Final Report

Refining Yellowtail Kingfish feeds and feed management

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Non-Technical Summary

2013/730: Refining Yellowtail Kingfish feeds and feed management

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PROJECT OBJECTIVES

1. Identify or improve production diets in a tank based setting using large (> 1.5 kg) Yellowtail Kingfish to provide information to reduce feed conversion ratios (FCRs) for Clean Seas Yellowtail Kingfish production by 0.45 units across the entire sea cage production cycle.
2. Identify or improve production diets in a tank based setting using large (> 1.5 kg) Yellowtail Kingfish to provide information to gain a 10% improvement in growth for Clean Seas Yellowtail Kingfish in sea cage production systems.
3. Identify or improve production diets in a tank based setting using large (> 1.5 kg) Yellowtail Kingfish to provide information to improve Yellowtail Kingfish health and reduce mortality by 2% in Clean Seas sea cage production systems.
4. Improve feeding strategies in a tank based setting using large (> 1.5 kg) Yellowtail Kingfish to provide information to improve Yellowtail Kingfish growth and feed utilisation by 2% in Clean Seas sea cage production systems during winter.

ABSTRACT

The sustainable and economically viable production of Yellowtail Kingfish (*Seriola lalandi*) relies on cost effective diets that have been formulated with a low level of marine ingredients due to economic and ecological issues associated with fish meal (FM) and fish oil (FO) production, while still providing a balanced combination of essential nutrients.

The key research findings described in this project addressed the highest nutritional research priorities identified by Clean Seas in 2013:

1. Establish reliable commercial formulations that contain sufficient essential and non-essential nutrients for the production of large (> 1.5 kg) Yellowtail Kingfish.

2. Improve the current status of knowledge on the energy requirements of large (> 1.5 kg) Yellowtail Kingfish.

Two trials (Trial 1 and Trial 2 lasting 5 and 3 months, respectively) were conducted over the summer months in the pool-farm facility at the South Australian Research and Development Institute's (SARDI's), South Australian Aquatic Sciences Centre (SAASC), West Beach, Adelaide, South Australia (SA). Both trials utilised eight different treatment combinations to investigate the growth performance, feed utilisation, health, survival and carcass composition of large (> 1.5 kg) Yellowtail Kingfish fed a range of commercially formulated diets that contained a range of FM and FO substitutions and energy levels. The trials were conducted in large tanks (24 × 5000 L; n = 3 replicate tanks treatment^{-1}) in a land-based

oxygenated semi-recirculating seawater system (100% water exchange day^{-1}). Fish in each trial were fed to apparent satiation and a selection of treatments were fed a sub-satiation ration of 80% apparent satiation. A third trial investigating growth performance and feed utilisation was conducted over the three winter months (average temp, 12.8 °C) in the pool-farm facility at the SAASC. Eight treatments were investigated. Fish were fed the newly formulated Ridley Clean Seas 2014 Pelagica diet (Ridley Auafeed trading as Ridley Agriproducts Pty Ltd) at seven feed rates from 0.10% body weight (BW) one day week^{-1} to apparent satiation six days week^{-1}. An additional diet comprised of thawed, diced Sardines (*Sardinops sagax*) was fed every second day to apparent satiation. All diets were fed in a single period between approximately 0930h and1130 h.

Overall, results suggest that growth performance of large Yellowtail Kingfish in the pool-farm facility at the SAASC was comparable to that achieved for the same fish cohort in the Clean Seas sea cage systems in Spencer Gulf, SA. These results indicate that this system is suitable for experimental work; including diet benchmarking and validation trials with large (> 1.5 kg) Yellowtail Kingfish, prior to scaling up to pilot scale sea cage validation trials at Clean Seas. Additionally, Yellowtail Kingfish grown in the recirculating tank system during summer (ranging from 17 °C - 26 °C in Trial 1; 20 °C - 26 °C in Trial 2) at the SAASC returned better FCR (Trial 1, 1.7 - 2.3; Trial 2, 1.6 - 1.7) than from the same cohort of fish grown in the Clean Seas sea cages (≥ 2.0); suggesting that factors, other than diet, may also be of interest to improve the growth of Yellowtail Kingfish in sea cages. In particular, it may be worthwhile to investigate the potential for the application of oxygenation to commercial sea cages to improve dissolved oxygen levels, growth, feed utilisation and resultant FCR and profits. With regard to winter growth performance and feed utilisation, feeding fish to apparent satiation once a day on two days week^{-1} appeared to be the minimum required ration to maintain positive growth (> maintenance ration, 0.2047% BW d^{-1}; 56.3 kJ fish^{-1} d^{-1}). However, better growth and feed utilisation was observed in fish fed once a day to apparent satiation six days week^{-1}. Although fish performed well, and it may be economically viable to feed Sardines in winter, the fish in-fish out ratio (tonnes of wild fish it takes to produce one tonne of farmed fish; Jackson, 2009) for Yellowtail Kingfish fed Sardines every second day was inferior to those fed the Ridley Clean Seas 2014 Pelagica diet to apparent satiation six days per week. Further refinements of feeding frequency and rates are required to optimise winter feeding.

Australian formulated and manufactured Yellowtail Kingfish diets performed as well as or better than an international Japanese Yellowtail diet. The manipulation of feeding strategies provided important information with regard to improving FCR and indicated potential monetary gains may be made with careful feed management during both summer and winter months. Fish meal (30%; FM) and fish oil (9%; FO) content of production diets for large Yellowtail Kingfish may be reduced significantly over previous levels, as long as essential long chain omega-3 polyunsaturated fatty acids LC n-3 PUFA (Σ eicosapentaenoic acid [EPA] + docosapentaenoic acid [DPA] + docosahexaenoic acid [DHA]) are maintained above 2 g 100 g^{-1} diet. Fish meal and FO substitution also improved sustainability by reducing the fish in-fish out ratio by 25%, to 50%. This should enhance consumer perception of cultured Yellowtail Kingfish and improve market access.

Based on results from the three tank-based trials, Clean Seas adopted the newly formulated Ridley Clean Seas 2014 Pelagica diet from this project for the commercial production of large Yellowtail Kingfish in their sea cages. In addition, feed management strategies were also adjusted on-farm. Clean Seas was also interested in using high energy diets for the production of large Yellowtail Kingfish next summer; which in turn, would also reduce the reliance on FM and FO, and contribute to reduce feed costs.

Further research is required into aspects of lipid and energy metabolism in larger fish, particularly under conditions of restrictive feeding practices during both summer and winter

periods. Overall, results from this project bode well for the future development of the Yellowtail Kingfish industry in Australia. However, dietary development work for this industry should not remain static, as important advancements will need to be ongoing to ensure the economically sustainable production of Australian Yellowtail Kingfish.

OUTCOMES ACHIEVED

The planned outcomes of the project was that Clean Seas attain:

- A 0.45 unit reduction in FCR averaged across the entire production cycle.
- A 10% improvement in growth.
- Improved fish health and a 2% reduction in mortality.
- Improve winter feeding strategies.
- Decreased cost of production.

In order to achieve the planned outcomes, four Objectives were established in consultation with Clean Seas and the participating feed company, Ridley Aquafeed, and a series of tank-based trials (Chapters 2, 3 and 4) were designed and carried out to completion. The four Objectives, along with the outcome for each, are provided below.

Objective 1.
Identify or improve production diets in a tank based setting using large fish (> 1.5 kg) to provide information to reduce FCRs for Clean Seas Yellowtail Kingfish production by 0.45 units across the entire sea cage production cycle.

Objective 2.
Identify or improve production diets in a tank based setting using large fish (> 1.5 kg) to provide information to gain a 10% improvement in growth for Clean Seas Yellowtail Kingfish in sea cage production systems.

The outcomes for Objectives 1 and 2 were achieved.

FCR generated in tank-based experiments during summer always exceeded (i.e. below) the target reduction of 0.45 units. New dietary formulations and the manipulation of feeding strategies provided important information with regard to improving growth, feed utilisation and FCR. The positive growth and feed utilisation obtained in Trial 1 and 2 (Chapters 2 and 3) resulted in improved FCR in the tank-based trials which were always ≤ 2.0. These FCR compared favourably to the FCR recorded for the same cohort of fish grown in Clean Seas sea cages over the corresponding period. The improvement of growth and FCR was not only attributed to improved diet formulations and feeding strategies, but also to increased levels of dissolved oxygen in the tank-based culture systems. To realise the same improvements on farm in sea cage systems the feasibility of oxygenation of sea cages needs to tested.

Increased FM and FO substitution in Yellowtail Kingfish diets should also reduce diet ingredient costs by ~\$100 t^{-1}. Fish meal and FO substitution will also improve environmental sustainability by reducing the fish in-fish out ratio by 25%, to 50%.

Objective 3.

Identify or improve production diets in a tank based setting using large fish (> 1.5 kg) to provide information to improved Yellowtail Kingfish health and reduce mortality by 2% reduction in Clean Seas cage production systems.

The outcomes from Objective 3 were achieved.

Results from the tank-based trials (Chapters 2 and 3) indicate that fish survival and health was positive in response to the diets tested. There were no visual signs of disease observed in either tank-based trial. Mortality of large Yellowtail Kingfish due to nutritional shortcomings may be reduced to practically zero if diets are formulated to provide the essential nutrients. However, compared to the tank-based environment, sea cage conditions are more stressful on fish. In order to validate tank results the new diets need to be tested on-farm over a growing season.

Objective 4.
Improve feeding strategies in a tank based setting using large (> 1.5 kg) Yellowtail Kingfish to provide information to improve Yellowtail Kingfish growth and feed utilisation by 2% in Clean Seas sea cage production systems during winter.

The outcome from Objective 4 was achieved.

With regard to winter growth performance and feed utilisation (Chapter 4), feeding Yellowtail Kingfish to apparent satiation once a day, two days week^{-1} (> maintenance ration, 56.3 kJ fish^{-1} d^{-1}) appeared to be the minimum required ration to maintain positive growth. However, fish fed to apparent satiation six days week^{-1} exhibited superior growth and feed utilisation than fish fed lower feed rates or frequencies. As a result of this research, Clean Seas adopted new winter feeding strategies.

Although fish performed well, and it may be more economical to feed Sardines in winter, Yellowtail Kingfish fed Sardines every second day exhibited an inferior fish in-fish out ratio compared to fish fed the Ridley Clean Seas 2014 Pelagica diet to apparent satiation six days per week. Further refinements of feeding frequency and rates when fed the Ridley Clean Seas 2014 Pelagica diet may further optimise winter feeding strategies.

Overall, information generated during this project should aid in reducing the cost of production for Yellowtail Kingfish producers. The Australian Yellowtail Kingfish producers now have the confidence to use newly formulated commercial diets, produced by Australian feed companies, to improve the weight gain, feed utilisation, FCR, survival and productivity of Yellowtail Kingfish. Improvements to winter feeding strategies were also realised. Additionally, Clean Seas, Ridley Aquafeed and research providers worked closely to achieve these goals. All groups have identified the importance of nutritional research in relation to growth, health and improved productivity for Yellowtail Kingfish production.

Of note, Australian Yellowtail Kingfish producers and researchers have agreed on the need to increase our understanding of Yellowtail Kingfish nutrition, physiology and health. This has led to the successful funding of a larger project investigating nutritional requirements, feeding strategies and nutrition health of Yellowtail Kingfish through the Fisheries Research and Development Corporation (FRDC) as part of the Rural Research and Development for Profit Programme, Australian Department of Agriculture and Water Resources. The Federal Government project commenced in September 2015 and will be comprised of industry and research partners from South Australia and New South Wales, with an aligned project through the FRDC broadening collaboration to involve a Western Australian industry and research

provider.

LIST OF OUTPUTS PRODUCED

This project was commercially oriented and designed to assist with the development of improved dietary formulations for Yellowtail Kingfish for use by Clean Seas and other farmers. The research described in this project has been extended directly to Clean Seas, Ridley Aquafeed and other industry stakeholders.
The most notable research outputs include:

Reports and publications

- One final report.

- A report on effects of diet on cellular immune parameters of Yellowtail Kingfish pre- and post-hypoxic stress (Appendix 1).

Commercial Yellowtail Kingfish diet formulations and feeding strategy and fish performance information

- Several new diet formulations for large Yellowtail Kingfish at summer water temperatures have been developed. The biochemical compositions of the formulations are available in Tables 2.1 and 3.1 in Chapters 2 and 3 of this report. The ingredient formulations remain commercial in-confidence to the project participants.

- Information pertaining to growth performance, feeding behaviour, fish composition and health (Chapters 2 and 3).

- Information regarding winter feeding rates and frequencies (Chapter 4).

Student training (Appendix 2)
There has been a considerable student training component to this project:

- Assisting in the training of greater than 50 undergraduate extra-mural work experience students from the School Biological Sciences at Flinders University and the University of Adelaide's Department of Animal and Veterinary Sciences.

- Training one Honours student from the School of Biological Sciences at Flinders University.

- Assisting in the training of five PhD students from the School of Biological Sciences at Flinders University.

A major benefit of the student training component is the output of new industry entrants, trained with relevant skills that will contribute to future industry development. Several students have already obtained work within the industry.

The pending peripheral student research associated with the project will also provide valuable insight into lipid metabolism and growth in relation to diet manipulation at optimal and sub-optimal water temperatures for Yellowtail Kingfish. This information is essential in improving feeding practices and productivity on-farm.

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Chapter 1. Introduction

Yellowtail Kingfish, *Seriola lalandi*, is a relatively new species to aquaculture in South Australia, Western Australia and New South Wales, with over 90% of production from Clean Seas (Spencer Gulf, South Australia). The production of Yellowtail Kingfish in South Australia was approximately ~3,000 t in 2010 and anticipated to reach 10,000 t by 2015 in the Spencer Gulf region (personal communication, Mr M. Thomson; Clean Seas). However, during the expansion process, production issues associated with nutrition and health led to high mortalities and this level of production has yet to be realised.

Clean Seas has assessed the situation, identified a solution to the pre-existing problem, and now wish to consolidate and expand Yellowtail Kingfish production in their sea cage operations. Clean Seas aimed to achieve this by expanding on the findings of several recently completed Australian Seafood Cooperative Research Centre (AS-CRC)/Clean Seas Yellowtail Kingfish production, nutrition and health projects, in combination with addressing some new R&D priorities. The AS-CRC and Clean Seas have investigated new methods and production options that have the potential to attain maximum growth rates and survival of Yellowtail Kingfish grown in sea cages. These new strategies are aimed at truncating the grow-out period of Yellowtail Kingfish (expose fish to one less winter) which will result in more fish produced per unit of capital investment, ultimately increasing production efficiency. Many of the potential improvements in growth and performance will come through better husbandry and feeding practices, as well as a better understanding of the impacts that environmental parameters, such as temperature, have on growth.

Some of the most influential issues affecting Yellowtail Kingfish growth in sea cages have already been identified and researched. For instance the AS-CRC/Clean Seas and AS-CRC project participants have investigated the effect of stocking size and temperature on growth and performance of small and large Yellowtail Kingfish (Understanding Yellowtail Kingfish; AS-CRC Project 2008/903). The outcomes of that work have assisted in making production management decisions. A recent AS-CRC/Clean Seas project (Sustainable feeds and feed management in Yellowtail Kingfish; AS-CRC Project 2009/728) also addressed several high priority research topics, such as improving the sustainability of Yellowtail Kingfish farming by investigating the maximum dietary inclusion levels of alternative protein (e.g. solvent extracted soybean meal and soy protein concentrate) and lipid sources (e.g. poultry oil and canola oil) to reduce the reliance on fish meal (FM) and fish oil (FO), at optimal (22 °C) and sub-optimal (18 °C) water temperatures. Information arising from the sustainable feeds project also identified potential shortfalls in the nutritional composition of commercial diets used in the production of Yellowtail Kingfish (Stone and Bellgrove, 2013; Stone and Bowyer, 2013). Bearing the above results in mind, Clean Seas present business model and future plan are to build a profitable Yellowtail Kingfish business.

To accomplish this, Clean Seas management identified two key areas which required urgent R&D investment:

1. The establishment of reliable commercial diet formulations containing sufficient essential and non-essential nutrients for the production of Yellowtail Kingfish.

2. The refinement of the current status of knowledge on the energy requirements of larger (> 1.5 kg) Yellowtail Kingfish.

Subsequent analysis of the nutrient profile of Yellowtail Kingfish production diets used over an extended period at Clean Seas operations (Stone, unpublished data), and a comparison with information provided from a literature review of the current status of the nutrient requirements of Yellowtail Kingfish (Sustainable Feeds and Feed Management for Yellowtail Kingfish; AS-CRC Project 2009/728) identified potential issues in some instances with the

provision of certain dietary nutrients such as taurine and potentially, histidine an essential amino acid, and other vitamins and minerals in current Yellowtail Kingfish commercial diet formulations (Stone and Bellgrove, 2013).

Taurine, a sulphonated organic acid, is essential for important physiological roles such as gut development, mucus production, cellular osmoregulation, anti-oxidative defence, bile production and lipid digestion and development of visual, muscular and neural systems in fish (Fang et al., 2002; Oliva-Teles, 2012). The closely related Japanese Yellowtail cannot synthesise taurine endogenously, therefore, the dietary provision of this sulphonated organic acid is conditionally essential (Stone and Bellgrove, 2013). Levels of 5 g kg^{-1} to 8 g kg^{-1} diet have been recommended for Yellowtail Kingfish when dietary FM levels of at least 30% are used. Moreover, when fish meal taurine levels are low, or when dietary FM substitution reduced dietary FM levels below 30% more dietary taurine is required. It is highly likely that Yellowtail Kingfish cannot synthesise taurine, and further work is needed to test diets supplemented with this conditionally essential nutrient.

Commercially available standard solvent extracted soybean meal (SESBM) has been tested in diets for Yellowtail Kingfish with varying success due to the presence of a range of anti-nutritional factors. Bowyer et al. (2013a; b) reported the inclusion of SESBM above 20% caused reductions in growth performance, and inclusions above 10% caused reductions in nutrient retentions and digestibility. Additionally, Bansemer et al. (2015) and Bansemer (2011) also examined the digestive tract of standard SESBM fed Yellowtail Kingfish and reported signs of mucus layer erosion and an increase in goblet cell abundance with increasing SESBM dietary inclusion, both early symptoms of enteritis. Therefore, based on these findings Stone and Bowyer (2013) suggested the use of 10% commercially available standard SESBM in diets for Yellowtail Kingfish. Further processing of standard SESBM to soy protein concentrate (SPC), which contains lower levels of anti-nutrients, resulted in improved growth at increased dietary inclusion levels (20%) in diets for Yellowtail Kingfish (Bowyer et al., 2013b). At the current price (> \$2,000 t^{-1}) SPC is considered cost prohibitive to use in diets for Yellowtail Kingfish. Recently, Ridley Aquafeed procured the sole rights to use a new strain of modified SESBM that contains low levels of anti-nutritional factors. This modified product is far cheaper than SPC, on a cost per unit protein basis, and only slightly more expensive to use than standard SESBM. Ridley Aquafeed has tested this product with FM replacement in Yellowtail Kingfish diets and reported good growth and health at FM substitution levels of 60% (~30% SBM inclusion), compared to a FM control diet (Smullen, 2013). This product is economical to use as a FM replacement, therefore, Clean Seas was interested in trialling it before using it in their commercial diet formulations.

When culturing fish to achieve maximum growth it is important to feed a diet that balances the dietary protein requirement with the dietary energy content (Halver and Hardy, 2002). Protein is recognised as the most expensive macro-nutrient in formulated fish diets (Halver and Hardy, 2002). The amount of dietary protein in aquafeeds may be optimised (“reduced”) by adding non-protein energy sources, such as lipid and carbohydrate. The addition of non-protein sources for energy may create a ‘protein sparing’ effect (Shiau and Lan, 1996; Halver and Hardy, 2002). This reduces the catabolism of protein for energy and improves protein retention efficiencies (Lupatsch et al., 2001; Halver and Hardy, 2002). However, not all fish species are tolerant of high dietary lipid or carbohydrate level and an imbalance of either nutrient class may affect growth efficiency and carcass composition. In Australia currently, commercial diets for Yellowtail Kingfish are based on salmonid/barramundi diets and Japanese Yellowtail diets (personal communication, Dr R. Smullen, Ridley Aquafeed). These grow-out diets typically contain a minimum of 45% protein and 20% lipid with an energy content of 23 MJ kg^{-1}, and are used throughout the entire sea cage production phase. Clean Seas was also interested in investigating the potential protein sparing effect of using higher lipid diets (30% lipid) for the larger Yellowtail Kingfish (> 1.5 kg). Slight reductions in dietary protein would equate to large savings in diet costs.

Limited information is available on the energy requirements of > 1.5 kg Yellowtail Kingfish (Stone and Bellgrove, 2013) and the way in which this species utilises lipid for energy (Booth et al., 2010), and further research is needed to formulate suitable diets for this size of fish. The energy requirements for smaller Yellowtail Kingfish have been reported to decrease with an increased body weight from 623 kJ DE Kg BW^{-1} d^{-1} at 50 g to 514 kJ DE Kg BW^{-1} d^{-1} at 100 g to 226 kJ DE Kg BW^{-1} d^{-1} at 2 kg (Booth et al., 2010). In contrast, Masumoto et al. (1997) reported the energy requirement for maximum growth of Japanese Yellowtail (initial BW 12 g, final BW 120 g) to be 773 kJ BW kg^{-1} d^{-1} which is much higher than the optimal dietary energy level for Yellowtail Kingfish of a comparable size (Booth et al., 2010). Additional knowledge in this aspect of Yellowtail Kingfish nutrition will aid in formulating diets to improve growth performance, feed efficiency, and health and product quality.

Clean Seas production managers were seeking further information on feeding regimes for Yellowtail Kingfish sea cages to improve fish weight gain and feed efficiency to gain the highest economic return. Early feeding practices used at Clean Seas for the production of Yellowtail Kingfish in sea cages involved feeding fish pellets twice daily to apparent satiation seven days $week^{-1}$. However, this may have resulted in under or overfeeding, leading to high feed conversion efficiencies and reduced dietary nutrient digestibility (Jobling, 1994). Japanese producers of *Seriola* spp. often feed larger fish on alternate days or only twice to three times a week in order to maximise economic return and or deal with seasonal changes in water temperature or unfavourable weather conditions (Nakada, 2002). Clean Seas was concerned about the efficiency, in terms of production costs and the environment, of their feeding practices and considered improvements in this area as a high priority. Hence, it was planned to compare a restricted feeding regime to a satiation feeding regime. These feeding regimes were compared in two separate trials, with the aim of improving feeding practices and feed efficiency for Yellowtail Kingfish.

Need

At the commencement of this project, the Clean Seas business model and future plan was to build a profitable Yellowtail Kingfish business by 2015 (personal communication, Dr C. Foster; Clean Seas). To accomplish this, the company identified key areas which required R&D investment. Using cost/benefit analyses to prioritise projects with the greatest payback on investment in the first year, Clean Seas developed a strategic, two year R&D plan encompassing internally funded/conducted research and research funded by the AS-CRC. Through this process it was proposed that two projects were to be developed for AS-CRC approval. This proposal focused on the second of those projects and encompassed research that will benchmark dietary formulations, assess feed rates and refine the knowledge of the dietary requirements for Yellowtail Kingfish.

Objectives

There were four objectives in this project:

1. Clean Seas identify or improve production diets in a tank based setting using large fish (> 1.5 kg) to provide information to reduce FCRs for Clean Seas Yellowtail Kingfish by 0.45 units across the entire sea cage production cycle.

2. Identify or improve production diets in a tank based setting using large fish (> 1.5 kg) to provide information to gain a 10% improvement in growth for Clean Seas Yellowtail Kingfish in sea cage production systems.

3. Identify or improve production diets in a tank based setting using large fish (> 1.5 kg) to provide information to improved Yellowtail Kingfish health and reduce mortality by 2% reduction in Clean Seas sea cage production systems.

4. Improve feeding strategies in a tank based setting using large (> 1.5 kg) Yellowtail Kingfish to provide information to improve Yellowtail Kingfish growth and feed utilisation by 2% in Clean Seas sea cage production systems during winter.

Note, after obtaining a project variation approval from the AS-CRC, an additional trial was added to this project (Chapter 4, Trial 3: Evaluation of different feeding strategies for the production of Yellowtail Kingfish at winter water temperatures). The trial was requested by Clean Seas and addresses the original objectives of this project.

Chapter 2. Trial 1: Improving production efficiency of large Yellowtail Kingfish using sustainable ingredients and manipulating feeding practices

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Abstract

The sustainable and economically viable production of Yellowtail Kingfish (*Seriola lalandi*) relies on the availability and efficient delivery of cost effective diets that have been formulated with a reduced reliance on marine ingredients and that provide a balanced combination of essential nutrients. This 5 month study utilised eight treatment combinations of fish meal (FM) and fish oil (FO) substitutions to investigate the growth performance, feed utilisation, stress and health of large (1.61 kg) Yellowtail Kingfish (*S. lalandi*) fed a range of commercially formulated diets. The study was conducted in a semi-flow-through oxygenated land-based system at summer water temperatures. One diet was formulated to contain a novel soybean meal (crude protein, 49%; crude lipid, 16%; and a gross energy level of 17.6 MJ kg^{-1}). All other diets were formulated to be iso-energetic (~19 MJ kg^{-1} gross energy) and contain crude protein and lipid levels of ~44% and 24%, respectively. One diet was formulated to be deficient in the essential long-chain omega-3 fatty acids (LC n-3 PUFA) to better understand the requirements by Yellowtail Kingfish. These diets were fed to apparent satiation once a day. To understand the effects of restricting the feed ration, one diet was fed to 80% of apparent satiation (sub-satiation) and compared to fish fed the same diet to apparent satiation. A short term trial to assess the immunological response to crowding stress was also conducted at the completion of the growth trial. Fish grew well and survival was positive in the land-based system at the South Australian Research and Development Institute (SARDI). Results for FO and FM substitution were encouraging. A new Yellowtail Kingfish production diet containing 30% FM and with 50% of the FO substituted with poultry oil (PO), performed well compared to the "gold standard" FM control diet. The diet containing the novel soybean meal did not support good growth or feed efficiency, nor did the diet deficient in LC n-3 PUFA. Arsenic and mercury levels in the whole fish samples were positively correlated to increasing dietary FM and FO levels. Results from the restricted feeding Treatment indicated that reducing feed rate improved FCR and improved protein retention. This suggests that with careful feed management, gains may be made in feed efficiency without compromising growth. With regard to the stress trial, blood biochemical and haematological parameters indicated that fish were stressed. Additionally, fish fed reduced levels of FM and FO appeared to mount a more pronounced white blood cell (granulocyte) response, compared to fish fed higher FM and FO inclusion levels. This may be indicative of a higher level of stress damage in these fish compared to the fish fed the high FM FO diet. Immunological results were inconclusive. Overall results suggest that growth performance of large Yellowtail Kingfish in the pool-farm system at SARDI was comparable to that achieved in the Clean Seas sea cage system for the same fish cohort. Fish meal (30%) and FO (9%) content of production diets for large Yellowtail Kingfish may be reduced significantly over previous levels used, as long as essential LC n-3 PUFA levels are maintained above 2 g 100 g^{-1} diet. This improvement, combined with savings from careful feed management practices should lead to savings in feed and feeding costs for Clean Seas. Improvements in the sustainable production of Yellowtail Kingfish should also enable wider market access for this quality product.

Introduction

Clean Seas is committed to improving the sustainable and economical production of Yellowtail Kingfish at their sea cage operations at Port Lincoln. Large Yellowtail Kingfish (> 1.5 kg) are currently produced at Clean Seas sea cage operations using a Ridley Clean Seas Pelagica 2013 diet which is formulated using considerable fish meal (FM) and fish oil (FO) substitution to contain a range of marine and terrestrial protein and lipid ingredients. Savings made on feed and feeding costs with larger fish should result in financial savings as the majority of the feed used in the production of Yellowtail Kingfish is fed during the later phases of production.

Clean Seas wished to benchmark the growth performance, feed utilisation and health of large Yellowtail Kingfish grown on the Ridley Clean Seas Pelagica 2013 diet against a "gold standard" marine diet formulated to contain high quality FM and FO as the primary protein and lipid sources. Both diets are formulated, based on data provided by Stone and Bellgrove (2013), to provide similar levels of crude protein, lipid and energy (crude protein ~45%; crude lipid ~23%; and gross energy ~19 MJ kg^{-1}) and other essential nutrients. However, the Pelagica diet was formulated to contain ~30% less FM and ~50% less FO than the "gold standard" diet, and is therefore, cheaper to manufacture. Concurrently, Clean Seas also planned to systematically assess the potential for even higher levels of FM and FO substitution with large Yellowtail Kingfish in this trial.

A diet that contained relatively large amounts of a new novel soybean meal ("Trifecta meal"), as a FM protein replacement, was also benchmarked in this trial. Previous research with soybean meal in Yellowtail Kingfish diets has demonstrated that growth, feed utilisation and digestive tract health is compromised once dietary inclusion levels exceed 10% (Bowyer et al., 2013a; b; Bansemer et al., 2015). However, the Trifecta soybean product has undergone a process that is reported to reduce the presence and activity of anti-nutritional factors and result in improved growth of Yellowtail Kingfish (Smullen, 2013).

Providing sufficient dietary essential long chain omega-3 poly unsaturated fatty acids (LC n-3 PUFA; eicosapentaenoic acid [EPA] + docosapentaenoic acid [DPA] + docosahexaenoic acid [DHA]) is one of the main limitations in replacing FO in diets for marine fish. The essential LC n-3 PUFA requirement for Yellowtail Kingfish has not been determined. However, the requirement for these nutrients has been shown to be ≥ 2 g 100 g^{-1} diet in the closely related Japanese Yellowtail (Deshimaru et al., 1982; Stone and Bellgrove, 2013), FO is rich in these fatty acids, while they are typically lacking in terrestrial ingredients (Bowyer et al., 2012). As FO substitution is an important aspect of attaining sustainable production, Clean Seas was also interested in determining the impact of sub-optimal levels of essential LC n-3 PUFA on fish growth and health of large Yellowtail Kingfish.

This trial also investigated the growth performance and health of large Yellowtail Kingfish in response to non-restrictive versus restrictive feeding practices to generate information, complement existing feed management tools, and facilitate better informed feed management decisions on-farm.

During this project, Clean Seas also wished to generate information on the performance of large fish cultured in the SARDI, South Australian Aquatic Science Centre (SAASC) tank-based system, to compare against the performance of the same fish cultured on-farm in sea cages.

Combined, the above research was designed to provide essential information that may contribute towards achieving the objectives of this project, which include: 1) identifying or developing improved production diets for large fish (> 1.5 kg) at summer water temperatures to provide information toward gaining a 10% improvement in growth; 2) a reduction of whole

cycle FCRs for Clean Seas Yellowtail Kingfish production by 0.45 units; and 3) maintaining health and improving survival to 98% in Clean Seas Yellowtail Kingfish sea cage production systems.

Aim

We aimed to:

1. Benchmark the growth performance and feed utilisation of large Yellowtail Kingfish (> 1.5 kg) fed the recently formulated Ridley Clean Seas Pelagica 2013 diet versus a high quality marine fish diet formulated to contain FM and FO as the main dietary ingredients;

2. Formulate and test commercially relevant production diets with varying levels of FM and FO substitution to determine if improvements in the production efficiency of large Yellowtail Kingfish in a tank-based setting can be achieved;

3. Determine if a deficiency in LC n-3 PUFA will reduce the growth of large Yellowtail Kingfish grown at summer water temperatures; and

4. Determine if growth performance and feed utilisation of large Yellowtail Kingfish can be maintained while restricting feed intake.

A short term crowding (hypoxic) stress trial was also conducted by Dr Jerome Delamare Debouteville of the University of Queensland at the end of this growth trial (Appendix A). The stress trial investigated differential sub-populations count, total mortality, phagocytic rate/capacity and oxidative activity of leucocytes isolated from the head-kidney of Yellowtail Kingfish fed with four different diets. These were assessed by flow cytometry pre- and post-hypoxic stress. The aim of this trial was to investigate the cellular immune function of Yellowtail Kingfish fed four experimental diets pre- and post-hypoxic stress.

Materials and Methods

Experimental treatments and feeding techniques
A total of six diets and eight treatment combinations were used to investigate the effect of varying levels of dietary FM and FO substitution, and feed rate on the growth performance, feed utilisation, health and immunology of Yellowtail Kingfish. The ingredient composition of the six diets remains confidential while their nutrient composition is displayed in Table 2.1.

Fish from Treatments 1, 3, 4, 5, 7 and 8 were fed to apparent satiation daily at 0830 h. Apparent satiation feeding was achieved by providing feed to the tank and monitoring feed intake of fish over a period of ~8 min tank^{-1}. Care was taken to minimise waste by dispersing feed evenly and slowly across each tank. Once small quantities of uneaten feed were observed on the tank bottom, fish were judged to have reached apparent satiation. Fish from Treatments 2 and 6 were fed to 80% sub-satiation. The feeding of the sub-satiation fed fish was determined as follows. At the start of the experiment fish were fed to apparent satiation for three consecutive days and the feed intake was recorded. Each respective tank was then fed 80% of the mean value recorded for the next 11 days. The sub-satiation feed rates were then readjusted using the same method. After one month the actual feed rate appeared to be greater than 90% of the fish fed to apparent satiation. Therefore, the feeding method was then modified as follows. Sub-satiation fed fish were fed to apparent satiation for four days and the average of the feed intake for the last three days of this period was used to calculate the 80% sub-satiation feed rate. Fish were then at this rate for 14 days at which point the feeding rate was re-established. This feeding method was used for the remainder of the trial.

The treatments investigated were:

- Treatment 1: Diet 1 fed to apparent satiation seven days week^{-1} (Gold standard diet consisting of ~57% FM and ~19% FO: planned composition [as fed], ~45% crude protein [CP], ~24% crude CL and a normal gross energy [GE] level of 19 MJ kg^{-1}).

- Treatment 2: Diet 1 fed to sub-satiation (80% of apparent satiation).

- Treatment 3: Diet 2 fed to apparent satiation seven days week^{-1} (Ridley Clean Seas 2013 Pelagica diet: 40% FM diet and normal CL level with FO substitution comprised of ~9% FO + ~9% PO; planned composition [as fed], ~44% CP, ~24% CL and a normal GE level of ~19 MJ kg^{-1}).

- Treatment 4: Diet 3 fed to apparent satiation seven days week^{-1} (Ridley Trifecta soybean diet: 20% FM diet [50% FM substitution using 30% of trifecta soybean meal] and low CL level with FO substitution of 50% [comprised of ~5% FO + ~5% PO]; planned composition [as fed], higher CP level of ~49%, low CL level of ~16% and a low GE level of ~17.6 MJ kg^{-1}).

- Treatment 5: Diet 4 fed to apparent satiation seven days week^{-1} (Reformulated Ridley Pelagica diet with 30% FM [25% FM substitution using alternative protein sources] and normal CL level with FO substitution of 70% [comprised of ~5% FO + ~15% PO]; planned composition [as fed], normal CP level of ~44%, normal CL level of 24% and a normal GE level of ~19 MJ kg^{-1}).

- Treatment 6: Diet 4 fed to sub-satiation (80% of apparent satiation).

- Treatment 7: Diet 5 fed to apparent satiation seven days week^{-1} (Reformulated Ridley Pelagica diet with 30% FM [25% FM substitution using alternative protein sources] and normal CL level with FO substitution of 50% [comprised of ~9% FO + ~9% PO]; planned composition [as fed], normal CP level of ~44%, normal CL level of 24% and a normal GE level of ~19 MJ kg^{-1}).

- Treatment 8: Diet 6 fed to apparent satiation seven days week^{-1} (Reformulated Ridley Pelagica diet with 20% FM [50% FM substitution using alternative protein sources] and normal CL level with FO substitution of 75% [comprised of ~5% FO + ~15% PO]; planned composition [as fed], normal CP level of ~44%, normal CL level of 24% and a normal GE level of ~19 MJ kg^{-1}). Nutrient profile balanced with Pelagica Diet 2 but with a lower LC n-3 PUFA level of 1.41 g 100 g^{-1} diet.

Table 2.1. The biochemical composition of the six test diets used in Trial 1.[1]

Item (as fed)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Analysed proximate composition						
Moisture (g kg^{-1})	69.0	58.0	82.0	56.0	60.0	77.0
Crude protein (g kg^{-1})	452.0	460.0	487.0	458.0	456.0	444.0
Crude lipid (g kg^{-1})	234.0	240.0	176.0	237.0	233.0	228.0
Ash (g kg^{-1})	87.0	88.0	91.0	90.0	90.0	91.0
Carbohydrate (g kg^{-1})[2]	158.0	154.0	164.0	159.0	161.0	160.0
Gross energy (MJ kg^{-1})	19.0	19.3	17.6	19.3	19.1	18.7
Cholesterol (g kg^{-1})	3.8	2.7	2.2	2.6	2.9	2.4
Analysed minerals (mg kg^{-1})						
Calcium	21000.0	24000.0	19000.0	23000.0	23000.0	20000.0
Choline	-	-	-	-	-	-
Copper	9.8	10.0	12.0	12.0	10.0	10.0
Iodide (Potassium Iodide) (µg kg^{-1})	4.0	20.0	21.0	26.0	25.0	22.0
Iron	250.0	270.0	320.0	360.0	400.0	340.0
Magnesium	1700.0	1600.0	1700.0	1600.0	1500.0	1600.0
Manganese	74.0	76.0	76.0	96.0	84.0	80.0
Phosphorus	14000.0	15000.0	16000.0	16000.0	16000.0	15000.0
Potassium	5700.0	5600.0	8900.0	5000.0	5000.0	5700.0
Selenium	1.9	1.9	1.6	1.9	1.6	1.8
Zinc	240.0	250.0	250.0	330.0	250.0	230.0
Analysed amino acids (g kg^{-1})						
Alanine	25.5	27.5	26.9	27.0	27.3	26.8
Arginine	20.9	22.3	22.9	20.4	20.8	21.5
Aspartic acid	47.5	48.3	53.5	48.6	47.7	49.8
Glutamic acid	58.5	62.5	64.3	57.1	57.1	60.0
Glycine	24.2	26.6	25.9	26.2	26.2	26.3
Histidine	15.1	16.7	16.0	16.1	18.1	16.1
Isoleucine	20.2	21.0	20.3	17.9	18.1	18.4
L cystine	3.5	4.0	4.1	3.7	3.5	3.7
Leucine	34.5	37.0	38.3	36.9	37.3	37.3
Lysine	33.0	27.7	34.3	31.2	30.2	29.9
Methionine	12.1	12.0	10.8	12.1	11.6	11.0
Phenylalanine	19.1	21.0	22.8	20.6	21.8	22.0
Proline	20.3	9.8	17.0	23.8	14.8	16.8
Serine	17.0	18.4	20.9	17.1	18.0	18.4
Taurine	16.3	14.6	12.7	12.8	12.1	14.0
Threonine	26.7	27.0	27.3	25.9	26.7	25.6
Tryptophan	5.0	4.9	5.3	5.1	5.1	4.7
Tyrosine	14.0	15.1	15.2	13.8	14.1	13.6
Valine	24.9	26.6	27.9	26.8	27.5	27.0

[1] Diets supplied by Ridley Aquafeeds (Narangba, QLD, Australia).
[2] Carbohydrate = 1000 - (moisture + lipid + protein + ash).

Table 2.1. Continued.

Item (as fed)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Analysed fatty acids (mg 100 g^{-1})						
Butyric C4:0	<10	<10	<10	<10	<10	<10
Caproic C6:0	<10	<10	<10	<10	<10	<10
Caprylic C8:0	<10	<10	<10	<10	<10	<10
Capric C10:0	<10	<10	<10	<10	<10	<10
Lauric C12:0	<10	<10	<10	<10	16	16
Trisdecanoic C13:0	<10	<10	<10	<10	<10	<10
Myristic C14:0	900	590	370	450	560	420
Pentadecanoic C15:0	130	87	55	66	82	62
Palmitic C16:0	4210	4900	3470	5090	4760	4870
Margaric C17:0	110	92	57	81	85	75
Stearic C18:0	940	1350	950	1460	1320	1370
Arachidic C20:0	54	42	31	43	45	40
Docosanoic C22:0	34	31	23	30	32	27
Tetracosanoic C24:0	23	19	<10	23	16	35
Decenoic C10:1	<10	<10	<10	<10	<10	<10
Myristoleic C14:1	59	58	37	58	57	52
Pentadecenoic C15:1	26	<10	<10	<10	<10	<10
Palmitoleic C16:1	1060	1120	750	1120	1080	1070
Heptadecenoic C17:1	93	69	44	56	66	53
Octadecenoic C18:1n-6	<10	<10	<10	<10	<10	<10
Octadecenoic C18:1n-7	730	640	450	590	610	560
Oleic C18:1n-9	4760	7170	5110	8050	7120	7800
Eicosenoic C20:1n-9	1390	820	460	440	700	410
Eicosenoic C20:1n-11,13	160	86	53	58	85	50
Eicosenoic C20:1 (total)	1550	820	510	490	780	460
Docosenoic C22:1n-9	190	98	61	58	91	53
Docosenoic C22:1n-11,13	<10	<10	<10	<10	<10	<10
Tetracosenoic C24:1	220	110	65	65	110	63
Linoleic C18:2n-6	830	2270	2750	2900	2310	2920
Gamma Linolenic C18:3n-6 (GLA)	43	36	25	35	36	34
Eicosadienoic C20:2n-6	65	45	29	35	41	32
Dihomo-gamma-linoleic C20:3n-6	31	28	19	26	28	24
Arachidonic C20:4n-6	230	170	110	140	150	120
Docosatetraenoic C22:4n-6	78	48	29	32	41	28
Docosapentaenoic C22:5n-6	64	37	<10	22	33	20
Alpha Linolenic C18:3n-3	200	300	340	340	290	340
Steridonic C18:4n-3	210	120	70	78	110	74
Eicosatrienoic C20:3n-3	49	29	18	19	28	17
Eicosatetraenoic C20:4n-3	600	310	190	170	290	160
Eicosapentanaeoic C20:5n-3 (EPA)	1430	800	480	500	710	450
Heneicosapentaenoic acid C21:5n-3	24	<10	<10	<10	<10	<10
Docosapentaenoic C22:5n-3	390	220	140	140	200	130
Docosahexaenoic C22:6n-3 (DHA)	2660	1530	900	940	1340	830
Octadecadienoic C18:2 Conjugated 9c 11t	<10	<10	<10	17	20	17
Octadectrenoic acid C18:3n-4	46	28	18	18	30	18
EPA + DPA + DHA	4480	2550	1520	1580	2250	1410
n-3 FA:n-6 FA	4.61	1.33	0.76	0.67	1.20	0.65

Experimental fish
Experimental work for Trial 1 was conducted in the pool-farm facility at the SARDI SAASC, South Australia. Yellowtail Kingfish (initial weight 1.61 ± 0.03 kg; initial fork length 483.0 ± 5.3 mm; mean ± standard deviation) were obtained from Clean Seas (Arno Bay, South Australia). Upon arrival at the SAASC experimental facility, Yellowtail Kingfish were transferred to 5000 L tanks supplied with partial flow-through/recirculating (100% system water exchange d^{-1}), sand filtered, UV treated, aerated sea water at ambient temperature and held for ~two weeks and fed the standard Ridley 2013 Pelagica diet (crude protein 46%; crude lipid 24%; gross energy 19.30 MJ kg^{-1}).

Skin and gill fluke treatments
Upon arrival at the SAASC, fish were inspected and observed to have a low burden of gill (*Zeuxapta seriola*) and skin (*Benedenia seriola*) flukes so treatment was deemed to be unnecessary. At the first weight check in January 2014 fish were observed to have a moderate burden of skin and gill flukes. At this stage fish were bathed in praziquantel (5 ppm for 20 min) according to the veterinary prescription (Dr Matt Landos, Future Fisheries Veterinary Service Pty Ltd.). Bathing in praziquantel was also practiced at the second weight check and flukes proved to be persistent, so fish were then exposed to two treatments of formalin (250 ppm for 60 min) over a two week period in March 2014 (Dr Matt Landos, Future Fisheries Veterinary Service Pty Ltd.). Subsequent tests of spare fish held in the same system under identical conditions revealed that the skin and gill fluke problem had been eradicated following the second treatment of formalin.

Experimental stocking
At the commencement of Trial 1, Yellowtail Kingfish were removed from their tank (n = 384), anaesthetised using AQUI-S® (AQUI-S® New Zealand Ltd., Lower Hutt, New Zealand) at a concentration of 14 mg L^{-1} of seawater. Sixteen fish were measured, weighed and stocked, using systematic interspersion, into one of the three replicate 5000 L tanks treatment $combination^{-1}$ (n = 24 tanks). The tanks supplied with partial flow-through/recirculating (100% system water exchange d^{-1}), sand filtered, UV treated sea water at ambient temperature. Aeration was provided to each tank for the first 8 weeks and then tanks were provided with aeration and oxygenation for the remainder of the trial.

Weight checks throughout the trial
At days 44, 87 and 117 post-stocking, fish were anaesthetised using AQUI-S® at a concentration of 14 mg L^{-1} of seawater. Fish were measured, weighed, visually inspected for skin and gill flukes and returned to their respective tanks. Operator error resulted in the loss of fish from tank M5 in mid-January (Replicate 1, Treatment 6); as a result this tank was excluded from all analyses. This resulted in 2 replicate tanks for Treatment 6.

Water quality and analyses
Water quality parameters were maintained throughout the study at appropriate levels for the acceptable growth of Yellowtail Kingfish (Table 2.2). Water temperature was measured daily using a thermometer (Figure 2.1). Dissolved oxygen (mg L^{-1} [Figure 2.2] and % saturation [Figure 2.3]) was measured daily using a dissolved oxygen meter (OxyGuard International A/S, Birkerød, Denmark). The pH was measured daily using a meter (Oakton pHtestr 20; Oakton Instruments, Vernon Hills, IL, USA). Salinity (g L^{-1}) was measured weekly using a portable salinity refractometer (model RF20, Extech Instruments, Nashua, NH, USA). Tanks were cleaned weekly by siphoning on a rotational basis.

Table 2.2. Summary of water quality for Trial 1.[1]

Item	Temperature (°C)	Dissolved oxygen (mg L^{-1})	Dissolved oxygen (% saturation)	pH	Salinity (mg L^{-1})
Mean[1]	21.7±1.70	6.7±0.8	92.9±10.2	7.94±0.27	36.4±1.0
Range	17.3-25.9	3.7-12.8	55.0-177.0	6.79-8.71	32.0-38.0

[1] Values means ± standard deviation.

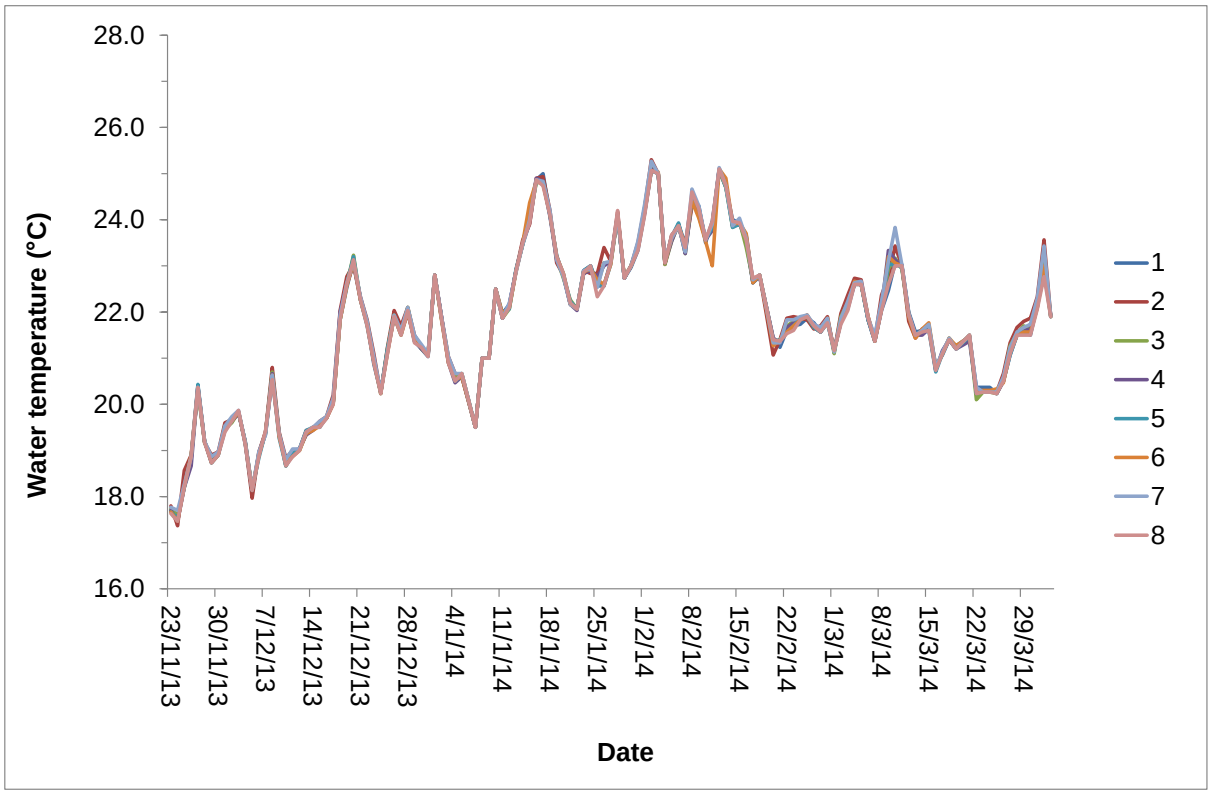

Figure 2.1. Daily average treatment water temperatures in Trial 1.
Values are expressed as mean for each day (n = 3).[1]
[1]Treatment 1 (Diet 1): Gold standard diet (57% fish meal [FM], Super-prime mackerel or anchovy meal and 18.6% fish oil [FO]) diet fed to apparent satiation;
Treatment 2 (Diet 1): Gold standard diet fed to sub-satiation (80% apparent satiation);
Treatment 3 (Diet 2): Existing Ridley Pelagica diet (crude protein [CP] level of 44%; 40% FM; minimum crude lipid [CL] level of 24%; 50% FO substituted with poultry oil) fed to apparent satiation;
Treatment 4 (Diet 3): Ridley Trifecta soya diet (20% FM, and ~50% of FM replaced with Trifecta soya meal (30%), CL level, 16%; with 75% FO substituted with poultry oil) fed to apparent satiation;
Treatment 5 (Diet 4): Reformulated Ridley Pelagica diet (30% FM and 25% FO; 75% PO) fed to apparent satiation;
Treatment 6 (Diet 4): Reformulated Ridley Pelagica diet fed to sub-satiation (80% apparent satiation);
Treatment 7 (Diet 5): Reformulated Ridley Pelagica diet (30% FM and 50% FO; 50% PO; nutrient profile balanced to that of the existing Pelagica diet) fed to apparent satiation;
Treatment 8 (Diet 6): Reformulated Ridley Pelagica diet (20% FM and 25 FO: 75 PO); nutrient profile (except EPA/DPA/DHA=1.41%) balanced to the existing Pelagica diet) fed to apparent satiation.

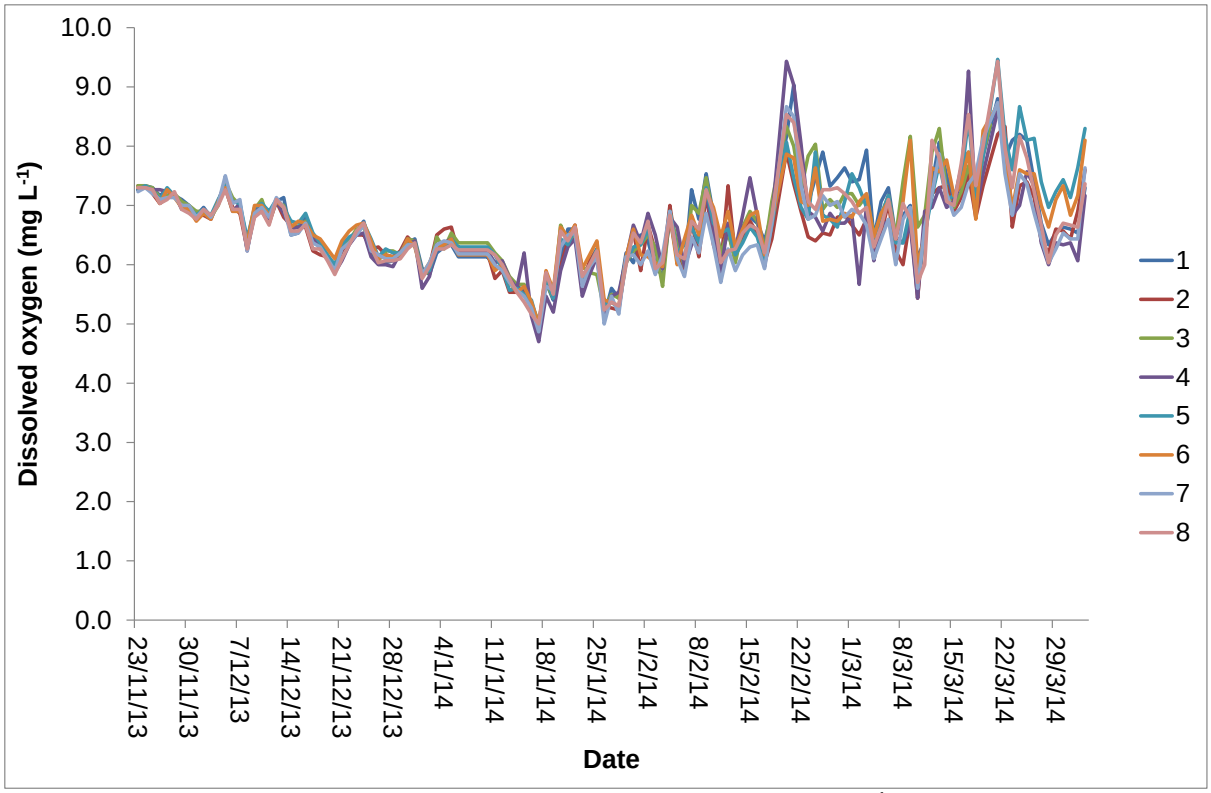

Figure 2.2. Daily average treatment dissolved oxygen levels (mg L^{-1}) in Trial 1.
Values are expressed as mean for each day (n = 3).[1]
[1]Treatment 1 (Diet 1): Gold standard diet (57% fish meal [FM], Super-prime mackerel or anchovy meal and 18.6% fish oil [FO]) diet fed to apparent satiation;
Treatment 2 (Diet 1): Gold standard diet fed to sub-satiation (80% apparent satiation);
Treatment 3 (Diet 2): Existing Ridley Pelagica diet (crude protein [CP] level of 44%; 40% FM; minimum crude lipid [CL] level of 24%; 50% FO substituted with poultry oil) fed to apparent satiation;
Treatment 4 (Diet 3): Ridley Trifecta soya diet (20% FM, and ~50% of FM replaced with Trifecta soya meal (30%), CL level, 16%; with 75% FO substituted with poultry oil) fed to apparent satiation;
Treatment 5 (Diet 4): Reformulated Ridley Pelagica diet (30% FM and 25% FO; 75% PO) fed to apparent satiation;
Treatment 6 (Diet 4): Reformulated Ridley Pelagica diet fed to sub-satiation (80% sub-satiation);
Treatment 7 (Diet 5): Reformulated Ridley Pelagica diet (30% FM and 50% FO; 50% PO; nutrient profile balanced to that of the existing Pelagica diet) fed to apparent satiation;
Treatment 8 (Diet 6): Reformulated Ridley Pelagica diet (20% FM and 25 FO: 75 PO); nutrient profile (except EPA/DPA/DHA=1.41%) balanced to the existing Pelagica diet) fed to apparent satiation.

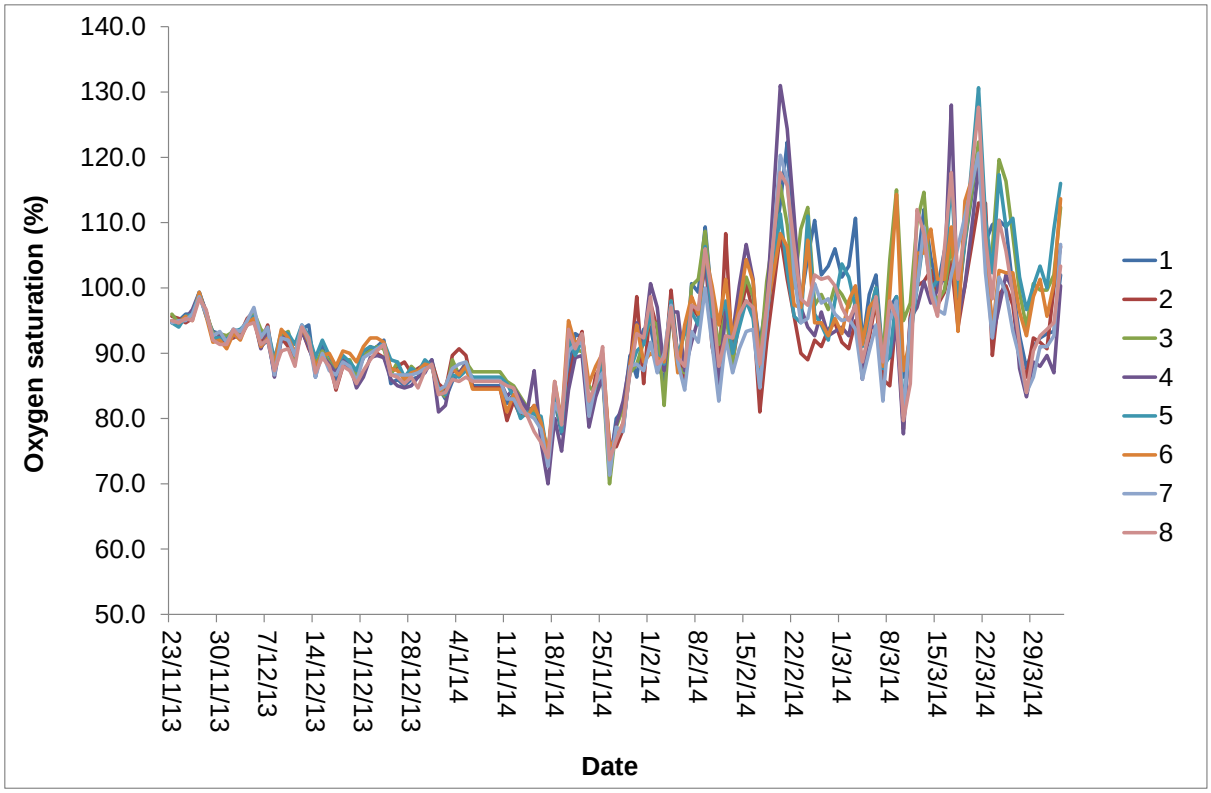

Figure 2.3. Daily average treatment dissolved oxygen levels (% saturation) in Trial 1.
Values are expressed as mean for each day (n = 3).[1]
[1]Treatment 1 (Diet 1): Gold standard diet (57% fish meal [FM], Super-prime mackerel or anchovy meal and 18.6% fish oil [FO]) diet fed to apparent satiation;
Treatment 2 (Diet 1): Gold standard diet fed to sub-satiation (80% apparent satiation);
Treatment 3 (Diet 2): Existing Ridley Pelagica diet (crude protein [CP] level of 44%; 40% FM; minimum crude lipid [CL] level of 24%; 50% FO substituted with poultry oil) fed to apparent satiation;
Treatment 4 (Diet 3): Ridley Trifecta soya diet (20% FM, and ~50% of FM replaced with Trifecta soya meal (30%), CL level, 16%; with 75% FO substituted with poultry oil) fed to apparent satiation;
Treatment 5 (Diet 4): Reformulated Ridley Pelagica diet (30% FM and 25% FO; 75% PO) fed to apparent satiation;
Treatment 6 (Diet 4): Reformulated Ridley Pelagica diet fed to sub-satiation (80% apparent satiation);
Treatment 7 (Diet 5): Reformulated Ridley Pelagica diet (30% FM and 50% FO; 50% PO; nutrient profile balanced to that of the existing Pelagica diet) fed to apparent satiation;
Treatment 8 (Diet 6): Reformulated Ridley Pelagica diet (20% FM and 25 FO: 75 PO); nutrient profile (except EPA/DPA/DHA=1.41%) balanced to the existing Pelagica diet) fed to apparent satiation.

Final harvest sampling
At the commencement of the trial, 12 fish were collected for tissue composition and blood samples. At the conclusion of the growth trial (day 131), three Yellowtail Kingfish were collected per tank for whole body proximate analyses and stored at -20°C prior to analysis. A further two Yellowtail Kingfish per tank were sampled for blood. In addition, the hindgut of two fish per replicate from Treatments 1, 4 and 7 were collected for histological investigation. One cm^2 hindgut samples were collected and opened before fixation in 10% seawater formalin. Histology samples were dehydrated to 100% ethanol, and embedded in paraffin wax. Tissue samples were cut at 5 µm and stained with Haematoxylin and Eosin (H & E). The villus height and area, lamina propria area and villus branching were measured for 10 villi $fish^{-1}$.

Biochemical and blood analyses
All tissue biochemical were analysed by AsureQuality Laboratories (Auckland, New Zealand). All blood samples were analysed by IDEXX Laboratories Pty Ltd (Unley, South Australia).

Calculation of performance indices
All data reported for each treatment for animal performance were based on the mean of the replicate tanks. All calculations using fish weight and diets were based on wet or as fed values:

- Weight gain = final weight - initial weight
- Biomass gain (g $tank^{-1}$) = (final weight + ∑mortality weight) - (initial weight + ∑replacement weight)
- Specific growth rate (SGR, % d^{-1}) = ([ln final weight - ln initial weight] / days) × 100
- Condition index = (fish weight [g] / fish length $[cm]^3$) × 100
- Apparent feed conversion ratio (FCR) = fish weight gain / feed consumed
- Apparent protein efficiency ratio (PER) = fish weight gain / protein consumed
- Apparent energy efficiency ratio (EER) = fish weight gain / energy consumed
- Apparent protein deposition = ([final soft body protein - initial soft body protein] / protein intake) × 100
- Apparent energy deposition = ([final soft body energy - initial soft body energy] / energy intake) × 100

Hypoxic stress test
The methods, results and discussion for this test are presented in detail in Appendix 1 and summarised here. After the final harvest (day 131) of the growth trial, the remaining fish were returned to their respective tanks and fed their respective diets for two weeks. Then a stress test was carried out on fish fed to apparent satiation with Diet 1 (Gold standard diet), Diet 2 (Existing Ridley Pelagica diet), Diet 3 (Ridley Trifecta soya diet) and Diet 5 (Reformulated Ridley Pelagica diet). The stress test comprised of lowering the water in the tanks to 15 cm depth and chasing the fish around for 15 min with a hand-held dip net. Tanks were then refilled and fish were fed as per normal treatment procedure. Three days later fish were captured by dip-net and blood and head kidney tissue samples were collected and analysed for blood biochemistry and haematology, and cellular immune parameters of head kidney samples (Appendix 1, Table 9).

Statistical analyses

IBM SPSS, Version 20 for Windows (IBM SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Homogeneity of variances and normality among mean values were assessed using Levene's test for equality of variance errors and the standardized residuals against the predicted mean plot, respectively. Differences between treatments for all variables were analysed using One-factor ANOVA. Additionally, difference between treatments for the feed rate (diet type by feed rate) and the stress test (diet type by stress type) were analysed using Two-factor ANOVA. When significant differences were observed, post-hoc tests were used to detect significant differences treatment combinations (Student Newman-Keuls). Weight and condition index distribution of fish fed Diet 1 and 4 to apparent satiation and sub-satiation were plotted, and data homogeneity or skewness were tested using the Shapiro Wilk test. A significance level of $P < 0.05$ was used for all statistical tests. All values are presented as means ± standard error (SE) of the mean unless otherwise stated.

Results

General observations
Fish fed actively during the experiment with no apparent differences observed between diet types. Apart from the presence of gill and skin flukes there were no apparent signs of disease observed. Apart from several jumping fish, and one tank (M5, Treatment 6, Diet 4) of fish that had the water flow turned off inadvertently, there were no mortalities.

Growth performance
The initial weight and fork length of fish were not statistically different between treatments (One-factor ANOVA; $P > 0.05$; Table 2.3). There were no significant differences between treatments for any of the growth performance parameters (One-factor ANOVA $P > 0.05$; Table 2.3). Growth performance parameters tended to be lower in the fish fed the Ridley Trifecta soya diet (Figure 2.4, Table 2.3, Treatment 4, Diet 3).

Apart from SGR, which was superior for fish fed Diet 1 compared to Diet 4 (Two-factor ANOVA; $P < 0.05$; Table 2.4), neither feed rate (satiation vs sub-satiation) or diet type (Diet 1 vs Diet 4) significantly effected growth performance parameters (Two-factor ANOVA; $P > 0.05$; Table 2.4).

Fish fed Diet 1 fed to apparent satiation or sub-satiation and Diet 4 fed to apparent satiation exhibited normal weight distribution ($P > 0.05$; Table A2.4.1; Appendix 2). The weight distribution of fish fed Diet 4 to sub-satiation was significantly negatively skewed ($P = 0.020$; -1.10). With regard to condition index, the condition index of fish fed Diet 4 to apparent satiation was significantly positively skewed ($P < 0.001$; 1.63). The condition index for fish fed Diet 1 fed to apparent satiation or sub-satiation and Diet 4 fed to sub-saltation was normally distributed ($P > 0.05$; Table A2.4.3; Appendix 2).

Feed utilisation
Feed consumption rates were significantly lower in Treatments 2 and 6 (Diets 1and 4, sub-satiation) (One-factor ANOVA; $P < 0.05$; Table 2.3). There were significant differences in FCRs between treatments (One-factor ANOVA, $P < 0.05$; Table 2.3). Generally, fish fed to sub-satiation exhibited superior FCRs. This is reinforced when analysed using a Two-factor ANOVA (Table 2.4) where feed consumption rate was significantly reduced and FCR was significantly superior in the fish fed to sub-satiation.

FCR tended to be higher in the fish fed the Ridley Trifecta soya diet to apparent satiation (Table 3, Treatment 4, Diet 3).

Whole fish proximate and energy composition
There were no significant differences between treatments for any of the soft tissue parameters (One-factor ANOVA $P > 0.05$; Table 2.3). However, moisture content appeared to be low (~70% expected). This may have been a result of re-freezing and grinding procedures.

Apart from lipid, which was higher for fish fed to apparent satiation compared to fish fed to sub-satiation (Two-factor ANOVA; $P < 0.05$; Table 2.4), neither feed rate (satiation vs sub-satiation) or diet type (Diet 1 vs Diet 4) significantly effected soft tissue composition parameters (Two-factor ANOVA; $P > 0.05$; Table 2.4).

Lipid and energy levels tended to be lower in the fish fed the Ridley Trifecta soya diet to apparent satiation (Table 2.3, Treatment 4, Diet 3).

Nutrient utilisation
Apparent protein and energy deposition were generally significantly higher in Treatments 2 and 6 (Diets 1 and 4, sub-satiation) (One-factor ANOVA; $P < 0.05$; Table 2.3). Apparent protein and energy deposition also tended to be lower in the fish fed the Ridley Trifecta soya diet to apparent satiation (Treatment 4, Diet 3).

Apparent protein deposition was superior for fish fed to sub-satiation compared to fish fed to apparent satiation (Two-factor ANOVA; $P < 0.05$; Table 2.4); while diet type had no significant effect on apparent protein deposition. In contrast, apparent energy deposition was effected by diet type (Diet 1 > Diet 4; Two-factor ANOVA; $P < 0.05$; Table 2.4); while there was no significant effect of feed rate on energy deposition.

Apparent energy deposition was generally significantly higher in Treatments 2 and 6 (Diets 1 and 4, sub-satiation) (One-factor ANOVA; $P < 0.05$; Table 2.3). Apparent protein deposition also tended to be lower in the fish fed the Ridley Trifecta soya diet to apparent satiation (Treatment 4, Diet 3).

Apparent protein deposition was superior for fish fed to sub-satiation compared to fish fed to apparent satiation (Two-factor ANOVA; $P < 0.05$; Table 2.4).

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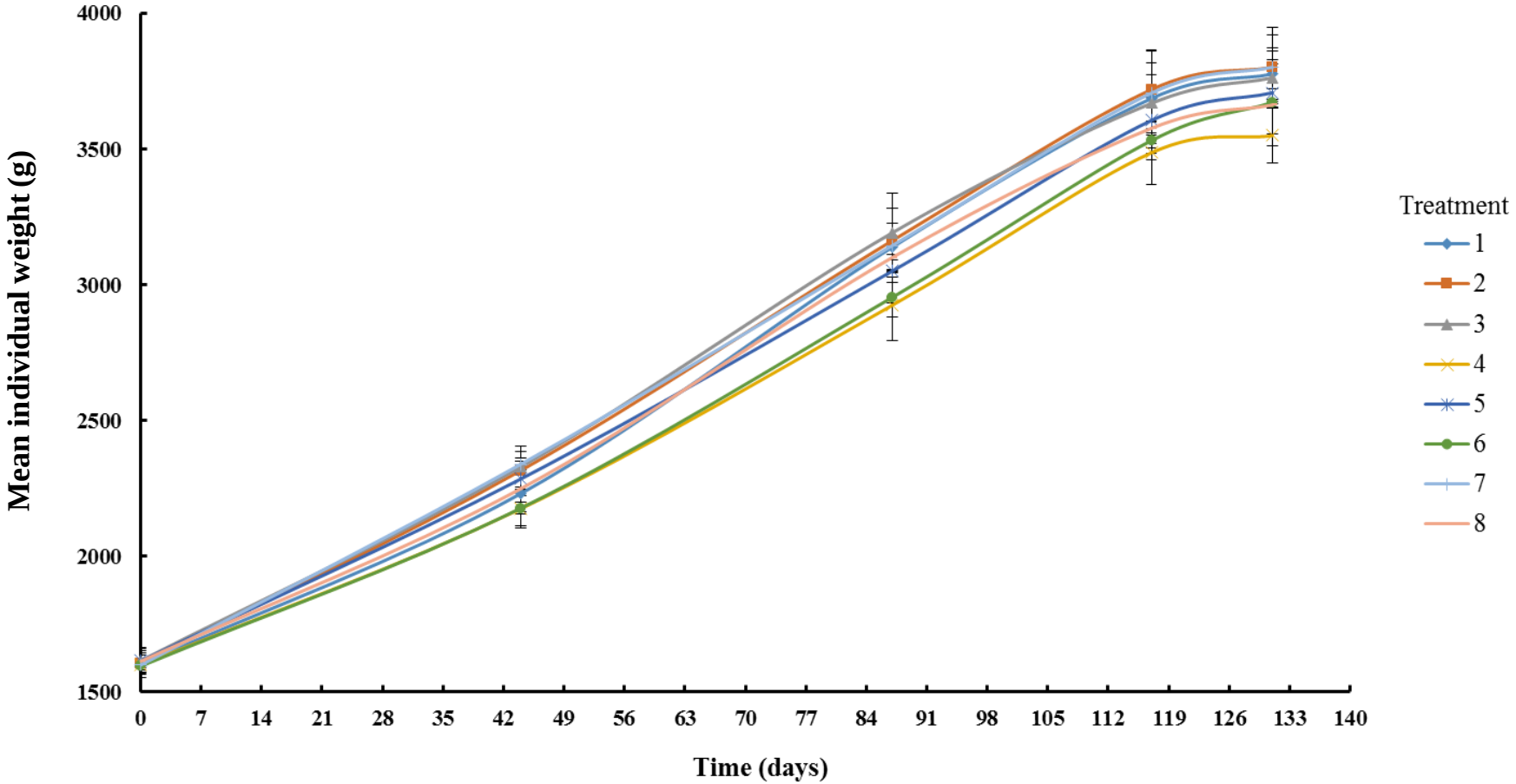

Figure 2.4. Mean individual weight of Yellowtail Kingfish in Trial 1 at 0, 44, 87, 117 and 131 days.[1, 2]

[1]Values are expressed as mean ± SD, n = 3 (except Treatment 6 from day 45 onwards, where n = 2 as tank M5 was removed from the study).

[2]Treatment 1 (Diet 1): Gold standard diet (57% fish meal [FM], Super-prime mackerel or anchovy meal and 18.6% fish oil [FO]) diet fed to apparent satiation;
Treatment 2 (Diet 1): Gold standard diet fed to sub-satiation (80% apparent satiation);
Treatment 3 (Diet 2): Existing Ridley Pelagica diet (crude protein level of 44%; 40% FM; minimum crude lipid [CL] level of 24%; 50% FO substituted with poultry oil) fed to apparent satiation;
Treatment 4 (Diet 3): Ridley Trifecta soya diet (20% FM, and ~50% of FM replaced with Trifecta soya meal (30%), CL level, 16%; with 75% FO substituted with poultry oil) fed to apparent satiation;
Treatment 5 (Diet 4): Reformulated Ridley Pelagica diet (30% FM and 25% FO; 75% PO) fed to apparent satiation;
Treatment 6 (Diet 4): Reformulated Ridley Pelagica diet fed to sub-satiation (80% apparent satiation);
Treatment 7 (Diet 5): Reformulated Ridley Pelagica diet (30% FM and 50% FO; 50% PO; nutrient profile balanced to that of the existing Pelagica diet) fed to apparent satiation; and
Treatment 8 (Diet 6): Reformulated Ridley Pelagica diet (20% FM and 25% FO: 75% PO); nutrient profile (except EPA/DPA/DHA=1.41%) balanced to the existing Pelagica diet) fed to apparent satiation.

Table 2.3. Growth performance, feed utilisation, proximate composition and nutrient retentions of Yellowtail Kingfish fed varying fish meal replacement and feed rates for 131 days in Trial 1.[1]

Diet	1	1	2	3	4	4	5	6	
Feed rate (%)	100	80	100	100	100	80	100	100	
Treatment	1	2	3	4	5	6	7	8	ANOVA[2]
Growth performance									
Initial weight (kg)	1.60±0.01	1.60±0.02	1.62±0.02	1.60±0.01	1.61±0.03	1.59±0.02	1.60±0.03	1.61±0.03	P = 0.993
Final weight (kg)	3.78±0.03	3.80±0.07	3.61±0.14	3.52±0.08	3.71±0.09	3.67±0.01	3.80±0.08	3.64±0.06	P = 0.215
Biomass gain (kg tank^{-1})	34.82±0.40	35.17±0.81	31.93±1.87	30.68±1.11	33.50±1.03	33.24±0.31	35.24±1.30	32.37±1.25	P = 0.117
SGR (% d^{-1})	0.66±0.01	0.66±0.01	0.65±0.01	0.61±0.01	0.63±0.01	0.64±0.01	0.66±0.02	0.63±0.03	P = 0.156
Initial fork length (mm)	483.9±3.56	482.3±3.19	484.6±3.33	481.8±0.68	482.9±3.97	483.3±1.09	483.4±4.55	481.3±5.49	P = 0.998
Final fork length (mm)	606.5±1.17	608.4±5.18	609.0±4.25	599.5±4.64	604.0±6.61	605.9±0.31	608.9±4.00	601.7±6.29	P = 0.784
Length growth rate (mm d^{-1})	0.94±0.02	0.96±0.02	0.95±0.01	0.90±0.04	0.92±0.02	0.94±0.01	0.96±0.03	0.92±0.05	P = 0.780
Condition factor	1.69±0.01	1.69±0.02	1.67±0.01	1.65±0.01	1.68±0.02	1.65±0.01	1.68±0.01	1.68±0.04	P = 0.512
Feed utilisation									
Feed consumption rate (g fish^{-1} d^{-1})	33.37±0.47^{a}	29.06±0.38^{b}	32.90±0.08^{a}	33.73±0.77^{a}	33.36±0.75^{a}	29.41±0.06^{b}	35.04±0.34^{a}	34.11±0.35^{a}	P < 0.001
Apparent FCR (as fed)	2.01±0.02^{abc}	1.73±0.03^{c}	2.17±0.12^{ab}	2.31±0.10^{a}	2.09±0.07^{ab}	1.85±0.01^{cb}	2.09±0.07^{ab}	2.22±0.10^{ab}	P = 0.003
Proximate composition									
Moisture (%)	55.6±1.4	57.7±0.7	56.7±0.4	59.1±0.8	57.7±0.5	58.5±2.3	56.7±0.8	57.5±1.4	P = 0.396
Protein (% wet)	19.3±0.4	20.0±0.6	21.6±0.5	20.6±0.6	19.9±0.0	20.2±0.5	20.3±0.2	20.0±0.5	P = 0.072
Lipid (% wet)	21.5±0.6	18.5±0.3	19.3±0.5	17.5±1.0	19.6±0.4	18.4±1.8	20.2±1.0	19.2±0.6	P = 0.061
Ash (% wet)	2.0±0.2	2.5±0.2	2.6±0.1	2.5±0.1	2.6±0.1	2.8±0.2	2.6±0.1	2.6±0.3	P = 0.147
Carbohydrate (% wet; by difference)	1.5±0.8	1.5±0.8	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	P = 0.051
Energy (MJ kg^{-1} wet)	11.40±0.36	10.50±0.17	10.83±0.15	9.98±0.27	10.63±0.12	10.23±0.78	10.90±0.38	10.49±0.32	P = 0.137
Nutrient retention									
Apparent PD	20.47±0.99^{ab}	25.38±1.62^{a}	23.01±2.28^{ab}	18.69±1.49^{b}	20.65±0.65^{ab}	23.68±0.76^{ab}	21.43±0.54^{ab}	20.15±0.44^{ab}	P = 0.044
Apparent ED	36.42±1.54^{ab}	37.47±1.30^{a}	31.49±1.66^{ab}	28.66±0.74^{b}	31.49±1.46^{ab}	33.35±3.68^{ab}	32.76±2.56^{ab}	30.18±0.88^{ab}	P = 0.030

[1] Values are mean ± SE; n = 3.
[2] A significance level of P < 0.05 was used for all statistical tests, where significant differences were observed post-hoc tests were used (Student Newman-Keuls test) to detect differences between treatments, values without a common superscript are significantly different ([a] indicates the highest value; P < 0.05).
Initial fish proximate composition (wet basis): Moisture 64.3%, protein 20.2%, lipid 12.3%, ash 2.2%, carbohydrate (by difference) <1.5%, energy 7.99 MJ kg^{-1}

Table 2.4. The effect of diet and feed rate on the growth performance, feed utilisation and nutrient retentions of Yellowtail Kingfish at the completion of the 131 day Trial 1.[1]

Diet	1	1	4	4	ANOVA[2]		
Feed rate (%)	100	80	100	80	Diet (A)	Feed rate (B)	A × B
Treatment	1	2	5	6	*P* value	*P* value	*P* value
Growth performance							
Initial weight (g)	1.60±0.01	1.60±0.02	1.61±0.03	1.59±0.02	*P* = 0.882	*P* = 0.695	*P* = 0.584
Final weight (g)	3.78±0.03	3.80±0.07	3.71±0.09	3.67±0.01	*P* = 0.132	*P* = 0.972	*P* = 0.655
Biomass gain (g tank^{-1})	34.82±0.40	35.17±0.81	33.50±1.03	33.24±0.31	*P* = 0.053	*P* = 0.924	*P* = 0.792
SGR (% d^{-1})	0.66±0.01	0.66±0.01	0.63±0.01	0.64±0.01	*P* = 0.007 (>)	*P* = 0.688	*P* = 0.881
Initial fork length (mm)	483.9±3.56	482.3±3.19	482.9±3.97	483.3±1.09	*P* = 0.982	*P* = 0.826	*P* = 0.786
Final fork length (mm)	606.5±1.17	608.4±5.18	604.0±6.61	605.9±0.31	*P* = 0.541	*P* = 0.670	*P* = 0.999
Length growth rate (mm d^{-1})	0.94±0.02	0.96±0.02	0.92±0.02	0.94±0.01	*P* = 0.251	*P* = 0.292	*P* = 0.703
Condition factor	1.69±0.01	1.69±0.02	1.68±0.02	1.65±0.01	*P* = 0.188	*P* = 0.302	*P* = 0.358
Feed utilisation							
Feed intake (g fish^{-1} d^{-1})	33.37±0.47	29.06±0.38	33.36±0.75	29.41±0.06	*P* = 0.766	*P* < 0.001 (<)	*P* = 0.747
Apparent FCR	2.01±0.02	1.73±0.03	2.09±0.07	1.85±0.01	*P* = 0.003 (<)	*P* < 0.001 (>)	*P* = 0.622
Proximate composition							
Moisture (%)	55.6±1.4	57.7±0.7	57.7±0.5	58.5±2.3	*P* = 0.275	*P* = 0.217	*P* = 0.562
Protein (% wet)	19.3±0.4	20.0±0.6	19.9±0.0	20.2±0.5	*P* = 0.320	*P* = 0.295	*P* = 0.569
Lipid (% wet)	21.5±0.6	18.5±0.3	19.6±0.4	18.4±1.8	*P* = 0.166	*P* = 0.028 (<)	*P* = 0.263
Ash (% wet)	2.0±0.2	2.5±0.2	2.6±0.1	2.8±0.2	*P* = 0.064	*P* = 0.065	*P* = 0.495
Carbohydrate (% wet; by difference)	1.5±0.8	1.5±0.8	<1.5	<1.5	*P* = 0.027 (>)	*P* = 1.00	*P* = 1.00
Energy (MJ kg^{-1} dry)	11.40±0.36	10.50±0.17	10.63±0.12	10.23±0.78	*P* = 0.139	*P* = 0.118	*P* = 0.502
Nutrient retention							
Apparent PD	20.47±0.99	25.38±1.62	20.65±0.65	23.68±0.76	*P* = 0.576	*P* = 0.004 (>)	*P* = 0.448
Apparent ED	36.42±1.54	37.47±1.30	31.49±1.46	33.35±3.68	*P* = 0.021 (>)	*P* = 0.439	*P* = 0.835

[1] Values are mean ± SE; n = 3.

[2] A significance level of $P < 0.05$ was used for all statistical tests; for variable with a significant effect of diet ($P < 0.05$) and no interaction, < or > denotes that Diet 1 was less than or greater than that measured for animals fed Diet 4; for variable with a significant effect of feed rate ($P < 0.05$) and no interaction, < or > denotes that sub-satiation (80% of apparent satiation) feed rate were less than or greater than that measured for animals in the apparent satiation feed rate Treatment.

Whole fish amino acid, taurine and choline composition
Apart from glycine, isoleucine, methionine, and tyrosine, there were no significant effects ($P > 0.05$; Table 2.5) of treatment on amino acid levels of Yellowtail Kingfish fed varying FM and FO replacement levels and feed rates for 131 days. Glycine levels tended to be lower in the fish fed Treatment 1 (Diet 1, apparent satiation; One-factor ANOVA, $P < 0.05$, Table 2.5). It was not possible to discern statistical difference between treatment means for isoleucine, methionine and tyrosine levels using the SNK post hoc test. However, isoleucine levels tended to be lower in the fish fed Treatments 1 and 7 (Diet 1, apparent satiation; Diet 5, apparent satiation, One-factor ANOVA, $P < 0.05$, Table 2.5). Methionine levels tended to be lower in the fish fed Treatment 1 (Diet 1, apparent satiation; One-factor ANOVA, $P < 0.05$, Table 2.5). Tyrosine levels tended to be lower in the fish fed Treatments 1 and 8 (Diet 1, apparent satiation; Diet 6, apparent satiation; One-factor ANOVA, $P < 0.05$, Table 2.5). There was no significant difference in taurine levels of fish between treatments (One-factor ANOVA, $P < 0.05$, Table 2.5). Choline levels tended to be lower in Treatment 7 (Diet 5, apparent satiation; One-factor ANOVA, $P < 0.05$, Table 2.5).

Whole fish fatty acid composition
There were numerous significant difference in fatty acid levels of fish between treatments (One-factor ANOVA, $P < 0.05$, Table 2.6). Most noteworthy, the EPA, DPA and DHA levels mirrored the levels found in the FO provided in the diets.

Whole fish mineral composition
Arsenic, caesium, cobalt, copper, iodine, iron, mercury, rubidium and selenium levels of fish were significantly influenced by treatments (One-factor ANOVA, $P < 0.05$, Table 2.7). Most noteworthy, arsenic and mercury levels found in the fish appeared to be related to the FM content of the diet. Selenium levels were lowest in Treatment 8 (Diet 6 to apparent satiation, 0.32 mg 100 g^{-1}) and highest in Treatment 1 (Diet 1 to apparent satiation, 0.45 mg 100 g^{-1}), which suggests that it could possibly be related to dietary FM and FO levels (Table 2.7). The selenium level of all fish cultured for 131 days were greater than or equal to selenium levels of the initial fish (Table 2.7).

Blood biochemistry and haematology
Apart from triglycerides, there was no significant effect ($P > 0.05$) of treatment on blood biochemistry and haematology of Yellowtail Kingfish fed varying FM and FO replacement levels and feed rates for 131 days. For triglycerides, Treatment 2 (Diet 1 at sub-satiation; 80% apparent satiation) levels tended to be lower than all other treatments ($P = 0.041$, Table 2.8). Results ranged from 0.92 for Treatment 2 (Diet 1 at sub-satiation; 80% apparent satiation) to 1.74 mmol L^{-1} for Treatment 5 (Diet 4 to apparent satiation). However, the SNK test could not detect a significant difference between Treatment 2 and the other treatments. Additionally, using a blood smear test, red and white cell were normal for all dietary treatments (IDEXX).

Stress test blood biochemistry and haematology and cellular immune parameters
As expected, over time, stress had a significant effect on many of the blood biochemical and haematological constituents of Yellowtail Kingfish fed to apparent satiation (Tables 2.9 and 2.10). Apart from granulocyte levels (Table 2.10), which were significantly elevated in Diet 2 and 5 compared to Diet 1 ($P = 0.02$; elevated levels suggests an immune response), there were no significant effects of diet type on any of the other constituents. For the blood smear tests, red and white cells were normal for all dietary treatments and at all sampling times before and after the stress test. Results for the cellular immune parameters from the head kidney, which are presented in Appendix 1, were variable and inconclusive.

Histological parameters
The villus height, villus area, lamina propria area, lamina propria area/total villus area and villus branching of fish in Treatments 1, 4 and 7 were not significantly different from each

other (One-factor ANOVA, $P > 0.05$). Villus height ranged from 737 to 1344 µm; villus area ranged from 82875 to 390418 μm^2; lamina propria area ranged from 22148 to 145557 μm^2; lamina propria area/total villus area ranged from 24 to 43%; and villus branching ranged from 1.20 to 8.30 branches per villus.

Table 2.5. Essential and non-essential amino acid composition (mg 100 g^{-1}), and taurine and choline concentrations of Yellowtail Kingfish fed diets containing varying fish meal replacement levels and feed rates for 131 days in Trial 1.[1]

Diet		1	1	2	3	4	4	5	6	
Feed rate (%)		100	80	100	100	100	80	100	100	
Treatment	Initial	1	2	3	4	5	6	7	8	ANOVA[2]
Essential										
Arginine	1258	1088±22	1158±16	1137±47	1154±35	1191±32	1178±23	1178±16	1155±8	*P* = 0.315
Histidine	1173	987±13	1057±15	990±28	1052±8	1094±62	1077±0	995±42	990±4	*P* = 0.118
Isoleucine	1117	911±21	984±10	933±28	973±18	985±9	973±11	912±28	923±1	*P* = 0.032[3]
Leucine	1676	1384±16	1425±71	1409±39	1481±21	1497±29	1478±27	1385±42	1386±15	*P* = 0.222
Lysine	1394	1403±85	1502±104	1390±45	1454±116	1423±44	1478±161	1259±71	1381±37	*P* = 0.601
Methionine	413	361±12	433±22	391±31	454±9	433±28	432±5	386±3	386±4	*P* = 0.033[3]
Phenylalanine	855	727±7	761±8	747±8	764±12	773±14	760±6	734±16	733±9	*P* = 0.097
Threonine	939	828±2	852±9	838±19	849±14	866±16	853±9	825±16	838±18	*P* = 0.506
Tryptophan	211	206±1	184±3	190±5	196±9	194±5	200±2	193±8	192±5	*P* = 0.292
Valine	1180	1141±54	1141±83	1161±50	1214±80	1146±100	1176±137	1091±94	1080±77	*P* = 0.954
Non-essential										
Alanine	1222	1096±20	1151±11	1161±64	1153±30	1184±40	1176±37	1220±16	1186±10	*P* = 0.347
Aspartic acid	2026	1733±25	1849±36	1884±22	1913±56	1878±62	1895±78	1812±20	1791±42	*P* = 0.125
L Cystine	225	195±6	196±8	184±3	207±6	199±8	215±9	190±16	182±6	*P* = 0.266
Glutamic acid	2695	2260±40	2405±51	2566±68	2536±95	2481±88	2500±67	2408±22	2362±87	*P* = 0.105
Glycine	968	958±42[b]	1056±12[ab]	1066±120 [ab]	1083±56 [ab]	1130±74 [ab]	1164±78 [ab]	1330±41[a]	1176±25 [ab]	*P* = 0.033
Proline	252	504±94	489±20	550±75	462±80	464±84	566±29	390±101	529±50	*P* = 0.796
Serine	778	673±15	714±8	675±24	720±20	730±20	735±10	715±9	697±3	*P* = 0.120
Tyrosine	764	597±18	652±10	608±23	653±13	663±17	652±12	604±13	597±3	*P* = 0.015[3]
Other										
Taurine	234	192±12	196±6	183±4	202±5	186±4	199±7	202±3	190±13	*P* = 0.555
Choline	1320	1306±28	1320±14	1246±31	1251±74	1268±37	1373±7	1180±54	1385±26	*P* = 0.046[3]

[1] Values are mean ± SE; n = 3.
[2] A significance level of $P < 0.05$ was used for all statistical tests, where significant differences were observed post-hoc tests were used (Student Newman-Keuls test) to detect differences between treatments, values within each row without a common superscript are significantly different ([a] indicates the highest value; $P < 0.05$).
[3] One-factor ANOVA detected a significant difference, but SNK test was unable to discern significant differences.

Table 2.6. Fatty acid composition (mg 100 g^{-1}) of Yellowtail Kingfish fed diets containing varying fish meal replacement levels and feed rates for 131 days in Trial 1.[1,2,3]

Diet		1	1	2	3	4	4	5	6	
Feed rate (%)		100	80	100	100	100	80	100	100	
Treatment	Initial	1	2	3	4	5	6	7	8	ANOVA[3]
Butyric (C4:0)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Caproic (C6:0)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Caprylic (C8:0)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Capric (C10:0)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Lauric (C12:0)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Trisdecanoic (C13:0)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Myristic (C14:0)	330	713±9^{a}	610±12^{b}	463±12^{c}	360±25^{d}	390±6^{d}	350±40^{d}	463±23^{c}	370±20^{d}	$P < 0.001$
Pentadecanoic (C15:0)	41	110±0^{a}	93±2^{b}	68±2^{c}	51±3^{d}	55±1^{d}	50±5^{d}	67±4^{c}	51±2^{d}	$P < 0.001$
Palmitic (C16:0)	1920	4067±102^{a}	3357±92^{b}	3620±110^{ab}	3100±210^{b}	3543±80^{ab}	3270±310^{b}	3680±159^{ab}	3403±152^{b}	$P = 0.012$
Margaric (C17:0)	46	93±2^{a}	81±1 ^{b}	69±2^{c}	54±3^{d}	63±1^{cd}	58±6^{cd}	69±4^{c}	58±2^{cd}	$P < 0.001$
Stearic (C18:0)	700	957±15^{a}	777±18^{b}	1043±27^{a}	940±61^{a}	1113±19^{a}	1035±95^{a}	1090±38^{a}	1080±60^{a}	$P = 0.001$
Arachidic (C20:0)	29	43±1^{a}	36±1^{b}	35±1^{b}	31±3^{b}	32±1^{b}	30±3^{b}	34±2^{b}	31±1^{b}	$P = 0.001$
Docosanoic (C22:0)	15	23±0^{a}	21±1^{ab}	19±1^{ab}	17±2^{b}	17±1^{b}	17±1^{b}	20±2^{ab}	17±0^{b}	$P = 0.014$
Tetracosanoic (C24:0)	22	41±2^{a}	40±1^{a}	32±1^{b}	30±1^{b}	29±1^{bc}	29±3^{bc}	33±2^{b}	23±1^{c}	$P < 0.001$
Saturated Fat (%m m^{-1})	3.1	6.0±0.1^{a}	5.0±0.01^{b}	5.4±0.01^{ab}	4.6±0.3^{b}	5.3±0.1^{ab}	4.9±0.5^{b}	5.4±0.2^{ab}	5.1±0.2^{b}	$P = 0.011$
Decenoic (C10:1)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Myristoleic (C14:1)	16	41±0^{a}	35±1^{b}	33±1^{b}	24±1^{d}	31±1^{bc}	28±3^{cd}	34±2^{b}	30±1^{bc}	$P < 0.001$
Pentadecenoic (C15:1)	<10	12±0^{a}	7±4^{b2}	<10^{c}	<10^{c}	<10^{c}	<10^{c}	<10^{c}	<10^{c}	$P < 0.001$
Palmitoleic (C16:1)	580	1130±15^{a}	977±18^{ab}	1040±35^{a}	840±51^{b}	1047±15^{a}	955±105^{ab}	1063±43^{a}	1033±47^{a}	$P = 0.007$
Heptadecenoic (C17:1)	33	86±24	88±1	69±2	53±3	59±1	55±6	71±3	57±2	$P = 0.084$
Octadecenoic (C18:1n-6)	<10	3±3[2]	<10	<10	<10	<10	<10	<10	<10	$P = 0.511$
Octadecenoic (C18:1n-7)	340	733±12^{a}	627±9^{b}	590±17^{bcd}	497±28^{d}	563±9^{bcd}	515±55^{cd}	607±26^{bc}	563±27^{bcd}	$P < 0.001$
Oleic (C18:1n-9)	3110	5680±100^{bc}	4780±76^{c}	6477±199^{ab}	5603±322^{bc}	7107±117^{a}	6605±655^{ab}	6747±299^{a}	7157±279^{a}	$P < 0.001$

[1] Values are mean ± SE; n = 3.

[2] One or two samples were below detectable range and were assigned the value of 0; if the values for all three replicates were below detectable range then they are reported here as below the detectable range (eg. <10).

[3] A significance level of $P < 0.05$ was used for all statistical tests, where significant differences were observed post-hoc tests were used (Student Newman-Keuls test) to detect differences between treatments, values within each row without a common superscript are significantly different (^{a} indicates the highest value; $P < 0.05$).

Table 2.6. Continued.

Diet		1	1	2	3	4	4	5	6	
Feed rate (%)		100	80	100	100	100	80	100	100	
Treatment	Initial	1	2	3	4	5	6	7	8	ANOVA[3]
Steridonic (C18:4n-3)	83	127±9[a]	120±0[a]	74±1[b]	61±4[bc]	58±6[bc]	54±6[bc]	74±6[b]	48±1[c]	*P* < 0.001
Eicosadienoic (C20:2n-6)	34	62±1[a]	54±1[b]	49±1[b]	49±2[b]	48±1[b]	45±6[b]	51±3[b]	45±2[b]	*P* = 0.001
Eicosatrienoic (C20:3n-3)	11	35±1[a]	32±0[b]	21±0[c]	18±1[cd]	16±0[d]	15±2[d]	21±1[c]	14±0[d]	*P* < 0.001
Dihomo-gamma-linoleic (C20:3n-6)	15	24±2	20±0	22±1	24±3	21±1	22±3	24±1	20±1	*P* = 0.497
Eicosatetracenoic (C20:4n-3)	120	507±19[a]	453±7[b]	243±3[c]	187±9[d]	157±3[d]	140±20[d]	243±20[c]	147±3[d]	*P* < 0.001
Arachidonic (C20:4n-6)	84	140±10[a]	130±0[a]	99±1[b]	86±3[bc]	89±6[bc]	89±8[bc]	103±7[b]	69±3[c]	*P* < 0.001
Eicosapentaenoic (C20:5n-3)	510	780±76[a]	750±15[a]	443±9[b]	367±22[bc]	347±41[bc]	330±40[bc]	453±47[b]	263±12[c]	*P* < 0.001
Heneicosapentaenoic acid (C21:5n-3)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Docosatetraenoic (C22:4n-6)	32	49±3[a]	43±1[a]	29±0[b]	23±1[bc]	23±1[bc]	22±2[bc]	27±3[b]	16±1[c]	*P* < 0.001
Docosapentaenoic (C22:5n-3)	180	343±35[a]	323±7[a]	200±0[bc]	177±7[bc]	163±15[bc]	155±25[bc]	213±17[b]	127±3[c]	*P* < 0.001
Docosapentaenoic (C22:5n-6)	26	40±5[a]	39±1[a]	23±1[b]	19±2[b]	17±2[b]	16±3[b]	23±2[b]	13±1[b]	*P* < 0.001
Docosahexaenoic (C22:6n-3)	800	1553±176[a]	1493±52[a]	847±32[b]	707±27[bc]	660±78[bc]	655±65[bc]	867±99[b]	460±35[c]	*P* < 0.001
Poly Unsaturated Fat (%m m^{-1})	4.7	5.7±0.4	5.2±0.1	4.9±0.1	5.1±0.2	4.9±0.2	4.6±0.5	5.1±0.4	4.4±0.1	*P* = 0.081
Trans Fat content (%m m^{-1})	0.1	0.3±0.0[a]	0.3±0.0[ab]	0.2±00[bcd]	0.1±0.0[d]	0.2±0.0[cd]	0.2±0.1[cd]	0.3±0.0[abc]	0.2±0.0[bcd]	*P* = 0.001
Cholesterol	115	95±4	95±1	100±2	98±4	96±3	99±1	103±1	103±1	*P* = 0.224

[1] Values are mean ± SE; n = 3.
[2] One or two samples were below detectable range and were assigned the value of 0; if the values for all three replicates were below detectable range then they are reported here as below the detectable range (e.g. <10), but were assigned the value of 0 for statistical analyses.
[3] A significance level of $P < 0.05$ was used for all statistical tests, where significant differences were observed post-hoc tests were used (Student Newman-Keuls test) to detect differences between treatments, values within each row without a common superscript are significantly different ([a] indicates the highest value; $P < 0.05$).

Table 2.7. Mineral composition (mg 100 g^{-1}) of Yellowtail Kingfish after 131 days in Trial 1.

Diet		1	1	2	3	4	4	5	6	
Feed rate (%)		100	80	100	100	100	80	100	100	
Treatment	Initial	1	2	3	4	5	6	7	8	*ANOVA*[3]
Aluminium	1.6	0.5±0.5[2]	<1	<1	<1	<1	<1	<1	<1	*P* = 0.511
Antimony	0.032	0.032±0.001	0.029±0.001	0.030±0.001	0.022±0.010	0.022±0.009	0.032±0.001	0.031±0.001	0.028±0.000	*P* = 0.655
Arsenic	0.41	0.52±0.01[a]	0.50±0.02[a]	0.35±0.01[b]	0.26±0.01[d]	0.26±0.02[d]	0.28±0.00[cd]	0.31±0.02[c]	0.24±0.00[d]	*P* < 0.001
Barium	0.230	0.153±0.020	0.123±0.013	0.463±0.318	0.064±0.024	0.184±0.094	0.060±0.002	0.126±0.034	0.723±0.539	*P* = 0.473
Bismuth	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-
Beryllium	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	-
Boron	<0.5	0.39±0.19[2]	<0.5	<0.5	<0.5	<0.5	<0.5	0.25±0.25	<0.5	*P* = 0.182
Caesium	0.029	0.017±0.000[ab]	0.017±0.000[ab]	0.016±0.001[ab]	0.019±0.001[a]	0.018±0.002[ab]	0.016±0.000[ab]	0.014±0.001[b]	0.016±0.000[ab]	*P* = 0.016
Cadmium	0.0086	0.0044±0.0004	0.0035±0.0002	0.0038±0.0001	0.0033±0.0003	0.0036±0.003	0.0030±0.0005	0.0038±0.0004	0.0031±0.0003	*P* = 0.180
Calcium	NR	3867±133	5800±58	7300±1266	5700±1513	5500±1124	4150±250	5700±208	6700±1150	*P* = 0.346
Chromium[2]	<0.1	0.20±0.05	0.04±0.04[2]	<0.1	<0.1	0.19±0.13	<0.1	<0.1	0.10±0.05[2]	*P* = 0.086
Cobalt	0.022	0.015±0.008[abc2]	0.007±0.007[bc2]	<0.02[c]	0.029±0.002[ab]	0.032±0.001[ab]	0.036±0.003[a]	0.018±0.009[abc2]	0.024±0.002[ab]	*P* = 0.004
Copper	0.69	0.70±0.01[ab]	0.57±0.01[b]	0.56±0.00[b]	0.59±0.06[b]	0.74±0.03[a]	0.62±0.03[b]	0.56±0.03[b]	0.58±0.02[b]	*P* = 0.004
Iodine (µg 100 g^{-1})	44	46±4[a]	42±1[ab]	39±2[abc]	41±1[abc]	33±4[cb]	41±0[abc]	36±1[abc]	31±1[c]	*P* = 0.005
Iron	45	44±8[a]	22±0[b]	22±1[b]	20±1[b]	25±3[b]	22±1[b]	22±1[b]	22±2[b]	*P* = 0.003
Lead	<0.01	0.004±0.004	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	*P* = 0.511
Lithium	0.029	0.029±0.001	0.035±0.003	0.037±0.003	0.032±0.004	0.036±0.002	0.032±0.001	0.037±0.002	0.039±0.002	*P* = 0.194
Magnesium	310	317±3	355±14[3]	373±24	347±27	370±12	315±5	333±17	353±28	*P* = 0.399
Manganese	NR[3]	0.73±0.06	0.91±0.77	0.84±0.13	0.68±0.15	0.74±0.13	0.56±0.01	0.67±0.04	0.92±0.19	*P* = 0.670
Molybdenum	<0.02	0.01±0.01[2]	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	*P* = 0.511
Mercury	0.047	0.120±0.006[a]	0.103±0.004[b]	0.081±0.001[c]	0.059±0.004[c]	0.061±0.007[c]	0.070±0.001[c]	0.079±0.010[c]	0.062±0.003[c]	*P* < 0.001
Nickel[2]	<0.1	0.04±0.04[2]	<0.1	<0.1	<0.1	<0.1	<0.1	0.07±0.07[2]	<0.1	*P* = 0.594
Potassium	3400	2867±120	2900±0	2800±58	3033±88	3233±233	2850±50	2667±88	2833±67	*P* = 0.082
Phosphorus	NR[3]	3600±58	4700±51[3]	5433±636	4700±862	4533±601	3800±200	4533±145	5167±667	*P* = 0.379
Rubidium	0.76	0.65±0.03[c]	0.71±0.00[bc]	0.72±0.03[bc]	0.93±0.03[a]	0.84±0.08[ab]	0.82±0.02[abc]	0.66±0.03[c]	0.72±0.01[bc]	*P* = 0.001
Selenium	0.34	0.45±0.01[a]	0.41±0.01[ab]	0.40±0.03[ab]	0.43±0.01[ab]	0.38±0.01[bc]	0.36±0.01[bc]	0.36±0.02[bc]	0.32±0.01[c]	*P* = 0.001
Sodium	840	897±35	877±13	887±39	930±15	1020±42	935±5	930±35	957±12	*P* = 0.065
Silver	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	-
Strontium	10	14±0	26±4	29±6	21±6	21±4	16±1	21±1	25±5	*P* = 0.266
Tin	2.5	1.45±0.70	0.26±0.04	0.42±0.07	0.17±0.03	0.57±0.16	0.58±0.05	0.53±0.01	0.16±0.01	*P* = 0.065
Thallium	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-
Uranium	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	-
Vanadium	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	-
Zinc	NR[3]	13±1	12±3[3]	14±0	13±1	13±2	13±1	13±1	15±1	*P* = 0.617

[1] Values are mean ± SE; n = 3.

[2] One or two samples were below the detectable range and were assigned the value of 0; if the values for all three replicates were below detectable range then they are reported here as below the detectable range (i.e. <x), but were assigned 0 for statistical analyses.

[3] A significance level of $P < 0.05$ was used for all statistical tests, where significant differences were observed post-hoc tests were used (Student Newman-Keuls test) to detect differences between treatments, values within each row without a common superscript are significantly different ([a] indicates the highest value; $P < 0.05$).

Table 2.8. Blood biochemistry and haematology of Yellowtail Kingfish fed diets containing varying fish meal replacement levels and feed rates for 131 days in Trial 1.[1, 2]

Diet	1	1	2	3	4	4	5	6	
Feed rate (%)	100	80	100	100	100	80	100	100	
Treatment	1	2	3	4	5	6	7	8	ANOVA[3]
Biochemistry									
Sodium (mmol L^{-1})	183.67±4.44	184.50±16.50	184.50±7.78	187.17±3.61	192.83±6.50	173.25±8.75	189.33±7.10	181.67±10.99	*P* = 0.924
Potassium (mmol L^{-1})	3.34±0.24	3.37±0.37	3.21±0.12	3.36±0.45	3.35±0.41	2.92±0.04	3.40±0.25	3.16±0.15	*P* = 0.970
Urea (mmol L^{-1})	5.7±0.5	5.6±0.3	5.2±0.1	6.2±0.6	6.9±1.5	5.2±0.5	5.6±0.7	6.2±0.7	*P* = 0.765
Creatinine (mmol L^{-1})	0.04±0.01	0.03±0.01	0.03±0.01	0.03±0.00	0.03±0.01	0.04±0.01	0.03±0.00	0.04±0.01	*P* = 0.904
Calcium (mmol L^{-1})	2.94±0.12	3.08±0.05	2.90±0.16	2.97±0.11	3.06±0.00	2.84±0.26	3.03±0.04	2.75±0.28	*P* = 0.741
Protein (g L^{-1})	48.00±5.06	53.00±5.97	52.17±1.48	49.33±1.64	48.50±5.20	49.25±2.75	48.83±3.11	51.17±4.92	*P* = 0.982
Albumin (g L^{-1})	18.00±2.57	19.83±4.34	19.67±1.92	17.67±0.44	16.83±1.92	21.00±0.50	16.00±0.58	20.17±3.28	*P* = 0.831
Globulin (g L^{-1})	30.50±2.29	33.17±1.92	32.50±2.60	31.67±1.88	31.67±3.56	28.25±3.25	32.83±2.59	31.00±2.02	*P* = 0.933
Total Bilirubin (mmol L^{-1})	0.47±0.02	0.62±0.17	0.32±0.06	0.20±0.05	0.43±0.10	0.48±0.03	0.55±0.14	0.35±0.13	*P* = 0.211
ALT (IU L^{-1})	28.50±5.68	38.67±20.33	27.17±4.92	24.67±4.44	23.33±11.37	61.50±14.00	16.83±3.94	28.50±7.94	*P* = 0.276
ALP (IU L^{-1})	1.33±1.33	9.50±4.51	4.83±4.11	3.83±2.32	4.33±3.38	2.50±2.50	4.50±2.29	1.67±1.01	*P* = 0.631
Magnesium (mmol L^{-1})	0.38±0.20	0.37±0.14	0.38±0.23	0.37±0.11	0.24±0.07	0.42±0.07	0.20±0.03	0.39±0.14	*P* = 0.946
Cholesterol (mmol L^{-1})	7.30±0.87	6.96±0.84	7.07±0.16	6.55±0.23	6.01±0.43	6.78±0.09	6.73±0.48	6.23±0.56	*P* = 0.740
Triglyceride (mmol L^{-1})	1.07±0.23	0.92±0.03	1.12±0.14	1.44±0.12	1.74±0.23	1.41±0.04	1.31±0.18	1.57±0.15	*P* = 0.041
Bile Acids (mmol L^{-1})	0.63±0.14	1.85±0.63	1.60±1.15	0.93±0.44	1.77±0.49	1.00±0.50	1.77±0.04	0.70±0.30	*P* = 0.578
Hematology									
RBC ($\times 10^{12}$)	3.75±0.14	3.42±0.14	3.55±0.31	3.62±0.44	3.62±0.10	3.61±0.18	3.50±0.19	3.09±0.49	*P* = 0.833
HGB (g L^{-1})	129.67±6.07	118.83±2.96	133.83±8.48	132.83±2.89	131.83±8.17	127.00±7.00	124.67±6.41	123.67±7.34	*P* = 0.697
PCV (L L^{-1})	0.54±0.05	0.52±0.05	0.53±0.01	0.53±0.01	0.52±0.03	0.55±0.03	0.50±0.02	0.54±0.05	*P* = 0.978
MCV (fl)	168.53±2.42	173.25±1.90	170.17±0.48	168.43±3.38	176.47±2.92	175.05±0.45	174.25±2.39	178.58±4.61	*P* = 0.156
MCH (pg)	34.57±0.80	34.82±1.18	38.93±4.25	39.01±5.59	36.35±1.40	35.23±0.23	35.62±0.62	42.08±5.33	*P* = 0.722
MCHC (g L^{-1})	205.17±2.73	201.00±5.63	230.67±25.94	231.67±31.83	205.83±5.05	201.00±1.50	204.33±3.77	236.00±26.06	*P* = 0.676
WBC ($\times 10^{9}$)	4.98±0.35	4.92±0.22	5.65±0.65	4.23±0.18	5.22±0.59	4.85±0.40	4.52±0.37	4.63±0.52	*P* = 0.475
Granulocytes (%)	4.67±0.17	7.33±2.09	6.83±0.44	5.33±0.73	6.00±0.50	5.50±0.50	6.50±1.32	5.83±0.44	*P* = 0.639
Lymph (%)	93.83±0.73	90.17±3.11	89.17±0.93	92.83±1.48	91.83±0.83	92.75±1.75	90.50±2.25	92.67±1.45	*P* = 0.561
Mono (%)	1.50±0.58	2.50±1.04	4.17±0.88	1.83±0.83	2.17±0.33	1.75±1.25	3.00±1.04	2.17±1.01	*P* = 0.497
Eosin (%)	0.00±0.00	0.17±0.17	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	*P* = 0.511
Baso (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	*P* = 1.000
Platelets ($\times 10^{9}$)	24.17±4.88	23.00±3.33	16.00±6.00	17.00±1.32	30.33±4.60	20.75±8.75	29.50±6.87	24.50±1.15	*P* = 0.362

[1] Values are mean ± SE; n = 3; [2] SE less than 0.01 are reported as "0.00"; [3]A significance level of $P < 0.05$ was used for all statistical tests.
ALT = alanine aminotransferase; ALP = alkaline phosphatase; Baso = basophil; Eosin = eosinophil; HGB = haemoglobin; Lymph = lymphocytes; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; Mono = monocytes; PCV = packed cell volume; RBC = red blood cell count; WBC = white blood cell count.

Table 2.9. Blood biochemistry of Yellowtail Kingfish fed varying fish meal replacement levels and feed rates at the conclusion of the growth study in Trial 1 or 3 days after 15 min of stress.[1,2]

Stressed	Not stressed				Stressed							
Diet	1	2	3	5	1	2	3	5	Stress (A)		Diet (B)	A × B
Dietary treatment	1	3	4	7	1	3	4	7	*P* value	> / <	*P* value	*P* value
Biochemistry												
Sodium (mmol L^{-1})	183.67±4.44	184.50±7.78	187.17±3.61	189.33±7.10	199.67±3.93	195.89±6.27	193.00±3.67	196.33±6.00	*P* = 0.009	(<)	*P* = 0.945	*P* = 0.793
Potassium (mmol L^{-1})	3.34±0.24	3.21±0.12	3.36±0.45	3.40±0.25	3.00±0.02	2.93±0.04	2.96±0.02	2.88±0.10	*P* = 0.008	(>)	*P* = 0.954	*P* = 0.944
Urea (mmol L^{-1})	5.7±0.5	5.2±0.1	6.2±0.6	5.6±0.7	2.71±0.48	3.33±0.35	3.12±0.26	3.39±0.25	*P* < 0.001	(>)	*P* = 0.734	*P* = 0.499
Creatinine (mmol L^{-1})	0.04±0.01	0.03±0.01	0.03±0.00	0.03±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00	*P* = 0.001	(>)	*P* = 0.335	*P* = 0.427
Calcium (mmol L^{-1})	2.94±0.12	2.90±0.16	2.97±0.11	3.03±0.04	3.04±0.01	3.05±0.01	3.06±0.00	3.04±0.01	*P* = 0.091	-	*P* = 0.869	*P* = 0.900
Protein (g L^{-1})	48.00±5.06	52.17±1.48	49.33±1.64	48.83±3.11	51.56±0.40	51.67±0.19	50.00±0.38	49.89±2.62	*P* = 0.453	-	*P* = 0.670	*P* = 0.863
Albumin (g L^{-1})	18.00±2.57	19.67±1.92	17.67±0.44	16.00±0.58	16.89±0.11	16.78±0.40	16.44±0.29	16.11±0.62	*P* = 0.136	-	*P* = 0.328	*P* = 0.668
Globulin (g L^{-1})	30.50±2.29	32.50±2.60	31.67±1.88	32.83±2.59	34.67±0.33	34.78±0.29	33.67±0.33	34.89±1.47	*P* = 0.027	(<)	*P* = 0.809	*P* = 0.913
Total Bilirubin (mmol L^{-1})	0.47±0.02 [b]	0.32±0.06 [b]	0.20±0.05 [b]	0.55±0.14 [b]	1.00±0.07 [a]	1.04±0.14 [a]	1.43±0.22 [a]	1.21±0.01 [a]	*P* < 0.001	-	*P* = 0.331	*P* = 0.034*
ALT (IU L^{-1})	28.50±5.68	27.17±4.92	24.67±4.44	16.83±3.94	12.44±0.73	11.44±1.64	14.89±3.89	11.56±3.38	*P* < 0.001	(>)	*P* = 0.372	*P* = 0.472
ALP (IU L^{-1})	1.33±1.33	4.83±4.11	3.83±2.32	4.50±2.29	9.78±1.06	10.33±0.67	10.67±0.58	9.89±1.64	*P* < 0.001	(<)	*P* = 0.724	*P* = 0.886
Magnesium (mmol L^{-1})	0.38±0.20	0.38±0.23	0.37±0.11	0.20±0.03	0.16±0.01	0.14±0.03	0.16±0.00	0.13±0.01	*P* = 0.020	(>)	*P* = 0.754	*P* = 0.870
Cholesterol (mmol L^{-1})	7.30±0.87	7.07±0.16	6.55±0.23	6.73±0.48	7.36±0.03	7.25±0.17	6.61±0.17	6.53±0.58	*P* = 0.915	-	*P* = 0.156	*P* = 0.974
Triglyceride (mmol L^{-1})	1.07±0.23	1.12±0.14	1.44±0.12	1.31±0.18	1.36±0.09	1.46±0.13	1.79±0.09	2.02±0.61	*P* = 0.026	(<)	*P* = 0.189	*P* = 0.836
Bile Acids (mmol L^{-1})	0.63±0.14	1.60±1.15	0.93±0.44	1.77±0.04	3.91±3.14	2.83±0.70	2.04±0.33	1.59±0.10	*P* = 0.102		*P* = 0.887	*P* = 0.579

[1] Values are mean ± SE; n = 3.

[2] SE less than 0.01 are reported as “0.00”.

[3] For variable with a significant effect of stress (*P* < 0.05) and no interaction, < or > denotes that non-stressed animals were less than or greater than that measured for stressed animals; [y, z] For parameters with a significant effect of diet and no interaction, values without a common letter are different (z indicated the highest value; *P* < 0.05); When significant interactions (A×B; *P* < 0.05) were observed difference in stressed treatments were compared across all diets (One-factor ANOVA, SNK test), values without a common superscript are significantly different ([a] indicates the highest value; *P* < 0.05).

ALT = alanine aminotransferase; ALP = alkaline phosphatase.

Table 2.10. Blood haematology of Yellowtail Kingfish fed varying fish meal replacement levels and feed rates at the conclusion of the growth study in Trial 1 or 3 days after 15 min of stress.[1,2]

Stressed	Not stressed				Stressed							
Diet	1	2	3	5	1	2	3	5	Stress (A)		Diet (B)	A × B
Dietary treatment	1	3	4	7	1	3	4	7	*P* value	> / <	*P* value	*P* value
Hematology												
RBC (×10^{12})	3.75±0.14	3.55±0.31	3.62±0.44	3.50±0.19	3.92±0.11	3.73±0.15	3.62±0.18	3.83±0.10	*P* = 0.259	-	*P* = 0.737	*P* = 0.909
HGB (g L^{-1})	129.67±6.07	133.83±8.48	132.83±2.89	124.67±6.41	142.22±0.22	139.89±1.13	139.00±2.41	146.11±3.56	*P* = 0.001	(<)	*P* = 0.992	*P* = 0.347
PCV (L L^{-1})	0.54±0.05	0.53±0.01	0.53±0.01	0.50±0.02	0.58±0.01	0.57±0.01	0.55±0.02	0.58±0.01	*P* = 0.002	(<)	*P* = 0.714	*P* = 0.649
MCV (fl)	168.53±2.42	170.17±0.48	168.43±3.38	174.25±2.39	172.84±0.88	175.52±1.93	176.53±2.71	172.31±1.91	*P* = 0.021	(<)	*P* = 0.706	*P* = 0.177
MCH (pg)	34.57±0.80	38.93±4.25	39.01±5.59	35.62±0.62	36.28±1.12	38.81±1.36	37.73±1.69	36.93±0.67	*P* = 0.818	-	*P* = 0.442	*P* = 0.939
MCHC (g L^{-1})	205.17±2.73	230.67±25.94	231.67±31.83	204.33±3.77	209.67±5.62	224.11±10.38	216.33±8.83	213.00±5.54	*P* = 0.836	-	*P* = 0.398	*P* = 0.866
WBC (×10^{9})	4.98±0.35	5.65±0.65	4.23±0.18	4.52±0.37	10.66±0.84	11.68±0.74	12.07±0.91	11.38±0.34	*P* < 0.001	(<)	*P* = 0.551	*P* = 0.320
Granulocytes (%)	4.67±0.17	6.83±0.44	5.33±0.73	6.50±1.32	6.00±0.67	8.67±1.50	7.22±0.68	8.67±0.51	*P* = 0.005	(<)	*P* = 0.020	*P* = 0.969
Lymph (%)	93.83±0.73	89.17±0.93	92.83±1.48	90.50±2.25	92.22±0.40	182.11±89.95	92.56±0.68	90.67±0.88	*P* = 0.332	-	*P* = 0.447	*P* = 0.386
Mono (%)	1.50±0.58	4.17±0.88	1.83±0.83	3.00±1.04	1.78±0.68	0.44±0.44	0.22±0.11	0.67±0.38	*P* = 0.003	(>)	*P* = 0.450	*P* = 0.061
Eosin (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-	-	-	-
Baso (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-	-	-	-
Platelets (×10^{9})	24.17±4.88	16.00±6.00	17.00±1.32	29.50±6.87	15.11±4.39	9.89±3.95	13.22±2.67	13.78±4.51	*P* = 0.015	(>)	*P* = 0.225	*P* = 0.609

[1] Values are mean ± SE; n = 3.

[2] SE less than 0.01 are reported as “0.00”.

[3] For variable with a significant effect of stress ($P < 0.05$) and no interaction, < or > denotes that non-stressed animals were less than or greater than that measured for stressed animals; [y, z] For parameters with a significant effect of diet and no interaction, values without a common letter are different (z indicated the highest value; $P < 0.05$); When significant interactions (A×B; $P < 0.05$) were observed differences in stressed treatments were compared across all diets (One-factor ANOVA, SNK test), values without a common superscript are significantly different ([a] indicates the highest value; $P < 0.05$).

Baso = basophil; Eosin = eosinophil; HGB = haemoglobin; Lymph = lymphocytes; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; Mono = monocytes; PCV = packed cell volume; RBC = red blood cell count; WBC = white blood cell count.

Discussion

Our overarching goal of Trial 1 was to improve the sustainability for Yellowtail Kingfish production by reducing the reliance of marine ingredients, and also to improve the current on-farm feed management practices. Information from this study was essential to achieve this goal, and is also valuable for the future development of on-farm feeding practices. In order to reduce the reliance of marine ingredients, investigation into diets formulated using highly palatable alternative ingredients at economically viable and realistic inclusion levels were carried out.

Yellowtail Kingfish fed actively on all diets throughout this trial, and growth rates were comparable to those observed at Clean Seas commercial sea cage facilities (personal communication, Dr T. D'Antignana; Clean Seas) and also to the growth rates observed in fish in the tank-based study in Trial 2 (Chapter 3, Table 3.3; Figure 3.1). Additionally, the survival of Yellowtail Kingfish was positive in this trial. Mortalities were low, and the only fish that died were due to operator error. Overall, based on the growth performance and survival obtained in this trial, data obtained from the tank system should be transferable to the sea cage production of Yellowtail Kingfish.

The first aim of this study, benchmarking the current Yellowtail Kingfish diet against the "gold standard" control diet, was successfully achieved. The growth performance and feed utilisation of large (> 1.5 kg) Yellowtail Kingfish fed the recently formulated Ridley Clean Seas Pelagica 2013 Yellowtail Kingfish production diet (Diet 2; Treatment 3) was not significantly different to fish fed the "gold standard" diet, which was formulated to contain high quality marine ingredients (FM and FO; Diet 1, Treatment 1).

With regard to improving our knowledge to improve feed management of Yellowtail Kingfish, fish fed to sub-satiation had significantly superior feed utilisation compared to fish fed to apparent satiation. Feed consumption rates were significantly lower in fish fed to sub-satiation (80% apparent satiation), but this did not compromise growth. In turn, this resulted in significantly superior FCRs, and improved protein and energy deposition for Yellowtail Kingfish fed to sub-satiation. These results suggest that with careful feed management, cost savings may be made to on-farm feed efficiency, without impairing growth. However, it should be noted that animals fed to apparent satiation had higher soft tissue lipid levels compared to fish fed to sub-satiation. Yellowtail Kingfish utilise lipids for energy (Booth et al., 2010), indicating that animals fed to sub-satiation may have utilised soft tissue lipids to satisfy energy requirements in the absence of sufficient dietary energy provisions. However, the energy requirements of > 1.5 kg Yellowtail Kingfish have not been determined experimentally (Stone and Bellgrove, 2013). It may be beneficial to investigate increasing dietary lipid and energy level in Yellowtail Kingfish fed to sub-satiation.

Clean Seas indicated that at the time of this trial, the targeted on-farm feeding regime for large fish in their sea cage systems was to feed to apparent satiety levels. However, when compared to feed intake data from Trial 1, it was discovered that Clean Seas were feeding sea cage fish to levels that were closer to 80% of apparent satiation (personal communication, Dr T. D'Antignana; Clean Seas). Information provided from Trial 1 has enabled Clean Seas to better calibrate feeding methods. This further demonstrates the importance in running controlled trials to improve feed management knowledge. It also needs to be noted that large Yellowtail Kingfish in Trial 1 were cultured in ideal water quality conditions, with plentiful dissolved oxygen, which without additional oxygenation may not be possible to maintain in commercial sea cage production systems.

Results for FM and FO substitution were encouraging. The Yellowtail Kingfish production diet (Diet 5 in Trial 1) containing 30% FM (25% reduction in FM) and with 50% of the FO substituted with poultry oil (PO) performed well compared to the "gold standard" FM control

diet. Bowyer et al. (2012) similarly concluded that the growth and feed utilisation of juvenile Yellowtail Kingfish (starting weight of 100 g) was not compromised when fed a diet that had FO substituted with PO, compared to a FO control diet. However, there was a significant amount of LC n-3 PUFA provided in these diets with the FM. In addition to the significant improvement to the sustainable production (improved fish in-fish out ratio) of Yellowtail Kingfish, market access for this top quality food product would also be increased, due to improved public perception (Bowyer et al., 2013a). Furthermore, arsenic and mercury levels were reduced when fish were fed reduced levels of dietary FM and FO. This needs to be considered due to human health implications associated with arsenic and mercury poisoning. Interestingly, maximum allowable limits in fish for arsenic and mercury are reported to be 2 mg kg^{-1} and 1 mg kg^{-1}, respectively (Australia New Zealand Food Standards, 2012). In Trial 1, mercury levels were exceeded for fish fed the "gold standard" diet high in FM and FO (Table 2.7). However, this is a purely experimental diet, which is never fed under commercial condtions. In contrast, when FM and FO substitution was practiced in Trial 1, all fish contained acceptable levels of mercury and arsenic levels were reduced (Table 2.7). Note, the mercury and arsenic were analysed in whole fish, and it is unlikely that mercury and arsenic levels would exceed acceptable standards if gilled and gutted fish were used for analyses.

It was hypothesised that reducing dietary FM and FO inclusion levels too far in Yellowtail Kingfish may compromise health and survival, particularly during stress events. This has been reported in other species such as Atlantic salmon (*Salmo salar*) (Oxley et al., 2010). With regard to the stress test, mortalities were non-existent. Results for immunological function showed significant dietary differences (Appendix 1), but differences due to FM and FO substitution were inconclusive. Haematological values were influenced by handling stress. In the stress challenge, the white blood cell count (granulocyte) tended to be higher in fish fed low FM and FO levels after the stress event. This may indicate a higher level of stress damage in fish fed low FM and FO diets, compared to fish fed high FM and FO diets. This needs to be considered on-farm, especially during on-farm stress events, including parasite bathing and cage transfer. This is an area of Yellowtail Kingfish physiology that requires further research. Based on the results of the stress test used in this study, it is recommended that a more sensitive bacterial challenge test is developed to assess the impacts of dietary alterations on the health and survival of Yellowtail Kingfish at the completion of future growth trials.

There is an opportunity to further reduce the dietary inclusions of FM and FO in production diets for large Yellowtail Kingfish. However, with respect to FO substitution, it is important to consider the dietary LC n-3 PUFA. Fish oil, and the inherent FO in FM, contain the essential LC n-3 PUFA: eicosapentaenoic acid (20:5n-3, EPA), docosapentaenoic acid (22:5n-3, DPA) and docosahexaenoic acid (22:6n-3, DHA) (Bowyer et al., 2012). These LC n-3 PUFA are important for numerous aspects of animal physiology including cellular membrane structure and function (Glencross, 2009). EPA, DPA and DHA are typically not found in terrestrial ingredients. The essential LC n-3 PUFA requirement for Yellowtail Kingfish has not been determined experimentally. However, the ∑LC n-3 PUFA requirement for a closely related Japanese Yellowtail has been shown to be ≥ 2 g 100 g^{-1} diet (Deshimaru et al., 1982; Stone and Bellgrove, 2013). In the current study, Diet 6 was formulated to be deficient in the essential LC n-3 PUFA (1.41 g $100g^{-1}$ diet). Yellowtail Kingfish fed Diet 6 grew poorly and the feed efficiency was reduced. Based on the previous research investigating the ∑LC n-3 PUFA requirements for Japanese Yellowtail (Deshimaru et al., 1982; Stone and Bellgrove, 2013) and results from the current study, we recommend that ∑LC n-3 PUFA are maintained above 2 g 100 g^{-1} diet in production diets for large Yellowtail Kingfish to ensure that dietary LC n-3 PUFA deficiencies do not limit growth.

A previous study reported good growth and health in Yellowtail Kingfish fed a diet containing ~30% novel SBM inclusion (60% FM substitution), compared to fish fed a FM control diet

(Smullen, 2013). In the current study however, the growth performance and feed efficiency of Yellowtail Kingfish fed the Ridley Trifecta soya diet (Diet 4), which also contained the novel soybean meal, tended to be inferior compared to all other diets. Dietary inclusions of soybean meal have previously been reported to limit the growth of Yellowtail Kingfish (Bowyer et al., 2013a). However, it was thought that this new strain of modified SESBM, which contained low levels of anti-nutritional factors, would not impair growth and feed utilisation to the same extent. This hypothesis was not supported by data in the current trial. Anti-nutritional factors in soybean meal are also thought to be involved in the development of sub-acute enteritis in Yellowtail Kingfish (Bansemer et al., 2015). The villus height, villus area, lamina propria area and branching were measured, and were not significantly different between treatments investigated. This result may indicate that this novel soybean meal may not induce sub-acute enteritis or other alterations to the mucosal architecture. It should be noted however, in previous studies the villus height, villus area, lamina propria area of juvenile Yellowtail Kingfish fed up to 30% SESBM were not significantly different to a control FM diet (Bowyer et al., 2013a; Bansemer et al., 2015). However, further investigation in the previous studies revealed an increased proliferation of goblet cells and alterations to the mucus layer thickness in Yellowtail Kingfish fed a 20% to 30% dietary inclusion of SESBM, particularly at sub-optimal water temperatures (<18 °C) (Bowyer et al., 2013a; Bansemer et al., 2015). Bansemer et al. (2015) hypothesised that this indicated the first sign of sub-acute enteritis. In the current trial, alterations to other gastrointestinal morphology parameters, including mucus layer thickness/composition, goblet cell numbers and microvilli, cannot be ruled out, and caution should be exercised interpreting these morphological parameters. Based on data from Trial 1, we recommend excluding this novel soybean meal product from Yellowtail Kingfish production diets until further research is undertaken.

Conclusions and Recommendations

In conclusion, information obtained from this trial improves our nutritional knowledge of Yellowtail Kingfish, and may be used to improve current on-farm feeding practices and reduce the reliance on the marine ingredients, FM and FO. Based on significantly lower feed consumption rates and superior FCR and good growth performance for Yellowtail Kingfish fed to sub-satiation (80% apparent satiation), we recommend that it may be beneficial to implement this feed strategy to on-farm production. Significant savings in feed and feeding costs are likely by careful feed management practices. In addition, we recommend that the dietary inclusion of FM may be reduced from 40% to 30%. Further reduction in FO usage, below ~9% may be possible. However, care must be exercised to ensure dietary LC n-3 PUFA are not limiting (≥ 2 g 100 g^{-1} diet). A more sensitive challenge test is required for the assessment of FM and FO substitution on fish health and survival.

Chapter 3. Trial 2: The effect of altering dietary energy levels and feed rate on growth and feed utilisation of large Yellowtail Kingfish during summer

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Abstract

In this 84 d trial, the effects of FM and FO replacement and high dietary energy levels on the growth, feed utilisation and health of large (1.87 kg) Yellowtail Kingfish (*Seriola lalandi*) were investigated. Yellowtail Kingfish fed the newly adopted Ridley Clean Seas 2014 Pelagica production diet (Diet 1; formulation arising from Trial 1 containing 30% FM, 9% FO and ~19 MJ kg^{-1} gross energy [GE]), was compared to diets that were formulated to contain varying protein, lipid and energy levels (Diet 2, Ridley trial Diet 2 containing 20% FM, 9% FO, 19 MJ kg^{-1} GE and ; Diet 3, Ridley high energy trial Diet 3 containing ~20.5 MJ kg^{-1} GE; Diet 4, Skretting high energy Yellowtail diet containing 22 MJ kg^{-1} GE; and Diet 5, Hayashikane Hiramasa commercial Japanese Yellowtail diet, 19 MJ kg^{-1} GE). Additionally, following on from the favourable results obtained in Trial 1, the interaction between three selected diet types (Diets 1, 4 and 5) and two feeding strategies (apparent satiation seven days $week^{-1}$ vs. sub-satiation [80% apparent satiation] six days $week^{-1}$) was also investigated. The trial was conducted over summer in the same land-based semi-recirculating system as described in Trial 1. The best growth performance and feed utilisation were observed in fish fed the Ridley high energy diet (Diet 3) and the Ridley Clean Seas 2014 Pelagica production diet (Diet 1) when fed to apparent satiation. This indicates the potential value of using cheaper high energy diets for Yellowtail Kingfish production. Fish fed the Hayashikane Hiramasa diet (Diet 5, using both feeding strategies) had the poorest growth performance and feed utilisation. With regard to feeding strategy, sub-satiation fed fish were fed close to the targeted 80% apparent satiation level over the entire trial. However, it was difficult to attain the desired level of 80% of apparent satiation. In future studies, to achieve 80% of apparent satiation using the current method restrictive feeding it may be desirable to use a multiplier of ~75% of Saturday's apparent satiation feeding level. With respect to fish performance, reducing feeding rate resulted in an improved FCR, but fish growth was compromised. Fish fed to sub-satiation had poorer growth performance than fish fed to apparent satiation. Blood urea, cholesterol and triglyceride levels were affected by diet type (particularly in the high energy diets) and/or feeding rate, indicating that metabolism is impacted by feeding level and energy level. Results from this study suggest feeding high energy diets may be economically beneficial in reducing the cost of Yellowtail Kingfish production during the summer growing season. Further investigation into improving on-farm feeding strategies is also warranted. The FCR of Yellowtail Kingfish grown in the recirculating tank system at the South Australian Research and Development Institute (SARDI) was better (1.6 - 1.7) than fish from the same cohort grown in the Clean Seas sea cages. These results suggest that factors, other than diet manipulation, may play an important role in the growth of Yellowtail Kingfish and deserve investigation to improve production. Dissolved oxygen is an important factor for fish growth and it may be worthwhile to investigate the application of oxygenation to sea cages to improve dissolved oxygen levels, growth, feed utilisation and profits. Further research is also required into aspects of lipid and energy metabolism in larger fish, particularly under conditions of restrictive feeding practices during both summer and winter.

Introduction

Following on from Trial 1, where diet benchmarking, fish meal (FM) and fish oil (FO) substitution to improve sustainability, and feed management issues were assessed, it was decided to further investigate these three important areas in Trial 2 with large Yellowtail Kingfish (1.87 kg). Clean Seas ascertained that improvements in these three key areas would lead to rapid improvements in commercial diet formulations. The company also believed that it would concurrently provide positive advancements towards the improved sustainable production of Yellowtail Kingfish, opening up, or maintaining a range of market access opportunities.

In this trial the modified Ridley Clean Seas 2014 Pelagica diet (based on Diet 5, Chapter 2, Trial 1, but with the FM level reduced to 30%), was used as the benchmark to compare the growth performance, feed utilisation and health of large Yellowtail Kingfish grown on a selection of high and low energy diets which contained varying levels of FM and FO substitution. A Japanese Yellowtail (*Seriola quinqueradiata*) commercial diet was also benchmarked against the Ridley Clean Seas 2014 Pelagica Diet 1 in this trial. The new Ridley Clean Seas 2014 Pelagica Diet 1 was formulated, based on data reported by Stone and Bellgrove (2013), to provide levels of crude 44% protein, ~24% lipid and ~19 MJ kg^{-1} gross energy. Several other commercial diets were also formulated to contain a higher FM replacement level, or higher energy levels combined with lower protein levels.

Further assessment of the growth performance and health of large Yellowtail Kingfish in response to non-restrictive versus restrictive feeding practices was also carried out to refine information to improve on-farm feeding practices. Combined with information generated in Trial 1 (Chapter 2), the above research was designed to provide essential information that would contribute towards achieving the objectives of this project. The objectives include identifying or developing improved production diets for large fish (> 1.5 kg) at summer water temperatures to provide information toward gaining a 10% improvement in growth, a 0.45 unit reduction across the whole of cycle FCRs while still maintaining health and improving survival to 98% in Clean Seas Yellowtail Kingfish sea cage production systems.

Aim

We aimed to expand on our previous findings in Trial 1 and:

1. Benchmark the growth performance and feed utilisation of large Yellowtail Kingfish (> 1.5 kg) fed the newly formulated Ridley Clean Seas Pelagica 2014 Yellowtail Kingfish production diet versus a Japanese Yellowtail production diet formulated to contain high levels of FM and FO.

2. Formulate and test a series of commercially relevant production diets with varying levels of energy, and FM and FO substitution, to determine if improvements in the production efficiency of large Yellowtail Kingfish in a tank-based setting can be achieved.

3. Determine if adequate levels of essential LC n-3 PUFA (Σ EPA + DPA + DHA ≥ 2 g 100 g^{-1} diet) will sustain the growth of large Yellowtail Kingfish grown at summer water temperatures when fed a diet containing 20% FM.

4. Further investigate if growth performance and feed utilisation of large Yellowtail Kingfish can be maintained while restricting the animals feed intake.

Materials and Method

Experimental treatments and feeding techniques
A total of five commercial diet formulations and two feeding strategies (unbalanced design) resulted in eight treatment combinations being used to investigate the effect of FM (substituted with a range of alternative protein sources) and FO (substituted with poultry oil; PO) replacement and varying dietary energy levels on the growth, feed utilisation and health of Yellowtail Kingfish. The biochemical composition of the five diets is displayed in Table 3.1. The actual ingredient composition of the diets is confidential and is not provided in this report.

Fish from Treatments 1 (Diet 1), 3 (Diet 2), 4 (Diet 3), 6 (Diet 4) and 7 (Diet 5) were fed for 4 min tank^{-1} to apparent satiation daily at 0830 h. Fish from Treatment 2 (Diet 1), 5 (Diet 3) and 8 (Diet 5) were targeted to be fed to sub-satiation (80% of apparent satiation) of Treatments 1, 4 and 7, respectively. The feeding of these fish was initially calculated as per Table 3.2. In brief, fish were fed to apparent satiation every Saturday for 4 min (which is slightly reduced from Trial 1 to further restrict feed intake), followed by a day of no feed on Sunday and then fed from Monday to Friday to the targeted sub-satiation level calculated from Saturday's apparent satiation feeding level. This cycle was repeated weekly. During the first half of the trial, and based on previous experience gained from Trial 1 (i.e. fish fed to 80% apparent satiation consumed 85% to 87% of satiation levels), we chose to feed fish 70% of what was fed to the 100% satiation fed fish on Saturdays (apparent satiation feeding level) for the remainder of the week in order to deliver the desired 80% apparent satiation ration. However, at the midpoint weight check of Trial 2 it was determined that fish from these treatments actually received <80% of satiation. Therefore, in order to meet our targeted level of 80% satiation we increased the multiplier up to 80% of the ration fed to the 100% satiation fed fish on Saturdays for the remainder of the study (Table 3.3).

The treatments investigated were:

- Treatment 1: Diet 1 (control) fed to apparent satiation seven days week^{-1} (Ridley trial Diet 1: Updated Ridley Clean Seas 2014 Pelagica diet with 30% FM and a normal crude lipid [CL] level comprised of 9% FO + 9% PO; planned dietary composition [as fed], ~44% crude protein [CP], ~24% CL and a normal gross energy [GE] level of 19 MJ kg^{-1}).

- Treatment 2: Diet 1 fed to sub-satiation (80% of apparent satiation).

- Treatment 3: Diet 2 fed to apparent satiation seven days week^{-1} (Ridley trial Diet 2: 20% FM diet [30% FM substitution using other alternative protein sources] and normal CL level with 50% FO substitution comprised of 9% FO + 9% PO; planned composition [as fed], ~44% CP, ~24% CL and a normal GE level of 19 MJ kg^{-1}; plus LC n-3 PUFA levels > 2 g 100 g $^{-1}$ diet).

- Treatment 4: Diet 3 fed to apparent satiation seven days week^{-1} (Ridley high energy trial Diet 3: High energy test diet with 30% FM and high CL level with 75% FO substitution comprised of ~7% FO + ~19% PO; planned composition [as fed], lower CP level of ~40%, a higher CL level of ~30% and a higher GE level of ~20.5 MJ kg^{-1}).

- Treatment 5: Diet 3 fed to sub-satiation (80% of apparent satiation).

- Treatment 6: Diet 4 fed to apparent satiation seven days week^{-1} (Skretting Yellowtail high energy diet: a low protein, high lipid and high energy diet; planned composition [as fed], lower CP level of ~40%, a higher CL level of ~35% and a higher GE level of ~22 MJ kg^{-1}).

- Treatment 7: Diet 5 fed to apparent satiation 7 days week^{-1} (Hayashikane Hiramasa Japanese Yellowtail diet: a normal protein, lipid and energy diet; planned composition [as fed], CP level of ~45%, a CL level of ~25% and a normal GE level of ~19 MJ kg^{-1}).

- Treatment 8: Diet 5 fed to sub-satiation (80% of apparent satiation).

Table 3.1. Biochemical composition of the 5 test diets used in Trial 2.[1]

Item (as fed)	Ridley 1	Ridley 2	Ridley 3	Skretting	Hayashikane Hiramasa
Analysed proximate composition (g kg^{-1})					
Moisture	72	66	51	58	84
Crude protein	438	433	420	407	442
Crude lipid	242	249	279	347	250
Ash	95	93	91	80	127
Carbohydrate[2]	153	159	159	108	97
Gross energy (MJ kg^{-1})	19.0	19.3	20.2	21.6	18.4
Cholesterol	3.0	3.0	3.1	3.0	2.6
Analysed minerals (mg kg^{-1})					
Calcium	23000.0	18000.0	16000.0	19000.0	30000.0
Choline	298.0	299.0	310.0	316.0	351.0
Copper	8.9	11.0	9.1	16.0	8.7
Iodide (Potassium Iodide) (µg kg^{-1})	1.0	1.4	1.4	3.7	3.0
Iron	450.0	570.0	730.0	760.0	480.0
Magnesium	2300.0	2200.0	2200.0	1500.0	2200.0
Manganese	43.0	45.0	45.0	53.0	33.0
Phosphorus	15000.0	14000.0	14000.0	15000.0	22000.0
Potassium	6400.0	5900.0	5400.0	5300.0	9800.0
Selenium	1.9	1.9	2.0	0.7	2.4
Zinc	160.0	150.0	150.0	320.0	93.0
Analysed amino acids (g kg^{-1})					
Alanine	28.0	27.3	27.0	23.8	27.3
Arginine	23.9	22.9	21.6	21.7	22.5
Aspartic acid	39.2	37.9	38.0	32.5	36.8
Glutamic acid	55.2	51.8	51.3	55.5	50.6
Glycine	29.6	26.7	24.4	25.9	29.0
Histidine	13.8	14.0	14.6	9.0	7.7
Isoleucine	14.5	12.8	12.4	10.7	12.2
L cystine	5.2	5.2	5.1	4.7	4.0
Leucine	35.3	35.5	35.6	28.5	29.0
Lysine	30.8	29.8	29.7	23.8	26.9
Methionine	11.3	11.7	11.0	10.7	11.2
Phenylalanine	21.4	21.7	21.7	16.3	15.5
Proline	22.3	21.3	19.8	12.7	12.1
Serine	18.5	18.0	17.8	16.1	15.9
Taurine	13.6	14.0	12.7	12.0	14.0
Threonine	17.6	17.0	17.1	14.0	14.8
Tyrosine	12.8	12.2	12.0	10.4	11.3
Valine	26.4	26.7	26.8	16.7	15.9

[1] Diets supplied by Ridley Aquafeeds (Narangba, QLD, Australia).
[2] Carbohydrate = 1000 - (moisture + lipid + protein + ash).

Table 3.1. Continued.

Item (as fed)	Ridley 1	Ridley 2	Ridley 3	Skretting	Hayashikane Hiramasa
Analysed fatty acids (mg 100 g^{-1})					
Butyric C4:0	<10	<10	<10	<10	<10
Caproic C6:0	<10	<10	<10	<10	<10
Caprylic C8:0	<10	<10	<10	<10	<10
Capric C10:0	<10	<10	<10	<10	<10
Lauric C12:0	20	21	21	33	<10
Trisdecanoic C13:0	<10	<10	<10	<10	<10
Myristic C14:0	930	990	920	1080	1210
Pentadecanoic C15:0	87	90	85	94	140
Palmitic C16:0	4860	5000	5610	7440	4950
Margaric C17:0	89	26	92	100	130
Stearic C18:0	1250	1270	1430	1960	2140
Arachidic C20:0	52	54	57	47	92
Docosanoic C22:0	34	37	39	27	65
Tetracosanoic C24:0	29	26	23	18	<10
Decenoic C10:1	<10	<10	<10	<10	<10
Myristoleic C14:1	56	58	62	90	<10
Pentadecenoic C15:1	<10	<10	<10	13	<10
Palmitoleic C16:1	1390	1460	1560	2120	1300
Heptadecenoic C17:1	52	53	55	54	80
Octadecenoic C18:1n-6	19	17	16	21	<10
Octadecenoic C18:1n-7	650	660	730	940	710
Oleic C18:1n-9	6470	6650	8230	11390	3540
Eicosenoic C20:1n-9	350	370	330	220	550
Eicosenoic C20:1n-11,13	61	64	62	70	590
Eicosenoic C20:1 (total)	410	430	390	290	1140
Docosenoic C22:1n-9	46	50	44	43	150
Docosenoic C22:1n-11,13	<10	<10	<10	<10	<10
Tetracosenoic C24:1	77	79	75	59	140
Linoleic C18:2n-6	2530	2630	3410	2530	820
Gamma Linolenic C18:3n-6 (GLA)	44	51	45	44	<10
Steridonic C18:4n-3	200	210	200	200	390
Eicosadienoic C20:2n-6	32	33	37	32	<10
Dihomo-gamma-linoleic C20:3n-6	33	34	34	33	<10
Arachidonic C20:4n-6	170	180	170	170	280
Docosatetraenoic C22:4n-6	58	51	47	58	110
Docosapentaenoic C22:5n-6	62	60	54	62	85
Alpha Linolenic C18:3n-3	350	360	450	350	230
Eicosatrienoic C20:3n-3	14	<10	<10	14	<10
Eicosatetraenoic C20:4n-3	260	270	230	260	1130
Eicosapentaenoic C20:5n-3 (EPA)	1380	1440	1330	1380	1890
Heneicosapentaenoic acid C21:5n-3	<10	<10	<10	<10	<10
Docosapentaenoic C22:5n-3 (DPA)	200	210	190	200	420
Docosahexaenoic C22:6n-3 (DHA)	1280	1240	1140	1280	2990
Octadecadienoic C18:2 Conjugated 9c 11t	<10	<10	<10	<10	<10
Octadectrenoic acid C18:3n-4	14	15	16	14	<10
EPA + DPA + DHA	2860	2890	2660	2860	5300
n-3 FA : n-6 FA	1.26	1.23	0.93	1.26	5.44

Table 3.2. Feeding technique for 80% sub-satiation fed fish for the first 43 days of Trial 2.[1]

Day	Fish fed
Saturday	Apparent satiation
Sunday	0% (feed withheld)
Monday	70% of Saturday
Tuesday	70% of Saturday
Wednesday	70% of Saturday
Thursday	70% of Saturday
Friday	70% of Saturday

[1] This method resulted in fish being fed < 80% of the respective satiation treatments and was adjusted as described in Table 3.3.

Table 3.3. Feeding technique for 80% sub-satiation fed fish for the last 41 days of Trial 2.

Day	Fish fed
Saturday	Apparent satiation
Sunday	0% (feed withheld)
Monday	80% of Saturday
Tuesday	80% of Saturday
Wednesday	80% of Saturday
Thursday	80% of Saturday
Friday	80% of Saturday

Experimental fish

Experimental work for Trial 2 was conducted in the pool-farm facility at the South Australian Research and Development Institute's (SARDI's) South Australian Aquatic Sciences Centre (SAASC); South Australia, Australia. Yellowtail Kingfish (mean ± standard deviation; initial weight 1.87 ± 0.05 kg; initial fork length 495.8 ± 3.6 mm) were obtained from Clean Seas (Arno Bay, Australia). Upon arrival at the SAASC experimental pool-farm facility (28/10/14), Yellowtail Kingfish were transferred to 5000 L tanks supplied with partial flow-through/recirculating, sand filtered, UV treated, aerated and oxygenated sea water at ambient temperature and held for ~4 weeks and fed the Ridley Clean Seas 2013 Pelagica diet (crude protein, 46%; crude lipid, 24%; Gross energy, 19.30 MJ kg^{-1}).

Skin and gill fluke treatment

Upon arrival at the SAASC, the fish were inspected and observed to have a skin flukes (*Benedenia seriola*) and gill flukes (*Zeuxapta seriola*) so treatment was deemed to be necessary. All treatments were prescribed by Dr Matt Landos (Future Fisheries Veterinary Service Pty Ltd.). Based on the successful treatment method of both skin and gill flukes used in Trial 1, fish were exposed to two treatments (3/11/14 and 11/11/14) of formalin (250 ppm for 60 min), each over a two week period. An intervening assessment of spare fish held under identical conditions, towards the end of the first half of Trial 2 (22/12/14), revealed that the skin and gill flukes had been eradicated following the first round of formalin treatment. However, there was visual evidence of skin flukes on some fish during the mid-term weight check (8/1/15) so fish were exposed to two further treatments of formalin on the 17/1/15 and 27/1/15. There was minimal evidence of skin and gill flukes at the final harvest (18/2/15).

Experimental stocking

At the commencement of Trial 2 (26/11/14), Yellowtail Kingfish were removed from their tank, anaesthetised using AQUI-S® (AQUI-S® New Zealand Ltd., Lower Hutt, New Zealand)

at a concentration of 14 mg L^{-1} of seawater. Nineteen fish were measured, weighed and stocked, using systematic interspersion, into one of the three replicate 5000 L tanks treatment combination^{-1} (n = 24 tanks). As per Trial 1, the tanks were supplied with partial flow-through (100% system water exchange d^{-1})/recirculating, sand filtered, UV treated sea water at ambient temperature. Aeration and oxygenation was provided to each tank for the entire trial.

Weight checks throughout the trial
At day 43 (8/1/15) animals were anaesthetised using AQUI-S® at a concentration of 14 mg L^{-1} of seawater. Animals were measured, weighed, visually inspected for skin and gill flukes and returned back to their respective tanks.

Water quality analyses
Water quality parameters were measured at 1130 h daily and maintained at appropriate levels for the acceptable growth of Yellowtail Kingfish throughout the study (Table 3.4). Water temperature was measured daily using a thermometer (Figure 3.1). Dissolved oxygen (% saturation [Figure 3.2]) was measured daily using a dissolved oxygen meter (OxyGuard International A/S, Birkerød, Denmark). The pH was measured daily using a meter (Oakton pHtestr 20; Oakton Instruments, Vernon Hills, IL, USA). Salinity (g L^{-1}) was measured weekly using a portable salinity refractometer (model RF20, Extech Instruments, Nashua, NH, USA). Tanks were cleaned weekly by siphoning on a rotational basis.

Table 3.4. Summary of water quality for Trial 2.

Item	Water temperature (°C)[1]	Dissolved oxygen (% saturation) [1]	pH[1]	Salinity (g L^{-1}) [2]
Mean ± SD	21.5±1.1	93.5±7.0	7.82±0.12	35.6±0.9
Range	19.5-26.0	74.0-124.0	7.06-8.18	34.0-37.0

[1] n = 84.
[2] n = 12.

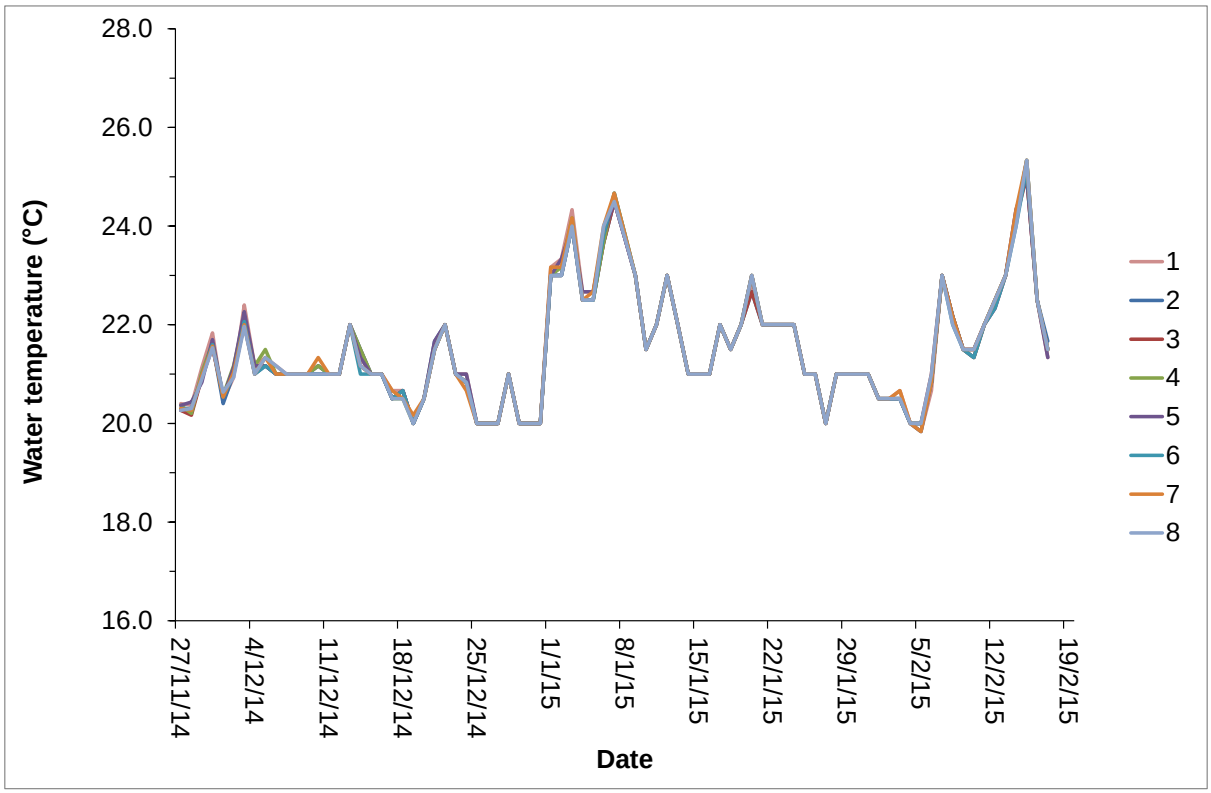

Figure 3.1. Average treatment water temperatures in Trial 2.[1]
Values for each day are expressed as mean, n = 3.
[1] 1: Ridley trial Diet 1 (Diet 1) fed to apparent satiation seven days $week^{-1}$ (Control);
2: Ridley trial Diet 1 (Diet 1) fed to sub-satiation (80% of apparent satiation);
3: Ridley trial Diet 2 (Diet 2; 20% FM) fed to apparent satiation seven days $week^{-1}$;
4: Ridley trial Diet 3 (Diet 3; High lipid lower protein) fed to apparent satiation seven days $week^{-1}$;
5: Ridley trial Diet 3 (Diet 3) fed to sub-satiation (80% of apparent);
6: Skretting trial diet (Diet 4; High lipid) fed to apparent satiation seven days $week^{-1}$;
7: Hayashikane Hiramasa trial diet (Diet 5) fed to apparent satiation seven days $week^{-1}$;
8: Hayashikane Hiramasa trial diet (Diet 5) fed to sub-satiation (80% of apparent satiation).

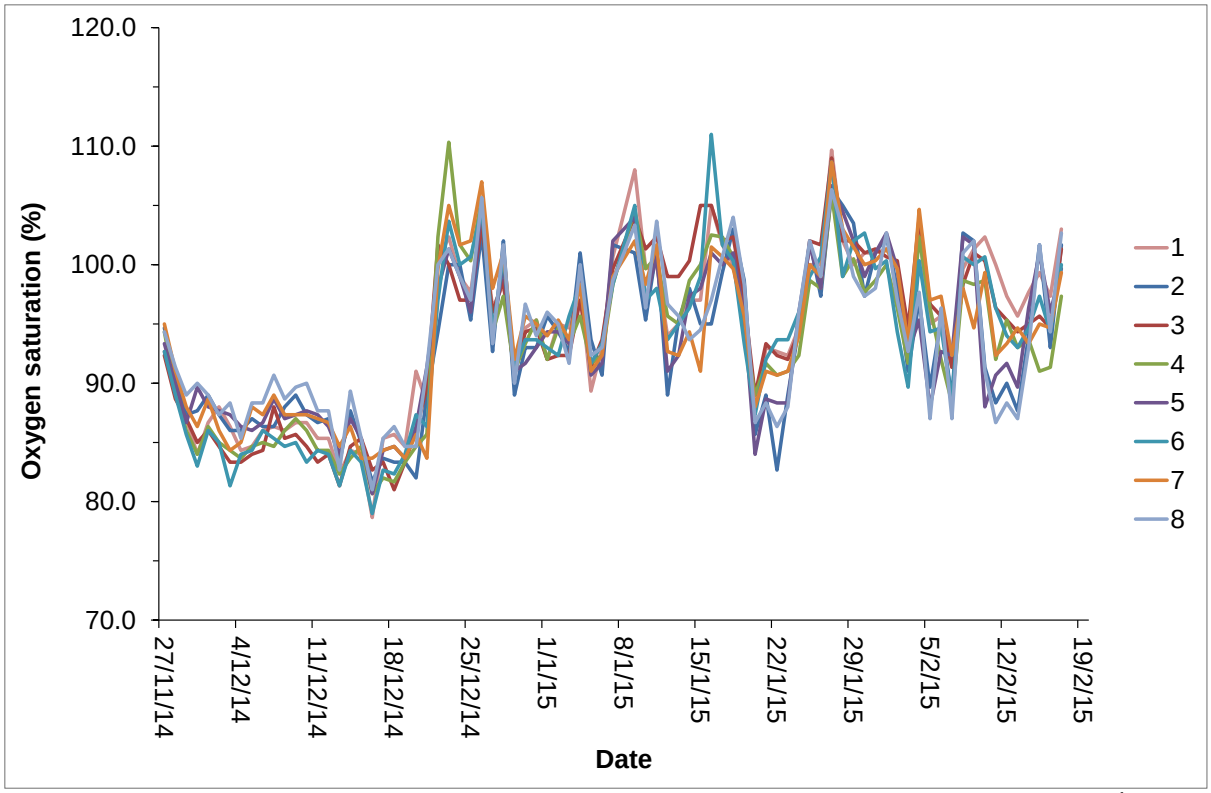

Figure 3.2. Average treatment water dissolved oxygen levels (% saturation) in Trial 2.[1]
Values for each day are expressed as mean, n = 3.
[1] 1: Ridley trial Diet 1 (Diet 1) fed to apparent satiation seven days week^{-1} (Control);
2: Ridley trial Diet 1 (Diet 1) fed to sub-satiation (80% of apparent satiation);
3: Ridley trial Diet 2 (Diet 2; 20% FM) fed to apparent satiation seven days week^{-1};
4: Ridley trial Diet 3 (Diet 3; High lipid lower protein) fed to apparent satiation seven days week^{-1};
5: Ridley trial Diet 3 (Diet 3) fed to sub-satiation (80% of apparent);
6: Skretting trial diet (Diet 4; High lipid) fed to apparent satiation seven days week^{-1};
7: Hayashikane Hiramasa trial diet (Diet 5) fed to apparent satiation seven days week^{-1};
8: Hayashikane Hiramasa trial diet (Diet 5) fed to sub-satiation (80% of apparent satiation).

Final harvest sampling
At day 84 (18/2/15) animals were anaesthetised using AQUI-S® at a concentration of 14 mg L^{-1} of seawater. All animals were visually inspected for skin and gill flukes and measured and weighed. Four fish from each tank were collected and stored at -20°C for subsequent whole body composition analyses. Three separate fish from each tank were also sampled for blood biochemistry and haematology and dissected (n = 9 fish treatment^{-1}). Livers were weighed in order to calculate hepatosomatic index (HSI; %). In addition, muscle and organ tissues samples were collected and stored in seawater buffered formalin for histological evaluation for student project (results pending).

Biochemical and pellet stability analyses
All diets were analysed for stability levels at the Lincoln Marine Science Centre (Port Lincoln, South Australia) by Dr Trent D'Antignana. All diets and sub-samples of whole initial and final fish (4 from each tank) were analysed for biochemical composition by AsureQuality Laboratories (Auckland, New Zealand). Blood biochemical and haematological levels were measured by IDEXX Laboratories (Unley, South Australia).

Calculation of performance indices
Performance indices were calculated as described in the corresponding section in the Materials and Methods section of Trial 1 provided in Chapter 2. In addition, haematocrit count and Hepatosomatic index (HSI; %) was also calculated:

- Haematocrit count (L L^{-1}) = red blood cell (mm) / total blood (red blood cell and plasma; mm) 100 × 100

- Hepatosomatic index (HSI; %) = wet liver wt × 100 / final wet fish wt

Statistical analyses
IBM SPSS, Version 22 for Windows (IBM SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Homogeneity of variances and normality among mean values were assessed using Levene's test for equality of variance errors and the standardized residuals against the predicted mean plot, respectively. Difference between treatments for all variables were analysed using One-factor ANOVA. Additionally, difference between treatments for the feed rate (diet type by feed rate) were analysed using Two-factor ANOVA. When significant differences were observed, post-hoc tests were used to detect significant differences between treatment combinations (Student Newman-Keuls). Weight and condition index distribution of fish fed Diets 1, 3 and 5 to apparent satiation and sub-satiation were plotted, and data homogeneity and skewness were determined using Shapiro Wilk test. A significance level of $P < 0.05$ was used for all statistical tests. All values are presented as means ± standard error (SE) of the mean unless otherwise stated.

Results

General observations
Fish all fed actively during the trial. However, fish fed the Hayashikane Hiramasa diet (Diet 5) did not appear to feed as actively as fish fed other diets. Fish did not show any signs of flashing due to skin or gill flukes, although a low prevalence of flukes was noted; there were no apparent signs of disease observed. Survival of fish was positive with only one mortality recorded in tank M16 (Treatment 5, Diet 3, sub-satiation). The weight and length of this fish was recorded, and no visible signs of disease were observed.

Pellet moisture content and stability
There were large differences measured in pellet moisture contents and pellet stability between diets. The Skretting diet (Diet 4) had the highest stability level (94.40%) followed by Ridley Diets 1 (89.42%), 2 (81.98%) and 3 (79.37%), respectively. The Hayashikane Hiramasa diet (Diet 5) recorded the lowest diet stability (32.85%). The moisture content for the Ridley and Skretting diets (Diets 1 to 4) ranged between 4.86% to 7.39%. The moisture content of the Hayashikane Hiramasa diet (Diet 5) was 17.09%.

Growth performance
Results are provided in Figure 3.3 and Table 3.5, 3.6 and 3.7 for One-factor ANOVA of all data for treatments and in Tables 3.8, 3.9 and 3.10 for Two-factor ANOVA of diet type and feed rate data for Diets 1 (Ridley Diet 1, control), 3 (Ridley Diet 3, high energy) and 5 (Hayashikane Hiramasa diet).

The initial weight and fork length of fish were not statistically different between treatments (One-factor ANOVA; $P > 0.05$; Table 3.5 and 3.7). There were significant differences between treatments for growth parameters (One-factor ANOVA; $P < 0.05$; Table 3.5). These differences were inclusive of final weight, condition factor, biomass gain, SGR and fork length growth rate at the mid-point of the trial. In general, Treatment 4 (Ridley Diet 3; high energy diet fed to apparent satiation) out-performed most diets followed by Treatment 1 (Ridley Diet 1; Pelagica control diet fed to apparent satiation) and Treatment 6 (Skretting diet; high energy diet). In all cases Treatments 7 and 8 (Hayashikane Hiramasa diet fed to apparent satiation and sub-satiation) were the poorest performing (One-factor ANOVA; $P < 0.05$; Table 3.5). Between the midpoint and the end of the trial final weight, biomass gain, SGR, final fork length, length growth rate and condition factor generally followed similar patterns as were observed in fish from the initial to midpoint sampling (Table 3.6).

At the completion of the trial, with the exception of fork length growth rate, all other growth parameters followed similar trends (Table 3.7). In general, Treatment 4 (Ridley Diet 3, high energy diet fed to apparent satiation) out-performed most diets followed by Treatments 1, 3 and 6. In all cases Treatments 7 and 8 (Hayashikane Hiramasa diet fed to apparent satiation and sub-satiation) were the poorest performing (One-factor ANOVA; $P < 0.05$; Table 3.7).

At the mid-point of the trial there was a significant influence of both diet type and feed rate on some of the growth performance parameters of Yellowtail Kingfish (Two-factor ANOVA; $P < 0.05$; Table 3.8). Growth performance parameters that were significantly influenced by diet type and feed rate included final weight, biomass gain, SGR and fork length growth rate. Condition factor was influenced by feed rate but not by diet type. In all cases fish fed to apparent satiation performed better than those fed to 80% satiation (Table 3.8). Fish fed Diets 1 and 3 performed better than fish fed Diet 5 (Table 3.8). However, there were significant interactions between the two factors for biomass gain and SGR. These interactions may be explained by a higher increase in growth for fish fed Diets 1 and 3 to apparent satiation compared to fish fed the respective diets to sub-satiation, when compared to the fish fed Diet 5 at both feeding rates (Table 3.8).

Between the mid-point to the end of the trial, final weight, biomass gain, SGR and final fork length followed similar patterns to fish from the initial to the mid-point of the trial in corresponding treatments (Table 3.8 and 3.9). In contrast, length growth rate and condition factor changed (Table 3.8 and 3.9). The length growth of the fish fed Diet 5 increased during the second half of the trial, whereas, fish fed Diets 1 and 3 decreased. For condition factor fish fed Diet 1 improved in condition factor compared to fish fed Diet 5 during the second half of the trial (Table 3.8 and 3.9).

At the completion of the trial final weight, biomass gain, SGR and condition factor were significantly affected by both diet type and feed rate (Table 3.10), whereas, final fork length and length growth rate were only affected by feed rate (Table 3.10).

The hepatosomatic index for Yellowtail Kingfish fed Diet 4 to apparent satiation (Treatment 6) was significantly higher than all other treatments (One-factor ANOVA; $P < 0.05$; Table 3.7). In addition, the hepatosomatic index for fish fed Diet 5 to sub-satiation (Treatment 8) was significantly lower than fish fed Diet 3 to apparent satiation (Table 3.7). Hepatosomatic index was also significantly influenced by diet ($P = 0.008$; D1 = D5 < D3) and feed rate ($P = 0.047$; satiation > sub-satiation), but not by the interaction between diet and feed rate (Two-factor ANOVA; $P > 0.05$; Table 3.10).

The weight distribution of Yellowtail Kingfish fed Diets 1, 3 and 5 to apparent or sub-satiation were not significantly skewed ($P > 0.05$; Table A2.5.1; Appendix 2). The condition index for fish fed Diet 5 to both sub-satiation ($P = 0.001$; 1.26; Table A2.5.3; Appendix 2) and apparent satiation (0.79; $P = 0.05$; Table A2.5.3; Appendix 2) was positively skewed. The condition index of fish fed Diet 1 and 3 to apparent and sub-satiation was normally distributed ($P > 0.05$).

Feed use
Results are provided in Table 3.5, 3.6 and 3.7 for One-factor ANOVA of all data for treatments and in Tables 3.8, 3.9 and 3.10 for Two-factor ANOVA of diet type and feed rate data for Diet 1 (Ridley Diet 1, Control), Diet 3 (Ridley Diet 3, high energy) and Diet 5 (Hayashikane Hiramasa diet).

At the mid-point of the trial, feed consumption (kg tank^{-1}) was significantly higher in Treatments 1 and 4 (Ridley Diet 1 and 3 fed to apparent satiation, respectively), and was lowest in Treatment 8 (Hayashikane Hiramasa Diet 5 fed to sub-satiation) (One-factor ANOVA, $P < 0.05$; Table 3.5). Feed intake (% bw d^{-1}) followed similar trends to feed consumption (Table 3.5). There was a significant difference in FCRs between treatments (One-factor ANOVA, $P < 0.05$; Table 3.5). For fish fed to apparent satiation, Treatment 7 (Diet 5; Hayashikane Hiramasa) exhibited the poorest FCR. Generally, fish fed to sub-satiation exhibited superior FCRs (Table 3.5).

In general, during the second half of the trial, feed utilisation followed similar patterns as observed during the first half of the trial, with the exception of fish fed Diet 5 (Hayashikane, Hiramasa) fed to apparent satiation (Table 3.5 and 3.6). The feed consumption and apparent feed intake for fish fed Diet 5 (Hayashikane, Hiramasa) to apparent satiation increased significantly compared to other treatments during the second half of the trial (Table 3.5 and 3.6).

At the completion of the trial, feed consumption (kg tank^{-1}) was significantly higher in Treatments 1, 3 and 4 (Diets 1, 2 and 3 fed to apparent satiation, respectively), and lowest in Treatments 2, 5 and 8 (Diets 1, 3 and 5 fed to sub-satiation) (Table 3.7). Feed consumption for fish fed Treatment 6 (Diet 4) and Treatment 7 (Diet 5) to apparent satiation was lower than fish fed Treatments 1, 3 and 4, but were significantly higher than the sub-satiation fed fish (Table 3.7). Feed intake (% bw d^{-1}) followed similar trends (Table 3.7). The FCR of fish

fed Treatments 2 (Diet 1, sub-satiation), Treatment 4 (Diet 3, apparent satiation), Treatment 5 (Diet 3, sub-satiation), and Treatment 8 (Diet 5, sub-satiation) were significantly superior to Treatment 7 (Diet 5, apparent satiation). Treatment 1 (Diet 1, apparent satiation) and Treatment 3 (Diet 2, apparent satiation) were similar to all other treatments (Table 3.7).

With regard to the interactive effects of diet type and feeding rate, at the mid-point of the trial, there was a significant influence of diet type and feed rate on the feed consumption (kg tank^{-1}), feed intake (% bw d^{-1}) and FCR of Yellowtail Kingfish (Two-factor ANOVA, $P < 0.05$; Table 3.8). Feed consumption and feed intake was highest in fish fed to apparent satiation and higher in fish fed Diets 1 and 3, compared to Diet 5 (Two-factor ANOVA, $P < 0.05$; Table 3.8). FCRs were lower in fish fed to sub-satiation and lower in Diets 1 and 3, compared to Diet 5 (Two-factor ANOVA, $P < 0.05$; Table 3.8).

During the second half of the trial there was no significant effect of diet on feed consumption, apparent feed intake and FCR (Table 3.9). During the same period, fish fed to apparent satiation followed the same trend as observed in the first half of the trial (Table 3.8 and 3.9).

At the conclusion of the trial, diet type and feed rate significantly influenced feed consumption (kg tank^{-1}), feed intake (% bw d^{-1}) and FCR of Yellowtail Kingfish (Two-factor ANOVA, $P < 0.05$; Table 3.10). Feed consumption and feed intake were highest in fish fed to apparent satiation, while feed consumption was higher in fish fed Diets 1 and 3, compared to Diet 5 (Two-factor ANOVA, $P < 0.05$; Table 3.10). Feed intake was numerically higher for fish fed Diet 1, compared to fish fed Diets 3 and 5 (Table 3.10). Fish fed to sub-satiation had lower FCR compared to fish fed to apparent satiation, and were lower in Diets 1 and 3, compared to Diet 5 (Two-factor ANOVA, $P < 0.05$; Table 3.10).

Difficulties were experienced achieving the targeted level of sub-satiation feeding desired (80% apparent satiation) during the trial. Based on the average daily feed intake (% bw d^{-1}), and using the multiplier of 70% (Table 3.2), the targeted feeding level was not reached during the first half of the trial (Treatment 2, Treatment 5 and Treatment 8 were 73.4, 73.6 and 74.9% bw d^{-1} of the apparent satiation fed fish, respectively). In contrast, the targeted feeding level of 80% sub-satiation was exceeded during the second half of the trial when the feed multiplier was increased to 80% of apparent satiation (Table 3.3) (Treatments 2, 5 and 8 were 88.2, 90.2 and 86.4% bw d^{-1} of the apparent satiation fed fish, respectively). However, on average across the entire trial, the 80% sub-satiation feeding level was achieved (Treatments 2, 5 and 8 were 80.1, 81.5 and 80.7% bw d^{-1}, respectively).

Whole fish proximate and energy composition

The tissue moisture content of fish fed Treatment 8 (Hayashikane Hiramasa Diet 5 fed to sub-satiation) was significantly higher than fish fed Treatments 3, 4 and 6 (Diets 2, 3 and 4 to apparent satiation, respectively) (Table 3.7; $P < 0.05$; One-factor ANOVA). Additionally, treatment significantly influenced tissue energy content (Table 3.7; $P < 0.05$; One-factor ANOVA). In general, fish fed Treatment 8 had significantly lower tissue energy content than fish fed Treatments 4 and 6 (Diet 3 and 4 to apparent satiation, respectively). In addition, fish fed Treatment 6 (Diet 4 to apparent satiation) had significantly higher tissue energy content to those fed Treatment 2 (Diet 1 fed to sub-satiation). Lipid tissue content was influenced by treatments ($P < 0.05$), but the SNK post-hoc test was unable to discern significant differences between treatment (One-factor ANOVA, $P > 0.05$ Table 3.7). There were no significant differences between treatments for protein, ash and carbohydrate tissue content (One-factor ANOVA; $P > 0.05$; Table 3.7).

Tissue moisture content was significantly influenced by diet type (Diet 1 = Diet 5 > Diet 3; $P < 0.05$) and feed rate (sub-satiation > satiation; $P < 0.05$), but no significant interaction existed (Two-factor ANOVA; $P > 0.05$; Table 3.10). Diet type, feed rate, and the interaction

between these variables did not significantly influence the tissue protein, fat, ash, carbohydrate and energy levels (Two-factor ANOVA; $P > 0.05$; Table 3.10).

Nutrient utilisation

Apparent protein deposition was not significantly different between treatments, and ranged from 27.08 to 31.61% (One-factor ANOVA; $P > 0.05$; Table 3.7). However, feed rate had a significant effect on the apparent protein deposition of Yellowtail Kingfish (sub-satiation > satiation; $P < 0.05$; Table 3.10). Apparent protein deposition was not significantly influenced by diet or the interaction between diet and feed rate (Two-factor ANOVA; $P > 0.05$; Table 3.10). Apparent energy deposition was not significantly different between treatments, and ranged from 37.35 to 43.51% (One-factor ANOVA; $P > 0.05$; Table 3.7). Apparent energy deposition was also not influenced by diet, feed rate or the interaction between these two factors (Two-factor ANOVA; $P > 0.05$; Table 3.10).

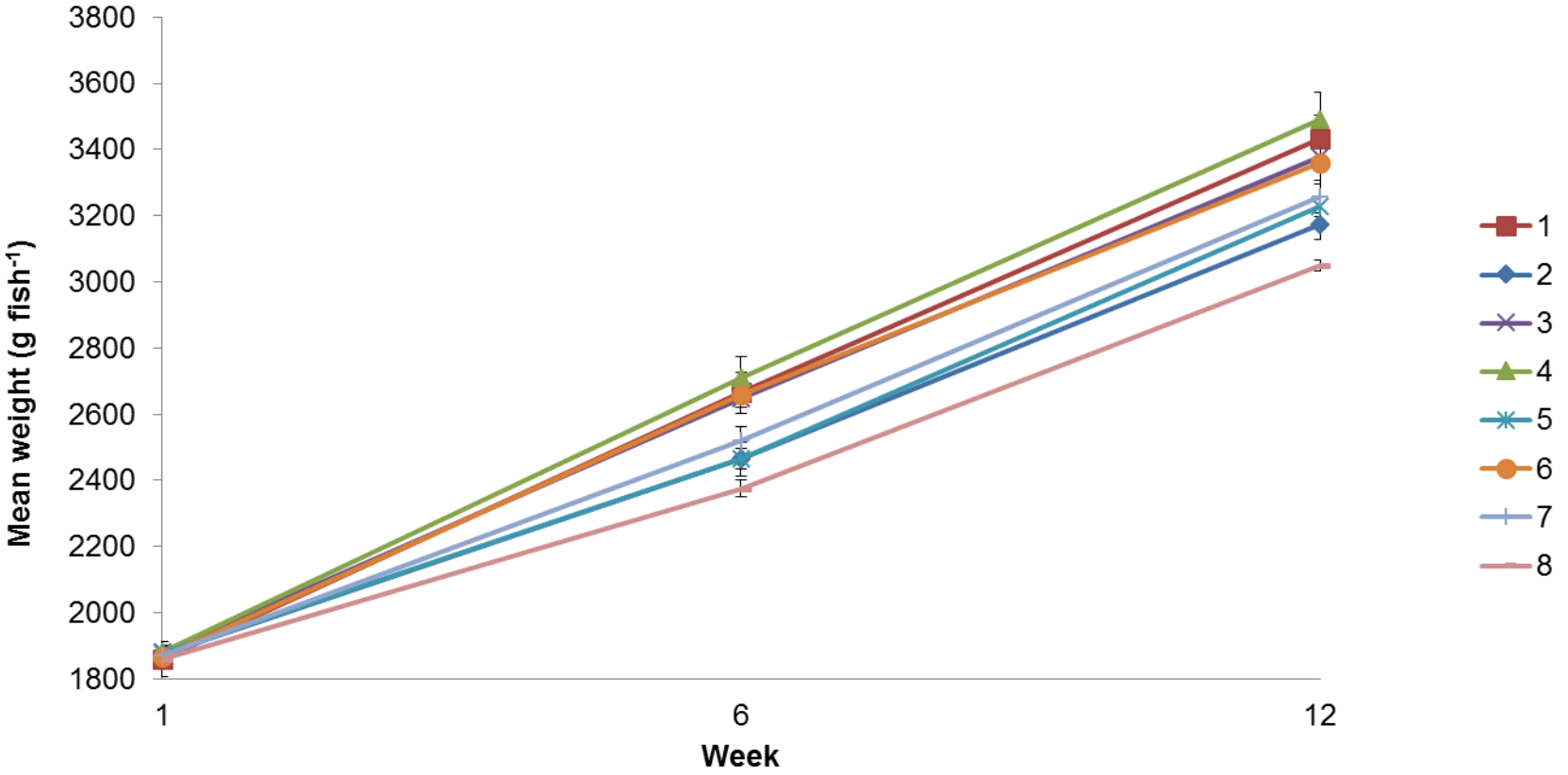

Figure 3.3. Mean individual weight of Yellowtail Kingfish in Trial 2 at 0, 43 and 84 days.
Values are expressed as mean ± SE, n = 3. [1]

[1] 1: Ridley trial Diet 1 (Diet 1) fed to apparent satiation seven days week^{-1} (Control);
2: Ridley trial Diet 1 (Diet 1) fed to sub-satiation (80% of apparent satiation);
3: Ridley trial Diet 2 (Diet 2; 20% FM) fed to apparent satiation seven days week^{-1};
4: Ridley trial Diet 3 (Diet 3; High lipid lower protein) fed to apparent satiation seven days week^{-1};
5: Ridley trial Diet 3 (Diet 3) fed to sub-satiation (80% of apparent satiation);
6: Skretting trial diet (Diet 4; High lipid) fed to apparent satiation seven days week^{-1};
7: Hayashikane Hiramasa trial diet (Diet 5) fed to apparent satiation seven days week^{-1};
8: Hayashikane Hiramasa trial diet (Diet 5) fed to sub-satiation (80% of apparent satiation).

Table 3.5. Growth performance, feed utilisation, proximate composition and nutrient retentions of Yellowtail Kingfish fed varying fish meal replacement and feed rates after 43 days in Trial 2.[1,2,3]

Diet	1	1	2	3	3	4	5	5	
Feed rate (%)	100	80	100	100	80	100	100	80	
Treatment	1	2	3	4	5	6	7	8	ANOVA
Growth performance									
Initial weight (kg)	1.86±0.05	1.88±0.04	1.88±0.02	1.88±0.03	1.88±0.03	1.87±0.03	1.86±0.02	1.89±0.03	$P = 0.999$
Final weight (kg)	2.67±0.06^{ab}	2.47±0.05^{c}	2.65±0.03^{ab}	2.71±0.06^{a}	2.46±0.03^{c}	2.66±0.02^{ab}	2.52±0.04^{bc}	2.38±0.02^{c}	$P = 0.000$
Biomass gain (kg tank^{-1})	15.32±0.19^{a}	11.21±0.26^{b}	14.59±0.32^{a}	15.75±0.60^{a}	11.00±0.37^{b}	15.08±0.28^{a}	12.30±0.39^{b}	9.84±0.31^{c}	$P = 0.000$
SGR (% d^{-1})	0.84±0.01^{a}	0.64±0.00^{abc}	0.80±0.02^{ab}	0.85±0.02^{a}	0.63±0.02^{bc}	0.82±0.02^{ab}	0.69±0.01^{bc}	0.57±0.002^{c}	$P = 0.000$
Initial fork length (mm)	493.42±4.53	496.58±2.13	495.35±1.78	498.07±1.36	496.23±1.73	496.05±2.30	496.67±1.27	494.04±1.46	$P = 0.871$
Final fork length (mm)	544.47±4.59	540.79±3.99	543.33±2.53	548.68±2.36	539.53±3.54	543.68±2.77	539.30±2.90	533.68±0.70	$P = 0.114$
Length growth rate (mm d^{-1})	1.19±0.01^{a}	1.03±0.04^{d}	1.12±0.02^{abc}	1.18±0.03^{ab}	1.01±0.06^{de}	1.11±0.05^{bc}	0.99±0.04^{c}	0.92±0.02^{e}	$P = 0.001$
Condition factor	1.65±0.01^{a}	1.56±0.00^{b}	1.65±0.02^{a}	1.64±0.02^{a}	1.57±0.01^{b}	1.65±0.02^{a}	1.61±0.01^{ab}	1.56±0.01^{b}	$P = 0.000$
Feed utilisation									
Feed consumption (kg tank^{-1})	22.26±0.16^{a}	15.70±0.41^{d}	21.16±0.10^{abc}	21.67±0.58^{ab}	15.04±0.10^{de}	20.63±0.53^{bc}	19.97±0.28^{c}	14.42±0.17^{e}	$P = 0.000$
Apparent feed intake (% bw d^{-1})	1.26±0.03^{a}	0.93±0.01^{c}	1.20±0.01^{ab}	1.21±0.01^{ab}	0.89±0.02^{c}	1.17±0.04^{b}	1.17±0.00^{b}	0.87±0.01^{c}	$P = 0.000$
Apparent FCR (as fed)	1.45±0.01^{b}	1.40±0.02^{b}	1.45±0.03^{b}	1.38±0.02^{b}	1.37±0.04^{b}	1.37±0.01^{b}	1.63±0.03^{a}	1.47±0.03^{b}	$P = 0.000$

[1] Values are mean ± SE; n = 3.
[2] A significance level of $P < 0.05$ was used for all statistical tests, where significant differences were observed post-hoc tests were used (Student Newman-Keuls test) to detect differences between treatments, values in the same row without a common superscript are significantly different (a indicates the highest value; $P < 0.05$).
[3] SE less than 0.01 are reported as “0.00”.

Table 3.6. Growth performance, feed utilisation, proximate composition and nutrient retentions of Yellowtail Kingfish fed varying fish meal replacement and feed rates between days 44 and 84 in Trial 2.[1,2,3]

Diet	1	1	2	3	3	4	5	5	
Feed rate (%)	100	80	100	100	80	100	100	80	
Treatment	1	2	3	4	5	6	7	8	ANOVA
Growth performance									
Initial weight (kg)	2.67±0.06^{ab}	2.47±0.05^{c}	2.65±0.03^{ab}	2.71±0.06^{a}	2.46±0.03^{c}	2.66±0.02^{ab}	2.52±0.04^{bc}	2.38±0.02^{c}	*P* = 0.000
Final weight (kg)	3.43±0.07^{ab}	3.17±0.05^{cd}	3.38±0.04^{abc}	3.49±0.08^{a}	3.20±0.03^{bcd}	3.36±0.06^{abc}	3.26±0.05^{abcd}	3.05±0.02^{d}	*P* = 0.000
Biomass gain (kg tank^{-1})	14.57±0.14	13.46±0.19	13.85±0.46	14.79±0.43	13.14±0.85	13.27±0.93	14.00±0.23	12.80±0.37	*P* = 0.166
SGR (% d^{-1})	0.62±0.01	0.62±0.02	0.59±0.02	0.62±0.01	0.64±0.02	0.57±0.03	0.63±0.01	0.61±0.02	*P* = 0.254
Initial fork length (mm)	544.47±4.59	540.79±3.99	543.33±2.53	548.68±2.36	539.53±3.54	543.68±2.77	539.30±2.90	533.68±0.70	*P* = 0.114
Final fork length (mm)	587.02±4.72	580.96±4.26	584.82±1.41	590.26±3.22	579.62±3.44	582.54±4.91	582.72±3.02	579.12±1.38	*P* = 0.373
Length growth rate (mm d^{-1})	1.04±0.01	0.98±0.01	1.01±0.05	1.01±0.03	0.98±0.01	0.95±0.05	1.06±0.00	1.11±0.04	*P* = 0.053
Condition factor	1.70±0.01^{a}	1.62±0.02^{b}	1.69±0.02^{a}	1.70±0.02^{a}	1.65±0.01^{ab}	1.70±0.01^{a}	1.65±0.01^{ab}	1.57±0.00^{c}	*P* = 0.000
Feed utilisation									
Feed consumption (kg tank^{-1})	25.78±0.14^{a}	21.04±0.04^{c}	24.85±0.16^{a}	25.64±0.25^{a}	20.77±0.22^{c}	23.14±0.68^{b}	25.48±0.18^{a}	20.68±0.34^{c}	*P* = 0.000
Apparent feed intake (% bw d^{-1})	1.09±0.02^{a}	0.96±0.02^{c}	1.06±0.01^{ab}	1.06±0.02^{ab}	0.96±0.03^{c}	0.99±0.03^{bc}	1.13±0.02^{a}	0.98±0.02^{bc}	*P* = 0.000
Apparent FCR (as fed)	1.77±0.01	1.56±0.02	1.80±0.05	1.74±0.05	1.59±0.11	1.75±0.09	1.82±0.04	1.62±0.03	*P* = 0.034

[1] Values are mean ± SE; n = 3.
[2] A significance level of $P < 0.05$ was used for all statistical tests, where significant differences were observed post-hoc tests were used (Student Newman-Keuls test) to detect differences between treatments, values in the same row without a common superscript are significantly different (a indicates the highest value; $P < 0.05$).
[3] SE less than 0.01 are reported as “0.00”.

Table 3.7. Growth performance, feed utilisation, proximate composition and nutrient retentions of Yellowtail Kingfish fed varying fish meal replacement and feed rates after 84 in Trial 2.[1,2,3]

Diet	1	1	2	3	3	4	5	5	
Feed rate (%)	100	80	100	100	80	100	100	80	
Treatment	1	2	3	4	5	6	7	8	ANOVA[2,3]
Growth performance									
Initial weight (kg)	1.86±0.05	1.88±0.04	1.88±0.02	1.88±0.03	1.88±0.03	1.87±0.03	1.86±0.02	1.89±0.03	P = 0.999
Final weight (kg)	3.43±0.07^{ab}	3.17±0.05^{cd}	3.38±0.04^{abc}	3.49±0.08^{a}	3.20±0.03^{bcd}	3.36±0.06^{abc}	3.26±0.05^{abcd}	3.05±0.02^{d}	P = 0.000
Biomass gain (kg tank^{-1})[4]	29.89±0.33^{a}	24.67±0.29^{cd}	28.44±0.75^{ab}	30.54±1.02^{a}	25.08±1.23^{cd}	28.35±1.16^{ab}	26.30±0.57^{bc}	22.64±0.61^{d}	P = 0.000
SGR (% day^{-1})	0.73±0.01^{a}	0.63±0.01^{cd}	0.70±0.02^{ab}	0.73±0.01^{a}	0.64±0.02^{cd}	0.70±0.02^{ab}	0.66±0.01^{bc}	0.59±0.02^{d}	P = 0.000
Initial fork length (mm)	493.42±4.53	496.58±2.13	495.35±1.78	498.07±1.36	496.23±1.73	496.05±2.30	496.67±1.27	494.04±1.46	P = 0.871
Final fork length (mm)	587.02±4.72	580.96±4.26	584.82±1.41	590.26±3.22	579.62±3.44	582.54±4.91	582.72±3.02	579.12±1.38	P = 0.373
Length growth rate (mm day^{-1})	1.11±0.01	1.00±0.03	1.07±0.02	1.10±0.03	0.99±0.03	1.03±0.05	1.02±0.02	1.01±0.01	P = 0.076
Condition factor	1.70±0.01^{a}	1.62±0.02^{b}	1.69±0.02^{a}	1.70±0.02^{a}	1.65±0.01^{ab}	1.70±0.01^{a}	1.65±0.01^{ab}	1.57±0.00^{c}	P = 0.000
Hepatosomatic index (HSI; %)	0.83±0.06^{bc}	0.75±0.10^{bc}	0.89±0.06^{bc}	0.97±0.18^{b}	0.85±0.06^{bc}	1.19±0.12^{a}	0.76±0.03^{bc}	0.70±0.04^{c}	P < 0.001
Feed utilisation									
Feed consumption (kg tank$^{-1)}$	48.04±0.29^{a}	36.73±0.39^{d}	46.01±0.19^{ab}	47.31±0.73^{ab}	35.81±0.32^{d}	43.77±1.20^{c}	45.45±0.24^{bc}	35.10±0.39^{d}	P = 0.000
Apparent feed intake (% bw d^{-1})	1.14±0.02^{a}	0.91±0.01^{c}	1.10±0.01^{ab}	1.10±0.01^{ab}	0.90±0.03^{c}	1.05±0.03^{b}	1.11±0.01^{ab}	0.90±0.01^{c}	P = 0.000
Apparent FCR (as fed)	1.61±0.01^{ab}	1.49±0.02^{b}	1.62±0.04^{ab}	1.55±0.03^{b}	1.49±0.07^{b}	1.55±0.03^{b}	1.73±0.03^{a}	1.55±0.03^{b}	P = 0.006
Proximate composition									
Moisture (%)	61.3±0.9^{ab}	61.4±0.7^{ab}	59.2±0.3^{b}	58.1±0.7^{b}	60.1±1.1^{ab}	58.4±0.6^{b}	60.5±0.4^{ab}	62.5±0.6^{a}	P = 0.004
Protein (% wet)	20.2±0.4	20.4±0.3	20.6±0.4	19.6±0.4	20.0±0.4	19.6±0.4	20.4±0.3	20.4±0.3	P = 0.389
Fat (% wet)	15.6±1.3	14.6±1.2	17.4±0.4	18.9±0.4	16.6±1.7t	19.1±0.7	16.0±1.1	14.2±0.4	P = 0.025[3]
Ash (% wet)	2.6±0.3	2.2±0.1	2.3±0.1	2.5±0.2	2.5±0.1	2.3±0.2	2.5±0.1	2.4±0.2	P = 0.648
Carbohydrate (% wet; by difference)	0.5±0.5[4]	0.8±0.8[4]	0.6±0.6[4]	<1.5	0.8±0.8[4]	0.7±0.7[4]	0.9±0.9[4]	0.8±0.8[4]	P = 0.989
Energy (MJ kg^{-1} dry)	9.28±0.46^{abc}	8.99±0.30^{bc}	10.04±0.12^{abc}	10.32±0.18^{ab}	9.71±0.55^{abc}	10.53±0.15^{a}	9.54±0.27^{abc}	8.85±0.02^{c}	P = 0.010
Nutrient retention									
Apparent PD	28.56±0.97	31.61±1.20	29.99±0.50	28.80±1.78	31.40±3.01	29.98±0.71	27.08±1.42	30.10±0.74	P = 0.458
Apparent ED	37.35±3.56	39.38±2.28	42.34±0.25	43.51±1.89	43.25±5.28	43.01±1.53	38.64±1.94	38.40±0.46	P = 0.516

[1] Values are mean ± SE; n = 3. Initial fish proximate composition (wet basis): Moisture 66.3%, Protein 20.2%, Fat 11.0%, Ash 2.2%, Carbohydrate (by difference) <1.5%, Energy 7.50 MJ kg^{-1}

[2] A significance level of P < 0.05 was used for all statistical tests, where significant differences were observed post-hoc tests were used (Student Newman-Keuls test) to detect differences between treatments, values without a common superscript are significantly different (a indicates the highest value; P < 0.05).

[3] One-factor ANOVA detected a significant difference, but SNK test was unable to discern significant differences.

[4] One or two samples were below detectable range and were assigned the value of 0; if the values for all three replicates were below detectable range then they are reported here as below the detectable range (e.g. <10), but were assigned 0 for statistical analyses.

Table 3.8. Two-factor ANOVA results for the effect of diet type and feeding rate on the growth performance and feed utilisation of Yellowtail Kingfish after 43 days in Trial 2.[1,2]

Diet	ANOVA		
Feed rate (%)	Diet (A)	Feed rate (B)	A × B
Treatment	*P* value	*P* value	*P* value
Growth performance			
Initial weight (g)	*P* = 0.898	*P* = 0.994	*P* = 0.906
Final weight (g)	*P* = 0.028 (D1 = D3 > D5)	*P* = 0.000 (S > RS)	*P* = 0.575
Biomass gain (g tank^{-1})	*P* = 0.000 (D1 = D3 > D5)	*P* = 0.000 (S > RS)	*P* = 0.027
SGR (% d^{-1})	*P* = 0.000 (D1 = D3 > D5)	*P* = 0.000 (S > RS)	*P* = 0.015
Initial fork length (mm)	*P* = 0.634	*P* = 0.824	*P* = 0.440
Final fork length (mm)	*P* = 0.840	P = 0.000 (S > RS)	*P* = 0.704
Length growth rate (mm d^{-1})	*P* = 0.003 (D1 = D3 > D5)	P = 0.001 (S > RS)	*P* = 0.372
Condition factor	*P* = 0.210	P = 0.000 (S > RS)	*P* = 0.173
Feed utilisation			
Feed consumption (kg tank^{-1})	*P* = 0.001 (D1 = D3 > D5)	*P* = 0.000 (S > RS)	*P* = 0.223
Apparent feed intake (% bw d^{-1})	*P* = 0.001 (D1 > D3 = D5)	*P* = 0.000 (S > RS)	*P* = 0.392
Apparent FCR	*P* = 0.000 (D1 = D3 < D5)	*P* = 0.005 (RS < S)	*P* = 0.034

[1] Values are mean ± SE; n = 3. A significance level of $P < 0.05$ was used for all statistical tests: RS denotes 80% sub-satiation feed rate; S denotes satiation feed rate.
[2] Refer to Table 3.5 for data and statistical analyses of interactions.

Table 3.9. Two-factor ANOVA results for the effect of diet type and feeding rate on the growth performance and feed utilisation of Yellowtail Kingfish between days 44 and 84 in Trial 2.[1,2]

Diet	ANOVA		
Feed rate (%)	Diet (A)	Feed rate (B)	A × B
Treatment	*P* value	*P* value	*P* value
Growth performance			
Initial weight (g)	*P* = 0.020 (D1 = D3 > D5)	*P* = 0.000 (S > RS)	*P* = 0.575
Final weight (g)	*P* = 0.006 (D1 = D3 > D5)	*P* = 0.000 (S > RS)	*P* = 0.776
Biomass gain (g tank^{-1})	*P* = 0.287	*P* = 0.001 (S > RS)	*P* = 0.805
SGR (% d^{-1})	*P* = 0.659	*P* = 0.815	*P* = 0.367
Initial fork length (mm)	*P* = 0.064	*P* = 0.049 (S > RS)	*P* = 0.704
Final fork length (mm)	*P* = 0.480	P = 0.024 (S > RS)	*P* = 0.607
Length growth rate (mm d^{-1})	*P* = 0.005 (D1 = D3 < D5)	P = 0.472	*P* = 0.060
Condition factor	*P* = 0.001 (D1 = D3 > D5)	P = 0.000 (S > RS)	*P* = 0.555
Feed utilisation			
Feed consumption (kg tank^{-1})	*P* = 0.292	*P* = 0.000 (S > RS)	*P* = 0.954
Apparent feed intake (% bw d^{-1})	*P* = 0.128	*P* = 0.000 (S > RS)	*P* = 0.520
Apparent FCR	*P* = 0.499	*P* = 0.000 (RS < S)	*P* = 0.796

[1] Values are mean ± SE; n = 3. A significance level of $P < 0.05$ was used for all statistical tests: RS denotes 80% sub-satiation feed rate; S denotes satiation feed rate.
[2] Refer to Table 3.6 for data and statistical analyses of interactions.

Table 3.10. Two-factor ANOVA results for the effect of diet and feeding rate on the growth performance and feed utilisation of Yellowtail Kingfish after 84 days in Trial 2.[1,2]

Diet	ANOVA		
Feed rate (%)	Diet (A)	Feed rate (B)	A × B
Treatment	*P* value	*P* value	*P* value
Growth performance			
Initial weight (kg)	*P* = 0.898	*P* = 0.994	*P* = 0.906
Final weight (kg)	*P* = 0.010 (D1 = D3 > D5)	*P* = 0.000 (S > RS)	*P* = 0.776
Biomass gain (kg tank^{-1})[3]	*P* = 0.001 (D1 = D3 > D5)	*P* = 0.000 (S > RS)	*P* = 0.392
SGR (% d^{-1})	*P* = 0.001 (D1 = D3 > D5)	*P* = 0.000 (S > RS)	*P* = 0.390
Initial fork length (mm)	*P* = 0.634	*P* = 0.824	*P* = 0.440
Final fork length (mm)	*P* = 0.506	*P* = 0.036 (S > RS)	*P* = 0.607
Length growth rate (mm d^{-1})	*P* = 0.306	*P* = 0.004 (S > RS)	*P* = 0.138
Condition factor	*P* = 0.002 (D1 = D3 > D5)	*P* = 0.000 (S > RS)	*P* = 0.555
Hepatosomatic index (HSI; %)	*P* = 0.008 (D1 = D5 < D3)	*P* = 0.047 (S > RS)	*P* = 0.858
Feed utilisation			
Feed consumption (kg tank^{-1})	*P* = 0.019 (D1 = D3 < D5)	*P* = 0.000 (S > RS)	*P* = 0.381
Apparent feed intake (% bw d^{-1})	*P* = 0.318	*P* = 0.000 (S > RS)	*P* = 0.817
Apparent FCR	*P* = 0.001 (D1 = D3 < D5)	*P* = 0.002 (RS < S)	*P* = 0.330
Proximate composition			
Moisture (%)	*P* = 0.014 (D1 = D5 > D3)	*P* = 0.045 (RS > S)	*P* = 0.379
Protein (% wet)	*P* = 0.163	*P* = 0.494	*P* = 0.828
Fat (% wet)	*P* = 0.072	*P* = 0.053	*P* = 0.840
Ash (% wet)	*P* = 0.809	*P* = 0.237	*P* = 0.504
Carbohydrate (% wet; by difference)	*P* = 0.831	*P* = 0.535	*P* = 0.797
Energy (MJ kg^{-1} dry)	*P* = 0.062	*P* = 0.066	*P* = 0.826
Nutrient retention			
Apparent PD	*P* = 0.556	*P* = 0.034 (RS > S)	*P* = 0.989
Apparent ED	*P* = 0.141	*P* = 0.825	*P* = 0.908

[1] Values are mean ± SE; n = 3. A significance level of *P* < 0.05 was used for all statistical tests: RS denotes 80% sub-satiation feed rate; S denotes satiation feed rate.
[2] Refer to Table 3.7 for data.
[3] n = 2 for treatment, due to mortality in M16 tank (Treatment 5, Diet 3, sub-satiation), tank M16 was omitted from analyses.

Whole fish amino acid, taurine and choline composition
Treatment had no significant effect on the amino acid, taurine or choline levels of Yellowtail Kingfish tissue after 84 days ($P > 0.05$; Table 3.11).

Whole fish fatty acid composition
There were numerous significant differences in fatty acid levels of fish between treatments (One-factor ANOVA, $P < 0.05$, Table 3.12). In contrast to Trial 1 (Chapter 2 of the current project), the EPA, DPA and DHA levels did not significantly mirror those found in the FO provided in the diets (Table 3.12). However, there was a tendency for these fatty acid tissue levels to be related to dietary levels.

Whole fish mineral composition
Yellowtail Kingfish selenium tissue concentration was significantly affected by treatment (One-factor ANOVA, $P < 0.05$, Table 3.13). In general, selenium tissue concentration was highest in fish fed Treatments 1 and 4, and lowest in those fed Treatments 6 and 7 ($P < 0.05$, Table 3.13). Other measured tissue mineral concentrations were not significantly influenced by treatment, or the respective post-hoc tests (SNK) were unable to discern differences between treatments ($P > 0.05$).

Blood biochemistry and haematology
Results are provided in Table 3.14 for One-factor ANOVA of data for all treatments and Table 3.12 for Two-factor ANOVA of diet type and feed rate values for Diets 1, 3 and 5.

At the completion of the trial, there was a significant difference in the blood triglyceride levels of fish fed different diets, with fish fed Treatment 6 (Skretting diet, apparent satiation; 2.40 mmol L^{-1}) having the highest levels and fish fed Treatment 1 having the lowest (Ridley Diet 1, apparent satiation; 1.66 mmol L^{-1}) (Table 3.14, $P < 0.05$; One-factor ANOVA). The blood triglyceride levels of fish from all of the other treatments ranged between these values and were not significantly different to Treatment 1 or 6 (Table 3.14; $P > 0.05$; One-factor ANOVA). There were no significant differences in any of the other blood biochemistry or haematology values for fish in different treatments (Table 3.14; $P > 0.05$; One-factor ANOVA).

At the conclusion of the trial, there were significant effects of diet type and/or feed rate on blood urea, cholesterol and triglyceride levels of Yellowtail Kingfish fed Diets 1, 3 and 5. There was a significant influence of diet type on blood urea levels (Diet 1 = Diet 5 > Diet 3), but there was no significant effect of feed rate or the interaction between the two factors (Table 3.14; $P < 0.05$; Two-factor ANOVA). In contrast, blood cholesterol levels were significantly affected by diet type ($P < 0.05$; Diet 1 = Diet 5 > Diet 3) and feed rate ($P < 0.05$; apparent satiation > sub-satiation), and there was no significant interaction between the two factors (Table 3.14; $P > 0.05$; Two-factor ANOVA). Blood triglyceride levels were only significantly affected by feed rate ($P < 0.05$; apparent satiation < sub-satiation) and there was no significant interaction between the two factors (Table 3.14; $P > 0.05$; Two-factor ANOVA). Haematocrit count was not significantly influenced by treatment, and ranged from 46.2 to 55.7 L L^{-1} (One-factor ANOVA; $P > 0.05$; Table 3.14). Diet, feed rate and the interaction between diet and feed rate did not significantly influence haematocrit count (Two-factor ANOVA; $P > 0.05$; Table 3.15). All other measured blood biochemical and haematological values were not significantly affected by diet type or feed rate (Table 3.12; $P > 0.05$; Two-factor ANOVA).

Table 3.11. Essential and non-essential amino acid composition (mg 100 g^{-1}), and taurine and choline concentrations of Yellowtail Kingfish fed diets containing varying fish meal replacement levels and feed rates for 84 days in Trial 2.[1]

Diet		1	1	2	3	3	4	5	5	
Feed rate (%)		100	80	100	100	80	100	100	80	
Treatment	Initial	1	2	3	4	5	6	7	8	ANOVA[2]
Essential										
Arginine	1065	1079±58	1111±26	1180±102	1082±59	1075±69	1091±107	1186±109	1103±17	*P* = 0.981
Histidine	931	953±38	1038±34	1026±93	1024±76	978±61	992±104	935±141	936±24	*P* = 0.959
Isoleucine	771	769±33	816±19	843±84t	811±55	778±49	828±79	872±88	816±10	*P* = 0.939
Leucine	1305	1297±49	1383±42	1434±161	1363±100	1304±79	1386±144	1454±163	1369±17	*P* = 0.962
Lysine	1572	1512±124	1688±91	1762±253t	1436±144	1473±160	1598±173	1617±217t	1563±20	*P* = 0.855
Methionine	452	453±20	449±43	457±29	415±29	420±41t	360±21	495±31	450±26	*P* = 0.192
Phenylalanine	693	694±33	749±44	775±93	735±72	691±44	761±90	802±97	716±2	*P* = 0.926
Threonine	810	797±23	811±35	854±52	802±24	820±46	807±52	852±50	856±9	*P* = 0.885
Valine	974	963±43	1052±61	1078±148	1024±108	958±67	1045±139	1098±157	1003±13	*P* = 0.970
Non-essential										
Alanine	1006	1052±108	1135±108	1185±191	1085±147t	991±72	1115±168	1284±202	1025±24	*P* = 0.855
Aspartic acid	1635	1573±42	1677±41	1723±197	1609±117	1590±114	1615±176	1615±176	1675±44	*P* = 0.760
L Cystine	183	161.1±5.4	161.3±8.1	160.3±11.3	148.1±3.2	161.3±10.0	153.9±7.7	152.0±8.1	166.0±1.2	*P* = 0.727
Glutamic acid	2095	2055±78	2156±39	2254±217	2084±83	2093±132	2086±176	2669±358	2240±48	*P* = 0.289
Glycine	1014	1114±207	1124±83	1235±189	1011±117	1004±102	1051±171	1340±183	990±52	*P* = 0.673
Proline	476	646±194	552±79	619±129	521±107	452±38	626±114	765±128	447±24	*P* = 0.538
Serine	702	695±14	722±25	748±38	702±23	720±43	693±47	722±58	754±11	*P* = 0.891
Tyrosine	606	562±38	584±27	582±58	546±39	585±44	579±15	581±16	650±7	*P* = 0.624
Other										
Taurine	192	179±13	175±8	144±4	164±3	174±9	162±13	179±8	184±2	*P* = 0.072
Choline	66	58±1	60±1	62±1	56±2	59±1	56±2	59±2	62±1	*P* = 0.059

[1] Values are mean ± SE; n=3 (mg 100 g^{-1})
[2] A significance level of $P < 0.05$ was used for all statistical tests.

Table 3.12. Fatty acid and cholesterol composition (mg 100 g^{-1}) of Yellowtail Kingfish fed diets containing varying fish meal replacement levels and feed rates for 84 days in Trial 2.[1]

Diet		1	1	2	3	3	4	5	5	
Feed rate (%)		100	80	100	100	80	100	100	80	
Treatment	Initial	1	2	3	4	5	6	7	8	ANOVA[3]
Butyric (C4:0)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Caproic (C6:0)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Caprylic (C8:0)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Capric (C10:0)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Lauric (C12:0)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Trisdecanoic (C13:0)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Myristic (C14:0)	270	473±27	547±48	633±69	660±50	467±19	587±42	590±6	470±15	$P = 0.012$[4]
Pentadecanoic (C15:0)	35	52±3	66±8	69±8	77±9	52±2	62±5	75±1	58±4	$P = 0.039$[4]
Palmitic (C16:0)	1710	2843±217^{ab}	3007±99^{ab}	3647±399^{ab}	3807±446^{a}	3027±92^{ab}	3913±266^{a}	2833±19^{ab}	2493±148^{b}	$P = 0.008$
Margaric (C17:0)	38	56±4	68±7	73±8	80±8	57±1	68±6	74±1	58±3	$P = 0.053$
Stearic (C18:0)	580	880±69^{ab}	937±39^{ab}	1127±128^{ab}	1167±139^{ab}	930±15^{ab}	1193±90^{a}	903±3^{ab}	790±31^{b}	$P = 0.018$
Arachidic (C20:0)	19	29±2^{b}	33±3^{ab}	37±4^{ab}	40±4^{a}	30±1^{ab}	30±2^{ab}	35±1^{ab}	29±1^{b}	$P = 0.025$
Docosanoic (C22:0)	12	18±1^{ab}	19±1^{ab}	22±2^{a}	22±2^{a}	18±0^{ab}	15±1^{b}	19±0^{ab}	16±0^{ab}	$P = 0.013$
Tetracosanoic (C24:0)	<10	3±3[2]	7±4[2]	8±4[2]	11±1[2]	3±3[2]	4±4[2]	7±3[2]	<10	$P = 0.318$
Saturated Fat (%m m^{-1})	2.7	4.5±0.3^{ab}	4.7±0.2^{ab}	5.7±0.6^{ab}	6.0±0.6^{a}	4.6±0.1^{ab}	6.0±0.5^{a}	4.7±0.0^{ab}	4.0±0.2^{b}	$P = 0.013$
Decenoic (C10:1)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Myristoleic (C14:1)	18	29±2^{b}	31±1^{b}	39±5^{ab}	39±4^{ab}	30±1^{b}	43±4^{a}	30±0^{b}	26±1^{b}	$P = 0.006$
Pentadecenoic (C15:1)	<10	<10	<10	4±4[2]	7±3[2]	<10	3±3[2]	<10	<10	$P = 0.249$
Palmitoleic (C16:1)	580	993±84^{b}	1060±25^{b}	1283±144^{ab}	1330±162^{ab}	1057±44^{b}	1473±104^{a}	993±9^{b}	897±58^{b}	$P = 0.006$
Heptadecenoic (C17:1)	37	49±4	61±6	65±7	73±8	50±2	60±6	70±2	54±3	$P = 0.036$[4]
Octadecenoic (C18:1n-6)	<10	<10	3±3[2]	8±4[2]	7±4[2]	<10	<10	4±4[2]	<10	$P = 0.186$
Octadecenoic (C18:1n-7)	320	497±43^{ab}	540±23^{ab}	650±72^{ab}	683±83^{a}	527±19^{ab}	693±50^{a}	523±3^{ab}	457±22^{b}	$P = 0.014$
Oleic (C18:1n-9)	3630	5587±650^{ab}	5397±364^{ab}	7083±790^{ab}	7080±1370^{ab}	6283±229^{ab}	8500±601^{a}	4270±47^{b}	4473±757^{b}	$P = 0.009$

[1] Values are mean ± SE; n=3 (mg 100 g^{-1})

[2] One or two samples were below detectable range and were assigned the value of 0; if the values for all three replicates were below detectable range then they are reported here as below the detectable range (eg. <10), but were assigned the value of 0 for statistical analyses.

[3] A significance level of $P < 0.05$ was used for all statistical tests, where significant differences were observed post-hoc tests were used (Student Newman-Keuls test) to detect differences between treatments, values without a common superscript are significantly different (^{a} indicates the highest value; $P < 0.05$).

[4] One-factor ANOVA detected a significant difference, but SNK test was unable to discern significant differences.

Table 3.12. Continued.

Diet		1	1	2	3	3	4	5	5	
Feed rate (%)		100	80	100	100	80	100	100	80	
Treatment	Initial	1	2	3	4	5	6	7	8	ANOVA3
Eicosenoic (C20:1n-9)	160	247±13^{b}	290±21^{ab}	337±38^{a}	347±23^{a}	247±9^{b}	230±17^{b}	303±3^{ab}	243±7^{b}	P = 0.002
Eicosenoic (C20:1n-11,13)	20	37±3	104±63	52±7	116±67	37±3	46±3	213±3	127±43	P = 0.037[4]
Eicosenoic (C20:1; total)	180	280±15	397±87	390±46	463±83	283±12	273±20	517±3	367±53	P = 0.026[4]
Docosenoic (C22:1n-9)	20	29±2^{ab}	40±7^{ab}	41±5^{ab}	46±7^{ab}	29±1^{ab}	27±2^{b}	50±0^{a}	35±5^{ab}	P = 0.018
Docosenoic (C22:1n-11, 13)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Tetracosenoic (C24:1)	26	37±1	48±5	49±7	58±9	35±1	37±6	57±3	39±5	P = 0.029[2]
Mono Unsaturated Fat (%m/m)	4.9	7.6±0.8^{ab}	7.8±0.2^{ab}	9.8±1.1^{ab}	10.0±1.6^{ab}	8.4±0.3^{ab}	11.3±0.8^{a}	6.7±0.0^{b}	6.5±0.8^{b}	P = 0.011
Linoleic (C18:2n-6)	1270	2033±292	1820±234	2537±275	2437±622	2377±94	2490±188	1243±24	1507±382	P = 0.067
Linoleic acid (C18:2n-9, 12)	1300	2083±292	1863±240	2593±284	2483±634	2423±97	2560±193t	1277±23	1540±391	P = 0.067
Alpha Linolenic (C18:3n-3)	160	263±38	257±15	330±38	333±64	303±12	377±27	213±3t	220±35	P = 0.035[4]
Gamma Linolenic (C18:3n-6)	19	31±2	34±1	41±5	40±5t	33±1	39±3	33±0	28±1	P = 0.060
Linolenic acid (total)	220	353±41t	350±15	453±52	463±74	397±15	490±36	320±0	310±35	P = 0.032[4]
Steridonic (C18:4n-3)	60	110±10	140±25	150±17	163±26	105±5	123±9	177±3	130±15	P = 0.065
Eicosadienoic (C20:2n-6)	23	33±2^{b}	39±3^{ab}	45±5^{ab}	51±7^{a}	35±2^{b}	39±2^{ab}	40±0^{ab}	33±1^{b}	P = 0.022
Eicosatrienoic (C20:3n-3)	<10	13±1	17±3	18±2	20±3	13±1	14±1	17±3	15±1	P = 0.642
Dihomo-gamma-linoleic (C20:3n-6)	16	24±2	24±5	32±3	33±4	24±1	33±2	26±0	23±1	P = 0.054
Eicosatetracenoic (C20:4n-3)	44	69±5	129±56	98±16	135±63	67±4	80±7	223±9	137±38	P = 0.070
Arachidonic (C20:4n-6)	89	117±9	143±15	157±20	165±20	113±3	157±12	160±0	127±9	P = 0.058
Eicosapentaenoic (C20:5n-3)	400	697±58	830±87	947±107	977±102	670±23	930±67	933±15	720±36	P = 0.025[4]
Heneicosapentaenoic acid (C21:5n-3)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Docosatetraenoic (C22:4n-6)	30	36±3^{b}	53±12^{ab}	52±3^{ab}	60±14^{ab}	35±0^{b}	41±4^{ab}	74±1^{a}	50±9^{ab}	P = 0.034
Docosapentaenoic (C22:5n-3)	160	220±15	290±42	307±32	337±43	210±6	287±18	330±6	250±25	P = 0.029[4]
Docosapentaenoic (C22:5n-6)	20	35±3	44±4	49±5	51±5	38±3	46±4	48±1	37±2	P = 0.051
Docosahexaenoic (C22:6n-3)	750	907±68	1310±282	1210±130	1463±331	867±12	1030±85	1740±36	1230±210	P = 0.054
Poly Unsaturated Fat (%m m^{-1})	3.2	4.7±0.5	5.5±0.4	6.3±0.7	6.5±0.8	5.1±0.2	5.9±0.4	5.7±0.2	4.7±0.1	P = 0.098
Cholesterol	112	107±4	101±1	108±2	104±2	107±3	108±3	112±4	115±2	P = 0.082

[1] Values are mean ± SE; n=3 (mg 100 g^{-1})
[2] One or two samples were below detectable range and were assigned the value of 0; if the values for all three replicates were below detectable range then they are reported here as below the detectable range (e.g. <10), but were assigned the value of 0 for statistical analyses.
[3] A significance level of $P < 0.05$ was used for all statistical tests, where significant differences were observed post-hoc tests were used (Student Newman-Keuls test) to detect differences between treatments, values without a common superscript are significantly different ([a] indicates the highest value; $P < 0.05$).
[4] One-factor ANOVA detected a significant difference, but SNK test was unable to discern significant differences.

Table 3.13. Mineral composition (mg 100 g^{-1}) of Yellowtail Kingfish after 84 days in Trial 2.

Diet		1	1	2	3	4	4	5	6	
Feed rate (%)		100	80	100	100	100	80	100	100	
Treatment	Initial	1	2	3	4	5	6	7	8	*ANOVA*[3]
Calcium	3600	6533±612	5200±889	5967±867	5333±267	5233±953	3900±473	3767±233	3733±233	*P* = 0.044[4]
Copper	0.66	0.60±0.01	0.80±0.09	1.26±0.53	0.77±0.17	0.65±0.02	0.65±0.04	0.71±0.02	0.80±0.02	*P* = 0.411
Iodine (µg 100 g^{-1})	20	23±1	20±3	19±3	26±1	22±2	29±6t	25±2	24±3	*P* = 0.414
Iron	22	18±0	24±2	21±1	21±2	19±1	24±3	20±2	28±6	*P* = 0.293
Magnesium	320	350±17	337±13	353±15	323±3	330±20t	307±9	313±3	327±7	*P* = 0.172
Manganese	0.53	0.65±0.07	0.54±0.03	0.73±0.15	0.59±0.04	0.52±0.06	0.47±0.06	0.46±0.05	0.51±0.06	*P* = 0.206
Potassium	3700	3200±58	3467±167	3133±33	3133±145	3200±0	3200±58	3167±67	3467±88	*P* = 0.085
Phosphorus	3700	5133±348	4400±416	4900±462	4433±176	4500±529	3667±233	3667±145	3767±88	*P* = 0.042[4]
Selenium	40	62±6[a]	58±2[ab]	56±7[ab]	62±1[a]	56±1[ab]	29±0[c]	34±4[c]	43±3[bc]	*P* < 0.001
Zinc	13	13±1	13±0	14±0	13±1	12±1	12±1	12±1	13±0	*P* = 0.509

[1] Values are mean ± SE; n=3 (mg 100 g^{-1})

[2] One or two samples were below detectable range and were assigned the value of 0; if the values for all three replicates were below detectable range then they are reported here as below the detectable range (e.g. <10), but were assigned 0 for statistical analyses.

[3] A significance level of $P < 0.05$ was used for all statistical tests, where significant differences were observed post-hoc tests were used (Student Newman-Keuls test) to detect differences between treatments, values without a common superscript are significantly different ([a] indicates the highest value; $P < 0.05$).

Table 3.14. Blood biochemistry and haematology of Yellowtail Kingfish after 84 days in Trial 2.[1,2,3,4]

Diet	1	1	2	3	3	4	5	5	
Feed rate (%)	100	80	100	100	80	100	100	80	
Treatment	1	2	3	4	5	6	7	8	ANOVA
Biochemistry									
Sodium (mmol L^{-1})	170±7	176±13	190±10	182±6	197±7	177±8	188±3	190±6	$P = 0.374$
Potassium (mmol L^{-1})	5.7±0.1	5.7±0.0	5.7±0.0	5.7±0.1	5.8±0.2	5.9±0.1	5.9±0.2	5.6±0.1	$P = 0.390$
Urea (mmol L^{-1})	7.7±0.3	7.4±0.2	6.8±0.5	6.3±0.2	5.9±0.2	6.7±0.5	6.8±0.6	6.9±0.4	$P = 0.105$
Creatinine (mmol L^{-1})	0.040±0.004	0.032±0.008	0.082±0.057	0.031±0.004	0.022±0.001	0.038±0.006	0.078±0.046	0.035±0.006	$P = 0.666$
Calcium (mmol L^{-1})	2.06±0.00	2.05±0.01	2.04±0.01	2.04±0.01	2.03±0.01	2.04±0.00	2.05±0.01s	2.05±0.00	$P = 0.442$
Protein (g L^{-1})	56±1	53±5	53±2	54±1	48±1	59±1	55±2	54±3	$P = 0.228$
Albumin (g L^{-1})	22±0	20±3	18±1	19±0	17±1	21±2	19±1	19±2	$P = 0.454$
Globulin (g L^{-1})	34±1	33±3	35±1	35±0	32±0	37±1	35±2	35±2	$P = 0.439$
Total Bilirubin (mmol L^{-1})	0.3±0.1	0.6±0.3	0.3±0.0	0.5±0.1	0.5±0.1	0.7±0.3	0.5±0.0	0.5±0.0	$P = 0.393$
ALT (IU L^{-1})	42±9	28±13	14±3	17±4	20±7	27±2	21±3	23±8	$P = 0.230$
ALP (IU L^{-1})	5±2	3±1	6±3	5±1	2±1	5±1	4±2	5±3	$P = 0.855$
Magnesium (mmol L^{-1})	0.37±0.03	0.30±0.10	0.27±0.05	0.23±0.02	0.22±0.04	0.24±0.03	0.27±0.04	0.28±0.04	$P = 0.508$
Cholesterol (mmol L^{-1})	8.21±0.14	7.79±0.58	7.68±0.42	7.56±0.03	6.81±0.12	7.70±0.10	8.18±0.17	7.79±0.43	$P = 0.130$
Triglyceride (mmol L^{-1})	$1.66±0.21^{b}$	$2.11±0.04^{ab}$	$1.67±0.09^{b}$	$2.00±0.12^{ab}$	$2.07±0.07^{ab}$	$2.40±0.26^{a}$	$1.80±0.15^{ab}$	$2.20±0.11^{ab}$	$P = 0.027$
Bile Acids (µmol L^{-1})	2.0±0.8	2.8±0.5	2.4±0.5	2.1±0.4	3.7±0.5	2.5±0.6	2.6±0.7	2.9±0.6	$P = 0.628$
Amylase (µmol L^{-1})	30±7	23±11	16±4	14±1	14±1	14±3	21±4	15±1	$P = 0.356$
GGT (IU L^{-1})	4±1	3±0	4±0	4±1	4±0	4±0	4±0	3±1	$P = 0.789$
Haematology									
RBC ($\times 10^{12}$)	3.29±0.13	3.18±0.30	2.98±0.33	3.16±0.09	2.84±0.17	3.46±0.10	3.25±0.22	3.01±0.19	$P = 0.535$
HGB (g L^{-1})	128±5	133±8	133±3	136±1	128±5	139±1	133±3	131±3	$P = 0.622$
PCV (L L^{-1})	0.58±0.03	0.60±0.03	0.59±0.02	0.59±0.02	0.55±0.01	0.61±0.01	0.59±0.02	0.57±0.01	$P = 0.464$
MCV (fl)	178.8±2.1	180.2±3.1	171.1±2.3	179.3±3.0	182.5±0.9	178.2±1.8	176.0±0.9	177.5±1.1	$P = 0.051$
MCH (pg)	39.2±0.2	39.9±0.2	39.5±0.9	43.0±2.1	42.0±0.8	40.2±1.5	38.2±1.2	42.3±1.7	$P = 0.135$
MCHC (g L^{-1})	222±5	224±4	221±2	242±13	233±7	226±6	219±4	228±2	$P = 0.257$
WBC ($\times 10^{9}$)	6.4±0.1	5.9±0.2	6.1±0.1	6.4±0.2	6.3±0.1	6.2±0.1	6.2±0.1	6.1±0.1	$P = 0.315$
Granulocytes (%)	8±1	8±1	7±0	6±1	8±1	7±1	7±0	9±1	$P = 0.479$
Lymph (%)	90±1	91±1	91±1	92±1	91±1	91±1	91±1	90±1	$P = 0.904$
Mono (%)	1±1	2±0	2±0	2±1	1±0	2±0	2±1	1±0	$P = 0.786$
Eosin (%)	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	-
Baso (%)	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	-
Platelets ($\times 10^{9}$)	20±6	16±3	22±3	26±7	19±1	27±7	15±2	26±2	$P = 0.473$
Haematocrit count (L L-1)[5]	48.6±2.8	48.8±4.0	50.1±3.3	50.9±1.4	46.2±1.2	55.7±2.6	50.4±1.3	49.7±2.3	$P = 0.403$

[1] Values are mean ± SE; n = 3. Smear content: red and white cell normal (IDEXX). [2] A significance level of $P < 0.05$ was used for all statistical tests, where significant differences were observed post-hoc tests were used (Student Newman-Keuls test) to detect differences between treatments, values in the same row without a common superscript are significantly different (a indicates the highest value; $P < 0.05$). [4] SE less than 0.01 are reported as "0.00". [5] Haematocrit analysed at SARDI with heparinised blood. ALP = alkaline phosphatase; ALT = alanine aminotransferase; Baso = basophil; Eosin = eosinophil; HGB = haemoglobin; Lymph = lymphocytes; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; Mono = monocytes; PCV = packed cell volume; RBC = red blood cell count; WBC = white blood cell count.

Table 3.15. Two-factor ANOVA results for the effect of diet type and feeding rate on blood biochemistry and haematology of Yellowtail Kingfish after 84 days in Trial 2. [1,2]

Diet	ANOVA[1,2]		
Feed rate (%)	Diet (A)	Feed rate (B)	A × B
Treatment	*P* value	*P* value	*P* value
Biochemistry			
Sodium (mmol L^{-1})	*P* = 0.079	*P* = 0.223	*P* = 0.729
Potassium (mmol L^{-1})	*P* = 0.687	*P* = 0.449	*P* = 0.167
Urea (mmol L^{-1})	*P* = 0.002 (D1 = D5 > D3)	*P* = 0.502	*P* = 0.667
Creatinine (mmol L^{-1})	*P* = 0.303	*P* = 0.221	*P* = 0.601
Calcium (mmol L^{-1})	*P* = 0.093	*P* = 0.364	*P* = 0.667
Protein (g L^{-1})	*P* = 0.331	*P* = 0.188	*P* = 0.650
Albumin (g L^{-1})	*P* = 0.117	*P* = 0.360	*P* = 0.683
Globulin (g L^{-1})	*P* = 0.458	*P* = 0.265	*P* = 0.732
Total Bilirubin (mmol L^{-1})	*P* = 0.873	*P* = 0.248	*P* = 0.197
ALT (IU L^{-1})	*P* = 0.100	*P* = 0.638	*P* = 0.526
ALP (IU L^{-1})	*P* = 0.750	*P* = 0.447	*P* = 0.502
Magnesium (mmol L^{-1})	*P* = 0.112	*P* = 0.542	*P* = 0.723
Cholesterol (mmol L^{-1})	*P* = 0.023 (D1 = D5 > D3)	*P* = 0.050 (S > RS)	*P* = 0.818
Triglyceride (mmol L^{-1})	*P* = 0.478	*P* = 0.011 (S < RS)	*P* = 0.304
Bile Acids (µmol L^{-1})	*P* = 0.743	*P* = 0.076	*P* = 0.567
Amylase (µmol L^{-1})	*P* = 0.101	*P* = 0.379	*P* = 0.783
GGT (IU L^{-1})	*P* = 0.944	*P* = 0.321	*P* = 0.699
Haematology			
RBC ($\times 10^{12}$)	*P* = 0.456	*P* = 0.151	*P* = 0.876
HGB (g L^{-1})	*P* = 0.941	*P* = 0.678	*P* = 0.409
PCV (L L^{-1})	*P* = 0.456	*P* = 0.617	*P* = 0.384
MCV (fl)	*P* = 0.132	*P* = 0.222	*P* = 0.889
MCH (pg)	*P* = 0.104	*P* = 0.250	*P* = 0.162
MCHC (g L^{-1})	*P* = 0.064	*P* = 0.923	*P* = 0.475
WBC ($\times 10^{9}$)	*P* = 0.438	*P* = 0.078	*P* = 0.394
Granulocytes (%)	*P* = 0.333	*P* = 0.207	*P* = 0.456
Lymph (%)	*P* = 0.387	*P* = 0.785	*P* = 0.889
Mono (%)	*P* = 0.830	*P* = 0.268	*P* = 0.503
Eosin (%)	-	-	-
Baso (%)	-	-	-
Platelets ($\times 10^{9}$)	*P* = 0.633	*P* = 1.000	*P* = 0.123
Haematocrit count (L L^{-1})	*P* = 0.794	*P* = 0.345	*P* = 0.568

[1] A significance level of $P < 0.05$ was used for all statistical tests: RS denotes 80% sub-satiation feed rate; S denotes satiation feed rate.
[2] Refer to Table 3.11 for data.
[3] Haematocrit analysed at SARDI with heparinised blood.
ALP = alkaline phosphatase; ALT = alanine aminotransferase; Baso = basophil; Eosin = eosinophil; HGB = haemoglobin; Lymph = lymphocytes; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume; Mono = monocytes; PCV = packed cell volume; RBC = red blood cell count; WBC = white blood cell count.

Discussion

Our goal in Trial 2 was to build on results from Trial 1 (Chapter 2), to further improve the sustainability and economic viability of Yellowtail Kingfish production. Throughout the trial, Yellowtail Kingfish fed actively on all diets, except for fish fed the Hayashikane Hiramasa diet (Diet 5, Treatments 7 and 8), which did not appear to feed as actively. Growth rates in tanks at the SAASC in Trial 2 were comparable or better to those observed at Clean Seas commercial sea cage facilities (personal communication, Dr T. D'Antignana; Clean Seas) and also to the growth rates observed in fish in the tank-based study in Trial 1 (Chapter 2, Table 2.3; Figure 2.4). Gill and skin fluke burdens throughout the trial were negligible and survival of Yellowtail Kingfish fed all of the diets was positive. Only one fish died during this trial; this fish was visually examined and did not show any signs of disease or skin loss due to irritation.

Yellowtail Kingfish grown in the recirculating tank system at the SAASC returned a better FCR (1.6 - 1.7) compared to fish from the same cohort grown in the Clean Seas sea cages during the same period (personal communication, Dr C. Foster; Clean Seas). Differences observed between FCR may not be attributed to dietary or feeding strategy alterations alone. FCR reductions in the sea cage cultured fish may have potentially been due to oxygen limitations and also higher skin and gill fluke burdens. Bowyer et al. (2013c) reported reduced growth and feed efficiency in Yellowtail Kingfish at sub-optimal dissolved oxygen levels. In addition, moderate skin and gill fluke burdens may also impact on feed utilisation and growth of large Yellowtail Kingfish cultured in sea cages (personal communication, Dr T. D'Antignana; Clean Seas). This would suggest, pending cost benefit analysis, that it may be worthwhile to investigate the application of oxygenation to commercial sea cages to improve dissolved oxygen levels, growth, feed utilisation and FCR, and resultant profits.

Yellowtail Kingfish fed the newly formulated Ridley Clean Seas 2014 Pelagica diet (Diet 1; Treatments 1 and 2) exhibited superior growth rates and feed utilisation compared to fish fed the Japanese Yellowtail production diet (Hayashikane Hiramasa diet; Diet 5, Treatments 7 and 8). The reason for the reduced growth rate for fish fed the Hayashikane Hiramasa diet is unknown, as the measured proximate compositions of diets, with the exception of ash, were similar as measured by AsureQuality (Table 3.1) and diets were stored under identical conditions prior to feeding. However, the moisture content of the Hayashikane Hiramasa diet was found to vary at different times; AsureQuality Laboratories 8.4% and SARDI 5.0%. This may reflect a varying proximate composition between pellets in this batch of feed. Furthermore, the pellet retention/stability of Hayashikane Hiramasa diet was low (32.6%), compared to the Ridley Clean Seas 2014 Pelagica (89.4%), which may have compromised feed acceptance and growth. The source of dietary ingredients may have also influenced results. For example, the ash content in Hayashikane Hiramasa diet was ~ 30% higher than in Diet 1, and may be indicative of the use of a poor quality high ash FM. However, without the actual ingredient formulation of Hayashikane Hiramasa diet it is difficult to draw further conclusions.

With regard to FO replacement and essential LC n-3 PUFA levels, there were large differences observed between Diet 1 and Diet 5 (Table 3.1). As previously mentioned, the ingredient composition of the Hayashikane Hiramasa diet is confidential but the measured LC n-3 PUFA levels indicate a high ∑LC n-3 PUFA level (5.3 g $100g^{-1}$ diet) indicative of the incorporation of high level of marine ingredients, particularly FM and FO. The high levels of LC n-3 PUFA would suggest it is most likely 100% FO was used in the Hayashikane Hiramasa diet. In contrast, the ∑LC n-3 PUFA level in the Ridley Clean Seas 2014 Pelagica diet (2.9 g $100g^{-1}$ diet) were approximately half that measured in the Hayashikane Hiramasa diet. Both diets contained the same levels of crude lipid (Table 3.1). As large Yellowtail Kingfish grew better on Diet 1 than Diet 5 in Trial 2, it would suggest that ∑LC n-3 PUFA levels in Diet 1 were not limiting (> 2 g 100 g $^{-1}$ diet). There may be no additional benefit of

using higher levels of FO in diets for large Yellowtail Kingfish. This result is promising as it will reduce the reliance of marine ingredients in Yellowtail Kingfish production diets. However, caution should be exercised when interpreting this conclusion due to the above-mentioned potential nutritional problems of the Hayashikane Hiramasa diet.

Fish meal replacement was also successful in this trial indicating the potential to further increase the sustainable production of Yellowtail Kingfish and increase market place acceptance. Yellowtail Kingfish fed Diet 2, containing 33% less FM than the newly formulated Ridley Clean Seas 2014 Pelagica diet (Diet 1), performed as well as fish fed Diet 1. It is worth noting that Diet 2 in Trial 2 was formulated to contain LC n-3 PUFA levels of 2.9 g 100 g^{-1}, which met the essential LC n-3 PUFA requirements for Japanese Yellowtail (Deshimaru et al., 1982; Stone and Bellgrove, 2013), whereas, the corresponding low FM diet (Diet 6) in Trial 1 contained deficient LC n-3 PUFA levels of 1.4 g 100 g^{-1} (Table 2.1). This highlights the importance of providing essential nutrients to Yellowtail Kingfish production diets. Further research in this important aspect of Yellowtail Kingfish nutrition and physiology is required to make further improvements in FM and FO substitution, sustainability and profits.

Protein is an expensive dietary component, and the first limiting factor for fish growth (Philips, 1972). The second growth limiting factor is dietary energy. Yellowtail Kingfish have been reported to have low levels and activities of α-amylase and a limited ability to digest and utilise dietary carbohydrates, and the energy requirements should ideally be satisfied by lipids (Bowyer et al., 2013a). Prior to Trial 2, there was limited information available on the energy requirements of large (> 1.5 kg) Yellowtail Kingfish (Stone and Bellgrove, 2013). Previous research conducted by Booth et al. (2010), reported the energy requirements of smaller (<1 kg) Yellowtail Kingfish decreased as body weight increased. The energy requirement of 50 g fish was 623 kJ DE Kg $BW^{-1}d^{-1}$ and decreased to 226 kJ DE Kg BW^{-1} d^{-1} for 2 kg fish (Booth et al., 2010). If dietary lipids are insufficient to supply energy for metabolism, animals deaminate protein for energy, rather than protein deposition and growth (Philips, 1972). This scenario should be avoided as supplying dietary protein is more expensive than dietary lipids. It was hypothesised by Clean Seas that a major area for dietary improvement was to increase the dietary lipid level, and subsequent energy level to achieve a “protein sparing effect.” Sparing protein by increased dietary energy has been successful in other culture freshwater and marine species including silver perch (*Bidyanus bidyanus*; Stone et al., 2003) and blunt snout bream (*Megalobrama amblycephala*; Li et al., 2012). In Trial 2, fish fed Diet 3 to apparent satiation (Ridley, high energy diet; Table 3.1) out-performed the growth of fish on most diets tested. Diet 3 contained ~7% FO + ~19% PO, which resulted in a crude lipid and protein level of 28% and 42%, respectively, and a gross energy level of 20.2 MJ kg^{-1}. In contrast, Diet 1 contained 24% crude lipid, 44% crude protein and a gross energy level of 19.0 MJ kg^{-1} (Table 3.1). It should also be noted that dietary lipid levels were increased by increasing the dietary inclusion of poultry oil, and not FO. However, the ∑LC n-3 PUFA was maintained at levels of > 2 g 100 g^{-1} diet. Based on the growth performance results from Trial 2, Yellowtail Kingfish may be fed a lower protein, but higher lipid, diet and achieve numerically or significantly superior growth. This indicates that increasing the dietary lipid level in Yellowtail Kingfish may result in a “protein sparing effect”. This information is vital to aid in formulating diets to improve growth performance and feed efficiency of Yellowtail Kingfish at the Clean Seas operations. However, this was not supported by protein and energy deposition, as all treatments exhibited statistically similar nutrient retention. Further research to optimise dietary lipid levels and energy (lipid) content, and to understand the effects on protein and energy utilisation may further benefit and improve Yellowtail Kingfish production.

When considering the results for restricted feeding practices, the FCR of fish fed to 80% sub-satiation was superior to fish fed to apparent satiation. However, the growth performance of fish fed to sub-satiation was significantly lower (Table 3.7 and 3.8). It should

be noted that fish fed to sub-satiation were underfed during the first period of the trial and overfed during the second period. However, on average, fish were fed close to the targeted 80% level over the entire duration of Trial 2. In order to achieve 80% sub-satiation using the current restrictive feeding method it may be desirable to use a multiplier of ~75% of Saturday's apparent satiation feeding level in future trials. In Trial 1, growth performance parameters for Yellowtail Kingfish were not different between feed rate treatments, but fish fed to 80% sub-satiation had significantly lower feed consumption rates and superior FCR, and protein and energy deposition compared to fish fed to apparent satiation (Tables 2.3 and 2.4). However, the 80% sub-satiation feed rate was determined differently between Trial 1 and 2 and it may be possible that the reduced feeding time of 4 min $tank^{-1}$ in Trial 2, as opposed to 8 min $tank^{-1}$ in Trial 1, may have compromised the feed intake and growth performance of Yellowtail Kingfish. Based on results from Trials 1 and 2, there appears to be a fine line between feeding fish to sub-satiation to improve feeding efficiency, and apparent satiation in order to not compromise growth rates. It may be beneficial in further studies to investigate feeding fish a range of different levels of sub-satiation, for example 80%, 85%, 90% and 95%. Additionally, as both Trials 1 and 2 were run during summer, different results would be expected during the cooler winter months. This requires further research.

Conclusions and Recommendations

In conclusion, this study adds to the nutritional knowledge of Yellowtail Kingfish. Based on results that demonstrated inferior growth and feed utilisation of Yellowtail Kingfish fed the Hayashikane Hiramasa Japanese Yellowtail production diet, based on the conditions tested in this trial we recommend that this diet is not fed to Yellowtail Kingfish. We further suggest that it may be beneficial to increase dietary lipid and energy levels in production diets for large (> 1.5 kg) Yellowtail Kingfish to achieve a "protein sparing effect" and increase profits. However, this also needs to be validated under commercial pilot scale conditions prior to adoption as a farming practices. Although care must be taken when comparing results between trials, based on results from Trial 2 and Trial 1 (Chapter 2), there appears to be a fine line between feeding fish to sub-satiation to improve feeding efficiency, and underfeeding fish, which in turn compromises growth. Further research is required to optimise these results. It is also worthwhile to investigate the potential for the application of oxygenation to commercial sea cages to improve dissolved oxygen levels, growth, feed utilisation and resultant FCR and profits. Fish meal and FO replacement also appeared to be successful in this trial, indicating the potential to further increase the sustainable production of Yellowtail Kingfish.

Chapter 4.Trial 3: Evaluation of different feeding strategies for the production of Yellowtail Kingfish at winter water temperatures

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Abstract

Farmed Australian Yellowtail Kingfish are primarily produced in sea cages in South Australia. During their production, fish are exposed to water temperatures from 24 °C in summer, to below 12 °C during winter. Recent research has primarily focused on optimising diet formulations and feeding regimes for Yellowtail Kingfish during summer. However, further research to optimise on-farm feeding practices during winter water temperatures is needed. In this 84 day trial, the growth performance, feed efficiency and physiology of Yellowtail Kingfish (1.44 kg) fed the newly formulated Ridley Clean Seas 2014 Pelagica diet (Diet 1 from Trial 2; referred to as Formulated diet) at seven feed rates from 0.1% body weight (BW) one day week^{-1} to apparent satiation six days week^{-1} at winter water temperatures were investigated. An additional dietary treatment, comprised of feeding thawed and diced Australian Sardines (*Sardinops sagax*) to apparent satiation every second day was also tested. The rations were fed at one period between 0930 h and 1100 h. With regard to fish fed the Formulated diet, Yellowtail Kingfish fed to apparent satiation six days week^{-1} exhibited significantly higher growth rates and numerically superior FCR, compared to fish fed the same diet at lower feed rates. The maintenance requirement for Yellowtail Kingfish (1.44 kg) fed the Formulated diet is 0.2047% BW d^{-1}(gross energy, 56.3 kJ fish^{-1}d^{-1}). In terms of feed management, in order to provide slightly above this rate to ensure positive growth, fish may be fed to apparent satiation once a day, two days week^{-1}. We recommend fish are not fed below this rate under commercial conditions during winter as this resulted in weight loss, which may manifest into nutrient deficiency symptoms, and also health problems. With regard to Yellowtail Kingfish fed Sardines, the growth and feed conversion ratio of Yellowtail Kingfish fed Sardines once a day, every second day and the Formulated diet to apparent satiation once a day, six days week^{-1} were similar. However, the fish in-fish out ratio (tonnes of wild fish it takes to produce one tonne of farmed fish), a measure of the sustainable utilisation of marine resources, for Yellowtail Kingfish fed Sardines (7.8) was 24% higher than animals fed the Formulated diet (6.3). Feeding Sardines may impact consumer perception and the marketability of Yellowtail Kingfish. Based on the results from this trial, we recommend that fish are fed the Formulated diet once a day to apparent satiation six days week^{-1} during winter months. However, further research focused on compensatory weight gain post-winter and/or essential nutrient requirements of Yellowtail Kingfish at winter water temperatures may allow fish to be fed a maintenance ration throughout winter. Improving the knowledge in these areas would likely further benefit and improve Yellowtail Kingfish production.

Introduction

In Australia, Yellowtail Kingfish (*Seriola lalandi*) are predominately cultured in South Australia, with over 90% of production from Clean Seas (Arno Bay, South Australia). Water temperature is an important environmental parameter that influences almost every aspect of Yellowtail Kingfish production (Miegel et al., 2010; Bowyer et al., 2013a; b; Bansemer et al., 2015). While the optimal water temperature for Yellowtail Kingfish growth was calculated to be 22.8 °C, cultured Yellowtail Kingfish are exposed to fluctuating water temperatures that range from 10 °C in winter to 24 °C in summer (Pirozzi and Booth, 2009; Miegel et al., 2010). Previous studies aimed to improve diet and feeding regimes have typically been investigated at water temperature ≥18 °C (Bowyer et al., 2013a; b; Bansemer et al., 2015; Chapter 2 and Chapter 3 from this project). At winter water temperatures (10 °C - 14 °C) however, Yellowtail Kingfish growth and metabolic rate is impaired, feed intake reduced and gut transit time prolonged, and the nutritional requirements and digestive physiology differ to fish at summer water temperatures (~22 °C) (Pirozzi and Booth, 2009; Miegel et al., 2010; Bowyer et al., 2013a; b; Bansemer et al., 2015). For example, Bowyer et al. (2013a) reported the feed intake for Yellowtail Kingfish (22.6 g) at 22 °C was two times greater than at 18 °C, and a 48% higher growth rate was observed in fish at 22 °C than at 18 °C, which are both further impaired as water temperature reduces (Pirozzi and Booth, 2009).

The sustainable and economically viable production of Yellowtail Kingfish relies on cost effective diets and feeding practices. In the early stages of commercial Yellowtail Kingfish production in Australia, summer feeding practices involved feeding fish twice daily to apparent satiation (Miegel et al., 2010). Using feeding practices developed for summer water temperatures during winter will result in inefficient feed utilisation, as the growth, feed intake, gut transit time, nutrient digestibility and numerous other aspects of Yellowtail Kingfish physiology are influenced by water temperature (Jobling, 1994). Recently, there is increased interest to optimise feeding rates and frequencies for aquaculture species, to improve the economical production efficiency (García-Mesa et al., 2014). This has the added benefit of reducing feed wastes and effluent, improving growth rates and food conversion ratios, and may also reduce fish-size variation at harvest (Güroy et al., 2006). Feed rates and frequencies have been optimised for a closely related *Seriola* sp., the Japanese Yellowtail (*Seriola quinqueradiata*) to maximise economic return (Nakada, 2002). Cultured Japanese Yellowtail are currently fed formulated diets on alternate days, or two to three times a week, depending on the water temperature (Nakada, 2002). Based on these results, there may also be considerable scope to improve on-farm feeding practices for Yellowtail Kingfish in Australia at winter water temperatures.

In addition to optimising feed rates and frequencies for Yellowtail Kingfish using formulated diets, there was also interest to assess Yellowtail Kingfish growth performance and feed utilisation by feeding Sardines throughout winter. Japanese Yellowtail producers currently feed fresh fish during the winter months (Nakada, 2008). While Sardines may closer resemble the natural diet of Yellowtail Kingfish, and as a result may provide a superior nutritional profile than commercial formulated diets at times, the nutritional composition of Sardines is dependent on source and season (Nakada, 2008). In contrast, commercial formulated diets are optimised to sustainably reduce the use of marine ingredients (Hardy and Tacon, 2002). Despite these issues, utilising Sardines as feed for Yellowtail Kingfish may improve growth and feed efficiency at winter water temperatures, which may also improve the sustainable use of marine ingredients (Tacon and Metian, 2008).

Aim

The aim of the current trial was to better understand winter feed management practices to improve the sustainable production of Yellowtail Kingfish during winter. More specifically, we aimed to:

1. Evaluate the growth performance and feed efficiency of Yellowtail Kingfish (~1.5 kg) fed a commercial production diet (Ridley Clean Seas 2014 Pelagica ["Formulated diet", Diet 1 from Trial 2]) at different feed rates and frequencies during low winter water temperatures.

2. Compare the growth performance and feed utilisation of Yellowtail Kingfish (~1.5 kg) fed a commercial production diet (Ridley Clean Seas 2014 Pelagica) to fish fed a Sardine diet during low winter water temperatures.

Materials and Method

Experimental treatments and feeding techniques
Two diets (biochemical composition Table 4.1) and eight treatment combinations were investigated in Trial 3.

A commercial diet formulation (Ridley Clean Seas 2014 Pelagica diet [30% FM; 9% FO]; referred to as the 'Formulated diet'), was manufactured according to the agreed formulation using a least cost ingredient profile, by Ridley Aquafeeds using cooking extrusion technology. The actual ingredient composition of the diet is confidential, and is not provided in this report. Fish fed the Formulated diet were fed one of seven feed rates based on % body weight or to apparent satiation, with the amount fed adjusted based on monthly weight checks (below). Freshly frozen Sardines (*S. sagax*) were supplied by Sardine Temptations Pty Ltd (Port Lincoln, South Australia). Sardines were thawed and diced prior to feeding. All feed rations were fed at one period between 0930 h and 1100 h, and the eight treatments combinations were:

- Treatment 1: Formulated diet fed to apparent satiation six days week^{-1}.

- Treatment 2: Formulated diet fed to apparent satiation two days week^{-1} (Monday and Thursday).

- Treatment 3: Formulated diet fed to apparent satiation one day week^{-1} (Monday).

- Treatment 4: Formulated diet fed at 0.10% body weight (BW) one day week^{-1} (Monday).

- Treatment 5: Formulated diet fed at 0.65% BW two days week^{-1} (Monday and Thursday).

- Treatment 6: Formulated diet fed at 0.35% BW two days week^{-1} (Monday and Thursday).

- Treatment 7: Formulated diet fed at 0.12% BW six days week^{-1} (Monday - Saturday).

- Treatment 8: Sardines fed to apparent satiation every second day.

Table 4.1. The biochemical composition of the Ridley Clean Seas 2014 Pelagica diet ("Formulated") and Sardines used in Trial 3[1].

	As fed		Dry basis	
Item	Ridley Clean Seas 2014 Pelagica	Sardines	Ridley Clean Seas 2014 Pelagica	Sardines
Analysed proximate composition (g kg^{-1})				
Moisture	68	721	0	0
Crude protein	451	189	484	677
Crude lipid	240	41	258	147
Ash	89	45	95	161
Carbohydrate[2]	152	4	163	14
Gross energy (MJ kg^{-1})	19.10	4.73	20.49	16.95
Crude protein:energy (g MJ^{-1})	23.6	40.0	25.3	143.2
Cholesterol	2.3	1.1	2.5	3.9
Analysed minerals (mg kg^{-1})				
Calcium	24000	6700	25751	24014
Choline	na	na	na	na
Copper	8.7	6.3	9.3	22.6
Iodide (Potassium Iodide) (µg kg^{-1})	1.8	0.69	1.9	2.5
Iron	290	34	311	122
Magnesium	1900	550	2039	1971
Manganese	na	na	na	na
Phosphorus	16000	5900	17167	21147
Potassium	4900	3600	5258	12903
Selenium	2.4	1.5	2.6	5.4
Zinc	150	31	161	111
Analysed amino acids (g kg^{-1})				
Alanine	24.40	10.68	26	38
Arginine	26.61	10.60	29	38
Aspartic acid	32.76	14.58	35	52
Glutamic acid	69.08	25.10	74	90
Glycine	26.46	10.78	28	39
Histidine	13.08	9.34	14	33
Isoleucine	16.94	8.27	18	30
L cystine	na	na	na	na
Leucine	33.57	14.42	36	52
Lysine	23.87	15.52	26	56
Methionine	10.21	4.31	11	15
Phenylalanine	19.14	7.60	21	27
Proline	28.08	7.34	30	26
Serine	17.65	6.91	19	25
Taurine	na	na	na	na
Threonine	17.19	8.16	18	29
Tryptophan	na	na	na	na
Tyrosine	14.15	6.00	15	22
Valine	21.95	9.25	24	33
Total amino acids	395	199	424	605

[1] The Ridley Cleanseas 2014 Pelagica diet was manufactured and supplied by Ridley Aquafeeds (Narangba, QLD, Australia); Sardines provided by Sardine Temptations Pty Ltd (Port Lincoln, South Australia).
[2] Carbohydrate = 1000 - (moisture + lipid + protein + ash).

Table 4.1. Continued.[1]

Item	As fed		Dry basis	
	Ridley Clean Seas 2014 Pelagica	Sardines	Ridley Clean Seas 2014 Pelagica	Sardines
Analysed fatty acids (mg 100 g^{-1})				
Butyric C4:0	<10	<10	<10	<10
Caproic C6:0	<10	<10	<10	<10
Caprylic C8:0	<10	<10	<10	<10
Capric C10:0	<10	<10	<10	<10
Lauric C12:0	21	<10	23	<10
Trisdecanoic C13:0	<10	<10	<10	<10
Myristic C14:0	950	250	1019	896
Pentadecanoic C15:0	88	56	94	201
Palmitic C16:0	4980	850	5343	3047
Margaric C17:0	46	52	49	186
Stearic C18:0	1310	230	1406	824
Arachidic C20:0	68	17	73	61
Heneicosanoic C21:0	<10	<10	<10	<10
Docosanoic C22:0	31	13	33	47
Tetracosanoic C24:0	26	<10	28	<10
Decenoic C10:1	<10	<10	<10	<10
Myristoleic C14:1	56	11	60	39
Pentadecenoic C15:1	13	<10	14	<10
Palmitoleic C16:1	1490	130	1599	466
Heptadecenoic C17:1	50	<10	54	<10
Octadecenoic C18:1n-6	20	<10	21	<10
Octadecenoic C18:1n-7	640	84	687	301
Oleic C18:1n-9	6430	220	6899	789
Eicosenoic (Gondoic) C20:1n-9	220	12	236	43
Eicosenoic C20:1n-11,13	30	<10	32	<10
Eicosenoic C20:1 (total)	250	18	268	65
Erucic C22:1n-9	30	<10	32	<10
Docosenoic C22:1n-11,13	<10	<10	<10	<10
Tetracosenoic C24:1	47	32	50	115
Linoleic C18:2n-6	2220	100	2382	358
Octadecadienoic C18:2 Conjugated 9c 11t	<10	<10	<10	<10
Alpha Linolenic C18:3n-3	350	80	376	287
Steridonic C18:4n-3	200	110	215	394
Octadectrenoic acid C18:3n-4	26	<10	28	<10
Gamma Linolenic C18:3n-6 (GLA)	42	<10	45	<10
Eicosadienoic C20:2n-6	31	14	33	50
Dihomo-gamma-linoleic C20:3n-6	34	<10	36	<10
Arachidonic C20:4n-6	210	47	225	168
Docosatetraenoic C22:4n-6	54	37	58	133
Docosapentaenoic C22:5n-6	59	11	63	39
Eicosatrienoic C20:3n-3	13	<10	14	<10
Eicosatetraenoic C20:4n-3	180	28	193	100
Eicosapentanaeoic C20:5n-3 (EPA)	1480	360	1588	1290
Heneicosapentaenoic acid C21:5n-3	<10	<10	<10	<10
Docosapentaenoic C22:5n-3 (DPA)	200	45	215	161
Docosahexaenoic C22:6n-3 (DHA)	1170	1020	1255	3656
Saturated fat (g 100 g^{-1})	7.6	1.5	8.2	5.4
Mono unsaturated fat (g 100 g^{-1})	9.2	0.6	9.9	2.2
Poly unsaturated fat (g 100 g^{-1})	6.7	1.8	7.2	6.5
Trans fat (g 100 g^{-1})	0.4	0.1	0.4	0.4
Omega 3 total	3590	1660	3852	5950
Omega 6 total	2630	220	2822	789
Omega 9 total	6730	270	7221	968
EPA + DPA + DHA	2850	1425	3058	5108
n-3 FA:n-6 FA	1.37	7.55	1.5	27.1

[1] The Ridley Cleanseas 2014 Pelagica diet was manufactured and supplied by Ridley Aquafeeds (Narangba, QLD, Australia); Sardines provided by Sardine Temptations Pty Ltd (Port Lincoln, South Australia).

Experimental fish
Experimental work for Trial 3 was conducted in the pool-farm facility at the South Australian Research and Development Institute's, South Australian Aquatic Science Centre at West Beach, South Australia. Yellowtail Kingfish (mean ± standard deviation; initial weight 1.44 ± 0.13 kg; initial fork length 461.6 ± 15.4 mm; n = 504) were obtained from Clean Seas (Spencer gulf, South Australia). Upon arrival at the SAASC (25/5/15), Yellowtail Kingfish were transferred to 5000 L tanks supplied with partial flow-through/recirculating (100% system water exchange d^{-1}), sand filtered, UV treated, aerated and oxygenated sea water at ambient temperature and held for ~4 weeks and fed the standard Ridley Clean Seas 2014 Pelagica diet (crude protein, 44%; crude lipid, 24%; gross energy, 19.30 MJ kg^{-1}).

Skin and gill fluke treatment
Upon arrival at the SAASC, the fish were inspected, and were observed to have a low burden of skin flukes (*Benedenia seriola*) and gill flukes (*Zeuxapta seriola*). Treatment was deemed necessary. Treatments were prescribed by Dr Matt Landos (Future Fisheries Veterinary Service Pty Ltd). Prior to the commencement of the trial, fish were treated for epitheliocystis with oxytetracycline in-feed (top coated on extruded feed) at 75mg kg^{-1} daily for five days. In addition, based on the successful treatment method of both skin and gill flukes used in Trial 1 and 2, fish were exposed to two treatments (18/06/15 and 14/07/15) of formalin (250 ppm for 60 min). The first treatment was prior to the commencement of the trial, and the second treatment was between stocking and the first weight check.

Experimental stocking
At the commencement of Trial 3 (25/6/15), Yellowtail Kingfish were removed from their tank, anaesthetised using AQUI-S® (AQUI-S® New Zealand Ltd., Lower Hutt, New Zealand) at a concentration of 14 mg L^{-1} of seawater. Twenty one fish were measured, weighed and stocked, using systematic interspersion, into one of the three replicate 5000 L tanks treatment $combination^{-1}$ (n = 24 tanks). Tanks were supplied with partial flow-through/recirculating (100% system water exchange d^{-1}), sand filtered, UV treated sea water at ambient temperature. All tanks were supplied with aeration and oxygenation throughout the trial. All treatment diets were fed to fish at 0830 h. Fish fed to apparent satiation, were fed for 4 min $tank^{-1}$. Fish fed restricted rations were fed until the allocation was consumed.

Weight checks throughout the trial
At day 28 (23/07/2015) and day 56 (20/08/2015), all fish were anaesthetised using AQUI-S® at a concentration of 14 mg L^{-1} of seawater. Yellowtail Kingfish were measured, weighed and visually inspected for skin and gill flukes, before fish were returned back to their respective tanks. These checks revealed that skin and gill flukes had been eradicated after the first formalin treatment.

Water quality analyses

Water quality parameters were measured at 1130 h daily, and maintained at appropriate levels for acceptable growth of Yellowtail Kingfish throughout the study (Table 4.2; Figure 4.1, 4.2 and 4.3). Water temperature was measured using an alcohol thermometer. Dissolved oxygen (mg L^{-1} and % saturation) was measured using a dissolved oxygen meter (OxyGuard International A/S, Birkerød, Denmark). The pH was measured daily using a meter (Oakton pHtestr 20; Oakton Instruments, Vernon Hills, IL, USA). Salinity (g L^{-1}) was measured weekly using a portable salinity refractometer (model RF20, Extech Instruments, Nashua, NH, USA).

Table 4.2. Summary of water quality for Trial 3.[2]

Item	Temperature (°C)	Dissolved oxygen (mg L^{-1})	Dissolved oxygen (% saturation)	pH	Salinity (mg L^{-1})
Mean[1]	12.8±0.8	8.6±0.3	102.2±3.1	8.12±0.05	36.9±1.3
Range	11.5-16.0	7.5-10.8	94.0-128.0	8.01-9.08	34.0-38.0

[1] Values means ± standard deviation.

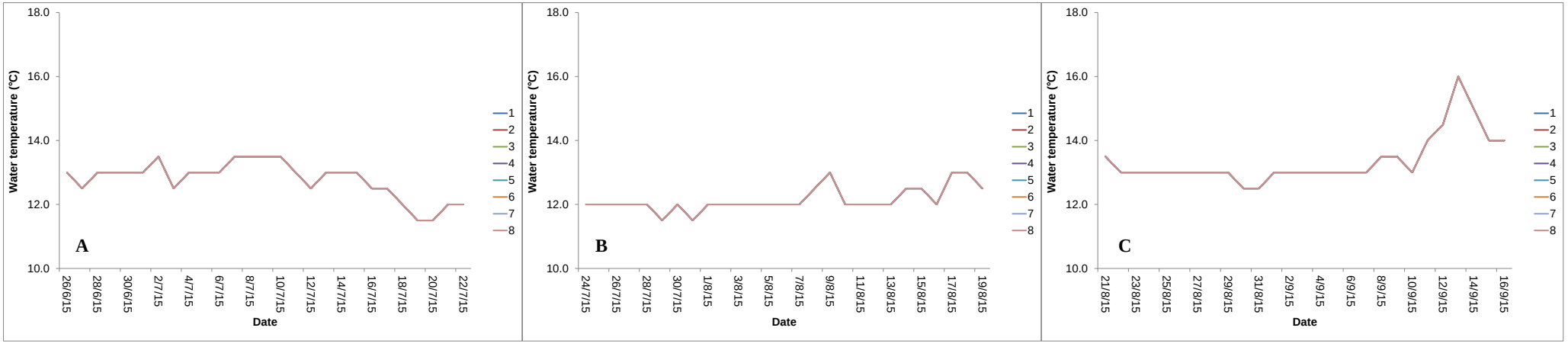

Figure 4.1. Daily average treatment water temperatures in Trial 3: A) from 26/06/15-22/07/15 (Day 1 to 27); B) 24/07/15-19/08/15 (Day 29 to 55); C) 21/08/15-16/09/15 (Day 57 to 83).[1,2]

[1] Values are expressed as mean for each day (n = 3).

[2] 1: Formulated diet (Ridley Clean Seas 2014 Pelagica) fed to apparent satiation six days week^{-1}.
2: Formulated diet fed to apparent satiation two days week^{-1} (Monday and Thursday).
3: Formulated diet fed to apparent satiation one day week^{-1} (Monday).
4: Formulated diet fed at 0.1% body weight (BW) one day week^{-1} (Monday).
5: Formulated diet fed at 0.65% BW two days week^{-1} (Monday and Thursday).
6: Formulated diet fed at 0.35% BW two days week^{-1} (Monday and Thursday).
7: Formulated diet fed at 0.12% BW six days week^{-1} (Monday - Saturday).
8: Sardines (thawed and diced) fed to apparent satiation every second day.

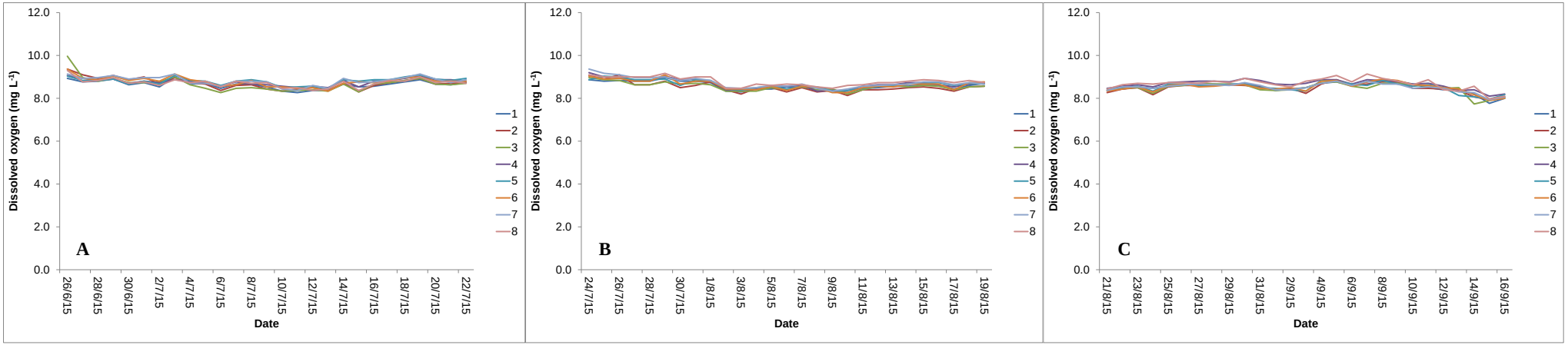

Figure 4.2. Daily average treatment dissolved oxygen levels (mg L^{-1}) in Trial 3: A) from 26/06/15-22/07/15 (Day 1 to 27); B) 24/07/15-19/08/15 (Day 29 to 55); C) 21/08/15-16/09/15 (Day 57 to 83).[1,2]

[1] Values are expressed as mean for each day (n = 3).

[2] 1: Formulated diet (Ridley Clean Seas 2014 Pelagica) fed to apparent satiation six days $week^{-1}$.
2: Formulated diet fed to apparent satiation two days $week^{-1}$ (Monday and Thursday).
3: Formulated diet fed to apparent satiation one day $week^{-1}$ (Monday).
4: Formulated diet fed at 0.1% body weight (BW) one day $week^{-1}$ (Monday).
5: Formulated diet fed at 0.65% BW two days $week^{-1}$ (Monday and Thursday).
6: Formulated diet fed at 0.35% BW two days $week^{-1}$ (Monday and Thursday).
7: Formulated diet fed at 0.12% BW six days $week^{-1}$ (Monday - Saturday).
8: Sardines (thawed and diced) fed to apparent satiation every second day.

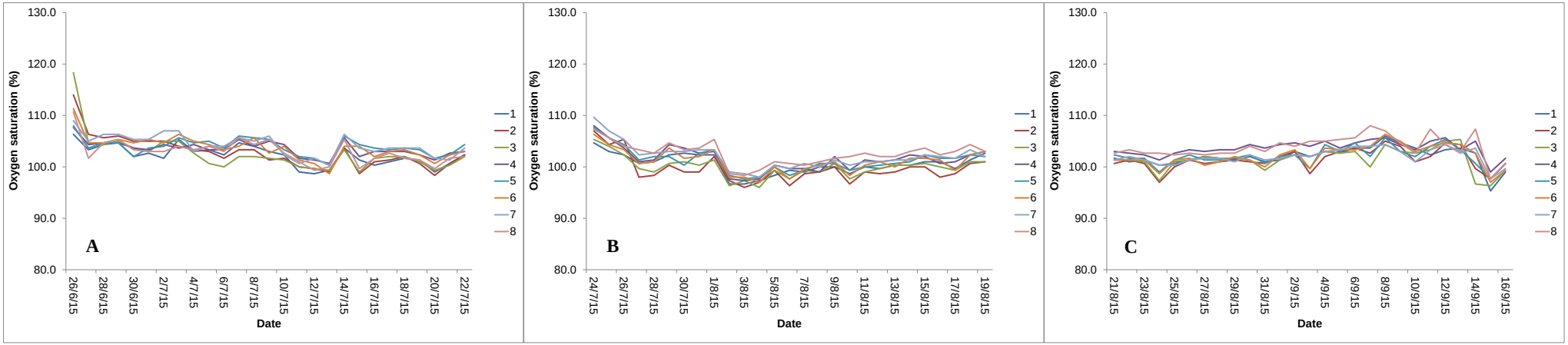

Figure 4.3. Daily average treatment dissolved oxygen levels (% saturation) in Trial 3: A) from 26/06/15-22/07/15 (Day 1 to 27); B) 24/07/15-19/08/15 (Day 29 to 55); C) 21/08/15-16/09/15 (Day 57 to 83).[1,2]

[1] Values are expressed as mean for each day (n = 3).

[2] 1: Formulated diet (Ridley Clean Seas 2014 Pelagica) fed to apparent satiation six days week^{-1}.
2: Formulated diet fed to apparent satiation two days week^{-1} (Monday and Thursday).
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6: Formulated diet fed at 0.35% BW two days week^{-1} (Monday and Thursday).
7: Formulated diet fed at 0.12% BW six days week^{-1} (Monday - Saturday).
8: Sardines (thawed and diced) fed to apparent satiation every second day.

Final harvest sampling
At day 84 (17/09/15) fish were anaesthetised using AQUI-S® (14 mg L^{-1} of seawater). All animals were visually inspected for skin and gill flukes, and measured and weighed. Four fish from each tank were collected and stored at -20°C for subsequent whole body composition analyses. Nine separate fish from Treatments 1, 4, 7 and 8 (n = 3 fish $tank^{-1}$) were sampled for blood haematocrit, and visceral and liver weight in order to calculate visceral index (VSI; %) and hepatosomatic index (HSI; %). These treatments were selected as they were thought to give a representation of feed rate and frequency restrictions investigated in the current study.

Biochemical analyses
Diet, and whole initial and final fish (n = 4 $tank^{-1}$) were analysed for proximate composition (moisture, protein, lipid, ash, total carbohydrate and energy), taurine and choline, fatty acids and minerals by AsureQuality Laboratories (Auckland, New Zealand). Diets were also analysed for cholesterol and amino acids profiles.

Calculation of performance indices
All data reported for each treatment for animal performance were based on the mean of the replicate tanks. All calculations using fish weights and diets were based on wet or as fed values:

- Weight gain = final weight - initial weight
- Biomass gain (g $tank^{-1}$) = (final weight + ∑mortality weight) - (initial weight + ∑replacement weight)
- Specific growth rate (SGR, % d^{-1}) = ([ln final weight - ln initial weight] / days) × 100
- Condition factor = (fish weight [g] / fish length $[cm]^3$) × 100
- Apparent feed conversion ratio (FCR) = feed consumed / fish weight gain
- Apparent protein efficiency ratio (PER) = fish weight gain / protein consumed
- Apparent energy efficiency ratio (EER) = fish weight gain / energy consumed
- Apparent protein deposition = ([final soft body protein - initial soft body protein] / protein intake) × 100
- Apparent energy deposition = ([final soft body energy - initial soft body energy] / energy intake) × 100
- Haematocrit count = red blood cell (mm) / total blood (red blood cell and plasma; mm) 100 × 100
- Visceral index (VSI; %) = wet visceral wt × 100 / final wet fish wt
- Hepatosomatic index (HSI; %) = wet liver wt × 100 / final wet fish wt

Statistical analyses
IBM SPSS, Version 22 for Windows (IBM SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Homogeneity of variances and normality among mean values were assessed using Levene's test for equality of variance errors and the standardized residuals against the predicted mean plot, respectively. Difference between treatments for all variables

were analysed using One-factor ANOVA. When significant differences are observed, post-hoc tests will be used to detect significant differences treatment combinations (Student Newman-Keuls). In addition, Pearson's correlation coefficient was used to determine the relationship between feed intake (% body weight d^{-1}) and specific growth rate (% d^{-1}) for fish fed the Formulated diet. Weight and condition index distribution of fish were plotted, and data homogeneity and skewness were tested using Shapiro Wilk test. A significance level of $P < 0.05$ was used for all statistical tests. All values are presented as means ± standard error (SE) of the mean unless otherwise stated.

Results

General observations
Fish fed actively during the experiment, with apparent feeding activity increasing dramatically as feed rate and frequency decreased. There were no mortalities during the trial and no apparent signs of disease observed. Moreover, there were negligible gill and skin fluke burdens observed throughout the trial.

Growth performance
The initial weight and fork length of fish were not statistically different between treatments (One-factor ANOVA; $P > 0.05$; Table 4.3). While dietary treatment had no significant effect on the final fork length of fish ($P > 0.05$), other growth performance parameters significantly differed between treatments (One-factor ANOVA; $P < 0.05$; Table 4.3). These growth performance parameters are inclusive of final weight, biomass gain, SGR, length growth rate and final condition factor. In general, Yellowtail Kingfish fed the Formulated diet to apparent satiation six days week^{-1} (Treatment 1) and Sardines fed to apparent satiation every second day (Treatment 8) outperformed other dietary treatments. The next best performing treatment was fish fed the Formulated diet to apparent satiation two days week^{-1} (Treatment 2). In contrast, fish fed the Formulated diet at other feed rates were not provided with enough feed to maintain weight (Figure 4.4). Generally, the growth performance of fish fed the Formulated diet at 0.1% BW one day week^{-1} (Treatment 4) was inferior to all other treatments investigated (Table 4.3).

In addition, there was a significant positive linear correlation between feed intake (% BW d^{-1}) and SGR (% d^{-1}) for Yellowtail Kingfish fed the Formulated diet ($y = 66.051x - 0.1352$, $R^2 = 0.961$, Pearson's correlation coefficient $P < 0.001$; Figure 4.5). The x-intercept (SGR = 0% d^{-1}) was 0.2047% BW d^{-1}.

Fish weight was normally distributed for all treatments investigated ($P > 0.05$; Table A2.6.1; Appendix 2). In contrast, the condition index distribution for fish fed Treatment 1 was significantly negatively skewed ($P = 0.008$; -0.918; Table A2. 6.3). The condition index for fish fed Treatments 3, 5 and 8 were positively skewed ($P < 0.05$, 1.153-1.661; Table A2.6.3; Appendix 2). Fish fed Treatments 2, 4, 6 and 7 exhibited a normally distributed condition index ($P > 0.05$; Table A2.6.3).

Feed utilisation
Feed consumption rates of Yellowtail Kingfish were significantly affected by treatment (One-factor ANOVA; $P < 0.05$; Table 4.3). Fish fed Sardines to apparent satiation every second day had significantly higher feed consumption rates compared to animals fed the Formulated diet at any feed rate ($P < 0.05$; Table 4.3). For fish fed the Formulated diet, the feed consumption rates of fish were generally related to the treatment feed rates. For example, fish fed to apparent satiation six days week^{-1} had the highest feed consumption rate, while animals fed 0.1% BW one day week^{-1} had the lowest.

Treatment significantly influenced the apparent FCR for Yellowtail Kingfish (One-factor ANOVA; $P < 0.05$; Table 4.3). Fish fed the Formulated diet to apparent satiation two or six days week^{-1} (Treatments 2 and 1, respectively) and Sardines to apparent satiation every second day (Treatment 8) exhibited positive FCR. The FCR of fish fed Treatment 1 was significantly higher than animals fed to apparent satiation one day week^{-1} (Treatment 3) and 0.65% BW two days week^{-1} (Treatment 5; $P < 0.05$) and numerically superior to other treatments. The FCR for Yellowtail Kingfish in Treatments 3, 4, 5, 6 and 7 were negative, due to the inherent weight loss of fish fed these feeding regimes.

Whole fish proximate and energy composition
Lipid and energy content of Yellowtail Kingfish in Treatments 1, 2 and 8 were significantly higher than Treatment 7 (One-factor ANOVA; Table 4.3; $P < 0.05$). There were no significant differences in lipid and energy content between all other treatments ($P > 0.05$). Furthermore, the tissue taurine content of fish were significantly higher for fish in Treatments 1 and 4, compared to animals fed Treatment 8 (One-factor ANOVA; Table 4.3; $P < 0.05$). There were no significant differences in taurine content between other treatments ($P > 0.05$). In contrast, the tissue moisture, protein, ash and carbohydrate content of fish were not significantly influenced by treatment (One-factor ANOVA; Table 4.3; $P > 0.05$). The tissue moisture, protein, ash and carbohydrate content of fish ranged from 65.4 to 69.2%, 19.65 to 20.78%, 2.3 to 2.8% and <1.5, respectively.

Nutrient utilisation
Apparent protein deposition for Yellowtail Kingfish was significantly affected by treatment (One-factor ANOVA; $P < 0.05$; Table 4.3). The protein deposition of fish in Treatment 4 was significantly lower than other treatments investigated ($P < 0.05$), which were not significantly different from each other ($P > 0.05$). Treatment also significantly influenced apparent energy deposition for Yellowtail Kingfish (One-factor ANOVA; $P < 0.05$; Table 4.3). Apparent energy deposition was significantly higher for fish in Treatments 1, 2 and 8, and significantly lower for fish in Treatment 4 (Two-factor ANOVA; $P > 0.05$; Table 3.10). However, fish in Treatments 3 and 6 exhibited statistically similar apparent energy depositions to those in Treatments 1, 2, 5 and 7 ($P > 0.05$).

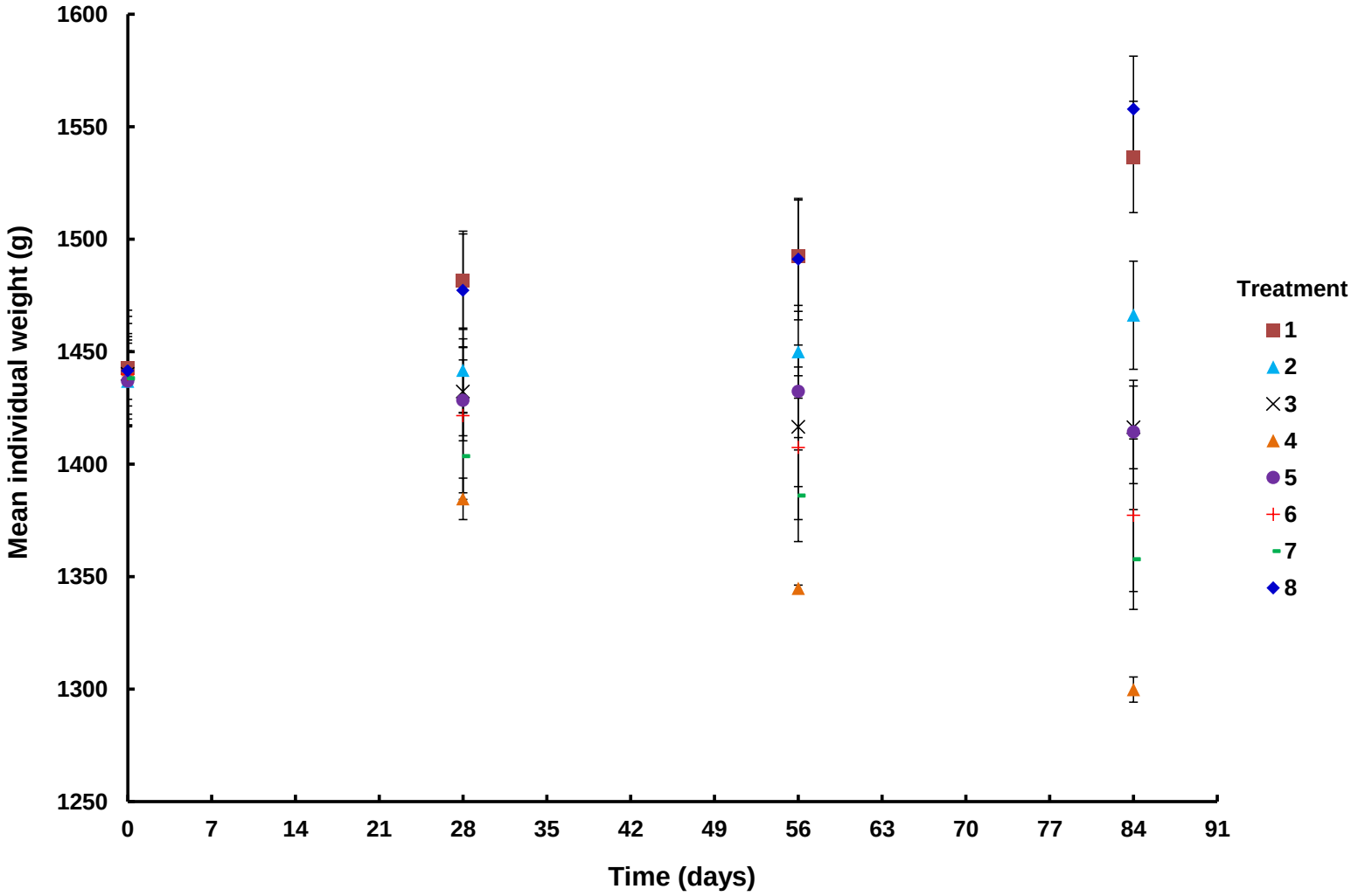

Figure 4.4. Mean individual weight of Yellowtail Kingfish in Trial 3 at 0, 28, 56, 84 days [1, 2].

[1] Values are expressed as mean ± SD, n = 3.

[2] 1: Formulated diet (Ridley Clean Seas 2014 Pelagica) fed to apparent satiation six days week^{-1}.
2: Formulated diet fed to apparent satiation two days week^{-1} (Monday and Thursday).
3: Formulated diet fed to apparent satiation one day week^{-1} (Monday).
4: Formulated diet fed at 0.1% body weight (BW) one day week^{-1} (Monday).
5: Formulated diet fed at 0.65% BW two days week^{-1} (Monday and Thursday).
6: Formulated diet fed at 0.35% BW two days week^{-1} (Monday and Thursday).
7: Formulated diet fed at 0.12% BW six days week^{-1} (Monday - Saturday).
8: Sardines (thawed and diced) fed to apparent satiation every second day.

Table 4.3. Growth performance, feed utilisation, proximate composition and nutrient retentions of Yellowtail Kingfish fed a commercial diet at varying feed rates and a Sardine diet for 84 days in Trial 3.[1]

Diet	1	1	1	1	1	1	1	2	
Treatment[2]	1	2	3	4	5	6	7	8	ANOVA[3]
Growth performance									
Initial weight (kg)	1.44±0.03	1.44±0.02	1.44±0.02	1.44±0.01	1.44±0.02	1.44±0.02	1.44±0.01	1.44±0.02	$P = 1.000$
Final weight (kg)	1.54±0.02^{ab}	1.47±0.02^{bc}	1.42±0.02^{cd}	1.30±0.01^{e}	1.41±0.02^{cd}	1.38±0.03^{cde}	1.36±0.02^{de}	1.56±0.02^{a}	$P < 0.001$
Biomass gain (kg tank^{-1})	1.97±0.18^{a}	0.62±0.10^{b}	-0.50±0.07^{c}	-2.99±0.18^{e}	-0.48±0.13^{c}	-1.32±0.24^{d}	-1.69±0.21^{d}	2.44±0.07^{a}	$P < 0.001$
SGR (% d^{-1})	0.08±0.01^{a}	0.02±0.00^{b}	-0.02±0.00^{c}	-0.12±0.01^{e}	-0.02±0.01^{c}	-0.05±0.01^{d}	-0.07±0.01^{d}	0.09±0.00^{a}	$P < 0.001$
Initial fork length (mm)	463.8±0.5	462.7±2.6	461.3±2.6	460.7±1.3	458.3±2.0	463.4±2.2	463.0±1.3	459.9±1.2	$P = 0.434$
Final fork length (mm)	474.5±0.9	470.9±1.3	469.3±2.7	465.8±0.8	466.2±2.5	470.5±2.6	468.9±1.3	471.1±1.2	$P = 0.074$
Length growth rate (mm d^{-1})	0.13±0.01^{a}	0.10±0.02^{b}	0.10±0.00^{b}	0.06±0.01^{c}	0.09±0.01^{b}	0.08±0.00^{bc}	0.07±0.01^{bc}	0.13±0.00^{a}	$P < 0.001$
Final condition factor	1.44±0.02^{b}	1.40±0.02^{bc}	1.37±0.01^{c}	1.29±0.01^{d}	1.40±0.00^{c}	1.32±0.01^{d}	1.32±0.01^{d}	1.49±0.01^{a}	$P < 0.001$
Feed utilisation									
Feed consumption rate (g as fed fish^{-1} d^{-1})	4.89±0.11^{b}	3.34±0.12^{c}	2.48±0.09^{d}	0.41±0.01^{f}	2.52±0.04^{d}	1.45±0.04^{e}	1.56±0.04^{e}	10.83±0.23^{a}	$P < 0.001$
Apparent FCR (as fed)	4.43±0.35^{ab}	9.92±1.14^{a}	-9.34±1.91^{c}	-0.24±0.01^{b}	-11.54±4.11^{c}	-2.14±0.55^{b}	-1.70±0.28^{b}	7.83±0.24^{a}	$P < 0.001$
Proximate composition									
Moisture (%)	65.4±0.8	66.0±1.6	68.0±1.1	67.9±0.9	67.2±1.5	68.3±1.0	69.2±0.1	65.7±0.9	$P = 0.213$
Protein (% wet)	19.83±0.34	19.65±0.44	20.66±0.57	19.82±0.21	20.50±0.57	20.69±0.48	20.78±0.87	20.14±0.17	$P = 0.587$
Lipid (% wet)	13.1±0.3^{a}	12.1±0.8^{a}	9.7±1.5^{ab}	9.8±0.5^{ab}	10.2±1.0^{ab}	9.7±1.2^{ab}	7.8±0.9^{b}	12.1±0.4^{a}	$P = 0.014$
Ash (% wet)	2.4±0.3	2.4±0.1	2.4±0.3	2.7±0.2	2.8±0.4	2.3±0.1	2.5±0.2	2.4±0.3	$P = 0.872$
Carbohydrate (% wet; by difference)	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	-
Energy (MJ kg^{-1} wet)	8.39±0.24^{a}	7.80±0.38^{a}	7.09±0.46^{ab}	6.99±0.15^{ab}	7.25±0.35^{ab}	7.12±0.36^{ab}	6.42±0.20^{b}	7.89±0.13^{a}	$P = 0.008$
Taurine (mg 100g^{-1})	281±6^{a}	265±7^{ab}	268±6^{ab}	242±8^{ab}	269±21^{ab}	256±9^{ab}	283±22^{a}	222±6^{b}	$P = 0.047$
Nutrient retention									
Apparent PD	11.84±2.19^{a}	4.76±5.09^{a}	11.62±9.38^{a}	-164.59±27.70^{b}	8.36±9.65^{a}	4.28±10.10^{a}	-0.21±19.41^{a}	17.98±1.26^{a}	$P < 0.001$
Apparent ED	34.66±4.23^{a}	24.75±10.11^{a}	-3.91±16.69^{ab}	-164.02±24.99^{c}	3.45±12.86^{b}	-14.52±24.68^{ab}	-56.44±9.18^{b}	49.89±5.15^{a}	$P < 0.001$

[1] Values are mean ± SE; n = 3. Initial fish proximate composition (wet basis): Moisture 66.7%, protein 19.62%, lipid 10.0%, ash 2.2%, carbohydrate (by difference) 1.5%, energy 7.04 MJ kg^{-1}, taurine 297mg 100g^{-1}. [2] Treatment 1: Formulated diet (Ridley Clean Seas 2014 Pelagica) fed to apparent satiation six days week^{-1}; Treatment 2: Formulated diet fed to apparent satiation two days week^{-1}; Treatment 3: Formulated diet fed to apparent satiation one day week^{-1}; Treatment 4: Formulated diet fed at 0.1% body weight (BW) one day week^{-1}; Treatment 5: Formulated diet fed at 0.65% BW two days week^{-1}; Treatment 6: Formulated diet fed at 0.35% BW two days week^{-1}; Treatment 7: Formulated diet fed at 0.12% BW six days week^{-1}. Treatment 8: Sardines (thawed and diced) fed to apparent satiation every second day.

[3]A significance level of $P < 0.05$ was used for all statistical tests, where significant differences were observed post-hoc tests were used (Student Newman-Keuls test) to detect differences between treatments, values without a common superscript are significantly different ([a] indicates the highest value; $P < 0.05$).

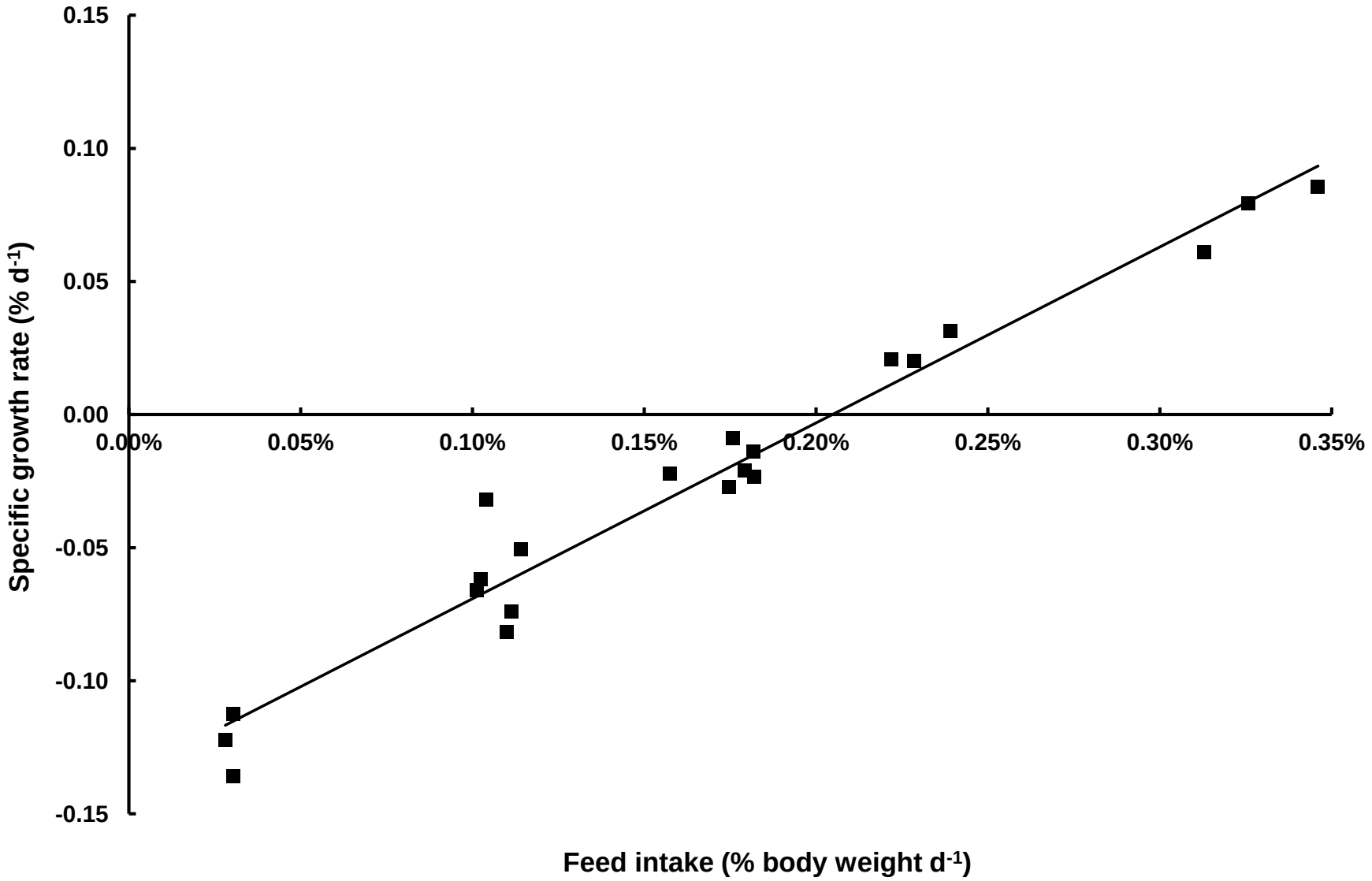

Figure 4.5. The relationship between feed intake (% body weight d^{-1}) and specific growth rate (% d^{-1}) for Yellowtail Kingfish fed the Formulated diet (Ridley Clean Seas 2014 Pelagica diet) after 84 days in Trial 3. Linear relationship: $y = 66.051x - 0.1352$, $R^2 = 0.961$, Pearson's correlation coefficient $P < 0.001$.

Whole fish fatty acid composition
There were numerous significant differences in fatty acid levels of fish between treatments (One-factor ANOVA, $P < 0.05$, Table 4.4). In general, the fatty acid level of fish in Treatments 1, 2 and 8 were significantly higher than those in Treatment 7 ($P < 0.05$), while the fatty acid level of fish in other treatments were similar ($P > 0.05$). These levels are consistent with the differences observed in lipid levels between treatments. The long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA; eicosapentaenoic acid [EPA], docosapentaenoic acid [DPA], docosahexaenoic acid [DHA]) were generally significantly higher in Yellowtail Kingfish fed Treatments 1 and 8 than Treatment 7.

Whole fish mineral composition
The tissue mineral levels (calcium, copper, iodine, iron, magnesium, manganese, potassium, phosphorus, selenium, zinc) of Yellowtail Kingfish were not significantly influenced by treatment (One-factor ANOVA, $P > 0.05$; Table 4.5).

Blood haematocrit, visceral and hepatosomatic index
For the four treatments sampled (Treatments 1, 4, 7 and 8), blood haematocrit of Yellowtail Kingfish were not significantly different between treatments, and ranged from 39.6 to 46.0% (One-factor ANOVA, $P > 0.05$, Table 4.6). For the same four treatments, treatment significantly influenced visceral index (VSI; %) and hepatosomatic index (HSI; %) of Yellowtail Kingfish (One-factor ANOVA, $P < 0.05$, Table 4.6). With regard to VSI, fish fed Treatments 1 and 8 had significantly higher VSI to animals fed Treatment 7 ($P < 0.05$), while the VSI of fish fed Treatment 4 was statistically similar to fish fed Treatments 1, 7 and 8 ($P > 0.05$). In addition, HSI was significantly higher in fish fed Treatments 1 and 8, compared to those fed Treatments 4 and 7 (One-factor ANOVA, $P < 0.05$, Table 4.6).

Table 4.4. Fatty acid composition (mg 100 g^{-1}) of Yellowtail Kingfish fed the Formulated diet at different feeding regimes and a Sardine diet for 84 days in Trial 3.[1,2,3]

Diet		1	1	1	1	1	1	1	2	
Treatment[3]	Initial	1	2	3	4	5	6	7	8	ANOVA[4]
Butyric (C4:0)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Caproic (C6:0)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Caprylic (C8:0)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Capric (C10:0)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Lauric (C12:0)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Trisdecanoic (C13:0)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Myristic (C14:0)	340	423±9^{a}	397±27^{ab}	310±52^{ab}	323±19^{ab}	343±38^{ab}	313±41^{ab}	253±28^{b}	403±13^{a}	*P* = 0.021
Pentadecanoic (C15:0)	36	45±1^{a}	42±3^{ab}	33±6^{ab}	34±2^{ab}	37±5^{ab}	33±4^{ab}	28±3^{b}	49±2^{a}	*P* = 0.008
Palmitic (C16:0)	1620	2063±34^{a}	1897±127^{ab}	1510±228^{ab}	1513±74^{ab}	1620±178^{ab}	1523±179^{ab}	1247±127^{b}	1880±61^{ab}	*P* = 0.016
Margaric (C17:0)	38	49±1^{ab}	46±4^{ab}	36±5^{bc}	36±2^{bc}	39±5^{bc}	35±4b^{c}	29±3^{c}	53±1^{a}	*P* = 0.003
Stearic (C18:0)	530	697±17^{a}	647±57^{a}	513±75^{ab}	527±27^{ab}	553±63^{ab}	513±55^{ab}	410±40^{b}	653±19^{a}	*P* = 0.013
Arachidic (C20:0)	17	23±0^{a}	22±2^{ab}	17±3^{ab}	19±1^{ab}	19±2^{ab}	18±2^{ab}	15±2^{b}	22±1^{ab}	*P* = 0.024
Heneicosanoic (C21:0)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Docosanoic (C22:0)[2]	10	14±1	13±1	4±4	11±0	13±0	12±1	<10	13±2	*P* = 0.043[5]
Tetracosanoic (C24:0)[2]	<10	7±7	5±5	4±4	4±4	4±4	5±5	<10	6±6	*P* = 0.986
Saturated Fat (g 100g^{-1})	2.6	3.4±0.1^{a}	3.1±0.2^{a}	2.5±0.4^{ab}	2.5±0.1^{ab}	2.7±0.3^{ab}	2.5±0.3^{ab}	2.0±0.2^{b}	3.1±0.1^{a}	*P* = 0.013
Decenoic (C10:1)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Myristoleic (C14:1)	17	22±1^{a}	20±1^{ab}	16±3^{ab}	16±1^{ab}	17±2^{ab}	16±2^{ab}	13±1^{b}	21±1^{ab}	*P* = 0.018
Pentadecenoic (C15:1)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Palmitoleic (C16:1)	620	787±22^{a}	727±47^{ab}	577±95^{ab}	567±32^{ab}	617±70^{ab}	570±76^{ab}	463±53^{b}	697±27^{ab}	*P* = 0.024
Heptadecenoic (C17:1)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Octadecenoic (C18:1n-6)	<10	3±3	<10	<10	<10	<10	<10	<10	<10	*P* = 0.466
Octadecenoic (C18:1n-7)	320	403±13^{a}	373±28^{a}	293±46^{ab}	293±15^{ab}	317±35^{ab}	290±35^{ab}	233±23^{b}	363±13^{ab}	*P* = 0.013
Oleic (C18:1n-9)	2730	3600±100^{a}	3330±239^{ab}	2700±393^{ab}	2690±150^{ab}	2777±307^{ab}	2673±328^{ab}	2163±214^{b}	3077±112^{ab}	*P* = 0.027
Eicosenoic C20:1 (total)	250	310±12	310±31	230±40	273±18	263±32	253±39	187±18	300±21	*P* = 0.077
Eicosenoic (C20:1n-9)	130	167±3	163±13	126±21	140±6	140±17	133±20	102±9	157±9	*P* = 0.067
Eicosenoic (C20:1n-11,13)	110	137±7	147±18	103±21	130±10	127±18	120±20	85±9	143±12	*P* = 0.129
Docosenoic (C22:1n-9)	22	28±0^{a}	28±2^{a}	21±3^{ab}	24±2^{ab}	22±3^{ab}	22±3^{ab}	16±1^{b}	27±1^{ab}	*P* = 0.035
Docosenoic (C22:1n-11, 13)	66	83±2	78±9	47±5	64±3	69±7	63±11	46±7	79±4	*P* = 0.172
Tetracosenoic (C24:1)	31	26±13	39±2	30±2	36±2	33±4	34±2	28±3	43±3	*P* = 0.351
Mono Unsaturated Fat (g 100g^{-1})	4.1	5.4±0.1^{a}	5.1±0.4^{a}	4.1±0.6^{ab}	4.1±0.2^{ab}	4.2±0.5^{ab}	4.0±0.5^{ab}	3.2±0.3^{b}	4.8±0.2^{ab}	*P* = 0.023

[1] Values are mean ± SE; n = 3; [2] One or two samples were below detectable range and were assigned the value of 0; if the values for all three replicates were below detectable range then they are reported here as below the detectable range (e.g. <10).

[3] Treatment 1: Formulated diet (Ridley Clean Seas 2014 Pelagica) fed to apparent satiation six days $week^{-1}$; Treatment 2: Formulated diet fed to apparent satiation two days $week^{-1}$; Treatment 3: Formulated diet fed to apparent satiation one day $week^{-1}$; Treatment 4: Formulated diet fed at 0.1% body weight (BW) one day $week^{-1}$; Treatment 5: Formulated diet fed at 0.65% BW two days $week^{-1}$; Treatment 6: Formulated diet fed at 0.35% BW two days $week^{-1}$; Treatment 7: Formulated diet fed at 0.12% BW six days $week^{-1}$. Treatment 8: Sardines (thawed and diced) fed to apparent satiation every second day.

[4] A significance level of $P < 0.05$ was used for all statistical tests, where significant differences were observed post-hoc tests were used (Student Newman-Keuls test) to detect differences between treatments, values within each row without a common superscript are significantly different (^{a} indicates the highest value; $P < 0.05$).

[5] One-factor ANOVA detected a significant difference, but SNK test was unable to discern significant differences.

Table 4.4. Continued.[1,2,3]

Diet		1	1	1	1	1	1	1	2	
Treatment[3]	Initial	1	2	3	4	5	6	7	8	ANOVA[4]
Linoleic (C18:2n-6)	960	1280±26^{a}	1167±74^{a}	960±136^{ab}	923±48^{ab}	967±94^{ab}	933±114^{ab}	760±81^{b}	1077±37^{ab}	*P* = 0.015
C18:2 CLA 9c, 11t	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
C18:2 CLA 10t, 12c	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Alpha Linolenic (C18:3n-3)	130	180±6^{a}	157±12^{a}	124±20^{ab}	120±6^{ab}	130±15^{ab}	120±17^{ab}	96±12^{b}	167±7^{a}	*P* = 0.005
Octadecatrienoic (C18:3n-4)	18	22±1^{a}	20±2^{a}	17±3^{ab}	16±1^{ab}	17±2^{ab}	16±2^{ab}	12±1^{b}	22±1^{a}	*P* = 0.012
Gamma Linolenic (C18:3n-6)	17	23±1^{a}	21±1^{a}	17±3^{ab}	17±1^{ab}	17±2^{ab}	17±2^{ab}	13±2^{b}	21±1^{a}	*P* = 0.008
Steridonic (C18:4n-3)	89	113±3^{a}	101±9^{ab}	76±14^{ab}	80±5^{ab}	84±8^{ab}	80±16^{ab}	61±9^{b}	120±6^{a}	*P* = 0.006
Eicosadienoic (C20:2n-6)	20	17±9	25±2	20±3	19±1	21±2	19±3	15±2	27±1	*P* = 0.396
Eicosatrienoic (C20:3n-3)[2]	<10	11±0^{a}	6±4^{b}	<10^{b}	<10^{b}	<10^{b}	3±3^{b}	<10^{b}	11±0^{a}	*P* < 0.001
Dihomo-gamma-linoleic (C20:3n-6)	15	20±1^{a}	18±1^{a}	15±2^{ab}	15±1^{ab}	15±2^{ab}	15±2^{ab}	11±1^{b}	19±1^{a}	*P* = 0.016
Eicosatetracenoic (C20:4n-3)	100	127±7	130±12	81±20	118±12	108±13	93±24	78±9	112±15	*P* = 0.169
Arachidonic (C20:4n-6)	89	117±3^{a}	110±6^{a}	90±11^{ab}	93±3^{ab}	91±5^{ab}	93±9^{ab}	73±8^{b}	110±6^{a}	*P* = 0.008
Eicosapentaenoic (C20:5n-3)	520	690±21	597±32	480±78	480±21	490±44	473±73	383±59	643±28	*P* = 0.007
Heneicosapentaenoic acid (C21:5n-3)	<10	<10	<10	<10	<10	<10	<10	<10	<10	-
Docosadienoic (C22:2)[2]	<10	<10	<10	8±8	<10	<10	5±5	<10	<10	*P* = 0.547
Docosatetraenoic (C22:4n-6)	32	44±1^{ab}	41±2^{ab}	33±4^{bc}	36±1^{bc}	34±3^{bc}	35±4^{bc}	27±3^{c}	47±1^{a}	*P* = 0.001
Docosapentaenoic (C22:5n-3)	170	250±10^{a}	227±17^{a}	187±26^{ab}	187±9^{ab}	187±15^{ab}	190±25^{ab}	143±13^{b}	233±9^{a}	*P* = 0.008
Docosapentaenoic (C22:5n-6)	31	36±2^{a}	32±1^{ab}	25±4^{ab}	26±1^{ab}	26±3^{ab}	25±4^{ab}	20±4^{b}	33±1^{a}	*P* = 0.011
Docosahexaenoic (C22:6n-3)	730	1003±44^{ab}	905±55^{bc}	736±93^{bc}	787±20^{bc}	777±56^{bc}	792±105^{bc}	619±78^{c}	1170±40^{a}	*P* = 0.001
EPA + DPA + DHA	1420	1943±69^{ab}	1728±101^{abc}	1403±197^{bc}	1454±46^{abc}	1454±112^{abc}	1456±201^{abc}	1146±150^{c}	2047±76^{a}	*P* = 0.003
Poly Unsaturated Fat (%m m^{-1})	3.0	4.0±0.1^{a}	3.6±0.2^{ab}	3.0±0.5^{ab}	3.0±0.1^{ab}	3.1±0.3^{ab}	3.0±0.4^{ab}	2.4±0.3^{b}	3.9±0.1^{a}	*P* = 0.009
Trans Fat content (%m m^{-1})	0.2	0.3±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.1±0.0	0.2±0.0	*P* = 0.062
Total Omega 3	1750	2367±81^{a}	2120±132^{ab}	1703±248^{ab}	1777±62^{ab}	1783±136^{ab}	1767±252^{ab}	1397±177^{b}	2470±93^{a}	*P* = 0.004
Total Omega 6	1150	1530±35^{a}	1393±85^{a}	1143±162^{ab}	1117±57^{ab}	1167±94^{ab}	1123±133^{ab}	907±97^{b}	1317±47^{ab}	*P* = 0.011
Total Omega 9	2920	3837±102^{a}	3560±253^{ab}	2880±422^{ab}	2890±161^{ab}	2973±326^{ab}	2863±350^{ab}	2310±225^{b}	3307±127^{ab}	*P* = 0.027

[1] Values are mean ± SE; n = 3

[2] One or two samples were below detectable range and were assigned the value of 0; if the values for all three replicates were below detectable range then they are reported here as below the detectable range (e.g. <10), but were assigned the value of 0 for statistical analyses.

[3] Treatment 1: Formulated diet (Ridley Clean Seas 2014 Pelagica) fed to apparent satiation six days week^{-1}; Treatment 2: Formulated diet fed to apparent satiation two days week^{-1}; Treatment 3: Formulated diet fed to apparent satiation one day week^{-1}; Treatment 4: Formulated diet fed at 0.1% body weight (BW) one day week^{-1}; Treatment 5: Formulated diet fed at 0.65% BW two days week^{-1}; Treatment 6: Formulated diet fed at 0.35% BW two days week^{-1}; Treatment 7: Formulated diet fed at 0.12% BW six days week^{-1}. Treatment 8: Sardines (thawed and diced) fed to apparent satiation every second day.

[4] A significance level of $P < 0.05$ was used for all statistical tests, where significant differences were observed post-hoc tests were used (Student Newman-Keuls test) to detect differences between treatments, values within each row without a common superscript are significantly different (^{a} indicates the highest value; $P < 0.05$).

Table 4.5. Mineral composition (mg kg^{-1}) of Yellowtail Kingfish after 84 days in Trial 3[1].

Diet		1	1	1	1	1	1	1	2	
Treatment[2]	Initial	1	2	3	4	5	6	7	8	*ANOVA[3]*
Calcium	4900	5400±2303	3233±788	4367±845	3867±1471	3900±458	4033±851	3333±977	4600±569	*P* = 0.914
Copper	0.72	0.82±0.06	0.92±0.09	0.78±0.03	0.65±0.04	0.82±0.04	0.75±0.05	0.73±0.05	0.79±0.06	*P* = 0.102
Iodine (mg 100g^{-1})	0.85	1.09±0.31	0.61±0.03	0.68±0.03	0.79±0.06	0.69±0.04	0.89±0.12	0.80±0.08	0.86±0.17	*P* = 0.355
Iron	19	33±8	22±1	22±1	23±1	21±2	21±1	24±1	25±6	*P* = 0.409
Magnesium	370	360±30	333±13	353±22	330±15	340±6	360±15	333±19	350±10	*P* = 0.830
Manganese	0.67	0.70±0.16	0.43±0.07	0.45±0.05	0.46±0.07	0.45±0.05	0.50±0.06	0.43±0.10	0.53±0.08	*P* = 0.435
Potassium	3700	3533±33	3600±0	3733±145	3633±33	3667±67	3667±33	3733±33	3467±33	*P* = 0.086
Phosphorus	4700	4867±1172	3767±470	4333±433	4000±656	3933±219	4267±467	3633±467	4367±219	*P* = 0.853
Selenium	0.61	0.69±0.02	0.59±0.05	0.68±0.03	0.62±0.02	0.61±0.01	0.63±0.04	0.60±0.04	0.69±0.04	*P* = 0.232
Zinc	14	14±1	11±0	12±1	13±1	12±1	11±0	13±1	12±1	*P* = 0.479

[1] Values are mean ± SE; n = 3

[2] Treatment 1: Formulated diet (Ridley Clean Seas 2014 Pelagica) fed to apparent satiation six days week^{-1}; Treatment 2: Formulated diet fed to apparent satiation two days week^{-1}; Treatment 3: Formulated diet fed to apparent satiation one day week^{-1}; Treatment 4: Formulated diet fed at 0.1% body weight (BW) one day week^{-1}; Treatment 5: Formulated diet fed at 0.65% BW two days week^{-1}; Treatment 6: Formulated diet fed at 0.35% BW two days week^{-1}; Treatment 7: Formulated diet fed at 0.12% BW six days week^{-1}. Treatment 8: Sardines (thawed and diced) fed to apparent satiation every second day.

[3] A significance level of $P < 0.05$ was used for all statistical tests, where significant differences were observed post-hoc tests were used (Student Newman-Keuls test) to detect differences between treatments, values within each row without a common superscript are significantly different ([a] indicates the highest value; $P < 0.05$).

Table 4.6. Visceral and hepatosomatic index, and blood haematocrit of Yellowtail Kingfish fed selected treatments after 84 days in Trial 3[1].

Diet	1	1	1	2	
Treatment[2]	1	4	7	8	*ANOVA[3]*
Blood haematocrit (%)	46.0±0.3	39.6±1.3	41.4±1.5	43.0±2.6	*P* = 0.105
Visceral index (%)	5.79±0.19[a]	4.92±0.23[ab]	4.53±0.23[b]	5.74±0.26[a]	*P* = 0.010
Hepatosomatic index (%)	1.11±0.02[a]	0.75±0.05[b]	0.78±0.08[b]	1.00±0.06[a]	*P* = 0.006

[1] Values are mean ± SE; n = 3

[2] Treatment 1: Formulated diet (Ridley Clean Seas 2014 Pelagica) fed to apparent satiation six days week^{-1}; Treatment 4: Formulated diet fed at 0.1% body weight (BW) one day week^{-1}; Treatment 7: Formulated diet fed at 0.12% BW six days week^{-1}. Treatment 8: Sardines (thawed and diced) fed to apparent satiation every second day.

[3] A significance level of $P < 0.05$ was used for all statistical tests, where significant differences were observed post-hoc tests were used (Student Newman-Keuls test) to detect differences between treatments, values within each row without a common superscript are significantly different ([a] indicates the highest value; $P < 0.05$).

Discussion

Our overarching goal was to improve current feed management practices and economic viability for Yellowtail Kingfish production during winter. To achieve this goal, the effect of restricting feed rates and frequencies for Yellowtail Kingfish during winter was investigated. Fish were fed the newly developed Ridley Clean Seas 2014 Pelagica diet (Diet 1; Trial 2, Chapter 3), at different feed rates from 0.1% BW one day week^{-1} to apparent satiation six days week^{-1}. In addition, the performance of fish fed this Formulated diet were also compared to those fed diced Sardines to apparent satiation every second day (Treatment 8). Yellowtail Kingfish fed actively on both diets, and apparent feeding activity increased dramatically as feed rate and frequency decreased.

Yellowtail Kingfish fed the Formulated diet to apparent satiation six days week^{-1} (Treatment 1) exhibited significantly higher growth rates and numerically superior FCR, compared to fish fed the Formulated diet at all other feed rates. Fish fed Treatment 1 had greater access to feed, and as a result consumed more feed and nutrients than those fed other feeding regimes. Results from the current study are not surprising as dietary protein and energy are the first growth-limiting factor for fish (Webster and Lim, 2002). Yellowtail Kingfish in the current study exhibited growth rates comparable to, or slightly better than, those observed at Clean Seas commercial sea cage facilities during the same period (personal communication, Dr T. D'Antignana; Clean Seas). Even though significant differences in growth rates were apparent in this study, Yellowtail Kingfish growth was markedly inferior to those in Trial 1 and 2 (Chapter 2 and 3) where fish were grown at summer water temperatures (17 °C - 26 °C in Trial 1; 20 °C - 26 °C in Trial 2). The SGR of fish fed the Formulated diet to apparent satiation once a day, six days week^{-1} at the colder water temperatures in the current study was 0.08% d^{-1}, while the SGR of fish fed a similar, but slightly modified diet in Trial 1, and Trial 2 to apparent satiation once a day, seven days week^{-1} was 0.63 and 0.84% d^{-1}, respectively. These differences were due to the temperature-dependent growth of Yellowtail Kingfish (Pirrozi and Booth, 2009; Bowyer et al., 2013a; b).

As Yellowtail Kingfish exhibited depressed growth rates during winter compared to summer, it may be beneficial to feed a maintenance ration through winter, or marginally above to avoid potential nutritional problems. Based on results from the current study, Yellowtail Kingfish (1.44 kg) fed the Formulated diet required 0.2047% BW d^{-1} to maintain weight at an average water temperature of 12.8 °C. Based on this feed rate, the initial weight of Yellowtail Kingfish (1.44 kg) and the dietary gross energy level of the Formulated diet (19.1 MJ kg^{-1}), each fish require 2.95 g of feed or 56.3 kJ d^{-1} at 12.8 °C. In contrast, Pirrozi and Booth (2009) modelled the routine metabolic rate (RMR), based on the oxygen consumption (MO_2), of smaller Yellowtail Kingfish (206 g) at 10, 15, 20, 25, 30 or 32.5 °C. Oxygen consumption was previously reported to be an accurate representation of metabolic rate (Withers, 1992). Pirrozi and Booth (2009) also used mass-specific data, which were scaled using the metabolic body mass exponent of 0.8 reported by Brett and Groves (1979). Based on this research, Pirrozi and Booth (2009) reported a temperature-dependant (T) routine metabolic rate of these smaller Yellowtail Kingfish (206 g) of $4.04T-13.14$ kJ kg$^{-0.8}$ d^{-1}. Using the average water temperature (12.8 °C) and initial larger fish weight (1.44 kg) in the current study, and the model developed by Pirrozi and Booth (2009), the RMR of Yellowtail Kingfish is 41.9 kJ d^{-1}. This RMR level is 26% lower than results from the current study (56.3 kJ d^{-1}), which highlights the need for further research focused on weight- and temperature-specific metabolic rates for larger Yellowtail Kingfish.

In order to feed just above the maintenance ration, Yellowtail Kingfish may be fed the Formulated diet to apparent satiation once a day, two days week^{-1}. However, other feed rates not investigated in the current study may also supply a maintenance ration to Yellowtail Kingfish, such as feeding fish once a day, every second day to apparent satiation. There are a number of important factors that need to be considered, before this feed management

strategy is implemented during winter. Firstly, the fish in-fish out ratio is not impaired. Secondly, it is important that the weight and condition of Yellowtail Kingfish is not compromised at harvest. Previous studies have reported that some fish species, including Channel Catfish (*Ictalurus punctatus*), Red Porgy (*Pagrus pagrus*), Barramundi (*Lates calcarifer*), and Rainbow Trout (*Oncorhynchus mykiss*) fed a restricted feed ration or fasted, exhibited compensatory growth once re-fed to apparent satiation (Kim and Lovell, 1995; Rueda et al., 1998; Tian and Qin, 2004; Nikki et al., 2004). For example, Barramundi at 28 °C exhibited compensatory growth when fed to 50% and 75% satiation for two weeks then re-fed to apparent satiation, compared to the control fed to apparent satiation (Tian and Qin, 2004). Specifically, Barramundi fed to 50% and 75% satiation caught up to control fish after two and four weeks, respectively. Fish fed to 0% and 25% satiation for two weeks however, did not catch up to control fish weight after five weeks (Tian and Qin, 2004). Although compensatory growth of some fish species during short durations of restricted feed ration is understood, this phenomenon is not reported in the scientific literature for Yellowtail Kingfish. It would be beneficial in future studies to investigate compensatory growth in Yellowtail Kingfish, particularly with regard to fish fed a maintenance ration throughout winter and then switched to apparent satiation daily during summer.

In addition, fish fed a maintenance ration (two days week^{-1} to apparent satiation) may not be supplied with adequate essential nutrients, particularly essential LC n-3 PUFA (EPA, DPA and DHA), cholesterol, taurine and vitamins and minerals. If these essential nutrients are not supplied in the diet, growth rates and health may be impaired and normal biochemical and physiological function may be compromised. The optimal dietary selenium requirements for Yellowtail Kingfish were reported by Le and Fotedar (2013). However, compared to other aquaculture species such as salmonoids, information pertaining to other essential nutritional requirements for Yellowtail Kingfish is lacking in the literature. As a result, current commercial diets for Yellowtail Kingfish are based on Japanese Yellowtail and salmonid/Barramundi diets (personal communication, Dr R. Smullen, Ridley Aquafeed). If Yellowtail Kingfish are fed weekly or twice weekly, over fortifying feed with essential nutrients, including LC n-3 PUFA, cholesterol, taurine and vitamins and minerals, may overcome potential nutritional problems. However, this practice would increase feed costs. It may be beneficial to optimise the nutritional profile of feed, particularly the aforementioned essential nutrients, prior to feeding maintenance rations to Yellowtail Kingfish under culture condition.

The second aim of the current study was to benchmark the performance of Yellowtail Kingfish fed the Formulated diet to fish fed a natural, 100% fish-based diet. For this purpose Yellowtail Kingfish were fed Sardines once a day, every second day to apparent satiation (Treatment 8). The growth and FCR of Yellowtail Kingfish fed the Formulated diet to apparent satiation once a day, six days week^{-1} (Treatment 1) and fish fed Sardines once a day, every second day (Treatment 8) were similar. There were also no significant differences in apparent protein and energy deposition between fish fed Treatments 1 and 8. However, Yellowtail Kingfish fed Treatment 8 exhibited numerically higher (52%) protein deposition (11.84 and 17.98%, respectively), and numerically higher (44%) energy deposition (34.66 and 49.89%, respectively), compared to fish fed Treatment 1. Despite these numerical improvements and a feed strategy that may be economical, feeding Sardines to Yellowtail Kingfish may not represent a sustainably viable option for the production of Yellowtail Kingfish. A common measure for the sustainable use of marine ingredients is the fish in-fish out ratio (Jackson, 2009). Jackson (2009) reported the fish in-fish out ratio to equal (level of FM in the diet + level of FO in the diet) / (yield of FM from wild fish + yield of FO from wild fish) × FCR, modified from earlier research by Tacon and Metian (2008). From 100 kg of fresh Sardines the average yield of FM and FO was reported to be 22.5% and 5%, respectively (Tacon and Metian, 2008; Jackson, 2009). Based on the FM (30%) and FO (9%) content of the Formulated diet, and an FCR of 4.43 for fish fed to apparent satiation six days week^{-1}, the fish in-fish out ratio over the duration of the current study was 6.3. In

contrast, over the duration of the current study Yellowtail Kingfish fed Sardines every second day had a fish in-fish out ratio of 7.8. Yellowtail Kingfish fed Sardines had a fish in-fish out ratio that was 24% higher than those fed the Formulated diet. The fish in-fish out ratio for Yellowtail Kingfish fed Sardines in this case was based on the (level of FM in the diet +level of FO in the diet) / (yield of FM from wild fish + yield of FO from wild fish) = 1, as fish are fed Sardines on an as-fed fresh basis and an FCR of 7.8. Furthermore, even over shorter periods, between weight check 1 and 2 (~12 °C; 23/07/15 to 20/08/15; Day 28 to 56), when water temperatures were at their coldest the fish in-fish out ratio for fish fed Sardines (20.3) was still 19% higher than those fed the Formulated diet (Treatment 1; 17.0). It should also be noted that the fish in-fish out ratio was negatively affected when fish were fed formulated diets at or below the maintenance ration.

Conclusions and Recommendations

In conclusion, this study adds to the nutritional knowledge of Yellowtail Kingfish. Results from the current study provide additional confidence to feed Yellowtail Kingfish the Ridley Clean Seas 2014 Pelagica diet developed in this project during winter. Clearly, Yellowtail Kingfish growth rates during winter are depressed. However, if farms aim to capitalise on limited fish growth during winter we recommend that farms feed Yellowtail Kingfish (~1.5 kg) the Formulated diet to apparent satiation once a day, six days week^{-1} throughout winter. In contrast, if the primary aim of farms is to reduce feed and feeding costs, and maintain fish weight throughout winter, Yellowtail Kingfish require 0.2047% BW d^{-1}. This feed rate is achieved, slightly to excess, by feeding fish the Formulated diet to apparent satiation once a day, two days week^{-1}. Fish fed below this rate resulted in weight loss, and we do not recommend feeding below this level. However, before these recommendations are adopted on-farm, results will need to be validated under commercial pilot scale conditions. As a result of this research, Clean Seas adopted new winter feeding strategies. If Yellowtail Kingfish are fed a maintenance rations, attention to the essential dietary nutrients levels are needed, particularly dietary LC n-3 PUFA, cholesterol, taurine, and vitamin and mineral levels. The growth performance of Yellowtail Kingfish fed Sardines once a day, every second day was similar to fish fed the Formulated diet to apparent satiation once a day, six days week^{-1}. However, Yellowtail Kingfish fed Sardines had a fish in-fish out ratio that was 24% higher than those fed the Formulated diet to apparent satiation six days week^{-1}. The current study provides a much clearer understanding of the optimal feed rates and frequencies for large adult Yellowtail Kingfish throughout winter.

Chapter 5. General Discussion

In this research project, there were four objectives that aimed to improve production diets for large Yellowtail Kingfish (> 1.5 kg) in a large tank based system for adoption into commercial sea cage culture:

1. Reduce FCRs for Yellowtail Kingfish production by 0.45 units across the entire sea cage production cycle.

2. Gain a 10% improvement in growth for Yellowtail Kingfish in Clean Seas sea cage production systems.

3. Provide information to improve Yellowtail Kingfish health and reduce mortality by 2% in Clean Seas sea cage production systems.

4. Improve feeding strategies in a tank based setting using large (> 1.5 kg) Yellowtail Kingfish to provide information to improve Yellowtail Kingfish growth and feed utilisation by 2% in Clean Seas sea cage production systems during winter.

These four project objectives were designed to address the two highest nutritional research priorities identified by Clean Seas in 2013, prior to the commencement of this project:

1. Reduce FCRs for Clean Seas Yellowtail Kingfish production by 0.45 units across the entire sea cage production cycle.

2. The refinement of the current status of knowledge on the energy requirements of large Yellowtail Kingfish (> 1.5 kg).

Results from this project were positive, and provided information that directly addressed the four project research objectives, and the two research priorities identified by Clean Seas in 2013. Information derived from this project was extended directly to Clean Seas, and was immediately adopted into commercial dietary formulations and on-farm production to provide several advancements.

In all three trials, the growth performance, feed utilisation, health and survival of large Yellowtail Kingfish were exceptional in the large partial recirculating seawater pool-farm system at the South Australian Research and Development Institute's South Australian Aquatic Sciences Centre (SAASC). Additionally, fish cultured at the SAASC exceeded the performance of fish from the same cohort cultured over the same period in Clean Seas sea cages (personal communication, Dr C. Foster; Clean Seas). This outcome was favourable, as it provided Clean Seas with confidence to use information derived from tank based trials at the SAASC in on-farm commercial sea cages with large Yellowtail Kingfish.

Significant improvements in FCR were observed in Yellowtail Kingfish at the SAASC, compared to sea cage cultured fish at Clean Seas. However, the improvement in FCR cannot be attributed to dietary alterations alone. The superior FCRs of Yellowtail Kingfish at the SAASC may be attributed to improved control over feeding in the smaller 5000 L tanks compared to sea cages. Additionally, large Yellowtail Kingfish grown in tanks at the SAASC were also provided with oxygen for half of Trial 1 and the whole of Trial 2 and 3 in order to maintain dissolved oxygen levels at ~100% saturation, while sea cage cultured fish were not provided with additional oxygenation. Yellowtail Kingfish are known to have high oxygen requirements at summer water temperatures, and reduced growth and feed utilisation were reported when dissolved oxygen levels are <90% saturation (Bowyer et al., 2013c). While other factors may have also influenced Yellowtail Kingfish performance, it may be worthwhile to investigate the application of oxygenation to commercial sea cages to improve growth and feed utilisation for Yellowtail Kingfish. In addition, fish were also subjected to efficient skin

and gill fluke treatment prior to the commencement of all three trials, as heavy burdens of gill and skin flukes also impact growth performance and feed utilisation of cultured Yellowtail Kingfish (personal communication, Dr T. D'Antignana; Clean Seas). The control of flukes may have also improved the growth and feed utilisation of large Yellowtail Kingfish at the SASC, compared to fish in sea cage systems at Clean Seas. Further research is also required to improve gill and skin fluke treatments particularly in the sea cage culture of Yellowtail Kingfish.

Several advancements in commercial dietary formulations for large Yellowtail Kingfish have resulted from this project. With regard to commercial diet benchmarking, results were positive for Australian Yellowtail Kingfish production diets. Australian Yellowtail Kingfish production diets were formulated according to the nutrient requirements suggested by Stone and Bellgrove (2013), and were manufactured by Ridley Aquafeeds. The Ridley Clean Seas 2013 Pelagica Diet (Diet 2 in Trial 1, Table 2.1) performed well against the "gold standard" FM and FO control diet (Diet 1, Trial 1, Table 2.1). Additionally, Yellowtail Kingfish fed the newly formulated Ridley high energy diet (Diet 3, Trial 2; Table 3.1) and the Ridley Clean Seas 2014 Pelagica diet (Diet 1, Trial 2, Table 3.1, developed based on results from Trial 1), exhibited superior growth rates and feed utilisation, compared to fish fed the Japanese Yellowtail production diet (Diet 5, Trial 2, Table 3.1). These outcomes are favourable, as they provide confidence for Clean Seas and other Yellowtail Kingfish producers to use Australian formulated and manufactured high energy diets, which bodes well for the future development of the Yellowtail Kingfish industry.

Fish meal replacement in Yellowtail Kingfish production diets was also improved during this project. In Trials 1 and 2, significant reductions in the dietary inclusion of FM and FO were successful, and the growth, health and survival of Yellowtail Kingfish were maintained. Fish meal inclusion level in commercial diets was successfully reduced by 50%. The dietary inclusion level of FM was reduced from 40% to 30% in Trial 1, and from 30% to 20% in Trial 2. Reductions in FM were attained in the current study by incorporating a range of less expensive terrestrial animal and plant protein sources, in combination with carefully balancing the dietary nutrient content to meet the suggested dietary requirements for Yellowtail Kingfish (reviewed by Stone and Bellgrove, 2013). Bearing in mind that the majority of feed is used in the production of large fish, the cost savings associated with this level of FM replacement should be significant for the Yellowtail Kingfish industry.

In Trials 1 and 2, a significant quantity of FO (50% - 75% substitution) was successfully replaced with poultry oil (PO), while the growth, feed efficiency, health and survival of Yellowtail Kingfish were maintained. For example, FO substitution levels of up to 75% were achieved in the Ridley high energy diet (Diet 3; Table 3.1; Trial 2; Chapter 3). However, it should be noted that based on the ∑long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA; eicosapentaenoic acid [EPA] + docosapentaenoic acid [DPA] + docosahexaenoic acid [DHA]) in the Ridley high energy 2013 diet, and results from the current study, we recommend that ∑LC n-3 PUFA are maintained above 2 g 100 g^{-1} diet in production diets for large Yellowtail Kingfish. This level would ensure that diets are not deficient in LC n-3 PUFA, and that dietary LC n-3 PUFA levels do not limit growth or impair health. However, further research is needed to ascertain the specific LC n-3 PUFA dietary requirements of cultured Yellowtail Kingfish, particularly at different life stages and water temperatures. This research would further improve the economical and sustainable production of this species.

With the current set of oil ingredients available to feed manufacturers, providing adequate levels of essential LC n-3 PUFA are the limiting factor for FO substitution in Yellowtail Kingfish diets. Based on the current research, a dietary inclusion of 7% FO and the small contribution of LC n-3 PUFA provided in the FO component of the ever diminishing levels of dietary FM is borderline sufficient to provide adequate LC n-3 PUFA (≥ 2 g 100 g^{-1} diet) to meet the requirements for Yellowtail Kingfish. In addition, when FO levels of 9% were

provided in the diet, Yellowtail Kingfish growth performance and feed utilisation was exceptional. Given that essential LC n-3 PUFA are predominantly derived from FO from the marine environment (Oliveira et al., 2008), our ability to improve the fish in-fish out ratio by replacing FO in diets for Yellowtail Kingfish is limited (Bowyer et al., 2012). In the near- to long-term future, commercially quantities of economically viable ingredients including microalgae and macroalgal oils, or genetically modified plant oils rich in LC n-3 PUFA may be utilised to further replace FO in Yellowtail Kingfish diets. At present however, these options are not commercially viable and the use of FO, rich in essential LC n-3 PUFA, combined with terrestrial plant oils and animal fats is the best option (Stone et al., 2011a; b).

Another important consideration relevant to FO substitution is the nutritional profile and health benefits of fish produced for human consumption. A current marketing advantage for the fisheries and aquaculture industries, over terrestrial animal agriculture, is that fish are a prime source of LC n-3 PUFA. Consumer perception and acceptance of fish cultured using alternative terrestrial oils lacking in LC n-3 PUFA may be lowered due to reduced LC n-3 PUFA levels in end products. However, the high lipid level of cultured fish may compensate for this by providing higher levels of EPA and DHA in the edible portion of fish, compared to wild fish (Stone et al., 2011a; b). The n-3:n-6 fatty acid (FA) ratio of fish fed terrestrial oils will be reduced, which would ultimately result in an inferior nutritional value and reduce the health benefits to the consumer (Stone et al., 2011a; b; Bowyer et al. 2012). A strategy to overcome the negative impacts of feeding terrestrial oils on LC n-3 PUFA levels and the ratio of n-3:n-6 FA is the phase feeding of a finishing diets rich in LC n-3 PUFA for a pre-determined period prior to harvesting. This approach has been validated and is successful for a range of salmonid species, and makes more efficient use of precious FO supplies (Stone et al., 2011a; b). Further research in this area is required as the duration of the “finishing off” period for Yellowtail Kingfish is unknown.

The good health and high survival of fish fed diets formulated with a range of FM and FO substitution levels in Trial 1 and 2 may be an indication that all commercial test diets were formulated to contain sufficient nutrients to support growth and health under culture conditions at the SAASC (Stone and Bellgrove, 2013). In addition, a shallow water stress challenge test (hypoxic stress test) was implemented at the end of Trial 1, to determine if FM and FO substitution impacted the health and survival of large Yellowtail Kingfish. A range of haematological and immunological functions were assessed pre- and post-hypoxic stress. While there were no mortalities post-stress test in Trial 1, immunological parameters were significantly influenced by diet and stress test. Differences associated with FM and FO substitution however, were inconclusive. Traditional haematological values (Table 2.9 and 2.10) were influenced by handling stress, particularly in relation to FM and FO substitution. Based on these results, we recommended that a wider range of immune parameters are assessed in future research. In addition, it may also be beneficial to develop a more sensitive bacterial challenge to assess the impacts of dietary FM and FO replacements on the health and survival of Yellowtail Kingfish at the completion of future trials. Improved health and survival of Yellowtail Kingfish under culture conditions, by improving dietary formulations will also enhance the reputation of Clean Seas as an ethical producer of quality seafood.

With regard to feeding strategies during summer (Trial 1 and 2), there appears to be a fine line in reducing feed rates to improve feed utilisation, and not limiting feed and impairing growth. In Trial 1, Yellowtail Kingfish fed to sub-satiation (targeted 80% apparent satiation; actual ~87%) exhibited similar growth, but significantly reduced feed consumption rates, significantly superior FCRs, and improved protein and energy deposition to fish fed to apparent satiation. In contrast in Trial 2, while the FCR of fish fed to sub-satiation was superior to fish fed to apparent satiation, the growth performance was significantly lower. In Trial 2 however, compared to the set feed rate of 80%, fish fed to sub-satiation were slightly underfed during the first period of the trial and slightly overfed during the second period,

which resulted in the targeted level of 80% on average over the duration of the trial. These results suggest that with careful feed management, cost savings may be made to feed efficiency on-farm, without impairing growth. We recommend further studies to investigate feeding Yellowtail Kingfish a range of different feed rate levels of sub-satiation, including 85%, 90% and 95%.

With regard to feeding strategies during winter, the growth of fish fed the Ridley Clean Seas 2014 Pelagica diet to apparent satiation once a day, six days week^{-1} was significantly higher than fish fed the same diet at lower feed rates. To capitalise on the limited fish growth during winter we recommend that farms feed Yellowtail Kingfish (~1.5 kg) the Ridley Clean Seas 2014 Pelagica diet to apparent satiation once a day, six days week^{-1}. However, as the growth rate of fish in the current project was markedly reduced during winter compared to summer, Clean Seas management may consider it to be beneficial to maintain fish weight through winter by feeding a maintenance ration. This practice would ultimately decrease feed costs and associated labour costs. In order to maintain fish weight through winter, Yellowtail Kingfish require 0.2047% BW d^{-1}, which is achieved, slightly to excess, by feeding fish the Ridley Clean Seas 2014 Pelagica diet to apparent satiation once a day, two days week^{-1}. If Yellowtail Kingfish are only fed a maintenance ration throughout winter however, further research focused on compensatory growth when switched to apparent satiation daily during summer is needed before this feeding strategy is implemented on-farm. In addition, fish fed to apparent satiation once a day, two days week^{-1} may not be supplied with adequate essential nutrients, including LC n-3 PUFA (EPA, DPA and DHA), cholesterol, taurine and vitamins and minerals which may result in the impairment of their immune-competence. Understanding the nutritional requirements of Yellowtail Kingfish fed a maintenance ration (e.g. two days week^{-1} to apparent satiation) is needed. We do not recommend feeding Yellowtail Kingfish below the maintenance ration during winter, as fish lost weight and may manifest in to health issue if fed for prolonged periods. However, before these recommendations are adopted on-farm, results will need to be validated under commercial pilot scale conditions.

Yellowtail Kingfish fed Sardines once a day, every second day to apparent satiation exhibited similar growth to fish fed the Ridley Clean Seas 2014 Pelagica diet to apparent satiation once a day, six days week^{-1}. However, feeding Sardines to Yellowtail Kingfish may not represent a sustainably viable practice for the production of Yellowtail Kingfish. A common measure of the sustainable use of marine ingredients is the fish in-fish out ratio, which was 24% inferior for Yellowtail Kingfish fed Sardines than the Ridley diet to apparent satiation six days week^{-1}.

In conclusion, the growth performance of large Yellowtail Kingfish in the pool-farm system at SAASC was comparable to that achieved in the Clean Seas sea cage system for the same fish cohort in Spencer Gulf. This indicates that the SAASC system is suitable for experimental work, including diet benchmarking and validation trials with large Yellowtail Kingfish (> 1.5 kg) prior to sea cage validation trials at Clean Seas. Additionally, Yellowtail Kingfish grown in the partial recirculating tank system at the SAASC returned better FCR (Trial 1, 1.7 – 2.3; Trial 2, 1.6 – 1.7) than the same cohort of fish grown in the Clean Seas sea cages. These results were not likely due to nutrition alone, and it may be worthwhile to investigate commercial sea cages oxygenation and feed management practices in relation to oxygen availability to improve Yellowtail Kingfish growth and feed utilisation, and ultimately profits.

Australian formulated and manufactured Yellowtail Kingfish diets performed as well or better than the international Japanese Yellowtail diet. The manipulation of feeding strategies provided information with regard to improving FCR and also indicated potential monetary gains may be made with careful feed management. Fish meal and FO substitution will also improve sustainability by reducing the fish in-fish out ratio by 25% to 50%. At the completion

of Trial 1 and 2, Clean Seas adopted the newly formulated Ridley Clean Seas 2014 Pelagica diet for the commercial production of large Yellowtail Kingfish in their sea cages. Additionally, Clean Seas altered winter feed management practices based on the results achieved in Trial 3. Prior to this research, Clean Seas fed Yellowtail Kingfish 0.1% BW d^{-1}, for fish of a similar size under culture condition during winter (personal communication, Dr C. Foster; Clean Seas). Based on this research, Clean Seas immediately increased the feed rate to > 0.2% BW d^{-1}. Further research is required into aspects of lipid and energy metabolism in larger fish, particularly under conditions of restrictive feeding practices during both summer and winter periods. During winter, we recommend that large Yellowtail Kingfish (~ 1.5 kg) are fed the Ridley Clean Seas 2014 Pelagica diet to apparent satiation, once per day, six days $week^{-1}$ to capitalise on the limited growth during winter, or no less than the maintenance ration (0.2047% BW d^{-1}) to ensure fish do not lose weight. However, as previously stated, before these recommendations are adopted on-farm, results will need to be validated under commercial pilot scale conditions. Results from this project bode well for the future development of the Yellowtail Kingfish industry in Australia. However, dietary development work for this industry should not remain static, as important advancements will need to be ongoing to ensure the economical and sustainable production of Yellowtail Kingfish in Australia.

Chapter 6. Benefits and Adoption

The key research findings described in this project addressed the two highest nutrition research priorities of Clean Seas in 2013:

1. Reduce FCRs for Clean Seas Yellowtail Kingfish production by 0.45 units across the entire sea cage production cycle.

2. The refinement of the current status of knowledge on the energy requirements of large Yellowtail Kingfish (> 1.5 kg).

Key research findings from Trials 1, 2 and 3 (Chapters 2, 3 and 4) have led to improved formulation and manufacture of production diets by Ridley Aquafeed and these diets have been used by Clean Seas, as they came to hand, to improve growth performance and feed utilisation for large Yellowtail Kingfish in summer and winter. Clean Seas are also adopting the use of high energy diets for the production of large Yellowtail Kingfish during summer. This is significant, as the majority of feed is fed during the later stages of production. In addition, the reduced reliance on FM and FO in Yellowtail Kingfish diets is also a major benefit, as in the ever increasing global economy, environmental sustainability impacts directly on market acceptance. The survival of Yellowtail Kingfish grown on these new feeds has also been improved, which benefits producers in regard to the ethical production of food and market acceptance.

Benchmarking of diet performance in Trials 1 and 2 has been beneficial to the Australian Yellowtail Kingfish industry. Australian commercially formulated diets performed as well or better than a “gold standard’ of international diets. This provides feed managers with more confidence to use domestically produced products and reduces reliance on internationally produced feeds, thereby enhancing control over dietary formulations and reducing biosecurity issues associated with feed importation.

Results have also been adopted by Clean Seas to refine feeding strategies and implement a sub-satiation feeding regime on-farm to improve FCR for the commercial production of large Yellowtail Kingfish in summer (Chapters 2 and 3) and have increased the feed rate of Yellowtail Kingfish during winter to >0.2% BW d^{-1}(Chapter 4). Results from both trials have also benefited Yellowtail Kingfish producers by highlighting the potential to improve FCR by oxygenating sea cage systems (Chapters 2 and 3). However, on-farm feasibility studies are still required before this technology is adopted by Yellowtail Kingfish producers.

There has been a large student training component to this project. This component has assisted in the training of:

- More than 50 undergraduate extra-mural work experience students from the School of Biological Sciences at Flinders University and the Department of Animal and Veterinary Sciences at University of Adelaide.

- Two Honours student from Flinders University (School of Biological Sciences).

- Five PhD students from the School of Biological Sciences at Flinders University.

A major benefit of the student training component is the output of new industry entrants, trained with relevant skills that will contribute to future industry development. Several students have already obtained work within the industry.

Chapter 7. Further Development

This research has improved our understanding of the dietary formulations with reduced levels of fish meal (FM) and fish oil (FO) for large Yellowtail Kingfish at summer water temperatures. It has also led to the development of information to improve on-farm feeding strategies at both summer and winter water temperatures. Results generated from the current research have been extended to industry and incorporated into commercial production, and also serve as a stepping-stone for future research. In the current research, there were several limitations and unsolved issues, or areas that were not covered in the experiments and trials, which require further investigation. Recommended areas for future research and development are:

- The experimental trials in this research were carried out within a limited time frame and in 5000 L tanks, which could have presented limitations to the validity of results when applied on-farm. We suggest using this baseline information to run longer-term, pilot commercial scale studies in sea cages on-farm. Results from such sea cage trials would likely provide more comparable data to the commercial situation as the Yellowtail Kingfish used would be stocked under commercially applicable densities and experience normal on-farm daily and seasonal environmental conditions. Some of the health aspects identified in the current research may manifest and become problematic under sea cage conditions. This would in turn, provide further insight on the precise causes and possible prevention and management of nutrition/health problems.

- Assessment of the effects of different feeding strategies on the production of large Yellowtail Kingfish at optimal and sub-optimal water temperatures. It may be beneficial in further studies to investigate feeding fish at a range of different levels of sub-satiation, for example 80%, 85%, 90% and 95%.Further research to determine the ∑long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA; eicosapentaenoic acid [EPA] + docosapentaenoic acid [DPA] + docosahexaenoic acid [DHA]) requirements of Yellowtail Kingfish is required to make further improvements in FM and FO substitution, and to improve sustainability and profits.

- Further research is required into aspects of lipid, cholesterol and energy metabolism in larger fish, particularly under conditions of restrictive feeding practices during both summer and winter.

- Further research into diet formulations may be beneficial to increase dietary lipid and energy levels in production diets for large (> 1.5 kg) Yellowtail Kingfish to achieve a “protein sparing effect” and increase profits.

- Histological examination of the morphology of the liver when fish were fed diets containing alternative lipids was not covered in this research as the main focus was on gastrointestinal tract histology. Therefore, a more comprehensive analysis of the liver to dietary and temperature effects is recommended.

- Investigate the application of oxygenation to commercial sea cages to improve dissolved oxygen levels, to improve growth, feed utilisation and FCR.

- Improvements in treating skin and gill fluke infestations in cultured Yellowtail Kingfish are also necessary.

Combined, new information provided from these areas of research may contribute to increased productivity and reduced operating costs of the Australian Yellowtail Kingfish aquaculture industry, thereby leading to its expansion.

Chapter 8. Planned Outcomes

The planned outcome of the project was to generate information from a series of tank based experiments to assist Clean Seas attain:

- A reduction in FCR of 0.45 units averaged across the entire production cycle.
- A 10% improvement in growth.
- Improved fish health and reduce mortality by 2%.
- Decreased cost of production by improving feed rates during winter by 2%.

In order to achieve the planned outcomes, four Objectives were established in consultation with Clean Seas, and the participating feed company Ridley Aquafeed. A series of tank-based trials (Chapters 2, 3 and 4) were designed and carried out to completion. The four Objectives, along with the outcome for each, are provided below.

Objective 1.
Identify or improve production diets in a tank based setting using large fish (> 1.5 kg) to provide information to reduce FCRs for Clean Seas Yellowtail Kingfish production by 0.45 units across the entire sea cage production cycle.

Objective 2.
Identify or improve production diets in a tank based setting using large fish (> 1.5 kg) to provide information to gain a 10% improvement in growth for Clean Seas Yellowtail Kingfish in sea cage production systems.

Objectives 1 and 2 were achieved.

- Feed conversion ratio generated in tank-based experiments always exceeded the targeted reduction in FCR of 0.45 units. New dietary formulations and the manipulation of feeding strategies provided important information with regard to improving growth feed utilisation and FCR. Results also indicated potential monetary gains may be made with careful feed management. The positive growth and feed utilisation obtained in Trials 1 and 2 (Chapters 2 and 3) resulted in improved FCR in the tank-based trials which were always ≤ 2.0. These FCR compared favourably to the FCR recorded for the same cohort of fish grown in Clean Seas sea cages over the corresponding period. Growth and FCR improvements were not only attributed to improved diet formulations and feeding strategies, but also to increased levels of dissolved oxygen in the tank-based culture systems. To realise the same improvements on farm in sea cage systems the feasibility of oxygenation of sea cages needs to tested.

- Increased FM and FO substitution in Yellowtail Kingfish diets should also reduce diet ingredient costs by ~\$100 t^{-1}. Fish meal and FO substitution should also improve environmental sustainability by reducing the fish in fish-out ratio by 25%, to 50%.

Objective 3.
Identify or improve production diets in a tank based setting using large fish (> 1.5kg) to provide information to improved Yellowtail Kingfish health and reduce mortality by 2% in Clean Seas sea cage production systems.

Objective 3 was achieved.

- Results from the tank-based trials (Chapters 2 and 3) indicated that fish health was good and survival was positive in response to the diets tested. There were no visual signs of disease observed in either tank-based trial. Mortality of large Yellowtail

Kingfish due to nutritional shortcomings may be reduced to practically zero if diets are formulated to provide essential nutrients. However, compared to the tank-based environment, sea cage conditions are more stressful on fish and in order to validate tank results the new diets need to tested on-farm over a growing season.

Objective 4.
Improve feeding strategies in a tank based setting using large (> 1.5 kg) Yellowtail Kingfish to provide information to improve Yellowtail Kingfish growth and feed utilisation by 2% in Clean Seas sea cage production systems during winter.

Objective 4 was achieved.

- Based on results from Trial 3 (Chapter 4), significant improvements in Yellowtail Kingfish growth performance and feed utilisation may be achieved by feeding the Ridley Clean Seas 2014 Pelagica diet to apparent satiation six days week^{-1} during winter, compared to lower feed rates investigated in this project. However, further improvements to feed management, by investigating other feed rates during winter (i.e. three to five days week^{-1} to apparent satiation) may also improve feed management and production costs. Clean Seas adopted new winter feeding strategies based on results from Trial 3.

Overall, information generated during this project should aid in reducing production costs for Clean Seas, and other Yellowtail Kingfish producers. The Australian Yellowtail Kingfish industry now has the confidence to use newly formulated commercial diets, produced by Australian feed companies, to improve the growth performance, feed utilisation, survival and productivity. Additionally, Clean Seas, Ridley Aquafeed, Skretting Australia and research providers worked closely to achieve these goals. All groups have identified the importance of nutritional research in relation to growth, health and improved productivity for Yellowtail Kingfish production. The confidence developed in gaining a significant return on their research and development investment during this project has resulted in Clean Seas management deciding to pursue this line of research into the future.

Of note, Australian Yellowtail Kingfish producers and researchers have agreed on the need to increase our understanding of Yellowtail Kingfish nutrition, physiology and health. This has led to the submission and successful funding of a larger project investigating nutritional requirements, feeding strategies and nutrition health of Yellowtail Kingfish through the Fisheries Research and Development Corporation (FRDC) as part of the Rural Research and Development for Profit Programme, Australian Department of Agriculture and Water Resources. The Federal Government project commenced in July 2015 and will be comprised of industry and research partners from South Australia and New South Wales, with an aligned project through the FRDC broadening collaboration to involve a Western Australian industry and research provider.

AS-CRC Output

Public Benefit Outcomes

The majority of the research described in this project has been extended to Clean Seas, other Yellowtail Kingfish producers, and Ridley Aquafeed. Project information was also extended to other members of the aquaculture and feed industry, government departments, the general public and members of the scientific community. Information will also be disseminated at domestic and international scientific conferences, AS-CRC and industry workshops and directly to feed company representatives.

There has been a large student training component in this project. This component has resulted in the training of more than 50 undergraduate extra-mural work experience students, Two Honours student (Appendix 2), and the partial training of five PhD students involved in running and sampling of fish from trials in this project. A major benefit of the student training component is the output of new industry entrants, trained with relevant skills that will contribute to future industry development. Several students have already obtained work within the industry, including Matthew Bansemer, who has recently graduated with a PhD from Flinders University, and has taken up a position with SARDI Aquatic Sciences involving further nutritional research of Yellowtail Kingfish.

Private Benefit Outcomes

The major private outcome of this project is the improved formulation and manufacture of diets for large Yellowtail Kingfish (> 1.5 kg). Clean Seas, in collaboration with commercial feed companies, have acted on the recommendations provided within this report, to provide significant improvements in FCR and survival for the production of Yellowtail Kingfish in sea cage operations.

All information from this project has been extended to Clean Seas and staff at Australian feed companies, as it has come to hand, and they have acted on it to improve the sustainable production of large Yellowtail Kingfish.

A large amount of information was also generated with regard to Yellowtail Kingfish growth, feed utilisation, feed management and survival at optimal summer water temperatures (Chapters 2 and 3) and sub-optimal winter water temperatures (Chapter 4). This information has been adopted by Clean Seas and has led to significant improvements in productivity. The information may also form the basis to design and evaluate strategies to improve the growth rate and feed utilisation of large Yellowtail Kingfish in oxygenated sea cage systems.

Peripheral research associated with the project will also provide an insight into lipid metabolism and physiology in relation to feeding strategies and energy levels in large Yellowtail Kingfish at optimal summer water temperatures and sub-optimal winter water temperatures. This information is essential in developing feeding practices on-farm.

Linkages with AS-CRC Milestone Outcomes

The outcomes from this project have addressed the following AS-CRC Milestone outcomes:

1.3. Removal or reduction of key production constraints in selected aquaculture systems;
   1.3.4. New low-cost aquaculture diets targeting improved feed conversion developed and evaluated.
   1.3.5. Production efficiency gains from genetic, health management and nutritional interventions quantified to inform long-term strategies and estimate commercial benefits.

1.5. Production interventions that add value to Australian Seafood:
   1.5.1. Diets contributing to enhanced product quality developed for at least two aquaculture species.

Chapter 9. Conclusion

In collaboration with Clean Seas, Ridley Aquafeed and Skretting Australia, the overarching outcome of this project was to develop diet formulations and feeding strategies that delivered improvements in growth, FCR, health and survival, to ultimately reduce production costs of large Yellowtail Kingfish (> 1.5 kg). The results presented in this report indicate that this project outcome was successfully achieved.

Domestic commercial diets formulated to meet the recommended nutritional requirements of Yellowtail Kingfish (Stone and Bellgrove, 2013) were successfully benchmarked against a highly regarded international Japanese Yellowtail diet. Results indicated positive performance of the locally formulated and manufactured diets, which provides confidence to feed Australian diets to Yellowtail Kingfish at Clean Seas, and at other producers of Yellowtail Kingfish.

Based on results from the project, dietary inclusion of fish meal can be successfully reduced for Yellowtail Kingfish by 50%, from a total dietary inclusion level of 40% to 20%. This was achieved while maintaining positive growth, health and survival of Yellowtail Kingfish. Furthermore, dietary fish oil levels can be reduced by 50% to 75% from a total dietary inclusion level to 7% by utilising poultry oil. It should be noted that the ∑long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) levels were maintained above 2 g 100 g^{-1} diet, as below this level Yellowtail Kingfish growth is impaired. We recommend a minimum dietary inclusion of 7% FO combined with the small amount of LC n-3 PUFA provided in the FO component of FM to supply adequate dietary LC n-3 PUFA (≥ 2 g 100 g^{-1} diet) and meet the dietary requirements for Yellowtail Kingfish.

In this project fish were fed their ration in one period between 0930 and 1100 h, the importance of careful feed rate management was highlighted. During summer, Yellowtail Kingfish may be fed to sub-satiation (80% apparent satiation) to capitalise on superior feed utilisation and nutrient deposition. It is important to note however, that Yellowtail Kingfish fed slightly below this rate (75% apparent satiation) for the first period of Trial 2 and then slightly above this rate (85% apparent satiation) exhibited inferior growth performance, compared to fish fed to apparent satiation. Therefore, if these feed management strategies are implemented on-farm to improve feed utilisation and nutrient deposition of large Yellowtail Kingfish, it is important that care is ensued to not compromise growth by under feeding fish. With regard to feed rate management during winter, Yellowtail Kingfish fed the Ridley Clean Seas 2014 Pelagica diet to apparent satiation six days $week^{-1}$ exhibited significantly higher growth rates and numerically superior FCR, compared to fish fed the same diet at lower feed rates. However, limited fish growth during winter in Trial 3 was observed, and it may be economically beneficial to feed a maintenance ration during the period of low growth. This may be achieved by feeding fish to apparent satiation two days $week^{-1}$. Before Yellowtail Kingfish are fed a maintenance ration under culture conditions, further research to understand the effect this would have on final harvest weight and nutritional deficiencies is needed.

With regard to feeding Sardines to Yellowtail Kingfish, while the growth and FCR of fish fed Sardines every second day and those fed the Ridley Clean Seas 2014 Pelagica diet to apparent satiation six days $week^{-1}$ were similar, feeding Sardines to Yellowtail Kingfish does not represent a sustainably viable option for the production of Yellowtail Kingfish. For example, the fish in fish-out ratio, a common measure for the sustainable use of marine ingredients (Jackson, 2009), was 24% higher for fish fed Sardines than those fed the Ridley Clean Seas 2014 Pelagica diet when fed to apparent satiation 6 d $week^{-1}$. Clean Seas promote their company as sustainably producing premium Yellowtail Kingfish; feeding Sardines does not align with these values, and may also reduce customer perceptions.

Further considerations of these issues are needed before feeding Sardines to Yellowtail Kingfish under commercial conditions.

Overall, this project has provided the Yellowtail Kingfish aquaculture industry with new information to achieve significant improvements in productivity across the entire grow-out period.

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Chapter 10. Appendices

Appendix 1. Effects of diet on cellular immune parameters of Yellowtail Kingfish pre- and post-hypoxic stress

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Abstract

Dietary deficiencies and replacement of fish meal (FM) and fish oil (FO) in diet formulation can have significant impacts on fish health. Differential sub-populations count, total mortality, phagocytic rate/capacity and oxidative activity of leucocytes isolated from the head-kidney of Yellowtail Kingfish fed with four different diets were assessed by flow cytometry pre- and post-hypoxic stress. Three sub-populations of leucocytes with increasing size and internal complexity were identified respectively as P1, P2 and P3. Cells size in sub-population P3 was significantly larger 48 h post-hypoxic stress for fish fed with Diets 1, 2 and 3 and cells granularity in sub-population P1 was significantly more complex for fish fed with Diets 2, 3 and 5. The effect of hypoxic stress on total leucocyte mortality, phagocytic rate and oxidative activity was considered significant. Total leucocytes mortality post-hypoxic stress was significantly lower for fish fed Diet 5 compared to Diet 1. While the total leucocyte mortality post-hypoxic stress was significantly lower in all diets except in Diet 1, the phagocytic rate only increased in Diet 1 and Diet 5 post-stress. No differences in the phagocytic capacity were observed pre- and post-hypoxic stress in any of the diets tested. The leucocyte respiratory burst activities in Yellowtail Kingfish fed with Diet 1 were significantly lower than fish fed with Diet 5 in normoxia condition. A decrease in the oxidative activity 48 h post-hypoxic stress was observed within each of the diets, although it was only significantly different in Diet 3 and Diet 5 formulations. Our results suggest a high sensitivity of Yellowtail Kingfish immune cells to hypoxia, which were dependent on the diet administrated, with marked effects observed with Diet 1 and Diet 5.

Background

Yellowtail Kingfish (*Seriola lalandi*) is an important species for Australian aquaculture (Padula et al., 2012). Kingfish are carnivorous and currently diets require high levels of high quality fish meal (FM) and fish oil (FO) for optimal performance (Booth et al., 2010). However, price and environmental sustainability are major drivers towards reduction of FM and FO in diets. Therefore, alternate protein and lipid sources are under investigation (Bowyer et al., 2013). Research into novel diet formulation is essential in order to maximize feed conversion ratio (FCR), whilst minimising any deleterious effects on fish health and flesh quality. Diet formulation based on terrestrial proteins and oils can substantially impact fish health. For example, in Atlantic salmon, some types of soy protein may cause gastrointestinal dysbiosis and mortality (Green et al., 2013; Uran et al., 2008a; Uran et al., 2008b) and in seabass, fish oil (FO) replacement with vegetable oils substantially impedes immune function (Montero et al., 2010; Montero et al., 2008). In the current study, the cellular immune function of Yellowtail Kingfish fed four experimental diets was measured pre- and post-hypoxic stress.

Material & Methods

Experimental diets
Diet 1 – Gold standard diet.
Diet 2 – Existing Ridley Clean Seas 2013 Pelagica diet.
Diet 3 – Ridley Trifecta soya diet.
Diet 5 – Reformulated Ridley Pelagica diet.

Experimental system
Yellowtail Kingfish from Trial 1 conducted at South Australian Research and Development Institute Aquatic Sciences Center (SARDI ASC, Adelaide, South Australia), were maintained in 5000 L tanks supplied with recirculating seawater. Fish had been fed to apparent satiation once daily for a period of 4.5 months on one of four experimental diets (named diet numbers 1, 2, 3 and 5), with each diet fed to fish in three replicate tanks. An acute hypoxic and crowding stress was applied by stopping the water supply, which lowered the water level to ~ 30 cm deep for a period of 15 min. Three fish were sampled per tank (nine fish diet^{-1}) to measure cellular immune parameters prior to hypoxic stress and 48 h post hypoxic stress to determine recovery response to stress.

Head-kidney isolation preparation
Fish were killed using an overdose of anesthetic (Aqui-S) and bled via the *vena caudalis* for serum and plasma collection. Gill arches were further sectioned to completely bleed the fish. The head-kidney (HK) (1 g sample) from the left side of each fish was removed aseptically and placed in 5 mL L-15 Leibovitz medium supplemented with 100 units mL^{-1} penicillin streptomycin (P/S), 2% foetal bovine serum (FBS) and 10 units mL^{-1} heparin. The tubes were placed on ice until the last fish was processed. The HK of each fish was cut into small piece and gently pushed through a 100 μL cell strainer with the plunger of a 1 mL tuberculin syringe into a sterile 50 mL tube to obtain a single cell suspension in supplemented medium. Each fish HK cell suspension was adjusted to a final concentration of 2 x 10^6 cell mL^{-1}.

Flow cytometry
Analysis of Head-Kidney Leucocyte (HKL) levels by flow cytometry
The BD FACSCanto II ™ cell analyzer flow cytometer instrument (BD Biosciences, NSW, Australia) was used to collect and analyse data on cellular functions from all samples used in this study. Voltages and thresholds were set up and optimized for each experiment before proceeding with experimental samples. Differential leucocyte sub-populations count was determined based on their forward and side scatters properties. A total of 25000 events were recorded for the phagocytosis and oxidative activity assays and 20000 events for the viability

assay. All assays incubation steps were carried out in dark condition at room temperature and samples put on ice to stop the reaction until being processed by flow cytometry.

Leucocyte characterization and mortality by flow cytometry
Morphological characteristics of the different sub-population of HKL were expressed as the forward scatter (FSC) and side scatter (SSC) values in arbitrary units (A.U.). FSC is related to the size of the particles, whereas SSC represents the internal complexity or granularity of the particles. Leucocyte mortality was measured according to Tumbol et al. (2009) and Aviles et al. (2013) with some modifications. Briefly cell viability was assayed using a dual stain of propidium iodide (PI) & SYBR Green I (Sigma, Castle Hill, Australia) that were added to each sample (300 μL) to a final concentrations of 20 μg mL^{-1} and 10X (1/1000 dilution of the DMSO-solubilized commercial solution), respectively. Samples were incubated for 2 h. HKL fixed with 4% formaldehyde was used as a positive control for dead cells stained with PI. Stained cells were analysed measuring green and red fluorescence emissions at 530 nm and 610 nm using the 488 and 561 nm excitation lasers respectively.

Flow cytometry assay of phagocytosis
The assessment of Yellowtail Kingfish HKL phagocytic rate and capacity was performed using a commercial preparation of 2 µm diameter latex fluorescent beads (Fluoresbrite TM plain Yellow Green 2.0 µm microspheres 2.5% solid-latex, Polysciences Inc). Cells were incubated with beads (1:500 dilution) for 2 h before analysis by flow cytometry. Phagocytic rate was calculated as the percentage of leucocytes that have ingested three or more fluorescent beads (Haugland et al., 2012). Phagocytic capacity was defined as the average number of fluorescent beads engulfed per leucocyte engulfing at least three beads.

Flow cytometry assay of respiratory burst
Oxidative activity of leucocytes isolated from the HK of Yellowtail Kingfish was evaluated pre- and post-hypoxic stress following incubation with a formalin killed *Photobacterium damselae* ssp. *damselae*, a gram-negative bacterium isolated from Yellowtail Kingfish in 2010. The bacteria were grown on tryptic soy agar (TSA) from stock, and then a single colony-forming unit (CFU) was inoculated in tryptic soy broth (THB) and grown overnight until late exponential phase. Optical density at 600 nm was fixed at 1 and formalin-killed bacterins prepared as previously described (Aviles et al., 2013). Bacteria were added to HKL at an approximate multiplicity of infection (MOI) of 1. To determine the leucocyte respiratory burst activity, linked to the presence of reactive oxygen species (ROS), a solution of the cell-permeable fluorogenic probe 2',7'-Dichlorodihydrofluorescein diacetate (DCFH-DA, Sigma–Aldrich) at a final concentration of 5 µM was added to 300 µL of leucocyte suspension (Hamoutene et al., 2009). Leucocytes stimulated with the specific activator of protein kinase C (PKC), phorbol myristate acetate (PMA) were used as a positive control. Mixtures were incubated for 2 h and DCF green fluorescence emission (530 nm), directly linked to the oxidative activity within each cell, was measured using a 488 nm excitation laser. Results are expressed as the median geometric fluorescence $cell^{-1}$ (in arbitrary units, A.U.). This activity was calculated for the entire HKL populations once debris and doublets were removed.

Statistical analyses
Data analyses was performed on GraphPad Prism version 6.0C for Mac OS X (GraphPad Software, San Diego California, USA). Data from the oxidative activity, phagocytosis, viability assays and the leucocyte characterisation were analysed by a Two-factor analyses of variance (ANOVA) with factor being diet (4 diets) and treatment (normoxia and hypoxia). Specific differences between treatments were identified using Tukey's *post-hoc* tests (between diets pre- and post-hypoxic stress) and using the Holm-Sidak's multiple comparison tests (within each diet before and after stress). Results were presented as mean ± standard error (viability and phagocytosis assays) and as the median ± standard error (oxidative activity assay).

Results

Immunological analyses
Leucocyte characterisation and viability
When analysed by flow cytometry, head-kidney leucocytes from Yellowtail Kingfish displayed three main cell populations with distinct forward and side scatter (FSC/SSC) characteristics (Figure A1).

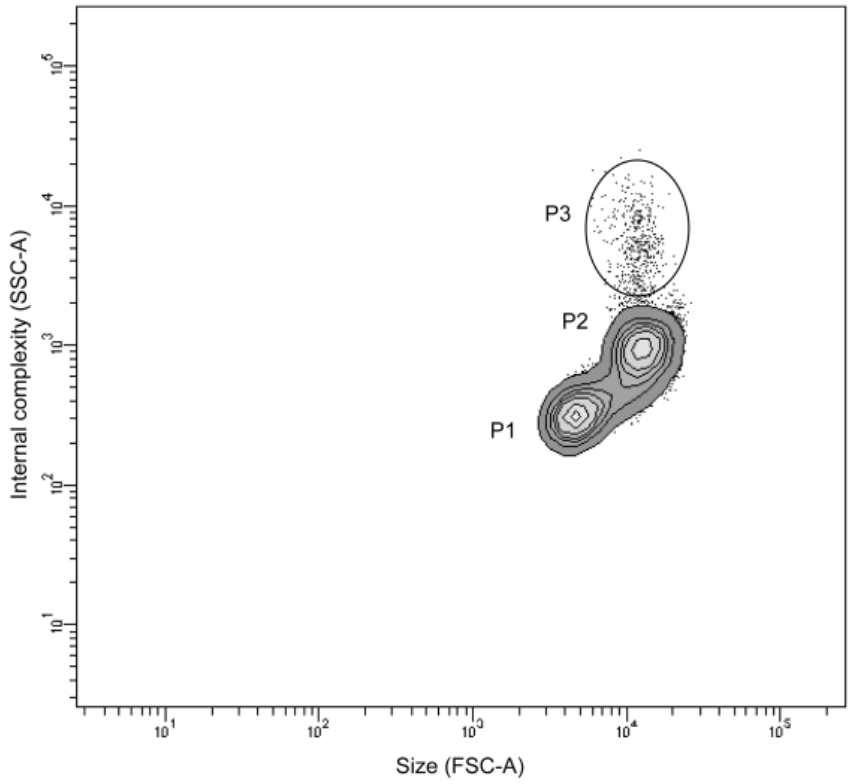

Figure A1. Representative dot plot analysis of Yellowtail Kingfish HKL based on the size (FSC-A value) and internal complexity (SSC-A value) obtained by flow cytometry. Three main populations of cells could be differentiated based on their scatter properties: P1, P2 and P3. The debris and doublets were removed from the analysis and are not displayed on the dot plot.

The small and less complex population (low FSC/SSC) was designated as P1 and the populations with increasing FSC/SSC values as P2 and P3 respectively (Figure A1). While total leucocyte count was slightly higher post-hypoxic stress in all diets (except for Diet 2), it was only significant for Diet 3 (P = 0.023) (Table A1). The proportion of P1 and P3 decreased post-hypoxic stress, in all the diets used in this study, although it was not statistically significant. P1 was the most abundant cell sub-population, averaged across all diets, comprising 60.6% and 52.1% of the leucocytes, pre- and post-hypoxic stress respectively (Table A1). P3 was the least abundant sub-population with an average of 3.6% and 2.5% before and after stress respectively (Table A1). The mean proportion of cells in P2 across all the diets increased from 35.3% and 45% pre- and post-stress respectively, and was considered only significant for fish fed with Diet 3 (P = 0.020) and Diet 5 (P = 0.017) (Table A1).

Table A1. Total leucocyte count (TLC); characterization of leucocytes sub-populations P1, P2 and P3: percentage of cell types, size (FSC-A), internal complexity (SSC-A) and leucocytes sub-population mortality for each diet before and 48 h after hypoxic stress. Values are expressed as the median ± standard error (SE) (n = 9). Bold and italic letter with grey background indicates a significant difference between fish fed a particular diet before and after stress ($P < 0.05$, One-factor ANOVA). * Represents $P < 0.05$, ** $P < 0.005$, *** $P = 0.001$ and **** $P < 0.0001$. A.U.: fluorescence arbitrary units.

		Before hypoxic stress				48h after hypoxic stress			
		Diet #1	Diet #2	Diet #3	Diet #5	Diet #1	Diet #2	Diet #3	Diet #5
TLC (total cell numbers)		8811.7 ± 904.7	10056 ± 504.9	***7847.9 ± 588.3****	9091.8 ± 976	9963.1 ± 316.1	9909.9 ± 312.8	***10265.7 ± 371.4****	10485 ± 399.3
Percentage of cell types (%)	P1	53.5 ± 6.6	60 ± 3.2	64 ± 5.9	65.2 ± 3.9	51.7 ± 1.8	54 ± 2.1	51 ± 2.1	51.7 ± 2.4
	P2	41.7 ± 4.9	36.1 ± 3.2	***32.1 ± 5.4****	***31.4 ± 3.7****	45 ± 1.8	43.3 ± 2.1	***46.1 ± 2.0****	***45.8 ± 2.3****
	P3	4.4 ± 1.9	3.5 ± 0.3	3.6 ± 0.8	3.0 ± 0.4	3.0 ± 0.5	2.4 ± 0.3	2.5 ± 0.3	2.2 ± 0.2
Size (A.U.)	P1	5561.8 ± 105.8	5365.4 ± 78.4	5383.4 ± 79.3	5436.1 ± 70.1	5502.3 ± 66.6	5603.1 ± 44.7	5527.4 ± 50.2	5567.3 ± 51.1
	P2	13841.3 ± 595.8	13164.9 ± 219.2	12929.7 ± 220.0	13015.6 ± 209.2	13629.9 ± 118.9	13589.3 ± 153.2	13884.9 ± 134.5	13878.8 ± 151.6
	P3	***12100.6 ± 283.3****	***12183.2 ± 320.3****	***12209.3 ± 406.3****	12477.7 ± 279.0	***13262.1 ± 106.3****	***13486.8 ± 243.2****	***13517.4 ± 275.1****	13087.0 ± 298.9
Internal complexity (A.U.)	P1	372.4 ± 4.5	***357.9 ± 5.3*****	***355.6 ± 3.5****	***354.8 ± 2.3****	377.7 ± 3.2	***377.7 ± 2.7*****	***373.2 ± 3.3****	***373.3 ± 2.9****
	P2	1085.2 ± 45.9	1126.8 ± 49.4	1061.3 ± 20.9	1041.4 ± 44.2	1092.0 ± 20.9	1178.8 ± 33.7	1147.8 ± 19.2	1147.0 ± 30.4
	P3	4254.2 ± 271	3936.4 ± 249.1	4207.3 ± 229.7	4091.0 ± 275.2	4702.2 ± 145.9	4328.3 ± 232.3	4557.9 ± 177.1	4444.8 ± 303.9
Total mortality (%)									
Percentage of cells types	P1	***27.1 ± 2.5*****	***19.6 ± 2.0****	***16.6 ± 1.8****	***18.8 ± 2.4****	***15.8 ± 2.4*****	***10.5 ± 1.4****	***8.8 ± 1.6****	***8.2 ± 1.4****
involved in mortality (%)	P2	53.7 ± 6.2	***50.7 ± 2.8****	***51.2 ± 3.1*****	***54.1 ± 4.9******	40.4 ± 4.2	***32.6 ± 5.0****	***25.4 ± 4.3*****	***22.5 ± 3.3******
	P3	97.9 ± 0.6	98.3 ± 0.4	***98.1 ± 0.7****	***98.2 ± 1.2****	95.5 ± 1.5	78.9 ± 8.9	***71.6 ± 10.2****	***72.4 ± 10.5****

P3 was composed of larger cells compared to cells in P2 and P1 and also displayed the highest internal complexity ($P < 0.001$; Figure A1, Table A1). Cells in P1 were significantly smaller than cells in P2 and P3 and had a lower internal complexity ($P < 0.001$; Figure A1, Table A1). The median size and internal complexity of cells in P1, P2 and P3 were not statistically different between diets, but slightly increased post-hypoxic stress across all diets, with significant differences for the size of cells in P3 post-hypoxic stress for fish fed Diet 1 ($P = 0.023$), Diet 2 ($P = 0.008$) and Diet 3 ($P = 0.008$). Internal complexity of cells in P1 increased post-hypoxic stress for fish fed Diet 2 ($P = 0.001$), Diet 3 ($P = 0.004$) and Diet 5 ($P = 0.002$). Overall the total HKL mortality decreased post-hypoxic stress. Cell mortality was the highest in P3, then in P2 and finally in P1 and were considered different from one another ($P < 0.001$).

Viability

The total head-kidney leucocyte mortality between the different diets during normoxia was not significantly different (ANOVA, Tukey $P > 0.05$) (Figure A2A). However 48 h post-hypoxic stress the percentage of dead leucocytes from fish fed Diet 1 and Diet 5 were significantly different from each other ($P = 0.018$) with higher cell mortality observed in Diet 1 compared to Diet 5 (Figure A2A). Significant differences before and after stress were observed for the mean percentage of dead cell for Diet 2 ($P = 0.028$), Diet 3 ($P = 0.026$) and Diet 5 ($P = 0.002$), with lower mortality recorded post-hypoxic stress in these diets. No statistical difference in cell mortality before and after stress was observed in Diet 1 ($P = 0.131$) (Figure A2A).

Source of variation with % of total variation and P values indicated:

- Interaction (2.1%, $P = 0.547$) – If there is no interaction overall, there is a 55% chance of randomly observing so much interaction in an experiment of this size. The interaction is considered not significant.
- Diet factor (9.818%, $P = 0.025$ *) – If Diet factor has no effect overall, there is a 2.5% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered significant.
- Stress factor (26.48%, $P < 0.001$ ****) – If Stress factor has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered significant.

Leucocyte phagocytosis rate and capacity

Yellowtail Kingfish HKL were capable of phagocytosis of 2 μm latex beads (Figure A2B/C). No significant differences were observed in terms of the phagocytic rates between treatment diets before and after stress (Figure A2B). However within two diets the phagocytic rate significantly increased 48 h post-hypoxic stress, in Diet 1 ($P = 0.044$) and Diet 5 ($P = 0.038$) (Figure A2B). Overall the tendency *observed* across all diets is an increase in the phagocytic rate 48 h post stress. Phagocytosis capacity was defined as the average number of fluorescent beads engulfed per leucocyte ingesting at least 3 beads. No significant differences were observed in term of the phagocytic capacity between the diets and within individual diets before and after stress (Figure A2C).

Source of variation with % of total variation and P values indicated:

- Interaction (1.707%, $P = 0.692$) – If there is no interaction overall, there is a 69% chance of randomly observing so much interaction in an experiment of this size. The interaction is considered not significant.
- Diet factor (3.798%, $P = 0.361$) – If Diet factor has no effect overall, there is a 36% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered not significant.

- Stress factor (19.97%, *P* < 0.001 ***) – If Stress factor has no effect overall, there is a 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered significant.

Leucocyte oxidative activity

The HKL median oxidative activity wasn't significantly different between treatment diets except between Diet 1 and Diet 5 before stress (*P* = 0.032) (Figure A2D), where HKL from fish fed with Diet 5 produced more oxygen radicals than fish fed with Diet 1. While the reactive oxygen species produced tends to decrease 48 h post-stress, the differences were only significant in Diet 3 (*P* = 0.004) and Diet 5 (*P* = 0.029) (Figure A2D).

Source of variation with % of total variation and P values indicated:

- Interaction (2.618%, P = 0.465) – If there is no interaction overall, there is a 46% chance of randomly observing so much interaction in an experiment of this size. The interaction is considered not significant.
- Diet factor (10.31%, *P* = 0.023 *) – If Diet factor has no effect overall, there is a 2.3% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered significant.
- Stress factor (22.33%, *P* < 0.001 ****) – If Stress factor has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered significant.

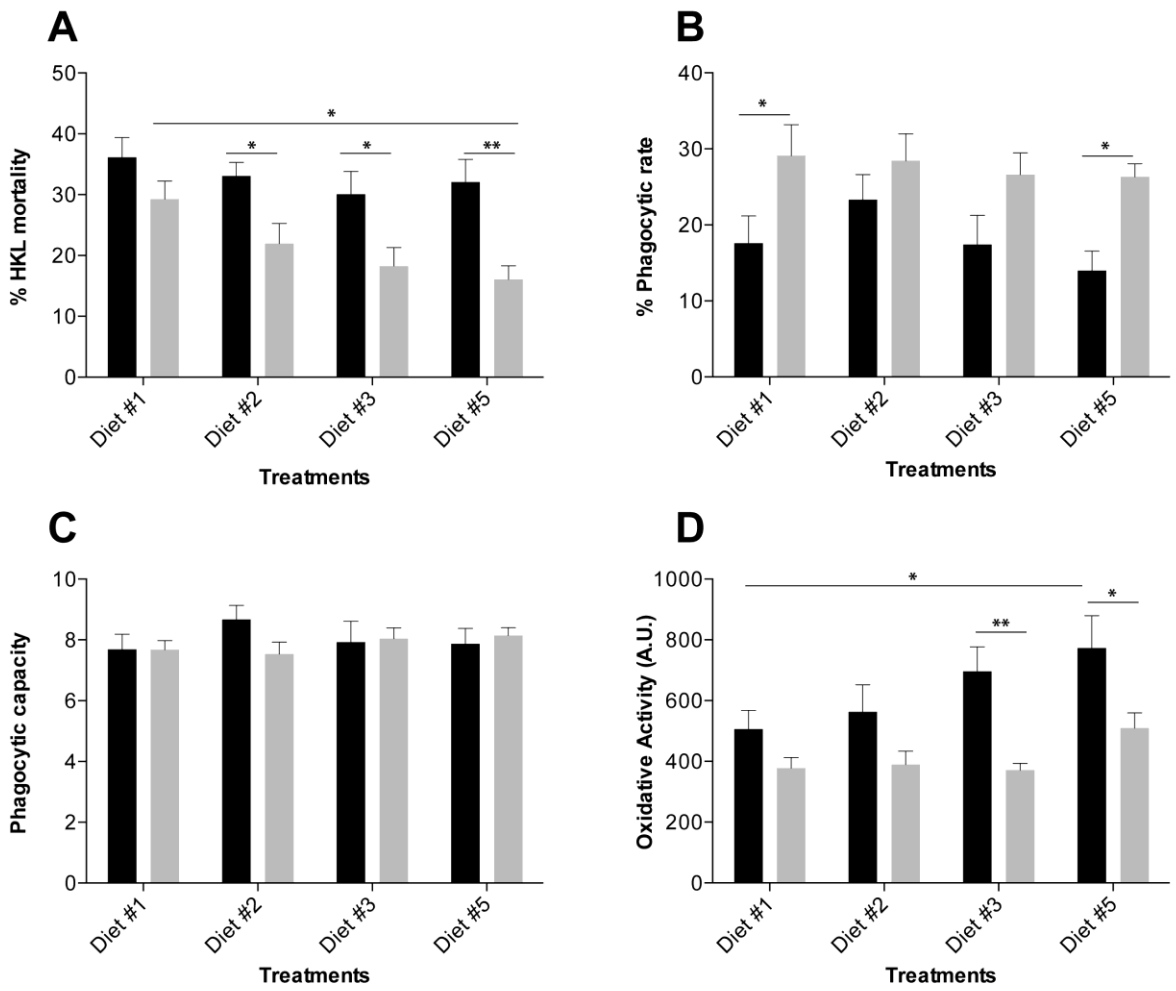

Figure A2. A. Mean percentage of head-kidney leucocytes mortality from fish fed with four different diets, pre- (black bars) and post-hypoxic stress (grey bars). **B.** HKL mean phagocytic rate from those same fish fed with four different diets. **C.** HKL mean phagocytic capacity from those same fish before and 48 h after stress. **D.** Median oxidative activity of the total HKL population from those same fish fed the different diets before and after stress in response to a formalin killed *Photobacterium damselae* sub sp. incubated for 2 h. Values are expressed as the mean ± standard error (SE) for the viability and phagocytosis assays. Oxidative activities are expressed as the median values and are expressed in flow cytometry arbitrary units (A.U). Bold stars ***** indicates a significant difference between treatments ($P < 0.05$, One-factor ANOVA). *Represents $P < 0.05$ and ** $P < 0.005$. Black bars = before stress and grey bars = after stress.

Discussion and tentative conclusions

In this study, we have demonstrated that flow cytometry can be used as a proxy to assess the long-term effect of various dietary formulations on some of the major immune parameters of Yellowtail Kingfish HKL before and after an acute stress (crowding and oxygen depletion). Differences in immune parameters, pre- and post-stress comprised variations in leucocyte sub-population count, morphology and mortality, which were diet dependent.

Stress is known to result in redistribution of immune cells in higher animals including humans (Dhabhar, 2012), with neutrophils being redistributed into the peripheral blood in all five vertebrate taxa (Davis et al., 2008). As neutrophils have a short lifespan (estimated at 120 h in zebrafish (Dixon et al., 2012)) they are likely to represent a significant proportion of dead cells during analysis of mixed leucocyte populations such as those found in the HK in fish. Therefore, their redistribution into peripheral blood from the haematopoietic tissues will affect the relative proportion of the different sub-populations types and reduce the numbers of dead cells detected in head-kidney as revealed in this study. In the future, HKL from Yellowtail Kingfish should be characterised by light microscopy and biochemically in order to confirm the nature of the cells in P1, P2 and P3.

Correlations were observed between the reduction of cell mortality with an increase of the phagocytic rate, as well as a reduction of the ROS production. In *Gadus morhua*, the respiratory burst of white blood cells was reduced after exposure to hypoxia (Hamoutene et al., 2009). Results strongly suggest that diet composition will have significant effects on some of the major non-specific immune functions of Yellowtail Kingfish such as the phagocytosis and oxidative burst activity, which are both critical defence mechanisms in teleost (Ellis, 1977; Secombes and Fletcher, 1992; Whyte, 2007). So we need to bear this in mind when developing new diet formulations for fish that are farmed in open systems where pathogens and environmental stressors can readily affect the animal and compromise their health status.

Dietary deficiencies of protein and FO in diet formulation can give rise to elevate production of ROS (Morales et al., 2004). In this study, during normoxia, fish fed with Diet 5 (reformulated Ridley Pelagica diet, composed of 30% FM and 50% FO) produced significantly more ROS than fish fed with Diet 1 (Gold standard diet, composed of full FM and FO).

Although ROS production by phagocytes is a critical immune defence in all taxa, excess production of free radicals (e.g., hydroxyl radicals and superoxide) and other reactive oxygen species such as hydrogen peroxide can have a deleterious effect on biomolecules (e.g., DNA, amino-acid and protein) and, therefore, must be matched by increased production of antioxidants to avoid damage to host cells (Pérez-Jiménez, 2009).

To conclude, the results from this study have shown that:

- Yellowtail Kingfish HKL mortality was significantly reduced 48 h post-hypoxic stress for fish fed all diets except Diet 1.
- Fish fed with Diet 5 had the lowest HKL mortality when compared with the HKL mortality from fish fed with Diet 1 (post-stress).
- The HKL phagocytic rate significantly increased 48 h post-hypoxic stress for fish fed Diet 1 and Diet 5.
- During normoxia, the HKL respiratory burst activity was significantly lower for fish fed Diet 1 compared to fish fed Diet 5 and was significantly reduced 48 h post-hypoxic stress for fish fed with Diet 3 and Diet 5.

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Appendix 2. Third year student project: Feeding rate, feeding frequency and feed type effects on Yellowtail Kingfish (*Seriola lalandi*) growth homogeneity.

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Abstract

Yellowtail Kingfish (*Seriola lalandi*) aquaculture in Australia is still relatively new. A major problem in culturing carnivorous fin fish in high density in a cages or tanks is intra-cohort aggression. This aggression can lead to size heterogeneity where dominant fish attack smaller subordinate fish, which may induce stress, and reduce the feed intake by smaller fish. Mortality and stress induced growth stunting have been reported in the culture of other fin fish. This research aimed to investigate the effect of reducing feed rate to save production cost, on fish size heterogeneity. Three trials were investigated (Trial 1, 4 treatments; Trial 2, 6 treatments; and Trial 3, 8 treatments). In Trial 1 and 2, the effect on weight, length and condition index distribution using different diets fed at apparent satiation and sub-satiation were investigated. In Trial 3, the weight, length and condition index using the same diet, but at different feeding rates and frequency were investigated. The null hypothesis that reducing feeding rate will result in broader fish size distribution is not entirely rejected as Trial 1 and 2 showed inconsistent result even when feed type was the same. In Trial 3, feed intake closer to or lower than the maintenance feed rate resulted in a more homogenous condition index distribution compared to fish fed above the maintenance feed intake. In conclusion, feeding Yellowtail Kingfish to sub-satiation does not impact on cohort heterogeneity.

Introduction

Yellowtail Kingfish (*Seriola lalandi*) production is becoming one of the main forms of aquaculture in Australia, alongside Southern Bluefin Tuna and Oysters (PIRSA, 2014). In 2009 to 2010, Yellowtail Kingfish aquaculture generated approximately AUD$25 million, compared to oyster aquaculture that generated AUD$37 million (PIRSA, 2014). As Yellowtail Kingfish aquaculture is a relatively recent development in Australia and New Zealand, further improvements in technique are likely (Moran et al, 2007; Love and Langenkamp, 2003).

One of the potential problems with cage cultured carnivorous fish is high death rate due to intra-cohort aggression. This type of behaviour has been recorded in Japanese fin fish culture with Greater Amberjack (*Seriola dummerili*; Papadroulakis et al., 2005) and Japanese Flounder (*Paralichthys olivaceus*; Sakakura and Tsukamoto, 1996). It is suggested that this aggression behaviour might be due to growth depression as size differences among initially similar sized fish increase (Kardi et al., 1996). Larger dominant fish may chase and attack smaller fish, in the form of nipping, which may lead to increased stress and stunted growth (Sloman and Armstrong, 2002). Feeding hierarchies will form in sea cages or tanks where fish density is high. During feeding time, subordinate fish may be denied access to feed as dominant fish dominate feeding activity (McCarthy et al., 1992), thus resulting in decreased access to feed and increased metabolic rates that may lead to reduction in growth (Winberg et al., 1992).

In addition to increase in size heterogeneity, physiological changes such as increase in blood cortisol and blood glucose levels can also occur in subordinate fish in response to stress (Sloman et al., 2001). These changes can affect disease resistance and competitive ability of a fish as blood cortisol level is linked to brain behaviour (Cutts et al., 1999).

All of these factors can be detrimental to fin fish aquaculture and may lead to more undesirable market sized fish per cohort, ultimately impacting profit. One of the techniques used to reduce size heterogeneity is fish grading to prevent dominance of smaller fish by larger fish. However, size grading can be labour intensive and therefore costly.

In this study, the effect of restricted feeding rate on size heterogeneity was investigated.

Materials and methods

Experimental design
Data from Trial 1, 2 and 3 (please refer to Chapters 2, 3, and 4 for precise methods) were used in the current study. Trial 1 used juvenile fin fish hatched from August 2012 and the trial ran from November 2013 to April 2014. Four different feeding treatments were investigated in trial 1 (n = 3 tanks treatment^{-1}) (Table A2.1), a total of 12 tanks and 16 Yellowtail Kingfish in each of the tank were used. Trial 2 used juvenile spawned from August 2013 and the trial ran from November 2014 to February 2015. Six treatments were tested in Trial 2 (n = 3 tanks treatment^{-1}) (Table A2.2), a total of 18 tanks and 19 fish in each tank were used. Trial 3 used juvenile spawned from August 2014, and the trial ran from July 2015 to September 2015. Eight treatments (n = 3 tanks treatment^{-1}) were tested in Trial 3 (Table A2.3), a total of 24 tanks and 21 fish in each tank were used. Trial 1 and 2 investigated the effect of different diets fed at apparent satiation and sub-satiation on size heterogeneity at summer water temperatures. Trial 3 investigated the effect of different feeding rates and frequencies on size heterogeneity of Yellowtail Kingfish at winter water temperatures. There was no separation of sex in all three trials, as Yellowtail Kingfish are not sexually dimorphic.

Table A2.1. Experimental design in Trial 1[1].

Treatment	Diet	Satiation
1	Gold Standard	Apparent satiation (100%)
2	Gold Standard	Sub-satiation (80%)
5	Reformulateed Ridley Pelagica Diet	Apparent satiation (100%)
6	Reformulateed Ridley Pelagica Diet	Sub-satiation (80%)

[1] Feeds offered to fish once daily.

Table A2.2. Experimental design in Trial 2[1].

Treatment	Diet	Feeding frequency
1	Reformulated Ridley Pelagica Diet	7 days per week to apparent satiation
2	Reformulated Ridley Pelagica Diet	Apparent satiation on Saturday, starve on Sunday, 80% satiation on Monday to Friday
4	Ridley (High fat / lower protein) Diet	7 days per week to apparent satiation
5	Ridley (High fat / lower protein) Diet	Apparent satiation on Saturday, starve on Sunday, 80% satiation on Monday to Friday
7	Hiramasa	7 days per week to apparent satiation
8	Hiramasa	Apparent satiation on Saturday, starve on Sunday, 80% satiation on Monday to Friday

[1] Feeds offered to fish once daily.

Table A2.3. Experimental design in Trial 3[1].

Treatments	Type of feed	Feed rate	Feeding frequency
1	Pellet	Appetite	Six days per week
2	Pellet	Appetite	Twice per week
3	Pellet	Appetite	Once per week
4	Pellet	0.1% BW per day	Once per week
5	Pellet	0.65% BW per day	Twice per week
6	Pellet	0.35% BW per day	Twice per week
7	Pellet	0.12% BW per day	Six days per week
8	Sardines	Appetite	Every second day

[1] Full ration provided at one feeding time.

Statistical analysis

The weight and length data analysed were from Trial 1, 2 and 3 (Chapter 2, 3 and 4). Fish weight and fork length were measured and condition index were calculated from the fish weight and length:

Condition index = (fish weight [g] / fish length [cm]^3) × 100

Data were analysed using SPSS version 20. Growth in terms of final fish weight, length and condition index were plotted. The Shapiro Wilk test was used to evaluate homogeneity of variance in each treatment based on the null hypothesis that reducing feeding rate and feeding frequency will result in a skewed distribution if $P > 0.05$.

Results

Trial 1

Treatments 1, 2 and 5 did not have significant effect of normality on weight data ($P > 0.05$; Table A2.4.1). Treatment 6 had a significant effect of normality of weight data ($P = 0.020$). All 4 of these treatments had negative skewness, with Treatment 6 having the greatest skewness, which was -1.10.

With regard to length, there was no significant difference between fin fish fed to apparent satiation and sub-satiation ($P > 0.05$; Table A2.4.2). The skewness of fish fed Diet 1 was lower than those fed Diet 2. On the other hand, fish fed Diet 2 to apparent satiation and sub-satiation showed a significant difference in distribution ($P<0.05$) (Table A2.4.2). The skewness ranged from 1.012 for apparent satiation to -1.051 for sub-satiation.

With regard to condition index, Treatment 5 had a significant effect on distribution ($P < 0.001$) (Table A2.4.3). This was due to a few fish that had a higher condition index (condition index value 2.0-2.2) compared to others within the same treatment. There was no significant difference in condition index distribution for the other three treatments. Treatments 1, 2 and 4 had a negative skewness of -0.196, -0.381 and -0.548, respectively (Table A2.4.3).

Trial 2

The weight distribution of Yellowtail Kingfish was not significantly different in Trial 2 ($P > 0.05$) (Table A2.5.1). The largest skewness for weight distribution was observed in fish fed Diet 1 to sub-satiation (-0.566; Table A2.5.1). The length distribution represented significant difference for Treatments 2, 4 and 7 ($P < 0.05$; Table A2.5.2). For example, fish fed Diet 1 and 5 to apparent satiation showed positive skewness, 0.160 and 0.972, while fish fed to sub-satiation showed negative skewness, -0.895 and -0.164, respectively (Table A2.5.2). The condition index distribution was not significantly different ($P > 0.05$), except for fish fed Treatment 8 ($P = 0.001$; Table A2.5.3). In terms of skewness, fish fed Diet 3 had a narrower distribution to those fed Diet 5.

Trial 3

There was no significant difference of weight distribution between treatments in Trial 3 ($P > 0.05$; Table A2.6.1). The length distribution of fish in Trial 3 was also not significantly influenced by treatment, except Treatment 2 ($P = 0.037$; Table A2.6.2). In contrast, the distribution for the condition index of the four treatments was significantly skewed ($P < 0.05$; Table A2.6.3). However, the skewness range varied among treatments. The greatest skewness was observed for Yellowtail Kingfish fed Treatment 8 (Sardine diet), which had a skewness of 1.661, while the smallest skewness was observed in fish fed Treatment 1 (pellet diet to apparent satiation six days week^{-1}), which had a skewness of -0.918.

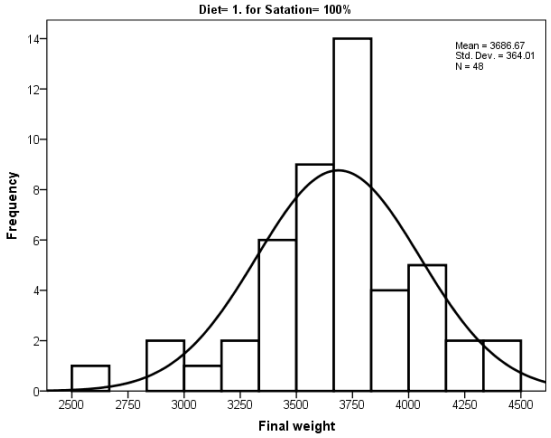

Figure A2.1.1. Weight distribution of fish fed with Diet 1 to apparent satiation in Trial 1. Distribution line shows the normal distribution curve.

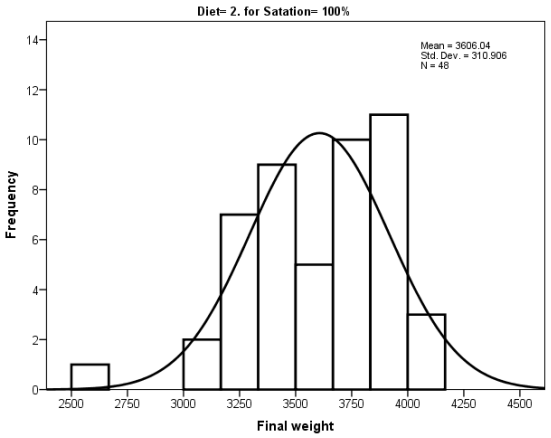

Figure A2.1.2. Weight distribution of fish fed Diet 2 to apparent satiation in Trial 1. Distribution line shows the normal distribution curve.

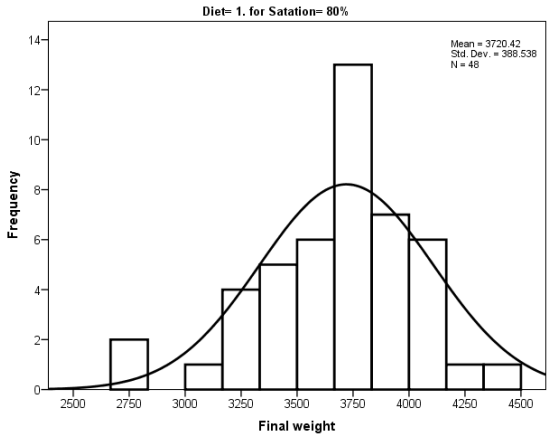

Figure A2.1.3. Weight distribution of fish fed Diet 1 to sub-satiation in Trial 1. Distribution line shows the normal distribution curve.

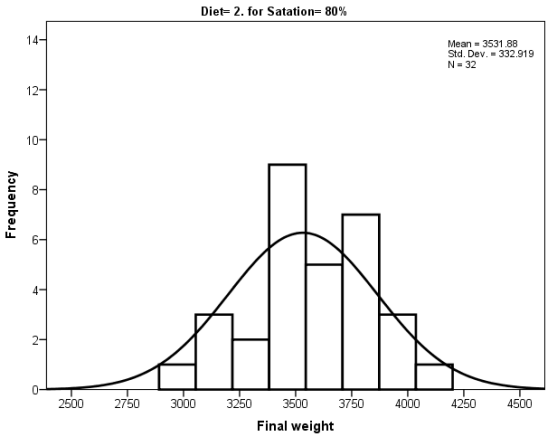

Figure A2.1.4. Weight distribution of fish fed with Diet 2 to sub-satiation in Trial 1. Distribution line shows the normal distribution curve.

Table A2.4.1. Shapiro-Wilk test and Skewness value for weight distribution of yellowtail kingfish fed Diet 1 and 2 to apparent satiation and sub-satiation in Trial 1.

Diet type	Diet name	Satiation	Shapiro-Wilk test		
			Df	Sig. value	Skewness
1	Gold Standard	Apparent	48	0.637	-0.337
		Sub	48	0.215	-0.406
2	Reformulated Ridley Pelagica Diet	Apparent	48	0.020	-0.720
		Sub	32	0.065	-1.103

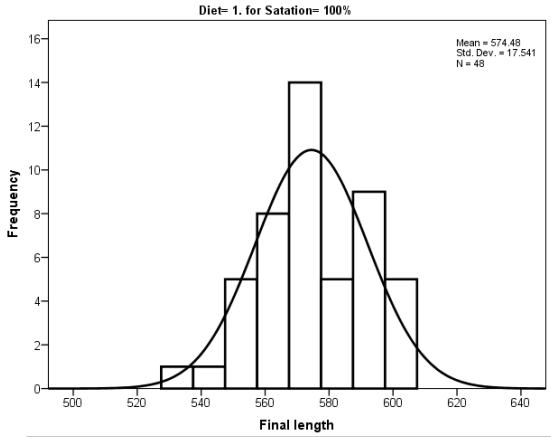

Figure A2.1.5. Length distribution of fish fed Diet 1 to apparent satiation in Trial 1. Distribution line shows the normal distribution curve.

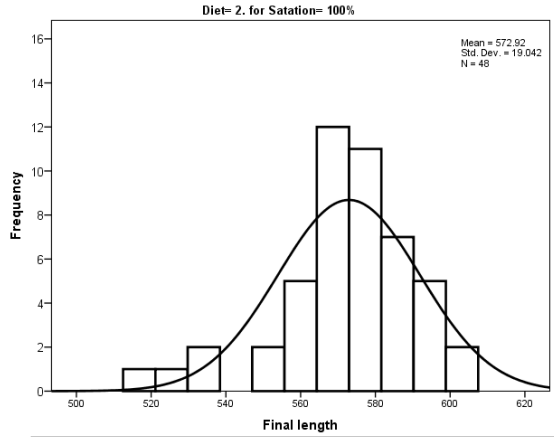

Figure A2.1.6. Length distribution of fish fed Diet 2 to apparent satiation in Trial 1. Distribution line shows the normal distribution curve.

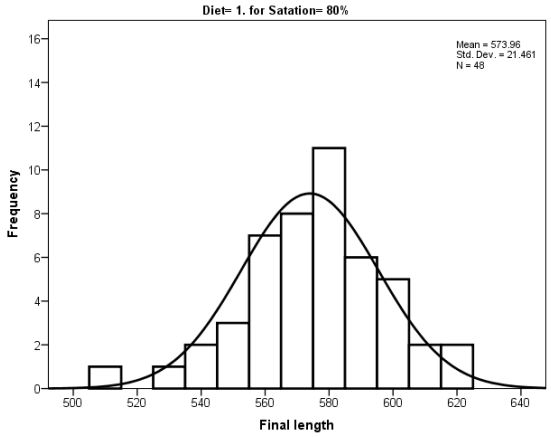

Figure A2.1.7. Length distribution of fish fed Diet 1 to sub-satiation satiation in Trial 1. Distribution line shows the normal distribution curve.

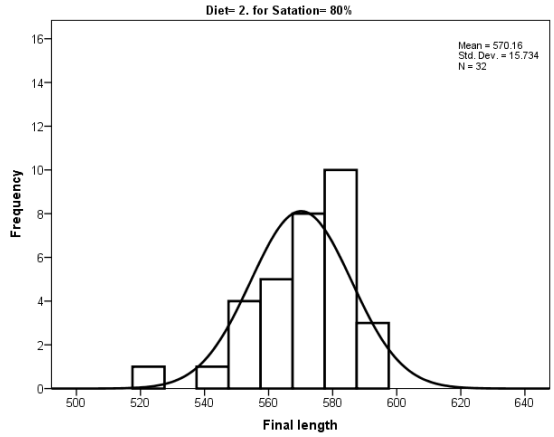

Figure 1.8. Length distribution of fish fed Diet 2 to sub-satiation satiation in Trial 1. Distribution line shows the normal distribution curve.

Table A2.4.2. Shapiro-Wilk test and Skewness value for length distribution of Yellowtail Kingfish fish fed Diet 1 and 2 to apparent satiation and sub-satiation in Trial 1.

Diet type	Diet name	Satiation	Shapiro-Wilk test		
			Df	Sig. value	Skewness
1	Gold Standard	Apparent	48	0.171	-0.110
		Sub	48	0.326	-0.499
2	Reformulated Ridley Pelagica Diet	Apparent	48	0.005	1.012
		Sub	32	0.039	-1.051

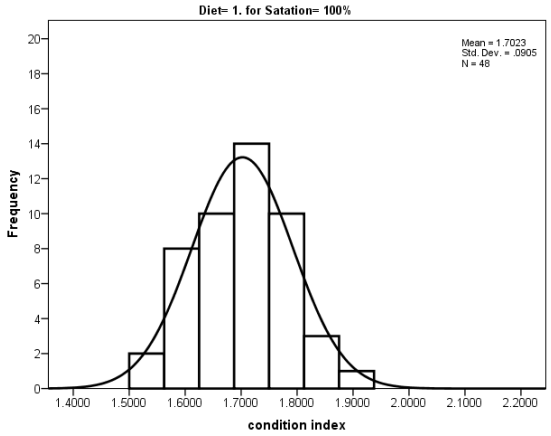

Figure A2.1.9. Condition index distribution of fish fed Diet 1 to apparent satiation in Trial 1. Distribution line shows the normal distribution curve.

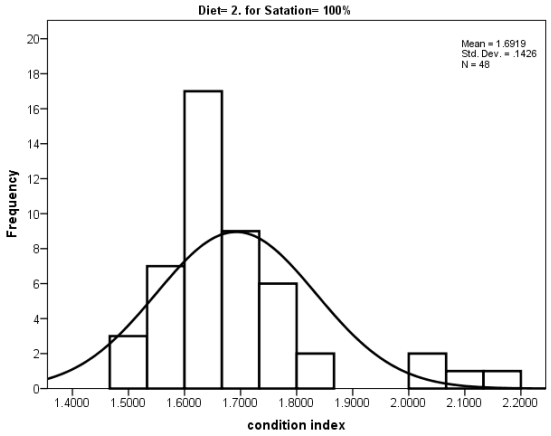

Figure A2.1.10. Condition index distribution of fish fed Diet 2 to apparent satiation in Trial 1. Distribution line shows the normal distribution curve.

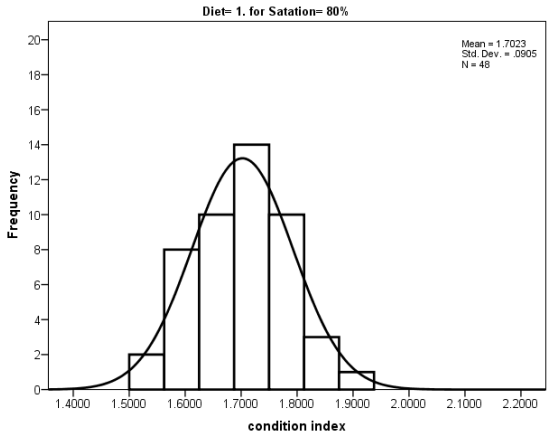

Figure A2.1.11. Condition index distribution of fish fed Diet 1 to sub-satiation in Trial 1. Distribution line shows the normal distribution curve.

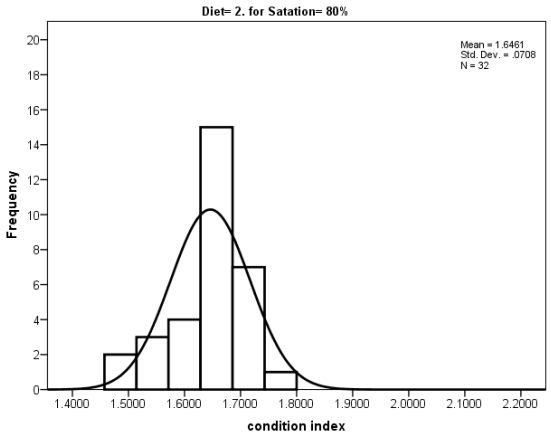

Figure A2.1.12. Condition index distribution of fish fed Diet 2 to sub-satiation in Trial 1. Distribution line shows the normal distribution curve.

Table A2.4.3. Shapiro-Wilk test and Skewness value for condition index distribution of Yellowtail Kingfish fed Diet 1 and 2 to apparent satiation and sub-satiation in Trial 1.

Diet type	Diet name	Satiation	Shapiro-Wilk test		
			Df	Sig. value	Skewness
1	Gold Standard	Apparent	48	0.954	-0.196
		Sub	48	0.410	-0.381
2	Reformulated Ridley Pelagica Diet	Apparent	48	<0.001	1.626
		Sub	32	0.375	-0.548

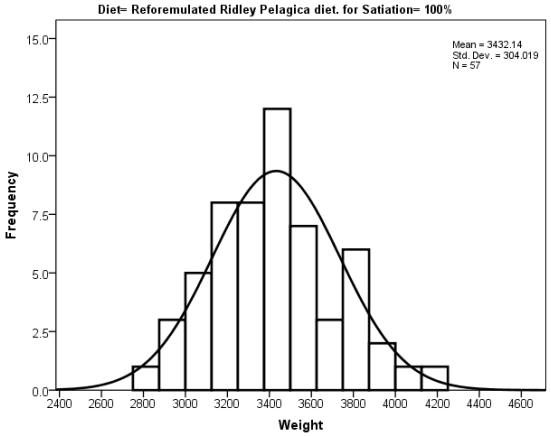

Figure A2.2.1. Weight distribution of fish fed Diet 1 to apparent satiation in Trial 2. Distribution line shows the normal distribution curve.

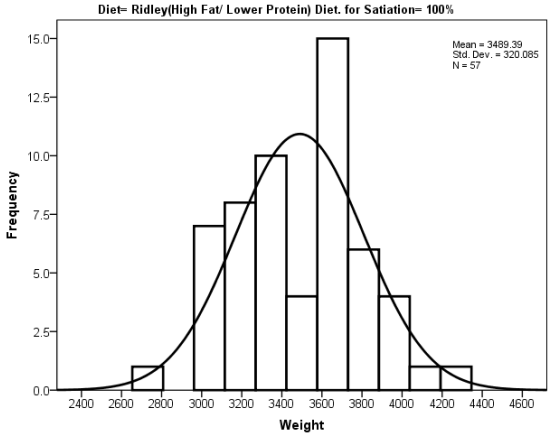

Figure A2.2.2. Weight distribution of fish fed Diet 3 to apparent satiation in Trial 2. Distribution line shows the normal distribution curve.

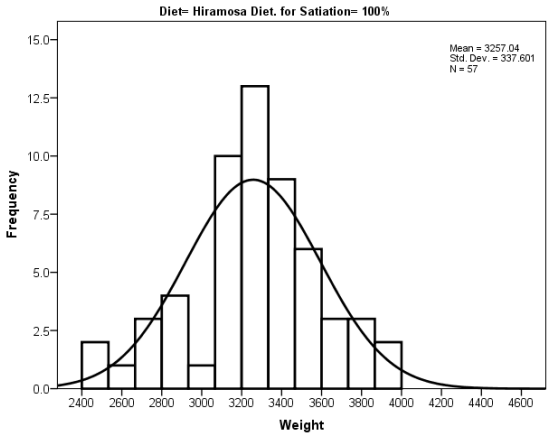

Figure A2.2.3. Weight distribution of fish fed Diet 5 to apparent satiation in Trial 2. Distribution line shows the normal distribution curve.

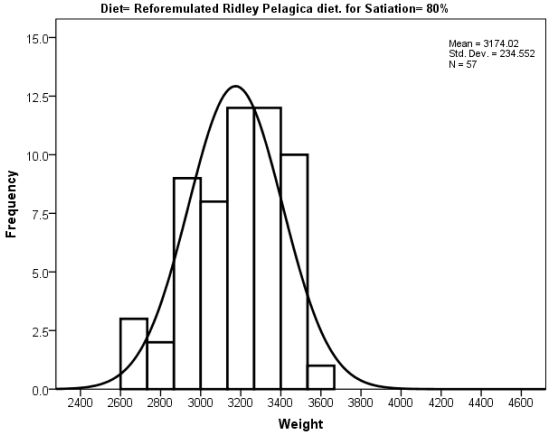

Figure A2.2.4. Weight distribution of fish fed Diet 1 to sub-satiation in Trial 2. Distribution line shows the normal distribution curve.

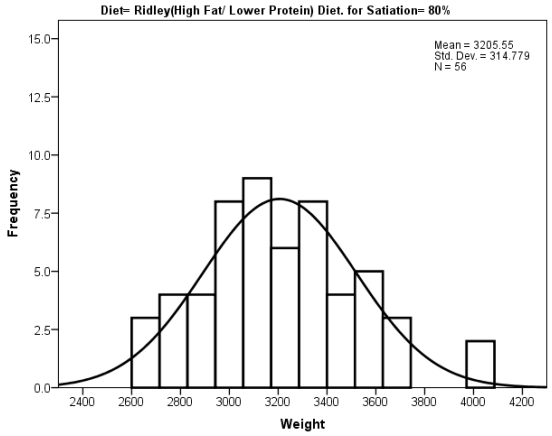

Figure A2.2.5. Weight distribution of fish fed Diet 3 to sub-satiation in Trial 2. Distribution line shows the normal distribution curve.

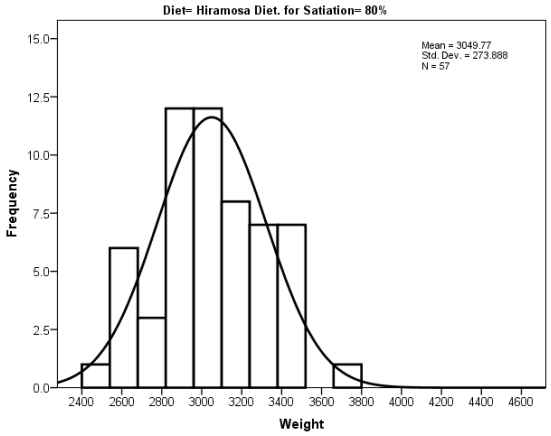

Figure A2.2.6. Weight distribution of fish fed Diet 5 to sub-satiation in Trial 2. Distribution line shows the normal distribution curve.

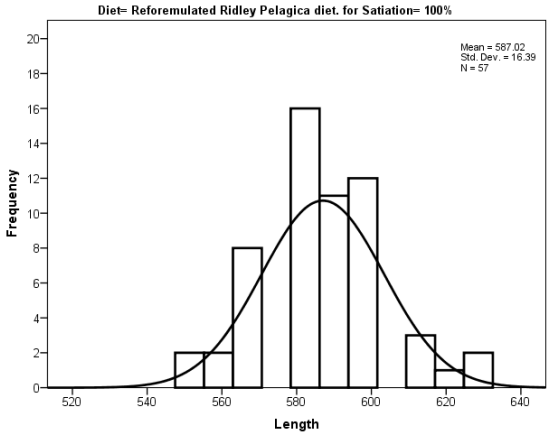

Figure A2.2.7. Length distribution of fish fed Diet 1 to apparent satiation in Trial 2. Distribution line shows the normal distribution curve.

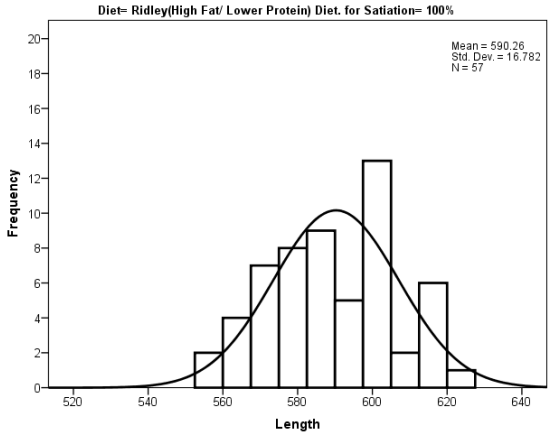

Figure A2.2.8. Length distribution of fish fed Diet 3 to apparent satiation in Trial 2. Distribution line shows the normal distribution curve.

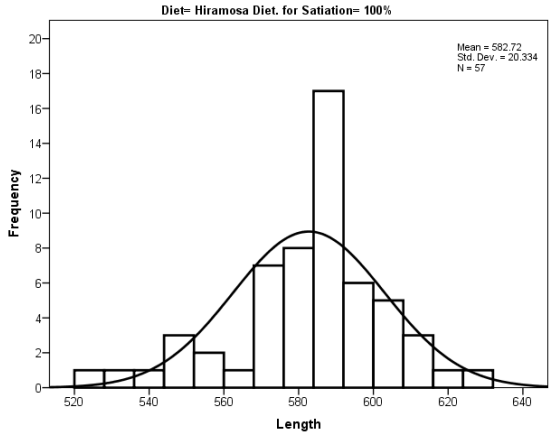

Figure A2.2.9. Length distribution of fish fed Diet 5 to apparent satiation in Trial 2. Distribution line shows the normal distribution curve.

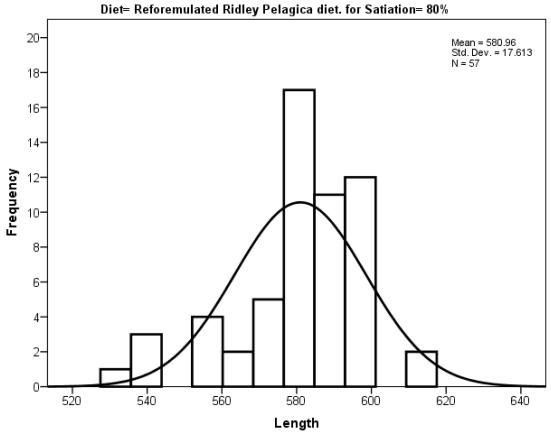

Figure A2.2.10. Length distribution of fish fed Diet 1 to sub-satiation in Trial 2. Distribution line shows the normal distribution curve.

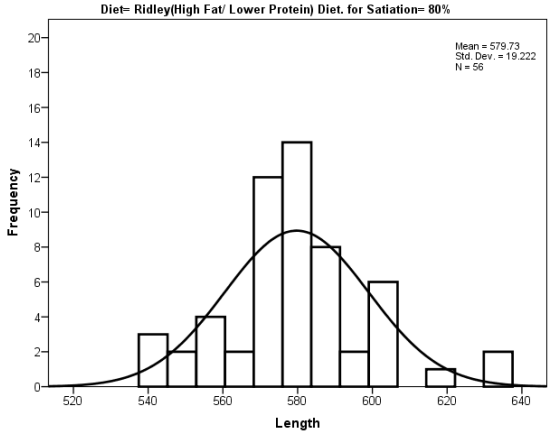

Figure A2.2.11. Length distribution of fish fed Diet 3 to sub-satiation in Trial 2. Distribution line shows the normal distribution curve.

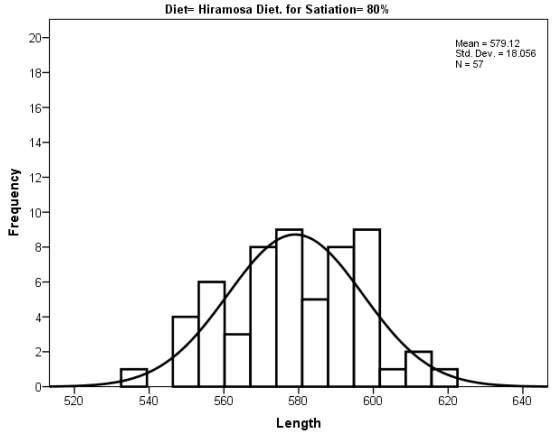

Figure A2.2.12. Length distribution of fish fed Diet 5 to sub-satiation in Trial 2. Distribution line shows the normal distribution curve.

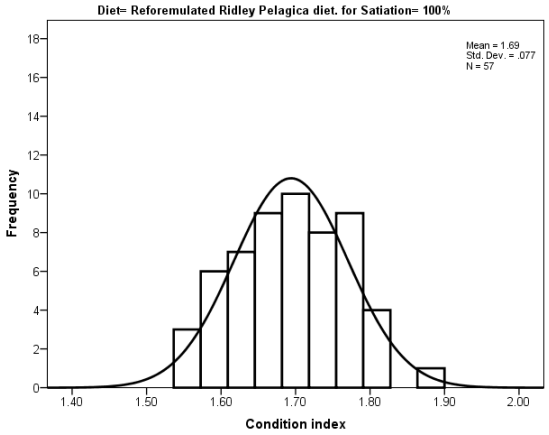

Figure A2.2.13. Condition index distribution of fish fed Diet 1 to apparent satiation in Trial 2. Distribution line shows the normal distribution curve.

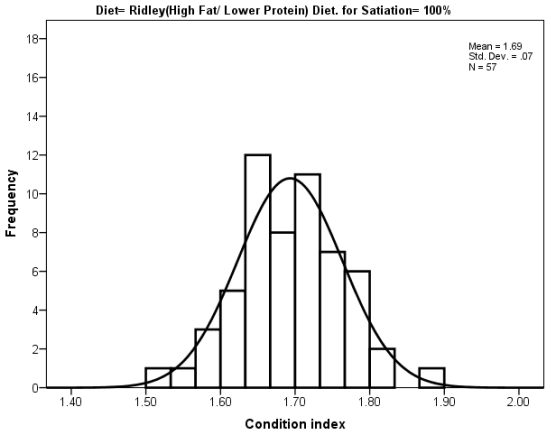

Figure A2.2.14. Condition index distribution of fish fed Diet 3 to apparent satiation in Trial 2. Distribution line shows the normal distribution curve.

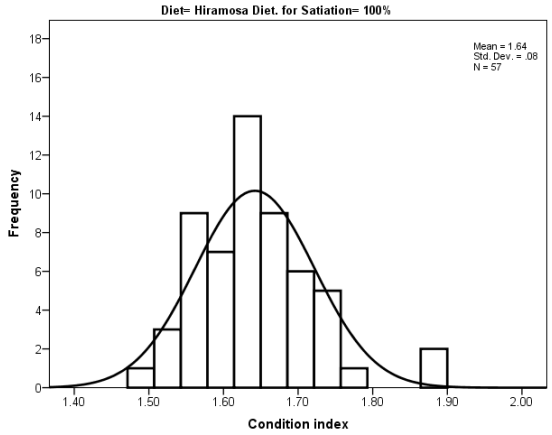

Figure A2.2.15. Condition index distribution of fish fed Diet 5 to apparent satiation in Trial 2. Distribution line shows the normal distribution curve.

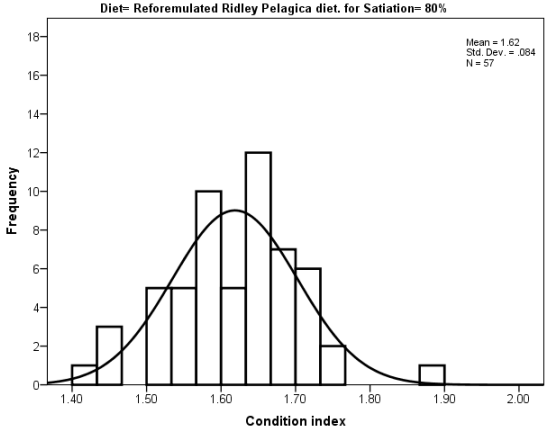

Figure A2.2.16. Condition index distribution of fish fed Diet 1 to sub- satiation in Trial 2. Distribution line shows the normal distribution curve.

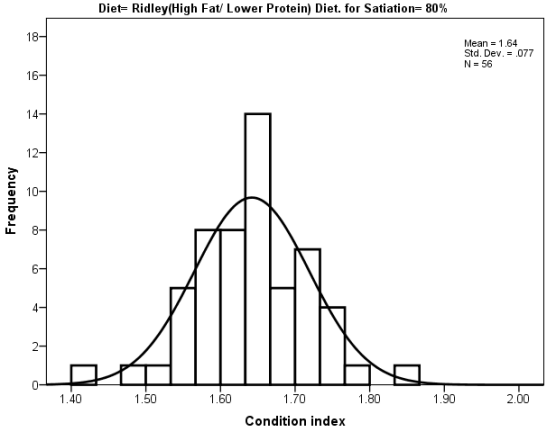

Figure A2.2.17. Condition index distribution of fish fed Diet 3 to sub- satiation in Trial 2. Distribution line shows the normal distribution curve.

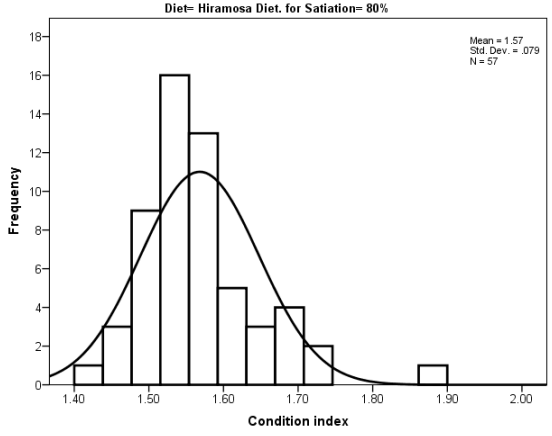

Figure A2.2.18. Condition index distribution of fish fed Diet 5 to sub-satiation in Trial 2. Distribution line shows the normal distribution curve.

Table A2.5.1. Shapiro-Wilk test and Skewness value for weight distribution of Yellowtail Kingfish fed Diets 1, 3 and 5 to apparent satiation and sub-satiation in Trial 2.

Diet type	Diet name	Satiation	Shapiro-Wilk test		
			Df	Sig. value	Skewness
1	Reformulated Ridley Pelagica Diet	Apparent	57	0.656	0.324
		Sub	57	0.060	-0.566
3	Ridley (High Fat/Low Protein) Diet	Apparent	57	0.477	0.138
		Sub	56	0.344	0.439
5	Hiramasa Diet	Apparent	57	0.268	-0.291
		Sub	57	0.772	0.132

Table A2.5.2. Shapiro-Wilk test and Skewness value for length distribution of Yellowtail Kingfish fed Diets 1, 3 and 5 to apparent satiation and sub-satiation in Trial 2.

Diet type	Diet content	Satiation	Shapiro-Wilk test		
			Df	Sig. value	Skewness
1	Reformulated Ridley Pelagica Diet	Apparent	57	0.132	0.160
		Sub	57	0.001	-0.895
3	Ridley (High Fat/Low Protein) Diet	Apparent	57	0.302	-0.006
		Sub	56	0.023	0.375
5	Hiramasa Diet	Apparent	57	0.005	0.972
		Sub	57	0.521	-0.164

Table A2.5.3. Shapiro-Wilk test and Skewness value for condition index distribution of Yellowtail Kingfish fed Diets 1, 3 and 5 to apparent satiation and sub-satiation in Trial 2.

Diet type	Diet content	Satiation	Shapiro-Wilk test		
			Df	Sig. value	Skewness
1	Reformulated Ridley Pelagica Diet	Apparent	57	.795	.027
		Sub	57	.615	.127
3	Ridley (High Fat/Low Protein) Diet	Apparent	57	.967	-.054
		Sub	56	.841	-.040
5	Hiramasa Diet	Apparent	57	.050	.788
		Sub	57	.001	1.258

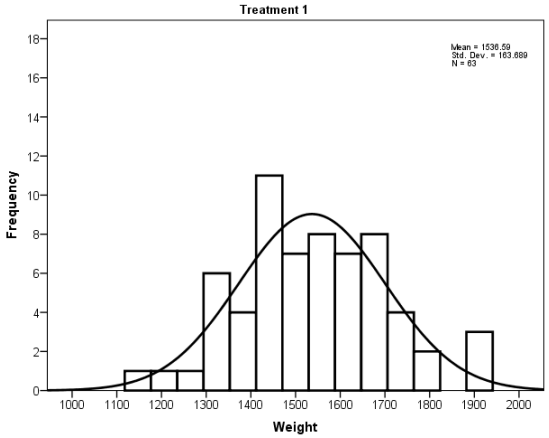

Figure A2.3.1. Weight distribution of fish fed Treatment 1 (Pellet, six days week^{-1} to apparent satiation). Distribution line shows the normal distribution curve.

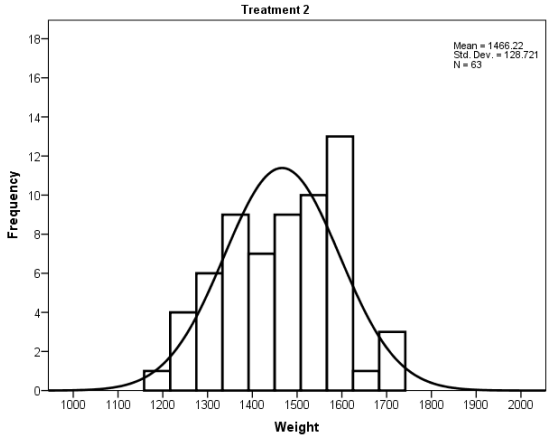

Figure A2.3.2. Weight distribution of fish fed Treatment 2 (Pellet, two days week^{-1} to apparent satiation). Distribution line shows the normal distribution curve.

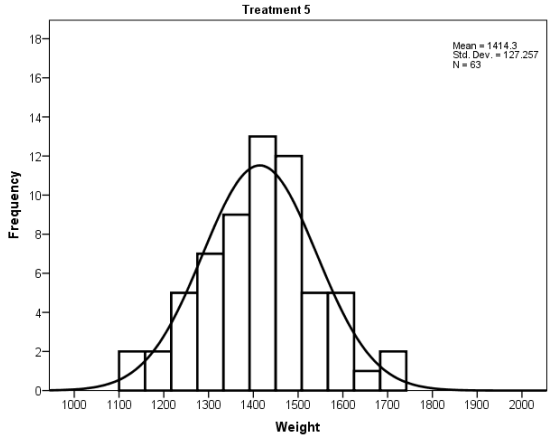

Figure A2.3.3. Weight distribution of fish fed Treatment 5 (Pellet, two days week^{-1} at 0.65% BW). Distribution line shows the normal distribution curve.

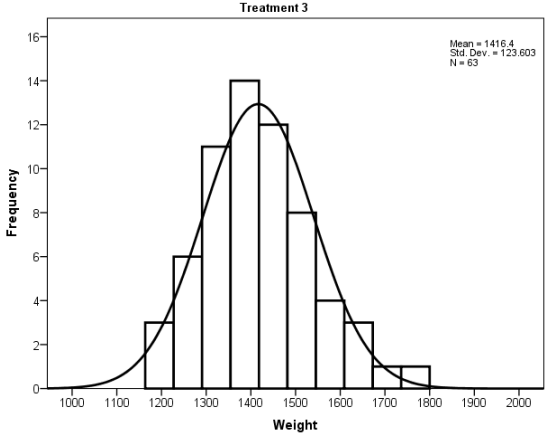

Figure A2.3.4. Weight distribution of fish fed Treatment 3 (Pellet, one day week^{-1} to apparent satiation. Distribution line shows the normal distribution curve.

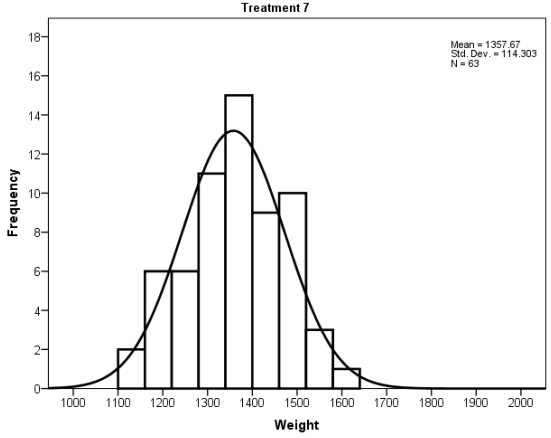

Figure A2.3.5. Weight distribution of fish fed Treatment 7 (Pellet, six days week^{-1} at 0.12% BW). Distribution line shows the normal distribution curve.

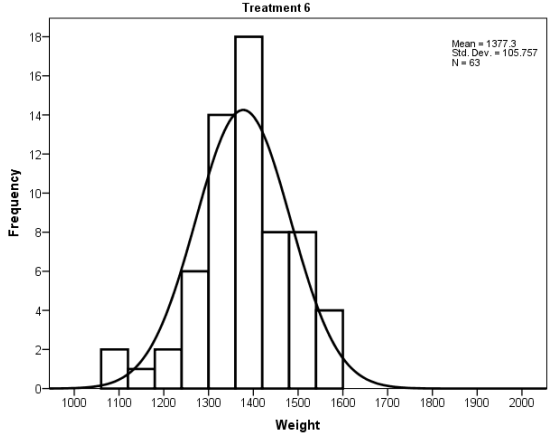

Figure A2.3.6. Weight distribution of fish fed Treatment 6 (Pellet, two days week^{-1} at 0.35% BW). Distribution line shows the normal distribution curve.

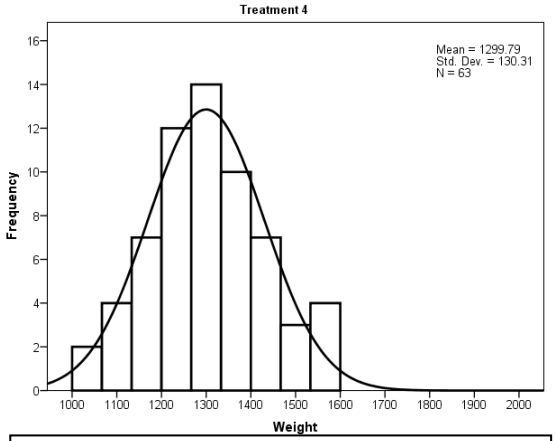

Figure A2.3.7. Weight distribution of fish fed Treatment 4 (Pellet, one day week^{-1} at 0.1% BW). Distribution line shows the normal distribution curve.

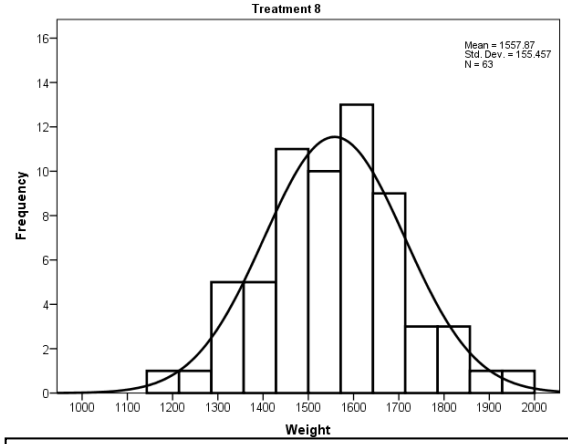

Figure A2.3.8. Weight distribution of fish fed Treatment 8 (Sardines, every second day to apparent satiation). Distribution line shows the normal distribution curve.

Table A2.6.1. Shapiro-Wilk Test and Skewness for weight distribution of Yellowtail Kingfish fed different feeding rates and frequencies in Trial 3 (Treatments arranged in descending order of fish feed intake (%BW d^{-1}); Treatment 8 was excluded from the order).

Treatment	Diet type	Feeding Frequency	Satiation	Feed intake (%BW d^{-1})	Shapiro-Wilk Test		
					Df	Sig. Value	Skewness
1	Pellet	Six days per week	Apparent	0.3283	63	0.832	0.110
2	Pellet	Twice per week	Apparent	0.2298	63	0.514	-0.116
5	Pellet	Twice per week	0.65% BW	0.1766	63	0.797	-0.026
3	Pellet	Once per week	Apparent	0.1737	63	0.289	-0.070
7	Pellet	Six days per week	0.12% BW	0.1118	63	0.966	0.080
6	Pellet	Twice per week	0.35% BW	0.1026	63	0.116	-0.472
4	Pellet	Once per week	0.1% BW	0.0296	63	0.832	0.193
8	Sardine	Every 2nd day	Apparent	0.7218	63	0.994	0.119

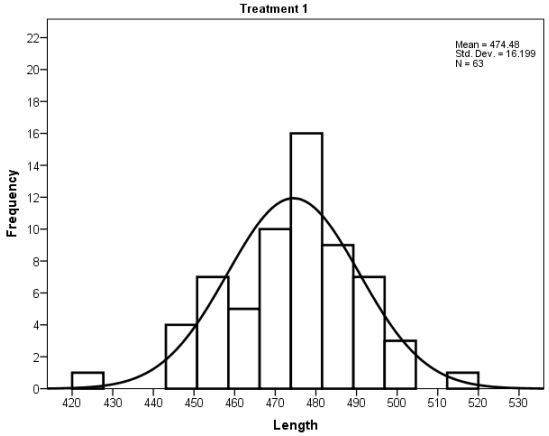

Figure A2.3.9. Length distribution of fish fed Treatment 1 (Pellet, six days week^{-1} to apparent satiation). Distribution line shows the normal distribution curve.

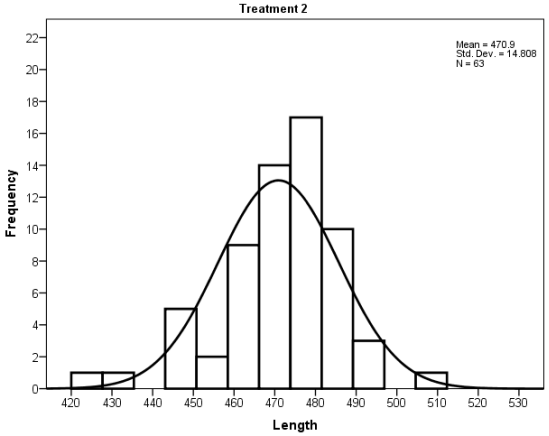

Figure A2.3.10. Length distribution of fish fed Treatment 2 (Pellet, two days week^{-1} to apparent satiation). Distribution line shows the normal distribution curve.

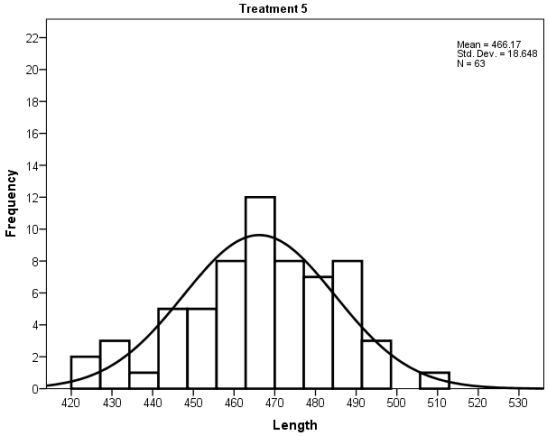

Figure A2.3.11. Length distribution of fish fed Treatment 5 (Pellet, two days week^{-1} at 0.65% BW). Distribution line shows the normal distribution curve.

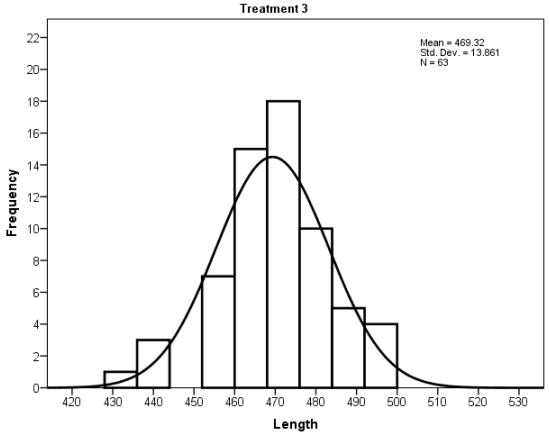

Figure A2.3.12. Length distribution of fish fed Treatment 3 (Pellet, one day week^{-1} to apparent satiation. Distribution line shows the normal distribution curve.

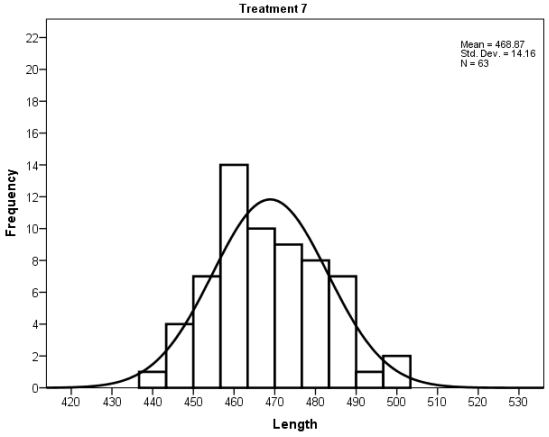

Figure A2.3.13. Length distribution of fish fed Treatment 7 (Pellet, six days week^{-1} at 0.12% BW). Distribution line shows the normal distribution curve.

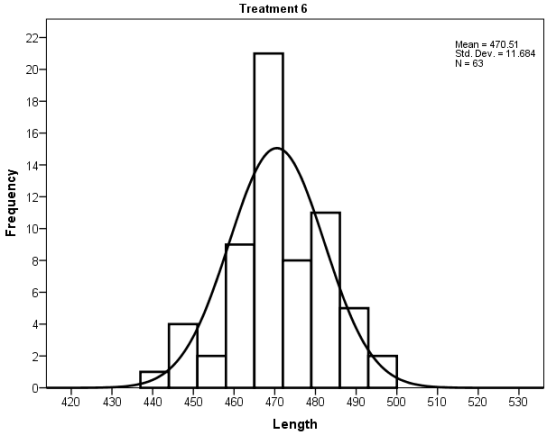

Figure A2.3.14. Length distribution of fish fed Treatment 6 (Pellet, two days week^{-1} at 0.35% BW). Distribution line shows the normal distribution curve.

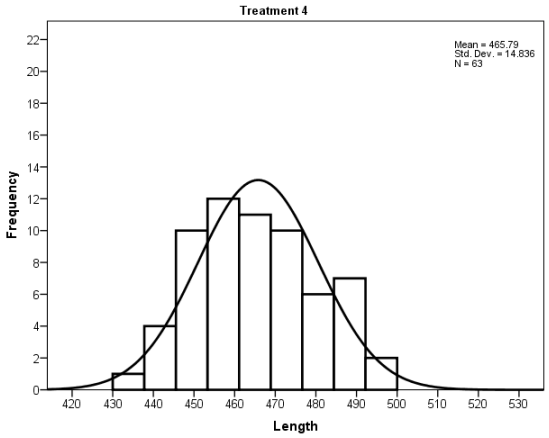

Figure A2.3.16. Length distribution of fish fed Treatment 4 (Pellet, one day week^{-1} at 0.1% BW). Distribution line shows the normal distribution curve.

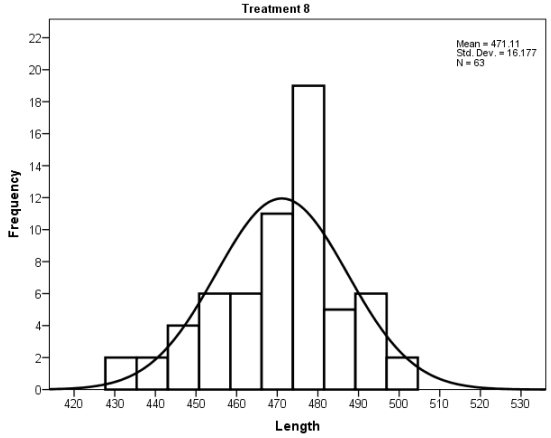

Figure A2.3.17. Length distribution of fish fed Treatment 8 (Sardines, every second day to apparent satiation). Distribution line shows the normal distribution curve.

Table A2.6.2. Shapiro-Wilk Test and Skewness for length distribution of Yellowtail Kingfish fed different feeding rates and frequencies in Trial 3 (Treatments arranged in descending order of fish feed intake (%BW d^{-1}); Treatment 8 was excluded out from the order).

Treatment	Diet type	Feeding Frequency	Feed rate	Feed intake (%BW d^{-1})	Shapiro-Wilk Test		
					Df	Sig. Value	Skewness
1	Pellet	Six days per week	Apparent satiation	0.3283	63	0.270	-0.252
2	Pellet	Twice per week	Apparent satiation	0.2298	63	0.037	-0.538
5	Pellet	Twice per week	0.65% BW	0.1766	63	0.514	-0.302
3	Pellet	Once per week	Apparent satiation	0.1737	63	0.081	-0.383
7	Pellet	Six days per week	0.12% BW	0.1118	63	0.565	0.185
6	Pellet	Twice per week	0.35% BW	0.1026	63	0.346	-0.315
4	Pellet	Once per week	0.1% BW	0.0296	63	0.509	0.053
8	Sardine	Every 2nd day	Apparent satiation	0.7218	63	0.105	-0.539

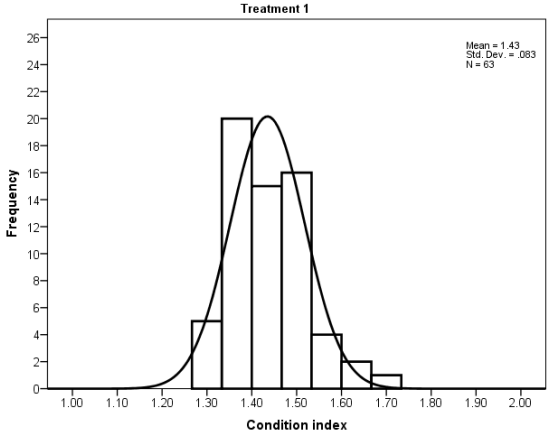

Figure A2.3.18. Condition index distribution of fish fed Treatment 1 (Pellet, six days week^{-1} to apparent satiation). Distribution line shows the normal distribution

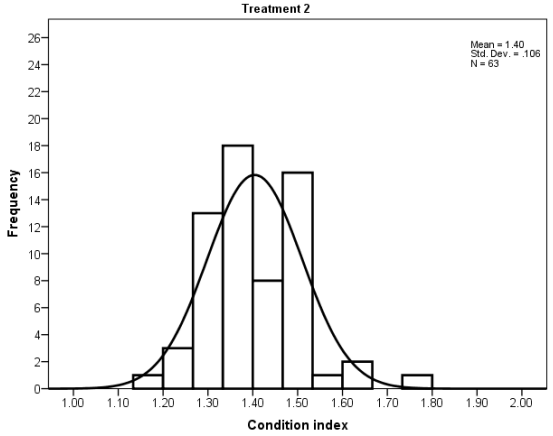

Figure A2.3.19. Condition index distribution of fish fed Treatment 2 (Pellet, two days week^{-1} to apparent satiation). Distribution line shows the normal distribution

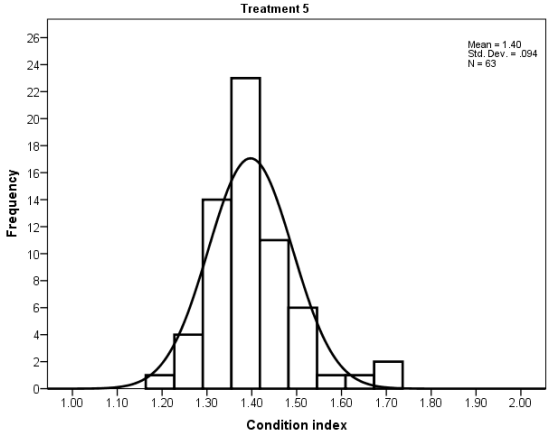

Figure A2.3.20. Condition index distribution of fish fed Treatment 5 (Pellet, two days week^{-1} at 0.65% BW). Distribution line shows the normal distribution

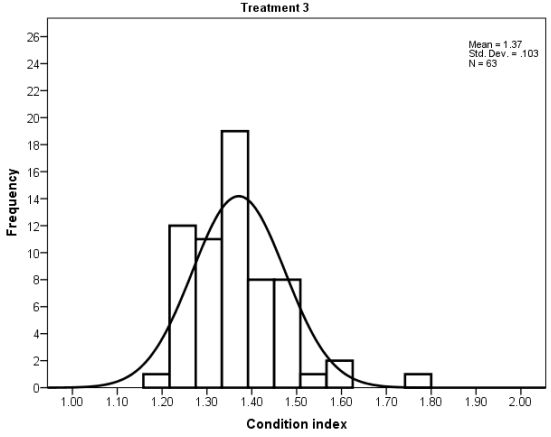

Figure A2.3.21. Condition index distribution of fish fed Treatment 3 (Pellet, one day week^{-1} to apparent satiation. Distribution line shows the normal distribution

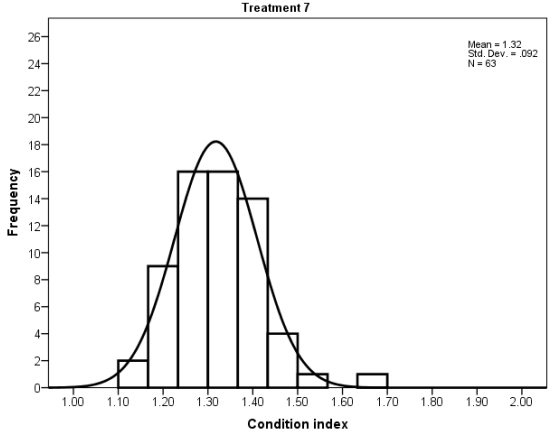

Figure A2.3.22. Condition index distribution of fish fed Treatment 7 (Pellet, six days week^{-1} at 0.12% BW). Distribution line shows the normal distribution curve.

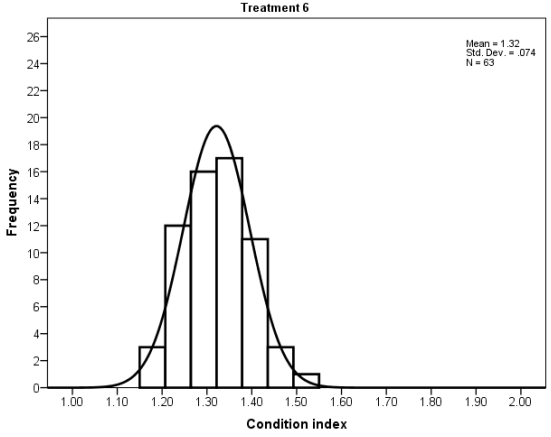

Figure A2.3.23. Condition index of fish fed Treatment 6 (Pellet, two days week^{-1} at 0.35% BW). Distribution line shows the normal distribution curve.

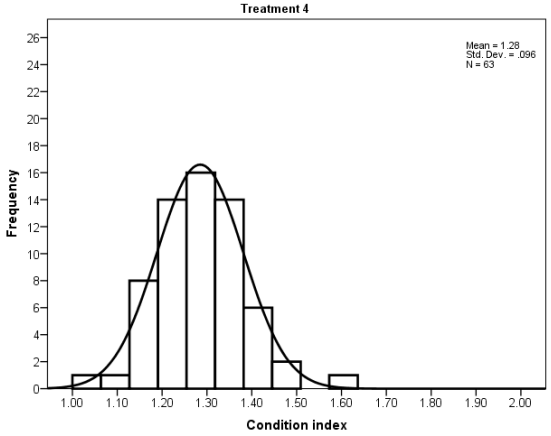

Figure A2.3.24. Condition index distribution of fish fed Treatment 4 (Pellet, one day week^{-1} at 0.1% BW). Distribution line shows the normal distribution curve.

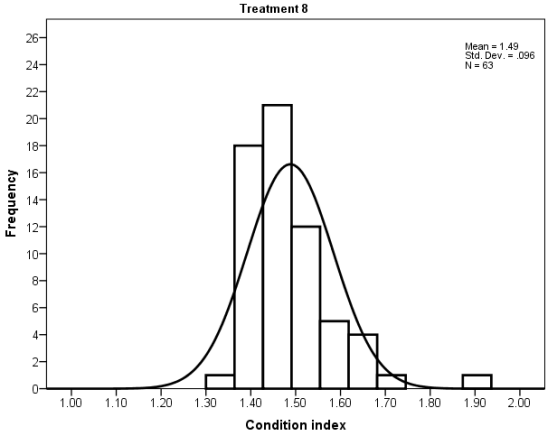

Figure A2.3.25. Condition index distribution of fish fed Treatment 8 (Sardines, every second day to apparent satiation). Distribution line shows the normal distribution curve.

Table A2.6.3. Shapiro-Wilk Test and Skewness for condition index distribution of Yellowtail Kingfish fed different feeding rates and frequencies in Trial 3 (Treatments arranged in descending order of fish feed intake (%BW d^{-1}); Treatment 8 was excluded out from the order).

Treatment	Diet type	Feeding Frequency	Feed rate	Feed intake (%BW/day)	Shapiro-Wilk Test		
					DF	Sig. Value	Skewness
1	Pellet	Six days per week	Apparent satiation	0.3283	63	.008	-.918
2	Pellet	Twice per week	Apparent satiation	0.2298	63	.186	.529
5	Pellet	Twice per week	0.65% BW	0.1766	63	.000	1.153
3	Pellet	Once per week	Apparent satiation	0.1737	63	.002	1.198
7	Pellet	Six days per week	0.12% BW	0.1118	63	.125	.603
6	Pellet	Twice per week	0.35% BW	0.1026	63	.714	.108
4	Pellet	Once per week	0.1% BW	0.0296	63	.510	.378
8	Sardine	Every 2nd day	Apparent satiation	0.7218	63	.000	1.661

Discussion

Some length distributions in Trials 1, 2 and 3 were skewed. However, as Yellowtail Kingfish are sold by weight and condition index is sometimes used to determine overall fish health (Richard et al., 2006), these two characteristics are commercially important. In the current study, the hypothesis that reducing feeding rate or frequency would increase distribution skewness was typically not supported, but not in all cases.

In Trial 1, normal size distribution was maintained even when fish were fed to sub-satiation. This occurred even in the presence of major differences in the ingredient composition of the diets.

In Trial 2, the condition index for Yellowtail Kingfish fed the reformulated Ridley Pelagica diet and Ridley high fat/low protein diet to apparent satiation or sub-satiation did not have a skewed distribution. This result is inconsistent with results from Trial 1, as fish fed with Reformulated Ridley Diet in Trial 1 exhibited a skewed distribution when fed to apparent satiation, suggesting that the effect of feed rates on size distribution may differ between fish cohorts. In contrast, the condition index of Yellowtail Kingfish fed the Hiramasa diet to sub-satiation exhibited a skewed distribution. The distribution graph for condition index showed that fish fed to apparent satiation had a higher condition index compared to fish fed to sub-satiation with the three diet types, even when the distribution was skewed. This is further supported by studies of feed rate on fin fish growth, which showed a significantly higher growth and feed conversion rate when the feeding rate was higher (Hung and Lutes, 1987).

In Trial 3, the effect of feeding rate is more obvious when the fish were fed the same diet. When fish had a lower feed intake, a large portion of the nutrients gained were likely used to maintain daily metabolic needs rather than used it for somatic growth (Hung and Lutes, 1987). Fish maintenance feed rate in this trial were reported in Chapter 4 (Trial 3) to be 0.2047% BW d^{-1}. When treatments were arranged in a descending order according to feed intake d^{-1} (%BW d^{-1}), fish that had a feed intake higher or close to the daily maintenance feed rate exhibited a skewed condition index, except for Treatment 2. Fish fed Treatment 2 had a feed intake of 0.2298% BW d^{-1}, which is close to the maintenance feed rate. This suggests that fish fed a maintenance ration may exhibit homogenous growth. In addition, fish fed a ration that was lower than the maintenance feed rate can lead to differences in size distribution. Some fish might be able to gain access to more feed compared to others, which was previously reported by Moran et al. (2007). The authors further reported size heterogeneity continued to occur even when fish were graded. This suggests that fish heterogeneity differences may not be entirely due to different diets, as per Trial 1 and Trial 2, but may also be dependent on feeding rate and frequency, which may lead to some fish consuming more feed than some other fish. Kardi et al. (1996) suggested that randomizing the time and space of when feed is presented to fish or tank may help in preventing some of the fish from hogging the feeding area, thus giving other fish higher chances of getting more food. Although some research can be done on using Sardines as feed as Sardine has the highest feed intake out of all the treatment and changing the feeding frequency might result in reduction of size heterogeneity.

In conclusion, by optimising diet formulations and controlling feeding rates, restricted feeding rates did not result in size heterogeneity. This suggests that it might be viable option to feed Yellowtail Kingfish to sub-satiation without impacting on cohort heterogeneity.

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Appendix 3. Student project details

A2.1: Student Projects

Benjamin Crowe (commenced February 2015). Honours project entitled “The influence of dietary lipid on liver and gut structure and enzyme activity in Yellowtail Kingfish (*Seriola lalandi*)". Faculty of Science and Engineering, School of Biological Sciences, Flinders University, South Australia, Australia. Supervised by Assoc. Prof. James Harris and Assoc. Prof. David Stone.
Benjamin was awarded the Playford Trust Honours Scholarship and the AJ & IM Naylon Honours Scholarships in 2015.

Leigh Kuerschner. Honours project entitled “Regulation of muscle growth in yellowtail kingfish (*Seriola lalandi*) under fasting and re-feeding conditions”. Faculty of Science and Engineering, School of Biological Sciences, Flinders University, South Australia, Australia. Supervised by Assoc. Prof. Kathryn Schuller and Assoc. Prof. David Stone. Commenced July 2015.

Keigo Kimura and Clement Kong, Flinders 3rd year student project entitled “Weight and length variation in cultured Yellowtail Kingfish in response to feed rate”. Faculty of Science and Engineering, School of Biological Sciences, Flinders University, South Australia, Australia. Supervised by Assoc. Prof. James Harris and Assoc. Prof. David Stone; August-December 2015.

Arif Malik PhD project entitled “Role of PGC-1 Transcriptional Co-activators during the Development of Regional Endothermy in Fish”. Faculty of Science and Engineering, School of Biological Sciences, Flinders University, South Australia, Australia. Supervised by Assoc. Prof. Kathryn Schuller and Assoc. Prof. David Stone. Commenced July 2015.