scoring for the presence (1) or absence (0) of fluid in the sinuses. Scale bar = 100 µm, magnification X 200. 4B) Gill leaflets showing examples of the scoring of damage to the columnar epithelial cells of the gill leaflet with scores of 0 (no damage) and 1 (damage), arrow (→) indicates damage location. Scale bars = 100 µm, magnification X 200.

2.10 Statistical analysis

Statistical analyses were undertaken using SPSS for Windows (Version 26, IBM Corp., Armonk, NY, USA). Data from each farm was analysed separately. Data were assessed for homogeneity of variance and normality using the Levene's test for equality of variance and Shapiro-Wilk test, respectively. Survival was analysed and compared using Kaplan-Meier, Log- Rank test and Cox proportional hazards regression analysis. Tukey's outlier test was used to assess the high variability of survival data for each treatment from the JTA Avalon trial. One-factor ANOVA was used to assess differences in growth performance and feed utilisation at the completion of the Yumbah trial. Histopathological data was assessed dependent on data type and trial location. For Tiger Abalone from JTA Avalon the Dunnett's 2-tailed test was used to assess differences in stomach and intestine epithelial thickness, mucous cells, and gill leaflet goblet cell numbers between initial abalone from the commencement of the trial (19/12/2019; day 11) and abalone fed the commercial diet, or the *Ulva* diet in January (22/01/2020; day 34), February (26/02/2020; day 69) and April (09/04/2020; day 112). Generalised linear mixed model (GLMM) was used to assess significant differences in histopathological scores (leaflet tip damage, sinus fluid, and columnar section damage) between initial abalone and abalone from other treatment groups (time x dietary treatment) at JTA Avalon, with pairwise comparisons used between each treatment group for all significant effects. Additionally, Pearson's correlation test was used to assess correlations between stomach and intestine epithelial thickness in relation to whole abalone weight or shell length. The histopathological data for each species (Greenlip Abalone, Tiger Abalone) from the Yumbah Narrawong trial was analysed separately (i.e., no statistical comparison was made between species). Two-factor ANOVA was used to assess the main effects of dietary treatment (commercial diet, *Ulva* diet), and time (January, 23/01/2020, day 49; February, 27/02/2020, day 84; April, 22/04/2020, day 139) on stomach and intestine epithelial thickness, mucous cells, and gill leaflet goblet cell numbers. Student Newman-Keuls (SNK) test was used to identify significant differences between treatment means. GLMM was used to assess the overall effect of dietary treatment and time

histopathological scoring, with pairwise comparisons used between each treatment group for all significant effects. Pearson's correlation test was also used to assess correlations between stomach and intestine epithelium thickness in relation to whole abalone weight or shell length. A significance level of $P < 0.05$ was used for all statistical tests. All values are presented as means ± standard error of the mean (SE), unless otherwise stated.

Results

3.1 Water and air temperature

Water and air temperature: Jade Tiger Abalone Avalon

The maximum AM and PM water temperature profiles and air temperature profile for JTA Avalon over the course of the trial are presented in Figure 5. PM water temperatures were generally higher than AM water temperatures, and peaks in AM and PM water temperatures usually followed peaks in air temperatures (Figure 5). During the trial, the highest water and air temperatures were recorded in January ([PM water temperatures {°C}: mean, 22.5; range, 19.5 - 25.5] [Air temperatures {°C}: mean, 26.9; range, 17.9 - 43.4]). Water and air temperatures remained relatively high throughout February ([PM water temperatures {°C}: mean, 21.7; range, 19.8 - 24.5] [Air temperatures {°C}: mean, 22.6; range 18.2 - 30.1]), and declined, thereafter, during March and April (Figure 5). The high water temperatures (> 22°C) observed during January and February, especially during the PM time, (Figure 5) would have been sufficient to potentially induce summer mortality, albeit at a lower level compared to higher waters temperatures experienced during more extreme summers typically witnessed at this farm site (Joshua McIntyre, Farm Manager, JTA Avalon, Pers. Comm.).

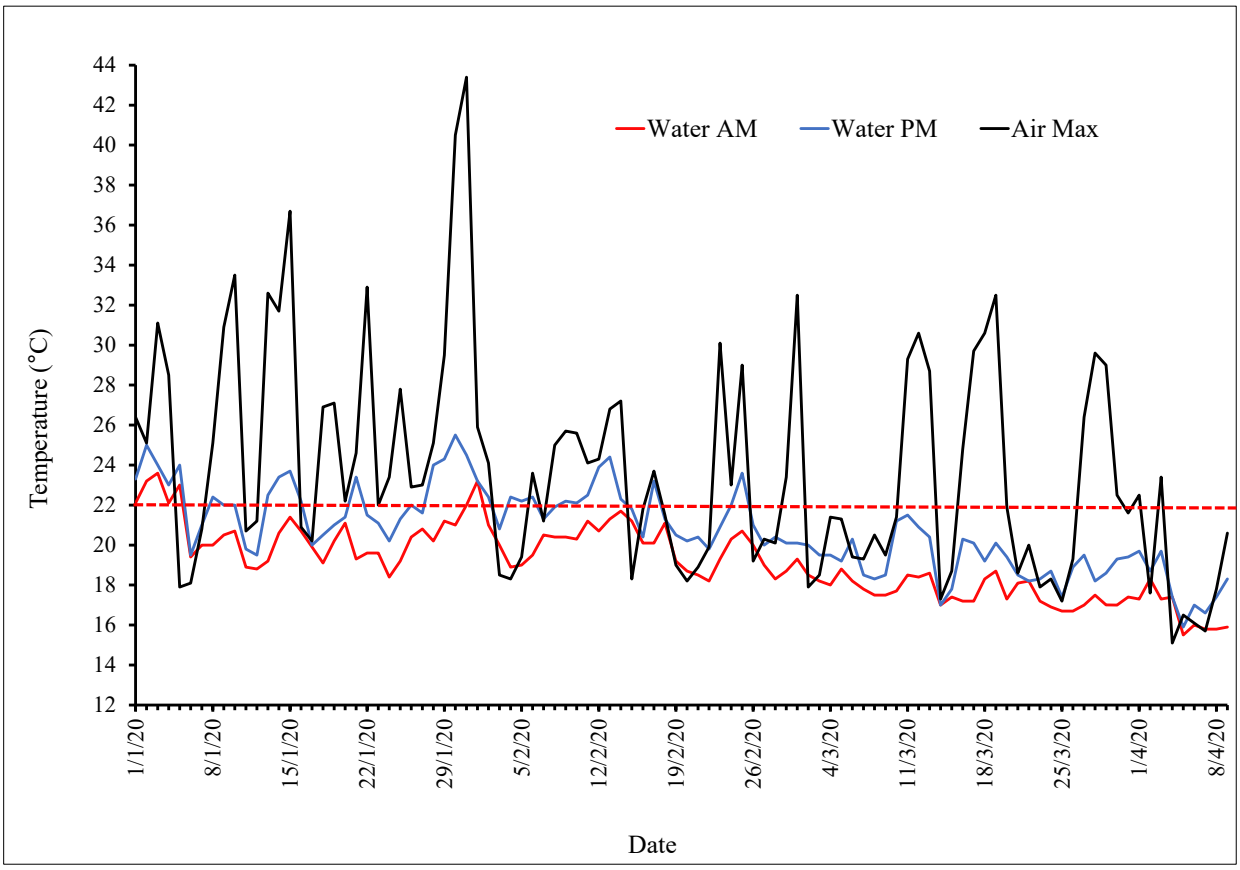


Figure 4. Maximum water temperatures and air temperature (maximum daily temperature) profiles (up to the 9/4/20) of experimental tanks during the Jade Tiger Abalone Avalon farm trial. Red dashed line indicates the theoretical critical temperature threshold level of > 22°C. AM water temperatures were recorded between 7:00 and 8:00 am and PM water temperatures were recorded between 3:00 and 4:00 pm daily. Daily maximum air temperatures were from the Bureau of Meteorology, Avalon airport, located adjacent to the JTA Avalon farm site.

Tiger Abalone at JTA Avalon experienced numerous peaks in water temperatures that exceeded 22°C throughout January and February during the trial (Figure 5). There were five periods during January 2020 where PM water temperatures reached or remained above 22°C in the culture tanks. The first period

occurred from the 1/1/20 to the 5/1/20 (PM water temperatures peaked at 25°C on the 2/1/20). The second period occurred between the 8/1/20 to 10/1/20 (PM water temperatures peaked at 22.5°C on the 8/1/20). The third period was observed from the 14/1/20 to 16/1/20 (PM water temperatures peaked at 23.7°C on the 15/1/20). The fourth period occurred on the 21/1/20 (PM water temperatures peaked at 23.4°C). While the fifth period was observed from the 27/1/20 to the 1/2/20 (PM water temperatures peaked at 25.5°C on the 30/1/20) (Figure 5).

PM water temperatures in culture tanks at JTA Avalon also exceeded 22°C on five more occasions in February 2020 (Figure 5). The first period occurred from the 1/2/20 to the 2/2/20 (PM water temperatures peaked at 23.2°C on the 1/2/20). The second period occurred between 4/2/20 to the 6/2/20 (PM water temperatures peaked at 22.4°C on the 4/2/20 and again on the 6/2/20). The third period occurred between 8/2/20 to the 14/2/20 (PM water temperatures peaked at 24.4°C on the 13/2/20). The fourth period occurred on the 17/2/20 (PM water temperatures peaked at 23.7°C). The fifth period was observed between the 24/2/20 to the 25/2/20 (PM water temperatures peaked at 23.6°C on the 25/2/20).

Water temperature: Yumbah Narrawong

Greenlip and Tiger Abalone at Yumbah Narrawong experienced mild water temperatures throughout the trial that ranged from 22.5°C on the 2/1/20 to 13°C on 5/5/2020 (Figure 6). Water temperatures exceeded 22°C on one day (22.5°C; 2/1/20) during the trial (Figure 6).

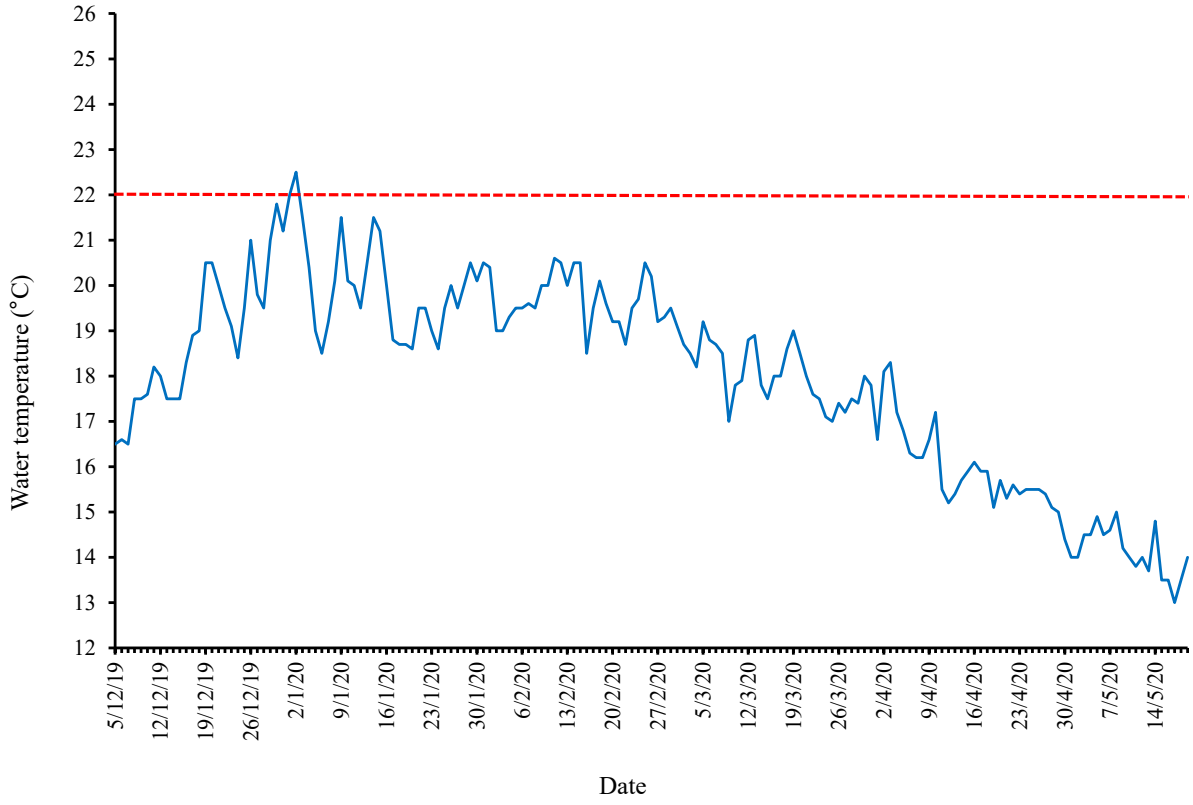


Figure 5. The maximum water temperature profile of experimental tanks during the Yumbah Narrawong farm trial. Red dashed line indicates critical theoretical temperature threshold of 22°C.

3.2 Survival

Survival: Jade Tiger Abalone Avalon

Overall, although with both being relatively high, the cumulative survival of Tiger Abalone fed the commercial diet (96.4%) during the JTA Avalon trial was significantly higher than those fed the *Ulva* diet (93.4%; $P < 0.001$; Kaplan-Meier; Log- Rank test; Figure 7). Tiger Abalone fed the *Ulva* diet were 1.56 times ($P < 0.001$) more likely to die over the period of the trial compared to abalone fed the commercial diet (Cox proportional hazards regression analysis).

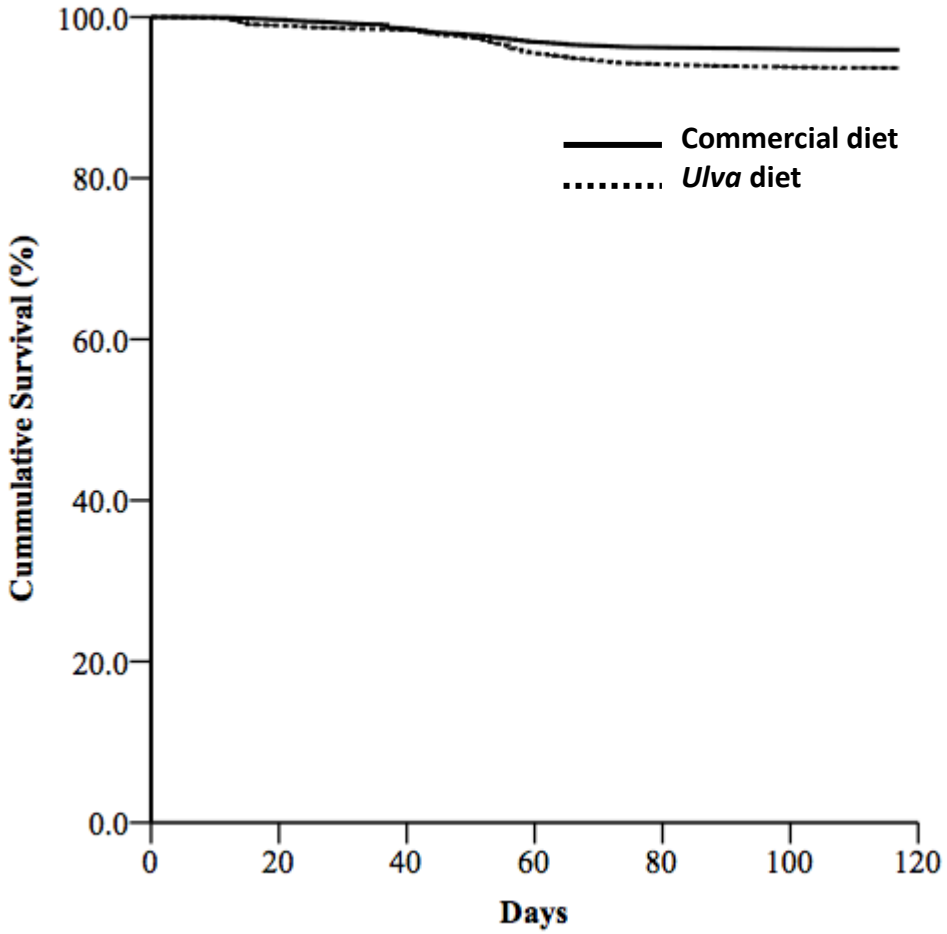


Figure 6. Kaplan-Meier survival curves of Tiger Abalone (*Haliotis laevigata* × *H. rubra*) fed the commercial diet, or the *Ulva* diet over 112 days (19/12/20 - 9/4/20) during the Jade Tiger Abalone Avalon farm trial. The cumulative survival of the Tiger Abalone fed the commercial diet (96.4%) was significantly higher than for the *Ulva* diet (93.4%) ($P < 0.001$; Kaplan-Meier; Log- Rank test; commercial diet, $n = 103,727$; *Ulva* diet, $n = 109,664$).

The cumulative mortality for Tiger Abalone during this trial was low and variable within treatment. On average 601 ± 1017 (stdev) and 989 ± 875 (stdev) abalone mortalities tank^{1} were recorded for the commercial and *Ulva* diet treatments, respectively. This equated to cumulative mortality of 3.6% ± 2.1% and 6.6% ± 2.3% for the commercial and *Ulva* diet treatments, respectively. The high levels of intra-treatment variability may be explained by high mortalities in some tanks, and not others. For example, for the commercial treatment, one tank (Tank B05, 4/1/20) recorded a cumulative mortality of 15.9%, while all other tanks from the same treatment recorded mortality levels of < 3.4%. The overall cumulative mortality data for tank B05 was determined to be an outlier (Appendix A Table 1-A).

Furthermore, two tanks from the *Ulva* diet treatment (Tank F04, 2/1/20; Tank B06, 3/2/20) recorded mortality rates of 14.8% and 15.1%, respectively while the remainder of the tanks from this treatment recorded mortality levels of < 7%. Due to the wider spread of cumulative mortality observed in the *Ulva* diet treatment, tanks F04 and B06 were not determined to be outliers (Appendix A Table 1-A).

The high cumulative mortality rates in tanks B05, F04 and B06 appeared to occur following periods of high-water temperatures and may be related to summer mortality (Figure 8). Cumulative mortality rates for both treatments declined as water temperatures declined towards the end of March and into April (Figures 7 and 8).

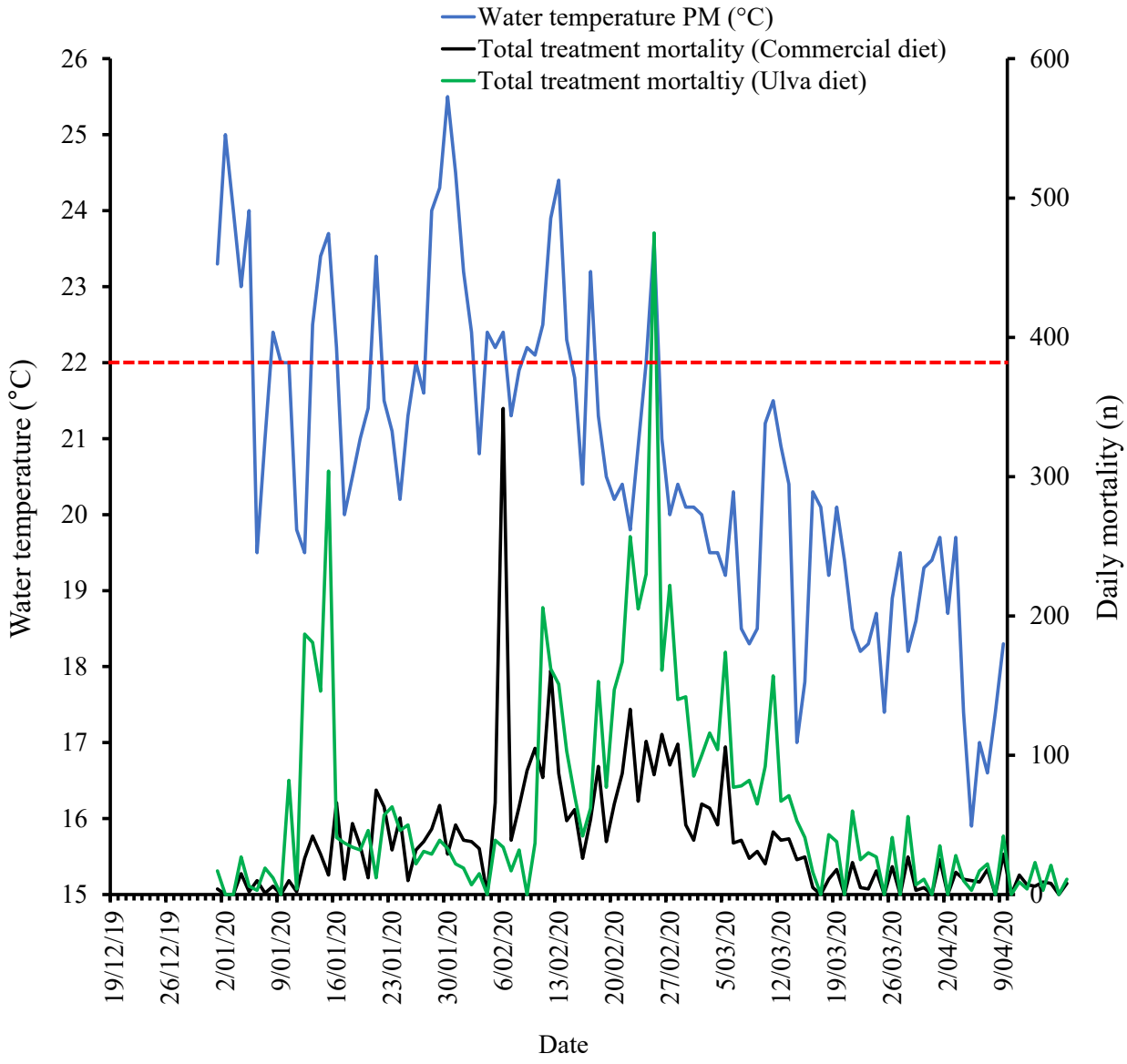


Figure 7. The afternoon (PM) maximum water temperature profile (primary vertical axis) and daily total treatment mortality (secondary vertical axis) of Tiger Abalone during the Jade Tiger Abalone Avalon farm trial. Red dashed line indicates the theoretical critical temperature threshold level of > 22°C. Maximum PM water temperatures were recorded between 3:00 and 4:00 pm daily.

Survival: Yumbah Narrawong

Overall, although being relatively high for both treatments, the survival of the combined Greenlip and Tiger Abalone fed the commercial diet (98.1%) was significantly higher than for those fed the *Ulva* diet (97.5%; $P < 0.001$; Kaplan-Meier; Log- Rank test; Figure 9). The abalone fed the *Ulva* diet were 1.34 times ($P < 0.001$) more likely to die over the period of the trial compared to abalone fed the commercial diet (Cox proportional hazards regression analysis; Figure 9).

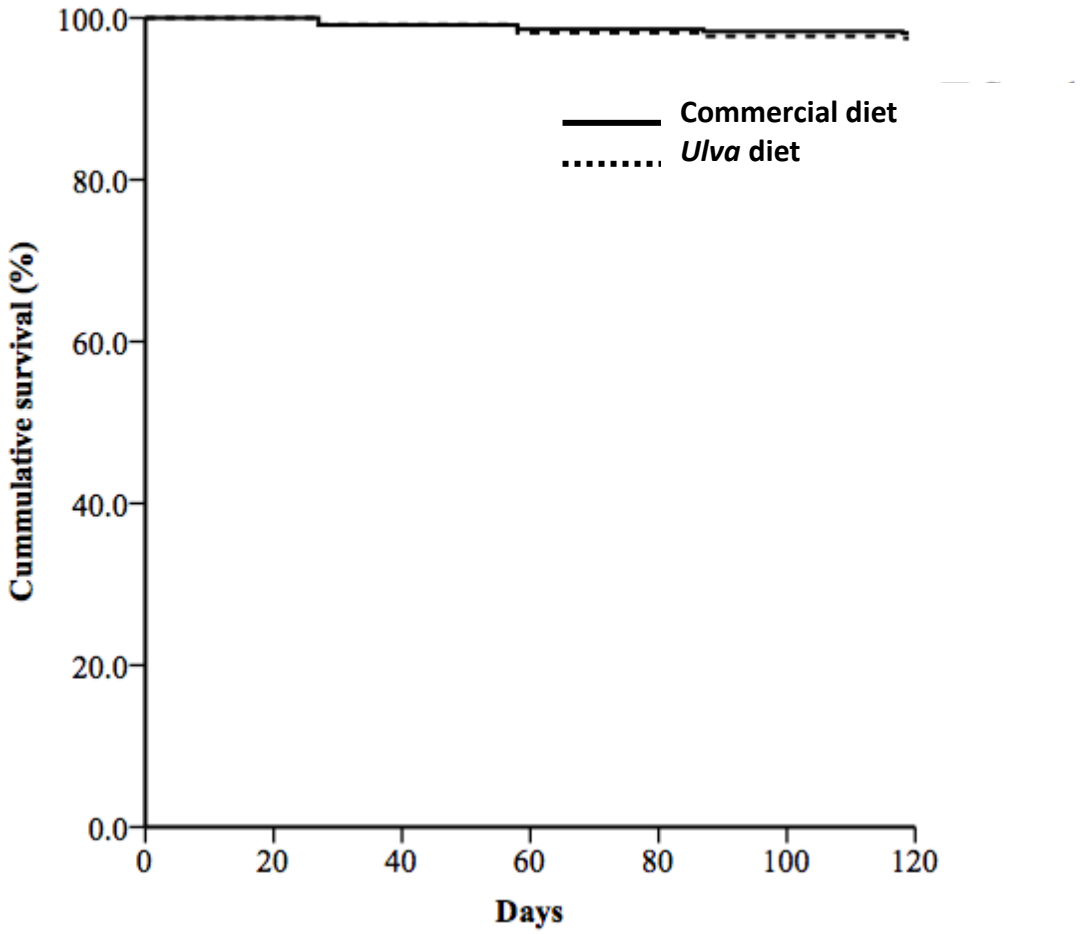


Figure 8. Kaplan-Meier survival curves of the combined group of Greenlip Abalone (*Haliotis laevigata*) and Tiger Abalone (*Haliotis laevigata × H. rubra*) fed the commercial diet, or the *Ulva* diet over 118 days (5/12/19 to 1/4/20) during the Yumbah Narrawong farm trial. The cumulative survival of abalone fed the commercial diet (98.1%) was significantly higher than for the *Ulva* diet (97.5%) ($P < 0.001$; Kaplan-Meier; Log- Rank test; commercial diet, $n = 39,629$; *Ulva* diet, $n = 43,539$).

Abalone mortalities in the Yumbah Narrawong trial occurred predominantly throughout periods of higher water temperatures during January and February (Figure 10). The monthly mortality for the combined Greenlip and Tiger Abalone during this trial was relatively low (Figure 10). On average 184 ± 54 (stdev) and 272 ± 61 (stdev) abalone mortalities slab tank^{-1} were recorded for the commercial and *Ulva* diet treatments, respectively. This equated to cumulative mortality of 1.9% ± 0.3% and 2.5% ± 0.3% for the commercial and *Ulva* diet treatments, respectively. It appears that regardless of the diet, the observed mortality patterns followed the rising and falling maximum daily water temperatures experienced throughout summer and autumn in this trial.

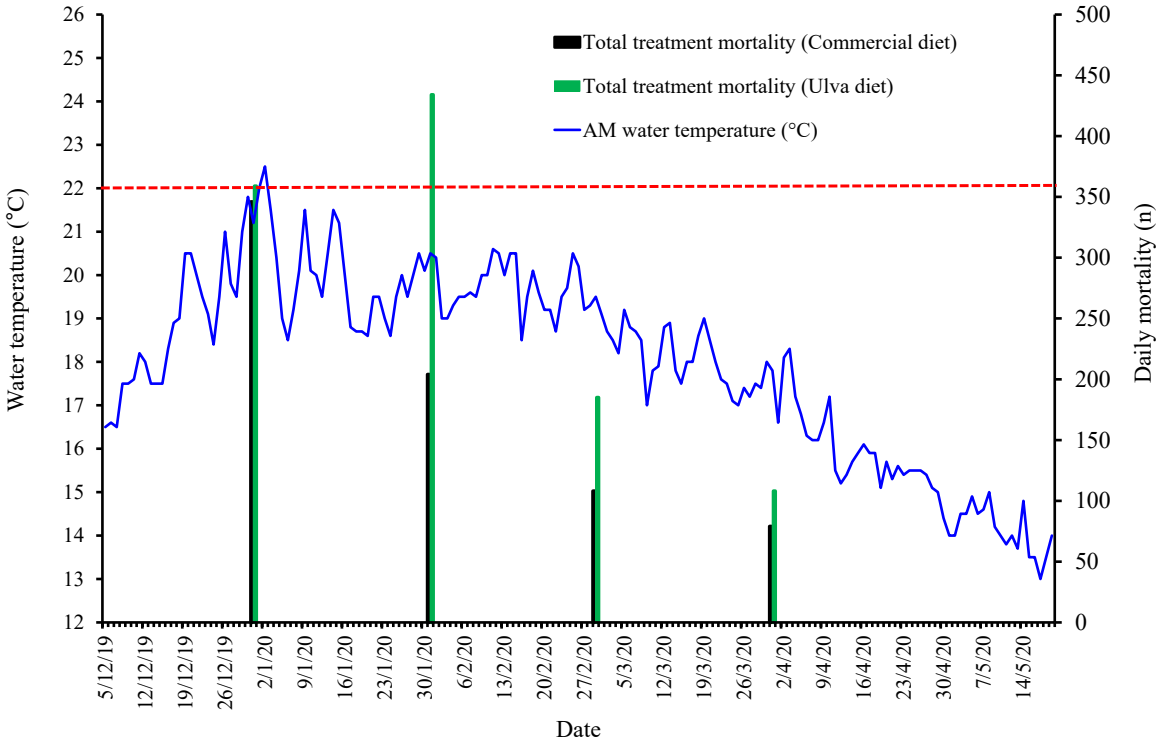


Figure 9. Daily maximum water temperatures (primary vertical axis) and total monthly treatment mortality (secondary vertical axis) for Tiger and Greenlip Abalone during the Yumbah Narrawong farm trial. Red dashed line indicates critical temperature threshold level of 22°C.

3.3 Growth performance and feed utilisation

Jade Tiger Abalone Avalon

There were no significant differences in initial tank biomass (P = 0.522) or initial individual weight (P = 0.167) of Tiger Abalone fed either diet in the study.

Tiger Abalone were observed to accept the commercial and *Ulva* diets equally well. Significantly more feed was offered to the Tiger Abalone fed the *Ulva* diet compared to those fed the commercial diet throughout the trial at JTA Avalon (Table 2; one-factor ANOVA; P = 0.025).

Table 2. Summary of stocked and final weights, lengths, condition factor, and total feed offered of Tiger Abalone (*Haliotis laevigata × H. rubra*) fed either the commercial diet or the *Ulva* diet at the completion of the Jade Tiger Abalone Avalon farm trial based on subsampling data (30 abalone per sampling event; 19/12/19 - 9/4/20; 112 d).

Item[1,2]	**Commercial diet**	***Ulva* diet**	***P* value**
Initial tank biomass (kg tank^{-1}) (19/12/19)[1]	609.5±37.3	578.7±37.3	0.522
Initial individual weight (g abalone^{-1}) (19/12/19)[1]	41.9±2.3	37.8±1.6	0.167
Initial shell length (mm)[4]	64.9±5.26	64.9±5.26	na
Total feed offered (g abalone^{-1})[3]	15.43±0.99[b]	18.09±0.34[a]	0.025

[1] Means values (± SE) for initial tank biomass and initial individual weight are not significantly different ($P > 0.05$; One-factor ANOVA; n = 7).
[2] Data based on limited samples derived from the n=mean value of 3 abalone from each tank (n = 7 tanks for each treatment).
[3] Mean (± standard deviation; n = 7) data used for total feed offered data was provided by JTA Avalon.
[4] Mean (± standard deviation; n = 21) data used for initial shell length data for both treatments was derived from 21 abalone randomly collected from stocked trial tanks on 19/12/19.
na = not applicable.

Growth performance, feed utilisation and basic economic analysis: Yumbah Narrawong

Greenlip Abalone and Tiger Abalone were observed to accept the diets well; however, observations by feed managers suggested that abalone tended to accept the commercial diet better than the *Ulva* diet; as observed by the higher level of uneaten feed remaining in the tanks on the day after feeding (Luke Thorpe, Farm Manager, Yumbah Narrawong, Pers. Comm.). The growth performance and feed utilisation component of this trial ran for 166 d (5.5 months; 5/12/19 – 19/5/20). There were no significant differences in initial biomass, final biomass, biomass gain or mortality weight of abalone fed the commercial diet or the *Ulva* diet (Table 3; one-factor ANOVA; $P > 0.05$). There was, however, a significant increase in SGR when abalone were fed the *Ulva* diet compared to the commercial diet (Table 3; one-factor ANOVA; $P = 0.004$).

With regards to feed utilisation, there was no significant difference in the amount of feed offered to abalone fed the commercial diet or the *Ulva* diet (Table 3; one-factor ANOVA; $P = 0.770$). There were, however, significant improvements in FCR ($P = 0.012$) and apparent protein efficiency ratio ($P = 0.038$) when abalone were fed the *Ulva* diet compared to the commercial diet (Table 3; one-factor ANOVA).

Table 3. Summary of combined growth performance of Greenlip Abalone and Tiger Abalone at the completion of the Yumbah Narrawong farm trial (5/12/19 - 19/5/20; 166 d).

Item[1]	Commercial diet	*Ulva* diet	*P* value
Initial biomass (kg tank^{-1}) (19/12/19)	405.8±2.6	405.3±3.3	0.910
Final biomass (kg tank^{-1}) (9/4/20)	695.6±25.2	730.5±17.5	0.300
Biomass gain (kg tank^{-1})	289.9±22.8	325.5±14.7	0.241
SGR (% biomass d^{-1})	0.47±0.01^{b}	0.53±0.01^{a}	0.004
Mortality weight (kg tank^{-1})[2]	9.13±1.40	12.38±1.40	0.151
Total feed offered (kg tank^{-1})	583.5±28.6	572.5±21.9	0.770
Apparent feed conversion ratio (FCR)	2.03±0.07^{b}	1.76±0.02^{a}	0.012
Apparent protein efficiency ratio (PER)	1.40±0.05^{b}	1.53±0.01^{a}	0.038

[1] Means (± SE) for each variable, apart for survival, in each row that do not share the same superscript are significantly different ($P < 0.05$; one-factor ANOVA; n = 4).
[2] Mortality weights were only recorded daily from stocking (5/12/19) until the 1/4/20 (118 d).

The basic economic analysis of abalone from the 166 d Yumbah Narrawong on-farm trial revealed an increase in basic sales revenue from $8, 717 slab tank^{-1} for the mixed population of Tiger and Greenlip Abalone fed the commercial diet to $9,446 slab tank^{-1} for those fed the *Ulva* diet. This $729 difference equated to an 8.4% increase in basic sales revenue per slab tank over the 166-d period when the abalone were fed the *Ulva* diet compared to the commercial diet.

3.4 Histopathological evaluation of the digestive tract (stomach and intestine) and gills

Histopathological evaluation: Jade Tiger Abalone Avalon

Histopathological comparisons of digestive tract and gill tissues from Tiger Abalone in January (day 34), February (day 69) and April (day 112), compared to the initial samples at stocking (day 11), indicated no significant alterations (Table 4; $P > 0.05$; Dunnett's 2-tailed test) to the structure of the stomach or intestinal tissues (stomach epithelial thickness, 80.43 ± 1.36 µm to 88.75 ± 3.33 µm; intestine epithelial thickness, 63.96 ± 3.03 µm to 78.91 ± 3.82 µm; and intestine mucous cell counts, 11.36 ± 1.56 to 14.14 ± 1.63 cells 1000 μm^{-1} of intestine). There was a weak and non-significant correlation between stomach epithelial thickness and whole abalone weight (52.07 ± 1.91 µm, $r = 0.151$, $P = 0.338$; n = 42, Pearson's correlation). The correlation between stomach epithelial thickness and shell length was also weak and not statistically significant (68.87 ± 0.67 mm, $r = 0.174$, $P = 0.271$; Pearson's correlation). There was a weak significant positive correlation between intestine epithelial thickness and whole abalone weight ($r = 0.321$; $P = 0.038$, $n = 42$). There was also a weak significant positive correlation between intestine epithelial thickness and stomach epithelial thickness ($r = 0.409$; $P = 0.003$, $n = 49$; Pearson's correlation).

Significant alterations between initial samples and gill tissue samples collected throughout the trial (January, day 34; February, day 69; April day, 112) were observed in all measured histopathological parameters in gill tissues of Tiger Abalone. The average number of goblet cells within the leaflet tips of Tiger Abalone gills in initial samples ranged from 2.14 ± 0.15 to 4.00 ± 0.26 cells leaflet tip^{-1} (Table 4). Significant increases in goblet cell numbers were observed between initial Tiger Abalone and those in February (Table 4; commercial diet, 50.9% increase, $P = 0.007$; *Ulva* diet, 47.6% increase, $P = 0.012$; Dunnett's 2-tailed test) and April (Table 4; commercial diet, 87.3% increase, $P < 0.001$; *Ulva* diet, 80.3% increase, $P = < 0.001$). There were no significant changes in goblet cell numbers between initial Tiger Abalone and those collected in January and fed either dietary treatment (Table 4; $P > 0.05$; Dunnett's 2-tailed test).

The leaflet tip damage score for individual treatment groups ranged between 0.030 ± 0.03 and 0.57 ± 0.04 $leaflet^{-1}$ (Table 5). When compared to the initial Tiger Abalone, significant decreases in leaflet tip damage scores were observed between Tiger Abalone fed the *Ulva* diet in January (Table 5; 19.5% decrease, $P = 0.006$; GLMM with pairwise comparisons), and between Tiger Abalone fed either diet in February (commercial diet, 34.7% decrease, $P < 0.001$; *Ulva* diet, 34.7% decrease, $P < 0.001$). A significant 23.9% increase in leaflet tip damage scores was observed between initial Tiger Abalone and abalone fed the commercial diet in April ($P = 0.001$). There was no significant difference in leaflet tip damage scores between initial samples and Tiger Abalone fed the commercial diet in January and those in April fed the *Ulva* diet ($P > 0.05$; GLMM).

The sinus fluid score for individual treatment groups range between 0.011 ± 0.03 and 0.45 ± 0.04 $leaflet^{-1}$ (Table 5). When compared to the initial Tiger Abalone, there were significant increases in sinus fluid scores observed in Tiger Abalone fed the commercial diet in January (Table 5; 100% increase, $P < 0.001$; GLMM with pairwise comparisons), February (54.5% increase, $P < 0.001$) and April (309% increase, $P < 0.001$). A significant 181.8% increase in sinus fluid score was also observed between Tiger Abalone fed the *Ulva* diet in April ($P = 0.010$) compared to the initial Tiger Abalone (Table 5). There were no significant differences in the sinus fluid scores between initial Tiger Abalone and those fed the *Ulva* diet in January and February (Table 5; $P > 0.05$; GLMM).

The columnar section damage score for individual treatment groups range between 0.055 ± 0.06 and 0.72 ± 0.03 $leaflet^{-1}$ (Table 5). There were significant decreases in columnar section damage scores in Tiger Abalone fed either diet in January (Table 5; commercial diet, 19.7% decrease, $P = 0.001$; *Ulva* diet, 22.5% decrease, $P = 0.005$; GLMM with pairwise comparisons), and the commercial diet in February (15.4% decrease, $P = 0.012$) when compared to initial Tiger Abalone. There was no significant difference in columnar section damage scores between initial Tiger Abalone and those fed the *Ulva* diet in February, or Tiger Abalone fed either dietary treatment in April (Table 5; $P > 0.05$; GLMM).

Table 4. Histopathological measurements of the digestive tract and gill leaflet tip goblet cell numbers for Tiger Abalone (*H. laevigata × H. rubra*) fed either the commercial diet or the *Ulva* diet over 112 days during the Jade Tiger Abalone Avalon trial [1].

Time	Initial[4]	January[4]		February[4]		April[4]	
Diet treatment	Commercial diet	Commercial diet	*Ulva* diet	Commercial diet	*Ulva* diet	Commercial diet	*Ulva* diet
Digestive tract							
Stomach epithelial thickness (µm)	86.01 ± 3.51	83.62 ± 4.21	85.94 ± 4.56	85.35 ± 4.32	82.18 ± 6.05	80.43 ± 1.36	88.75 ± 3.33
Intestine epithelial thickness (µm)	66.75 ± 2.37	63.96 ± 3.03	72.21 ± 6.54	64.15 ± 2.87	71.07 ± 2.26	76.04 ± 3.51	78.91 ± 3.82
Mucous cells (cells 1000 μm^{-1} of intestine)	12.29 ± 1.59	14.14 ± 1.63	12.10 ± 1.90	13.05 ± 2.04	12.67 ± 2.30	11.83 ± 1.57	11.36 ± 1.56
Gills							
Leaflet tip goblet cell (cells $leaflet^{-1}$)[2,3]	2.14 ± 0.15	2.49 ± 0.19	2.63 ± 0.21	3.23 ± 0.27*	3.16 ± 0.26*	4.00 ± 0.26**	3.86 ± 0.12**

[1] Data presented as means ± standard error (SE) for individual treatments (n = 7).

[2] Mean values marked with an asterisk (*) denote a significance difference of $P < 0.05$ when compared to the corresponding initial mean value for abalone fed the commercial diet (Dunnett's two-tailed test).

[3] Mean values marked with an asterisk (**) denote a significance difference of $P < 0.001$ when compared to the corresponding initial mean value for abalone fed the commercial diet (Dunnett's two-tailed test).

[4] Average individual Tiger Abalone weight for each treatment group was 41.9±2.3 (Initial commercial), 37.8±1.6 (Initial *Ulva* diet); 39.70 ± 3.74 g (January, commercial); 39.49 ± 1.58 g (January, *Ulva* diet), 54.92 ± 2.02 g (February, commercial); 51.54 ± 3.21 g (February, *Ulva* diet); and 66.10 ± 3.11 g (April, commercial), 60.70 ± 3.23 g (April, 10% *Ulva* diet).

Table 5. Histopathological scoring in gills of Tiger Abalone (*H. laevigata × H. rubra*) fed either the commercial diet or the *Ulva* diet over 112 days during the Jade Tiger Abalone Avalon trial[1,2,3].

Time	Initial	January		February		April	
Diet treatment	Commercial diet	Commercial diet	*Ulva* diet	Commercial diet	*Ulva* diet	Commercial diet	*Ulva* diet
Leaflet tip damage score ($leaflet^{-1}$)	0.46 ± 0.05	0.44 ± 0.05	0.37 ± 0.03*	0.30 ± 0.03**	0.30 ± 0.04**	0.57 ± 0.04*	0.52 ± 0.07
Sinus fluid score ($leaflet^{-1}$)	0.11 ± 0.03	0.22 ± 0.05**	0.17 ± 0.05	0.19 ± 0.02**	0.20 ± 0.05	0.45 ± 0.04**	0.31 ± 0.04*
Columnar section damage score ($leaflet^{-1}$)	0.71 ± 0.06	0.57 ± 0.06*	0.55 ± 0.09*	0.60 ± 0.03*	0.66 ± 0.05	0.72 ± 0.03	0.71 ± 0.06

[1] Data presented as means ± standard error (SE) for individual treatments (n = 7).

[2] Mean values marked with an asterisk (*) denote a significance difference of $P < 0.05$ when compared to the corresponding initial mean value for abalone fed the commercial diet (Generalized linear mixed model pairwise comparisons).

[3] Mean values marked with an asterisk (**) denote a significance difference of $P < 0.001$ when compared to the corresponding initial mean value for abalone fed the commercial diet (Generalized linear mixed model pairwise comparisons).

Histopathological evaluation: Yumbah Narrawong

Greenlip Abalone

For Greenlip Abalone, stomach epithelial thickness ranged between 75.08 ± 6.45 µm and 93.89 ± 9.81 µm (Table 6). There was no significant effect of dietary treatment on stomach epithelial thickness (Table 6; $P = 0.563$; two-factor ANOVA). There was also no significant effect of time ($P = 0.174$) and no significant interaction between the two factors ($P = 0.681$). There was a moderate and non-significant correlation between stomach epithelial thickness and whole abalone weight (63.90 ± 4.29 g, $r = 0.404$, $P = 0.050$, $n = 24$). There was also a weak and non-significant correlation between stomach epithelial thickness and shell length (74.16 ± 1.30 mm, $r = 0.352$, $P = 0.092$, $n = 24$; Pearson's correlation).

For Greenlip Abalone, the intestine epithelial thickness ranged between 73.27 ± 1.90 µm and 79.98 ± 4.61 µm (Table 6). There was no significant effect of dietary treatment (Table 6; $P = 0.970$; two-factor ANOVA), or time ($P = 0.846$) on intestine epithelial thickness and no significant interaction between the two factors ($P = 0.798$). There was a weak and non-significant ($r = -0.021$, $P = 0.925$, $n = 23$) correlation between intestine epithelial thickness and whole abalone weight. There was also a weak and non-significant correlation ($r = -0.031$, $P = 0.889$, $n = 23$) between intestine epithelial thickness and shell length. There was also a weak and non-significant correlation between intestine epithelial thickness and stomach epithelial thickness ($r = -0.038$, $P = 0.865$, $n = 23$; Pearson's correlation).

There was no significant effect of dietary treatment (Table 6; $P = 0.861$; two-factor ANOVA), or time ($P = 0.147$) on mucous cell counts of Greenlip Abalone. There was also no significant interaction between the two factors ($P = 0.520$). The number of mucous cells per individual treatment group ranged from 3.88 ± 1.30 to 9.29 ± 1.38 cells 1000 μm^{-1} of intestine (Table 6).

There was no significant effect of dietary treatment (Table 6; $P = 0.726$; two-factor ANOVA) or time ($P = 0.180$) on the number of goblet cells in gill leaflet tips of Greenlip Abalone. There was no significant interaction between the two factors ($P = 0.242$). The number of goblet cells for individual treatment groups ranged between 2.12 ± 0.26 and 2.83 ± 0.15 cells $leaflet^{-1}$ (Table 6).

The leaflet tip damage scores of Greenlip Abalone for individual treatment groups ranged between 0.037 ± 0.02 and 0.66 ± 0.09 $leaflet^{-1}$ (Table 7). There was no significant effect of dietary treatment on the gill leaflet tip damage score in January (Table 7; $P = 0.139$; GLMM), February ($P = 0.945$) or April ($P = 0.636$). A significant effect of time was observed in leaflet tip damage scores for abalone fed either the commercial diet (35.1% decrease, $P < 0.001$; January > February = April; GLMM with pairwise comparisons) or the *Ulva* diet (37.8% decrease, $P < 0.001$; January > February = April).

There was no significant effect of dietary treatment on sinus fluid scores in gill leaflets of Greenlip Abalone in January (Table 7; $P = 0.153$; GLMM), February ($P = 0.846$) or April ($P = 0.817$). A significant effect of time was recorded on sinus fluid scores for abalone fed either the commercial diet (93.9% increase, $P < 0.001$; January = February < April) or the *Ulva* diet (190.4%, increase $P < 0.001$; January < February < April; GLMM with pairwise comparisons). The sinus fluid score for individual treatments ranged between 0.21 ± 0.04 and 0.64 ± 0.02 $leaflet^{-1}$ (Table 7).

There was a mixture of significant effects of 10% dried *Ulva* sp. meal inclusion in diets on the columnar section damage scores in gill leaflets of Greenlip Abalone in January (Table 7; 55.1% increase, $P < 0.001$; commercial diet < *Ulva* diet; GLMM with pairwise comparisons), February (22.2% decrease, $P = 0.003$; commercial diet > *Ulva* diet) and April (20.63% increase, $P = 0.015$; commercial diet < *Ulva* diet). There was a significant effect of time on the columnar section damage scores for abalone fed either the commercial diet (46.9% increase, $P < 0.001$; January < April < February; GLMM with pairwise comparisons) or the *Ulva* diet (26.3% decrease, $P < 0.001$; January = April > February). The columnar section damage scores for individual treatments ranged between 0.49 ± 0.08 and 0.76 ± 0.11 $leaflet^{-1}$ (Table 7).

Tiger Abalone

Stomach epithelial thickness in Tiger Abalone ranged between 65.98 ± 2.84 µm and 82.24 ± 8.91 µm (Table 6). There was no significant effect of dietary treatment (Table 6; $P = 0.388$; two-factor ANOVA), or time ($P = 0.052$) on stomach epithelial thickness and no significant interaction between the two factors ($P = 0.885$). There was a weak and non-significant correlation between stomach epithelial thickness and whole abalone weight (67.88 ± 2.25 g, $r = -0.064$, $P = 0.766$, $n = 24$). There was also a weak and non-significant correlation between stomach epithelial thickness and shell length (75.37 ± 0.79 mm, $r = -0.392$, $P = 0.058$, $n = 24$; Pearson's correlation).

There were no significant effects of dietary treatment (Table 6; $P = 0.983$; two-factor ANOVA), or time ($P = 0.159$) on intestine epithelial thickness of Tiger Abalone. There were also no significant interactions between the two factors ($P = 0.877$). Intestine epithelial thickness ranged between 71.87 ± 4.83 µm and 84.09 ± 7.61 µm (Table 6). There was a weak and non-significant ($r = 0.306$, $P = 0.146$, $n = 24$) correlation between intestine epithelial thickness and whole abalone weight. There was also a weak and non-significant correlation ($r = 0.089$, $P = 0.678$, $n = 24$) between intestine epithelial thickness and shell length. There was also a weak and non-significant correlation between intestine epithelial thickness and stomach epithelial thickness ($r = 0.340$, $P = 0.105$, $n = 24$; Pearson's correlation).

There was no significant effect of dietary treatment (Table 6; $P = 0.579$; two-factor ANOVA), or time ($P = 0.777$) on mucous cell counts of Tiger Abalone. There was also no significant interaction between the two factors ($P = 0.085$). The number of mucous cells per individual treatment group ranged from 2.58 ± 1.02 to 8.63 ± 2.98 cells 1000 μm^{-1} of intestine (Table 6).

There was no significant effect of dietary treatment (Table 6; $P = 0.896$; two-factor ANOVA) on the number of goblet cells in the leaflet tips in Tiger Abalone. There was a significant effect of time, with a 41.3% increase in goblet cell numbers in April compared to January and February (P = 0.001; January = February < April; two-factor ANOVA, SNK), and there was no significant interaction between the two factors ($P = 0.441$). The number of goblet cells for individual treatment groups ranged between 2.20 ± 0.25 and 3.39 ± 0.15 cells $leaflet^{-1}$ (Table 6).

The leaflet tip damage score for individual treatment groups of Tiger Abalone range between 0.037 ± 0.06 and 0.61 ± 0.06 $leaflet^{-1}$ (Table 7). There was no significant effect of dietary treatment on the gill leaflet tip damage score in January (Table 7; $P = 0.231$; GLMM) or April ($P = 0.370$); However, there was a positive significant effect of dietary treatment in February (37.8% decrease, $P = 0.038$; commercial diet > *Ulva* diet; GLMM with pairwise comparisons). A positive significant effect of time was observed in leaflet tip damage score for abalone fed either the commercial diet (21.3% decrease, $P < 0.001$; January > February = April; GLMM with pairwise comparisons) or the *Ulva* diet (30.1% decrease, $P < 0.001$; January > February = April).

There was a negative significant effect of dietary treatment on sinus fluid scores in gill leaflets of Tiger Abalone in January (Table 7; 100% increase, $P = 0.006$; commercial diet < *Ulva* diet; GLMM with pairwise comparisons). There was no significant effect of dietary treatment in February ($P = 0.127$) or April ($P = 0.080$). There was a negative significant effect of time on sinus fluid scores for abalone fed either the commercial diet (657.1% increase, $P < 0.001$; January < February < April; GLMM with pairwise comparisons) or the *Ulva* diet (221.4% increase, $P < 0.001$; January < February < April). The sinus fluid scores for individual treatments ranged between 0.07 ± 0.01 and 0.53 ± 0.05 $leaflet^{-1}$ (Table 7).

There was no significant effect of dietary treatment on the columnar section damage score in gill leaflets of Tiger Abalone in January (Table 7; $P = 0.527$; GLMM), February ($P = 0.587$) or April ($P = 0.622$). There was a significant negative effect of time on the columnar section damage scores for abalone fed either the commercial diet (21.7% increase, $P < 0.001$; January < February = April; GLMM with pairwise comparisons) or the *Ulva* diet (22.8% increase, $P = 0.001$; January < February = April). The columnar section damage scores for individual treatments ranged between 0.69 ± 0.06 and 0.86 ± 0.04 $leaflet^{-1}$ (Table 7).

Table 6. Histopathological measurements of the digestive tract and gill leaflet tip goblet cell numbers in Greenlip Abalone (*H. laevigata*) and Tiger Abalone (*H. laevigata × H. rubra*) fed either commercial diet or *Ulva* diet over 139 days of the Yumbah Narrawong trial.[1]

Time	January		February		April		Two-factor ANOVA (*P* values)[2]		
Diet treatment	Commercial diet	*Ulva* diet	*Commercial diet*	*Ulva* diet	Commercial diet	*Ulva* diet	Diet (A)	Time (B)	Interaction (AxB)
Greenlip Abalone[3]									
Digestive Tract									
Stomach epithelial thickness (µm)	75.08 ± 6.45	78.40 ± 4.32	82.80 ± 9.15	78.18 ± 1.12	93.89 ± 9.81	85.49 ± 4.32	0.563	0.174	0.681
Intestine epithelial thickness (µm)	73.27 ± 1.90	77.25 ± 5.71	79.98 ± 4.61	76.15 ± 4.93	74.81 ± 3.32	75.21 ± 9.46	0.970	0.846	0.798
Mucous cells (cells 1000 μm^{-1} of intestine)	9.29 ± 1.38	8.58 ± 2.78	6.08 ± 1.21	8.28 ± 3.25	6.21 ± 0.43	3.88 ± 1.30	0.861	0.147	0.520
Gills									
Leaflet tip goblet cells ($leaflet^{-1}$)	2.80 ± 0.14	2.78 ± 0.34	2.24 ± 0.33	2.83 ± 0.15	2.46 ± 0.21	2.12 ± 0.26	0.726	0.180	0.242
Tiger Abalone[4]									
Digestive Tract									
Stomach epithelial thickness (µm)	74.13 ± 7.05	69.85 ± 0.56	71.83 ± 3.69	65.98 ± 2.84	82.24 ± 8.91	81.33 ± 2.17	0.388	0.052	0.885
Intestine epithelial thickness (µm)	71.87 ± 4.83	74.61 ± 4.01	76.84 ± 4.20	75.98 ± 4.36	84.09 ± 7.61	81.94 ± 3.49	0.983	0.159	0.877
Mucous cells (cells 1000 μm^{-1} of intestine)	8.63 ± 2.98	2.58 ± 1.02	4.08 ± 1.40	5.25 ± 1.23	3.17 ± 1.46	5.42 ± 2.45	0.579	0.777	0.085
Gills									
Leaflet tip goblet cells ($leaflet^{-1}$)	2.49 ± 0.30	2.20 ± 0.25	2.20 ± 0.29	2.52 ± 0.19	3.39 ± 0.15	3.28 ± 0.21	0.896	0.001 (J=F<A)	0.441

[1] Data presented as means ± standard error (SE) for individual treatments (n = 8). There was no statistical comparison between species.

[2] Values in parentheses indicate significant differences in effect time (J = January, F = February, A = April), ($P < 0.05$; two-factor ANOVA; SNK).

[3] Average individual Greenlip Abalone weight for each treatment group was 44.34 ± 1.92 g (January, commercial diet), 45.17 ± 0.86 g (January, *Ulva* diet), 58.77 ± 2.73 g (February, commercial diet), 52.10 ± 3.81 g (February, *Ulva* diet), 93.03 ± 2.30 g (April, commercial diet), 90.03 ± 2.80 g (April, *Ulva* diet).

[4] Average individual Tiger Abalone weight for each treatment group was 66.31 ± 5.32 g (January, commercial diet), 57.90 ± 3.88 g (January, *Ulva* diet), 79.08 ± 1.83 g (February, commercial diet), 62.95 ± 2.23 g (February, *Ulva* diet), 73.35 ± 7.88 g (April, commercial diet), 76.14 ± 5.40 g (April, *Ulva* diet).

Table 7. Mean values for histopathological scoring of gills for Greenlip Abalone (*H. laevigata*) and Tiger Abalone (*H. laevigata × H. rubra*) fed either the commercial diet or the *Ulva* diet over 139 days at Yumbah Narrawong[1].

Time	January		February		April		GLMM (P values)[2]				
Diet treatment	*Commercial diet*	*Ulva* diet	*Commercial diet*	*Ulva* diet	*Commercial diet*	*Ulva* diet	Diet			Time	
							January	February	April	*Commercial diet*	*Ulva* diet
Greenlip Abalone											
Leaflet tip damage score (leaflet^{-1})	0.57 ± 0.02	0.66 ± 0.09	0.37 ± 0.06	0.41 ± 0.09	0.37 ± 0.02	0.40 ± 0.05	0.139	0.945	0.636	<0.001 (J>F=A)	<0.001 (J>F=A)
Sinus fluid score (leaflet^{-1})	0.33 ± 0.07	0.21 ± 0.04	0.29 ± 0.06	0.30 ± 0.09	0.64 ± 0.02	0.61 ± 0.04	0.153	0.846	0.817	<0.001 (J=F<A)	<0.001 (J<F<A)
Columnar section damage Score (leaflet^{-1})	0.49 ± 0.08	0.76 ± 0.11	0.72 ± 0.04	0.56 ± 0.05	0.63 ± 0.05	0.76 ± 0.11	<0.001 (C<U)	0.003 (C>U)	0.015 (C<U)	<0.001 (J<A<F)	<0.001 (J=A>F)
Tiger Abalone											
Leaflet tip damage score (leaflet^{-1})	0.61 ± 0.06	0.53 ± 0.08	0.48 ± 0.09	0.37 ± 0.06	0.40 ± 0.09	0.37 ± 0.07	0.231	0.038 (C>U)	0.370	<0.001 (J>F=A)	<0.001 (J>F=A)
Sinus fluid score (leaflet^{-1})	0.07 ± 0.01	0.14 ± 0.08	0.21 ± 0.09	0.29 ± 0.05	0.53 ± 0.05	0.45 ± 0.05	0.006 (C<U)	0.127	0.080	<0.001 (J<F<A)	<0.001 (J<F<A)
Columnar section damage Score (leaflet^{-1})	0.69 ± 0.06	0.70 ± 0.11	0.84 ± 0.04	0.86 ± 0.04	0.84 ± 0.07	0.80 ± 0.05	0.527	0.587	0.622	<0.001 (J<F=A)	0.001 (J<F=A)

[1] Data presented as means ± standard error (SE) for individual treatments (n = 8). There was no statistical comparison between species.

[2] Values in parentheses indicate significant differences of $P < 0.05$ due to diet (C = commercial diet, U = *Ulva* diet), time (J = January, F = February, A = April) ($P < 0.05$; Generalized linear mixed model (GLMM) with pairwise comparisons).

Discussion

The project's main objective was to investigate if dietary intervention, using a diet containing 10% dried *Ulva* sp. meal, could be used to improve survival of Greenlip Abalone (*Haliotis laevigata*) and Tiger Abalone (*H. laevigata × H. rubra)* exposed to summer water temperatures on-farm under commercial conditions. Both survival and histopathological evaluation of select digestive tract and gill tissues through summer, and in response to diet, were investigated. The project's secondary objective was to determine if feeding a diet containing 10% dried *Ulva* sp. meal would improve growth performance, feed utilisation and ultimately production cost across a summer to autumn grow-out period in two separate farm trials for Greenlip and Tiger Abalone grown under commercial conditions. Overall, the results achieved during this project, although not positive for improvements in survival at high summer water temperatures (Figures 7 and 9), indicated the potential usefulness of dried *Ulva* meal as a commercial dietary ingredient for abalone production during mild summer temperature growing conditions based on historical temperature data at Yumbah, Narrawong.

Water temperatures and survival

To put the discussion of survival in response to water temperature in context in this report, it is worth reiterating that the selection of the theoretical temperature threshold (> 22°C) considered applicable to potentially induce abalone deaths related summer mortality for the two the farm trials was based on discussions about historical water temperature records, previous summer mortality events (Nick Savva, EO, AAGA, Joshua McIntyre, Farm Manager, JTA Avalon, Pers. Comm.; Luke Thorpe, Farm Manager; Yumbah Narrawong, Pers. Comms.), investigations of the literature (Vandepeer, 2006; Hooper et al., 2011; Hooper et al., 2014a, 2014b; Lange et al., 2014; Stone et al., 2014a), and the geographical locations of the farms in Victoria.

The water temperature profiles recorded throughout each farm trial differed considerably, both within and between, each trial. The JTA Avalon trial experienced water temperatures exceeding 22°C on ten occasions, with temperatures exceeding 24°C on several occasions during January and February (peaked at 25.5°C, during early January) (Figure 5), whereas the Yumbah Narrawong trial experienced relatively mild water temperatures (Figure 6) that peaked at 22.5°C for one day in early January (2/1/20) and did not exceed 22°C for the remainder of the trial. In the laboratory, Lange et al. (2014) exposed Greenlip Abalone to a constant challenge of 26°C for a period of 38 days, as opposed to the lower and fluctuating water temperatures (January ([PM water temperatures {°C}: mean, 22.5; range 19.5 - 25.5]; February ([PM water temperatures {°C}: mean, 21.7; range 19.8 - 24.5]) that the Tiger Abalone were exposed to during the summer period of the JTA Avalon trial. Combined with differences in species, genetics, culture conditions and stocking densities employed in each trial, the water temperatures recorded during the JTA Avalon trial may be considered sufficient to induce summer mortality in stocks, whereas the water temperatures recorded during the Yumbah Narrawong trial were unlikely to be sufficient to induce summer mortality (Vandepeer, 2006; Hooper et al., 2011; Hooper et al., 2014a, 2014b).

The survival rate of abalone fed the *Ulva* diet was significantly lower at each farm compared to that of abalone fed the commercial diet. While no further direct comparisons will be made between farm trials, the Tiger Abalone in the JTA Avalon farm trial tested under higher water temperatures recorded slightly lower total survival rates (94.6%) than the those of the mixed population of Tiger and Greenlip Abalone (97.8%) tested under cooler water temperatures during the Yumbah Narrawong trial. The overall survival levels recorded at Yumbah Narrawong were considered high and consistent with historical levels for comparable seasons (Luke Thorpe, Farm Manager, Yumbah Narrawong, Pers. Comms.). Whereas the overall survival levels recorded at JTA Avalon were considered lower than for years of higher water temperatures but were still considered to be problematic when compared to survival levels experienced during cooler summers (Joshua McIntyre, Farm Manager, JTA Avalon & Luke Thorpe, Farm Manager, Yumbah Narrawong, Pers. Comms.). While there was a significant 0.6% reduction in survival between the abalone fed the *Ulva* diet compared to the commercial diet at Yumbah Narrawong, the difference was minor and may be considered economically irrelevant as the abalone fed the Ulva diet exhibited a ~9.6% improvement in SGR. Whereas the observed difference in survival between treatments at JTA Avalon were slightly larger, and upon closer inspection, the cumulative mortality of Tiger Abalone during the JTA Avalon trial was 3.6% ± 2.1% and 6.6% ± 2.3% for the commercial and *Ulva* diet treatments, respectively (Figure

7). However, it was also apparent that mortality during the JTA Avalon trial was variable and sporadic between tanks, both within and between dietary treatments. For example, for the commercial treatment, one tank (Tank B05, 4/1/20) recorded a cumulative mortality of 15.9%, while all other tanks from the same treatment recorded mortality levels of < 3.4%. Furthermore, two tanks from the *Ulva* diet treatment (Tank F04, 2/1/20; Tank B06, 3/2/20) recorded mortality rates of 14.8% and 15.1%, respectively, while the remainder of the tanks from this treatment recorded mortality levels of < 7%. The high mortalities in tanks B05, F04 and B06 appeared to occur following periods of high-water temperatures (>22°C) and may be examples of summer mortality (Figure 8). Interestingly, there were no apparent differences in histopathological results for the gills and digestive tract tissues of Tiger Abalone from tanks B05, F04 and B06 sampled in January and February compared to abalone sampled from all of the other tanks during same period. This suggests that the histopathological parameters measured in this study may not be sensitive indicators of summer mortality for Tiger Abalone. However, it must be noted that individual samples sizes of abalone tested from each replicate tank were small (n =3) and a larger, more targeted, sampling effort during the actual temperature spikes and mortality events may have been able to better discriminate within treatment differences within and between treatment replicate tanks.

Given the variable and sporadic nature of the mortalities observed during the JTA Avalon trial with Tiger Abalone, it may well be that there was not enough heat stress on these animals to promote higher mortality and thus no significant difference were observed between treatments. For example, in a laboratory trial, Lange et al. (2014) reported a positive response in survival from 50% to 82% when Greenlip Abalone were held at 26°C and fed a diet containing 30% dried *Ulva* sp. meal compared to abalone fed a pelleted commercial diet containing no *Ulva* meal. Lange et al. (2014) suggested antioxidants and other bioactive compounds present in the dried *Ulva* sp. meal may have had beneficial effects on feed acceptance, cellular anti-oxidant capacity, immune response and ultimately the survival of Greenlip Abalone exposed to heat stress. Differences in survival rates between the laboratory trial of Lange et al. (2014) and the JTA Avalon trial may be due to several factors including species and strain differences, differences in dietary inclusion level of dried *Ulva* sp. meal (30% vs 10%), differences in culture conditions and practices or differences in the duration and peak temperatures attained during the different trials.

Differences in survival (Figures 7 and 8) observed between dietary treatments during the JTA Avalon farm trial may have also been, in part, due to differences in feed input and the potential effect on water quality. The total tank feed input of the *Ulva* diet was significantly higher (17%, Table 2) than for the commercial diet tanks during the JTA Avalon trial. This was despite there being a ~5% lower biomass in the *Ulva* diet treatment tanks at stocking. Unfortunately, water temperature was the only water quality variable measured in either farm trial during the study. To enable a better assessment of specific physiological responses of abalone to treatments, it is recommended that closer attention is given to the measurements of water quality parameters in future farm trials, including temperature, dissolved oxygen, ammonia, and salinity.

Histopathological evaluation of the digestive tract and gills

Histopathological analysis of the digestive tract and gills of abalone allows for the characterisation of their structure and function and may be a useful tool to assist management with decision making during production of abalone. To put the results of the histopathological evaluation of the digestive tract and gill tissues from this project in context with the dietary inclusion of 10% dried *Ulva* sp. meal and the occurrence of summer mortality it is important to consider that abalone were exposed to different water temperature profiles during each farm trial. Over the course of the JTA Avalon trial, Tiger Abalone experienced water temperatures > 22°C frequently during January and February (Figures 5 and 8). These high temperatures were consistent with previous research to induce summer mortality (Vandepeer, 2006; Hooper et al., 2011; Hooper et al., 2014a, 2014b; Lange et al., 2014; Stone et al., 2014a), and potentially cause histopathological alterations to the digestive tract or gills. In contrast, water temperatures during the Yumbah Narrawong Trial were below the threshold level of 22°C for all but one day of the trial. Therefore, water temperatures in this trial were likely to have been insufficient to induce summer mortality and related histopathological alterations to the digestive tract and gill tissues. Feeding the *Ulva* diet also led to increased mortality in abalone in both farm trials, although at a much lower level for the Yumbah Narrawong trial compared to the JTA Avalon trial.

Gill histopathology

Abalone gills are important for respiration and osmoregulation and are a point of contact for external pathogens (Hooper et al., 2014a, 2014b). The gill histopathological parameters measured in this study were chosen as they are indicators of the effects of temperature and handling stress on gill epithelial damage in abalone (Mouton, 2003; Hooper et al., 2014a, 2014b; Pedler et al. accepted; Thomson, unpublished data). In the current study, in both farm trials more significant histopathological alterations were observed in the gill tissues as opposed to the digestive tract tissues, particularly in the gill leaflet tips of Greenlip and Tiger Abalone. The majority of these alterations were negative and may have major implications for gas and ion exchange, osmotic balance and potential infection, growth and ultimately survival.

At JTA Avalon, there was significant evidence of gill damage (increased leaflet tip goblet cell numbers, increased leaflet tip damage scores and increased sinus fluid scores) in Tiger Abalone fed both diets over the course of the trial. Columnar section damage scores remained relatively unchanged (Table 4 and 5). As abalone from each treatment were cultured under the same conditions, with routine tank cleaning and no grading once the trial was underway, it is reasonable to suggest the main stress experienced by abalone during the trial was imparted as combination of heat and culture stressors. The heat stress component of the combined stressors, represented by exceeding the 22°C threshold, was present during January and February and not during March and April.

Measured gill parameters were typically negatively altered by exposure to high water temperatures and associated culture stressors in January and February, with limited recovery to initial values observed by the completion of the trial in April. The presence of gill epithelial damage is not surprising in this study as previous studies with farmed Tiger Abalone have also reported this response. Based on results from two separate studies investigating anaesthesia stress and handling and movement stress, Hooper et al (2014a; 2014b) concluded that some level of background damage to the gill epithelial layer of both healthy and stressed farmed Tiger Abalone is always present. Their conclusion would suggest that the gills of abalone are sensitive to physical damage associated with traditional on-farm abalone culture practices, such as tank cleaning and grading.

The significant increase in gill leaflet tip damage score in April, as well as continuous increases in gill leaflet tip goblet cell numbers and sinus fluid scores throughout the trial at JTA Avalon, suggest the epithelium of the gill leaflets tips of Tiger Abalone are more sensitive to damage than the columnar section of the gill leaflet. This is not unexpected, as the gill leaflet tips are more exposed to potential physical damages associated with culture practices than the more protected columnar section of the gill leaflet. The increased sensitivity to damage observed in the current study is consistent with earlier studies with cultured abalone exposed to heat stress under laboratory conditions (Hooper et al., 2014a; Pedler et al., accepted). In the current study, alterations of the gill leaflet tip were examined more closely by measuring changes in the leaflet tip section compared to the columnar section, as opposed to whole gill leaflets. Hooper et al. (2014a) reported increases in histopathological damage scores in entire gills leaflets of Tiger Abalone in response to seven days exposure to water temperatures of 26°C; the histopathological damage had not recovered by the end of the exposure period. In the same study, Hooper et al. (2014a) also observed a trend for fluid accumulation in entire gill leaflets to increase in heat stressed Tiger Abalone. Pedler et al. (accepted) developed a scoring protocol for the standard quantification of gill lesions in *Haliotis laevigata* and reported a significant increase in epithelial lifting in entire gill leaflets of Greenlip Abalone held at 25°C compared to 22°C for 47 days. Pedler et al. (accepted) also assessed epithelial hypertrophy, focal epithelial hyperplasia, goblet cell hyperplasia, lamellar fusion, epithelial atrophy, and epithelial necrosis in a range of gill tissues and reported no significant alterations of these histopathological parameters in response to heat stress.

The elevated leaflet tip damage score, goblet cell numbers, and sinus fluid scores measured in the gill leaflet tips of Tiger Abalone from the JTA Avalon trial showed no sign of returning to initial levels measured in abalone prior to the elevation of water temperatures. This suggests that Tiger Abalone were unable to recover from the heat stress exposures experienced during January and February, and even as water temperatures decreased during March and April. The observed lack of recovery is problematic for the industry as the damage may compromise the health, growth, and survival of abalone. A longer period of

investigation may be required to determine if and when recovery is possible. Further research is required in this area.

At Yumbah Narrawong, the addition of 10% dried *Ulva* sp. meal to the diet did not lead to improvements in any of the gill parameters of Greenlip and Tiger Abalone compared to the commercial diet, however as water temperature decreased from January-February to March-April, significant histopathological alterations to leaflet tip damage scores, goblet cell numbers and sinus fluid scores were observed, while columnar section damage scores remained relatively unchanged (Table 7). Gill leaflet tip damage scores improved (Greenlip Abalone, 37.8% decrease; Tiger Abalone, 30.1% decrease) in both Greenlip and Tiger Abalone at Yumbah Narrawong as water temperatures decreased after summer (Figures 6 and 10). These results were in contrast to the negative alterations observed for the gill leaflet tip damage scores (increased by up to 34.7%) of Tiger Abalone consistently exposed to heat stress (> 22°C; Figure 5 and 8) at JTA Avalon. Results suggest constant exposure to heat stress will result in increased epithelial damage in gill leaflet. This also suggests gill leaflet tip damage scores may be a reliable health indicator of heat stress in farmed abalone, however its use as a mortality indicator may be limited. For example, there was no apparent difference detected in this parameter between replicate tanks, within or between treatments that experienced low versus high mortality rates (B05, commercial diet treatment; F04 and B06, *Ulva* diet treatment) in the JTA Avalon trial. Further research more closely monitoring moribund abalone is required to assess the parameters sensitivity in relation to mortality.

Even though the abalone were not exposed to heat stress during the Yumbah Narrawong trial, negative histopathological alterations in the form of increasing gill leaflet tip goblet cell numbers (Tiger Abalone only, 41% increase) were observed. These results were consistent with those measured in heat stressed Tiger Abalone during the JTA Avalon trial and indicate heat stress may not be the only stressor abalone were exposed to in the current study. Thomson (unpublished data) also reported a significant increase in gill leaflet tip goblet cell number as water temperatures increased from 10°C in winter to 19°C in mid-summer in commercially cultured Tiger Abalone at Southern Ocean Mariculture (SOM), Victoria. In contrast, Hooper et al. (2014b) reported no alteration to gill leaflet tip goblet cell numbers in commercially cultured Tiger Abalone following anaesthesia and handling stress. Whereas, Tiger Abalone exposed to heat stress (26 °C) in a laboratory showed an initial decrease in gill leaflet tip goblet cell numbers after two days with recovery to initial values after 7 days (Hooper et al., 2014a). The contrasting results of increased goblet cell numbers in the gills of abalone exposed to a commercial environment (Thomson, unpublished data) and decreased numbers in abalone exposed to heat stress in a laboratory environment (Hooper et al., 2014a) suggest this parameter may be negatively affected by a range of different stress factors associated with seasonal commercial culture conditions. This parameter may lack the sensitivity to discriminate between heat stress and other stressors, however it may be a useful indicator of general health and survival for abalone exposed to the combined stressors associated with commercial culture.

During the Yumbah Narrawong trial, negative alterations were also observed in gill leaflet tip sinus fluid scores over the course of the trial for both species of abalone. Greenlip Abalone gill leaflet tip sinus fluid scores increased by up to 190%, while the corresponding scores for Tiger Abalone increased by up to 657%. These negative responses were consistent with the ~300% increase in gill leaflet tip sinus fluid scores recorded in Tiger Abalone from the JTA Avalon trial. The large responses observed for this parameter during both farm trials also suggests that gill leaflet tip sinus fluid scores may be affected by factors other than heat stress. In fact, Mouton (2003) reported increases in fluid accumulation (as observed by the presence or absence of fluid) in the entire gill leaflet of commercially cultured South African Abalone (*Haliotis midae*) due to live transport stress (held out of the water for 30-42 hours). Similarly, Hooper et al. (2014b) reported a visual increase in the presence of fluid in gill leaflet tips in commercially cultured Tiger Abalone exposed to anaesthesia and handling stress. However, Hooper et al. (2014b), while utilising a more refined graded scoring system, reported no significant changes in the level of fluid in gill leaflet tips of Tiger Abalone exposed to the same stressors. The increased presence of fluid in the gills of abalone during both farm trials in the current study, and in commercially cultured South African Abalone and Tiger Abalone exposed to transport, handling, and anaesthesia stress (Mouton 2003; Hooper et al., 2014b) suggests this parameter may be negatively affected by a range of different stress factors associated with a range of seasonal commercial culture conditions. This parameter may also be

useful as a general health and survival indicator for abalone exposed to combined stress factors during commercial culture.

Digestive tract histopathology

The digestive tract is important to abalone health and growth as it is the location of digestion and absorption of nutrients (assisted by the microbiome present), osmoregulation and acts as an external barrier against pathogens (Starck 2003). The addition of 10% dried *Ulva* sp. meal to the diet did not lead to any demonstrable alterations in histopathology of the stomach and intestine as indicated by epithelial thickness and intestinal mucous cell counts in Tiger Abalone and Greenlip Abalone over the course of either farm trial. Further investigation is recommended to confirm this.

Limited published research has been carried out on the effects of combined heat and culture stressors on the digestive tract health of abalone. Gao et al. (2009) reported decreased total gut masses in sea cucumber (*Apostichopus japonicus*) held at water temperatures of 21°C or 28°C compared to 14°C and 17°C over the course of a 40-day laboratory trial. Indeed, heat stress is also a problem for terrestrial animals and has been reported to lead to damage to the structural integrity of the intestinal epithelium in broiler chickens (Vesco et al., 2020). Considered together, heat damage to the structural integrity of the digestive tract has been shown to impact nutrient absorption, osmoregulation and barrier function against external pathogens and result in decreased growth, health and survival in heat stressed animals (Starck 2003). In contrast, minimal histopathological alterations were observed in the digestive tract of Tiger Abalone and Greenlip Abalone in response to heat stress and other on-farm culture stressors over the course of both farm trials This suggests that the chosen histopathological parameters may not be sensitive enough to detect alterations in digestive tract tissues due to repeated exposure to water temperatures above 22°C. Additionally, the sample size used for this analysis may have been too small to pick up such changes.

In summary, Schaefer et al. (2013) and Thomson (unpublished) suggest that severity of exposure to high water temperature could be an important factor that influence the integrity of the abalone digestive tract. Further research, with more targeted sampling using larger sample sizes during and immediately following temperature spikes, is recommended to ascertain if histopathological alterations occur in the digestive tract of abalone in response to combined heat and culture stressors.

The economic benefits of *Ulva* meal inclusion: Ingredient and diet cost, growth performance and feed utilisation.

The secondary project objective was to determine if feeding a diet containing 10% dried *Ulva* sp. meal would improve growth performance, feed utilisation and ultimately production cost across a summer to autumn grow-out period in two separate farm trials for Greenlip and Tiger Abalone grown under commercial conditions. In practical terms, the usefulness of dried algal meal is influenced by cost, nutritional value, dietary inclusion level and ultimately, how well it supports nutrient utilisation, growth, and productivity. The dried *Ulva* meal ingredient used in this study was relatively expensive (~\$5000 t^{-1}) when compared to the plant based dietary ingredients it replaced (between \$750 - \$860 t^{-1}, Aquafeeds Pty Ltd, 2019). The dried *Ulva* meal ingredient appeared to be of good quality and nutritionally, was high in protein (~31%), low in lysine (~2%) and methionine (~0.5%), low in total fat (~1%) and contained a low level of long chain omega 3 fatty acids (∑LC n-3 PUFA = ~0.1%).

The price (~\$5,000 $tonne^{-1}$) and availability of the domestically produced *Ulva* sp. meal used in the current study were limiting factors that resulted in the selection of the 10% dietary inclusion content. The 30% inclusion level of *Ulva* meal, as tested by Lange et al. (2014), was considered cost prohibitive at the time of diet manufacture for this project (November/December 2019) and supplies were limited to quantities sufficient to make 4 tonnes of the *Ulva* diet (2 tonnes for each farm). The inclusion of 10% dried *Ulva* sp. meal in the *Ulva* diet, at the exclusion of some cereal and pulse ingredients, resulted in a \$950 $tonne^{-1}$ increase in the dietary cost alone. The price of dried high quality, domestically produced *Ulva* sp. meal is forecast to drop in future as production levels increase. For example, in 2016 *Ulva* sp. meal produced primarily for human applications by Venus Shell Systems sold for > AUD\$ 20 kg^{-1}; however, this price was based on a three tonne per annum production capacity. Venus Shell Systems envisage that once production levels increase to in excess of 100 tonne per annum a short-term future price of AUD\$ 3 kg^{-1} will be attained by 2025 (Dr Pia Winberg, Owner Manager; Venus Shell Systems Pty. Ltd.; Pers. Comm.).

There was evidence to suggest the *Ulva* diet supported equivalent or slightly better growth and feed utilisation in both Tiger Abalone and Greenlip Abalone during the Yumbah Narrawong farm trial compared to the commercial diet (Table 3). In fact, the specific growth rate, and feed and nutrient utilisation of a mixed population of Greenlip Abalone and Hybrid Abalone were significantly improved by feeding the *Ulva* diet during the Yumbah Narrawong Trial (Table 3). This agrees with laboratory results that reported the dietary inclusion of dried macroalgae meals in formulated diets supported equivalent growth (Bates et al., 2017) or improved growth of Greenlip Abalone (Bansemer et al., 2016b). However, given that there were no significant differences in final biomass or biomass gain at the completion of the Yumbah Narrawong trial, caution must be exercised when interpreting growth results when relying on SGR alone (~9.6% difference), as a Type 1 error (false positive) may have been induced, given the low replication (*n* = 4 tanks) used during the Yumbah Narrawong trial. Numerous studies have demonstrated the importance of sufficient replication to ascertain a true statistical difference in growth without inducing Type 1 (false positive) or Type 2 (false negative) errors due to low experimental power and the high degree of variability in growth rates observed between individual abalone culture units (Britz, 1996a; 1996b; Coote et al., 2000; Stone et al., 2013; Stone et al., 2014b, 2014c; Stone et al., 2016). Based on historical growth data from a range of commercial abalone culture units derived from three separate Australian farm trials, Stone et al. (2014 b, 2014c, 2016) recommended that when conducting farm trials a minimum of eight culture unit replicates should be used to provide sufficient statistical power (≥ 0.8) to discern a 10% meaningful and reliable statistical difference between growth rates for different dietary treatments. Given that feed utilisation data is dependent on growth data, a high degree of replication is also required to ascertain differences in feed and nutrient utilisation data (Stone et al., 2016).

Feed efficiency and protein conversion efficiency are important economic indicators for aquaculture production. Lange et al. (2014) reported that the diet containing 30% dried *Ulva* sp. meal enhanced the feed acceptance rate of Greenlip Abalone compared to the commercial diet in the face of the 26°C water temperature challenge. Lange et al. (2014) suggested antioxidants and other bioactive compounds present in the dried *Ulva* sp. meal may have had beneficial effects on feed acceptance and feed utilisation, and ultimately survival of Greenlip Abalone exposed to heat stress. Both the commercial diet and the *Ulva* diet was observed to be palatable to abalone tested in both farm trials (Joshua McIntyre, Farm Manager, JTA Avalon, Pers. Comm.; Luke Thorpe, Farm Manager, Yumbah Narrawong, Pers. Comm.). Further research is recommended to investigate the potential beneficial effects on growth, product quality and survival of the inclusion of a range of dried algal meals in commercial production diets for cultured abalone.

As previously mentioned, the dried *Ulva* meal ingredient used in this study was relatively expensive (~\$5000 t^{-1}) when compared to the plant based dietary ingredients it replaced (between \$750 - \$860 t^{-1}, Aquafeeds Pty Ltd, 2019), and when included at the 10% in the diet, it contributed a \$950 $tonne^{-1}$ cost premium to the total cost of the *Ulva* diet when compared to the cost of the commercial diet. Ultimately, although it did not improve survival at the inclusion level of 10% tested at either farm in this study, this resulted in an \$729 or 8.4% increase in basic sales revenue per slab tank over the 166-d period of the Yumbah Narrawong trial when the mixed population of Tiger and Greenlip Abalone were fed the *Ulva* diet compared to the commercial diet. This outcome is economically beneficial and suggests further research using well replicated trials is required to assess the potential benefits of the inclusion of dried macro algae meals into diets on the productivity for farmed abalone.

Overall, there are numerous commercial benefits of producing larger abalone more quickly and cheaply. Of most relevance to the current study, based on a ~9.6% improvement in SGR achieved with economically irrelevant differences in survival as recorded for the *Ulva* diet treatment at Yumbah Narrawong, the overall duration of a typical three-year production cycle for Tiger or Greenlip Abalone in Australia may be shortened by ~3.5 months by feeding the *Ulva* diet. A reduced production cycle may also enable farmers to harvest abalone sooner, which may reduce the exposure of the larger more valuable stock to one less summer. This factor alone would result in significantly improved productivity and cost savings, and when combined with savings made with increased growth rates and feed efficiency and a reduced cost of production, a significant improvement in productivity across the entire grow-out period for abalone may be achieved by feeding the *Ulva* diet.

Conclusion

The primary project objective was to determine if dietary intervention, utilising 10% dried *Ulva* sp. meal in a practical formulated feed, could improve survival of Greenlip Abalone (*Haliotis laevigata*) and Tiger Abalone (*H. laevigata × H. rubra)* exposed to high summer water temperatures on-farm under commercial conditions.

To achieve the primary objective, firstly a suitable commercial type test diet containing 10% dried *Ulva* sp. meal had to be formulated and manufactured. The 10% dried *Ulva* sp. meal was successfully formulated and manufactured into an extruded commercial type diet which exhibited good water stability. Abalone were observed to readily accept this diet.

On mainland southern Australia, summer mortality typically occurs during periods of high summer water temperatures (> 22°C) and may also occur at even lower temperatures on Tasmanian farms. Abalone in both farm trials experienced mild summers and associated water temperatures. However, despite this, differences in water temperature profiles were still evident between the two farm trials during the trials. JTA Avalon experienced several instances where water temperatures exceeded 22°C during January and February, whereas during the Yumbah Narrawong trial water temperatures rarely exceeded 22°C (maximum, 22.5°C in early January) and remained relatively low compared to those recorded at JTA Avalon. Mortality patterns associated with summer mortality were observed during the farm trial at JTA Avalon, whereas, water temperatures were too low during the Yumbah Narrawong trial to induce mortalities associated with summer mortality. Unfortunately, the dietary inclusion of 10% dried *Ulva* sp. meal did not result in any significant improvement in survival during either farm trial. In fact, survival appeared to be lower during both farm trials (JTA Avalon survival: commercial diet survival 96.4% vs. 93.4% for the *Ulva* diet; Yumbah Narrawong survival: commercial diet 98.1% vs 97.5 % for the *Ulva* diet) when 10% dried *Ulva* sp. meal was included in the diets.

Several histopathological parameters were chosen to investigate the effects of the dietary inclusion of 10% dried *Ulva* sp. meal and heat stress during periods of high summer water temperatures on the digestive tract and gill health of Greenlip and Tiger Abalone cultured on-farm under commercial conditions. The inclusion of 10% dried *Ulva* sp. meal did not lead to any significant histopathological alteration in digestive tract or gill structure of abalone in either trial. The absence of any detectable alterations in digestive tract histopathology implied that abalone in the current study were not adversely affected by the inclusion of 10% dried *Ulva* sp. meal to formulated diets. This outcome provides industry with the confidence to continue to use macroalgae meals safely within formulated abalone diets. Further investigation is recommended with increased inclusion (up to 30%) of macroalgae in diets for commercially cultured abalone.

Over the course of the trials heat stress during the commercial production of abalone appeared to be one of the main drivers associated with histopathological alterations, which were chiefly noted in the gill leaflet tips of Tiger Abalone. Gill leaflet tip damage scores appeared to be useful indicators of the combined effects of culture stress and heat stress in abalone, whereas gill goblet cell numbers and gill sinus fluid scores appeared to more useful as indicators of general stressors. Based on the histopathological results obtained from the current study, we would recommend that future studies continue to investigate histopathological changes to the gill structure of abalone in response to heat stress and summer mortality. It is worth noting, only tissue samples from surviving abalone were assessed from each farm trial during this project; therefore, the discussion of the results presented in this report may represent characteristics either associated with survival or progression towards mortality. Future studies should attempt to incorporate more targeted samples from moribund abalone to gain a better insight into the changes that occur immediately leading up to death.

The secondary project objective was to determine if feeding a diet containing 10% dried *Ulva* sp. meal would improve growth performance, feed utilisation and ultimately production cost across a summer to autumn grow-out period in two separate farm trials for Greenlip and Tiger Abalone grown under commercial conditions. During the Yumbah Narrawong trial the dietary inclusion of 10% dried *Ulva* sp. meal improved growth performance, feed and nutrient utilisation for a mixed population of Tiger and Greenlip Abalone, and compared to the commercial diet ultimately led to a $729 or 8.4% increase in basic sales revenue per slab tank for the 166-d trial period. Unfortunately, due to logistical constraints, data pertaining to growth performance was not available from the JTA Avalon trial. However, the increase in basic sales revenue recorded at Yumbah Narrawong was a significant finding, and suggests further research is necessary to assess

the potential benefits of the inclusion of dried macro algae meals into diets on the productivity for farmed abalone.

Overall, there are numerous commercial benefits of producing larger abalone more quickly and cheaply. Of most relevance to the current study, based on a ~9.6 % improvement in SGR achieved with economically irrelevant differences in survival as recorded for the *Ulva* diet treatment at Yumbah Narrawong, the overall duration of a typical three-year production cycle for Tiger or Greenlip Abalone in Australia may be shortened by ~3.5 months by feeding the *Ulva* diet. A reduced production cycle may also enable farmers to harvest abalone sooner, which may reduce the exposure of the larger more valuable stock to one less summer. This factor alone would result in significantly improved productivity and cost savings, and when combined with savings made with increased growth rates and feed efficiency and a reduced cost of production, significant improvements in productivity across the entire grow-out period for abalone may be achieved by feeding the *Ulva* diet.

Implications

The impacts of the outcomes on end user from this study are significant and are as follows:

- The project demonstrated that the prices of dried algal meals produced domestically, although still expensive, are now approaching commercial reality for use in formulated commercial abalone feeds. Prices of dried algal meal will fall further as production levels increase. This implies that to improve growth performance it is now time for abalone farmers to demand that feed companies start to incorporate higher levels of dried macroalgal meals in abalone feeds.
- Although the dietary inclusion of 10% dried *Ulva* sp. meal did not improve survival of abalone at either farm in this study, it did result in a $729 or 8.4% increase in basic sales revenue per slab tank over the 166-d period when the mixed population of Tiger and Greenlip Abalone were cultured under mild summer conditions and fed the *Ulva* diet compared to the commercial diet during the trial at Yumbah Narrawong. This outcome is significant and suggests further research to assess if the inclusion of a range of different dried macro algae meals into diets would be beneficial for productivity of abalone farms.
- Results from the project support the concept that diets for improved abalone production should not be formulated on a least cost basis, but rather on an ingredient quality and abalone and economic performance basis. As demonstrated on a commercial basis in the current project, in terms of growth performance and differences in basic sales revenue, a diet that costs $950 tonne^{-1} more to produce, outperformed a well performing commercial diet by between 8 to 10%. Feed manufactures and abalone producers need to be open to paying a premium price for high quality ingredients used to formulate improved production diets to obtain optimum performance and increased profits for their cultured abalone.
- Overall, there are numerous commercial benefits of producing larger abalone more quickly and cheaply. Of most relevance to the current study, based on a ~9.6 % improvement in SGR achieved with economically irrelevant differences in survival as recorded for the *Ulva* diet treatment at Yumbah Narrawong, the overall duration of a typical three-year production cycle for Tiger or Greenlip Abalone in Australia may be shortened by ~3.5 months by feeding the *Ulva* diet. A reduced production cycle may also enable farmers to harvest abalone sooner, which may reduce the exposure of the larger more valuable stock to one less summer. This factor alone would result in significantly improved productivity and cost savings, and when combined with savings made with increased growth rates and feed efficiency and a reduced cost of production and significant improvements in productivity across the entire grow-out period for abalone may be achieved by feeding the *Ulva* diet.
- Currently, the importation of dried macroalgal meals into Australia for use in aquafeeds is restricted because of biosecurity risks. The positive outcome for growth and the economics of production demonstrated by this study showed that domestically produced dried macroalgae meals may be viable alternatives.
- The inclusion of levels of > 5% macroalgal meals into commercial abalone diets has previously been considered difficult, as algal meals are considered to impart moderate to high levels of negative functionality during the extrusion pelleting process which may negatively impact pellet water stability. Commercial quantities of water stable extruded diets containing 10% dried *Ulva* sp. meal were formulated and manufactured during this project. This technical demonstration will provide confidence for feed manufacturers to go forward and include higher levels of macroalgal meals to produce commercial abalone diets.
- The lack of alterations in the digestive tract tissues measured in this study indicates the suitability of dried *Ulva* sp. meal as an ingredient for inclusion in commercial abalone feeds.
- In the current study gills appeared to be more sensitive to histopathological alterations in response to the combined effects of culture stressors and heat stress than the digestive tract. This result demonstrates the importance of the inclusion of histopathological examination of the gills to assess abalone health in response to seasonal water temperature changes in future trials.

Recommendations

A point by point list of recommendations on the activities or other steps that may be taken to further develop, disseminate or to exploit commercially the results from this project is provided below:

- Although the 10% dried *Ulva* sp. meal was successfully formulated and manufactured into an extruded commercial type diet which exhibited good water stability during the current project, as the supply of dried algal meals is expected to become more commercially viable in the near future we recommend further research to optimise higher inclusion levels of dried algal meal, whilst still maintaining adequate diet water stability.
- Mortality patterns associated with summer mortality were observed during the farm trial at JTA Avalon, whereas, water temperatures were too low during the Yumbah Narrawong trial to induce mortalities associated with summer mortality. Bearing this in mind we would recommend well replicated farm trials that are designed to study summer mortality in the future should be appropriately placed on farms that traditionally experience high summer water temperatures routinely.
- The dietary inclusion of 10% dried *Ulva* sp. meal did not result in any significant improvement in survival during either farm trial. However, based on results from a previous laboratory trial where diets containing 30% dried *Ulva* sp. meal significantly reduced mortality in Greenlip Abalone exposed to heat stress (25°C), we recommend that once dried algal meals production increases and supplies become more available, studies investigating the effects of higher inclusion levels of ≥ 20% macroalgae on improving survival should be undertaken on-farm during summer.
- The $729 or 8.4% increase in basic sales revenue per slab tank for the 166-d trial period recorded at Yumbah Narrawong for the abalone fed the *Ulva* diet compared to those fed the commercial diet during mild summer growing conditions suggests further research is necessary to assess the potential benefits of the inclusion of dried macro algae meals into diets on the productivity for farmed abalone. Australian cultured abalone have been reported to grow better when fed certain species of red macroalgae (*Gracilaria cliftonii*) compared to green species of macroalgae (*Ulva* sp.). However, currently dried meals derived from *G. cliftonii* or other red macroalgae are not available in commercial quantities domestically, therefore, we recommend that in the short term, nutrition research is applied to investigate the potential of domestically produced commercially available dried meals derived from *Ulva* sp. until meals from red species become commercially available.
- Given the sensitivity of the gills in response to the combined effect of culture stressors and heat stress, as exemplified by alterations to gill leaflet tip structure and function, it is recommended that future studies relating to mortality due to exposure to high summer water temperatures with abalone, are designed to include the examination of histopathological alterations to the gills.
- We also recommend that histological investigation of the digestive tract tissues may be more beneficial in determining aspects of histopathological alterations to the epithelial layer in longer term trials where abalone may be exposed to an extended and wider range of seasonal and culture fluctuations.
- We also recommend that histopathological investigation of the digestive tract is always carried out when testing new ingredients in diets for abalone, especially when investigating higher inclusion levels of macroalgae of up to 30%. This approach will give the feed industry and abalone producers more confidence to incorporate new ingredients into commercial production diets.
- Future studies should incorporate more targeted sampling from moribund abalone to gain a better insight into the histopathological alterations that occur immediately leading up to death.

Further development

The current project has provided industry stakeholders with valuable information to assist the Australian abalone aquaculture industry improve productivity and profits. Overall, results from this project bode well for the future development of the abalone aquaculture industry in Australia. However, dietary development work for this industry should not remain static, as important advancements in our knowledge of the inclusion of macroalgae into diets for abalone will need to be ongoing to ensure the economically sustainable and healthy production of Australian abalone and a flourishing industry. Throughout the project opportunities for further research and development were identified and include:

- Improve strategic approaches to summer mortality management based on understanding which treatments are best in which circumstances.
- Attend a workshop involving all abalone aquaculture industry participants following completion of the project to identify and prioritise future needs to further drive the development of this industry.
- Refine our approach based on results from this commercial project and other projects and run another trial with higher macroalgae inclusion levels, more replication, and other types of macroalgae meal to improve abalone survival over summer.
- Further investigation of bioactive components of macroalgae to enhance the survival of abalone during summer.
- Further work evaluating the optimum types of seaweed for inclusion into feeds to improve growth and health should be undertaken for juvenile and sub-adult abalone to advance the sustainable performance of abalone.
- Further investigate and identify the growth promoting components of macroalgae meals for abalone.
- Further improve the sustainable production of abalone by investigating the potential of macroalgal meals as fish meal replacement to reduce the industry's current reliance on the use of in-feed marine products.
- Further investigate and identify general stressors that impact histopathological parameters in gills of abalone. This includes water quality (pH, ammonia, nitrite, dissolved oxygen) and farm practices (tank cleaning, tank stocking density).
- Histopathological analysis of multiple abalone species to identify and determine species specific health parameters within the digestive tract and gills that can be utilised to assess health status due to heat stress and other general stressors.
- Development and adoption of new scoring methods for gills histopathological parameters in response to heat stress, such as those put forward by Pedler et al. (accepted).
- Ensure that the results of the ongoing PhD project indirectly linked to this project are captured and disseminated to industry (PhD candidate: Nicole Thomson; Project: An assessment of abalone gills and digestive tract: identifying changes due to species, size, season and diet; School of Animal and Veterinary Sciences, University of Adelaide, South Australia, Australia. Supervised by Prof. Gordon Howarth, A/P David Stone, Dr Rebecca Forder).

Extension and Adoption

Extension

The application was developed in direct collaboration with members of the Australian Abalone Growers' Association (AAGA), Aquafeeds Pty, and other research providers to meet a specific industry need. This project comprised an array of collaborators which included participants from a number of private companies (Jade Tiger Abalone Avalon; Yumbah Narrawong; Aquafeeds Pty Ltd; Thomson Image Analysis Services) and organisations (AAGA), universities (Flinders University; University of Adelaide) and government departments (SARDI; PIRSA). Of particular importance to this work was the strong collaborative networks that were forged with the relevant industry participants and the feed company. Their continued support throughout the project and in generating the R&D within this report was paramount in the project's success.

SARDI, PIRSA and AAGA, the participating farm managers (Joshua McIntyre, Jade Tiger Abalone Avalon; Luke Thorpe, Yumbah Narrawong), Joel Scanlon of Aquafeeds Pty Ltd and participating co-investigative researchers communicated by e-mail, telephone, and face-to-face meetings, on a frequent ad-hoc basis. The primary purposes of these communications were to engage industry in the development and design of research, coordinate sampling and the supply of materials, provide updates on the progress of each individual research component undertaken and discuss the interpretation and use of research results. These discussions are ongoing.

This project also conducted complimentary activities aligned with another FRDC/AAGA project entitled "Risk factors and management strategies associated with summer mortality in Australian abalone (FRDC Project No: 2019-147)". Dr Matthew Bansemer (PIRSA) who was the Principal Investigator of the FRDC Project (No: 2019-147), participated in regular on-farm sampling events for the trials in the current project to assist with sampling and final report writing. Matthew also conducted complementary activities within his own project whilst on the farm visits. This arrangement enabled Matthew Bansemer a direct and immediate insights into the results from the current project as they came to hand.

Apart from this final report, to date no publications have arisen from this project. However, a chapter related to the histopathological component of this project will be included in a PhD thesis, with a publication in a peer reviewed to follow (Nicole Thomson; Project: An assessment of abalone gills and digestive tract: identifying changes due to species, size, season and diet; School of Animal and Veterinary Sciences, University of Adelaide, South Australia, Australia. Supervised by Prof. Gordon Howarth, A/P David Stone, Dr Rebecca Forder).

Industry Adoption

There is an enhanced appetite by AAGA members for the inclusion of suitable and economical macroalgae meals in diets for cultured abalone.

Joel Scanlon of Aquafeeds Pty Ltd is currently investigating the use of higher commercial inclusion levels of several different types of dried macroalgae meals in diets for abalone production.

Information pertaining to the growth performance benefits of a current commercial diet (Go2 diet, Aquafeed Pty Ltd) used as the control in this study has led to the extended use of this diet in production systems.

Project materials developed

Apart from this final report, to date no publications have arisen from this project. However, a chapter related to the histopathological component of this project will be included in a PhD Thesis, with a publication in a peer reviewed to follow (Nicole Thomson; Project: An assessment of abalone gills and digestive tract: identifying changes due to species, size, season and diet; School of Animal and Veterinary Sciences, University of Adelaide, South Australia, Australia. Supervised by Prof. Gordon Howarth, A/P David Stone, Dr Rebecca Forder).

Images (JPEG) of the histological slides of digestive tract and gill tissues from the abalone sampled within this project may be obtained by request from the project's Principal Investigator at SARDI and from AAGA.

Appendices

Appendix 1: Stocking numbers and mortality data from each farm trial

Table A1-1. Number of abalone stocked and mortality data for each treatment for the Jade Tiger Abalone farm trial.

Tank	Treatment	Opening number per tank	Mortality number per tank	Mortality %
B05	Control	18,126	2,887	15.9%*
C01	Control	13,283	316	2.4%
D06	Control	12,989	27	0.2%
E01	Control	19,033	352	1.8%
E06	Control	14,136	99	0.7%
F05	Control	12,206	382	3.1%
G07	Control	13,954	142	1.0%
Mean		*14,818*	*601*	*3.6%*
Standard deviation		*2,660*	*1,017*	*5.5%*
B06	Ulva	15,693	2,370	15.1%
C02	Ulva	14,003	366	2.6%
D05	Ulva	17,714	72	0.4%
E02	Ulva	17,345	631	3.6%
E05	Ulva	14,269	347	2.4%
F04	Ulva	12,922	1,916	14.8%
G06	Ulva	17,718	1,221	6.9%
Mean		*15,666*	*989*	*6.6%*
Standard deviation		*1,978*	*875*	*6.1%*

*Denotes a significant outlier (Tukey's outlier test; 95% confidence intervals: lower bound-1.52%, upper bound, 8.69%)

Table A1-2. Number of abalone stocked and mortality data for each treatment for the Yumbah Narrawong farm trial.

Tank	Treatment	Opening number per tank	Mortality number per tank	Mortality %
A31	Control	9,844	256	2.60
A32	Control	9,396	178	1.89
C29	Control	9,864	177	1.79
C30	Control	10,525	126	1.20
Mean		*9,907*	*184*	*1.87*
Standard deviation		*465*	*54*	*0.58*
A29	Ulva	11,566	343	2.97
A30	Ulva	11,282	201	1.78
C31	Ulva	10,266	247	2.41
C32	Ulva	10,425	295	2.83
Mean		*10,885*	*272*	*2.50*
Standard deviation		*637*	*61*	*0.53*

Appendix 2: List of researchers and project staff

A/P David A.J. Stone (Principal Investigator): South Australian Research and Development Institute (SARDI) Aquatic Sciences: West Beach, South Australia

Mr Joel Scanlon (Co-Investigator): Owner / Manager Aquafeeds Pty Ltd, Mount Barker, South Australia

A/P James O. Harris (Co-Investigator): College of Science & Engineering, Flinders University, Adelaide, South Australia

Prof. Gordon S. Howarth (Co-Investigator), School of Animal and Veterinary Sciences, University of Adelaide (Roseworthy Campus), South Australia

Mr Tim Rudge (Manager): Yumbah Narrawong, Allestree, Victoria.

Mr Luke Thorpe (Farm Manager): Yumbah Narrawong, Allestree, Victoria.

Mr Joshua McIntyre (Farm Manage): Jade Tiger Abalone, Avalon, Victoria

Dr Matthew Bansemer (Project participant): Department of Primary Industries and Regions (PIRSA), Fisheries and Aquaculture, West Beach, South Australia

Nicole L. Thomson (Consultant and PhD candidate), Thomson Image Analysis service, Adelaide, South Australia, and School of Animal and Veterinary Sciences, University of Adelaide (Roseworthy Campus), South Australia

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