Understanding lipid utilisation in large (> 2 kg) Yellowtail Kingfish (*Seriola lalandi*)

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Declaration

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	25/06/2019
Samantha Naomi Chown	Date

Dedication

For Caroline Radvanyi, my grandmother and the most beautiful person that I ever had the joy of loving. I wish that you could be here to share this with.

Table of Contents

Abstract

Yellowtail Kingfish (*Seriola lalandi*) (YTK) are carnivorous marine finfishes that are commercially farmed in Australia. YTK present the greatest opportunity for expansion of the Australian aquaculture industry, but improved diet formulations and feed conversion ratios are essential for production gains and economic upscaling.

Lipids constitute a major cost component of aquafeeds but lipid composition has not been optimised for YTK. The purpose of this research was to increase understanding of how YTK utilise dietary lipids, and to improve feed conversion efficiency and product quality for human consumers. Fish oil (FO) as a dietary lipid source is central to this research as YTK require dietary omega 3 (n-3) long chain polyunsaturated fatty acids (LC PUFA) from FO for healthy development and growth, but FO is limited and less economically sustainable than other types of oil/lipid.

The first study presented in this thesis sought to benchmark the fatty acid composition of wild YTK compared to aquacultured YTK. Tissue total lipid content was on average 4-times higher in aquacultured than wild YTK, with significantly higher concentrations of total saturated, omega 9, omega 7 and omega 6 fatty acids in tissues, but n-3 LC PUFA concentrations were not significantly different in the white muscle of wild and aquacultured YTK.

The second and third studies were carried out with YTK grown in tanks using aquafeeds with varying lipid formulations. Generally, the fatty acid composition of aquacultured fish is reflective of the composition of aquafeeds, however this was not always the case for YTK in the following experiments. The key findings were that YTK have the capacity to spare in full Docosahaexonoic Acid (DHA) in white muscle at the expense of oleic acid (18:1n-9) when dietary levels of n-3 LC PUFA were <1.6 g 100 g^{-1} feed and that the digestibility of saturated

fatty acids decreased with increasing chain length. Both of these findings could be used to manipulate dietary formulations and improve utilisation of n-3 LC PUFA.

The fourth study investigated the potential for finishing diets to be utilised to modify the tissue fatty acid composition of YTK prior to harvest. Results showed significant changes in white muscle n-3 LC PUFA over 33 days at warm water temperatures, however further research was recommended to optimise the duration of finishing periods under a range of conditions. It was also recommended that the n-3 LC PUFA content of YTK feeds be closely monitored with strict lower limits set to ensure optimal product quality.

The fifth and final study validated a method for the quantification of bioactive free fatty acid and oxylipin levels in YTK blood plasma. The approach was then used to determine the effects of dietary levels of n-3 LC PUFA on plasma free fatty acids and oxylipin bioactives. This method provides a new tool for aquaculture nutritionists to assess the impact of changes to YTK aquafeed formulations.

In summary, this thesis has provided insight into the factors that affect fatty acid utilisation in YTK which have the potential to positively influence future aquafeed formulations, while also providing new methods to investigate lipid metabolism in the future.

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Chapter 1 – General introduction and literature review

1.1. Background and purpose

This research was undertaken as part of the national Kingfish for Profit (K4P) project, which was funded by industry partners and the Australian Department of Agriculture and Water Resources under the rural research and development (R&D) for profit programme. The aim of the K4P project was to develop more cost-effective Yellowtail Kingfish (*Seriola lalandi*) (YTK) feeds and feeding strategies, drive immediate production gains for YTK aquaculture, and build a YTK aquaculture R&D network to strengthen industry adoption of research outcomes.

Yellowtail Kingfish farming presents the greatest opportunity to expand the aquaculture industry in Australia. Industry leaders in YTK farming, Clean Seas Seafood Ltd. (based in South Australia), are in the processes of expanding their production volume with the addition of a fourth production site in Whyalla, in regional South Australia. Meanwhile, Huon Aquaculture have recently invested in YTK farming as part of the K4P project, with trial YTK sea-cage farming in Port Stephens, New South Wales and the acquisition of new lease sites in regional Western Australia. The production of YTK in Australia is predicted to increase by 34,000 tonnes in the next decade, at a value of $440 million, which will require an additional 68,000 tonnes of aquafeeds (Stone et al., 2019a). To enable the expansion of the YTK aquaculture industry the K4P project had 3 key objectives: 1) to improve diet formulations and the economic sustainability of feeds, 2) to improve feeding strategies to increase the profitability of commercial farming operations, and 3) to improve the nutritional health to boost production profitability (Stone et al., 2019a). The body of work presented in this thesis assisted in achieving these objectives by focusing on the utilisation of dietary lipids by large (> 2kg) YTK. In relation to the first and third objectives, an in-depth understanding of the range of

interactions that dietary lipids and their fatty acids have within the body of farmed YTK will aid in the development of more functional and sustainable diets and has the potential to bolster the nutritional health of farmed YTK.

Formulating diets with a lipid composition that can be utilised with the greatest efficiency will be pivotal in reducing the cost of aquaculture feeds (aquafeeds). Dietary lipids constitute a major cost component of aquafeeds, yet their composition and the potential for their manipulation have not been fully explored. Previous research efforts have predominantly focused on YTK nutritional demands during larval, fingerling and small grow-out stages; during these stages optimal nutrition is paramount as it is a key factor affecting survival and minimising rates of deformities (Kolkovski and Sakakura, 2004; Abbink et al., 2012). In contrast, the efficiency of dietary lipid utilisation in large, grow-out stage YTK has received less attention, regardless of the extensive quantity of aquafeed required to grow YTK through this production stage. For decades, the scientific community and aquaculture practitioners have been aware that aquacultured fish achieve optimal growth, maintain superior health and attain superior product quality when sustained on feeds rich in fish oil (FO), compared to feeds supplemented with alternative oils. However, the growth of aquaculture worldwide has resulted in the industry using greater quantities of FO in aquafeeds which is economically unsustainable.

The use of FO in aquafeeds is hereafter reviewed, with specific focus on: 1) the replacement of FO in aquafeeds, 2) species-specific dietary omega 3 (n-3) long chain polyunsaturated fatty acid (LC PUFA) requirements, and 3) methods for modifying the n-3 LC PUFA concentrations of aquacultured species prior to harvest to benefit human consumers. Furthermore, the state of knowledge specifically concerning dietary FO and n-3 LC PUFA utilisation by YTK will be evaluated.

1.2. Fish oil use in aquaculture

In 1990 global aquaculture production was at 13 million tonnes per year, increasing by 75 million tonnes to 88 million tonnes per year in 2016 (FAO, 2016). On the other hand, total global wild capture fisheries production only increased by 6.23 million tonnes per year during the same period, indicating that aquaculture is fulfilling a substantial and increasing proportion of global demand for seafood (FAO, 2016 - Figure 1.1). Problematically for the aquaculture industry, the harvests from wild capture fisheries that supply fish meal and FO for aquafeeds have remained relatively static over the same period, meaning that the supply of key ingredients for aquafeeds are becoming limiting. Specifically, the global quantity of FO has peaked at approximately one million tonnes per year (Finco et al., 2016) and a large percentage of this is consumed by the aquaculture industry. In the future, as aquaculture production continues to increase, there will be a need to reduce the quantity of FO used per unit of aquafeed.

Over the last 20 years a vast amount of research has focused on quantifying the effect of aquaculture on global supplies of FO, reducing the inclusion levels of FO and finding suitable replacements for FO in commercial aquaculture feeds that satisfy the cultured species requirements for n-3 LC PUFA, including Eicosapentaenoic Acid (EPA), Docosapentaenoic Acid (DPA) and Docosahexaenoic Acid (DHA). With dwindling wild fisheries stocks the cost of FO has increased dramatically in the past 20 years (from approximately $600USD to $1,450USD per tonne between 1995 and 2015; FAO, 2016). This has caused a flow-on increase to the cost of aquafeeds and the overall cost of production for commercial aquaculture.

The use of marine resources in the aquaculture industry has been reviewed numerous times over the past 20 years. Most recently Turchini et al. (2019) reviewed the use of marine resources with the perspective of realigning key issues and contemplating future directions. That review outlined the necessity for understanding the way that raw ingredients complement each other and collectively fulfil the nutrient requirements of the target species, rather than

focusing on replacing unsustainable or costly ingredients with alternatives. Prior to this Tocher (2015) reviewed the use of n-3 LC PUFA sourced from FO in the aquaculture industry. That review outlined the key changes in nutritional aquaculture research over the previous 20 years and the ways in which n-3 LC PUFA requirements can be defined. Specifically, that they can be set by either: 1) meeting the minimum essential fatty acid (EFA) requirements of the target species, 2) determining the level that results in maximal growth and health of the target species, or 3) at a level which results in a similar product quality profile to the wild counterpart of the cultured species. In that review it was concluded that the aquaculture industry needs to find suitable new sources of n-3 LC PUFA and, indeed, set a challenge for the aquaculture industry to become a net producer of n-3 LC PUFA via *de novo* synthesis of n-3 LC PUFA.

Additionally, a number of other reviews have previously addressed similar issues (Trushenski et al., 2006, Miller et al., 2008, Turchini et al., 2009). The general consensus within these reviews was that the aquaculture industry has expanded with such exponential growth that the resources that it relies on are no longer capable of supporting it, specifically the demand for FO. Furthermore, it is widely agreed that the majority of aquacultured species are limited by their essential demand for n-3 LC PUFA, from FO, to maintain proper biological function and as such the only option for sustaining the continual growth of the industry is to find alternate dietary sources of these n-3 LC PUFA.

From the perspective of minimising negative interactions with the environment, mainly overexploiting natural resources such as FO, Naylor et al. (2000) and Naylor et al. (2009) have provided reviews addressing the effects of aquaculture on world fish supplies and highlighted the issue of finite resources in aquafeeds. These reviews assessed the challenges which the aquaculture industry would face into the future. The authors discussed the strides which had been made by the aquaculture industry and also the limitations that it would face. A particular challenge is that human consumers desire aquaculture products that are produced in an

environmentally friendly manner, but still require the same nutritional benefits in terms of n-3 LC PUFA content when compared to wild caught fish products. This means that the aquaculture industry is limited by its reliance on wild fisheries as a source of FO and specifically n-3 LC PUFA. The inclusion rate of FO in aquafeeds is low, indeed the quantity of FO in salmon diets has decreased from 30% to 10%. However, the immense quantity of aquafeed required worldwide means that the aquaculture industry is consuming most of the global supply of FO and consequently that in the future the aquaculture industry will be responsible for either conserving or depleting wild fisheries.

The authors of these reviews stated a clear way forward: 1) utilising n-3 LC PUFA from terrestrial sources such as genetically modified canola or 2) commercially developing single cell organisms, such as algae, to provide n-3 LC PUFA. However, both of these options are currently limited, the former by consumer acceptance of genetically modified organisms and the latter by the high cost of production. As the aquaculture industry and the human population continue to expand, the requirement for nutritionally beneficial seafood will increase and it is likely that these challenges will be overcome as consumer opinions change and production techniques improve.

Encouragingly since theses reviews have been published it is apparent that the aquaculture industry has made positive strides in managing FO use. With the sustainable use of by-product lipid sources, terrestrial lipid sources and reducing the overall FO inclusion in aquafeeds the predicted negative impacts of worldwide aquaculture have not been realised.

It is clear that the future success of the aquaculture industry will be determined by its ability to overcome its reliance on FO as a raw ingredient and to carefully manage all of the resources that it relies on. Importantly, as an industry there is a clear path forward with a number of viable alternatives to the unsustainable use of FO and as a sector it is obvious that collaboration and ongoing research is essential to future success.

1.3. Replacement of dietary FO in aquafeeds

A vast quantity of research has endeavoured to increase our understanding of how FO replacement in aquafeeds affects the cultured animals consuming such feeds. The topic of FO replacement has been reviewed by Turchini et al. (2010) and Turchini et al. (2009). Once again, the key issues identified within these reviews were: potentially restricted expansion of the aquaculture industry due to reliance on wild fisheries for FO, altered product quality of farmed fish due to dietary FO replacement and the requirement for the industry to find new sources of dietary lipids and n-3 LC PUFA. These authors state that future replacements for dietary FO in aquafeeds need to be competitively priced, readily available and have minimal concentrations of linoleic acid (18:2n-6). This last point, concerning linoleic acid, is not considered with the same weighting elsewhere but is important in relation to fish and human nutrition. Omega 6 fatty acids have pro-inflammatory downstream bioactive metabolites (free fatty acids and oxylipins) and for fish and humans the ratio of dietary n-3 to omega 6 fatty acid has implication for nutrient metabolism and bioconversion. Furthermore, omega 6 fatty acids are already abundant in the human diet, main due to the consumption of terrestrial plant oils and as such their minimization in seafood products is preferable.

Fish oil replacement has also been discussed in depth by Glencross et al. (2007), Nasopoulou and Zabetakis (2012) and Oliva-Teles (2012), who address issues such as ingredient quality, animal nutrition and health, and the benefits of plant derived oils. The key point of difference of the Glencross et al. (2007) review was the necessity for refined experimental designs which are capable of addressing specific diet composition queries. This is vital because of the interactive nature of dietary ingredients and the fact that it is often difficult to discern the source of impacts if experimental diets are not formulated correctly. The Nasopoulou and Zabetakis (2012) review differed from others because it solely focused on plant originated oil as a replacement for FO in aquafeeds. Furthermore, those authors discussed

the implication of growing terrestrial oil crops for aquafeeds in the context of climate change and an increasing shortage of freshwater for agriculture. Lastly, Oliva-Teles (2012) discussed the reduced immunity of farmed fish when dietary FO was reduced, noting that reduced disease resistance could be driven by changes to the ratio of dietary fatty acids and also by essential fatty acids not being supplied in adequate quantities. These three reviews demonstrate that FO replacement is a multifactorial problem and that each factor needs to be carefully considered.

Dietary FO is currently replaced in aquafeeds by four groups of alternative oils: terrestrial derived animal oils and tallows, terrestrial vegetable and plant derived oils, oils derived from marine algae and oils derived from terrestrial crops that have been genetically modified to produce n-3 LC PUFA. Generally, terrestrial animal derived oils and tallows, primarily poultry oil (PO) but also beef tallow, have been used most broadly and successfully as a FO replacement in aquafeeds.

1.3.1. Terrestrial derived animal oils and tallows

Poultry oil has been shown to be a viable partial or total FO replacement in the aquafeeds for Yellowtail Kingfish (100% replacement) (Bowyer et al., 2012a), European Sea Bass (*Dicentrarchus labrax*) (75% replacement) (Monteiro et al., 2018) and Barramundi (*Lates calcarifer*) (100% replacement) (Ahmad et al., 2013, Salini et al., 2015) without affecting growth or feed conversion efficiency, however the fatty acid profile the fish is consistently reflective of the dietary fatty acid profile. Beef tallow has successfully replaced FO in the feeds for Atlantic Salmon (*Salmo salar*) (Emery et al., 2016) and Rainbow Trout (*Oncorhynchus mykiss*) (Gause and Trushenski, 2013) without affecting growth or feed conversion efficiency. However, beef tallow as a FO replacement has been shown to be detrimental in Silvery Black Porgy (*Sparidentex hasta*), which exhibited reduced growth and feed conversion efficiency (Mozanzadeh et al., 2016) and in Cobia, where growth was unaffected but feed conversion

efficiency was inferior (Woitel et al., 2014a, Woitel et al., 2014b). As with the majority of FO replacements the major bottleneck for terrestrial derived animal oils and tallows is the modification to the fatty acid profile of the fish, generally resulting in tissues that are high in saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and omega 6 fatty acids and low in n-3 LC PUFA. Comparatively, the fatty acid profile of wild-caught seafood is generally abundant in n-3 LC PUFA and scarce in omega 6 fatty acids (Yearsley et al., 1998). Reduced n-3 LC PUFA concentrations in fish flesh resulting from dietary oil replacement can be mitigated with a partial replacement strategies (including a small amount of FO in the diet), but the high levels of SFA, MUFA and omega 6 fatty acids can be difficult to reduce in fish flesh. Omega 6 fatty acids are already in great abundance in non-fish protein sources in the western diet (Givens and Gibbs, 2006; Zambiazi et al., 2007) and they should be limited in the diet. Reducing their content in fish products would likely be beneficial to the human consumer.

The general consensus from recent research into terrestrial derived animal oils and tallows as a FO replacement are that these alternative oils are capable of producing comparable growth and feed efficacy in fish as when they are reared on FO diets. The cost reduction associated with alternative oils currently make these sources viable for commercial aquafeeds. While a substantial portion of FO can be replaced with these alternative oils, some dietary FO is still generally required to assure n-3 LC PUFA requirements of the species are met and n-3 LC PUFA inclusion is the flesh is adequate for human consumers. Terrestrial animal-derived oils and tallows only provide the part of the solution to replacing FO in aquafeeds and in the future they will need to be used in conjunction with the efficient use of FO and finishing diets to improve fatty acid composition and provide human consumers with the superior nutritional quality found in wild caught fish or in cultured fish reared on FO rich diets.

1.3.2. Terrestrial derived plant oils

The most consistently utilised terrestrial derived plant oil is arguably canola oil (CO). Canola oil has been used to successfully replace FO in Red Hybrid Tilapia (*Oreochromis sp.*) (100% replacement) (Teoh and Ng, 2016), Atlantic Salmon (80% replacement) (Liland et al., 2013) and Rainbow Trout (75% replacement) (Turchini et al., 2013) aquafeeds without negatively affecting growth or feed conversion efficiency. However, in YTK high dietary CO at an inclusion above 50% affected growth and feed conversion efficiency and also downregulated trypsin and lipase enzyme activities, which could impact nutrient digestibility (Bowyer et al., 2012b). Conversely, in Red Hybrid Tilapia, dietary CO has a positive effect, upregulating n-3 LC PUFA biosynthetic pathways resulting in greater quantities of n-3 LC PUFA in the flesh, compared to diets where FO was replaced with sunflower oil (Teoh and Ng, 2016). This was attributed to the increased quantities of precursor 18:3n-3 alpha-linoleic acid in the CO diet, compared to the sunflower oil diet, that were able to be readily bio-converted in to n-3 LC PUFA by Nile Red Tilapia. When comparing the two species it is evident that vast differences exist in the capacity of different species to bio-convert precursor fatty acids into n-3 LC PUFA and as such while CO might be a viable alternative lipid source for some species of farmed fish it will not be suitable for all.

A range of other terrestrial derived plant oils have been successfully trialled recently in feeds for a range of aquacultured species. In Barramundi, rice bran oil can 100% replace FO (Glencross et al., 2016), in Atlantic Salmon olive oil can replace 80% FO (Liland et al., 2013), in Greater Amberjack (*Seriola dumerili*) palm oil can 50% replace FO and linseed oil can 100% replace FO (Monge-Ortiz et al., 2018), in Cobia (*Rachycentron canadum*) soybean oil can replace 67% FO (Trushenski et al., 2011), in Murray Cod (*Maccullochella peelii peelii*) FO can be replaced with palm oil, olive oil, sunflower oil or linseed oil (Turchini et al., 2011), in

Rainbow Trout FO can be 75% replaced with sunflower oil or soybean oil (Turchini et al., 2013), and in all cases growth and feed conversion efficiency were unaffected by FO replacement. However, other effects were observed in European Sea Bass, where replacing dietary FO with terrestrial derived plant oils resulted in changes to the anterior and posterior gut morphology and microbiota (Torrecillas et al., 2017). In Murray Cod fed terrestrial derived plant oils, the abundant MUFA and SFA were catabolised at the expense of n-3 LC PUFA, creating a n-3 LC PUFA sparing effect (Turchini et al., 2011). The sparing of n-3 LC PUFA refers to a mechanism that is responsible for the n-3 LC PUFA concentration in various tissue regions being preserved while other fatty acids are utilised for energy and metabolic processes (Trushenski et al., 2011). When n-3 LC PUFA is not being spared in fish it generally appears that all fatty acids are utilised for energy and metabolic processes without prejudice. The sparing of n-3 LC PUFA generally occurs in fish when dietary levels of n-3 LC PUFA are low, however it remains to be elucidated how fish are able to discriminate between these fatty acids to achieve these processes. Lastly in Rainbow Trout, FO replacement with terrestrial derived plant oils caused fish to actively bio-convert EPA to DHA (Turchini et al., 2013). These authors did however note that this bio-conversion also occurred in their FO control group, they thereafter indicated that the ratio of EPA to DHA of 1.8 was likely not ideal. They concluded that close attention needed to be paid to the ratio of individual n-3 LC PUFA in aquafeeds for Rainbow Trout to minimise the metabolic effort required for such processes. This last study brings attention to the need to closely monitor all factors that can be affected by modifications to aquafeeds formulations and manage them such that results of feed trials are not limited by inadequate nutrient supply.

Using terrestrial derived plant oils as a replacement for FO has similar associated problems to terrestrial derived animal oils with alterations to the flesh fatty acid profile. However, these plant derived alternative oils do appear to trigger some beneficial biological

reactions with regards to n-3 LC PUFA sparing and bio-conversion of precursor fatty acids into n-3 LC PUFA by some species. However, given that this sparing was at the expense of MUFA and SFA, it is likely that the same reactions could be triggered with the inclusion of terrestrial derived animal oil replacements. It appears to be necessary to consider the capacity of each individual species to spare and bio-convert n-3 LC PUFA when fed different alternative dietary lipids in order to maximise the utilisation of aquafeeds.

1.3.3. Marine algal products

Recently marine algal products have become more widely available for experimental use in aquafeeds. Algal products have the added benefit of being a naturally rich source of important n-3 LC PUFA and are in fact the source of LC PUFA in all marine fish. The marine algae *Tisochrysis lutea* and *Tetraselmis suecica* have been successfully used to substitute for FO in the feeds for European Sea Bass (Cardinaletti et al., 2018). Similarly, *Aurantiochytrium sp.* has been utilised in the feeds for Totoaba (*Totoaba macdonaldi*) to counteract the reduction in n-3 LC PUFA deposition resulting from replacing dietary FO with PO and beef tallow (Mata-Sotres et al., 2018). *Arthrospira sp.* and *Schizochytium limacinum* have also shown potential as algal products capable of replacing dietary FO in aquafeeds for Red Drum (*Sciaenops ocellatus*) (Perez-Velazquez et al., 2018).

The primary bottleneck for the use of marine algal oils in large-scale aquaculture operations is high cost and limited availability. The potential for marine algal products to replace dietary FO in aquafeeds has only recently been realised. As with the development of any new product the process of refining and upscaling production can be complex. However, over time, as production techniques improve and costs decrease, it is expected that marine algal products will become more available and affordable, and as such will be used more readily in commercial aquafeeds.

1.3.4. Oils derived from terrestrial transgenic crops

Lastly, transgenics provides an interesting opportunity to modify terrestrial crops to produce a new source of n-3 LC PUFA and fill the gap between supply and demand of these critically important fatty acids. Recently, the Commonwealth Scientific and Industrial Research Organisation (CSIRO) successfully bioengineered a strain of canola which is capable of producing n-3 LC PUFA (CSIRO, 2019). This was achieved by introducing a set of eight genes from marine algal sources into canola DNA to extend its fatty acid biosynthetic pathway and allow it to produce n-3 LC PUFA. The fatty acids profile of the oil from these canola crops are exceptionally abundant in DHA and have an ideal n-3 to omega 6 ratio. Similar strides have been made in producing transgenic flaxseed oil (*Linum usitatissimum*) crops capable of producing n-3 LC PUFA (Lu and Kang, 2008).

Recently, Napier et al. (2015), Sayanova and Napier (2011) and Robert (2006) have reviewed the capacity for oils derived from terrestrial transgenic crops to meet the growing demand for n-3 LC PUFA, both as a direct source for humans and also for aquaculture. These authors consistently note the same challenges that must be overcome if terrestrial transgenic oil seed crops are going to alleviate the pressure on wild fisheries as a source of n-3 LC PUFA. Firstly, they identify the need to obtain regulatory approval to grow these crops and then utilise their products in aquafeeds. Secondly, there is a need to obtain a thorough understanding of the agronomy of these crops to ensure their efficient production. Thirdly, it will be important to understand the capacity of farmed fish to utilise n-3 LC PUFA from this new source and any secondary factors that may present as a result of FO being replaced with oils derived from terrestrial transgenic crops, for example, changes to gut microbiota or morphology, which could have flow on effects for fish health. And lastly, there is the challenge of consumer acceptance. The general public will likely require a thorough understanding of the benefits of

oils derived from terrestrial transgenic crops in order to accept their incorporation in food products.

Utilising oils derived from terrestrial transgenic crops will not be as challenging as utilising marine algal products in relation to high cost or availability, but other challenges may be difficult to overcome. As previously mention the production of terrestrial transgenic crops is currently limited by regulatory restrictions, but approval for production appears to be imminent in Australia and the United States of America (Napier et al., 2019). The recent primary research utilising oils from transgenic terrestrial crops has focussed on the use of transgenic *Camelina sativa* in the feeds of Atlantic Salmon (Betancor et al., 2015a, Betancor et al., 2015b) and Gilthead Sea Bream (*Sparus aurata*) (Betancor et al., 2016). In both species, genetically modified oils from terrestrial transgenic crops, high in n-3 LC PUFA, where shown to be suitable replacements for dietary FO. Therefore, the likely final challenge will be overcoming negative consumer preconceptions about genetically modified products and educate them on the need for such products in the expanding aquaculture industry.

1.3.5. Fish oil replacement in YTK feeds

As previously mentioned, dietary FO can be replaced for juvenile YTK 100% by PO or 50% by CO, as long as the minimum n-3 LC PUFA requirement is met. Furthermore, Bowyer et al. (2012a) and Meigel et al. (2010) found that temperature influenced the digestibility of dietary fatty acids in juvenile YTK. In large YTK, FO is also replaced in significant proportions, often by PO, after the n-3 LC PUFA specification has been fulfilled with an adequate inclusion of FO, but further research is required to fully understand the range of effects that PO as a dietary lipid source has on YTK.

In YTK the use of PO as a dietary lipid source appears to have negligible effects on growth or feed conversion efficiency, however, there is an impact on the resulting product

quality. As observed with other species, the fatty acid profile of YTK is reflective of the dietary fatty acid profile. When PO replaces dietary FO, this results in higher quantities of SFA, MUFA and omega 6 fatty acids compared to the quantities observed in wild YTK or YTK reared on high FO, more 'natural' diets. Canola oil is not widely used as a FO replacement in the diets of large YTK given its inferior results in terms of growth and feed conversion efficiency in juvenile YTK.

Marine algal products and oils derived from terrestrial transgenic crops that are high in n-3 LC PUFA have not yet been trialled for YTK, however, once these lipid sources become more widely available and affordable it will be worthwhile investigating their suitability for this species.

1.4. Dietary n-3 LC PUFA requirements

To sustainably manage FO as a resource it will continue to be important to understand species-specific n-3 LC PUFA requirements and how they can change with age, environmental parameters and/or disease status. Defining these requirements for commercially important aquaculture species will allow the minimal quantity of FO to be used to attain maximal growth and feed efficacy without being wasteful.

Specific dietary n-3 LC PUFA requirements have been established for juvenile Japanese Yellowtail (*Seriola quinqueradiata*) (3.9 g 100 g^{-1} feed (Ishihara and Saito, 1996)), adult Japanese Yellowtail (2.0 g 100 g^{-1} feed (Deshimaru et al., 1982)), juvenile Meagre (*Argyrosomus regius*) (2.0 g 100 g^{-1} feed (Carvalho et al., 2018)), Grouper (*Epinephelus coioides*) (1.83 g 100 g^{-1} feed (Chen et al., 2017)), and Atlantic Salmon (> 2.0 g 100 g^{-1} feed - ref 17). In terms of net production of n-3 LC PUFA, Nile Tilapia and Eurasian Perch (*Perca fluviatilis*), have been shown to be efficient bio-converters of linoleic acid (LOA) to n-3 LC PUFA (Henrotte et al., 2011, Chen et al., 2018), while Grouper and Barramundi have been

shown to be inefficient converters to n-3 LC PUFA. In Barramundi excess inclusion of alpha linoleic acid (ALA) may inhibit DHA synthesis.

Recently, in Atlantic Salmon and Rainbow Trout the addition of dietary micronutrients and coenzymes has been observed to improve n-3 LC PUFA biosynthetic pathways (Lewis et al., 2013, Giri et al., 2016). The timing of feeding has also been investigated in relation to the efficiency of n-3 LC PUFA deposition in Rainbow Trout (Brown et al., 2010), Gilthead Sea Bream and European Sea Bass (Eroldoğan et al., 2018). In all cases feeding in the afternoon had a positive effect on fatty acid deposition, suggesting that circadian patterns can be exploited to improve feeding efficiency.

Lastly, some studies have sought to understand whether all individual n-3 LC PUFA are required for cultured fish. Research in this area had been limited due to the limited availability of purified forms of EPA, DPA, DHA and Arachidonic Acid (ARA). In California Yellowtail (*Seriola dorsalis*), it appears that only DHA and ARA are required for optimal growth and health and when they can be supplemented into the diet at adequate quantities, 100% FO can be replaced in the diet (Rombenso et al., 2016). Similarly, in juvenile Cobia, dietary DHA was required while EPA was largely expendable (Trushenski et al., 2012).

As mentioned above, species-specific n-3 LC PUFA requirements are likely to change based on a number of factors (age, sexual maturity, environmental parameters and/or disease status) but are also likely to be influenced by changes to other dietary components and feeding strategies. The complex nature of n-3 LC PUFA species-specific requirements has likely limited progress in this area. The lack of defined requirements for all commercially important species is indicative that further research is required in this area.

1.4.1. Dietary n-3 LC PUFA requirements for YTK

The dietary n-3 LC PUFA requirement for YTK had previously been conservatively estimated based on the requirement reported for the closely related Japanese Yellowtail at 2.0 g 100 g^{-1} feed (Deshimaru et al., 1982; Stone and Bellgrove, 2013). Based on results from the recently completed K4P project this requirement has since been revised to 2.12 – 2.26 g 100 g^{-1} feed for sub-adult Yellowtail Kingfish (Stone et al., 2019b). This requirement was established based on the optimal growth and feed conversion of large sub-adult YTK (> 2 kg) during an 84-day experiment conducted at warm water temperatures. This requirement will be discussed further in Chapter 3.

1.5. Modifications to n-3 LC PUFA concentrations of aquacultured species prior to harvest to benefit human consumers

Phase feeding and finishing/harvest diets have been utilised in the aquaculture industry for a number of years. While they are not a long-term solution to minimising the use of FO in aquafeeds, they are a means of reducing the quantity of FO required to produce a product that is as nutritionally beneficial to the human consumer as a fish reared on high FO diets throughout the production cycle. The basic premise of phase feeding and finishing/harvest diets is that farmed fish can be reared during the majority of the production cycle on a cheaper and more sustainable low FO diet, then in the weeks/months prior to harvest the diet is substituted for high FO diet. The high FO diet is abundant in n-3 LC PUFA and improves the fatty acid profile of the fish relative to a particular benchmark (generally the fatty acid profile of fish reared throughout production on the equivalent of the high FO diet or the fatty acid profile of a wild caught fish of the same species).

The use of finishing diets has been investigated for Rainbow Trout (Thanuthong et al., 2011, Stone et al., 2011a, Stone et al., 2011b, Thanuthong et al., 2012) Atlantic Salmon (Bell

et al., 2003, Codabaccus et al., 2012), Gilthead Sea Bream (Benedito-Palos et al., 2009), Turbot (*Psetta maxima*) (Regost et al., 2003), Senegalese Sole (*Solea senegalensis*) (Reis et al., 2014), Jade Perch (*Scortum barcoo*) (Van Hoestenberghe et al., 2013) and Red Hybrid Tilapia (Ng et al., 2013). In all cases a finishing diet rich in FO resulted in a significant increase in tissue n-3 LC PUFA concentration. However, there were slight differences in the experimental designs utilised, with finishing periods varying from 2 weeks (Van Hoestenberghe et al., 2013) up to 20 weeks (Bell et al., 2003) and in some cases a food deprivation period was implemented prior to the finishing period (Codabaccus et al., 2012, Thanuthong et al., 2012). Generally, shorter finishing periods did not restore the fatty acid profile to the same degree as longer finishing periods and were not able to fully restore the fatty acid profile compared to a control treatment fed dietary FO throughout the growth period. Van Hoestenberghe et al. (2013) achieved a 25% recovery of n-3 LC PUFA in 2 weeks, while in Bell et al. (2003) achieved approximately 80% recovery in 20 weeks. Also, from a fatty acid perspective, omega 6 (n-6) fatty acids remained consistently higher in fish reared on non-FO diets and then switched to finishing diets, compared to those reared throughout the growth period on FO control diets. The high n-6 fatty acid concentrations in these fish is problematic, because n-6 fatty acids are abundant in the western diet and their consumption should be limited in humans (Simopoulos, 2002), and as such their excess quantity in aquacultured fish is not a desirable trait.

In more recent studies, a finishing period was preceded by a food deprivation period, which aimed to reduce flesh fat content to improve n-3 LC PUFA restoration. In Codabaccus et al. (2012) food deprivation prior to the finishing period improved the efficiency of n-3 LC PUFA restoration in the flesh of Atlantic Salmon. However, Thanuthong et al. (2012) found that this strategy resulted in only minor improvements in tissue n-3 LC PUFA content and a loss of weight gain due to the 2 weeks of food deprivation. The shorter food deprivation period utilised by Codabaccus et al. (2012) had reduced negative implications for weight gain but in

parallel likely reduced total lipid loss during this time and would have implications for the degree of n-3 LC PUFA restoration that was possible. Furthermore, in both studies, food deprivation did not result in significantly different contents of n-3 LC PUFA in the flesh compared to a standard finishing diet fed without prior food deprivation. These results indicate that the same level of recovery could be achieved in a shorter period of time when the food deprivation method was employed, specifically, 1 or 2 weeks of food deprivation followed by 4 or 6 weeks respectively of feeding of the finishing diet, compared to a standard 5 or 8 weeks of continual feeding of a finishing diet result in the same muscle content of n-3 LC PUFA. This is promising as a smaller quantity of high FO feed is required to produce a fish with the same nutritional benefits (n-3 LC PUFA), meaning that producers can reduce FO use and aquafeed costs, and improve environmental sustainability. Conversely, withholding feed has substantial associated risk, such as reduced health status of fish and reduced product quality if finishing periods are not successful. The practicality of such strategies would need to be carefully considered and extensively research before implementation.

1.5.1. Modification of n-3 LC PUFA concentration in YTK prior to harvest

Finishing diets have not yet been assessed for YTK, but they do present an opportunity to alter the fatty acid profile of YTK in a positive manner prior to harvest. A short period of feeding of a high FO diet prior to harvest, with or without a period of food deprivation, could assist in providing human consumers with a product that is higher in n-3 LC PUFA than that which is available with current commercial YTK aquaculture practices. This is investigated further in Chapter 5.

1.6. Thesis objectives and aims

The primary objective of this research was to expand on the current state of knowledge of lipid utilisation by YTK, with a specific focus on optimising dietary lipids for YTK during

712 the grow-out stage of production (when fish were > 2kg). Implications for fish health, human
713 consumers, commercial YTK producers and economic and environmental sustainability were
714 considered throughout each stage of investigation.

715 To understand the baseline or normal lipid and fatty acid composition of YTK this
716 research compared the composition of aquacultured and wild YTK. And although the sample
717 size of wild fish was small (n = 6) and the average fish weight was two times greater (6.7 kg)
718 than that of the YTK tested in the current tank-based trial, differences existed between the two
719 groups likely due to differences between their respective diets. Understanding the effects of
720 compounded aquafeeds and commercial feeding practices on the lipid and fatty acid
721 composition of aquacultured YTK compared to 'natural' diets and feeding behaviours of wild
722 YTK has provided a key starting point for improving biological functioning and product quality
723 of aquacultured YTK, while also improving the profitability and sustainability of YTK
724 production. Thereafter, methods to manipulate and improve the utilisation of n-3 LC PUFA in
725 aquacultured YTK were investigated. The aim of these experiments was to investigate how
726 dietary n-3 LC PUFA, the critical group of fatty acids provided by the inclusion of FO in YTK
727 aquafeeds, could be reduced without negatively impacting fish health or fatty acid digestibility
728 and could be manipulated during the final stages of production to benefit human consumers.
729 Lastly, this research aimed to validate a new method to quantify the downstream bioactive
730 products of dietary fatty acids and investigate the effect of changes to dietary fatty acid
731 composition on their relative abundance. This line of investigation has provided a new tool for
732 aquaculture nutrition studies and has the potential to increase our understanding of dietary fatty
733 acids are utilised once within the bodies of YTK.
734

1.7. References

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1.8. Figure

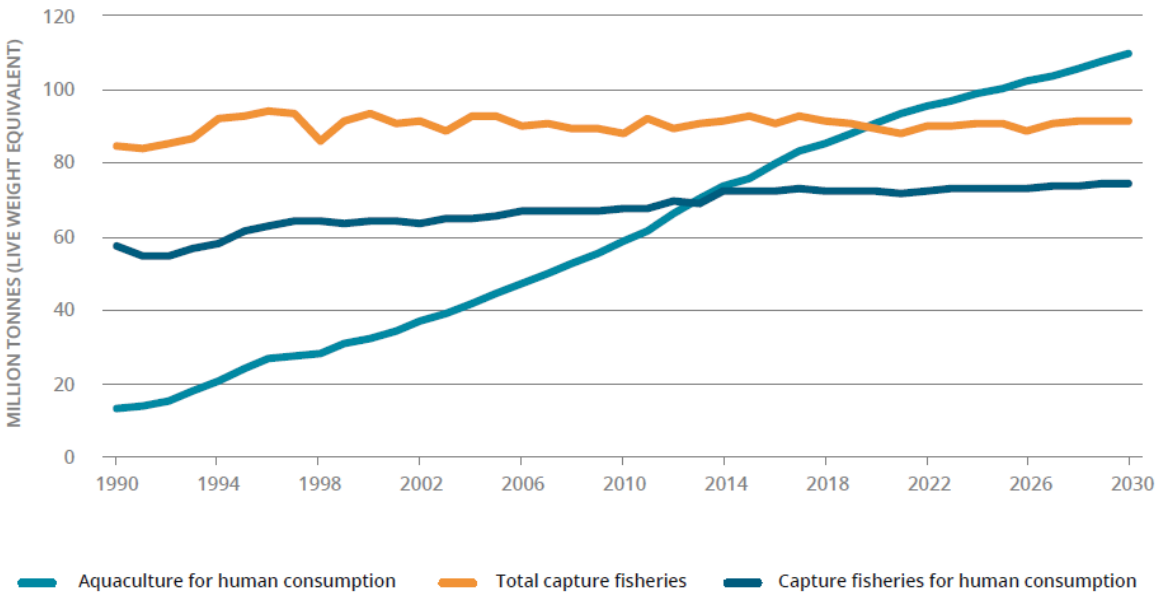

Figure 1.1: Global capture fisheries and aquaculture production from 1990 and estimated through to 2030 (Source: FAO, 2016 – Figure 34).

Chapter 2 – Statement of authorship

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Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Resources, methodology, writing – review & editing, supervision, project administration and funding acquisition (2.5%)		
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Chapter 2: Comparison of the fatty acid composition of aquacultured versus wild Yellowtail Kingfish (*Seriola lalandi*) from South Australia

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Abstract

Consumers often set benchmarks for the product quality of aquacultured fish based on a comparison to wild fish, and arguably one of the most important product quality attributes for fish is its omega 3 (n-3) long chain (LC) polyunsaturated fatty acid (PUFA) content, due to its benefits for human health. In the present study, we investigated the differences in total lipids and fatty acid content between wild and aquacultured Yellowtail Kingfish (*Seriola lalandi*) (YTK) from South Australia, where most farmed Australian YTK is grown. Tissue samples (white muscle, red muscle, liver and visceral adipose) were taken from tank-reared aquacultured (n = 9; average weight = 3.79 kg) and wild YTK (n = 6; average weight = 6.77 kg). Tissue total lipid content of the aquacultured YTK was on average 4-times higher compared to the wild YTK (e.g. 6.8% vs 1.5% for white muscle). There was a significantly higher content of total saturated, omega 9, omega 7, omega 6 and omega 3 fatty acids ($P < 0.001$) in the white muscle and liver of the aquacultured fish, however, the total n-3 LC PUFA content of the aquacultured fish was not significantly different to the wild fish ($P > 0.05$). In red muscle and adipose tissue, the total n-3 LC PUFA was significantly lower in aquacultured YTK. There were also significant differences in the relative abundances of each of the individual n-3 LC PUFAs (DHA > EPA > DPA) in the tissues of the aquacultured and wild YTK. Importantly, for consumers to obtain their recommended daily intake of 500 mg of n-3 LC PUFA a 72 g portion of white muscle from either the aquacultured or wild YTK would be sufficient. Commercial feed manufacturers and YTK producers can utilise this information to improve feed formulations and manage product quality.

Keywords

Yellowtail Kingfish, wild, aquaculture, omega 3 (n-3) long chain (LC) polyunsaturated fatty acids (PUFA) and product quality.

Highlights

1. The fatty acid composition of the white muscle, red muscle, liver and adipose tissue of wild and aquacultured South Australian YTK was determined.
2. The quantity of n-3 LC PUFA in the white muscle of the wild and aquacultured South Australian YTK did not differ significantly.
3. A 72 g portion of white muscle from either the aquacultured or wild YTK would be sufficient for human consumers to obtain their recommended daily intake of 500 mg of n-3 LC PUFA.
4. Fat content was significantly higher in aquacultured than wild South Australian YTK.

2.1. Introduction

Commercial farming of Yellowtail Kingfish (*Seriola lalandi*) (YTK) is expanding in Australia, with lease areas now allocated in South Australia and Western Australia, and production outputs expected to increase substantially in the coming years (Norwood, 2017). From a product quality perspective, it is desirable that aquacultured YTK are nutritionally at least as beneficial to consumers as their wild caught counterparts, given that consumers could reasonably set a benchmark expectation for the nutritional quality of aquacultured fish based on equivalency with the wild fish (Yearsley et al., 1998). This can be challenging for aquaculturists to achieve as they try to minimise the costs of production, improve the sustainable use of marine ingredients (fish oil) in the fish diets, maximise growth rates, and carefully manage fish health and product quality. Furthermore, since regular consumption of fish products is recommended to meet human daily intake requirements for omega 3 (n-3) long chain (LC) polyunsaturated fatty acids (PUFA) (e.g. The International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommends a daily intake of 500 mg n-3 LC PUFA per day (ISSFAL, 2004)) consumers and producers want to know the serving size of the aquacultured fish product that contributes toward this target.

Formulated aquaculture diets require a certain amount of fish oil (FO) as it provides essential n-3 LC PUFA for growth and development, energy for metabolism and also adds to the palatability of the diet (Sargent et al., 1999, Miller et al., 2008). However, FO and likewise fish meal (FM) are limited resources and high global demand has made them some of the most expensive macro-ingredients in aquaculture feeds (Naylor et al., 2000, Naylor et al., 2009, Tacon and Metian, 2008). Moving forward, there is a need to ensure global supply of marine fish for human food. The measure of sustainability most commonly used in the aquaculture industry is the fish-in fish-out ratio, which equates the quantity of fish and fish products (FO and FM) required to produce the same quantity of aquacultured fish (Terpstra, 2015). For these

reasons, considerable effort has been made to reduce the FO and FM content of aquaculture diets by replacing a portion of it with more sustainable and cheaper protein sources and terrestrial lipids such as poultry meal, poultry oil, canola oil, soybean oil, corn oil and to a lesser extent, meat tallows (Turchini et al., 2009, Naylor et al., 2009). These terrestrial lipid sources have a different fatty acid profile to wild FO, with higher omega 6 (n-6) PUFA, monounsaturated (omega 9, n-9) and/or saturated fatty acids, depending on the type of oil (Burton et al., 2004). Importantly, the saying "you are what you eat" is largely true for fish in regard to lipids and substitution of FO with terrestrial oils is known to affect the fatty acid composition of the aquacultured fish (Turchini et al., 2009).

For this reason, differences in the fatty acid composition of aquacultured and wild YTK are likely to exist. Recently O'Neill et al. (2015) studied the fatty acid composition of aquacultured and wild YTK from the southern region of Africa, concluding that there was no significant difference in n-3 fatty acid content between them, although, aquacultured fish had significantly higher quantities of n-6 PUFA and thus a higher ratio of n-6 to n-3 fatty acids. Similar differences are likely to exist between Australian aquacultured and wild YTK.

In Australia, while there has been substantial research effort to define parasite interactions between wild and aquacultured YTK (Hutson, 2007), differentiating between wild and aquacultured YTK with natural element signatures and otolith analyses (Gillanders and Joyce, 2005) and to define the fatty acid composition of aquacultured YTK (Bowyer et al., 2012, Stone et al., 2016, Australian Seafood CRC, 2018, Chapter 3; Stone et al., 2019), there is currently little available information on the fatty acid composition of wild YTK (Yearsley et al., 1998). The aim of the current study, based on limited spatial and temporal sampling, was to obtain a preliminary understanding of the fatty acid composition of wild and aquacultured YTK from South Australian stocks.

2.2. Methods and Materials

2.2.1. Sample collection

A total of 6 wild YTK, 3 females and 3 males, with an average weight of 6.77 ± 1.37 kg (mean ± SE, range: 2.85 - 12.40 kg), were collected off the south west coast of the Eyre Peninsula in South Australia in February 2018. Fish were captured utilizing standard recreational fishing practices including lures and baited hooks. Once on-board the fishing vessel, each fish was stunned, bled and tissue samples collected including: white muscle, red muscle, liver and adipose. White muscle was collected from the dorsal fillet adjacent to the dorsal fin, red muscle was collected from along the lateral line posterior to the pectoral fin, the whole liver was collected, and visceral fat was collected from around the visceral mass. Samples were frozen and stored at -20 °C until analysis. Additionally, for each fish length and weights (including total, visceral mass, visceral fat, stomach, gonad and liver) were recorded, stomach contents and gonads were collected, and reproductive stage was scored.

Aquacultured YTK were collected as part of a tank-based feed trial that investigated the optimum dietary inclusion levels of n-3 LC PUFA 100 g^{-1} on the growth of large sub-adult YTK in May 2016 (Stone et al., 2019). The optimum growth rate resulted from using a commercial diet containing 2.14 g n-3 LC PUFA 100 g^{-1} using 5% FO and 15% poultry oil coated onto a pellet that contained 7% lipid (from fishmeal and other ingredients) (20% fish meal; 40% crude protein, 27% crude lipid and a gross energy level of approximately 21 MJ kg^{-1};Stone et al., 2019). Given that this feed composition was recommended to commercial YTK feed producers, this data set was selected to be utilized to represent the aquacultured YTK for comparisons. Thus, matching tissue samples from 9 YTK, with an average weight of 3.79 ± 0.01 kg (mean ± SE, range: 3.59 - 4.34 kg), that had been reared on a commercial diet

containing 2.14 g n-3 LC PUFA 100 g^{-1} feed for 12-weeks were collected, frozen and stored at -20 °C until analysis.

2.2.2. Total lipid analysis

Tissue total crude lipid (as a percentage of wet weight) was estimated utilizing the gravimetric approach (Folch et al., 1957). Briefly, samples were homogenised in 0.9% saline in a 10 mL glass centrifuge tube, thereafter lipids were extracted into a 4:1 chloroform: isopropanol solution and then centrifuged at 3000 RPM for 10 minutes. The chloroform layer was removed, placed into a pre-weighed vial and then evaporated using nitrogen gas leaving only the lipid component behind; the vial was then reweighed.

2.2.3. Fatty acid analysis

Fatty acid profiling was conducted for all samples. The lipid component (extracted during total lipid analysis) was transmethylated with 1 % H_2SO_4 in MeOH at 70 °C for 3 hours, then cooled to room temperature, after which fatty acid methyl esters (FAME) were extracted in to 2 mL of heptane. The heptane was transferred to a gas chromatography (GC) vial with approximately 30 mg of anhydrous sodium sulphate, sealed and stored at -20 °C until analysis by GC. Samples were processed on a Hewlett-Packard 6890 GC (Hewlett-Packard, CA, USA) with a flame ionization detector, a split injector and a BPX-70 capillary column (internal diameter of 50 m × 0.32 mm) with a 0.25 µm film thickness (SGE, Victoria, Australia). Gas chromatography operating conditions were as described previously (Tu et al., 2010) and peaks were identified with GLC 463 external standard (Nu-Chek Prep Inc., MN, USA). Data output was processed with Agilent ChemStation (version Rev: B.01.03) (Agilent Technologies, CA, USA).

2.2.4. Calculations

Condition index was calculated using the following equation:

- Condition index = (fish weight (kg) / fork length (m^3)) / 10

Hepatosomatic index was calculated using the following equation:

- Hepatosomatic index (HSI, %) = (liver weight (g) / fish weight (g)) × 100

Visceral somatic index was calculated using the following equation:

- Visceral somatic index (VSI, %) = (wet visceral weight (g) / fish weight (g)) × 100

2.2.5. Statistics

Statistical analysis was performed using IBM SPSS (version 24). Homogeneity of variance was assessed using Levene's test, whilst normality was assessed with Kolmogorov-Smirnov test. Differences were analysed using a one-way ANOVA where fish source (aquacultured or wild) was a factor and data were separated by tissue region. An alpha level of 0.05 was used for all statistical tests. Results are presented as means ± standard error (SE).

2.3. Results

2.3.1. General observations

All aquacultured YTK readily accepted their feed and appeared to be in good health at the time of sample collection. All wild fish collected appeared healthy, minimal external parasite infection was observed, and no internal signs of compromised health were present. Although the sample size of wild YTK was small and the size range was large, minimal differences total lipid or fatty acid composition were observed between fish, creating increased confidence despite experimental limitations. Furthermore, wild YTK had little visceral fat, could be described as lean and had a condition index of 1.16 ± 0.03 compared to a condition index of 1.73 ± 0.04 for aquacultured YTK (Table 2.1). The adipose tissue collected from wild YTK was membranous and scarce, whereas visceral fat was abundant in aquacultured YTK.

2.3.2. Tissue total lipid content

Total lipid content of all tissues examined was more than 4 times higher for aquacultured YTK than for their wild counterparts (Tables 2.2 – 2.5). Notably, aquacultured YTK had 6.8% lipid in the white muscle compared to 1.5% in the wild YTK ($P < 0.001$). In the liver, there was 32.9% lipid in aquacultured YTK compared to 6.4% in wild YTK ($P < 0.001$).

2.3.2.1. Fatty acid composition of white muscle

With the exception of total n-3 fatty acids, all totals for major groups of fatty acids were substantially more abundant in the aquacultured YTK. In the aquacultured YTK total n-6 fatty acids were 13.5 times higher, total omega 7 (n-7) fatty acids were 8.1 times higher, total n-9 fatty acids were 12.5 times higher and total saturated fatty acids were 3.6 times higher, than in wild fish (Figure 2.1; all $P < 0.001$). All individual fatty acids, except DHA, were significantly more abundant in the aquacultured group (Table 2.2). On the other hand, there was no

significant difference in the quantity of n-3 LC PUFA in the white muscle between groups, overall average 705 mg 100 g^{-1} muscle (one-factor ANOVA; $P = 0.182$; Table 2.2). However, there was significantly higher levels of DHA in the wild YTK, with 575 mg 100 g^{-1} muscle compared to 422 mg 100 g^{-1} muscle in the aquacultured YTK (one-factor ANOVA; $P < 0.001$; Table 2.2). In contrast, the quantities of EPA and DPA were significantly higher in the white muscle of the aquacultured YTK, with 78 mg EPA and 32 mg DPA 100 g^{-1} tissue in wild fish, compared to 227 mg EPA and 76 mg DPA 100 g^{-1} muscle in aquacultured fish.

The n-3: n-6 ratio in the white muscle was significantly different between groups (one-factor ANOVA; $P < 0.001$). In wild YTK for every unit of n-3 present there was 0.1 unit of n-6, while in aquacultured YTK this increased to 0.9 units of n-6 (Table 2.2).

2.3.2.2. Fatty acid composition of red muscle

Total n-6, n-7, saturated fatty acids and individual fatty acids were significantly more abundant in the aquacultured group (Tables 2.3). Aquacultured YTK had a significantly higher content of n-3 LC PUFA in their red muscle compared to their wild counterparts, with 3179 mg and 2871 mg 100 g^{-1} muscle respectively (one-factor ANOVA; $P < 0.001$; Table 2.3). Similar to the white muscle, DHA was significantly more abundant in red muscle of wild YTK (one-factor ANOVA; $P < 0.001$) and EPA and DPA were both significantly more abundant in the aquacultured YTK (one-factor ANOVA; $P < 0.001$ for both). The n-3: n-6 ratio in the red muscle was significantly different between groups (one-factor ANOVA; $P < 0.001$, Table 2.3) and consistent with observations made in the white muscle.

2.3.2.3. Fatty acid composition of liver

All major groups of fatty acids and individual fatty acids were present in significantly greater quantities in the aquacultured YTK group (Table 2.4), with the exception of total n-3

fatty acids, DHA and tetracosenoic acid (24:1n-9), which were not significantly different between groups.

There were significantly higher quantities of DHA (one-factor ANOVA; $P < 0.001$) in the liver of the wild YTK but significantly higher levels of EPA and DPA in the aquacultured YTK (one-factor ANOVA; $P < 0.001$ for both), which resulted in total n-3 LC PUFA levels that were not significantly different between groups (Table 2.4).

Differences in the n-3: n-6 ratio were more exacerbated in the liver compared to the muscles, for every 1 unit of n-3 there was 1.6 units of n-6 in the aquacultured YTK, while in the wild YTK for every 1 unit of n-3 there was 0.1 units of n-6 (Table 2.4).

2.3.2.4. Fatty acid composition of adipose tissue

With the exception of total n-3 fatty acids, all totals for major groups of fatty acids, total n-6, n-7, n-9 saturated fatty acids, were all substantially more abundant in the adipose tissue of aquacultured YTK (Table 2.5). In aquacultured YTK, total n-3 fatty acids, DHA and n-3 LC PUFA were significantly less abundant compared to the wild YTK (one-factor ANOVA; $P < 0.001$ on all accounts; Table 2.5). On the other hand, all other individual fatty acids were present in significantly higher quantities in the aquacultured YTK than in the wild YTK, with the exception of tetracosanoic acid (24:0) and tetracosenoic acid (24:1n-9) which did not differ significantly between groups (Table 2.5).

2.4. Discussion

The data presented here describe the full fatty acid profile of wild South Australian YTK and provide a comparison to aquacultured South Australian YTK. Summary data for key fatty acids in wild Australian YTK flesh were previously described by Yearsley et al. (1998). Total lipid content, arachidonic acid and EPA values reported by Yearsley et al. (1998) and the current study were similar, however DHA values were substantially higher in wild fish in the current study. The higher DHA content could be explained by differences in numerous factors between the two studies; environmental (water temperature, capture location, feed source, etc.), physical (age, sexual maturity, etc.) or sample location (whole body verses fillet). Without further information regarding these factors from the Yearsley et al. (1998) study the reason for the difference in DHA content of wild YTK could not be discerned.

The most recent comparable study that investigated the differences between wild and farmed YTK was from African stocks (O'Neill et al., 2015). Data reported by O'Neill et al. (2015) were for the whole fillet, skin and bones removed and fish were collected in March 2009 for both wild and sea-cage farmed fish. The key findings from the O'Neill et al. (2015) study and the current study were similar; in the fillet (O'Neill et al., 2015) and in the white muscle (current study) total n-3 LC PUFA was not significantly different between wild and aquacultured YTK, DHA was significantly higher in the wild YTK, but EPA and n-6 PUFA were significantly higher in the aquacultured YTK.

O'Neill et al. (2015) didn't find any significant difference in total fat content of the fillet between aquacultured and wild YTK (3.72% vs 4.29% respectively). Interestingly, the commercial diet utilized by O'Neill et al. (2015) had a dietary lipid level of 15% compared to 27% in the current study, and this would have resulted in a lower total lipid and gross energy value in the farmed South African diet and perhaps this was why the fillet lipid level was less than in Australian farmed fish. In contrast, in the current study the fat content in all tissues

collected from the aquacultured YTK were significantly higher than their wild counterparts. This difference was attributed to aquacultured YTK being raised in intensive culture conditions with feeding to visual satiation occurring daily designed to maximise growth rate (Stone et al., 2016). Furthermore, commercial YTK feed in Australia typically consists of 6.8% moisture, 45.1% crude protein, 24% crude lipid, 15.2% carbohydrates and 19.1 MJ gross energy kg^{-1} (Stone et al., 2016), which provides a substantially higher content of lipid than the aquacultured YTK feed from the O'Neill et al. (2015) study. In comparison, wild YTK need to hunt and capture mostly live prey items (such as fish and squid) and are likely to consume a substantially smaller portion of food on a daily basis and expend a lot more energy to obtain their food. A standard prey item for wild YTK is the South Australian sardine (*Sardinops sagax*; a plentiful baitfish species in South Australian waters) and its composition has been reported as 72.1% moisture, 18.9% crude protein, 4.1% crude lipid, 0.4% carbohydrates and only 4.73 MJ gross energy kg^{-1} (Stone et al., 2016). Therefore, the quantity and nutrient density of foods, as well as differences in energy expenditure to capture that food, are likely driving the differences observed in the quantitative aspect of lipid deposition in all tissues in the present study.

Furthermore, the qualitative fatty acids composition of aquacultured YTK was substantially different to their wild counterparts. There were significantly higher content of non n-3 LC PUFA fatty acids, particularly n-6, n-9 and saturated fatty acids, in aquacultured compared to wild YTK, which could be considered an undesirable quality to human consumers. The Food and Agriculture Organization of the United Nations (FAO) recommends limiting dietary intake of saturated and n-6 PUFA due to associated negative health outcomes (FAO, 2010). In the current study, on average, saturated and n-6 PUFA are 3.6 and 12.9 times more abundant in aquacultured YTK than in their wild counterparts, due to their high concentrations in aquafeeds. When consuming aquacultured YTK this translates to an additional intake of 1.2 g or 0.7 g 100 g^{-1} white muscle of saturated and n-6 fatty acids respectively. Saturated fatty

acids are abundant in animal products, such as meat or dairy (Givens and Gibbs, 2006), while n-6 fatty acids are abundant in plant-based oils, such as canola or sunflower oil (Zambiazi et al., 2007), all of which are generally consumed in greater quantities in the western diet than fish products. Since saturated and n-6 fatty acids should be limited in the diet and are already highly abundant in other food products, reducing their content in fish products would likely be beneficial to the human consumer. However, when comparing both aquacultured and wild YTK to other commonly consumed protein sources, such as beef, chicken or pork, the fatty acid profile of YTK (both aquacultured and wild) appears to be superior in relation to n-6 PUFA and saturated fatty acids being less abundant (Table 2.6).

In relation to n-3 LC PUFA, which was not significantly different between the wild and aquacultured YTK, human consumers would require a 72 g portion of white muscle from either aquacultured or wild YTK to obtain their recommended daily intake of 500 mg of n-3 LC PUFA. However, this was the case when aquacultured YTK were fed a level of n-3 LC PUFA that maximised fish growth, whereas white muscle n-3 LC PUFA concentration of aquacultured YTK could be further increased with a period feeding a high n-3 LC PUFA finishing diet prior to harvesting (see Chapter 5), making the n-3 LC PUFA concentration higher than the wild YTK.

For individual n-3 LC PUFA, DHA was significantly higher in all tissues measured in the wild YTK. For human consumers DHA is highly nutritionally beneficial, playing an important role in brain and vision development during infancy and in minimizing risk of cardiovascular issues and reducing inflammation in adults (FAO, 2010). Marine fish are recognized for being an excellent source of DHA (Ackman, 2008) and are often strongly marketed on this basis, however these results suggest that aquacultured YTK aren't reaching the benchmark of wild YTK in concern to this key fatty acid. Given that n-3 LC PUFA are digested with equal efficacy (see Chapter 4), it is likely that dietary content of individual n-3

LC PUFA was responsible for this difference. In wild marine fish, such as the sardines that wild YTK consumer, DHA approximately twice as abundant as EPA and this dietary pattern then gets reflected in the YTK body tissues. Comparatively, in the diets of aquacultured YTK (see Chapter 3 diets), DHA is only approximately 1.5 times more abundant than EPA, similarly, this dietary pattern then gets reflected in the YTK body tissues and likely accounts for this difference in DHA observed between aquacultured and wild YTK. Human daily intake requirements for DHA are not currently available, only n-3 LC PUFA requirements are available, as the individual benefits of consuming EPA and DPA are not fully understood. Until recently, purified forms of these fatty acids have not been available in sufficient quantities to undertake such studies and this has been a limiting factor for research (Kris-Etherton et al., 2009). Once the individual benefits of these n-3 LC PUFA are elucidated, it may be possible to more specifically modify their content and proportion in seafood products. However, given the current state of knowledge it is important to note that in terms of n-3 LC PUFA these wild and aquacultured YTK are not significantly different, regardless of the proportional differences in EPA, DHA and DPA.

The product characteristics of aquacultured and wild YTK, in terms of sensory attributes, are also likely to be driven by differences in the tissue lipid content. The difference in crude fat content between aquacultured and wild YTK would likely be discernible when consuming white and red muscle both as sashimi and when cooked. Sensory evaluation panels would need to be conducted to assess whether higher fat content is considered to be a positive or negative attribute, however the preference would likely differ among consumer groups. The significantly higher fat content of aquacultured YTK also results in an increased caloric intake for the human consumer. Specifically, consuming a 100 g portion of white muscle from aquacultured or wild YTK would result in an intake of either 1.5 g or 6.8 g of fat, respectively; this means that consuming aquacultured YTK results in an additional 48 kilocalories per 100

g serve. When considering the 2500 and 2000 kilocalories per day recommendations for adult men and women respectively, this could impact consumer selection. However, when considered in the context of other foods, such as processed meats, which are substantially higher in fat it would be likely that this difference would be trivial.

High fat content in aquafeeds has previously been observed to have some negative implications for YTK and aquaculturists. Firstly, feeds with high lipid contents more readily degrade before they are able to be consumed by the fish (during transport and storage, etc.), resulting in fish being fed oxidized or rancid lipids (Goddard, 2012). This has mostly been overcome by including antioxidants in aquafeeds (Hertrampf and Piedad-Pascual, 2012), however, it is an ongoing issue that requires careful management. Secondly, from the perspective of both aquaculturists and human consumers, high lipid feeds are wasteful in terms of lipid and specifically n-3 LC PUFA being incorporated in to the less commonly consumed portions (liver and adipose) of the fish. During summer months YTK growth rates substantially increase and feed rates increase in parallel to meet increased metabolic demand (Bowyer et al., 2012). However, not all of the nutrients are utilized for growth or energy and as such adipose tissue is accumulated in the body, often this adipose tissue is utilised for energy in winter months were reduced water temperature coincides with reduced feed intake. Importantly, aquacultured YTK do not appear to preferentially distribute different fatty acids among functionally different tissues (Chapter 3), meaning that fat storage/ non-edible tissues, such as visceral adipose, have the same amount of n-3 LC PUFA per gram of fat as the white muscle, which appears to be very wasteful, unless the processing of by-products can help to reclaim these nutrients.

2.5. Conclusions

This study has provided important information on the fatty acid profile of wild Australian YTK that can be used to inform seafood consumers and the commercial YTK aquaculture industry. Aquacultured YTK had higher crude fat levels in their tissues relative to wild fish (e.g. 6.8% vs 1.5% for white muscle, respectively) and proportionally higher levels of n-3 LC PUFA. However, when n-3 LC PUFA is calculated as mg 100 g^{-1} white muscle, the average content was 705 mg 100 g^{-1}, and not significantly different between wild and aquacultured YTK. Therefore, for consumers to obtain their recommended daily intake of 500 mg of n-3 LC PUFA a 72 g (recommended serving size of 100g) portion of white muscle from either aquacultured or wild YTK would be sufficient.

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2.8. Tables and figures

Table 2.1: Mean length (cm), weight (kg), condition index, hepatosomatic index (HSI) (%) and visceral somatic index (VSI) (%) wild (n = 6) and aquacultured (n = 9) Yellowtail Kingfish (*Seriola lalandi*) from South Australia (mean ± standard error).

	Length (cm)	Weight (kg)	CI	HSI (%)	VSI (%)
Wild	81.9 ± 6.27	6.77 ± 1.37	1.16 ± 0.03	1.23 ± 0.14	6.39 ± 0.30
Aquacultured	60.6 ± 0.58	3.79 ± 0.08	1.73 ± 0.04	1.30 ± 0.05	6.27 ± 0.23

Table 2.2: Total lipid content (%), fatty acid composition (mg 100 g^{-1} tissue) and ratio of omega 3 (n-3) to omega 6 (n-6) fatty acids of white muscle of wild (n = 6) and aquacultured (n = 9) Yellowtail Kingfish (*Seriola lalandi*) from South Australia (mean ± standard error).

	Wild	Aquacultured	*P* =
Lipid content (%)	1.5 ± 0.3	6.8 ± 0.6	< 0.001
t18:1n-9 (Palmitelaidic acid)	1.4 ± 0.1	15.8 ± 0.4	< 0.001
t18:1n-7 (Elaidic acid)	0.0 ± 0.0	21.7 ± 2.5	< 0.001
14:0 (Myristic acid)	29.9 ± 4.0	148.2 ± 8.0	< 0.001
15:0 (Pentadecanoic acid)	9.4 ± 0.7	17.7 ± 0.7	< 0.001
16:0 (Palmitic acid)	300.9 ± 4.0	1119.3 ± 7.9	< 0.001
17:0 (Margaric acid)	12.2 ± 0.3	26.1 ± 0.5	< 0.001
18:0 (Stearic acid)	110.1 ± 0.1	355.3 ± 3.2	< 0.001
20:0 (Arachidic acid)	2.4 ± 0.1	10.2 ± 0.4	< 0.001
22:0 (Docosanoic acid)	0.6 ± 0.2	4.5 ± 0.2	< 0.001
24:0 (Tetracosanoic acid)	0.3 ± 0.1	2.5 ± 0.1	< 0.001
18:3n-3 (Alpha Linolenic acid- ALA)	8.2 ± 1.0	106.7 ± 1.6	< 0.001
20:5n-3 (Eicosapentaenoic acid- EPA)	78.4 ± 3.3	227.4 ± 18.1	< 0.001
22:5n-3 (Docosapentaenoic acid- DPA)	31.5 ± 1.5	76.3 ± 3.5	< 0.001
22:6n-3 (Docosahexaenoic acid- DHA)	575.2 ± 28.6	421.7 ± 24.6	< 0.001
18:2n-6 (Linoleic acid- LOA)	20.2 ± 1.2	14.2	< 0.001
18:3n-6 (Gamma Linolenic acid)	1.7 ± 0.2	9.1 ± 0.2	< 0.001
20:2n-6 (Eicosadienoic acid)	4.6 ± 0.2	11.8 ± 0.1	< 0.001
20:3n-6 (Dihomo-gamma-linoleic acid)	1.8 ± 0.1	10.0 ± 0.1	< 0.001
20:4n-6 (Arachidonic acid)	26 ± 2.2	51.0 ± 1.7	< 0.001
22:4n-6 (Docosatetraenoic acid)	3.0 ± 0.3	8.3 ± 0.2	< 0.001
16:1n-7 (Palmitoleic acid)	37.4 ± 5.5	400.5 ± 5.1	< 0.001
18:1n-7 (Octadecenoic acid)	35.8 ± 2.1	190 ± 1.1	< 0.001
18:1n-9 (Oleic acid- OLA)	179.1 ± 17.8	2369.1 ± 50.0	< 0.001
20:1n-9 (Eicosenoic acid)	8.5 ± 0.8	56.3 ± 1.4	< 0.001
22:1n-9 (Docosenoic acid)	0.9 ± 0.1	6.4 ± 0.3	< 0.001
24:1n-9 (Tetracosenoic acid)	6.3 ± 0.5	10.5 ± 0.4	< 0.001
Total trans	1.5 ± 0.1	45.7 ± 2.5	< 0.001
Total saturated	466.5 ± 4.0	1684.4 ± 15.1	< 0.001
Total Omega 3	693.3 ± 24.4	832.2 ± 43.2	< 0.001
Total Omega 6	57.3 ± 1.9	771.4 ± 12.8	< 0.001
Total Omega 7	73.2 ± 7.4	590.5 ± 6.0	< 0.001
Total Omega 9	194.9 ± 19.2	2442.3 ± 48.5	< 0.001
n-3 LC PUFA	685.1 ± 25.4	725.4 ± 16.2	0.182
n-3 FA: n-6 FA	0.1 ± 0.0	0.9 ± 0.0	< 0.001

Table 2.3: Total lipid content (%), fatty acid composition (mg 100 g^{-1} tissue) and ratio of omega 3 (n-3) to omega 6 (n-6) fatty acids of red muscle of wild (n = 6) and aquacultured (n = 9) Yellowtail Kingfish (*Seriola lalandi*) from South Australia (mean ± standard error).

	Wild	Aquacultured	*P* =
Lipid content (%)	6.8 ± 0.8	29.3 ± 1.2	< 0.001
t18:1n-9 (Palmitelaidic acid)	8.3 ± 0.2	79.8 ± 3.8	< 0.001
t18:1n-7 (Elaidic acid)	0.0 ± 0.0	86.4 ± 4.8	< 0.001
14:0 (Myristic acid)	193.3 ± 9.0	612.8 ± 25.2	< 0.001
15:0 (Pentadecanoic acid)	53.5 ± 1.1	80.1 ± 2.4	< 0.001
16:0 (Palmitic acid)	1196.6 ± 9.4	4947.2 ± 30.5	< 0.001
17:0 (Margaric acid)	67.5 ± 1.9	138.8 ± 6.9	< 0.001
18:0 (Stearic acid)	528 ± 19.1	1647.1 ± 21.5	< 0.001
20:0 (Arachidic acid)	15.8 ± 1.0	51.2 ± 6.9	< 0.001
22:0 (Docosanoic acid)	5.2 ± 0.6	25.1 ± 0.8	< 0.001
24:0 (Tetracosanoic acid)	0.0 ± 0.0	16.2 ± 0.7	< 0.001
18:3n-3 (Alpha Linolenic acid- ALA)	50.7 ± 2.3	399.5 ± 10.9	< 0.001
20:5n-3 (Eicosapentaenoic acid- EPA)	378.7 ± 12.1	882.5 ± 60.5	< 0.001
22:5n-3 (Docosapentaenoic acid- DPA)	171.8 ± 2.2	410.0 ± 19.7	< 0.001
22:6n-3 (Docosahexaenoic acid- DHA)	2320.0 ± 46.1	1886.6 ± 122.4	< 0.001
18:2n-6 (Linoleic acid- LOA)	119.5 ± 2.2	2870.3 ± 58.4	< 0.001
18:3n-6 (Gamma Linolenic acid)	13.1 ± 0.4	77.8 ± 7.1	< 0.001
20:2n-6 (Eicosadienoic acid)	27.3 ± 1.0	56.2 ± 1.7	< 0.001
20:3n-6 (Dihomo-gamma-linoleic acid)	9.0 ± 0.2	42.3 ± 0.5	< 0.001
20:4n-6 (Arachidonic acid)	98.8 ± 5.1	204.6 ± 4.6	< 0.001
22:4n-6 (Docosatetraenoic acid)	14.8 ± 1.0	39.5 ± 0.9	< 0.001
16:1n-7 (Palmitoleic acid)	219.0 ± 19.4	1614.0 ± 24.2	< 0.001
18:1n-7 (Octadecenoic acid)	200.9 ± 3.8	872.9 ± 11.3	< 0.001
18:1n-9 (Oleic acid- OLA)	1006.3 ± 28.7	11434.3 ± 206.0	< 0.001
20:1n-9 (Eicosenoic acid)	54.4 ± 2.8	220.2 ± 2.9	< 0.001
22:1n-9 (Docosenoic acid)	6.4 ± 0.8	32.8 ± 0.8	< 0.001
24:1n-9 (Tetracosenoic acid)	41.9 ± 2.7	61.9 ± 2.3	< 0.001
Total trans	9.6 ± 0.3	265.3 ± 19.6	< 0.001
Total saturated	2064.6 ± 16.2	7521.2 ± 59.4	< 0.001
Total Omega 3	2921.2 ± 45.9	3578.5 ± 192.3	< 0.001
Total Omega 6	282.5 ± 7.4	3290.7 ± 55.7	< 0.001
Total Omega 7	419.9 ± 20.6	2486.9 ± 28.7	< 0.001
Total Omega 9	1108.9 ± 28.3	11749.2 ± 202.3	< 0.001
n-3 LC PUFA	2870.5 ± 46.6	3179.0 ± 39.1	< 0.001
n-3 FA: n -6 FA	0.1 ± 0.0	0.9 ± 0.0	< 0.001

Table 2.4: Total lipid content (%), fatty acid composition (mg 100 g^{-1} tissue) and ratio of omega 3 (n-3) to omega 6 (n-6) fatty acids of liver tissue of wild (n = 6) and aquacultured (n = 9) Yellowtail Kingfish (*Seriola lalandi*) from South Australia (mean ± standard error).

	Wild	Aquacultured	*P* =
Lipid content (%)	6.4 ± 0.3	32.9 ± 1.7	< 0.001
t18:1n-9 (Palmitelaidic acid)	7.7 ± 0.5	81.5 ± 1.9	< 0.001
t18:1n-7 (Elaidic acid)	0.0 ± 0.0	97.5 ± 4.0	< 0.001
14:0 (Myristic acid)	60.3 ± 2.1	434.3 ± 27.1	< 0.001
15:0 (Pentadecanoic acid)	22.8 ± 2.5	68.6 ± 4.6	< 0.001
16:0 (Palmitic acid)	1426.5 ± 57.8	5077.5 ± 76.8	< 0.001
17:0 (Margaric acid)	43.7 ± 6.2	136.3 ± 5.6	< 0.001
18:0 (Stearic acid)	483.9 ± 23.7	1833.9 ± 26.8	< 0.001
20:0 (Arachidic acid)	9.1 ± 1.9	21.4 ± 0.8	< 0.001
22:0 (Docosanoic acid)	0.0 ± 0.0	7.9 ± 0.5	< 0.001
24:0 (Tetracosanoic acid)	0.0 ± 0.0	7.6 ± 0.6	< 0.001
18:3n-3 (Alpha Linolenic acid- ALA)	21.4 ± 2.3	465.4 ± 12.6	< 0.001
20:5n-3 (Eicosapentaenoic acid- EPA)	264.1 ± 31.7	615.6 ± 98.6	< 0.001
22:5n-3 (Docosapentaenoic acid- DPA)	153.8 ± 17.9	501.378.2	< 0.001
22:6n-3 (Docosahexaenoic acid- DHA)	1788.2 ± 184.3	952.4 ± 139.1	< 0.001
18:2n-6 (Linoleic acid- LOA)	61.7 ± 6.1	3592.9 ± 79.3	< 0.001
18:3n-6 (Gamma Linolenic acid)	8.6 ± 0.7	50.9 ± 3.4	< 0.001
20:2n-6 (Eicosadienoic acid)	16.9 ± 1.7	145.0 ± 8.3	< 0.001
20:3n-6 (Dihomo-gamma-linoleic acid)	3.9 ± 1.3	72.8 ± 3.1	< 0.001
20:4n-6 (Arachidonic acid)	141.9 ± 11.8	219.0 ± 16.4	< 0.001
22:4n-6 (Docosatetraenoic acid)	13.6 ± 1.2	40.9 ± 2.9	< 0.001
16:1n-7 (Palmitoleic acid)	211.3 ± 43.3	1536.9 ± 43.1	< 0.001
18:1n-7 (Octadecenoic acid)	211.6 ± 14.8	1520.1 ± 34.0	< 0.001
18:1n-9 (Oleic acid- OLA)	1378.1 ± 168.9	14164.3 ± 395.8	< 0.001
20:1n-9 (Eicosenoic acid)	45.6 ± 5.8	354.4 ± 20.3	< 0.001
22:1n-9 (Docosenoic acid)	0.0 ± 0.0	19.3 ± 1.3	< 0.001
24:1n-9 (Tetracosenoic acid)	0.0 ± 0.0	24.0 ± 1.4	0.659
Total trans	7.7 ± 0.5	210.8 ± 5.4	< 0.001
Total saturated	2046.3 ± 44.8	7643.1 ± 98.0	< 0.001
Total Omega 3	2227.4 ± 231.8	2534.7 ± 312.9	0.139
Total Omega 6	246.6 ± 20.3	4121.5 ± 66.4	< 0.001
Total Omega 7	422.8 ± 56.6	3057.0 ± 49.7	< 0.001
Total Omega 9	1445.8 ± 175.1	14561.9 ± 410.1	< 0.001
n-3 LC PUFA	2206.0 ± 229.6	2069.3 ± 43.6	0.488
n-3 FA: n -6 FA	0.1 ± 0.0	1.6 ± 0.0	< 0.001

Table 2.5: Total lipid content (%), fatty acid composition (mg 100 g^{-1} tissue) and ratio of omega 3 (n-3) to omega 6 (n-6) fatty acids of adipose tissue of wild (n = 6) and aquacultured (n = 9) Yellowtail Kingfish (*Seriola lalandi*) from South Australia (mean ± standard error).

	Wild	Aquacultured	*P* =
Lipid content (%)	27.9 ± 7.8	92.4 ± 1.8	< 0.001
t18:1n-9 (Palmitelaidic acid)	36.1 ± 0.9	273.7 ± 12.7	< 0.001
t18:1n-7 (Elaidic acid)	0.0 ± 0.0	0.0 ± 0.0	< 0.001
14:0 (Myristic acid)	989.7 ± 51.9	2565.0 ± 108.9	< 0.001
15:0 (Pentadecanoic acid)	263.7 ± 7.5	282.4 ± 9.6	0.043
16:0 (Palmitic acid)	5459.4 ± 95.0	15688.1 ± 127.0	< 0.001
17:0 (Margaric acid)	282.3 ± 10.4	413.6 ± 12.3	< 0.001
18:0 (Stearic acid)	1904.9 ± 73.5	5180.2 ± 42.0	< 0.001
20:0 (Arachidic acid)	72.9 ± 3.6	163.4 ± 4.6	< 0.001
22:0 (Docosanoic acid)	16.3 ± 5.4	84.6 ± 13.2	< 0.001
24:0 (Tetracosanoic acid)	0.0 ± 0.0	4.8 ± 0.0	0.435
18:3n-3 (Alpha Linolenic acid- ALA)	260.3 ± 8.5	1579.0 ± 24.5	< 0.001
20:5n-3 (Eicosapentaenoic acid- EPA)	1887.6 ± 50.1	3385.3 ± 223.0	< 0.001
22:5n-3 (Docosapentaenoic acid- DPA)	665.4 ± 24.1	1003.2 ± 37.2	< 0.001
22:6n-3 (Docosahexaenoic acid- DHA)	8173.3 ± 179.4	3740.4 ± 226.8	< 0.001
18:2n-6 (Linoleic acid- LOA)	539.6 ± 10.9	10329.8 ± 147.0	< 0.001
18:3n-6 (Gamma Linolenic acid)	52.6 ± 3.2	170.9 ± 6.5	< 0.001
20:2n-6 (Eicosadienoic acid)	117.1 ± 8.2	167.0 ± 5.1	< 0.001
20:3n-6 (Dihomo-gamma-linoleic acid)	41.1 ± 0.8	145.2 ± 4.9	< 0.001
20:4n-6 (Arachidonic acid)	361.0 ± 15.4	552.2 ± 20.5	< 0.001
22:4n-6 (Docosatetraenoic acid)	52.7 ± 2.5	106.6 ± 3.4	< 0.001
16:1n-7 (Palmitoleic acid)	1058.1 ± 78.2	6166.4 ± 70.9	< 0.001
18:1n-7 (Octadecenoic acid)	823.2 ± 19.1	3058.7 ± 59.1	< 0.001
18:1n-9 (Oleic acid- OLA)	4367.2 ± 102.9	34404.0 ± 621.9	< 0.001
20:1n-9 (Eicosenoic acid)	226.2 ± 12.3	1092.3 ± 25.7	< 0.001
22:1n-9 (Docosenoic acid)	0.0 ± 0.0	136.0 ± 6.2	< 0.001
24:1n-9 (Tetracosenoic acid)	169.3 ± 7.6	153.9 ± 5.2	0.075
Total trans	40.4 ± 1.8	410.6 ± 34.6	0.001
Total saturated	143.1	24712.7 ± 248.4	< 0.001
Total Omega 3	10986.6 ± 203.0	9707.8 ± 474.1	< 0.001
Total Omega 6	1164.1 ± 24.0	11471.6 ± 132.8	< 0.001
Total Omega 7	1881.3 ± 91.4	9225.1 ± 94.4	< 0.001
Total Omega 9	4762.8 ± 107.3	35786.2 ± 606.2	< 0.001
n-3 LC PUFA	10726.3 ± 201.5	8128.8 ± 127.5	< 0.001
n-3 FA: n -6 FA	0.1 ± 0.0	1.2 ± 0.0	< 0.001

Table 2.6: Fatty acid composition (g 100 g^{-1} tissue) of wild and aquacultured Yellowtail Kingfish (*Seriola lalandi*) (YTK) white muscle, compared to commonly consumed portions of beef, chicken, lamb and pork.

	YTK (wild; white muscle)	YTK (aquacultured; white muscle)	Beef (fillet steak, lean)	Chicken (breast, lean)	Lamb (chop, lean)	Pork (chop, lean)
Lipid content	1.50	6.80	5.20	1.60	4.30	1.80
Total Saturates	0.47	1.68	2.13	0.53	1.41	0.68
Total n-3	0.69	0.83	0.16	0.04	0.12	0.03
Total n-6	0.06	0.77	0.43	0.28	0.42	0.27
Total n-7	0.07	0.59	0.17	0.05	0.07	0.07
Total n-9	0.19	2.44	1.99	0.66	1.90	0.73
n-3 LC PUFA	0.69	0.73	0.10	0.02	0.06	0.02
n-3: n-6	0.09	0.93	2.65	7.21	3.46	9.87
n-3: n-9	0.28	2.94	12.35	17.08	15.82	27.13

*Beef, chicken, lamb and pork values sourced from Nuttab 2010 (FSANZ, 2011).

*All portions were analysed raw

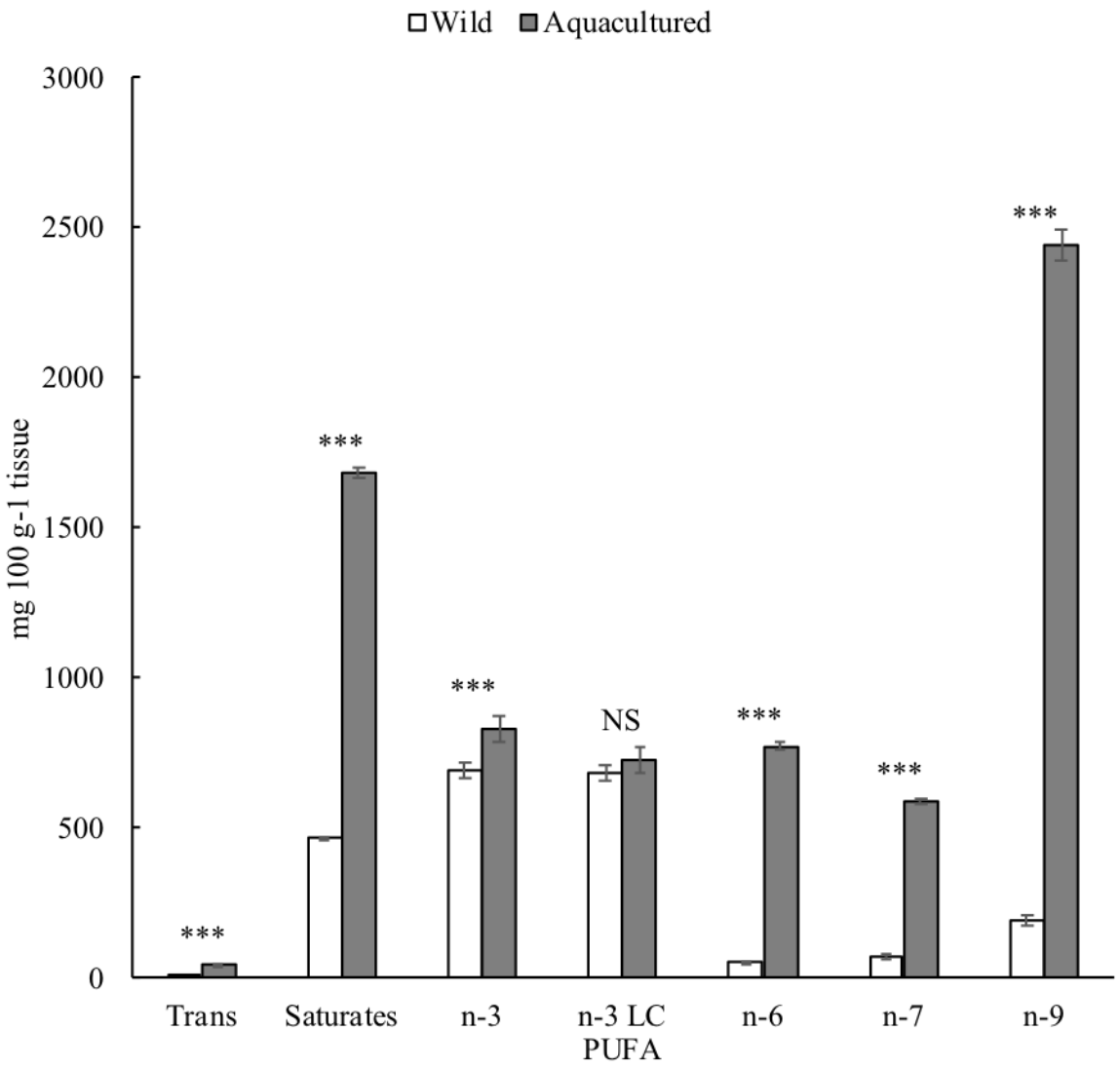

Figure 2.1: Comparison of major fatty acid groups; total trans, saturates, omega 3 (n-3) omega 3 long chain polyunsaturated fatty acids (n-3 LC PUFA), omega 6 (n-6), omega 7 (n-7) and omega 9 (n-9) (mg $100g^{-1}$ white muscle), from wild (n = 6) and aquacultured (n = 9) Yellowtail Kingfish (*Seriola lalandi*) (mean ± standard error). Levels of significance defined as: *** $P < 0.001$; NS = non-significant.

2.9. Statement to link Chapter 2 and Chapters 3 and 4

After examining the differences in the fatty acid composition between wild and aquacultured YTK (fed an optimal dietary concentration of n-3 LC PUFA) in Chapter 2, it became apparent that the fatty acid profile of the flesh from aquacultured YTK was quantitatively and qualitatively different to that of wild YTK. These differences were mainly attributed to quantitative and qualitative differences in the fatty acid composition of the diets of the two groups. Knowing that dietary fatty acid composition was so influential for flesh fatty acid composition, further investigation into a number of different commercially relevant YTK diets was warranted. As such, diets with graded inclusions of n-3 LC PUFA were formulated to cover a range from deficient to excess of the expected requirement of YTK. The effect of these diets on fatty acid utilisation and the composition of YTK are addressed in Chapters 3 and 4.

Chapter 3 – Statement of authorship

Title of Paper	Optimising omega 3 long chain polyunsaturated fatty acids in formulated diets for harvest size Yellowtail Kingfish (*Seriola lalandi*) - is there a trade-off between omega 3 and omega 9 fatty acid deposition in red and white muscle tissues?
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Principal Author

Name of Principal Author (Candidate)	Samantha N Chown		
Contribution to the Paper	Methodology, formal analysis, investigation, data curation, writing original draft, writing – review and editing and visualisation.		
Overall percentage (%)	90%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	24/06/2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Signature		Date	24/06/2019

Chapter 3: Optimising omega 3 long chain polyunsaturated fatty acids in formulated diets for harvest size Yellowtail Kingfish (*Seriola lalandi*) - is there a trade-off between omega 3 and omega 9 fatty acid deposition in red and white muscle tissues?

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Abstract

Yellowtail Kingfish (*Seriola lalandi*) (YTK) require dietary omega 3 (n-3) long chain polyunsaturated fatty acids (LC PUFA) for healthy development and growth. Dietary n-3 LC PUFA are typically provided by incorporating fish oil into aquafeeds, but because of cost and sustainability issues, attempts to reduce the use of fish oil has led to partial replacement with cheaper terrestrial plant and animal oils. However, interactions between dietary fatty acids (especially omega 6) have been observed to influence the incorporation of n-3 LC PUFA in YTK muscle tissues. This may have consequences for the nutritional value of fish to the consumer. In the present study, we investigated the effects of reducing dietary n-3 LC PUFA on white and red muscle fatty acid composition of harvest size sub-adult YTK. This was achieved by partially replacing dietary fish oil with poultry oil, which is particularly high in omega 9 (n-9) fatty acids. Eight diets were formulated to cover a range from deficient to excessive levels of n-3 LC PUFA (0.8 to 3.0 g n-3 LC PUFA 100 g^{-1} feed). Diets were fed to large YTK (3.77 ± 0.04 kg; mean final weight ± SE; n = 480) to apparent satiation daily for 12 weeks. At the conclusion of the trial, white and red muscle samples were collected for fatty acid analysis. The fatty acid composition of the red muscle lipids correlated with the dietary fatty acid profile, with the proportions of n-3 LC PUFA decreasing and n-9 fatty acids increasing with dietary fish oil replacement. However, in the white muscle there was an apparent trade-off between n-9 (primarily oleic acid; OLA; 18:1n-9) and n-3 LC PUFA (primarily docosahexaenoic acid; DHA; 22:6n-3) when dietary fish oil inclusion was low and n-3 LC PUFA was supplied at less than 1.6 g 100 g^{-1} feed, below this level white muscle DHA content was maintained at the expense of OLA. This result is positive for the consumer because the n-3 LC PUFA content of the fillet is maintained at a higher level than would otherwise be the case when fish are fed diets containing low n-3 LC PUFA. However, it also demonstrates that OLA may not be neutral in regard to DHA deposition in YTK white muscle. Further

research to investigate this relationship will enhance our understanding of fatty acid metabolism in YTK and may be beneficial to aid in development of sustainable production diets for this species.

Keywords

Yellowtail Kingfish; aquaculture; omega 3 (n-3) long chain (LC) polyunsaturated fatty acids (PUFA); Oleic acid (18:1n-9); product quality.

Highlights

1. An interaction between DHA and OLA in the white muscle of Yellowtail Kingfish was observed when dietary n-3 LC PUFA was supplied at concentrations below 1.6 g 100 g^{-1} feed.
2. The fatty acid composition of the red muscle correlated with the dietary fatty acid profile, with the proportions of n-3 LC PUFA decreasing and n-9 fatty acids increasing with gradual dietary fish oil replacement
3. Recommendations were made for OLA to be considered in dietary formulations as it plays a role in DHA deposition in Yellowtail Kingfish.

3.1. Introduction:

Carnivorous marine finfish, such as Yellowtail Kingfish (*Seriola lalandi*) (YTK) have an essential dietary requirement for omega 3 (n-3) long chain polyunsaturated fatty acids (LC PUFA) (NRC, 2011, Stone et al., 2019). These n-3 LC PUFA, including eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA), are necessary for healthy cellular metabolism and maintaining cell membrane structure and integrity in fish (Sargent et al., 1999a, Miller et al., 2008) and also play a vital role in human nutrition (McCann and Ames, 2005, Eilander et al., 2007). In commercial aquaculture, dietary n-3 LC PUFA are primarily supplied by dietary inclusions of fish oil (FO) (NRC, 2011). However, the increasing use of wild fisheries-sourced FO is unsustainable, and future growth and success of the aquaculture industry depends on the successful replacement or reduction of wild fisheries sourced FO in commercial diets.

Numerous studies have investigated the implications of reducing or replacing dietary FO with poultry oil (PO) and canola oil in aquaculture diets (reviewed in Miller et al., 2008 and Tocher, 2015). Importantly, in YTK dietary FO has been 100% replaced by poultry oil (PO) or 50% replaced by canola oil without reducing growth, so long as the minimum n-3 LC PUFA requirements were met (Bowyer et al., 2012a). The major advantage of FO replacement with terrestrially sourced animal or plant oil is that these sources are perceived to be more sustainable, reliably available and relatively inexpensive (Miller et al., 2008). However, the key challenge for FO replacement with terrestrial-sourced oils is their lack of n-3 LC PUFA and the high concentrations of omega 6 (n-6), omega 9 (n-9) and/ or saturated fatty acids, which skews the fatty acid composition of the aquacultured fish and thus diminishes the nutritional benefits for the human consumer (Turchini et al., 2009).

Understanding the mechanisms that affect fatty acid deposition and thus the final fatty acid profile of harvested fish is an important part of responsibly utilising FO. Interactions

between dietary fatty acids, particularly relating to n-3 LC PUFA, have been observed in terrestrial animals, including piglets and rats (Leece and Allman, 1996, Blank et al., 2002) and in humans (Schmitz and Ecker, 2008, Gibson et al., 2011). In these cases, high dietary inclusion of n-6 linoleic acid (LOA) inhibited the incorporation of n-3 LC PUFA into tissues and consequently diets low in n-6 fatty acids are recommended to increase n-3 LC PUFA tissue concentrations. Furthermore, but less commonly reported, there are interactions between dietary n-3 and n-9 fatty acids, with abundant dietary concentrations of n-9 fatty acids, primarily oleic acid (18:1n-9; OLA), having an impact on the rate at which n-3 alpha linolenic acid (ALA) and n-3 LC PUFA were deposited in chicken and mouse tissues (Picklo et al., 2017, Elkin et al., 2018). In commercial aquaculture feeds FO is routinely partially replaced with PO. However, PO has a substantially higher content of n-6 and n-9 fatty acids than FO and the relationship between these fatty acids and the utilisation of n-3 LC PUFA in fish is unknown.

The dietary requirements for n-3 LC PUFA to achieve optimal growth for small and harvest size sub adult YTK have been established (Bowyer et al., 2013; Stone et al., 2019), however the effects of reducing dietary n-3 LC PUFA on fatty acid deposition in the tissues have not been fully investigated. Therefore, the aim of the current study was to investigate the effect of reducing n-3 LC PUFA (from FO) with a concomitant increase in the concentration of n-6 and n-9 (from PO) in the diets of large sub-adult YTK during the grow-out phase of production on white and red muscle tissue fatty acid composition as these are the tissues eaten by consumers.

3.2. Methods and Materials

3.2.1. Experimental location and animals

Animal ethics approval for this work was granted by the University of Adelaide animal ethics committee (Approval number: S-2016-127). The experiment was conducted at the South Australian Research and Development Institute (SARDI) South Australian Aquatic Science Centre (SAASC) (West Beach, South Australia, Australia). Yellowtail Kingfish were supplied by Clean Seas Seafood Ltd. (Port Lincoln, South Australia, Australia). Prior to the experiment, fish were housed in 18 × 5000 L tanks supplied with partial flow-through/recirculating (100% system water exchange day^{-1}), sand filtered, UV treated, aerated sea water at ambient temperature and held for ~3.5 months. During this period fish were fed a 9 mm commercial diet (Ridley Pelagica diet; crude protein 46%; crude lipid 24%; gross energy 19.30 MJ kg^{-1}; Narangba, Queensland, Australia) to apparent satiation once daily.

3.2.2. Experimental diets

The diet kernels, FO and PO used in the experimental diets were supplied by Skretting Australia. The diet formulations were based on Skretting Australia's YTK diet (20% fish meal; 40% crude protein, 30% crude lipid and a gross energy level of approximately 21 MJ kg^{-1}) (Stone et al., 2019). The diet kernels contained a base level of 10% crude lipid and were then top coated with an additional 17% lipid (graded blends of FO and PO; total crude lipid level 27%) at Aquafeeds Australia (Mount Barker, South Australia). The main effect of substituting FO with PO was a decrease in n-3 LC PUFA with an increase in n-9 fatty acids (mostly OLA). Eight experimental diets were formulated with n-3 LC PUFA levels ranging from 0.8 to 3.0 g 100 g^{-1} of feed (Table 3.1). Diets were coded according to dietary n-3 LC PUFA inclusion (e.g. DIET0.8 has 0.8 g of n-3 LC PUFA 100 g^{-1} of feed and DIET3.0 has 3.0 g of n-3 LC PUFA 100 g^{-1} of feed).

3.2.3. Experimental housing and animal care

At the start of the 12-week feed trial, YTK were anaesthetised in 5000 L tanks (total water volume 2500 L) using AQUI-S® (AQUI-S® New Zealand Ltd., Lower Hutt, New Zealand) at a concentration of 14 mg L^{-1} of seawater. Fish were randomly distributed into 24 × 5000 L recirculating aquaculture tanks (20 fish per tank) and randomly assigned one of the 8 experimental diets (3 replicate tanks $diet^{-1}$). Initial weight of fish was 2.67 kg. Fish were fed their experimental diet once daily to apparent satiation and intake was recorded as grams consumed per tank per day. Apparent satiation was defined as the point at which ~90% of the population had ceased feeding. Water quality parameters were measured daily and maintained within the accepted optimal levels for YTK (Bowyer et al., 2014). Temperature (ºC) was measured with a thermometer. Dissolved oxygen (mg L^{-1} and percentage saturation) was measured using a dissolved oxygen meter (OxyGuard International A/S, Birkerød, Denmark). The pH was measured using a multi-parameter meter (Oakton pHtestr 20; Oakton Instruments, Vernon Hills, IL, USA). Ammonia (ppm) was measured using an Aquarium Pharmaceuticals ammonia test kit (Mars Fishcare, North America). Salinity (g L^{-1}) was measured weekly using a portable salinity refractometer (model RF20, Extech Instruments, Nashua, NH, USA).

3.2.4. Sample collection

After 12 weeks the fish were anaesthetised, removed from their tank, measured and weighed. Three randomly selected fish from each tank were humanely euthanised by percussive stunning, and the white and red muscle was sampled. White muscle was collected from the dorsal fillet adjacent to the dorsal fin and red muscle was collected from along the lateral line posterior to the pectoral fin. Tissue samples were immediately frozen by immersion in dry ice and thereafter stored at -20 ºC prior to analysis.

3.2.5. Total lipid analysis

Tissue total crude lipid (as a percentage of wet weight) was estimated for white and red muscle samples utilizing the gravimetric approach (Folch et al., 1957). Briefly, samples were homogenised in 0.9% saline in a 10 mL glass centrifuge tube, thereafter lipids were extracted into a 4:1 chloroform: isopropanol solution and then centrifuged at 3000 RPM for 10 minutes. The chloroform layer was removed, placed into a pre-weighed vial and then evaporated using nitrogen gas leaving only the lipid component behind.

3.2.6. Fatty acid analysis

Fatty acid profiling was conducted for white and red muscle samples. The lipid component (extracted during total lipid analysis) was transmethylated with 1% H_2SO_4 in MeOH at 70 °C for 3 hours, then cooled to room temperature, after which fatty acid methyl esters (FAMES) were extracted into 2 mL of heptane. The heptane was transferred to a gas chromatography (GC) vial with 30 mg of anhydrous sodium sulphate, sealed and stored at -20 °C until analysis by GC. Samples were processed on a Hewlett-Packard 6890 GC (Hewlett-Packard, CA, USA) with a flame ionization detector, a split injector and a BPX-70 capillary column (50 m × 0.32 mm) with a 0.25 µm film thickness (SGE, Victoria, Australia). Gas chromatography operating conditions were as described previously (Tu et al., 2010) and peaks were identified with GLC 463 external standard (Nu-Chek Prep Inc., MN, USA). Data output was processed with Agilent ChemStation (version Rev: B.01.03) (Agilent Technologies, CA, USA).

3.2.7. Statistics

Statistical analysis was performed using IBM SPSS (version 24). Homogeneity of variance was assessed using Levene's test, whilst normality was assessed with the

Kolmogorov-Smirnov test. Where data met prior requirements, differences were analysed using a one-way ANOVA where diet was a factor. Where significant differences were detected, post-hoc comparisons were made via Tukey's tests. An alpha level of 0.05 was used for all statistical tests. Results are presented as means ± standard error (SE) unless otherwise stated.

3.3. Results

3.3.1. General observations

The mean water temperature during the experimental period was 19.7 ± 0.03 °C (range: 15.5 – 24.5 °C). Experimental diets were readily accepted by YTK with no rejection of feed observed. Overall YTK survival for the duration of the experiment was 98.5%. Fish behaviour and gross pathology (data not shown) were typical of healthy fish suggesting there were no negative impacts of dietary treatments (Stone et al., 2019). The mean final weight of YTK was 3.77 ± 0.04 kg (n = 480) and there were significant differences in specific growth rate (SGR) and feed conversion ratio (FCR) between groups. Maximal SGR was achieved in the DIET2.4 group and an optimal FCR was achieved in the DIET2.1 group (Table 3.2 extracted form Stone et al., 2019).

3.3.2. Total lipid and fatty acid profiles

Total lipid content was not significantly influenced by experimental diet; average total lipid concentration was 6.4% and 29.0% for white muscle and red muscle, respectively (one-factor ANOVA; $P > 0.05$ for both; Table 3.3).

3.3.2.1. Fatty acid composition of white muscle

The fatty acid composition of the white muscle was significantly affected by dietary treatments (Table 3.4). Notably, there was a 44.4% increase in white muscle n-3 LC PUFA between DIET0.8 and DIET3.0 (one-factor ANOVA; $P < 0.001$; Table 3.3). However, this increase primarily occurred between DIET1.6 and DIET3.0, or above a n-3 LC PUFA dietary concentration of 1.6 g 100 g^{-1} feed (one-factor ANOVA; $P < 0.001$); below this threshold the white muscle n-3 LC PUFA remained consistent at an average of 0.6 g 100 g^{-1} tissue (between DIET0.8 and DIET1.6) (Figure 3.1). This pattern was primarily driven by DHA; the

concentration of DHA in white muscle reached a minimum of 0.35 g 100 g^{-1} with DIET1.6 and then did not significantly change as dietary n-3 LC PUFA concentration continued to decrease (one-factor ANOVA; $P < 0.001$, Table 3.4). Total n-6 fatty acid concentration consistently and significantly decreased from DIET0.8 to DIET3.0 (one-factor ANOVA; $P < 0.001$; Figure 3.1). Total n-9 fatty acids in the white muscle followed the same but opposite pattern to n-3 LC PUFA with decreasing concentrations between DIET1.8 and DIET3.0 and remained constant between DIET0.8 and DIET1.6 (one-factor ANOVA; $P < 0.001$; Figure 3.1). This was primarily driven by OLA, increased with increasing dietary inclusion to a maximum of 2.5 g 100 g^{-1} tissue in the DIET1.3 group, thereafter no significant changes were observed between DIET1.3 and DIET0.8 (one-factor ANOVA; $P < 0.001$, Table 3.4).

3.3.2.2. Fatty acid composition of red muscle

The fatty acid composition of the red muscle was significantly affected by dietary treatments in a similar way to the white muscle. A 56% increase in red muscle n-3 LC PUFA resulted from the 291% increase in dietary n-3 LC PUFA (one-factor ANOVA; $P < 0.001$; Table 3.3). Red muscle n-3 LC PUFA concentrations increased consistently and significantly between DIET0.8 and DIET3.0 (one-factor ANOVA; $P < 0.001$; Figure 3.2). Similarly, total n-6 fatty acid concentration consistently and significantly decreased from DIET0.8 to DIET3.0 (one-factor ANOVA; $P < 0.001$; Figure 3.2). In contrast to what was observed in the white muscle, the red muscle DHA concentration continually and significantly decreased from treatment DIET3.0 to DIET0.8 (one-factor ANOVA; $P < 0.001$; Table 3.5), while OLA concentrations increased from treatment DIET3.0 to DIET1.6 with no further significant change from DIET1.6 to DIET0.8 (one-factor ANOVA; $P < 0.001$, Figure 3.2).

3.4. Discussion

As expected, the fatty acid composition of the muscle tissues was affected by the fishes' dietary fatty acid intake, but there were some surprising results. Importantly, the results indicate a novel interaction between n-9 fatty acids (particularly OLA) and n-3 LC PUFA (particularly DHA) in white muscle of YTK. When n-3 LC PUFA was supplied at an inclusion above 1.6 g 100 g^{-1} feed, white muscle concentrations of DHA and OLA were reflective of dietary concentrations. However, when dietary n-3 LC PUFA was supplied in quantities less than 1.6 g 100 g^{-1} feed, white muscle DHA and OLA concentrations remained fixed in spite of dietary concentrations of DHA decreasing and OLA increasing (Figure 3.3). While competitive interactions for metabolism and incorporation between n-6 and n-3 fatty acids have been well documented in a range of finfish species (Sargent et al., 1999a, Sargent et al., 1999b, Glencross, 2009), interactions between n-9 fatty acids and n-3 LC PUFA have not previously been documented in YTK. These results could suggest that YTK differ from other finfish species, as an interaction between n-6 fatty acids and n-3 LC PUFA was absent in this study while an interaction between n-9 fatty acids and n-3 LC PUFA was observed. However, the interaction of n-9 instead of n-6 fatty acids with n-3 LC PUFA could also have been driven by the relative abundance of these fatty acid groups in the diet, with n-9 fatty acids being 4 times more abundant than n-6 fatty acids. The implications of the relative magnitude of n-9 fatty acids to n-3 LC PUFA has not been evaluated in fish feeds before, so this hypothesis remains to be tested.

In the current study the relationship between OLA and DHA in the YTK white muscle may suggest preferential absorption, incorporation, conversion and/ or sparing of DHA at the expense of OLA. These findings are supported by a recent study demonstrating that dietary OLA is not neutral in regard to the process by which ALA is absorbed, metabolized and deposited in the egg yolks of Hy-Line W-36 laying hens (Elkin et al., 2018). While there was

no obvious competition between ALA and OLA for metabolic enzymes, Elkin et al. (2018) suggested that the triacylglycerol structure of the dietary oils, particularly the higher quantity of OLA (85 mol%), compared to ALA (63 mol%) in the sn-2 position of dietary triglycerides, which was expected to be more conserved during absorption, was likely to be the mechanism by which OLA outcompeted ALA for absorption and therefore incorporation into tissues. While Elkin et al. (2018) attributed their differences to a potential higher rate of absorption of OLA than ALA, this explanation does not fit with the data presented for YTK in the current study. Analysis of faecal material from experimental animals (Refer to Chapter 5) indicated a mean of 96.1 ± 0.1% and 96.6 ± 0.2% (mean ± SE) absorption of OLA and DHA respectively, which did not differ significantly among treatments.

Another recent study by Picklo et al. (2017), observed that high OLA diets reduced tissue content of ALA in mice. Those authors suggested that competitive inhibition of ALA uptake into cells by OLA was a possible cause for reduced ALA content in tissues. A plausible explanation for the relationship between DHA and OLA in YTK in the current study is competition for incorporation and more specifically selective incorporation of DHA when dietary concentrations are low at the expense of OLA. Reduction of dietary OLA could thus potentially reduce competition for incorporation between DHA and OLA, resulting in increased accumulation of n-3 LC PUFA in YTK muscle. Therefore, further investigation of the impacts of high OLA diets for other fish species is recommended as a means of better understanding the mechanisms affecting the utilization dietary n-3 LC PUFA.

The maintenance of white muscle DHA at low dietary inclusion rates could also have been driven by conversion of EPA or DPA to DHA. Conversion of ALA to n-3 LC PUFA is known to be limited in marine fish (Tocher, 2003, Strobel et al., 2012) and it is likely that the conversion of EPA and DPA to DHA is also limited. Generally, n-3 LC PUFA are accumulated up the food chain after being produced by phytoplankton, rather than being converted or

interconverted in higher order consumers such as YTK. In the current study, the ratio of EPA: DPA: DHA in the muscle was reflective of the dietary ratios of these fatty acids, indicating that the interconversion of n-3 LC PUFA was either not necessary or not occurring even at the lowest dietary inclusion rate. Furthermore, the *de novo* biosynthesis of longer chain fatty acids such as the conversion from EPA to DHA is known to be supressed by excess intake of n-6 fatty acids and n-3 LC PUFA in fish, due to excess competition for the Δ6 desaturase enzyme system (Sargent et al., 1993, Glencross, 2009). Therefore, it is unlikely that interconversion of n-3 LC PUFA was responsible for conservation of white muscle DHA concentrations.

In the current study the sparing of DHA was observed at dietary inclusion of less than 1.6 g n-3 LC PUFA 100 g^{-1} feed. The recommended dietary inclusion of n-3 LC PUFA for optimal growth of large YTK has been defined as between 2.1 and 2.3 g 100 g^{-1} feed (Stone et al., 2019) so with an adequate dietary inclusion sparing of DHA should not be observed. However, developing an understanding of the mechanisms by which DHA can be spared in YTK could have positive repercussions for maximizing dietary n-3 LC PUFA utilization. Sparing, selective accumulation and selective retention of DHA, has been reported in a range of marine fish including YTK (Ishihara and Saito, 1996, Saito et al., 1996, Bowyer et al., 2012b, Codabaccus et al., 2012, Rombenso et al., 2015) and high utilization of O;A has previously been observed in YTK, which was attributed to the ease with which O;A was catabolized by β oxidation (Bowyer et al., 2012b). Similarly, the interaction observed between DHA and O;A in the current study could be attributed to conservation of DHA by selectively increasing the rate at which O;A is utilized for energy. If DHA can be spared at the expense of O;A, supplying dietary O;A at an adequate concentration may ensure the maintenance of muscle DHA concentrations. However, if there is a persistently low dietary concentration of DHA throughout a period of rapid growth, it is likely that DHA concentrations in muscle tissue would

decrease. This would have negative implications for fish growth and health and nutritional value for human consumers.

Interestingly, large increases in the dietary n-3 LC PUFA content (3.9-fold increase) between DIET0.8 and DIET3.0 did not result in equally or even comparatively large increases in n-3 LC PUFA content in the white muscle (1.4-fold increase) or the red muscle (1.6-fold increase). While these results could suggest inefficiency in n-3 LC PUFA accumulation by YTK, it is more likely that the magnitude of change was limited by the quantity of growth (approximately 50% increase in body weight) achieved during the 12-week experimental period. Tissue compositional changes are achieved more rapidly during the accumulation of new tissue rather than the turnover of existing tissue lipids. This has been described in a previous study which proposed a dilution model for fatty acid compositional changes, over time, when dietary lipid composition is altered (Jobling, 2003). In the current study, the relative change in fatty acid profile would likely be more reflective of the dietary composition if the weight gain achieved during the experimental period was greater.

The difference in n-3 LC PUFA accumulation between tissue regions could be attributed to differences in the functional role of each region. The fatty acid profile of the red muscle was influenced to a greater degree by experimental diet than the white muscle (Table 3.3). In YTK the red muscle is primarily responsible for routine swimming activity and requires a large energy reserve which can be readily utilized (Tsukamoto, 1984). Therefore, utilization of tissue lipids for energy in combination with growth and incorporation of new tissue lipids likely accelerated tissue compositional changes. Comparatively, the vast majority of lipid in the white muscle is in the form of phospholipids, which form the structural components of cells and are not readily utilized for energy (Tsukamoto, 1984). Therefore, compositional changes to the white muscle fatty acid profile would be more limited and occurring primarily in response to the accumulation of new tissue. From a grow-out perspective, the difference in the

rate of change of tissue fatty acid composition should be considered in relation to finishing diets for improved product quality during the final stages of growth before harvest, with a particular focus on the limited changes possible in the white muscle as this is the main tissue eaten by consumers.

Concentrations of n-3 LC PUFA in the white muscle ranged from 0.6 (DIET0.8) to 0.9 (DIET3.0) g 100 g^{-1} tissue. Importantly, in relation to seafood consumption, fish from all of the treatment groups had the potential to meet the recommended daily intake requirements of n-3 LC PUFA for human consumers. In Australia, the Nation Health and Medical Research Council (NHMRC) recommends a dietary intake target of n-3 LC PUFA of 0.43 g per day for adult females and 0.61 g per day for adult males (NHMRC, 2006), while the International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommends a daily intake of 0.50 g per day of n-3 LC PUFA (ISSFAL, 2004). Therefore, a 100 g serving of YTK white muscle from any of these treatment groups would exceed the 0.50 g n-3 LC PUFA per day requirement, by 19% (DIET0.8) to 72% (DIET3.0) (Table 3.3). When YTK is served as sashimi or as a skin-on portion of fillet, a portion of the red muscle is also consumed. This would contribute additional n-3 LC PUFA toward the daily recommended intake (e.g. only 13 g or 20 g of red muscle from DIET3.0 or DIET0.8 respectively, would satisfy the 0.50 g n-3 LC PUFA per day requirement).

3.5. Conclusions

This study has revealed an interaction between DHA and OLA when dietary n-3 LC PUFA concentrations are significantly reduced (below 1.6 g 100 g^{-1} feed). These results suggest that OLA plays a role in regard to DHA deposition in YTK white muscle and should be considered when formulating commercial diets. Two possible explanations for this interaction are proposed: 1) competition between DHA and OLA for incorporation into lipid molecules, and 2) selective conservation of DHA at the expense of OLA. These explanations would result in conflicting recommendations for future diet formulations for YTK. Further research is required to elucidate the mechanisms driving this interaction and should focus on the effects of increasing or decreasing dietary OLA on DHA deposition in YTK white muscle.

Given that a 100 g serving of YTK white muscle from any of the treatment groups would exceed the recommended daily intake requirements for n-3 LC PUFA it is suggested that the primary factors to consider when setting n-3 LC PUFA requirements for YTK should be cost and availability of dietary fats, maximizing growth rate, minimizing feed conversion ratio and maintaining optimal health of the fish. However, further research should additionally focus on optimizing the incorporation and conservation of n-3 LC PUFA in YTK muscle such that consumers can reap maximum nutritional benefits.

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3.8. Tables and figures

Table 3.1: Total dietary lipid content (%) and fatty acid composition (mg 100 g^{-1} feed) of eight experimental diets

Item (as fed)	DIET0.8	DIET1.0	DIET1.3	DIET1.6	DIET1.8	DIET2.1	DIET2.4	DIET3.0
Lipid content (%)	26.2	26	26.9	26.6	27.1	26.9	26.8	27.1
Analysed fatty acids (mg 100 g^{-1})								
t18:1n-9 (Palmitelaidic acid)	83	76	74	73	74	73	72	72
t18:1n-7 (Elaidic acid)	140	131	127	121	121	119	115	108
14:0 (Myristic acid)	420	480	540	620	660	730	760	900
15:0 (Pentadecanoic acid)	53	59	66	69	74	77	77	89
16:0 (Palmitic acid)	5930	5880	5890	5860	5780	5760	5570	5550
17:0 (Margaric acid)	89	90	93	95	98	100	100	110
18:0 (Stearic acid)	1870	1860	1840	1810	1790	1770	1690	1670
20:0 (Arachidic acid)	36	44	48	44	47	51	49	53
22:0 (Docosanoic acid)	25	23	24	26	28	30	31	31
24:0 (Tetracosanoic acid)	13	14	14	15	15	16	16	18
18:3n-3 (Alpha Linolenic acid- ALA)	550	540	530	520	490	490	460	430
20:5n-3 (Eicosapentaenoic acid- EPA)	270	400	530	680	790	930	1060	1350
22:5n-3 (Docosapentaenoic acid- DPA)	63	72	83	100	110	130	140	160
22:6n-3 (Docosahexaenoic acid- DHA)	420	540	680	830	930	1080	1190	1440
18:2n-6 (Linoleic acid- LOA)	3150	3040	2960	2900	2730	2650	2500	2300
18:3n-6 (Gamma Linolenic acid)	31	30	35	36	37	41	38	43
20:2n-6 (Eicosadienoic acid)	28	28	30	30	31	32	33	36
20:3n-6 (Dihomo-gamma-linoleic acid)	24	27	25	28	28	35	33	33
20:4n-6 (Arachidonic acid)	110	110	120	140	140	50	160	170
22:4n-6 (Docosatetraenoic acid)	16	17	18	19	19	20	21	23
16:1n-7 (Palmitoleic acid)	1450	1440	1490	1540	1530	1560	1570	1610
18:1n-7 (Octadecenoic acid)	640	640	650	660	650	660	650	670
18:1n-9 (Oleic acid- OLA)	11050	10580	10310	10080	9530	9290	8700	8020
20:1n-9 (Eicosenoic acid)	130	140	150	140	150	150	156	160
22:1n-9 (Docosenoic acid)	11	12	14	16	18	20	22	26
24:1n-9 (Tetracosenoic acid)	20	24	24	32	35	41	37	48
Total trans	223	207	201	194	196	192	187	180
Total saturated	8436	8450	8515	8539	8492	8534	8293	8421
Total Omega 3	1303	1552	1823	2130	2320	2630	2850	3380
Total Omega 6	3359	3252	3187	3153	2985	2829	2785	2605
Total Omega 7	2090	2080	2140	2200	2180	2220	2220	2280
Total Omega 9	11211	10756	10498	10268	9733	9501	8915	8254
n-3 LC PUFA	753	1012	1293	1610	1830	2140	2390	2950
n-3 FA: n -6 FA	2.58	2.10	1.75	1.48	1.29	1.08	0.98	0.77
n-3 FA: n -9 FA	8.60	6.93	5.76	4.82	4.20	3.61	3.13	2.44

Table 3.2. Growth performance, feed utilisation, proximate composition and nutrient retention Yellowtail Kingfish (*Seriola lalandi*) from fish fed eight experimental diets for 12 weeks extracted from Stone et al. (2019). (D0.75 = Diet0.8, D1.01= Diet 1.0, D1.29 = Diet1.3, D1.61 = Diet 1.6, D1.83 = Diet1.8, D2.13 = Diet2.1, D2.39 = Diet2.4 and D2.95 = Diet3.0)

Diet[1]	D2.95	D2.39	D2.13	D1.83	D1.61	D1.29	D1.01	D0.75	ANOVA[2]
Growth performance									
Initial weight (kg)	2.67±0.02	2.67±0.02	2.66±0.01	2.67±0.01	2.67±0.01	2.66±0.02	2.66±0.02	2.67±0.02	$P = 0.994$
Final weight (kg)	3.77±0.04^{ab}	3.84±0.06^{a}	3.79±0.01^{ab}	3.84±0.04^{a}	3.81±0.05^{ab}	3.75±0.02^{ab}	3.71±0.04^{ab}	3.61±0.07^{b}	$P = 0.036$
Biomass gain (kg tank^{-1})	21.88±0.85^{ab}	23.28±0.80^{a}	22.59±0.11^{a}	23.44±0.78^{a}	22.84±0.93^{a}	21.90±0.46^{ab}	20.92±1.09^{ab}	18.70±1.09^{b}	$P = 0.017$
SGR (% d^{-1})	0.41±0.02^{a}	0.43±0.01^{a}	0.42±0.00^{a}	0.43±0.01^{a}	0.42±0.01^{a}	0.41±0.01^{a}	0.39±0.02^{ab}	0.35±0.02^{b}	$P = 0.016$
Initial fork length (mm)	559.2±1.3	556.3±3.0	554.1±0.6	555.3±1.7	558.0±1.4	556.7±0.3	555.0±0.7	556.2±1.2	$P = 0.370$
Final fork length (mm)	610.6±0.9^{ab}	612.6±2.1^{a}	607.2±0.8^{ab}	608.8±1.8^{ab}	610.0±1.4^{ab}	608.3±2.5^{ab}	605.1±1.6^{ab}	602.0±2.9^{b}	$P = 0.028$
Length growth rate (mm d^{-1})	0.61±0.02	0.67±0.01	0.63±0.00	0.63±0.02	0.61±0.01	0.61±0.03	0.59±0.02	0.54±0.02	$P = 0.014$
Final Condition factor	1.65±0.01	1.67±0.01	1.69±0.00	1.70±0.01	1.68±0.01	1.67±0.02	1.67±0.02	1.65±0.01	$P = 0.146$
Feed utilisation (as fed)									
Apparent feed consumption (kg tank$^{-1)}$	45.99±0.49	47.23±0.70	47.58±0.78	48.34±0.76	48.45±1.99	47.45±0.49	45.98±1.21	45.26±1.45	$P = 0.394$
Apparent feed intake (% BW d^{-1})	0.88±0.01	0.89±0.01	0.90±0.01	0.91±0.02	0.92±0.03	0.91±0.01	0.89±0.02	0.88±0.01	$P = 0.629$
Apparent FCR	2.11±0.09^{b}	2.03±0.05^{b}	2.11±0.04^{b}	2.07±0.05^{b}	2.12±0.06^{b}	2.17±0.03^{b}	2.20±0.07^{b}	2.43±0.07^{a}	$P = 0.008$
Proximate composition (wet basis)									
Moisture (%)	59.1±1.1	58.8±0.4	59.2±0.4	58.8±0.4	59.6±0.8	58.7±0.4	59.0±0.3	60.0±0.2	$P = 0.751$
Protein (%)	20.06±0.25	19.58±0.14	20.35±0.16	19.88±0.13	20.19±0.43	20.69±0.33	20.76±0.16	20.11±0.35	$P = 0.093$
Lipid (%)	19.1±1.0	18.7±0.5	19.0±0.7	19.4±0.2	18.2±0.9	17.9±0.7	18.8±0.4	17.6±0.3	$P = 0.500$
Ash (%)	2.0±0.1	2.6±0.4	2.3±0.4	2.7±0.2	2.4±0.4	2.3±0.2	2.2±0.2	2.2±0.2	$P = 0.708$
Carbohydrate (%)	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	<1.5	$P = 1.000$
Energy (MJ kg^{-1})	10.48±0.31	10.28±0.19	10.50±0.23	10.57±0.09	10.16±0.25	10.17±0.23	10.47±0.13	9.94±0.14	$P = 0.401$
Nutrient retention (%)[3]									
Apparent PD	21.14±0.56	20.12±0.51	22.34±0.41	21.02±0.58	21.72±1.54	22.95±1.16	22.86±1.22	18.51±1.05	$P = 0.067$
Apparent ED	31.30±3.30	30.39±2.12	30.93±2.35	32.05±0.19	27.90±1.99	27.52±2.00	29.77±0.63	23.20±0.61	$P = 0.088$

[1] Values are mean ± SE; $n = 3$. Initial fish proximate composition (wet basis): Moisture 61.8%, protein 20.41%, lipid 16.5%, ash 2.2%, carbohydrate (by difference) 1.5%, energy 9.57 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 ED = energy deposition; PD = protein deposition.

Table 3.3: Total lipid content (%), fatty acid composition totals (mg 100 g^{-1} tissue) and ratio of omega 3 (n-3) to omega 6 (n-6) and omega 9 (n-9) fatty acids of white and red muscle of Yellowtail Kingfish (*Seriola lalandi*) from fish fed eight experimental diets for 12 weeks (values presented as mean ± standard error, n = 3, different subscripts denote significant differences between treatments; $P < 0.05$).

	DIET0.8	DIET1.0	DIET1.3	DIET1.6	DIET1.8	DIET2.1	DIET2.4	DIET3.0	*P* =
White muscle									
Lipid content	5.3 ± 0.6	6.0 ± 1.1	6.0 ± 0.5	6.9 ± 1.0	6.7 ± 0.8	6.8 ± 0.6	6.8 ± 0.7	6.7 ± 0.6	0.798
Total trans	49.3 ± 1.2	44.5 ± 3.1	46.7 ± 2.0	43.6 ± 0.8	45.1 ± 2.1	45.7 ± 2.5	49.9 ± 0.5	43.9 ± 2.7	0.204
Total saturated	1629.5 ± 16.7 a	1642.4 ± 19.3 ab	1644.0 ± 15.5 ab	1641.5 ± 12.9 ab	1676.0 ± 9.8 bc	1684.4 ± 15.1 bc	1707.8 ± 10.6 c	1719.1 ± 14.4 c	< 0.001
Total Omega 3	704.1 ± 28.4 a	714.9 ± 33.5 ab	707.4 ± 33.3 ab	721.4 ± 22.8 ab	793.8 ± 21.9 bc	832.2 ± 43.2 cd	874.9 ± 42.1 d	961.4 ± 31.3 e	< 0.001
Total Omega 6	841.8 ± 10.2 a	822.4 ± 7.3 ab	814.0 ± 13.4 b	804.7 ± 10.3 b	783.1 ± 11.4 c	771.4 ± 12.8 cd	757.3 ± 12.3 de	740.2 ± 15.3 e	< 0.001
Total Omega 7	567.9 ± 6.3 ab	562.2 ± 7.1 a	572.7 ± 8.4 ab	590.2 ± 5.3 bc	583.8 ± 8.3 abc	590.5 ± 6.0 bc	592.8 ± 9.2 bc	607.4 ± 8.2 c	< 0.001
Total Omega 9	2575.6 ± 35.8 a	2579.9 ± 39.5 a	2584.3 ± 37.7 a	2566.8 ± 29.9 ab	2484.7 ± 25.6 bc	2442.3 ± 48.5 cd	2383.0 ± 47.6 d	2288.4 ± 35.3 e	< 0.001
n-3 LC PUFA	595 ± 24.1 a	604.4 ± 23.9 ab	596.1 ± 19.2 a	608.4 ± 11.7 ab	684.9 ± 18.0 bc	725.4 ± 16.2 cd	771.2 ± 23.1 d	859.3 ± 12.3 e	< 0.001
n-3 FA: n -6 FA	1.2 ± 0.1 a	1.2 ± 0.0 a	1.2 ± 0.0 a	1.1 ± 0.0 a	1.0 ± 0.0 b	0.9 ± 0.0 b	0.9 ± 0.0 bc	0.8 ± 0.0 c	< 0.001
n-3 FA: n -9 FA	3.7 ± 0.2 a	3.6 ± 0.1 a	3.7 ± 0.1 a	3.6 ± 0.1 ab	3.1 ± 0.1 bc	2.9 ± 0.1 c	2.7 ± 0.1 cd	2.4 ± 0.0 d	< 0.001
Red muscle									
Lipid content	27.8 ± 2.4	29.0 ± 1.9	28.8 ± 2.2	28.8 ± 1.7	33.4 ± 1.9	29.3 ± 1.2	28.0 ± 1.8	26.7 ± 1.3	0.340
Total trans	338.8 ± 27.9 a	315.2 ± 10.9 ab	318.3 ± 12.2 ab	281.6 ± 26.1 ab	246.0 ± 27.0 a	265.3 ± 19.6 ab	287.0 ± 20.8 ab	247.9 ± 15.1 b	0.008
Total saturated	7285.3 ± 74.7 a	7327.1 ± 59.0 ab	7366.3 ± 64.5 abc	7343.4 ± 64.9 ab	7483.6 ± 39.8 abcd	7521.2 ± 59.4 bcd	7558.9 ± 50.1 cd	7617.9 ± 59.1 d	< 0.001
Total Omega 3	2895.7 ± 112.9 a	2991.8 ± 113.8 ab	3136.9 ± 139.2 b	3186.2 ± 69.6 bc	3419.4 ± 114.7 cd	3578.5 ± 192.3 de	3748.2 ± 197.3 e	4218.0 ± 184.2 f	< 0.001
Total Omega 6	3624.4 ± 57.4 a	3530.2 ± 32.6 ab	3469.6 ± 71.5 bc	3413.2 ± 46.8 cd	3315.2 ± 48.2 de	3290.7 ± 55.7 e	3246.0 ± 59.6 ef	3154.6 ± 66.3 f	< 0.001
Total Omega 7	2404 ± 9.7 ab	2430.9 ± 27.1 abc	2379.8 ± 24.2 a	2436.5 ± 28.9 abc	2487.6 ± 27.5 bcd	2486.9 ± 28.7 bcd	2518.7 ± 28.4 cd	2559.3 ± 32.1 d	< 0.001
Total Omega 9	12359.2 ± 127.1 a	12307.0 ± 132.0 a	12232.6 ± 147.3 a	12241.7 ± 89.4 a	11946.5 ± 106.3 b	11749.2 ± 202.3 bc	11525.9 ± 180.4 c	11086.6 ± 190.4 d	< 0.001
n-3 LC PUFA	2465.1 ± 57.3 a	2547.5 ± 29.4 ab	2712.8 ± 44.0 ab	2773.0 ± 66.6 bc	3012.1 ± 42.2 cd	3179 ± 39.1 de	3342.5 ± 63.0 e	3835.7 ± 87.7 f	< 0.001
n-3 FA: n -6 FA	1.3 ± 0.0 a	1.2 ± 0.0 ab	1.1 ± 0.0 bc	1.1 ± 0.0 c	1.0 ± 0.0 d	0.9 ± 0.0 de	0.9 ± 0.0 e	0.8 ± 0.0 f	< 0.001
n-3 FA: n -9 FA	4.3 ± 0.1 a	4.1 ± 0.0 ab	3.9 ± 0.1 b	3.9 ± 0.1 b	3.5 ± 0.0 c	3.3 ± 0.0 cd	3.1 ± 0.1 d	2.6 ± 0.1 e	< 0.001

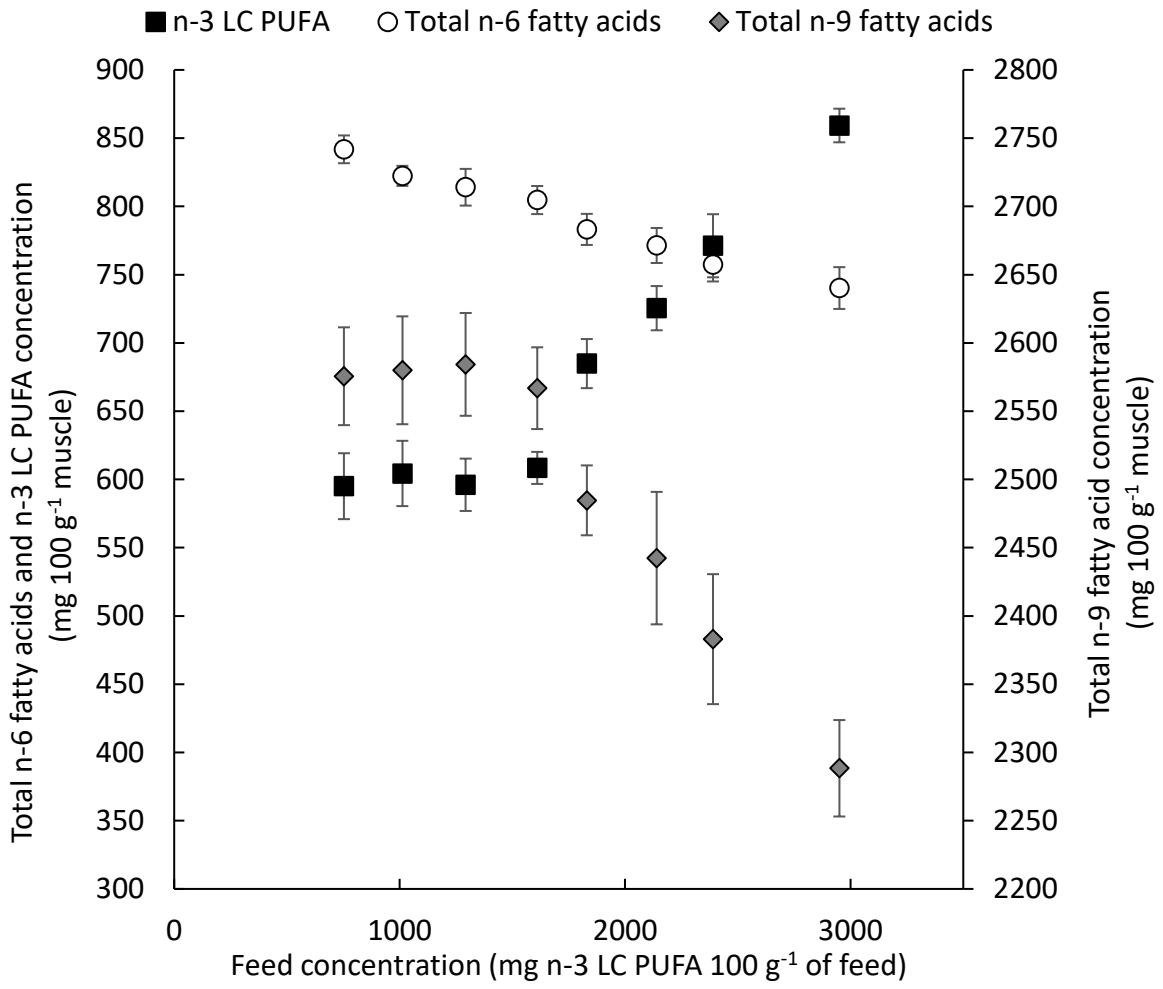
Table 3.4: Fatty acid composition (mg 100 g^{-1} tissue) of white muscle of Yellowtail Kingfish (*Seriola lalandi*) from fish fed eight experimental diets for 12 weeks (values presented as mean ± standard error, n = 3, different subscripts denote significant differences between treatments; $P < 0.05$).

Fatty acid	DIET0.8	DIET1.0	DIET1.3	DIET1.6	DIET1.8	DIET2.1	DIET2.4	DIET3.0	*P* =
t18:1n-9 (Palmitelaidic acid)	16.3 ± 0.4	16.8 ± 0.4	16.8 ± 0.5	15.9 ± 0.2	16.4 ± 0.3	15.8 ± 0.4	15.9 ± 0.3	15.7 ± 0.2	0.157
t18:1n-7 (Elaidic acid)	23.0 ± 0.4	20.7 ± 0.7	23.1 ± 0.5	21.6 ± 0.5	22.1 ± 1.2	21.7 ± 2.5	22.1 ± 0.2	21.8 ± 0.6	0.837
14:0 (Myristic acid)	123.1 ± 4.2 a	118.8 ± 4.0 a	126.8 ± 7.5 ab	139.1 ± 5.4 bc	142.3 ± 7.1 cd	148.2 ± 8.0 cd	156.0 ± 8.1 d	174.1 ± 7.5 e	< 0.001
15:0 (Pentadecanoic acid)	16.3 ± 0.4 ab	15.4 ± 0.4 a	16.2 ± 0.7 ab	16.9 ± 0.5 ab	17.2 ± 0.6 bc	17.7 ± 0.7 bc	18.5 ± 0.7 c	20.1 ± 0.6 d	< 0.001
16:0 (Palmitic acid)	1096.0 ± 9.8 a	1106.6 ± 11.9 ab	1108.0 ± 9.6 ab	1094.5 ± 6.3 a	1111.2 ± 5.1 ab	1119.3 ± 7.9 ab	1130.0 ± 5.0 b	1128.5 ± 8.1 b	0.004
17:0 (Margaric acid)	25.0 ± 0.4 ab	24.3 ± 0.4 b	25.0 ± 0.5 ab	25.6 ± 0.5 ab	26.2 ± 0.7 bc	26.1 ± 0.5 bc	27.4 ± 0.5 cd	27.9 ± 0.5 d	< 0.001
18:0 (Stearic acid)	352.9 ± 4.7	361.3 ± 8.3	351.3 ± 3.9	348.5 ± 3.3	361.3 ± 3.9	355.3 ± 3.2	357.5 ± 5.0	348.5 ± 5.4	0.416
20:0 (Arachidic acid)	9.5 ± 0.2 ab	9.1 ± 0.2 a	9.6 ± 0.3 ab	9.8 ± 0.3 abc	10.1 ± 0.3 bc	10.2 ± 0.4 bc	10.6 ± 0.3 c	11.5 ± 0.4 d	< 0.001
22:0 (Docosanoic acid)	4.1 ± 0.1 b	4.2 ± 0.1 bc	4.3 ± 0.2 bc	4.3 ± 0.1 abc	4.4 ± 0.1 bc	4.5 ± 4.7 bc	4.7 ± 0.1 cd	5.1 ± 0.2 d	< 0.001
24:0 (Tetracosanoic acid)	2.3 ± 0.1 b	2.5 ± 0.1 bc	2.4 ± 0.1 ab	2.3 ± 0.1 b	2.5 ± 0.1 bc	2.5 ± 0.1 bc	2.6 ± 0.0 bc	2.7 ± 0.1 c	0.003
18:3n-3 (Alpha Linolenic acid- ALA)	109.0 ± 2.3 ab	110.5 ± 2.0 ab	111.3 ± 1.7 a	112.9 ± 1.6 a	108.9 ± 1.5 abc	106.7 ± 1.6 abc	103.7 ± 1.8 bc	102.1 ± 1.4 c	< 0.001
20:5n-3 (Eicosapentanaeoic acid- EPA)	164.8 ± 8.4 a	165.7 ± 7.6 a	176.8 ± 15.6 ab	196.5 ± 11.6 bc	216.3 ± 12.5 cd	227.4 ± 18.1 de	239.7 ± 16.5 e	288.0 ± 17.3 f	< 0.001
22:5n-3 (Docosapentaenoic acid- DPA)	64.1 ± 2.8 a	62.6 ± 1.8 a	62.8 ± 3.7 a	66.1 ± 2.9 ab	72.6 ± 2.2 bc	76.3 ± 3.5 c	77.6 ± 3.8 cd	85.0 ± 2.7 d	< 0.001
22:6n-3 (Docosahexaenoic acid- DHA)	366.1 ± 21.6 a	376.1 ± 29.0 a	356.4 ± 20.0 a	345.9 ± 12.1 a	395.9 ± 15.7 ab	421.7 ± 24.6 abc	453.9 ± 27.7 bc	486.2 ± 15.5 c	< 0.001
18:2n-6 (Linoleic acid- LOA)	753.4 ± 10.9 a	733.3 ± 8.9 ab	728.9 ± 15.0 b	720.1 ± 11.4 b	693.1 ± 11.7 c	681.2 ± 14.2 cd	663.9 ± 12.9 de	643.1 ± 16.4e	< 0.001
18:3n-6 (Gamma Linolenic acid)	9.3 ± 0.1	8.5 ± 1.3	8.5 ± 0.9	9.6 ± 0.3	9.5 ± 0.2	9.1 ± 0.2	10.8 ± 0.8	10.6 ± 0.5	0.101
20:2n-6 (Eicosadienoic acid)	11.8 ± 0.2	11.8 ± 0.2	11.7 ± 0.2	11.7 ± 0.1	11.9 ± 0.2	11.8 ± 0.1	11.4 ± 0.1	11.7 ± 0.2	0.650
20:3n-6 (Dihomo-gamma-linoleic acid)	9.5 ± 0.2 a	10.0 ± 0.1 ab	9.6 ± 0.2 a	9.7 ± 0.1 a	9.9 ± 0.2 ab	10.0 ± 0.1 ab	10.1 ± 0.1 ab	10.4 ± 0.2 b	< 0.001
20:4n-6 (Arachidonic acid)	49.6 ± 1.9 abc	50.4 ± 1.8 abc	47.4 ± 1.7 ab	45.7 ± 1.1 a	50.3 ± 1.2 abc	51.0 ± 1.7 abc	52.7 ± 2.0 bc	55.8 ± 0.9 c	< 0.001
22:4n-6 (Docosatetraenoic acid)	8.2 ± 0.3 ab	8.4 ± 0.2 ab	7.9 ± 0.2 ab	7.8 ± 0.2 a	8.4 ± 0.1 ab	8.3 ± 0.2 ab	8.4 ± 0.2 ab	8.7 ± 0.2 b	0.033
16:1n-7 (Palmitoleic acid)	383.3 ± 5.7 ab	378.5 ± 6.5 a	388.6 ± 6.8 ab	403.9 ± 3.9 bc	396.2 ± 7.0 abc	400.5 ± 5.1 abc	401.7 ± 7.4 bc	416.8 ± 7.1 c	< 0.001
18:1n-7 (Octadecenoic acid)	184.7 ± 1.5 ab	183.7 ± 2.1 b	184.1 ± 2.1 ab	186.3 ± 1.9 ab	187.7 ± 1.8 ab	190.0 ± 1.1 ab	191.1 ± 2.0 a	190.6 ± 1.9 ab	0.003
18:1n-9 (Oleic acid- OLA)	2504.2 ± 36.3 ab	2511.6 ± 39.0 a	2513.7 ± 39.3 ab	2496.1 ± 31.7 ab	2412.2 ± 26.2 bc	2369.1 ± 50.0 cd	2309.7 ± 48.7 d	2209.9 ± 36.4 e	< 0.001
20:1n-9 (Eicosenoic acid)	55.2 ± 1.0	52.6 ± 1.6	54.5 ± 1.4	55.0 ± 1.5	55.6 ± 1.5	56.3 ± 1.4	55.5 ± 1.3	59.0 ± 1.5	0.120
22:1n-9 (Docosenoic acid)	6.2 ± 0.2 a	5.9 ± 0.2 a	6.1 ± 0.3 a	6.1 ± 0.3 a	6.5 ± 0.2 ab	6.4 ± 0.3 ab	6.7 ± 0.3 ab	7.5 ± 0.4 b	0.001
24:1n-9 (Tetracosenoic acid)	10.0 ± 0.4 a	9.8 ± 0.4 a	10.0 ± 0.5 a	9.6 ± 0.5 a	10.4 ± 0.3 ab	10.5 ± 0.4 ab	11.1 ± 0.4 ab	11.9 ± 0.4 b	< 0.001

Table 3.5: Fatty acid composition (mg 100 g^{-1} tissue) of red muscle of Yellowtail Kingfish (*Seriola lalandi*) from fish fed eight experimental diets for 12 weeks (values presented as mean ± standard error, n = 3, different subscripts denote significant differences between treatments; $P < 0.05$).

Fatty acid	DIET0.8	DIET1.0	DIET1.3	DIET1.6	DIET1.8	DIET2.1	DIET2.4	DIET3.0	*P* =
t18:1n-9 (Palmitelaidic acid)	107.5 ± 6.4 a	100.4 ± 4.5 ab	97.8 ± 5.9 abc	95.8 ± 3.8 abc	89.1 ± 4.6 bc	79.8 ± 3.8 c	97.7 ± 6.0 abc	80.3 ± 4.9 c	< 0.001
t18:1n-7 (Elaidic acid)	110.8 ± 4.5	99.6 ±7.9	96.8 ± 5.7	101.4 ± 6.5	86.1 ± 4.5	86.4 ± 4.8	106.4 ± 6.8	98.1 ± 7.0	0.071
14:0 (Myristic acid)	526.4 ± 12.4 a	540.7 ± 17.9 a	528.0 ± 23.4 a	566.5 ± 19.2 a	609.1 ± 19.4 b	612.8 ± 25.2 b	654.5 ± 26.0 c	709.4 ± 26.9 d	< 0.001
15:0 (Pentadecanoic acid)	73.6 ± 1.6 a	72.5 ± 1.4 a	72.8 ± 2.0 a	74.8 ± 1.9 ab	78.6 ± 1.8 bc	80.1 ± 2.4 cd	83.4 ± 2.4 d	88.7 ± 2.5 e	< 0.001
16:0 (Palmitic acid)	4844.1 ± 50.5	4862.7 ± 34.0	4912.1 ± 45.9	4856.4 ± 38.2	4933.6 ± 29.1	4947.2 ± 30.5	4953.5 ± 31.8	4970.2 ± 42.6	0.086
17:0 (Margaric acid)	137.1 ± 8.8	140.9 ± 5.9	139.6 ± 6.7	130.0 ± 7.4	130.7 ± 7.1	138.8 ± 6.9	140.6 ± 5.1	137.8 ± 5.9	0.930
18:0 (Stearic acid)	1596.0 ± 24.4	1601.0 ± 26.2	1620.7 ± 24.6	1622.1 ± 26.5	1632.8 ± 19.2	1647.1 ± 21.5	1616.3 ± 24.2	1605.0 ± 26.0	0.831
20:0 (Arachidic acid)	68.5 ± 9.6	69.4 ± 7.4	51.9 ± 6.2	51.6 ± 8.6	55.3 ± 7.8	51.2 ± 6.9	65.0 ± 4.0	58.7 ± 4.3	0.377
22:0 (Docosanoic acid)	23.5 ± 0.8 ab	23.0 ± 0.9 a	23.6 ± 0.8 ab	24.0 ± 0.9 ab	25.4 ± 0.6 abc	25.1 ± 0.8 abc	26.4 ± 0.8 bc	27.6 ± 1.0 c	< 0.001
24:0 (Tetracosanoic acid)	14.2 ± 0.6 a	14.5 ± 0.4 ab	15.4 ± 0.6 ab	15.6 ± 0.5 ab	15.4 ± 0.5 ab	16.2 ± 0.7 ab	16.2 ± 0.8 ab	17.5 ± 0.5 b	0.001
18:3n-3 (Alpha Linolenic acid- ALA)	430.6 ± 16.5 ab	444.2 ± 7.4 a	424.0 ± 10.5 ab	413.2 ± 15.1 ab	407.4 ± 15.4 ab	399.5 ± 10.9 ab	405.7 ± 12.0 ab	382.3 ± 11.0 b	0.014
20:5n-3 (Eicosapentanaeoic acid- EPA)	648.1 ± 34.4 a	678.9 ± 32.3 ab	701.0 ± 48.4 ab	747.9 ± 44.7 b	844.6 ± 44.9 c	882.5 ± 60.5 cd	945.1 ± 61.6 d	1085.8 ± 61.8 e	< 0.001
22:5n-3 (Docosapentaenoic acid- DPA)	343.1 ± 14.6 a	347.1 ± 14.4 a	358.9 ± 17.3 ab	362.0 ± 9.1 ab	391.3 ± 8.7 bc	410.0 ± 19.7 cd	419.9 ± 20.4 c	471.7 ± 19.0 d	< 0.001
22:6n-3 (Docosahexaenoic acid- DHA)	1474.0 ± 82.5 a	1521.5 ± 78.6 a	1653.0 ± 75.6 ab	1663.1 ± 39.1 ab	1776.1 ± 83.1 bc	1886.6 ± 122.4 c	1977.4 ± 136.3 c	2278.2 ± 109.8 d	< 0.001
18:2n-6 (Linoleic acid- LOA)	3198.8 ± 51.6 a	3110.1 ± 34.9 ab	3049.5 ± 74.8 b	3005.6 ± 43.7 bc	2908.8 ± 46.3 cd	2870.3 ± 58.4 d	2819.9 ± 58.6 de	2722.5 ± 71.5 e	< 0.001
18:3n-6 (Gamma Linolenic acid)	95.1 ± 10.2	94.3 ± 5.4	88.2 ± 8.8	79.3 ± 9.7	66.8 ± 9.4	77.8 ± 7.1	82.8 ± 8.9	73.8 ± 6.3	0.187
20:2n-6 (Eicosadienoic acid)	57.3 ± 2.4	53.7 ± 0.8	54.2 ± 0.9	54.6 ± 0.9	56.1 ± 1.0	56.2 ± 1.7	53.9 ± 0.6	52.9 ± 1.3	0.242
20:3n-6 (Dihomo-gamma-linoleic acid)	40.3 ± 0.6 a	41.0 ± 0.7 ab	40.3 ± 0.5 a	40.7 ± 0.4 a	42.4 ± 0.5 ab	42.3 ± 0.5 ab	41.8 ± 0.5 ab	43.1 ± 0.9 b	0.001
20:4n-6 (Arachidonic acid)	194.0 ± 3.0 a	192.8 ± 4.5 a	198.2 ± 4.6 ab	194.4 ± 2.5 ab	201.8 ± 4.7 ab	204.6 ± 4.6 ab	208.8 ± 5.1 bc	222.3 ± 4.4 c	< 0.001
22:4n-6 (Docosatetraenoic acid)	38.8 ± 0.6	38.3 ± 0.7	39.2 ± 0.7	38.6 ± 0.5	39.4 ± 0.6	39.5 ± 0.9	38.7 ± 0.7	40.1 ± 0.7	0.620
16:1n-7 (Palmitoleic acid)	1553.4 ± 12.1 ab	1579.3 ± 24.7 abc	1531.5 ± 21.4 a	1595.7 ± 27.4 abcd	1635.3 ± 27.4 bcd	1614.0 ± 24.2 abcd	1654.5 ± 25.3 cd	1685.0 ± 29.9 d	< 0.001
18:1n-7 (Octadecenoic acid)	850.6 ± 7.2 ab	851.5 ± 6.9 ab	848.4 ± 9.7 ab	840.8 ± 6.2 a	852.3 ± 6.5 ab	872.9 ± 11.3 ab	864.2 ± 7.1 ab	874.3 ± 8.9 b	0.014
18:1n-9 (Oleic acid- OLA)	12071.7 ± 127.2 a	11949.9 ± 138.5 a	11882.0 ± 177.3 a	11885.9 ± 95.3 a	11583.4 ± 88.2 b	11434.3 ± 206.0 bc	11204.4 ± 183.7 c	10702.1 ± 193.4 d	< 0.001
20:1n-9 (Eicosenoic acid)	199.7 ± 6.3	268.2 ± 31.8	260.5 ± 37.8	263.5 ± 5.9	268.2 ± 32.1	220.2 ± 2.9	223.8 ± 3.5	274.4 ± 3.1	0.114
22:1n-9 (Docosenoic acid)	30.9 ± 0.6 ab	31.0 ± 0.7 ab	30.2 ± 1.2 a	31.3 ± 1.2 ab	32.6 ± 0.7 ab	32.8 ± 0.8 ab	33.6 ± 1.0 bc	36.5 ± 1.1 c	< 0.001
24:1n-9 (Tetracosenoic acid)	56.9 ± 1.6 a	57.9 ± 1.7 a	59.9 ± 3.0 a	61.0 ± 2.1 ab	62.3 ± 1.7 ab	61.9 ± 2.3 ab	64.0 ± 2.5 ab	68.7 ± 1.6 b	0.001

2014



2015

2016 **Figure 3.1:** Concentration (mg 100 g^{-1} tissue) of n-3 LC PUFA, total omega 6 (n-6) and total
2017 omega 9 (n-9) fatty acids in white muscle of Yellowtail Kingfish (*Seriola lalandi*) compared
2018 to feed concentration of n-3 LC PUFA (mg 100 g^{-1} feed) after 12 weeks of feeding (values
2019 presented as mean ± standard error, n = 3).

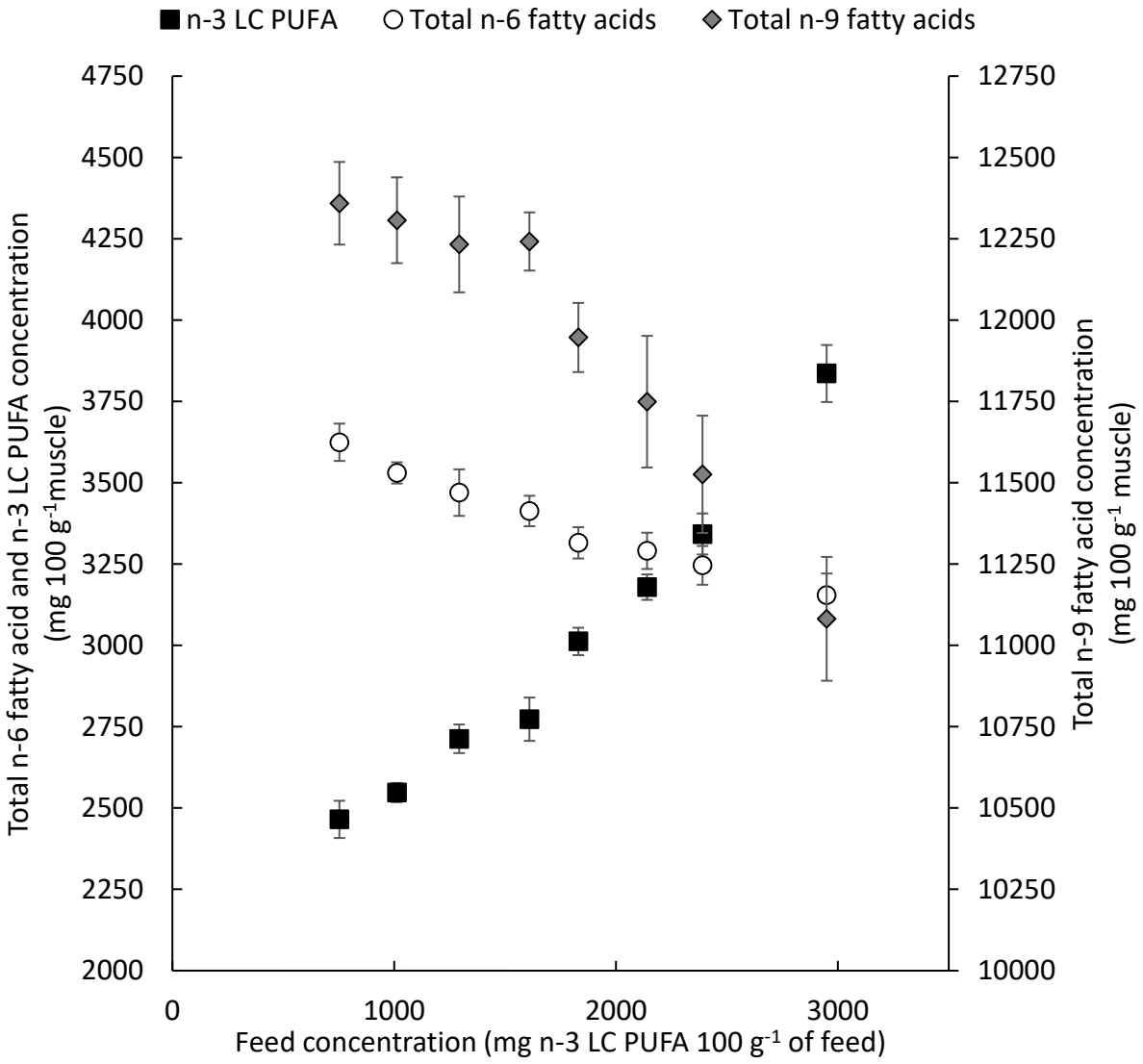


Figure 3.2: Concentration (mg 100 g^{-1} tissue) of n-3 LC PUFA, total omega 6 (n-6) and total omega 9 (n-9) fatty acids in red muscle of Yellowtail Kingfish (*Seriola lalandi*) compared to feed concentration of n-3 LC PUFA (mg 100 g^{-1} feed) after 12 weeks of feeding (values presented as mean ± standard error, n = 3).

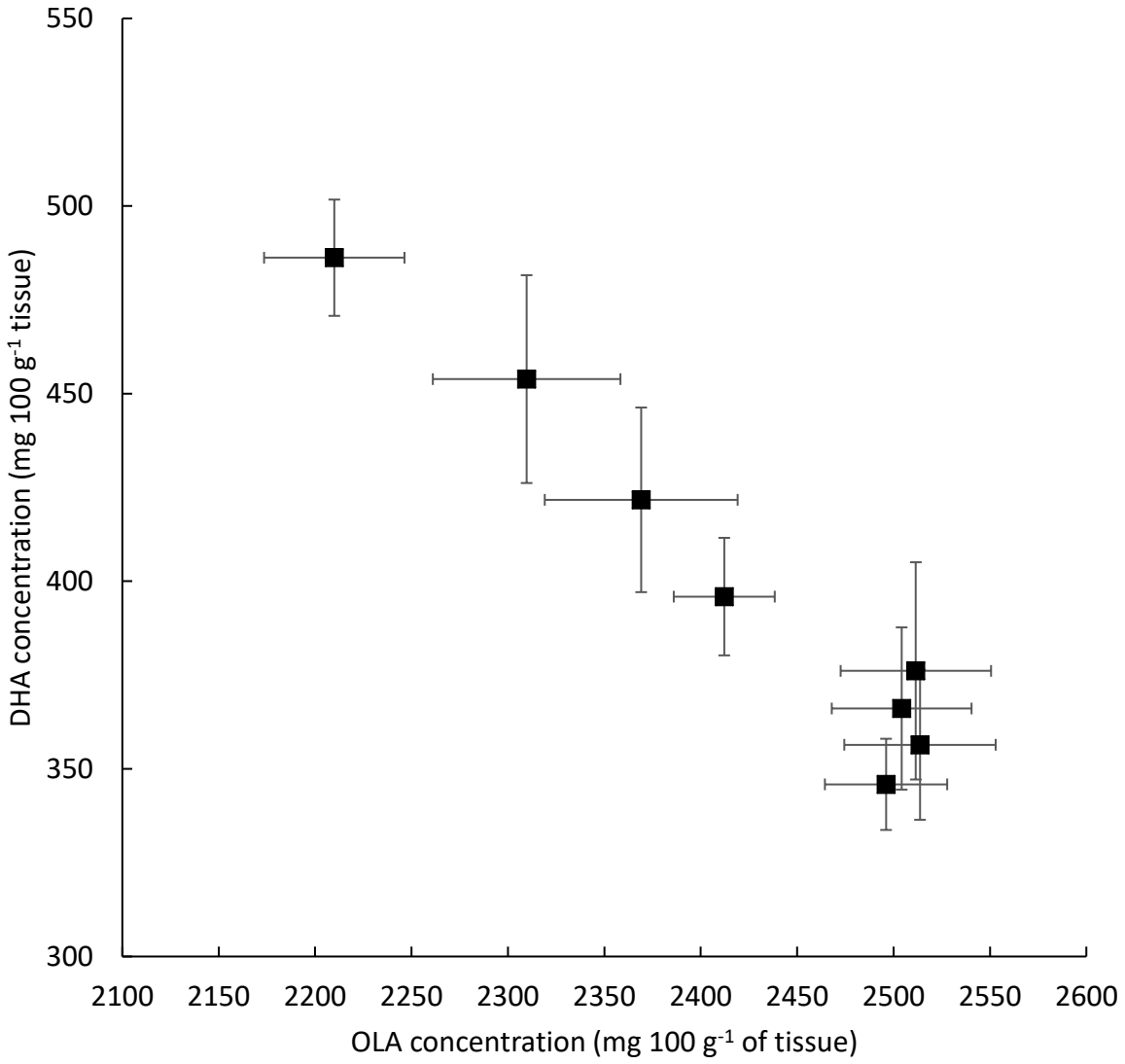


Figure 3.3: White muscle concentration of docosahexaenoic acid (DHA) compared to white muscle concentration of oleic acid (OLA) (mg 100 g^{-1} tissue) in Yellowtail Kingfish (*Seriola lalandi*) after 12 weeks of feeding (values presented as mean ± standard error, n = 3).

3.9. Statement to link Chapter 3 and Chapter 4

In Chapter 3 and in conjunction with the Stone et al., (2019) study, it became apparent that YTK reared on diets with deficient dietary n-3 LC PUFA (high poultry oil/ low fish oil) had slower growth and poorer feed conversion efficiency than YTK fed on the optimal 2.1 – 2.4 g n-3 LC PUFA 100 g^{-1} feed, and YTK fed dietary levels > 2.4 g n-3 LC PUFA (low poultry oil/ high fish oil) did not achieve any further improvements to growth and feed conversion. The fatty acid profile of the flesh was generally reflective of the respective dietary compositions (with the exception of DHA and OLA in low n-3 LC PUFA diets), which suggested that lipids from dietary fish oil and poultry oil were incorporated with equal efficiency. However, assessing the digestibility of lipids from diets with different lipid compositions would provide more clarity about lipid utilisation, this was addressed in Chapter 4.

Chapter 4 – Statement of authorship

Title of Paper	Do differences in the digestibility of dietary lipids and fatty acids explain differences in growth and FCR in fish oil replacement trials with Yellowtail Kingfish (*Seriola lalandi*)?
Publication Status	Manuscript prepared
Publication Details	N/A

Principal Author

Name of Principal Author (Candidate)	Samantha N Chown		
Contribution to the Paper	Methodology, formal analysis, investigation, data curation, writing original draft, writing – review and editing and visualisation.		
Overall percentage (%)	90%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	24/06/2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Resources, writing – review and editing, supervision (2%)		
Signature		Date	24/06/2019

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Contribution to the Paper	Resources, writing – review & editing, supervision, project administration and funding acquisition (1%)		
Signature		Date	24/06/2019

Chapter 4: Do differences in the digestibility of dietary lipids and fatty acids explain differences in growth and FCR in fish oil replacement trials with Yellowtail Kingfish (*Seriola lalandi*)?

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Abstract

Yellowtail Kingfish (*Seriola lalandi*) (YTK) require dietary omega 3 (n-3) long chain polyunsaturated fatty acids (LC PUFA) for healthy development and growth. Dietary n-3 LC PUFA are typically provided by incorporating fish oil (FO), however FO is routinely partially replaced with poultry oil (PO) in aquafeeds. Replacing too much FO with PO has been shown to reduce the n-3 LC PUFA content in flesh and negatively affect growth and performance of YTK. The mechanism responsible has not been elucidated but likely results from either inferior digestion or inferior utilisation of dietary fatty acids from PO compared to those from FO. Three diets were formulated by incrementally replacing FO with PO: 0.8 (DIET0.8), 2.1 (DIET2.1) and 3.0 (DIET3.0) g n-3 LC PUFA 100 g^{-1} of feed and following an 84-day feed trial these diets produced significantly different growth and performance results (reported by Stone et al. (2019)). By measuring the lipid and fatty acid composition of the feeds and stripped faecal material the apparent assimilation efficiency of these components was estimated. The results indicated that the overall lipid component of the diets and the majority of fatty acids were digested with high and equal efficiency. This suggested that digestibility of fatty acids from PO was not the responsible for inferior growth and performance, so the cause is likely further downstream – in the ability of the YTK to metabolise these lipids. While the results of the current study do not resolve the question of the mechanism, another interesting trend was observed in relation to the digestibility of saturated fatty acids by YTK. The percentage of saturated fatty acids digested from YTK feeds decreased with increasing chain length. Specifically, 89.1% of dietary 14:0 (myristic acid) was digested compared to only 74.5% of dietary 22:0 (docosanoic acid). This suggested that replacing FO with alternative oils that have a higher proportion of shorter chain saturated fatty acids, compared to longer chain saturated fatty acids, should improve lipid digestion and utilisation in YTK.

Keywords

Yellowtail Kingfish (*Seriola lalandi*); aquaculture; fish oil; poultry oil; fatty acid digestibility.

Highlights

1. Yellowtail Kingfish appear to digest lipids and fatty acids from dietary poultry oil and fish oil with equal efficiency.
2. Yellowtail Kingfish digest shorter chain saturated fatty acids with greater efficiency than longer chain saturated fatty acids.
3. Replacement oils in aquafeeds for YTK should have a greater proportion of shorter chain saturated fatty acids (<16:0) than longer chain saturated fatty acids (>17:0) to ensure >80% digestion of this group of fatty acids.

4.1. Introduction

The composition of dietary lipids in aquafeeds for commercially reared finfish has changed extensively over the last 20 years (Tocher, 2015). Increasing pressure to reduce the quantity of fish oil (FO) used in commercial aquaculture has led to large proportions of lipids now being supplied by poultry or beef tallow and/or vegetable oils, such as canola, sunflower or palm oil (Gatlin et al., 2007, Tacon and Metian, 2008, Naylor et al., 2009, Turchini et al., 2009). The incremental replacement of dietary FO with alternative oils significantly increases the proportions of omega 6 (n-6), omega 9 (n-9) and saturated fatty acids and reduces the proportion of omega 3 (n-3) long chain (LC) polyunsaturated fatty acids (PUFA) (Turchini et al., 2009). While in the majority of cases the alternative oils are able to fulfil the energy requirements of the finfish, their interactions within their digestive system are different from FO. As a consequence, dietary FO replacement has been shown to impact lipid digestibility, growth, fat deposition, enzyme expression, feed conversion ratio (FCR) and product quality in various marine fish (Menoyo et al., 2003, Seno-o et al., 2008, Thanuthong et al., 2011, Bowyer et al., 2012a, Bowyer et al., 2012b, Yılmaz et al., 2016).

Menoyo et al., (2003) determined that the lipid metabolism of Atlantic Salmon (*Salmo salar*) was affected by dietary fatty acids according to their degree of unsaturation and chain length. In both Atlantic Salmon and YTK saturated fatty acids were observed to be preferentially utilised for energy via β oxidation (Menoyo et al., 2003, Bowyer et al., 2012b). As such, the replacement of dietary FO with alternative oils in both species impacted lipid metabolism and utilisation. Fish oil replacement was also shown to affect fatty acid bioconversion in Rainbow Trout (*Oncorhynchus mykiss*) and European Sea Bass (*Dicentrarchus labrax*) (Thanuthong et al., 2011, Yılmaz et al., 2016). In fish, reduced bioconversion of dietary lipids due to changes in composition generally manifests as less growth with the same quantity of feed, which can be problematic as it increases production

costs. In YTK, FCR, growth and product quality have been shown to diminish with increasing replacement of FO with alternative oils (Bowyer et al., 2012a, Stone et al., 2019).

For YTK a common dietary FO replacement that has been widely and successfully used is poultry oil (PO) (Moran et al., 2009, Bowyer et al., 2012a, Bowyer et al., 2013, Collins et al., 2014). In these studies, partial replacement of dietary FO with PO either has either had no significant effect or indeed had a slight positive effect on growth but the fatty acid composition of the muscle tissues has generally been reflective of the PO profile, which has implications for YTK product quality. However, in a recent FO replacement trial conducted by Stone et al. (2019), significant decreases in growth and diminished FCR were observed in YTK with increasing replacement of FO with PO. The deposition of fatty acids in the flesh of YTK for this study were also reported in Chapter 3, which showed that the dietary lipid profile was reflected in the flesh fatty acid profile. Differences in the growth and FCR of YTK in Stone et al., (2019) were speculated to be due to poorer digestibility and metabolism of PO by YTK, however this was not quantified. In order to understand the reason for inferior growth and FCR the current experiment was designed to determine whether dietary lipids from FO and PO were digested with equal efficacy. Therefore, the aim of the current study was to quantify the digestibility of lipids and fatty acids by YTK fed graded levels of FO and PO to determine whether inferior growth was linked to reduced digestibility of PO as a dietary lipid source.

4.2. Methods and Materials

4.2.1. Experimental location and animals

Animal ethics approval for this work was granted by the University of Adelaide animal ethics committee (Approval number: S-2016-127). The experiment was conducted at the South Australian Research and Development Institute (SARDI) South Australian Aquatic Science Centre (SAASC) (West Beach, South Australia, Australia). Yellowtail Kingfish were supplied by Clean Seas Seafood Ltd. (Port Lincoln, South Australia, Australia). Prior to the experiment, fish were housed in 18 × 5000 L tanks supplied with partial flow-through/recirculating (100% system water exchange day^{-1}), sand filtered, UV treated, aerated sea water at ambient temperature and held for ~3.5 months. During this period fish were fed a 9 mm commercial diet (Ridley Pelagica diet; crude protein 46%; crude lipid 24%; gross energy 19.30 MJ kg^{-1}; Narangba, Queensland, Australia) to apparent satiation once daily.

4.2.2. Experimental diets

The diet kernels, FO and PO used in the experimental diets were supplied by Skretting Australia. The diet formulations were based on Skretting Australia's YTK diet (20% fish meal; 40% crude protein, 30% crude lipid and a gross energy level of approximately 21 MJ kg^{-1}) (Stone et al., 2019). The diet kernels contained a base level of 10% crude lipid and were then top coated with an additional 17% lipid (graded blends of FO and PO; total crude lipid level 27%) at Aquafeeds Australia (Mount Barker, South Australia). The main effect of substituting FO with PO was a decrease in n-3 LC PUFA with an increase in n-9 fatty acids (mostly oleic acid, 18:1n-9, OLA). Three experimental diets were formulated with n-3 LC PUFA contents of 0.8 (DIET0.8), 2.1 (DIET2.1) and 3.0 (DIET3.0) g 100 g^{-1} of feed (Table 4.1).

4.2.3. Experimental housing and animal care

At the start of the feed trial, YTK were anaesthetised in 5000 L tanks (total water volume 2500 L) using AQUI-S® (AQUI-S® New Zealand Ltd., Lower Hutt, New Zealand) at a concentration of 14 mg L^{-1} of seawater. Fish were randomly distributed into 9 × 5000 L recirculating aquaculture tanks (13 fish per tank) and randomly assigned one of the 3 experimental diets (3 replicate tanks $diet^{-1}$). Fish were fed their experimental diet for 14 weeks, with feeding once daily to apparent satiation and intake was recorded as grams consumed per fish per day. Water quality parameters were measured daily and maintained within the accepted optimal levels for YTK (Bowyer et al., 2014). Fish growth and FCR were determined after 12 weeks and faecal samples were collected at 13 and 14 weeks for digestibility analysis.

4.2.4. Faecal sample collection

In the 24 hours prior to sample collection fish were fed their respective experimental diet to apparent satiation twice (0900 and 1600 h) to ensure that feed intake was not a limiting factor affecting digestibility. Faecal samples were collected on 2 occasions (13 and 14 weeks) to ensure that sufficient quantity of material could be obtained for analysis. To collect samples, the fish were anaesthetised, removed from their tank and faecal material was manually stripped from each fish following the procedure outlined by Stone et al. (2008). Faecal samples were pooled by tank and immediately frozen by immersion in dry ice and thereafter stored at -20 ºC prior to analysis.

4.2.5. Total lipid analysis

Total crude lipid (as a percentage of wet weight) was estimated for feed and faecal samples utilizing the gravimetric approach (Folch et al., 1957). Briefly, weighed samples were homogenised in 0.9% saline, thereafter lipids were extraction into a 4:1 chloroform: isopropanol solution and the chloroform: isopropanol component was then transferred to a pre-

weighed glass scintillation vial and evaporated to dryness using nitrogen gas leaving only the lipid component behind. The vial was re-weighed to get the weight of the extracted crude fat.

4.2.6. Fatty acid analysis

Fatty acid profiling was conducted for white and red muscle samples. The lipid component (extracted during total lipid analysis) was transmethylated with 1% H_2SO_4 in MeOH at 70 °C for 3 hours, then cooled to room temperature, after which fatty acid methyl esters (FAME) were extracted into 2 mL of heptane. The heptane was transferred to a gas chromatography (GC) vial with 30 mg of anhydrous sodium sulphate, sealed and stored at -20 °C until analysis by GC. Samples were processed on a Hewlett-Packard 6890 GC (Hewlett-Packard, CA, USA) with a flame ionization detector, a split injector and a BPX-70 capillary column (50 m × 0.32 mm) with a 0.25 µm film thickness (SGE, Victoria, Australia). Gas chromatography operating conditions were as described previously (Tu et al., 2010) and peaks were identified with GLC 463 external standard (Nu-Chek Prep Inc., MN, USA). Data output was processed with Agilent ChemStation (version Rev: B.01.03) (Agilent Technologies, CA, USA).

4.2.7. Calculations

The total feed intake for each tank was recorded and feed consumption was assumed to be equal between fish. Faecal output per tank was recorded as the quantity of faecal material pooled from all fish per tank and it was assumed that excretion was equal among individual fish. Thereafter the average faecal output was determined as a proportion of the assumed feed intake for each fish.

Digested fatty acids were estimated both as apparent assimilation efficiency (%) and as grams of fatty acids removed during digestion, both in relation to the quantity of lipids and fatty acids in the feeds.

- Apparent assimilation efficiency (%) = (mg of fatty acid present in faecal material / mg of fatty acid consumed) × 100
- Mass of fatty acids removed during digestion = mg of fatty acid consumed – mg of fatty acid present in faecal material

4.2.8. Statistics

Statistical analysis was performed using IBM SPSS (version 24). Homogeneity of variance was assessed using Levene's test, whilst normality was assessed with the Kolmogorov-Smirnov test. Differences were analysed using a one-way ANOVA where diet was a factor. Where significant differences were detected, post-hoc comparisons were made via Tukey's tests. An alpha level of 0.05 was used for all statistical tests. Results are presented as means ± standard error (SE) of n= 3 tanks per treatment unless otherwise stated.

4.3. Results

4.3.1. Feed trial performance

The mean water temperature during the experimental period was 19.7 ± 0.03 °C (range: 15.5 – 24.5 °C). Experimental diets were readily accepted by YTK with no rejection of feed observed. Overall survival for the duration of the experiment was 98.5%. Fish behaviour and gross pathology (data not shown) were typical of healthy fish suggesting there were no negative impacts of dietary treatments. The mean final weights of YTK were significantly different between groups, with DIET0.8 weighing significantly less than DIET2.1 or DIET3.0 fish, for DIET0.8, DIET2.1 and DIET3.0 fish weighed 3.61 ± 0.07, 3.79 ± 0.01 and 3.77 ± 0.04 kg (total n = 117) respectively (See table 3.2 extracted from Stone et al. (2019)). The FCR achieved by YTK improved from 2.43 to 2.11 as dietary n-3 LC PUFA levels increased from 0.8 to 3.0 g $100g^{-1}$ (DIET0.8 to DIET3.0) when dietary FO increased, and PO decreased (See table 3.2 extracted from Stone et al. (2019)).

4.3.2. Total lipid and fatty acid profiles

Mean lipid content of feed was 26.7 ± 0.27 g 100 g^{-1} feed (Table 4.1). Mean total lipid content of the faeces was 2.4 ± 0.19 g 100 g^{-1} and was not significantly different between treatments (one-factor ANOVA; $P = 0.122$; Table 4.1). The apparent assimilation efficiency of total lipid during digestion was 91.1 ± 0.19 %, 89.1 ± 1.81 % and 92.6 ± 1.81 % for DIET0.8, DIET2.1 and DIET3.0 respectively and was not significantly different between treatments (one-factor ANOVA; $P = 0.121$; Table 4.1).

4.3.3. Fatty acid composition of feed and faeces

Fatty acid composition of the diets was reflective of the dietary lipid sources as FO was incrementally replaced with PO (from DIET3.0 to DIET0.8); total n-6 and n-9 fatty acids

2253 increased, total n-3 fatty acids decreased, and total saturated fatty acid remained relatively
2254 constant (Table 4.1).

2255 Abundant dietary fatty acids including oleic acid, EPA, DPA and DHA that varied
2256 among treatments were absorbed with equal efficiency. Specifically, the percentage of fatty
2257 acids removed from feeds were not significantly different between treatments (Table 4.1).
2258 Saturated fatty acids were the most abundant group present in the faecal material, accounting
2259 for 62% of total fatty acids (Table 4.1). When quantified as mg 100 g^{-1} faeces, saturated fatty
2260 acid content differed among treatment groups, with myristic acid (14:0), pentadecanoic acid
2261 (15:0), margaric acid (17:0), arachidic acid (20:0) and docosanoic acid (22:0) all significantly
2262 less abundant in DIET0.8 faeces than that of DIET2.1 or DIET3.0 (Table 4.1). However, when
2263 the apparent assimilation efficiency was calculated, there were no significant differences in
2264 14:0, 17:0, 20:0 and 22:0 between treatment groups (Table 4.1). On the other hand, there was
2265 a significant difference in the apparent assimilation efficiency of 15:0 with a higher percentage
2266 of 15:0 being extracted from DIET3.0 than DIET2.1 or DIET0.8.

2267 When considering the apparent assimilation efficiency (pooled dietary treatments) it
2268 was noted that longer chain saturated fatty acids were not digested with the same apparent
2269 efficiency as shorter chain fatty acids (Figure 4.1). Specifically, 89.1% of dietary 14:0 (myristic
2270 acid) was digested from YTK feeds which reduced to 74.5% of dietary 22:0 (docosanoic acid),
2271 as chain length increased.

4.4. Discussion

This study aimed to quantify the digestibility of lipids and fatty acids by YTK fed graded levels of dietary FO incrementally replaced by PO and investigate whether compromised growth was linked to reduced digestibility of PO as a dietary lipid source. The results indicate that the overall lipid component of the diets and all the essential n-3 LC PUFA were digested with equal efficiency. Thus, fish from each diet group extracted the same quantity of energy from their dietary lipids, regardless of that fact that dietary lipid composition was different. These lipids were then deposited in to the flesh with equal efficiency (Chapter 3) but yet there was still a reduction in growth and FCR (Stone et al., 2019). This strongly suggests that while YTK are capable of digesting and depositing dietary fatty acids from PO, they were not capable of utilising them with the same efficiency as those from FO, indicating that they require the specific fatty acids supplied by dietary FO for optimal growth.

Given that the reduced growth and FCR observed in FO replacement trials could not be explained by inferior digestibility of dietary lipids and fatty acids from PO as an alternative lipid source, further investigation is recommended to elucidate the mechanisms responsible. While decreasing digestibility of alternative dietary lipids would have been a reasonable explanation for inferior growth and FCR, it appears that the cause is likely further downstream in the ability of the YTK to metabolise these lipids.

Lipid metabolism, as a process, is facilitated by specific enzymes in fish. The abundance of these enzymes (and expression levels of the genes that code for them) could be affected by changes in dietary lipid sources and this could therefore have an impact on the metabolic value to fish. In relation to enzyme activity, a previous study by Bowyer et al., (2012b) showed that lipase activity was not affected by the inclusion of PO in small YTK diets, indicating that it was not necessary for YTK to upregulate their enzyme activity to adequately metabolise PO in the place of FO. It would be expected that if increased enzyme production

and thus an increase in the energy required to metabolise lipids was causing reduced growth and FCR that an increase in lipase activity would have been observed in that study. Therefore, it is unlikely that the cause of reduced growth and FCR in YTK was increased energy required to metabolise dietary lipids and that the cause it a decreased capacity to utilise the nutritional products from PO as efficiently as those from FO.

Changes to the expression of genes associated with lipid metabolism has previously been observed in Atlantic Salmon and Rainbow Trout that were fed diets with manipulated lipids compositions (Panserat et al., 2008; Martinez-Rubio et al., 2013). Specifically, Panserat et al., (2008) reported a lower level of the expression of genes for energy metabolism in Rainbow trout when dietary FO was replaced with alternative lipids. While Martinez et al., (2013) showed that decreasing dietary lipid content lead to the upregulation of biosynthetic pathways and altered the expression of key genes associated with lipid and fatty acids metabolism in Atlantic Salmon. It is possible that decreased gene expression in YTK when reared on dietary PO could be responsible for the decreased growth and FCR observed in FO replacement studies with YTK. Indeed, a recent review by Jobling (2016) discusses the role of fatty acids in regulating lipid metabolism and modulating gene expression, with individual fatty acids having differing effects. Therefore, the increased quantity of dietary n-6 and n-9 fatty acids and decrease quantity of dietary n-3 LC PUFA could lead to differences in the efficiency of fatty acid metabolism/ beta oxidation to make energy.

An interesting result observed during the current study was the pattern of varied digestibility of saturated fatty acids by YTK. Specifically, YTK were observed to digest shorter chain saturated fatty acids (<16:0) with greater efficiency than longer chain saturated fatty acids (>17:0). Digestibility of nutrients was previously estimated for YTK by Miegel et al. (2010), however that study only reported total crude lipid of digesta and faecal material without reporting on the fatty acid profile. Greater efficiency in the digestion of shorter chain saturated

fatty acids, compared to longer chain (>17 carbon) saturated fatty acids, has been well documented in a range of other species including; rats (Carroll, 1958), humans (Ramırez et al., 2001) and a number of aquacultured fish (Cravedi et al., 1987, Sigurgisladottir et al., 1992, Olsen et al., 1998, Johnsen et al., 2000, Menoyo et al., 2003, Francis et al., 2007). Fatty acids each have their own chemical and physical properties which impact their interaction with the gastrointestinal system. Fatty acid digestion and absorption in fish is known to be affected by the chain length, its degree of unsaturation and its melting point of the lipid (Turchini et al., 2009). Importantly, there is increased digestibility of short and medium chain saturated fatty acids as they are hydrophilic and as such can be absorbed with greater ease than longer chain saturated fatty acids. These data demonstrate how considerations of the fatty acid content of dietary lipids can be utilized to manipulate fatty acid digestion by cultured YTK and thus improve the utilization of diets, potentially improve FCR and reduce the cost of YTK production. Specifically, for YTK, given their ability to absorb 14 – 16 carbon length saturated fatty acids with the greatest efficiency, dietary lipids that have a greater proportion of these fatty acids and less 20 and 22 carbon length saturated fatty acids are recommended for investigation for use in YTK feeds. Moreover, saturated fatty acids are readily utilized for energy in YTK (Bowyer et al., 2012b) and as such replacing FO with alternative oils high in short chain saturated fatty acids, should provide a digestible lipid that is an efficient source of energy for YTK.

4.5. Conclusions

Differences in the digestibility of dietary lipids and fatty acids did not explain the differences in growth and FCR observed in FO replacement trials in large sub-adult YTK. While decreasing digestibility of alternative dietary lipids would have been a reasonable explanation for inferior growth and FCR, it appears that the cause is likely further downstream in the ability of the YTK to metabolise these dietary lipids.

The finding of decreased digestibility of saturated fatty acids with increasing chain length in YTK does not address the initial aim of this study, however, it does provide interesting new knowledge for this species. This knowledge could assist YTK feed producers in selecting advantageous alternative oils to replace dietary FO while mitigating negative impacts for productivity and product quality for YTK.

4.6. Acknowledgements

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4.8. Tables and figures

Table 4.1: Total dietary lipid content (%) and fatty acid composition (mg 100 g^{-1}) feed and faeces and percentage of lipid and fatty acids absorbed from feed (%) of three experimental diets (Data presented as mean ± SE; n = 3).

	Feed composition			Faecal composition				Percentage of lipid and fatty acid removed from feed			
	DIET0.8%	**DIET2.1%**	**DIET3.0%**	**DIET0.8%**	**DIET2.1%**	**DIET3.0%**	**P =**	**DIET0.8%**	**DIET2.1%**	**DIET3.0%**	***P =***
Lipid content (%)	26.2	26.9	27.1	2.3 ± 0.1	2.9 ± 0.5	2.0 ± 0.1	0.122	91.1 ± 0.5	89.1 ± 1.8	89.1 ± 0.4	0.121
Analysed fatty acids (mg 100 g-1)											
t18:1n-9 (Palmitelaidic acid)	83	73	72	2.8 ± 0.1	2.9 ± 0.1	3.0 ± 0.2	0.443	92.2 ± 0.6	88.2 ± 2.0	91.6 ±0.6	0.090
t18:1n-7 (Elaidic acid)	140	119	108	7.8 ± 0.3	7.2 ± 0.5	7.4 ± 0.2	0.510	87.1 ± 0.8	83.2 ± 1.5	86.4 ± 0.7	0.051
14:0 (Myristic acid)	420	730	900	20.4 ± 0.7a	31.0 ± 1.2b	41.7 ± 0.4c	< 0.001	88.7 ± 0.7	88.0 ± 1.4	90.7 ± 0.4	0.141
15:0 (Pentadecanoic acid)	53	77	89	4.1 ± 0.1a	4.8 ± 0.3a	6.0 ± 0.1b	< 0.001	81.8 ± 1.2a	82.8 ± 1.4a	86.4 ± 0.7b	0.028
16:0 (Palmitic acid)	5930	5760	5550	421 ± 16.8	374.2 ± 29.4	411.8 ± 6.8	0.245	83.5 ± 1.0	82.2 ± 1.2	85.1 ± 0.8	0.177
17:0 (Margaric acid)	89	100	110	8.0 ± 0.4a	9.0 ± 0.7a	10.9 ± 0.2b	0.002	79.2 ± 1.3	75.5 ± 1.7	80.0 ± 1.3	0.096
18:0 (Stearic acid)	1870	1770	1670	167.7 ± 6.4	151.3 ± 15.2	174.4 ± 3.1	0.256	79.2 ± 1.4	77.0 ± 1.1	79.0 ± 1.2	0.407
20:0 (Arachidic acid)	36	51	53	4.0 ± 0.2a	4.6 ± 0.5a	6.2 ± 0.2b	< 0.001	74.4 ± 1.6	75.6 ± 1.1	76.3 ± 1.3	0.600
22:0 (Docosanoic acid)	25	30	31	2.5 ± 0.1a	3.0 ± 0.3a	4.1 ± 0.1b	0.001	76.6 ± 1.9	73.1 ± 1.1	73.6 ± 1.6	0.251
24:0 (Tetracosanoic acid)	13	16	18	1.2 ± 0.1a	1.3 ± 0.1ab	1.7 ± 0.1b	0.021	78.2 ± 3.3	77.2 ± 1.4	80.4 ± 1.8	0.604
18:3n-3 (Alpha Linolenic acid- ALA)	550	490	430	7.2 ± 0.7	8.7 ± 1.5	6.2 ± 0.2	0.213	96.9 ± 0.4	94.1 ± 2.1	97.1 ± 0.2	0.184
20:5n-3 (Eicosapentanaeoic acid- EPA)	270	930	1350	3.4 ± 0.3a	13.3 ± 2.9b	11.9 ± 0.9b	0.002	97.0 ± 0.4	95.1 ± 2.0	98.2 ± 0.2	0.192
22:5n-3 (Docosapentaenoic acid- DPA)	63	130	160	1.9 ± 0.3a	3.2 ± 0.4b	3.1 ± 0.2b	0.010	92.9 ± 1.4	92.2 ± 2.3	96.1 ± 0.3	0.212
22:6n-3 (Docosahexaenoic acid- DHA)	420	1080	1440	13.1 ± 1.9a	26.2 ± 2.9b	27.6 ± 1.6b	< 0.001	92.6 ± 1.3	92.3 ± 2.2	96.2 ± 0.3	0.160
18:2n-6 (Linoleic acid- LOA)	3150	2650	2300	52.0 ± 4.5	57.8 ± 7.6	42.7 ± 1.9	0.154	96.1 ± 0.5	92.9 ± 2.2	96.3 ± 0.2	0.162
18:3n-6 (Gamma Linolenic acid)	31	41	43	2.4 ± 0.3	2.5 ± 0.2	3.4 ± 0.3	0.062	82.1 ± 2.3	83.4 ± 1.6	84.6 ± 1.2	0.619
20:2n-6 (Eicosadienoic acid)	28	32	36	0.8 ± 0.1	1.0 ± 0.1	0.9 ± 0.0	0.173	93.2 ± 0.6	90.9 ± 2.2	94.8 ± 0.2	0.137
20:3n-6 (Dihomo-gamma-linoleic acid)	24	35	33	2.2 ± 0.2	2.0 ± 0.2	2.6 ± 0.1	0.111	78.8 ± 3.0	83.8 ± 2.0	84.3 ± 0.6	0.157
20:4n-6 (Arachidonic acid)	110	50	170	2.6 ± 0.4	3.6 ± 0.3	3.0 ± 0.2	0.086	94.4 ± 0.9a	77.3 ± 6.2b	96.4 ± 0.3a	0.004
22:4n-6 (Docosatetraenoic acid)	16	20	23	0.8 ± 0.1	0.7 ± 0.0	0.7 ± 0.1	0.956	88.7 ± 2.5	89.3 ± 2.0	93.4 ± 0.7	0.191
16:1n-7 (Palmitoleic acid)	1450	1560	1610	22.5 ± 1.5	30.8 ± 4.6	25.6 ± 0.7	0.148	96.4 ± 0.4	93.6 ± 2.1	96.8 ± 0.2	0.167
18:1n-7 (Octadecenoic acid)	640	660	670	16.1 ± 0.8	19.9 ± 1.7	18.4 ± 0.4	0.089	94.1 ± 0.5	90.6 ± 2.5	94.5 ± 0.3	0.146
18:1n-9 (Oleic acid)	11050	9290	8020	227.4 ± 15.3	230.0 ± 26.7	173.8 ± 4.6	0.074	95.2 ± 0.5	92.1 ± 2.3	95.7 ± 0.2	0.172
20:1n-9 (Eicosenoic acid)	130	150	160	3.9 ± 0.2a	5.2 ± 0.4b	5.3 ± 0.2b	0.004	93.0 ± 0.5	89.3 ± 2.6	93.4 ± 0.3	0.166
22:1n-9 (Docosenoic acid)	11	20	26	0.7 ± 0.1a	0.8 ± 0.0ab	1.1 ± 0.1b	0.029	83.4 ± 3.0a	88.5 ± 1.5a	91.8 ± 0.4b	0.025
24:1n-9 (Tetracosenoic acid)	20	41	48	1.3 ± 0.1a	2.1 ± 0.1b	2.9 ± 0.1c	< 0.001	85.0 ± 0.6	85.7 ± 1.6	87.8 ± 0.6	0.198
Total trans	223	192	180	10.6 ± 0.4	10.1 ± 0.5	10.4 ± 0.3	0.729	89.0 ± 0.7	85.1 ± 1.7	88.4 ± 0.6	0.050
Total saturated	8436	8534	8421	629.0 ± 24.2	579.3 ± 47.7	656.9 ± 9.7	0.241	82.7 ± 1.1	81.5 ± 1.2	84.3 ± 0.8	0.192
Total Omega 3	1303	2630	3380	25.6 ± 2.9a	51.4 ± 7.5b	48.8 ± 2.7b	0.004	95.4 ± 0.7	93.6 ± 2.1	97.1 ± 0.2	0.190
Total Omega 6	3359	2829	2605	60.7 ± 5.1	67.6 ± 7.8	53.4 ± 2.2	0.224	95.7 ± 0.5	92.4 ± 2.3	95.9 ± 0.2	0.144
Total Omega 7	2090	2220	2280	38.6 ± 2.4	50.7 ± 6.3	44.0 ± 1.1	0.130	95.7 ± 0.4	92.7 ± 2.2	96.1 ± 0.2	0.160
Total Omega 9	11211	9501	8254	233.4 ±15.4	238.1 ± 27.0	183.2 ± 4.8	0.092	95.1 ± 0.5	92.0 ± 2.3	95.6 ± 0.2	0.172
n-3 LC PUFA	753	2140	2950	18.4 ± 2.5	42.7 ± 6.0	42.6 ± 2.6	0.068	94.2 ± 1.0	93.5 ± 2.1	97.1 ± 0.2	0.169

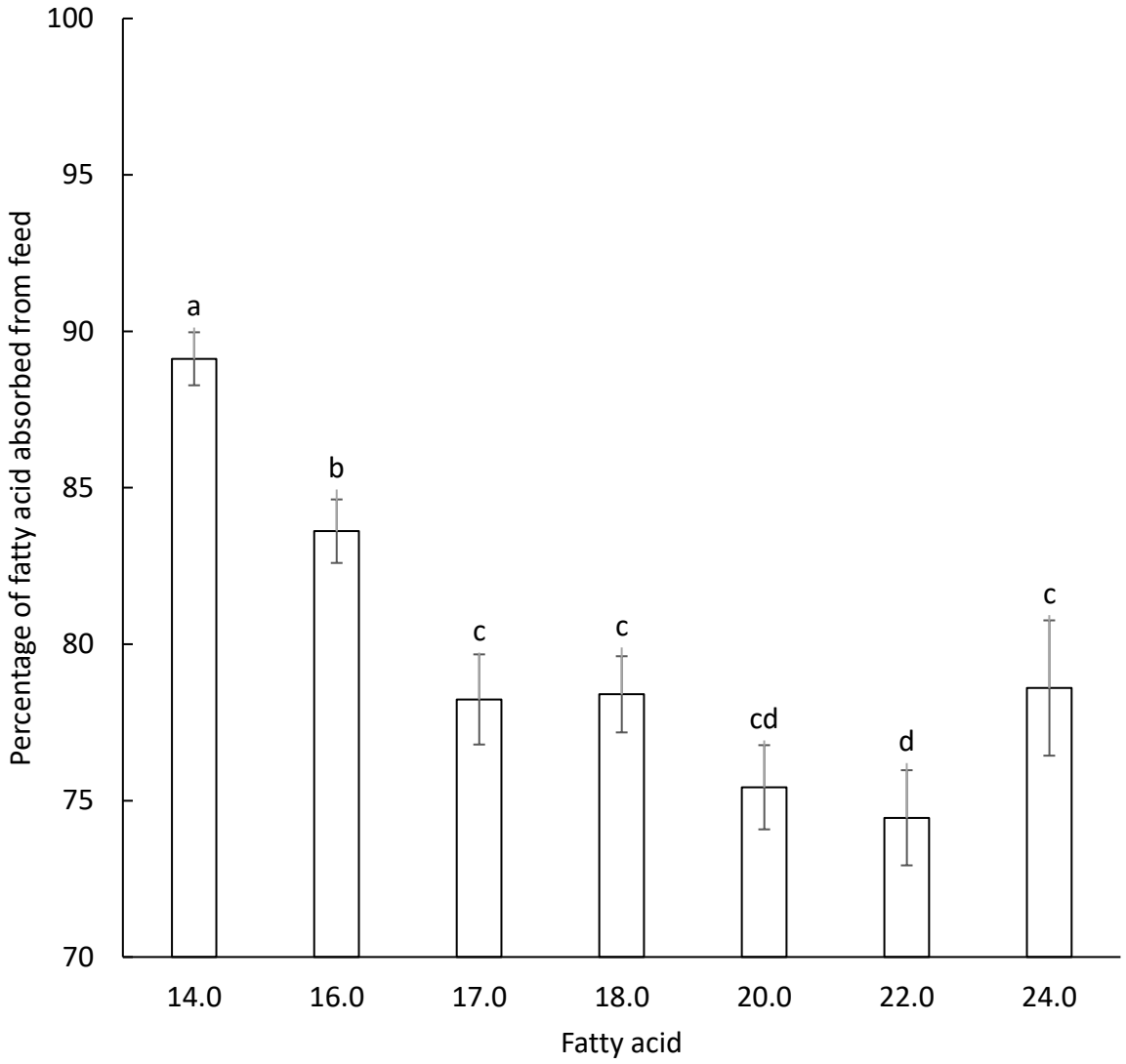

Figure 4.1: Percentage of saturated fatty acids absorbed from feed by Yellowtail Kingfish (*Seriola lalandi*) (Data presented as mean ± SE; n = 3, subscripts denote significant differences, one-way ANOVA; $P < 0.001$)

4.9. Statement to link Chapter 3 and Chapter 5

In Chapter 3 and in conjunction with the Stone et al. (2019) study, it became apparent that YTK could be reared on diets deficient in n-3 LC PUFA without a detrimental impact on survival. Growth, feed conversion efficiency and flesh fatty acid composition were affected by dietary n-3 LC PUFA concentration, but these losses could potentially be counteracted if the financial cost of lost growth and feed conversion efficiency was outweighed by the cost savings in aquafeed production due to reduced inclusion of expensive fish oil. If this approach was financially viable it would be necessary to understand the rate at which the flesh fatty acid profile could be restored prior to harvesting YTK. With this in mind, the rate at which YTK could accumulate n-3 LC PUFA in the flesh following a change in diet was investigated. As an additional investigation, the rate at which n-3 LC PUFA was diluted in YTK flesh following a reduction in diet FO was assessed as a means of further understanding the time course for changes in flesh n-3 LC PUFA.

Chapter 5 – Statement of authorship

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Name of Principal Author (Candidate)	Samantha N Chown		
Contribution to the Paper	Conceptualization, methodology, formal analysis, investigation, data curation, writing original draft, writing – review and editing and visualisation.		
Overall percentage (%)	92%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	24/06/2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Writing – review & editing, supervision, resourcing, project administration and funding acquisition (1%)		
Signature		Date	24/06/2019

Chapter 5: Accumulation and dilution of n-3 LC PUFA in the white muscle of large Yellowtail Kingfish (*Seriola lalandi*) following a change in dietary fish oil inclusion level

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Abstract

Finishing diets have been extensively researched and utilised in the aquaculture industry to improve the nutritional quality of commercially farmed fish for human consumers. Marked changes to omega 3 long-chain polyunsaturated fatty acid (n-3 LC PUFA; including eicosapentaenoic acid- EPA; docosapentaenoic acid - DPA and docosahexaenoic acid- DHA) concentrations in the edible portion of the Yellowtail Kingfish (*Seriola lalandi*) (YTK) were observed as a result of changes to dietary fish oil (FO) intake were investigated at warm water temperatures (>20 °C). The aim of the study was to better understand how quickly such changes could happen and to make recommendations to industry about the length of time required to obtain significant increases in white muscle n-3 LC PUFA by feeding a high n-3 LC PUFA diet. In parallel, the rate at which n-3 LC PUFA diminished in YTK white muscle was also investigated, with the aim of understand the rate at which the feeding diets deficient in n-3 LC PUFA can negatively impact white muscle fatty acid profiles. Four diets were formulated: two preconditioning diets which were either moderate or high in dietary FO (at 1.42 and 3.34 g n-3 LC PUFA 100 g^{-1} feed, respectively) and 2 finishing diets that were high and low in dietary FO (at 2.85 and 0.71 g n-3 LC PUFA100 g^{-1} feed, respectively). These diets were fed in succession to investigate the effects of the finishing diets relative to their starting points, forming two treatment groups (MOD/HIGH and HIGH/LOW) to address the two aims. Preconditioning diets were fed for 84 days, after which the fatty acid profile of the white muscle of the two treatment groups was measured before finishing diets were fed for an additional 33 days, with the fatty acid profile measured again after 19 and 33 days of feeding of finishing diets. With 33 days of feed of finishing diets, white muscle n-3 LC PUFA increased by 48 mg 100 g^{-1} (17% increase) and decreased by 51 mg 100 g^{-1} (14% decrease) with HIGH and LOW finishing diets, respectively. Apparent feed conversion ratio (FCR) was impacted by dietary treatment, with a superior FCR of 1.71 achieved by the MOD/HIGH group compared to 1.85

by the HIGH/LOW group. A minimum 33 day finishing period was recommended for commercial YTK producers to ensure a significant increase in white muscle n-3 LC PUFA content. It was also recommended that the dietary n-3 LC PUFA content of YTK feeds should be closely monitored with strict lower limits set with feed producers to ensure no diminishment to product quality of feed conversion efficiency in commercially farmed YTK.

Keywords

Yellowtail Kingfish (*Seriola lalandi*), omega 3 (n-3) long chain (LC) polyunsaturated fatty acids (PUFA), product quality, accumulation and dilution.

Highlights

1. The n-3 LC PUFA concentration of YTK white muscle was increased by 48 mg 100 g^{-1} (17% increase) by increasing the dietary concentration of n-3 LC PUFA by 1.42 g 100 g^{-1} feed in a finishing period of 33 days.
2. Conversely, the n-3 LC PUFA concentration of YTK white muscle was decreased by 51 mg 100 g^{-1} (14% decrease) by decreasing the dietary concentration of n-3 LC PUFA by 2.63 g 100 g^{-1} feed in a period of 33 days.
3. The rate at which individual n-3 LC PUFA assimilate in YTK was not equal, EPA was observed to be more readily altered in the white muscle than DHA.
4. Apparent FCR was influenced by a change in dietary n-3 LC PUFA concentration over the 33-day finishing period, specifically a superior FCR of 1.71 was achieved by the MOD/HIGH group compared to 1.85 by the HIGH/LOW group.

5.1. Introduction

Finishing or washout diets have been extensively researched and utilised in the aquaculture industry in the past 20 years (Bell and Sargent, 2003, Bell et al., 2003, Ng et al., 2004, Mourente and Bell, 2006, Naylor et al., 2009, Turchini et al., 2009, Tocher, 2015). The basic premise of the finishing diet is that fish can be reared through the grow-out period on one diet (either a less expensive or more sustainable formulation) and then given a second diet (e.g. with flesh pigmenting additives or high in dietary lipid) prior to harvest. In some instances, the grow-out diet is high in less expensive terrestrial plant or animal-based ingredients and the finishing diet replaces those with fish meal and fish oil (FO). This change in composition results in increased flesh content of omega 3 (n-3) long chain polyunsaturated fatty acids (LC PUFA) including eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) (Naylor et al., 2009). The target for n-3 LC PUFA concentration at harvest varies, but has been benchmarked to many different levels including: a comparable level to wild fish, fish which have been reared on high FO diets throughout grow-out, parity with what is achieved by other aquaculture species or to meet recommended dietary intake levels for the human consumer (Einen et al., 1999). Regardless of the reason, the finishing diet strategy has the potential to provide benefits for the producer in terms of reduced cost of production.

Reducing consumption of FO is global priority for the aquaculture industry. A review of feed demands for aquaculture by Tocher (2015) estimated that global aquaculture production of marine fish would increase to 3.675 million tonnes per year by 2020 and this would be responsible for consuming 4.998 million tonnes of feed per year. If the aquaculture industry is going to continue to grow and supply the expanding human population with nutritious seafood the careful management of finite FO supplies is imperative (Naylor et al., 2009). The optimal inclusion of n-3 LC PUFA (determined by maximal growth rate and lowest food conversion

ratio, FCR) for Yellowtail Kingfish (*Seriola lalandi*) (YTK) during grow-out is approximately 2.1 – 2.6 g n-3 LC PUFA 100 g^{-1} feed (Stone et al., 2019). At an average FCR of 1.6 this equates to a consumption of 102.4 – 124.8 g of FO to produce every kilogram of YTK (based on FO with 33% n-3 LC PUFA). Any decrease that can been made in dietary n-3 LC PUFA inclusion for YTK from FO without compromising consumer nutrition will be beneficial for the environment and specifically the diminishing supply of FO.

In the human diet, n-3 LC PUFA are nutritionally important, having anti-inflammatory and cardio-protective effects as well as being highly important in infant development (Wood et al., 2015). The international society for the study of fatty acids and lipids (ISSFAL) recommends a daily intake of 500 mg of n-3 LC PUFA per day (ISSFAL, 2004). In YTK dietary n-3 LC PUFA content is reflected in the flesh, and the recently determined optimal dietary content of 2.1 g n-3 LC PUFA 100 g^{-1} feed (Stone et al, 2019) results in 725 ± 16 mg of n-3 LC PUFA 100 g^{-1} white muscle (Chapter 3). The daily recommended intake of n-3 LC PUFA is achievable with a 69 g portion of white muscle, however any further increases to YTK flesh n-3 LC PUFA could still benefit the human consumer, as a smaller portion or less frequent consumption of fish would be required. Another possibility is for YTK to be reared on diets with less than 2.1 g n-3 LC PUFA 100 g^{-1} feed, and then finished on diets with greater than 2.1 g n-3 LC PUFA 100 g^{-1} feed to restore the fatty acid profile of the flesh. This strategy would, however, impact growth and FCR and detailed cost benefit analysis would be required before implementing such strategies.

Since finishing diets have potential benefits for YTK producers, consumers and the environment the aim of the current study was to investigate how quickly n-3 LC PUFA could change in the muscle tissue of large YTK following either an increase or decrease in dietary FO level.

5.2. Methods and Materials

5.2.1. Experimental location and animals

Animal ethics approval for this work was granted by the University of Adelaide animal ethics committee (Approval number: S-2017-103). The experiment was conducted at the South Australian Research and Development Institute (SARDI) South Australian Aquatic Science Centre (SAASC) (West Beach, South Australia, Australia). Yellowtail Kingfish were supplied by Clean Seas Seafood Ltd. (Port Lincoln, South Australia, Australia). Prior to the experiment, fish were housed in 18 × 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 ~3.5 months. During this period fish were fed a 9 mm commercial diet (Ridley Pelagica diet; crude protein 44%; crude lipid 24%; gross energy 19.30 MJ kg^{-1}; Narangba, Queensland, Australia) to apparent satiation once daily.

5.2.2. Experimental diets

The diet kernels, fish oil and poultry oil used in the experimental feed were supplied by Ridley (Narangba, Queensland, Australia). The formulations were based on Ridley's Yellowtail Kingfish diet (30% fish meal; 44% crude protein, 25% crude lipid and a gross energy level of approximately 21 MJ kg^{-1}). Experimental diets were produced with a diet kernel which contained a base level of 8% crude lipid, the kernels were top coated with an additional 14 - 17% lipid (graded blends of fish oil and poultry oil to give varying levels of n-3 LC-PUFA, reaching a target 22 - 25% dietary lipid inclusion level).

5.2.3. Experimental design

The experimental fish used in this study were fed with a diet that was either moderate (MOD) or high (HIGH) in n-3 LC PUFA for 84 days (the preconditioning phase), they were

then switched to a different diet for a further 33 days, giving us two treatment groups, a MOD/HIGH group and a HIGH/LOW group. The MOD/HIGH group's diet changed from 1.4 to 2.85 g n-3 LC PUFA 100 g^{-1} feed and the HIGH/LOW group's diet switched from 3.3 to 0.7 g n-3 LC PUFA 100 g^{-1} feed. (Table 5.1).

5.2.4. Animal housing and care

At the conclusion of the 84-day preconditioning period, fish were anaesthetised in 5000 L tanks (total water volume 2500 L) using AQUI-S® (AQUI-S® New Zealand Ltd., Lower Hutt, New Zealand) at a concentration of 14 mg L^{-1} of seawater. Fish were weighed, measured (fork length) and returned to their 9 × 5000 L recirculating aquaculture tanks (13 fish per tank) and switched to their secondary experimental diets (3 replicate tanks $diet^{-1}$). Fish were fed their experimental diet once daily to apparent satiation and intake was recorded as grams consumed per tank per day. Water quality parameters were measured daily and maintained within the accepted optimal levels for YTK (Bowyer et al., 2014). Water temperature (ºC) was measured with a thermometer. Dissolved oxygen (mg/ L and percentage saturation) was measured using a dissolved oxygen meter (OxyGuard International A/S, Birkerød, Denmark). The pH was measured using a multi-parameter meter (Oakton pHtestr 20; Oakton Instruments, Vernon Hills, IL, USA). Ammonia (ppm) was measured using an Aquarium Pharmaceuticals ammonia test kit (Mars Fishcare, North America). Salinity (g L^{-1}) was measured weekly using a portable salinity refractometer (model RF20, Extech Instruments, Nashua, NH, USA).

5.2.5. Sample collection

Tissue samples were collected from 3 fish per tank at 0, 19 and 33 days after being anesthetised as previously described. At each sample time all fish were anaesthetised, measured and weighed. Three randomly selected fish from each tank were humanely euthanised by percussive stunning, and a section of white muscle was collected from the dorsal fillet adjacent

to the dorsal fin. Remaining fish were returned to their respective tanks. Muscle samples were immediately frozen by immersion in dry ice and thereafter stored at -20 ºC prior to analysis.

5.2.6. Growth and feed efficacy

Growth and feed efficacy indices were calculated using the following equations:

- Weight gain (g $fish^{-1}$) = (total tank final biomass – total tank initial biomass) / number of fishes
- Condition factor = (fish weight (g) / fish fork length $(cm)^3$) × 100
- Specific growth rate (SGR, % day^{-1}) = ((ln final tank average fish weight − ln initial tank average fish weight) / days) × 100
- Apparent feed conversion ratio (Apparent FCR) = weight of total tank feed consumed (dry weight)/ total tank fish weight gain (wet weight)

5.2.7. Total lipid analysis

Tissue total crude lipid (as a percentage of wet weight) was estimated utilizing the gravimetric approach (Folch et al., 1957). Briefly, weighed muscle samples were homogenised in 0.9% saline, thereafter muscle lipids were extraction in to a 4:1 chloroform: isopropanol solution and the chloroform: isopropanol component was then evaporated in a pre-weighed vial using nitrogen gas leaving only the lipid component behind.

5.2.8. Fatty acid analysis

Fatty acid profiling was conducted for all samples. The lipid component (extracted during total lipid analysis) was transmethylated with 1% H_2SO_4 in MeOH at 70 °C for 3 hours, then cooled to room temperature, after which fatty acid methyl esters (FAMES) were extracted in to 2 mL of heptane. The heptane was transferred to a gas chromatography (GC) vial with 30 mg of anhydrous sodium sulphate, sealed and stored at -20 °C until analysis by GC. Samples were processed on a Hewlett-Packard 6890 GC (Hewlett-Packard, CA, USA) with a flame

ionization detector, a split injector and a BPX-70 capillary column (internal diameter of 50 m × 0.32 mm) with a 0.25 µm film thickness (SGE, Victoria, Australia). Gas chromatography operating conditions were as described previously (Tu et al., 2010) and peaks were identified with GLC 463 external standard (Nu-Chek Prep Inc., MN, USA). Data output was processed with Agilent ChemStation (version Rev: B.01.03) (Agilent Technologies, CA, USA).

5.2.9. Statistics

Statistical analysis was performed using IBM SPSS (version 24). Homogeneity of variance was assessed using Levene's test, whilst normality was assessed with the Kolmogorov-Smirnov test. Where data met prior requirements, differences were analysed using a one-way ANOVA (across time) or t-tests (between treatments). Two lines of inquiry were followed: firstly, data were separated by treatment group and differences were assessed for significance across time, and secondly, data were separated by sampling episode and differences between treatments at each time point were assessed for significance. Where significant differences were detected, post-hoc comparisons were made via Tukey's tests. An alpha level of 0.05 was used for all statistical tests. Results are presented as means ± standard error (SE).

5.3. Results

5.3.1. General observations

The mean water temperature during the experimental period was 20.8 ± 0.2 °C (mean ± SE, range: 19.0 – 23.0 °C). Experimental diets were readily accepted by YTK with no rejection of feed observed. Overall survival for the duration of the experiment was 100%. Fish behaviour and gross pathology (data not shown) were typical of healthy fish suggesting there were no negative impacts of dietary treatments over the 33-day study.

5.3.2. Growth and feed efficacy

Weight and length increased significantly over time for both treatments (one-factor ANOVA; $P < 0.001$ for all; Table 5.2), while condition factor did not differ significantly for either treatment (one-factor ANOVA; MOD/HIGH $P = 0.927$ and HIGH/LOW $P = 0.437$; Table 5.2). Total weight gain was not significantly different between treatment groups; but trends showed the MOD/HIGH treatment group gaining quantitatively more weight (one-factor ANOVA; $P = 0.244$; Table 5.3). During the 33-day experimental period the MOD/HIGH treatment group gained 615.8 ± 27.7 g and increased their initial body weight by 29.9%, while the HIGH/LOW treatment group only gained 490.0 ± 71.9 g and increased their initial body weight by 22.2% (Table 5.3). Specific growth rate was not significantly different between treatment groups. Average SGR was 0.76 and 0.65 % day^{-1} for the MOD/HIGH and HIGH/LOW treatment groups respectively (Table 5.3). Apparent FCR was significantly different between treatment groups, average FCRs were 1.71 and 1.85 for the MOD/HIGH and HIGH/LOW treatment groups respectively (one-factor ANOVA; $P = 0.029$; Table 5.3). While FCR for the HIGH/LOW treatment group was relatively consistent between the separate time periods (1.89 during days 0 – 19 and 1.93 during days 20 – 33), the same could not be said for the MOD/HIGH treatment group. The FCR for the MOD/HIGH was 2.27 during days 0-19,

then reduced to 1.21 during the subsequent time period, this trend was driven by less weight gain in the first period.

5.3.3. Total lipid content white muscle

Total lipid content of the white muscle did not differ significantly across time for either treatment (one-factor ANOVA; MOD/HIGH: $P = 0.370$ and HIGH/LOW: $P = 0.746$; Table 5.4 and Table 5.5 respectively). However, the HIGH/LOW treatment group did show a trend for decreased total lipid content over time.

5.3.4. Fatty acid profile of the white muscle

5.3.4.1. MOD/HIGH treatment group

In the MOD/HIGH treatment group there was a significant increase in total omega 3 fatty acids (one-factor ANOVA; $P = 0.020$; Table 5.4), a significant decrease in total omega 6 fatty acids (one-factor ANOVA; $P < 0.001$; Table 5.4) and no significant change in total omega 9 fatty acids (one-factor ANOVA; $P = 0.747$; Table 5.4) over time.

Total n-3 LC PUFA remained consistent for the first 19 days, then by day 33 there was an increase of 45 mg 100 g^{-1} white muscle (one-factor ANOVA; $P = 0.012$; Figure 5.1). When considering the individual fatty acids, EPA increased significantly after 19 days (one-factor ANOVA; $P = 0.009$; Table 5.4), however, DPA and DHA did not significantly increase until day 33 (one-factor ANOVA; $P = 0.006$ and $P = 0.017$ respectively; Table 5.4).

5.3.4.2. HIGH/LOW treatment group

For the HIGH/LOW treatment group there was a significant decrease in total omega 3 fatty acids (one-factor ANOVA; $P = 0.013$; Table 5.5) and significant increases in total omega 6 fatty acids and total omega 9 fatty acids (one-factor ANOVA; $P = 0.001$ and $P = 0.007$ respectively; Table 5.5) over time.

Total n-3 LC PUFA decreased by 51 mg 100 g^{-1} in white muscle during the 33 days (one-factor ANOVA; $P = 0.023$; Figure 5.2). When considering the individual fatty acids, EPA steadily significantly decreased over time (one-factor ANOVA; $P < 0.001$; Table 5.5) and DPA decreased from 0 to 19 days (one-factor ANOVA; $P < 0.001$; Table 5.5), while DHA did not differ significantly over time (one-factor ANOVA; $P = 0.201$; Table 5.5).

5.4. Discussion

The data presented here demonstrate that substantial increases or decreases in dietary n-3 LC PUFA can have significant impacts on feed conversion and the fatty acid profile of white muscle in large YTK in a relatively short (33-day) period of time. In 33 days, white muscle n-3 LC PUFA increased 48 mg 100 g^{-1} muscle (17% increase) and decreased 51 mg 100 g^{-1} muscle (14% decrease) with switches to HIGH and LOW experimental diets, respectively. Apparent FCR was also impacted by dietary treatment, with a superior FCR of 1.71 achieved by the MOD/HIGH group compared to 1.85 by the HIGH/LOW group. These results have implications for human consumers and commercial fish producers, with changes to dietary n-3 LC PUFA having repercussions for product quality (varied nutrient content for consumers), the sustainability and efficiency of production for commercial producers.

Finishing diets, high in n-3 LC PUFA have been trialled for a range of other commercially farmed fish, most notably with Atlantic Salmon (*Salmo salar*). In Atlantic salmon finishing diets have been trialled for various durations from 12 weeks up to 24 weeks (Bell et al., 2003, Bell et al., 2004, Ng et al., 2004). Bell et al. (2004) fed a 100% fish oil finishing diet for 24 weeks to fish which had previously been fed a 100% linseed oil diet and was able to restore muscle n-3 LC PUFA concentrations to 80% of those that were present in fish fed a 100% FO diet throughout the whole growth period. A relative increase in muscle n-3 LC PUFA concentration of 453 mg 100 g^{-1} muscle was achieved in 24 weeks. Data presented by Bell et al. (2003) showed similar results, with preconditioning linseed and rapeseed oil trials followed by a 100% FO diet over a 20-week finishing period. The magnitude of the increase of muscle n-3 LC PUFA achieved in those salmon was substantially greater than in YTK. This can be attributed to a combination of factors. Firstly, initial linseed and rapeseed oil diets were lower in n-3 LC PUFA thus the salmon muscle n-3 LC PUFA concentration began at a lower starting point than YTK. Secondly, the high fat content in the muscle of salmon compared to

YTK (9.6% in salmon compared to 1.9% in YTK) would allow the finishing strategy to be more efficient in salmon, as with a higher fat content there is a greater potential for quantitative increases in n-3 LC PUFA. Lastly, the duration of the finishing period was 4 times longer in salmon compared to YTK. Currently minimal data exists for the accumulation of n-3 LC PUFA in YTK muscle as finishing diets have not been widely researched for this species. The current and previous studies for other commercially farmed fish demonstrate that finishing diets can be an efficient means of improving the fatty acid profile of aquacultured fish. However, by comparing the quantitative increases in flesh n-3 LC PUFA between YTK and Atlantic salmon, it was evident that the duration of the finishing period, the composition of preconditioning and finishing diets, and biological differences between species influence the effectiveness of the finishing diet.

Similarly, a number of studies have investigated the impact of feeding low n-3 LC PUFA diets on flesh fatty acid composition, growth and feed conversion efficiency. Diets low in n-3 LC PUFA have been trialled for Japanese Yellowtail (*Seriola quinqueradiata*), a species that is closely related to YTK (Seno-o et al., 2008). While the primary aim of that study was to investigate the effect of replacing dietary FO with olive oil on the product quality and shelf stability of filleted products, it does provide a comparison group for the HIGH/LOW treatment group in the current study. After 40 days a decreased dietary n-3 LC PUFA level of 1.94 g n-3 LC PUFA 100 g^{-1} feed had no effect on growth or the proximate composition of Japanese Yellowtail. Similar to the current study, white muscle fatty acid composition was reflective of dietary fatty acid composition (low in n-3 LC PUFA and high in omega 9 fatty acids from olive oil) and feed conversion efficiency decreased with decreasing dietary n-3 LC PUFA. Specifically, a decrease in dietary n-3 LC PUFA of 1.94 g n-3 PUFA 100 g^{-1} feed (from 3.33 g to 1.39 g) resulted in a decrease of 53 mg n-3 LC PUFA 100 g^{-1} in white muscle. This was comparable the current study where a decrease of 51 mg n-3 PUFA 100 g^{-1} in white muscle

was observed after 33 days. These two closely related species have similarities in dietary fatty acid requirements (Deshimaru et al., 1982, Stone et al., 2019) and fatty acid utilisation patterns (Masumoto, 2002, Hilton et al., 2008, Booth et al., 2010, Chapter 3) so this similarity was expected.

Changes to dietary n-3 LC PUFA were observed to have an effect on feed conversion efficacy in YTK and could have implications for commercial YTK farmers. In the 33-day experimental period a significant difference in apparent FCR was observed, with the fish in the MOD/HIGH group having a superior FCR compared to those in the HIGH/LOW group. Similar finishing diet studies have reported no differences in growth between treatments and have not reported apparent FCR (Bell et al., 2003, Bell et al., 2004, Ng et al., 2004). In the current study, growth was not different between treatment groups, but feed conversion was significantly different, demonstrating the need to report both values. The pattern for inferior FCR in the HIGH/LOW group was expected, given that the dietary n-3 LC PUFA concentration in the finishing diet for this group was substantially lower than the recommended dietary concentration (2.1g n-3 LC PUFA 100 g^{-1} feed) for YTK of this size (Stone et al., 2019). Interestingly, a study utilizing juvenile YTK (approximately 100 g), carried out under similar conditions (water temperature >20° C), which reared fish on diets that were low or high in n-3 LC PUFA (0.57 g and 2.02 g n-3 LC PUFA 100 g^{-1} respectively) for 5 weeks, recorded no significant differences in apparent FCR between groups (Bowyer et al., 2012a). There are however a number of challenges when comparing these 2 size classes of YTK. Juvenile YTK are known to have superior FCR compared to adult YTK (Pirozzi and Booth, 2009), diet composition and nutrient requirements differ over the size classes and could impact FCR, and the quality of the feeds utilised in the two studies could have also impacted FCR. While n-3 LC PUFA dilution is not consistent over the lifecycle of YTK, the diminishment of white muscle n-3 LC PUFA in large YTK fed diets low in n-3 LC PUFA has substantial implications

2822 for commercial producers. The results of the current study indicate that with a short period of
2823 feeding of inadequate dietary n-3 LC PUFA, a reduced FCR can be observed for large YTK
2824 during the grow-out period with feed not being converted to growth with the same efficiency
2825 as when adequate dietary n-3 LC PUFA is supplied. This information will be valuable for
2826 commercial YTK producers as reduced feed conversion efficiency will decrease the
2827 profitability of production. These data demonstrate the necessity of setting minimum inclusion
2828 levels for dietary n-3 LC PUFA and closely monitoring their content in feeds to ensure that
2829 specifications are being met.

2830 An additional finding of the current study was that the rate of change of the individual
2831 n-3 LC PUFAs over time was not equal. Specifically, concerning the MOD/HIGH treatment
2832 group, white muscle EPA was observed to increase in the first 19 days, while DPA and DHA
2833 did not significantly increase until 33 days. Interestingly it appears that DHA takes longer to
2834 accumulate in YTK white muscle. In the MOD/HIGH treatment group DHA increased by 41
2835 mg 100 g^{-1} white muscle between day 19 and 33, indicating this fatty acid was slower to
2836 respond, while EPA levels changed faster. By comparison, in the HIGH/LOW treatment group
2837 white muscle EPA continually decreased from 0 to 33 days, DPA decreased in the first 19 days
2838 and DHA did not differ significantly over time. Previous studies have suggested that DHA is
2839 preferentially conserved (Bowyer et al., 2012b, Chapter 3) and the results of the current study
2840 support this hypothesis. Changes in the flesh fatty acid profile of fish are known to be affected
2841 by the growth achieved over the experimental period and the rate of turnover of stored fatty
2842 acids, specifically in the forms of triglycerides (slow exchange rate) and phospholipids (more
2843 readily exchangeable). While the relative changes to individual n-3 LC PUFA provide insight
2844 into the capacity of YTK to preserve and utilise specific fatty acids, for producers it's more
2845 important to note that no increase in muscle n-3 LC PUFA was observed until after 19 days of
2846 feeding of high n-3 LC PUFA diet. This indicates that the minimum finishing period, under

2847 these conditions (warm water >20 °C), required to achieve a significant increase in muscle n-
2848 3 LC PUFA is somewhere between 20 and 33 days for YTK. Therefore, a minimum finishing
2849 period of 33 days prior to harvest is recommended to commercial YTK producers, without
2850 further research to refine the required duration. It would also be worthwhile considering the
2851 capacity for further increases to muscle n-3 LC PUFA with higher water temperature, as growth
2852 rates would be higher and thus a faster and/or greater increase could be achieved.

2853 The current study differed from the majority of other finishing diet studies, with the
2854 primary aim focusing on short term changes to n-3 LC PUFA concentrations in the edible
2855 portion of the fish, rather than full restoration of a 'normal' fatty acid profile achieved with
2856 100% FO diets. The International Society for the Study of Fatty Acids and Lipids (ISSFAL)
2857 recommends a daily intake of 500 mg per day of n-3 LC PUFA for humans (ISSFAL, 2004)
2858 and any improvement to the YTK muscle content of n-3 LC PUFA would make this target
2859 more achievable for consumers. Increasing dietary content of n-3 LC PUFA in the current study
2860 for 33 days resulted in an increase of 48 mg n-3 LC PUFA 100 g^{-1} white muscle (17% increase),
2861 which would be significant for consumers. Further research is warranted to investigate the time
2862 required for to reach full restoration of n-3 LC PUFA tissue levels.

5.5. Conclusions

This study has demonstrated that the n-3 LC PUFA concentration of the white muscle of YTK can be significantly increased in a 33-day finishing period. An additional 48 mg n-3 LC PUFA 100 g^{-1} white muscle was deposited when dietary n-3 LC PUFA was increased by 1.92 g 100 g^{-1} feed and so this would be made available to the human consumer. While significant gains were achieved with the 33-day finishing period, further research was recommended for commercial YTK producers to define appropriate finishing period under a range of conditions.

Given that decreasing dietary n-3 LC PUFA concentration had significant negative impacts for white muscle n-3 LC PUFA concentration and feed conversion it was also recommended that YTK producers set strict minimum specifications for dietary n-3 LC PUFA with feed manufacturers and closely monitor dietary n-3 LC PUFA content in their feed products to ensure maximum profitability in production.

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5.8. Tables and figures

Table 5.1: Total dietary lipid (%) and n-3 LC-PUFA concentration (mg 100 g^{-1} feed) for preconditioning and finishing diets.

	Preconditioning diets		Finishing diets	
Item (as fed)	**MOD**	**HIGH**	**HIGH**	**LOW**
Lipid content (%)	24.9	25.3	22.03	21.97
Analysed fatty acids (mg 100 g^{-1})				
t18:1n-9 (Palmitelaidic acid)	0.06	0.07	0.06	0.07
t18:1n-7 (Elaidic acid)	0.11	0.10	0.07	0.06
14:0 (Myristic acid)	0.41	0.67	0.66	0.37
15:0 (Pentadecanoic acid)	0.07	0.11	0.10	0.05
16:0 (Palmitic acid)	6.21	5.70	4.44	4.95
17:0 (Margaric acid)	0.12	0.12	0.10	0.09
18:0 (Stearic acid)	1.88	1.55	1.16	1.51
20:0 (Arachidic acid)	0.05	0.07	0.03	0.04
22:0 (Docosanoic acid)	0.02	0.03	0.05	0.03
24:0 (Tetracosanoic acid)	0.05	0.08	0.07	0.04
18:3n-3 (Alpha Linolenic acid- ALA)	0.61	0.46	0.35	0.51
20:5n-3 (Eicosapentanaeoic acid- EPA)	0.39	0.96	0.91	0.27
22:5n-3 (Docosapentaenoic acid- DPA)	0.11	0.31	0.28	0.06
22:6n-3 (Docosahexaenoic acid- DHA)	0.92	2.07	1.66	0.39
18:2n-6 (Linoleic acid- LOA)	3.55	2.56	1.74	2.79
18:3n-6 (Gamma Linolenic acid)	0.05	0.07	0.06	0.04
20:2n-6 (Eicosadienoic acid)	0.04	0.07	0.06	0.03
20:3n-6 (Dihomo-gamma-linoleic acid)	0.03	0.05	0.04	0.03
20:4n-6 (Arachidonic acid)	0.14	0.20	0.16	0.10
22:4n-6 (Docosatetraenoic acid)	0.02	0.04	0.04	0.02
16:1n-7 (Palmitoleic acid)	1.44	1.42	1.15	1.16
18:1n-7 (Octadecenoic acid)	0.64	0.77	0.74	0.59
18:1n-9 (Oleic acid)	7.71	6.41	6.73	8.55
20:1n-9 (Eicosenoic acid)	0.22	1.00	1.03	0.15
22:1n-9 (Docosenoic acid)	0.02	0.16	0.18	0.02
24:1n-9 (Tetracosenoic acid)	0.03	0.16	0.16	0.02
Total trans	0.16	0.17	0.13	0.13
Total saturated	8.85	8.41	6.60	7.08
Total Omega 3	2.02	3.79	3.20	1.22
Total Omega 6	3.83	2.99	2.09	3.01
Total Omega 7	2.07	2.19	1.89	1.75
Total Omega 9	7.95	7.56	8.09	8.74
Total n-3 LC PUFA	1.42	3.34	2.85	0.71
n-3 FA: n-6 FA	0.53	0.79	0.65	2.47

Table 5.2: Weight (kg), fork length (m) and condition factor of Yellowtail Kingfish (*Seriola lalandi*) following a change in dietary n-3 LC PUFA level (MOD/HIGH and HIGH/LOW) for 33 days (Mean ± standard error; subscripts denote significant difference across time; statistical test: One-way ANOVA; n = 3).

	Day 0	Day 19	Day 33	*P*=
Weight (kg)				
MOD/HIGH	2.07 ± 0.08 a	2.48 ± 0.07 b	2.68 ± 0.05 b	< 0.001
HIGH/LOW	2.21 ± 0.03 a	2.35 ± 0.08 a	2.69 ± 0.10 b	< 0.001
Length (m)				
MOD/HIGH	0.50 ± 0.00 a	0.53 ± 0.01 b	0.54 ± 0.00 b	< 0.001
HIGH/LOW	0.51 ± 0.00 a	0.52 ± 0.01 ab	0.54 ± 0.01 b	< 0.001
Condition factor				
MOD/HIGH	1.68 ± 0.02	1.69 ± 0.03	1.70 ± 0.03	0.927
HIGH/LOW	1.66 ± 0.02	1.64 ± 0.05	1.71 ± 0.04	0.437

Table 5.3: Initial weight (g), final weight (g), total weight gain (g), percentage body weight increase (%), specific growth rate and apparent feed conversion ratio of Yellowtail Kingfish (*Seriola lalandi*) following a change in diet (MOD/HIGH and HIGH/LOW n-3 LC PUFA) for 33 days (Mean ± standard error; statistical test: One-way ANOVA; n = 3).

	MOD/HIGH	HIGH/LOW	P =
Initial weight (kg)	2068.7 ± 79.8	2201.9 ± 27.3	0.255
Final weight (g)	2684.4 ± 52.1	2691.9 ± 99.3	0.952
Weight gain (g)	615.8 ± 27.7	490.0 ± 71.9	0.244
Percentage body weight increase (%)	29.9 ± 2.5	22.2 ± 3.0	0.187
Specific growth rate (% day^{-1})	0.79 ± 0.06	0.61 ± 0.07	0.194
Apparent feed conversion ratio	1.71 ± 0.02	1.85 ± 0.00	0.032

Table 5.4: Total lipid content (%) and fatty acid composition (mg 100 g^{-1}) of white muscle from Yellowtail Kingfish (*Seriola lalandi*) which were subjected to the MOD/HIGH dietary change treatment for 33 days (Mean ± standard error, n = 3).

	Day 0	Day 19	Day 33	*P* =
Lipid content (%)	2.0 ± 0.3	2.4 ± 0.2	2.0 ± 0.2	0.370
Analysed fatty acids (mg 100 g^{-1})				
t18:1n-9 (Palmitelaidic acid)	3.3 ± 0.1 ab	3.0 ± 0.2 a	3.6 ± 0.0 b	0.005
t18:1n-7 (Elaidic acid)	0.0 ± 0.0 a	3.3 ± 0.2 b	0.7 ± 0.3 a	< 0.001
14:0 (Myristic acid)	33.1 ± 1.4 a	37.5 ± 1.0 b	31.5 ± 1.1 a	0.005
15:0 (Pentadecanoic acid)	4.9 ± 0.1 a	5.5 ± 0.1 b	5.0 ± 0.1 a	0.001
16:0 (Palmitic acid)	335.9 ± 2.3 a	353.1 ± 2.5 b	346.5 ± 2.2 b	< 0.001
17:0 (Margaric acid)	5.0 ± 0.5 a	5.5 ± 0.1 a	6.8 ± 0.0 b	< 0.001
18:0 (Stearic acid)	112.3 ± 3.3 ab	105.4 ± 2.0 a	114.8 ± 2.5 b	0.049
20:0 (Arachidic acid)	3.7 ± 0.1 a	3.6 ± 0.1 a	2.3 ± 0.0 b	< 0.001
22:0 (Docosanoic acid)	1.0 ± 0.1 a	0.0 ± 0.0 b	1.2 ± 0.1 a	< 0.001
24:0 (Tetracosanoic acid)	7.5 ± 0.4 a	0.0 ± 0.0 b	0.7 ± 0.0 b	< 0.001
18:3n-3 (Alpha Linolenic acid- ALA)	35.6 ± 1.2 a	36.0 ± 0.7 a	29.2 ± 0.9 b	< 0.001
20:5n-3 (Eicosapentanaeoic acid- EPA)	50.7 ± 0.8 a	54.6 ± 0.9 b	55.2 ± 1.2 b	0.009
22:5n-3 (Docosapentaenoic acid- DPA)	26.9 ± 0.9 a	26.8 ± 0.5 a	30.2 ± 0.9 b	0.006
22:6n-3 (Docosahexaenoic acid- DHA)	202.4 ± 12.9 a	201.8 ± 5.8 a	242.8 ± 12.2 b	0.017
18:2n-6 (Linoleic acid- LOA)	241.6 ± 3.8 a	233.5 ± 2.2 a	204.9 ± 3.7 b	< 0.001
18:3n-6 (Gamma Linolenic acid)	3.1 ± 0.1 a	3.8 ± 0.2 b	2.9 ± 0.0 a	< 0.001
20:2n-6 (Eicosadienoic acid)	4.1 ± 0.1 a	4.4 ± 0.1 a	4.8 ± 0.1 b	< 0.001
20:3n-6 (Dihomo-gamma-linoleic acid)	3.1 ± 0.0	3.0 ± 0.0	3.1 ± 0.0	0.383
20:4n-6 (Arachidonic acid)	21.8 ± 1.2	20.8 ± 0.4	22.7 ± 1.0	0.361
22:4n-6 (Docosatetraenoic acid)	3.1 ± 0.1 a	3.1 ± 0.1 a	3.5 ± 0.1 b	0.006
16:1n-7 (Palmitoleic acid)	102.0 ± 3.5 a	106.8 ± 2.1 a	88.4 ± 2.5 b	< 0.001
18:1n-7 (Octadecenoic acid)	54.5 ± 0.7 a	58.3 ± 0.5 b	60.0 ± 0.5 b	< 0.001
18:1n-9 (Oleic acid)	643.4 ± 12.5	617.5 ± 6.3	620.8 ± 11.4	0.195
20:1n-9 (Eicosenoic acid)	17.5 ± 3.3 a	35.2 ± 0.9 b	41.4 ± 1.7 b	< 0.001
22:1n-9 (Docosenoic acid)	7.2 ± 0.4	6.9 ± 1.3	5.1 ± 0.3	0.162
24:1n-9 (Tetracosenoic acid)	2.6 ± 0.2 a	2.6 ± 0.3 a	4.6 ± 0.2 b	< 0.001
Total Trans	3.3 ± 0.1 ab	6.3 ± 0.3 c	4.3 ± 0.3 b	< 0.001
Total Saturates	506.3 ± 4.2	511.4 ± 3.7	509.3 ± 3.5	0.644
Total Omega 3	315.6 ± 12.8 a	319.2 ± 5.7 ab	357.4 ± 12.5 b	0.020
Total Omega 6	276.9 ± 3.4 a	268.7 ± 1.9 a	241.8 ± 3.1 b	< 0.001
Total Omega 7	156.5 ± 4.0 ab	165.2 ± 2.3 a	148.4 ± 3.0 b	0.004
Total Omega 9	670.6 ± 11.0	662.1 ± 5.3	671.9 ± 11.2	0.747
Total n-3 LC PUFA	280.0 ± 13.9 a	283.2 ± 6.3 a	328.2 ± 13.3 b	0.012
n-3 FA: n-6 FA	890.2 ± 40.7 a	844.5 ± 21.0 a	684.9 ± 28.2 b	< 0.001
n-3 FA: n-9 FA	2165.0 ± 124.0	2082.2 ± 56.8	1909.2 ± 93.5	0.168

Table 5.5: Total lipid content (%) and fatty acid composition (mg 100 g^{-1}) of white muscle from Yellowtail Kingfish (*Seriola lalandi*) which were subjected to the HIGH/LOW dietary change treatment for 33 days (Mean ± standard error; n = 3).

	Day 0	Day 19	Day 33	*P* =
Lipid content (%)	1.9 ± 0.3	1.7 ± 0.2	1.6 ± 0.3	0.746
Analysed fatty acids (mg 100 g^{-1})				
t18:1n-9 (Palmitelaidic acid)	3.4 ± 0.1 a	3.0 ± 0.1 b	3.4 ± 0.1 a	0.002
t18:1n-7 (Elaidic acid)	0.0 ± 0.0 a	3.1 ± 0.2 b	0.2 ± 0.2 a	< 0.001
14:0 (Myristic acid)	35.9 ± 1.7 a	35.5 ± 1.2 a	28.4 ± 1.3 b	0.001
15:0 (Pentadecanoic acid)	5.7 ± 0.2 a	5.6 ± 0.2 a	4.6 ± 0.2 b	< 0.001
16:0 (Palmitic acid)	349.9 ± 5.0 a	365.0 ± 2.4 b	354.6 ± 3.4 ab	0.028
17:0 (Margaric acid)	4.4 ± 0.9 a	5.8 ± 0.1 ab	6.6 ± 0.1 b	0.011
18:0 (Stearic acid)	112.3 ± 3.8	111.3 ± 3.0	122.2 ± 3.9	0.076
20:0 (Arachidic acid)	3.5 ± 0.1 b	3.8 ± 0.1 a	2.3 ± 0.1 c	< 0.001
22:0 (Docosanoic acid)	1.1 ± 0.1 a	0.0 ± 0.0 b	1.2 ± 0.0 a	< 0.001
24:0 (Tetracosanoic acid)	8.5 ± 0.3 a	0.0 ± 0.0 c	0.7 ± 0.0 b	< 0.001
18:3n-3 (Alpha Linolenic acid- ALA)	30.1 ± 1.3	31.2 ± 1.1	30.0 ± 1.1	0.744
20:5n-3 (Eicosapentanaeoic acid- EPA)	64.9 ± 1.5 a	53.7 ± 1.1 b	46.9 ± 1.4 c	< 0.001
22:5n-3 (Docosapentaenoic acid- DPA)	33.9 ± 0.8 a	30.4 ± 0.7 b	28.4 ± 0.8 b	< 0.001
22:6n-3 (Docosahexaenoic acid- DHA)	259.9 ± 12.2	230.4 ± 9.7	232.3 ± 14.4	0.201
18:2n-6 (Linoleic acid- LOA)	203.8 ± 4.0 a	214.5 ± 2.4 ab	219.2 ± 2.6 b	0.005
18:3n-6 (Gamma Linolenic acid)	3.2 ± 0.1	3.6 ± 0.2	3.2 ± 0.1	0.105
20:2n-6 (Eicosadienoic acid)	4.3 ± 0.0 a	4.6 ± 0.1 b	4.9 ± 0.1 c	< 0.001
20:3n-6 (Dihomo-gamma-linoleic acid)	3.1 ± 0.0	3.0 ± 0.1	3.1 ± 0.1	0.642
20:4n-6 (Arachidonic acid)	24.5 ± 0.9	22.0 ± 0.9	22.6 ± 1.5	0.321
22:4n-6 (Docosatetraenoic acid)	3.5 ± 0.1	3.3 ± 0.1	3.4 ± 0.1	0.500
16:1n-7 (Palmitoleic acid)	96.2 ± 4.1	97.8 ± 3.1	86.9 ± 3.6	0.089
18:1n-7 (Octadecenoic acid)	57.4 ± 0.8	58.3 ± 0.6	57.5 ± 0.6	0.558
18:1n-9 (Oleic acid)	582.9 ± 11.6 a	595.9 ± 7.4 ab	629.9 ± 13.5 b	0.019
20:1n-9 (Eicosenoic acid)	16.4 ± 5.3 a	39.9 ± 0.8 b	32.3 ± 0.9 b	< 0.001
22:1n-9 (Docosenoic acid)	10.8 ± 0.3 a	7.2 ± 1.8 ab	4.0 ± 0.1 b	< 0.001
24:1n-9 (Tetracosenoic acid)	3.3 ± 0.4	3.4 ± 0.5	4.0 ± 0.1	0.353
Total Trans	3.4 ± 0.1 a	6.1 ± 0.3 b	3.6 ± 0.2 c	< 0.001
Total Saturates	525.9 ± 6.8	527.7 ± 3.9	521.0 ± 5.7	0.678
Total Omega 3	388.8 ± 11.6 a	345.7 ± 8.9 b	337.6 ± 14.3 b	0.013
Total Omega 6	242.5 ± 3.4 a	251.0 ± 1.6 b	256.3 ± 1.2 b	0.001
Total Omega 7	153.6 ± 4.8	156.1 ± 3.6	144.5 ± 4.2	0.138
Total Omega 9	613.5 ± 10.4 a	646.4 ± 8.0 ab	670.2 ± 14.4 b	0.007
Total n-3 LC PUFA	358.7 ± 12.8 a	314.5 ± 9.9 ab	307.6 ± 15.4 b	0.023
n-3 FA: n-6 FA	629.6 ± 25.3 a	730.6 ± 21.6 ab	772.7 ± 36.0 b	0.006
n-3 FA: n-9 FA	1594.6 ± 71.1 a	1883.8 ± 67.7 ab	2033.8 ± 129.3 b	0.012

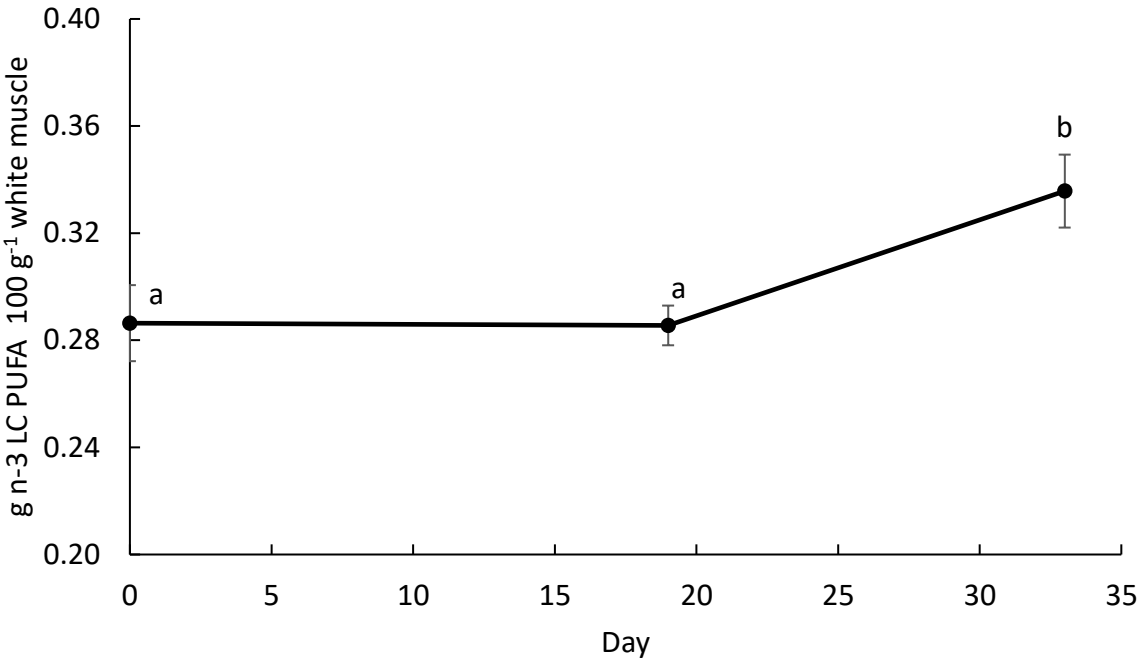

Figure 5.1: Quantity (g 100 g^{-1} of tissue) of long-chain omega 3 polyunsaturated fatty acids (n-3 LC PUFA; EPA + DPA + DHA) in the white muscle of Yellowtail Kingfish (*Seriola lalandi*) which were subjected to the MOD/HIGH dietary change treatment for 33 days (Mean ± standard error, subscripts denote significant difference; One-way ANOVA; $P = 0.016$; n = 3)

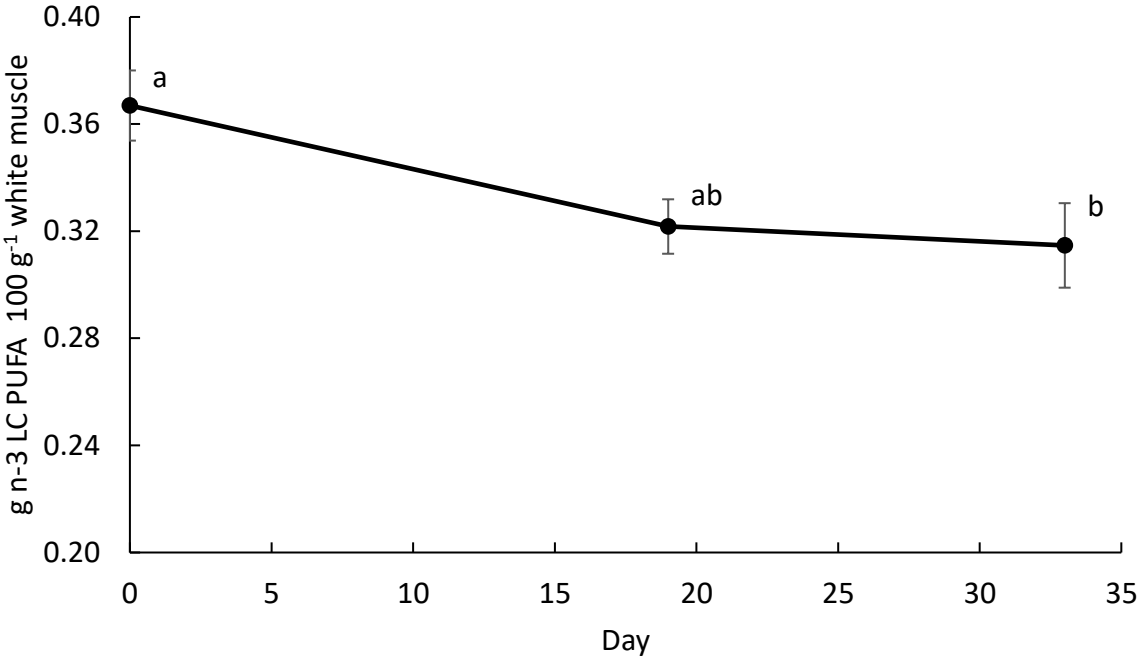

Figure 5.2: Quantity (g 100 g^{-1} of tissue) of long-chain omega 3 polyunsaturated fatty acids (n-3 LC PUFA; EPA + DPA + DHA) in the white muscle of Yellowtail Kingfish (*Seriola lalandi*) which were subjected to the HIGH/LOW dietary change treatment for 33 days (Mean ± standard error, subscripts denote significant difference; One-way ANOVA; $P = 0.021$; n = 3)

5.9. Statement to link Chapters 2 – 5 to Chapter 6

Throughout this body of work (Chapters 2 – 5) the effects of dietary fatty acid composition, particularly the effects of dietary n-3 LC PUFA and n-6 fatty acids, on product quality, metabolism, growth and feed conversion efficiency have been addressed. In Chapter 6, the aim was to expand further on the role of these fatty acids within the biological systems of YTK. Specifically, the aim was to study the effects of the bioactive components of these fatty acids and their downstream products once they are circulating in the blood of YTK. These include free fatty acids and oxylipins and in Chapter 6 a method to quantify these free fatty acids was validated and used to quantify them among different dietary treatments in the hours following a feeding episode.

Chapter 6 – Statement of authorship

Title of Paper	Measuring free fatty acids and oxylipins in blood plasma of large Yellowtail Kingfish (*Seriola lalandi*) fed different levels of n-3 LC PUFA
Publication Status	Manuscript prepared
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Principal Author

Name of Principal Author (Candidate)	Samantha N Chown		
Contribution to the Paper	Conceptualization, methodology, formal analysis, investigation, data curation, writing original draft, writing – review and editing and visualisation.		
Overall percentage (%)	94%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	24/06/2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Chapter 6: Measuring free fatty acids and oxylipins in blood plasma of large Yellowtail Kingfish (*Seriola lalandi*) fed different levels of n-3 LC PUFA

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Abstract

Yellowtail Kingfish (*Seriola lalandi*) (YTK) require dietary omega 3 (n-3) long chain polyunsaturated fatty acids (LC PUFA) for healthy development and growth. However, the fatty acid profile of the aquafeeds for many commercially cultured fish has changed extensively over time as dietary fish oil (FO) is replaced with alternative oils. A variety of methods have been utilised to assess the effects of replacing dietary FO in aquafeeds for a number of species, mostly consisting of routine growth and feed conversion efficiency parameters. Despite intensive research, the exact mechanisms by which fatty acids influence the growth and development of fish (and many other organisms) are largely unknown. The current study aimed to validate recently developed liquid chromatography and tandem mass spectroscopy (LC-MSMS) methods for the detection and quantification of free fatty acids and oxylipins in human blood samples for use with YTK blood plasma. The study then further aimed to use the technique to measure the effects of an acute feeding response following consumption of deficient, target or excessive quantities of dietary n-3 LC PUFA on the composition of free fatty acids (FFA) and oxylipins in the blood plasma of YTK. The results indicated that the LC-MSMS methods could be adapted for use with YTK blood plasma and that n-3 and omega 6 free fatty acids (FFA) and their oxylipin derivatives were able to be detected and quantified. Thereafter it was shown that differences in FFA and oxylipin profiles were detected in fish on 3 types of diet, with increased dietary concentrations of n-3 LC PUFA being reflected in significantly increased concentrations of n-3 FFA in YTK blood plasma. Similarly, increased dietary concentrations of n-6 linoleic acid (18:2n-6) (LOA) translated into significantly increased concentrations of free LOA in YTK blood plasma. Interestingly, plasma levels of n-3 FFA peaked at 3 hours post feed whilst n-6 LOA did not peak until 9 hours post feed. Omega 6 derived oxylipin abundance in YTK blood plasma also followed the same trend of the parent dietary fatty acids concentration, however, levels of the n-3 derived oxylipin 4HDHA were not

affected by variable dietary levels of n-3 LC PUFA. The methods used to measure FFA and oxylipins in the current study had only previously been utilised for human biological samples and this study demonstrates for the first time that these methods can be applied to acquire similar information from YTK blood plasma samples. Further expansion of this technique to include measuring omega 9 and saturated FFA and their downstream oxylipins would be highly beneficial and future research should aim to address these knowledge gaps.

Keywords

Yellowtail Kingfish; omega 3 (n-3) long chain (LC) polyunsaturated fatty acids (PUFA); free fatty acids (FFA); oxylipins; aquaculture.

Highlights

1. A new high throughput, highly sensitive and robust method for measuring FFA and oxylipins in human blood utilising LC-MSMS was adapted and validated for use with YTK blood plasma.
2. Changes were observed in the concentration of omega 3 and omega 6 FFA present in YTK blood plasma concurrent with alterations in dietary n-3 LC PUFA concentrations.
3. Omega 3 and omega 6 FFA were observed to peak in blood plasma at different times after a feeding episode, free DHA and EPA peaked at 3 hours post feed while free LOA did not peak until 9 hours post feed.
4. Omega 6 derived oxylipin abundance reflected the relative abundances of the parent FFA in the blood plasma.
5. Abundance of the DHA-derived oxylipin 4HDHA was not reflective of the relative abundances on free DHA in YTK blood plasma, suggesting that at this inclusion level, variation in the dietary quantity of this key fatty acid does not influence the abundance of its downstream oxylipin.

6.1. Introduction

Yellowtail Kingfish (*Seriola lalandi*) (YTK) have a nutritional requirement for omega 3 (n-3) long chain polyunsaturated fatty acids (LC PUFA), specifically they achieve optimal growth and feed conversion efficiency when supplied with 2.1 g of n-3 LC PUFA 100 g^{-1} aquafeed (Stone et al., 2019). These n-3 LC PUFA, including eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA), are necessary for healthy cellular metabolism and maintaining cell membrane structure and integrity in fish (Sargent et al., 1999a, Miller et al., 2008) and also play a vital role in human nutrition (McCann and Ames, 2005, Eilander et al., 2007). In commercial aquaculture, dietary n-3 LC PUFA are primarily supplied by dietary inclusions of fish oil (FO) (NRC, 2011). The fatty acid composition of commercial aquafeeds has varied substantially over time as dietary fish oil (FO) has been incrementally replaced with alternative oil, such as terrestrial plant oils (e.g. canola oil) or animal by-product oils (e.g. poultry oil). As FO is incrementally replaced with alternative oils, the quantity of omega 6 (n-6), omega 9 (n-9) and saturated fatty acids in aquafeeds have increased (Turchini et al., 2009), which is likely to have an extensive effect on the biological functioning of YTK and other farmed fish.

Despite intensive research, the exact mechanism by which fatty acids work to influence the growth and development of fish and many other organisms is largely unknown, although various hypotheses have been put forward (Caldwell, 2009, Bruins et al., 2013, Hewawasam et al., 2017, Hewawasam et al., 2018). One mechanism that has received traction in animal and human studies is that dietary fats can be stored/incorporated in tissues, largely as phospholipids and triglycerides, and as required for basic physiological needs or in response to trauma can be liberated as free fatty acids (FFA). These in turn can be oxidised to form highly reactive compounds such as prostaglandins, leukotrienes, resolvins, protectins and maresins known collectively as oxylipins (Samuelsson et al., 1987, Buckley et al., 2014).

Oxylipins have a range of known activities spanning from pro- to anti-inflammatory actions (Jira et al., 1997, Jira et al., 1998, Duffield et al., 2006, Aoki et al., 2008), however, very little is known about the role that oxylipins play in the normal physiology of fish. Recently a method has been developed that is capable of preserving the integrity of fatty acids in dried blood spots (DBS) which provides an ideal collection device for monitoring the fatty acid status of fish in the field and in feed trials (Liu et al., 2014). More recently, Hewawasam et al. (2017) and Hewawasam et al. (2018) have reported that the DBS system could be utilised to measure both the FFA and the oxylipins present in human blood. Given these recent developments and the lack of understanding of the presence, abundance and role of FFA and oxylipins in fish, this method presents an interesting opportunity to better understand the mechanisms by which dietary fatty acids might affect growth and development in fish.

Therefore, the aim of the current study was to validate the methods described by Hewawasam et al. (2017) and Hewawasam et al. (2018) for use with YTK blood plasma and then to measure the effects of feeding deficient, target or excessive dietary quantities of n-3 LC PUFA on the composition of free fatty acids and oxylipins in the blood plasma of YTK.

6.2. Methods and Materials

6.2.1. Feeding trial and sample collection

6.2.1.1. Experimental location and animals

Animal ethics approvals for this work was granted by the University of Adelaide (Approval number: S-2016-127) animal ethics committee. The experiment was conducted at the South Australian Research and Development Institute (SARDI) aquatic sciences pool farm, West Beach, South Australia. Yellowtail Kingfish were supplied by Clean Seas Seafood Ltd. in Port Lincoln, South Australia. Prior to being subjected to experimental protocol fish were housed in 9 × 5000 L recirculating seawater tanks and fed a 9 mm commercial diet (Pelagica formulation) made by Skretting Australia (Cambridge, TAS, Australia) to apparent satiation once per day for a 4-week acclimation period. Fish were exposed to a natural photoperiod and ambient seawater temperatures throughout.

6.2.1.2. Experimental diets

The diet kernels, FO and PO used in the experimental diets were supplied by Skretting Australia. The diet formulations were based on Skretting Australia's YTK diet (20% fish meal; 40% crude protein, 30% crude lipid and a gross energy level of approximately 21 MJ kg^{-1}) (Stone et al., 2019). The diet kernel contained a base level of 10% crude lipid and it was then top coated with an additional 17% lipid (graded blends of FO and PO to give varying levels of n-3 LC PUFA and n-9 fatty acids; total crude lipid level 27%) at Aquafeeds Australia (Mount Barker, SA). Three experimental diets were formulated with n-3 LC PUFA contents of 0.8 (DIET0.8), 2.1 (DIET2.1) and 3.0 (DIET3.0) g 100 g^{-1} of feed (Table 6.1).

6.2.1.3. Animal housing and care

At the start of the feed trial, YTK were anaesthetised in 5000 L tanks (total water volume 2500 L) using AQUI-S® (AQUI-S® New Zealand Ltd., Lower Hutt, New Zealand) at a concentration of 14 mg L^{-1} of seawater. Fish were randomly distributed into 9 × 5000 L recirculating aquaculture tanks (13 fish per tank) and randomly assigned one of the 3 experimental diets (3 replicate tanks $diet^{-1}$). Fish were fed their experimental diet for 12 weeks, with feeding once daily to apparent satiation and intake was recorded as grams consumed per tank per day. Water quality parameters were measured daily and maintained within the accepted optimal levels for YTK (Bowyer et al., 2014).

6.2.1.4. Sample collection

At the conclusion at the experiment, fish were fed their experimental diets once more to satiation after which blood samples were collected at 0, 3 and 9 hours post feed (HPF). In order to collect blood samples fish were crowded and netted out of their tank one at a time (3 fish per tank per time point). Thereafter fish were secured by being wrapped in a damp towel, and then a blood sample was drawn from the caudal vein using an 18-gauge 1.5-inch needle and a 10 mL syringe. Importantly, all fish were sampled within 3 minutes from the initial introduction of the sampling stress. Each blood sample was immediately deposited into a 6 mL EDTA vacutainer and refrigerated until the sample could be separated. The vacutainers were centrifuged at 4 °C at 3000 rpm for 10 minutes and the plasma portion was separated, with 2 × 30 μL samples of plasma deposited on to the PUFAcoat™ paper designed as part of the DBS system (Liu et al., 2014) and the remainder of the plasma frozen at -80 °C.

6.2.2. Laboratory methods

The methods used for analysis of free fatty acids and oxylipins are outlined in Hewawasam et al. (2018) and are briefly described below.

6.2.2.1. Standards and reagents

LC–MS grade methanol and acetonitrile were from Merck (VIC, Australia). America Chemical Society grade formic acid and analytical standard grade 3,5–di–tert–4–butylhydroxytoluene (BHT) were sourced from Sigma–Aldrich (NSW, Australia). Analytical standards (≥98% purity) for oxylipins, 4–hydroxydocosahexaenoic acid (4–HDHA), 13–hydroxyoctadecadienoic acid (13–HODE), 5–hydroxyicosatetraenoic acid (5–HETE), 8–hydroxyeicosatetraenoic acid (8–HETE), 9–hydroxyeicosatetraenoic acid (9–HETE), 11–hydroxyeicosatetraenoic acid (11–HETE), 12–hydroxyeicosatetraenoic acid (12–HETE), 15–8,9–epoxyeicosatrienoic acid (8(9)–EET), leukotriene B4 (LTB4) and free fatty acids eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), linoleic acid (LOA) and arachidonic acid (AA) were purchased from Cayman Chemical Company (Michigan, USA). Deuterated internal standards (≥99% purity) d5–EPA, d5–DHA, d4–LA and d8–AA, d4–13–HODE, d4–LTB4 and d8–12–HETE, were purchased from Cayman Chemical Company (Michigan, USA).

6.2.2.2. Sample extraction from PUFAcoat™ paper

Lipid extraction was preformed following a method described by Hewawasam et al. (2017). Firstly, a 3mm disc was removed from each dried PUFAcoat plasma sample and placed in a 96–well plate. Extraction solvent (150 µL of 80% methanol) containing deuterated internal standard mix (stock was prepared in methanol at 0.01 ng μL^{-1} of d4–13–HODE, d4–LTB4 and d8–12–HETE) was added to each well, and the plate was covered, then gently shaken on a plate shaker for 30 min at room temperature. The extract from each well was transferred to a second 96 well plate, sealed and analysed by UPLC–MS/MS.

6.2.2.3. Instrument parameters

Instrument parameters for the mass spectrometer were set as outlined in Hewawasam et al. (2017) and Hewawasam et al. (2018). Briefly, all analysis was conducted with an Agilent 1290 Infinity LC system (Agilent Technologies, VIC, Australia) fitted with a binary pump and thermostated autosampler held at 4 °C, connected to a 5500 triple quadrupole mass spectrometer (AB Sciex, VIC, Australia), using electrospray ionisation in negative mode.

6.2.2.4. Standard curve preparation

For data interpretation standard curves were prepared following the methods outlined in Hewawasam et al. (2018), with the exception that human blood samples were substituted with YTK blood plasma samples.

6.2.3. Statistics

Statistical analysis was performed using IBM SPSS (version 24). Homogeneity of variance was assessed using Levene's test, whilst normality was assessed with Kolmogorov-Smirnov test. Where data met prior requirements, differences were analysed using two-way ANOVAs, where HPF and diet were factors. Where significant differences were detected, post-hoc comparisons were made via pairwise comparisons. An alpha level of 0.05 was used for all statistical tests. Results are presented as means ± standard error (SE).

6.3. Results

6.3.1. General observations

Experimental diets were readily accepted and palatable for YTK with no rejection of feed observed. Blood samples were able to be collected within 3 minutes of sampling stress being induced and fish which had not been sampled appeared to return to routine swimming behaviour within 5 minutes of the end of each sampling episode.

6.3.2. Standard curves

Standard curves in YTK blood plasma were developed for free fatty acids including; EPA, DPA, DHA, AA and LOA ($R^2 > 0.85$ for all, Figure 6.1), and for oxylipins; 9-HODE, 13-HODE, 5-HETE, 8-HETE, 11-HETE, 12-HETE, 4-HDHA, 8(9)-EET and LTB4 ($R^2 > 0.98$ for all, Figure 6.2).

6.3.3. Free fatty acids

For all free fatty acids measured there was no significant interaction between diet and HPF (two-factor ANOVA; $P > 0.050$ for all; Figure 6.3), however significant differences were observed among diets and HPF for each free fatty acid.

The concentration of EPA was observed to be significantly different across diets and HPF (two-factor ANOVA; $P = 0.001$ and $P < 0.001$ respectively; Figure 6.3A). The mean concentration of EPA in YTK blood plasma was significantly lower in fish fed DIET0.8 compared to DIET2.1 or DIET3.0 (two-factor ANOVA – pairwise comparisons; $P = 0.019$ and $P < 0.001$, respectively). The mean concentration of EPA in YTK blood plasma was significantly higher at 3 and 9 HPF compared to 0 HPF (two-factor ANOVA – pairwise comparisons; $P < 0.001$ and $P < 0.001$ respectively; Figure 6.3A).

Docosahexaenoic acid concentrations were observed to be significantly different across diets and HPF (two-factor ANOVA; $P < 0.001$ for both; Figure 6.3B). The mean concentration

of DHA in YTK blood plasma was significantly lower in fish fed DIET0.8 compared to DIET2.1 or DIET3.0 and for DIET2.1 compared to DIET3.0 (two-factor ANOVA – pairwise comparisons; $P = 0.002$ $P < 0.001$ and $P = 0.039$ respectively). The mean concentration of DHA in YTK blood plasma was significantly higher at 3 and 9 HPF compared to 0 HPF (two-factor ANOVA – pairwise comparisons; $P < 0.001$ and $P < 0.001$ respectively).

Arachidonic acid concentrations were observed to be significantly different only across HPF (two-factor ANOVA; $P < 0.001$; Figure 6.3C). The mean concentration of AA in YTK blood plasma was significantly higher at 3 and 9 HPF compared to 0 HPF (two-factor ANOVA – pairwise comparisons; $P < 0.001$ for both).

The concentration of LOA was observed to be significantly different across diets and HPF (two-factor ANOVA; $P = 0.039$ and $P = 0.049$ respectively; Figure 6.3D). The mean concentration of LOA in YTK blood plasma was significantly lower in fish fed DIET3.0 compared to DIET0.8 (two-factor ANOVA – pairwise comparisons; $P = 0.012$). The mean concentration of LOA in YTK blood plasma was significantly higher at 9 HPF compared to 0 HPF and 3 HPF (two-factor ANOVA – pairwise comparisons; $P = 0.026$ and $P = 0.041$ respectively).

6.3.4. Oxylipins

For all oxylipins measured there was no significant interaction between diet and HPF (two-factor ANOVA; $P > 0.050$ for all; Figures 6.4 and 6.5), however significant differences were observed among diets and HPF for each free fatty acid.

6.3.4.1. DHA derived oxylipins

The concentration of 4HDHA in YTK blood plasma was observed to vary significantly across HPF but was not affected by diet (two-factor ANOVA; $P < 0.001$ and $P = 0.966$ respectively; Figure 6.4C). At 3 and 9 HPF mean 4HDHA concentration was significantly

higher in YTK blood plasma than at 0 HPF (two-factor ANOVA – pairwise comparisons; $P <$ 0.001 and $P = 0.002$ respectively).

6.3.4.2. LOA derived oxylipins

The concentration of 9HODE in YTK blood plasma was observed to vary significantly across diet and with HPF (two-factor ANOVA; $P = 0.005$ and $P < 0.001$ respectively; Figure 6.4A). The mean 9HODE concentration was significantly higher in YTK blood plasma at 3 and 9 HPF compared to 0 HPF, and at 3 HPF compared to 9 HPF (two-factor ANOVA – pairwise comparisons; $P < 0.001$, $P < 0.001$ and $P = 0.014$ respectively). The mean concentration of 9HODE was significantly higher in the blood plasma of fish fed DIET0.8 compared to DIET2.1 and DIET3.0 (two-factor ANOVA – pairwise comparisons; $P = 0.010$ and $P = 0.002$ respectively).

The concentration of 13HODE in YTK blood plasma was observed to vary significantly across diet and with HPF (two-factor ANOVA; $P = 0.034$ and $P < 0.001$ respectively; Figure 6.4B). The mean 13HODE concentration was significantly higher in YTK blood plasma at 3 and 9 HPF compared to 0 HPF, and at 3 HPF compared to 9 HPF (two-factor ANOVA – pairwise comparisons; $P < 0.001$, $P < 0.001$ and $P = 0.008$ respectively). The mean concentration of 13HODE was significantly higher in the blood plasma of fish fed DIET0.8 compared to DIET2.1 and DIET3.0 (two-factor ANOVA – pairwise comparisons; $P = 0.043$ and $P = 0.015$ respectively).

6.3.4.3. AA derived oxylipins

The concentration of 5HETE in YTK blood plasma was observed to vary significantly across diet and with HPF (two-factor ANOVA; $P = 0.016$ and $P < 0.001$ respectively; Figure 6.5A). At 3 and 9 HPF mean 5HETE concentration was significantly higher in YTK blood plasma than at 0 HPF (two-factor ANOVA – pairwise comparisons; $P < 0.001$ for both). The

mean concentration of 5HETE was significantly higher in the blood plasma of fish fed DIET0.8 compared to DIET3.0 (two-factor ANOVA – pairwise comparisons; $P = 0.004$).

The concentration of 8HETE in YTK blood plasma was observed to vary significantly across diet and with HPF (two-factor ANOVA; $P = 0.005$ and $P < 0.001$ respectively; Figure 6.5B). The mean 8HETE concentration was significantly higher in YTK blood plasma at 3 and 9 HPF compared to 0 HPF, and at 3 HPF compared to 9 HPF (two-factor ANOVA – pairwise comparisons; $P < 0.001$, $P < 0.001$ and $P = 0.002$ respectively). The mean concentration of 8HETE was significantly higher in the blood plasma of fish fed DIET0.8 compared to DIET2.1 and DIET3.0 (two-factor ANOVA – pairwise comparisons; $P = 0.010$ and $P = 0.003$ respectively).

The concentration of 11HETE in YTK blood plasma was observed to vary significantly only across HPF and not with diet (two-factor ANOVA; $P < 0.001$ and $P = 0.111$ respectively; Figure 6.5C). The mean 11HETE concentration was significantly higher in YTK blood plasma at 3 and 9 HPF compared to 0 HPF, and at 3 HPF compared to 9 HPF (two-factor ANOVA – pairwise comparisons; $P < 0.001$, $P = 0.001$ and $P = 0.032$ respectively).

The concentration of 12HETE in YTK blood plasma was observed to vary significantly only across HPF and not with diet (two-factor ANOVA; $P < 0.001$ and $P = 0.075$ respectively; Figure 6.5D). The mean 12HETE concentration was significantly higher in YTK blood plasma at 3 and 9 HPF compared to 0 HPF, and at 3 HPF compared to 9 HPF (two-factor ANOVA – pairwise comparisons; $P < 0.001$, $P = 0.002$ and $P = 0.043$ respectively).

The concentration of 8(9)EET in YTK blood plasma was observed to vary significantly across diet and with HPF (two-factor ANOVA; $P = 0.024$ and $P < 0.001$ respectively; Figure 6.5E). The mean 8(9)EET concentration was significantly higher in YTK blood plasma at 3 and 9 HPF compared to 0 HPF (two-factor ANOVA – pairwise comparisons; $P < 0.001$ for both). The mean concentration of 8(9)EET was significantly higher in the blood plasma of fish

fed DIET0.8 compared to DIET2.1 and DIET3.0 (two-factor ANOVA – pairwise comparisons; $P = 0.035$ and $P = 0.010$ respectively).

The concentration of LTB4 in YTK blood plasma was observed to vary significantly only across HPF and not with diet (two-factor ANOVA; $P < 0.001$ and $P = 0.056$ respectively; Figure 6.5F). The mean LTB4 concentration was significantly higher in YTK blood plasma at 3 and 9 HPF compared to 0 HPF (two-factor ANOVA – pairwise comparisons; $P < 0.001$ for both).

6.4. Discussion

The methods used to measure free fatty acids and oxylipins in the current study have only previously been utilised for human biological samples using dried blood spot technology (Hewawasam et al., 2017, Hewawasam et al., 2018). The results presented here demonstrate for the first time that these methods can be applied to acquire similar information from YTK blood plasma samples, with detectable differences in free fatty acid and oxylipin concentrations across dietary treatments and in the hours following a feeding episode.

The oxidation of dietary fatty acids mainly by the enzymatic or chemical formation of fatty acid hydroperoxides drives many processes within the body. Importantly, oxylipins, which are biologically important lipid mediators, are derived enzymatically from free fatty acids and have a substantial role in regulating inflammatory processes (Samuelsson et al., 1987, Buckley et al., 2014). In humans, n-3 LC PUFA and their downstream oxylipins have anti-inflammatory actions (Duffield et al., 2006, Aoki et al., 2008), while n-6 PUFA and their downstream oxylipins generally have pro-inflammatory actions (Jira et al., 1997, Jira et al., 1998, Grapov et al., 2012, Zivkovic et al., 2012). Given that the quantity, ratio and types of n-3 LC PUFA and n-6 PUFA are readily manipulated in commercial aquaculture diets and differ substantially from those in 'natural' fish diets, the investigation of the production of oxylipins in aquacultured fish could provide a novel opportunity for better understanding and improving fish nutrition.

In the current study, when free fatty acids and oxylipins were measured in fish plasma after 24 hours fasting, significant differences were detectable in EPA and DHA between dietary treatments. These n-3 LC PUFA were strategically altered in the diets of the treatment groups, demonstrating that changes to dietary fatty acids do indeed affect the circulating free fatty acid pool in YTK. As dietary n-3 LC PUFA decreased dietary LOA increased, but while plasma free LOA was not significantly different between diet treatments after 24 hours fasting (0HPF),

free LOA was significantly higher in DIET0.8 overall, specifically free LOA was higher in DIET0.8 at 9 HPF, and free LOA derived oxylipins were also observed to peak in DIET0.8. These findings support the hypothesis that increased dietary n-6 PUFA will drive increased n-6 free fatty acids and their derived oxylipins, which could be a mechanism by which growth and feed efficacy in YTK fed excess potentially proinflammatory dietary n-6 PUFA is mediated. Arachidonic acid was only present in the diets in very small quantities and as such diet had no effect on the level of free AA in YTK plasma. However, there was a significant effect on the AA derived oxylipins such that the highest level of AA derived oxylipins was seen in the DIET0.8 group, which also had the lowest dietary n-3 LC PUFA concentration. The only n-3 LC PUFA derived oxylipin measured in the current study was 4HDHA and it was not observed to be significantly different between treatment groups, however for all treatment groups there was an increase in 4HDHA at 3 HPF. While low concentrations of dietary n-3 LC PUFA are known to reduce growth and feed conversion efficiency in YTK (Stone et al., 2019), it appears that even at these low dietary n-3 LC PUFA concentrations, YTK are still capable of producing anti-inflammatory oxylipins.

The interpretation of the actions of n-3 LC PUFA and n-6 PUFA free fatty acids and derived oxylipins are based on their known bioactive roles in humans and land animals but their mode of action in fish is still to be confirmed. However, given the known effects of reduced growth and feed efficacy in a range of commercially farmed fish due to low n-3 LC PUFA/ high n-6 PUFA diets, it is likely that their role is similar. The current study was exploratory in nature, and it is the first report of free fatty acids and oxylipins in the plasma of YTK, although oxylipins have been reported in flesh of other fish species (Flaskerud et al., 2017). The actual role of oxylipins in fish physiology remains to be determined, but the finding that we could not detect differences in n-3 LC PUFA derived oxylipins in YTK plasma despite the highly variable levels of n-3 LC PUFA in the diets, is intriguing. Clearly further work

remains to be done in this important arena, to understand the role and function of free fatty acids and oxylipins in YTK and to better inform nutritional studies.

6.5. Conclusions

This study was the first to utilise LC-MSMS technology to assess the free fatty acid and oxylipin concentrations in YTK blood plasma, in the hours post feed and to detect differences driven by changes to dietary fatty acid intake. Free fatty acids, including EPA, DHA and AA, in YTK blood plasma was observed to peak within 3 hours of a feeding episode, while LOA did not peak until 9 HPF. Differences in the concentration of plasma free fatty acids followed differences in dietary composition, which is similar to the pattern for utilisation of total dietary fatty acids in YTK. Omega 6 derived oxylipin abundance in YTK blood plasma also followed the same trend of the parent dietary fatty acids composition, however, n-3 derived 4HDHA was not affected by highly variable dietary levels of n-3 LC PUFA. The role of oxylipins in the physiology of fish remains unknown but there is ample opportunity for it to provide productive avenues for aquaculture nutrition research.

6.6. Acknowledgements

This project was supported by funding from the Australian Government Department of Agriculture and Water Resources as part of its Rural R&D for Profit programme, the Fisheries and Research and Development Corporation (FRDC) and other project participants (DAWR Grant Agreement RnD4Profit-14-01-027). The authors would also like to acknowledge the support of the South Australian Research and Development Institute (SARDI) for the provisions of the SARDI SAASC experimental facilities at West Beach, South Australia. We would also like to thank Dr Richard Smullen, Dr Michael Salini and Dr Simon Tabrett of Ridley and Dr Trent D'Antignana of Nutrisea Pty Ltd for their input into experimental design, Dr Nicole Ruff and Dr Matthew Bransden of Skretting Australia for their input into experimental design, experimental diet formulation and manufacture. Thanks to Dr Matt Landos (Future Fisheries Veterinary Service Pty Ltd.) for veterinary services. We also thank Paul Skordas, Leigh Kuerschner, Krishna-Lee Currie, Jessica Buss, Nicole Thompson, Filipa Isabel and Aaron Teoh for their technical assistance during the experiment, and Kristina Hickson, and Ela Zielinski from Waite Lipid Analysis Services (WLAS). Finally, we thank Dr Ge Liu and Dr Erandi Hewawasam from The University of Adelaide for their technical support and expertise in use of DBS and LC-MSMS for measuring FFA and oxylipins.

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6.8. Tables and figures

Table 6.1: Total dietary lipid content (%) and fatty acid profile (mg 100 g^{-1} feed) of three experimental diets used in the free fatty acid and oxylipin validation study.

Item (as fed)	DIET0.8%	DIET2.1%	DIET3.0%
Lipid content (%)	28.44	26.71	26.73
Analysed fatty acids (mg 100 g^{-1})			
t18:1n-9 (Palmitelaidic acid)	83	73	72
t18:1n-7 (Elaidic acid)	140	119	108
14:0 (Myristic acid)	420	730	900
15:0 (Pentadecanoic acid)	53	77	89
16:0 (Palmitic acid)	5930	5760	5550
17:0 (Margaric acid)	89	100	110
18:0 (Stearic acid)	1870	1770	1670
20:0 (Arachidic acid)	36	51	53
22:0 (Docosanoic acid)	25	30	31
24:0 (Tetracosanoic acid)	13	16	18
18:3n-3 (Alpha Linolenic acid- ALA)	550	490	430
20:5n-3 (Eicosapentanaeoic acid- EPA)	270	930	1350
22:5n-3 (Docosapentaenoic acid- DPA)	63	130	160
22:6n-3 (Docosahexaenoic acid- DHA)	420	1080	1440
18:2n-6 (Linoleic acid- LOA)	3150	2650	2300
18:3n-6 (Gamma Linolenic acid)	31	41	43
20:2n-6 (Eicosadienoic acid)	28	32	36
20:3n-6 (Dihomo-gamma-linoleic acid)	24	35	33
20:4n-6 (Arachidonic acid)	110	50	170
22:4n-6 (Docosatetraenoic acid)	16	20	23
16:1n-7 (Palmitoleic acid)	1450	1560	1610
18:1n-7 (Octadecenoic acid)	640	660	670
18:1n-9 (Oleic acid)	11050	9290	8020
20:1n-9 (Eicosenoic acid)	130	150	160
22:1n-9 (Docosenoic acid)	11	20	26
24:1n-9 (Tetracosenoic acid)	20	41	48
Total trans	223	192	180
Total saturated	8436	8534	8421
Total Omega 3	1303	2630	3380
Total Omega 6	3359	2829	2605
Total Omega 7	2090	2220	2280
Total Omega 9	11211	9501	8254
Total n-3 LC PUFA	753	2140	2950
n-3 FA: n-6 FA	2.58	1.08	0.77
n-3 FA: n-9 FA	8.60	3.61	2.44

Table 6.2: Concentration of fatty acids in Yellowtail Kingfish diets (mg 100 g^{-1}), total fatty acids in white muscle tissue (mg 100 g^{-1}), free fatty acids in blood plasma (ng μL^{-1}) and oxylipins in blood plasma (ng μL^{-1}), that were fed three experimental diets for 84 days, samples collected after 24 hours of fasting (Data is presented as mean ± SE; difference subscripts denote significant differences between treatment groups, $P > 0.05$, n = 3).

	Diet (mg 100 g^{-1})			**Tissue - White muscle (mg 100 g^{-1})**				**FFA and oxylipins in plasma (ng μL^{-1})**			
	DIET0.8	DIET2.1	DIET3.0	DIET0.8	DIET2.1	DIET3.0	*P* =	DIET0.8	DIET2.1	DIET3.0	*P* =
EPA	270	930	1350	164.8 ± 8.4 a	227.4 ± 18.1 b	288.0 ± 17.3 c	< 0.001	4.34 ± 0.27 a	5.95 ± 0.34 b	6.45 ± 0.48 b	0.002
DHA	420	1080	1440	366.1 ± 21.6 a	421.7 ± 24.6 b	486.2 ± 15.5 c	< 0.001	10.45 ± 0.66 a	16.63 ± 1.56 b	18.56 ± 1.25 b	< 0.001
DHA derivative											
- 4HDHA								0.147 ± 0.019	0.164 ± 0.026	0.163 ± 0.014	0.798
AA	110	50	170	49.6 ± 1.9 a	51.0 ± 1.7 a	55.8 ± 0.9 b	< 0.009	1.35 ± 0.08	1.66 ± 0.10	1.68 ± 0.13	0.103
AA derivatives											
- 5HETE								0.342 ± 0.046	0.267 ± 0.041	0.25 ± 0.022	0.212
- 8HETE								0.017 ± 0.003	0.013 ± 0.003	0.012 ± 0.001	0.373
- 11HETE								0.026 ± 0.006	0.022 ± 0.006	0.020 ± 0.003	0.703
- 12HETE								0.016 ± 0.003	0.013 ± 0.003	0.012 ± 0.002	0.677
- 8(9)EET								0.008 ± 0.001	0.006 ± 0.001	0.006 ± 0.001	0.340
- LTB4								0.024 ± 0.009	0.013 ± 0.005	0.015 ± 0.004	0.414
LOA	3150	2650	2300	753.4 ± 10.9 a	681.2 ± 14.2 b	643.1 ± 16.4 c	< 0.001	9.85 ± 0.53	9.82 ± 1.44	8.17 ± 0.82	0.399
LOA derivatives											
- 9HODE								0.139 ± 0.030	0.081 ± 0.021	0.076 ± 0.019	0.139
- 13HODE								0.144 ± 0.033	0.088 ± 0.024	0.082 ± 0.022	0.217

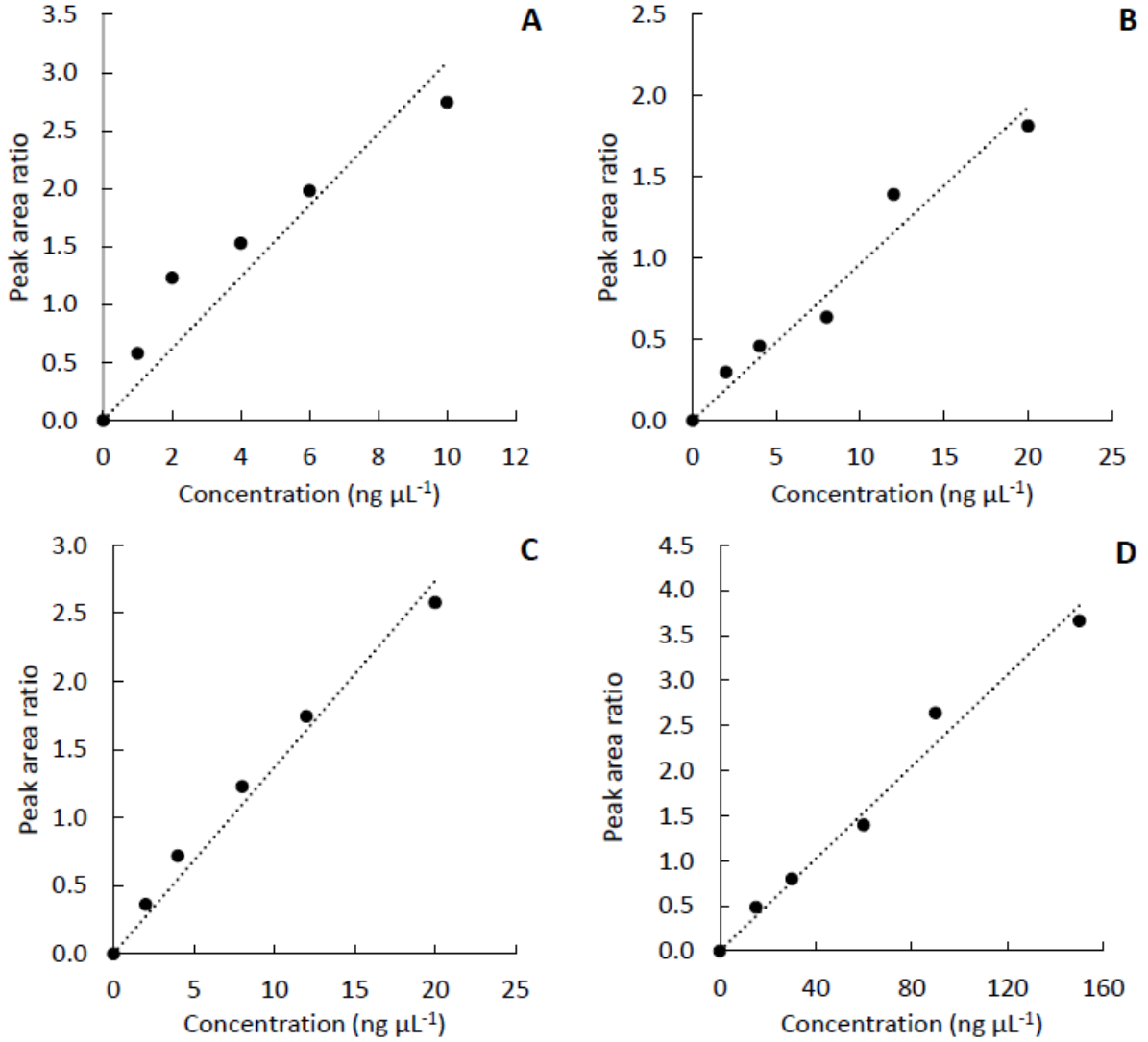

Figure 6.1: Standard curves, concentration of free fatty acid (ng μL^{-1}) verses peak area ratio, for; A) EPA (R^2= 0.8606 and y= 0.3089x), B) DHA (R^2= 0.9572 and y= 0.0964x), C) AA (R^2= 0.9800 and y= 0.1368x) and D) LOA (R^2= 0.9819 and y= 0.0255x) in Yellowtail Kingfish blood plasma.

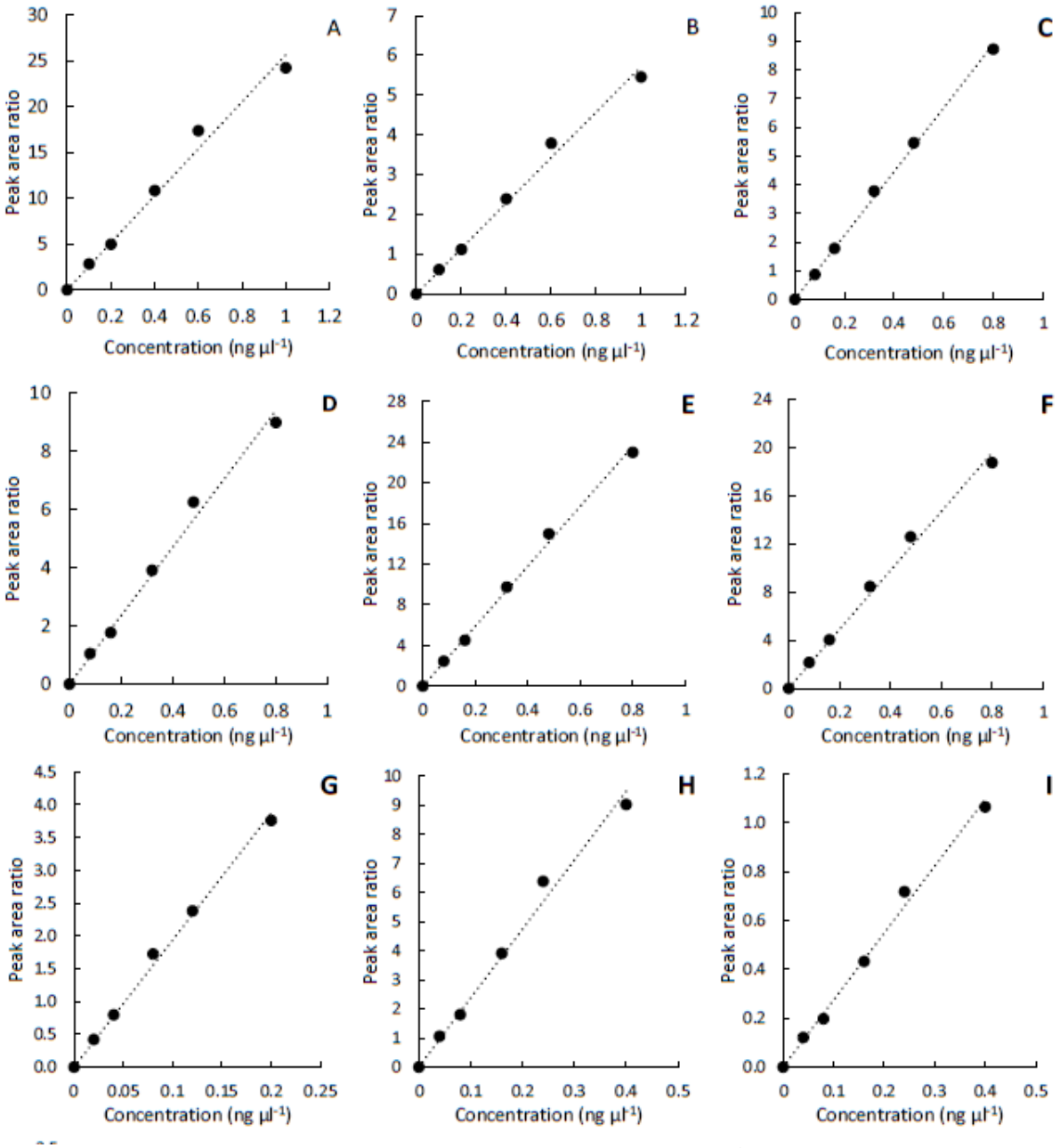

Figure 6.2: Standard curves, concentration of oxylipins (ng μL^{-1}) verses peak area ratio, for; A) 9-HODE (R^2= 0.9852 and y= 25.642x), B) 13-HODE (R^2= 0.9903 and y= 5.7149x), C) 5-HETE (R^2= 0.9983 and y= 11.108x), D) 8-HETE (R^2= 0.9898 and y= 11.758x), E) 11-HETE (R^2= 0.9968 and y= 29.484x), F) 12-HETE (R^2= 0.9927 and y= 24.432x), G) 4-HDHA (R^2= 0.9950 and y= 19.372x), H) 8(9)-EET (R^2= 0.9879 and y= 23.708x) and I) LTB4 (R^2= 0.9935 and y= 2.7411x) in Yellowtail Kingfish blood plasma.

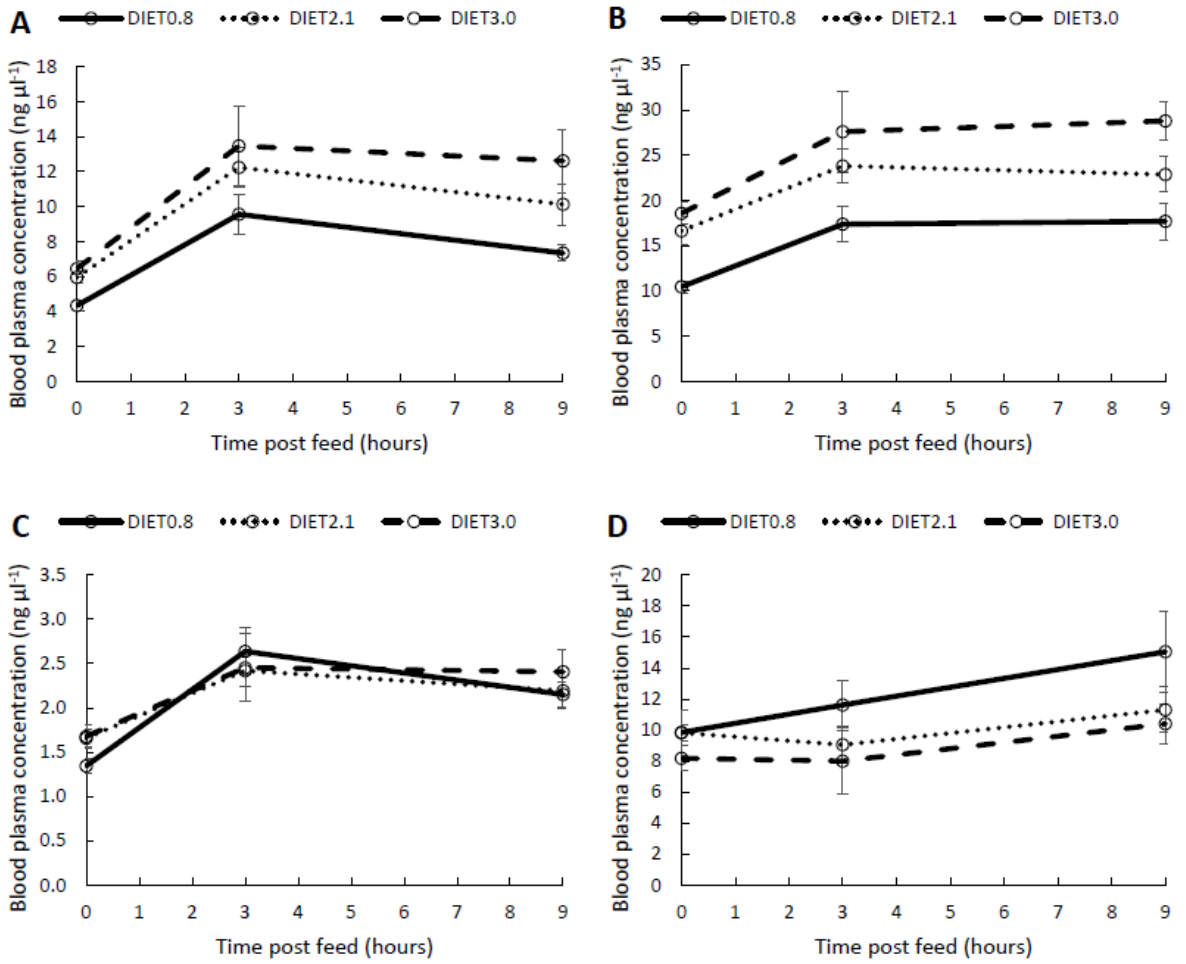

Figure 6.3: Concentration of free fatty acids (ng μL^{-1}) in blood plasma of Yellowtail Kingfish (*Seriola lalandi*) fed three diets that contained different levels of fish oil resulting in n-3 LC PUFA concentrations of 0.8 (DIET0.8), 2.1 (DIET2.1) and 3.0 (DIET3.0) g $100g^{-1}$ feed at time 0, 3 hr and 9 hr post feeding; A) EPA, B) DHA, C) AA and D) LOA. (Data is presented as mean ± SE; n = 3)

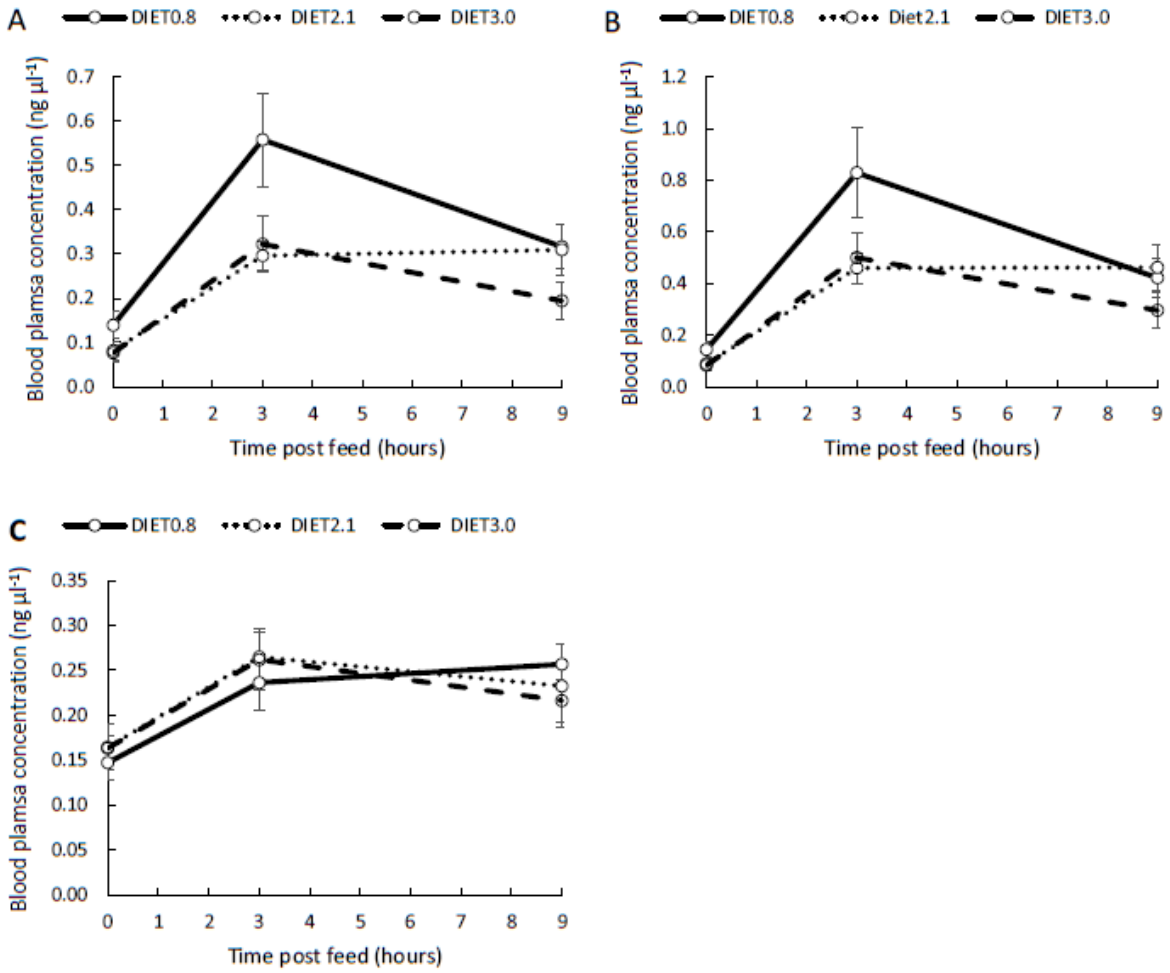

Figure 6.4: Concentration of linoleic acid (LOA) and docosahexaenoic acid (DHA) derived oxylipins (ng μL^{-1}) in blood plasma of Yellowtail Kingfish (*Seriola lalandi*) fed three diets that contained different levels of fish oil resulting in n-3 LC PUFA concentrations of 0.8 (DIET0.8), 2.1 (DIET2.1) and 3.0 (DIET3.0) g $100g^{-1}$ feed at time 0, 3 hr and 9 hr post feeding; A) 9-HODE, B) 13-HODE and C) 4-HDHA (Data are presented as mean ± SE; n = 3).

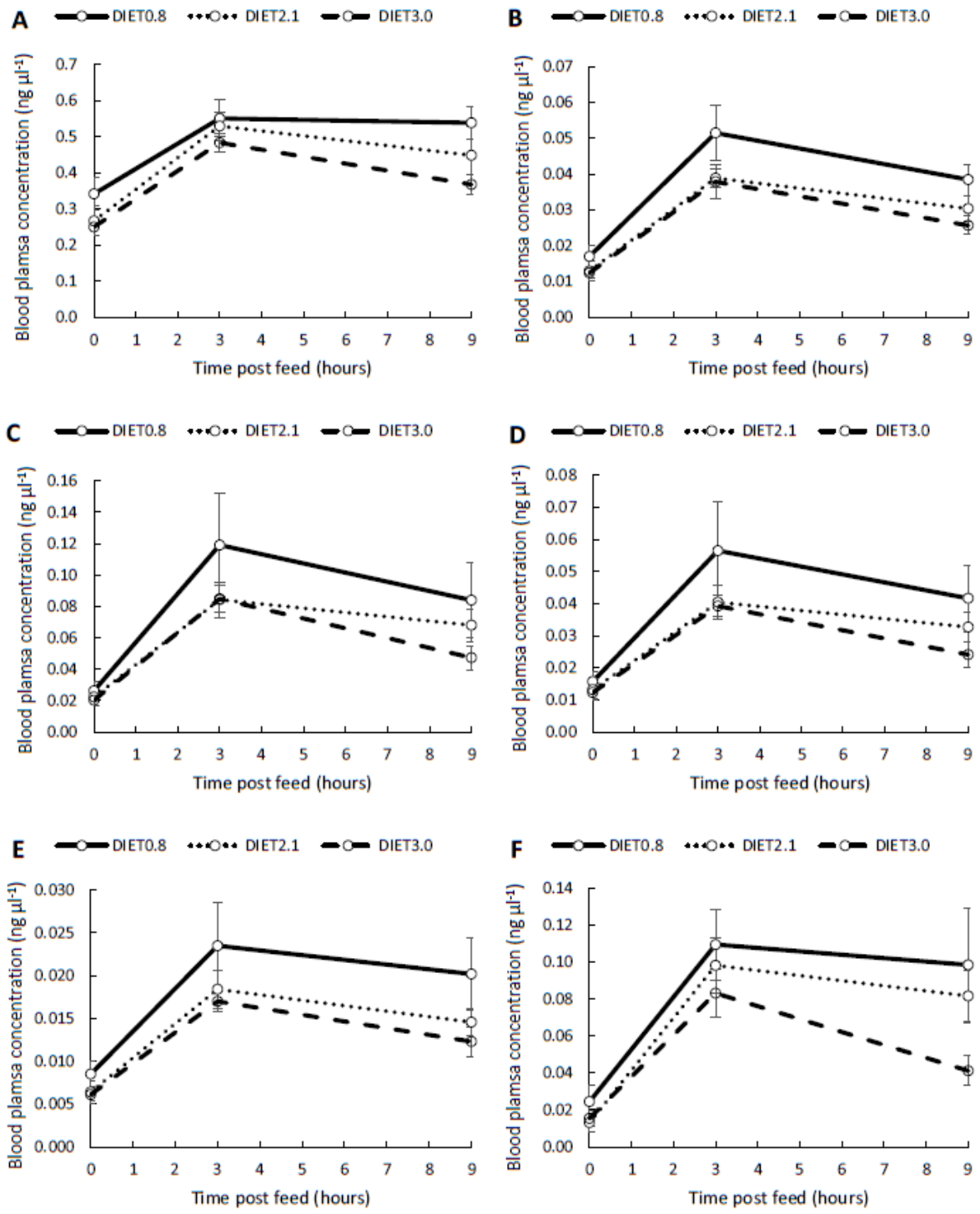

Figure 6.5: Concentration of arachidonic acid (AA) derived oxylipins (ng μL^{-1}) in blood plasma of Yellowtail Kingfish (*Seriola lalandi*) fed three diets that contained different levels of fish oil resulting in n-3 LC PUFA concentrations of 0.8 (DIET0.8), 2.1 (DIET2.1) and 3.0 (DIET3.0) g $100g^{-1}$ feed at time 0, 3 hr and 9 hr post feeding; A) 5-HETE, B) 8-HETE, C) 11-HETE, D) 12-HETE E) 8(9)-EET and F) LTB4 (Data are presented as mean ± SE; n = 3).

Chapter 7 – General discussion

7.1. Purpose

During the course of this research program the primary focus was to increase our understanding of Yellowtail Kingfish (*Seriola lalandi*) (YTK) utilisation of dietary lipids, with a view to manipulating aquafeeds to improve the health, growth, feed conversion efficiency and product quality of aquacultured YTK. Within the experimental chapters, results and conclusions were considered in the context of benefits to the human consumer and also commercial YTK farmers. In this general discussion the major findings and contribution to the field of aquaculture nutrition, the limitations of the research and proposed future research will be discussed.

7.2. Major findings and contribution to the field of aquaculture nutrition

In order to create a benchmark for the lipid and fatty acid tissue profile of commercially cultured YTK, wild YTK from South Australia were captured and their tissue lipid and fatty acid profile investigated. While this portion of the research was limited by a relatively small number of wild fishes, it did provide initial data that was able to be used throughout the remainder of this research. Key differences were identified between wild and aquacultured YTK. Wild YTK were found to have significantly less total lipid in each of the tissue regions measured. Wild YTK were found to have significantly less total omega 6, omega 7, omega 9, saturated and trans fatty acids which are commonly associated with terrestrial protein sources, when compared to aquacultured YTK, no doubt reflecting the diet of farmed fish. Conversely, no significant difference in n-3 LC PUFA content in the white muscle was observed between the wild and aquacultured YTK, and it was concluded that in regard to these vital fatty acids, either source of YTK would be equally beneficial to human consumers. It is relevant that similar conclusions were made in wild and aquacultured YTK from southern Africa (O'Neill et al., 2015). Overall, these findings are positive for both YTK consumers and commercial

producers: consumers can have increased confidence in the nutritional benefits of aquacultured YTK and commercial producers can use these values to benchmark their products.

The next three chapters focused on increasing our understanding of the factors that affect fatty acid digestion, assimilation and deposition in aquacultured YTK, with a specific emphasis on n-3 LC PUFA, due to their necessity for YTK and human nutrition. Reducing dietary n-3 LC PUFA in aquafeeds is a high global priority (Naylor et al., 2009), however in order to achieve this we need to understand the effects that reduced dietary n-3 LC PUFA content has on YTK growth, health and product quality. The study described in Chapter 3 identified a key relationship which affects n-3 LC PUFA deposition, with DHA being spared at the expense of oleic acid in the white muscle (but not the red muscle) and recommended that the level of oleic acid needs to be carefully considered in YTK diet formulations. This is important because any natural mechanism that could assist in better utilisation of dietary n-3 LC PUFA would increase the economic and environmental sustainability of YTK production. In Chapter 4 results indicated that YTK absorb n-3 LC PUFA and oleic acid with equal efficiency even when dietary concentrations are changed. Furthermore, YTK do not absorb all saturated fatty acids with equal efficiency, specifically shorter chain saturated fatty acids (< 16:0) were absorbed more efficiently than long chain saturated fatty acids (> 17:0). In the context that saturated fatty acids are readily utilised for energy by YTK, this was an important finding and could be used to inform commercial YTK diet formulations. By ensuring that the majority of saturated fatty acids in YTK feeds is present in these more absorbable forms there is potential to improve feed efficacy and decrease production costs. In the Chapter 5 study the rate of n-3 LC PUFA assimilation by YTK was investigated, with an aim to better understand the effects of dietary changes and make recommendations concerning the required duration of finishing periods where YTK are fed diets rich in n-3 LC PUFA. Finding that an additional 17% n-3 LC PUFA could be deposited into the white muscle in less than 5 weeks of feeding

of a diet rich in n-3 LC PUFA was again a positive for consumers and commercial producers. Consumers could obtain an increased quantity of n-3 LC PUFA and commercial producers could achieve increased product quality (in relation to muscle n-3 LC PUFA concentration) with minimal expense. While each key finding from Chapters 3 – 5 only represents a small opportunity to improve commercial YTK production, when implemented in combination their impact could be meaningful. Furthermore, each finding assists researchers and producers in understanding the complex nature of nutrient utilisation in YTK.

In Chapter 6 a method was presented which had previously not been utilised for aquaculture nutrition investigations, indeed the method has only recently been optimised as a tool for human nutrition studies. In this chapter a high throughput, high precision method for quantifying free fatty acids and oxylipins in YTK blood plasma was validated. Importantly, differences between dietary treatments and across time following a feeding event were detectable. As these free fatty acids and oxylipins are the bioactive products of digested dietary lipids, and have pro- and anti-inflammatory actions within the body, they will likely be a useful tool to assess the impacts of dietary changes on the biological functioning of fish in future nutrition studies.

7.3. Limitations

7.3.1. Wild YTK sampling

The sample of wild YTK collected during this research was limited to six individual fish, collected from one geographical location, during a single sampling event. It is well known that the lipid and fatty acid composition of YTK varies due to water temperature/ seasonality, sex, sexual maturity and across geographical locations. During winter, when water temperature decreases, the total lipid content of YTK reduces as they utilise their lipid stores for energy (Bowyer et al., 2012a). When YTK mature and prepare to spawn their gonadal mass is known to increase substantially in size, diminishing lipid reserves from other body compartments, and

the timing of this process and the extent of its effect on lipid and fatty acid composition differs between the sexes (Poortenaar et al., 2001). Geographical location is likely to affect lipid and fatty acid composition of YTK, due to the availability and type of prey species, as well as the temperature of the water (O'Neill et al., 2015).

With a sample size of only six fish, confidence in the data in Chapter 2 is limited, however the variance in the data was minimal. Furthermore, gonads were visually scored during sampling in order to limit the effect of this factor on the data set and no individual appeared to be preparing for spawning or increasing their gonadal mass relative to the rest of their body mass. Data were also separated by sex, in order to identify if this was influencing the results and no differences were identified between the sexes (data not included in manuscript). As such the effects of small sample size, sex and sexual maturity were able to be mitigated however the effects of water temperature/ seasonality and geographical location were unable to be overcome. Additional wild YTK samples were not obtained due to the costly nature of sampling. Numerous other sampling strategies were considered to obtain additional wild YTK samples, however, samples needed to be obtained from fish immediately after their removal from the ocean to ensure that sample collection was consistent, and samples were not degraded. This restricted samples to those collected first hand by researchers, eliminating the possibility to engage recreational fishers. Additionally, the target size range for aquacultured YTK (2 - 4 kg, 50 - 70cm) falls partially below the minimum size limit for recreational fishing of YTK in South Australia (>60cm), however ministerial approval was required to catch and keep a maximum of 10 wild YTK below the minimum recreational size limit. After a failed attempt to capture any wild YTK on the first sampling occasion, a recreational fishing charter boat was engaged to assist in obtaining wild YTK, while this strategy was effective it was costly and as such could not be repeated to increase sample size or eliminate the effects of water temperature/ seasonality and geographical location.

7.3.2. Diet formulations

The experimental diets used in Chapters 3 – 5 were limited to formulations which could be realistically utilised by commercial YTK producers. This research was conducted under funding provided by the Kingfish for Profit project (RnD4Profit-14-01-027), with 50% of the funding for the project provided by industry partners who were focused on manipulation of diets within the limitation of the commercial environment. This affected the current study primarily by restricting the upper and lower dietary n-3 LC PUFA limits. In Chapter 3, this meant that the effects of low dietary n-3 LC PUFA on DHA sparing in YTK white muscle could not be further explored. In Chapter 5, the rate at which n-3 LC PUFA was shown to accumulate and dilute in YTK flesh could likely be increased with diets with n-3 LC PUFA significantly higher or lower respectively in FO, but such diets could not be trialled. Restricting the upper and lower limits of dietary n-3 LC PUFA inclusion rates has provided important information for industry partners but constrained the scientific questions which might have been addressed by these experiments. Future research should endeavour to understand the time required for the full restoration of tissue n-3 LC PUFA during the washout period after feeding diets low in FO.

7.3.3. Feed trial duration

The trial schedule for the Kingfish for Profit project was designed to obtain the maximal information during its 3-year duration. Previously, feed trials for YTK have demonstrated that the effects of dietary changes can be observed within 12 weeks of feeding (Booth et al., 2011). As such, the feeding trials were scheduled for 12-week durations, with adequate time between trials for the tank system to be cleaned, and new fish to be transferred in and acclimated, with few opportunities for modification to the schedule. While this strategy was effective for the

majority of experiments, it did limit research presented in Chapter 3. While DHA sparing at the expense of oleic acid in the white muscle was quantifiable within the 12-week feed trial, it would have been beneficial to extend the feed trial to observe the capacity of YTK to continue sparing DHA in the white muscle when dietary n-3 LC PUFA was lower than 1.6 g n-3 LC PUFA 100 g^{-1} feed. It is likely that at some point the fish would reach a breakpoint where it was no longer able to spare DHA and tissue levels would begin to deplete, which could have negative flow on effects for fish health and growth. Future research should endeavour to understand the rate at which this will occur, and the factors that might be influential.

7.4. Proposed future research

7.4.1. Collection of additional wild YTK samples

During this study, time and resources limited the number of wild YTK caught but it has provided a starting point for future data to build on. It would be beneficial to have a thorough understanding of the lipid and fatty acid profiles of wild YTK throughout the lifecycle and during different environmental conditions. Currently our understanding of what is 'normal' for YTK is limited and we proposed that future research should aim to address this by collecting additional wild YTK samples.

7.4.2. DHA sparing in YTK white muscle

Further research is also required to investigate the capacity of YTK to spare DHA at the expense of oleic acid in the white muscle when dietary n-3 LC PUFA is supplied at less than 1.6 g 100 g^{-1} feed for extended periods of time. While this trade-off was observed during an 84-day feed trial it is plausible that a more prolonged deficiency of dietary n-3 LC PUFA could have more negative effects as YTK may not have the capacity to continue sparing DHA. It is also possible that under the conditions described above, the utilisation of the DHA and other n-3 LC PUFA in storage adipose may be observable.

7.4.3. Oxylipins and free fatty acid measurements as a tool for nutrition research

The number of oxylipins and free fatty acids which could be quantified during this research was limited due to the extensive work required to validate the method for use with YTK blood plasma. We targeted specific n-3 LC PUFA and omega 6 fatty acids which were likely to be important in YTK but being able to quantity the full range of free fatty acids and their respective oxylipins will likely be important for aquaculture nutrition studies. Given the abundance of saturated and omega 9 fatty acids in YTK feed it would be of great benefit to be able to measure oxylipins produced from these parent fatty acids.

7.5. Relevant knowledge gaps that could not be addressed within the scope of this research program

There are a number of important aspects that have a role in lipid metabolism in YTK that were not able to be able to be assessed within research program but could provide various means of improving lipid utilisation in aquacultured YTK. There is an ongoing need to assess new commercial dietary lipid/ n-3 LC PUFA sources as they become available. Whilst vast strides have been made towards upscaling algal oil production for use in aquafeeds and producing genetically modified terrestrial crops that are able to produce n-3 LC PUFA, there is substantial requirement for ongoing research to assess their suitability to various commercially cultured fish.

At the start of this project there was a plan to assess the effects of dietary emulsifiers on lipid metabolism in YTK, however, as time went on this was not possible. The addition of emulsifiers to aquafeeds may have the potential to improve lipid digestibility (Dickinson, 1993), thereby increasing the efficacy of feed conversion and reducing the cost of production. In this PhD (Chapter 4), it was seen that a proportion of dietary lipid was not absorbed from feeds. If this could be improved dietary lipid content could be reduced, creating meaningful savings for producers.

The effect of water temperature of dietary lipid metabolism was not investigated here but there was an aim to assess the suitability of various alternative lipid sources, selected based on their melting point, for use in winter YTK diets. Yellowtail Kingfish digestion, lipid metabolism and growth are negatively affected by the reduced winter water temperatures recorded in South Australia. One possible way to mitigate these negative effects is to increase the digestibility of dietary lipids. This could potentially be achieved by replacing poultry oil (which is solid at winter water temperatures) with alternative lipid sources that are liquid at winter water temperatures. Little research has focused on understanding how the physical properties of dietary lipids affect their biochemical interactions within the digestive system of cultured fish. This remains an area that could be addressed in future studies.

Lastly, there is still a substantial amount of research required to fully understand the role of enzymes and specific genes associated with lipid metabolism in YTK. For example, in Atlantic Salmon dietary fatty acid composition has been suggested to be a driving factor for the regulation of genes involved in lipid metabolism, specifically the ratio of dietary n-3 LC PUFA and n-6 fatty acids, with lack of n-3 LC PUFA increasing gene expression (Martinez-Rubio et al., 2013). This upregulation of gene expression is likely to be metabolically costly to the fish and as such an understanding of the full range of factors affecting such processes will be beneficial. Similarly, in small YTK dietary FO replacement with canola oil has been shown to downregulate lipase activity (Bowyer et al., 2012b). This reduced enzyme activity would also be a driving factor for reduced lipid metabolism, but research has not yet fully investigated whether large YTK have the capacity to regulate their digestive enzymes to sufficiently cope with the pressure of challenging dietary lipid compositions. Understanding the full range of factors influencing lipid utilisation in YTK will allow future diets to be formulated to positively interact with all YTK biological systems and fully exploit all aquafeed ingredients.

7.6. References

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