Understanding Yellowtail Kingfish Overall Summary

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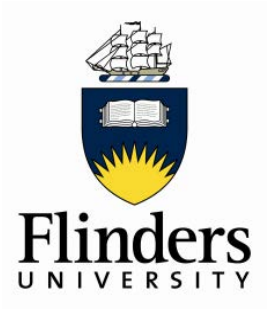
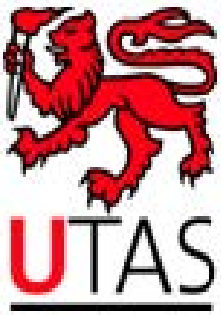
Flinders University

Project No. 2008/903

AUSTRALIAN SEAFOOD COOPERATIVE RESEARCH CENTRE





This project was conducted by Flinders University, University of Tasmania, South Australian Research and Development Institute, and New South Wales Department of Primary Industries in collaboration with Clean Seas Tuna Ltd.

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ABBREVIATIONS

CST	Clean Seas Tuna Ltd
DO	dissolved oxygen
GSI	gonadosomatic index
HOGG	head on, gilled and gutted
NSW DPI	New South Wales Department of Primary Industries
QIM	quality index method
SARDI	South Australian Research and Development Institute
TBARS	thiobarbituric acid reactive substances
UTas	University of Tasmania
YTK	Yellowtail Kingfish

NON-TECHNICAL SUMMARY

PROJECT 2008/903 Understanding Yellowtail Kingfish

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PROJECT OBJECTIVES:

1. To determine and quantify the interactive effects of fish sizes and water temperature on survival, weight gain, feed intake, feed conversion ratio and carcass composition of juvenile YTK.
2. To provide YTK producers and consumers detailed information on the nutritional and biochemical composition of YTK.
3. To identify how flesh composition, post-harvest processing methods and cold chain management techniques influence the shelf-life stability of the processed YTK product.
4. To characterise the occurrence of sexual maturation and identify the hormonal cues which initiate it.
5. To develop farm management strategies which improve flesh quality attributes and production and increase market access.

OUTCOMES ACHIEVED

This broad ranging study sought to increase our understanding about the performance of Yellowtail Kingfish (YTK) in sea cages and the quality attributes of YTK products for consumption.

The first of the planned outcomes was:

- *Managers will have new information on the effects of fish size and temperature on the performance of juvenile and sub-adult YTK which will allow them to determine the most appropriate time to stock YTK into sea cages. New stocking regimes will enhance survival of YTK and increase profitability.*

This outcome has been achieved. The mortality and growth rate issues associated with small fish going into cold water temperatures are now clear, and the SOP for transfer of fingerlings to sea cages has changed to reflect this. Specifically, this project has demonstrated:

- It is not advisable to rear juvenile YTK between 6 to 40g in bodyweight at water temperatures ≤12°C. Fish within this size range reared at 12°C became moribund, lost equilibrium, had difficulty feeding and died.
- Irrespective of water temperature, juvenile YTK subjected to chronic low dissolved oxygen concentrations (between 40 and 70% saturation) exhibited 14-20% less growth and 21-38% lower feed intake than normoxic (71 to 106% saturation) controls.
- The growth rate of juvenile YTK ≤ 1000g at water temperatures between 10°C and 29°C can be predicted from a growth model prepared as part of this study.

- Growth rate of juvenile YTK (from 50g to 100g) is optimal at water temperatures between 23-24°C.
- YTK grown in water temperatures below 17°C, or above 28°C, will achieve less than 50% of the growth rate of fish reared at 23-24°C.
- This model will assist farm managers in forecasting growth rates of YTK based on actual ambient water temperatures at a site.

Another outcome was:

- *Develop assessment techniques and identify sampling sites necessary for the development of an ongoing quality control monitoring program.*

This outcome has been achieved. Different areas of the fillet differ in crude lipid content and no one sampling site reflects that variability. A method for objectively measuring YTK flesh colour was developed. Freshness (K value) was a useful indicator of the quality of chilled product, but TBARS was not. Microbiological techniques needs to be applied for future product quality, spoilage and shelf life studies.

Another outcome was:

- *To maximise the quality and consistency of the YTK product by identifying periods or groups of fish which are undesirable to harvest and changing stocking and harvesting strategies or differentiating the product to suit particular markets.*

This outcome has been achieved. The slightly reduced product quality (fillet fat and % yield) attributes associated with hatchery run 2 and 3 fish, and those that are graded small, were demonstrated. At the time of writing (May 2012), the business has not been in a position to introduce strategies (e.g. dietary modifications or a longer grow out time/size) to address the issue. Specifically, this project demonstrated:

- Fish that have a bodyweight greater than 1.2kg have a 2-3 fold higher ($p<0.001$) level of crude fat in the ventral area (belly flap) than in the dorsal fillet area. Ventral crude fat reached a maximum of 35% of fillet weight whereas the dorsal fat level was more commonly 10-12% of fillet weight.
- CST usually produce 3 batches of fingerlings each year for stocking at sea, with each batch (or hatchery run) being 4-6 weeks after the previous one. This means that the fingerlings from hatchery run 1 have longer in the seacages during their first summer and grow faster in the warmer seawater. Fish that were from hatchery run 1 grow faster and have higher levels of fillet crude fat than fish from hatchery runs 2 or 3, and those from hatchery run 1 that were graded small. This may put hatchery run 1 fish at an advantage when water temperatures cool and they have to rely more on their body reserves.
- The nutritional attributes of YTK fillet are very similar to that of a familiar and widely accepted farmed species (Atlantic salmon) in the marketplace. The flesh is typically ~23% protein and 6-15.5% lipid (depending on the fillet area). Levels of omega-3 fatty acids are between 1.1-2.8% of the total fatty acids, with EPA and DHA levels (1,600 to 2,500mg) in a small (100g) serve of YTK fillet easily meeting the Australian National Health and Medical Research Council guidelines for recommended daily intake (RDI) for adult males (610mg) and females

(430mg).

- Vacuum packed chilled fillets produced in this project had a shelf life of 12-15 days post-processing when stored at 4^{o}C. Shelf-life of the chilled vacuum packed fillets was mainly determined by the detection of a strengthening 'fishy' odour when the packet was opened suggesting microbial activity. This coincided with a freshness (K value) score of 60-70%. Lipid oxidation and fillet colour did not change during vacuum packed shelf life.

Another outcome was:

- *The extent of maturation and its impact upon product quality will be determined – this will help identify the best approaches to future mitigation strategies. The cues controlling maturation will be determined, information which will be essential in developing an appropriate management strategy if maturation is of economic importance.*

This outcome has been achieved. Male YTK produced sperm in November – December at both age 1 and 2, but testes size were small and of little consequence. Female fish showed no reproductive development at these ages/sizes. There was no deleterious effect of reproductive maturity on product yield or quality attributes for these fish. There is no need to introduce any mitigation strategy for YTK up to this age/size. This project established that:

- 100% of male fish undergo spermatogenesis as in their first and second years of life, but the extent of sexual maturation (gonad growth as measured by gonadosomatic index - GSI, and milt volume) is limited (maximum mean GSI of 1.2% of bodyweight).
- Female fish do not show any signs of vitellogenesis in their first or second years of life.
- The incidence or extent of sexual maturation did not differ between fish that were derived from hatchery runs 1, 2 or 3 in each spawning year, and the sex ratio of the fish was 50:50.
- Sex of the fish did not affect growth rate or condition factor (i.e. the weight of the fish in relation to the body length).
- There were data indicating that 2 year old male fish (about 4kg in bodyweight) had a significantly ($p<0.05$) higher fillet yield than females (i.e. 64% vs 63% of body weight, respectively) at the when gonad size was largest (Nov-Dec).
- Together, these findings indicate that the production performance implications of sexual maturation on YTK to age 2 years and bodyweight of 4-5kg are minimal.
- As long as this product specification remains the focus for the industry no steps are needed to affect sex ratios, induce sterility or manipulate environmental factors that cue sexual development or maturation.

The final outcome was:

- *To improve market acceptance both domestically and overseas and to stimulate the growth of the industry, improving revenue for the YTK industry, seafood processors and others in the supply chain.*

This ambitious outcome has been partly achieved. Markets domestically and internationally are more aware of the attributes of Australian farmed YTK, and farm gate prices have increased as a consequence of this and the readjustment of production volumes to match market demand. However, difficult global financial circumstances have had much more bearing on the size and growth potential of the Australian YTK sector. This project demonstrated that:

- The results from data loggers used in domestic and international supply chains showed that temperature remained within the specified range of -1 to +4^{o}C at all times.
- Fibre board boxes proved to be suitable alternatives to polystyrene boxes in a 36-48hr domestic supply chains, but did not provide equivalence of temperature control in a simulated export shipment when two periods of warm external temperature were applied. The best proof of this was the proportion of the gel packs that remained frozen at the end of the simulation. The polystyrene boxes gel packs were 90% frozen, the best of the fibre board boxes was less than 30% frozen, and two of the others were completely thawed.

This information is being used by CST managers to improve the efficiency, quality and reduce variability of YTK supplied to domestic and overseas markets.

LIST OF OUTPUTS PRODUCED

No outputs have been produced to date (May 2012).

NON TECHNICAL SUMMARY

This broad ranging project took a subproject approach to examine several issues that ultimately relate to product yield and quality attributes of farmed Yellowtail Kingfish (YTK) grown in sea cages. Within this project, the performance (survival and growth) of fish stocked into sea cages was assessed in regards to (a) fish size and water temperature at the time of transfer to the sea, and (b) environmental conditions (i.e. water temperature and dissolved oxygen levels) during grow out. A model was developed for predicting growth rate of YTK from 50g to 1kg as a function of water temperature. Fillet yield as a percentage of whole carcass weight, biochemical composition of fillets and nutritional content of fillets were determined as fish grew from 1 to 4.2kg. These parameters were considered with regards to spawning year (i.e. whether the fish were produced in 2007 or 2008), hatchery run (i.e. if the fish came from the first, second or third batch of eggs in a particular year), and degree of sexual maturation in male and female fish. Fish size and time of year of harvest was correlated with the shelf life of chilled vacuum packed fillets as determined using drip loss, freshness (K value), lipid oxidation (TBARS), quality index method, changes in fillet colour measured by a digital imaging technique developed during this project, and microbiology. Temperature data loggers were placed inside YTK, inside and outside polystyrene transport boxes during one trip to Sydney by road, and six air cargo export shipments to the EU and USA. The effect of five types of alternative fibre board boxes on fillet quality and microbial load was compared to polystyrene boxes during a simulated export shipment.

The findings from each of these subprojects have been used by Clean Seas Tuna Ltd management across the production, postharvest and marketing areas of the business to improve efficiency and more consistently deliver high quality YTK products to the market.

KEYWORDS: Yellowtail Kingfish, environmental requirements, seasonality, product quality, product yield, fillet shelf life

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Dr John Carragher (Logifish Consulting) for co-authoring, editing and compiling the final report.

1. Background

The farming of Yellowtail Kingfish (YTK) is a new form of aquaculture in Australia, with fish being grown in sea cage operations in NSW and South Australia since 2000. Though the production of YTK in NSW is negligible, production in South Australia mostly from Clean Seas Tuna Ltd (CST) is estimated to be 2,500 tonnes in 2009 (Miegel et al., 2010). Most of the product is sold fresh (chilled) as whole fish, head-on gilled and gutted fish, and vacuum packed fillets both domestically in food service (restaurants and catering) and in retail outlets, and following export to a variety of countries in Asia, Europe, Middle East and North America. The product specification and production costs incurred in YTK aquaculture mean that the product has to be positioned toward the premium quality and price end of the market spectrum. The aims of this project were to reduce production and processing costs, and improving product quality and consistency, in order to increase margins and sales volume in a very competitive marketplace.

Aquaculture of YTK in South Australia occurs in inshore waters that experience a wide seasonal variation in water temperature from 13-24°C, whereas in NSW water temperatures range from 16-25°C (Bureau of Meteorology Sea Surface Temperatures, August 2005-August 2007). The optimal growing temperature for YTK is not well documented but temperatures below 18°C and above 28°C are known to cause reductions in feed intake (Kohbara et al. 2003). Recent work by Pirozzi and Booth (2009) investigated the effects of increasing water temperature (10, 15, 20, 25, 30 or 32.5°C) on the fasting routine metabolic rate (RMR) of juvenile YTK. The results indicated that the thermo-sensitivity (i.e. temperature range over which metabolism is least dependant on temperature) occurred between 20-25°C. While not strictly stenotherms, the increased thermo-sensitivity of RMR outside these ranges suggests that YTK have a narrow temperature range for optimal metabolic function. These data suggest that growth of YTK will only approach an optimal level for short periods of time each year at either the NSW or South Australian grow out sites.

Current industry practice in Australia is to stock 5-10g YTK fingerlings to sea cages starting in September to coincide with rising seawater temperatures. However, production constraints mean that 3 hatchery runs are usually required to meet target fingerling numbers with these different runs of fingerlings being put to sea at 4-6 week intervals thereafter. The fish are then on-grown to a target harvest weight of approximately 4-5kg over a period of 24 months. This grow out period encompasses two winters during which the growth of YTK slows considerably. Farm data suggest

that fish that are put to sea later in the season (ie hatchery runs 2 and 3) often fail to put on much condition in the short summer period and struggle with the colder winter water temperatures, with increased mortality rates and a slow growth response in the following spring when water temperatures start to increase. These obviously add to the cost of YTK production.

One of the strategies that could reduce the mortality rate and slow growth observed following stocking at sea is to on-grow fingerling YTK in warmer water temperature nursery systems prior to stocking them into sea cages at a larger size than is currently the case. Stocking more robust juveniles should reduce mortality rates and increase at sea growth rates, potentially shortening the overall grow-out period and consequently, reducing the costs of YTK production. To develop their thinking on this strategy any further, aquaculturists need to know the optimum fish size and water temperature conditions that would favour this approach.

As mentioned above, YTK is currently sold to domestic, Asian, Middle Eastern, US and EU markets where the most commonly recognised quality indicators are fat content, flesh colour and texture (pers. comm. Tim Graham, Marketing and Sales manager, CST). In-market research indicates that sashimi customers prefer the Japanese produced YTK due to its consistently higher fat content. However the Australian YTK is favoured as a cooked product due to its milder flavour and firmer texture. Considering the importance of fat content in YTK it is necessary to understand how the fat content in the YTK carcase changes with fish size and time of the year. Knowledge about these differences will enable YTK producers the opportunity to tailor product specifications to meet market preferences and achieve more consistency in product quality. Differences in distribution of fat within the fillet may allow carcases to be portioned based on their fat content with the various cuts accordingly targeted to different markets.

In relation to this, anecdotal comments and direct feedback from some customers that buy whole chilled YTK has been that the flesh can be soft in November-December, and the shelf life (see below) of those fish/fillets is shorter than in the remainder of the year. Large wild YTK are known to spawn in SA waters about this time, and aquaculture broodstock are induced to mature and spawn out of season by environmental manipulations that mimic springtime conditions. In previous years some YTK were retained in growout cages for a third winter and there was noticeably developed gonads (testes and ovaries) when they were harvested in October to December (see Carragher et al., 2009). The coincidence between YTK sexual maturity and the reported issues with fillet quality and shelf life need to be investigated as sexual maturation of

aquaculture stock is a constraining factor with other species including: salmonids (Atlantic salmon, Chinook salmon, coho salmon, rainbow trout, brown trout), tilapia, carp, Atlantic cod, and barramundi amongst others (eg Thorpe et al., 1990, Hansen et al., 2000).

Sexual maturation can lead to three issues: (1) a diversion of energy and nutrients away from somatic (muscle) growth because of gonad growth and energy being spent on associated social interactions, and (2) deterioration in flesh quality (soft flesh, reduced flesh lipid and pigment levels) and (3) the development of secondary sexual characters (changes in skin pigmentation) which can render stock unmarketable. This limits the harvest season or at the very least, results in significant seasonal fluctuations in product volume and quality, which adversely affect product value and producer cash-flows. Consequently, aquaculturists manage unwanted maturation using a number of different strategies, with the particular strategy chosen depending on several factors – the main cue(s) for initiating maturation, the size of the marketable product and the amount of manipulative control possible with the culture system being used. These management strategies include:

- Single sex stocks (only the non- maturing sex is farmed; or sexual maturity only occurs when the sexes are mixed)
- Triploidy (all fish are sexually sterile)
- Genetics (populations that mature later are used as broodstock)
- Environmental manipulation (overriding the cues for initiating sexual maturation).

The costs and timeframes for developing and fine-tuning each of these strategies needs to be balanced against the costs of maturity observed in the growout animals. Consequently the first goal is to characterise the occurrence of maturation, this means:

- The sex exhibiting sexual maturity (males, females, both)
- The extent of maturity shown (amount of gonad growth, loss of somatic growth, loss of somatic condition, development of secondary sexual characteristics)
- The timing and duration of sexual maturity
- The proportion of the population exhibiting the condition
- The impact of maturation on product yield and quality attributes.

Consumers who identify as "health conscious" purchasers are now becoming a significant proportion of the marketplace, and make purchases based of the nutritional content of food (Danenberg and Mueller, 2011). However, the nutritional content of YTK fillet product and how

that might change at different times of the year, or in different fillet areas, is incomplete. For this, and legislative product labelling requirements in different markets, it is important to accumulate a larger body of that information in order to assist with marketing YTK product.

Product shelf life is a critical factor for marketers, customers and consumers as it is a primary determinant of where the product can be distributed, how often shipments need to be sent to those markets, how much risk the customer carries by having unsold product at the end of that shelf life period, and the extent of the food safety risk for the consumer. Clearly, product that has a longer shelf life will be perceived as less risky in the marketplace, but a producer / processor must put a use by date on a product that reflects the realistic, rather than an aspirational target, and one based on reliable and appropriate data.

Product shelf life can be affected many factors, some pre-harvest (such as the size of the fish, harvest season and composition of the fillet tissue, as described above), some post-mortem (a cascade of changes in cellular metabolic and enzymatic processes due to the intense activity associated with harvest and the lack of blood circulation that will affect oxygen and metabolite levels in tissues); and many that are processing or post-harvest related. The latter includes: sanitary practices affecting the microbial flora on the product; product contact with fish blood, slime, processing water and/or air; and storage temperature (Huss, 1995; Bremner 2002). A better understanding of the factors affecting product shelf life would help to identify strategies to extend it to take advantage of the marketing benefits that would ensue.

South Australian YTK producers are isolated from both major domestic and international marketplaces and thus product quality and shelf life are at risk from inappropriate cold chain management practices along the supply chain. Although CST has undertaken some previous temperature monitoring trials, and the various supply chain partners all have quality assurance practices and processes in place, no comprehensive cold chain monitoring study has been undertaken.

There is widespread reliance through the seafood industry on polystyrene boxes for product shipments of any duration (local, interstate or export). Polystyrene boxes are light, good insulators, strong and inexpensive. However, they occupy a lot of space because they need to be delivered and stored pre-assembled, they are not recycleable, and they are prone to breaking on impact. Consequently, there is interest in the use of alternative boxes that don't have the

disadvantages, and yet keep the advantages of polystyrene. To date, none of these alternative boxes have been trialled with chilled shipments of YTK products.

1.1 Need

The Australian YTK aquaculture industry needs to produce products that are competitive in terms of quality and price in both the domestic and international marketplaces. A key requirement is to reduce the post-transfer mortality rate and increase the at-sea growth rate to reduce production costs and improve margins. Marketing of YTK relies on positioning the species as a high quality and high value product, and these expectations need to reflect the customer experience. When this project was initiated YTK producers did not have a sufficiently comprehensive knowledge base or understanding of the extent and variation in product specification to always meet this expectation. This was the reason for this study and the various subproject research activities.

1.2 Objectives

1. To determine and quantify the interactive effects of fish sizes and water temperature on survival, weight gain, feed intake, feed conversion ratio and carcass composition of juvenile YTK.
2. To provide YTK producers and consumers detailed information on the nutritional and biochemical composition of YTK.
3. To identify how flesh composition, post-harvest processing methods and cold chain management techniques influence the shelf-life stability of the processed YTK product.
4. To characterise the occurrence of sexual maturation and identify the hormonal cues which initiate it.
5. To develop farm management strategies which improve flesh quality attributes and production and increase market access.

1.3 Design and Structure of this Final Report

The multi-component nature of this project, with each of three contributing groups being responsible for a different subproject, and the way in which the different activities were contracted to each collaborating organisation by Seafood CRC, have affected how this final report is constructed.

The contributions from the three collaborating parties have each been retained as complete documents, included as Appendices A, B and C. They have only been edited and formatted to make them appear to be generally consistent with each other, but also, and more importantly, so as not to affect the authenticity of the researcher contributions, and potentially misconstrue the expert scientific interpretation of the data, by 'cutting and pasting' all of the supplied material into single document.

The initial section of the report will take a 'whole of project' approach and has been put together by the Principal Investigators. This section of the report will cross-reference to the appropriate data in the various Appendices, as and when required, in order to bring together the key findings and outcomes of the project.

Note:

The work described in subprojects 1 and 2 of this report was carried out in the 2008, 2009 and 2010 YTK farming seasons, therefore the procedures described reflect the YTK farming practices at that time. As a result of this project, and others carried out by CST and various CRC collaborators, some of these practices and procedures will since have been modified as necessary.

2. KEY FINDINGS

The approach taken in this Overall Summary report is to present and consider in a logical order through production to post- harvest the key research activities carried out in the 3 subprojects. This means that the work done in the 3 different subprojects is not presented sequentially. The order in which the topics are presented, the subproject and the Research Agency that did the work, and the Appendix in which the detailed results can be found are summarised in the following Table.

Topic	Subject	Subproject	Research Agency	Appendix
1.	The relationship between fish size and water temperature on survival and growth of fingerling YTK	3	NSW DPI	C
2.	Development of a model to predict YTK growth from 50g to 1kg as a function of water temperature	3	NSW DPI	C
3.	The proximate composition of whole YTK carcases	1	Flinders University	A
4.	Flesh quality attributes and the nutritional profile of fillets	1	Flinders University	A
5.	Determining the shelf life of chilled vacuum packed YTK fillets	1	Flinders University	A
6.	Determining the nature and extent of sexual maturation of YTK during growout	2	SARDI and University of Tasmania	B
7.	An examination of temperature control within key domestic and export supply chains	1	Flinders University	A
8.	An evaluation of alternative box types within domestic and stimulated export supply chains	1	Flinders University	A

2.1 Fingerling size and temperature tolerance (Subproject 3 – Appendix C)

Two manipulative experiments were done with juvenile yellowtail kingfish (*Seriola lalandi*) (YTK) to provide new information that will support development of an "ideal production strategy" for YTK aquaculture operations in temperate Australian waters. The first experiment was designed to investigate the implications of stocking fingerling fish into water temperatures of 12 and 15°C; temperatures that are typical of September / October in the Spencer Gulf when juvenile YTK are stocked into sea-cages. The second experiment investigated the interactive effects of higher water temperatures (21, 24, or 27°C) and dissolved oxygen regime to examine the impacts of normoxia and hypoxia on survival growth and feed conversion.

Two indoor recirculating tank systems (set to 12 and 15°C, respectively) each with 12 identical 200L tanks each holding either 30 × 6g fingerlings, or 20 × 44g fingerlings, were used. Three replicate tanks were used for each size of fish with each of two different diets: the 6g fingerlings were fed either 2mm Ridley Start or Skretting Nutra Alpha feeds, the 44g fingerlings received either 3mm Ridley Start or Skretting Nova ME feeds. Feeding was to apparent satiation once or twice daily. The experiment with the 6g fingerling was run for 28 days, the 44g fish experiment ran for 64 days. Survival, growth and feed conversion ratio (FCR) was measured for fish in all tanks.

The results clearly demonstrated that even following an acclimation regime that decreased the initial water temperature from 22^{o}C by approximately 1°C day^{-1} it is not advisable to rear 6g or 44g juvenile YTK at 12°C. After about 11 days the fish reared at 12°C started to become moribund; they lost equilibrium, had difficulty feeding and died. The 6g fish fared worst with only 25-65% of fish in different tanks surviving to day 28 (Figure 1b). At this point the study with 6g fish was terminated according to NSW DPI Fisheries animal care and ethics requirements. The 44g fish fared better than the 6g fish, but still only 45-60% of these individuals survived to day 64 (Figure 1a). In contrast, survival of 6g and 44g fish at 15°C was extremely high (≈ 99%) and growth rate and apparent feed conversion were both acceptable (Figures 1a and 1b). Diet type did not have any effect on the mortality rate of either size of fish.

Based on this work we do not recommend stocking fingerling YTK up to 44g in bodyweight into sea-cages when water temperatures are lower than 15°C.

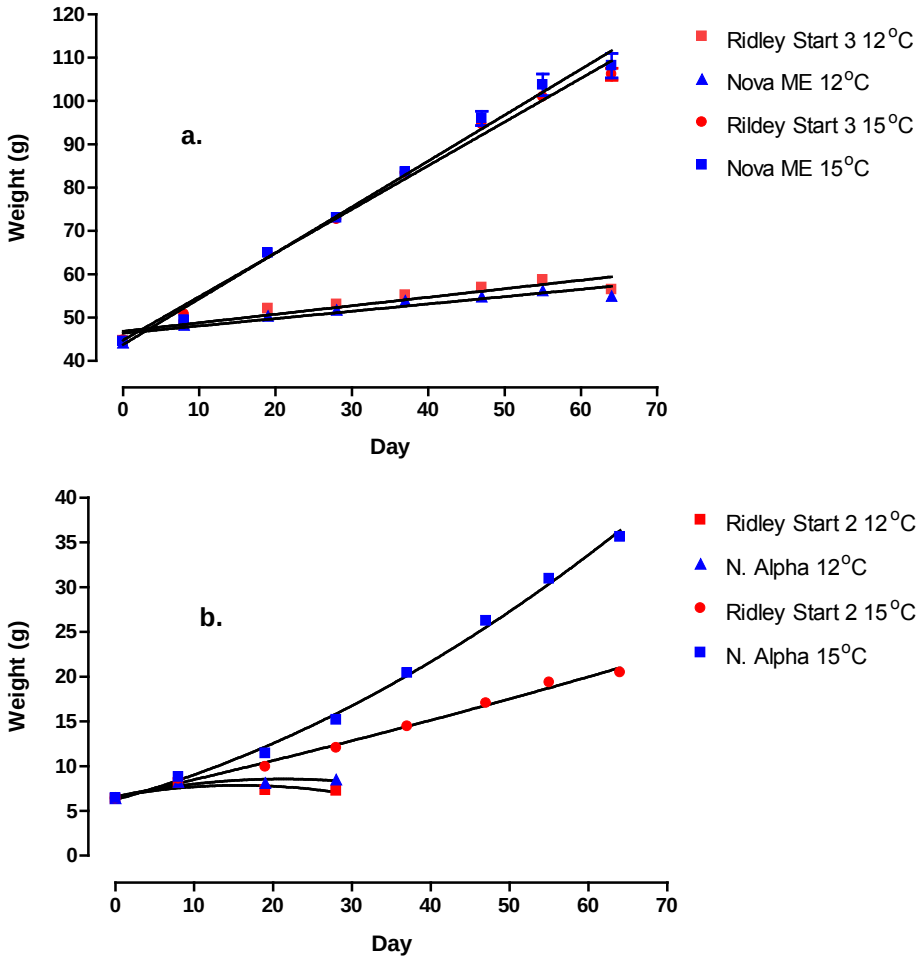

Figure 1. Mean growth of juvenile YTK reared under a 12°C or 15°C temperature regime and fed different commercial diets: a) 44g fish reared at 12°C or 15°C and b) 6g fish reared at 12°C or 15°C. Note; the study with 6g fish reared at 12°C was terminated after 28 days. Data points and error bars indicate mean±sem; in most cases the error bars are hidden by the symbol.

The 44g fish grew faster at 15°C than at 12°C and there were no differences in growth rate (Figure 1a) or apparent FCR (Ridley 2.49; Nova ME 2.40) between the two types of diet. In contrast, there was a significant difference in growth rate of the 6g fish on the two diets at 15°C, with fish on Nutra Alpha performing better (Figure 1b); they also had a better apparent FCR (Ridley 1.90; Nutra Alpha 0.91).

These results indicate that water temperature is critical for the survival and performance of YTK. Small (6g) fish could not tolerate 12°C and quickly succumbed; larger fish (44g stocking weight) were more robust, but survival and growth were still considerably affected by the colder water temperature.

The results indicated that YTK are reasonably tolerant of hypoxic conditions typically experienced at high stocking densities and feed rates. However, subjecting juvenile fish to the hypoxic conditions dramatically reduced growth potential by 13%, 20% and 17% at 21°, 24°C or 27°C, respectively compared to fish reared under normoxic conditions. This study also confirmed that water temperatures between 22-24°C promote optimal growth in juvenile YTK. Growth and temperature data from both experiments was combined with similar data from previous research on YTK to develop a temperature dependent growth model for fish < 1000g. Information on size at stocking and the effects of water temperature and hypoxia on performance of juvenile YTK will improve management decisions surrounding production of this species. In addition, growth and performance can now be benchmarked against a temperature dependent model developed specifically for this species and reared under South Australian conditions.

Another experiment used the 24 × 200L tank indoor recirculating system to determine the effect of different water temperatures (21, 24 and 27^{o}C) with dissolved oxygen (DO) levels that were either consistently normal (5-7mg/l; 70-100% sat; normoxic), or consistently low (3-5mg/l; 40-70% sat; hypoxic). The YTK fingerlings used in this experiment had a mean starting bodyweight of 8.7g, four replicate tanks of 30 fish were used for each temperature/DO treatment combination, and the experiment was run for 35 days.

Survival at all temperatures was high in the normoxic groups, but was significantly lower in the hypoxic group at 27^{o}C (90% compared to 99%; $p<0.05$). Growth rate was significant affected by water temperature ($p<0.0001$) being highest at 24^{o}C, followed by 27^{o}C and 21^{o}C (Figure 2a; and see section 2.2). Growth rate was slightly, but significantly ($p<0.0001$), lower in the hypoxic groups (Figure 2a). Apparent FCR was also significantly affected by water temperature and level of dissolved oxygen, with higher FCR values (ie worse growth-feed consumed performance) at 27^{o}C compared to 24 or 21^{o}C, irrespective of the dissolved oxygen level (Figure 2b).

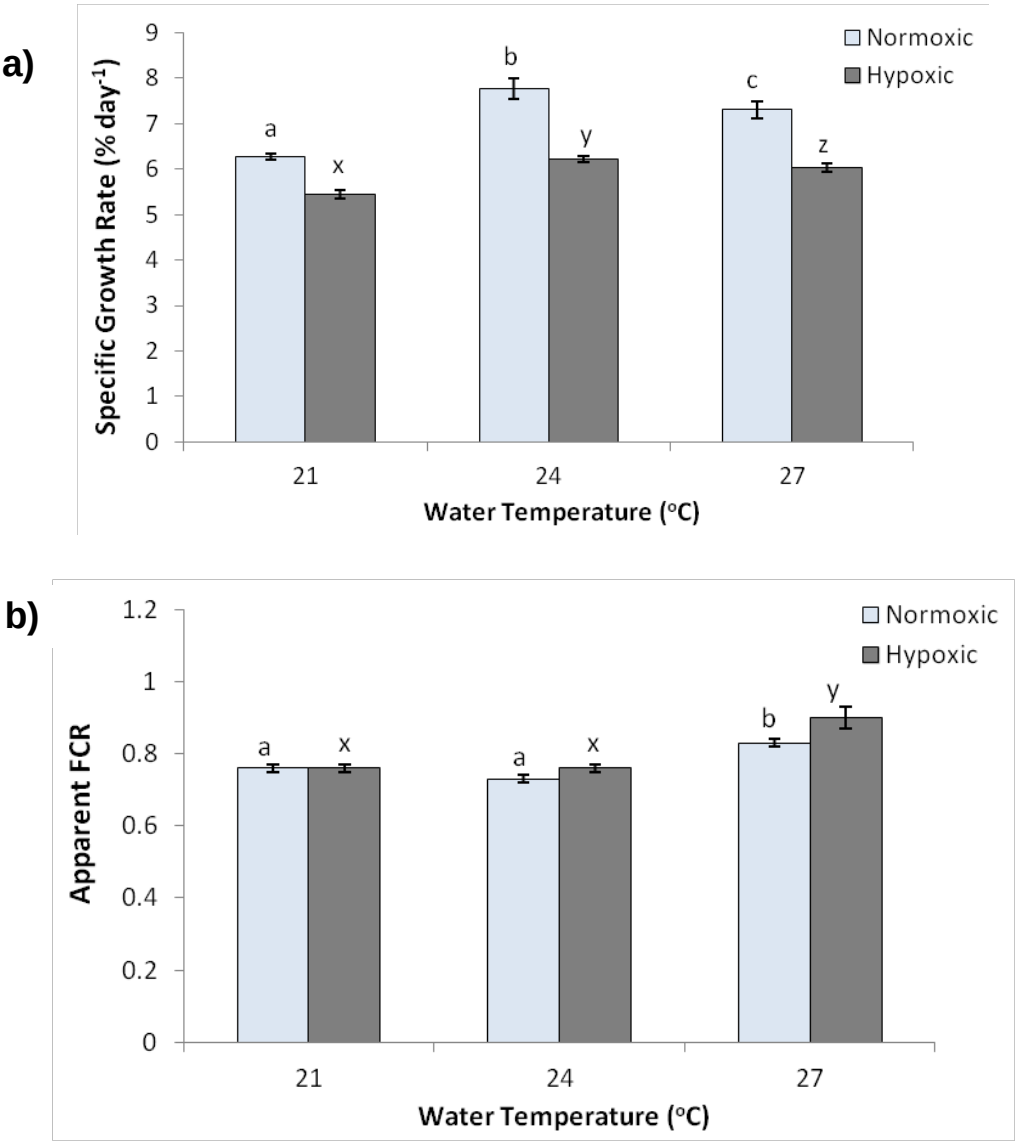

Figure 2. Mean ± SD (n=4) (a) specific growth rate (SGR; %day^{-1}) and (b) apparent feed conversion rate (FCR) of 8.7g fingerling Yellowtail Kingfish reared at different water temperatures and with consistently normal (normoxic) or low (hypoxic) dissolved oxygen levels over 35 days. Different letters above the bars within a dissolved oxygen treatment group (normoxic or hypoxic) denote statistically significant values ($p<0.05$).

2.2 A temperature dependent model for YTK growth (Subproject 3 – Appendix C)

Growth data from the two experiments described above was combined with those from several other experiments carried out at the NSW DPI facility over several different water temperatures using a range of sizes of YTK. A non-linear regression model was used to fit these data and the analysis gave the following growth model equation:

$$\text{Daily gain (g day}^{-1}) = [(1.354)+(0.144)*(\text{Temp})+(0.003)*(\text{Temp})^2] * (\text{GMBW})^{0.48}$$

Where:

Temp = water temperature in ^{o}C, and

geometric mean body weight (GMBW) = (initial weight (g) x final weight (g))$^{0.5}$

A simplified two dimensional representation of the growth model (converted to daily growth as a percentage of bodyweight) is presented in Figure 3. From a biological perspective the model predicts that growth will be optimised when water temperature is 23°C. Growth rate of fish at any size declines on either side of this temperature. The model also indicates that weight gain decreases with increasing body weight for a given temperature, however, the response becomes asymptotic as fish become larger. Thus, at a water temperature of 23^{o}C a 50g YTK will grow at 5% per day (=2.5g) but a 1000g fish will grow at about 1% per day (=10g).

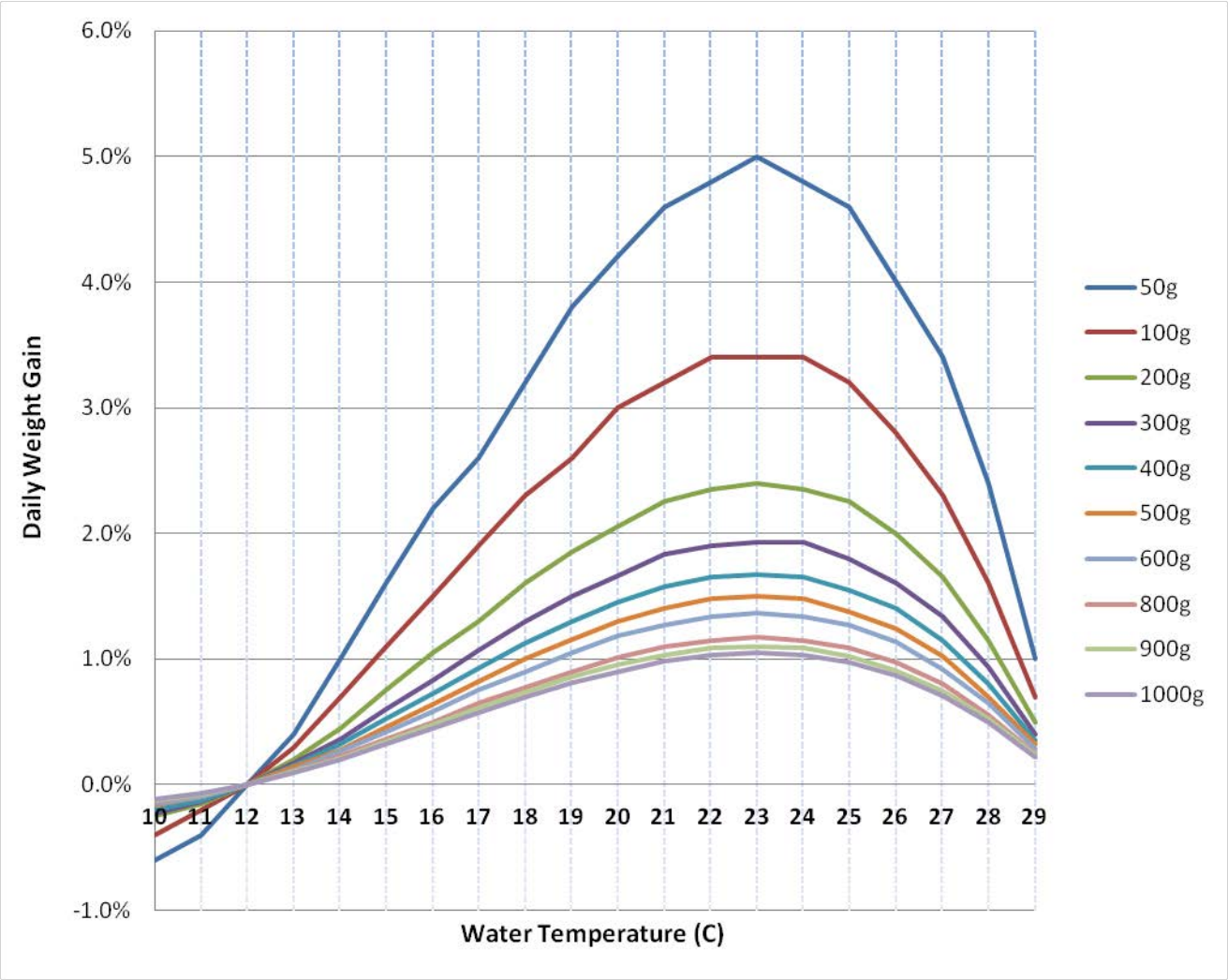

Figure 3. Two dimensional representation of the temperature dependent growth model (% bodyweight per day) for juvenile Yellowtail kingfish (*Seriola lalandi*).

2.3 Whole carcase proximate composition (Subprojects 1 & 2 – Appendices A and B)

YTK from three different hatchery runs in the same year were harvested at four times over an 11 month period. Each fish was minced and the homogenised tissue was analysed for proximate composition (Figure 4). The protein level was between 19 and 21% and the proportion of ash increased from 2 to 3% as the study progressed. The level of whole carcass lipid (and the inversely correlated moisture content) showed most variation, with values between 7 and 16%. Levels of fat were lower in September and January than in May/June. As with other fish, there was no appreciable carbohydrate in the carcass.

The breakdown of how that carcase was comprised (i.e. what amount of the fish was fillet, viscera and head and frame) was determined in subproject 2 and is shown as a function of bodyweight in Figure 5a, and as a percentage of the carcass in Figure 5b. The frame was generally around 30% of the bodyweight, the viscera about 10% and about 60% was fillet for the range of YTK sampled (1.3 to 4.2kg).

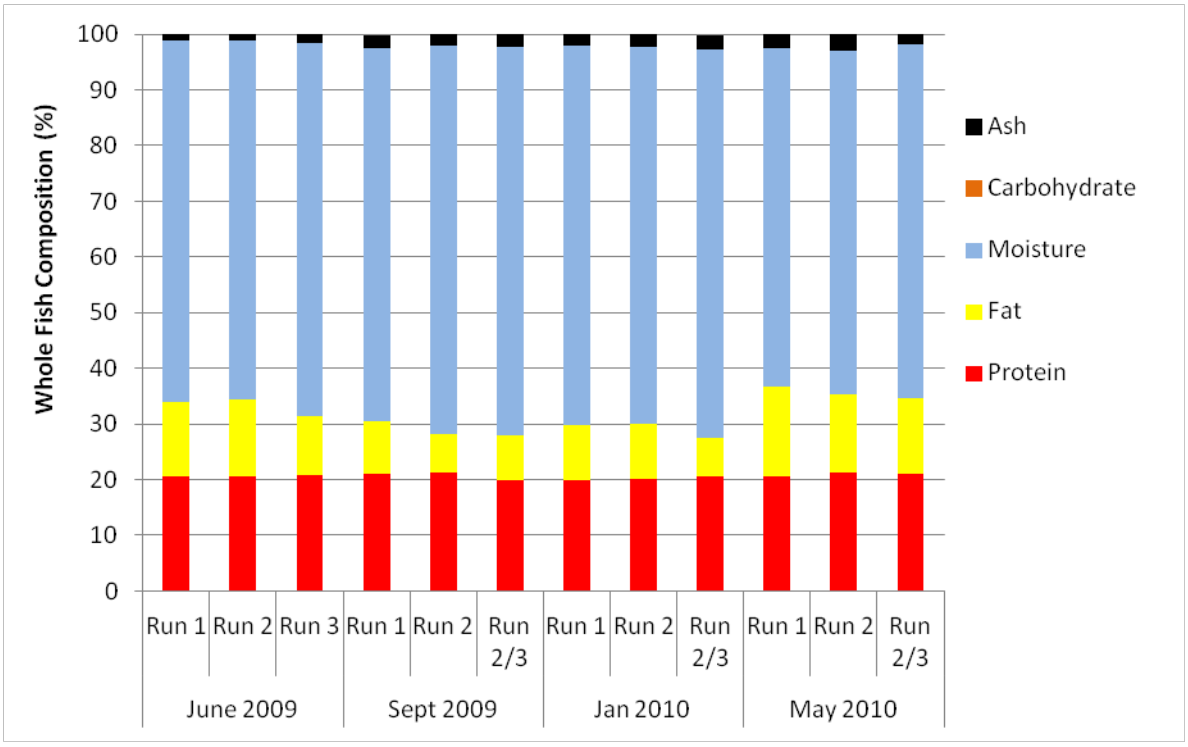

Figure 4. Stacked chart showing whole carcase mean percent proximate composition of Yellowtail kingfish (*Seriola lalandi*) (spawned 2008) harvested from June and September 2009 and January and May 2010.

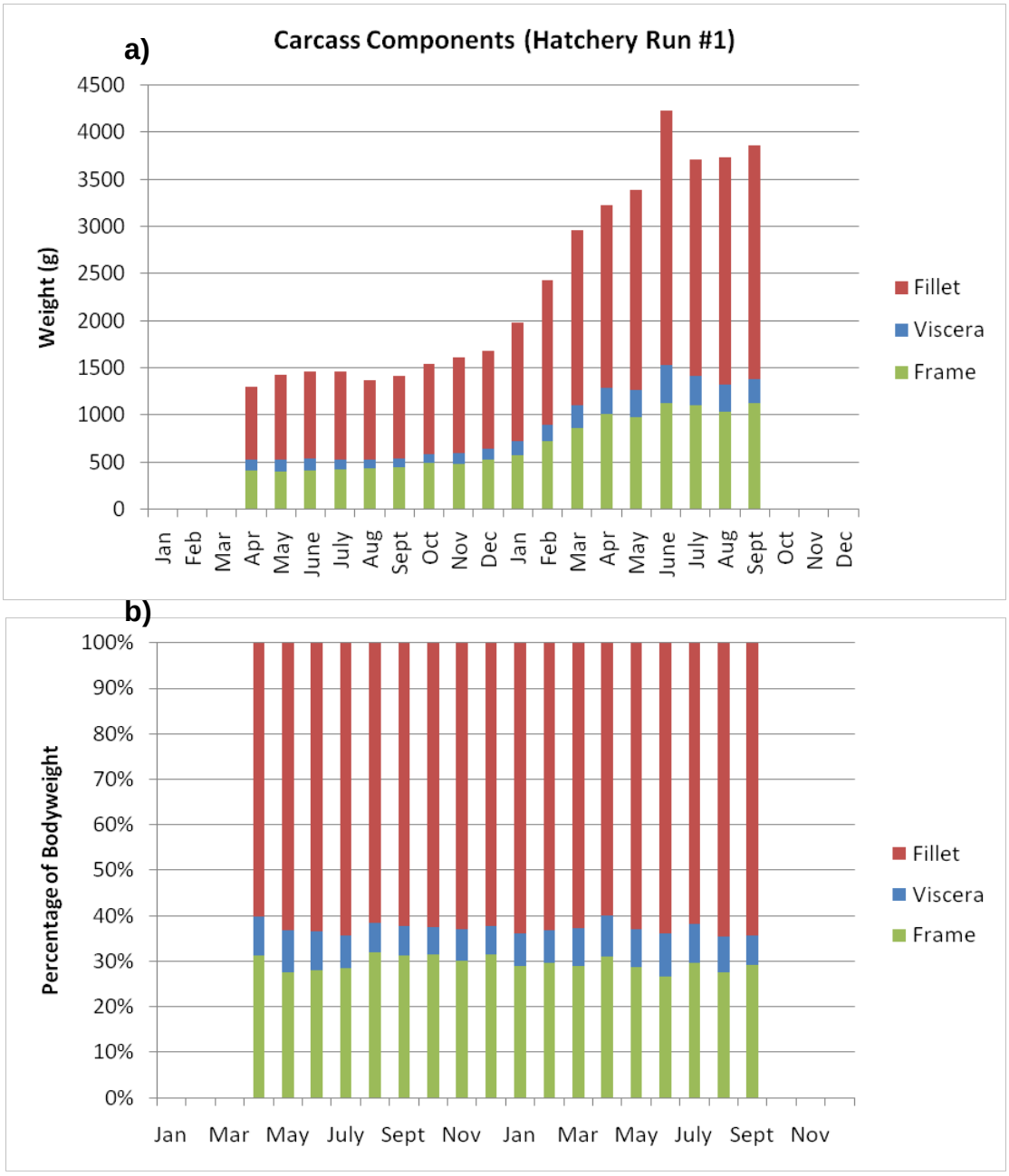

Figure 5. Carcass composition as (a) fish grow, and (b) as a percentage of body weight through the production cycle. These data are from hatchery run 1 fish in the 2007 year class of Yellowtail kingfish (*Seriola lalandi*). The frame includes head, backbone, most fins (dorsal, caudal, anal) gills, kidney, swinbladder and heart.

2.4 Fillet quality attributes and nutritional composition (Subproject 1 – Appendix A)

Fillet composition was analysed for proximate and nutritional attributes by fillet area (dorsal vs ventral) and in summer and winter. Figure 6 shows that fillet protein content was 21-24% and ash was 1-2%, with slightly higher levels in the ventral area. Crude lipid again showed the greatest variability with values in the dorsal areas of 7 to 9% in winter and summer, respectively; and 12.7 to 15.5% in the ventral area (winter and summer, respectively). Again, there was no detectable carbohydrate in the fillet.

Crude fat can be characterised into different lipid classes; when represented proportionally, the different lipid classes were not substantially different in the dorsal/ventral areas of the fillet, or in winter/summer (Figure 7). Thus, saturated fats were about 25%, *trans* fats were very low (0 to 0.2%), polyunsaturates were 30-35% and the remaining 40-45% were monounsaturates. The omega-3 polyunsaturates were high in both areas of the fillet, but highest in the ventral area, and maximal in summer (Figure 8). Table 1 shows how the nutritional attributes of YTK compare to another aquacultured finfish (Atlantic salmon) and the Australian dietary guidelines (NHMRC, 2006). Overall, YTK flesh is very similar to farmed Atlantic salmon and a 100g portion contains substantially more omega-3 fatty acids than the recommended daily intake (RDI).

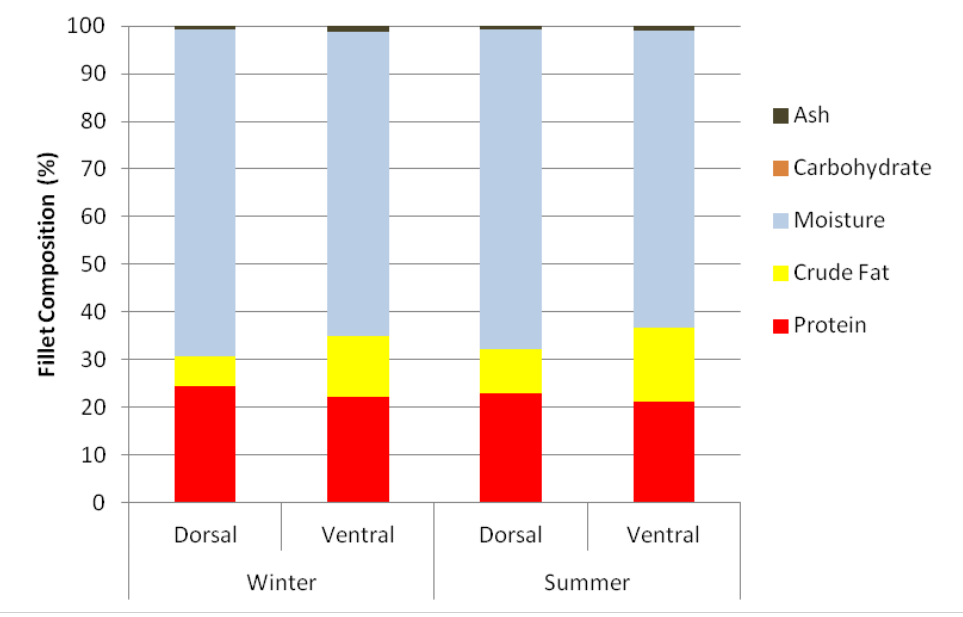

Figure 6. Stacked chart showing mean proximate composition (as a percentage) of dorsal and ventral areas of the fillet of Yellowtail kingfish (*Seriola lalandi*) (2007 hatchery run 2) harvested from September 2009 (Winter) and January 2010 (Summer).

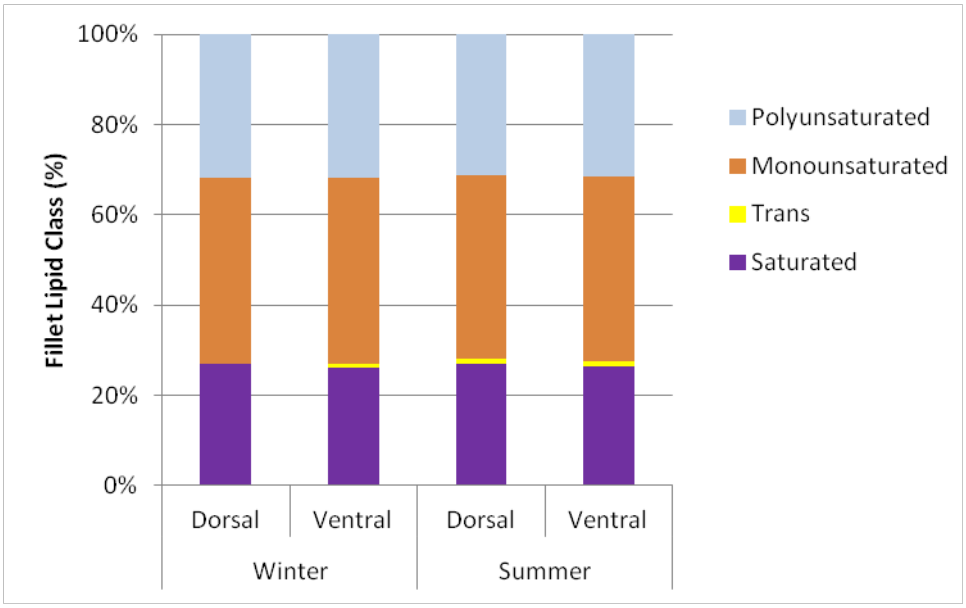

Figure 7. Stacked chart showing mean lipid class composition (as a percentage of all lipid) in dorsal and ventral areas of the fillet of Yellowtail kingfish (*Seriola lalandi*) (2007 hatchery run 2) harvested from September 2009 (Winter) and January 2010 (Summer).

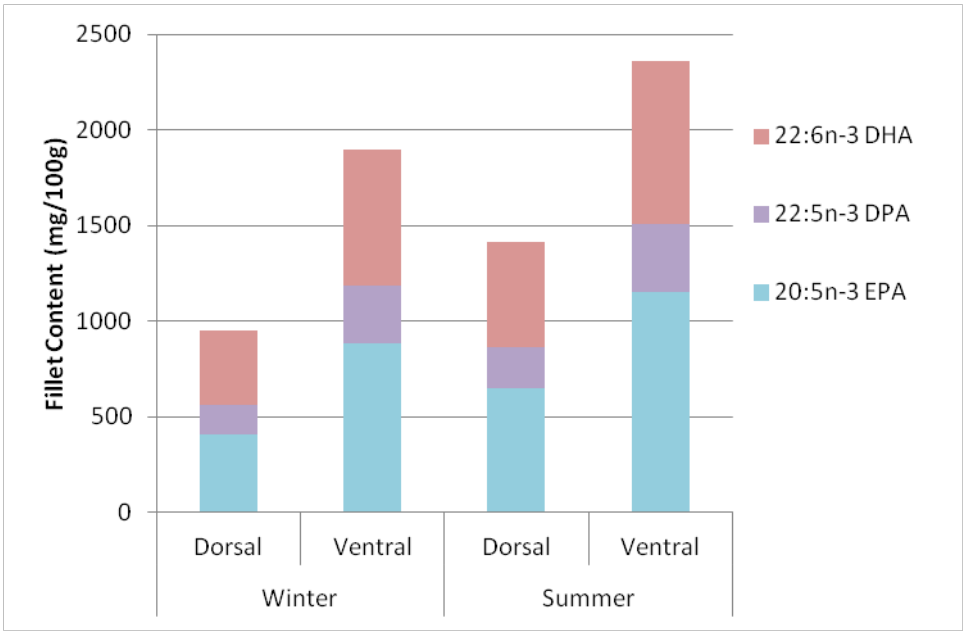

Figure 8. Stacked chart showing mean omega-3 fatty acid content (EPA, DPA and DHA; mg/100g) in dorsal and ventral areas of the fillet of Yellowtail kingfish (*Seriola lalandi*) (2007 hatchery run 2) harvested from September 2009 (Winter) and January 2010 (Summer).

Table 1. Mean summer/winter values of nutritional attributes for Yellowtail kingfish (*Seriola lalandi*) dorsal and ventral fillet areas compared to published values for raw farmed Atlantic salmon fillets (USDA SR 24) and recommended daily intake (RDI) and adequate daily intake (ADI) for adults aged 31-50 from NHMRC (2006).

	YTK		Atlantic Salmon	Adult age 31-50 RDI (mg/day)		Adult age 31-50 ADI (mg/day)	
	Dorsal	**Ventral**	**Fillet**	**Male**	**Female**	**Male**	**Female**
Proximates (g/100g)							
Protein	23.0	21.7	20.4				
Crude Fat	9.2	14.1	13.4				
Moisture	67.1	63.1	64.9				
Carbohydrate	0	0	0				
Ash	1.6	1.6	1.2				
Energy (kJ/100g)	760	920	862				
Lipid types (g/100g)							
Saturated	2.50	3.70	3.05				
Trans	0.10	0.15	0.00				
Polyunsaturated	2.90	4.45	3.77				
Monounsaturated	3.80	5.80	3.77				
Unsaturated fatty acids (mg/1000g)							
Omega 6 FA	1,000	1,600	666				
Omega 3 FA	1,600	2,500	2,260	610	430		
20:5n-3 EPA	648	1,021	690				
22:6n-3 DHA	552	777	1,457				
Micronutrients and minerals (mg/100g)							
Fe	0.40	0.45	0.34	8	18		
Cu	0.10	0.05	0			1.7	1.7
Zn	0.50	0.55	0.36	14	8		
Mn	0	0.01	0			5.5	5
Ca	5.80	6.90	9.00	1,000	1,000		
Na	31	45	59			460-920	
Mg	32	29	27	420	320		
P	244	252	240	1,000	1,000		
K	422	373	363			3,800	2,800
Vitamins (mg/100g)							
Vit C	3.5	3.5	3.6	45	45		
Vit E	2.6	2.6	3.6			10	7

2.5 Fillet shelf life (Subproject 1 – Appendix A)

Substantial data were gathered in relation to the chemical, physical and sensory attributes of vacuum packed YTK fillets stored at 4°C and readers are referred to Appendix A for the detailed presentation and discussion therein. Results from the TBARS assay (indicator of lipid oxidation) are not shown in this summary as levels were low and did not change over chilled storage out to 30 days (at which time the fillets had a noticeable fishy odour). Drip loss was one of the parameters that changed during the shelf life trials, with higher levels of drip loss evident throughout the shelf life period in January 2010 compared to the other 3 harvest times (Figure 9). The possible reason(s) for this result are not known, but it does not relate to a higher level of fillet moisture as crude fat levels were higher in January.

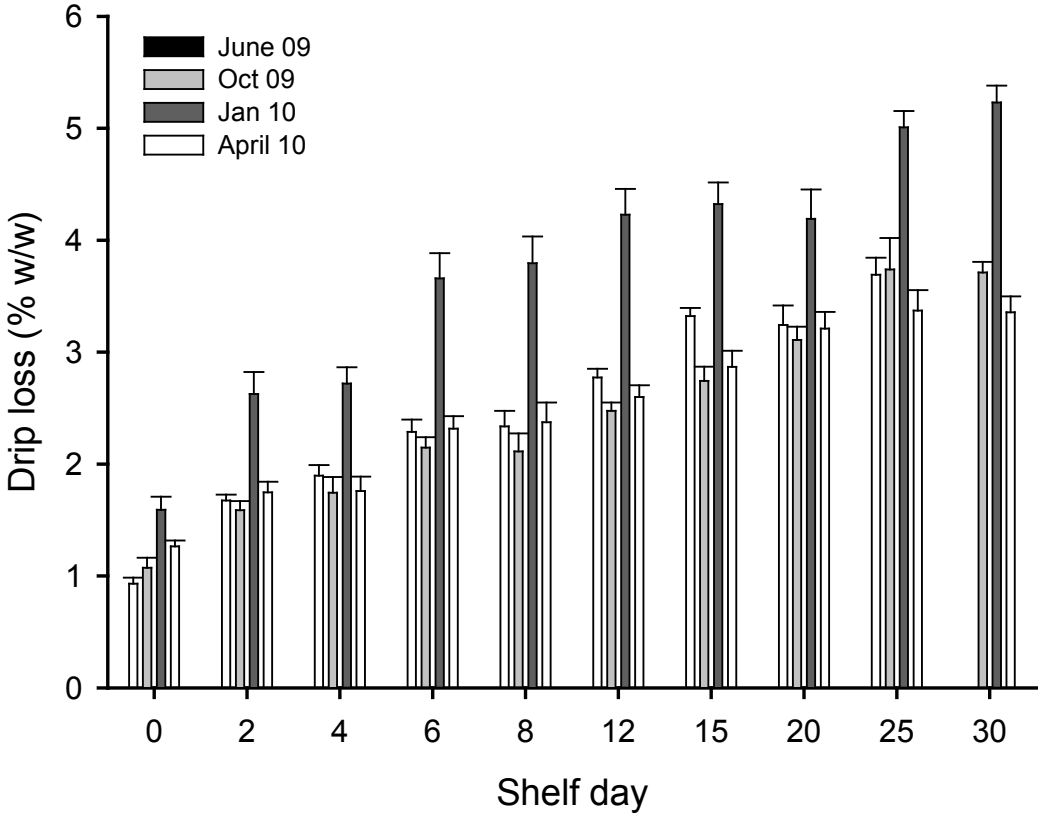

Figure 9. Mean (± se) fillet moisture loss in Yellowtail kingfish (*Seriola lalandi*) harvested in June 2009, October 2009, January 2010 (hatchery run 1, August 2007) and April 2010 (hatchery run 2, September 2007). Fillets were vacuum packed (Day 0) then held at approximately 4°C for up to 30 days. n = 10.

A technique for objectively measuring YTK fillet colour was developed (based on getting a single representative value from each individual fish ("blur individual"), and then getting mean colour

value from all the fish in the treatment group ("blur average")) and used to measure changes after different storage times, and for up to 3 days after those vacuum packed fillets were opened (see Appendix A for full details of the method). As well as giving a visual estimation of the average colour of the flesh (Figure 10), the "blur individual" values were converted to L*a*b* colour coordinates using Photoshop™ image analysis software (see below). These coordinates could be statistically analysed to identify treatment effects. There were no visually identifiable trends in fillet colour changing over storage time (e.g. Figure 10), although the statistical analysis of L*a*b* values did identify significant differences.

Figure 10. Colour swatches for Yellowtail kingfish (*Seriola lalandi*) hatched as run 1, (August 2007) and harvested in October 2009 (Harvest 2). Fillets were vacuum packed (day 0) then held at approximately 4°C for up to 30 days (only some of the days are shown). *n* = 9-10.

Changes in flesh freshness as shown by K-value results were consistent and large during the shelf life trials. Figure 11 shows the K-value results from four seasonal harvests, with vacuum packed fillets being opened on days 0, 4, 8, 15 and 30 of storage at 4°C. After the packs were opened the fillets were retained for another 3 days and remeasured to see if exposure to the air caused any significant changes in freshness. As expected for good quality fish, the K-value levels are initially low and increase steadily over time in storage. A K-value of 60-70% was attained after 8-15 days, depending on the harvest/season; this coincided with the time the fillets had a slight fishy odour when the packs were first opened. There was no major change in K-value after the packs had been opened for 3 days.

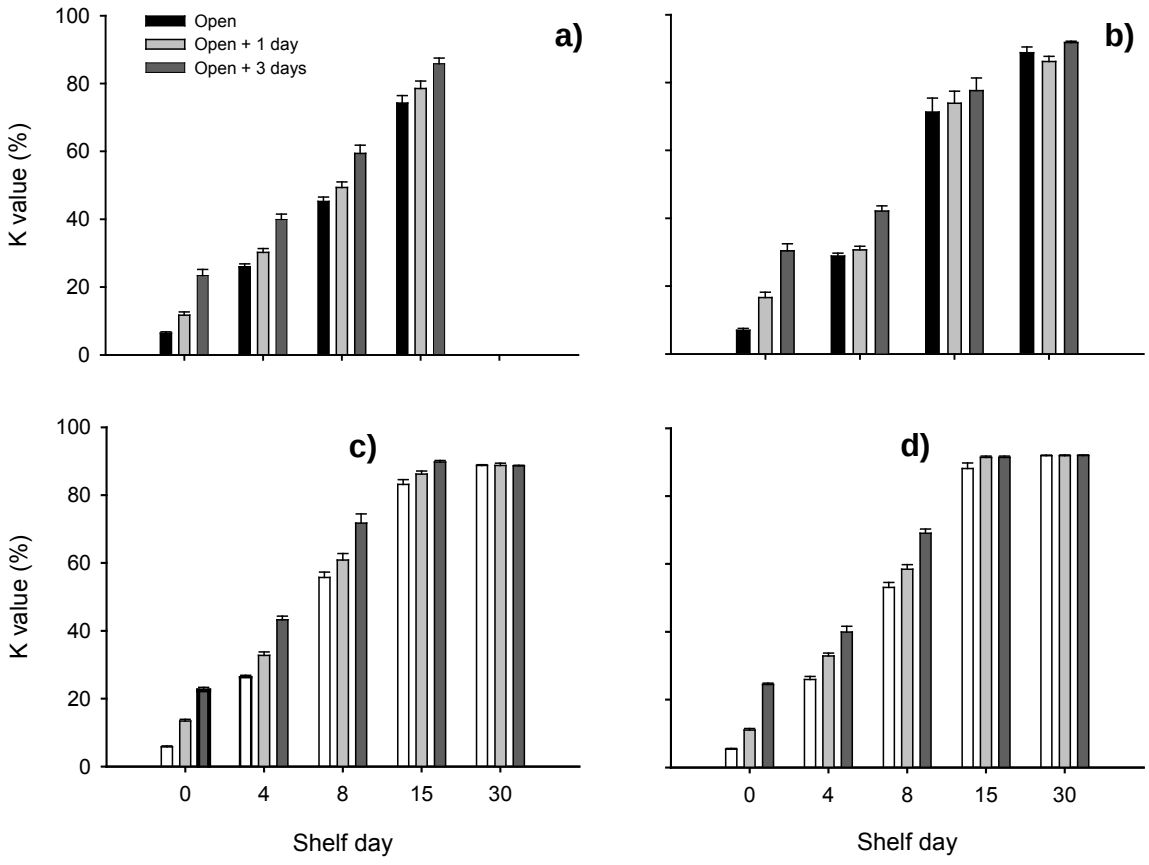

Figure 11. Mean (± se) ventral fillet K value in farmed Yellowtail kingfish (*Seriola lalandi*) harvested in a) June 2009, b) October 2009, c) January 2010 and d) April 2010, from fish spawned in 2008, hatchery run 1 (August). Fillets were vacuum packed (day 0) then held at 4°C for up to 30 days. K value was measured on the day of vacuum pack opening and then 1 and 3 days post opening. *n* = 10.

L*a*b* coordinates for fillet colour on the day the vacuum packs were opened and again 3 days later are show in Figure 12. The statistical analysis indicates that the open and filled circles on each plot are significantly different from each other, meaning that the fillets have changed colour. The direction of the change (all of them slight) is that L* (lightness) has increased, as has b* (redness) and a* has decreased (yellowness); however, as stated above, these differences are hard to identify by looking at the colour swatches that represent the mean L*a*b* values.

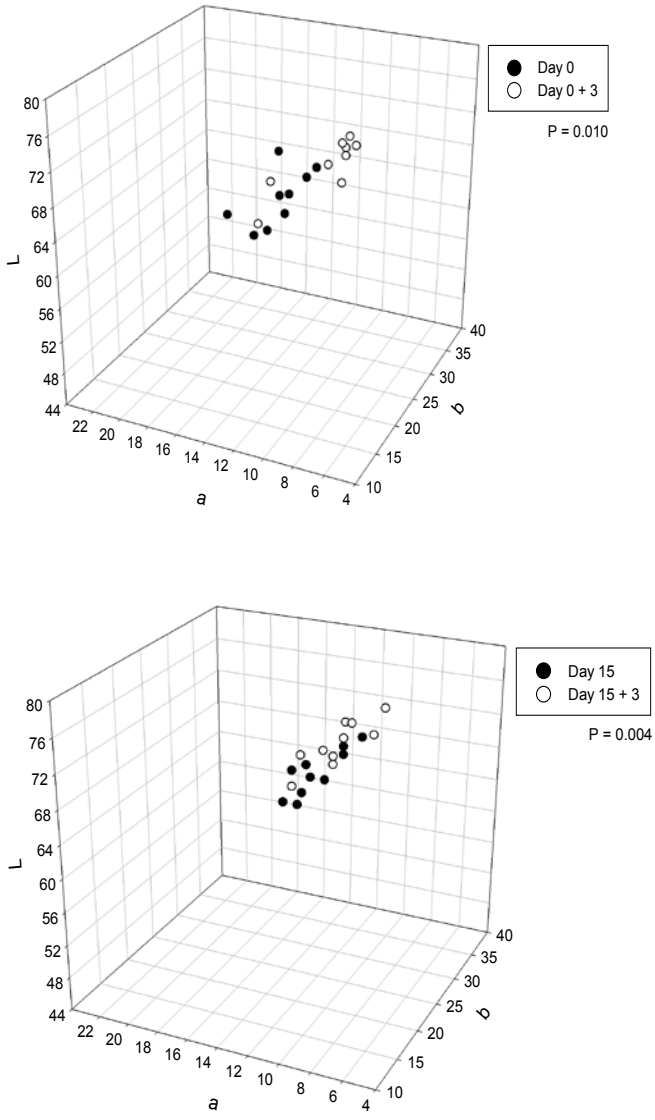

Figure 12. L*a*b* coordinates for individual fillets from Yellowtail kingfish (*Seriola lalandi*) hatched as run 1 (August 2007) and harvested in October 2009 (Harvest 2). Fillets were vacuum packed (day 0) then held at approximately 4°C for up to 30 days. Once opened the fillets were retained for an extra 3 days and re-sampled. Only data from days 0 and 15 are shown. n = 9-10.

In summary, the shelf life of vacuum packed fillets appeared to be typically 12-15 days at 4^{o}C. K-value increased as storage time progressed, and was about 60-70% when fillets first had a slightly fishy odour on opening. Fillet colour did not appear (to the eye) to change substantially with vacuum packed storage, but the instrumentally attributed L*a*b* coordinates did show statistically significant differences. Lipid oxidation did not appear to be a feature of chilled fillet shelf life. Microbiological spoilage was not carried out during these trials, and any future work should include this parameter.

2.6 Sexual maturation in YTK and effects on product yield and quality (Subproject 2 – Appendix B)

Fish from 3 hatchery runs in each of three hatchery seasons (2008, 2009 and 2010) were sampled monthly for 12 months. When the data on gonad size (as a proportion of body weight; GSI) was analysed by whether the fish were male or female, the results were very interesting (Figure 13). Female fish had GSI values that increased steadily month by month reaching about 0.4% of bodyweight after they had been at sea for about 2 years (harvest size 4kg), these levels, although low, were higher than male fish GSI values for almost all that same time (0.1-0.2%). Only in the October-December as 2 year old fish, did the testes size increase to about 1.2% of bodyweight. Histological examination of the testes showed that 100% of males were producing sperm at this time. Interestingly, 12 months beforehand, about 80% of male fish were also producing sperm, albeit in very small amounts from very small testes. Histologically, only 1 of the 500 or so female fish (a fish of 4kg) showed any signs of sexual maturation, and that was very early changes in a few oocytes. It was concluded that female fish do not become sexually mature for another year (eg a bodyweight of 7kg).

Fillet yield from 2 year old male and female fish (3.6-4.2kg) in the October-December period when male fish had larger testes, indicated that male fish had slightly higher fillet yields than females (by 1%). This suggests that there is not deleterious effect of male sexual maturation on fillet yield in YTK at ages up to 2 years (at about 4-5kg bodyweight).

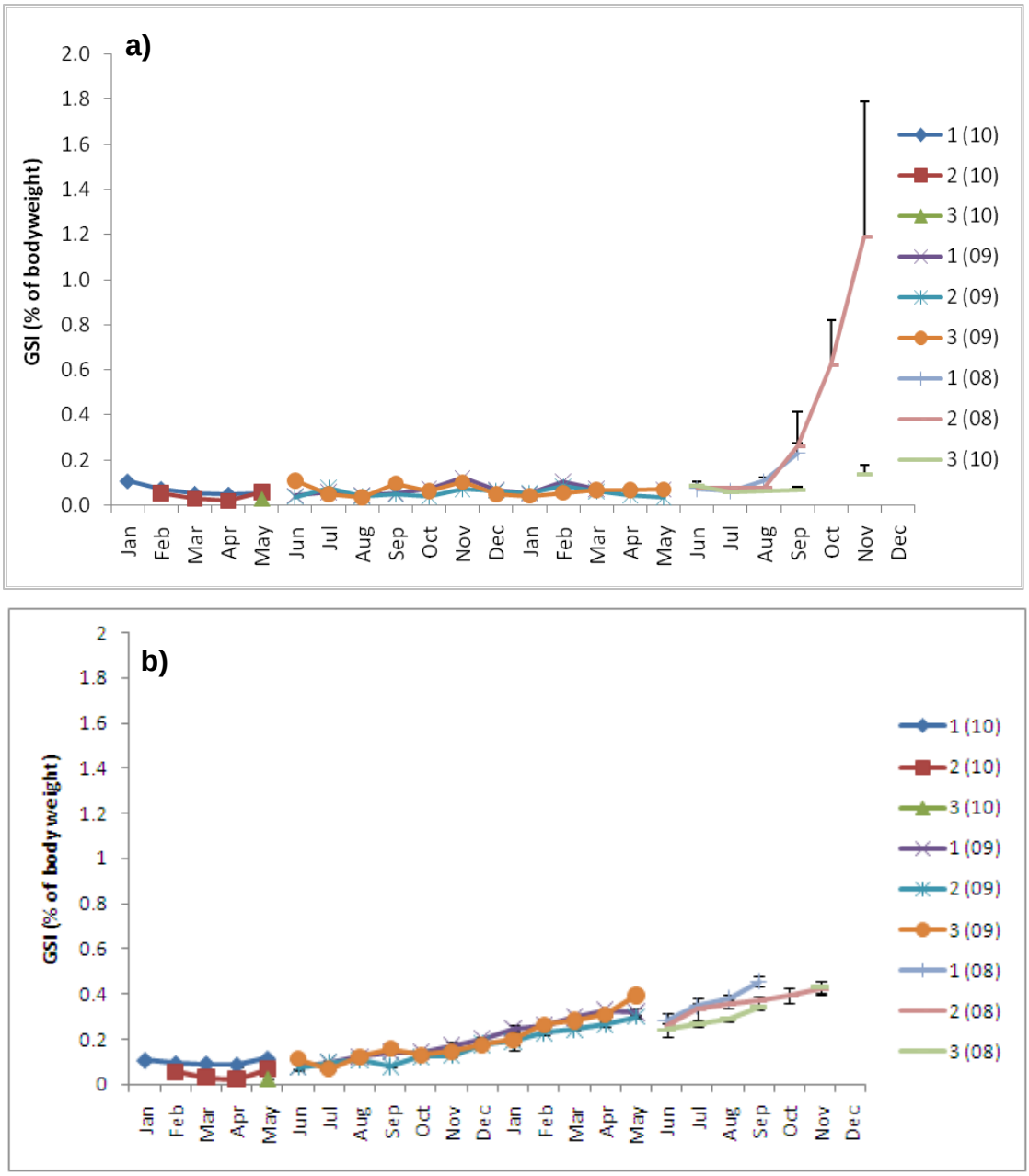

Figure 13. Mean ± sem gonad mass in (a) males, and (b) females as a per centage of body weight. Numbers of fish sampled in each month are given in Table 5. Legend code indicates hatchery run (1st, 2nd or 3rd) and year fingerlings were introduced to sea- cages ('08, '09 or '10).

2.7 Performance of the cold chain for chilled YTK products (Subproject 1 – Appendix A)

Seven commercial shipments of chilled whole/head on gilled and gutted (HOGG) YTK carcases were equipped with temperature logging probes. The individually identified probes were inside the body cavity of the fish, outside of the fish but inside the box and outside of the box. All of the export shipments were done using the regular polystyrene fish box. The domestic shipment from Port Lincoln to Sydney (34 hours) was carried out using the regular polystyrene box and five fibre board alternative boxes. Key data from the six export shipments are shown in Tables 2 and 3. During transit and in storage at the destination the temperature inside the boxes remained low and constant, whilst the temperature range was greater outside of the boxes (Table 2).

Table 2. Minimum and maximum temperatures measured inside and outside the fish boxes during both the transit period and in storage at the destination before the loggers were retrieved from the boxes.

	In Transit				In Storage			
	Min (^{o}C)		Max (^{o}C)		Min (^{o}C)		Max (^{o}C)	
Destination	**Inside**	**Outside**	**Inside**	**Outside**	**Inside**	**Outside**	**Inside**	**Outside**
Rome	1.1	3.0	2.3	12.5	-1.0	-0.5	3.8	3.8
Philadelphia	-1.0	-0.8	2.5	9.0	2.5	2.5	3.0	12.5
Los Angeles	-0.5	-0.5	1.5	1.5	1.0	2.8	2.2	10.0
Zurich	1.0	1.3	3.1	8.7	2.2	2.9	4.0	10.0
Philadelphia	0	0.5	3.1	12.0	1.5	0.3	2.0	17.0
Amsterdam	-0.5	0	2	5.5	1.5	1.5	2.0	5.5

Temperatures inside the fish were also low and stable, and no temperature violations (above 4^{o}C) were recorded during transport (Table 3). Fish temperatures during a prolonged period of storage in Rome, however, were sometimes higher (between 3.0 and 5.1^{o}C; Table 3), despite adequate external temperature control (-1 to +3.8^{o}C; Table 2) which suggests that the chill packs had completely thawed. The Ice Days method (a technique for assessing quality deterioration or estimating the remaining shelf life of the fish in comparison to the spoilage of the fish if it were iced at 0^{o}C) was used to compare the time × internal fish temperature circumstances of each shipment (Table 3). Most shipments were achieved within 3-5 Ice Days, however the Rome shipment had a value of about 14 Ice Days, with 10.9 of those coming during the time in storage at the destination. This suggests those fish would have shown greater post-mortem deterioration and had shorter additional shelf-life than could have been the case had temperature control been better. The actual time for whole/HOGG YTK to spoil/reach the end of shelf life remains to be

determined. The data from the shipment to Zurich was used as the basis for a simulated export trial using the alternative fish boxes (see below).

Table 3. Minimum and maximum temperatures measured inside the YTK carcase during the transit period (duration in days) and in storage at the destination (duration in days) before the loggers were retrieved from the boxes. Ice Days were calculated for each phase, and as a total.

	In Transit				In Storage				Total
Destination	Time (days)	Min (°C)	Max (°C)	Ice Days	Time (days)	Min (°C)	Max (°C)	Ice Days	Ice Days
Rome	2.00	2.3	3	**3.2**	6.00	3	5.1	**10.9**	**14.1**
Philadelphia	2.50	-1	2.5	**3.9**	0.75	2.5	2.8	**1.2**	**5.1**
Los Angeles	1.33	-0.2	2	**1.5**	1.25	1	1.8	**1.6**	**3.1**
Zurich	1.83	1.5	2.2	**2.6**	0.25	2.6	3.2	**0.4**	**3.1**
Philadelphia	3.13	0.2	2.5	**3.9**	0.50	1.5	2	**0.7**	**4.6**
Amsterdam	2.00	1	2.5	**2.8**	0.29	2.6	2.8	**0.4**	**3.2**

2.8 Alternative packaging for chilled YTK products (Subproject 1 – Appendix A)

Five fibre board boxes were used in a trial to ship YTK by refrigerated truck to Sydney, and their performance was compared to the standard polystyrene box. As in the export shipments, temperature loggers were put inside the fish, inside the box and outside the box from the processing factory in Port Lincoln, to when the fish were unpacked at the wholesaler in Sydney (Figure 14). Temperature outside the boxes was well regulated throughout the journey to Sydney, but was about 10°C for several hours at SFM awaiting pickup. All the boxes held low and stable temperatures during the road trip, but it was noticeable that despite being so low, the polystyrene box was about 1°C cooler. This difference was exaggerated when the boxes were shifted to the 10°C holding area, and the temperatures in all of the fibre board boxes increased by 1.5 to 3°C, whereas the change in the polystyrene box was less than 1°C. Temperatures inside the fish were more constant, and were between -1 and +1°C in all the boxes throughout the trip. Not surprisingly, the quality index score (QIM), K-value and microbiological assessment of the fish from all of the boxes were not significantly different at unpacking.

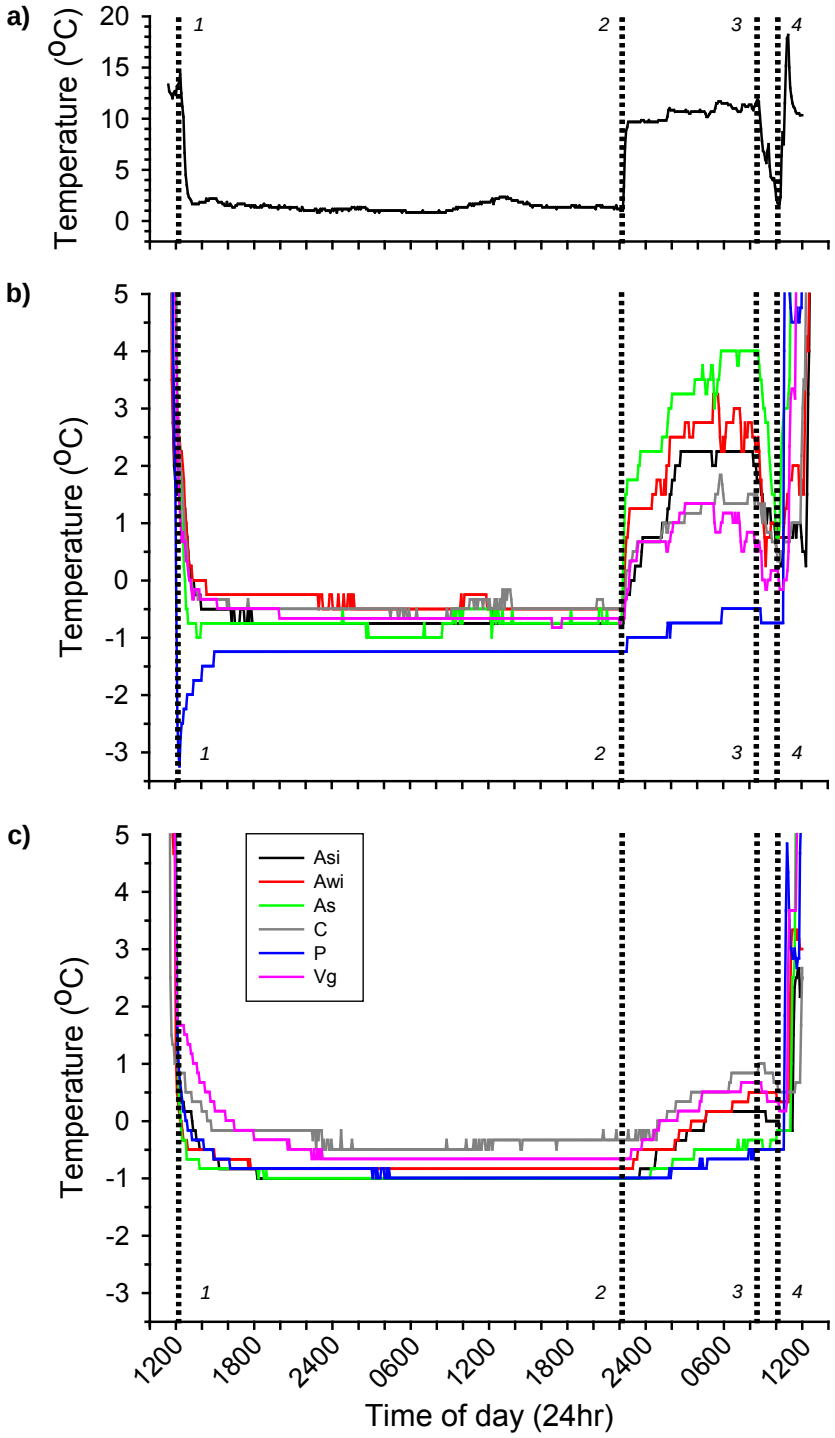

Figure 14. Mean temperature profile of domestic shipment of fresh, whole Yellowtail Kingfish (*Seriola lalandi*) from Port Lincoln, South Australia to the Sydney Fish Markets, Pyrmont, New South Wales (*n* = 3) (1 = Load truck in Port Lincoln, 2 = Unload truck at Sydney Fish Markets, Pyrmont, 3 = Box transferred to Joto chiller, Botany, 4 = Retrieve loggers). Temperature profile were for outside the box (a), inside the box (b) and inside the fish (c) when shipped in six packaging systems (polystyrene (P), Chillquest (C), Visy gusset (Vg), Amcor silver lined (As), Amcor silver insert (Asi) and Amcor white insert (Awi)).

A simulated export shipment was performed using the alternative fibre board boxes in a temperature controlled room. To challenge the ability of the boxes to maintain a non-violating temperature profile, the equivalent times when the product was on the ground (simulated) in Sydney and Singapore, the external box temperature was raised to 20-28^{o}C for up to 3.5 hr

As was observed in the Sydney shipment, the fibreboard boxes did not perform as well as the polystyrene box, especially during and after the periods of temperature abuse. This was reflected in the proportion of the frozen gel packs that were not thawed at the end of the trial (Figure 15).

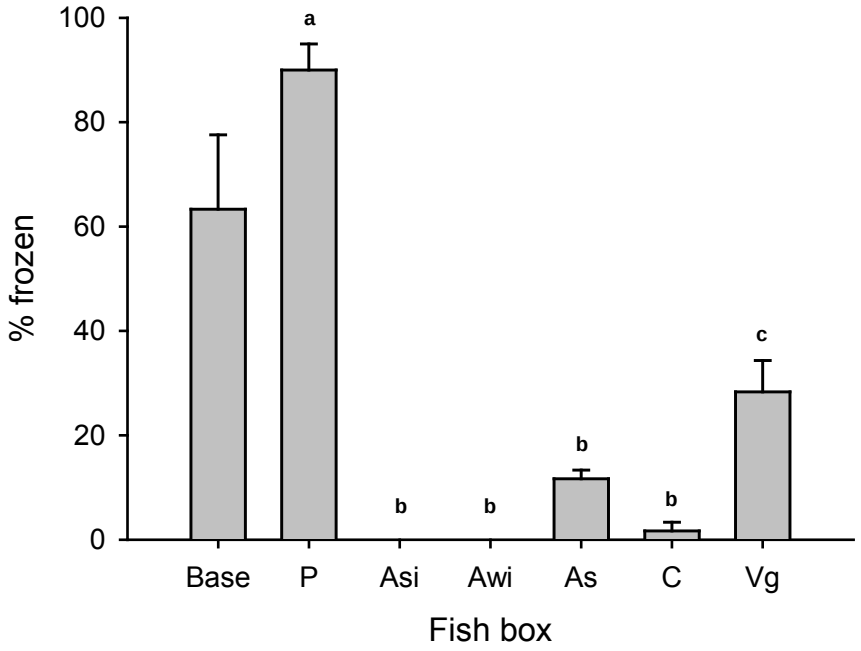

Figure 15. Mean (± se) proportion of gel packs that were frozen (*n* = 3) after simulated export shipment packed in six different packaging systems (polystyrene (P), Chillquest (C), Visy gusset (Vg), Amcor silver lined (As), Amcor silver insert (Asi) and Amcor white insert (Awi)). Baseline (Base) group were P boxes held at constant 4^{o}C for same period. Significance is indicated by different superscripts between groups ($p<0.05$) (Base not included in statistical analysis).

So, whilst fibre board boxes performed reasonably well on the domestic shipment where there were fewer opportunities for temperature abuse due to fewer transfers between supply chain partners, they did not offer equivalence in the export scenario. A decision to change packaging system needs to be made considering performance and costs. A preliminary comparison of the metrics that come into such an exercise is shown in Table 4. This Table takes account of the different box volumes (and therefore the number of fish they each hold), the weight of the box, the amount of ice needed to cover the fish in the box, the number of boxes and weight of packaging

(boxes+ ice) to ship 100 fish. This simple example shows that some of the fibreboard box types offer a 20% weight advantage over polystyrene boxes. Factors that have not been considered here include: the relative costs of each box, the amount of labour need to assemble, fill and seal each type of box, and whether freight costs are charged per kg or per unit volume.

Table 4. Pack-out weight of alternative packaging systems for whole, fresh Yellowtail kingfish (*Seriola lalandi*; average 3.4kg) without and with ~10 litre of flake ice to cover fish for domestic shipments. Sorted by packaging wt per 100 fish.

Box type*	Capacity (pieces)	Box wt (kg)	Ice (kg) per box	Packaging (kg/box)	Boxes per 100 fish	Packaging wt (kg) per 100 fish
Awi	8	1.64	4.6	6.24	13	78.0
Asi	8	1.6	4.9	6.50	13	81.3
As	8	2.4	4.2	6.60	13	82.5
Vg	7	2.14	4.9	7.04	14	100.6
P	5	0.68	4.5	5.18	20	103.6
C	5	1.36	4.7	6.06	20	121.2

* (P) Polystyrene, (Awi) Amcor white insert, (Asi) Amcor silver insert, (As) Amcor silver lined, (Vg) Visy gusset, (C) Chillquest

3. Benefits and Adoption

This entire project was carried out in very close consultation and collaboration with CST and its commercial partners in various processing and supply chain operations. The findings from each of the subprojects have been reported back to CST in a timely way and have been used by CST senior management, financial controller, production, harvest and post-harvest manager and the marketing team to:

- Understand the consequences of decisions to stock fingerlings to sea when water temperatures are still low
- Improve the growout operations area to better anticipate fish growth rates and feed requirements using the temperature-dependent growth model
- Identify a commercial feed that promoted rapid growth in small YTK (6g) for use in the CST hatchery and sea-cages
- Demonstrate the effects of temperature and hypoxia on juvenile YTK to highlight the potential impacts of sub-optimal environmental parameters
- Determine that reproductive maturation of farmed YTK harvested at weights up to 5kg and at age 2 years has negligible impact on production parameters or product yield and quality characteristics
- Help address customer complaints and other enquiries about product attributes
- Develop more comprehensive product specification information
- Address requirements in the nutritional labelling for YTK products
- Improve the basis for claims regarding the shelf life of chilled YTK fillet products
- Identify high priority issues and differentiate them from lower priority issues that relate to perceptions of YTK product quality in the marketplace.

4. Further Development

As always with projects of this nature, as many questions have been raised as have been answered. The topics for further research include:

- Evaluation of juvenile YTK survival and performance at water temperatures between 12°C and 15°C.
- The critical oxygen limits of different sized YTK reared in sea cage environments should be determined.
- Additional data on larger YTK (> 1000g) is required to extend the utility of temperature dependent growth models for this species.
- The nutrition of juvenile YTK requires further investigation.
- The microbiological spoilage patterns and profiles of YTK fillets need to be assessed.
- Spoilage profiles (physical, chemical, sensory and microbiological) of whole and HOGG YTK need to be determined.
- The shelf lives of YTK fillets and whole fish need to be conducted with respect to Ice Days.
- Differences in YTK product attributes (including assessment of shelf life by microbiological approaches) between harvest times (season) should be carried out with fish of different ages to try to separate any seasonal component from any age/growth component.
- A full cost analysis of the different packaging and freight options needs to be carried out, as this is critical to a decision to stay with polystyrene or switch to an alternative fibre board box.

5. Planned Outcomes

Public and Private Benefit Outcomes

The planned outcomes were:

> ***Managers will have new information on the effects of fish size and temperature on the performance of juvenile and sub-adult YTK which will allow them to determine the most appropriate time to stock YTK into sea cages. New stocking regimes will enhance survival of YTK and increase profitability.***

This outcome has been achieved. The mortality and growth rate issues associated with small fish going into cold water temperatures are now clear, and the SOP for transfer of fingerlings to sea cages has changed to reflect this.

> ***Develop assessment techniques and identify sampling sites necessary for the development of an ongoing quality control monitoring program.***

This outcome has been achieved. Different areas of the fillet differ in crude lipid content and no one sampling site reflects that variability. A method for objectively measuring YTK flesh colour has been developed. Freshness (K value) was a useful indicator of the quality of chilled product, but TBARS was not. Microbiological techniques needs to be applied for future product quality, spoilage and shelf life studies.

> ***To maximise the quality and consistency of the YTK product by identifying periods or groups of fish which are undesirable to harvest and changing stocking and harvesting strategies or differentiating the product to suit particular markets.***

This outcome has been achieved. The slightly reduced product quality (fillet fat and % yield) attributes associated with hatchery run 2 and 3 fish, and those that are graded small, were demonstrated. At the time of writing (February 2012), the business has not been in a position to introduce strategies (e.g. dietary modifications or a longer grow out time/size) to address the issue.

> ***The extent of maturation and its impact upon product quality will be determined – this will help identify the best approaches to future mitigation strategies. The cues controlling maturation will be determined, information which will be essential in developing an appropriate management strategy if maturation is of economic importance.***

This outcome has been achieved. Male YTK produced sperm in November – December at both age 1 and 2, but testes size were small and of little consequence. Female fish showed no reproductive development at these ages/sizes. There was no deleterious effect of reproductive

maturity on product yield or quality attributes for these fish. There is no need to introduce any mitigation strategy for YTK up to this age/size.

To improve market acceptance both domestically and overseas and to stimulate the growth of the industry, improving revenue for the YTK industry, seafood processors and others in the supply chain.

This ambitious outcome has been partly achieved. Markets domestically and internationally are more aware of the attributes of Australian farmed YTK, and farm gate prices have increased as a consequence of this and the readjustment of production volumes to match market demand. However, difficult global financial circumstances have had much more bearing on the size and growth potential of the Australian YTK sector.

Linkages with CRC Milestones

This project has addressed several Seafood CRC Milestones, including:

1.3.5 Milestone

Production efficiency gains from management and nutritional interventions quantified to inform long-term strategies and estimate commercial benefits

1.5.2 Milestone

Management systems for improved and more uniform condition of selected aquaculture species at harvest developed

2.8.1 Milestone

Microbiological, physical and biochemical spoilage mechanisms determined through chain

2.8.6 Milestone

Harvest, post-harvest and processing practices evaluated and enhanced to maximise and protect quality attributes and nutritional properties

6. Conclusion

This broad ranging study sought to increase our understanding about the performance of YTK in sea cages and the quality attributes of YTK products post-harvest, with the overall aim of improving product marketability. The three subprojects in this study have contributed to this by demonstrating:

- It is not advisable to rear juvenile YTK between 6 to 40g in bodyweight at water temperatures ≤12°C. Fish within this size range reared at 12°C became moribund, lost equilibrium, had difficulty feeding and died.
- Irrespective of water temperature, juvenile YTK subjected to chronic low dissolved oxygen concentrations (between 40 and 70% saturation) exhibited 14-20% less growth and 21-38% lower feed intake than normoxic (71 to 106% saturation) controls.
- The growth rate of juvenile YTK ≤ 1000g at water temperatures between 10°C and 29°C can be predicted from a growth model prepared as part of this study.
- Growth rate of juvenile YTK (from 50g to 100g) is optimal at water temperatures between 23-24°C.
- YTK grown in water temperatures below 17^{o}C, or above 28^{o}C, will achieve less than 50% of the growth rate of fish reared at 23-24^{o}C.
- This model will assist farm managers in forecasting growth rates of YTK based on actual ambient water temperatures at a site.
- Fish that have a bodyweight greater than 1.2kg have a 2-3 fold higher ($p<0.001$) level of crude fat in the ventral area (belly flap) than in the dorsal fillet area. Ventral crude fat reached a maximum of 35% of fillet weight whereas the dorsal fat level was more commonly 10-12% of fillet weight.
- CST usually produce 3 batches of fingerlings each year for stocking at sea, with each batch (or hatchery run) being 4-6 weeks after the previous one. This means that the fingerlings from hatchery run 1 have longer in the seacages during their first summer and grow faster in the warmer seawater. Fish that were from hatchery run 1 grow faster and have higher levels of fillet crude fat than fish from hatchery runs 2 or 3, and those from hatchery run 1 that were graded small. This may put the faster growing hatchery run 1 fish at an advantage when water temperatures cool and they have to rely more on their body reserves.
- The nutritional attributes of YTK fillet are very similar to that of a familiar and widely accepted farmed species (Atlantic salmon) in the marketplace. The flesh is typically ~23% protein and 6-15.5% lipid (depending on the fillet area). Levels of omega-3 fatty acids are between 1.1-2.8% of the total fatty acids, with EPA and DHA levels (1,600 to 2,500mg) in a small (100g)

serve of YTK fillet easily meeting the Australian National Health and Medical Research Council guidelines for recommended daily intake (RDI) for adult males (610mg) and females (430mg).

- Vacuum packed chilled fillets produced in this project had a shelf life of 12-15 days post-processing when stored at 4^{o}C. Shelf-life of the chilled vacuum packed fillets was mainly determined by the detection of a strengthening 'fishy' odour when the packet was opened suggesting microbial activity. This coincided with a freshness (K value) score of 60-70%. Lipid oxidation and fillet colour did not change during vacuum packed shelf life.
- 100% of male fish undergo spermatogenesis as in their first and second years of life, but the extent of sexual maturation (gonad growth as measured by gonadosomatic index - GSI, and milt volume) is limited (maximum mean GSI of 1.2% of bodyweight).
- Female fish do not show any signs of vitellogenesis in their first or second years of life.
- The incidence or extent of sexual maturation did not differ between fish that were derived from hatchery runs 1, 2 or 3 in each spawning year, and the sex ratio of the fish was 50:50.
- Sex of the fish did not affect growth rate or condition factor (i.e. the weight of the fish in relation to the body length).
- There were data indicating that 2 year old male fish (about 4kg in bodyweight) had a significantly ($p<0.05$) higher fillet yield than females (i.e. 64% vs 63% of body weight, respectively) at the when gonad size was largest (Nov-Dec).
- Together, these findings indicate that the production performance implications of sexual maturation on YTK to age 2 years and bodyweight of 4-5kg are minimal.
- As long as this product specification remains the focus for the industry no steps are needed to affect sex ratios, induce sterility or manipulate environmental factors that cue sexual development or maturation.
- The results from data loggers used in domestic and international supply chains showed that temperature remained within the specified range of -1 to +4^{o}C at all times.
- Fibre board boxes proved to be suitable alternatives to polystyrene boxes in a 36-48hr domestic supply chains, but did not provide equivalence of temperature control in a simulated export shipment when two periods of warm external temperature were applied. The best proof of this was the proportion of the gel packs that remained frozen at the end of the simulation. The polystyrene boxes gel packs were 90% frozen, the best of the fibre board boxes was less than 30% frozen, and two of the others were completely thawed.

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Appendix 1 – Intellectual property

There was no new intellectual property arising from this project.

Appendix 2 – Staff

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Dr Erin Bubner

Mark Thomas

Claudio Giordano

Andre Smith

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NSW DPI – Dr Mark Booth, Dr Geoff Allan, Dr Stewart Fielder, Ian Russell, Mitchell Elkins

Appendix A

Understanding Yellowtail Kingfish: Sub-project 1

Trent D'Antignana, Erin Bubner, Mark Thomas and John Carragher

Project No. 2008/903.10

AUSTRALIAN SEAFOOD COOPERATIVE RESEARCH CENTRE

This project conducted by Flinders University

Title: Understanding Yellowtail Kingfish: Sub-project 1

Authors: Trent D'Antignana[1], Erin Bubner[1], Mark Thomas[1] and John Carragher[2]

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Non-Technical Summary

2008/903.10 – Understanding Yellowtail Kingfish: Sub-project 1

PRINCIPAL INVESTIGATORS: Dr Trent D'Antignana and Dr Erin Bubner

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OBJECTIVES:

1. To provide YTK producers and consumers detailed information on the nutritional and biochemical composition of YTK to improve market access.
2. To identify how flesh composition, post-harvest processing methods and cold chain management techniques influence the shelf-life stability of the processed YTK product.
3. To develop farm management strategies, which improve flesh quality attributes and production levels and increase market access.

NOTE: This project was undertaken as part of an overarching project "2008/903 Understanding Yellowtail Kingfish", which was comprised of three stand-alone sub-projects. This final report presents activities carried out within sub-project 1.

NON-TECHNICAL SUMMARY

In a comprehensive series of sampling events and trials, this project addressed a number of important questions and perceptions relating to product specification and product shelf life of YTK fish and fillets produced by Clean Seas Tuna (CST) during 2008-10. The main findings included:

- Fish that have a bodyweight greater than 1.2kg have a 2-3 fold higher ($p<0.001$) level of crude fat in the ventral area (belly flap) than in the dorsal fillet area. Ventral crude fat reached a maximum of 35% of fillet weight whereas the dorsal fat level was more commonly 10-12% of fillet weight.
- Fillets from fish larger than 1.2kg bodyweight could be processed into dorsal and ventral portions, with the latter possibly being sold as differentiated product if the market opportunity was established;
- CST usually produce 3 batches of fingerlings each year for stocking at sea, with each batch (or hatchery run) being 4-6 weeks after the previous one. This means that the fingerlings from hatchery run 1 have longer in the seacages during their first summer and grow faster in the warmer seawater. Fish that were from hatchery run 1 grow faster and have higher levels of fillet crude fat than fish from

hatchery runs 2 or 3, and those from hatchery run 1 that were graded small. This may put the faster growing hatchery run 1 fish at an advantage when water temperatures cool and they have to rely more on their body reserves.

- The nutritional attributes of YTK fillet are very similar to that of a familiar and widely accepted farmed species (Atlantic salmon) in the marketplace. The flesh is typically around 23% protein and 6-15.5% lipid (depending on the fillet area). Levels of omega-3 fatty acids are between 1.1-2.8% of the total fatty acids, with EPA and DHA levels (1,600 to 2,500mg) in a small (100g) serve of YTK fillet easily meeting the Australian National Health and Medical Research Council guidelines for recommended daily intake (RDI) for adult males (610mg) and females (430mg).
- Vacuum packed chilled fillets produced in this project had a shelf life of 12-15 days post-processing when stored at 4^{o}C. Shelf-life of the chilled vacuum packed fillets was mainly determined by the detection of a strengthening 'fishy' odour when the packet was opened suggesting microbial activity. This coincided with a freshness (K value) score of 60-70%. Lipid oxidation and fillet colour did not change during vacuum packed shelf life. Drip loss increased with time to be about 3% of the fillet weight at day 15 of storage.
- During the project an objective method for measuring flesh colour in YTK fillets was developed and validated.

This study also determined whether the supply chains for chilled YTK products practiced proper cold chain management that will protect the shelf life and product quality attributes in major domestic and international destinations. The main findings included:

- The results from data loggers used in domestic and international supply chains showed that fish temperature remained within the specified range of -1 to +4^{o}C at all times during transport.
- Fibre board boxes proved to be suitable alternatives to polystyrene boxes in a 36-48hr domestic supply chains, but did not provide equivalence of temperature control in a simulated export shipment when two periods of warm external temperature were applied. The best proof of this was the proportion of the gel packs that remained frozen at the end of the simulation. The polystyrene boxes gel packs were 90% frozen, the best of the fibre board boxes was less than 30% frozen, and two of the others were completely thawed.

The findings of this study have been utilized by CST by improving their knowledge and understanding of the attributes and specifications, including the shelf life limitations, of YTK sold as chilled fresh vacuum packed fillets. Furthermore, the demonstrated effectiveness of the chilled product supply chains both domestically and internationally, has given CST marketers more confidence that those supply chain partners have the appropriate cool chain management practices to protect the quality of YTK products.

KEYWORDS: Yellowtail kingfish, product quality, shelf-life, post harvest, cold chain management

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- Mike Thomson, Joe Ciura and Clean Seas Tuna Ltd farm operations staff for kingfish husbandry and assistance in sample collection.
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- Beverley Stephens, Helen Rammers and Clinton Wilkinson, South Australian Shellfish Quality Assurance Program, for fillet shelf life microbiology.

1. Introduction

Seafood is widely perceived as being a tasty, nutritious, healthy and high quality food choice, and many consumers would like to eat more (Danenberg and Mueller, 2011). Despite this, however, sales of seafood are constrained by a number of market factors, including: competition from other types of meat that compare very favourably in $/kg; convenient, value-added meal options that offer a near restaurant quality eating experience without the chef knowhow; a shrinking proportion of the population that is knowledgeable and confident in buying or cooking seafood; and, even within the seafood offer, a great deal of competition between products for the customers spend (Olsen, 2004). With so many less expensive, more convenient, easier to cook and more enduring (longer shelf life) alternative food choices, a seafood product needs to offer 'something special' to make the sale, particularly at the 'premium' end of the market.

Much of the 'something special' about the offer of aquacultured finfish is the potential to consistently supply high quality fish to the market. Consistency is gauged on several metrics; in terms of quantity (volume) and quality of product supply, and operates at the scale of day-to-day, week-to-week, and season to season. Inconsistency in any of these measures might drive customers to select other product choices. A producer of aquacultured finfish needs to be aware of these market requirements for consistency, and do everything possible to ensure that those standards are met. However, for a number of reasons that are seasonal, production- and/or post-harvest-related, this is easier said than done. One of the essential aspects of an aquaculture operation is the need to know what these factors are, what their consequence(s) could be, and how they can be mitigated. Consistency in product quality is the focus of the current project.

This study was comprised of six activities, each with distinct aims:

1. To examine the effects of season on crude fat distribution in fillets from YTK.
2. Determine the nutritional profile of commercially harvested YTK in winter and summer so there is more detailed nutritional information known about these products, and this can be passed on to customers.
3. To examine how whole body proximate composition, vitamin content and distribution in the flesh could change during the grow-out period, and if there are differences between spawning runs.
4. To determine whether fillet shelf life is influenced by season, and whether dorsal

and ventral areas of the fillet have different shelf life profiles.

5. Identify whether the shelf life of vacuum packed fillets is different depending on whether the fillet has been shallow skinned, or skin on.
6. Investigate the cold chain management of chilled YTK products to major domestic and international markets, and to determine the suitability of five alternative fibre board boxes to replace the standard polystyrene box for shipping chilled YTK to domestic and international markets.

1.1 Need

When this project was initiated YTK production was increasing and substantial new market opportunities were being developed for YTK product. During the project, global economic conditions deteriorated and the YTK production strategy was changed to reflect more realistic sales target. Essentially, both strategies rely on the business marketing products that are consistently of the highest possible quality. This is only attainable if the business has good knowledge and understanding of the quality attributes, shelf-life and nutritional content of the products, and, as a consequence, the business is able to tailor products to specific market requirements. The majority of YTK is marketed internationally, so substantial packaging and freight costs have to be incorporated into the price being charged in those markets, and this can impact on sales volumes and margins. Cost-effective alternative packaging systems that offer equivalent (or better) cold chain performance to the current system need to be considered based on scientifically collected data. Performance of all supply chain partners needs to be monitored to ensure that practices and processes are not compromising the quality and shelf life of the product when it arrives in the market.

1.2 Objectives

1. To provide YTK producers and consumers detailed information on the nutritional and biochemical composition of YTK to improve market access.

2. To identify how flesh composition, post-harvest processing methods and cold chain management techniques influence the shelf-life stability of the processed YTK product.

3. To develop farm management strategies, which improve flesh quality attributes and production levels and increase market access.

2. Methods

Fish sampled in this study were managed according to normal commercial practice. Thus, factors such as feeding regime, feed type, fish health management, pontoon relocation, grading and splitting, and harvest schedule were outside the control of this project. The impact of these factors on pontoons that needed to be harvested seasonally was minimised where possible.

2.1 Activity one: fillet crude fat distribution

2.1.1 Experimental fish

Two cohorts of fish stocked in two separate sea cages were sampled in this activity. One cage was stocked with fish spawned in August 2008 (run 1) and the other cage contained fish originating from spawning events in both September (run 2) and October 2008 (run 3).

2.1.2 Sampling collection and analysis

Ten fish were harvested, bled and held in ice slurry as per industry practice from each cohort at each of six regular intervals: September 2009, January 2010, April 2010, August 2010, October 2010 and January 2011. January and October were considered warmer months, April, August and September were cooler months. Within one to two days after harvest the chilled fish were taken to a commercial processing facility in Port Lincoln. Lengths and whole wet weights were recorded. YTK were filleted as per "Japanese cut" (head and tail removed, gutted, left and right whole fillets produced with pectoral fins, rib and pin bones left intact) then vacuum packed, held in insulated boxes and kept cold with frozen gel packs. On the same day as processing, the chilled fillets were taken to the Lincoln Marine Science Centre (LMSC) in Port Lincoln for sub-sampling. For fat mapping a ~20g cross section of muscle was taken from each of four distinct sites on the right side fillet as illustrated in Figure 1. To try to represent the average whole fillet fat level a "Quality Cut "cross section of dorsal and ventral muscle was taken from the left side fillet as illustrated in Figure 2 (it was assumed there was no difference in fat content or distribution between left and right fillets given the symmetrical muscle plan). Sub samples were held at -20˚C until analysis was performed (see section 2.7.1).

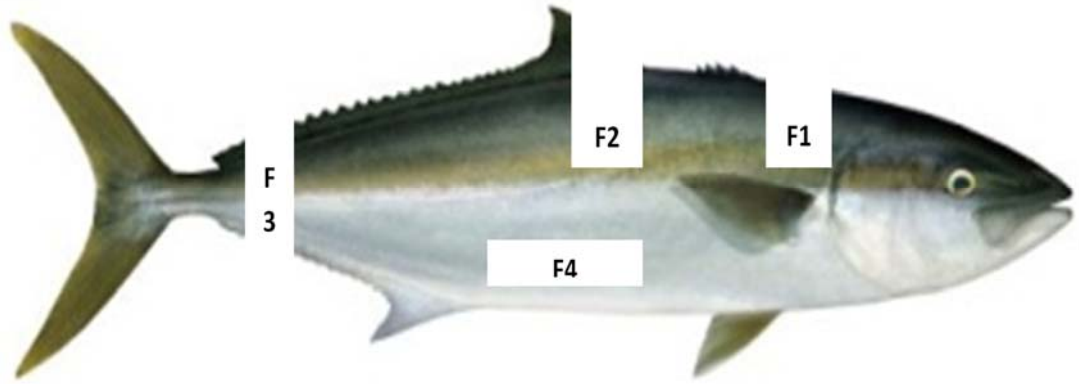

Figure 1. Approximation of Yellowtail kingfish (*Seriola lalandi*) right fillet sampling sites for mapping of crude fat. F1 and F2 are dorsal fillet cuts, F3 is a tail cut including both dorsal and ventral meat and F4 is a ventral or "belly" cut.

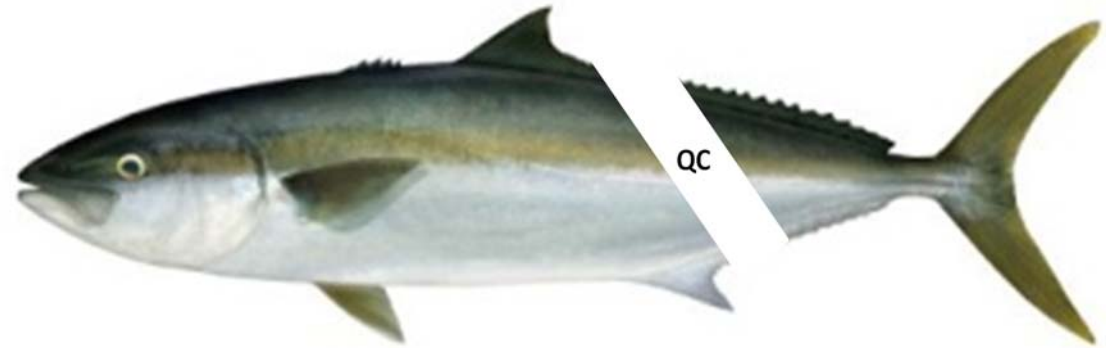

Figure 2. Approximation of Yellowtail kingfish (*Seriola lalandi*) left fillet sampling site for the quality cut (QC), a cross section of both dorsal and ventral meat to represent whole fillet crude fat.

2.1.3 Statistical analysis

To test for effects of season and hatchery run on fillet crude fat distribution a three way analysis of variance (ANOVA) was performed in Sigma Plot 11/0.0.75. Independent factors were season, hatchery run and fillet sampling site (i.e. position on fillet) while the dependant variable was crude fat. To test for effects of season and hatchery run on crude fat content of YTK quality cut a two way ANOVA was performed. Independent factors were season (i.e. month fish were harvested) and hatchery run while the dependant variable was crude fat. Where assumptions of normality and / or equal variance were not met data were log transformed. P value was set at 0.05.

2.2 Activity two: nutritional composition of YTK fillets

2.2.1 Experimental Fish

One cohort of fish (spawned in August 2007, hatchery run 1) held in sea cages offshore of Port Lincoln, South Australia were sampled in this activity in September 2009 (winter) and January 2010 (summer).

2.2.2 Sampling collection and analysis

At each sampling event 10 fish were harvested by hook, bled and held in ice slurry as per industry practice. Within one to two days after harvest the chilled fish were taken to a commercial processing facility in Port Lincoln. Lengths and whole wet weights were recorded. YTK were filleted and the fillets were further separated into dorsal and ventral loins with skin and bones removed. On the same day as processing, the chilled loins were taken to the LMSC in Port Lincoln. There, the loins were individually homogenised to a fine mince in a domestic food processor and a sub-sample (~100 g) was immediately stored at -80°C until sent for analyses.

Frozen samples were shipped on dry ice to the IANZ (International Accreditation New Zealand) accredited laboratories of Assure Quality Ltd. Auckland, New Zealand for analyses. Nutritional parameters measured were proximates (crude fat, protein, moisture and ash), fatty acids, minerals (elements) and energy (kJ). Analyses were conducted by mass spectrometry, titration, gas chromatography and gravimetric methods. Detailed laboratory methodology is commercial in confidence.

2.2.3 Statistical analysis

To test for effects of season and loin cut on nutritional parameters two way ANOVA was performed in Sigma Plot 11/0.0.75. Independent factors were season (summer and winter) and loin cut (dorsal and ventral). There were 25 dependant variables separately tested covering proximates, minerals and energy. Where assumptions of normality and / or equal variance were not met data were log transformed. P value was set at 0.05.

2.3 Activity three: whole body proximate composition

2.3.1 Experimental fish

Fish from three distinct hatchery runs were sampled on four occasions over an 11 month period in this activity. Separate cages stocked with fish spawned in August 2008 (hatchery run 1), September 2008 (hatchery run 2) and October 2008 (hatchery

run 3) were sampled in June 2009. However, due to factors outside of the control of this project, in September 2009, January 2009 and May 2010 fish were sampled from hatchery runs 1, 2 and a cage that held a mixture of similar size-graded fish from runs 2 and 3.

2.3.2 Sampling collection and analysis

Ten fish were harvested, bled and held in ice slurry as per industry practice at each sampling point and from each cohort of fish. Length and whole wet weight were recorded. These fish were retained as whole fish (i.e. no onboard processing) and transported back to the LMSC, Port Lincoln in insulated boxes kept cold with frozen gel ice packs. Within 24 hours of harvest each whole fish was individually homogenised using an industrial meat mincing machine and duplicate sub-samples (approximately 100g) from each fish were retained at -80°C until required for analysis of concentration of vitamin E
(α-tocopherol) and C (ascorbic acid) (see section 2.7.2 and 2.7.3, respectively). One frozen sub-sample was shipped on dry ice to the IANZ (International Accreditation New Zealand) accredited laboratories of Assure Quality Ltd. Auckland, New Zealand for analysis of whole body proximates. Nutritional parameters measured were crude fat, protein, moisture and ash. Detailed laboratory methodologies are commercial in confidence.

2.3.3 Statistical analysis

To test for effects of season and hatchery run on whole body proximate composition and vitamin content two way ANOVA was performed in Sigma Plot 11/0.0.75. The independent factors were season (i.e. month fish were harvested) and hatchery cohort (run 1, 2, 3 or 2/3). The dependant variables were the proximate variables (crude fat, protein, moisture and ash) and vitamins (α-tocopherol and ascorbic acid). Where assumptions of normality and / or equal variance were not met data were log transformed. P value was set at 0.05.

2.4 Activity four: fillet shelf life

This activity was comprised of two components. The first component was to develop a quantitative method of measuring colour changes in fillets of YTK. This was carried out using two groups of fish known to have visually distinct fillet colour; these were harvested as part of a separate confidential study conducted for CST. The second component was designed to determine (a) the vitamin content and distribution in the flesh, (b) whether the dorsal and ventral loins of a fillet exhibit differences in shelf life

and (c) if either the age/size of fish and/or seasonal factors influence fillet shelf life. The methods used for these components are described below.

2.4.1 Component one – developing quantitative method to measure colour change in fillets

Nine farmed YTK were harvested, euthanised in carbon monoxide (CO) infused seawater then bled and held in ice slurry. Another group of 30 YTK were taken from a standard commercial harvest (using percussive stunning and bleeding) and held in ice slurry. On the following day all fish were taken to a commercial processing facility in Port Lincoln. Length and whole weights were recorded, fish were "Japanese cut" filleted (head and tail removed, gutted, left and right whole fillets produced with skin on and bone in), and the separate fillets were vacuum packed and held in insulated boxes chilled with frozen gel packs. On the same day as processing, the vacuum packed fillets were taken to the LMSC in Port Lincoln and held for up to 14 days at 4°C. Vacuum packed fillets from both CO (n=3) and commercially harvested (n=10) groups were opened periodically to assess colour differences between groups and colour changes over time (see section 2.7.4).

2.4.2. Component two – shelf life of fillets and vitamin content and distribution over grow-out

2.4.2.1 Experimental fish

Fish from hatchery run 1 (spawned August 2007) were sampled during commercial harvests in June 2009, October 2009 and January 2010. It was intended to continue sampling from the same cohort in April 2010 but for commercial reasons that sampling was instead conducted on a cohort spawned in September 2007 (run 2).

2.4.2.2. Sampling collection and analysis

One hundred fish were harvested, bled and held in ice slurry as per industry practice at each of four commercial harvests (see *2.4.2.1*). The fish were taken from the start of a commercial harvest to minimise any variations in flesh quality attributes that may be due to harvest stress. Within one to two days after harvest the chilled fish were taken to a commercial processing facility in Port Lincoln. Length and whole wet weights were recorded. The fish were filleted as per "Japanese cut" (head and tail removed, gutted, left and right fillets produced, skin on, bones in). A sample of flesh was excised using a 15 mm diameter stainless steel coring tool from the dorsal and ventral regions of the right side fillet, and the cores were wrapped, labelled and packed onto dry ice for transport back to the LMSC. The left fillet from each fish was

weighed, and then both the left and right side fillets from each fish were vacuum packed (flesh down on absorbent paper), stacked into insulated boxes with frozen gel packs and transported to LMSC.

Immediately after arriving at the LMSC, the flesh samples excised from the dorsal and ventral regions of the right fillet were transferred to -80°C storage prior to analysis of vitamin E and C (see section 2.7.2 and 2.7.3, respectively). The vacuum packed fillets were stored at 4°C for up to 30 days. Ten fillets were opened on each of 10 fillets at 0, 2, 4, 6, 8, 12, 16, 20, 25 and 30 days refrigerated storage, with flesh colour, drip-loss, TBARS and K value being assessed (see section 2.7.4; 2.7.5; 2.7.6 and 2.7.7, respectively).

Fillet colour, drip-loss, TBARS and K value were also determined for fillets that had been stored in vacuum packs that had been opened and stored inside the plastic pack at 4^{o}C for 1 and 3 days (i.e. open +1 day and open +3 days). These packs were opened at 0, 4, 8, 15 and 30 days storage.

2.4.3 Statistical analysis

All analyses for this activity were conducted by two way ANOVA in Sigma Plot 11/0.0.75. To test for effects of season and fillet area on fresh (day 0) fillet vitamin content the independent factors were season (i.e. month fish were harvested) and fillet area (i.e. dorsal and ventral). The dependant variables were fillet vitamins (α-tocopherol and ascorbic acid). Owing to the considerable time and expense of conducting the various biochemical shelf life analyses, the effects of fillet area and shelf storage were tested to determine if it was necessary to assay all of the tissues that were collected. To this end the independent factors were fillet area (dorsal, ventral) and shelf life (days post filleting) while the dependant variable was K value. To test for the effects of season and shelf life on biochemical quality indicators, season (harvest event) and shelf life (days post filleting) were independent factors and K value, TBARS and drip loss were dependant variables. Where assumptions of normality and / or equal variance were not met data were log transformed. P value was set at 0.05.

To test for shelf storage effects on fillet L*a*b* colour, MANOVA (multiple analysis of variance) was conducted in SPSS 19. The independent factor was shelf storage (days post filleting) while L*a*b* were co-variates. This test was used because the intercept of the co-variates represented the L*a*b* position in the 3 dimensional

colour space. Each test was a single comparison between Day 0 and subsequent days. The tests were performed separately on each seasonal sampling event.

2.5 Activity five: fillet skin on / skin off shelf life

2.5.1. Experimental fish

One cohort of fish (spawned September 2008, hatchery run 2) held in sea cages offshore of Port Lincoln, South Australia, were sampled in this activity in February 2010 (summer) and August 2010 (winter).

2.5.2. Sample collection and analysis

Sixty fish were harvested, bled and held in ice slurry as per industry practice. Within one to two days after harvest the chilled fish were taken to a commercial processing facility in Port Lincoln. Length and whole wet weights were recorded. The fish were then filleted as per "Japanese cut" (head and tail removed, gutted, left and right fillets produced and bone in). One fillet from each fish was shallow skinned by a skilled fish processor, while the other fillet was left with the skin on. The individual fillets were then vacuum packed (skin (or skinned side) up, with an absorbent pad), stacked into insulated boxes and transported to the LMSC. The fillets were then stored at 4°C for up to 30 days.

Ten skin on, and ten skin-off, matched fillets were opened at 0, 5, 10, 15, 20 and 30 days of refrigerated storage to measure TBARS. Additional sampling was carried out after the packs had been opened for 1 and 3 days (i.e. open +1 day and +3 days) with 4^{o}C refrigeration between determinations. At each sampling event a flesh sample was excised using a 15 mm diameter cylindrical stainless steel coring tool from the ventral and dorsal region of each fillet (Figure 3) and homogenised separately using a domestic blender. Homogenized samples were stored at -80°C until required for analysis. Analysis of oxidative state was done according to methods outlined in section 2.7.6.

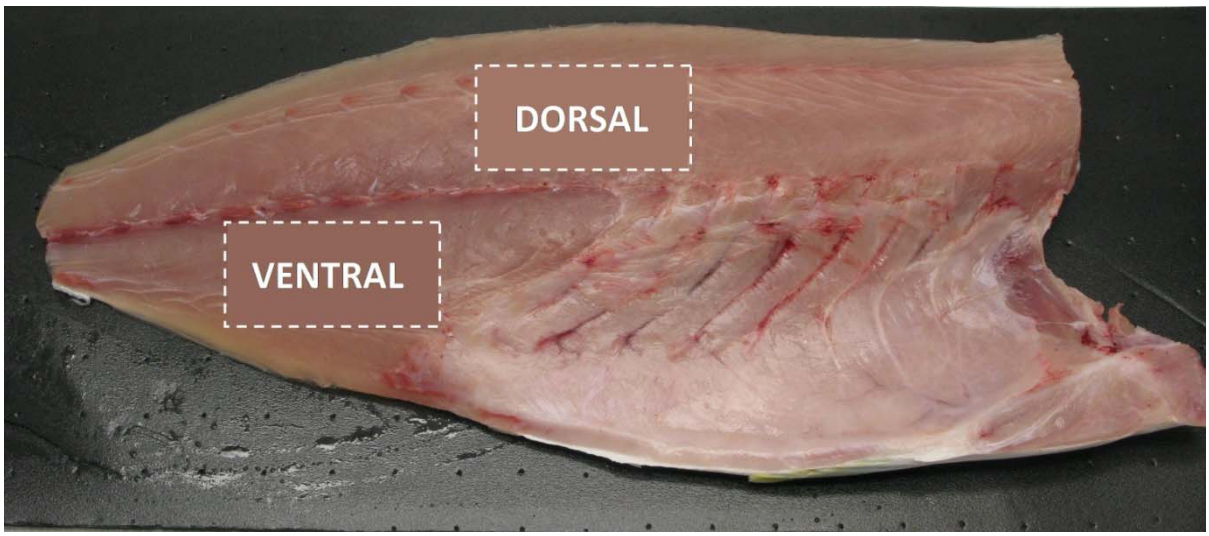

Figure 3. Pre-determined location of sub-sample of tissue from the ventral and dorsal region of each Yellowtail kingfish (*Seriola lalandi*) fillet.

2.5.3 Statistical analysis

To test for effects of shelf storage and fillet skinning on fillet quality a two way ANOVA was conducted in Sigma Plot 11/0.0.75. The independent factors were shelf life (i.e. days post filleting) and skin status (i.e. skin on and skin off) while the dependant variable was TBARS. Tests were conducted separately for dorsal and ventral areas in fish sampled in two distinct seasons. Where assumptions of normality and / or equal variance were not met data were log transformed. P value was set at 0.05.

2.6 Activity six: effect of temperature fluctuations on fillet shelf life

2.6.1 Cold chain management practices

The temperature profiles of chilled shipments of whole YTK to Thailand, USA, Italy, Switzerland and the Netherlands were examined (Table 1). To access the temperature profile of each route, three temperature loggers (Dallas semi-conductor DS1921G Thermochron) were assigned to each box: one was placed inside the body cavity of one fish, another was located between the plastic liner and the outside of the box, and the final one was attached to the outside end wall of the box. For each shipment five polystyrene boxes were examined, which were placed strategically within each shipment. At the destination the loggers were retrieved and posted to the LMSC for downloading and analysis.

Table 1. Shipments of whole, fresh Yellowtail kingfish (*Seriola lalandi*) that were tracked from Australia to Thailand, USA, Italy, Switzerland and Netherlands.

Date	Origin	Destination
February 2010	Port Lincoln, Australia	Rome, Italy
May 2010	Port Lincoln, Australia	Bangkok, Thailand
May 2010	Whyalla, Australia	Philadelphia, USA
July 2010	Port Lincoln, Australia	Los Angeles, USA
July 2010	Port Lincoln, Australia	Zurich, Switzerland
July 2010	Port Lincoln, Australia	Philadelphia, USA
November 2010	Port Lincoln, Australia	Amsterdam, Netherlands

2.6.2 Alternative packaging systems

A variety of fibre board box prototypes were sourced from the Australian packaging companies Amcor Ltd, Chillquest Pty Ltd, and Visy Industries as potential alternatives to traditional polystyrene fish boxes. The boxes assessed in this activity are shown in Figure 4 with brief descriptions as follows:

1. **Polystyrene (P)** – standard 20 kg long box with lid. Thick walls. Pre-assembled. 32 litres.
2. **Chillquest (C)** (manufactured by Visy) – Fibre board carton with lid. Thin walls. Self assembly. 32 litres.
3. **Visy gusset (Vg)** – Fibre board carton. Water resistant white lined inner. Gusset outer. Thick walls. Self assembly. 43 litres.
4. **Amcor silver lined (As)** – Fibre board carton. Water resistant silver lined inner. Medium thickness. Hot glue or packing tape bottom self-assembly. 53 litres.
5. **Amcor silver insert (Asi)** – Fibre board carton. Water resistant / reflective silver inner and outer walls. Insert lid. Outer lid. Thick walls. Self assembly. 49 litres.
6. **Amcor white insert (Awi)** – Fibre board carton. Water resistant white inner and outer walls. Insert lid. Outer lid. Thick walls. Self assembly. 49 litres.

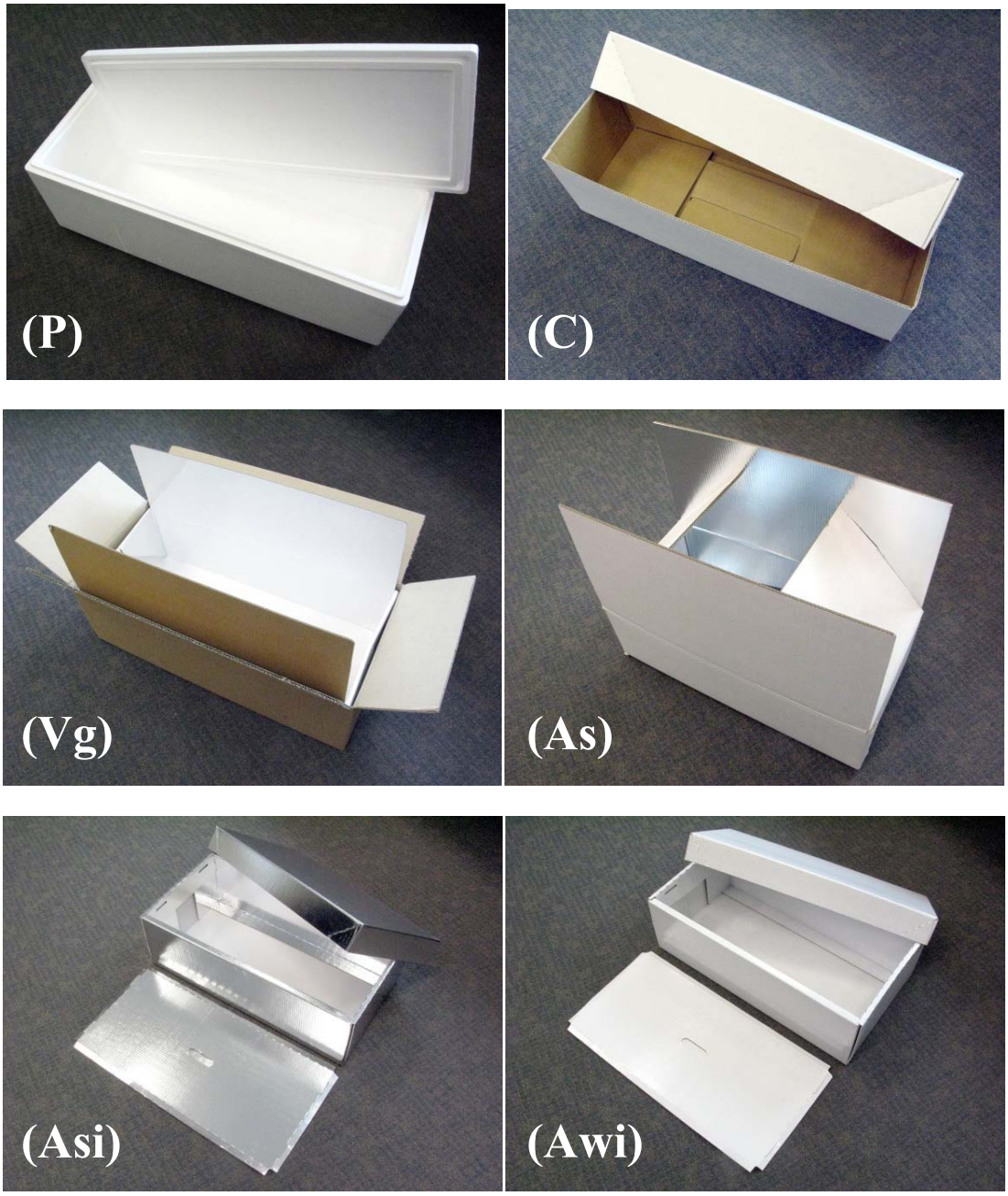

Figure 4. Packaging system: Polystyrene (P), Chillquest (C) (manufactured by Visy), Visy gusset (Vg), Amcor silver lined (As), Amcor silver insert (Asi), Amcor white insert (Awi).

After packing, polystyrene boxes are conventionally taped around the lid perimeter and then strapped laterally. The Visy gusset (Vg) and Amcor silver lined (As) fibre board cartons have an open flap lid design, the other cartons have separate lids. All fibre board cartons required strapping but taping was not necessary.

2.6.2.1 Domestic shipping

At one day post-harvest, whole YTK (mean individual weight 3.4 kg) were commercially processed and packed for domestic shipping at Australian Bight Seafoods Ltd, Port Lincoln, South Australia. Ten fish were randomly selected from the processing line and assessed for Quality Index (QI) using a modified version of the YTK method outlined in Boulter et al. (2009), where scores were given to a range of visual and sensory qualities, which were inversely related to product freshness (Table 2). On completion of QI assessment fish were returned to the processing line. Each of the six box types was packed with five YTK inside a standard domestic plastic liner bag (40µm thick) in random order (n = 3 per box type) on the processing line. Three fish from each box were fitted with an identifying tag and an anterior dorsal flesh sample was excised with a 15 mm diameter stainless steel coring tool. The flesh sample was immediately placed on dry ice until transferred to -80°C storage until required for analysis of freshness (K value) (see section 2.7.7). Three pre-programmed temperature loggers were assigned to each box: (1) inserted into the body cavity of one of the tagged fish; (2) located between the plastic liner and inner wall of the box; and (3) attached to an outer end wall of the box. Approximately 10 litres of flake ice was poured over the contents of each box before the liner was closed and the box secured with lateral straps using an automated strapping machine. All experimental boxes were stacked on one pallet and within approximately one hour the boxes departed for Joto Fresh Fish, Botany, NSW (via distribution at SFM, Pyrmont, NSW) by refrigerated road transport.

Table 2. Quality Index Scheme for Yellowtail kingfish (modified from Boulter et al. (2009))

	Quality Parameter	Description	Score
Skin	**Colour/ appearance**	Bright &/or iridescent. Strong blue or blue-green upper with yellow band on midline, white-silver below. Lower fins bright yellow. Lateral line indistinct when viewed from ventral.	**0**
		Loss of brightness &/or iridescence. Prominent scale pattern. Olive band on midline. Viewed from dorsal - Pink tone on 2nd operculum & base of pectoral and ventral fins. Lateral line becoming evident.	**1**
		Dull &/or matt. Bronze tinge. Lateral line bronze. Pink tone on 2nd operculum & base of pectoral and ventral fins. Green tinge on body.	**2**
	Scales	Attached	**0**
		Loose / missing	**1**
	Slime (if present)	Clear / absent	**0**
		Slightly cloudy / brown	**1**
		Milky or opaque	**2**
	Odour	Fresh sea / neutral	**0**
		Not so fresh or cabbage or sour	**1**
		Off or rotten	**2**
	Texture of flesh	Firm, bounce when pressed	**0**
		Slightly soft, slow bounce back	**1**
		Soft, finger mark remains over 3 seconds	**2**
	Rigor	Pre	**0**
		In Rigor	**1**
		Post	**2**
Eyes	**Form**	Convex	**0**
		Flat	**1**
		Concave	**2**
	Cornea/jelly	Clear	**0**
		Cloudy	**1**
		Fully opaque	**2**
	Pupils/Iris	Pupil - black. Iris - yellow.	**0**
		Pupil - dull black. Iris - yellow-black.	**1**
		Pupil - cloudy / grey. Iris - pale bronze / grey	**2**
Gills	**Colour/ appearance**	Red/dark red	**0**
		Brown-red &/or some discolouration	**1**
		Brown &/or discoloured	**2**
	Mucus	Milky/cloudy	**0**
		Cloudy brown	**1**
		Brown	**2**
	Odour	Fresh seawater/seaweed.	**0**
		Not so fresh, stale	**1**
		Sour, vegetable, meaty, chemical, oily, rancid	**2**
		Rotten	**3**
Quality Index			**0-24**

The shipment arrived at Joto Fresh Fish approximately 44 hours after processing. At approximately 46 hours after processing the boxes were unpacked in random order. From each box, temperature data loggers were retrieved, QI measurements were taken from all tagged fish as previously described and anterior dorsal flesh cores were excised from the opposite fillet to those sampled at processing in Port Lincoln. Flesh samples were immediately placed on dry ice until transferred to -80˚C storage until required for analysis of freshness (K value) (see section 2.7.7).

2.6.2.2 Export shipping

A simulated export shipment was conducted at the LMSC. The packing and sampling of the fish was the same as that reported in 2.6.2.1, except an additional three polystyrene boxes were included and kept at a constant (4^{o}C) temperature to provide baseline (control) data. In addition, the other differences were that two gel ice packs per box were used instead of the flake ice and an additional three dorsal flesh samples (skin on, approximately 1 cm^{3}) from two fish per box were taken for microbiological analysis (i.e. total plate counts) at the start and conclusion of the experiment (see section 2.7.8). After packing, the boxes were transported to the LMSC in a refrigerated truck where they were then held in a controlled environment. The simulated export shipment was based on a typical shipment to Zurich, Switzerland, determined from the first component of activity six (see section 3.6.1 and Appendix 3: Figure A16). However, the temperature regime assumed poor product handling in Singapore (i.e. boxes exposed to 20-28°C for 3.5 hours at the appropriate time point) so as to provide data on a worst case scenario (Table 3). The control polystyrene boxes were held at 4°C for the remainder of the experiment (i.e. from event 4 to 12).

Table 3. Simulated export shipment conditions of whole, fresh yellowtail kingfish (*Seriola lalandi*).

Event no.	Description of event	Period (hour)	Temperature (°C)
1	Factory floor	3	13-15
2	Factory chiller	1	4
3	Non-refrigerated van	1	18
4	Road / air freight	24	1
5	Singapore ground	3.5	20-28
6	Singapore chiller	4.5	6
7	Air freight	11	1
8	Zurich ground	3	10
9	Zurich chiller	5	4
10	Out of chiller	4	10
11	Market	3	18
12	Retrieve logger	-	-

At the completion of the simulated export event the temperature data loggers were retrieved, the proportion of each gel pack that remained frozen was determined, Quality Index measurements were taken from all tagged fish (as described in section 2.6.2.1) and anterior dorsal flesh cores were excised from the opposite side of the fish to that sampled at the processors and sent for immediate microbiological analysis (see section 2.7.8), or placed on dry ice then transferred to -80˚C storage until required for analysis of freshness (K value) (see section 2.7.7).

2.6.2.3 Packing weight and packaging system durability

One of each of the six types of boxes was lined with a plastic bag and weighed. Each box was then packed to capacity with YTK (i.e. five to eight fish, depending on the size of the box) (mean individual fish weight approximately 3.4 kg) and either ~10 litres of flake ice (enough to cover the fish) or two gel ice packs, and re-weighed.

Eight fish (mean individual weight approximately 2.2 kg) and two gel packs were packed in to each box type and secured by packing tape. They were then dropped from a height of 1.6 metres onto a concrete floor. The drops were recorded to video for slow motion viewing to assess relative box strength under a typical impact scenario.

2.6.3 Statistical analysis

To test for differences in temperature profiles between boxes over the transport period, area under the curve (AUC) was calculated on time series data from each box type in the statistical/data analysis package NCSS 2007 07.1.21. The AUC values were then analysed by one way ANOVA and Student-Newman-Keuls post-hoc multiple comparison test in SPSS 19. P value was set at 0.05. The statistical design was identical in both domestic and export experiments. To test for differences in quality indicators (i.e. K value, Quality Index and total plate count) between fish held in different box types one way ANOVA was performed in Sigma Plot 11/0.0.75. One way ANOVA was also used to test for differences in the proportion of the gel pack frozen between box types at the completion of the transport period. Where assumptions of normality and / or equal variance were not met data were log transformed. P value was set at 0.05.

2.7 Sample analysis

2.7.1 Crude fat determination

The crude fat content of the flesh was determined with an ethyl acetate extraction method based on the Norwegian Standard method (NS 9402 E) (NSA, 1994). Immediately prior to analysis, frozen tissue samples were allowed to partially thaw at 19°C, any skin and bone in the sample were removed and then the flesh was homogenised to a fine mince in a domestic food processor. A weighed sample of approximately 10 g of homogenised flesh, 40 g of anhydrous sodium sulphate and 80 ml of ethyl acetate were put into a clean polypropylene bag and agitated in the stomacher mixer (IUL Instruments) for three minutes. The resulting extract was filtered (Whatmans GF/C filter papers). Then 40 ml filtrate was decanted into a pre-weighed plastic beaker and placed in a fume hood overnight by which time all the solvent had evaporated. The beakers were then placed in an oven at 80°C for approximately 60 minutes to remove any traces of moisture and then weighed to determine the fat weight (± 0.001 g), which was expressed as a percentage of the muscle wet weight (g) using the following formula:

$$Crude\ fat\ content\ \% = \frac{\left(\frac{Extracted\ fat\ (g)}{Dilution\ Factor}\right)}{Extracted\ Muscle\ wet\ wt\ (g)} \times$$

2.7.2 α-tocopherol (vitamin E) determination

The α-tocopherol (vitamin E) content of the flesh was determined by HPLC method, which was based on Huo et al. (1999). Briefly, vitamin E was double extracted from

homogenised muscle in 60 ml of methanol (meth) - butylhydroxytoluene (BHT) solution (1ml of meth to 1 mg of BHT). Homogenisation of the tissue depended on the form of the sample. If the sample was a -80°C frozen muscle core it was crushed to a powder by mortar and pestle on dry ice, 0.95 – 1.05g was added to 40ml meth/BHT in a 50ml centrifuge tube then vortexed for the first extraction. If the sample was -80°C frozen minced muscle, 0.95 – 1.05g was sectioned off and added to 40ml meth/BHT then homogenised by Omni-Prep (Omni International, USA) for the first extraction. First extraction continued for three hours on ice with hourly re-suspension by 15 second vortex. After three hours the homogenate was centrifuged for four minutes at 4500 rpm. 3.9 ml of the resulting supernatant was pipetted into a 10 ml tube and set aside on ice. The remaining supernatant was discarded and the tissue pellet re-suspended by vortex in 20 ml meth-BHT solution for the second extraction. After 30 minutes on ice, the homogenate was centrifuged as previously and 2.1 ml of the supernatant was combined with the 3.9ml set aside from the first extract. The combined extracts were mixed by vortex for 30 seconds then filtered (0.45 µm). Samples were then eluted through a Prevail C18 5µm column (Waters, 150 mm x 4.6 mm) with methanol:distilled water (98:2) as the mobile phase. The α-tocopherol was detected using a Waters 2475 fluorescent detector (filters: 296 nm excitation and 340 nm emission) connected to a Waters 2695 separations module refrigerated at 4°C. Using an α-tocopherol standard of known concentration the concentration of vitamin E (mg.kg^{-1} wet weight) in the tissue was calculated using the following equation:

$$Vitamin\ E = \frac{\left(\left(\frac{[Area\ of\ vit\ E\ std]}{Area\ of\ sample}\right) \times Dilution\ factor\right)}{(Extracted\ Muscle\ ww\ g)}$$

2.7.3 Ascorbic acid (vitamin C) determination

Ascorbic acid (vitamin C) was determined by a technique based on the HPLC fluorometric method of Brown and Miller (1992), which was adapted to use with fish muscle at the LMSC with the cooperation of CSIRO Hobart Tasmania. As in the vitamin E method, tissue was, depending on its sample form, homogenised by mortar and pestle or Omni-Prep. A sample of 1.5 g of muscle tissue was combined with 5 ml of ice cold metaphosphoric acid (MPA) solution containing 20 µl of 0.2 M glutathione - ethylene glycol tetra-acetic acid (EGTA) solution in a 10 ml tube and vortexed for 15 seconds. The tube was mixed by vortex again after five minutes and then left for two hours on ice. After two hours, the tube was centrifuged for 15 minutes at 4000 rpm and 1.5 ml of the resultant supernatant was combined with 400 µl of 4.2 M sodium acetate in another 10 ml tube and vortexed. Prepared samples and standards were then placed in a 37°C water bath for 10 minutes. Vitamin C was reduced to de-hydro ascorbic acid with the addition of ascorbic oxidase (Roche Diagnostics, Germany) and 250 µl of 0.1 % o-phenylene diamine was added to induce the florescence of de-hydro ascorbic acid. Following 30 minutes at 37°C, samples were filtered (0.45 µm) and eluted using a Altima C18 5µm column (Waters, 250 mm x 4.6 mm) with 0.08 M di-potassium phosphate in 20 % methanol as the mobile phase (pH 7.8). De-hydro ascorbic acid was detected using a Waters 2475 Fluorescent detector (filters: 355 nm excitation and 425 nm emission) connected to a Waters 2695 separation module. The concentration of vitamin C (µg of vit C g^{-1} wet weight of tissue) was then calculated using the following equation:

$$Vitamin\ C = \frac{\left(\left(\frac{[Area\ of\ vit\ C\ std]}{Area\ of\ sample}\right) \times Dilution\ factor\right)}{(Extracted\ Muscle\ ww\ g)}$$

2.7.4 Fillet colour

At each assessment event individual left fillets were placed meat side up with the anterior end to the left in a purpose built light box with matte white interior. The light source was four parallel fluorescent tubes (cool white, 18W) fixed above the subject. The fillet was blotted with paper towel to reduce reflection then photographed with a Canon G9 *Powershot* digital still camera from directly above. Manual camera settings were saved to custom as follows: 12 mega pixels, compression superfine, custom white balance (with matte white card), film speed ISO 200, shutter speed 1/30 sec, aperture F 8.0, macro, no flash and the focal length was fixed with the zoom adjusted to fit each fillet comfortably in the frame. These settings were found to deliver close to true colour (as assessed by eye) under these lighting conditions. The fillets were kept

on the black backing board they were placed on in the vacuum pack to minimise handling of the flesh and make it easier to do the photography.

After consultation with a professional graphic designer a process was developed in Adobe Photoshop™ CS5 to apply a colour "blur average" of all pixels in fixed representative sample areas on each fillet (Figure 5). Blur average was subsequently applied to groups of blur averages to provide one averaged colour for each group for the purposes of visual comparison. To quantify colour, measurements were generated from the blur averages in Photoshop™. For the purposes of meat colour assessment the L*a*b* colour model (Photoshop™ the CIELAB D50 protocol) was chosen as it approximates what is seen by the human eye. L*a*b* are coordinates that can be plotted as single points on a three dimensional chart. These data can be statistically analysed by multivariate analysis of variance (MANOVA).

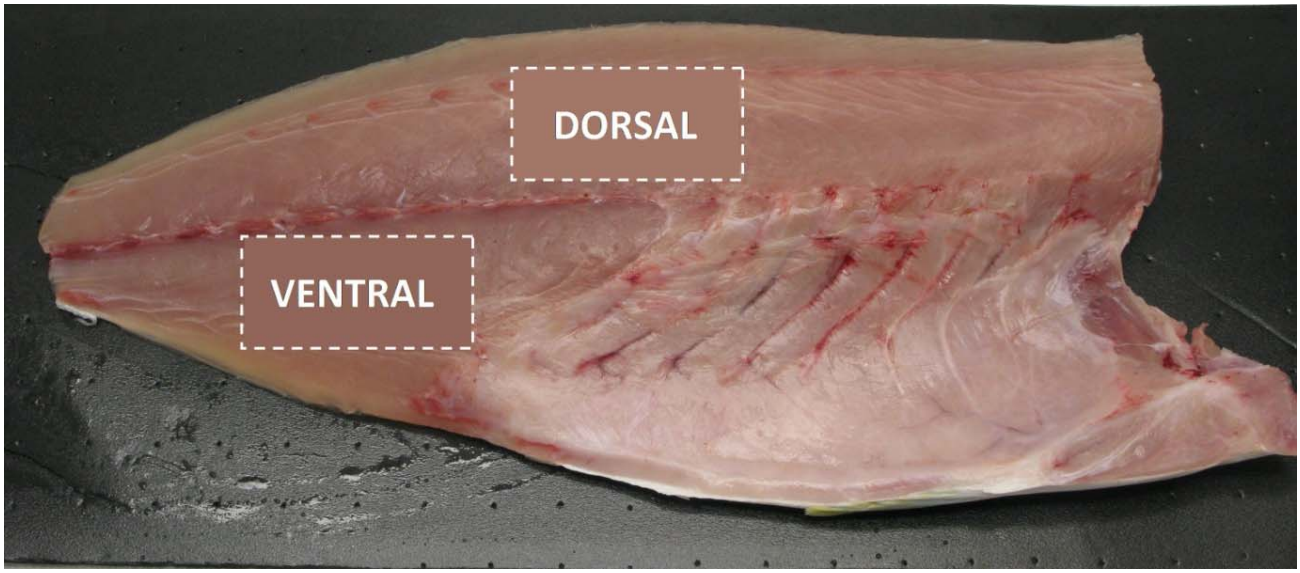

Figure 5. A digital photograph of a Yellowtail kingfish (*Seriola lalandi*) fillet taken under controlled conditions and imported into Photoshop™. A "Blur Average" in Photoshop™ has then been performed on a prescribed area of the dorsal and the ventral loin of the fillet (indicated on the image). The blurred areas yield average, quantitative colour values (L*,a*, b* or R,G,B) for the fillet, which can be used to statistically assess treatments.

2.7.5 Drip loss

At each time point 10 fillets were removed from their vacuum packs, blotted dry and individually weighed. Drip loss was then determined by the change in the weight of the fillet over time.

2.7.6 Lipid oxidation (2-thiobarbituric acid reactive substances, TBARS)

TBARS expressed as malonaldehyde concentration in fish muscle was determined using a spectrophotometric method modified after Wong et al. (1991) on a Multiskan Ascent (Thermo Labsystems) multi-plate reader (D'Antignana, 2007). Cored flesh samples were crushed to a powder by mortar and pestle on dry ice then a weighed amount (~1g) was added to a 10 ml centrifuge tube containing 5 ml of cold 0.6 M perchloric acid and vortexed for 15 seconds. The samples were then allowed to extract on ice. After 20 minutes, samples were centrifuged for 5 minutes at 4000 rpm. The supernatant was then filtered (0.45 µm) into 2ml cryogenic vials for storage at -80^{o}C until analysis.

Extracted samples were removed from -80°C storage and thawed at a cool room temperature. A serial dilution of tetraethoxypropane (malonaldehyde inclusive) standards (Sigma) appropriate to the expected range of sample results was prepared and 500 µl of 0.02M 2-thiobarbituric acid solution was added to 500 µl of sample extract or the standards in 10 ml vials. The vials were vortexed and then incubated in a 100°C water bath for 35 minutes. The vials were removed and allowed to stand for 15 minutes at room temperature. Duplicate 300 µl aliquots were transferred from each vial to a 96 well micro plate and their absorbance measured at 540 nm. TBARS (mg.kg^{-1} wet weight flesh) was calculated from the standard curve using the following formula:

$$X = \left(\frac{1000}{A}\right) \times \left(\frac{B}{C}\right) \times 0.00072$$

where *A* = weight of flesh extracted (g), *B* = volume of perchloric acid the flesh was extracted in, *C* = volume used in assay, and *X* = concentration of malonaldehyde in the tissue (mg.kg^{-1} wet weight flesh).

2.7.7 K value

The breakdown products of the nucleotide ATP were analysed by the HPLC methods of Ryder (1985), Van der Boon et al. (1992) and Ozogul et al. (2000). Nucleotides were extracted in 0.6M perchloric acid as per the TBARS method (section 2.7.6).

Filtered (0.45 µm) extracts were analysed by HPLC in a phosphate buffer / acetonitrile gradient mobile phase. ATP breakdown components were eluted on a Prevail C18 5µm column (Waters, 150 mm x 4.6 mm) and identified by UV detector at 254nm.

K value was calculated as the percentage of end products in the total pool of ATP products as per Huss (1995):

$$K\ (\%) = \frac{[Hx] + [HxR]}{[ATP] + [ADP] + [AMP] + [IMP] + [IHx] + [HxR]} \times 100$$

where *ATP* = adenosine triphosphate, *ADP* = adenosine diphosphate, *AMP* = adenosine monophosphate, *IMP* = inosine monophosphate, *Hx* = hypoxanthine and *HxR* = inosine.

2.7.8 Total plate count

Muscle tissue samples (skin on) were excised from pre-determined positions on the carcase/fillet using sterile scalpel blades and forceps and transferred into separate sterile containers in an esky with ice packs for transportation to the South Australian Shellfish Quality Assurance Program laboratory, Port Lincoln for analysis. Sampling was carried out using good aseptic technique.

Total plate counts were performed according to standard AS 5013.5-2004 (ISO 4833:2003), AS 5013.1-204, AS 5013.14-2004, ISO 6887-3-2003, AS 4276.3.1-2007 and AS/NZS 2-031.2001. Upon arrival at the laboratory (<8 hours after the samples were taken) the flesh samples were diced with scalpel blades, weighed and transferred into a stomacher bag. Peptone saline water was added to the bag to make a 1 in 10 dilution and the tissue was homogenised for 2 minutes at 230 rpm. The resultant homogenate was then serially diluted with peptone saline water out to the desired point (i.e. 1 in 100, 1 in 1000, 1 in 10^5 and 1 in 10^6). Each dilution was then plated by pipetting 1 ml of dilution into replicate petri dishes. Approximately 15 ml of agar, which had been melted and brought to 45°C, was added into each petri dish. The dishes were gently rotated by hand to distribute the agar which was then allowed to solidify. After complete solidification, the plates were inverted and incubated at 25°C for 96 hours. After the incubation period, the number of colonies in each dish (maximum 300 colonies) was counted. The number (N) of colonies per gram (cfu.g^{-1}) of muscle was calculated using the following formula:

$$N = \frac{\sum C}{V \times [n_1 + (0{,}1 \times n_2)] \times d}$$

Where $\sum C$ is the sum of the colonies counted on all the dishes retained from two successive dilutions, and where at least one contains a minimum of 15 colonies, *V* is the volume of inoculums applied to each dish in ml, n_1 is the number of dishes retained in the first dilution, n_2 is the number of dishes retained in the second dilution and *d* is the dilution factor corresponding to the first dilution retained.

3. Results

3.1 Activity one: fillet crude fat distribution

Fillet crude fat content varied with fillet site, season and hatchery run (Figure 6). In general, at most sampling events the ventral belly fillet site (F4) had a crude fat content 2-3-fold higher (8-35%) than in the other fillet sites (3-10%). Fillet crude fat level (all sites) appeared to be higher in cooler seasons, and hatchery run 1 fish had ~30% higher crude fat levels than fish from hatchery runs 2 and 3 sampled at the same time, except in January 2011 where a 'graded small' cohort of run 1 fish was sampled. It is not possible to separate the effects of season and fish size/growth from this study. Compared with hatchery run 1 there appeared to be a 2-3 month lag (the resolution of the sampling protocol) in peak fat content in the hatchery run 2/3 group. The lag in peak fat between run 1 and run 2/3 corresponded with the two month difference between spawning dates of the fish in the two groups.

A three way ANOVA indicated a significant difference was evident in YTK fillet crude fat content between hatchery run (F_1=16.492, P < 0.001), season (F_5 = 91.750, P <0.001) and fillet area (F_3 = 340.078, P < 0.001) (Figure 6). A significant interaction was also evident between hatchery run and season (F_5 = 19.484, P < 0.001) and hatchery run, season and fillet area (F_{15} = 4.210, P < 0.001) (Figure 6). The significant interactions indicate that the effect of one factor is not consistent at all combinations of the two other factors and therefore it is not possible to clearly interpret the influences of the main effects (i.e. hatchery run, season and fillet area) on crude fat content.

Crude fat content of the quality cut was generally similar to the mean level from fillet sites F1, F2 and F3 (compare Figure 6 and 7). Crude fat content of the quality cut generally increased over the sampling period in both hatchery runs from approximately 4% in September 2009 to approximately 10% in October 2010, and again there appeared to be a two month lag in the peak of crude fat content in hatchery runs 2/3 compared to run 1, which corresponded with the difference in spawning dates between the runs (Figure 7). The quality cut crude fat content was significantly affected by hatchery run (F_1 = 13.250, P = <0.001), season (F_5 = 22.747, P = <0.001) and the interaction of hatchery run and season (F_5 = 4.151, P = 0.002) (Figure 7).

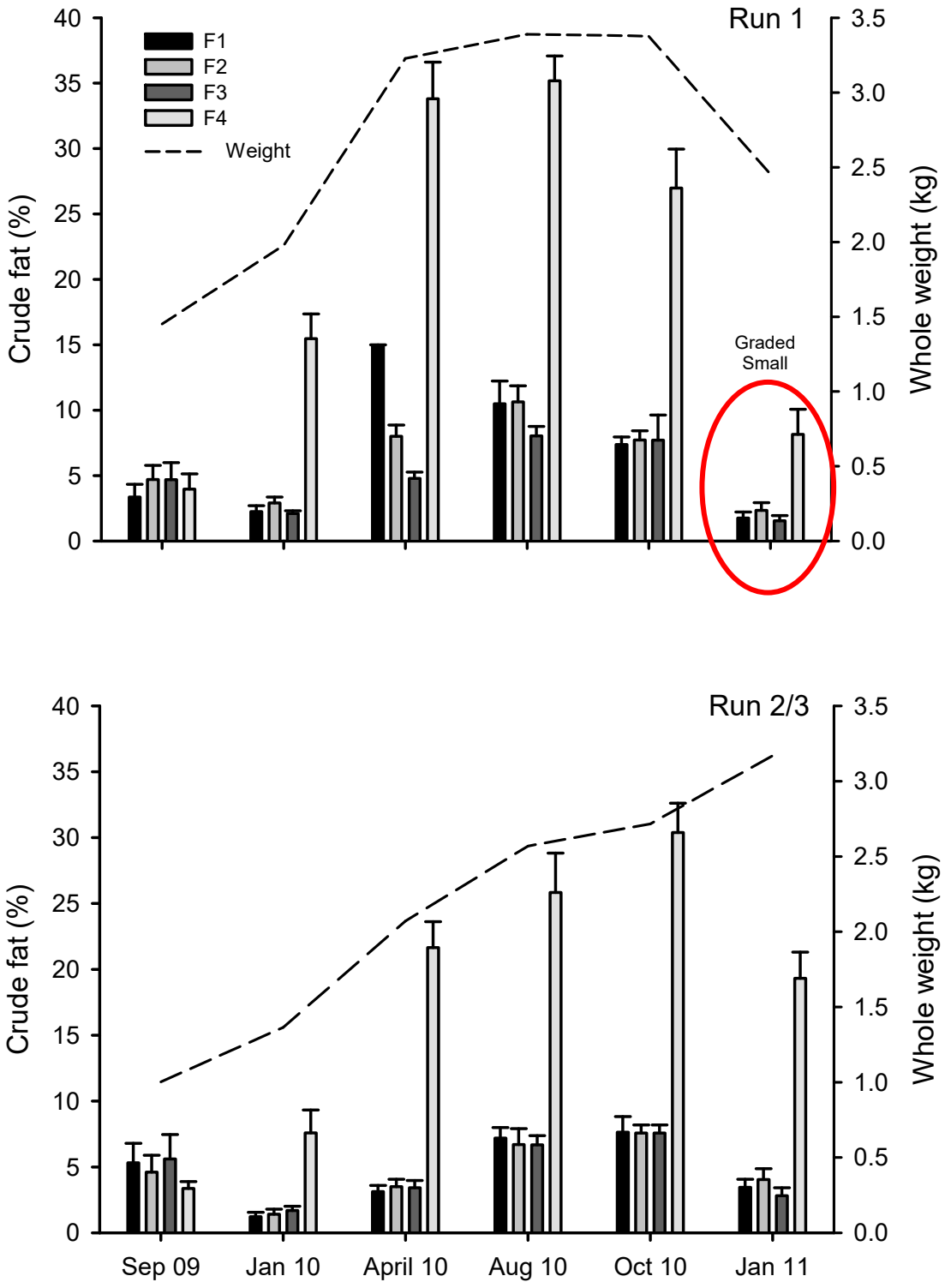

Figure 6. Mean (±se) fillet crude fat distribution and mean whole weight in farmed Yellowtail kingfish (*Seriola lalandi*) (graded medium) spawned in August 2008 (hatchery run 1) and September /October 2008 (combined hatchery runs 2 and 3). Fish sampled in January 2011 from run 1 were from a different cohort (graded small) to previous months. *n* = 10.

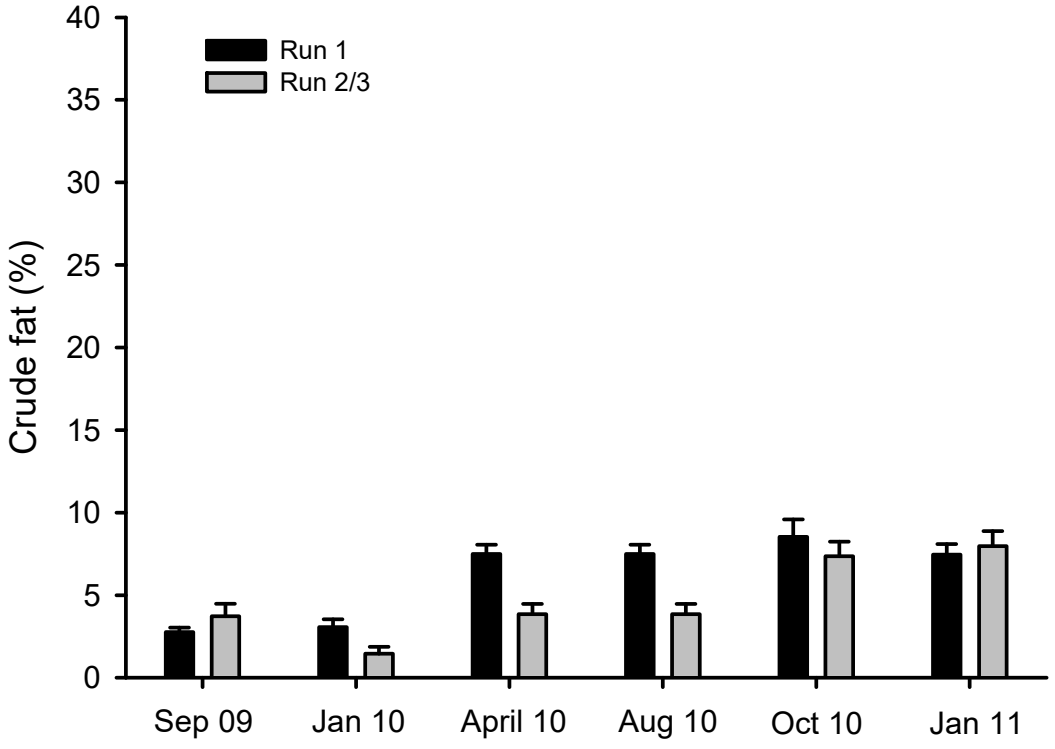

Figure 7. Mean (±se) quality cut crude fat (%) in farmed Yellowtail kingfish (*Seriola lalandi*) (graded medium) spawned in August 2008 (hatchery run 1) and September / October 2008 (combined hatchery run 2 and 3). Fish sampled in January 2011 from run 1 were from a different cohort (graded small) to previous months. *n* = 10.

3.2 Activity two: nutritional composition of YTK fillets

Fillet composition varied between seasons and fillet regions, with most of the changes being due to differences in crude fat and the inverse relationship between fat and moisture (Table 4). Fillet protein levels were between 21-24%; being significantly higher in the dorsal flesh compared to the ventral area, and slightly, but again significantly, higher in winter than in summer. The analytical method detected no carbohydrate in the flesh. Ash content could not be analysed statistically, but it was low and consistent (1.4 to 1.6%) across seasons and fillet regions. The energy content of the flesh varied (677 to 964 kJ/100g) with season and fillet area in a way that reflected the crude fat composition of the meat.

There were significant effects of season and fillet area (i.e. dorsal and ventral) on fillet crude fat, saturated fat, mono- and polyunsaturated fats, omega-9, omega-6, omega-3, EPA, DPA and DHA fatty acid levels (Table 4 and Figure 8). In each case,

the trend was for higher values in summer compared with winter, and higher values in the ventral area compared to dorsal area of the fillet.

Lipid classes were proportionally similar in the dorsal and ventral areas, and did not change with season (Figure 9). About 30% of the lipid was saturated, *trans* fats were very low (<1%), about 40% was monounsaturates and 30% was polyunsaturates. Of the polyunsaturates, about 65% was omega-3 (Table 4); comprised mostly of EPA (408 to 1155 mg/100g), with DHA (394 to 846 mg/100g) and DPA (152 to 356 mg/100g) making up the remainder (Figure 10). A sum of these 3 fatty acids gives a total flesh LCPUFA omega-3 level between 954 and 2,357 mg/100g.

The mineral content of the fillet meat is shown in Table 4 and Figures 11-13. Although there were some statistically significant effects of season, or interaction between season and fillet area, most of the differences across regions and seasons were relatively slight. The relevance of the mineral and the omega-3 fatty acid values will be discussed later, when they are in context and compared to levels found in other species and with reference to nutritional dietary requirements.

Table 4. Yellowtail kingfish (*Seriola lalandi*) (2007 hatchery run 2) harvested from September 2009 (Winter) and January 2010 (Summer). Skinned and bone-out dorsal and ventral areas of fillet analysed (*n* = 10). Two way ANOVA tested for main and interaction effects of season and fillet (P = 0.05). *Fail = assumptions of ANOVA not met.

Nutritional component	***Winter***		***Summer***		***P value***		
	Dorsal	**Ventral**	**Dorsal**	**Ventral**	**Season**	**Fillet**	**Season x Fillet**
Proximates							
Protein (%)	24.4	22.2	23.0	21.2	<0.001	<0.001	
Crude Fat (%)	6.4	12.7	9.2	15.5	<0.001	<0.001	
Moisture (%)	68.6	63.9	67.1	62.3	<0.01	<0.001	
Carbohydrate (%)	0.0	0.0	0.0	0.0	n/a	n/a	n/a
Ash (%)	1.4	1.6	1.6	1.5			*Fail**
Energy (kJ/100g)	677	875	760	964	<0.001	<0.001	
Lipid types							
Saturated fat (%)	1.7	3.3	2.5	4.1	<0.001	<0.001	
Trans fat (%)	0.0	0.1	0.1	0.2			*Fail**
Polyunsaturated fat (%)	2.0	4.0	2.9	4.9	<0.001	<0.001	
Monounsaturated fat (%)	2.6	5.2	3.8	6.4	<0.001	<0.001	
Unsaturated fatty acids							
Omega 9 FA (%)	1.9	3.8	2.7	4.5	<0.001	<0.001	
Omega 6 FA (%)	0.7	1.5	1.0	1.7	<0.01	<0.001	
Omega 3 FA (%)	1.1	2.2	1.6	2.8	<0.001	<0.001	
20:5n-3 EPA (mg/100g)	408	887	648	1155	<0.001	<0.001	
22:6n-3 DHA (mg/100g)	394	708	552	846	<0.001	<0.001	
22:5n-3 DPA (mg/100g)	152	301	214	356	<0.01	<0.001	
Micronutrients and minerals							
Fe (mg/100g)	0.4	0.5	0.4	0.4			
Ca (mg/100g)	6.6	7.6	5.8	6.2	<0.05		
P (mg/100g)	250	271	244	233			*Fail**
Na (mg/100g)	35.2	58.8	30.9	30.2			<0.001
K (mg/100g)	425	384	422	361			0.047
Cu (mg/100g)	0.1	0.1	0.1	0.0			<0.001
Zn (mg/100g)	0.6	0.6	0.5	0.5			*Fail**
Mg (mg/100g)	32.6	32.8	32.1	26.1			*Fail**
Mn (mg/100g)	0.000	0.012	0.004	0.004			*Fail**
S (mg/100g)	278	231	218	216			<0.001

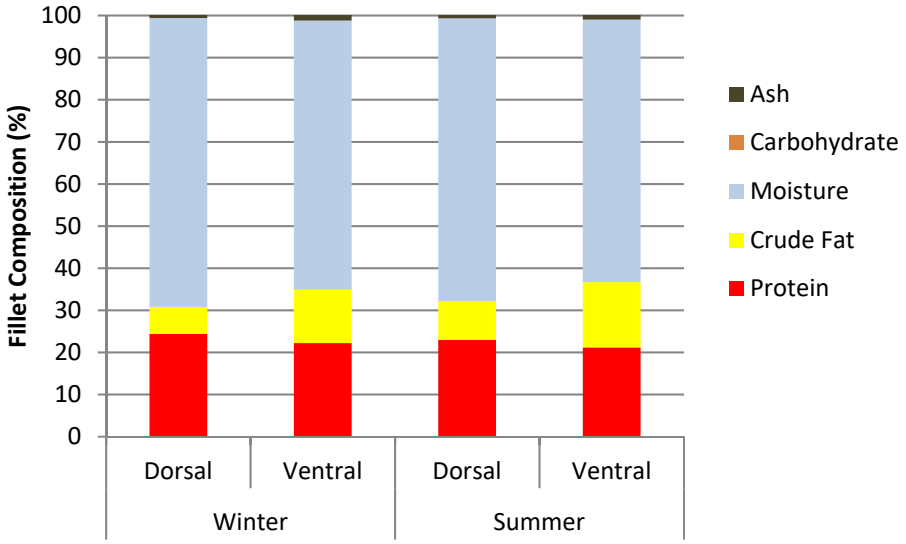

Figure 8. Stacked chart showing mean % proximate composition of dorsal and ventral areas of the fillet of Yellowtail kingfish (*Seriola lalandi*) (2007 hatchery run 2) harvested from September 2009 (Winter) and January 2010 (Summer).

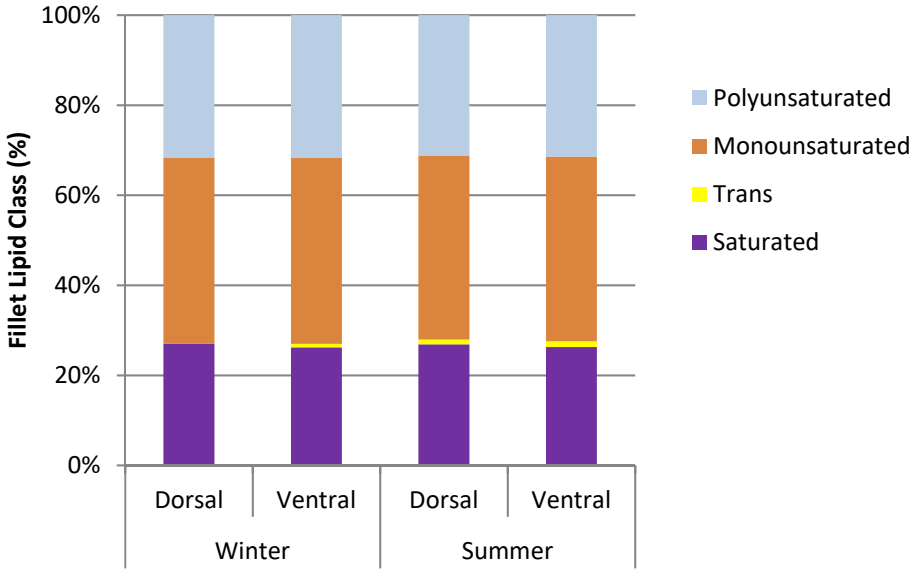

Figure 9. Stacked chart showing mean % lipid class composition in dorsal and ventral areas of the fillet of Yellowtail kingfish (*Seriola lalandi*) (2007 hatchery run 2) harvested from September 2009 (Winter) and January 2010 (Summer).

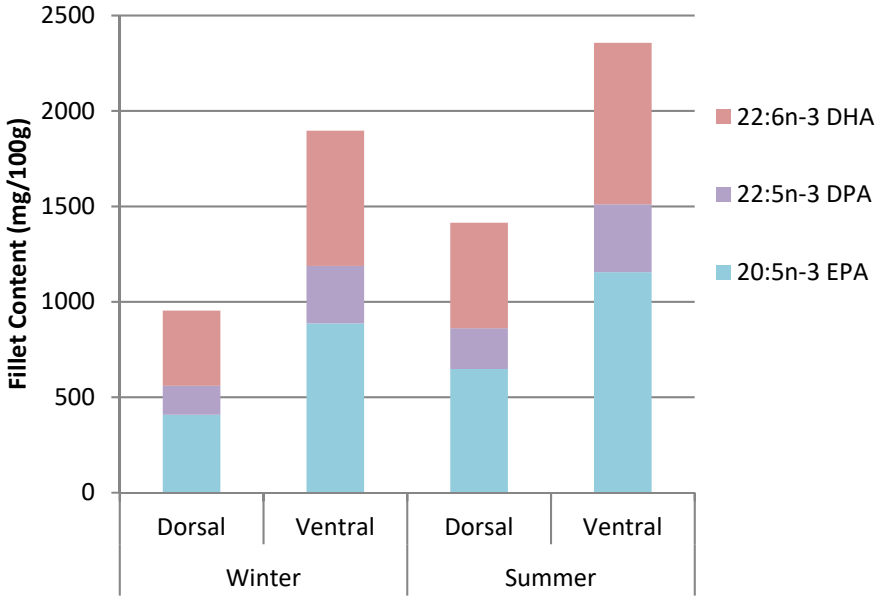

Figure 10. Stacked chart showing mean omega-3 fatty acid content (EPA, DPA and DHA; mg/100g) in dorsal and ventral areas of the fillet of Yellowtail kingfish (*Seriola lalandi*) (2007 hatchery run 2) harvested from September 2009 (Winter) and January 2010 (Summer).

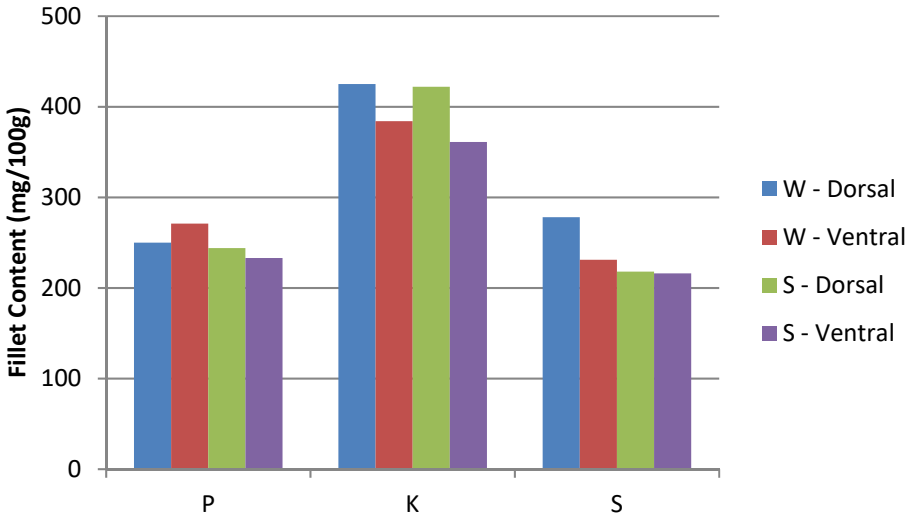

Figure 11. Mean element content (Phosphorus, Potassium and Sulphur; mg/100g) in dorsal and ventral areas of the fillet of Yellowtail kingfish (*Seriola lalandi*) (2007 hatchery run 2) harvested from September 2009 (Winter) and January 2010 (Summer).

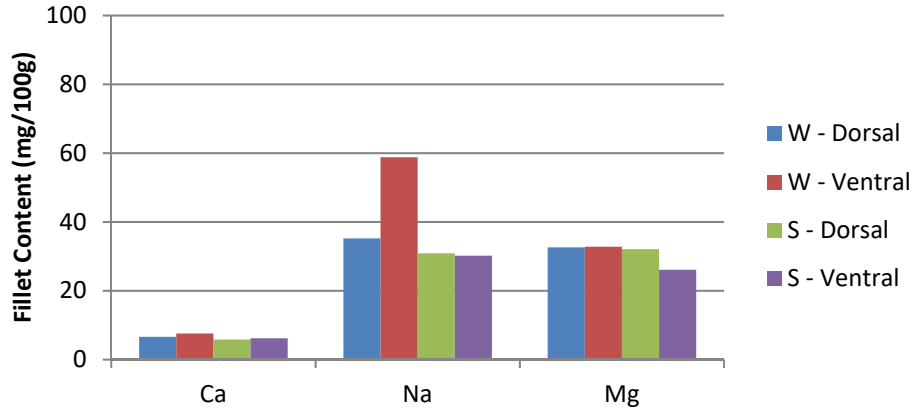

Figure 12. Mean element content (Calcium, Sodium and Magnesium; mg/100g) in dorsal and ventral areas of the fillet of Yellowtail kingfish (*Seriola lalandi*) (2007 hatchery run 2) harvested from September 2009 (Winter) and January 2010 (Summer).

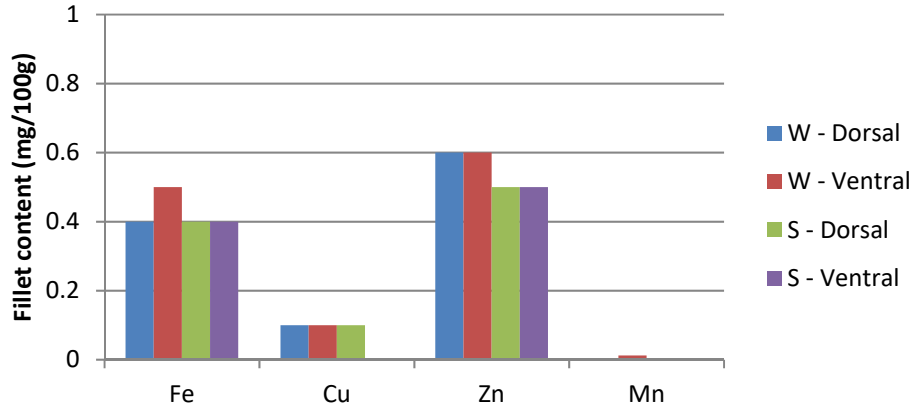

Figure 13. Mean element content (Iron, Copper, Zinc and Manganese; mg/100g) in dorsal and ventral areas of the fillet of Yellowtail kingfish (*Seriola lalandi*) (2007 hatchery run 2) harvested from September 2009 (Winter) and January 2010 (Summer).

3.3 Activity three: whole body proximate composition

These analyses were carried out on whole fish (scales, skin, skeleton, visceral organs and flesh) and not just the edible flesh component of the fillet (as was the case in activity two). Carcase yield values would suggest that the whole fish is comprised of 60-65% fillet and 20-30% viscera, with the remaining 10-15% being the head, backbone, fins and skin. Changes in the weight of various visceral organs, especially the liver, gonad and visceral fat, occur as the fish grow and/or with seasonal conditions, and these will be reflected in the results of whole body biochemical analysis.

Whole body proximate analysis showed that protein concentration varied between 19.8 and 21.5%, and was significantly affected by season ($F_3 = 3.143$, $P = 0.028$) and by the interaction of season and hatchery run ($F_6 = 2.779$, $P = 0.015$) (Figure 14). The main effects (i.e. season and hatchery run) cannot be properly interpreted, as the concentration of protein present within the season varied depending on the hatchery run.

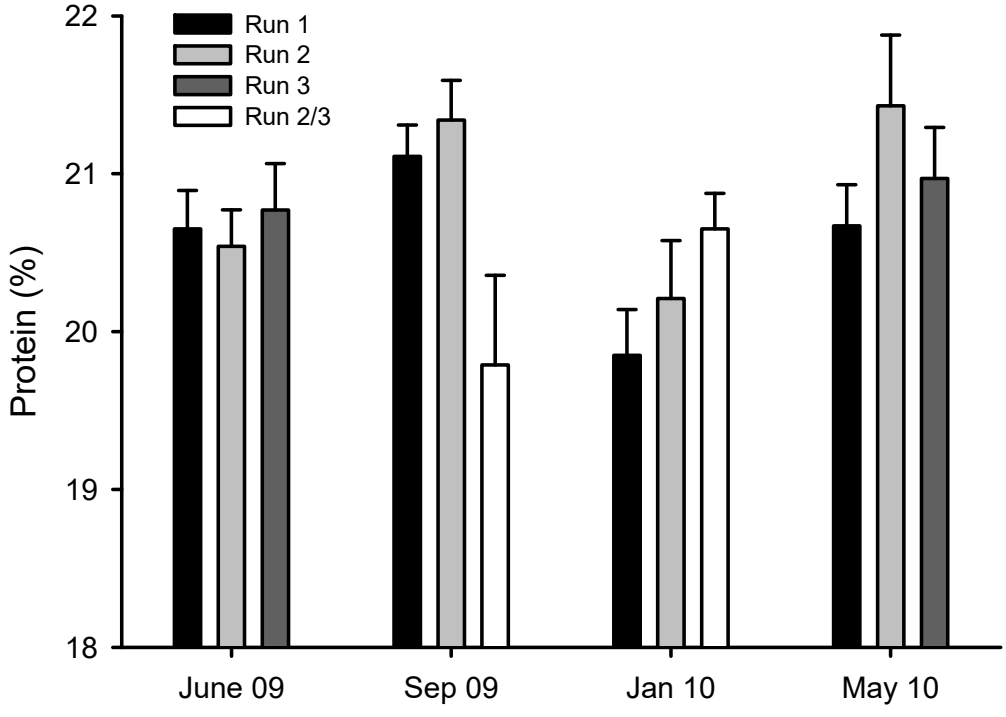

Figure 14. Mean (± se) whole body protein (%) in Yellowtail kingfish (*Seriola lalandi*) sampled over 11 month period from fish spawned in 2008, hatchery runs 1 (August), 2 (September) and 3 (October). *n* = 10.

Whole body crude fat content varied between 7 and 16%, with levels in hatchery run 1 being 1-3% higher than run 3 or run 2/3 ($F_2 = 8.111$, $P < 0.001$) (Figure 15).

Seasonal effects were also significant (F_3 = 35.178, P < 0.001), with similar low levels in September and January, and the highest levels in May 2010. The significant difference in whole carcase crude fat levels between June 2009 and May 2011 could be related to changes due the growth/age of the fish, rather than season *per se*.

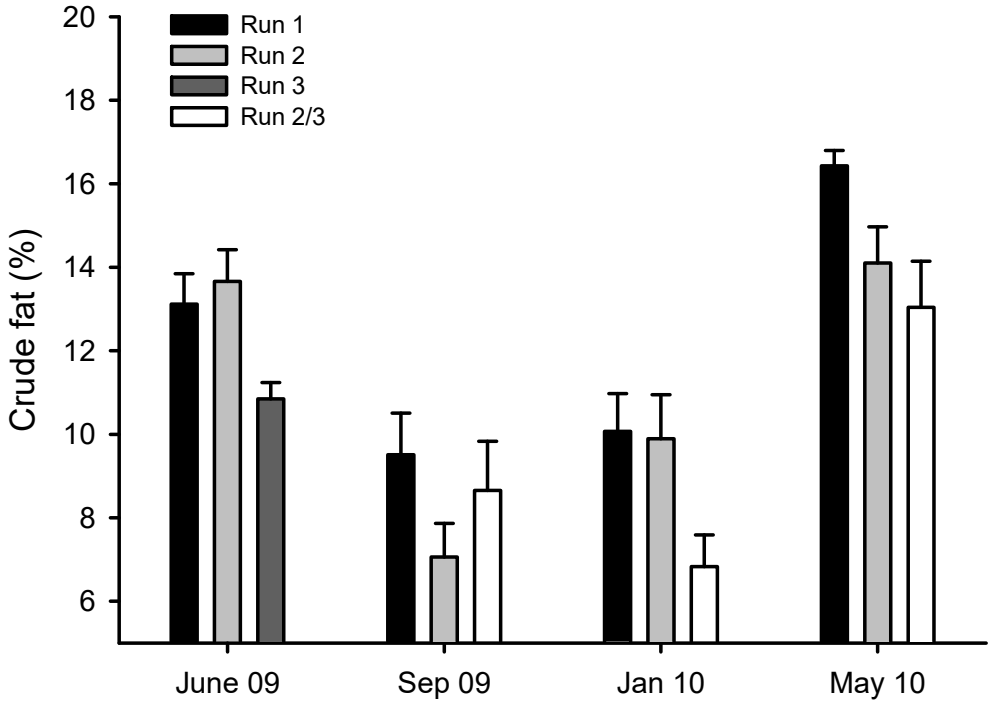

Figure 15. Mean (± se) whole body crude fat (%) in Yellowtail kingfish (*Seriola lalandi*) sampled over 11 month period from fish spawned in 2008, hatchery runs 1 (August), 2 (September) and 3 (October). *n* = 10.

Whole body moisture level showed the greatest variability through this study with values between 61 and 70%. Whole body moisture was significantly affected by hatchery run (F_2 = 9.031, P < 0.001) and season (F_3 = 47.232, P < 0.001), but not by the interaction of run and season (F_6 = 0.895, P = 0.501) (Figure 16). Moisture levels were significantly different between all months except September and January, with the highest levels recorded in those months. Significant differences were also detected between hatchery run 1 and 3 and 2 and 3, but not between hatchery runs 1 and 2, with moisture levels typically higher in hatchery run 3. The pattern in moisture results is inversely proportional to the patterns shown in crude fat levels. This finding is expected and is dealt with in more detail in Subproject 2.

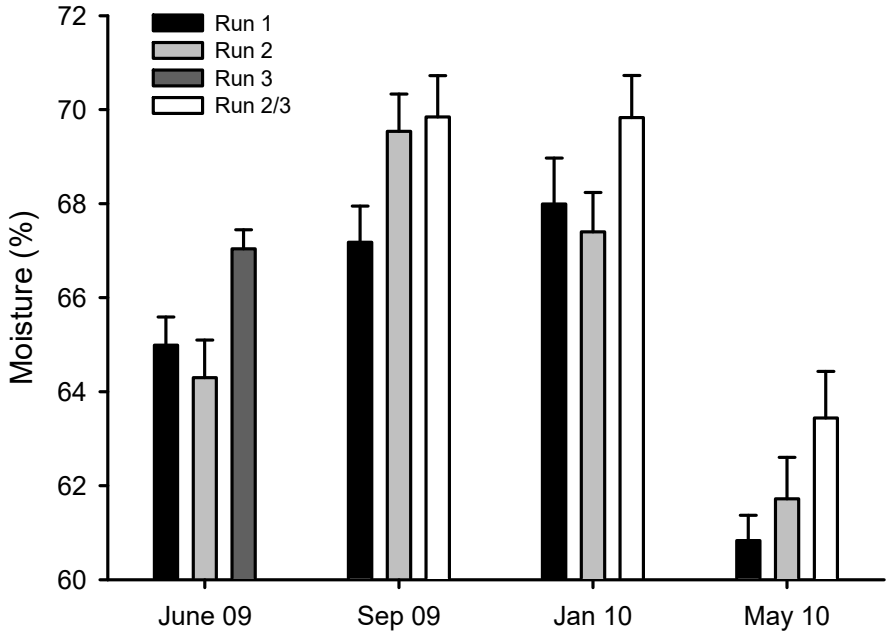

Figure 16. Mean (± se) whole body moisture (%) in Yellowtail kingfish (*Seriola lalandi*) sampled over 11 month period from fish spawned in 2008, hatchery runs 1 (August), 2 (September) and 3 (October). *n* = 10.

Whole body ash content significantly increased over the course of the sampling period, with lowest concentration observed in June 2009 at approximately 2.2%, and a peak at approximately 3.1% in May 2010 (F_3 = 43.3, P = <0.001) (Figure 17). Interestingly, ash content was significantly higher in hatchery run 3 or 2/3, than in runs 1 and 2 (F_2 = 4.6, P = 0.01).

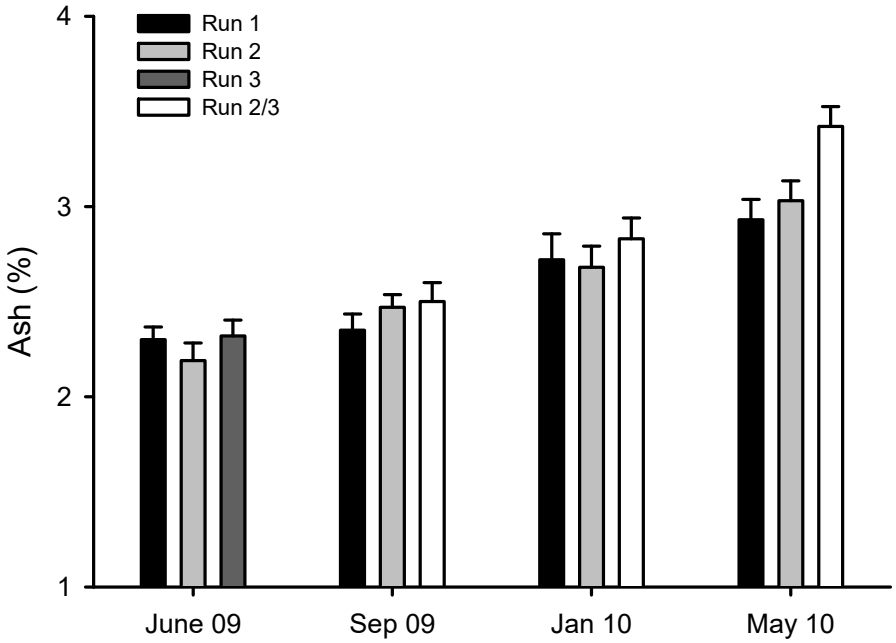

Figure 17. Mean (± se) whole body ash (%) in Yellowtail kingfish (*Seriola lalandi*) sampled over 11 month period from fish spawned in 2008, hatchery runs 1 (August), 2 (September) and 3 (October). *n* = 10.

Whole body vitamin C (ascorbic acid) concentration varied between 8 and 38 $mg.kg^{-1}$ with significantly higher levels (~30$mg.kg^{-1}$) being found in June and September, and lower levels (~10$mg.kg^{-1}$) in January and May ($F_3 = 140.308$, $P < 0.001$). There was also a significant interaction effect between season and hatchery run ($F_6 = 6.320$, $P < 0.001$) (Figure 18). The main effects (i.e. season and hatchery run) cannot be properly interpreted, as the concentration of vitamin C present within the season depends on the hatchery run; however, it is clear that ascorbic acid concentration was overall much lower in fish sampled in January and May compared to those sampled in June and September.

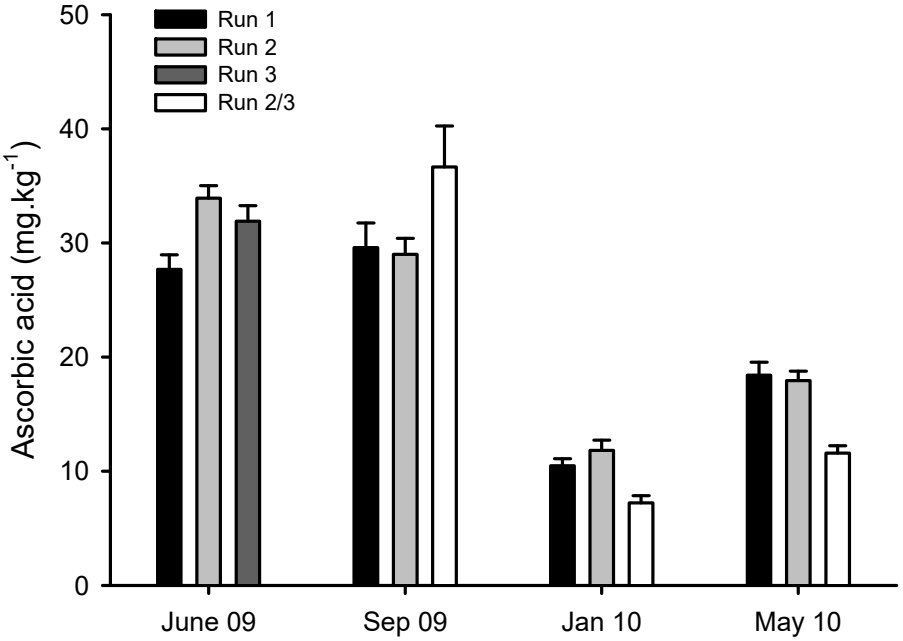

Figure 18. Mean (± se) whole body concentration ($mg.kg^{-1}$) of vitamin C (ascorbic acid) in Yellowtail kingfish (*Seriola lalandi*) sampled over 11 month period from fish spawned in 2008, hatchery runs 1 (August), 2 (September) and 3 (October). *n* = 10.

Whole body vitamin E (α-tocopherol) concentrations fluctuated greatly (from 22 to 85 $mg.kg^{-1}$), with seasonal ($F_3 = 6.5$, $P = <0.001$) and between hatchery runs ($F_2 = 17.1$, $P = <0.001$) effects being significant. The interaction between hatchery run and season was also significant ($F_6 = 26.1$, $P = <0.001$) (Figure 19).

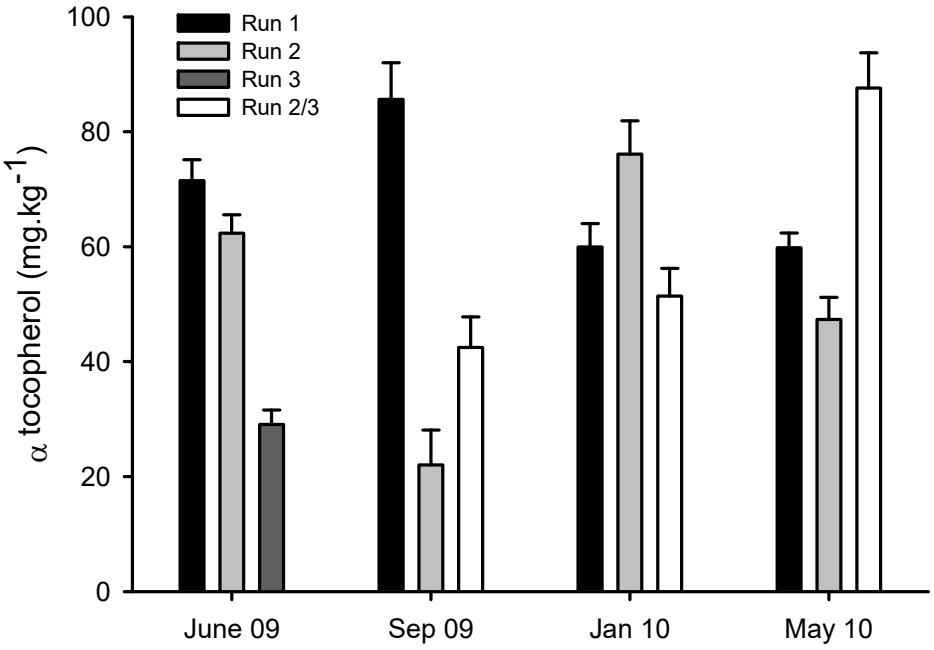

Figure 19. Mean (± se) whole body concentration (mg.kg^{-1}) of vitamin E (α-tocopherol) in Yellowtail kingfish (*Seriola lalandi*) sampled over 11 month period from fish spawned in 2008, hatchery runs 1 (August), 2 (September) and 3 (October). *n* = 10.

Specific subsamples from each different batch of feed that was fed to these pontoons of fish during this trial were not retained for vitamin or mineral analyses.

3.4 Activity four: fillet shelf life

3.4.1 Component one – quantitative method to measure colour change in fillets

The quantitative method developed in this activity to measure colour change in YTK fillets allows fillet colour to be displayed visually (Figure 20 and 21), and/or with the L*a*b* co-ordinates plotted on a three dimensional chart (Figure 22). Figure 20 illustrates the large variation in fillet colour between fish within a treatment. A "blur average" step can then applied to the pooled colour swatches (excluding any that are blank/white) to get an average colour swatch, as shown in Figure 21. This can be used to visually compare the average colour of fillets between treatments. Individual fish colour coordinate values can be generated in Photoshop for each of the treatments from the colour swatches and plotted on a three dimensional chart, as shown in Figure 22. This form of graphing can be used to illustrate separation in colour between treatments.

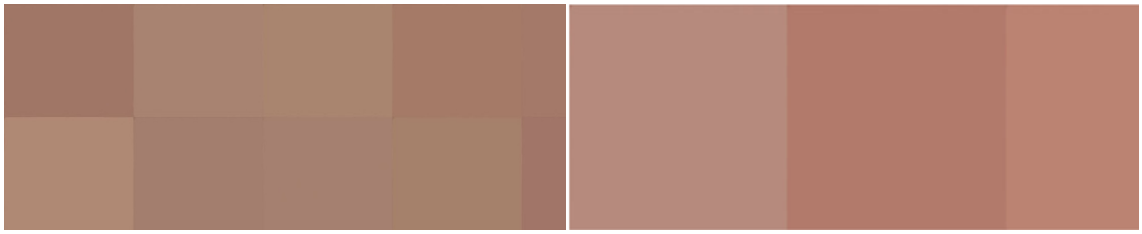

Figure 20. Individual "blur" colour swatches derived from the dorsal fillets of Yellowtail kingfish (*Seriola lalandi*) from non-CO treatment (left, n = 10) and CO treatment (right, n = 3).

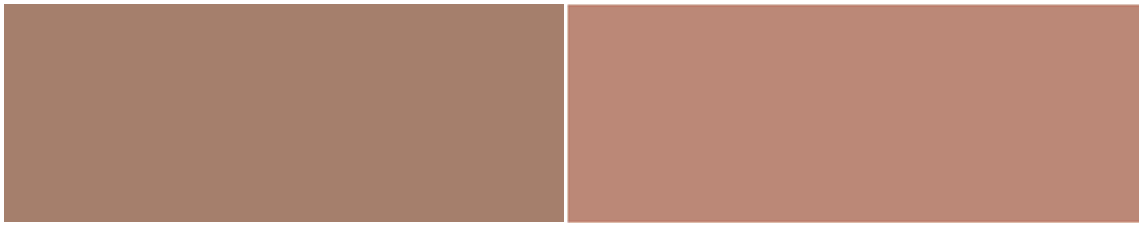

Figure 21. Treatment average colour swatches derived from the "blurring" of the individual "blur colour swatches" of Yellowtail kingfish (*Seriola lalandi*) fillets from non-CO treatment (left, n = 10) and CO treatment (right, n = 3).

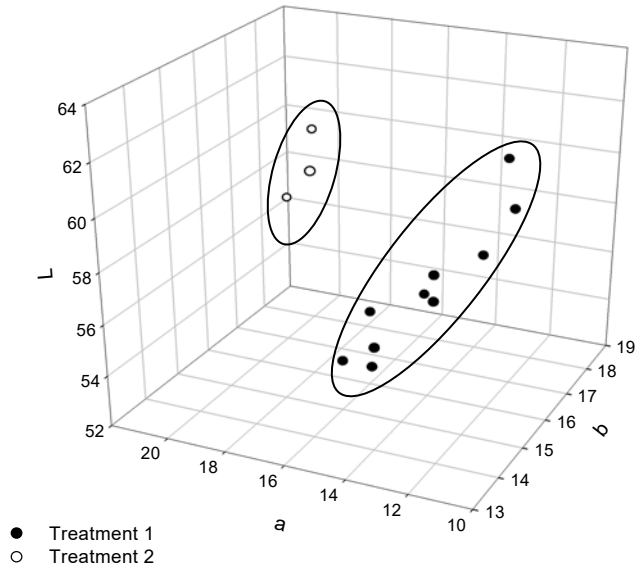

Figure 22. *L*,a* and b** colour space values of Yellowtail kingfish (*Seriola lalandi*) fillets applying a Photoshop™ "blur average" to the dorsal sampling site at day 0 from treatment group one (non-CO) (*n*=10) and treatment group two (CO) (n = 3) harvested fish.

3.4.2 Component two - shelf life of fillets and vitamin content and distribution over grow-out

3.4.2.1 Shelf life of fillets

Vacuum packed fillets were stored in a temperature controled room at 4°C for up to 30 days. Ten fillets were opened on each of six sampling day for assessment of several physical and biochemical flesh quality characteristics. One attribute that was not measured objectively was fillet odour, but notes were made of subjective assessments of fillet smell. Fresh fillets, and those vacuum packed for up to 15 days has no, or a slight seawater/seaweed/fishy odour; but as storage duration increased, there was an increase in the frequency and intensity of the fishy odour. Indeed, the intensity of the fishy odour of some of the day 25 or 30 fillets at +3 days after opening was such that they were considered as not suitable for consumption. Unfortunately, no parallel microbiological analyses were carried out on the fillets in the shelf life trials. However, the olfactory results suggest that the shelf life of the chilled (4°C) vacuum packed fillet as processed in the manner described in this project is in the order of 20 days.

Samples taken from the ventral region of the fillets were selected for analysis of oxidative state (i.e. TBARS) during fillet storage. This was due to the typically higher

fat content observed in this region of the fillet (see Figure 6), a factor that could result in greater deleterious changes as storage progresses. The TBARS results were extremely low and somewhat variable, with values between 0.04 and 0.25 mg.kg^{-1}, and no clear trends were evident with shelf life or season. The low TBAR values obtained in this trial meant that statistical analysis of these data was unnecessary (Figure 23). Similarly, with TBAR values being so low in ventral areas, it was decided there was little point in measuring levels in the lower fat dorsal areas.

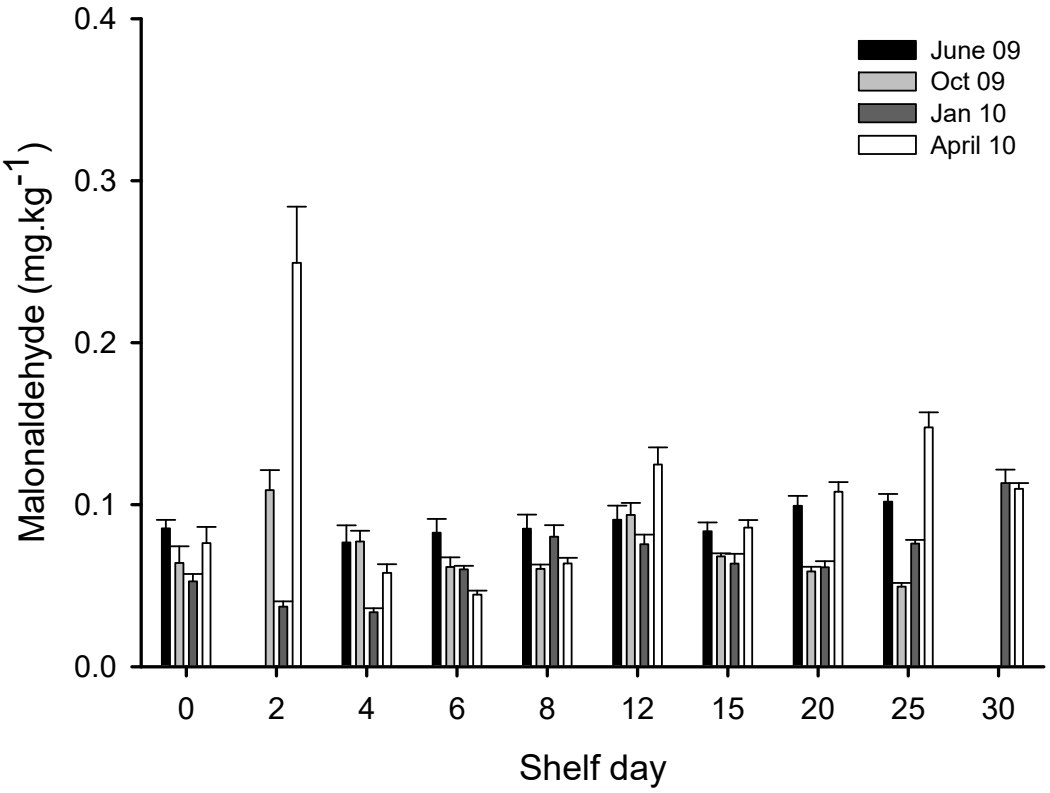

Figure 23. Mean (± se) ventral fillet malonaldehyde concentration (mg.kg^{-1}) in Yellowtail kingfish (*Seriola lalandi*) harvested in June 2009, October 2009, January 2010 (hatchery run 1, August 2007) and April 2010 (hatchery run 2, September 2007. Fillets were vacuum packed (day 0) then held at approximately 4˚C for up to 30 days. *n* = 10.

K value analysis of both the ventral and dorsal fillet areas was only completed for the first sampling event (June 2009) to determine initially if there was a difference between the two fillet regions. The results indicated that K value significantly increased with increasing shelf life (F_7 = 875.757, P < 0.001), but there was no significant difference between the dorsal and ventral areas of the fillet (F_1 = 0.199, P = 0.656) (Figure 24). Consequently, K value was only analysed for the ventral area samples in the remaining sampling events (October 2009, January 2009 and April 2010) (see Figure 25).

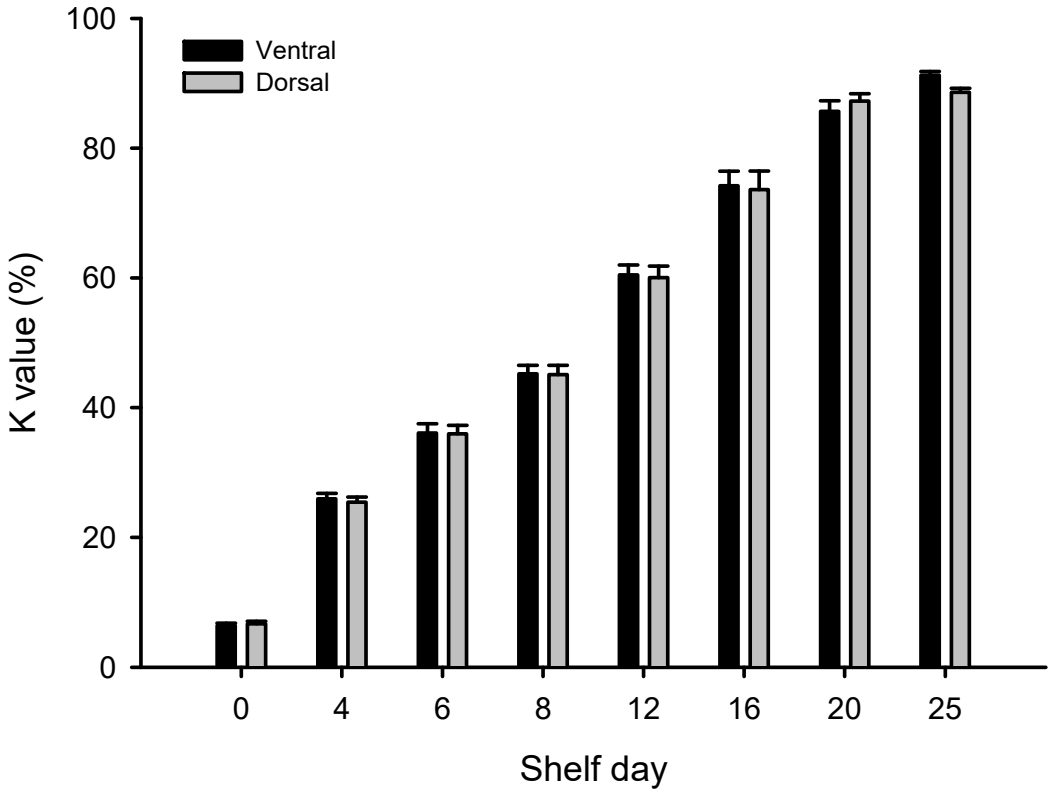

Figure 24. Mean (± se) ventral and dorsal fillet K value in Yellowtail kingfish (*Seriola lalandi*) harvested in June 2009, spawned in August 2007. Fillets were vacuum packed (Day 0) then held at approximately 4˚C for up to 30 days. *n* = 10. Mean whole fish weight 3.4kg.

K value of the ventral area of the fillet was significantly affected by shelf life (F_7 = 1560.727, P < 0.001), season (F_3 = 73.799, P < 0.001) and by the interaction between shelf life and season (F_{21} = 9.197, P < 0.001) (Figure 25). The results showed K value significantly increased with increasing shelf life until 20 days, where it plateaus at ~90%. It is difficult to properly interpret the main effects (i.e. effect of shelf life and season) on K value as a significant interaction between shelf life and season was present (i.e. the effect of shelf life on K value depends on the season). However, it is clear YTK sampled in October had a higher K value on shelf days 6, 8 and 12 compared to YTK sampled in the other months.

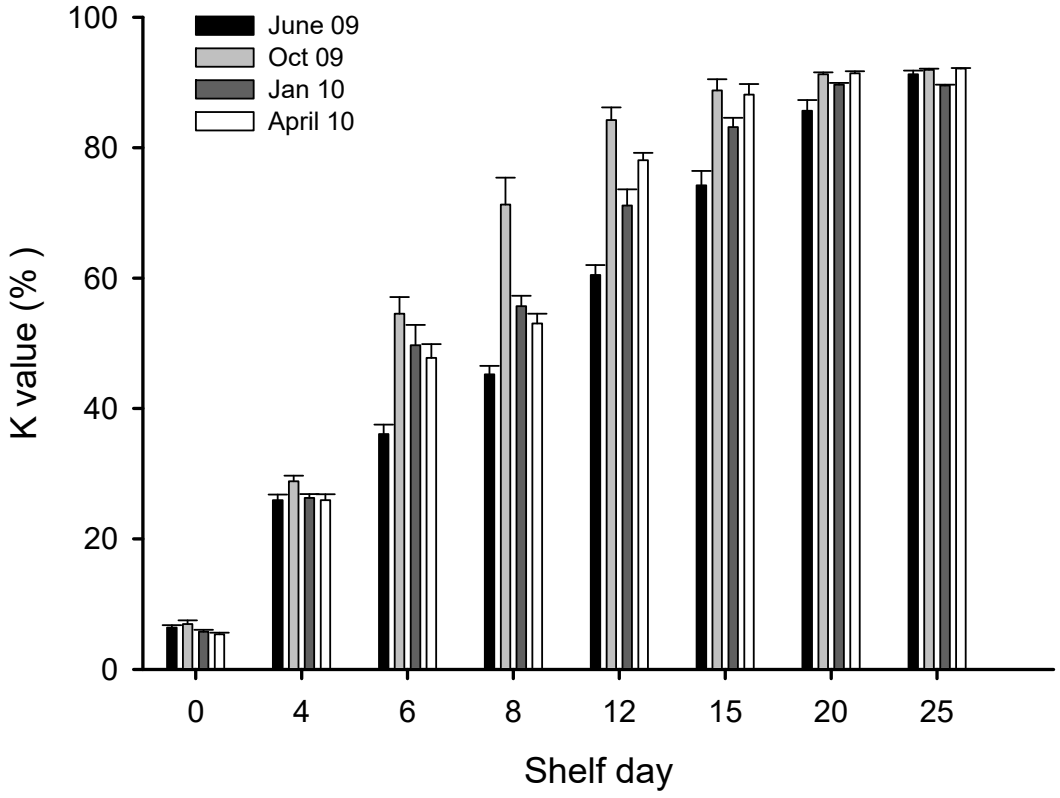

Figure 25. Mean (± se) ventral fillet K value in Yellowtail kingfish (*Seriola lalandi*) harvested in June 2009, October 2009, January 2010 (hatchery run 1, August 2007) and April 2010 (hatchery run 2, September 2007. Fillets were vacuum packed (Day 0) then held at approximately 4˚C for up to 30 days. *n* = 10.

Drip-loss from the fillet was lowest on day 0 (~1%) and increased to 3-5% by day 30 of storage. This pattern was consistent in the different harvest events, but the January harvested fillets lost up to 1.5% more drip than the other three harvests, and this higher level of drip loss started at day 0. Statistical analysis indicated a number of significant effects; shelf life ($F_8 = 132.178$, $P < 0.001$), season ($F_3 = 144.263$, $P < 0.001$) and by the interaction of shelf life and season ($F_{24} = 2.124$, $P = 0.002$) (Figure 26). It is difficult to interpret main effects (i.e. shelf life and season) on drip-loss, as a significant interaction between shelf life and season was present.

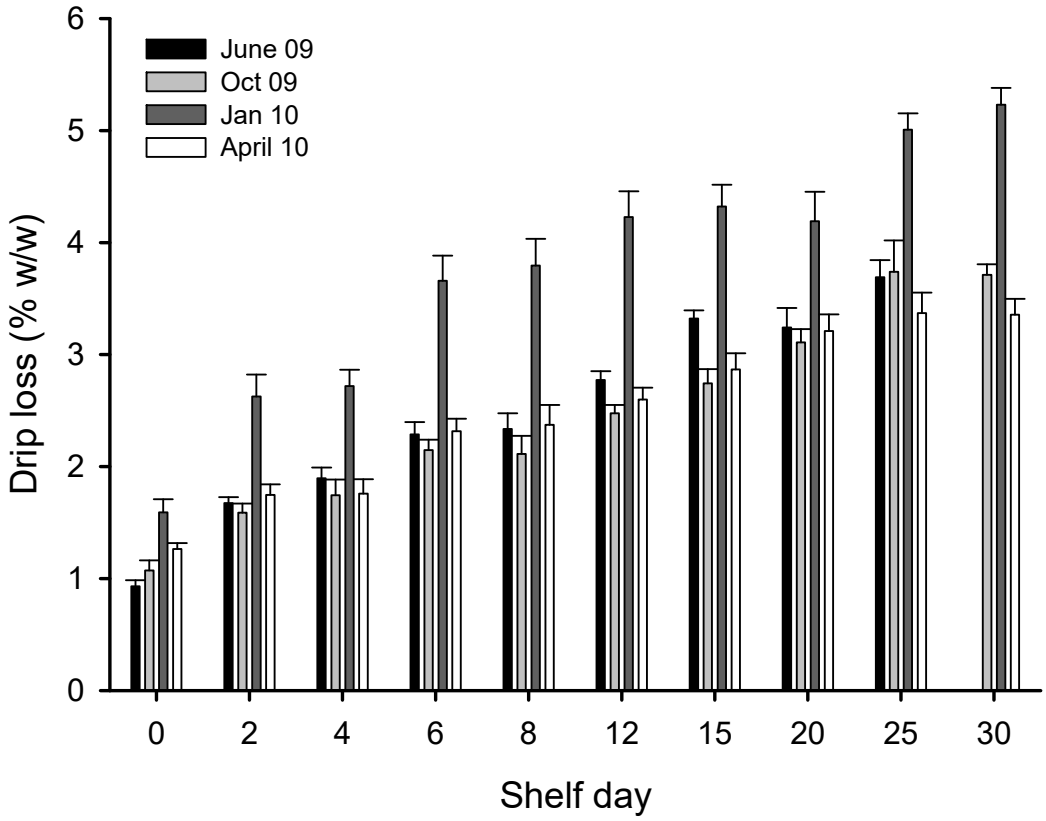

Figure 26. Mean (± se) fillet moisture loss in Yellowtail kingfish harvested in June 2009, October 2009, January 2010 (hatchery run 1, August 2007) and April 2010 (hatchery run 2, September 2007). Fillets were vacuum packed (Day 0) then held at approximately 4˚C for up to 30 days. n = 10.

A typical colour swatch for the individual fillets and the average colour swatch of each of the shelf life days in one (harvest 2, October 2010) of the four different seasonal sampling events is shown in Figure 27 (results from the other three harvests are shown in Appendix Figures A1-A3). The L*a*b* co-ordinates derived from the individual fillet colour swatches were plotted on a three dimensional chart and compared to the values of fillets sampled on day 0 (Figure 28 shows the data from harvest 2, the results for the other 3 seasonal sampling events are shown in Appendix Figures A4-A6).

It is important to note that the method for measuring fillet colour was still being refined in June 2009 when Harvest 1 was carried out, thus the colour results for that particular trial should be viewed with caution. Consequently, as a result of the 'work in progress' nature in method development during Harvest 1 the flesh colour appears to be darker with a purple tone, and within- and between- day variation in flesh colour between fillets appears to be significant. In contrast, images taken from Harvests 2-4 were lighter (higher L*), slightly less red (lower a*) and more yellow (higher b*), and overall tended to be much less variable.

In harvest events 2, 3 and 4 there were slight, if any, noticeable differences in average fillet colour over the 30 days of the trial, and no trend in fillet darkening, lightening, reddening or yellowing was visible to the eye (Figure 27). In contrast, the MANOVA analysis of the L*a*b* coordinate data did show significant differences between the Day 0 and subsequent shelf life fillet colour (Figure 28). The general trend was that the fillets stored for longer had slightly higher L* values and slightly lower a* values compared to Day 0. The industry or consumer relevance of the barely perceptible nature of any change in visual colour, must be balanced against the small, but statistically significant, numerical results.

Figure 27. Colour swatches for Yellowtail kingfish (*Seriola lalandi*) hatched as run 1, (August 2007) and harvested in October 2009 (Harvest 2). Fillets were vacuum packed (day 0) then held at approximately 4˚C for up to 30 days. *n* = 9-10.

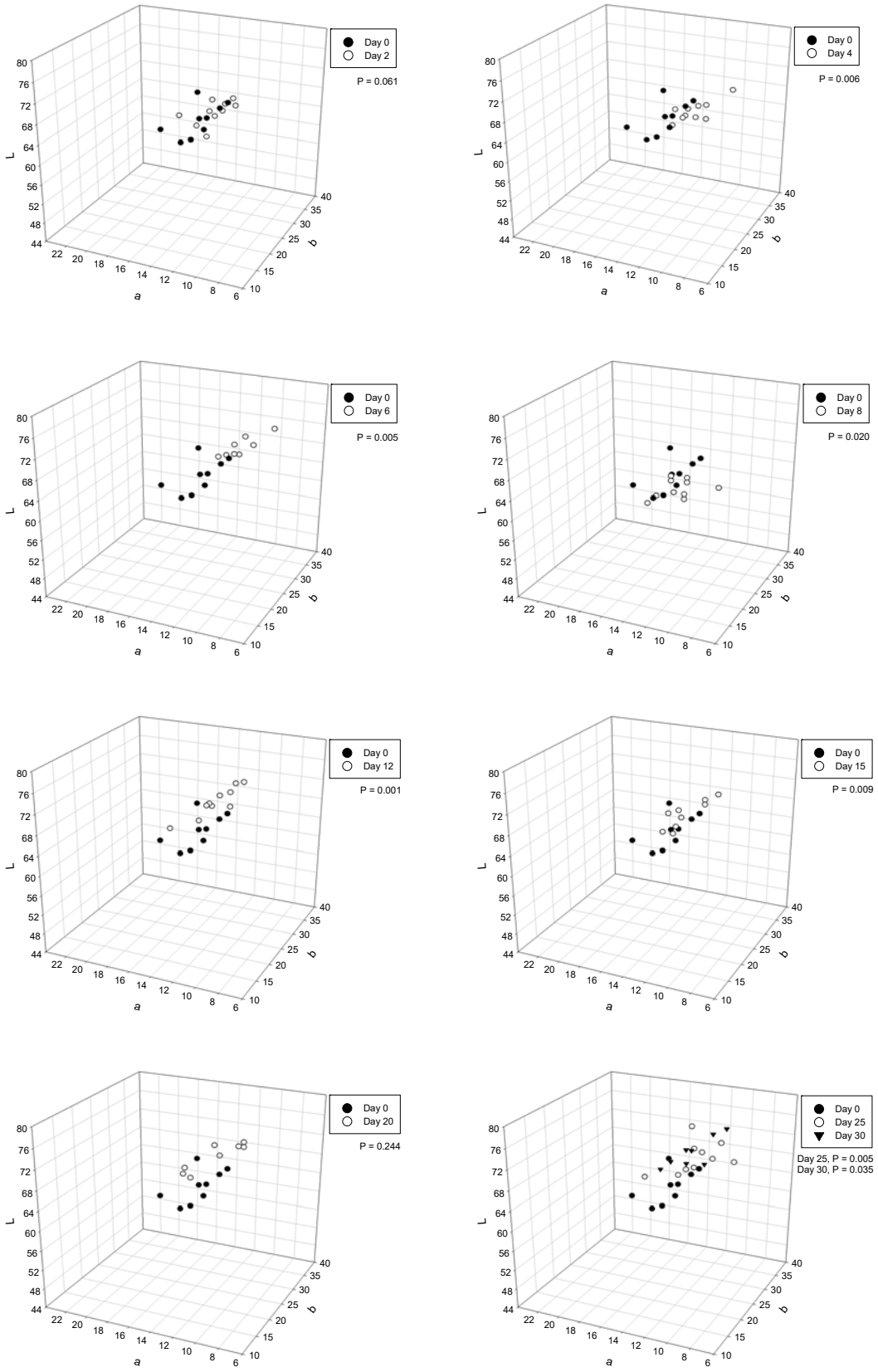

Figure 28. L*a*b co-ordinate plots for Yellowtail kingfish (*Seriola lalandi*) hatched as run 1 (August 2007) and harvested in October 2009 (Harvest 2). Fillets were vacuum packed (day 0) then held at approximately 4°C for up to 30 days. n = 9-10.

On some of the days (0, 4, 8, 15 and 25/30) on which vacuum packs of fillets were opened, the open packs were retained in the chiller and the fillets were re-sampled on days +1 and +3 after opening. TBARS analysis showed that fillet values were low during vacuum packed shelf life, and did not change substantially, nor consistently, in the 4 days after the packs were opened (Figure 29). TBARS levels were so low that no statistical analyses were performed on the data.

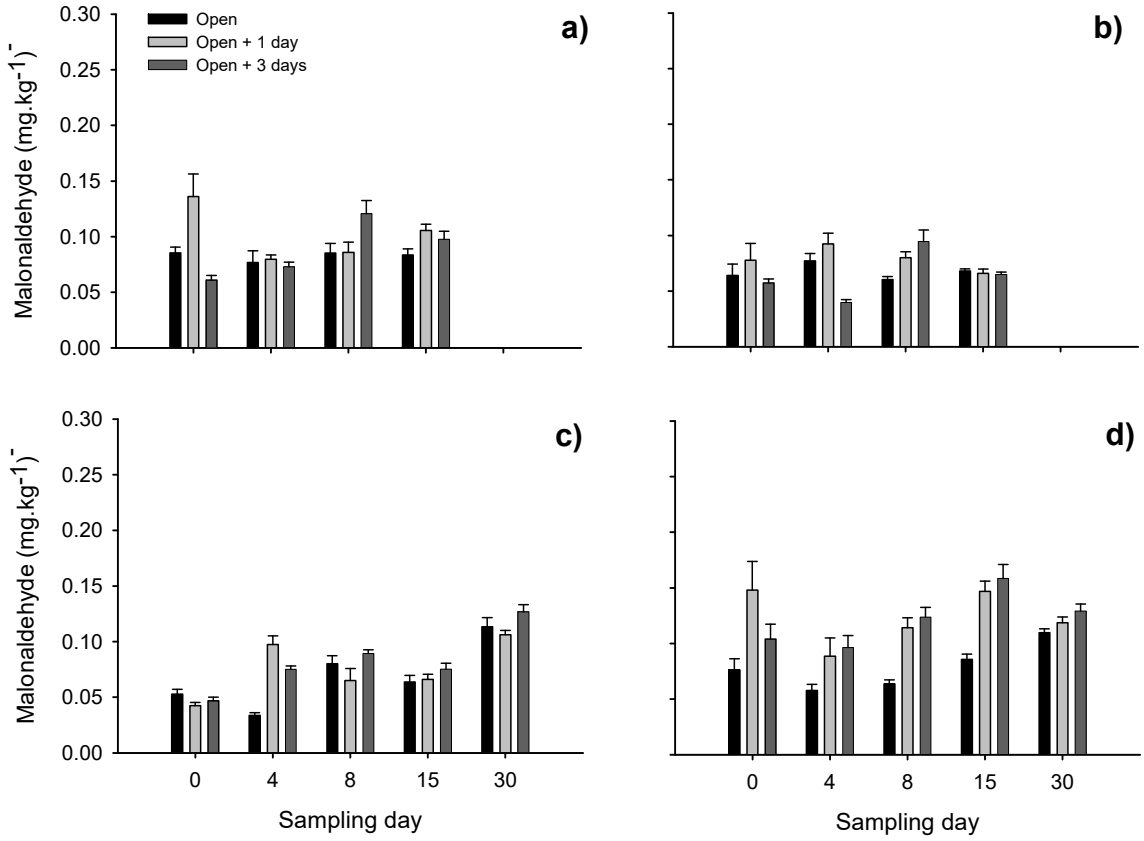

Figure 29. Mean (± se) ventral fillet malonaldehyde concentration (mg.kg^{-1}) in farmed Yellowtail kingfish (*Seriola lalandi*) harvested in a) June 2009, b) October 2009, c) January 2010 (hatchery run 1, August 2007) and d) April 2010 (hatchery run 2, September 2007). Fillets were vacuum packed (day 0) then held at 4˚C for up to 30 days. Malonaldehyde concentration was measured on the day of vacuum pack opening and then 1 and 3 days post opening. *n* = 10.

In contrast to the TBARS results, a clear upward trend was observed in the drip loss (1 to 5%) and K value (10 to 80%) data over storage days (Figure 30 and 31, respectively). There were also consistent trends for moisture loss and K value to increase with time after the vacuum packs were opened. Interestingly, because of the design of this study, the day +3 observations from day 0 and 4 opened packs were made the day before the day 4 and 8 vacuum packs were opened. It is therefore possible to compare the trends to see if vacuum packing or air exposure affected the

results obtained (Figures 30 and 31). Whilst no statistical analyses were performed on these data, the general observation seems to be that changes in moisture loss and K value are mostly driven by storage time, not packaging method or exposure to air.

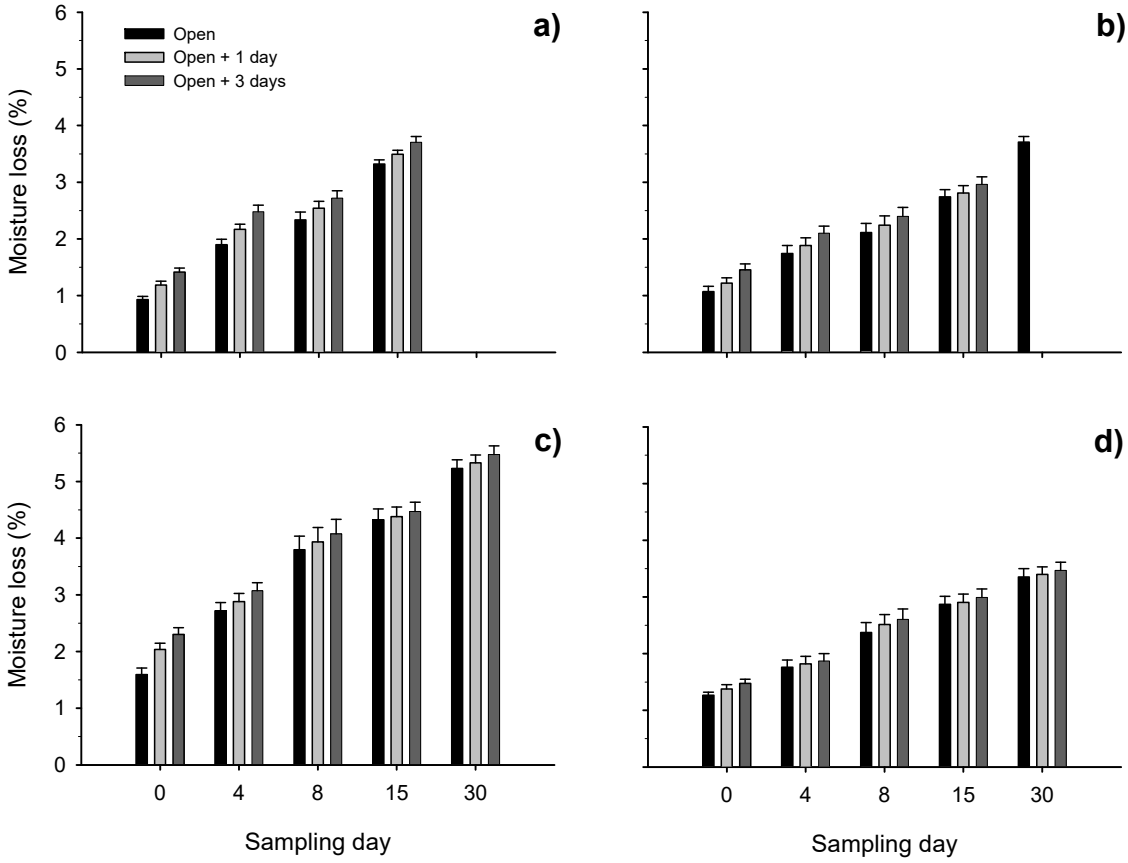

Figure 30. Mean (± se) fillet moisture loss (%) in farmed Yellowtail kingfish (*Seriola lalandi*) harvested in a) June 2009, b) October 2009, c) January 2010 and d) April 2010, from fish spawned in 2008, hatchery run 1 (August). Fillets were vacuum packed (day 0) then held at 4˚C for up to 30 days. Moisture loss was measured on the day of vacuum pack opening and then 1 and 3 days post opening. *n* = 10.

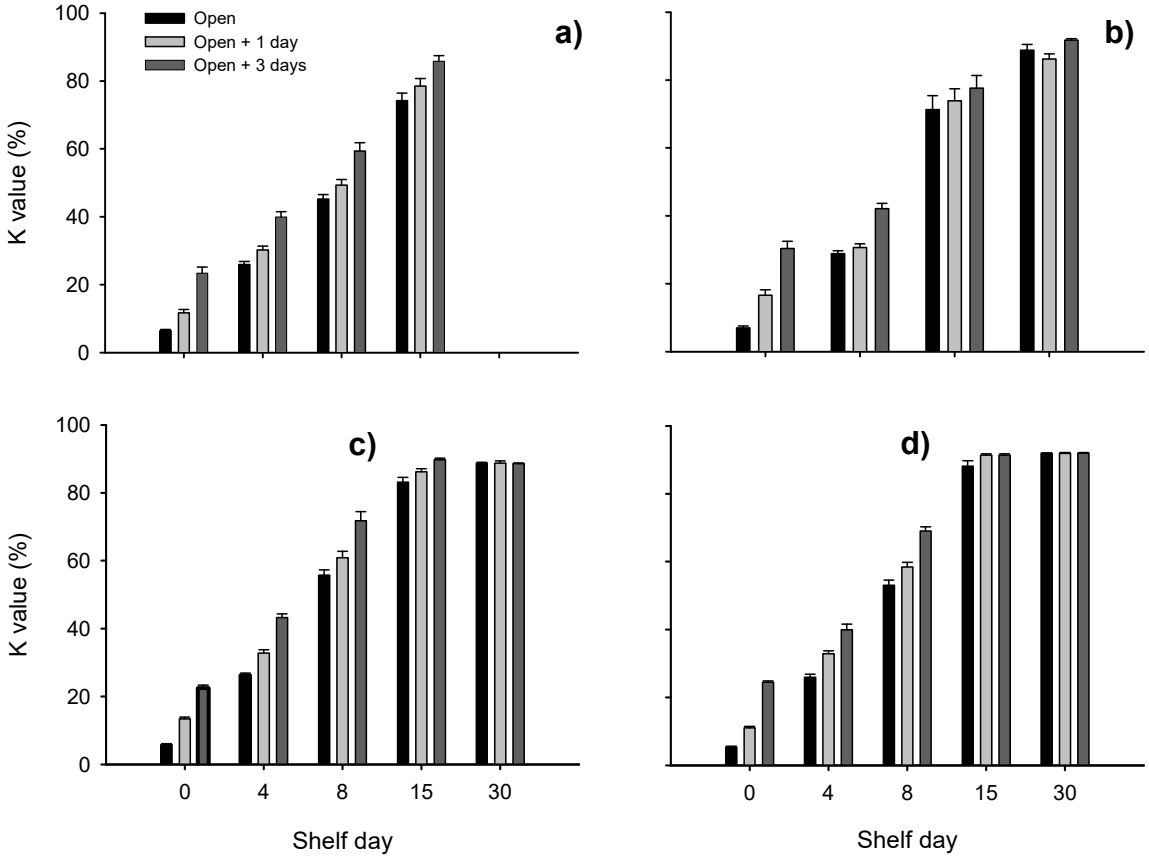

Figure 31. Mean (± se) ventral fillet K value in farmed Yellowtail kingfish (*Seriola lalandi*) harvested in a) June 2009, b) October 2009, c) January 2010 and d) April 2010, from fish spawned in 2008, hatchery run 1 (August). Fillets were vacuum packed (day 0) then held at 4°C for up to 30 days. K value was measured on the day of vacuum pack opening and then 1 and 3 days post opening. *n* = 10.

Colour analysis was performed on fillets on the day the vacuum packs were opened and the same fillets at +3 days. The average blurred colour swatch results from the day of opening and day +3 are shown in Figure 31. In 2 cases the fillets that were opened on day 25 or 30 had deteriorated to a point at day +3 to be too offensive in odour to be photographed. To the eye, the colour change of the fillets after the packs had been open for 3 days was difficult to detect (Figure 31), but when the average colours were converted to L*a*b* values in Photoshop™ there were statistically significant differences between days (e.g. for Harvest 2; Figure 32). The L*a*b* values for fillets 3 days after the packs were opened showed that there were slight increases in L* (lightness) and b* (redness), and a slight decrease in a* (yellowness). The industry or consumer relevance of the barely perceptible nature of any change in visual colour, must be balanced against the small, but statistically significant, numerical results.

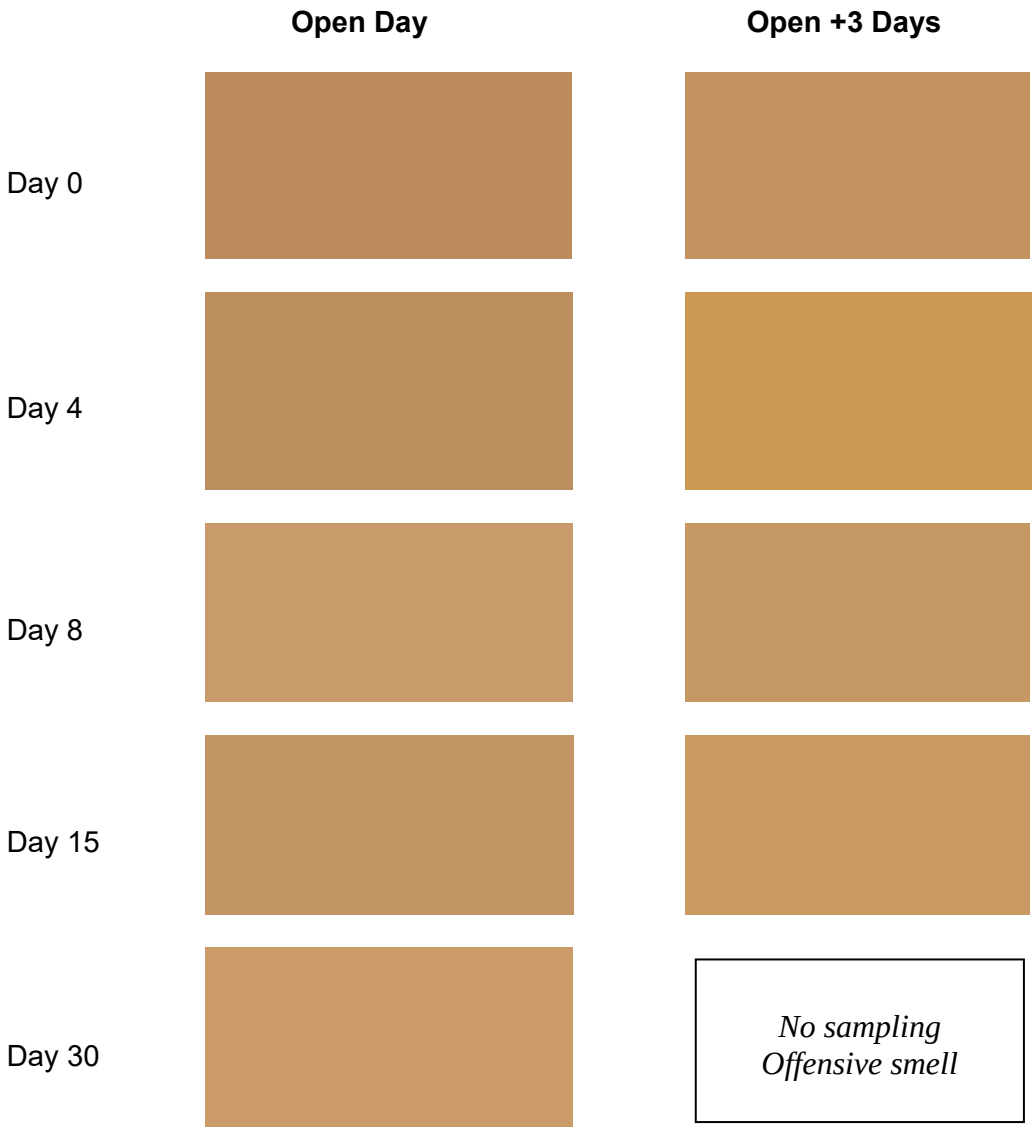

Figure 32. Mean blurred colour swatches for fillets from Yellowtail kingfish (*Seriola lalandi*) hatched as run 1 (August 2007) and harvested in October 2009 (Harvest 2). Fillets were vacuum packed (day 0) then held at approximately 4°C for up to 30 days. Once opened the fillets were retained for an extra 3 days and re-sampled (where possible). n = 9-10.

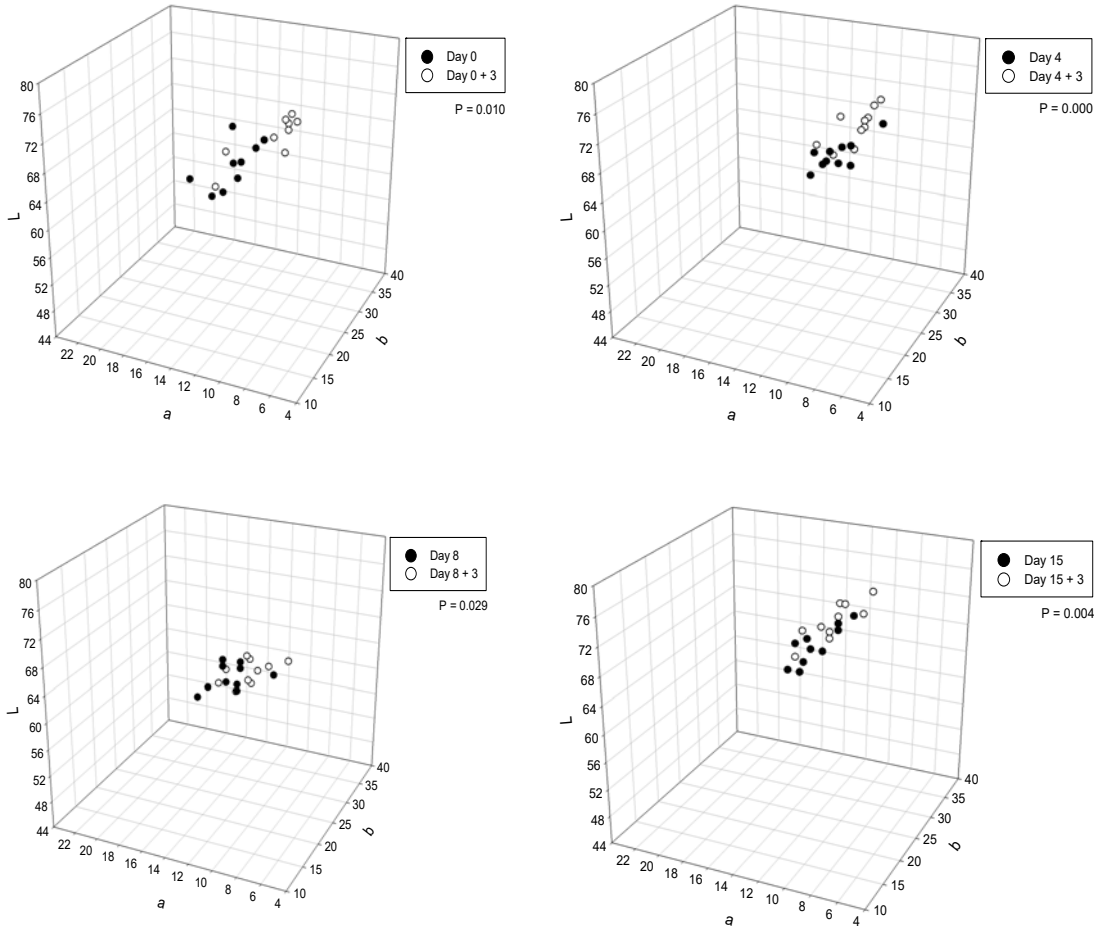

Figure 33. L*a*b* coordinates for individual fillets from Yellowtail kingfish (*Seriola lalandi*) hatched as run 1 (August 2007) and harvested in October 2009 (Harvest 2). Fillets were vacuum packed (day 0) then held at approximately 4°C for up to 30 days. Once opened the fillets were retained for an extra 3 days and re-sampled. n = 9-10.

3.4.2.2 Vitamin content and distribution over grow-out

3.4.2.2 Vitamin content and distribution over grow-out

Fillet vitamin C (ascorbic acid) concentration was significantly affected by season (F_3 = 20.759, P < 0.001), but not by fillet area (i.e. dorsal or ventral) (F_1 = 0.948, P = 0.332) or by the interaction of fillet cut and season (F_3 = 1.176, P = 0.321) (Figure 34a). Vitamin C concentrations ranged between approximately 20 and 25 mg.kg^{-1} in June 2009, October 2009 and January 2010, increasing significantly to approximately 45mg.kg^{-1} in April 2010. Fillet vitamin E (α-tocopherol) concentration was also significantly affected by season (F_3 = 12.7, P = <0.001), but not affected by fillet area (i.e. dorsal or ventral) (F_1 = 1.1, P = 0.3) or by the interaction of cut and season (F_3 = 1.8, P = 0.1) (Figure 34b). Vitamin E concentrations were lowest in June 2009 and April 2010 (22 to 25 mg.kg^{-1}) and higher in October 2009 and January 2010 at approximately 28 and 31 mg.kg^{-1}, respectively.

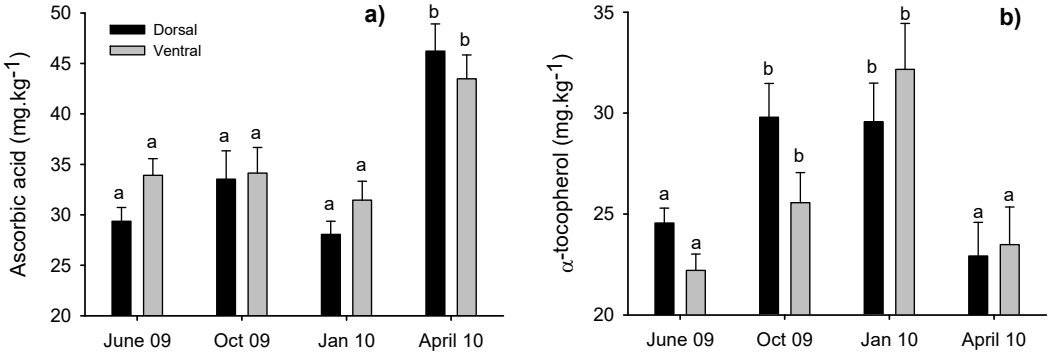

Figure 34. Mean (± se) dorsal and ventral fillet day 0 (a) vitamin C (ascorbic acid) and (b) vitamin E (α-tocopherol) in Yellowtail kingfish (*Seriola lalandi*) sampled over 10 month period from fish spawned in August / September 2007. n = 10.

3.5 Activity five: fillet skin on / skin off shelf life

TBARS levels in this trial were relatively low (mostly <0.2 $mg.kg^{-1}$) and did not show substantial or consistent trends between the February and August harvest events with respect to changes over storage time, and/or depending on whether the fillet was skinned or not (Figure 35).

When detailed statistical analyses were applied to the TBARS results from each trial (February or August), some differences were identified. Thus, for fish sampled in February 2010, malonaldehyde concentration in the dorsal area of the fillet was significantly affected by shelf day (F_5 = 12.829, P < 0.001) and by the interaction of shelf day and fillet presentation (i.e. skin on or skin off) (F_5 = 5.032, P < 0.001), but was not affected only by whether the fillet was skinned or not (F_1 = 0.567, P = 0.453) (Figure 35a). Malonaldhyde concentration in the ventral area of the fillets from February 2010 was significantly affected by shelf day (F_5 = 49.879, P < 0.001), fillet presentation (F_1 = 34.878, P < 0.001) and by the interaction of shelf day and fillet presentation (F_5 = 3.970, P = 0.002) (Figure 35b). For fish sampled in August 2010, malonaldehyde concentration in the dorsal area was significantly affected by shelf day (F_5 = 22.934, P < 0.001), but not by fillet presentation (F_1 = 0.423, P 0.517) or by the interaction of shelf day and fillet presentation (F_5 = 1.857, P = 0.108) (Figure 35c). In August 2010, malonaldehyde concentration in the ventral area was significantly affected by shelf day (F_5 = 40.663, P < 0.001) and by the interaction between shelf day and fillet presentation (F_5 = 9.540, P < 0.001), but not by fillet presentation (F_1 = 0.00586, P = 0.939) (Figure 35d).

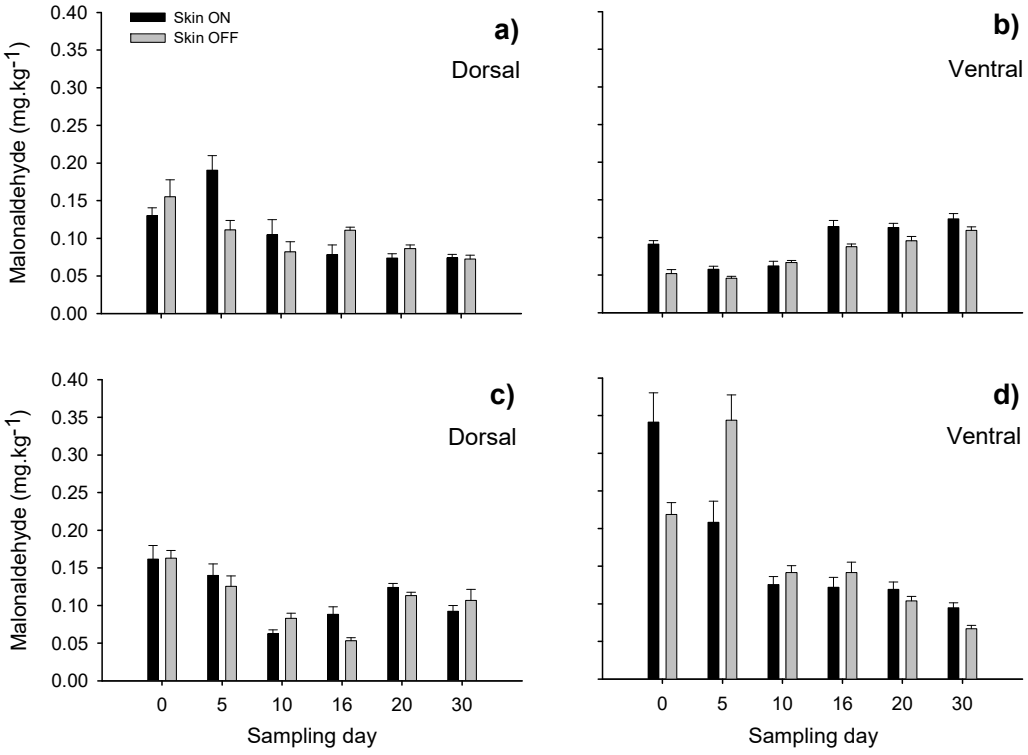

Figure 35. Mean (± se) malonaldehyde concentration (mg.kg^{-1}) in the dorsal and ventral fillet area of Yellowtail kingfish (*Seriola lalandi*) hatched in 2009 and harvested in (a) and (b) February 2010, and (c) and (d) August 2010, with either skin on or skin off. Fillets were vacuum packed (day 0) then held at approximately 4˚C for up to 30 days. *n* = 10.

3.6 Activity six: effect of temperature fluctuations on fillet shelf life

3.6.1 Cold chain management practices

The return rate of temperature loggers shipped with whole, fresh YTK to the various destinations overseas was generally good, with the exceptions being one of the shipments to the USA and one shipment to Thailand. None of the temperature profiles showed evidence of temperature violations (i.e. >4°C) inside the boxes, or in the fish, in any of the shipments examined (see Appendix 3: Figures A13-A18). Some large temperature variations were observed outside the box (up to approximately 20°C and as low as -18°C) for short periods of time; however, the temperature inside the box and inside the fish remained low and comparatively constant (Tables 5 and 6).

Table 5. Minimum and maximum temperatures measured inside and outside the fish boxes during both the transit period and in storage at the destination before the loggers were retrieved from the boxes.

Destination	**In Transit**				**In Storage**			
	Min (ºC)		**Max (ºC)**		**Min (ºC)**		**Max (ºC)**	
	Inside	**Outside**	**Inside**	**Outside**	**Inside**	**Outside**	**Inside**	**Outside**
Rome	1.1	3.0	2.3	12.5	-1.0	-0.5	3.8	3.8
Philadelphia	-1.0	-0.8	2.5	9.0	2.5	2.5	3.0	12.5
Los Angeles	-0.5	-0.5	1.5	1.5	1.0	2.8	2.2	10.0
Zurich	1.0	1.3	3.1	8.7	2.2	2.9	4.0	10.0
Philadelphia	0	0.5	3.1	12.0	1.5	0.3	2.0	17.0
Amsterdam	-0.5	0	2	5.5	1.5	1.5	2.0	5.5

Table 6. Minimum and maximum temperatures measured inside the YTK carcase during the transit period (duration in days) and in storage at the destination (duration in days) before the loggers were retrieved from the boxes. Ice Days were calculated for each phase, and as a total.

Destination	**In Transit**				**In Storage**				**Total**
	Time (days)	**Min (ºC)**	**Max (ºC)**	**Ice Days**	**Time (days)**	**Min (ºC)**	**Max (ºC)**	**Ice Days**	**Ice Days**
Rome	2.00	2.3	3	**3.2**	6.00	3	5.1	**10.9**	**14.1**
Philadelphia	2.50	-1	2.5	**3.9**	0.75	2.5	2.8	**1.2**	**5.1**
Los Angeles	1.33	-0.2	2	**1.5**	1.25	1	1.8	**1.6**	**3.1**
Zurich	1.83	1.5	2.2	**2.6**	0.25	2.6	3.2	**0.4**	**3.1**
Philadelphia	3.13	0.2	2.5	**3.9**	0.50	1.5	2	**0.7**	**4.6**
Amsterdam	2.00	1	2.5	**2.8**	0.29	2.6	2.8	**0.4**	**3.2**

Ice days during the transit phase were relatively low and generally within +50% of the actual transit duration. Most of the fish were retrieved within 24 hr of the shipment arriving at the destination, and their additional Ice Days were suitably low as well. The shipment of YTK that was sent to Rome, however, was quite different. The fish were not retrieved for 6 days and the fish temperature was 3-5^{o}C. This resulted in a calculation of an extra 10.9 Ice Days, with a total of 14.1 Ice Days. This value is less than the ~30 Ice Days that was typical of the vacuum packed fillets in activity four; however, it remains to be determined if whole YTK fish and fillets have similar spoilage profiles.

3.6.1.1 Domestic shipping

During the domestic shipment, the carriage conditions were consistent with low (+2^{o}C) temperature during 35 hr of road freight from Port Lincoln to Sydney. In Sydney the shipment was stored at 10^{o}C for 11 hours before it went for a short (1 hour) journey to Botany in a refrigerated vehicle (Figure 36a). Box type did not significantly affect the temperature inside the box (F_5=1.3, P=0.4) or inside the fish (F_5=1.4, P=0.3) (Figure 36b and c). However, inside box temperature statistics should be viewed with some caution as sample sizes of the treatment groups were unequal due to several missing data loggers, and it should also be noted that due to practical constraints the box sample size was limited to three. This is considered less than ideal in terms of performing robust statistical tests (pers. comm, Skuza, P).

Despite these factors, the temperatures inside the boxes remained between -1 and +3.5^{o}C for the entire trip; similarly, the temperatures inside the fish were between -1 and +1^{o}C for the journey. This suggests that the combination of icing of the product in the boxes, and the well-regulated temperature regimes during transport and storage, are sufficient to keep the whole YTK sufficiently chilled during domestic shipments.

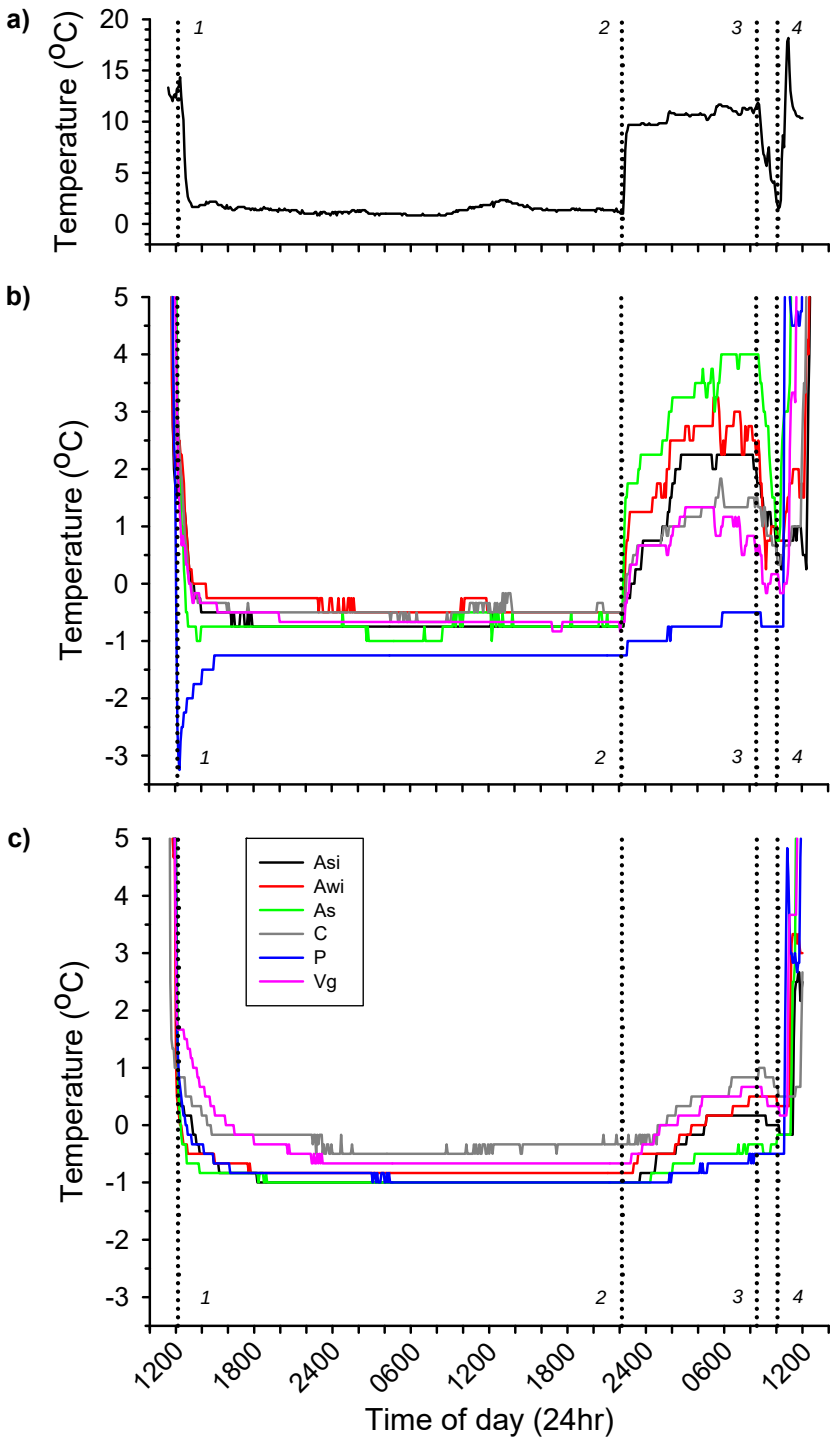

Figure 36. Mean temperature profile of domestic shipment of fresh, whole Yellowtail kingfish (*Seriola lalandi*) from Port Lincoln, South Australia to the Sydney Fish Markets, Pyrmont, New South Wales (*n* = 3) (1 = Load truck in Port Lincoln, 2 = Unload truck at Sydney Fish Markets, Pyrmont, 3 = Box transferred to Joto chiller, Botany, 4 = Retrieve loggers). Temperature profile were for outside the box (a), inside the box (b) and inside the fish (c) when shipped in six packaging systems (polystyrene (P), Chillquest (C), Visy gusset (Vg), Amcor silver lined (As), Amcor silver insert (Asi) and Amcor white insert (Awi)).

Box type did not significant affect the QI score of the fish (F_5=1.1, P=0.4) or freshness (K value) of the muscle (F_5=1.3, P=0.4) when assessed on arrival at the destination wholesaler (Figure 37 and 38, respectively). A QI score of 3-4 indicates the fish are still in very good condition, and this is confirmed by the low K values (<20).

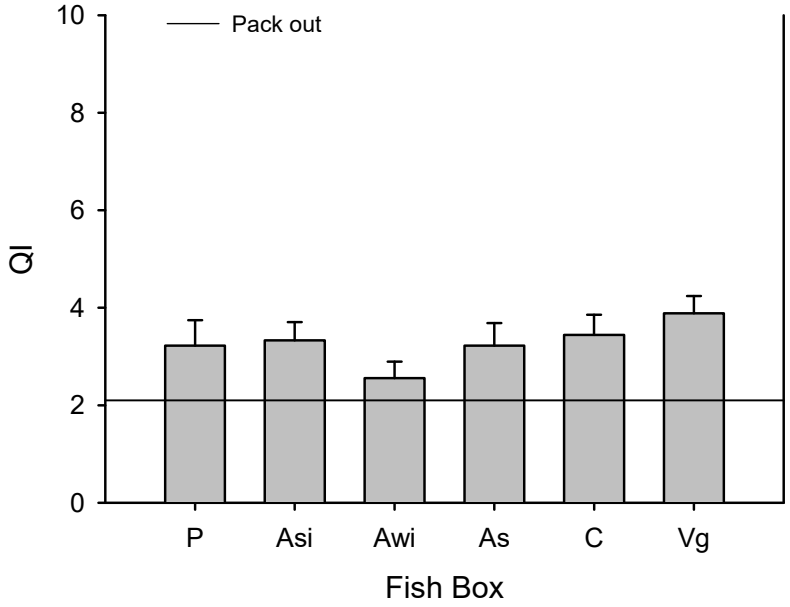

Figure 37. Mean (± se) quality index (QI) of fresh, whole Yellowtail kingfish (*n* = 3) at pack out and domestic destination wholesaler when shipped in six different packaging systems: Polystyrene (P), Amcor silver insert (Asi), Amcor white insert (Awi), Amcor silver lined (As), Chillquest (C) and Visy gusset (Vg).

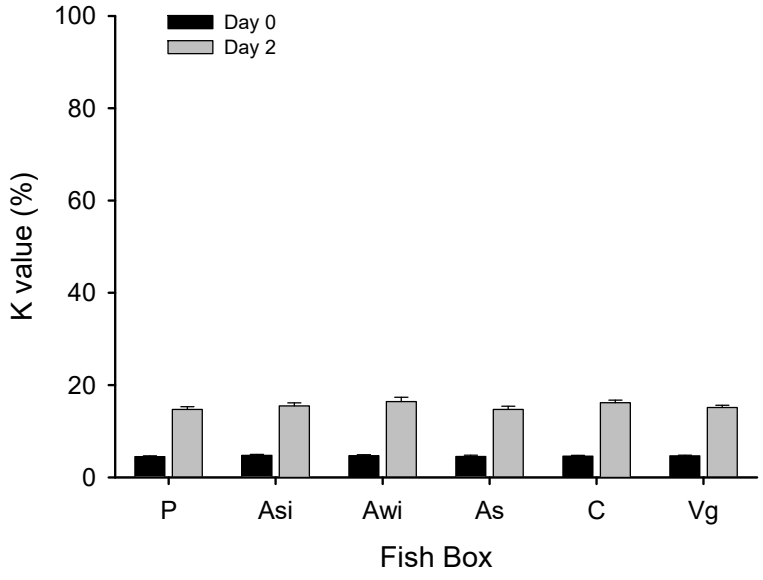

Figure 38. Mean (± se) K value of fresh, whole Yellowtail kingfish (*Seriola lalandi*) (*n* = 3) at pack-out (day 0) and at domestic destination wholesaler (day 2) when shipped in six different packaging systems: Polystyrene (P), Amcor silver insert (Asi) and Amcor white insert (Awi), Amcor silver lined (As), Chillquest (C) and Visy gusset (Vg).

3.6.1.2 Export shipping simulation

During the simulated export shipment, box type had a significant effect on the temperature inside the box (F_5=23.6, P=0.00) and inside the fish (F_5=13.7, P=0.00) (Figure 39). The temperature inside the box was significantly lower (by 1-7^{o}C) in the polystyrene box (P) compared to all other box types throughout the trial. The temperature inside the Visy gusset box (Vg) was also significantly lower than the Amcor silver lined box (As) and the Chillquest box (C); however, there was no significant difference between the Visy gusset (Vg), Amcor silver insert box (Asi) or the Amcor white insert box (Awi) or between the Amcor silver insert box (Asi), Amcor white insert box (Awi), Amcor silver lined box (As) or the Chillquest box (C). Most importantly, the temperature inside the fish was significantly lower (by 1.5-6^{o}C) in the polystyrene box (P) compared to all other box types throughout the simulated journey. When these profiles were converted to Ice Days the polystyrene boxes gave a result of 2.9 Ice Days, whereas a midrange fibre board box gave a value of 4.4 calculated Ice Days.

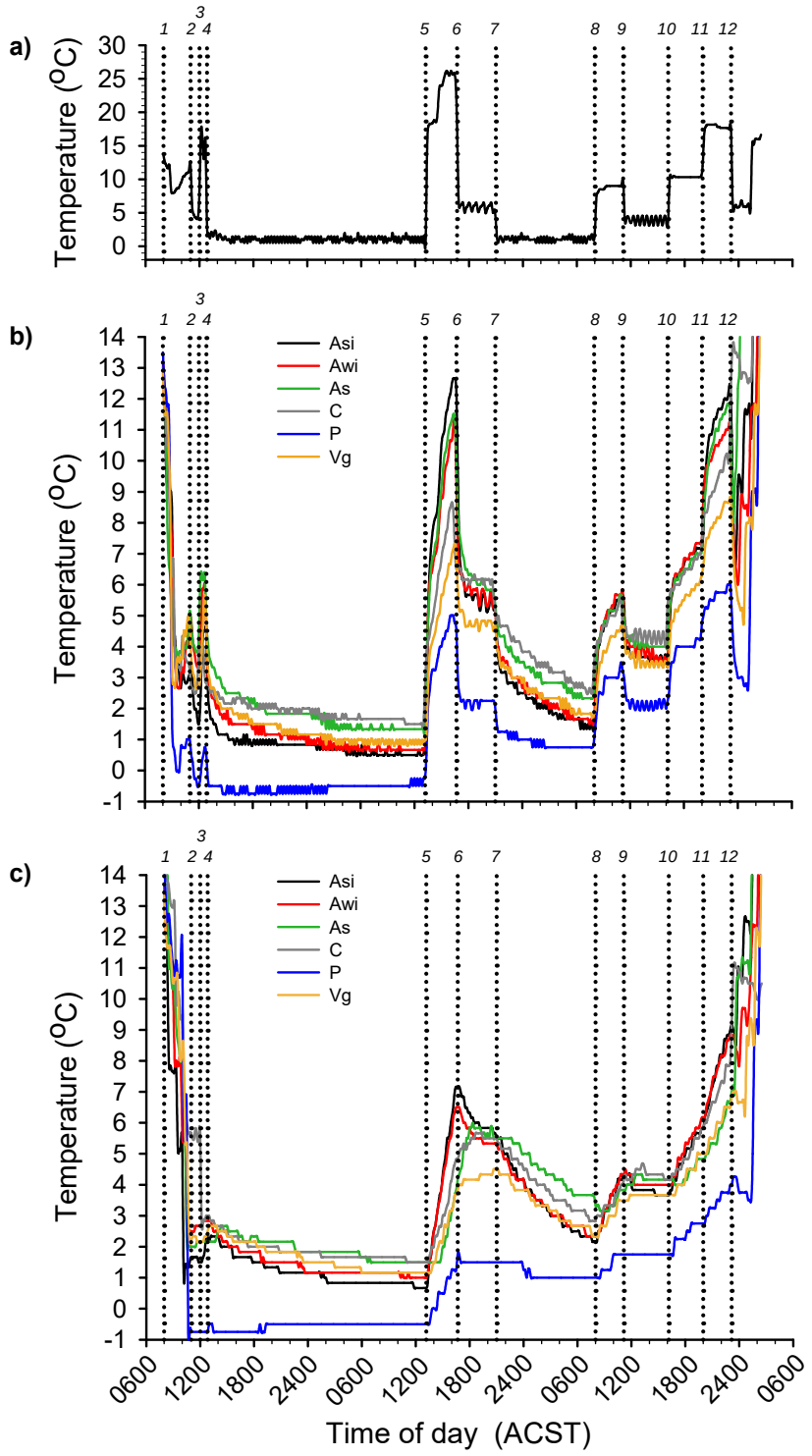

Figure 39. Mean temperature profile outside the box (a), inside the box (b) and inside the fish (c) during simulated export shipment of fresh, whole Yellowtail kingfish (*Seriola lalandi*) in six different packaging systems (polystyrene (P), Chillquest (C), Visy gusset (Vg), Amcor silver lined (As), Amcor silver insert (Asi) and Amcor white insert (Awi)) (*n* = 3). (Critical ambient temperature sequence is overlayed as vertical dotted lines and numbered: 1 = factory floor, 2 = factory chiller, 3 = insulated van, 4 = road / air freight, 5 = Singapore ground, 6 = Singapore chiller, 7 = air freight, 8 = Zurich ground, 9 = Zurich chiller, 10 = out of chiller, 11 = market, 12 = retrieve loggers and experiment ends).

The temperature profile outside the box, inside the box and inside the fish for the control polystyrene boxes (i.e. baseline) is shown in Figure 40. The profile shows a fairly stable temperature throughout the simulated transport period. Even after 2.5 days the temperature inside the box and the fish was ~2°C below the external temperature.

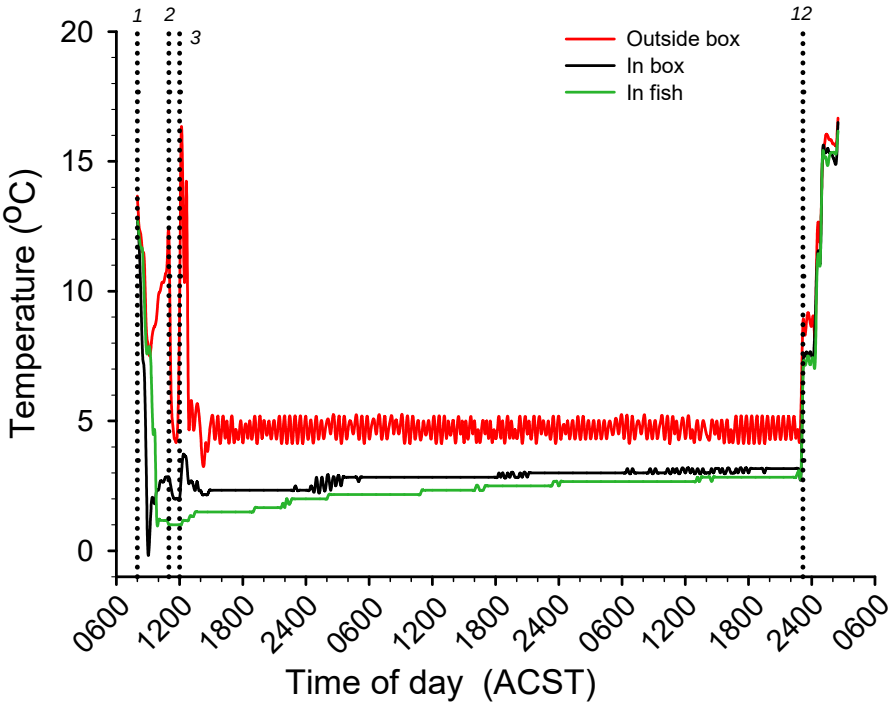

Figure 40. Mean (*n* =3) temperature profile outside the box, increase the box and inside whole, fresh Yellowtail kingfish (*Seriola lalandi*) packed into polystyrene fish boxes held in a constant temperature room set at 4°C. (Critical ambient temperature events other than constant temperature room storage are overlayed as vertical dotted lines and numbered: 1 = factory floor, 2 = factory chiller, 3 = insulated van, 12 = retrieve loggers and experiment ends).

Box type did not significantly affect total plate count (P = 0.077), QI score of the fish (P = 0.063) or freshness (K value) of the meat (F_5=0.9, P=0.5) assessed at the completion of the simulated export shipment period (Figure 41 to 43, respectively).

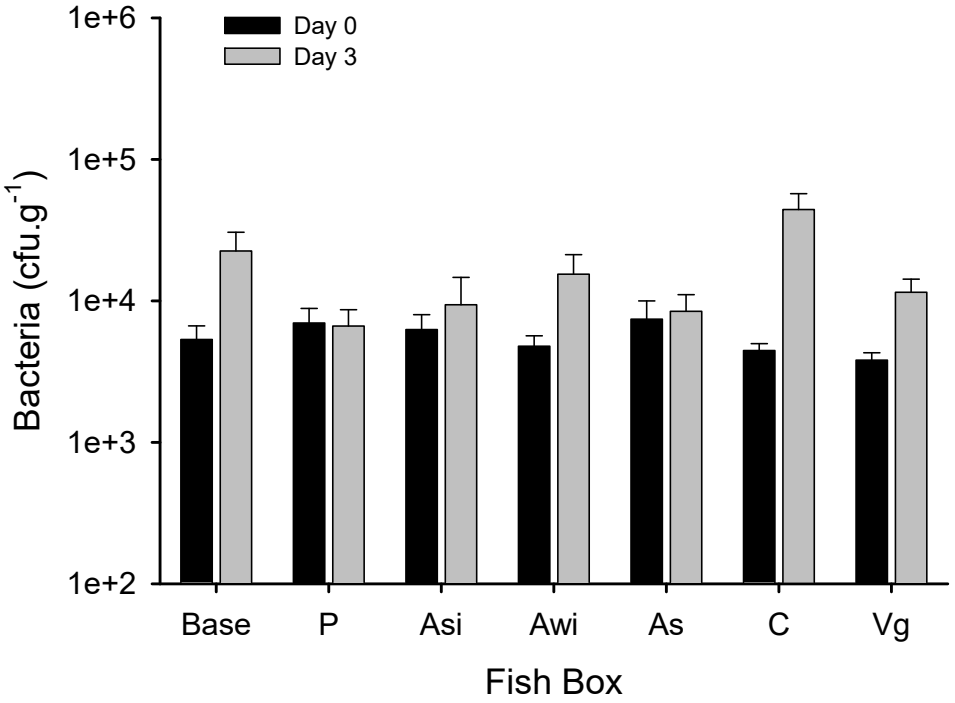

Figure 41. Mean (± se) total plate count (note the log scale) from whole, fresh Yellowtail kingfish (*Seriola lalandi*) (*n* = 3) packed in six different packaging systems for simulated export shipment (polystyrene (P), Amcor silver insert (Asi), Amcor white insert (Awi), Amcor silver lined (As), Chillquest (C) and Visy gusset (Vg)). Baseline (Base) group were P boxes held at constant 4ºC for the same period.

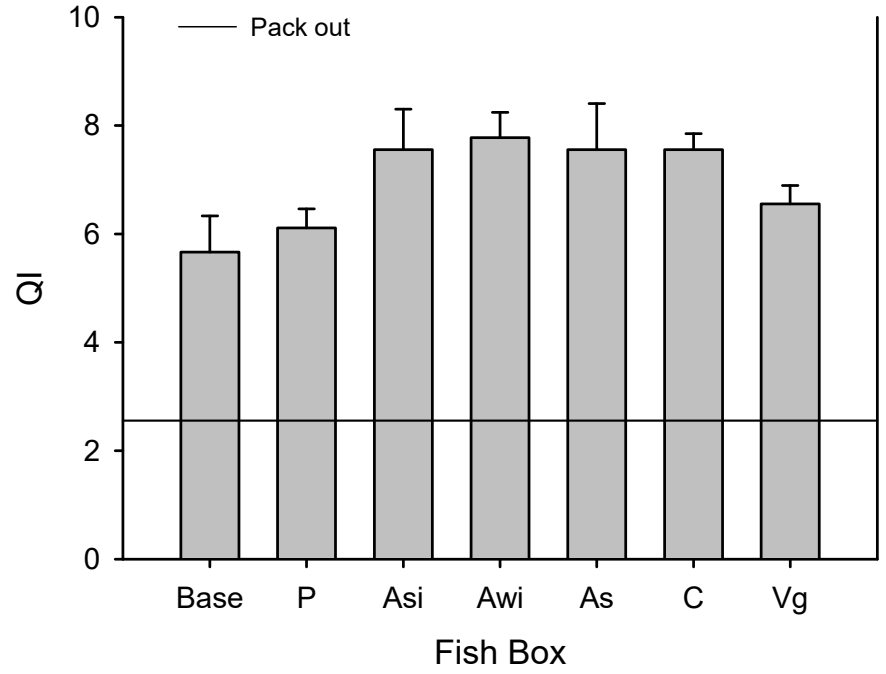

Figure 42. Mean (± se) quality index (QI) of fresh, whole Yellowtail kingfish (*Seriola lalandi*) (*n* = 3) at pack out (day 0) and after simulated export shipment in six different packaging systems (polystyrene (P), Amcor silver insert (Asi), Amcor white insert (Awi), Amcor silver lined (As), Chillquest (C) and Visy gusset (Vg)). Baseline (Base) group were P boxes held at constant 4ºC for same period.

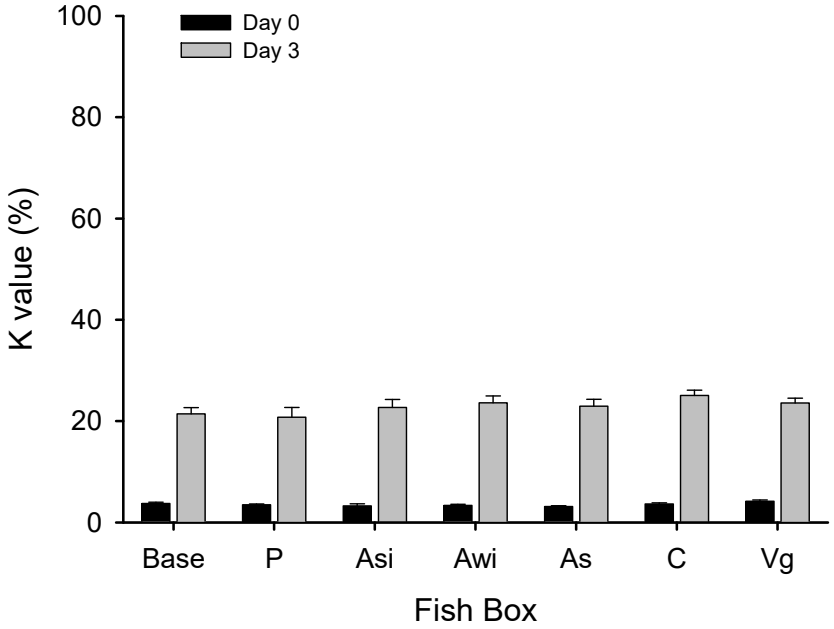

Figure 43. Mean (± se) K value in fillet sample from fresh, whole Yellowtail kingfish (*Seriola lalandi*) (*n* = 3) packed in six different packaging systems for simulated export shipment (polystyrene (P), Amcor silver insert (Asi), Amcor white insert (Awi), Amcor silver lined (As), Chillquest (C) and Visy gusset (Vg)). Mean K value at factory pack out (Day 0) is displayed but is not included in analysis. Baseline (Base) group were P boxes held at constant 4°C for same period.

The proportion of the gel pack frozen at the completion of the simulated transport period was significant affected by box type (F_5=99.4, P=<0.001) (Figure 44). A significantly higher proportion of the gel pack was still frozen in the polystyrene box (90%; P), compared to all other box types (0-10%) and the Visy gusset box (30%; Vg) had a significantly higher proportion of the gel pack still frozen compared to all other non-polystyrene box types.

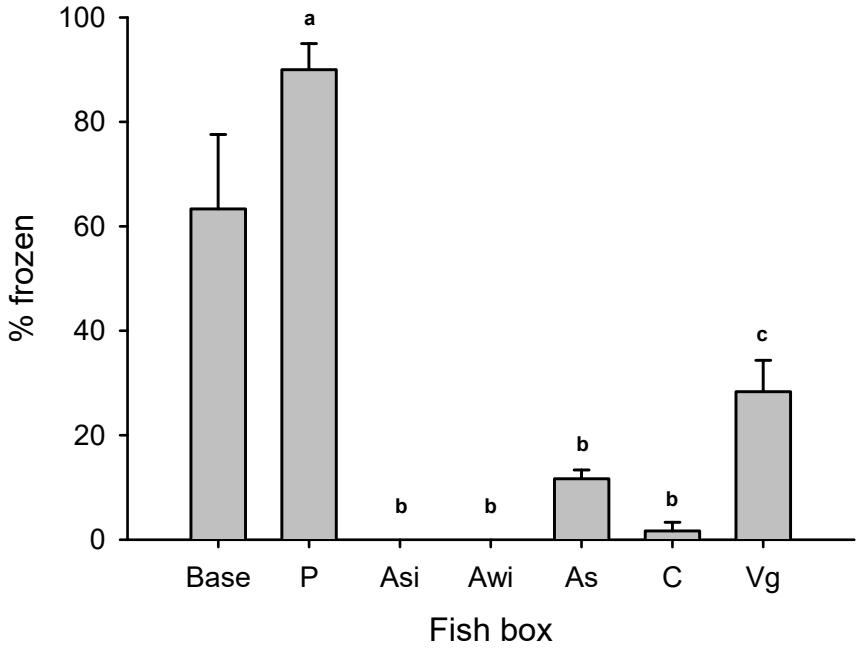

Figure 44. Mean (± se) proportion of gel packs that were frozen (*n* = 3) after simulated export shipment packed in six different packaging systems (polystyrene (P), Chillquest (C), Visy gusset (Vg), Amcor silver lined (As), Amcor silver insert (Asi) and Amcor white insert (Awi)). Baseline (Base) group were P boxes held at constant 4°C for same period. Significance is indicated by different superscripts between groups ($p<0.05$) (Base not included in statistical analysis).

3.6.1.2 Packing weight and packaging system durability

The pack-out weights of the alternative packaging system for domestic shipment (weight of the box and ~10 litres of flake ice that is enough to cover the fish) and for export shipment (weight of the box and two gel packs) are shown in Table 7 and 8, respectively. Prior to packing with fish and gel packs / ice, the polystyrene (P) box was the lightest compared to all other box types, with the Amcor silver lined (As) being the heaviest followed by the Visy gusset (Vg), Amcor white insert (Awi), Amcor silver insert (Asi) and Chillquest (C). Compared to the P box, all the other boxes were between two and three times heavier. The capacity of the Amcor silver lined box (As) has a larger capacity (eight fish) compared to the polystyrene box (five fish), along with the Amcor silver insert (Asi) and Amcor white insert (Awi). When these results were extrapolated to a hypothetical shipment of 100 fish the differences in box number, weight of packaging (boxes + ice) became more 'real'. The larger capacity boxes were ~20-25% lighter than the polystyrene standard boxes+ice, which worked out to be ~2.5% of the overall shipment weight (340kg fish + 78 to 104kg packaging). In this measure the Awi box came out slightly ahead at 78kg, the Vg and P boxes were similar at 100-103kg but the heaviest was the C box at 121kg. Cost of the

boxes per 100 fish needs to be entered into the calculation, as do the road freight charges (especially whether they are levied per pallet or per tonne).

Table 7. Pack-out weight of alternative packaging systems for whole, fresh Yellowtail kingfish (*Seriola lalandi*; average 3.4kg) without and with ~10 litre of flake ice to cover fish for domestic shipments. Sorted by packaging wt per 100 fish.

Box type*	Capacity (pieces)	Box wt (kg)	Ice (kg) per box	Packaging (kg/box)	Boxes per 100 fish	Packaging wt (kg) per 100 fish
Awi	8	1.64	4.6	6.24	13	78.0
Asi	8	1.6	4.9	6.50	13	81.3
As	8	2.4	4.2	6.60	13	82.5
Vg	7	2.14	4.9	7.04	14	100.6
P	5	0.68	4.5	5.18	20	103.6
C	5	1.36	4.7	6.06	20	121.2

* (P) Polystyrene, (Awi) Amcor white insert, (Asi) Amcor silver insert, (As) Amcor silver lined, (Vg) Visy gusset, (C) Chillquest

When the same exercise was carried out for the export shipment, the only difference was that the variable ice weight was replaced with a constant weight of two gel packs (Table 8). Again, the three fibre board boxes capable of holding eight fish came out ~20% ahead of the P box. Aircraft AV containers are charged by volume, so these values must be adjusted by the number of each type of box that can fit into an AV.

Table 8. Pack-out weight of alternative packaging systems for whole, fresh Yellowtail kingfish (*Seriola lalandi*; average size 3.4kg) without and with two gel packs for export shipments. Sorted by packaging wt per 100 fish.

Box type*	Capacity (pieces)	Box wt (kg)	Gelpack (kg) / box	Packaging (kg/box)	Boxes per 100 fish	Packaging wt (kg) per 100 fish
Asi	8	1.6	2.7	4.30	13	53.8
Awi	8	1.64	2.7	4.34	13	54.3
As	8	2.4	2.7	5.10	13	63.8
P	5	0.68	2.7	3.38	20	67.6
Vg	7	2.14	2.7	4.84	14	69.1
C	5	1.36	2.7	4.06	20	81.2

* (P) Polystyrene, (Awi) Amcor white insert, (Asi) Amcor silver insert, (As) Amcor silver lined, (Vg) Visy gusset, (C) Chillquest

The durability test showed that when all packaging systems when packed with eight whole, fresh YTK and two gel packs were dropped from a height of approximately 1.6 metres onto a concrete surface the polystyrene box cracked severely and all other

box types remained intact (Figure 45). The bottom flaps of the Chillquest box did loosen; however, the box still remained intact.

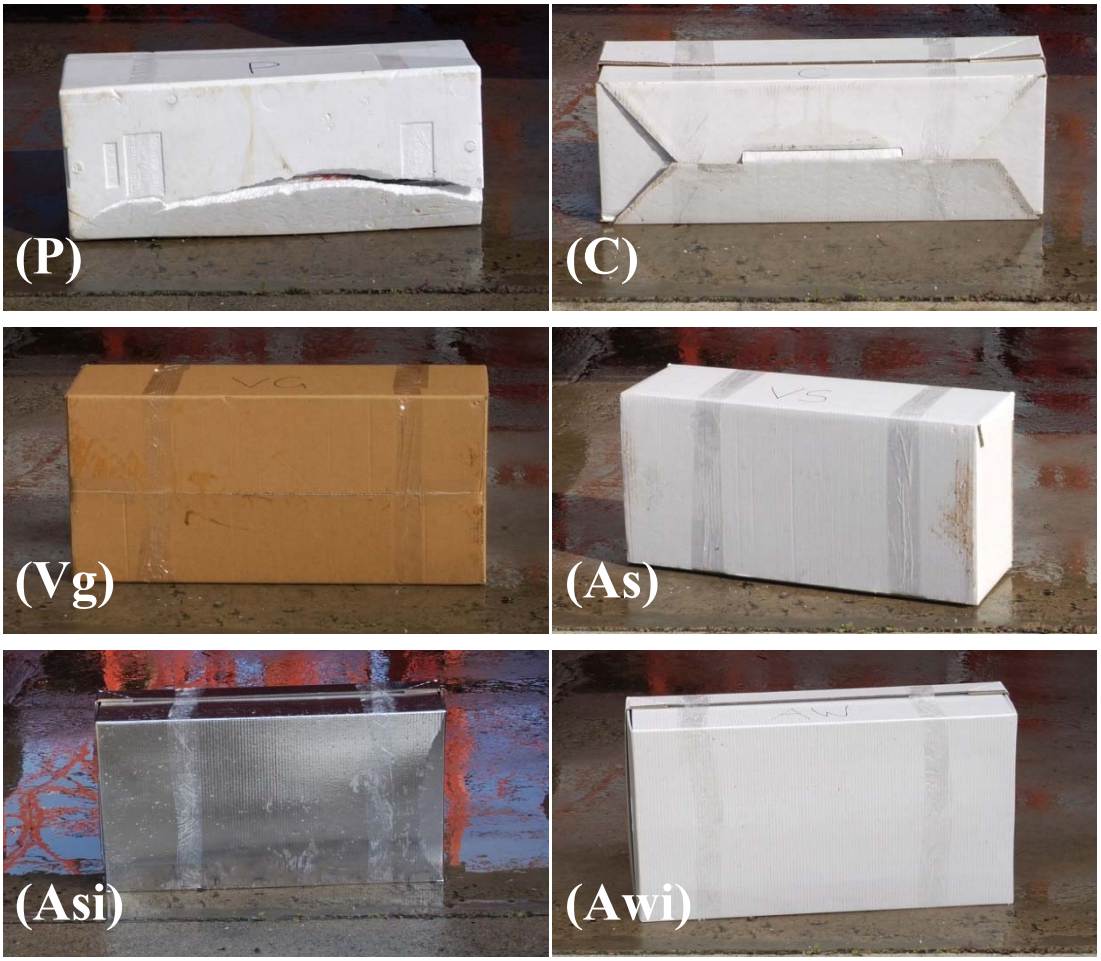

Figure 45. Packaging systems following the durability test: Polystyrene (P), Chillquest (C) (manufactured by Visy), Visy gusset (Vg), Amcor silver lined (As), Amcor silver insert (Asi), Amcor white insert (Awi). Boxes were packed with eight whole fresh kingfish and two gel packs and dropped from a height of approximately 1.6 metres onto a concrete surface.

4. General Discussion

This project was carried out during 2009-11 and the results and conclusions reflect the situation as it was at the time. The fish that were sampled during this study were from the 2007 and 2008 YTK hatchery seasons, and their bodyweights varied as the fish grew from about 1.1 to 3.4kg. This size range was representative of commercially harvested fish at this time. No attempt was made to influence the on-farm management of the fish sampled in this study; thus, feeding ration, feed type, stocking density, cage location, fish grading and harvesting decisions were outside of the responsibility of the research staff. Despite this, most research activities were not greatly impacted by these factors. Due to the practical limitations of the study any seasonal changes in carcase and fillet characteristics reported in this document could also be due to the effects of increasing age/size of the sampled fish.

4.1 Activity one: fillet crude fat distribution

It's known that the biochemical composition of muscle tissue/flesh/meat will vary between sites within a carcass and over time (season and/or size) (Grigorakis, 2007). One of the most common observations is that the flesh from the ventral area of the fillet (sometimes called the 'belly flap') is higher in lipid than the flesh from the dorsal area. Thus, in dorsal and ventral areas the crude lipid levels in rainbow trout, sea bass and gilthead seabream were 4.0 vs 6.6%, 4.5 vs 13.0% and 8.6 vs 14.4%, respectively (Testi et al., 2006), and in Atlantic salmon it varied between 2.4 and 18.6% in the same fillet (Katikou et al., 2001). In temperate waters this difference can be overlayed by a seasonal pattern in lipid deposition/mobilisation, with lipid being accumulated in summer when food is more plentiful, and then being mobilized and being used for energy in winter when food is less plentiful, and/or for growth in the following spring when water temperature begins to increase. Lipid reserves can also be mobilized if required for seasonal activities such as migrations or spawning (MacKinnon, 1972); however, neither of these factors are unlikely to affect young YTK in aquaculture conditions. Changes in the amount of lipid in a tissue are usually mirrored by an opposing change in the amount of moisture, with the levels of protein and ash not usually changing very much (Grigorakis, 2007).

Lipid is also very important in affecting the post-harvest attributes of the flesh. Lipid affects flavour, mouth-feel and colour of the product, with high fat cuts of species like tuna and salmon being favoured for high quality sashimi (Hyldig and Nielsen, 2007). Fish flesh that is high in lipid may be more prone to rapid and/or more noticeable

deterioration due to the highly unsaturated nature of the fatty acids contributing to the development of rancidity due to oxidation.

Thus, for a number of reasons it is important for a producer of YTK to know how much lipid there is in different parts of the carcase, so that they can choose to take advantage of the opportunity to differentiate higher-value high lipid specification cuts of YTK in the marketplace, and also to be aware of factors that can impinge on the quantity and/or quality of that lipid (and therefore the flesh) in order to mitigate their impact.

The aim of this activity was to examine the effects of season on crude fat distribution in fillets from YTK. Fish from two different spawning runs were assessed in September 2009, January 2010, April 2010, August 2010, October 2010 and January 2011 with meat samples being taken from four distinct sites on the fillet (F1, 2, 3 and 4), in addition to a "Quality Cut" (QC) that represented an average fillet fat content. Overall, the results of this activity showed: 1) that there were seasonal changes occurring in the fat content of the fish over the season; 2) that there were distinct differences in fat content between the different fillet locations; and 3) that there were differences in fat content between different spawning runs over the season.

Fish had higher fillet crude fat content during winter (i.e. April, August and October) in all fillet areas (F1-3, the "belly flap" F4 and the "quality cut"), in both hatchery run groups. However, this pattern may appear exaggerated because at the start of the trial (September 2009) the fish did not have differentially higher lipid levels in the belly flap area (F4), and also the run 1 fish in January 2011 (the last sampling date) were 'graded small', and fish growth rate/size may a factor in the apparent 'hatchery run' effect on flesh lipid levels (see below). Higher lipid levels in fillet region F4 were measured from January 2010 onwards, and reached about 35% of flesh weight (3-4 fold higher than the other areas of the fillet). This suggests that fish larger than ~2kg could be marketed with a differentiated 'high belly fat' specification. The yield of the belly flap area, as a proportion of the whole fillet, should be determined for fish from 2kg and larger to see when the high lipid portion is larger enough to be marketable.

Generally similar patterns in flesh lipid in the different fillet areas were observed in hatchery run 1 and runs 2/3 fish, but the latter group was slower to show the trends. Both groups had very similar levels of fillet lipid in September 2009, so it does not appear as though they were coming from different starting points; instead, it appears

as though the run 1 fish were more quickly able to respond to the warming water conditions to put on body weight and differentially deposit lipid in the belly flap area. Whether this was because they were larger (1.5 vs 1.1kg), older or managed differently from the run 2/3 fish, cannot be determined from this study. What is clear though, is that the average run 2/3 fish, whilst only 2-3 months younger than the run 1 fish, do not reach the same average bodyweight or fillet lipid levels as the slightly older fish during the remainder of that calendar year. Similarly, the 'graded small' run 1 fish are substantially inferior to even the run 2/3 fish (graded medium). This finding does have implications for managing production and harvest schedules; the negatives (smaller bodyweight, lower carcase lipid levels) need to be considered with the extra costs of production (a longer time at sea with the greater implications for FCR and health issues), and a decision made as to whether small graded fish should be culled, and more focus be put onto the better growing fish, for maximal market return.

The "quality cut" appeared to have a crude fat level that most closely reflected the dorsal and caudal flesh (areas F1-F3), and did not show any influence from the belly flap area (F4). In this respect the "quality cut" fails to represent the whole carcase. Indeed, the level of lipid measured in the "quality cut" appears slightly lower than in areas F1, F2 and perhaps F3, suggesting that there may be an anterior-posterior decrease in fillet lipid. There is probably little commercial application for this observation.

4.2 Activity two: nutritional composition of YTK fillets

It has been long known that seafood is a healthy eating option due to its highly digestible protein, low levels of saturated fat, and high levels of several micronutrients. In addition, seafood is the richest and best natural source of long chain omega-3 fatty acids, a group of essential dietary lipids that our bodies cannot function properly without (Mozaffarian and Rimm, 2006). A growing section of the consumer market is concerned about how healthy their diets is, and these people make, at least partly, their purchase decisions based on the nutritional labelling of different foods (Danenberg and Mueller, 2011). Knowledge of the nutritional profile of seafood products can be extremely valuable with the information able to be used in many different ways to develop and maintain a products position in the market, add value to a product and/or target a niche market (pers. comm. J McCowan).

Different seafood species have slightly different nutritional profiles; thus, some fish are low in fillet lipid (e.g. flathead 0.7%) and others have a lot (e.g. Atlantic salmon 11.2%; Soltan and Gibson, 2008). As well as variation between species, there can be variation in nutritional profile within a species due to location, age or diet (Grigorakis, 2007). Thus, for an aquaculture company growing fish in a particular location and with particular diets, it is important to gather data from their own fish, and not rely on data that has been gathered from analysing wild fish, or another farm.

Thus, the aim of activity two was to determine the nutritional profile of commercially harvested YTK in winter and summer so that CST can confidently communicate nutritional information about their products to the marketplace.

The results showed that season (i.e. winter and summer) and fillet area (i.e. dorsal and ventral region) significantly influenced various nutritional components of YTK fillets. Whilst statistically significant, most of the seasonal and fillet area differences in proximate (protein), macro- and micronutrients (vitamins and minerals) were relatively small. The largest differences were related to the levels of crude fat, lipid types and fatty acids in the different fillet areas.

How do the levels in the different fillet areas of YTK compare to other seafood species, and, where appropriate, to the recommended daily intake of these nutrients? Table 9 puts the YTK values into context. The comparison shows that the YTK product (dorsal and ventral areas of the fillet) has a very similar product specification to the farmed Atlantic salmon. Thus, proximate (protein, lipid, moisture and ash (and carbohydrate)) composition are essentially the same, as are the levels of most micronutrients and vitamin C. YTK fillet does appear to be slightly higher than Atlantic salmon in iron (Fe), zinc (Zn) and potassium (K), but none of these are high enough to affect their contribution to the %RDI, so they cannot be seen as being substantial differences.

Most of the difference between YTK and Atlantic salmon is in the breakdown of the lipid types, with YTK having more omega-6 (1,000 vs 666 mg/100g), slightly higher levels of the omega-3 fatty acid EPA (850 vs 690 mg/100g) and less than half the level of omega-3 DHA (660 vs 1,457 mg/100g) than salmon. The carcase lipid profile is mostly affected by the types (sources) of lipid found in the diet, and these high omega-6 and low DHA results suggest that the YTK diet was higher in terrestrial (plant and/or animal tallow) sources of lipid than the salmon diet which was higher in

marine fish oils. Despite this, a serve of 100g of YTK flesh clearly meets the daily recommended omega-3 lipid intake for an adult. All of these data confirm that farmed YTK is a highly nutritious product and if this message is communicated properly it should appeal to the health-conscious customers in the marketplace and result in more sales of the product.

Table 9. Mean summer/winter values of nutritional attributes for Yellowtail kingfish (*Seriola lalandi*) dorsal and ventral fillet areas compared to published values for raw farmed Atlantic salmon fillets (USDA SR 24) and recommended daily intake (RDI) and adequate daily intake (ADI) for adults aged 31-50 from NHMRC (2006).

	YTK		Atlantic Salmon	Adult age 31-50 RDI (mg/day)		Adult age 31-50 ADI (mg/day)	
	Dorsal	**Ventral**	**Fillet**	**Male**	**Female**	**Male**	**Female**
Proximates (g/100g)							
Protein	23.0	21.7	20.4				
Crude Fat	9.2	14.1	13.4				
Moisture	67.1	63.1	64.9				
Carbohydrate	0	0	0				
Ash	1.6	1.6	1.2				
Energy (kJ/100g)	760	920	862				
Lipid types (g/100g)							
Saturated	2.50	3.70	3.05				
Trans	0.10	0.15	0.00				
Polyunsaturated	2.90	4.45	3.77				
Monounsaturated	3.80	5.80	3.77				
Unsaturated fatty acids (mg/1000g)							
Omega 6 FA	1,000	1,600	666				
Omega 3 FA	1,600	2,500	2,260	610	430		
20:5n-3 EPA	648	1,021	690				
22:6n-3 DHA	552	777	1,457				
Micronutrients and minerals (mg/100g)							
Fe	0.40	0.45	0.34	8	18		
Cu	0.10	0.05	0			1.7	1.7
Zn	0.50	0.55	0.36	14	8		
Mn	0	0.01	0			5.5	5
Ca	5.80	6.90	9.00	1,000	1,000		
Na	31	45	59			460-920	
Mg	32	29	27	420	320		
P	244	252	240	1,000	1,000		
K	422	373	363			3,800	2,800
Vitamins (mg/100g)							
Vit C	3.5	3.5	3.6	45	45		
Vit E	2.6	2.6	3.6			10	7

This activity shows differences in some of the nutritional attributes of YTK fillets and CST's marketing team may find this information extremely useful in devising new marketing strategies. With knowledge of the nutritional profile of YTK fillets based on season and fillet region the dorsal area (i.e. top loin) could be sent to one market where its attributes are highly desirable and the ventral region (i.e. belly loin, trimmed) sent to an alternative market seeking that particular high-fat specification (pers. comm. J McCowan). This would potentially secure a higher farm-gate price than could be realised from an undifferentiated product (as is currently practiced by the company).

CST promotes that its aquaculture products are consistent year round so adjusting the nutritional profile on the labels of products to account for seasonal variation may have a negative effect in the marketplace (although knowledgeable customers generally understand that there can be seasonal differences). Such changes might instil the thought in some customers that the product is better (or conversely, not as good) at different times of year, and this may drive a pattern of seasonal demand that may not fit with the production schedule of the company. For this reason the company may prefer to present average values for the various nutritional attributes on product labels. Legislative requirements in different marketplaces can require that producers report average values on their products, but the label must indicate the seasonal variability of the product, which prevents the consumer of being misled (FSANZ, 2011). This activity has clearly shown seasonal variation of some nutritional components and therefore such statement must continue to be included on the labelling of YTK products.

Indeed, the nutritional information determined from this activity has been supplied to CST for immediate use on their nutritional content labelling of retail packs, on brochures and other marketing materials (printed and online).

4.3 Activity three: whole body proximate composition

Various studies have shown that water temperature and seasonal changes can influence the proximate composition of fish (Grigorakis, 2007). However, to date there have been no detailed studies conducted investigating this for YTK. With fillet yield in YTK of 60-65% (see Carragher and Wilkinson, 2012 – Subproject 2 of this study), the data gathered from activity two cannot be extrapolated to the whole of the fish since the relative growth of bone, scales and various visceral organs (especially liver, gonad) and the amount of visceral fat can and will change as the fish grow, develop and mature. Many studies that try to determine optimal diet formulations for a new aquaculture species begin by analysing the composition of the species from the wild, and use that as an approximate starting point for choosing a diet (Shearer, 1994). With formulated feed being such a large cost for YTK production, and the pronounced seasonal growth rates in South Australian sea cages, it is important for CST to understand how the composition of their product change across seasons and between spawning runs and to provide feeds that match the requirements for the growth of the whole carcase. Thus, the aim of this activity was to examine how YTK whole body proximate composition changed during the grow-out period and identify any differences between spawning runs.

The results showed that as the fish grew the amount of ash (representing the elements and minerals, largely derived from the bones and scales) increased as a proportion of total body weight. The 1% increase, whilst not large, does indicate that these skeletal structural tissues become more mineralised as the fish age/grow, and that the elements involved in the mineralisation (not determined, but almost certainly calcium, phosphorous, magnesium etc) need to be provided either in the formulated diet and/or in the seawater. Mineralisation also requires that fish have sufficient vitamins for this process to happen effectively. This study does not reveal whether any of the minerals or vitamins were limiting.

Whole body protein levels were 19.5-21.5%, slightly lower than the fillet values of 21-24%, reflecting the difference in mineralisation between fillet (no bones) and the remainder of the carcase. Seasonal and hatchery run differences in whole body protein were observed and could be due to several factors including management activities (changes in feeding regimes or specifications), site differences in water temperature and/or the growth pattern/phase of that cohort of fish. What is commonly reported is that when fish enter a growth spurt phase the first increase in length (skeletal growth), and then they increase in weight as the muscle tissue grows to

take up the 'space' offered in the extension. Sometimes the growth is supported by an increase in nutrient intake (appetite, food quantity or quality) or a mobilization of energy and nutrient reserves from other tissues (oftentimes lipid stored in the flesh and/or liver). Compared to some species, such as those that undertake pronounced migrations or extended spawning activities that require significant energetic and nutrient mobilisations, the differences observed in this study are relatively minor. The pronounced seasonal effect on whole body vitamin C concentrations; however, does suggest that during the warmer months (January and May), the levels are 66% lower and perhaps a greater dietary level is needed at that time. Again, from the study carried out here it is not possible to deduce how the fish were growing, and/or where the resources came from to facilitate that growth.

For various reasons outside the control of this project, not enough samples of the commercial feeds that were being fed to the fish over the sampling period had the proximate composition analysed. The availability of this information would have allowed for a more in depth interpretation of the results to be performed. For example, studies have shown a strong link between dietary lipid level and levels high levels of lipid in the fillets (e.g. Regost et al., 2001). Therefore, the findings presented here only assume that there was no difference in the composition of the feed fed throughout the sampling period and cannot be considered an influential factor in the whole body proximate composition of the YTK.

4.4 Activity four: fillet shelf life

The marketability of seafood is greatly affected by its freshness, with (real or perceived) fresh seafood being highly sought after and gaining higher prices in the marketplace. Compared to many other fresh meat products, seafood can spoil quickly and it does so with sensory changes (texture, smell and/or appearance) that are pronounced and easily identified. Products that only have a short shelf life are more risky for the supply chain (producer, wholesaler, retailer, food service outlet or consumer) to carry, as the window to sell it or eat it could elapse, and the entity with the product is left carrying that financial cost (wastage). Clearly, products that have a longer shelf life carry much less risk and they offer the opportunity to be marketed further away from the site of production, reaching new customers and keeping prices high in all markets.

Chilled seafood product shelf life is affected by many things, some are intrinsic properties of the product, others are related to production and/or harvest processes,

and others are more affected by post-harvest processes. It is important to know what these factors are, and how they can be managed to extend the shelf life, and thus maximise the marketability of the product.

Whole books (very good ones at that), have been written on defining and measuring seafood 'quality' (Huss, 1995 ; Bremner, 2002). It is not the intention of this report to debate or repeat the detailed wisdom in those texts; nevertheless, it is important to clarify the terms and describe the attributes that are associated with the product(s) under consideration. In this regard, there has been market feedback that YTK, once filleted, has a short shelf life with a the flesh changing colour from a semi-translucent white/pale pink to an opaque mid-yellow colour within 2-3 days.

The aims of this activity were to 1) develop a quantitative method of measuring colour changes in fillets of YTK; 2) determine whether fillet shelf life is influenced by the time of harvest during grow-out; 3) if dorsal and ventral regions of the fillet have a different shelf life and; 4) determine whether the vitamin content and distribution in the flesh changes during grow-out. The activity was divided into two components to meet these aims.

4.4.1. Component one - quantitative method to measure colour change in fillets

The new method developed in component one provides the potential to quantitatively assess YTK fillet colour differences and change using inexpensive, commonly available and easy to master digital photography and Photoshop™ techniques. The YTK that were used to initially develop the colour measurement technique were from two groups that were harvested using quite different approaches, and it was already known that they would have different flesh colour with one treatment clearly having a redder flesh than the other. Indeed, the initial digital photography and Photoshop™ methodology did detect the colour separation both as blur averaged colour swatches and L*a*b* plots. However, there was considerable variation within the 10 fish in the non CO group that was not consistent with visual observations of the fillets. A closer examination of the digital photographs revealed there was some variability in the baseline colour balance; in that the white areas surrounding the fillet on its black backboard occasionally exhibited a slight pink or off white hue. This suggests that, despite using fixed camera settings, the set up that was used (camera and/or lighting) may not always give consistently repeatable images. Alternatively, the issue may be with the fillets themselves, they are inherently moist and have a non-flat

surface, both attributes that would reflect the direct light in this trial differently, potentially contributing to some colour variation.

Despite this, the results of the first trial suggest that the initial methodology was capable of detecting major colour differences in YTK fillets, but was probably less sensitive to subtle changes, and may, in fact, have contributed to some of the apparent colour variation. The extent of this variation could not be established until the technical artefacts were eliminated, and this was not possible in the time between component one and two of this activity. Thus, it was necessary to continue with the same methodology for the duration of component two.

4.4.2. Component two – shelf life of fillets and vitamin content and distribution over grow-out

The shelf life of YTK fillets was determined by using changes in several biochemical, physical and sensory attributes that have been used in other species. Since some customers have indicated that the colour shelf life of the fillet is their main issue, most of the attributes measured were either directly or indirectly linked to processes that could affect fillet colour. These included: fillet colour itself (measured directly by digital photography and Photoshop™), lipid oxidation (measured indirectly via TBARS), freshness (measured by determining the degradation of ATP and it's metabolites) and by odour (subjective assessment). No assessment was made of microbial spoilage in this trial.

4.4.2.1 Oxidative state - TBARS

The low level of TBARS measured during this trial, even at timepoints where subjective odour assessments clearly indicated the fillet was past it's marketable threshold, suggests that lipid oxidation is probably not a significant factor affecting the shelf life of YTK. When a finding like this is made, questions have to asked about the validity of the assay; but the TBARS assay used in this laboratory has been used in several other projects and has worked well, with values of 0.5-5 $mg.kg^{-1}$ measured in fresh and aged, freezer damaged and/or spoiled seafood products, respectively (D'Antignana et al., 2008). Indeed, TBARS levels of 5-8 $mg.kg^{-1}$ have been proposed as being indicative of spoiled flesh in other studies (Arashisar et al., 2004). Thus, it appears as though the finding that TBARS levels are low, and show no signs of change even when the fish is spoiled, would strongly suggest that lipid oxidation is not a prevalent process occuring in YTK fillets produced, packaged and stored as described in this trial. TBARS analysis may be better suited to assess lipid oxidation

in YTK products that have been frozen long term (i.e. two years) where oxidation could happen slowly.

4.4.2.2 Freshness - K value

K value has been used as an indicator of meat freshness for several decades, but values for product acceptability vary between species and according to the analytical technique used (Mørkøre et al., 2008). The Japanese guideline for freshness of tuna meat based on K value is: sashimi grade < 20 %, moderately fresh < 50 % and not fresh > 70 % (Ehira, 1976). European researchers have suggested that a K value of 70-80%, is the threshold for consumption of flesh (other than tuna) that will be cooked (Erikson et al., 1997). Based on the results of this trial, YTK fillets could be consumed as sashimi grade within three days of storage at 4°C, or less than three days after opening vacuum packs on day one of storage. Fillets that were vacuum packed reached a K value of 60-70% between days 12-15 in 3 of the 4 harvest events, but after only 8-12 days in the October 2009 harvest. The day 15-20 period corresponded when the subjective fillet odour scores were noted. Whether the short shelf life in October 2009 was a true seasonal, or a specific harvest and/or processing effect, is not known. What it does indicate though, is that the freshness (and thus the shelf life) of YTK fillets can be reduced and this could have negative consequences in the marketplace. It is essential to carry out further work to, firstly, understanding why and when this occurs, and secondly, develop mitigation strategies to prevent or minimise such accelerated deteriorations in product freshness from happening again. The correspondence between increasing K value (decreasing freshness), odour acceptability and microbial spoilage should be further examined.

4.4.2.3 Drip loss

Considering that the moisture content of flesh is inversely related to fat content, we would expect moisture loss to be lower in fish with a higher fat content. Crude fat content was higher in the fillets of fish sampled during the cooler months (i.e. winter) compared to those harvested in warmer months (i.e. summer) (see activity one) and therefore suggests that fish harvested in the cooler months should have a lower tendency to lose moisture. This was reflected in the results.

The other finding was that the longer vacuum packed fillets were kept in chilled storage, the more moisture they lost on opening such that by day 15 (when fillet smell/freshness was reaching the threshold of unacceptability), the fillet was losing 3% of its weight as moisture. Drip loss is an issue for the consumer because of the

perception that it is expensive water. Extrapolating to a 15 day old 1kg fillet, 3% drip loss is 30ml, at a retail fillet price of $30/kg this is effectively $0.90 'down the sink'. Minimising drip loss is probably not the highest company priority, but it is something that should be monitored to ensure it does not increase any further.

4.4.2.4 Changes in the fillet after the vacuum packaging is opened

Somewhat surprisingly, there was relatively little evidence that after a vacuum pack was opened the YTK fillet would deteriorate more quickly. Indeed, the experimental design was such that there were control vacuum packed fillets opened the day after some of the +3 day measurements were made. In each case, the K value and drip loss values were similar, and fillet colour was statistically, but not visually, slightly different. What might this indicate? It could suggest that the factor most affecting shelf life is time-temperature of storage, and that vacuum packing/exposure to air are relatively negligible. However, further experiments would be required to reveal what might be driving spoilage (including microbial spoilage).

4.4.2.5 Vitamin content over season

Interestingly, the fat soluble vitamin E was not higher in the high-lipid ventral area of the fillet compared to the leaner dorsal area. This differs from what has been reported in other studies (D'Antignana, 2007). In activity three (whole body composition) in the present study, the whole body vitamin C and E concentration was determined. The values obtained then are somewhat different to the fillet concentrations measured in activity 4 (vitamin C, 10-30 $mg.kg^{-1}$ whole body vs 30-45 $mg.kg^{-1}$ fillet; vitamin E, 20-80 $mg.kg^{-1}$ whole body vs 25-35 $mg.kg^{-1}$ fillet). This suggests that vitamin C is mainly stored in the fillet, and there is a sizeable non-fillet reserve of vitamin E. Furthermore, there were different seasonal profiles for concentrations of vitamins C and E in whole body and fillet, suggesting that there is differential control of abundance; that is, the fillet and the remainder of the carcase are decoupled. The physiological or functional significance of this is not known.

4.4.2.6 Summary

Overall, most of the results of component two, activity four, suggest that the time of harvest (i.e. season) and / or fillet area, had little effect on shelf life. Exceptions were drip loss (which was highest in January) and K value (which increased more rapidly in October), compared to all other months examined. Although not specifically tested, the benefits of vacuum packaging of fillets (other than the undoubtedly more convenient handling), were not especially evident in this study. Lipid oxidation did not

appear to be an issue for chilled and vacuum packed YTK fillets, and the reported yellowing of YTK fillets was not observed. Using the vacuum packaging and 4°C chilled storage conditions the deterioration in K value and subjective odour scoring, suggested the shelf life of YTK fillets was typically 12-15 days, but was 8-12 days in one trial.

Applying the 'Ice Days' equation (e.g. Doyle 1989; after Bremner) to these findings:

$$r = (0.1 \times T_{storage} + 1)^2$$

and $r \times t_{days}$ = ice days

where: r is the relative rate of spoilage, $T_{storage}$ is in °C and t_{days} is time in days

(ignoring the storage time-temperature after harvest and before processing as this will likely be constant) if we use $T_{storage}$ = 4°C and t_{days} is 8 or 15, we have a storage time to spoilage of between 16 to 30 ice days. The benefit of using the ice days principle is that we can see the effects of changing the storage temperature on the actual shelf life of the product. Consequently, if we hold the YTK fillets at 2°C instead of 4°C we get to 30 ice days in about 21 actual days instead of 15, thereby adding an extra 6 days to the actual shelf life.

4.5 Activity five: fillet skin on / skin off shelf life

It is well known that fatty fish are more prone to lipid oxidation that can spoil the flesh by producing rancid odours or flavours. The skin can provide protection to the lipid in the flesh because it is a relatively impermeable barrier and reduce the rate of oxygen diffusion into the flesh and thereby slows down the oxidative processes. On the other hand, the intense metabolic activities that happen during harvest and others that progress in the tissues post-mortem, can generate free radicals that can similarly oxidise fatty acids in the tissues. Thus, skinning the fish and removing the subcutaneous layer of lipid that is more prone to oxidation can also be employed as a mitigation strategy. Which fillet presentation strategy might work best for YTK, a fish known to have relatively high fillet fat levels?

The aim of this activity was to identify whether the vacuum packed skin-on YTK fillet presentation had a different oxidative shelf life (as measured by TBARS) to YTK fillet that was shallow skinned before packing. The sampling also sought to determine if there was a difference in the incidence or rate of oxidative deterioration of fillets between YTK harvested in summer and winter.

The results of this activity showed that levels of lipid oxidation in the fillets (skin on or

shallow-skinned) were low and did not change much with storage at 4°C, suggesting that this particular mechanism is not a concern for chilled vacuum packed YTK fillets. This finding was consistent with the results of activity four (component two). Thus, it appears that fillet presentation format did not impact on oxidative shelf-life of this product type, and therefore presentation format (skin on or skin off) need only be dictated by customer preference, not shelf life concerns. However, whether lipid oxidation could be a more important factor in other product forms (e.g. long-term frozen storage of fillets) still needs to be addressed.

4.6 Activity six: effect of temperature fluctuations on fillet shelf life

Temperature is arguably the single greatest determinant of seafood shelf life, with rates of physical, biochemical and microbiological spoilage processes all being affected (Huss, 1995; Bremner, 2002). Even short periods of elevated temperature can allow one or more of those spoilage processes to be accelerated, and even if temperature control is re-established, the consequences of those spoilage changes will remain or persist in the product (e.g. Cyprian et al., 2008). Those consequences include: a shortened product shelf life in the hands of the customer, restrictions on how long the product can be shipped (i.e. where the product can be sent) to expand market opportunities, increased wastage of spoiled products, perceptions of this being a low quality product, perceptions that poor quality products are symptomatic of a poorly managed company, and, most worryingly, potential for issues of food safety. One incident of any of the above issues can have long-term and broadly felt consequences for that product, that business or even the whole sector, in the marketplace.

The issue about poor temperature control is that it can happen anywhere in the supply chain, from harvest through to when it gets to the customer or consumer. Depending on where the customer or consumer are, that supply chain could have between 4 and more than 10 parties, any of which could have been responsible for the problem. A quality focussed business needs to know that it has the equipment, protocols, people and record-keeping systems to prevent this from occurring, and if it does happen, the ability to change things to make sure it doesn't happen again. A company producing temperature-sensitive products needs to ensure that all the parties in the supply chain share and work by that ethos.

The aims of this activity were to collect data on the cold chain management practices

in the current (2009-10) shipments of chilled YTK product to key domestic and export markets, and to investigate the suitability of five alternative packaging systems to the standard polystyrene box for shipping whole fresh YTK to domestic and international markets. This activity was divided into two components to address these aims.

4.6.1 Cold chain management practices

CST are currently shipping whole, chilled YTK to a number of overseas destinations, including Rome, Bangkok, Philadelphia, Los Angeles, Zurich and Amsterdam. The objective of this component was to collect data to validate the current cold chain management practices during chilled shipments using polystyrene boxes to these destinations. The results showed that on these shipments good cold chain management practises were employed, with no temperature violations recorded (i.e. >4°C) either inside the polystyrene box or inside fish. There were larger variations in temperature observed outside the boxes; however, the highest temperatures were less than 15^{o}C and the lower temperatures was -7^{o}C, but these were only recorded for short durations (an hour at most) and the polystyrene box was able to insulate the product from any significant warming or threat of freezing.

The extremes in outside box temperature generally occurred when the product was being transferred from one part of the supply chain to the next, with temperatures being consistently low and well controlled whilst the product was en route. In some of the shipments (e.g. Los Angeles in July 2010); however, there were signs that the end-user did not keep the product in a sufficiently chilled environment (~7^{o}C) for ~16 hours. During this time the inside box and inside fish temperatures remained below 2^{o}C, suggesting that the in-box chill packs were still largely frozen and thereby protecting the fillets from violating temperature thresholds.

These findings suggest that the supply chain partners used by CST for chilled shipments of YTK to major domestic and export centres were effective in their adherence to cold chain principles and practices.

4.6.2 Alternative packaging systems

Polystyrene boxes are a popular option for shipment of seafood products: they have excellent insulation properties, they are light weight and relatively inexpensive. However, there are several disadvantages too: because they cannot be flat-packed they are expensive to freight empty, they are fragile to crushing impacts, and they are not made from biodegradable components and therefore not perceived as being

environmentally friendly. Consequently, there has been a lot of interest in moving to alternative packaging systems, and several packaging companies are working toward solutions that they hope may replace the polystyrene box.

Most of the alternative packaging systems overcome the shortcomings to polystyrene boxes: they are supplied as flat pre-cut and crease-folded fibre board sheets that stack as a compact, dense load reducing freight and storage requirements, they offer greater resilience to crush impacts and they are fully recyclable. They are also lightweight and relatively inexpensive to purchase. However, the big questions are 'how easy/expensive are they to assemble' and 'do they insulate as well (or better) than polystyrene'?

CST sends most of its product to markets both domestically and overseas in polystyrene boxes and, like most businesses, is looking for efficiency improvements in its operations. The costs of packaging, and the weight/volume implications of that packaging on freight costs, are significant additions to pricing of product in the marketplace. Any modifications that can reduce the cost without compromising product quality (in this case, through temperature control and physical integrity of boxes as a result of impact damage) will improve the margins for the business.

This investigation showed some very promising results for the use of alternative packaging systems for shipping whole, fresh YTK, especially domestically. During the domestic shipping trial, although polystyrene boxes maintained the lowest inside and in-fish temperature, there was no significant difference in temperatures inside the box or inside the fish, and QI and K values were not significantly different when the product reached the destination using the alternative box types. It therefore appears as though these boxes will give 'equivalency' to polystyrene for domestic shipments, so the next question is are there cost-savings by using one or more of the alternative packaging types? This will be discussed more below.

However, for the simulated export shipment, there were significant differences in the temperature profiles evident from inside the box and inside the fish for the different box types, with polystyrene performing best.

Why were the findings for 'equivalency' of the alternative box types different in the domestic trial and the simulated export shipment? This could be related to a number of factors: 1) boxes were exposed to different temperature profiles during domestic

and export shipment (in particular, the simulated export shipment used a 'worst case' scenario with elevated outside temperatures of 20-28^{o}C at the beginning and halfway through the trial); 2) different durations of shipment; 3) different means of in-box cooling (i.e. gel packs vs flake ice). It is interesting that, right from the outset of both trials, the inside box and inside fish temperatures of the different alternative box types were 1.0 to 2.5^{o}C higher than the polystyrene box. This strongly indicates that the difference in performance is not due to the difference in durations of the trials, nor the in-box cooling media. Instead, it suggests that the various fibre board boxes have different insulating properties from polystyrene boxes. This conclusion is reinforced when the outside temperature increases and internal fibre board box temperatures rise faster and higher than they did in the polystyrene. The final piece of evidence is the proportion of gel packs that remained frozen at the end of the simulated export shipment: polystyrene boxes far outperformed the fibre board alternatives, with the best of those appearing to be the Visy gusset box type.

At the end of the day, the decision as to what box(es) to go with will depend on many factors, including performance and expense (direct and indirect costs). The direct cost is largely made up of the price per box, but allowances need to be made for the different volumes of the boxes, and thus the price of the box per fish (or per kg of fish) is probably the best measure. The costs of the ice and/or gel pack, tape, strapping and the costs of labour to assemble, pack, tap and strap the different boxes need to be considered in this process too. The indirect costs would include the volume of storage space needed to maintain a stock of boxes sufficient to cope with the production schedule, the labour needed to stack and unstack that store, the configuration of the boxes on a pallet and/or AV container for export. That sort of information needs to be collected by the business and considered in-house, it is not the sort of commercial-in-confidence information that should be presented here.

5. Benefits and Adoption

This project has been carried out in very close consultation and collaboration with CST and its commercial partners in various processing and supply chain operations. The findings from this project have been reported back to CST in a timely way and have been used by CST senior management, financial controller, harvest and post-harvest manager and the marketing team to:

- help address customer complaints and other enquiries,
- develop more comprehensive product specification information,
- address requirements in the nutritional labelling for YTK products,
- improve the basis for claims regarding the shelf life of chilled YTK fillet products,
- identify high priority issues and differentiate them from lower priority issues that relate to perceptions of YTK product quality in the marketplace.

As further proof of this, the Principal Investigator of this project was seconded to work for CST full-time in the role as harvest and post-harvest manager, to utilise the greater understanding gained by this and other research projects within business operations.

6. Further Development

As is the case with almost every research project, the work reported here answered many questions, but raised several others. Probably the most important of these is to focus on the microbiological spoilage patterns and mechanisms of YTK fillets. It is clear from this project that fillet colour and lipid oxidation were not useful indicators of product spoilage of YTK, and that K value and subjective odour were more useful parameters to measure. Consequently, any microbiological work should happen in parallel with K value and odour assessment.

Differences in YTK product attributes (including assessment of shelf life by microbiological approaches) between harvest times (season) should be carried out with fish of different ages to try to separate any seasonal component from any age/growth component.

The business needs to do a full cost analysis of the different packaging and freight options, particularly for domestic markets where the shorter shipment durations and fewer transfers between supply chain partners, mean that the fibre board boxes

perform as well as the polystyrene boxes. With 'equivalence' in their cold chain performance, the decision to stay with polystyrene or switch to an alternative fibre board box is essentially financial. That decision needs to be made after consideration of all the direct and indirect cost-benefits of the different alternatives.

7. Planned Outcomes

The main outcomes of this project have been realised by CST, especially in their improved understanding of the attributes and specifications of the YTK it harvests and sells as chilled fresh vacuum packed fillets. Furthermore, the demonstrated effectiveness of the chilled product supply chains both domestically and internationally, gives CST marketers more confidence that those supply chain partners have the appropriate cool chain management ethos and practices to protect the quality of their products. The other outcome is that the CST marketers have greater knowledge and understanding of the opportunity to value-add to the YTK product range by offering differentiated high fat ventral portions/loins if the market demand is there.

8. Conclusion

In a comprehensive series of sampling events and trials, this project addressed a number of important questions and perceptions relating to product specification and product shelf life of YTK fish and fillets produced by Clean Seas Tuna (CST) during 2008-10. The main findings included:

- Fish that have a bodyweight greater than 1.2kg have a 2-3 fold higher ($p<0.001$) level of crude fat in the ventral area (belly flap) than in the dorsal fillet area. Ventral crude fat reached a maximum of 35% of fillet weight whereas the dorsal fat level was more commonly 10-12% of fillet weight.
- Fillets from fish larger than 1.2kg bodyweight could be processed into dorsal and ventral portions, with the latter possibly being sold as differentiated product if the market opportunity was established;
- CST usually produce 3 batches of fingerlings each year for stocking at sea, with each batch (or hatchery run) being 4-6 weeks after the previous one. This means that the fingerlings from hatchery run 1 have longer in the seacages during their first summer and grow faster in the warmer seawater. Fish that were from hatchery run 1 grow faster and have higher levels of fillet crude fat than fish from hatchery runs 2 or 3, and those from hatchery run 1 that were graded small. This may put the faster growing hatchery run 1 fish at an advantage when water temperatures cool and they have to rely more on their body reserves.
- The nutritional attributes of YTK fillet are very similar to that of a familiar and widely accepted farmed species (Atlantic salmon) in the marketplace. The flesh is typically around 23% protein and 6-15.5% lipid (depending on the fillet area). Levels of omega-3 fatty acids are between 1.1-2.8% of the total fatty acids, with EPA and DHA levels (1,600 to 2,500mg) in a small (100g) serve of YTK fillet easily meeting the Australian National Health and Medical Research Council guidelines for recommended daily intake (RDI) for adult males (610mg) and females (430mg).
- Vacuum packed chilled fillets produced in this project had a shelf life of 12-15 days post-processing when stored at 4^{o}C. Shelf-life of the chilled vacuum packed fillets was mainly determined by the detection of a strengthening 'fishy' odour when the packet was opened suggesting microbial activity. This coincided with a freshness (K value) score of 60-70%. Lipid oxidation and fillet colour did not change during vacuum packed shelf life. Drip loss increased with time to be about 3% of the fillet weight at day 15 of storage.

- During the project an objective method for measuring flesh colour in YTK fillets was developed and validated.

This study also determined whether the supply chains for chilled YTK products practiced proper cold chain management that will protect the shelf life and product quality attributes in major domestic and international destinations. The main findings included:

- The results from data loggers used in domestic and international supply chains showed that fish temperature remained within the specified range of -1 to +4^{o}C at all times during transport.
- Fibre board boxes proved to be suitable alternatives to polystyrene boxes in a 36-48hr domestic supply chains, but did not provide equivalence of temperature control in a simulated export shipment when two periods of warm external temperature were applied. The best proof of this was the proportion of the gel packs that remained frozen at the end of the simulation. The polystyrene boxes gel packs were 90% frozen, the best of the fibre board boxes was less than 30% frozen, and two of the others were completely thawed.

The findings of this study have been utilized by CST by improving their knowledge and understanding of the attributes and specifications, including the shelf life limitations, of YTK sold as chilled fresh vacuum packed fillets. Furthermore, the demonstrated effectiveness of the chilled product supply chains both domestically and internationally, has given CST marketers more confidence that those supply chain partners have the appropriate cool chain management practices to protect the quality of YTK products.

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Appendix 1: Intellectual Property

No new intellectual property has been generated by this project.

Appendix 2: Staff

Flinders University
Dr Trent D'Antignana
Dr Erin Bubner
Mark Thomas
Claudio Giordano
Andre Smith

Clean Seas Tuna
Mike Thomson
Joe Ciura

Appendix 3

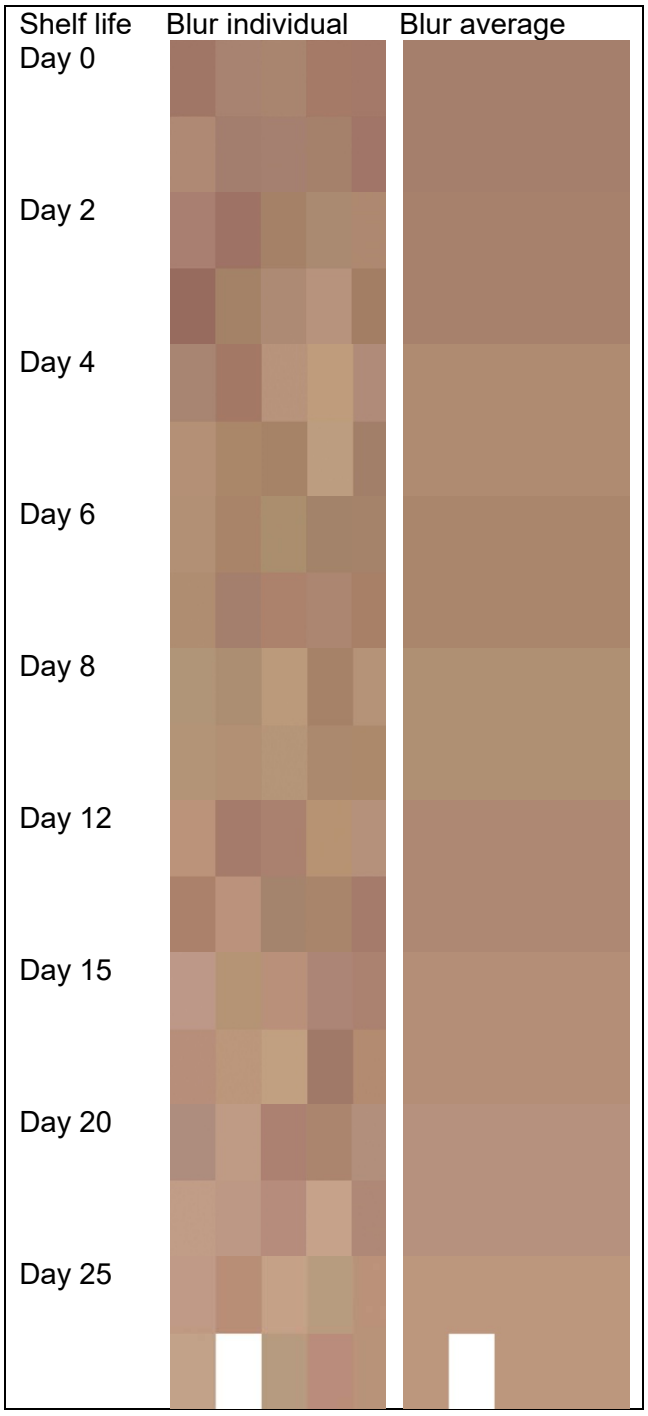


Figure A1. Colour swatches for yellowtail kingfish (*Seriola lalandi*) hatched as run 2 (September) 2008 and harvested in June 2009 (Harvest 1). Fillets were vacuum packed (day 0) then held at approximately 4˚C for up to 25 days. *n* = 9-10. Note: the photography conditions and specifications were still being developed at this time. The results may not be comparable with those from subsequent Harvests.

Figure A2. Colour swatches for yellowtail kingfish (*Seriola lalandi*) hatched as run 2 (September) 2008 and harvested in January 2010 (Harvest 3). Fillets were vacuum packed (day 0) then held at approximately 4˚C for up to 25 days. *n* = 9-10.

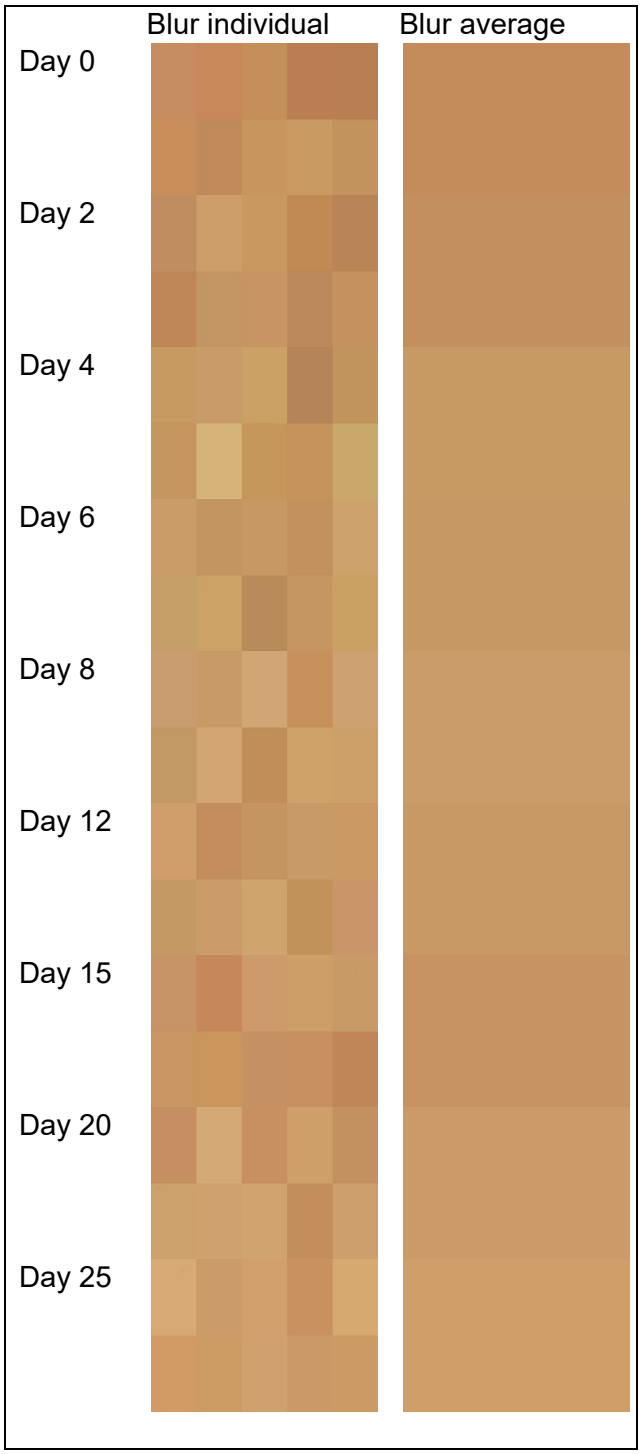


Figure A3. Colour swatches for yellowtail kingfish (*Seriola lalandi*) hatched as run 2 (September) 2008 and harvested in April 2010 (Harvest 4). Fillets were vacuum packed (day 0) then held at approximately 4˚C for up to 25 days. *n* = 10.

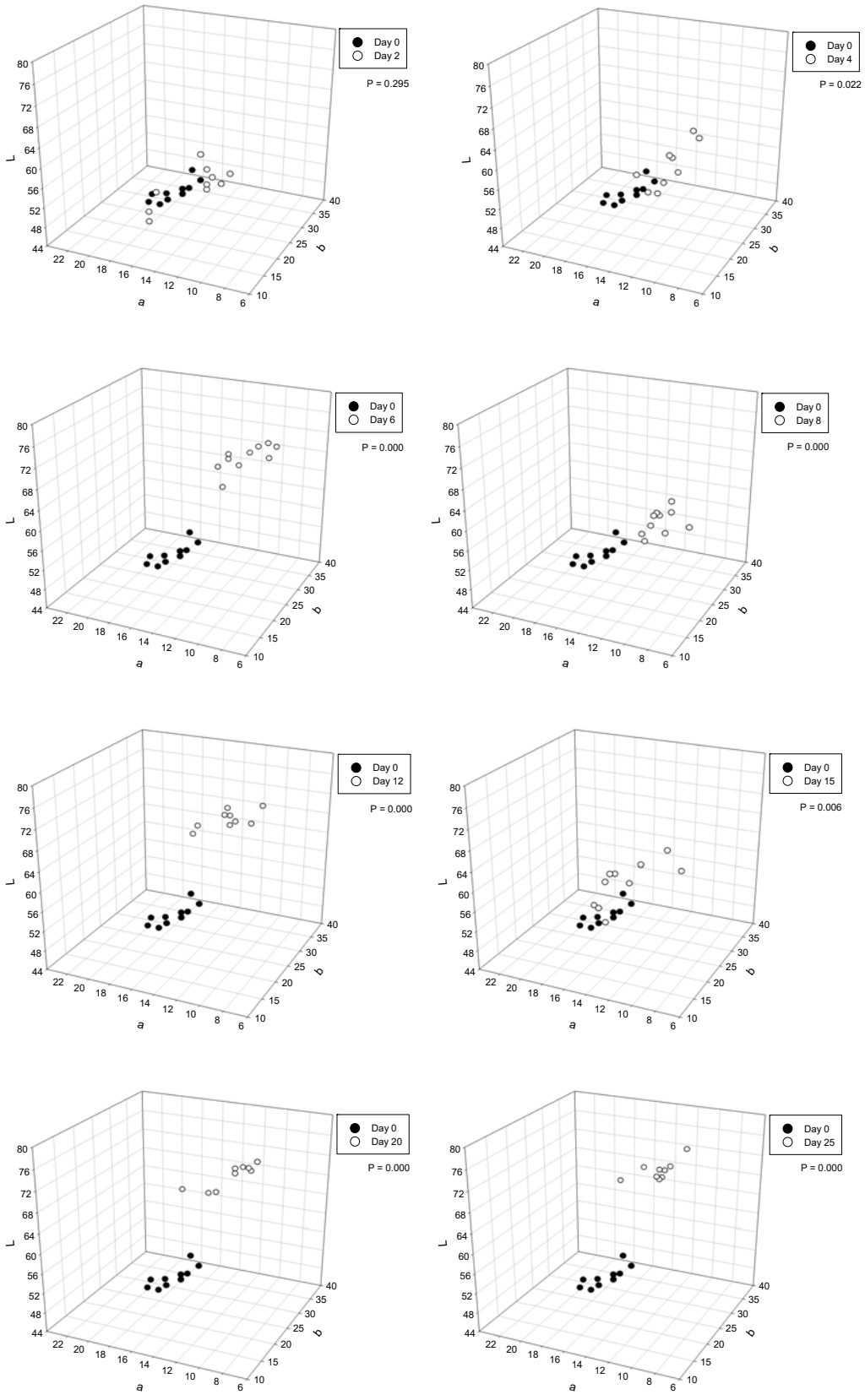
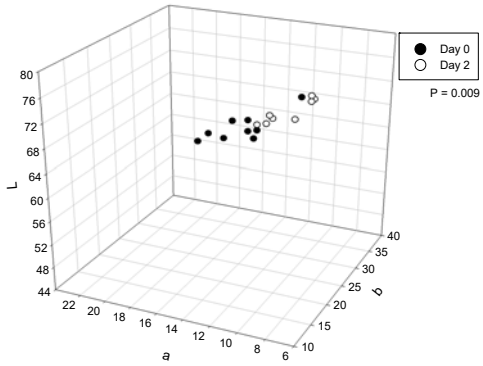
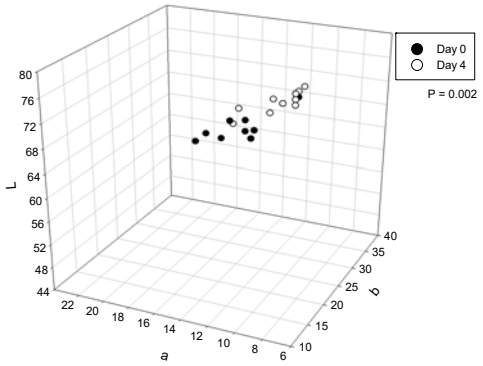
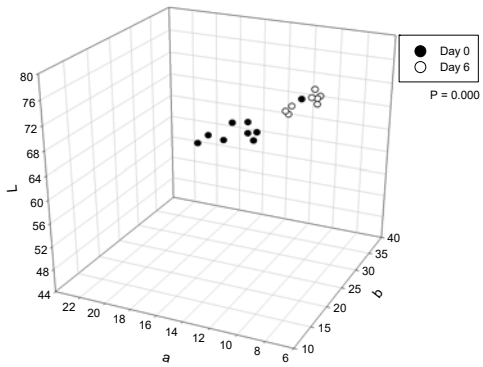


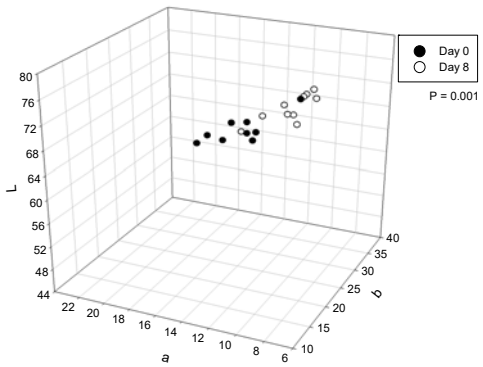
Figure A4. L*a*b co-ordinate plots for yellowtail kingfish (*Seriola lalandi*) hatched as run 2 (September) 2008 and harvested in June 2009 (Harvest 1). Fillets were vacuum packed (day 0) then held at approximately 4˚C for up to 25 days. *n* = 9-10.

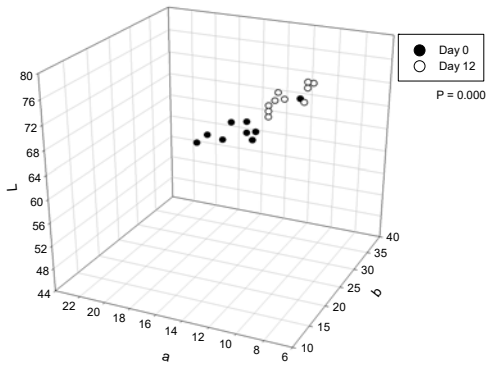
Note: The photography conditions were still being developed at this time and the results may not be directly comparable to other Harvests.

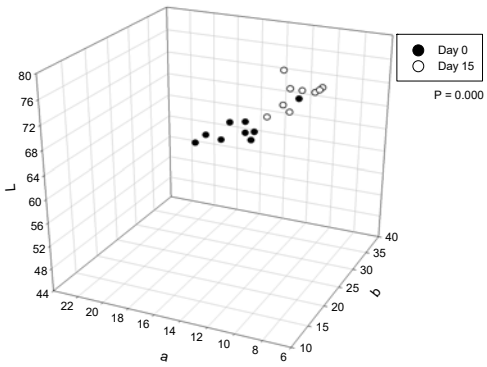


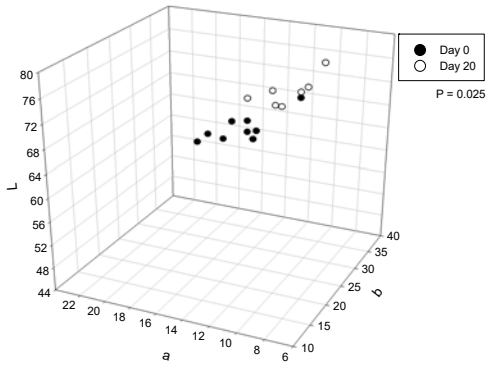












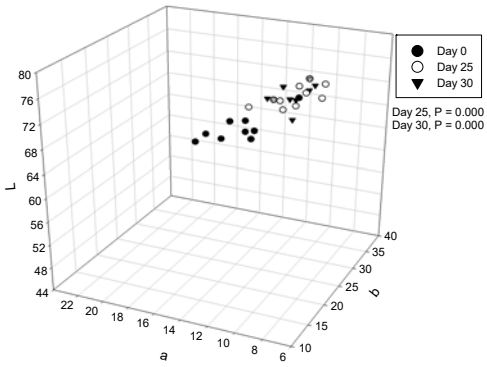


Figure A5. L*a*b co-ordinate plots for yellowtail kingfish (*Seriola lalandi*) hatched as run 2 (September) 2008 and harvested in January 2010 (Harvest 3). Fillets were vacuum packed (day 0) then held at approximately 4˚C for up to 30 days. *n* = 9-10.

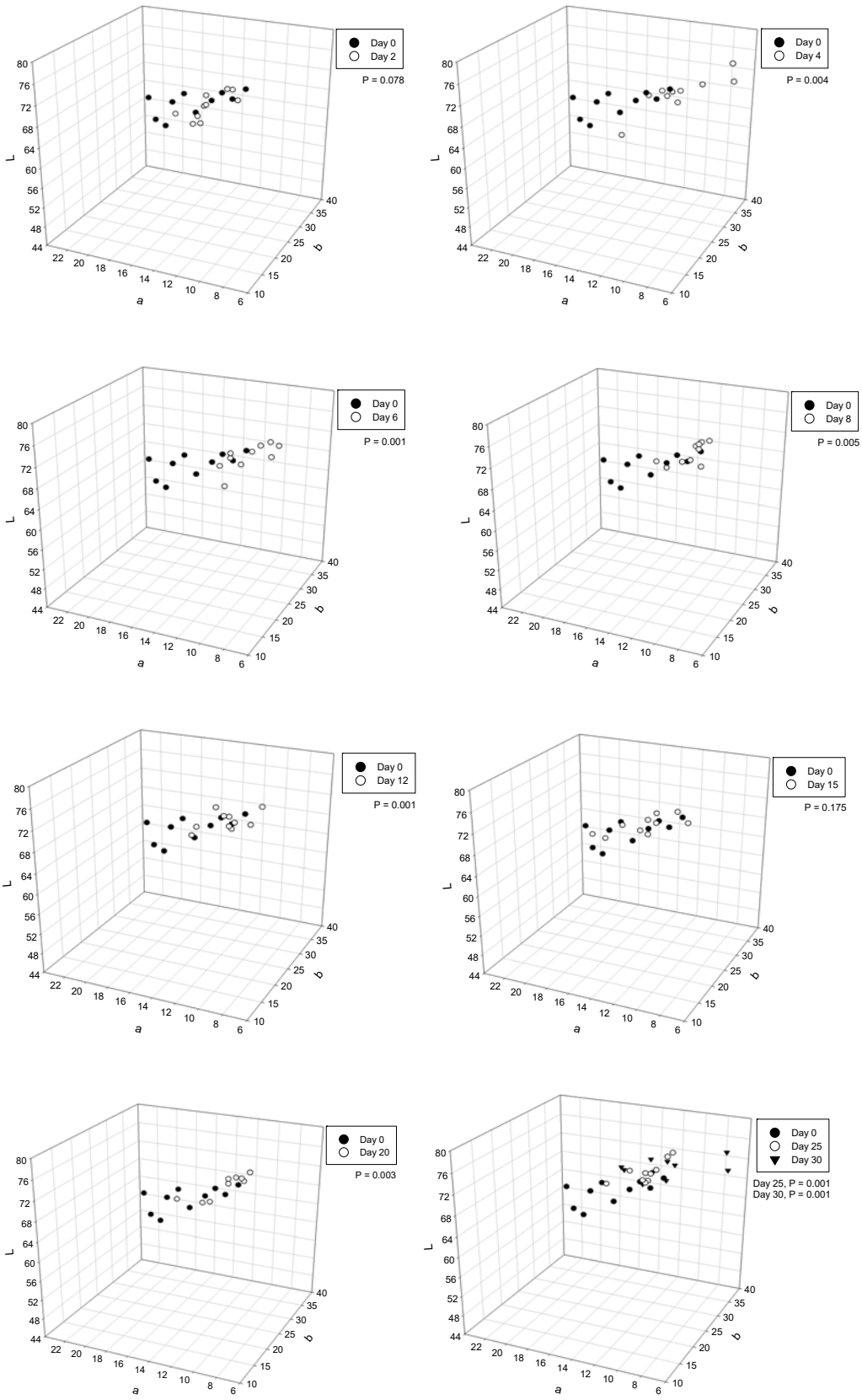


Figure A6. L*a*b co-ordinate plots for yellowtail kingfish (*Seriola lalandi*) hatched as run 2 (September) 2008 and harvested in April 2010 (Harvest 4). Fillets were vacuum packed (day 0) then held at approximately 4˚C for up to 30 days. *n* = 10.

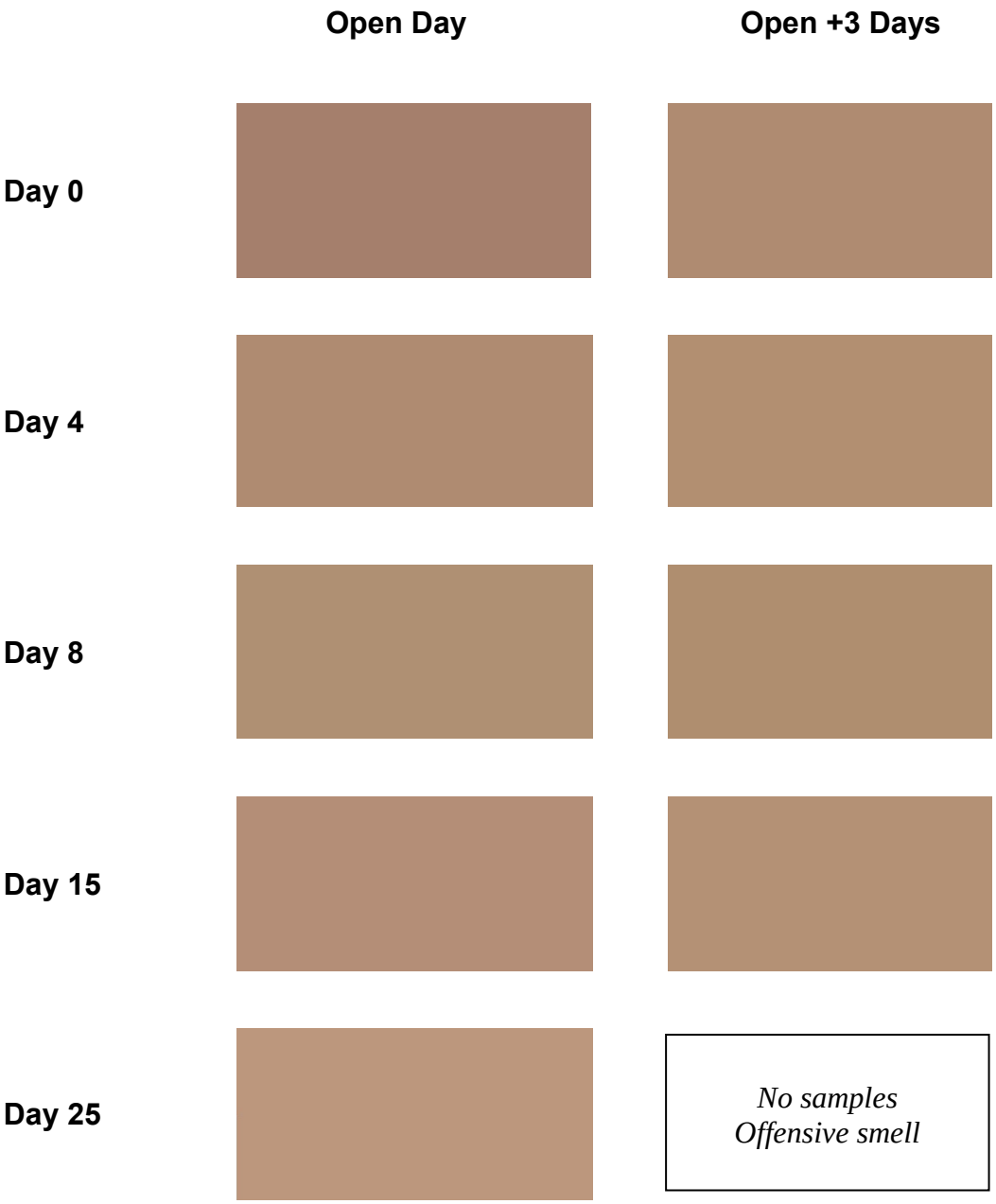


Figure A7. Colour swatches for yellowtail kingfish (*Seriola lalandi*) hatched as run 2 (September) 2008 and harvested in June 2009 (Harvest 1). Fillets were vacuum packed (day 0) then held at approximately 4˚C for up to 25 days. Packs were opened on Days 4, 8, 15 or 25 (Open Day) and kept open for another 3 days at 4ºC (Open + 3 Days). *n* = 9-10. Note: the photography conditions and specifications were still being developed at this time. The results may not be comparable with those from subsequent Harvests.

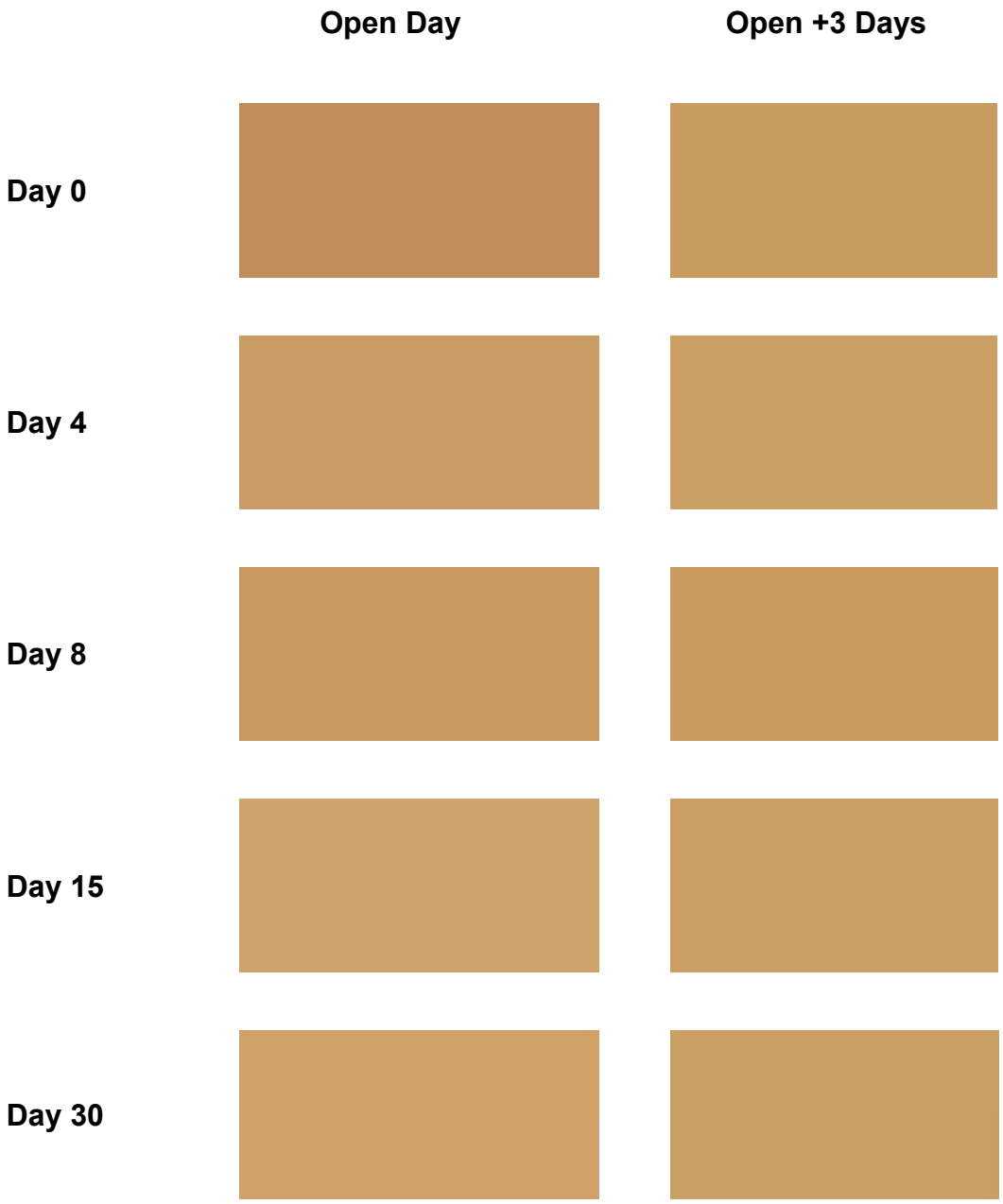


Figure A8. Colour swatches for yellowtail kingfish (*Seriola lalandi*) hatched as run 2 (September) 2008 and harvested in January 2010 (Harvest 3). Fillets were vacuum packed (day 0) then held at approximately 4˚C for up to 25 days. Packs were opened on Days 4, 8, 15 or 30 (Open Day) and kept open for another 3 days at 4ºC (Open + 3 Days). *n* = 10.

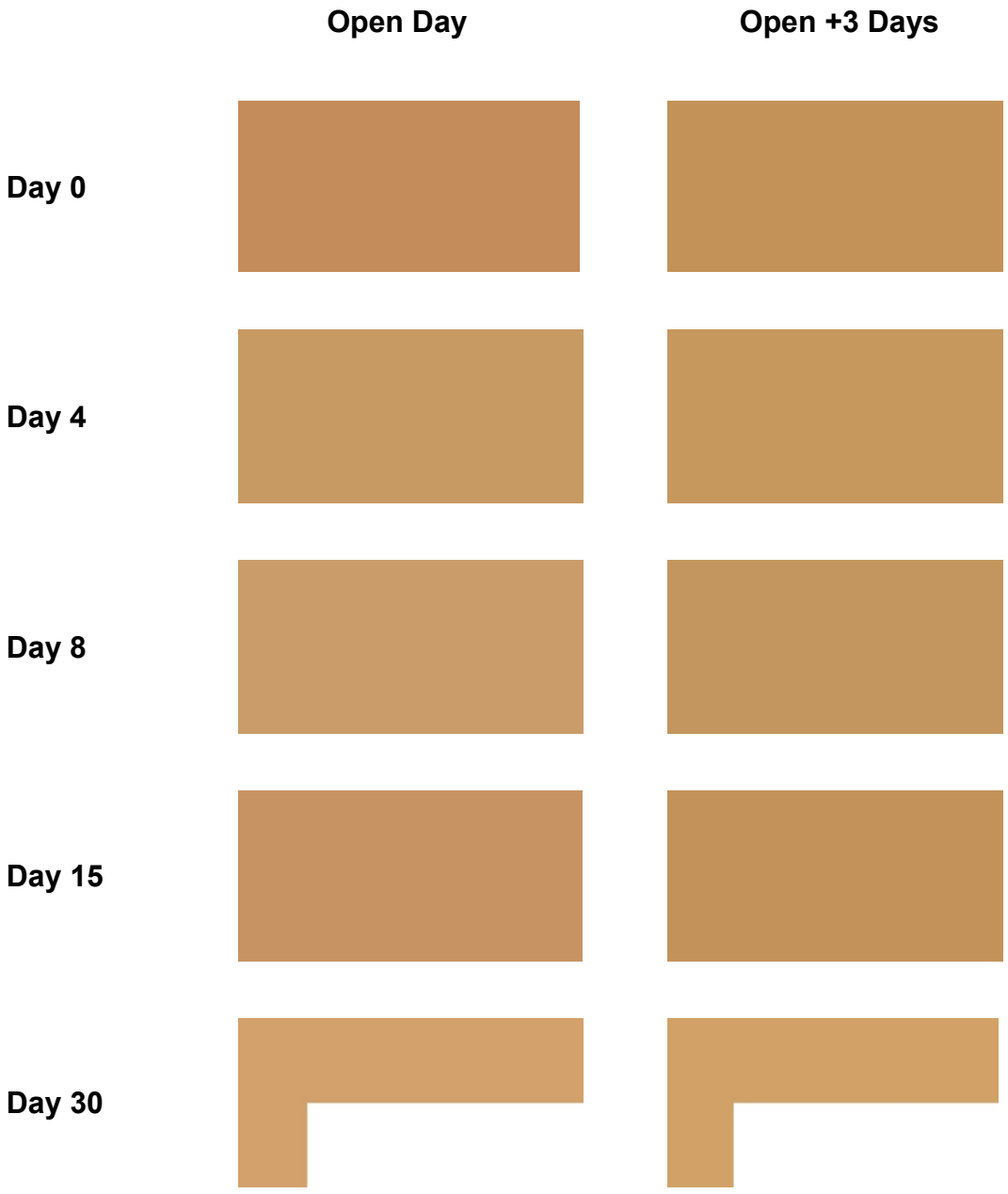


Figure A9. Colour swatches for yellowtail kingfish (*Seriola lalandi*) hatched as run 2 (September) 2008 and harvested in April 2010 (Harvest 4). Fillets were vacuum packed (day 0) then held at approximately 4˚C for up to 25 days. Packs were opened on Days 4, 8, 15 or 30 (Open Day) and kept open for another 3 days at 4ºC (Open + 3 Days). *n* = 6 (Day 30) or 10.

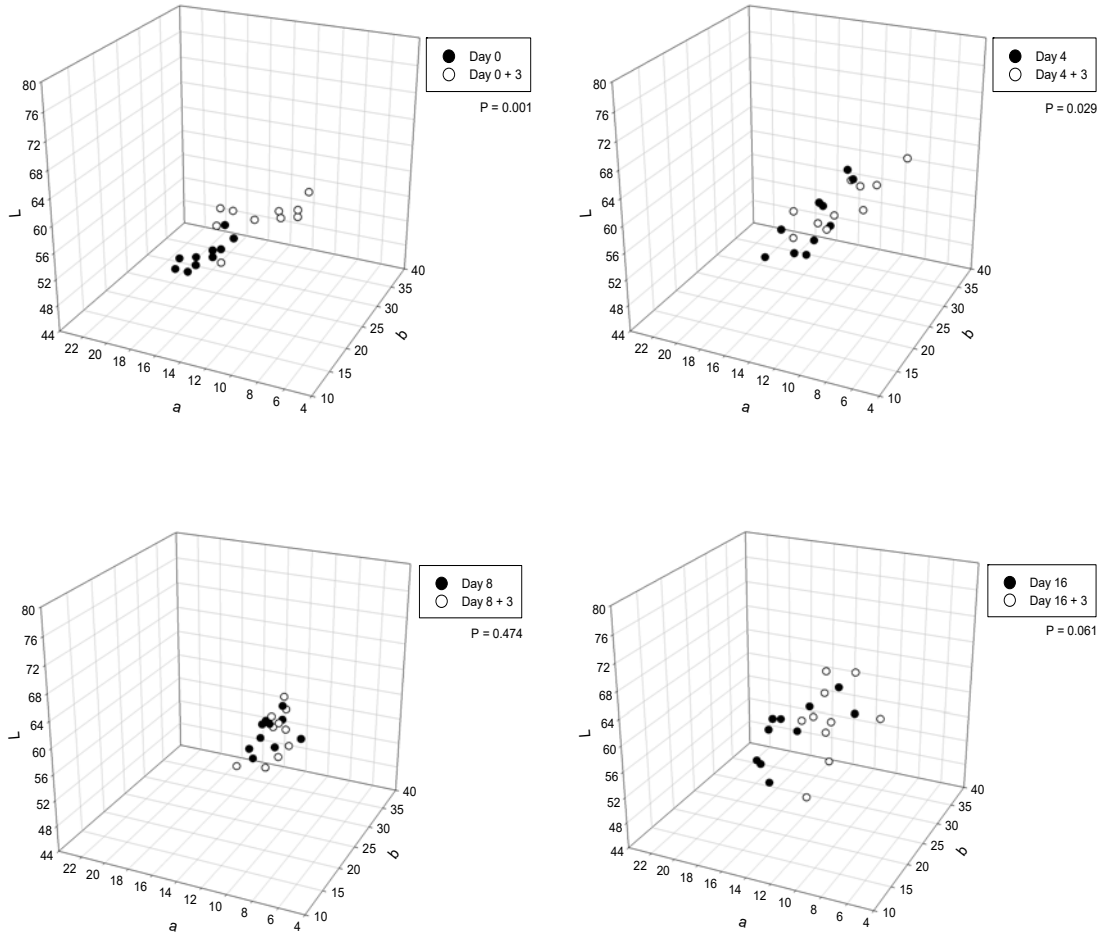


Figure A10. L*a*b* coordinates for Yellowtail Kingfish (*Seriola lalandi*) hatched as run 2 (September) 2008 and harvested in June 2009 (Harvest 1). Fillets were vacuum packed (day 0) then held at approximately 4˚C for up to 25 days. Packs were opened on Days 0, 4, 8, or 15 and kept open for another 3 days at 4ºC (+ 3 Days). *n* = 9-10. Note: the photography conditions and specifications were still being developed at this time. The results may not be comparable with those from subsequent Harvests.

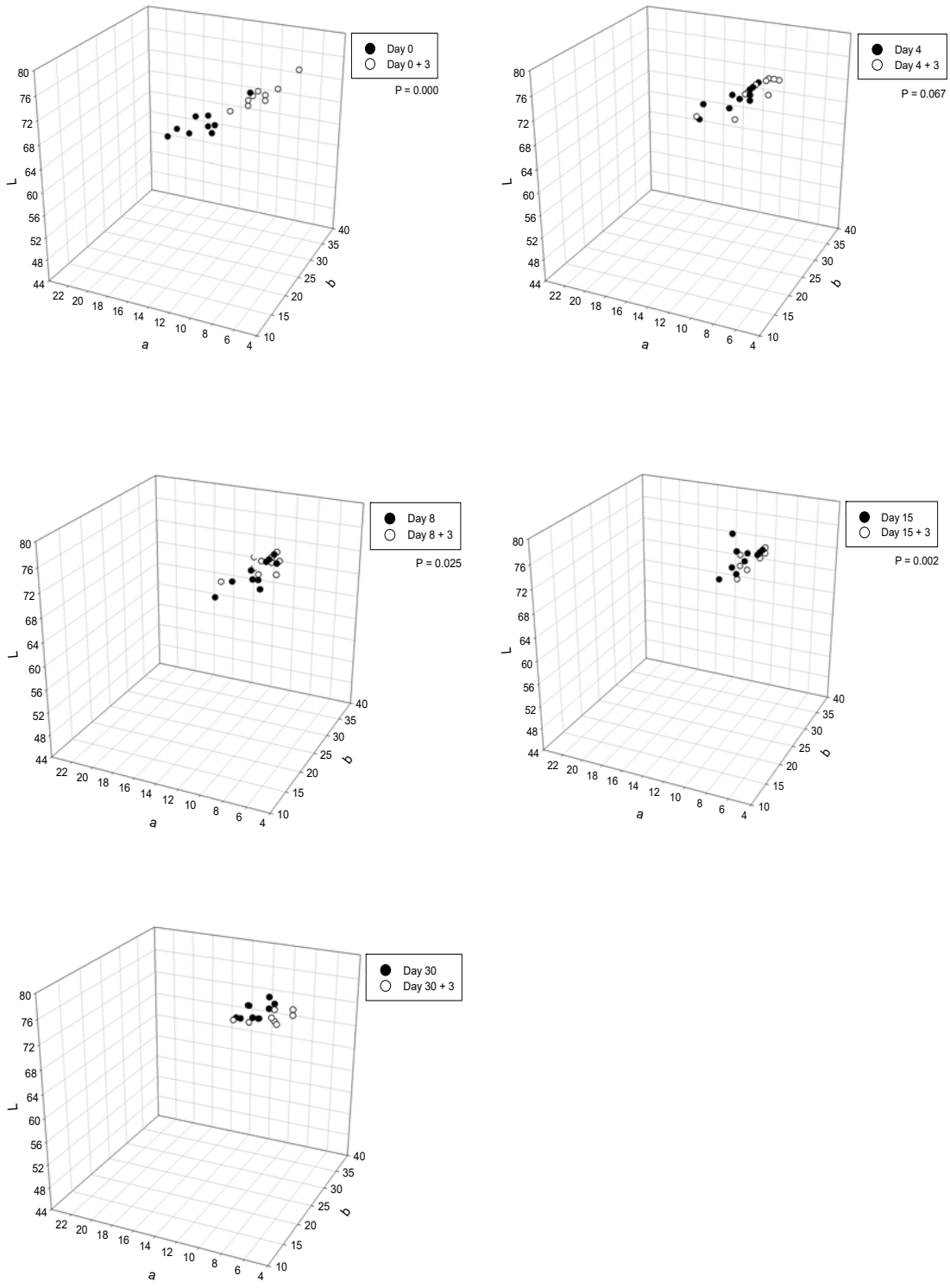


Figure A11. L*a*b* coordinates for Yellowtail Kingfish (*Seriola lalandi*) hatched as run 2 (September) 2008 and harvested in January 2010 (Harvest 3). Fillets were vacuum packed (day 0) then held at approximately 4˚C for up to 30 days. Packs were opened on Days 0, 4, 8, 15 or 30 and kept open for another 3 days at 4ºC (+ 3 Days). *n* = 9-10.

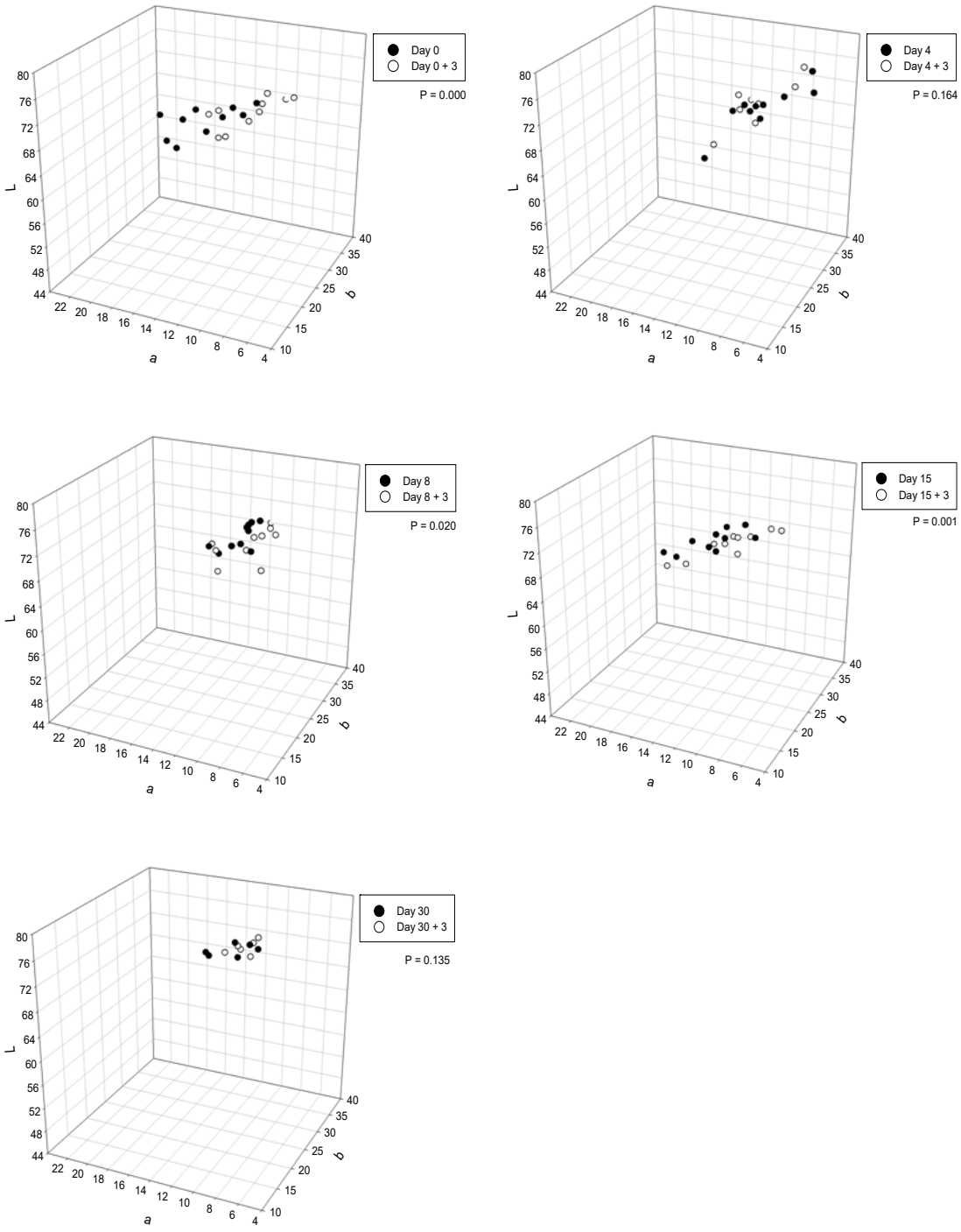


Figure A12. L*a*b* coordinates for Yellowtail Kingfish (*Seriola lalandi*) hatched as run 2 (September) 2008 and harvested in April 2010 (Harvest 4). Fillets were vacuum packed (day 0) then held at approximately 4˚C for up to 30 days. Packs were opened on Days 0, 4, 8, 15 or 30 and kept open for another 3 days at 4ºC (+ 3 Days). *n* = 6-10.

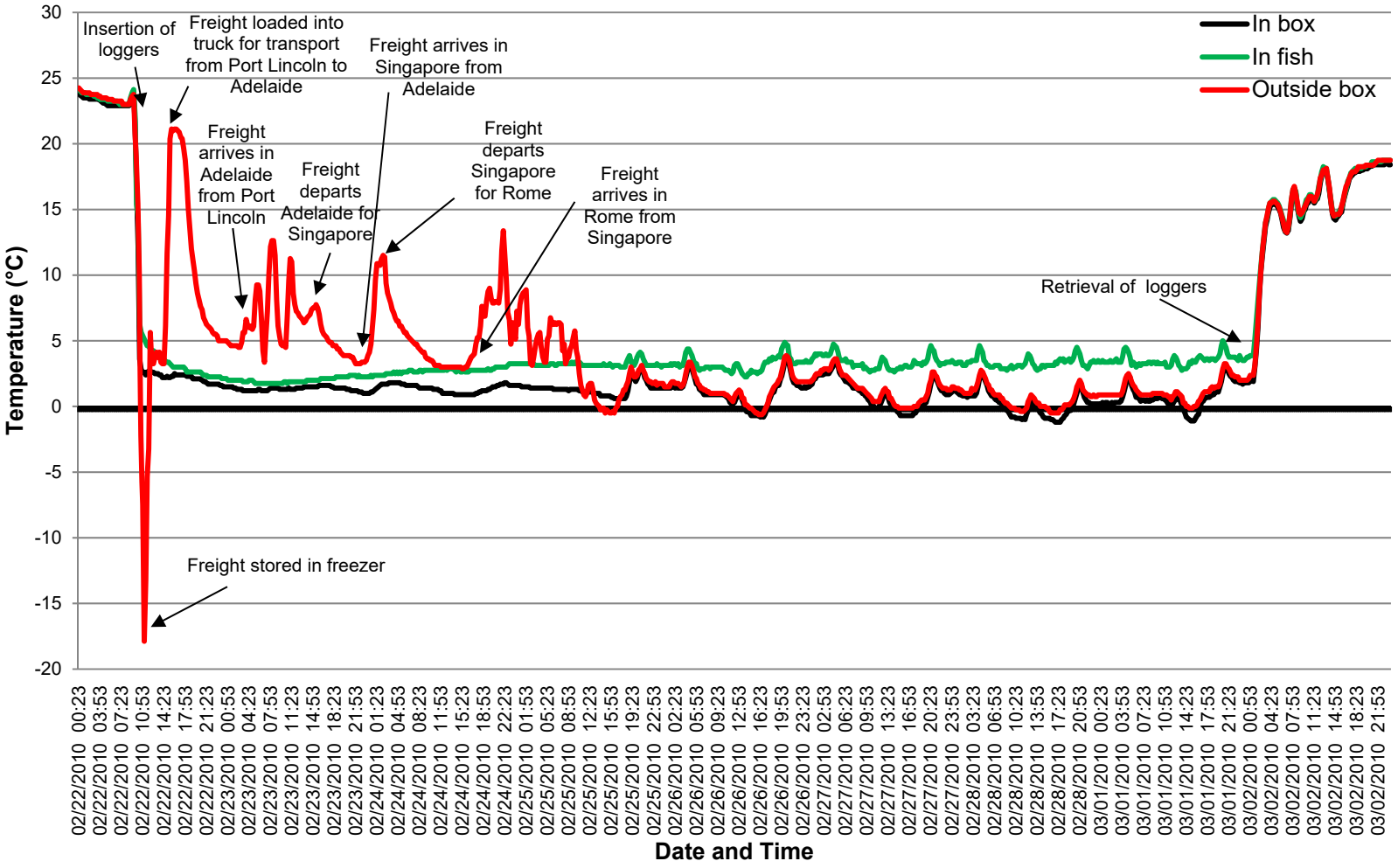


Figure A13. Mean temperature profile of shipment of whole, fresh YTK from Port Lincoln, South Australia to Rome, Italy in February 2010.

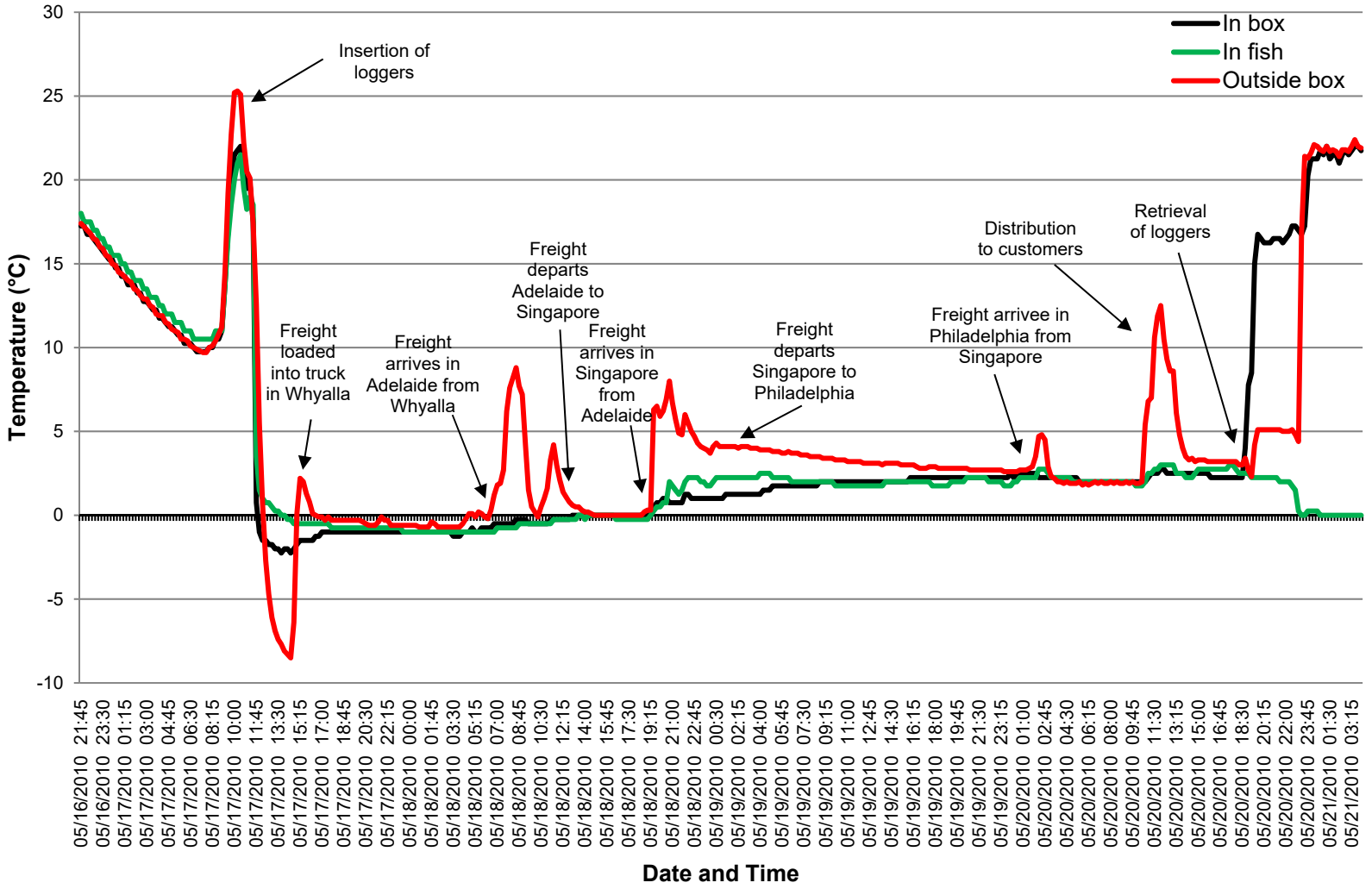


Figure A14. Mean temperature profile of shipment of whole, fresh YTK from Whyalla, South Australia to Philadelphia, United States of America in May 2010.

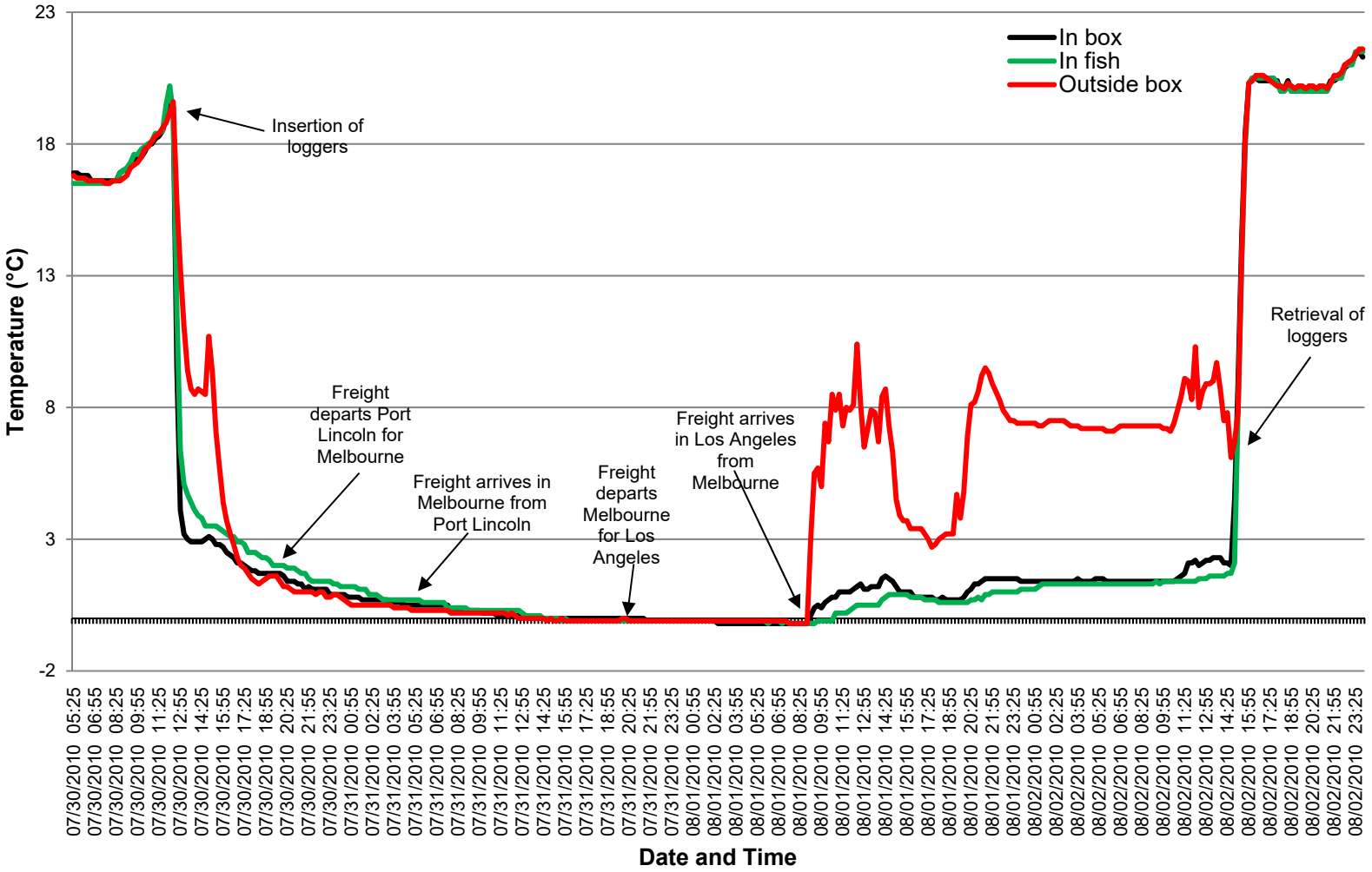


Figure A15. Mean temperature profile of shipment of whole, fresh YTK from Port Lincoln, South Australia to Los Angeles, United States of America in July 2010.

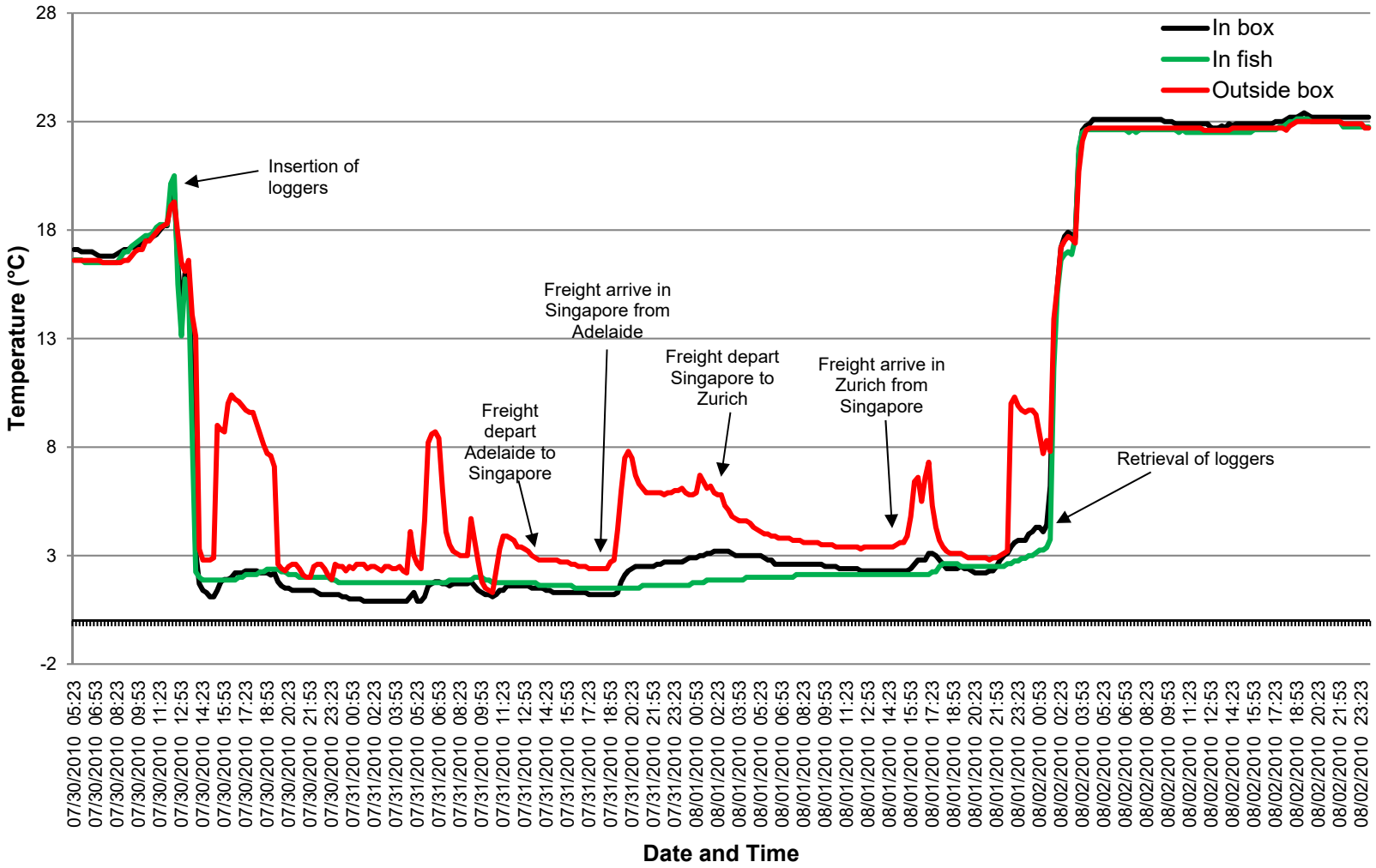


Figure A16. Mean temperature profile of shipment of whole, fresh YTK from Port Lincoln, South Australia to Zurich, Switzerland in July 2010.

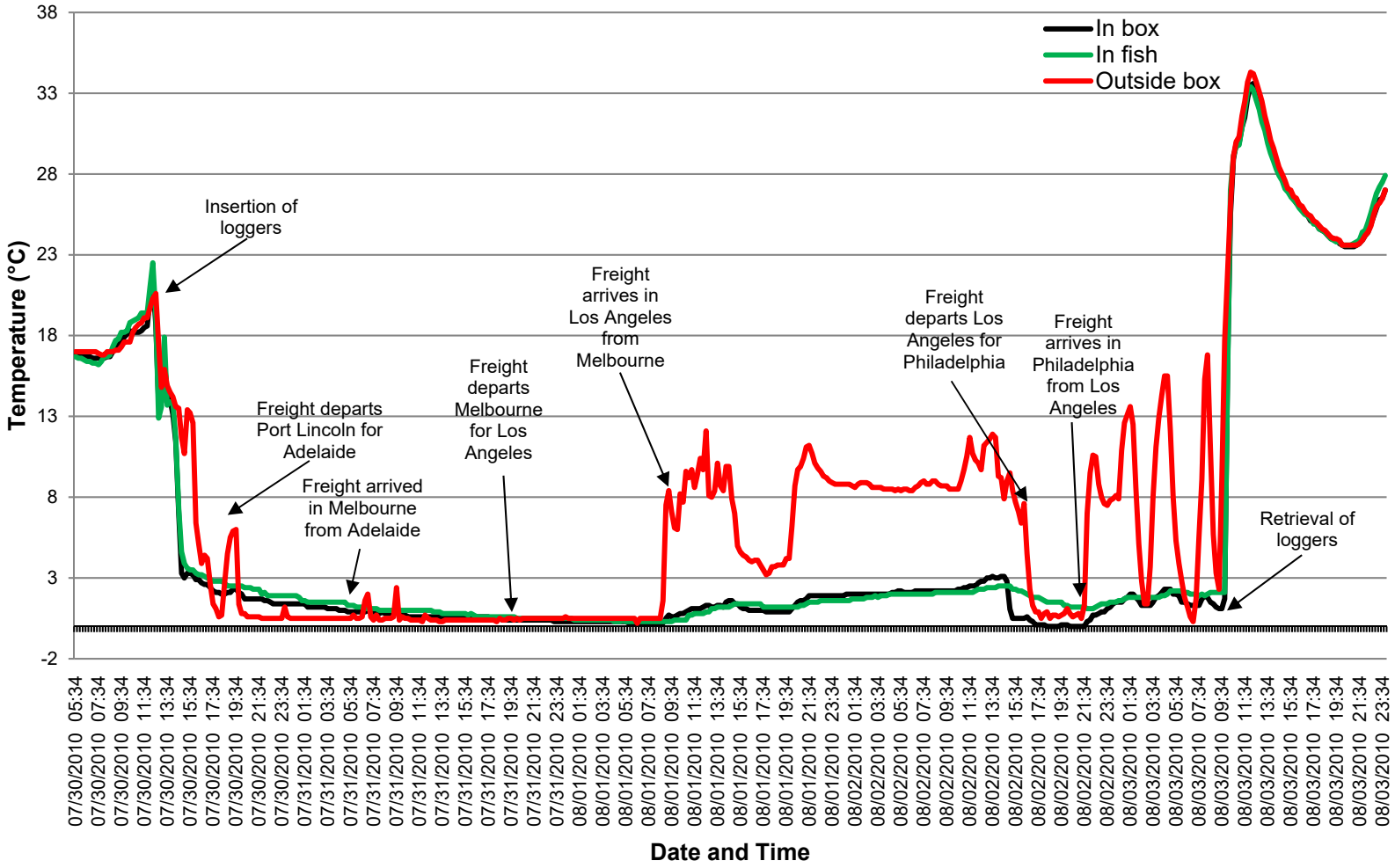


Figure A17. Mean temperature profile of shipment of whole, fresh YTK from Port Lincoln, South Australia to Philadelphia (via Los Angeles), United State of America in July 2010.

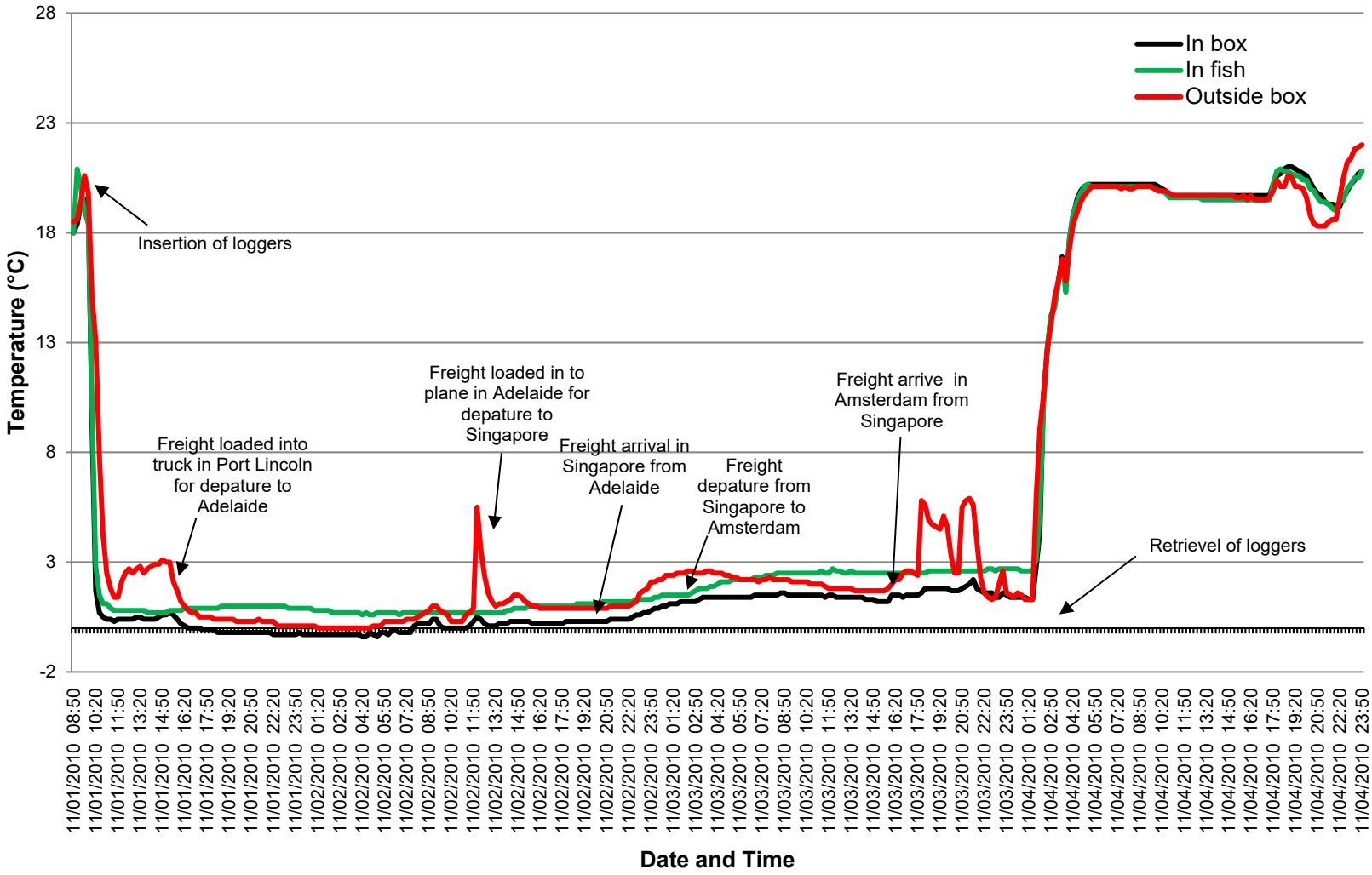
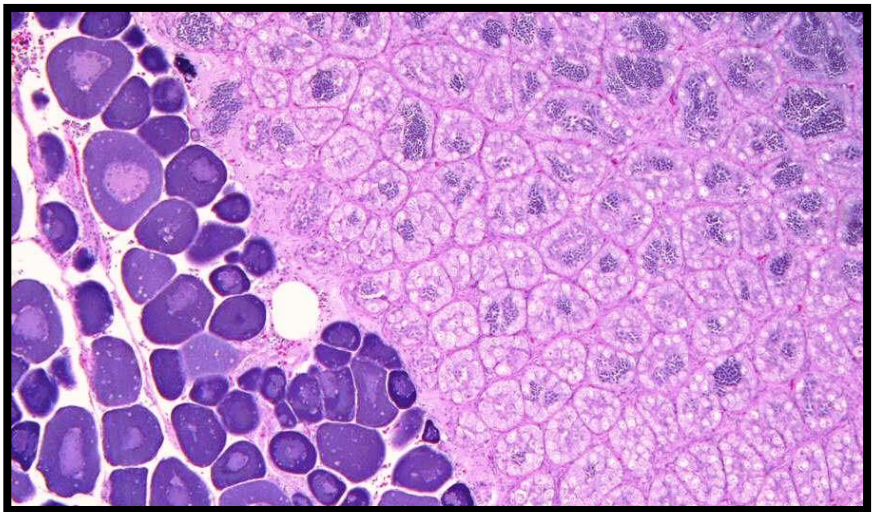


Figure A18. Mean temperature profile of shipment of whole, fresh YTK from Port Lincoln, South Australia to Amsterdam, Netherlands in November 2010

ay AUSTRALIAN SEAFOOD COOPERATIVE RESEARCH CENTRE

Understanding Yellowtail Kingfish: Sub-project 2 – Characterising Maturation

Appendix B



Dr John Carragher and Dr Ryan Wilkinson

Project No. 2008/903.20

This project was conducted by the South Australian Research and Development Institute (SARDI) and the University of Tasmania, Australian Maritime College in collaboration with Clean Seas tuna Ltd.

Title: SEAFOOD CRC PROJECT NUMBER: 2008/903.20 “Understanding Yellowtail Kingfish: Sub-project 2 – Characterising Maturation”

Authors: John Carragher[1], Ryan Wilkinson[2]

Authors Affiliations:

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Reviewed by: Dr Richard Musgrove (SARDI) and Erin Bubner (Flinders University)
Approved for distribution by: Dr Andrew Barber
Date: October 2011

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Non-Technical Summary

2008/903.20 - Understanding Yellowtail Kingfish: Sub-project 2 – Characterising Maturation

PRINCIPAL INVESTIGATOR: Dr John Carragher

ADDRESS: SARDI Innovative Foods
SA Food Centre,
PO Box 1671, Adelaide SA 5001

OBJECTIVES:

1. To characterise the occurrence of sexual maturation and identify the hormonal cues which initiate it.
2. To identify the extent of maturation in the population and its economic impacts.

NOTES:

1. This project was undertaken as part of an overarching project "2008/903 Understanding Yellowtail Kingfish", which was comprised of three stand-alone sub-projects. This final report presents activities carried out within sub-project 2.
2. Fish ages are commonly indicated as 0+, 1+ or 2+ to indicate fish in its first, second or third year of life, respectively. An annual spawning fish approaching reproductive maturity will do so at a time around its birthday. So a 0+ fish approaching its 1st birthday will be a rising 1+ fish, as it approaches its 2nd birthday it is called a rising 2+ fish etc.

OUTCOMES ACHIEVED TO DATE

This study has determined the development of sexual maturation in aquacultured Yellowtail Kingfish (YTK) from the time the fish were put into a sea-cage at 4-5 months of age, until they were harvested for commercial sale as 2+ fish (and 3.5 - 5kg bodyweight). Fish from 3 separate hatchery runs in each of the 2007, 2008 and 2009 spawning seasons (the 2008, 2009 and 2010 stocking-at-sea cohorts, respectively) were sampled on a monthly basis, with 20 fish per pontoon sampled from 0+ groups, and 10 fish per pontoon from 1+ and 2+ groups. Despite the early appearance of spermatogenesis (present even in 4-5 month old fish), and the high prevalence of male maturation (95% and 100% were spermiating as rising 1+ and rising 2+ fish, respectively),the relatively low gonad weight (GSI values of 0.1% and 1.2% of bodyweight in rising 1+ and 2+ males, respectively), the complete lack of vitellogenesis (yolk deposition) in any rising 1+ or 2+ females, and the identical growth profile, carcass breakdown and fillet yield of maturing male and non-maturing female fish, indicates that sexual maturation has negligible deleterious impact on product yield or quality for farmed Yellowtail Kingfish up to the age 2+ or size of 5kg. Therefore, while 2+ and 5kg are the maximum age and size of Yellowtail Kingfish intended for market there is no need for the company to attempt to mitigate the process of sexual maturation through expensive genetic or environmental management approaches.

NON TECHNICAL SUMMARY:

Sexual maturation is known to affect fillet yield, flesh quality characteristics and product shelf life in several species of fish. This study was undertaken to determine the extent of sexual maturation on product yield and quality attributes of farmed Yellowtail Kingfish (*Seriola lalandi*) (YTK) at the Clean Seas Tuna Ltd farm sites.

Sampling was carried out on three different cohorts of fish (the 1st, 2nd and 3rd hatchery runs) in each of the 2008, 2009 and 2010 'at-sea' year classes. Monthly sampling was undertaken between June 2009 and May 2010. Twenty (age 0+) or ten (1+) fish were sampled at random from each pontoon each month. It was not possible to select equal numbers of fish based on their sex.

Fish were sampled for blood, carcass and flesh parameters. The parameters measured included reproductive hormones (plasma oestradiol and testosterone), insulin-like growth factor I (IGF-I), somatic and reproductive parameters (body weight, fork length, condition factor, sex, reproductive stage (macroscopic and microscopic determination), viscera, liver, gonad and visceral fat mass relative to body weight, carcass composition, fillet yield (skin on and bone in the 'Japanese cut' fillet) and fillet crude fat and moisture levels).

None of the female fish sampled showed signs of sexual maturation (vitellogenesis). Ovary weight increased in a steady trend over the grow-out period, and for all but September – November as rising 2+ fish, the female GSI was larger than the male GSI. Maximum average ovary size was only 0.4% of body weight. In contrast, all 0+ males were undergoing spermatogenesis (starting as early as January, at 4-5 months of age) and producing small amounts of thick milt as rising 1+ fish in October-November. Similarly, 100% of rising 2+ males were producing milt, but with less viscosity and in greater amounts. None of the male fish were producing enough milt such that it could be expressed by hand-stripping. The average size of the testes in rising 1+ and 2+ fish was only 0.1% and 1.2% of body weight, respectively; with the fish with the heaviest testes having a GSI of 3.3%.

There were no differences in fork length, body weight, condition factor or fillet yield between male and female fish from the same hatchery run during the majority of the production cycle. However, males had a 1% greater fillet yield (64 versus 63% of body weight) than females in November-December as rising 2+ fish.

Taken together, these maturation data suggest that if YTK are harvested at an age up to 2+ and a body weight less than 5kg, then sexual maturation has little, if any, deleterious impact on the quantity or quality of the product. There is no evidence to suggest that there is any need to investigate any type of approach (such as all female stock, sterile fish or environmental manipulation) to disrupt the maturation process in the current production model.

KEYWORDS: Yellowtail Kingfish, sexual maturation, product yield, product quality

Acknowledgements

The authors wish to thank:

All the folks from Clean Seas Tuna, especially Mike Thomson, Allan Mooney and Joe Cuira, plus the crews on the various feeding, fish health and harvest vessels at Whyalla, Arno Bay and Port Lincoln for patience and cheerful help with fish sampling. Thanks also to Maria and Gail for keeping track of the paperwork.

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John Carragher wishes to thank Andrew Barber and Andrew Pointon at SARDI for allowing him to finish this project on a part-time contract.

Richard Musgrove at SARDI and Erin Bubner at Flinders University for reviewing and comments on the draft report.

1. Background

Unwanted (and sometimes precocious) sexual maturation of stock is a constraining factor in aquaculture world-wide (e.g. Thorpe et al., 1990, Hansen et al., 2000). Species demonstrating unwanted maturation include salmonids (Atlantic salmon, Chinook salmon, coho salmon, rainbow trout, brown trout), tilapia, carp, Atlantic cod, and barramundi amongst others. Unwanted maturation leads to two problems in aquaculture: (a) diversion of energy and nutrients away from somatic (muscle) growth because of gonad growth and energy being spent on associated social interactions, and (b) deterioration in flesh quality (e.g. soft flesh, reduced flesh lipid and pigment levels) and the development of secondary sexual characteristics (e.g. changes in skin pigmentation), which can render stock unmarketable. This limits the harvest season or at the very least, results in significant seasonal fluctuations in product volume and quality, all of which adversely affect product value and producer cash-flows.

Unwanted maturation can be evident in one, or both sexes, depending on the species and culture system. Unwanted maturation can be caused by a number of factors such as:

- genetics,
- nutritional state (i.e. the quantity and quality of food supplied),
- body size as a consequence of faster growth rates and
- environment (i.e. day/length, water temperature, social conditions).

In some instances, only some of the individuals within a cohort of fish will become sexually mature (precocious sexual maturation), the remainder will mature at the ‘normal’ size or age. The seasonality of occurrence of precocious maturation is usually the same as for normal maturing fish of the species.

Aquaculturists manage unwanted maturation using a number of different strategies and the particular strategy chosen will depend on several factors – the main cue(s) for initiating maturation, the size of the marketable product and the amount of manipulative control possible with the culture species and system used. These management strategies may include:

- single sex stocks (only the non-maturing sex is farmed; or maturity only occurs when the sexes are mixed),
- triploidy (all fish are sexually sterile),
- genetics (populations that show low levels of precociousness are used as

broodstock) and

- environmental manipulation (the cues for initiating sexual maturation are overridden).

The costs and timeframes for developing and fine-tuning each of these strategies needs to be balanced against the costs of maturity observed in the grow-out animals. Consequently the first goal is to characterise the occurrence of maturation, which means identifying the:

- sex exhibiting maturity (males, females, both),
- extent of maturity shown (amount of gonad growth, loss of somatic growth, loss of somatic condition, extent of secondary sexual characteristics),
- timing and duration of precocious maturity,
- proportion of the population exhibiting the condition and
- impact of maturation on product quality and value.

Studies have looked at reproductive development in Yellowtail Kingfish (YTK) (*Seriola lalandi*), particularly from a fisheries management perspective (Australian study (New South Wales): Gillanders et al., 1999), but also with an eye on their aquaculture potential (New Zealand study: Poortenaar et al., 2001). Both of these studies sampled fish that were caught by commercial and recreational fishers and thus the smallest fish sampled were 350mm and 450mm in fork length, respectively. These studies showed that Australian (New South Wales) YTK matured at a smaller size and younger age than New Zealand YTK, and that in both populations males matured at a smaller size and younger age than did females (Table 1).

Table 1. Summary of information on size (fork length) and age on manifestation of sexual maturity in populations of wild YTK from Australia (Gillanders et al., 1999) and New Zealand (Poortenaar et al., 2001).

	Males		**Females**	
	Australian	**New Zealand**	**Australian**	**New Zealand**
1st maturity	<300mm (<1 yr)	750mm (4 yrs)	698mm (3 yrs)	775mm (4 yrs)
50% maturity	471mm (<1 yr)	812mm (5 yrs)	834mm (4-5 yrs)	944mm (7-8 yrs)

A previous Seafood CRC project had a preliminary look at the effects of sexual maturation on product quality of aquacultured YTK in South Australia (Carragher et al., 2009). In that study, albeit with limited numbers of fish and at only one time point (December 2007), 88% of 600-700mm fork length 2+ males had testes that produced

milt when they were sectioned, whereas only 4% of females from the same cohort had vitellogenic oocytes. Thus, it appears as though these limited observations on aquacultured YTK in South Australia are consistent with the findings of the study by Gillanders et al., (1999) conducted in New South Wales.

Obvious (large, >200g) and maturing gonads were reported when some large (3+ and 7kg) YTK harvested from sea-cages in South Australia were produced and processed during October – December 2007. Many processors, wholesalers and food service end-users are aware of the negative impacts of maturation on product quality in other species (particularly salmonids as mentioned above), and therefore they raised the question of whether the same consequences affect sexually mature YTK. End-users were also concerned that the large size of the maturing gonad they were removing from these fish was a significant cost that they would have to pass on to the consumer as a higher price/kg of gutted or filleted product. Changes in farm and marketing management mean that only a relative few YTK are retained from each year class and allowed to reach this age or size as potential future broodstock.

Poortenaar et al. (2001) measured reproductive hormones in a limited number (n= 5-13) of male and female YTK as characterised by their stage of gonadal development. There were significant differences in levels of oestradiol, testosterone and 17,20β-progesterone between the females at different stages of gonad development, and in levels of testosterone and 11-keto-testosterone in males at different stages of gonad development. Despite this, there was not enough temporal information on when these hormone changes happened in relation to increases in relative gonad weight (GSI), or progressive changes in gonad development as assessed by histology, to determine what the cues for reproductive development might be for wild YTK.

This study was the first to characterise reproductive development in aquacultured YTK from an early age (4-5 months after hatch), through to harvest size (~4kg) at 24-30 months. Gonad development was assessed macroscopically and microscopically (i.e. histological analysis), and in relation to body weight, and was compared to changes in blood reproductive hormone and growth factor levels to determine when the fish begin the process to become sexually mature. These reproductive data were considered in light of the information gathered from the same fish on the somatic (flesh and viscera) growth and partitioning during the production cycle. If sexual maturation is identified as a significant concern in the production schedule of <5kg and up to 2+ fish, the hormone information may allow researchers and producers to

consider implementing strategies to overcome maturation possibly resulting in significant economic benefits and improved product consistency.

1.1 Need

Through existing farming practices it is apparent that there are several factors which may be limiting production, and ultimately revenue. This sub-project examined on one of these factors.

Maturation may be one constraining factor in YTK production, reducing somatic growth. There are also flesh quality concerns, which threaten sales of YTK harvested in November-December, a period when males exhibit maturation. However, preliminary analysis of work carried out in Seafood CRC project 2008/901 suggests that male maturity did not have a significant deleterious effect on the flesh quality at that time. Though there are numerous strategies to manage maturation it is initially necessary to characterise the occurrence of maturation and the factors controlling it. With this information, appropriate mitigation strategies can be introduced resulting in economic benefits to the YTK industry.

1.2 Objectives

1. To characterise the occurrence of sexual maturation and identify the hormonal cues which initiate it.
2. To identify the extent of maturation in the population and its economic impacts.

2. Methods

2.1 Experimental fish

Monthly sampling of YTK commenced in June 2009 and was completed in May 2010. Fish were sampled from Clean Seas Tuna Ltd sea-cages near Port Lincoln, Arno Bay and Whyalla, South Australia. The sampling regime allowed development to be tracked in three separate cohorts of fish transferred into sea-cages in 2008, 2009 and 2010 (three hatchery runs per cohort) with 20 fish from each hatchery run sampled for fish in their first year at sea through to December (i.e. to an average body weight of ~1.5 to 1.8kg), and 10 fish per hatchery run thereafter. Fish were sampled randomly and therefore numbers of each sex were not balanced.

Fish in each pontoon were managed according to normal commercial practice. Factors such as feeding regime, feed type, fish health management, pontoon relocation, grading and splitting, and harvest schedule were outside the control of this project. However, with very few exceptions, the farm staff did everything in their power to ensure fish could be sampled from the same cages in successive months. When this wasn't possible an age-matched pontoon was offered as a substitute.

2.2 Sample collection and analysis

Fish were sampled early in the morning usually before feeding (manual or automatic). Fish were harvested from pontoons by: 1) poling using a hook and teaser; 2) a handline with a weighted lure; 3) quickly dip netting fish that were responding to feed pellets thrown on the water surface; or 4) brail netting fish from a larger group confined in a harvest net. The method used was based on fish behaviour, water temperature and equipment available at the time (Figure 1). At each sampling point the selection of fish was random.

a)  b) 

Figure 1. Harvesting fish by (a) handline and lure and (b) brailing from a harvest net.

After capture, fish were transferred into a tank of seawater containing Aqui-S to anaesthetise them for handling. The fish were anaesthetised within 2 to 5 minutes and blood was taken from the caudal vessels using 18 or 16 gauge syringe needles on 6mL syringes that had been pre-rinsed with a saturated solution of EDTA (Figure 2). The needle was then removed from the syringe and the blood was gently dispensed into a labelled test tube, which was then capped, inverted several times to allow mixing, and stored on ice. The fish was identified with a numbered plastic tag held in place with a cable tie, and put into ice slurry for transport to the Lincoln Marine Science Centre (LMSC), Port Lincoln for later processing.

Figure 2. Photograph showing blood sampling from the caudal vessels.

Once back at the LMSC laboratory (usually within 6 hours after sampling) blood samples were centrifuged and the plasma separated. Individual aliquots of plasma from each fish were frozen (-20^{o}C) for later analysis of sex steroids (testosterone and oestradiol) and growth factors (insulin-like growth factor-I and -II) at the University of Tasmania, Launceston, Tasmania. Additional aliquots of plasma samples were collected for growth hormone, gonadotropin, vitellogenin and melatonin assays, if required.

2.2.1 Somatic data

Each tagged fish was processed for somatic data, which included fork length (mm), body weight (g), viscera weight (g), liver weight (g), gonad weight (g), and visceral fat weight (g), and, later, the fillet weight – Japanese cut (g). Viscera weight included the liver, spleen, gonad, stomach (including contents), pyloric caecae, digestive tract, gall bladder, bile duct, urinary bladder and visceral fat. It did not include the heart, gills, kidney or swim bladder. If, during dissection, a fish stomach contained more than the usual 10-20 food pellets the weight of the food was subtracted from the body weight and visceral weight. Visceral fat was removed from the surfaces of the visceral organs by a scraping action with the knife blade. When the fish were too small for quantitative fat measurement (<500g bodyweight), a simple qualitative relative scoring system was used with 0 = no visceral fat, + = some visceral fat coverage, ++ = more visceral fat coverage (data not shown). Morphological indices (condition factor, viscerosomatic index, hepatosomatic index, gonadosomatic index), fillet yield and carcass and viscera composition were then calculated.

Condition factor was calculated using the formula:

$$Condition\ factor = \frac{wt\ (g)}{fork\ length\ (mm)^3} \times 100{,}000$$

Hepatosomatic, gonadosomatic and viscerosomatic indices and fillet yield (%) were calculated using the formula:

$$Index = \frac{parameter\ weight}{body\ weight} \times 100$$

Visceral composition (% of the viscera that is comprised of liver, gonad, fat, digesta, including spleen, gall bladder and bile duct) was calculated using the formula:

$$Visceral\ composition = \frac{weight\ of organ}{weight\ of\ viscera} \times 100$$

Gutted fish (still with their tag) were stored in ice slurry for up to 48 hr until they could be taken to Southern Waters Marine Products, Port Lincoln, South Australia for filleting by a professional fish processor. The combined weight of the two trimmed 'Japanese cut' fillets was recorded.

2.2.2 Crude fat and moisture determination

A 75-100g dorso-ventral section of flesh ('quality cut') from just behind the vent (Figure 3) was removed from one of the fillets, placed in a labelled plastic bag and frozen (-20^{o}C) for later crude fat and moisture determinations.

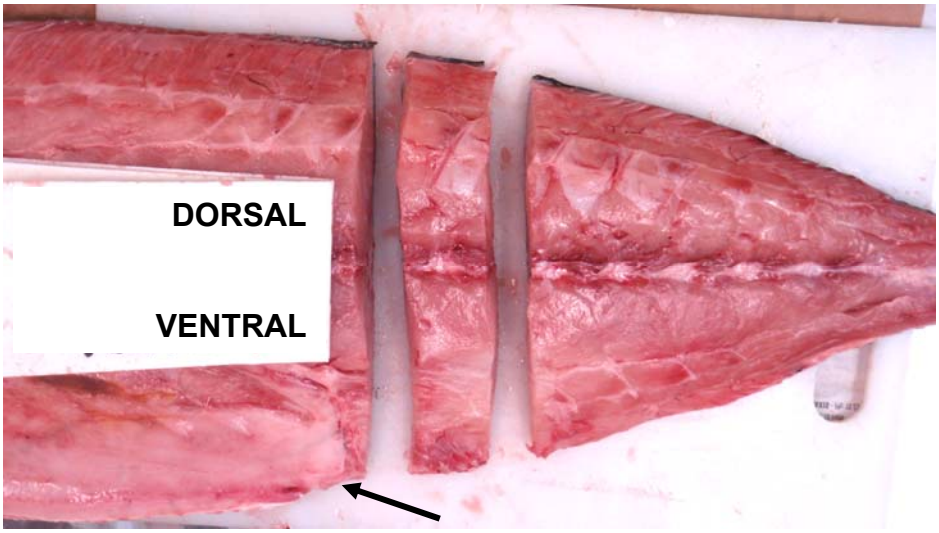


Figure 3. Relationship of the dorso-ventral section slice of flesh removed for crude fat and moisture content to the rest of the fillet. The arrow shows the position of the vent.

Fillet crude fat and moisture were taken from skinned flesh from the 'quality cut' homogenised in a domestic blender. Crude fat was measured using a modified Norwegian standard method developed for high fat cuts from southern bluefin tuna (D'Antignana., 2008). A weighed amount (~10g) of homogenised flesh was taken and mixed with 40g anhydrous sodium sulphate (to absorb water) and 80ml of ethyl acetate in a sealed plastic bag. The tissue was agitated by a stomacher machine (IUL Instruments) for 3 minutes, following this the ethyl acetate was filtered (Whatmans GF/C filter papers) into a conical flask. Forty ml of ethyl acetate was

transferred to a weighed glass beaker by pipette. The ethyl acetate was left to evaporate in a fume cupboard overnight. The crude fat residue left in the beaker was weighed the next day and the % fat of the original tissue calculated using the following formula:

$$Crude\ fat\ content\ \% = \frac{\left(\frac{Extracted\ fat\ (g)}{Dilution\ Factor}\right)}{Extracted\ Muscle\ wet\ wt\ (g)} \times 100$$

Moisture was measured by drying a weighed amount (~50g) of homogenised flesh in a drying oven at 60°C overnight to constant weight. The amount of weight lost was used to calculate the moisture level in the original tissue.

2.2.3 Assessment of reproductive maturity

Reproductive maturity was determined by macroscopic and microscopic assessment of the gonad from each fish using pre-determined criteria (see Tables 2, 3 and 4). First, the gonad was dissected from the body cavity of the fish and macroscopically staged according to the criteria shown in Table 2 (after Poortenaar et al., 2001).

Table 2. Male and female macroscopic gonad staging criteria.

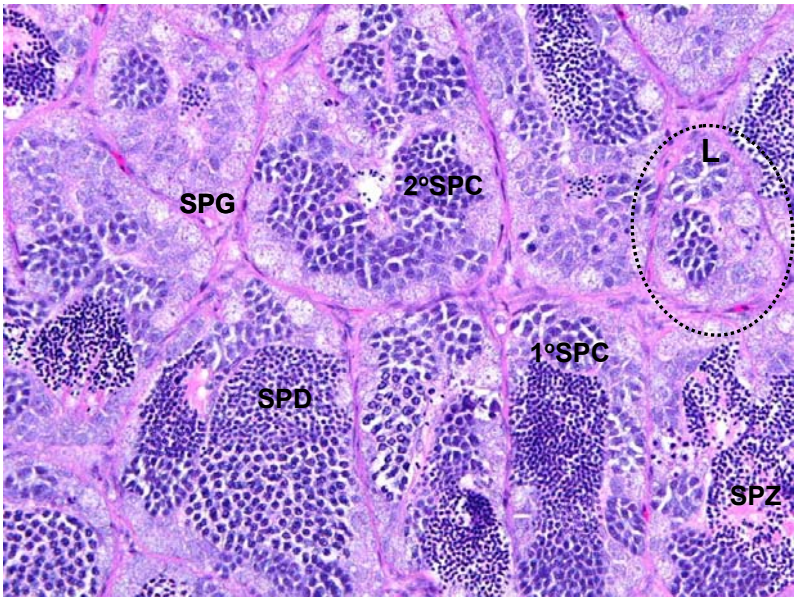
Stage	Classification	Description
Males		
1	Immature	Testes short, thin (~2mm Ø) translucent threads, cream/white lobes
2	Spermatogenic	Testes elongating and thickening (~5 mm Ø), cream/white in colour
3	Partially spermiating	Testes larger (~10mm Ø), ivory in colour, small volume of viscous white milt when testes cut
4	Fully spermiating	Testes large (>12mm Ø), ivory in colour, copious white milt when testes cut (classified as 'spermiating' when fish is stripped)
5	Spent	Testes shrivelled, dark grey/black in colour, small volume of watery grey exudate when testes cut
Females		
1	Immature	Ovaries short and thin (~2mm Ø) translucent threads, pale pink/orange in colour
2	Regressed	Ovaries elongating and thickening (~5 mm Ø), orange in colour, no granularity apparent in matrix when cut
3	Vitellogenic	Ovaries larger (>15mm Ø), orange in colour, obvious granular appearance of matrix when cut (ie vitellogenic oocytes are visible). Not seen
4	Final oocyte maturation	Large ovary, clear hydrated oocytes visible through epithelium. Not seen
5	Ovulated	Oocytes freely expressed from oviduct. Not seen
6	Spent	Ovary bloody and flaccid. Not seen

Subsequently, whole small gonads, or transverse slices (3-4mm thick) were taken from the mid-section of one or two gonad lobes (depending on size) from each fish, secured inside a labelled histology cassette and placed in 10% buffered formalin solution. Fixed samples were embedded in paraffin wax, sectioned at 8μm, stained with haemotoxylin and eosin and examined under a microscope equipped with a digital camera. Each fish was assessed for microscopic gonad stage based on the types of cells observed (males: Table 3 and Figure 4; females: Table 4 and Figure 5).

Table 3. Histological staging of YTK testes (after Poortenaar et al., 2001).

Stage	Classification	Description
1	Immature	Connective stroma, spermatogonia (SPG) present
2	Spermatogenic	SPG, primary spermatocytes (1^{o}SPC), secondary spermatocytes (2^{o}SPC), spermatids (SPD), spermatozoa (SPZ) in lobules
3	Partially spermiating	All germ cell stages present, SPZ common in the lumen
4	Fully spermiating	All germ cell stages present (but not often found), SPZ predominate, sperm ducts filled with SPZ
5	Spent	Lumen mostly empty, residual SPZ in central lumen, other germ cell stages in edges of lobules

a)



b)

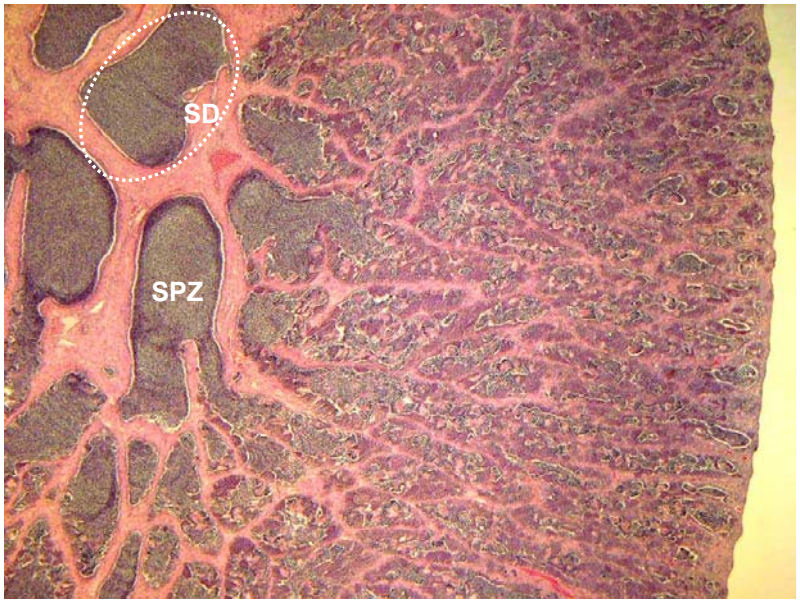


Figure 4. Germ cell identification from (a) 0+ 1st hatchery run fish in August (under x40), and (b) a 1+ 3rd hatchery run fish in October (under x4). Germ cell stages are: spermatogonia (SPG), primary spermatocytes (1°SPC), secondary spermatocytes (2°SPC), spermatids (SPD), spermatozoa (SPZ). Lobules (L) and sperm ducts (SD) are indicated by dotted lines.

Table 4. Histological staging of YTK ovary (after Poortenaar et al., 2001).

Stage	Classification	Description
1	Immature	Connective stroma, chromatin nucleolus (CN), perinucleolus (PN) present
2	Regressed	CN, PN and cortical alveoli (CA) present
3	Vitellogenic	CN, PN, CA and vitellogenic oocytes (V)
4	Final oocyte maturation	CN, PN, CA, V and germinal vesicle breakdown (GVM). Not seen
5	Ovulated	CN, PN, CA, V, GVM, hydrated (H) and post-ovulatory follicles (PF). Not seen
6	Spent	CN, PN, CA, with V, H and PF mostly atretic. Not seen

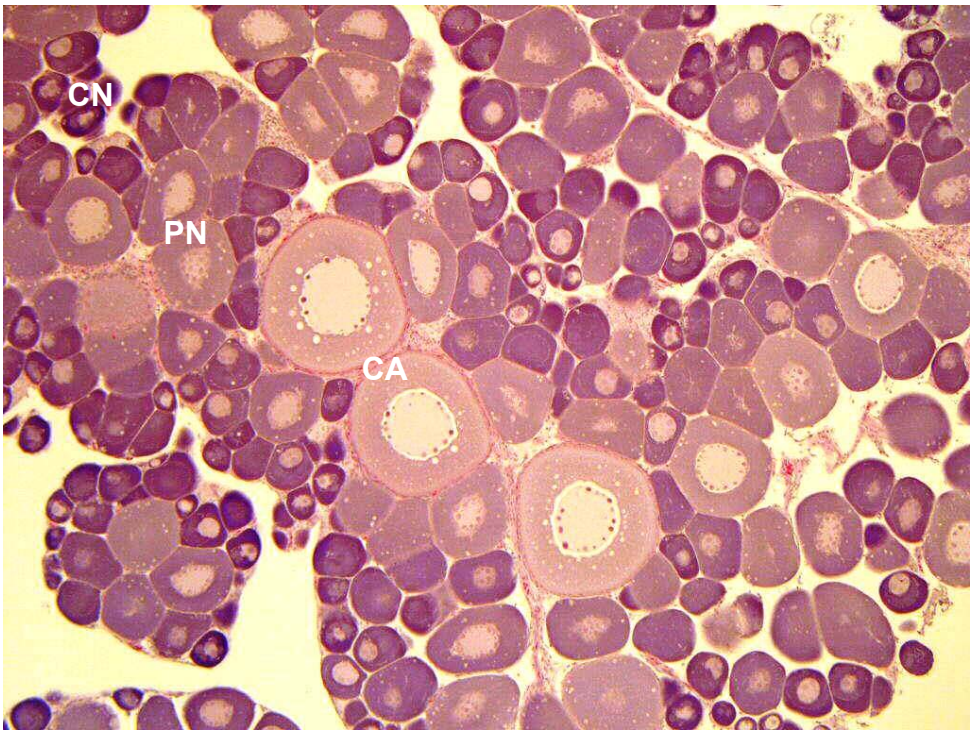


Figure 5. Female germ cell identification from a 1+ 3rd hatchery run fish in November (under x20). Germ cell stages are: chromatin nucleolus (CN), perinucleolus (PN) and cortical alveoli (CA).

The stage was assigned according to the majority of the visible section, or by the most advanced stage that is seen, as development of the YTK gonad (particularly a testis) is not synchronous. For example, it is possible to look across or along a whole section and see several hundred lobules containing only spermatogonia (Stage 1), except for one lobule where there are 1° or 2° spermatocytes (the nuclei are densely stained) and spermatogenesis is underway (Stage 2) (Figure 6a). Alternatively, it may be that all of the lobules contain only spermatogonia (Stage 1) but there is a

reasonable amount of spermatozoa in the sperm ducts – is this 'old' sperm from a previous maturation (Stage 5), or is there another part of the testis where spermiation is active (Stage 3 or 4)(Figure 6b)? In this study, stage was assigned from the most advanced cell type observed in the lobules (not the lumen).

a) b)

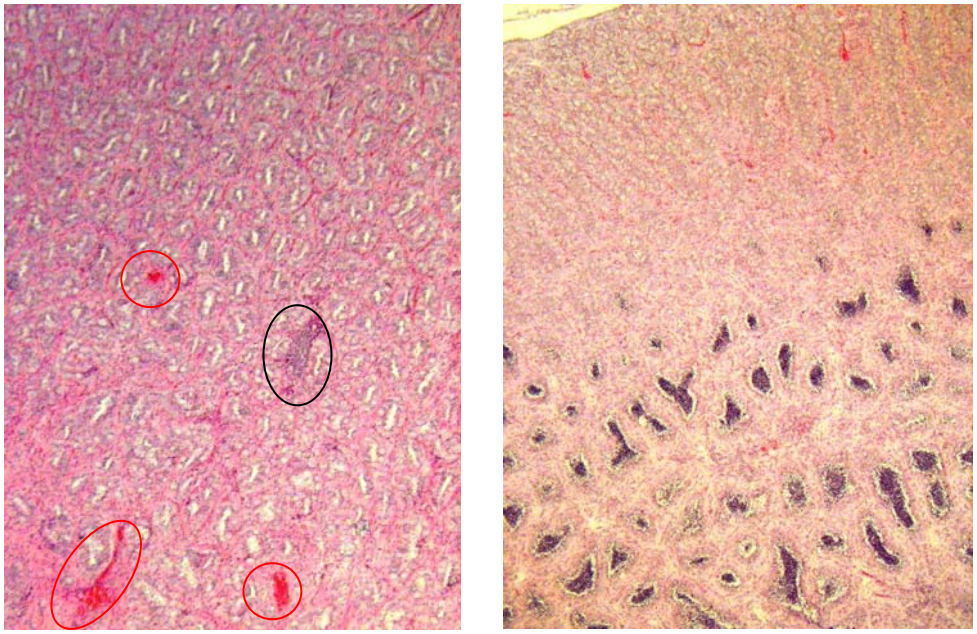


Figure 6. Issues in assigning microscopic stage. Photograph (a) shows a 1+ 3rd hatchery run May fish testes, the lubules all contain spermatogonia except the one in the black circle where spermatocytes are present (stage 2). The red circles show blood vessels. Photograph (b) shows a 2+ 3rd hatchery run November fish testis. The lobules only contain pink staining spermatogonia cells, but the lumens of centrally located sperm ducts contain dense, dark staining residual spermatozoa (stage 1).

Each fish was assigned a sex and stage twice, once according to the macroscopic inspection at the time of dissection, and again following preparation of histology slides, at a microscopic level. The proportion of males and females scored at each developmental stage in each hatchery run in each month was calculated.

2.2.4 Hormone assays

The levels of oestradiol (E_2) and testosterone (T) in thawed plasma (typically 200µL aliquots) were measured by radioimmunoassay (RIA) following extraction with ethyl acetate using the reagents and protocols described by Pankhurst and Carragher (1992). Extraction efficiency (mean recovery of ^{3}H-labelled steroid from triplicates of a plasma pool) was 91.21% for the E_2 RIA and 90.35% for the T RIA and values for each steroid were adjusted accordingly. The minimum detection limits were typically 25.5 pg/mL for E_2 and 110 pg/mL for T (NOTE – limits of detection can vary from assay-to-assay). Inter-assay variability measured using aliquots of a pooled internal standard were 7.2% and 10.3% for E_2 and T, respectively.

Circulating levels of insulin-like growth factor-I (IGF-I) were determined using commercially available RIA reagents (GroPep Ltd., Adelaide, Australia) as described in Shimizu et al. (2000) and further in Dyer et al. (2004a), following acid-ethanol extraction of thawed plasma. The typical mid-point (calculated as the EC_{50}) of the recombinant IGF-I standard curve was 0.7-1.0 ng/mL with a minimum detection limit of 0.15 ng/mL.

Commercial enzyme-linked immunosorbent assay (ELISA) reagents were sourced (Gropep Ltd., Adelaide, Australia) for the determination of insulin-like growth factor-II (IGF-II) levels in YTK plasma. The reagents were developed for determination of IGF-II levels in salmonids and results from several trials carried out during the present study showed there was limited cross-reactivity with YTK plasma, indicating that these reagents are not suitable for use with YTK. As a result, the plasma samples collected in the current study could not be analyzed for IGF-II.

As there is currently no commercially available assay for the determination of plasma growth hormone (GH) levels in YTK a novel RIA was developed for this project. This assay utilised gilthead seabream GH standard and ^{125}I-labelled tracer (Sapphire Biosciences) and Australian black bream GH antibody (GroPep Ltd, Adelaide, Australia). These reagents were selected as gilthead seabream and Australian black bream GH molecules share 97% and 86% amino-acid sequence homology with YTK GH, respectively. Therefore it was anticipated that these reagents would be suitable for the accurate determination of GH levels in the plasma of YTK. A series of preliminary assays were completed to test the performance of the YTK GH assay. These tests determined that the limit of detection of the GH RIA was 15.6 ng/mL. Further attempts using new batches of reagents failed to improve (or even replicate)

the assay sensitivity previously observed. Due to time and financial constraints, further development of this GH RIA was not attempted.

2.3 Data Presentation

Data presentation was discussed with project PI and Clean Seas Ltd co-investigators and the decision was made to show the data (that was collected over 12 months) as if it were a 24 month grow-out timeframe, representing the commercial production cycle, but with each different year class (2008, 2009 and 2010) and hatchery run (1, 2 or 3) shown by different coloured lines as described in the legend. The lines do not join between May and June in both years as this is the beginning/end of the 12 months of sampling. Results are shown as mean ± standard error measurement (sem). Where the error bars are not visible they are smaller than the symbols used to signify the mean.

Some data points are missing from the graphs. For example, due to an oversight fork length was not collected for 0+ 3rd hatchery run fish in July (and consequently there is also no condition factor for this group), the blood samples for the 1+ 3rd hatchery run fish sampled in January were lost during transit, and due to misidentification of a pontoon the 1+ 3rd hatchery run fish were not sampled in October.

Fillet weight was not collected from fish that had a mean body weight <1kg. It was not possible to collect quantitative visceral data from the smallest fish, so a qualitative scoring approach was used for visceral fat.

It was not possible to sample fish from pontoons that were being harvested for market, so the 1+ datasets end in September (1st hatchery run) and November (2nd and 3rd hatchery runs).

3. Results and Discussion

3.1 Somatic data

The somatic characteristics of the three hatchery cohorts of fish from each of the 2008, 2009 and 2010 at-sea year classes sampled in this study are shown in Figures 7 to 11.

3.1.1 Fish size

The fork length and body weight of the fish were most different between the three hatchery runs in the first 6 months, with differences becoming less as the production cycle progressed (Figure 7 and 8, respectively). Absolute differences in fork length and body weight of fish between each of the hatchery runs within a year class were not solely related to time of spawning, as there were also differences in husbandry practises (i.e. food type, feeding regime, cage stocking, splitting and grading) and environmental factors (i.e. water temperature), within the three growing areas used by Clean Seas Tuna Ltd. Also, some fish were moved between grow-out areas during the study that could account for some of the differences shown. It was not possible to control for these factors in the experimental design due to farm management constraints.

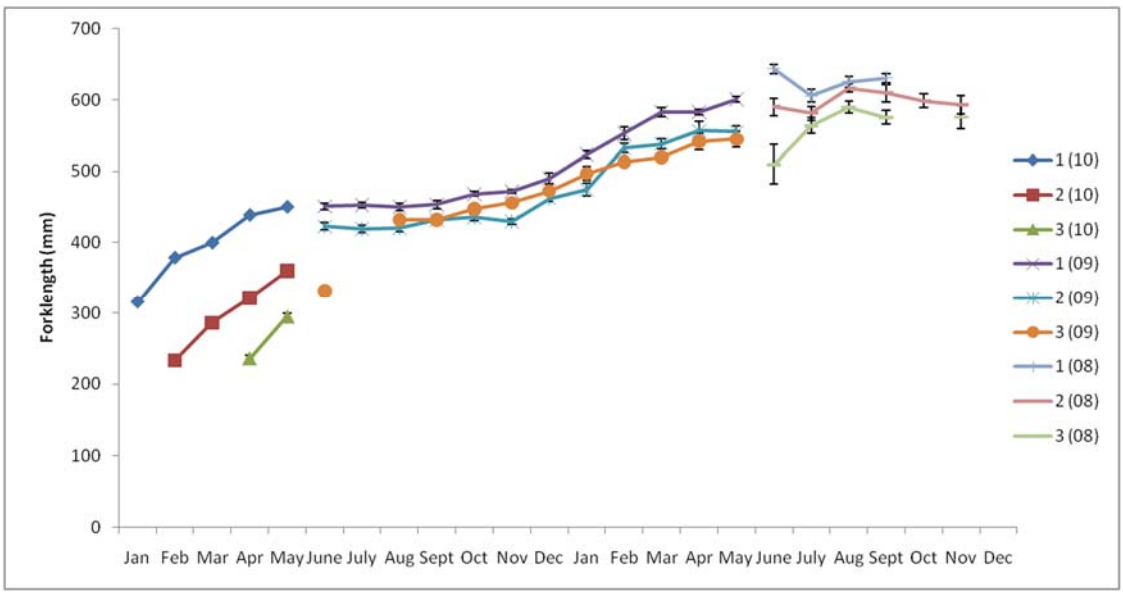


Figure 7. Mean ± sem fork length (mm) of fish sampled each month (male and female fish combined). January to December (year 1, n=20), January to November (year 2, n=10). Legend code indicates hatchery run (1st, 2nd or 3rd) and year fingerlings were introduced to sea-cages (’08, ’09 or ’10).

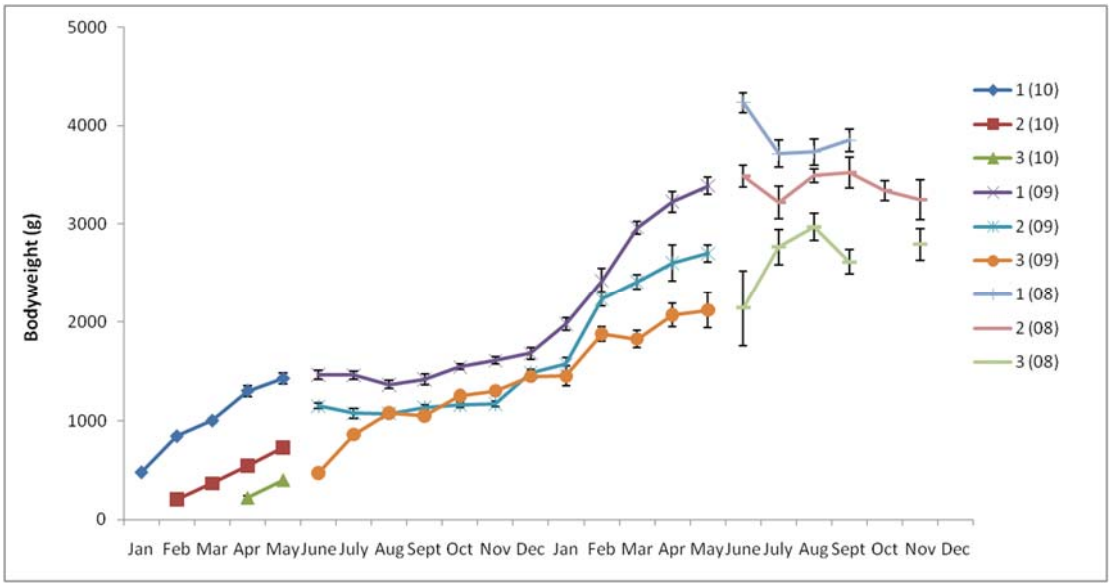


Figure 8. Mean ± sem body weight (g) of fish sampled each month (male and female fish combined). January to December (year 1, n=20), January to November (year 2, n=10). Legend code indicates hatchery run (1st, 2nd or 3rd) and year fingerlings were introduced to sea-cages (’08, ’09 or ’10).

Despite the caveat on the constraints of experimental design, it is reasonably clear that the first hatchery run fish remained on average larger and heavier than the 2nd and 3rd hatchery run fish (Figure 7 and 8, respectively). The 3rd hatchery run fish in the 2010 and 2008 cohorts were on average shorter and lighter than the 2nd hatchery run fish, but for the 2009 cohort they overlapped for much of the winter-spring period. It is not possible to deduce how much of this was due to husbandry or environmental factors; however, it is relevant that hatchery runs 1 and 2 were grown in Port Lincoln and hatchery run 3 was grown in Arno Bay from June-September and in Whyalla from October. The initial increase in body weight in run 3 fish was observed in August, before the move from Arno Bay to Whyalla and the growth rate of run 1 and 2 fish in located in Port Lincoln accelerated in February-April, where the Whyalla fish did not.

It is also interesting that whilst the fork length and body weight lines between the 1st and 3rd hatchery runs of the 2010 and 2009 fish in the May-June transition trend together, while the 2nd hatchery runs do not. This again suggests that the 2nd hatchery run fish in the two year classes may not have had similar hatchery (e.g. spawning date, live food availability, weaning success) or stocking at sea experiences (e.g. age, size, water temperature, stocking density).

There are larger discrepancies in the transition of data from May-June of the 2009

and 2008 fish, with the suggestion being that the 2008 fish were larger and heavier than the 2009 fish. Again, it is not possible to say how much this may have been related to husbandry or environmental conditions. On balance, it appears that the 2009 year class (hatchery runs 1, 2 and 3) were not the best performers compared to 2008 and earlier year classes (Clean Seas, farm data), with slow growth for much of the winter-spring as they were rising 1+. It is not possible to deduce whether this was due to hatchery, husbandry or environmental constraints. Farm management records would better reveal the extent and possible causes of this observation.

3.1.2 Condition factor

Condition factor was generally less variable between hatchery runs and between year classes, with only the 3rd hatchery run of the 2009 cohort showing less condition than the other fish (Figure 9). Essentially this reflects that these fish were skinny for their length. Again, whether this was due to hatchery, husbandary or environmental factors is not deducable from this dataset. Farm management records would be able to identify at what age this trait appeared and how long it persisted. In all other respects, the condition factor of YTK during growout is relatively stable, with (ignoring 2009 hatchery run 3) perhaps a suggestion that it is slightly higher in autumn-early winter (April-July), indicating deposition of some sort of visceral reserves that could be metabolised through the cooler months as appetite decreases. Again, study of farm management records could reveal whether this trend is consistantly found.

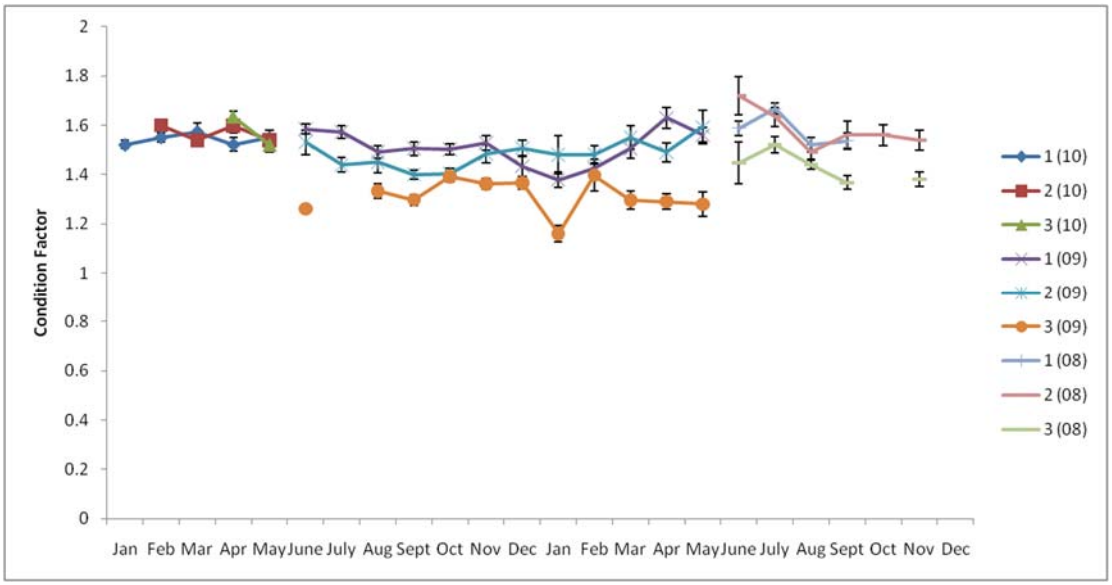


Figure 9. Mean ± sem condition Factor of fish sampled each month (male and female fish combined). January to December (year 1, n=20), January to November (year 2, n=10). Legend code indicates hatchery run (1st, 2nd or 3rd) and year fingerlings were introduced to sea-cages (’08, ’09 or ’10).

3.1.3 Total visceral mass and viscerosomatic index

Visceral mass is between 6-10% of the body weight of the fish, with indications that it peaks in late autumn (May-June) and decreases during the winter (Figure 10a, b). This is consistant with the hypothesis that the fish accummulate a depot of reserves in readyness for the cooler water temperatures and reduced appetite. There is no indication of any differences between hatchery runs in the percentage of visceral mass they accumulate, suggesting that all fish undergo this accummulation and that they are being supplied with enough nutrient to allow this process to occur. Those involved in the marketing of farmed YTK should be aware that customers may report a higher percentage of wastage from fish harvested in April-June; indeed, the relative size of the visceral mass can vary by up to 100% between seasons. Data shown below reveal whether the fillet yield is similarly affected by season.

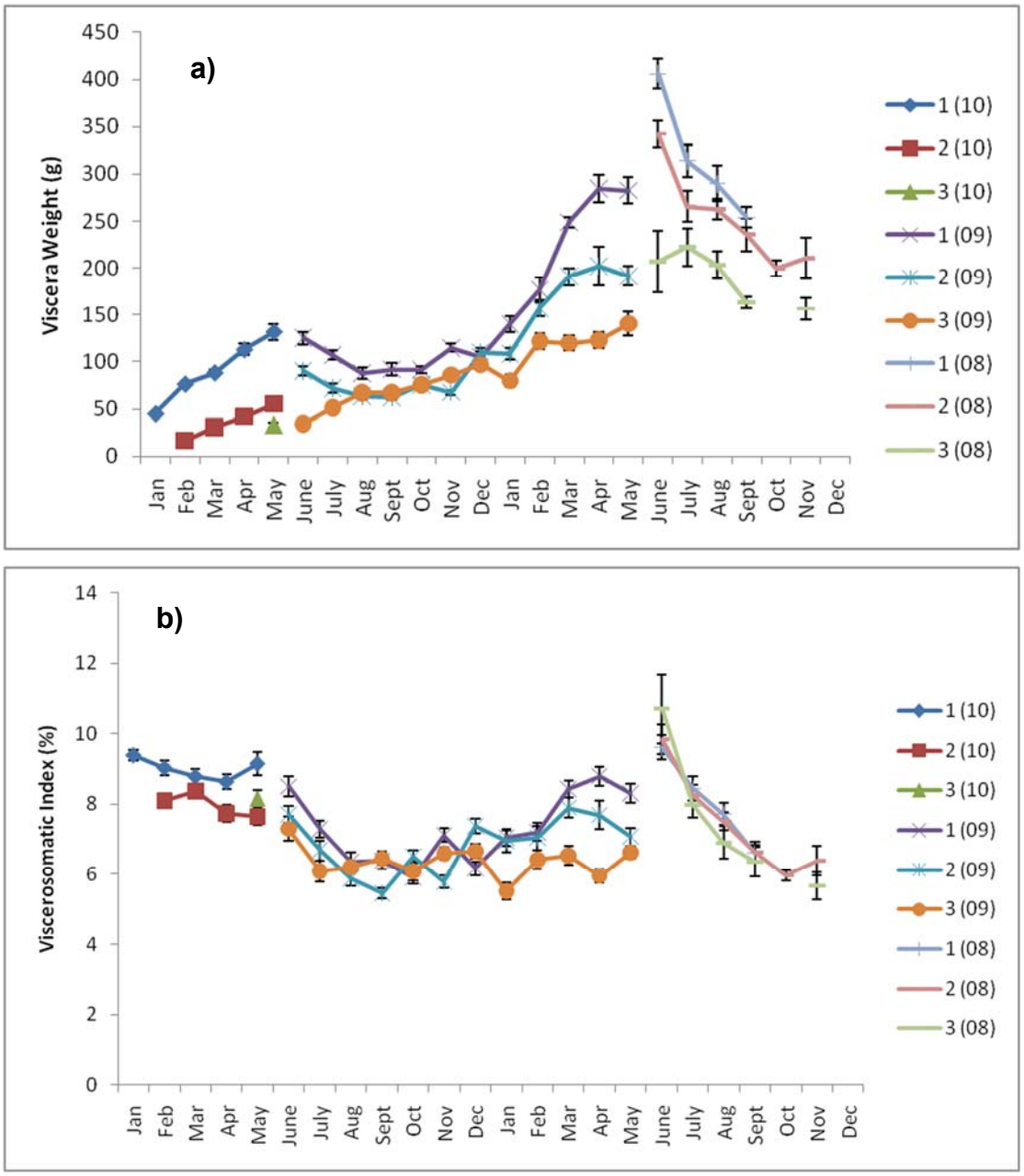


Figure 10. Mean ± sem visceral mass by (a) gross weight, and (b) as a percentage of body weight, of fish sampled each month (male and female fish combined). January to December (year 1, n=20), January to November (year 2, n=10). Legend code indicates hatchery run (1st, 2nd or 3rd) and year fingerlings were introduced to sea-cages ('08, '09 or '10). The viscera includes the stomach, pyloric caecae, intestine, liver, gall bladder and bile duct, gonads, spleen, and overlying fat. It does not include the heart, gills, swimbladder, kidney and fat lining the peritoneal cavity wall.

3.1.4 Liver mass and hepatosomatic index

One component of the viscera that can change in relative size due to seasonal and/or reproductive factors is the liver. The liver size is farmed YTK varied from 0.7 to 1.9% (more commonly 1.6%) of body weight (Figure 11a,b). Larger livers were generally pale in colour, sometimes even cream-yellow in patches, and histologically showed large vacuoles left when lipid was dissolved away by the alcohols used during the histology process (Figure 12 shows a section through one such liver collected opportunistically and fixed, processed and stained as per the gonad samples). So called 'fatty liver' is well described in farmed fish and has several possible causes (Manera et al., 2003). There was no suggestion that in these 0+ and 1+ fish liver size differed by sex as has been reported for other species where females undergoing vitellogenesis have larger livers due to the manufacture of the egg yolk precursor molecule in the hepatocytes (Aristizabal, 2007).

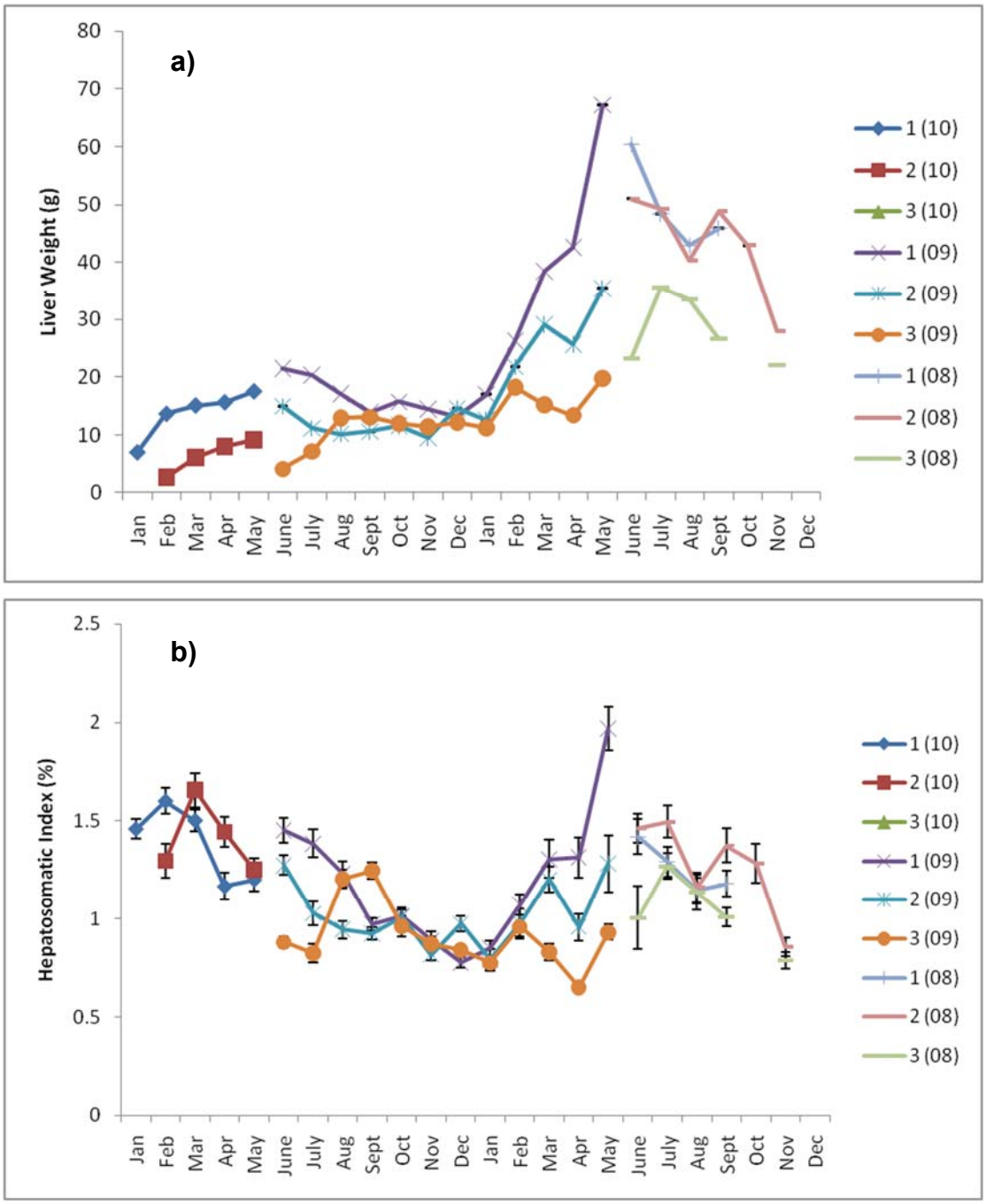


Figure 11. Mean ± sem liver mass by (a) gross weight, and (b) as a percentage of body weight, of fish sampled each month (male and female fish combined). January to December (year 1, n=20), January to November (year 2, n=10). Legend code indicates hatchery run (1st, 2nd or 3rd) and year fingerlings were introduced to sea-cages ('08, '09 or '10).

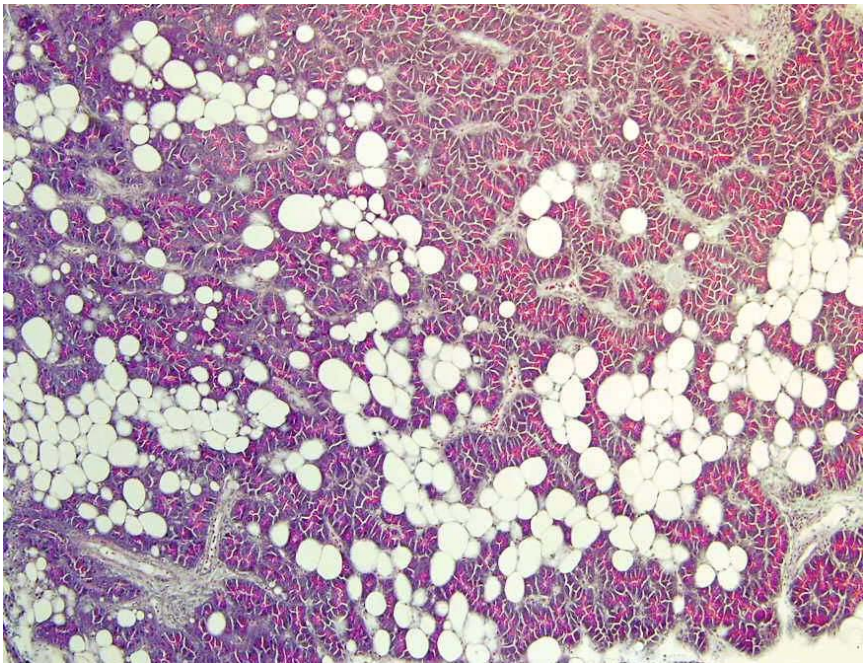


Figure 12. Section through the liver of a rising 1+ (2nd hatchery run) fish in November showing the purple-pink hepatocytes and multiple vacuoles that contained lipid. Magnification at x10.

3.1.5 Gonad mass and gonadosomatic index

Relative gonad size can also change as a fish undergoes reproductive maturation. Average gonad mass (male and female fish data combined) was approximately 0.1 - 0.2% of body weight for much of the production cycle, with an increase in September, October and November as fish were rising 2+ (Figure 13). The peak GSI value was 0.8%, but with a higher level of variability than observed at other timepoints (Figure 13). The peak value was observed in hatchery run 2 fish, with hatchery run 3 fish showing no rapid increase in GSI at this time. Hatchery run 1 fish had been harvested by this time.

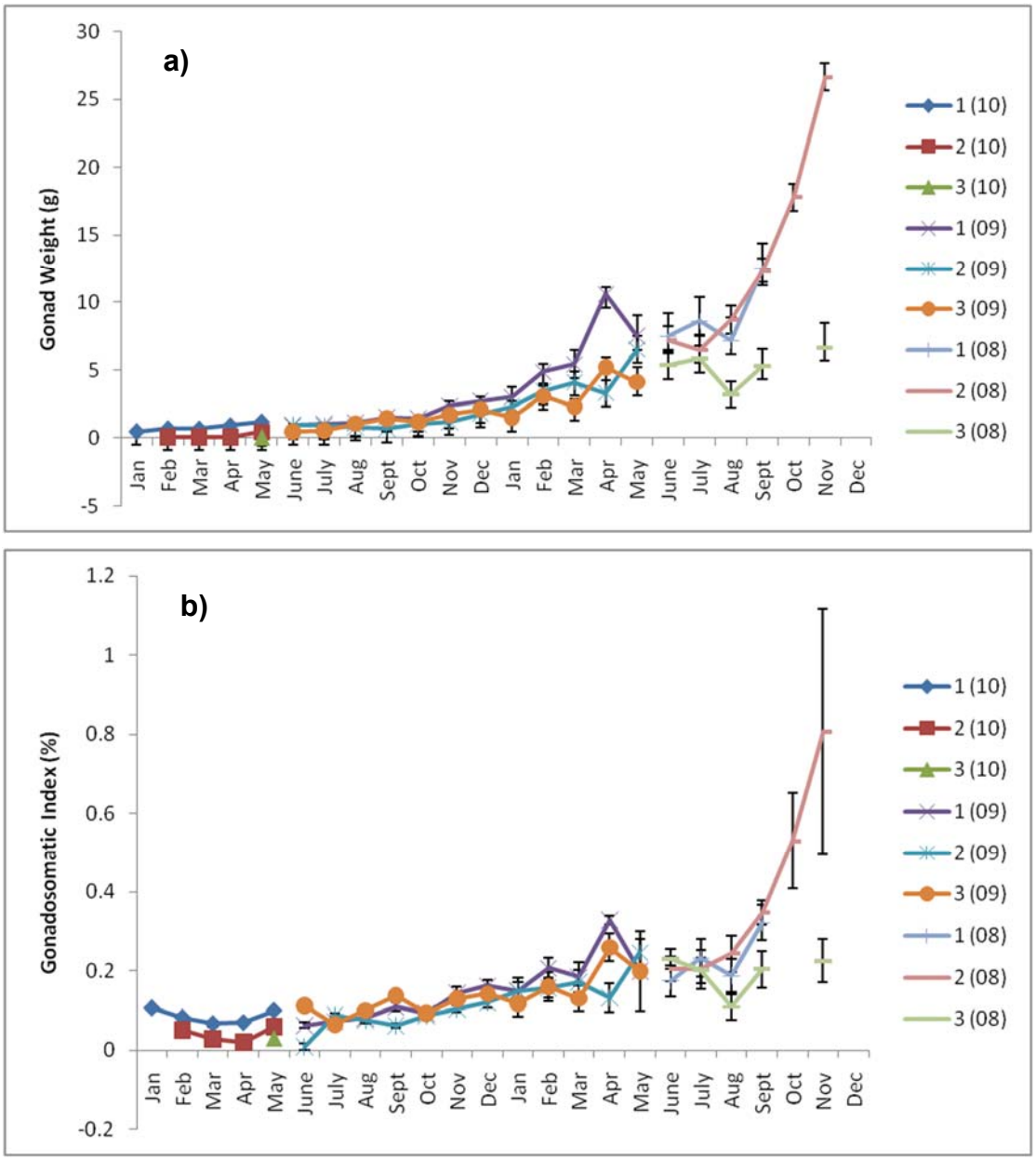


Figure 13. Mean ± sem gonad mass by (a) gross weight, and (b) as a percentage of body weight, of fish sampled each month (male and female fish combined). January to December (year 1, n=20), January to November (year 2, n=10). Legend code indicates hatchery run (1st, 2nd or 3rd) and year fingerlings were introduced to sea-cages ('08, '09 or '10).

Assessing maturation by sex, it is clear that the males were driving the increase in gonad mass in September-November as rising 2+ fish (Figure 14a, b and Table 5). Average GSI of male fish increased approximately 10-fold from 0.1% to 1.2% in three months, in comparison to females that had a consitsent 0.05% increase in gonad size each month. The variiability in testes mass between hatchery run 2 rising 2+ males in November was also large, with the indivudal fish minima and maxima GSI values of 0.06 and 3.34% (101g) respectively. Female GSI was plotted on same scale as male

GSI. Note the GSI values for female fish were actually greater than males from April in the first year through to October of the second year.

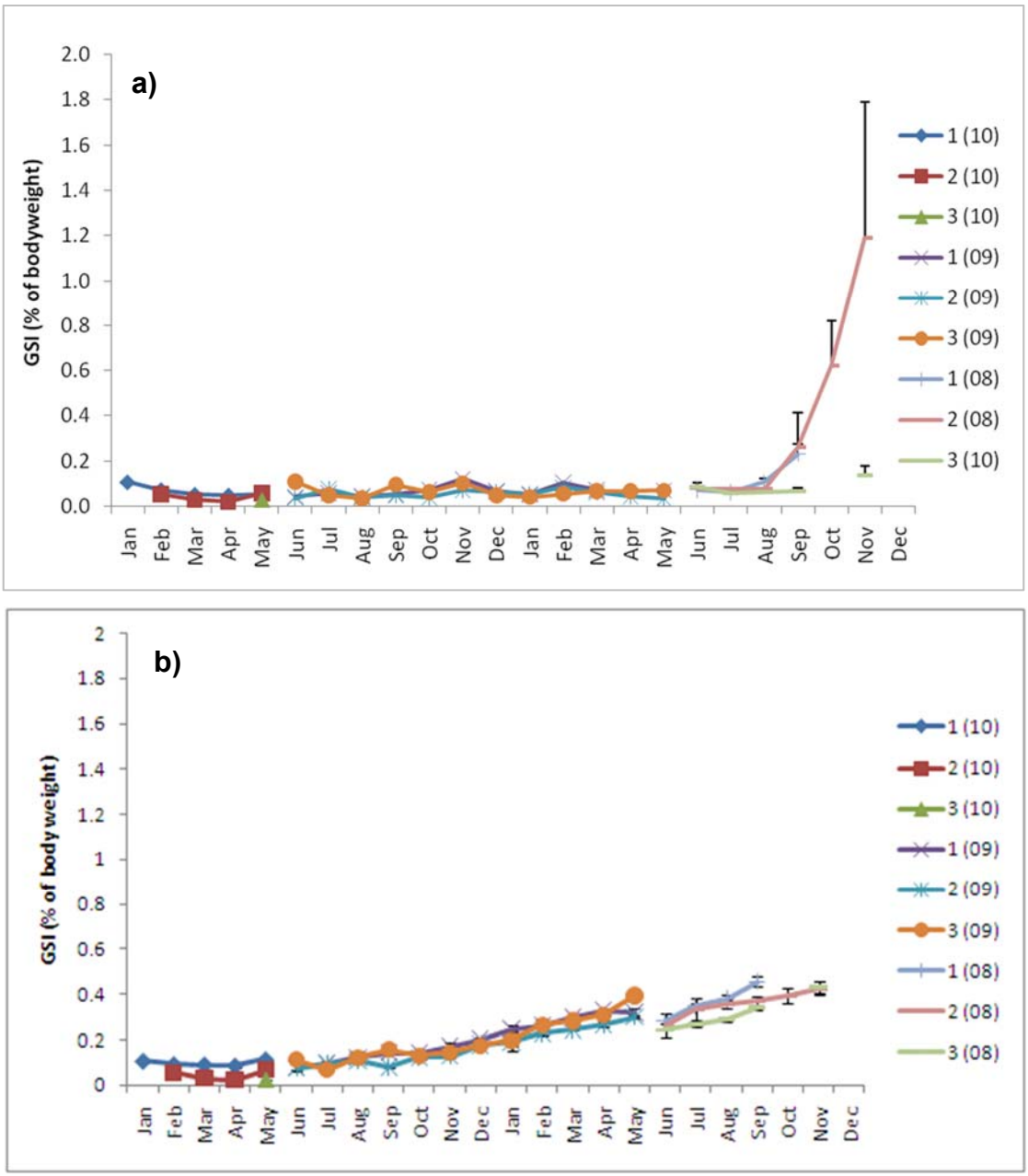


Figure 14. Mean ± sem gonad mass in (a) males, and (b) females as a percentage of body weight. Numbers of fish sampled in each month are given in Table 5. Legend code indicates hatchery run (1st, 2nd or 3rd) and year fingerlings were introduced to sea- cages (’08, ’09 or ’10).

Table 5. Numbers of male and female fish sampled in each hatchery run from each month as 0+ and 1+ fish. Where no number is shown the fish were not sampled in that month.

Sex	Age	Run	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
Males	0+	1	9	7	14	8	10	11	12	9	7	14	9	6
		2		13	12	11	12	8	8	8	11	8	10	10
		3				13	13	6	16	5	6	11	8	5
	1+	1	5	4	5	0	4	5	4	7	6			
		2	3	5	4	6	2	3	5	4	2	6	5	
		3	5	5	7	2	6	2	3	8	5		7	
Females	0+	1	11	13	6	12	10	9	8	11	13	6	11	14
		2		7	8	9	8	12	12	12	9	12	10	10
		3				7	7	14	4	15	14	9	12	15
	1+	1	5	6	5	10	6	5	6	3	4			
		2	7	5	6	4	8	7	5	6	8	4	5	
		3	5	5	3	8	4	8	7	2	5		3	

No published study has examined YTK as small or young as those in the present study, or, if they did, these data were excluded as the main focus was on the mature or spawning fish within the population. In studies by Gillanders et al. (1999) and Poortenaar et al. (2001) they reported maximum mean GSI in YTK (>700mm in fork length) from November to January; however, both mention unpublished data that suggest that this may vary with geographical location outside their particular sampling region. Poortenaar et al. (2001) reported maximal GSI for both males and females of 3-4%. Gillanders et al. (1999) reported weight of the gonad only, rather then GSI. However, extrapolating from their length data we can estimate an approximate maximum GSI of 5% (i.e. female ovaries were 300g and male testis were 400g, fish body weight was assumed to be >7 kg). The maximal GSI values (3-5%) reported by Poortenaar et al. (1999) and Gillanders et al. (1999) in >700mm wild YTK were higher than the maximum values found in the current study (males 1.2% and female 0.4%). This suggests that the degree of maturation shown by the smaller (<700mm) farmed YTK sampled in the current study is much less likely to impact on the energetics of somatic growth and product yield, than it would in larger YTK. The NZ and NSW studies reported that testes were heavier than ovaries for similarly sized fish, an observation that was also evident in the current study. If the farming system changed, or market demands changed, such that older and/or larger fish (rising 3+ or >7kg) were grown, there may be some impact of maturation on product yeild and this would need to be assessed. However, with current farming practices the impacts are seemingly insignificant.

The numbers and percentages of male and female fish for each hatchery run in each year class is shown in Table 6. The overall percentage of male fish was 47.3% and females 52.7%. Three fish (0.3% of total number of fish sampled) were found with both male and female tissues in gonad histology, but in all cases the minor gonad type was only a small fraction of the organ, thus, these fish were classified to sex according to the majority of the gonad type present. Overall, there does not appear to be a significant deviation from a 1:1 sex ratio for these fish.

Table 6. Numbers and percentages of male and female fish sampled in each hatchery run from each year class of fish.

	Run	2008	2009	2010
Males	1	22 (55%)	86 (45%)	48 (48%)
	2	25 (42%)	83 (44%)	48 (48%)
	3	25 (50%)	82 (43%)	26 (65%)
Females	1	18 (45%)	104 (55%)	52 (52%)
	2	35 (58%)	107 (56%)	52 (52%)
	3	25 (50%)	108 (57%)	14 (35%)

3.1.6 Visceral fat mass and index

The amount of fat removed from the viscera was highly variable in comparison to all other visceral components (Figure 15a, b). The weight of visceral fat ranged from zero through to a maximum average of 110g, or 3.5% of body weight (Figure 15a).

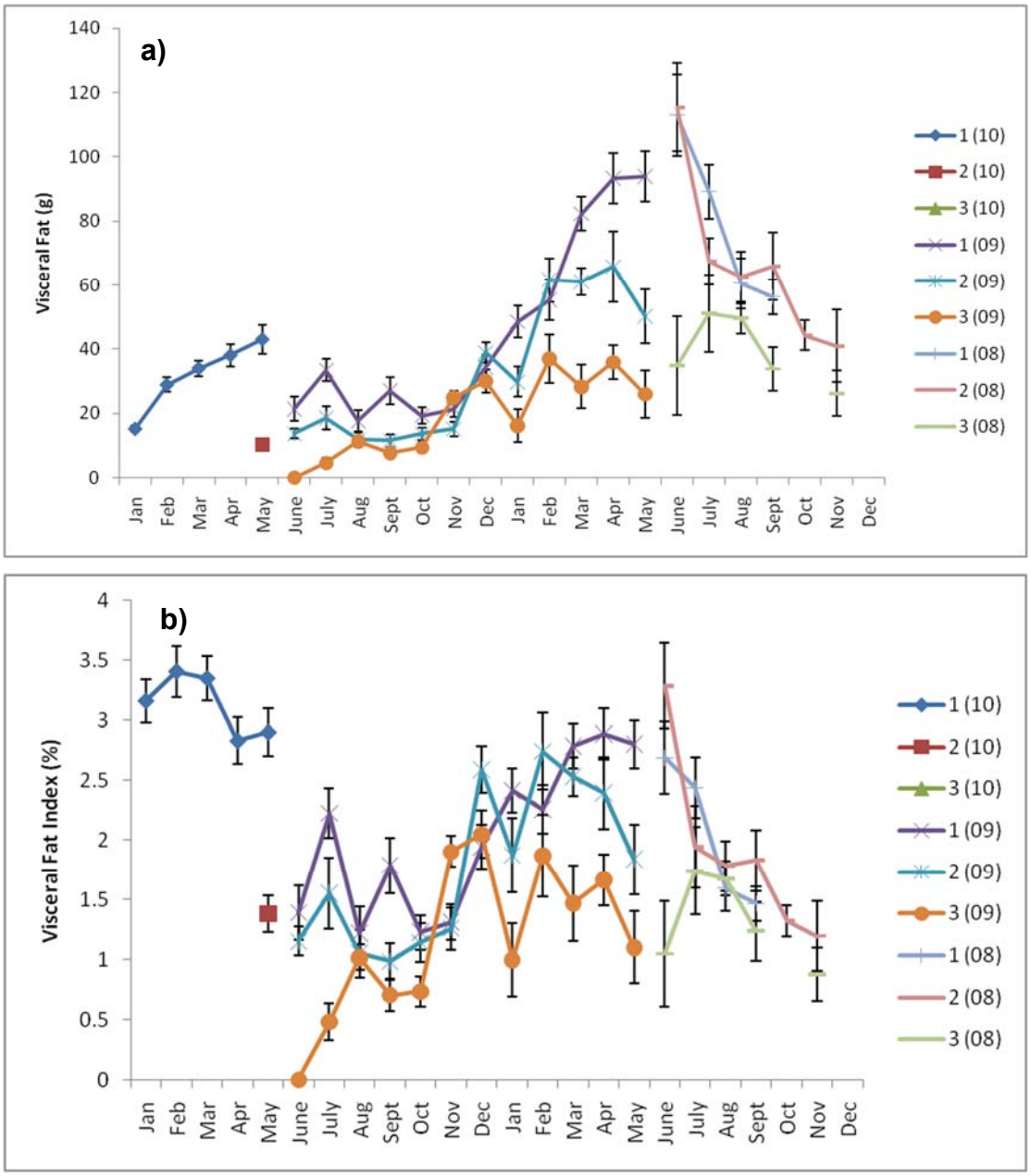


Figure 15. Mean ± sem visceral fat mass by (a) weight, and (b) as a percentage of body weight, of fish sampled each month (male and female data combined). January to December (year 1, n=20), January to November (year 2, n=10). Legend code indicates hatchery run (1st, 2nd or 3rd) and year fingerlings were introduced to sea-cages ('08, '09 or '10). Visceral fat is all the fat that can be physically separated from the surface of the viscera, especially the pyloric caecae and intestine.

A seasonal change in the amount of visceral fat was noted, with fat mass appearing to increase during summer, and decrease in autumn-winter. Changes in fat mass appeared to largely drive the changes in visceral mass shown in Figure 10. The maximum relative amount of fat mass in the 3rd hatchery run of the 2009 year class (2%) was lower than that observed in hatchery run 2 (2.5%) and hatchery run 1 (3%).

The onset of the observed autumn-winter decrease in fat mass appeared to affect the different hatchery runs of fish in the same order (i.e. 3, 2 and then 1), but even in the 2009 year class, the minimum average visceral fat mass was still >1% of body weight. Thus, there is no reason to believe these fish were in danger of starvation. However, the data raised the possibility that a 3rd hatchery run (if produced late in the year) may risk fish not spending enough time at sea in summer to accumulate adequate fat reserves to allow them to metabolise through winter, or perhaps get as early a start to growth at the onset of spring water temperatures.

3.1.7 Fillet mass and yield

Fillet yield is of major interest to customers as this directly reflects the processed value of this product. Changes in fillet mass and subsequent fillet yield as fish go through the production cycle are shown in Figure 16a ,b. Fillet yield in fish over 1.1kg body weight was 55-65% (Figure 17). Ignoring the trend observed in smaller fish (<1.5kg), there did not appear to be a seasonal variation in fillet yield. Variability in yield was greater for the 3rd hatchery run fish, and all fish harvested in April produced a lower yield percentages than in either March or May, which may suggest there was a processing error (although the same filleting team was doing the work). This suggests that customers should know or be made aware by the marketing department that they will get a very reliable year-round yield of saleable product from their purchase of farmed YTK up to the age/size examined in the current study. Although they may see seasonal variability in the amount of viscera removed from the fish, there is negliable impact on the fillet yield.

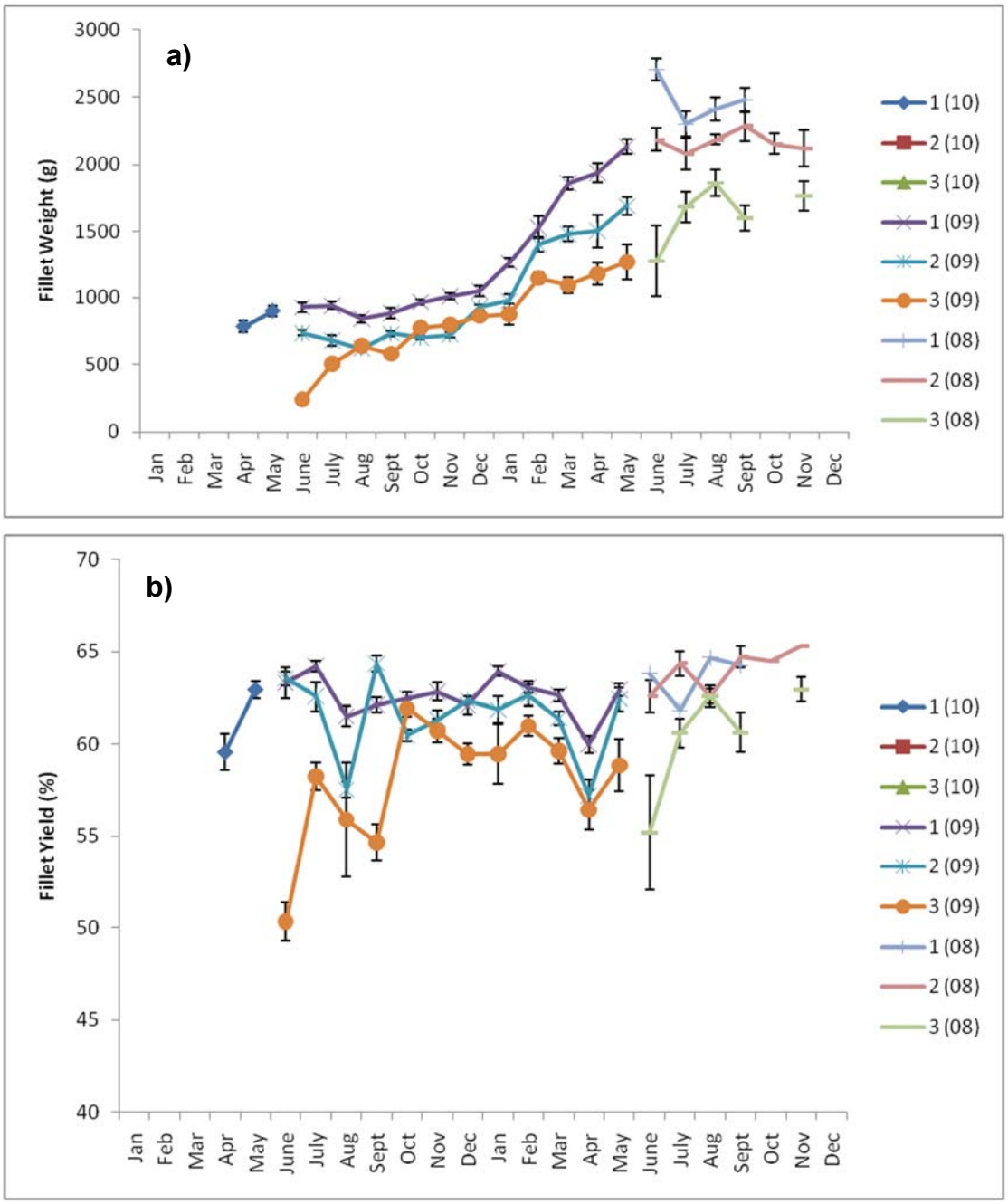


Figure 16. Mean ± sem fillet yield by (a) weight, and (b) as a percentage of body weight, of fish sampled each month (male and female data combined). January to December (year 1, n =20), January to November (year 2, n=10). Legend code indicates hatchery run (1st, 2nd or 3rd) and year fingerlings were introduced to sea-cages ('08, '09 or '10). The fillet was a 'Japanese cut' removed and trimmed to commercial specifications.

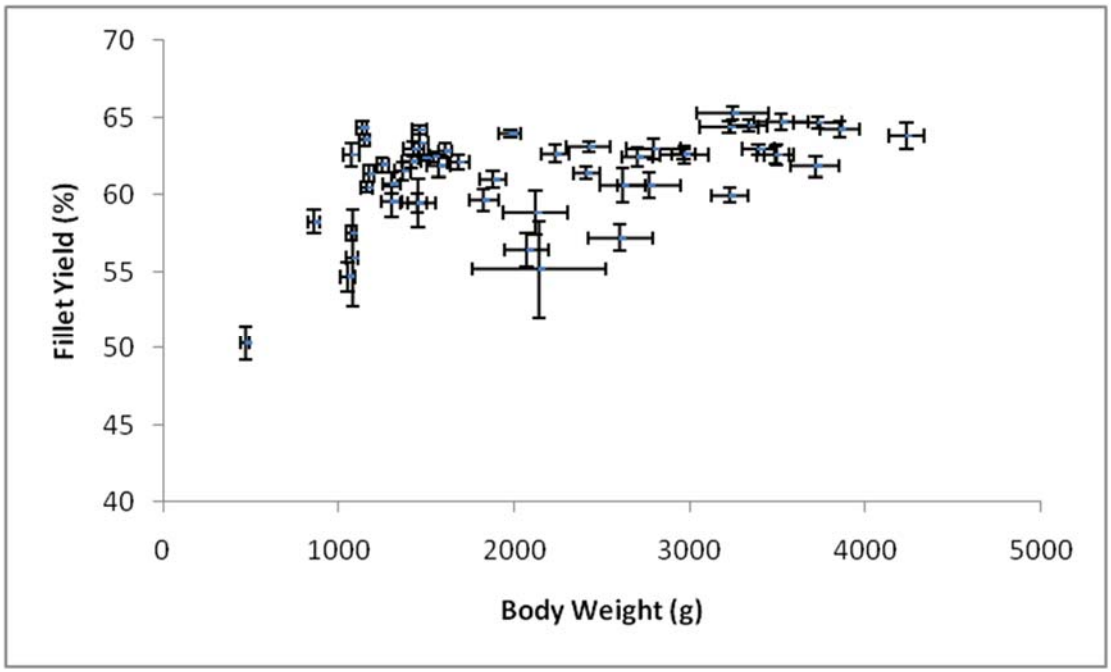


Figure 17. Fillet yield (%) plotted against body weight (g) from fish (average >1000g) sampled each month (both sexes combined). Mean ± sem is shown, n=20 for 0+, n=10 for 1+ fish. The fillet is a 'Japanese cut' removed and trimmed to commercial specifications.

3.2 Crude fat and moisture

The flesh fat content of 0+ and early 1+ fish was about 4%, which increased in 1+ fish (over 2kg in body weight) from February (mid summer) to peak at around 8-9% in May-June, whereafter it decreased to about 5% by October-November (Figure 18). Hatchery run 3 fish appeared to have a consistantly lower fillet fat content than earlier hatchery run fish, but at the time these were being harvested (October-November) the fillet fat levels were similar to hatchery run 2 fish. The relationship between the fat and moisture content measured in this 'quality cut' sample, and the remainder of the carcase is work that is being carried out in sub-project 1. The changes in fillet fat content was inversely related to fillet moisture, as shown in Figure 19.

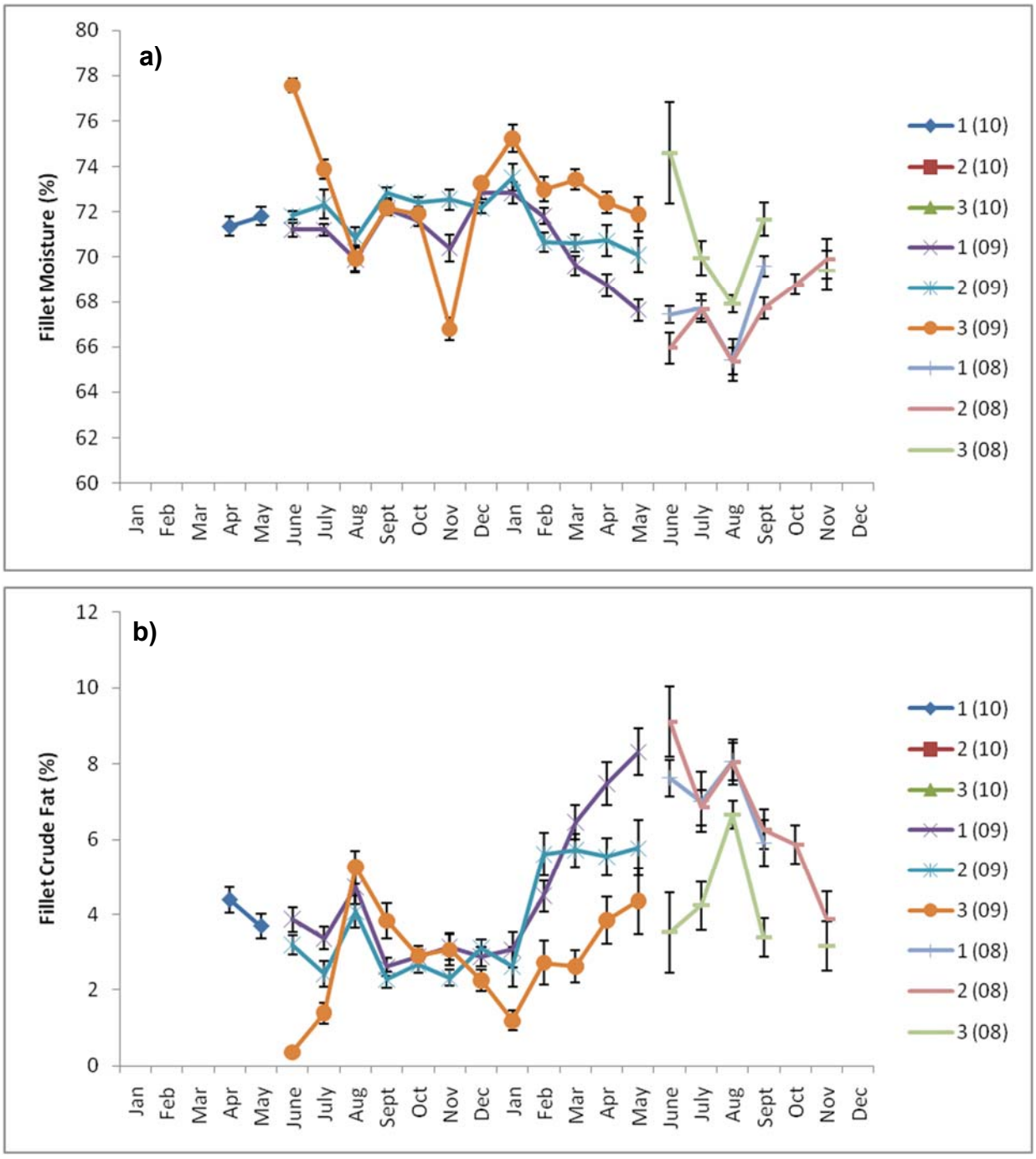


Figure 18. Mean ± sem fillet (a) moisture and (b) crude fat content as a percentage of wet weight of all fish filleted each month (both sexes combined). January to December (year 1, n=20), January to November (year 2, n=10). Legend code indicates hatchery run (1st, 2nd or 3rd) and year fingerlings were introduced to sea-cages (’08, ’09 or ’10). The fillet portion sampled was a dorso-ventral strip taken from just behind the vent.

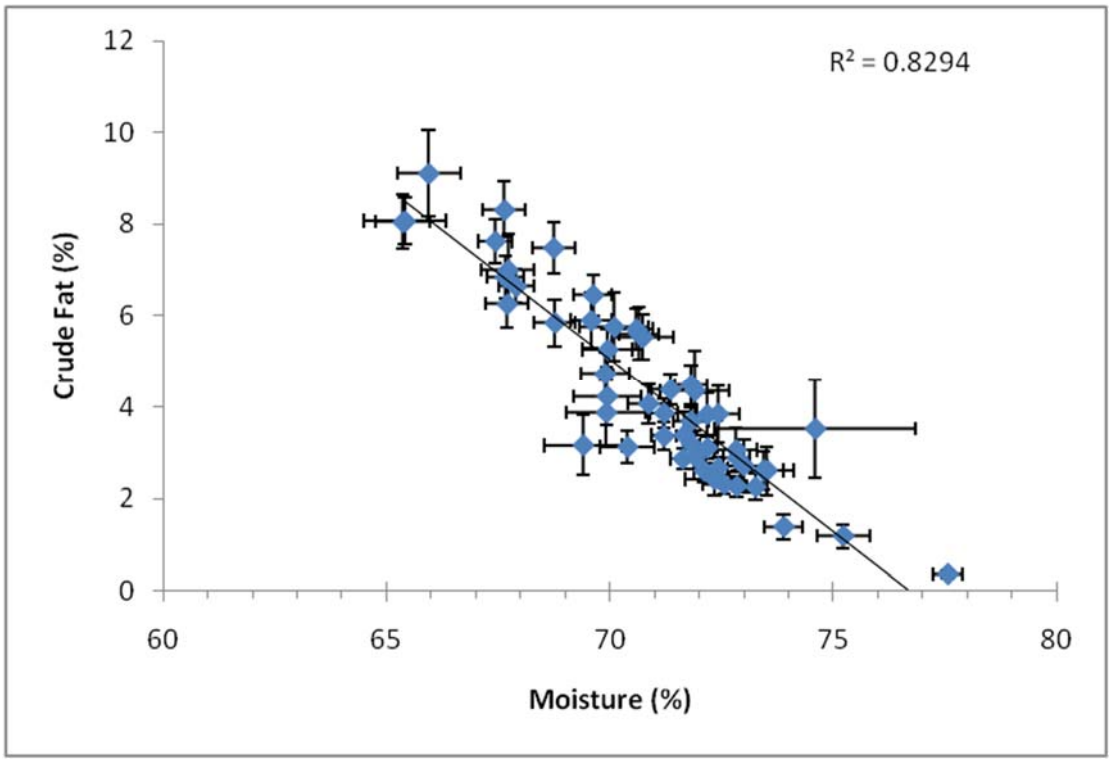


Figure 19. Fillet crude fat (%) plotted against fillet moisture (g) from fish (average bodyweight >1000g) sampled each month (both sexes combined). The dorso-ventral fillet section used in the analysis was removed from just behind the vent.

3.3 Carcass Composition

With any product that has pronounced growth/size and/or seasonal related changes in the relative composition it is very important to understand how these factors affect the final product the customer is offered. Composition (fillet yield, viscera and frame component) of whole YTK changes with the age/size of the fish (Figure 20a, b). Only data from the first hatchery run of each year class are shown, as similar trends were also found for later hatchery runs. When each component is expressed as a percentage of total body weight it is clear that there is little change in the relative proportion of the three components during the production cycle (Figure 20b). Fillet yield was 60-65%, viscera 7-9% and frame 27-33%. On the other hand, the composition of the viscera does change substantially over time (Figure 21). Visceral fat changes the most (from 15-40% of visceral mass, with over 100g of fat found in some 3.5-4kg fish), followed by the digesta (35-55%), liver (12-20% mass) and gonad (1-4% of the visceral mass). The only seasonal trend appears to be that the fat mass appears to accumulate over the warmer months, and decrease during the colder months, suggesting that it acts as an energy or fatty acid depot at a time when the fish's appetite is low due to the colder water temperature.

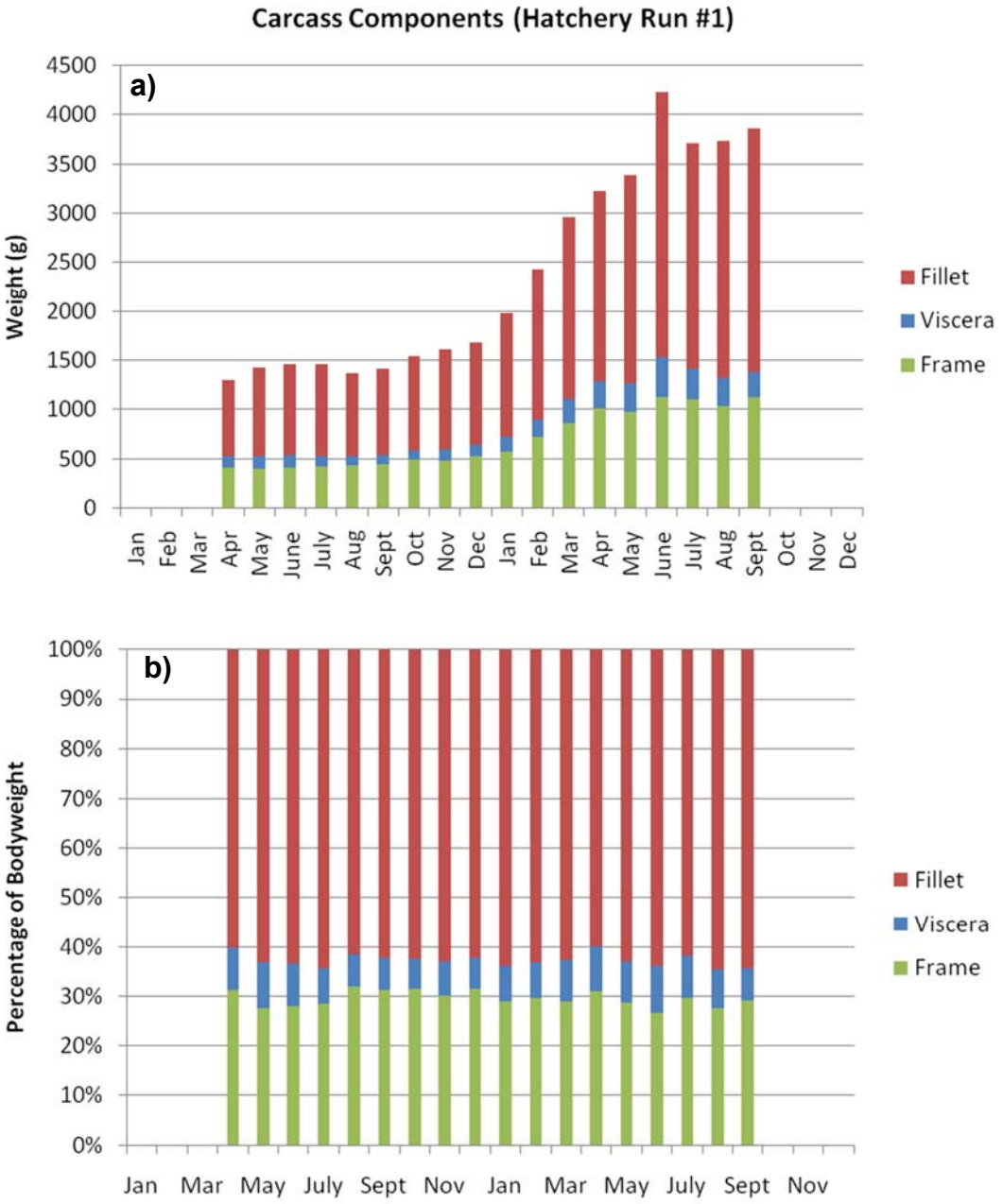


Figure 20. Carcass composition as (a) fish grow, and (b) as a percentage of body weight through the production cycle. These data are from hatchery run 1 fish in the three year classes sampled during this study. The frame includes head, backbone, most fins (dorsal, caudal, anal) gills, kidney, swinbladder and heart.

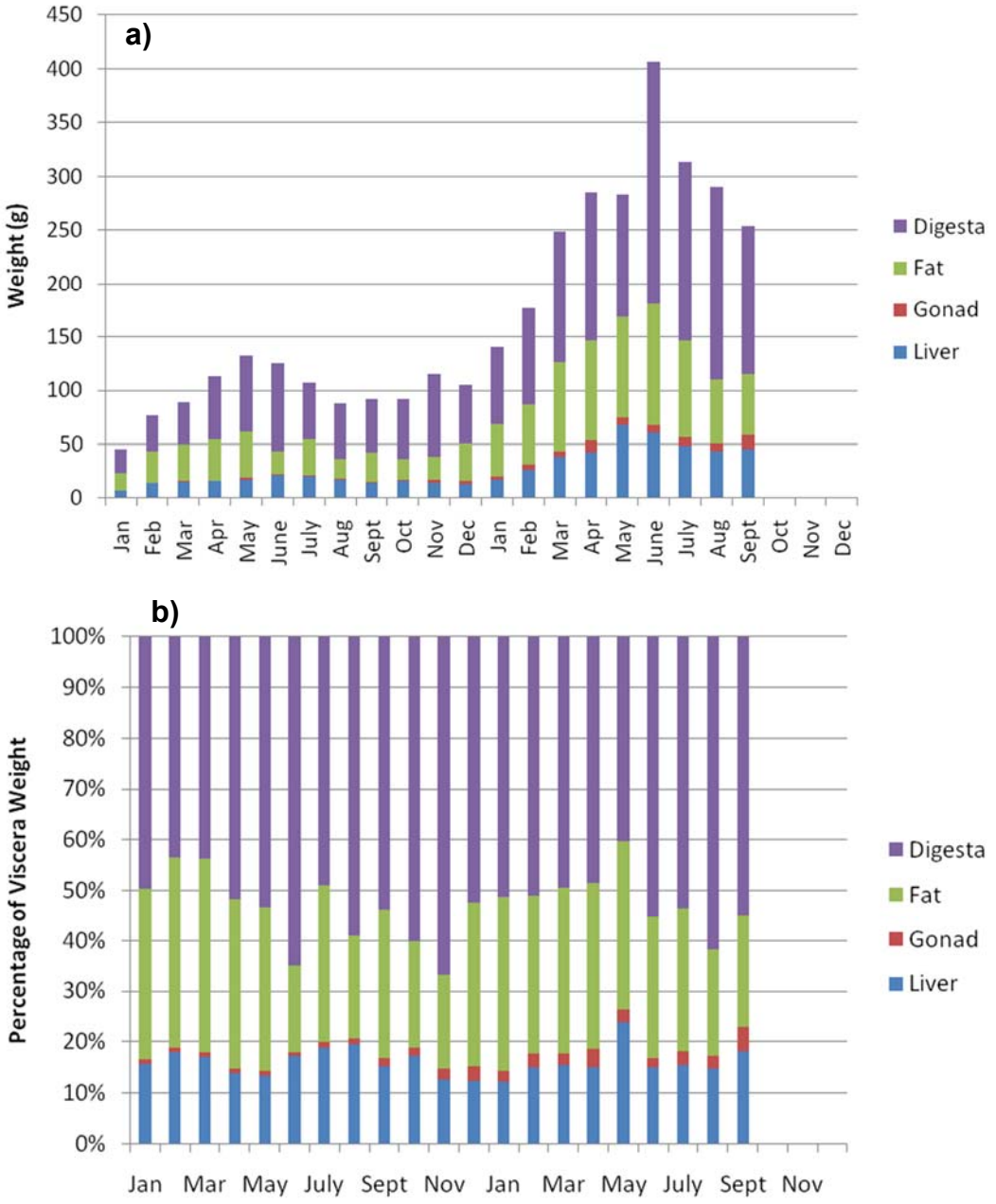


Figure 21. Viscera composition as (a) fish grow, and (b) as a percentage of total viscera weight through the production cycle. These data are from hatchery run 1 fish in the three year classes sampled during this study. Digesta includes stomach, pyloric caecae, intestine, gall bladder and bile duct, and spleen.

3.4 Reproductive Maturity

3.4.1 Sex Ratios

The number/proportion of male and female fish caught using the random sampling approach varied from 100% female (from a group of 10 fish), through to 80% males (from groups of 10 and 20 fish). The sex ratios found for most sampling events fell between 30 and 70% males. As would be expected, the variability in sex ratios was greater in 1+ fish because only 10 fish were sampled from each pontoon each month as opposed to 20 fish for 0+ fish. The same pontoons were resampled in successive months and there was no indication that any particular pontoon had a persistant sex ratio bias (Figure 22).

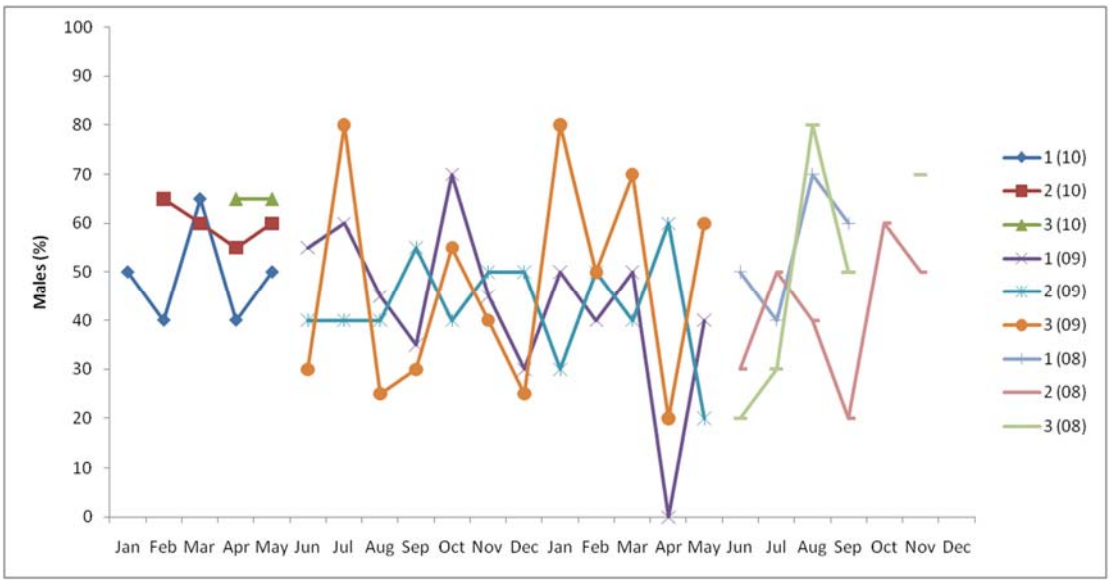


Figure 22. Percentage males (as assessed by histological analysis) of each pontoon sampled each month. January to December (year 1, n=20), January to November (year 2, n=10). Legend code indicates hatchery run (1st, 2nd or 3rd) and year fingerlings were introduced to sea-cages (’08, ’09 or ’10).

3.4.2 Macroscopic staging - female fish

A few females were scored as stage 1 by the macroscopic appearance of the gonad; these, as would be expected, were observed in the smallest and youngest fish sampled in each hatchery cohort in the 0+ year class (Figure 23). All other females were assessed as stage 2, consequently no female was classified as a stage 3 (using the criterion that no oocytes/granularity were/was visible in a cross-section of the ovary).

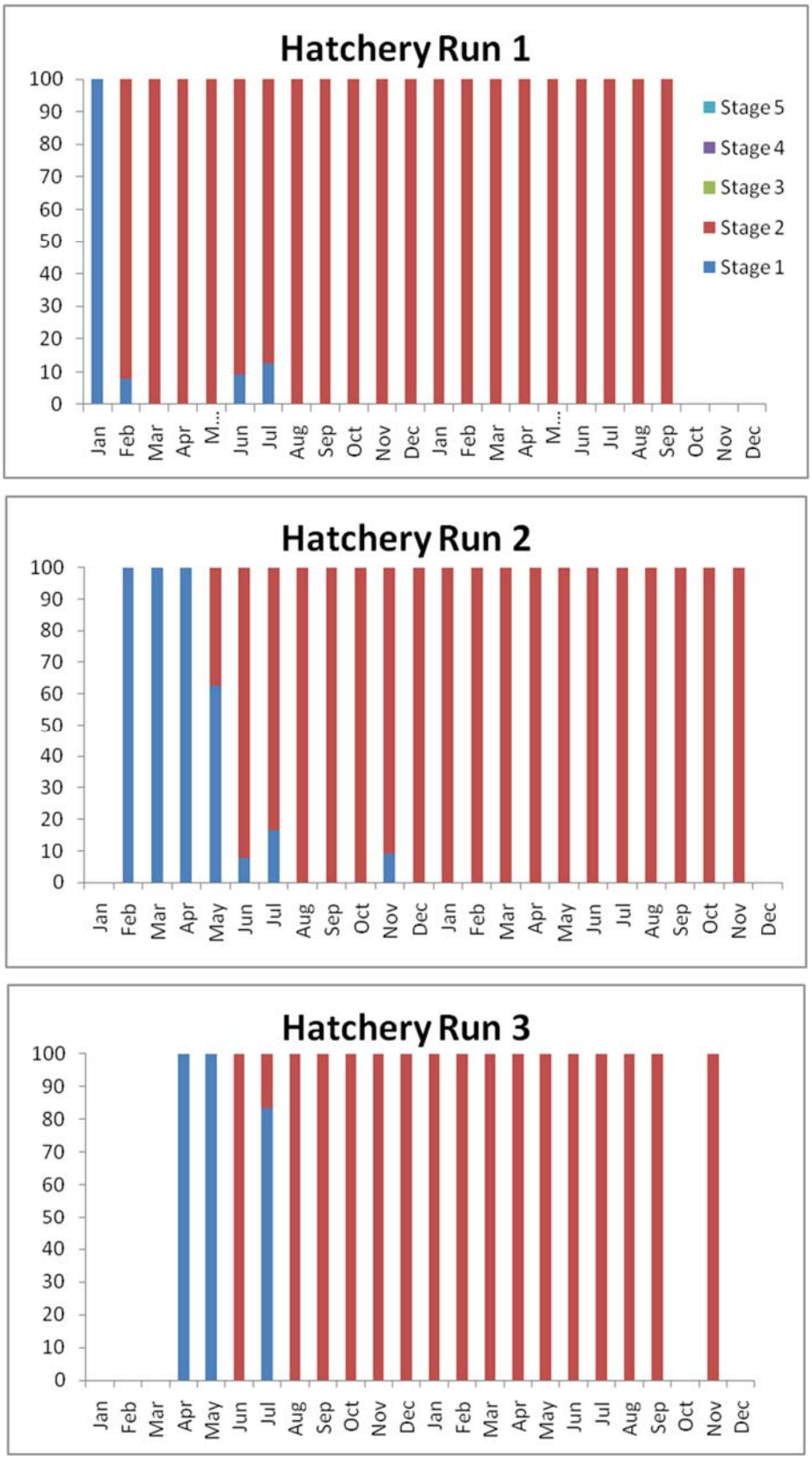


Figure 23. Proportion of female fish in hatchery runs 1, 2 and 3 assigned to reproductive stage by macroscopic assessment in each month during the production cycle.

3.4.3 Macroscopic staging – male fish

All hatchery runs showed similar patterns of macroscopic maturation (Figure 24), with hatchery run 1 fish slightly more advanced than run 2 and 3 fish. Stage 1 and 2 testes dominating from January until September-October. After this time all 0+ males were stage 3. No testes were assessed as Stage 4. After their first maturation there were no 'spent' (Stage 5) fish found, suggesting the maturation 'effort' of 0+ fish was not sufficient to require major 'recycling' of the tissues. Perhaps this is why many 1+ males carried small volumes of sperm in the lumen until May, leftovers from the October-December peak of maturation (5-7 months previously). Stage 3 1+ fish (maturing for the second time) first appeared in September-October, with hatchery run 1 and 2 appearing to be advanced relative to run 3 fish (Figure 24).

Comparison of the relative timing of reproductive development between the 3 hatchery runs is best shown by Figure 25. Despite the variability inherent in random sampling resulting in unbalanced numbers of male fish in each month and between hatchery runs it is clear that there are very similar patterns of reproductive staging between the 3 hatchery runs. The Stage 3 data suggest that hatchery run 1 fish start oozing milt slightly earlier than the other runs, but run 2 and 3 fish were similar to each other.

Figure 24. Proportion of male fish in hatchery runs 1, 2 and 3 assigned to reproductive stage by macroscopic assessment in each month during the production cycle.

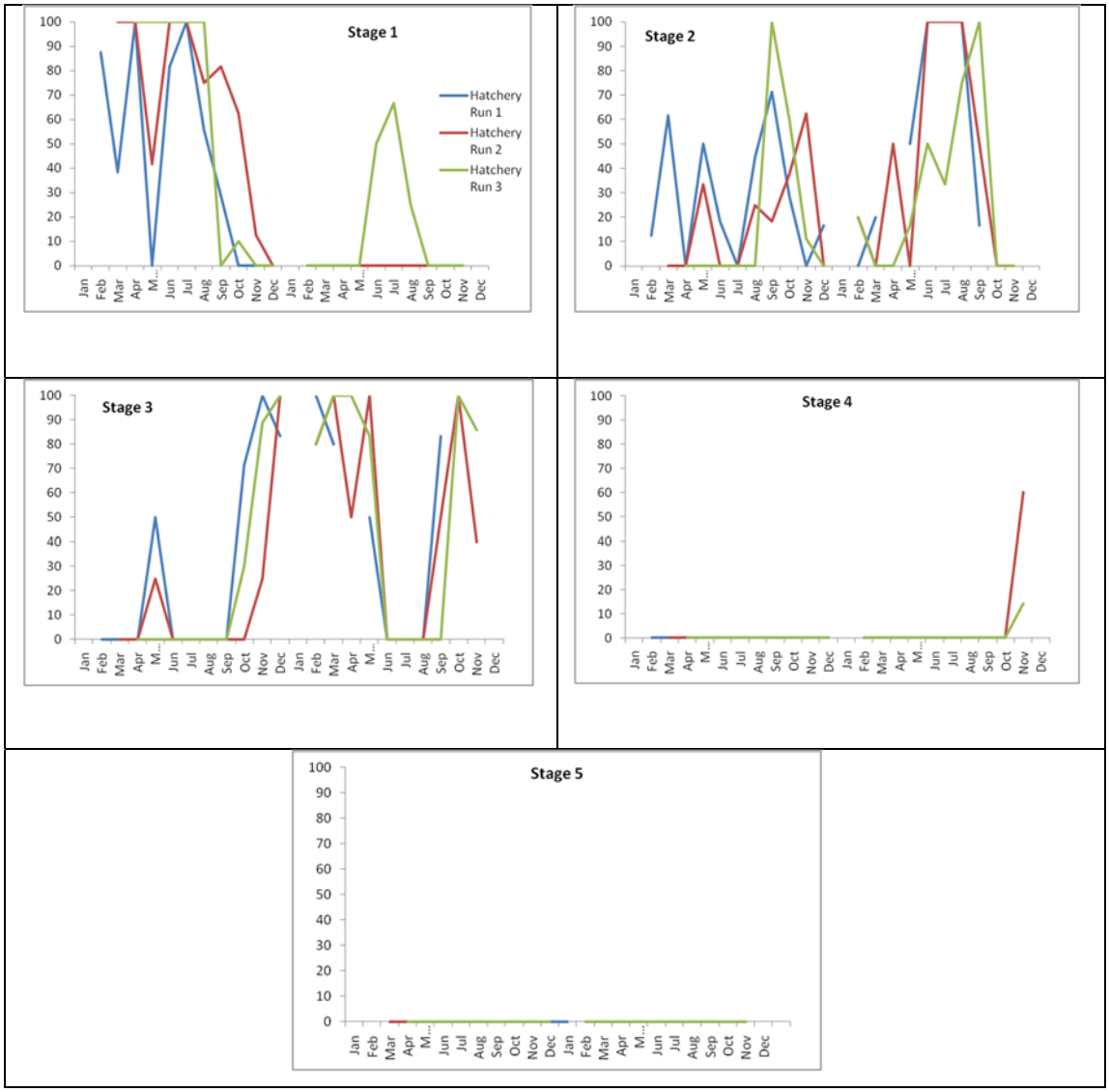


Figure 25. Proportion of male fish assigned to each reproductive stage by macroscopic assessment in each month during the production cycle from hatchery runs 1, 2 and 3.

3.4.4 Microscopic staging - female fish

Histological analysis confirmed that no female fish sampled were vitellogenic. Primary and secondary oocytes appear to be continually recruited throughout the first and second years of life (Figure 26). Between fish variability in the ratio of primary and secondary oocytes present in the section was largest in the younger fish, as fish grew the population become more synchronised. The largest and most developed oocytes (at the cortical alveolar stage) measured in any section were ~150μm in diameter. They showed some development of follicular layers, but no thickening of the zona radiata (oocyte membrane) or evidence of yolk vesicles (Figure 27).

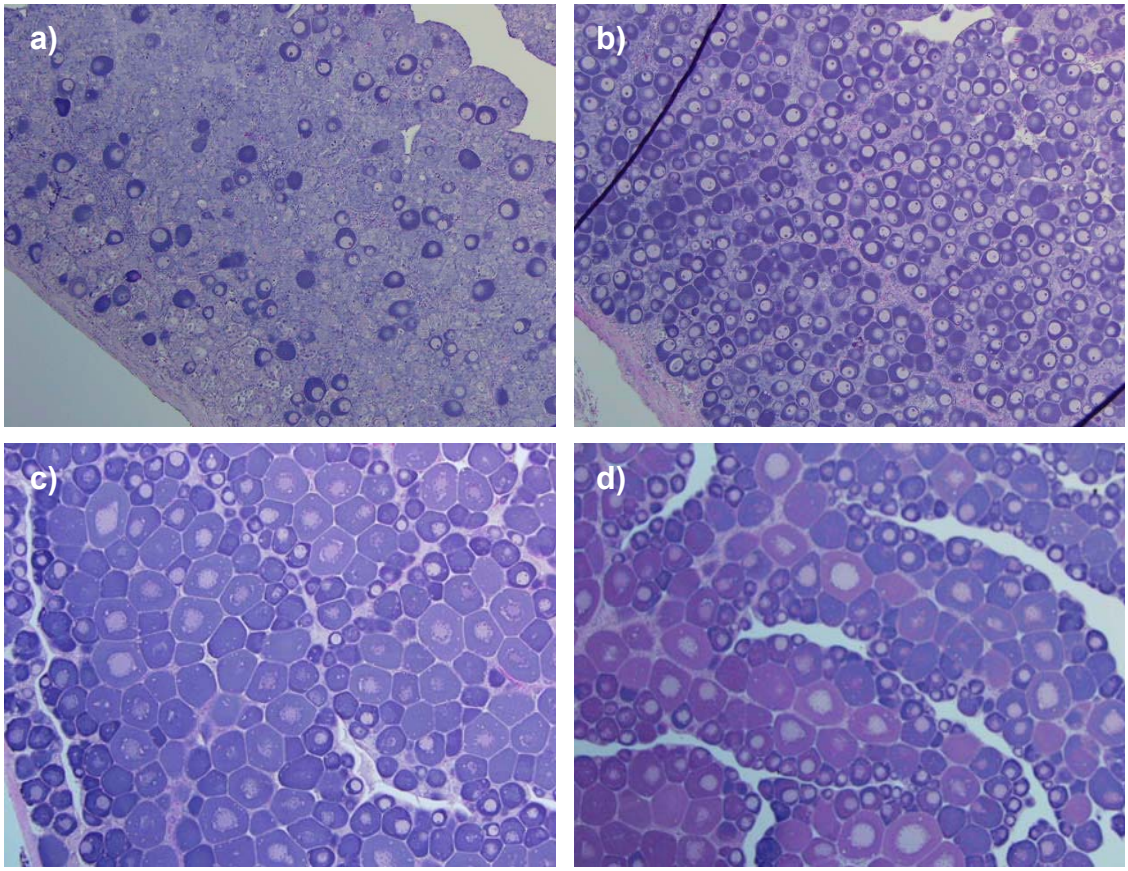


Figure 26. Photographs of H&E stained sections through ovaries. (a) and (b) are from 0+ hatchery run 1 fish in February. (c) and (d) from 1+ run 1 fish in September. All photographs are at the same magnification (x20).

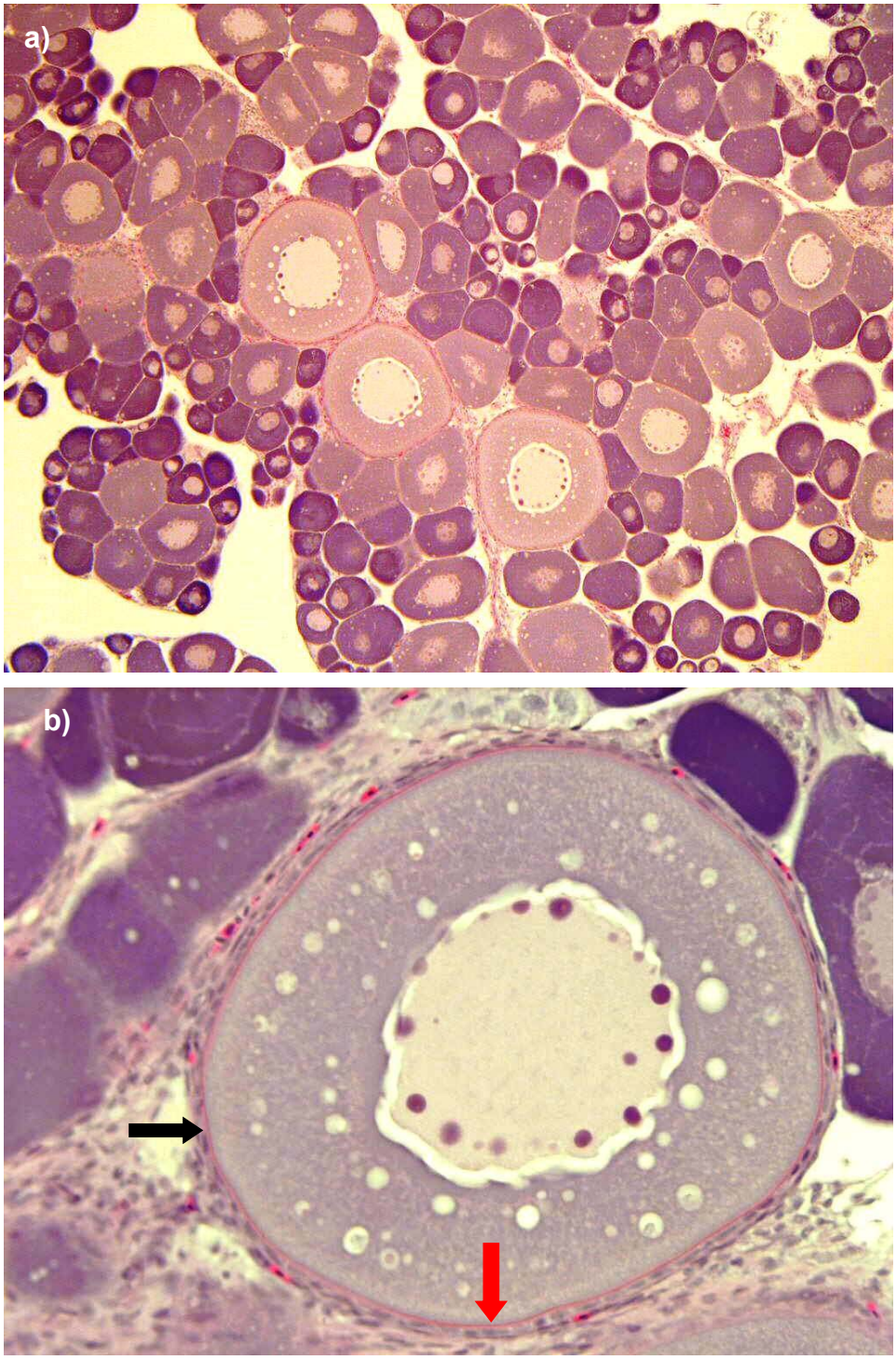


Figure 27. Photographs of the most advanced oocytes observed in this study. The fish was harvested in November and was from hatchery run 3. The (a) low power (x10 objective) photograph shows three oocytes developed to the cortical alveolar stage within the background of more numerous chromatin nucleolus and perinucleolus oocytes. The (b) higher power (x40 objective) shows detail of one cortical alveolus stage oocyte, the zona radiata is just showing some thickening and red colouration (red arrow), outside of the oocyte it's possible to see a layer of small follicle cells with dark nuclei (black arrow). The largest oocytes are ~150μm in diameter.

All but one of the female fish were scored as Stage 1 by the microscopic appearance of the gonad histology. The only fish assessed as Stage 2 represented 33% of the 1+ females from hatchery run 3 sampled in November. The fish assessed as Stage 2 only had a few oocytes from the hundreds visible in the section that showed the transition to the cortical alveolus stage, suggesting this fish had only just begun to progress to this stage of reproductive maturity. It is believed that there is no possibility that this fish would have undergone a rapid period of vitellogenesis to become sexually mature as a 1+ (or rising 2+) fish .

3.4.5 Microscopic staging - male fish

In contrast to female fish, male fish showed characteristics of reproductive development in fish at very early age/size, with spermatogenesis initiating in February of the first year (i.e. 5 months after hatch) (Figure 28). All males were sexually mature in November (i.e. at 1 year of age); however, testis size and milt volumes were small (GSI <0.2% of body weight) and limited, respectively. Sperm squeezed from the cut testis of 0+ and 1+ males could be activated by seawater suggesting they were physiologically capable of fertilization.

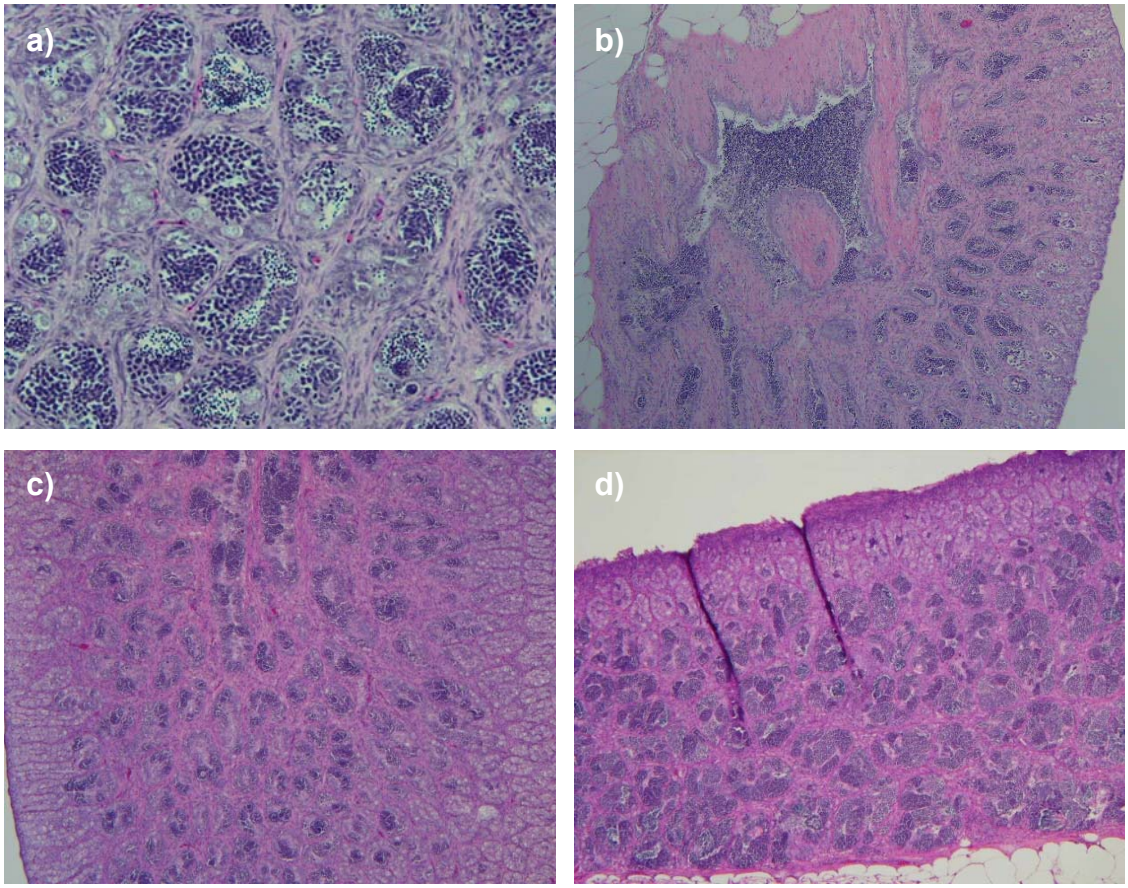


Figure 28. Photographs from testes sections taken from 0+ fish (a) and (b) in February, and (c) and (d) in September. Photograph A is high magnification and shows active spermatogenesis. Photo B is low magnification and shows areas (normally towards the core of the testis) in active spermatogenesis, and spermatozoa (dense blue-black nuclei) in the lumen. Photos C and D show moderate levels of active spermatogenesis.

Following their first maturation the 1+ males all undergo a period of testicular resorbtion (Figure 29). They appeared to begin the process of spermatogenesis for their second maturation opportunity in June, later than the 0+ males. Not surprisingly GSI and milt volumes were greater for 1+ males, though still relatively small compared to 2+ YTK (and some other species of aquacultural importance, especially salmonid species).

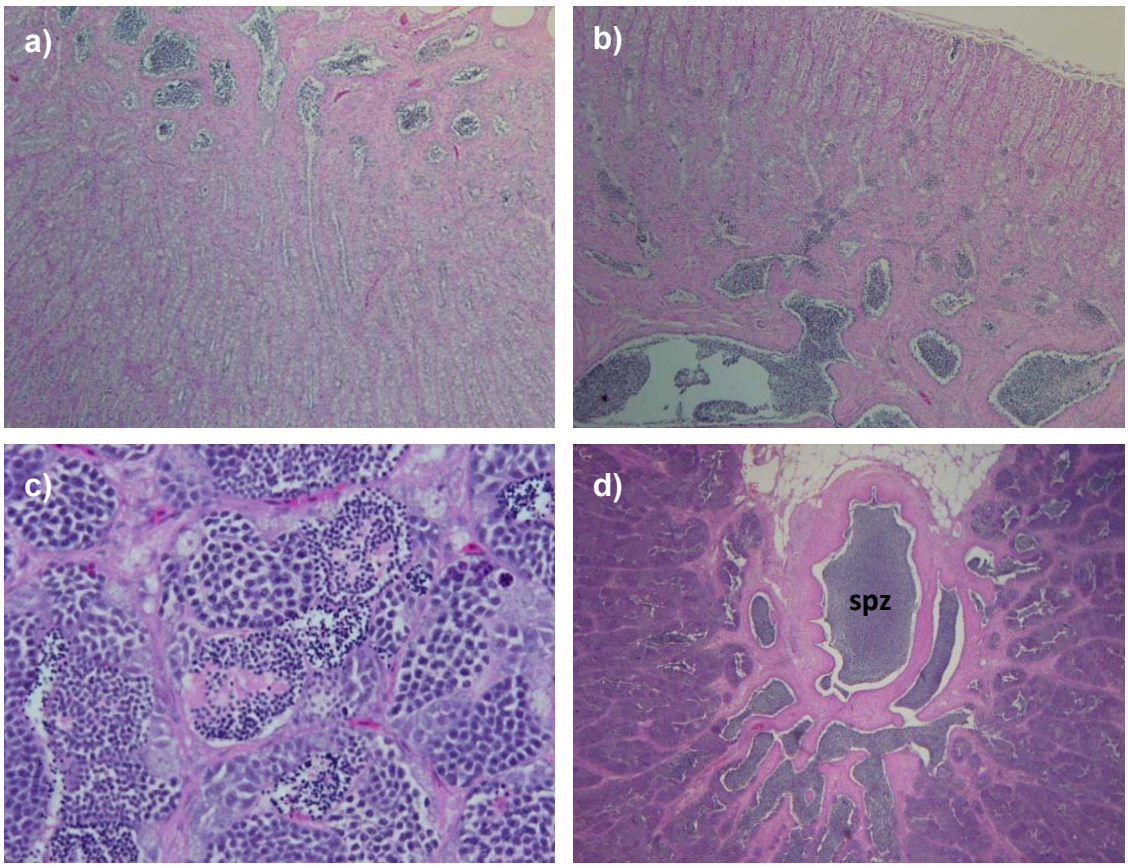


Figure 29. Photographs of H&E sections through testes from 1+ fish. (a) and (b) are fish in June. Photo (a) shows residual spermatozoa in lumen from maturation 6 months earlier, with little spermatogenesis occuring in the tubules. Photo (b) is a low magnification of a different testis but at a similar stage of re-maturation. Photos (c) and (d) are 1+ fish in September. Photo (c) is a high power view and shows all cysts at all stages of spermatogenesis with pink tails on spermatozoa (spz). Photo (d) is low power and shows the accumulating spermatozoa (dense blue-black cells) in the lobules and ducts at the centre of the organ. Connective tissue is pink.

Staging based on microscopic cellular assessment gave maturity estimates that were more advanced than the staging assessed by macroscopic criteria. Very few fish were histologically assessed as Stage 1, and more fish were assessed as Stage 4 and Stage 5, compared to the macroscopic assessment. Spermatogenesis was underway in many fish at an estimated age of 4-5 months (hatchery run 1 fish were hatched in September and first sampled in January) (Figure 30). Up to 50% of hatchery run 1 and 2 males were producing small amounts of spermatozoa that were accumulating in the tubules from February to July; however, this appeared to be a premature 'try-out' of the spermatogenic mechanism in some fish, as this stage was not apparent in August. The majority of 0+ males were assessed as Stage 3 in September-October, with Stage 4 males present from November to February, and a few spent fish present in January-April. More Stage 1 fish were observed in the 1+

males compared to the 0+ fish. Spermatogenesis for the second maturation seemed to be initiated in August-September.

The histological data suggested that hatchery run 1 males showed a lower extent of sexual maturity than run 2 or run 3 fish (Figure 31). This was evident through fewer Stage 3 and 4 fish being sampled, and an earlier disappearance of these stages from that cohort. It may be that hatchery run 1 male fish were more synchronised in their peak of reproductive maturity than later hatchery runs. Only further fine-scale sampling could answer this question, but in the whole scheme of the production cycle, it is probably only of minor commercial interest.

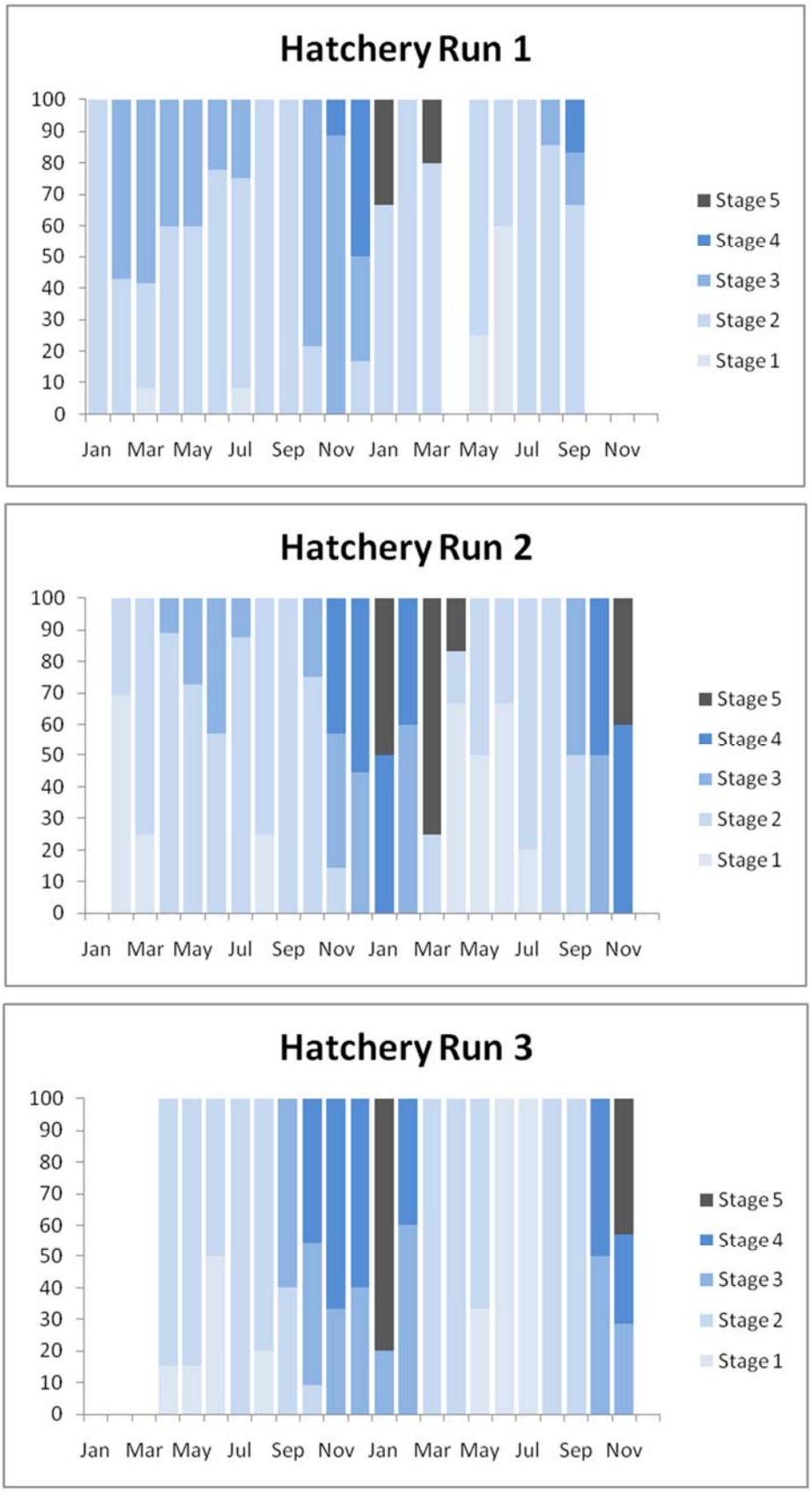


Figure 30. Proportion of male fish in hatchery runs 1, 2 and 3 assigned to reproductive stage by microscopic assessment in each month during the production cycle.

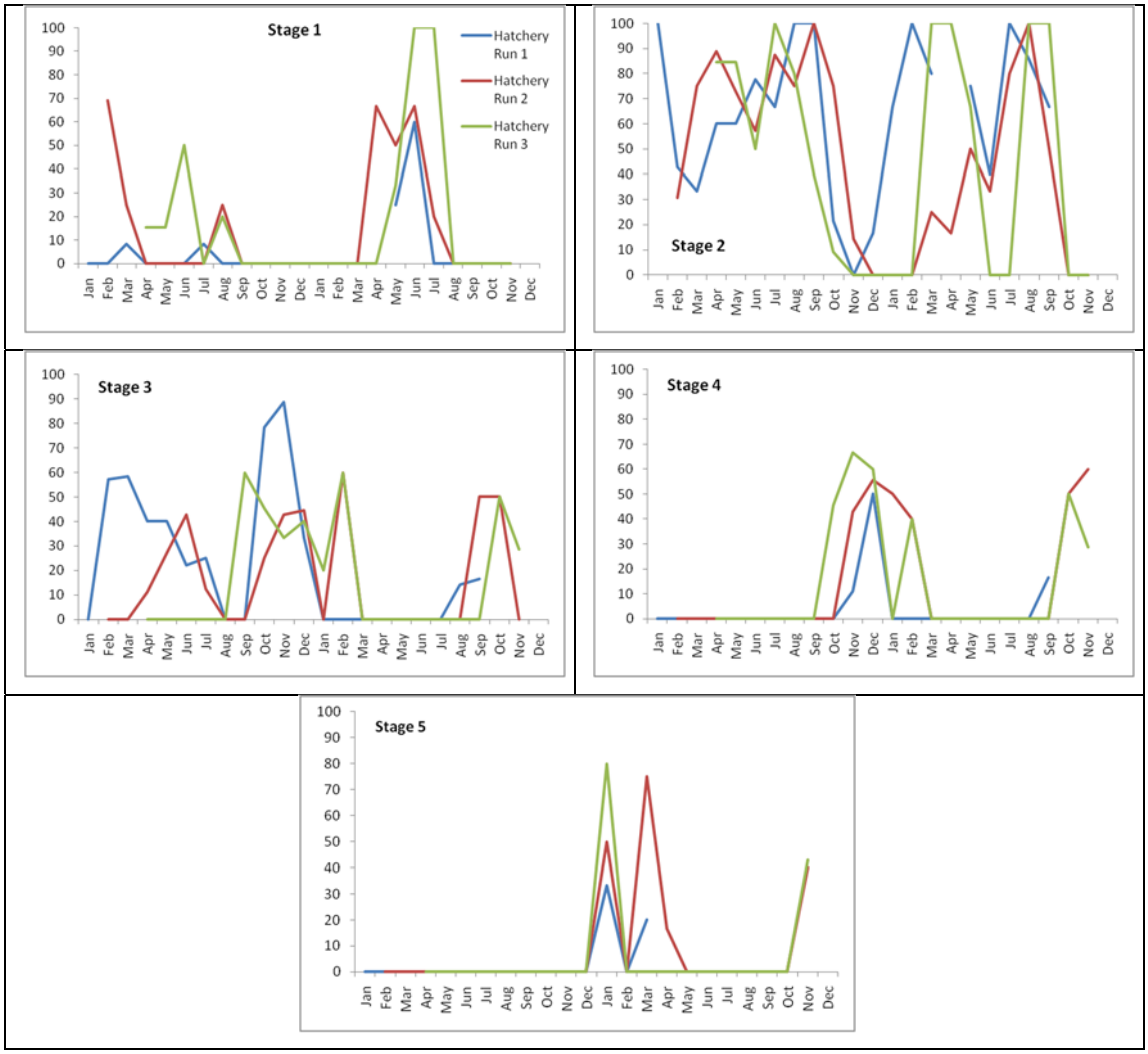


Figure 31. Proportion of male fish assigned to each reproductive stage by microscopic histological assessment in each month during the production cycle from hatchery runs 1, 2 and 3.

3.4.6 Plasma testosterone and oestradiol levels

Blood plasma (male and female fish) was assayed for testosterone levels for all fish sampled. The data for the two sexes are presented separately. Testosterone was not measureable (i.e. below the detection limit of the assay; BDL) in 50% of males. The monthly pattern shown by males that were above the detection limit of the assay is shown in Figure 32. For most of the first 18 months at sea, levels of testoerone were low (below 300pg/ml) with no obvious differences between the three hatchery runs. There was an increase in testosterone in some 0+ hatchery run 2 fish in July and August, with average values above 500pg/ml. These averages were strongly influenced by two single fish that measured 1,400 and 1,800 pg/ml respectively. These samples were re-assayed and again produced high values. There was no justification to exclude the data on the basis of any other parameter; in all other senses they were typical of their cohort. There is no suggestion that plasma

testosterone values in 0+/rising1+ males were increased in October-December in association with the presence of milt in the testes. The highest testosterone values were obtained in the last three sampling events with mean values of 1,000 to 1,500pg/ml in hatchery run 1 and 2 fish in September, October and November (when sampled). This coincides with the period of most rapid testes growth associated with re-maturation as 1+/rising 2+ males (see Figure 14). It was interesting that the 3rd hatchery run males did not show this testosterone increase, and this matched their lack of increase in GSI (see Figure 14).

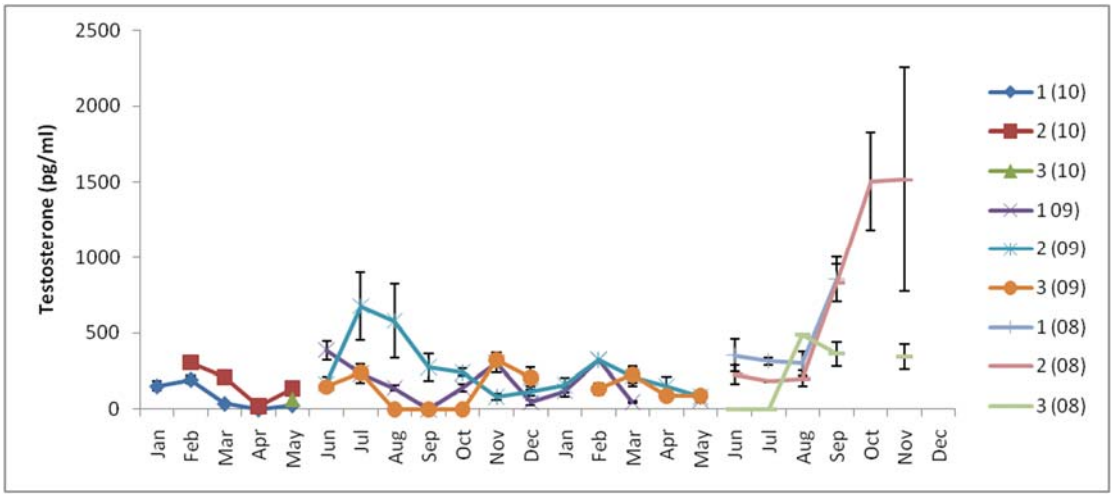


Figure 32. Mean ± SD plasma testosterone values for male YTK that were above the detection limit of the assay by month and by hatchery run during the production cycle.

The proportion of males in each hatchery run in each month classified as BDL (i.e. <300 pg/ml) are shown in Figure 33. A higher percentage of males in hatchery run 3 (60%), had testosterone values that were classified as BDL compared to run 1 (44%) and run 2 (36%).

There was a tendency for testosterone values to be higher in Stage 3 and 4 male fish (those with more copious volumes of spermatozoa present in the lumen of the tubles) compared to Stage 1 and 2 males (no spermatozoa), and Stage 5 males (spent) (Figure 34).

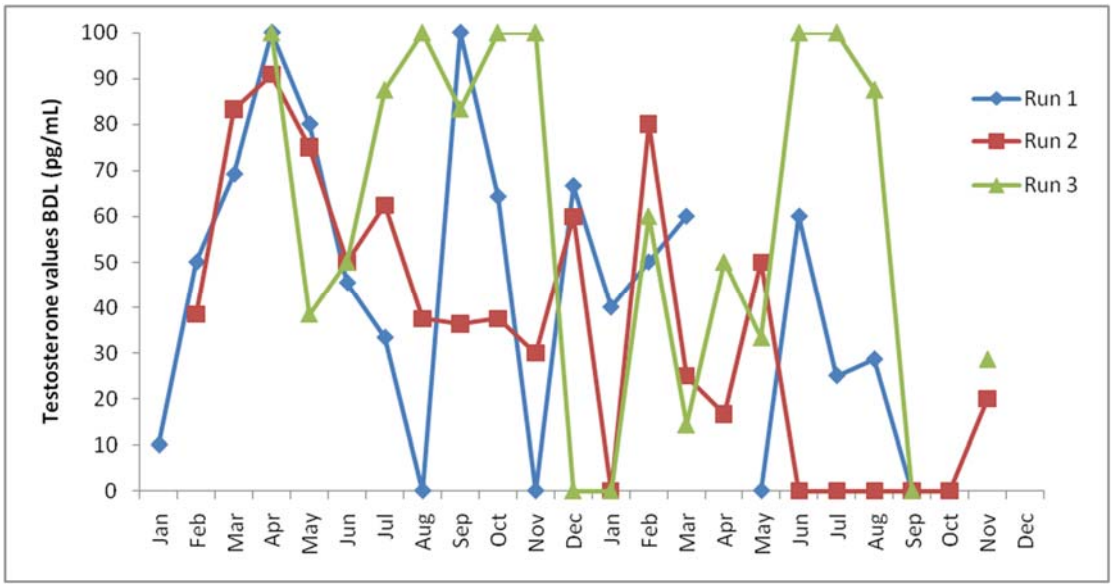


Figure 33. Percentage of plasma testosterone values for male YTK for each month and hatchery run that were below the detection limit (BDL) of the assay (<300 pg/ml).

However, despite this apparent relationship, the proportion of males with testosterone levels BDL were 48, 50, 54, 46 and 38% in stages 1 to 5 respectively, indicating that even in the most mature stage 4 males there was close to 50% of testosterone values that were BDL. This suggests that plasma testosterone value is not a good indicator of testes reproductive stage.

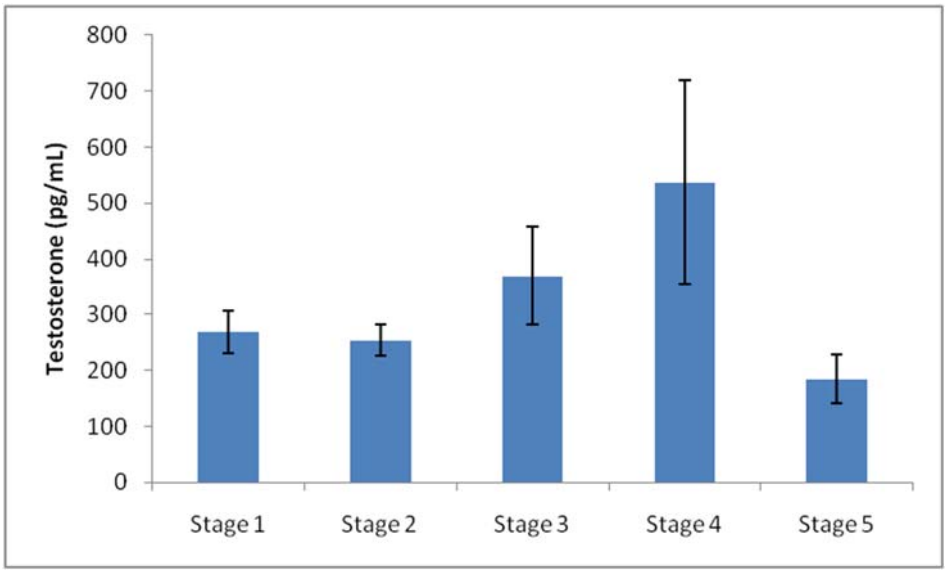


Figure 34. Mean ± SD testosterone values for male YTK above the detection limit of the assay by reproductive stage assessed by histology (n=22,112, 41, 22 and 10 for stages 1 to 5 respectively).

The highest plasma tetosterone value recorded for female fish was in a 1+ hatchery run 1 female in June at 2,900 pg/ml (also high on re-assay) (Figure 35). There was nothing else about this fish that was atypical for the group. Since there was little sign of reproductive maturation observed in female fish, analysis of female plasma testosterone level by ovary stage was not performed. The single fish that was classified through histology as a stage 2 had 350 pg/ml of testosterone, which was similar to many other fish throughout the production cycle.

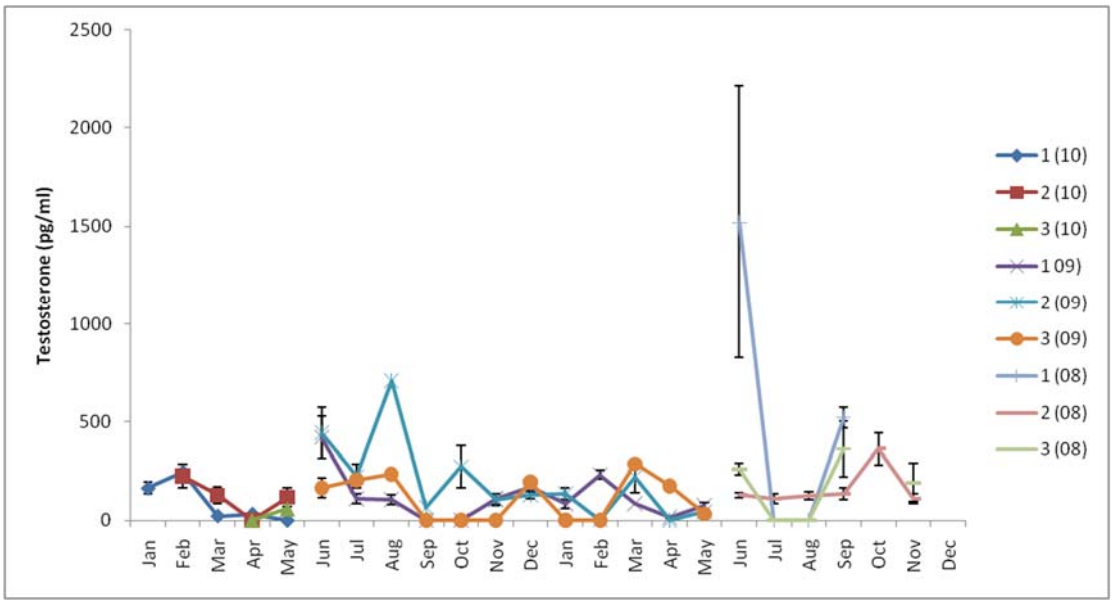


Figure 35. Mean ± SD plasma testosterone values from female YTK for samples that were above the detection limit of the assay by month and by hatchery run during the production cycle.

Similarly to the male fish, testosterone levels in a large number of the the female fish were BDL of the assay (<300 pg/ml) (Figure 36), with the occasional higher value influenced by single fish within sample groups. Compared to the males (50%) a higher percentage of female testerone values were BDL (57%) (Figure 36). Hatchery run 2 had proportionally fewer BDL values (47%) than hatchery run 1 or 3 females (both 62%) (Figure 36).

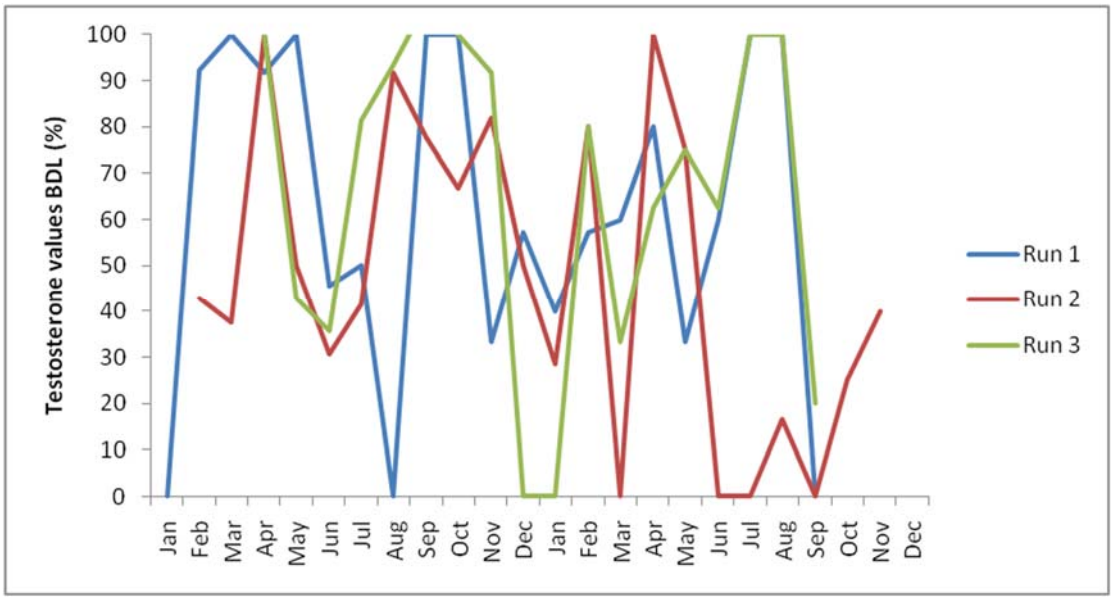


Figure 36. Percentage of plasma testosterone values for female YTK that were below the detection limit (BDL) of the assay for each month and hatchery run.

Plasma oestradiol levels in females typically increase when the fish is producing vitellogenin in the liver to be transported to the ovary where it is taken up by the larger oocytes to be deposited as yolk (Wallace and Selman, 1981). Oestradiol is mostly secreted from the follicle cells that surround a developing oocyte and it's production is usually triggered when the oocyte cells are ready to begin vitellogenesis. In the current study, most plasma oestradiol values were below 100 pg/ml (Figure 37), and, as seen in the male and female testosterone data there were several fish which had higher values that affected the monthly mean. However, there did appear to be similar trends in all hatchery runs that suggests seasonal or developmental patterns. Thus, even from the outset with the smallest and youngest females in January-March there appears to be a period of relatively elevated oestradiol levels, these decrease in the winter months only to increase again in summer-autumn, with another decrease in the following winter.

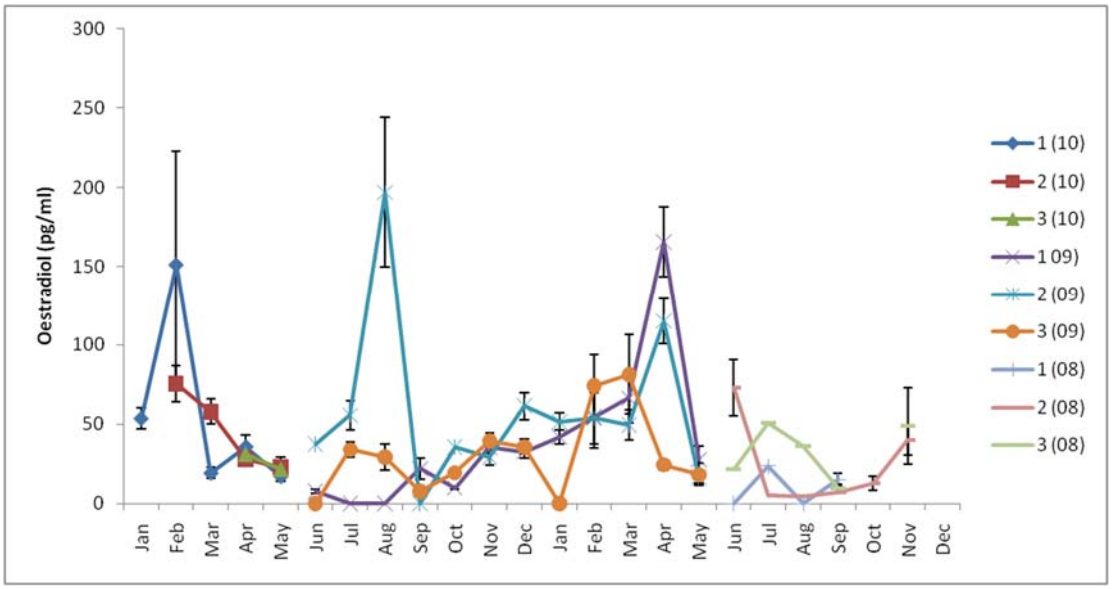


Figure 37. Mean ± SD plasma oestradiol values for female YTK that were above the detection limit of the assay for each month and by hatchery run during the production cycle.

A clear and complementary pattern was seen in the proportion of females that had oestradiol values recorded as BDL (Figure 38). When mean measureable plasma oestradiol values were low (generally winter), the proportion of fish that were assigned as BDL was high, and vice versa. This suggests a reasonable degree of synchrony between females, though because there was no corresponding changes in reproductive development it's difficult to conclude that the oestradiol was functioning in this capacity.

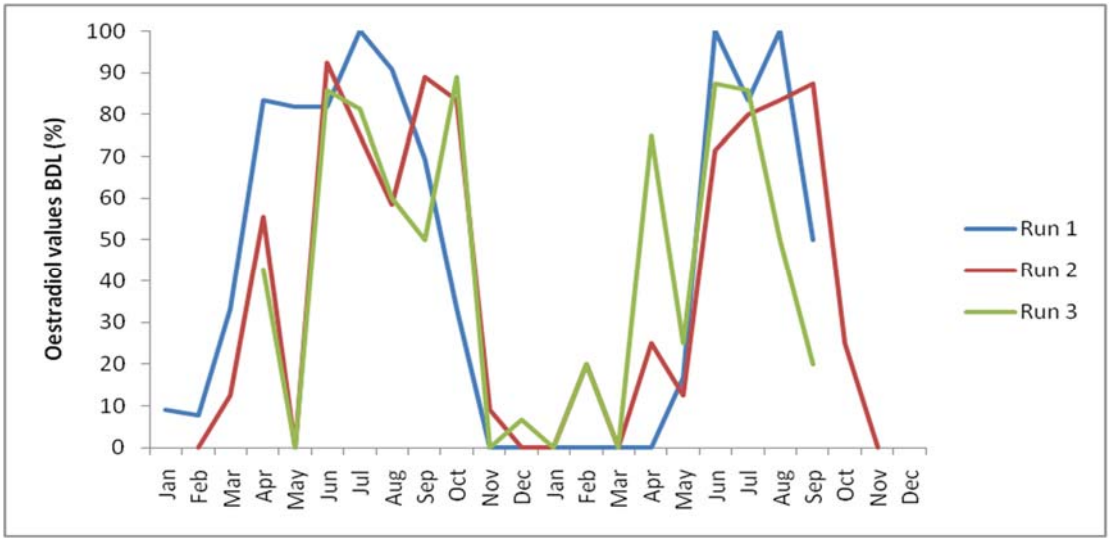


Figure 38. Percentage of plasma oestradiol values for female YTK that were below the detection limit (BDL) of the assay for each month and hatchery run.

The plasma testosterone and oestradiol levels and patterns observed in this study can be compared to those reported by Poortenaar et al. (2001). However, the fish sampled in the present study were smaller, younger and less mature than the range sampled by Poortenaar et al. (2001), and so the female data from the present study can only be compared with the stages they called F1 and F2 (the pre-vitellogenic stages) during the maturation season (October to January). The detection limit of the testosterone and oestradiol assays in Poortenaar et al. (2001) were 300 pg/ml, somewhat higher than those used in the present study (testosterone 110 pg/ml; estradiol 25 pg/ml). The mean testosterone and oestradiol levels in the F1 and F2 stages in the study by Poortenaar et al. (2001) were low (testosterone ~300 pg/ml and oestradiol ~500 pg/ml), similar to the concentrations measured in the present study, except that our testosterone levels tended to be higher than oestradiol values. It is not specifically mentioned whether Poortenaar et al. (2001) had some F1 and F2 samples that assayed below the detection limit, but with the mean values around 300 to 500 pg/ml it would be likely that some fish were BDL. How different researchers deal with samples that are BDL in the data analysis can vary. Thus, rather than put zero values in the dataset, which makes statistical analysis difficult, some researchers use a value that is half of the detection limit of the assay. These differences may help to explain some of the discrepencies between studies.

The main point we can draw from a comparison of the two studies is that in female fish both testosterone and oestradiol concentrations were significantly higher in F3 stage fish – those that were vitellogenic. Their maximum mean values were 6,000 pg/ml for testosterone and 3,500 pg/ml for oestradiol, much higher than any individual values measured in the present study. This is further evidence to support the claim that the female fish sampled in the present study were not vitellogenic, and therefore female fish grown to this size/weight (<5kg) and age (rising 2+) have no problems with undesirable reproductive maturation.

The male fish in both studies can be directly compared because all reproductive stages were found in the population and categorisation of reproductive stages were the same. Stage M1 fish in the study by Poortenaar et al. (2001) had mean testosterone levels around 300 pg/ml, M2 fish around 500 pg/ml, M3 fish (some milt present in the sectioned testes) were significantly higher (2,500 pg/ml), and stage M4 (copious runny milt) was higher again at 3,000 pg/ml. Spent fish (M5) had a much lower value of 500 pg/ml. The general pattern seen in the two studies is similar, but peak values measured in our study were lower. This could again be due to any of the

several differences between the studies such as in the time/months when samples were taken, the size/age of the fish and the effect of this on their GSI, and/or the way we each dealt with values that were BDL.

3.4.7 Insulin-like Growth Factor (IGF-I)

Plasma levels of IGF-I were above the detection limit in all fish sampled in all months. The variability in range of IGF-I values between fish (as shown by the small SEM bars) was less than was observed for the sex steroids (Figure 39). 0+ and early 1+ fish had mean values that varied between 20 and 120 ng/ml, and whilst there doesn't appear to be any clear seasonal trends there does appear to be a possible degree of consistency between the three hatchery runs from one month to another (Figure 39). Specifically, the 2009 year class hatchery run 1 and 2 lines parallel each other very closely from June to May, and hatchery run 3 often shows a similar monthly increase/decrease. There was no difference in IGF-I values between males and females, nor between different reproductive stages in males (data not shown). The higher IGF-I values were measured in the 2008 year class fish from June to August (maximum mean values of 100-150 ng/ml) and though there was not any close synchrony in the pattern between hatchery runs, there was a trend that the high values all decreased to 40-60 ng/ml by September. It is interesting that the maximal IGF-I values occurred in such different months/seasons in the different cohorts (February and June-August; acknowledging that the sampling design only captured the whole year for the 2009 cohort, and 2 to 6 months from the other cohorts).

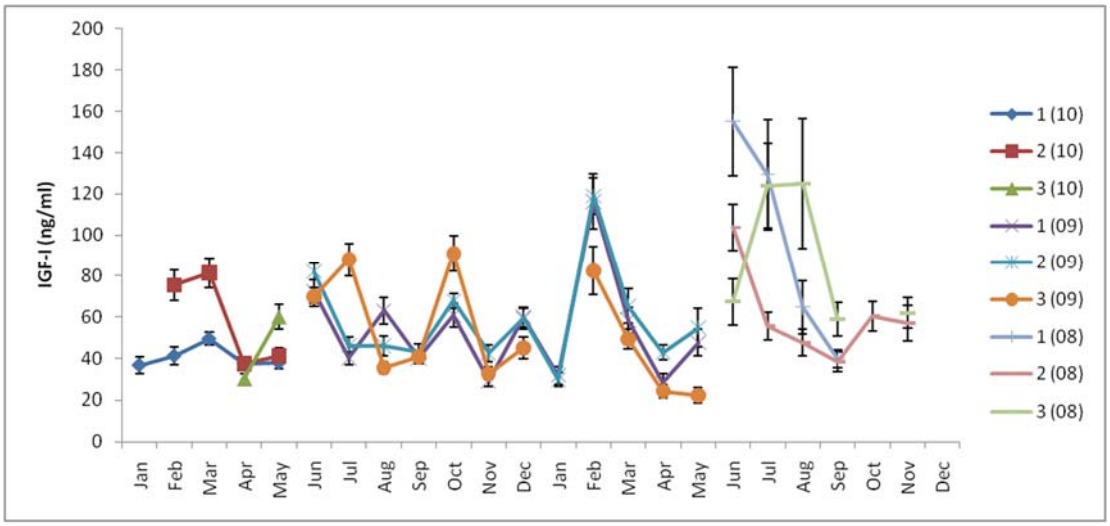


Figure 39. Mean ± SD plasma insulin-like growth factor-I (IGF-I) values for fish (males and female data combined) sampled in each month and by hatchery run during the production cycle.

IGF-I has previously been shown to be associated with the 'instantaneous' growth rate of fish (Dyer et al., 2004b; Cooper et al., 2004). Due to the sampling strategy used we do not have growth rate data on individual fish in the current study, so we can only estimate growth rate based on the mean percentage change in body weight of each hatchery run between months (where available). There was no relationship between mean plasma IGF-I value and the estimated growth rate of that cohort of fish in the previous month. Indeed, higher IGF-I values were associated with groups of fish that showed positive and negative monthly growth (Figure 40). It may be that the IGF-I is involved in two (or more) different functions (eg stimulating growth in February, and stimulating lipolysis – metabolism of body fat reserves – in June to August). To see if there was any evidence of this the mean plasma IGF-I level was plotted against both the mean visceral fat index (% of bodyweight), and the change in mean visceral fat index between months where the data were available (Figures 41 and 42). There were no clear relationships between IGF-I levels and visceral fat index or change in visceral fat index, suggesting that this is not the case.

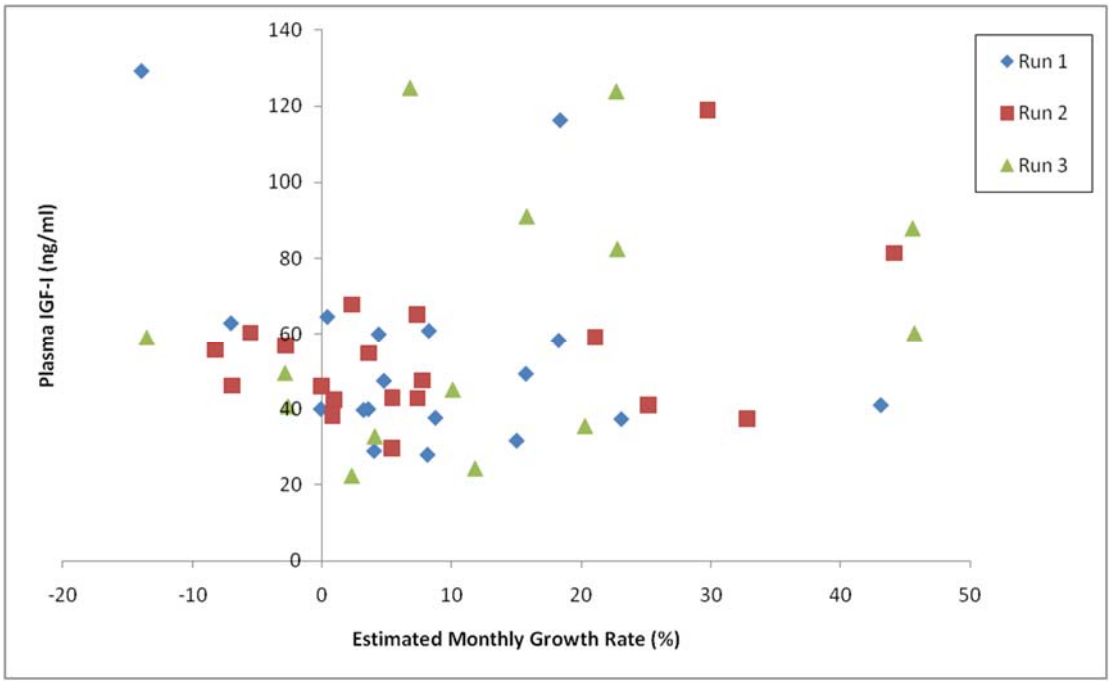


Figure 40. Mean plasma IGF-I plotted against the estimated previous monthly growth rate (% of bodyweight), for each hatchery run (male and female data combined),

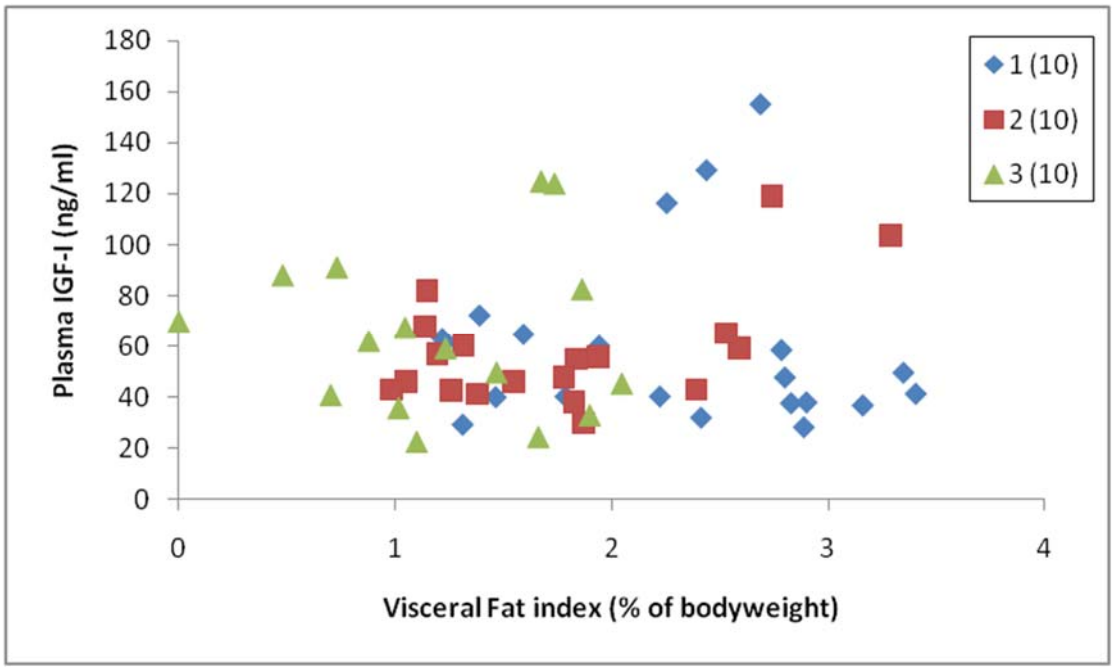


Figure 41. Mean plasma IGF-I plotted against mean visceral fat index (% of bodyweight), for each hatchery run (both sexes combined),

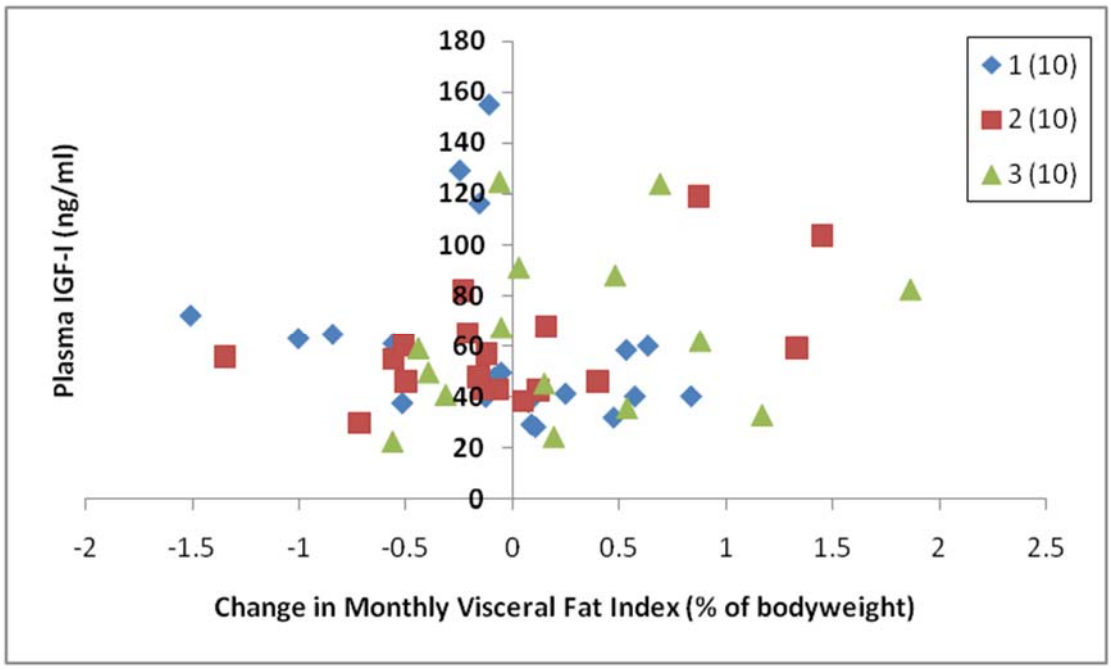


Figure 42. Mean plasma IGF-I plotted against change in mean visceral fat index (% of bodyweight) from the previous month, for each hatchery run (both sexes combined),

3.5 Differences in somatic characteristics between male and female YTK

One of the most common questions asked when one sex matures at a particular age/size and the the other one does not, is whether there is any impact (usually negative) of maturation on the quality or quantity of product/yield. The somatic data from each hatchery run sampled in this study have been analysed on the basis of fish sex, only the data from hatchery run 1 are shown as they are typical of runs 2 and 3. There was no indication that there was any difference in fish size (fork length; Figure 43) (body weight; Figure 44), condition factor (Figure 45) or fillet yield (Figure 46) between the male and female fish sampled in this study (up to age rising 2+ and 4-5kg body weight).

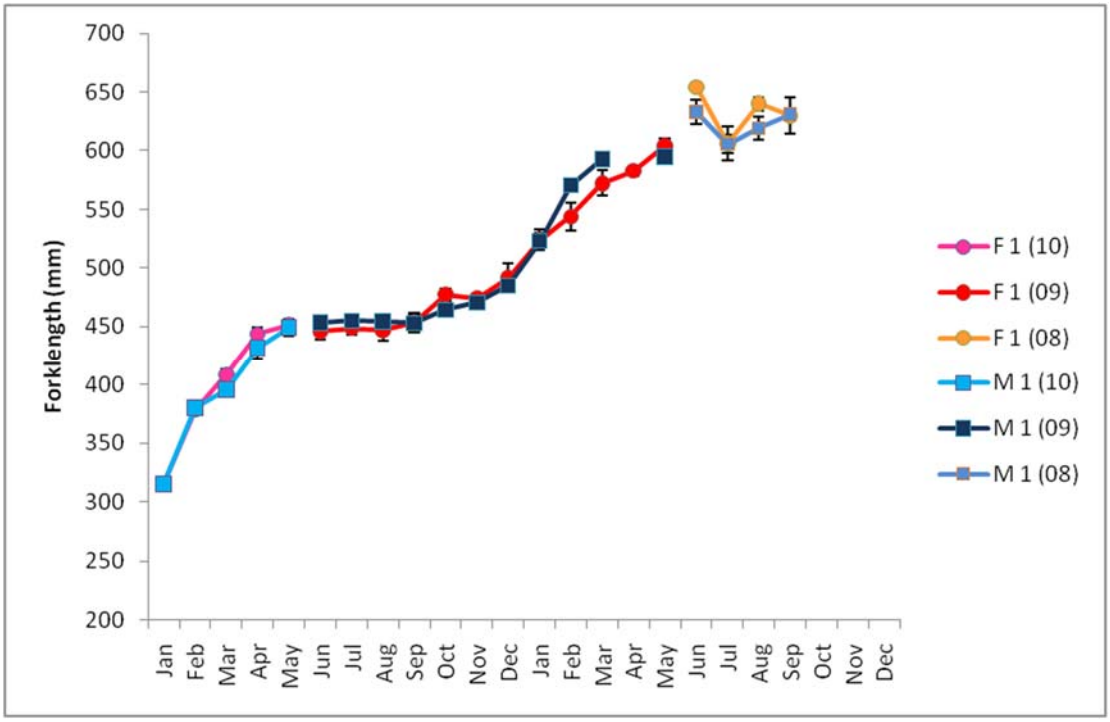


Figure 43. Fork length (mm, mean ± sem) of male and female YTK from hatchery run 1 fish throughout the production cycle. The legend indicates F (female) or M (male) fish in the three year classes of fish sampled in the study (2008, 2009, and 2010 at-sea cohorts).

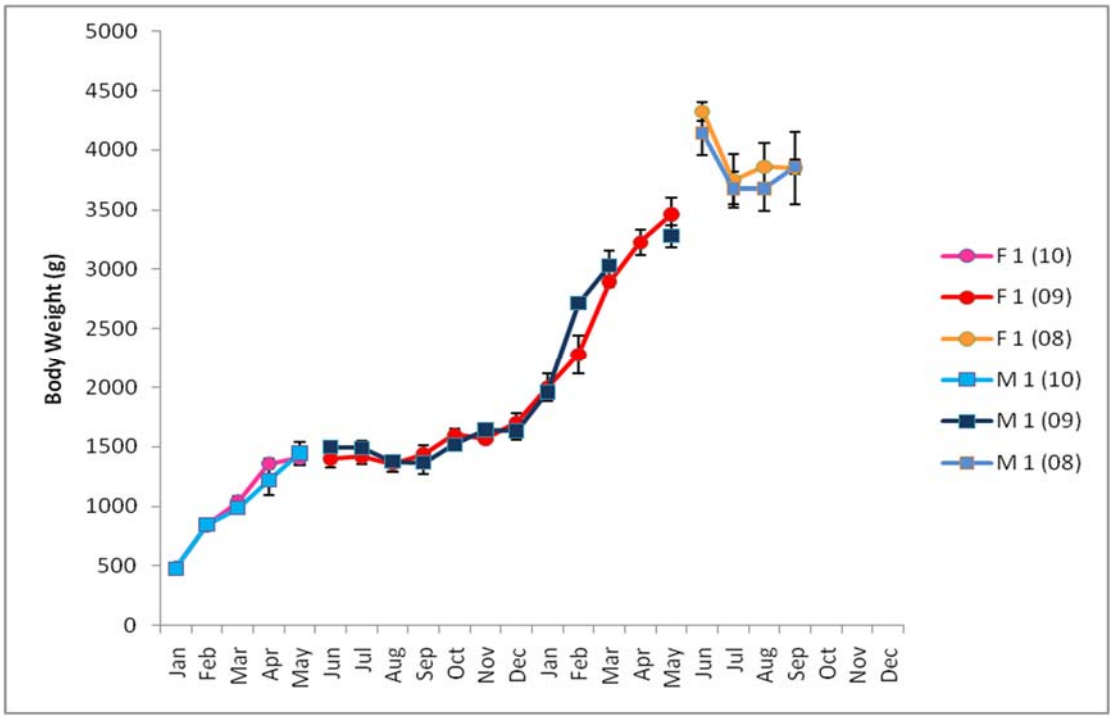


Figure 44. Body weight (g, mean ± sem) of male and female YTK from hatchery run 1 fish throughout the production cycle. The legend indicates F (female) or M (male) fish in the three year classes of fish sampled in the study (2008, 2009, and 2010 at-sea cohorts).

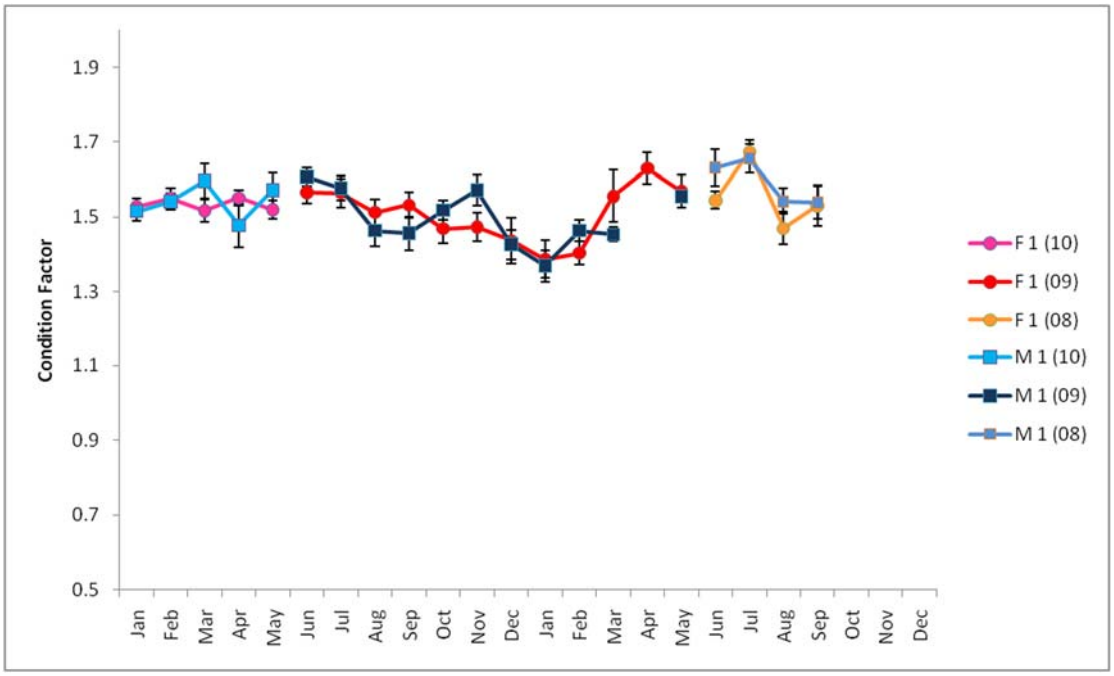


Figure 45. Condition factor (mean ± sem) of male and female YTK from hatchery run 1 fish throughout the production cycle. The legend indicates F (female) or M (male) fish in the three year classes of fish sampled in the study (2008, 2009, and 2010 at-sea cohorts).

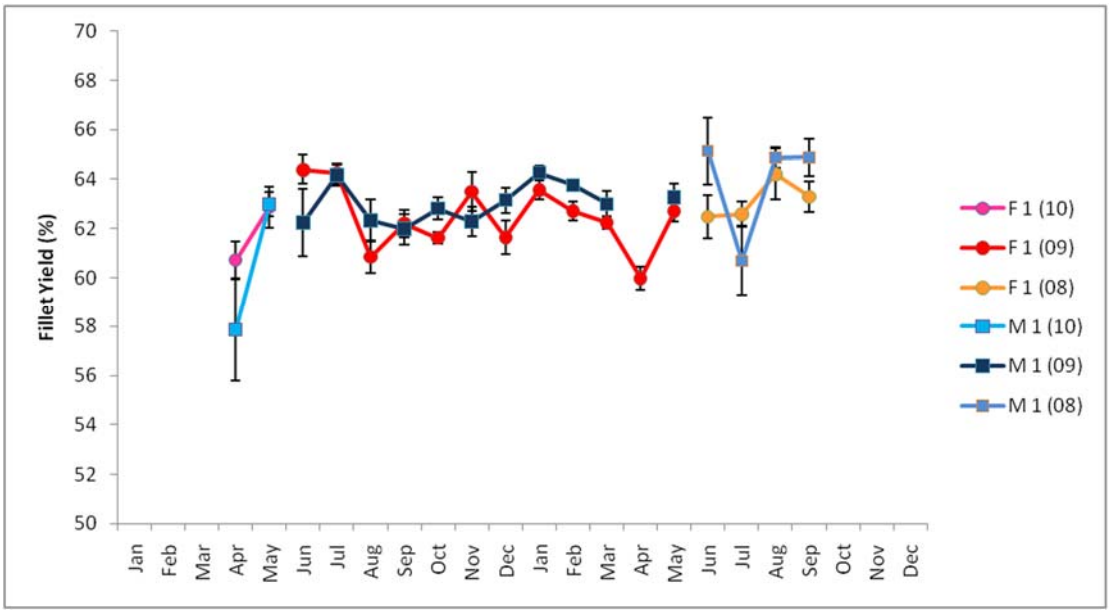


Figure 46. Fillet yield (% of body weight, mean ± sem) of male and female YTK from hatchery run 1 fish throughout the production cycle. The legend indicates F (female) or M (male) fish in the three year classes of fish sampled in the study (2008, 2009, and 2010 at-sea cohorts).

The data suggest that as long as production is aimed to harvest YTK as or before they reach 4-5kg (as rising 2+ fish) there is no evidence that product quantity or yield will be affected by the sex of the fish. Due to the small numbers of rising 2+ fish sampled (n=10 per hatchery run) in the monthly sampling study, additional sampling of commercially harvested YTK was carried out in November and December in the processing factory (see section 3.6).

3.6 Processing factory sampling of commercially harvested rising 2+ fish

A total of 108 rising 2+ YTK (51 females and 57 males) were selected to be filleted from the commercial harvest as the fish were being washed, packed and boxed for distribution to wholesalers and customers in a processing facility. The fish used for filleting were selected by the processing staff and whilst not intentionally biased towards a particular size criterion, they would not have selected any fish that had a low condition factor. The fish were measured (fork length and body weight), and fillet weight (Japanese cut) recorded. The gonad was weighed and sex and macroscopic stage determined. There were no significant differences in fork length, body weight, condition factor or GSI between the sexes (Table 8); however, male fish tended to be lighter than the females ($P=0.07$). However, fillet yield was significantly different between the sexes, with males having ~1% greater yield than females ($P<0.01$).

Unfortunately, due to the requirement to minimise interference with commercial processing practice the viscera was not removed and weighed, so it's not possible to determine whether the non-significant tendency for female fish to be heavier and have a higher condition factor, but yet have a lower fillet yield, was due to them having a slightly larger visceral mass.

Table 8. Somatic and reproductive characteristics of rising 2+ female (n=51) and male (n=57) Yellowtail Kingfish. Statistical analysis was a Students T-Test (2-tailed) using the Data Analysis Tool Pack in Excel 2007.

	Female	**Male**	**p value**
Fork length (mm)	640 ± 3	634 ± 3	0.13
Body weight (g)	3,727 ± 57	3,577 ± 60	0.07
Condition factor	1.42 ± 0.02	1.40 ± 0.02	0.56
Fillet yield	65.9 ± 0.3	66.9 ± 0.3**	0.01
GSI (%)	0.45 ± 0.01	0.47 ± 0.06	0.61

These results suggest that sexual maturation (all of the males were assessed as stage 3 or 4, all females as Stage 2 – consistent with the findings of the monthly sampling study) of males does not negatively impact on fish growth or product quantity. Indeed, the male fish have a 1% higher fillet yield ($P<0.01$). Interestingly, the same tentative conclusion was drawn from the very limited sampling of rising 2+ YTK in the study by Carragher et al. (2009).

4. Benefits and Adoption

This work has determined that reproductive maturation of farmed YTK harvested at weights up to 5kg and at age rising 2+ has negligible impact on production parameters or product yield and quality characteristics. This information has two benefits. Firstly, farmers of YTK can now dismiss the suggestion that reproductive maturation of YTK at this age and size is something that needs to be mitigated by techniques such as all female stock, sterile stock or environmental manipulation. Secondly, producers of YTK can more readily anticipate and address comments in regards to product quality and yield that might come from the marketplace in October-December as males develop obvious testes. Both benefits should increase the production certainty and market acceptability of this premium product.

5. Further Development

The findings of this study indicate that there is a very limited effect of reproductive maturation on product yield or quality of farmed YTK harvested at up to 5kg and an age of 2+. Therefore, no further work needs to be carried out on this topic. However, if the market demand for YTK changes, such that larger and/or older fish have to be factored into the production cycle, it is likely that additional work will be required. It is likely that the impacts of reproductive maturation on the yield and product quality of ~7kg and rising 3+ farmed YTK would be significantly greater than observed for the rising 2+ fish in the present study.

6. Planned Outcomes

The planned outcomes of this project were:

1. The extent of maturation and its impact upon product quality will be determined – this will help identify the best approaches to future mitigation strategies.
2. The hormonal cues controlling maturation will be determined, information which will be essential in developing an appropriate management strategy if maturation is of economic importance.

In regard to planned outcome one the extent of maturation and its impact on product yield and quality have been determined. The limited impact of male reproductive maturation (up to age rising 2+ and 3.5-5 kg body weight) means that no mitigation strategy needs to be considered.

In regard to planned outcome two hormone (plasma testosterone) data was not particularly well correlated to what was happening in the testes of 0+ males. From histological assessment of the testes it appears as though many 0+ males had already initiated the process of spermatogenesis around the time they were introduced to sea cages at 4-5 months of age. Whether this is driven by changes in water temperature and/or light regime from the hatchery to the open water is not known, but controlling it by light manipulation at sea would be difficult logistically, and not worthwhile unless there was also benefit for fish growth or survival.

Re-maturation of the males as 1+/rising 2+ fish appeared to occur later than in the previous year, with spermatogenesis not getting underway until August-September. This may be triggered by increasing day-length from the winter minimum. Again, any

attempt to influence this by controlling light regime during winter would be logistically difficult, and again, unless there was associated benefit from improved growth rate or survival, the expense of doing this to perhaps delay male maturation would not be worthwhile. There was a better correlation between plasma testosterone and testes development in 1+/rising 2+ males.

7. Conclusion

From the extensive and comprehensive sampling of YTK from stocking at sea at 4-5 months of age through to a size of 4-5kg as rising 2+ fish it can be concluded that:

- 100% of male fish undergo spermatogenesis as 0+ and 1+ fish
- the extent of sexual maturation (GSI, milt volume) as 0+ and 1+ fish is limited (maximum mean GSI of 1.2%)
- female fish do not show any signs of vitellogenesis as 0+ or 1+ fish
- there is no suggestion that the extent of sexual maturation differs between hatchery runs 1, 2 and 3
- there is no evidence that the sex ratio of different hatchery runs differs significantly from 50:50
- there is no suggestion that the sex of the fish affects growth rate or condition factor
- there are some data to suggest that rising 2+ male fish have a slightly (~1%) higher fillet yield than females at this time
- these findings indicate that the production performance implications of sexual maturation on YTK to 4-5kg and age rising 2+ are minimal, and intervention is not warranted
- the hormonal control of sexual reproductive development in 0+ males is not highly synchronised, environmental cues and/or endogenous mechanisms triggering the onset of reproductive development are acting very early, certainly by an age of 4-5 months. This coincides with summer (January-March), so there is little opportunity to manipulate environmental conditions to prevent maturation
- the re-maturation process of 1+ males is more synchronised, with spermatogenesis starting in August-September. It may be that this is due to the increasing daylength at this time. Experimental manipulation of daylength, by lighting cages during winter months, may disrupt the re-maturation process but may not be very effective as the fish may override the lack of environmental cues by an endogenous annual clock (having matured 12 months previous). The

logistical difficulties and expense of illuminating pontoons through two winters would be significant

- the current production cycle for YTK that is geared to harvest 4-5kg fish at an age of rising 2+, minimises the impacts of sexual maturation on production performance and product yield. As long as this remains the focus for the industry no steps are needed to affect sex ratios, sterility or manipulate environmental factors.

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Appendix 1 – Intellectual property

There was no intellectual property arising from this project.

Appendix 2 – Staff

John Carragher – SARDI, Innovative Foods

Mark Thomas – Flinders University, Lincoln Marine Science Centre

Ryan Wilkinson – University of Tasmania, Launceston

Flinders University

Trent D'Antignana

Claudio Giordano

Maria Jedensjö

Erin Bubner

Clean Seas

Mike Thomson

Jo Cuira

Allan Mooney

Maria Jedensjö

The Fish Health, Feeding and Farm Management teams on vessels at the Whyalla, Arno Bay and Port Lincoln growout sites

Processors

Southern Waters Marine Products

Australian Hiramasa

Understanding Yellowtail Kingfish: Sub-project 3

Appendix C

Mark Booth, Geoff Allan, Ian Russell, Mitchell Elkins and Jenna Bowyer

Project No. 2008/903.30

AUSTRALIAN SEAFOOD COOPERATIVE RESEARCH CENTRE

This project was conducted by New South Wales Department of Primary Industries, Port Stephens Fisheries Institute

Title: Understanding Yellowtail Kingfish: Sub-project 3

Authors: M.A. Booth[1], G.L. Allan[1], I. Russell[1], M. Elkins & J. Bowyer[2]

Authors Affiliations: [1] NSW Department of Primary Industries Port Stephens Fisheries Institute Locked Bag 1, Nelson Bay 2315, NSW, [2] Flinders University, School of Biological Sciences, GPO Box 2100, Adelaide 5001, South Australia

The Australian Seafood CRC is established and supported under the Australian Government's Cooperative Research Centres Programme. Other investors in the CRC are the Fisheries Research and Development Corporation, Seafood CRC company members, and supporting participants.

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Non-Technical Summary

2008/903.30 – Understanding Yellowtail Kingfish: Sub-project 3

PRINCIPAL INVESTIGATOR: Dr Mark Booth

ADDRESS: NSW Department of Primary Industries
Port Stephens Fisheries Institute
Locked Bag 1, Nelson Bay NSW 2315

OBJECTIVE:

1. Determine and quantify the interactive effects of fish size and water temperature on survival, weight gain, feed intake and feed conversion ratio of juvenile YTK.
2. Determine and quantify the interactive effects of water temperature and hypoxia on survival, weight gain, feed intake and feed conversion ratio of juvenile YTK fed a single high performing commercial diet
3. Develop a temperature dependent growth model for juvenile YTK.

NON TECHNICAL SUMMARY:

Two manipulative experiments were carried out with juvenile Yellowtail Kingfish (*Seriola lalandi*) (YTK) to provide Clean Seas Tuna (CST) with new information that will support development of an "ideal production strategy' for their YTK operation in South Australia. The first experiment in this study was designed to investigate the implications of stocking juvenile fish into suboptimal water temperatures (i.e. 12 and 15°C). These suboptimal temperatures are typical of the long run water temperatures recorded in September / October in the Spencer Gulf and are generally coincident with the Spring stocking of juvenile YTK into sea-cages. The results of experiment 1 demonstrated that even following an acclimation rate of approximately 1°C day^{-1} it is not advisable to rear small (6g) or large (44g) juvenile YTK at 12°C. Fish reared at 12°C became moribund, lost equilibrium, had difficulty feeding and died. Small fish reared at 12°C were removed from the study according to NSW DPI Fisheries animal care and ethics requirements' with only 25-65% of small individuals surviving to day 28. It is highly likely that the majority of small fish would have succumbed to the 12°C regime well before the end of the experiment. Longer term survival and rate of mortality of the 44g animals was better than that of smaller juveniles at 12°C, but only 45-60% of larger individuals survived to day 64. In contrast, survival of 6g and 44g fish at 15°C was extremely high (≈ 99%) and growth rate and apparent feed conversion were acceptable. Based on this work we do not recommend stocking small juveniles between 5-40g into sea-cages when water temperatures are lower than 14-15°C.

The second experiment investigated the interactive effects of higher water temperatures (21, 24, or 27°C) and dissolved oxygen regime, to examine the impacts of normoxia and hypoxia on feed intake, growth and feed conversion. The results indicated that YTK are reasonably tolerant of hypoxic conditions typically experienced at high stocking densities and feed rates. However, subjecting juvenile fish to the hypoxic conditions dramatically reduced growth potential by 13%, 20% and 17% at 21°, 24°C or 27°C, respectively compared to fish reared under normoxic conditions. This study also confirmed that water temperatures between 22-24°C promote optimal growth in juvenile YTK.

Growth and temperature data from both experiments was combined with similar data from previous research on YTK to develop a temperature dependent growth model for fish < 1000g at water temperatures between 10°C and 29°C. Growth rate of juvenile YTK (from 50g to 100g) is optimal at water temperatures between 23-24°C. YTK grown in water temperatures below 17^{o}C, or above 28^{o}C, will achieve less than 50% of the growth rate of fish reared at 23-24^{o}C. Information on size at stocking and the effects of water temperature and hypoxia on performance of juvenile YTK will improve management decisions surrounding production of this species. In addition, growth and performance can now be benchmarked against a temperature dependent model developed specifically for this species and reared under South Australian conditions.

KEYWORDS: Yellowtail Kingfish, water temperature, stocking size, dissolved oxygen, growth, survival

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1. Introduction

Yellowtail Kingfish (*Seriola lalandi*) (YTK) are currently grown in sea cage operations in NSW, South Australia and Western Australia. Production of YTK in NSW is negligible, however, annual production in South Australia is reported to range between 1250–2250 tonnes (Miegel et al., 2010). Production in Western Australia is restricted to pilot scale enterprises. Aquaculture of YTK in South Australia occurs in the Spencer Gulf predominantly at Port Lincoln and Arno Bay. These sites experience a wide range of seasonally or diurnally affected water temperatures. For example, at Port Lincoln water temperature ranges from 14-24˚C while at Arno Bay water temperature can range between 13-23˚C (Bureau of Meteorology (BOM) Sea Surface Temperatures – August 2005 - August 2007).

Temperature is one of the most important extrinsic factors influencing metabolic rate in ectotherms, directly governing the speed at which biochemical and physiological processes proceed (Clarke & Johnston, 1999) as well as having a direct influence on activity (Jobling, 1982). The relationship between temperature and metabolic rate is strongly linked to the temperature dependence of enzymatic reactions (Hochachka & Somero, 2002). Optimal or preferred growing temperature for YTK is not well documented but temperatures below 18˚C and above 28˚C are known to cause reductions in feed intake (Kohbara et al., 2003). Nakada et al. (2002) categorised growth of *Seriola spp.* according to the range of water temperatures experienced by grow-out facilities in Japan. These authors classified high growth areas as regions where the annual mean water temperature is around 20-24˚C, mid range growth areas as regions where the annual mean temperatures are 17-19˚C and low growth areas as regions which experience water temperatures <17˚C. According to their review, fish experienced optimum growing temperature in the high, mid and low temperature regions for approximately 75, 60 and 50% of the year, respectively. Juvenile *S. quinqueradiata* (stock weight < 200g) stocked into high temperature regions can expect to reach 6kg after about 2 years, however juveniles stocked into mid and low temperature regions will normally take 3 and >3 years to reach harvest weight, respectively (Nakada, 2002). Recent investigations on the effects of water temperature (10, 15, 20, 25, 30 and 32.5˚C) on the fasting routine metabolic rate (RMR) of juvenile YTK (Pirozzi & Booth, 2009) indicated that RMR was least thermally sensitive at approximately 22.7˚C. This evidence and that from the

Japanese experience suggests that growth of farmed YTK at South Australian grow-out sites will only approach an optimal level for short periods of time each year.

Current industry practice in South Australia is to stock 5-10g YTK fingerlings into sea-cages in September / October to coincide with rising seawater temperatures. Fish are then on-grown to a target harvest weight of approximately 4-6kg over a period of 24 months. This grow-out period encompasses two winters, during which the growth of YTK slows considerably. As a consequence feed intake falls and feed conversion ratio (FCR) typically worsens. Extremely low water temperatures may also affect the nutritional and immune status of YTK and as a result farms often experience increased rates of mortality during winter months, especially in juvenile stock, which have failed to significantly increase their condition during the previous spring / summer.

Industry has identified several strategies that may reduce the overall production costs of YTK farming through increased survival of stock. One strategy is the perceived benefit of stocking larger rather than smaller juvenile YTK into sea-cages at the start of each production cycle when water temperature is still below optimum levels. Stocking larger, better conditioned fingerlings from hatcheries may reduce mortality rates. In addition, stocking YTK at larger sizes may significantly truncate the grow-out period, avoiding the need for farms to carry stock through a second winter. Use of larger juveniles may also allow farm managers to stock YTK at different times of the year in order to take advantage of environmental or market conditions. A strategy such as this is closely linked to brood-stock management, feed management and a better understanding of the growth potential of juvenile and sub-adult at optimal and sub-optimal water temperatures.

Clean Seas Tuna (CST) record high levels of juvenile mortality in small (5-10g) newly stocked YTK reared in sea-cages. This acute mortality may be related to the combined effects of high water temperature and low dissolved oxygen concentrations (hypoxia) inside sea-cages, especially after heavy feeding. Hypoxia is best defined as any level of dissolved oxygen low enough to negatively impact the behaviour and (or) physiology of an organism (Pollock et al., 2007). All fish have some ability to cope with fluctuations in dissolved oxygen concentration and during mild hypoxia are generally able to maintain a constant metabolic rate by increasing respiratory volume. But, below a critical dissolved oxygen level most fish are unable to maintain metabolic rate and oxygen consumption declines linearly with dissolved oxygen level.

If the hypoxic condition persists, the fish will eventually die (Fitzgibbon et al., 2007). This response is more critical for fish at higher water temperatures and higher salinities due to the low solubility of oxygen under these conditions (Barnes et al., 2011).

It is known YTK have a high routine metabolic rate compared to more sedentary species such as mulloway and that their metabolic rate and thus demand for oxygen increases dramatically in response to feeding, feeding activity and increases in water temperature (Clark & Seymour, 2006; Pirozzi & Booth, 2009). Although dissolved oxygen within the sea-cage environment may be at normoxic levels for fish during routine activity, the increased oxygen demand associated with feeding and feeding activity may, depending on stocking densities, also induce periods of chronic oxygen debt. Chronic hypoxia within the sea-cage environment might also result from poor water exchange due to bio-fouling, dodge tides and overstocking. The long term effects of chronic hypoxia, regardless of the cause will likely manifest in reduced voluntary feed intake, reduced growth potential, increased susceptibility to disease and infection and reduced survival (Kestemont & Baras, 2001; Fitzgibbon et al., 2007).

This report presents the results of two manipulative experiments which were designed to improve our understanding of the effects of stocking size, water temperature and oxygen concentration on the growth and survival of juvenile YTK. The specific aims of each experiment were to:

1) determine the effects of fish size and water temperature on survival, weight gain, feed intake and feed conversion ratio of juvenile YTK fed a number of commercial diets.

2) determine the effects of water temperature and hypoxia on survival, weight gain, feed intake and feed conversion ratio of juvenile YTK fed a single high performing commercial diet.

In addition to the aforementioned aims, growth data from both experiments as well as data on the growth of juvenile YTK recorded in previous Aquafin CRC research was combined to develop a temperature dependent growth model for juvenile YTK.

1.1 Need

Through existing farming practices it is apparent that there are several factors that may be limiting production of YTK and ultimately revenue. Firstly, there is a need to identify an "ideal production strategy" to achieve forecast production levels. As temperature and oxygen significantly affect YTK growth, feed conversion ratios and health, it is essential that the relationship between growth, temperature and oxygen concentration is investigated in fish of various sizes. This information will enable CST to make informed decisions on size and time of stocking YTK into sea-cages and improve management decisions when the threat of hypoxia is expected. It is anticipated this information will improve production efficiencies by reducing the mortality of juvenile or mature stock and ultimately by shortening the production cycle. Together these outcomes will reduce the standing on-farm biomass of undersized stock, ultimately increasing farm profitability.

1.2 Objectives

1. Determine the effects of fish size and water temperature on survival, weight gain, feed intake and feed conversion ratio of juvenile YTK.
2. Determine the effects of water temperature and hypoxia on survival, weight gain, feed intake and feed conversion ratio of juvenile YTK fed a single high performing commercial diet.
3. Develop a temperature dependent growth model for juvenile YTK.

2. Methods

2.1 Experiment one: effect of water temperature and stocking size on survival and growth of juvenile YTK

2.1.1 Fish stocks

Two separate cohorts of juvenile YTK were produced for this experiment from progeny of wild captured YTK brood-stock held at PSFI. Brood-fish were reared on natural foods (fresh frozen pilchards and squid) and were induced to spawn using temperature and photoperiod cues; spawning was timed in order that the planned experiment could be started with fish weighing approximately 5 and 40g, respectively. Fertilised eggs were obtained from a single 20kL spawning tank holding 12 brood-fish, however, the sex ratio of brood-fish is unknown. Viable eggs and larvae were then reared according to protocols described by Fielder and Heasman

(2010). Larval fish were weaned onto a specialised Japanese micro diet (Otohime) and were transferred to 10kL nursery tanks (PSFI greenhouse) when they reached approximately 0.5-0.8g in body weight. Both weight cohorts were held in separate nursery tanks and fed a range of commercial diets until they were required for the experiment. Water temperature in nursery tanks was controlled to 20-22°C using an in-line reverse cycle heater / chiller unit (Haden Pty Ltd).

2.1.2 Stocking procedures

Large and small fish cohorts were captured from nursery tanks and transferred to the laboratory where they were placed into 200L holding tanks. Single tanks of fish were then lightly sedated (Aqui-S; 10-15ppm) in order to grade and select individual fish for stocking into experiment tanks. Selected fish were weighed and measured in small groups of 10 and systematically distributed to their allocated experiment tanks. Twenty large fish and 30 small fish were placed into each of 12 x 200L white cylindrical, flat bottom polyethylene tanks, respectively (i.e. total of 24 experiment tanks). Average ± standard deviation (sd) weight of individual large fish at stocking was 44.5 ± 6.2g while the average ± sd weight of individual small fish at stocking was 6.4 ± 0.7g. All fish were given a light settling feed after conclusion of stocking procedures. During the first week in the laboratory fish were gradually acclimated from their nursery temperature of 21°C to their relevant experimental temperature regimes of 12°C or 15°C at approximately 1°C day^{-1}.

2.1.3 Experimental system

The experiment was performed in a laboratory that housed 24 x 200L white cylindrical, flat bottom polyethylene tanks (Plate 1). Water flow to each experiment tank was controlled by small polyvinylchloride (PVC) taps that provided a centripetal current within each tank which promoted the removal of settled waste through a central, overhead standpipe (32mm diameter) fixed approximately 10mm above the floor of the tank. All 200L experiment tanks were supplied with compressed air via a mini in-line tap which was connected to a single 100mm air stone diffuser. Black plastic was wrapped around each of the tanks and a black plastic lid, which covered half the tank opening, was fitted to minimise external disturbance of fish. Black 10mm ‘bird-mesh’ was stretched and secured under the cover of each experiment tank to prevent fish escaping.

Two isolated, but mechanically identical rearing systems were used to provide the nominal temperature treatments. Each system consisted of a 1500L rectangular

sump, a proprietary protein skimmer (Aquasonic Pty Ltd), a 500L rotating biological filter (B-cell media) and a twin cartridge particle filter. Each system was connected to a refrigeration unit capable of chilling circulating water temperature to 8°C. Water temperature was precisely controlled in each system using a 2.4kw immersion heater operating antagonistically against the chilling unit. Influent saltwater (≈ 34‰) was pumped (2kW pool pump) to individual tanks in each system from the sump via a 50mm manifold and each tank received influent water at approximately 7-8L min^{-1}. Effluent water returned to the sump under gravity flow via a 200mm PVC pipe where it was continuously pumped through the associated particle and biological filters. Fresh, top-up salt water was drawn from a common storage tank (10kL) and bled into the system sumps via ball floats connected to a 50mm manifold. A high dissolved oxygen regime was maintained in all tanks by diffusing industrial grade oxygen (BOC) into the manifold intake located within each sump.

Plate 1. Experiment tanks used to rear juvenile YTK at PSFI.

2.1.4 Feeding protocols and weighing procedures

During the temperature acclimation phase all small and large fish were fed a ration of 2mm or 3mm starter diet, respectively (Ridley Aquafeed Pty Ltd; 54% crude protein, 10% fat). After the acclimation phase was complete (≈ 7 days), fish were switched to their respective dietary treatments. Large fish were given 3mm pellets (Skretting Pty Ltd, Nova ME or Ridley Pty Ltd Start 3) and small fish were given 2mm pellets (Skretting Chile, Nutra Alpha or Ridley Pty Start 2). Fish were maintained on these diets for the remainder of the trial. Proximate and amino acid composition of these diets is presented in Table 1 while fatty acid composition is presented in Table 2. Fish were fed to apparent satiation twice daily (0900h and 1430h) from Monday to Friday and once on Saturdays and Sundays (0900h). Fish were weighed individually at the start of the experiment and then bulk weighed every seven to ten days to monitor growth rate. Fish were not fed in the morning prior to bulk weight check procedures, but were fed in the afternoon after they had recovered from the effects of anaesthetic. All fish were starved for 48h prior to the conclusion of the experiment, killed with an overdose of anaesthetic (Aqui-S), individually weighed and measured. Note: five fish were removed from each experiment tank at the first weight check to

obtain samples for a related experiment. During the experiment mortalities were weighed and recorded but fish were not replaced.

Table 1. Measured proximate, energy and amino acid composition of commercial diets fed to juvenile YTK.

	Diet			
	Ridley Start 2[1]	Ridley Start 3[2]	Nutra Alpha[3]	Nova ME[4]
Dry matter (g kg^{-1})	925.00	916.00	919.00	913.00
Ash (g kg^{-1})	101.00	94.00	116.00	99.00
Crude protein (g kg^{-1})	558.13	569.38	556.88	518.75
Total lipid (g kg^{-1})	150.74	134.48	252.37	225.18
NFE (g kg^{-1})	190.13	202.14	74.75	157.07
GE (MJ kg^{-1})	21.99	21.73	23.53	23.17
Amino acids (g kg^{-1})				
Alanine	34.42	30.59	26.59	27.71
Arginine	33.91	35.92	30.82	36.16
AsparticAcid	49.91	51.17	36.42	40.78
Cystine	5.08	7.39	5.73	8.08
GlutamicAcid	71.17	71.75	75.86	66.43
Glycine	32.71	25.64	27.10	34.08
Histidine	20.85	17.65	15.99	13.44
isoLeucine	15.56	19.67	19.39	19.23
Leucine	43.64	42.58	34.14	35.22
Lysine	37.80	37.90	31.35	28.16
Methionine	10.56	12.79	11.95	8.42
Phenylalanine	25.75	24.17	20.51	21.10
Proline	25.56	21.79	25.68	28.23
Serine	23.74	22.23	20.22	26.25
Threonine	22.46	21.60	20.75	21.09
Tryptophan	7.02	6.66	6.34	5.38
Tyrosine	16.09	16.21	16.05	15.44
Valine	30.13	26.91	23.07	25.20

[1] Ridley Start 2 2mm; product contains restricted animal material, product code 875000220, run # 90403.
[2] Ridley Start 3 LC 3mm; product contains restricted animal material, product code 875000420, run # 90540.
[3] Nutra Alpha 2.3mm; Skretting Chile, lot # 2028205; fishmeal, fish oil, wheat, milling by-products, vegetable protein concentrates, crustacean meal, vitamins, minerals, astaxanthin.
[4] Nova ME 3mm; Skretting Australia, lot # 9013206, mammalian product free, fishmeal, plant protein meal, poultry protein meals, wheat, fish oil, poultry oil, vitamins, minerals.

Table 2. Measured fatty acid composition of commercial diets fed to juvenile YTK.

	Diet			
	Ridley Start 2[1]	Ridley Start 3[2]	Nutra Alpha[3]	Nova ME[4]
Fatty acid class (g kg^{-1} dry sample)				
14	4.74	3.47	11.53	6.78
14:1n-5	-	-	-	-
15	0.51	0.31	0.86	0.62
16	21.36	19.04	34.92	35.39
16:1n-7	5.69	4.73	14.08	11.03
17	0.60	0.44	0.79	0.80
17:1n-8	-	-	-	-
18	6.91	5.17	7.08	9.69
18:1n-9	20.31	21.30	17.66	43.29
18:1n-7	2.80	2.45	6.12	4.62
18:2n-6	12.45	16.71	6.42	14.02
19	-	-	-	-
18:3n-3	1.72	2.56	1.39	2.29
18:4n-3	1.68	1.12	4.21	2.15
20	1.09	0.69	1.19	1.42
20:1n-11	-	-	-	-
20:1n-9	1.97	0.49	2.66	1.03
20:1n-7	-	-	0.44	-
20:2n-6	-	0.30	0.33	0.31
20:3n-6	-	-	-	-
20:4n-6	0.92	0.60	1.96	1.45
20:3n-3	-	-	-	-
20:4n-3	-	-	-	-
20:5n-3 (EPA)	10.06	7.06	32.89	16.27
22	0.30	0.25	0.24	-
22:1n-11	-	-	-	-
22:1n-9	0.23	-	0.38	-
22:1n-7	-	-	-	-
23	0.50	0.34	1.46	0.73
22:4n-6	-	-	-	-
22:5n-6	0.31	0.18	-	0.43
24	-	-	-	-
22:5n-3	1.62	1.09	4.52	2.30
22:6n-3 (DHA)	9.86	7.10	20.43	11.18
24:1n-9	0.37	0.23	0.83	0.35
Total fatty acids	106.03	95.62	172.37	166.13

Note: hyphen "-" indicates <0.01 g kg^{-1}

[1] Ridley Start 2 2mm; product contains restricted animal material, product code 875000220, run # 90403.

[2] Ridley Start 3 LC 3mm; product contains restricted animal material, product code 875000420, run # 90540.

[3] Nutra Alpha 2.3mm; Skretting Chile, lot # 2028205; fishmeal, fish oil, wheat, milling by-products, vegetable protein concentrates, crustacean meal, vitamins, minerals, astaxanthin.

[4] Nova ME 3mm; Skretting Australia, lot # 9013206, mammalian product free, fishmeal, plant protein meal, poultry protein meals, wheat, fish oil, poultry oil, vitamins, minerals.

2.1.5 Performance criteria

Performance criteria were first calculated using measurements of individual fish housed in each replicate tank using the following equations:

Weight gain (g $fish^{-1}$) = harvest weight (g) – stocking weight (g)
Geometric mean body weight (GMBW) = (initial weight (g) x final weight (g))$^{0.5}$
Apparent FCR = feed consumption per tank (g) / wet weight gain per tank (g)
Specific growth rate (%day^{-1}) = (ln(Fw) – ln(Iw))/days x 100

Unless otherwise noted, the average tank value for specific performance criterion was subsequently used in tables, figures and statistical analysis.

2.1.6 Water quality monitoring

General water quality was monitored in sumps and experiment tanks on a daily basis during the experiment using a Horiba water quality analyser (Model U10). Average dissolved oxygen, salinity and pH were 8.0mgL^{-1}, 34.2mg L^{-1} and 8.0 pH units, respectively. Total ammonia nitrogen (TAN) was measured regularly at the beginning of the experiment and then at longer intervals once TAN levels had stabilised. Recorded TAN levels were ≤ 1.0 mgL^{-1}. Water temperature regimes were recorded during the experiment using data logging equipment submerged in each sump (Tinytag Model TG-4100 Aquatic 2, Hastings Data Loggers, Port Macquarie, NSW, Australia).

2.1.7 Chemical analyses

Proximate, amino and fatty acid analysis of diets was done by Agri-Science Queensland Department of Employment, Economic Development and Innovation (DEEDI, QLD Government). All analyses were conducted according to specific in-house laboratory methods or recent AOAC methods (AOAC, 2005). The crude protein content of diet samples was determined by multiplying the measured nitrogen content of each sample by 6.25.

2.1.8 Statistics

The experiment was designed for evaluation in two parts using two-factor analysis of variance (ANOVA) due to the fact that diet type was not orthogonal. In the first instance the performance of large stocked fish at harvest was analysed using water temperature (2 levels; 12°C or 15°C) and diet type (2 levels; Ridley Start 3 or Nova ME) as the two fixed factors. In the second instance the performance of small stocked fish at day 28 was analysed using water temperature (2 levels; 12°C or 15°C) and diet type (2 levels; Ridley Start 2 or Nutra Alpha) as the two fixed factors. As small fish allocated to the 12°C were euthanased, a one-way ANOVA was used to analyse the difference between harvest weights of small fish fed the Ridley Start 2 or

Nutra Alpha diet. Performance criteria were first calculated using measurements of individual fish housed in each replicate tank. The average tank value for specific performance criterion was used in statistical analysis. All statistical analysis was performed using Statgraphics Plus Version 4 software (Manugistics Inc. 1998). Alpha was set to 0.05. If ANOVA was significant, Turkey's HSD multiple comparison procedure was used to separate treatment means at the 95% confidence interval. Cochran's C-test was used to check if the standard deviations (sd) among treatments were similar prior to ANOVA. If treatment sd were found to be heterogeneous appropriate transformations were applied to the raw data before ANOVA was conducted.

2.2 Experiment two: effect of water temperature and hypoxia on growth, feed intake and survival of juvenile YTK.

2.2.1 Fish stocks

Fish for use in this experiment were progeny of CST YTK brood-stock. Fertilised eggs were treated with ozone, carefully placed into 20L polystyrene bags containing filtered sea water and thoroughly oxygenated. Bags were housed in polystyrene boxes and air freighted (≈ 8h) from the CST hatchery to the Marine Finfish Hatchery at Primary Industries NSW PSFI. On arrival eggs were placed into dedicated 2000L larval rearing tanks and subjected to rigorous rearing protocols as described in Fielder and Heasman (2010). Newly hatched fish were weaned onto a specialised Japanese weaning diet (Otohime) and were transferred to 10kL nursery tanks (PSFI greenhouse) when they reached approximately 0.5-0.8g in body weight. Following transfer to nursery tanks fish were weaned onto Nutra-Alpha (2mm; see Table 1 and 2) in preparation for use in the experiment.

2.2.2 Stocking procedures

Prior to stocking a selection of fish from the greater population of fish in one nursery tank were captured and transferred to the laboratory where they were placed into 200L tanks. Single tanks of fish were then lightly sedated (Aqui-S; 10ppm) in order to grade and select individual fish for stocking into experiment tanks. Individual fish were weighed and measured in groups of 15 and systematically distributed to each of 24 x 200L white cylindrical, flat bottom polyethylene tanks until each experiment tank contained 30 fish (i.e. 2 rounds). Average ± sd weight of individual fish at stocking was 8.7 ± 1.6g. All fish were given a light settling feed (Nutra-Alpha) in the afternoon after conclusion of stocking procedures. During the first week in the laboratory fish

were gradually acclimated from their nursery temperature of 18°C to their relevant experimental temperature regimes of 21, 24 or 27°C, respectively.

2.2.3 Experimental system

The experiment was performed in a laboratory that housed 24 x 200L white cylindrical, flat bottom polyethylene tanks. Water flow to each experiment tank was controlled by small polyvinylchloride (PVC) taps that provided a centripetal current within each tank which promoted the removal of settled waste through a central, overhead standpipe (32mm diameter) fixed approximately 10mm above the floor of the tank. All 200L experiment tanks were supplied with compressed air via a mini in-line tap which was connected to a single 100mm air stone diffuser (note: although air stones were present in tanks allocated to the low saturation treatments, no air was provided). Black plastic was wrapped around each of the tanks and a black plastic lid, which covered half the tank opening, was fitted to minimise external disturbance of fish. Black 10mm 'bird-mesh' was stretched and secured under the cover of each experiment tank to prevent fish escaping.

Three isolated, but mechanically identical rearing systems were used to provide the nominal temperature treatments. Each system consisted of a 1500L rectangular sump, a proprietary protein skimmer (Aquasonic Pty Ltd), a 500L rotating biological filter (B-cell media) and a twin cartridge particle filter. Each system was connected to a computer controlled reverse-cycle refrigeration unit capable of controlling circulating water temperature between 10-30°C. Influent saltwater (≈ 31‰) was pumped (2kW pool pump) to individual tanks in each system from the sump via a 50mm manifold and each tank received influent water at approximately 7-8L min^{-1} (note: flow rates to tanks allocated to the low saturation treatments were adjusted during the experiment as tank biomass increased). Effluent water returned to the sump under gravity flow via a 200mm PVC pipe where it was continuously pumped through the associated particle and biological filters. Fresh, pre-heated top-up water was drawn from a common storage tank (10kL) and bled into the system sumps via ball floats connected to a 50mm manifold.

A high saturation regime was maintained by diffusing industrial grade oxygen (BOC) into experiment tanks using an additional fine bubble diffuser. Flow of oxygen in tanks was controlled using a combination medical grade oxygen regulator and flowmeter (400kPa Ezi-Flow Series O; Comweld Medical, Cigweld Pty Ltd, Preston Victoria, Australia). Flow of oxygen and compressed air in each of the experiment

tank allocated to the high saturation treatment was adjusted during the course of the experiment to ensure saturation levels within each temperature treatment remained > about 70% as fish biomass increased. A low saturation regime was maintained in experiment tanks by shutting off the supply of compressed air and adjusting / reducing the flow rate of influent water. Flow rates in low saturation tanks also varied according to temperature regime and were regularly adjusted to ensure saturation levels remained < about 70% as fish biomass increased. No effort was made to control the natural variation in dissolved oxygen concentration during or after feeding sue to increases in post-prandial metabolism.

2.2.4 Feeding protocols and weighing procedures

All fish were fed a 2.3mm commercial pellet for the duration of the trial (Nutra-Alpha; Skretting Chile). This feed was chosen due to the superior performance of juvenile YTK reared on this diet in an earlier growth trial at PSFI (see experiment 1 for nutrient and energy composition). Fish were fed to apparent satiation twice daily (0900h and 1430h) from Monday to Friday and once on Saturdays and Sundays (0900h). Fish were weighed individually at the start of the experiment and then bulk weighed once weekly (i.e. every Tuesday) to monitor growth rate. Fish were not fed in the morning prior to bulk weight check procedures, but were fed in the afternoon after they had recovered from the effects of anaesthetic. All fish were starved for 48h prior to the conclusion of the experiment, killed with an overdose of anaesthetic (Aqui-S), individually weighed and measured.

2.2.5 Performance criteria

Performance criteria were first calculated using measurements of individual fish housed in each replicate tank using the following calculations:

Weight gain (g $fish^{-1}$) = harvest weight (g) – stocking weight (g)
Geometric mean body weight (GMBW) = (initial weight (g) x final weight (g))$^{0.5}$
Apparent FCR = feed consumption per tank (g) / wet weight gain per tank (g)
Specific growth rate (%day^{-1}) = (ln(Fw) – ln(Iw))/days x 100
Relative feed intake (g $kgBW^{-0.8}day^{-1}$) = g intake $fish^{-1}$/(GMBW/1000)$^{0.8}$)/days

Unless otherwise noted, the average tank value for specific performance criterion was subsequently used in tables, figures and statistical analysis.

2.2.6 Water quality monitoring

In order to manually control in-situ tank concentrations, dissolved oxygen was regularly monitored during the experiment using a luminescent dissolved oxygen probe (Hach LDO™ Sensor, Model HQ30d, Hach Company). Dissolved oxygen was normally measured at between 0830 and 0900h each day prior to feeding to ensure nominal dissolved oxygen regimes were being maintained. In-situ dissolved oxygen concentrations were also monitored over extended periods during the trial in order to establish the minimum and maximum level of dissolved oxygen experienced by different treatments and how dissolved oxygen was affected by post-prandial metabolism.

General water quality was monitored in sumps and experiment tanks on a daily basis during the experiment using a Horiba water quality analyser (Model U10). Average salinity and pH were 30.7mg L^{-1} and 7.74 pH units, respectively. Total ammonia nitrogen (TAN) was measured regularly at the beginning of the experiment and then at longer intervals once TAN levels had stabilised. Recorded TAN levels were ≤ 0.63 mgL^{-1}.

Water temperature regimes were recorded during the experiment using data logging equipment submerged in each sump (Tinytag Model TG-4100 Aquatic 2, Hastings Data Loggers, Port Macquarie, NSW, Australia).

2.2.7 Chemical analyses

Proximate, amino and fatty acid analysis of the commercial diet (Nutra Alpha; Skretting Chile) was done by Agri-Science Queensland Department of Employment, Economic Development and Innovation (DEEDI, QLD Government). All analyses were conducted according to specific in-house laboratory methods or recent AOAC methods (AOAC, 2005). The crude protein content of the diet sample was determined by multiplying the measured nitrogen content by 6.25.

2.2.8 Statistics

The experiment was designed for evaluation using two-factor ANOVA. The fixed factors were temperature (3 levels; nominal 21, 24 or 27°C) and oxygen saturation level (2 levels; suboptimal, 50-60% saturation or optimal > 90% saturation). Four replicate tanks (n=4) were provided for each of the 6 experimental treatments. Performance criteria were first calculated using measurements of individual fish housed in each replicate tank. The average tank value for specific performance

criterion was used in statistical analysis. All statistical analysis was performed using Statgraphics Plus Version 4 software (Manugistics Inc. 1998). Alpha was set to 0.05. If ANOVA was significant, Tukey's HSD multiple comparison procedure was used to separate treatment means at the 95% confidence interval. Cochran's C-test was used to determine if the sd among treatments were similar prior to ANOVA. If treatment sd were found to be heterogeneous appropriate transformations were applied to the raw data before ANOVA was conducted.

2.3 Development of a temperature dependent growth model for juvenile YTK

Growth data from the first and second experiments presented in this report and growth data obtained from previous research conducted on YTK at PSFI was amalgamated in order to develop a temperature dependent growth model for juveniles < 1000g. The specific data set is tabulated in Appendix 1. Amalgamated data on juvenile growth that appeared sub-optimal with respect to performance was eliminated from the data set prior to fitting the chosen model (e.g. growth data for small juvenile fish reared on Ridley Start 2 (experiment one) and growth data for fish reared under hypoxic conditions (experiment two)).

Numerous mathematical models are available for constructing temperature dependent growth models and most have certain advantages and disadvantages (Glencross, 2008; Lupatsch, 2009). Regardless, the primary aim of a reliable growth model is to predict the response variable of interest, in this case growth rate, with a high degree of accuracy. It is obvious therefore that the quality of the data used to construct a growth model needs to be of the highest standard. The temperature dependent growth model used in this analysis is an adaptation of a polynomial model used to model temperature and size dependent growth in barramundi *Lates calcarifer* (Glencross, 2008). The adapted model was;

Daily growth rate (g day^{-1}) = $(A+B*Temperature+C*Temperature^2)*(GMBW^D)$

The model parameters A, B, C and D are the respective coefficients and exponents iteratively fit to the data set. The abbreviation GMBW is the geometric mean body weight of YTK in grams with respect to the daily growth rate recorded at a particular temperature. Temperature is in degrees Celsius.

The non linear regression option in NCSS (Hintze, 2006) was used to iteratively fit the selected model to the amalgamated data set. This program estimates the

parameters in nonlinear models using the Levenberg-Marquardt nonlinear least-squares algorithm. The starting value of the lambda parameter as defined in Marquardt's procedure was set to 0.0001. Parameter estimates were given initial minimum, starting and maximum values of -10000, 0 and 10000, respectively and the maximum number of iterations was set to 1000. Alpha of significance tests was set to 0.05.

3. Results

3.1 Experiment one: effect of water temperature and stocking size on survival and growth of juvenile YTK

3.1.1 Water temperature regimes

Water temperatures recorded at three hourly intervals during and after the acclimation phase until the end of the experiment are presented in Figure 1. Actual water temperatures were slightly different to nominal values of 12 or 15°C, however, a 3°C difference in temperature between treatments was generally well maintained (Figure 1).

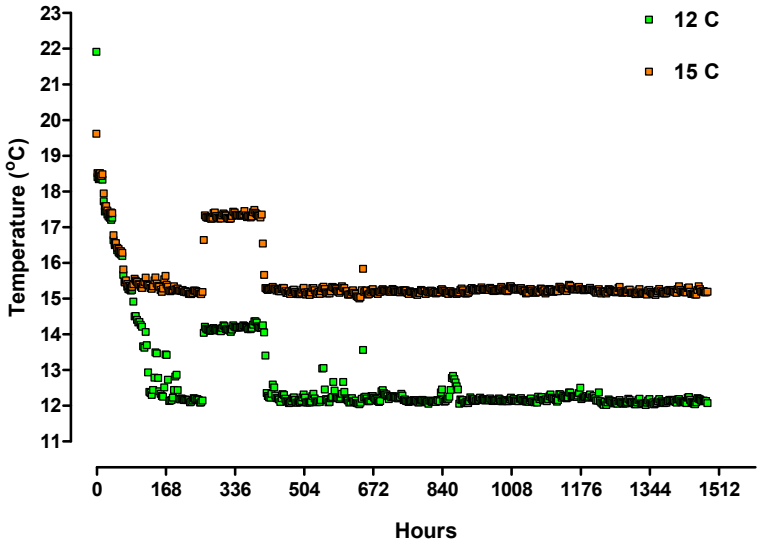


Figure 1. Temperature profile of 12 and 15°C systems during experiment with juvenile YTK. Aberration in steady state reflects equipment failure during experiment.

3.1.2 Survival

A significant number of small fish acclimated to the 12°C regime exhibited unusual vertical swimming behaviour, had difficulty feeding and eventually became moribund and died (Figure 2a). Mortality in small fish increased significantly around day 11 and due to ethical requirements all small fish reared in the 12°C system were euthanized

after 28 days. Many large fish stocked into the 12°C regime also exhibited vertical swimming behaviour and appeared to have trouble maintaining buoyancy. These fish also had difficulty ingesting pellets as a consequence of a “lock jaw” (similar to tetany) and lost condition before becoming moribund. Although survival of larger fish stocked into the 12°C system was higher than small fish stocked at the same temperature, large fish also began to succumb to the low temperature regime around day 11 and mortalities continued until the end of the trial (Figure 2b). Mortality in either small or large fish stocked at 12°C appeared to be unrelated to diet type. No such aberrations in normal swimming or feeding behaviours were observed or recorded in any fish reared in the 15°C system and with the exception of one mortality at day 49 (i.e. Tank 46, Ridley diet), all fish reared under the 15°C regime survived.

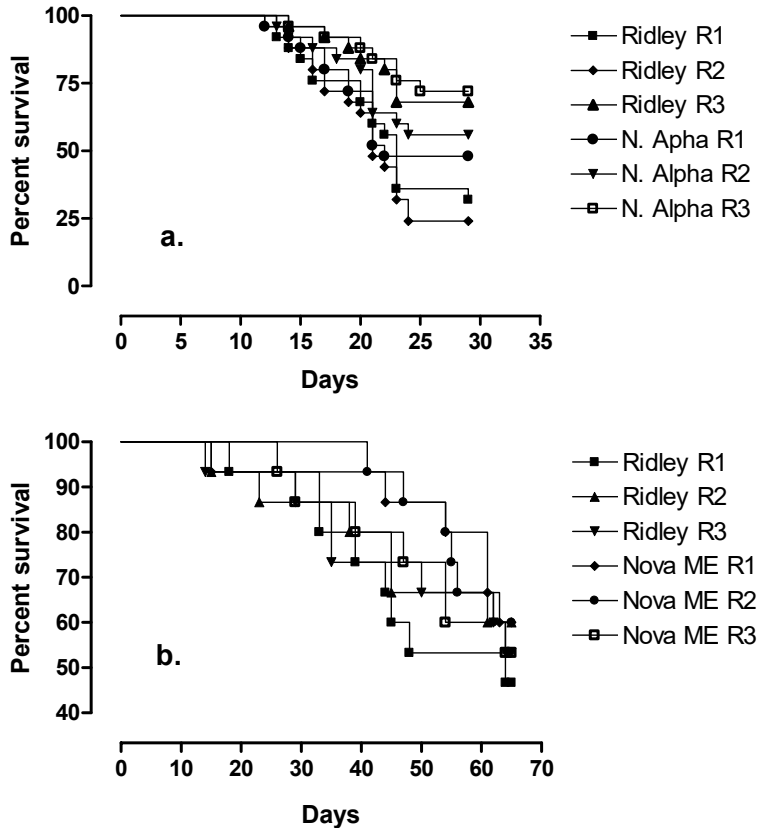


Figure 2. Survival plots of individual replicate tanks assigned to dietary treatments (e.g. R1, R2 or R3 = replicate 1, 2 or 3): a) small YTK reared at 12°C and b) large YTK reared at 12°C. Note; small fish were euthanized after 28 days and surviving large fish were reared until the conclusion of the experiment.

3.1.3 Growth

Weight change of juvenile YTK at consecutive weight checks is presented in Table 3 and graphically in Figure 3. Weight increments were minimal in small and large fish reared at 12°C. In contrast growth rate of fish reared at 15°C was much higher and both small and large fish continued to increase in body weight throughout the trial.

Table 3. Weight (mean ± sd) trajectory of small and large stocked juvenile YTK reared at 12°C or 15°C.

		Days elapsed							
		Stocking	8	19	28	37	47	55	64
12°C temperature regime									
		(g)	(g)	(g)	(g)	(g)	(g)	(g)	(g)
Ridley Start 3	Large	44.7	48.2	52.1	53.0	55.2	56.9	58.7	56.5
		0.2	*1.8*	*0.1*	*0.2*	*1.3*	*1.2*	*1.0*	*1.3*
Nova ME	Large	44.2	48.3	50.4	51.8	53.9	54.9	56.3	55.0
		0.4	*0.7*	*0.2*	*0.6*	*0.7*	*1.2*	*1.1*	*0.9*
Ridley Start 2	Small	6.3	8.1	7.3	7.3	na.	na.	na.	na.
		0.1	*0.3*	*0.4*	*0.5*				
Nutra Alpha	Small	6.4	8.2	8.1	8.5	na.	na.	na.	na.
		0.0	*0.1*	*0.4*	*0.4*				
15°C temperature regime									
Ridley Start 3	Large	44.8	50.8	64.7	72.7	83.4	94.6	100.9	106.1
		0.3	*0.4*	*0.2*	*0.1*	*0.4*	*0.9*	*1.6*	*2.4*
Nova ME	Large	44.5	49.4	64.9	73.0	83.6	96.0	103.7	108.2
		0.4	*0.8*	*1.1*	*0.6*	*1.8*	*2.9*	*4.3*	*4.9*
Ridley Start 2	Small	6.4	8.6	9.9	12.0	14.5	17.0	19.3	20.5
		0.1	*0.1*	*0.1*	*0.1*	*0.0*	*0.4*	*0.1*	*0.4*
Nutra Alpha	Small	6.4	8.7	11.4	15.1	20.4	26.2	31.0	35.6
		0.2	*0.8*	*0.7*	*0.7*	*0.9*	*1.0*	*0.6*	*0.7*

Note: small fish allocated to the 12°C temperature regime were removed from the study on day 28.

Two way ANOVA indicated that $SGR_{Harvest}$ of large stocked fish was significantly affected by temperature ($F_{1,8}$=3.11, P<0.0001), but not by diet type ($F_{1,8}$=0.0002, P=0.75) nor the interaction of temperature and diet type ($F_{1,8}$=1.62, P=0.24). The comparison of marginal means (pooling across diet) indicated that large fish had significantly higher SGR at 15°C (1.37%day^{-1}, n=6) compared to fish reared at 12°C (0.35%day^{-1}, n=6).

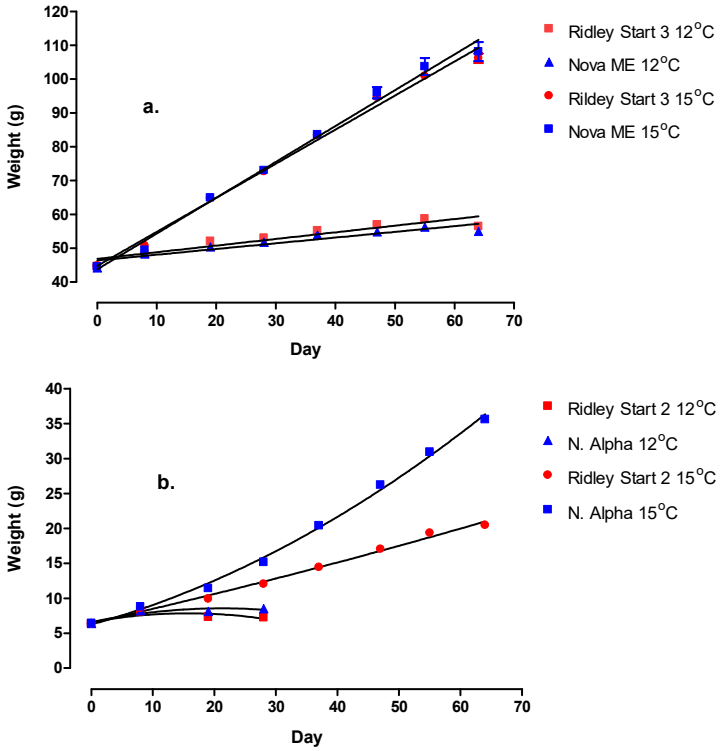


Figure 3. Mean growth of juvenile YTK reared under a 12°C or 15°C temperature regime and fed different commercial diets: a) large fish reared at 12°C or 15°C and b) small fish reared at 12°C or 15°C. Note; small fish were euthanized after 28 days. Data points and error bars indicate mean±sem.

Two way ANOVA indicated that $SGR_{Day\ 28}$ of small fish surviving at day 28 was significantly affected by temperature ($F_{1,8}$=717.81, *P*<0.0001) and by diet type ($F_{1,8}$=85.41, *P*<0.001), but was not affected by the interaction of temperature and diet type ($F_{1,8}$=3.66, *P*=0.09). Comparison of marginal means indicated that the growth rate of small fish was significantly higher at 15°C (2.66%day^{-1}, n=6) than 12°C (0.76%day^{-1}, n=6) and the growth rate of small fish fed Nutra Alpha (2.04%day^{-1}, n=6) was significantly higher than fish fed Ridley Start 2 (1.38%day^{-1}, n=6).

Oneway ANOVA indicated the $SGR_{Harvest}$ of small fish reared at 15°C and fed on Nutra Alpha was significantly higher than small fish fed on Ridley Start 2 (i.e. 2.68 vs 1.82%day^{-1}, $F_{1,4}$=2260, *P*<0.0001) (Table 4).

3.1.4 Feed intake and apparent FCR

Observed feed intake was sporadic in fish reared in the 12°C system over the experimental period. In addition, calculated AFCR values were negative in large fish allocated to the 12°C system fed the Ridley Start 3 and Nova ME diets and negative in small fish allocated to the 12°C system fed the Ridley Start 2 or Nutra Alpha diets

as mortalities were recorded in these treatment groups (Table 4). Therefore, statistical comparisons of feed intake and AFCR were not performed on these data. In contrast, the AFCR of small and large stocked fish reared at 15°C was positive (Table 4).

Oneway *ANOVA* indicated there was no significant difference ($P>0.05$) in the SGR_{28}, $SGR_{Harvest}$ or $AFCR_{Harvest}$ of large fish fed on Nova ME or Ridley Start 3 reared at 15°C. However, fish consumed significantly more of the Nova ME feed than the Ridley Start 3 on a per tank basis ($P<0.022$; Table 4).

Oneway *ANOVA* indicated the SGR_{28}, $SGR_{Harvest}$ and $AFCR_{Harvest}$ of small fish fed on Nutra Alpha diet were all significantly better ($P<0.0001$) than small fish reared on Ridley Start 2. Despite the apparently higher feed intake of small fish fed on Nutra Alpha, oneway ANOVA did not find any significant difference between intake of Nutra Alpha and Ridley Start 2 ($P>0.05$) (Table 4). It should be noted that neither the Ridley Start diets (R. Smullen Ridley Aquafeed; *pers. comm.*) nor the Nutra Alpha diet was formulated specifically for *S. lalandi* (Atlantic salmon formulation).

A power function describing the fit between increasing geometric mean body weight and daily feed intake of fish reared at 15°C was highly significant and could be described by the equation:

Daily feed intake (g) = $0.0512\pm0.0187 \times GMBW(g)^{0.8001\pm0.0842}$; $R^2=0.88$.

Table 4. Average specific growth rate (SGR), feed intake and apparent feed conversion (AFCR) in small and large stocked juvenile Yellowtail Kingfish (*Seriola lalandi*) reared at 12°C or 15°C.

	Diets			
	Ridley Start 3	Nova ME	Ridley Start 2	Nutra Alpha
Large fish 12°C				
SGR_{Day28}	0.61	0.56	-	-
$SGR_{Harvest}$	0.37	0.34	-	-
Feed $intake_{Harvest}$	654.1	821.9	-	-
$AFCR_{Harvest}$	-1.50	-2.30	-	-
Large fish 15°C				
SGR_{Day28}	1.73	1.77	-	-
$SGR_{Harvest}$	1.35	1.39	-	-
Feed $intake_{Harvest}$	1629.0	1749.6	-	-
$AFCR_{Harvest}$	2.49	2.40	-	-
Small fish 12°C				
SGR_{Day28}	-	-	0.49	1.02
Feed $intake_{Day28}$	-	-	83.0	96.1
$AFCR_{Day28}$	-	-	-0.44	-0.50
Small fish 15°C				
SGR_{Day28}	-	-	2.27	3.07
$SGR_{Harvest}$	-	-	1.82	2.68
Feed $intake_{Harvest}$	-	-	607.9	658.8
$AFCR_{Harvest}$	-	-	1.90	0.91

Note: small fish allocated to the 12°C temperature regime were removed from the study on day 28. Average feed intake = grams feed per tank. Apparent FCR (AFCR) calculated by dividing feed input per tank by weight gain per tank. AFCR calculation is negative in fish reared at 12°C due to loss of biomass from ongoing mortalities.

The function can be used to calculate absolute or relative feeding rates for fish growing between approximately 7 to 100g at 15°C when fed similar diets to those used in this trial (Figure 4).

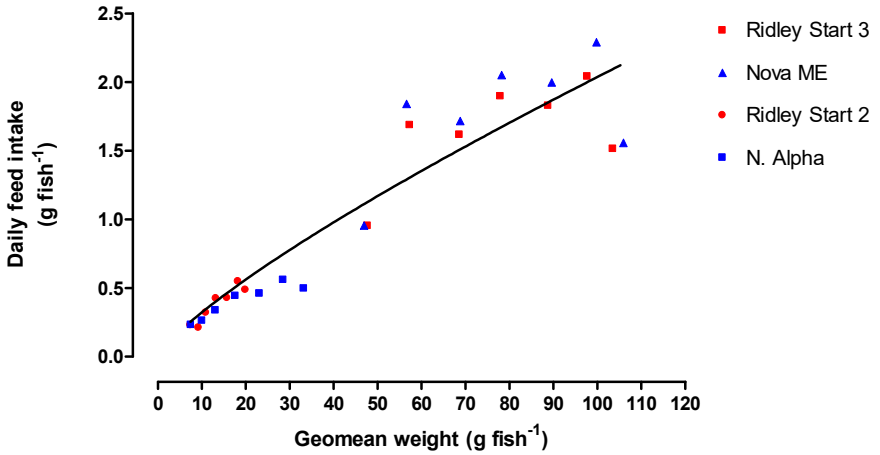


Figure 4. Relationship between body weight and daily feed intake of juvenile YTK reared under a15°C temperature regime and fed different commercial diets. Power function; Daily feed intake = 0.0512±0.0187 x GMBW(g) $^{0.8001\pm0.0842}$; R^2=0.88.

3.2 Experiment two: effect of water temperature and hypoxia on growth, feed intake and survival of juvenile YTK.

3.2.1 Maintenance of water temperature regimes

Average ± sd water temperatures recorded after the acclimation phase until the end of the experiment in the low, mid and high water temperature treatments were 20.55 ± 0.94°C, 23.51 ± 0.74°C and 27.03 ± 0.30°C, respectively. Actual water temperatures were slightly different to nominal values of 21°C, 24°C or 27°C, however, the 3°C difference in temperature between the treatments was generally well maintained.

3.2.2 Dissolved oxygen regimes

Low and high dissolved oxygen regimes were established immediately after the temperature acclimation phase was completed. Indicative dissolved oxygen levels are shown in Figure 5. The experimental protocols clearly established separation between the levels of dissolved oxygen experienced by fish reared at different water temperatures. However, as expected the dissolved oxygen concentration in tanks declined after feeding due to post-prandial metabolism. Dissolved oxygen concentration fell rapidly following the morning feed at 0900h and declined further following the afternoon feed at 1400h.

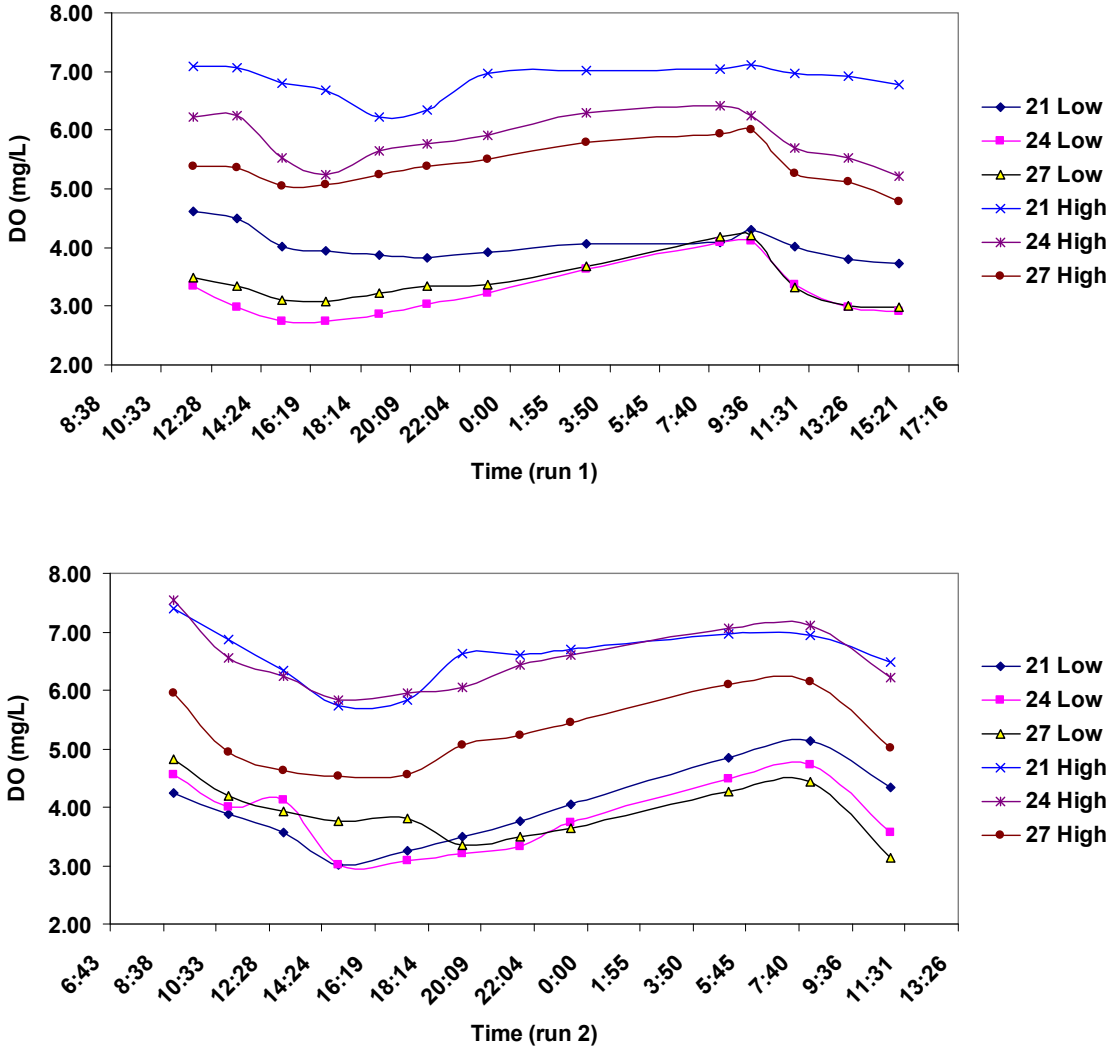


Figure 5. Snapshot of fluctuation in mean dissolved oxygen readings taken on the 29.6.11 to 30.6.11 (run 1) and the 7.7.2011 to 8.7.2011 (run 2); n=4 replicate tanks per data point for each temperature (21°C, 24°C or 27°C). Low = low dissolved oxygen regime; High = high dissolved oxygen regime.

Peak dissolved oxygen concentration in tanks (governed by the prevailing experimental conditions) was not reached for another 12h (Figure 5).

Minimum and maximum dissolved oxygen concentration and oxygen saturation levels recorded during run 1 and run 2 are presented in Table 5.

Table 5. Range of average dissolved oxygen concentrations recorded on two occasions in the different temperature systems over an extended 24h period. Run 1 (29.6.11 to 30.6.11) and run 2 (7.7.2011 to 8.7.2011); n=4 replicate tanks per temperature x dissolved oxygen treatment.

		Run 1		Run 2	
Temperature	DO regime	minimum	maximum	minimum	maximum
Dissolved oxygen (mgL^{-1})					
21°C	low	3.72	5.385	3.02	5.14
24°C	low	2.76	5.16	3.01	4.72
27°C	low	2.97	4.99	3.12	4.82
21°C	high	6.22	7.22	5.73	7.41
24°C	high	5.21	6.98	5.84	7.55
27°C	high	4.78	6.18	4.54	6.14
Oxygen saturation (%)					
21°C	low	49.38	70.93	41.18	66.30
24°C	low	38.78	70.90	43.03	65.23
27°C	low	44.35	72.98	46.45	72.10
21°C	high	83.05	95.55	78.25	98.53
24°C	high	73.73	95.73	83.43	106.18
27°C	high	71.53	92.03	67.95	92.05

3.2.3 Growth

Fish grew rapidly during the trial with specific growth rates reaching 7.5% day^{-1} for fish in the normoxic regime (Table 6). Two-way ANOVA indicated that $SGR_{Harvest}$ was significantly affected by dissolved oxygen regime ($F_{1,18}$=471.48, P<0.0001), water temperature ($F_{2,18}$=145.43, P<0.0001) and the interaction of dissolved oxygen and water temperature ($F_{2,18}$=14.66, P=0.0002). The interaction was driven by the slightly smaller magnitude in growth rate between fish reared under the normoxic or hypoxic regimes at 21°C compared to differences in growth rates at 24°C and 27°C. Growth was clearly improved under normoxic conditions and reduced under hypoxic conditions, regardless of temperature (Figure 6a). Oneway *ANOVA* indicated there were significant differences between the $SGR_{Harvest}$ of fish reared at different temperatures and the effect was the same irrespective of saturation regime. $SGR_{Harvest}$ could be grouped independently according to Tukeys HSD such that SGR was highest at 24°C > 27°C > 21°C.

Table 6. Performance criteria (mean±sd) for YTK reared for 35 days; n=4 replicate tanks per temperature x dissolved oxygen treatment.

	Days elapsed							
	Stocking	7	14	21	28	Harvest data (day 35)		
High dissolved oxygen regime								
	(g)	(g)	(g)	(g)	(g)	SGR (%day^{-1})	Daily FI (g fish^{-1})	AFCR
21°C	8.53	15.23	25.05	37.21	49.25	6.26	0.86	0.76
	0.28	*0.51*	*0.66*	*0.87*	*1.34*	*0.07*	*0.02*	*0.01*
24°C	8.42	16.04	31.62	53.11	73.93	7.76	1.35	0.73
	0.31	*0.25*	*1.09*	*2.06*	*2.68*	*0.23*	*0.06*	*0.01*
27°C	8.58	16.26	31.43	49.63	66.27	7.30	1.36	0.83
	0.17	*0.21*	*0.77*	*1.38*	*2.69*	*0.18*	*0.04*	*0.01*
Low dissolved oxygen regime								
21°C	8.75	14.85	22.76	31.62	40.12	5.44	0.68	0.76
	0.64	*1.00*	*1.60*	*1.79*	*1.99*	*0.10*	*0.04*	*0.01*
24°C	8.25	14.01	24.25	35.46	46.95	6.21	0.84	0.76
	0.12	*0.36*	*0.62*	*0.83*	*1.17*	*0.06*	*0.01*	*0.01*
27°C	8.43	14.80	23.48	34.54	45.60	6.03	0.88	0.90
	0.15	*0.25*	*0.66*	*1.43*	*0.65*	*0.09*	*0.02*	*0.03*

3.2.4 Feed intake and apparent FCR

Two-way ANOVA indicated that relative feed intake was significantly affected by dissolved oxygen regime ($F_{1,18}$=546.80, P<0.0001), water temperature ($F_{2,18}$=256.64, P<0.0001) and the interaction of dissolved oxygen and water temperature ($F_{2,18}$=28.74, P<0.0001). Once again the interaction was driven by the slightly smaller magnitude in relative feed intake between fish reared under the normoxic or hypoxic regimes at 21°C compared to differences in feed intake at 24°C and 27°C. Reducing tests to simple ANOVA indicated that feed intake was significantly affected by temperature, regardless of saturation regime. The pattern in relative feed intake according to Tukeys HSD was such that under normoxic conditions relative feed intake was similar at 24°C and 27°C but significantly lower at 21°C. Under hypoxic conditions relative feed intake was significantly different at each temperature, being highest at 27°C > 24°C > 21°C (Figure 6b).

Two-way ANOVA indicated that $AFCR_{Harvest}$ was significantly affected by dissolved oxygen regime ($F_{1,18}$=28.49, P<0.0001), water temperature ($F_{2,18}$=133.08, P<0.0001) and the interaction of dissolved oxygen and water temperature ($F_{2,18}$=7.59, P=0.0041). Apparent FCR was highest (i.e. worst) at 27°C and lowest (i.e. best) at

21°C and 24°C, irrespective of whether the environment was normoxic or hypoxic (Figure 6c).

3.2.5 Survival

Overall survival was extremely high and no mortality was recorded in fish reared under hypoxic conditions at the cooler temperatures of 21°C or 24°C (Figure 6d). As no mortality (and no variance) was recorded in fish reared at 21°C or 24°C under hypoxic conditions only survival data for normoxic conditions was evaluated. Oneway ANOVA indicated there was no significant difference between the survival of fish at the different temperatures (Figure 6c). Mortality in normoxic treatments was restricted to one or two individual fish from different tanks and was not related to treatments effects. Survival of juvenile fish at 27°C under hypoxic conditions (90±2.7%; mean ± sd) was significantly lower than survival of fish reared at 27°C under normoxic conditions (99.1±1.7; mean ± sd) (Figure 6d).

3.3 Temperature dependent growth model for juvenile YTK

The growth model estimated from the nonlinear regression was:

Daily gain (g day^{-1}) = ((1.353758)+(.1437899)*(Temperature) +(.0029999) *$(Temperature)^2$) * $((GMBW)^{(.4804985)})$.

Parameter estimates, standard errors and upper and lower 95% confidence intervals for each parameter are presented in Table 7.

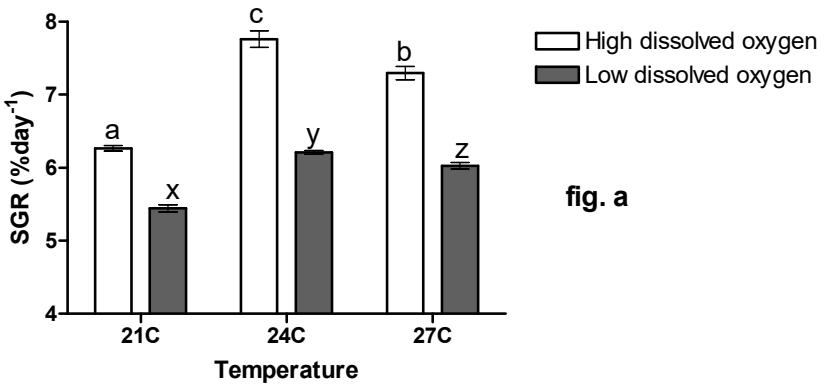
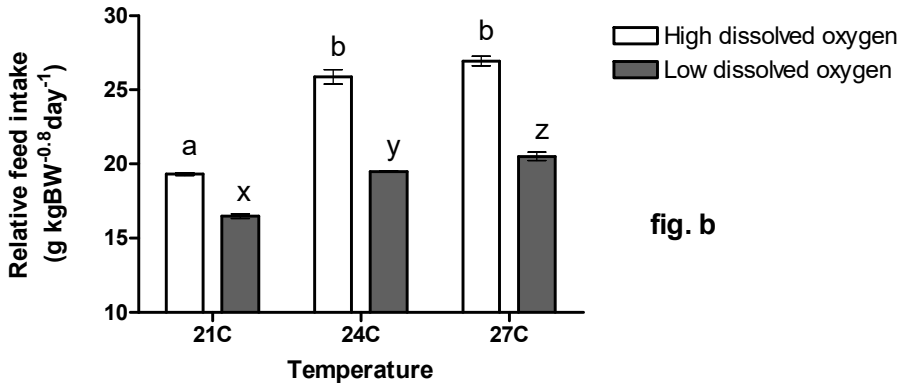


fig. a



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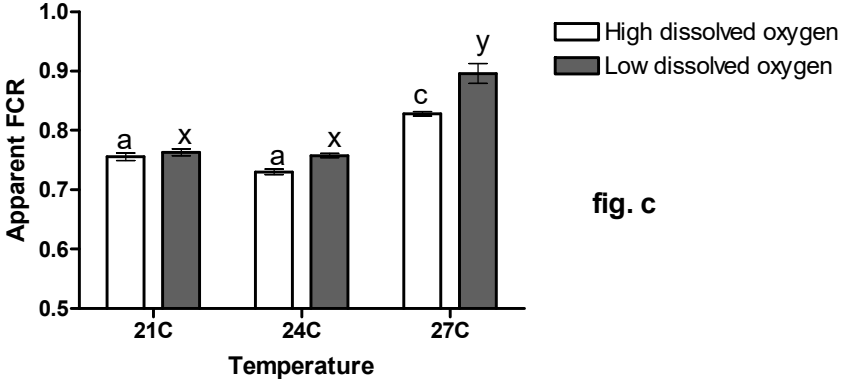

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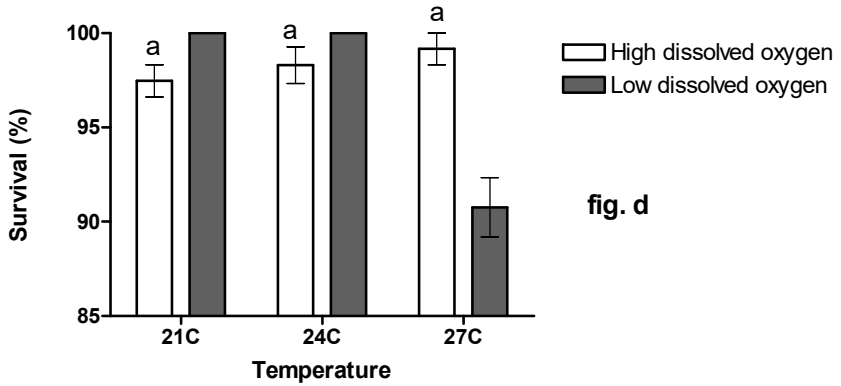

fig. d

Figure 6. Mean ± sd performance criteria (n=4) of juvenile YTK reared at different temperatures and subjected to a low or high dissolved oxygen regime for 35 days. Letters a, b or c denote statistical comparisons between high dissolved oxygen treatments. Letters x, y or z denote comparisons between low dissolved oxygen treatments (i.e. oneway *ANOVA*; Tukeys HSD, α set to 0.05).

Table 7. Parameter estimates of nonlinear regression analysis

Parameter name	Parameter estimate	Asymptotic stand. error	Lower 95% C.L.	Upper 95% C.L.
A	-1.353758	0.1395081	-1.628076	-1.079439
B	0.1437899	1.490175E-02	0.1144881	0.1730916
C	-0.0029999	3.543831E-04	-3.696733E-03	-2.303067E-03
D	0.4804985	0.0129253	0.4550831	0.5059138

Estimated R^2=0.89

Iterations 17

The model was used to predict and tabulate the daily growth rate of different size YTK at different water temperatures (Table 8). Table 8 also includes predicted daily weight gains for YTK according to a growth model presented by Booth et al. (2010).

Table 8. Estimate of increment in daily weight gain of juvenile Yellowtail Kingfish (*Seriola lalandi*) based on fitted model parameters.

	Fish weight (g)									
	50	100	200	300	400	500	600	800	900	1000
Temperature (°C)										
10	-0.3	-0.4	-0.5	-0.7	-0.8	-0.8	-0.9	-1.1	-1.1	-1.2
11	-0.2	-0.2	-0.3	-0.4	-0.5	-0.5	-0.6	-0.6	-0.7	-0.7
12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13	0.2	0.3	0.4	0.5	0.6	0.7	0.7	0.8	0.9	0.9
14	0.5	0.7	0.9	1.1	1.3	1.4	1.6	1.8	1.9	2.0
15	0.8	1.1	1.5	1.8	2.1	2.3	2.5	2.9	3.1	3.2
16	1.1	1.5	2.1	2.5	2.9	3.2	3.5	4.0	4.3	4.5
17	1.3	1.9	2.6	3.2	3.7	4.1	4.5	5.2	5.5	5.7
18	1.6	2.3	3.2	3.9	4.5	5.0	5.4	6.2	6.6	7.0
19	1.9	2.6	3.7	4.5	5.2	5.8	6.3	7.2	7.7	8.1
20	2.1	3.0	4.1	5.0	5.8	6.5	7.1	8.1	8.6	9.0
21	2.3	3.2	4.5	5.5	6.3	7.0	7.6	8.8	9.3	9.8
22	2.4	3.4	4.7	5.7	6.6	7.4	8.0	9.2	9.8	10.3
23	2.5	3.4	4.8	5.8	6.7	7.5	8.2	9.4	9.9	10.5
24	2.4	3.4	4.7	5.8	6.6	7.4	8.0	9.2	9.8	10.3
25	2.3	3.2	4.5	5.4	6.2	6.9	7.6	8.7	9.2	9.7
26	2.0	2.8	4.0	4.8	5.6	6.2	6.8	7.8	8.2	8.7
27	1.7	2.3	3.3	4.0	4.6	5.1	5.5	6.4	6.7	7.1
28	1.2	1.6	2.3	2.8	3.2	3.5	3.9	4.4	4.7	5.0
29	0.5	0.7	1.0	1.2	1.4	1.6	1.7	2.0	2.1	2.2
Booth et al.[1]	*2.0*	*2.9*	*4.2*	*5.2*	*6.0*	*6.8*	*7.5*	*8.7*	*9.2*	*9.7*

[1]. Comparative estimates of daily weight gain in YTK at temperatures between 20-25°C predicted from a growth model for this species presented in Booth et al. 2010.

A three dimensional representation of the growth model is presented in Figure 7. From a biological perspective the model predicts that growth will be optimised when water temperature is 23°C. Growth rate of fish at any size declines on either side of this temperature. The model also indicates that absolute weight gain increases with increasing body weight for a given temperature, however, the response becomes asymptotic as become larger.

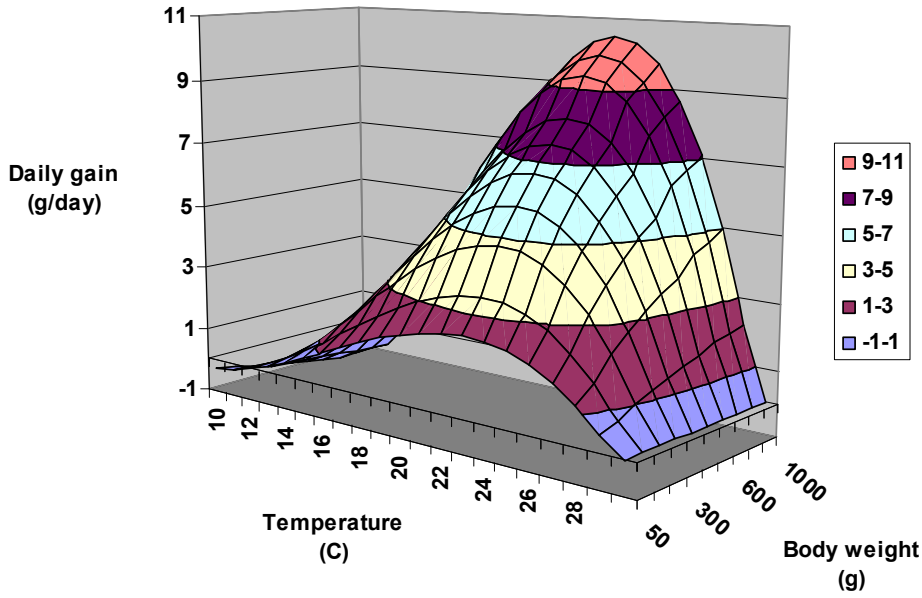

Figure 7. Three dimensional representation of the temperature dependent growth model for juvenile YTK.

4. General Discussion

4.1 Effect of stocking size and water temperature on survival and growth of juvenile YTK

Yellowtail Kingfish is a high performance pelagic marine carnivore and being a thermoconfomer its body temperature reflects that of the surrounding water. As such, water temperature has a profound effect on the physiology and performance of this animal through its effect on the regulation of biochemical reactions (Withers, 1992). In practical terms YTK can probably be classed as eurythermal in nature as it tolerates a wide range of rearing temperatures (e.g. 10 – 28°C). However, recently published evidence suggests that the metabolic rate of juvenile YTK is least thermally sensitive at around 22°C. At this temperature metabolic losses are minimised and a

greater proportion of energy can be channelled into productive growth (Clark & Seymour, 2006; Pirozzi & Booth, 2009). Consequently, at body temperatures either side of the preferred temperature performance is reduced (Magendans et al., 2010). This has implications for YTK held in sea-cages because they are subject to ambient fluctuations in water temperature (and other physicochemical changes) and are therefore unable to thermoregulate to their preferred body temperature by behavioural means.

The first experiment in this study was designed to investigate the implications of stocking juvenile fish into suboptimal water temperatures (i.e. 12 and 15°C). These suboptimal temperatures are typical of the long run water temperatures recorded in September / October in the Spencer Gulf, South Australia, and are generally coincident with the Spring stocking of juvenile YTK into sea-cages. The results of experiment 1 demonstrated that even following an acclimation rate of approximately 1°C day^{-1} it is not advisable to rear small (6g) or large (40g) juvenile YTK at 12°C. Fish reared at 12°C became moribund, lost equilibrium, had difficulty feeding and died. Longer term survival and rate of mortality of larger animals was better than that of smaller juveniles, but only 45-60% of larger individuals survived to day 64. Small fish reared at 12°C were removed from the study according to NSW DPI Fisheries animal care and ethics requirements, with only 25-65% of small individuals surviving to day 28. It is highly likely that the majority of small fish would have succumbed to the 12°C regime well before the end of the experiment.

Growth rate was negligible and survival, feed intake and AFCR were poor in small and large fish reared at 12°C, irrespective of diet type. In combination, these results confirm that juvenile YTK should not be reared at temperatures ≤ 12°C. In addition, stocking events where ambient water temperature is expected to approach 12°C should be avoided. Many factors may have influenced the survival of fish reared at 12°C including the acclimation rate used to bring fish from their previous holding temperature of 21°C down to 12°C as well as a lack of adaptability in the biochemical machinery of fish within this size range (e.g. enzyme structure, function and activity and the function of lipid bilayer membranes (Withers, 1992)).

Notwithstanding these recommendations, our study did not determine if water temperatures between 12°C and 15°C are appropriate for increasing the survival of juvenile YTK. Given the positive response of fish reared at 15°C, it is likely that survival of 5g and 40g fish would be improved at water temperatures between 12°C and 15°C providing no other environmental stressors were present. Larger YTK may

also cope better than smaller individuals at low water temperatures. For example, Pirozzi & Booth (2009) successfully acclimated 200g YTK to water temperatures between 10°C and 32.5°C in order to study the effect of temperature on routine metabolic rate. They did not record mortality within this range, but did observe fish regurgitating food at temperatures closer to 33°C. In Japan, *S. quinqueradiata* generally experience mass mortality when water temperature falls below 10°C (Nakada, 2000). Larger YTK may be more tolerant of low water temperatures, but the economics of holding and then transporting large numbers of advanced juveniles from hatcheries to sea-cages remains to be investigated.

In contrast, small and large fish reared at 15°C had high survival, reasonable growth rates and low (i.e. better) AFCR. In addition, growth rate and AFCR was superior in small fish fed the imported Nutra Alpha compared to that of small fish reared on the Ridley Start 2. The crude protein of these diets was similar but there were marked differences between their lipid (25% vs 15%) and carbohydrate (7% vs 19%) content. It should be noted that neither the Ridley Start diet (R. Smullen Ridley Aquafeed; *pers. comm.*) nor the Nutra Alpha diet (Atlantic salmon) was formulated specifically for YTK. Nonetheless, the imported feed promoted far superior performance in small fish. One hypothesis for the superior growth and FCR may be the elevated levels of the long chain HUFAs EPA and DHA contained in the lipid fraction of the Nutra Alpha diet compared to the Ridley Start 2. These essential fatty acids have been shown to be critical for rapid growth in larval *S. quinqueradiata* (Furuita et al., 1996) and likely play a vital role in the nutrition of small juveniles. Alternatively, the Nutra Alpha diet also contained some form of crustacean meal. It is highly probable that this was krill meal, a known feed attractant. This may have increased feed intake resulting in higher weight gain for the YTK fed this diet. Both krill meal and squid meal extracts have been shown to increase protein digestibility in 500g *S. quinqueradiata* (Kofuji et al., 2006), however, further research is required to determine why the Nutra Alpha diet used in this experiment promoted superior weight gain and AFCR in small YTK.

Dietary effects were largely absent in 40g fish reared at 15°C despite differences in the nutrient and energy content of the tested diets. The lack of difference between growth rate, AFCR and feed intake in larger fish may indicate that all nutritional requirements of fish this size and reared at 15°C were being adequately met by either diet. In terms of proximate composition the Ridley Start 3 diet was higher in protein (57% vs 52%) and carbohydrates (20% vs 16%), but lower in total lipid (13% vs 23%) and gross energy (22MJ kg^{-1} vs 23MJ kg^{-1}) than the Nova ME. Interestingly, growth rate of slightly larger New Zealand YTK (body weight range 400-1200g) was

similar when fed a series of three commercially available diets known as SalmoFeed ESF, Skretting Nova ME and Reliance salmon food. However, the biological FCR was best (i.e. lowest) on the Salmofood ESF, which was higher in energy density than either of the other commercial feeds as a result of more dietary fish oil (26%) (Moran et al., 2009).

4.2 Effect of water temperature and hypoxia on growth, feed intake and survival of juvenile YTK

Aerobic metabolism predominates in fish so the oxygen concentration of the culture water is potentially a limiting factor (Kestemont & Baras, 2001). The natural solubility of oxygen in water depends on its partial pressure (pO_2) and its solubility coefficient. The solubility coefficient is itself dependent on water temperature and salinity, decreasing at higher water temperatures and elevated salinity (Campbell, 1990; Withers, 1992). Temporary oxygen depletion in wild environments usually results in fish moving to areas of higher dissolved oxygen (Pollock et al., 2007). In captive environments such as sea-cages, localised depletion of dissolved oxygen can result from heavy feeding events, overcrowding (i.e. high stocking density) or reduced water exchange due to bio-fouling or dodge tides. It is evident therefore that hypoxic conditions can be intermittent or chronic in nature. The interactive effects of temperature and oxygen have important influences on respiration in fish, especially as increases in water temperature tend to increase metabolic rate due to the imposition of a higher body temperature while at the same time the solubility and availability of oxygen decreases. Unfortunately, both effects increase respiratory demands and place many hypoxia intolerant species under great physiological stress (Barnes et al., 2011).

The second experiment clearly demonstrated that growth rate, AFCR and relative feed intake of juvenile YTK are dramatically and negatively affected under hypoxic conditions. In addition, the results indicate that growth rate and feed conversion of juvenile YTK was maximised at 24°C when oxygen concentrations were maintained at or near normoxic levels (≥ 5mg L^{-1}) by infusing industrial oxygen gas directly into experiment tanks. Growth rate was also maximised at 24°C in the low saturation regime suggesting this temperature is close to the preferred or optimum temperature for this species and is in agreement with Pirozzi and Booth (2009). These results emphasise the importance of providing normoxic conditions in sea-cages for juveniles and have wider implications for the water quality management and production outcomes of larger stock. Importantly, although juvenile YTK appear to be somewhat tolerant of short term hypoxia, it is evident that long term exposure to

hypoxic conditions in sea-cages will severely retard growth potential, worsen FCR and probably result in increased mortality. Similarly, results from experiments 1 and 2 indicate that culture temperatures below 23°C and above 24°C will lead to decreases in growth potential and aberrations in AFCR. Together these outcomes will significantly increase production costs and decrease profitability. It should be noted that some juvenile production of YTK occurs at Whyalla and Port Augusta in the far northern reaches of the Spencer Gulf, South Australia (M. Thomsen CST; *pers. comm.*). These areas of the Gulf experience elevated water temperature and salinity, which are typically higher than the levels recorded at more southerly locations. From the preceding discussion on the effects of high water temperature and salinity on oxygen solubility in water, it is clear that the water quality experienced in the northerly regions of the Spencer Gulf are not conducive to rearing juvenile YTK.

Recent research with mulloway (*Argyrosomus japonicus*) showed their metabolic capacity was impaired by even mild hypoxic conditions (i.e. 75% saturation) and that their critical oxygen concentration is around 1.8mg L^{-1}. At oxygen concentrations below this critical level mulloway became oxygen conformers and lost their ability to maintain their routine metabolic rate (Fitzgibbon et al., 2007). Barnes and colleagues (2011) recently investigated the effect of progressive hypoxia on Tasmanian strains of Atlantic salmon (*Salmo salar*) (150g body weight) to assess if fish were able to regulate there metabolic rate at optimal and supra-optimal water temperatures. The authors found that critical oxygen threshold increased from about 3.42 to 4.59 mg L^{-1} as temperature increased from 14°C or 18°C to 22°C. As far as we can determine critical oxygen limits have not been determined for YTK, however, there were incidences of individual mortality in several tanks allocated to the 27°C hypoxic regime where dissolved oxygen concentration dropped to 2.6mg L^{-1} (≈ 39.5% saturation). These fish were found with flared gill operculum and gill lamellae were anaemic. The duration of hypoxia was not accurately measured in our study and survival is likely to be affected by duration as well as critical threshold limits. More research is needed to determine the critical oxygen threshold limits for juvenile YTK.

The swimming behaviour of YTK juveniles varied dramatically in the hypoxic regime, especially after the second daily feeding event when dissolved oxygen levels were lowest. At these times fish could be seen swimming rapidly in a circular motion around the tank with a wide mouth gape. Individuals often swam in the same direction. This behaviour attenuated to normal swimming behaviour over time and as dissolved oxygen concentrations returned to pre-feeding levels (Figure 5). The swimming response of fishes to hypoxia has been shown to be closely linked with

their niche. For example pelagic fish such as skipjack tuna (*Katsuwonus pelamis*) (Pollock et al., 2007) and southern bluefin tuna (*Thunnus maccoyii*) (Fitzgibbon et al., 2008) increase their swimming speed under hypoxic conditions, apparently as a mechanism for increasing ventilation volume. Some tanks of YTK held under the 27°C hypoxic regime refused to consume any pellets offered at the afternoon feed. On these occasions acute saturation levels were as low as 35-40%. To meet increased demand for oxygen many active fish such as YTK and tuna use ram ventilation as an alternate means of gill ventilation (Clark & Seymour, 2006; Fitzgibbon et al., 2008). Paradoxically, for YTK increasing levels of activity (and feeding) result in increased respiratory demands and thus additional energy expenditure (Clark & Seymour, 2006).

Respiratory systems in fish generally have a set of pacemaker neurons located in the medulla, which act as detector and effector system. This “centre” modulates/regulates metabolic demands based on oxygen availability (pO_2) (i.e. the rate of ventilation must increase when oxygen demand is high or water is hypoxic). Increasing blood flow through the gills is important in keeping lamella pO_2 low, thus increasing oxygen uptake from the water by diffusion (Withers, 1992). An increased gill ventilation rate is therefore the primary respiratory response to increased metabolic demand. Many fish also increase both heart rate and stroke volume to meet increased demand for oxygen. Absolute and factorial aerobic metabolic scope has been studied in larger YTK (2000g) by employing swim tunnel respirometers (Clark & Seymour, 2006). Clark & Seymour (2006) found that maximum metabolic rate for larger YTK (MO_{2Max}; 10.93-13.32 mg min^{-1} kg^{-1}) was induced at swim speeds close to 2.3 body lengths sec^{-1} and was in the upper range of other active fish such as salmon (about $14 \cdot mg \cdot min^{-1} \cdot kg^{-1}$) but below the maximum metabolic rate predicted for tunas (about $27–45 \cdot mg \cdot min^{-1} \cdot kg^{-1}$) (based on other citations by Clark & Seymour, 2006). However, while the absolute aerobic scope of YTK remained fairly constant at approximately $9.5 \cdot mg \cdot min^{-1} \cdot kg^{-1}$ at 20°C and 25°C, the factorial aerobic scope declined by a factor of 1.75 at the higher water temperature. This reduction was due in part to the higher routine metabolic rate of YTK at 25°C, which effectively resulted in a reduction in the available metabolic scope. This led the authors to speculate that YTK has a bell-shaped relationship between absolute aerobic scope and water temperature, such as that reported for species of salmon and trout. This hypothesis may partly explain the increased performance of YTK reared at near optimal temperature under both normoxic and hypoxic conditions in our study. Further, it appears that YTK meet increased metabolic demands by increasing tissue oxygen extraction rate, as neither increases in heart rate nor cardiac stroke volume appear to

contribute significantly towards meeting increased metabolic demands (Clark & Seymour, 2006). Hypoxia may also influence protein metabolism as some fish have been shown to down regulate protein synthesis, or down regulate and (or) modify certain regulatory enzymes in the anaerobic and aerobic pathways to ameliorate hypoxic conditions (Pollock et al., 2007).

As seen in the present study the prevailing concentration of oxygen in the water column has profound effects on the growth potential of YTK. Subjecting juvenile fish to the hypoxic conditions we generated reduced growth potential by 13, 20 and 17% at 21, 24 or 27°C, respectively. These reductions are likely influenced by the low $p0_2$ in experimental tanks, as well as circulatory and cellular interactions of $p0_2$ and temperature on the affinity of haemoglobin for oxygen. Oxygen binding to respiratory pigments is an exothermic process and high temperatures almost universally decrease the oxygen affinity of haemoglobin. It promotes unloading of oxygen at the tissues but inhibits oxygen loading at the respiratory surface (Withers, 1992). Hypoxia and hypercapnia are often highly correlated and the effects of either condition on physiology and survival are difficult to separate. We did not measure CO_2 in the present study, but juvenile YTK were shown to be capable of tolerating and surviving CO_2 concentrations close to 75mg L^{-1} (38mm Hg) for short periods during transport confinement and appear to be reasonably robust with respect to acute CO_2 exposure (Moran et al., 2008). More work is needed to fully understand the physiological and haematological consequences of hypoxia and hypercapnia in farmed YTK.

4.3 Temperature dependent growth model for YTK

Amalgamation of data from the experiment 1 and 2 presented in this report along with other reliable data from experiments carried out on YTK at PSFI were used to construct a mathematical model to predict daily weight gain from known water temperatures and the body weight of fish. The derived model was a reasonably good fit and returned an R^2 value of 0.89. According to the model, growth rate in juvenile fish is maximised at 23°C. Earlier predictions of the growth rate of YTK at temperatures of 20-25°C were made by Booth et al. (2010) in constructing a bio-energetic model for this species. Their model was limited to fish weighing less than 2000g and it did not incorporate a temperature function. The bio-energetic model was based on the function $y = a*GMBW^b$:

i.e. Daily gain (g day^{-1}) = $0.268\pm0.033 \bullet GMBW^{0.52\pm0.02}$ $R^2 = 0.95$

Comparison of the predicted daily weight gain from this model and that of the temperature dependent model at 20°C is reasonably close and indicates the earlier data set of Booth et al. (2010) might be suitable for inclusion into the more recent data set. The effect of temperature regime on the growth rate of juvenile YTK (500-1300g) was also explored by Moran et al. (2009) who found that specific growth rate was almost double (SGR = 1.23% day^{-1}) at temperatures between 17-22°C compared to the growth rate at temperatures between 14-17°C (SGR = 0.64% day^{-1}).

In its present form the temperature dependent model for YTK is somewhat limited in its utility because it does not allow reliable prediction of growth rates over the entire size range under South Australia environmental conditions (e.g. 10-5000g). Incorporation of reliable farm data for both smaller and larger fish will improve the temperature dependent model and allow prediction of growth over the entire production cycle. Nonetheless, the temperature dependent model will be extremely useful as a farm management tool for use with fish up to approximately 1000g. Manipulation of the model will allow forecasting of on farm biomass according to prevailing water temperatures and serve as a benchmark for growth performance. However, it is clear from the research on hypoxia that even subtle changes to key environmental variables are likely to affect the potential growth rate of YTK and these impacts will need to be fully understood when developing a wider ranging growth model using farm data or benchmarking actual growth against predicted growth.

5. BENEFITS & ADOPTION

- New data on the growth rate of juvenile YTK at different water temperatures has led to the development of a temperature growth model. This model will allow managers to reliably predict and benchmark growth of juvenile at different sites and at different times of the year. This model will assist in planning different production stages and may be extended to assist in forecasting feed demand.
- Growth data determined in this research will and is being amalgamated with new farm data provided by CST for use in other Seafood CRC projects (e.g. Feed Management for YTK 2009/728), increasing the utility of the juvenile model.
- Growth data and models determined in this study have been used to assist CST hatchery staff (Arno Bay) plan hatchery runs of YTK to homogenise the size of fish held in the hatchery and transferred to sea-cages.
- Identification of a commercial feed that promoted rapid growth in small YTK (6g) has been adopted for use in the CST hatchery and sea-cages.

- Data and results on the effects of temperature and hypoxia on juvenile YTK has been presented to CST hatchery and production staff in informal technical exchanges to educate and highlight staff to the potential impacts of sub-optimal environmental parameters.

6. FURTHER DEVELOPMENT

- Evaluation of juvenile YTK survival and performance at water temperatures between 12°C and 15°C should be evaluated.
- The critical oxygen limits of different size YTK reared in seacage environments should be determined.
- The potential economic losses of rearing YTK in conditions where acute or chronic hypoxia are prevalent should be examined.
- Additional data on larger YTK (> 1000g) is required to extend the utility of temperature dependent growth models for this species.
- The nutrition of juvenile YTK requires further investigation.

7. PLANNED OUTCOMES

1. Managers will have new information on the effects of fish size, water temperature and dissolved oxygen on the performance of juvenile YTK which will allow them to determine the most appropriate time to stock YTK into sea cages.
2. New stocking regimes will enhance survival of YTK and increase profitability.

The work presented in this section of the final report details a series of manipulative experiments which were designed to address the planned outcomes of project 2008/903.30 – Understanding Yellowtail Kingfish: Sub-project 3. The planned outcomes relevant to the work presented by NSW DPI were that farm managers would have new information on the combined effects of fish size, water temperature and dissolved oxygen on the performance of newly stocked juvenile YTK. This knowledge was sought in order that CST could improve its current faming practices in South Australia and move towards an ‘ideal production strategy’ for juvenile YTK. The premise behind the ‘ideal production strategy’ is that the success of the YTK grow-out phase in South Australia depends heavily on the quality and condition of juvenile YTK entering their first winter. The larger, more vigorous and healthy these juveniles are, the more likely they will reach harvest weight prior to the end of a second summer at sea. Any biological factors, extrinsic environmental or nutritional factors that reduce this vigour will ultimately effect performance and reduce

production efficiency and profit. The work we have undertaken on the effects of fish size, water temperature and hypoxia has clearly demonstrated that survival of juvenile YTK weighing less than 40g is extremely poor at water temperatures around 12°C and even slight reductions in normal levels of dissolved oxygen reduce growth potential and increase FCR. In contrast, an elevation of water temperature by 2-3°C leads to significantly increased survival and improved growth rate. Managers can now use this information to make better informed decisions about the potential risks of stocking new fingerlings into sea-cages; especially when the current or expected water temperatures approach 12°C. This work will also encourage better management of the in-situ dissolved oxygen concentration in juvenile and grow-out sea-cages, especially at time of heavy feeding and high stocking density. The cumulative economic losses of chronic hypoxia are likely to be high (especially at elevated water temperatures), therefore better management of dissolved oxygen regimes will increase profitability through higher growth rates and lower (better) FCR.

8. Conclusions

- It is not advisable to rear juvenile YTK between 6 to 40g in bodyweight at water temperatures ≤12°C. Fish within this size range reared at 12°C became moribund, lost equilibrium, had difficulty feeding and died.
- Irrespective of water temperature, juvenile YTK subjected to chronic low dissolved oxygen concentrations (between 40 and 70% saturation) exhibited 14-20% less growth and 21-38% lower feed intake than normoxic (71 to 106% saturation) controls.
- The growth rate of juvenile YTK ≤ 1000g at water temperatures between 10°C and 29°C can be predicted from a growth model prepared as part of this study.
- Growth rate of juvenile YTK (from 50g to 100g) is optimal at water temperatures between 23-24°C.
- YTK grown in water temperatures below 17^{o}C, or above 28^{o}C, will achieve less than 50% of the growth rate of fish reared at 23-24^{o}C.
- This model will assist farm managers in forecasting growth rates of YTK based on actual ambient water temperatures at a site.

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Appendix 1 – Intellectual Property

There was no new IP arising from this project.

Appendix 2 – Staff

The following people have been instrumental in ensuring the success of the research undertaken on juvenile YTK at PSFI with respect to Australian Seafood Cooperative Research Centre Project 2008/903.30.

Dr Mark Booth NSW DPI
Dr Stewart Fielder NSW DPI
Mr Mitchell Elkins NSW DPI
Mr Luke Cheviot NSW DPI
Ms Jenna Bowyer FLINDERS
Dr Mike Thomsen CST
Ms Erin Bubner FLINDERS
Dr Richard Smullen (RIDLEY)
Mr Adam Pytko (DEEDI)
Dr Geoff Allan NSW DPI
Mr Ian Russell NSW DPI
Mr James Mumby (visiting student)
Mr Luke Vandenberg NSW DPI
Dr David Stone SARDI
Dr Trent D'Antignana FLINDERS
Dr Gavin Shaw (SKRETTING)
Dr Peter Martin (DEEDI)

Appendix 3 – Growth Data used to Build Model

Growth data recorded for YTK from several experiments performed at Port Stephens Fisheries Institute (Aquafin CRC and Seafood CRC data).

Block 1.

Geometric mean Body Weight (g)	Daily Gain (d/day)	Temperature	Geometric mean Body Weight (g)	Daily Gain (d/day)	Temperature	Geometric mean Body Weight (g)	Daily Gain (d/day)	Temperature
47.11	0.58	15.9	7.7	0	12	53.74	1.47	23.1
46.82	0.56	15.9	6.87	-0.03	12	54.82	1.17	23.1
46.37	0.47	15.9	7.26	0.02	12	44.26	0.9	23.1
46.64	0.58	15.9	8.29	0.06	12	46.62	1.02	23.1
45.68	0.49	15.9	7.91	0.04	12	49.94	1.26	23.1
7.34	0.23	15.9	8.64	0.03	12	55.04	1.41	23.1
7.08	0.23	15.9	68.51	0.91	14.8	52.7	1.22	23.1
7	0.2	15.9	68.79	0.87	14.8	53.65	1.47	23.1
7.26	0.24	15.9	68.52	0.88	14.8	88.47	1.75	21.5
7.23	0.22	15.9	69.49	0.87	14.8	82.69	1.57	21.5
7.25	0.23	15.9	69.15	0.89	14.8	69.3	1.76	21.5
47.55	0.66	16.1	67.86	0.96	14.8	75.43	1.72	21.5
47.59	0.81	16.1	12.74	0.42	14.8	82.1	1.93	21.5
47.85	0.77	16.1	13.93	0.42	14.8	89.99	1.82	21.5
47.38	0.64	16.1	12.77	0.41	14.8	86.93	2.12	21.5
46.81	0.71	16.1	53.26	0.09	11.9	88.14	1.87	21.5
46.5	0.5	16.1	54.68	0.34	11.9	110.09	2.6	20
7.36	0.28	16.1	54.34	0.28	11.9	104.38	3.54	20
7.32	0.27	16.1	53.45	0.23	11.9	90.77	3.96	20
7.5	0.27	16.1	52.39	0.18	11.9	96.6	2.67	20
7.12	0.23	16.1	52.63	0.3	11.9	107.34	4.46	20
8.01	0.38	16.1	78.1	1.24	14.7	114.62	3.36	20
7.33	0.26	16.1	77.86	1.16	14.7	113.33	3.91	20
50.83	0.25	13.2	77.63	1.16	14.7	112.53	4.15	20
50.6	0.28	13.2	78.97	1.25	14.7	714.77	8.01	20
48.99	0.53	13.2	78.67	1.24	14.7	703.3	8.31	20
49.42	0.21	13.2	76.83	1.03	14.7	705.08	8.03	20
49.03	0.25	13.2	17.13	0.57	14.7	610.97	2.17	15
8.01	-0.06	13.2	18.48	0.61	14.7	681.26	2.33	15
7.51	-0.1	13.2	17.13	0.57	14.7	687.47	3.41	15
7.5	-0.06	13.2	55.58	0.39	11.9	707.62	6.97	25
8.15	-0.03	13.2	56.97	0.15	11.9	737.5	8.03	25
7.96	-0.04	13.2	55.59	-0.01	11.9	741.77	9.03	25
8.37	0.02	13.2	55.21	0.14	11.9	883	7.89	20
56.95	1.3	16	53.37	0.03	11.9	862	6.52	20
57.55	1.28	16	54.61	0.12	11.9	875	8.1	20
57.46	1.24	16	89.51	1.17	14.6	651	1.59	15
57.3	1.42	16	88.78	1.14	14.6	712	0.55	15
56.98	1.41	16	88.2	1.07	14.6	739	1.39	15
55.58	1.38	16	91.25	1.34	14.6	20.62	1.09	22.6
9.44	0.26	16	90.58	1.27	14.6	21.41	1.22	22.6
10.86	0.23	16	86.96	1.11	14.6	21.97	1.17	22.6
9.67	0.23	16	22.7	0.6	14.6	21.14	1.17	22.6
52.53	0.07	12	24.21	0.59	14.6	58.57	0.26	11.9
52.65	0.11	12	22.51	0.56	14.6	58.35	0.16	11.9
52.54	0.12	12	20.42	1.16	22.6	56.56	0.26	11.9
51.49	0.21	12	22.65	1.28	22.6	56.36	0.11	11.9
50.86	0.16	12	19.57	1	22.6	270	6	22
50.84	0.1	12	20.04	1.02	22.6	315	6	22

Block 2.

Geometric mean Body Weight (g)	Daily Gain (d/day)	Temperature	Geometric mean Body Weight (g)	Daily Gain (d/day)	Temperature	Geometric mean Body Weight (g)	Daily Gain (d/day)	Temperature
859	7.44	25	213	2	16	55.29	0.44	11.9
904	7.72	25	195	2	16	55.12	-0.03	11.9
918	7.5	25	205	1	16	97.99	0.62	14.6
213	3	16	208	4	22	98.6	1	14.6
210	2	16	195	2	22	96.62	0.73	14.6
210	3	16	214	4	22	102.84	1.19	14.6
244	7	22	279	4	16	100.7	0.91	14.6
237	7	22	258	2	16	95.83	0.8	14.6
230	6	22	275	3	16	28.16	0.59	14.6
198	2	16	402	8	22	29.4	0.54	14.6
215	3	16	394	8	22	27.94	0.65	14.6
197	2	16	386	8	22	4.85	0.33	23
220	5	22	237	2	16	125.29	4.76	23
230	6	22	279	3	16	117.38	3.63	23
221	5	22	240	2	16	5.33	0.45	23
195	2	16	332	6	22	121.54	3.99	23
217	2	16	384	9	22	5.56	0.5	23
203	2	16	335	5	22	128.58	5.35	23
236	6	22	243	3	16	129.7	5.01	23
232	6	22	264	2	16	5.3	0.45	23
228	6	22	240	2	16	5.8	0.58	23
195	2	16	341	4	22	5.02	0.34	23
202	3	16	372	7	22	120.66	4.09	23
200	2	16	361	7	22	119.11	3.5	23
248	7	22	236	2	16	5.45	0.48	23
239	6	22	263	3	16	5.6	0.52	23
228	6	22	253	3	16	5.19	0.45	23
208	2	16	413	9	22	127.22	4.84	23
197	2	16	378	6	22	4.75	0.31	23
211	2	16	350	5	22	124.43	4.44	23
236	6	22	264	3	16	5.38	0.45	23
228	6	22	249	3	16	124	4.53	23
220	5	22	271	3	16	128.57	4.84	23
197	2	16	364	6	22	8.97	0	23
194	2	16	358	6	22	10.34	0.57	23
188	2	16	332	5	22	184.82	4.23	23
230	5	22	168.54	4.52	23	13.02	0.85	23
232	6	22	13.24	0.85	23	190.42	4.87	23
238	6	22	172.94	3.95	23	9.91	0.52	23
348	6	22	14.62	1.03	23	100.96	0.42	14.7
374	8	22	201.93	6.06	23	32.84	0.52	14.7
384	8	22	195.66	5.15	23	33.92	0.53	14.7
260	2	16	13.35	0.89	23	32.79	0.5	14.7
232	2	16	16.33	1.17	23	11.44	0.12	14.7
241	2	16	10.91	0.63	23	11.15	0.13	14.7
282	3	22	175.4	4.45	23	11.28	0.11	14.7
237	2	16	171.89	5.1	23	11.9	0.99	21
241	2	16	13.99	0.92	23	11.2	0.97	21
226	2	16	14.61	0.94	23	11.41	0.97	21

Block 3.

Geometric mean Body Weight (g)	Daily Gain (d/day)	Temperature	Geometric mean Body Weight (g)	Daily Gain (d/day)	Temperature
185.2	5.09	23	11.59	1.09	24
13.26	0.86	23	11.71	1.12	24
179.54	3.84	23	11.97	1.1	27
189.11	4.33	23	11.94	1.11	27
12.49	0.78	23	11.78	1.08	27
17.87	0.89	23	11.56	1.1	27
230.36	4.37	23	20.32	1.45	21
219.2	5.09	23	19.35	1.38	21
24.41	1.31	23	19.45	1.35	21
219.63	5.01	23	19	1.43	21
28.47	1.67	23	22.17	2.03	24
262.82	5.22	23	22.08	2.2	24
246.84	4.36	23	22.73	2.31	24
25.07	1.38	23	23.1	2.36	24
31.67	1.79	23	22.52	2.06	27
19.35	1.01	23	23.02	2.26	27
229.19	5.9	23	22.7	2.26	27
221.62	4.07	23	22.17	2.09	27
26.62	1.55	23	31.62	1.77	21
27.15	1.5	23	30.28	1.74	21
24.45	1.39	23	29.96	1.66	21
245.42	5.58	23	30.27	1.78	21
17.25	0.93	23	39.25	2.9	24
232.97	3.68	23	40.5	3.1	24
24.85	1.4	23	41.45	3.01	24
220.12	3.81	23	42.73	3.27	24
242.37	5.96	23	38.36	2.4	27
23.26	1.35	23	40.45	2.65	27
58.64	-0.21	11.9	39.84	2.54	27
57.84	-0.25	11.9	39.3	2.81	27
56.32	-0.28	11.9	44.32	1.82	21
55.87	-0.21	11.9	42.63	1.73	21
56.49	-0.14	11.9	41.76	1.68	21
54.55	-0.1	11.9	42.53	1.65	21
103.87	0.76	14.7	59.93	2.89	24
105.15	0.55	14.7			
101.48	0.42	14.7			
110.08	0.53	14.7			
106.78	0.54	14.7			
11.06	0.9	21			
11.94	1.05	24			
11.25	1.09	24			
62.8	3.13	24			
62.35	2.8	24			
65.57	3.07	24			
54.84	2.2	27			
59.31	2.65	27			
56.95	2.22	27			
58.28	2.44	27			