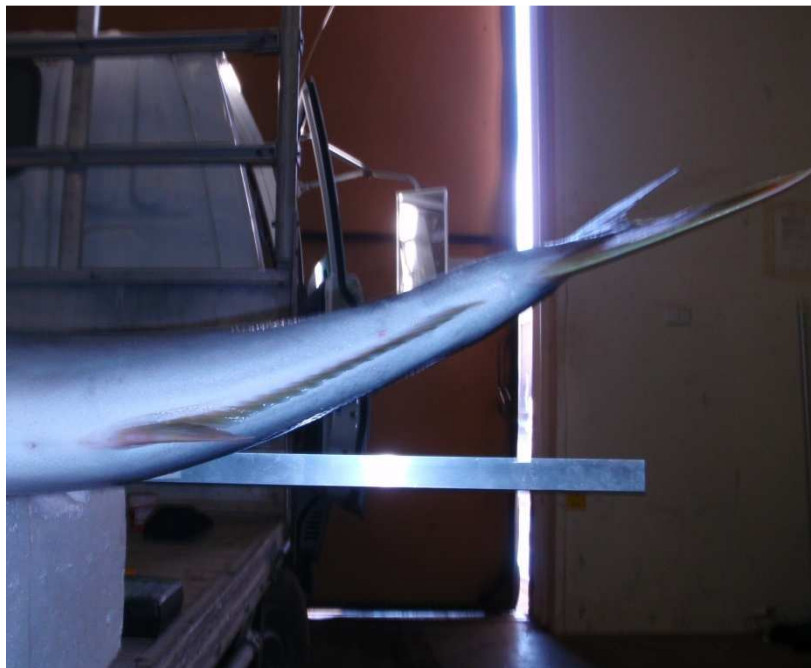# Australian Seafood CRC Project 2008/901

Yellowtail Kingfish Product Quality: Effects of Maturation and Harvest Stress

John Carragher[1], Mark Thomas[2] and Ryan Wilkinson[3]

[1]South Australian Research & Development Institute, Innovative Foods
[2]Flinders University, Lincoln Marine Science Centre
[3]University of Tasmania

DECEMBER 2009

Australian Seafood CRC

Cover photo: Rigor index being measured in a Yellowtail Kingfish at the processing factory.

Disclaimer

The authors do not warrant that the information in this document is free from errors or omissions. The authors do not accept any form of liability, be it contractual, tortious, or otherwise, for the contents of this document or for any consequences arising from its use or any reliance placed upon it. The information, opinions and advice contained in this document may not relate, or be relevant, to a readers particular circumstances. Opinions expressed by the authors are the individual opinions expressed by those persons and are not necessarily those of the publisher, research provider or the CRC.

Table of Contents

2008/901	Yellowtail Kingfish Product Quality: Effects of Maturation and Harvest Stress

PRINCIPAL INVESTIGATOR: Dr John Carragher
ADDRESS: SARDI Innovative Foods
SA Food Centre, Regency Park
PO Box 1671
Adelaide SA 5001
Telephone: 08 8348 2472   Fax: 08 8348 2484

OBJECTIVES:

1. Identify, characterise and quantify the effects of sexual maturity on defined product quality attributes of farmed YTK

2. Determine the extent and nature of the impact of "dead haul" harvest processes and practices on product quality attributes of farmed YTK

NON TECHNICAL SUMMARY:

OUTCOMES ACHIEVED TO DATE

This preliminary study has determined the stress response of 2+ Yellowtail Kingfish harvested by a commercial vessel using a shallow net to crowd the fish, then a fish pump to lift the fish onto the boat, shortly afterwards they voluntarily swim into a chute where they are pneumatically stunned and bled. The information has been used by the company to improve aspects of the process to ensure the fish are harvested as efficiently as possible with little deleterious impact on product quality and shelf-life. The extent of impact by reproductive maturation on product quality and shelf-life has been assessed at a single time (mid-December, near the end of the maturation window) and the information will be used by Yellowtail Kingfish producers to help decide if the limited deleterious consequences of male maturation need to be mitigated through management approaches.

Harvest stress and sexual maturation are known to affect the flesh quality and product shelf life in several species of fish. This preliminary study was undertaken to determine the extent of any impact of harvest stress and/or sexual maturation of males on product quality and shelf life attributes of 2+ farmed Yellowtail Kingfish (*Seriola lalandi*) at the Clean Seas Tuna Whyalla farm site.

Sampling was carried out on the 15-16 December 2008 from the Clean Seas harvest vessel "*Ulysses*". Twenty eight random fish were sampled immediately after they were delivered to the bleed table following crowding, pumping, pneumatic stunning and bleeding as per normal commercial practice ("commercial harvest" treatment). Other fish were sampled after harvest in different ways: 15 fish were hook harvested from the pontoon without being crowded ("hook

harvest" treatment), and 4 fish were netted from the crowded part of the harvest pontoon because they had a swimbladder deformity which results in them rising to the surface of the water ("floaters" treatment). Approximately equal numbers of male and female fish were sampled, however it was not possible to select equal numbers of fish based on their degree of reproductive maturity.

Fish were sampled for blood, carcass and flesh parameters at the time of harvest, then daily for 3 days, and at days 8/9 as fillets. The parameters measured included indicators of stress (blood pH, flesh pH, plasma lactate and cortisol), as well as somatic and reproductive parameters (sex, reproductive stage, fillet yield (skin on and bone in ie the 'Japanese cut' fillet), visceral mass, liver, gonad and visceral fat weight, fillet crude fat and moisture levels), and physical aspects of shelf life and quality (rigor index, carcass and fillet firmness, flesh colour and smell, fillet drip loss).

Only 1 (4%) female fish sampled showed signs of sexual maturation (vitellogenesis), in contrast 67% of males were producing copious amounts of runny milt, and a further 21% were already spent. Thus, 88% of males were demonstrating sexual maturity. However, the male fish with the heaviest testes only had a GSI of 2.9%.

There were few significant differences between male and female fish. Males tended to be slightly heavier (4.46 ± 0.09 vs 4.32 ± 0.09 kg) and have a slightly greater condition factor (1.46 ± 0.03 vs 1.43 ± 0.02) than females. Fillet yield from males was slightly, but significantly, greater than females (3.03 ± 0.06 vs 2.89 ± 0.07 kg; or 67.9 vs 66.8% of bodyweight). This was probably because females had a significantly larger visceral index (308.4 ± 8.7 vs 275.8 ± 8.8 g; or 7.16 vs 6.18% of bodyweight), with significantly more visceral fat (50.4 ± 6.7 vs 26.0 ± 3.2 g; or 1.17 vs 0.58% of bodyweight) than males. There were no differences in liver size (32g; 0.73%), gonad size (24g; 0.56%), fillet crude fat (8.4%) or fillet moisture (66.8%), between the sexes. There were no significant differences between males and females in their physiological stress indicators at harvest, or the physical responses of the carcass post-harvest.

Thus, whilst sex significantly affected carcass attributes with male fish yielding an additional 138g (1.1% of bodyweight; $2.10 per fish at a fillet price of $15/kg) of fillet weight compared to females, it did not appear to deleteriously affect the shelf life of fillet product in this trial.

Despite the small number of Stage 5 (spent) males sampled (n=5), there were significant differences in some product attributes that were related to the degree of maturation with spent fish having a 1.7% lower fillet yield (= 79 g), a 2% lower level of fillet crude fat with a 1.5% higher level of fillet moisture, and showing a tendency to produce proportionally more browner

coloured fillets than running ripe male fish (ie 60% vs 15%) on days 4/5 post-filleting (ie 8/9 days post-harvest).

Physiological stress indicators measured at the time of harvest (eg plasma lactate and cortisol) indicated that commercially harvested fish had higher stress levels than hook harvested fish. It is likely that factors associated with the pre-harvest crowd (high levels of activity, probably limited dissolved oxygen, an ability of most fish to swim against the pump for some time) combine to cause this stress response. Interestingly, however, there were no signs that the stress of the crowd increased with duration suggesting either that the fish cope reasonably well with the process or the stress responses is constant and/or maximal. In contrast to the significant differences measured in stress parameters between the different harvest treatment groups, there were few significant differences in product quality attributes or shelf life. Thus, even the "floater" fish that had the highest levels of stress indicators at time of harvest had flesh quality characteristics (pH, firmness, drip loss, colour) that were similar to hook harvested (low stress) fish.

At this stage it is not possible to draw any conclusions about the relative stressfulness of the commercial harvest procedure employed in this preliminary study. It is not appropriate to extrapolate absolute stress response parameter values from Yellowtail Kingfish to other species where there is more data and understanding (eg salmonids) to try to 'calibrate' the magnitude of stress response. Instead, additional experiments would be required whereby Yellowtail Kingfish are exposed to various acute and chronic stressors (confinement, crowding, poor water quality) in order to increase the understanding of stress responses in this species.

Taken together, these product quality data suggest that Yellowtail Kingfish is a robust fish that, although stressed by harvest procedures, shows little, if any, deleterious impact on the quality and shelf life of the resulting chilled product.

KEYWORDS: Yellowtail Kingfish, product quality, harvest stress, maturation.

Acknowledgements

Background

Harvest stress and sexual maturation are known to affect the flesh quality and product shelf life in several species of fish (harvest stress: Erikson et al., 1997; Jerrett et al., 1996; Lowe et al., 1993; Ostenfeld et al., 1995; Robb et al., 2000; Roth et 2006; Thomas et al., 1999; and maturation: Aksnes et al., 1986; Bilinski et al., 1984; Reid et al., 1993; Roth et al., 2007).

Customer feedback to Clean Seas Tuna's (CST) Yellowtail Kingfish (YTK) farming operations have reported a consistent dip in product quality attributes (mostly soft flesh and poor shelf-life) in November-December, and in 2008 the extent of this problem was more noticeable than over the previous 2 years. The problem is being manifested in an increase in customer complaints and in particular from existing customers producing sashimi and sushi, who are the most discerning of customers. Product quality complaints include reports of flesh appearing "old" in appearance, soft and gaping, which have been previously described in similar episodes with salmon and halibut in Northern Europe where the procedures immediately pre-harvest were sub-optimal or where maturing males have been implicated (Mike Thomson, pers. comm.).

The reason for the drop in product quality has not been clearly identified, however there are several factors which need to be considered:

1 - a greater volume of fish are being harvested and sent to a greater number of customers

2 - the extent of sexual maturation of harvest-size YTK in 2008 seems to be greater than previous years and

3 - the company has introduced a new harvesting process.

The new harvesting process is known as a "dead haul" process, which is increasingly recognised as one of the most efficient harvest systems being actively introduced by major salmon farming companies in Norway, Scotland, Canada and Australia. It is a process that involves killing the fish at the cage side, followed by rapid cooling of the product in tanks or bins on the vessel or a barge tied alongside and transport of a larger volume of fish than was possible with the more traditional "live haul" method. At CST, a percussive stunning machine delivers a blow to the head in order to render the fish insensible before the main blood vessels exiting the heart and gills are cut to kill the fish by exsanguination. In order to gain efficiencies, a fish pump is used to transport the fish from the cage side into the harvest system instead of the traditional brail net method.

The company needed to determine to what extent each of these factors could be affecting the quality of the product as perceived by the customers.

This project addressed the 2nd and 3rd factors - maturation and harvest stress.

5

Need

Ultimately, the need is to improve the quality of the product at the time of year when mature males occur (October-December) and when sea temperatures begin to peak. If mature males are shown to contribute significantly to product quality issues at this time, then remedial measures can be investigated which are proportional to the scale of the problem.

Information on the extent of the impacts of maturation and/or harvest stress on product quality of YTK will help all involved in growing, buying and selling YTK in domestic and overseas markets.

Objectives

1. Identify, characterise and quantify the effects of sexual maturity on defined product quality attributes of farmed YTK

2. Determine the extent and nature of the impact of "dead haul" harvest processes and practices on product quality attributes of farmed YTK

Methods

Experiment 1 - The effects of sexual maturity on product quality attributes of farmed YTK

The only way to adjudge reproductive status of commercially harvested YTK whilst alive or immediately after they are euthanised is to determine whether the fish are spermiating (white fluid, which may be runny and copious or viscous and slight, is released from the gonopore when the flanks of the belly are massaged and stripped by hand; in which case they are mature males) or not (in which case they could be immature males, immature females or mature females).

YTK from 2+ class (ie spawned in October-November 2006)(n= 15 comprising 8 spermiating, and 7 non-spermiating fish) were hook harvested from at sea pontoons and killed within 30 seconds with a spike to the brain to avoid the stressors involved in the 'dead haul' harvesting approach. Another group of 12 fish (again 6 spermiating, 6 non-spermiating) were hooked out and put into a deck tank for 20 hours to try to exert maximum stress. Unfortunately, despite running deck hoses through the tank, the fish were dead the next morning and no sampling was possible. The hooked fish data would be useful as 'unstressed' controls for Experiment 2.

Brain spiked fish were immediately sampled as described below ("on board sampling") to measure physiological stress indicators and subsequent product quality attributes. After sampling, all the experimental fish were tagged through the mouth/operculum with a two-headed cable tie furnished with a unique colour-number code tag, looped onto a fixed line (to aid their relocation) and put into a chilled deck tank for several hours.

The experimental fish were unloaded from the deck tank into an ice slurry bin at the same time as the remainder of the commercial harvest when the *Ulysses* arrived at the Whyalla marina. They were then driven to the processing factory and remained in the ice slurry bins until resampled the following morning. Additional measurements, described below ("rigor sampling"), were taken from each carcass at the factory and for 3 days afterwards. Between the daily sampling episodes the fish were returned to the ice slurry. Following the 4th day rigor sampling the carcasses were processed to skin on/bone-in "Japanese cut' fillets by an experienced commercial YTK filleter and additional physical parameters were measured (see "morphometric sampling" below). Filleting was carried out on day 4 for 2 reasons: (1) to allow rigor changes to develop and then resolve, and (2) because most YTK will be send to wholesalers/processors as chilled whole fish, and filleting will happen 3-7 days post-harvest immediately before sale. One of the resulting fillets was refrigerated for a further 4-5 days and more sampling carried out ("fillet shelf life sampling").

It was intended that Experiment 1 would be replicated on successive days with the intention of pooling the data for analysis if there were no significant differences between days. However the plan failed because on the first experimental day (after 3 fish had already been hooked and sampled) the fish that had been crowded for the 'dead haul' harvest (Experiment 2 – see below) but not used, were released back into the section of the harvest cage that had been less disturbed. It was therefore not possible to ensure that only 'unstressed fish' were hooked on that day.

Experiment 2 - The extent and nature of the effects of "dead haul" harvest and post-harvest processes and practices on product quality attributes of farmed YTK

YTK from the 2+ year class were harvested using the 'dead haul' harvesting approach on the vessel *Ulysses*. YTK were drawn up from the net crowd by the fish pump, delivered to the whirlpool in preparation for their subsequent rapid descent though the percussive stunner and gill bleeder. Random fish were then selected from the 4 hour harvest to see how crowding time/conditions affected physiological stress indicators and subsequent product quality attributes. After "on board sampling" measurements (described below), the fish were tagged and put on the conveyor to go through the normal dry/wet bleed and deck tank cooling processes. As carried out in Experiment 1, the sampled fish were unloaded with the commercial harvest when the *Ulysses* docked at Whyalla marina and placed in ice slurry in fishbins. "Rigor sampling", "morphometric sampling", and "fillet shelf-life sampling" was carried out as per Experiment 1.

On the first sampling day 20 YTK were sequentially harvested and sampled from the beginning to the end of the commercial harvest. On the second harvest day it was decided to only sample 10 fish from the last hour of the commercial harvest process. The commercial harvest process took approximately the same number of fish and amount of time on each day.

On Board Sampling

Immediately after the brain was spiked (Experiment 1) or the fish came out onto the dry bleed conveyor (Experiment 2), a series of samples were taken and measurements made on each fish. They were:

- Blood samples (analysed for pH, lactate and cortisol levels)
- Flesh pH
- Flesh lactate
- Rigor index
- Carcass firmness (by penetrometer and Durofel meter)
- Spermiating (yes/no)

Rigor Sampling (Days 1, 2, 3 and 4 post-harvest)

- Flesh pH
- Rigor index
- Carcass firmness (by penetrometer and Durofel meter)
- Eye cloudiness (subjective score)

Morphometric Sampling

On day 4 post harvest the carcasses were processed by an experienced YTK filleter and the following parameters were measured.

- Fork Length (cm) and whole weight (kg; to calculate Condition Index)
- Fillet weight (% yield)
- Viscera weight (to calculate a gut loss %)
- Sex (by visual inspection of the gonads)
- Gonad weight (to calculate GSI)
- Gonad stage (macroscopic staging)
- Visceral fat weight (to see if maturing fish are utilizing energy stores)
- Liver weight (to calculate HSI)
- Fillet crude fat and moisture (%)

Fillet Shelf Life Sampling (Days 4 and 8/9 post-harvest)

Product quality attributes:

- Fillet firmness (penetrometer, Durofel meter and subjective score)
- Fillet gaping (subjective score)
- Fillet drip loss (%)
- Fillet pH
- Fillet colour (digital camera and subjective score)
- Fillet smell (subjective score)

Carcass Temperature Logging

Product quality and shelf life can be greatly influenced by post harvest handling, particularly poor temperature control. To determine if this was a problem in the present study small programmable temperature loggers (iButton^{TM}; programmed to record temperature every 2 minutes) were placed inside ziplock bags and affixed to a two-headed cable tie, and pushed down the oesophagus of selected fish (from the start, middle and end of each day's sampling) into the stomach. The loggers were identified and retrieved using the protruding cable tie when the fish were filleted on day 4. Additional loggers were cable-tied to the fixed nylon lines that were deployed in the deck tanks and ice slurry bins containing the experimental fish during the same times.

Methodological Details

pH – Flesh and blood pH were measured using a spear probe (Ionode 573) connected to a TPS meter (model WP80). Each day prior to use the probe and meter were calibrated using two pH standards (pH 4.0 and 7.0). The tip of the probe was washed and/or wiped between samples. Blood pH was measured twice, once immediately after the sample was transferred from the syringe to the storage tube, the second time was 12-14 hours later after the tube had been uncapped for 30 minutes. The aim of measuring blood pH twice is to determine the relative contributions of lactic acid and carbonic acid to the initial measurement. Allowing the carbonic acid to de-gas (as carbon dioxide) before the second measurement leaving just the lactic acid helps us understand the metabolic condition of the fish at the time of harvest. Flesh pH was measured through a slit made in the skin of the fish midway between the lateral line and the dorsal fin behind the head. The probe was pushed into the musculature until the reading stabilised.

Flesh and fillet firmness – a hand-held penetrometer (model FT 327 Fruit Tester with a flat tip 15 mm in diameter made by Instituto di Coltivazioni Arboree, Università di Milano, Italy) was loaned from Steve Slattery (Queensland DPIF). The penetrometer works by measuring the force required to compress a spring. The force is shown on a gauge and a sweep needle indicates the appropriate value. Depending on the firmness of the sample being tested, the penetrometer plate can penetrate the material.
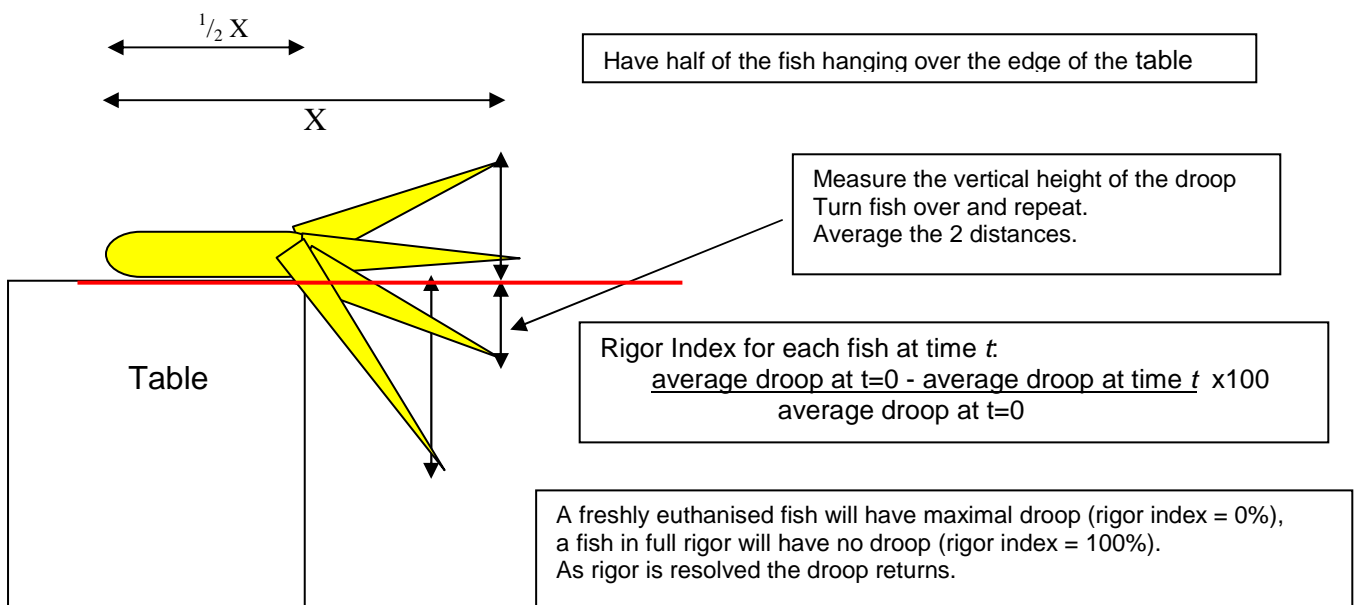
Flesh and fillet firmness – the Durofel (Setop Giraud-Technologie, France) is an electronic meter connected to a probe with a stainless steel retractable head. It was initially designed to measure the firmness of fruit such as cherries. When the probe is placed onto the surface being tested, the force needed to make the head retract a fixed distance is recorded. To allow for wide differences in product firmness, the Durofel has several heads each with a different surface area that can be fitted. Two heads were used in the present study, the $0.10cm^2$ head for whole fish, and the $0.50cm^2$ head for fillets. The main advantages of the Durofel are that it does not penetrate the flesh (and is therefore non-destructive) and that it can accurately measure the firmness of softer materials than can a penetrometer. Durofel and penetrometer values are similar in that, the lower the value, the softer the material being tested.

Both types of flesh firmness meters can become inaccurate due to material (in this case fish mucus, scales and clotting blood) affecting the free travel of the head or piston. Several times during the study the meters had to be taken off-line until they were properly cleaned.

Plasma cortisol – was measured by tritiated radioimmunoassay using a commercially available antibody (Sirosera™ C-3368 supplied by Krius Pty. Ltd.; Pankhurst and Sharples, 1992). Frozen plasma was thawed, extracted with ethyl acetate and assayed in duplicate with overnight incubation. Separation was with ice cold charcoal solution. Supernatant was decanted, mixed with scintillation cocktail and counted in a beta counter. Extraction efficiency was 92% and the minimum detectable concentration was 1.25 ng/ml.

Plasma lactate – lactate concentrations were determined from duplicate 1 or 5 µl plasma samples using an Analox GM7 Analyser (Series II) as per manufacturers instructions. The detection limit using this method was 0.3 mmol l^{-1}.

Rigor index – This was measured using the 'droop' method (Lowe et al., 1993) as described in the diagram below.

Have half of the fish hanging over the edge of the table

Measure the vertical height of the droop
Turn fish over and repeat.
Average the 2 distances.

Rigor Index for each fish at time t:
$$\frac{\text{average droop at t=0 - average droop at time } t}{\text{average droop at t=0}} \times 100$$

A freshly euthanised fish will have maximal droop (rigor index = 0%), a fish in full rigor will have no droop (rigor index = 100%). As rigor is resolved the droop returns.

Eye clarity – A subjective assessment of eye clarity/opacity was made on both eyes of each fish by one researcher. The scale used was none (0), some (1), moderate (2) and dense (3). Typical examples of each of the categories are shown in Fig 1.

Fillet drip loss – One fillet from each fish was selected and tagged. The fillet was weighed to the nearest gram and then put into a plastic bag (open at one end) and laid flat in a walk-in cold room. On subsequent sampling days the fillet was removed from the bag, blotted dry with paper towel, and reweighed. The fillet was then photographed and assessed for gaping before being returned to the plastic bag and re-refrigerated.

Subjective Fillet Attributes – An experienced independent assessor (Mr Mohan Raj, 15 years experience in seafood processing including 6 years as plant manager in Nile Perch processing plants exporting chilled fillets to the EU and US) evaluated each fillet on the last day of the study. His scoring scheme for each attribute is shown below.

- o gaping – 3 levels – none, some evident, obviously evident (see Fig 2)
- o colour – 3 levels – good, slight browning, brown; particularly in the anterior/dorsal (thickest) part of the fillet (see Fig 3)
- o smell – 2 levels – good, stronger
- o flesh firmness – 4 levels – soft, slightly soft, firm, very firm

Fillet crude fat and moisture – A 2 cm wide strip of flesh was cut dorso-ventrally from one fillet, just posterior to the vent (Fig 4). The 2cm wide strip of tissue was homogenised in a blender and samples taken for fat and moisture analysis.

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 minced 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 mins, 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.

Moisture was measured by drying a weighed amount of minced fillet (~50g) in a drying oven at 60°C overnight. The amount of weight lost was used to calculate the moisture level in the original tissue.
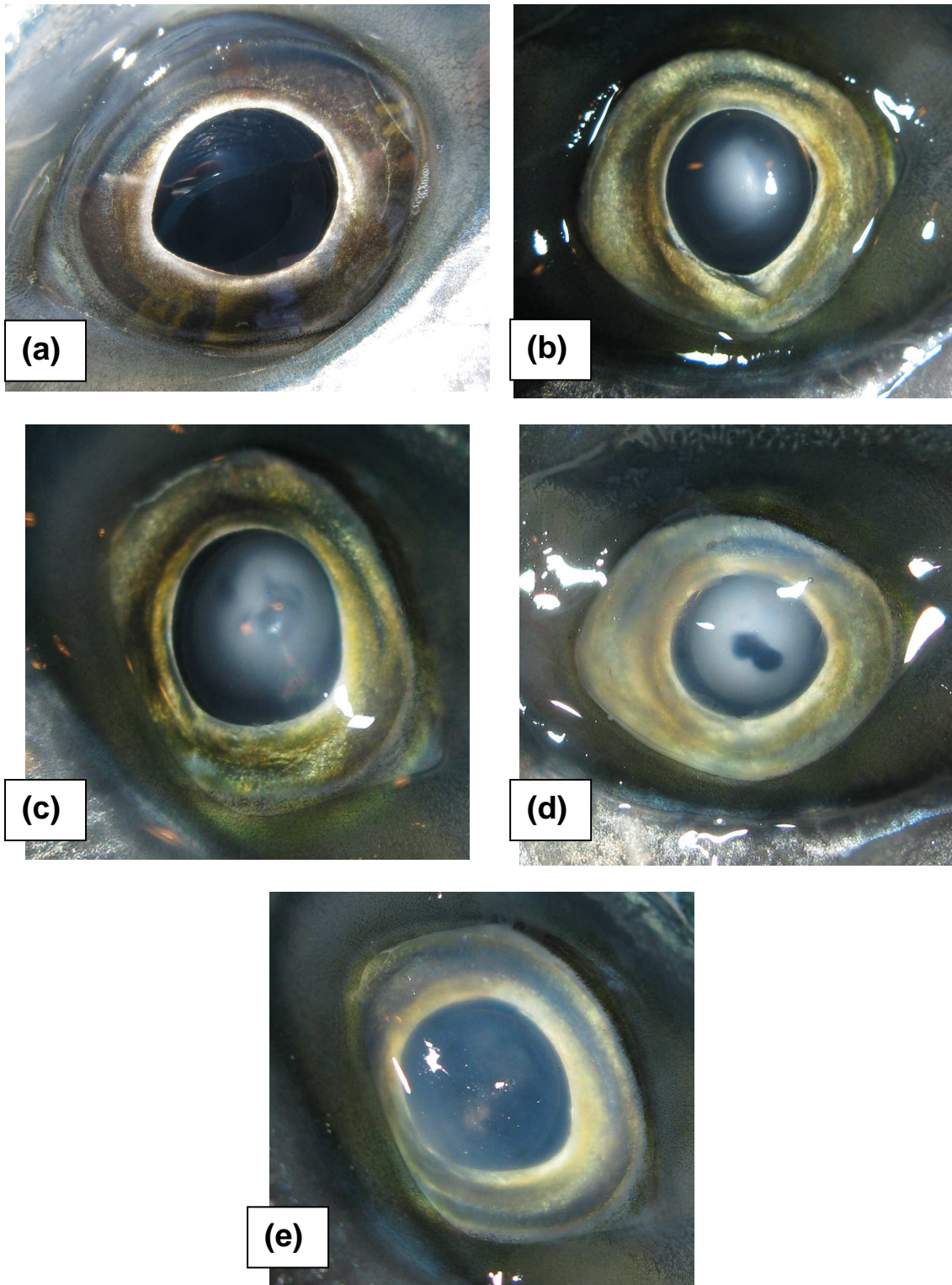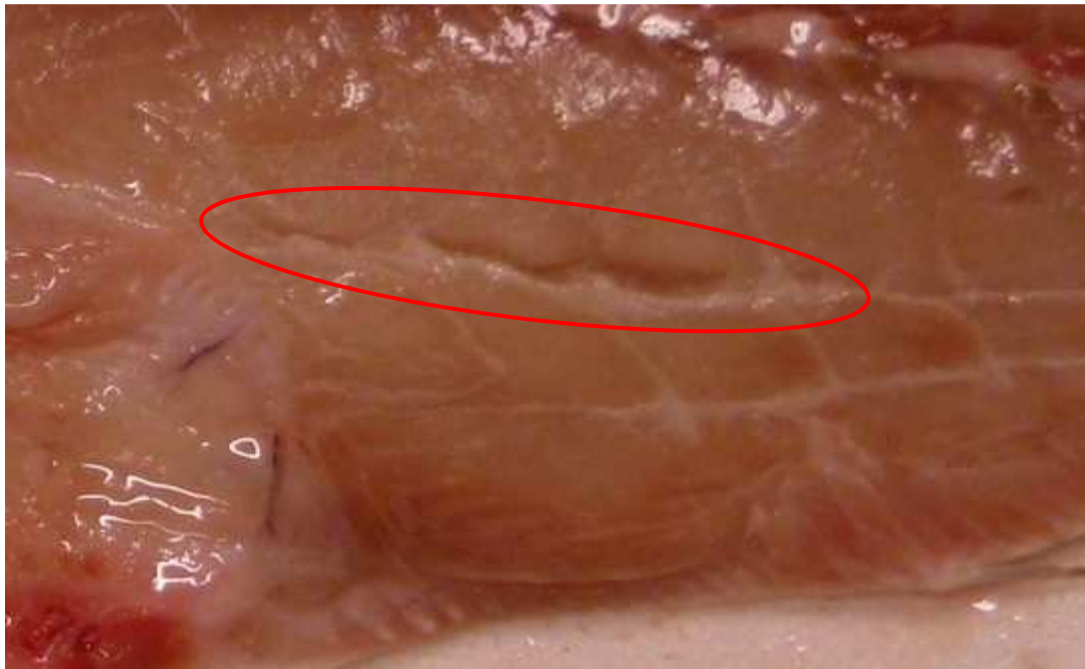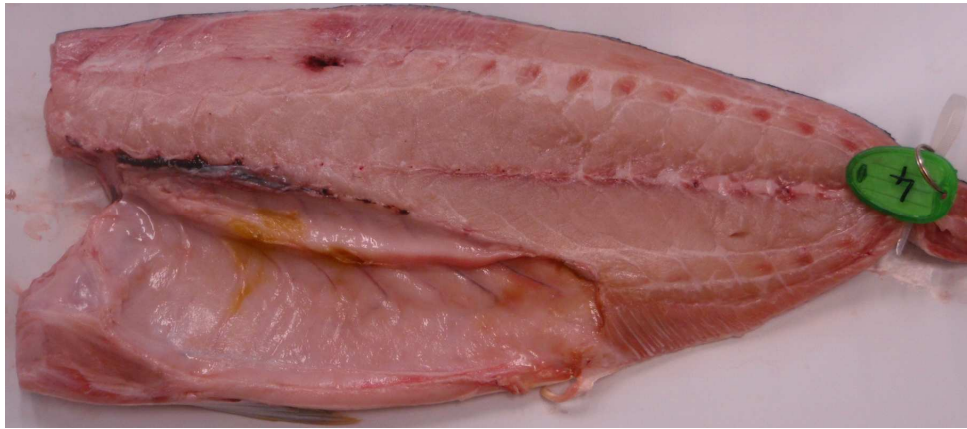
Figure 1. Eye Clarity. Typical photographs of eyes that were scored as (a) 0 – none, (b) 1 – some, (c) 2 – moderate, and (d) 3 – dense cloudiness. Picture (e) shows the eye after the cold cataract has largely resolved.
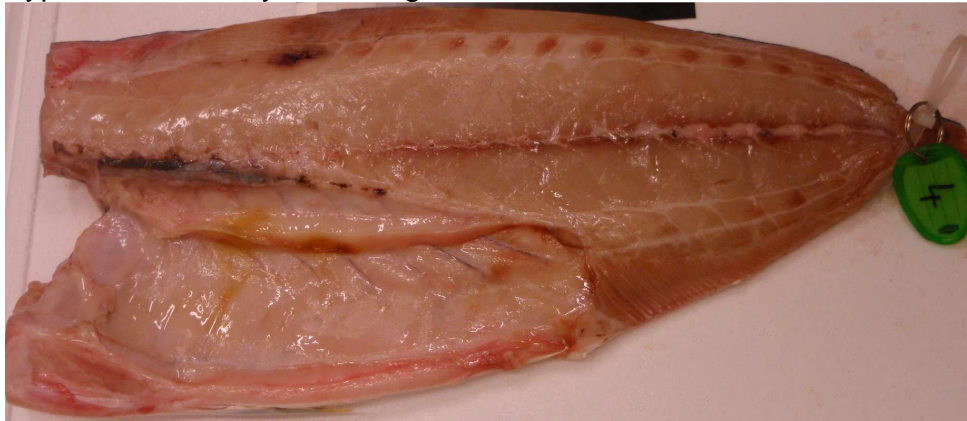
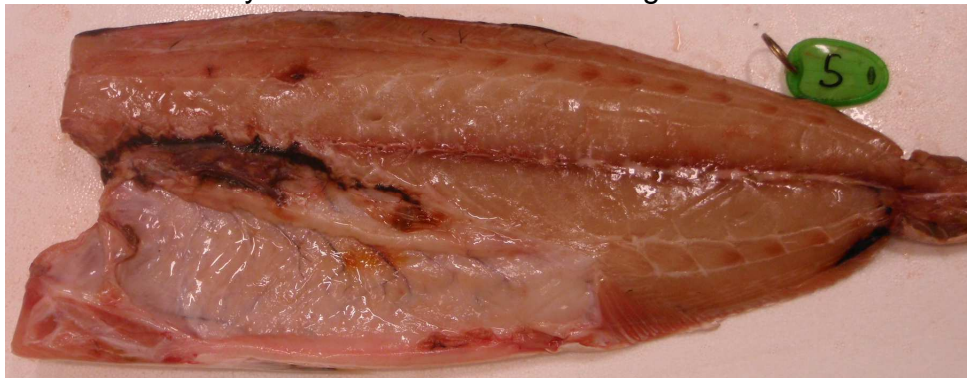Gaping – Score "some evident" – area in red oval.

Figure 2. Gaping scores. Gaping – Score "obviously evident" – area in red oval. Flesh coring tool made the indentation shown in blue circle.
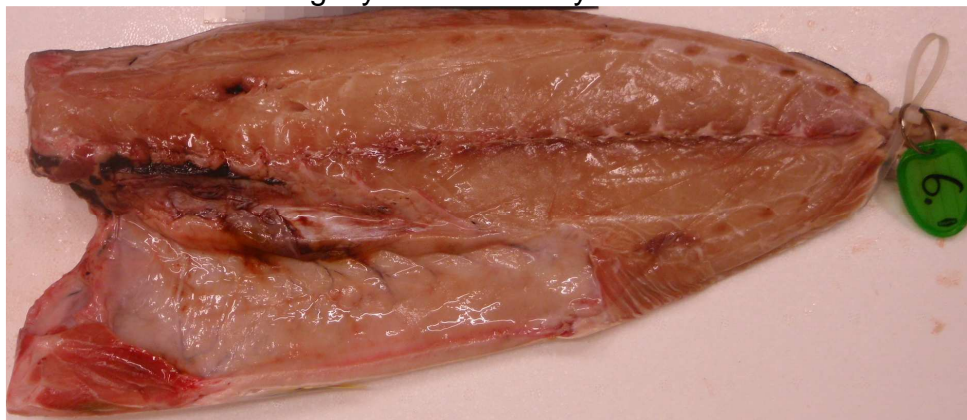
Typical fillet on day of filleting

Same fillet on day 8/9 – colour assessed as "good"

Fillet assessed as "slightly brown" on day 8/9

Fillet assessed as "brown" on day 8/9

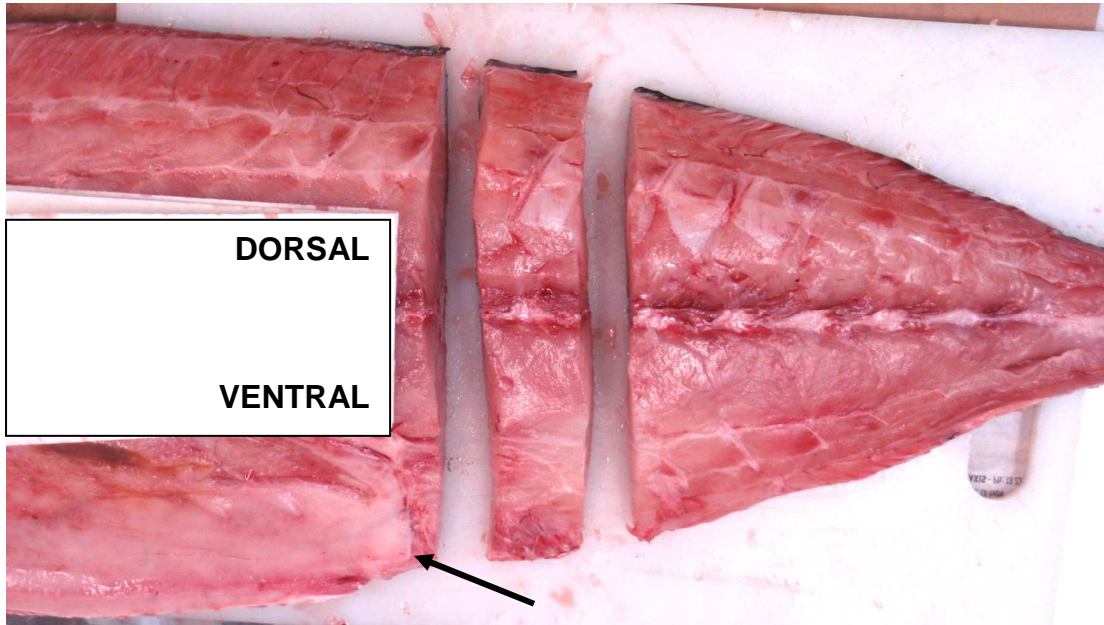**Figure 3.** Fillet colour score.

Figure 4. 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.

Macroscopic gonad staging – The following staging system was used (after Pankhurst, 1987).

Males

Stage	Description
1	Testes short, thin (~2mm Ø) opaque threads, pink in colour
2	Testes elongating and thickening (~5 mm Ø), pink/ivory in colour
3	Testes larger (~10mm Ø), ivory in colour, small volume of viscous white milt when testes cut
4	Testes large (>15mm Ø), ivory in colour, copious white milt when testes cut (classified as 'spermiating' when fish is stripped)
5	Testes shrivelled, dark grey/black in colour, small volume of watery grey exudate when testes cut

Females

Stage	Description
1	Ovaries short and thin (~2mm Ø) translucent threads, pale pink/orange in colour
2	Ovaries elongating and thickening (~5 mm Ø), orange in colour, no granularity apparent in matrix when cut
3	Ovaries larger, orange in colour, obvious granular appearance of matrix when cut (ie vitellogenic oocytes are visible)
4	Not seen
5	Not seen
6	Not seen

At the time the study was conducted (mid-December) it was late in spawning window (Oct-Dec; Joe Cuira pers. comm.; Clean Seas Tuna, unpublished data), so it can be considered that males classified as Stage 1 or 2 were immature, and Stages 4 and 5 were mature. Similarly, with the females, Stages 1 and 2 were immature, Stage 3 was mature.

Histology – Transverse slices (3-4mm thick) were taken from the mid-section of one or two gonads (depending on size) from each fish, secured inside a 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 gonad stage based on the types of cells and characteristic development observed.

Statistical Analysis

Data were analysed by T-tests, one- and/or two-way analysis of variance (ANOVA). Two-way ANOVA was used to determine the affect of harvest type and sex on the results obtained. Regression analyses were performed to determine if there was a significant relationship between stress and product quality attributes due to sampling order (sequential commercial harvest), between carcass attributes (eg fillet crude fat and moisture) and biochemical parameters (eg plasma lactate and blood pH values). All data are shown as mean ± standard error of the mean (sem). Probability (P) values are given in the figure legends. Statistical significance was $P > 0.05$ and significant differences are shown in bold text. All analyses were carried out using the Data Analysis toolpack in the Microsoft package Excel 2000.

Results

Somatic Data

Based on macroscopic appearance of the gonads 45 of the 47 fish sampled during the study could be sexed; 23 female and 22 male fish. The other two fish (4%) had to be sexed by histology of the gonad that indicated they were males. Although some efforts were made to try to get even numbers of each sex, particularly for the hook harvest groups where it was difficult to identify spermiating males, the sex ratio in the overall population being harvested at this time did appear to be close to 1:1.

Macroscopic and histological appearance of ovaries led to 22 females being classified as Stage 2, and 1 was classified as Stage 3 (Table 1). Stage 2 females had an average GSI of 0.45%, the Stage 3 fish was 2.2% (ovary weight of 106g; Fig 5). The GSI of the Stage 3 female was larger than the GSIs of the Stage 4 and 5 males (compare Tables 1 and 2). Gonadal development data from the male fish is shown in Table 2, but is described in more detail in the following section.

Table 1. Reproductive development stage, number of fish sampled and % of total, and mean gonadosomatic index (GSI % ± sd) of the female fish sampled in the 2 days of the experiment.

Stage	Maturity	Number (%)	GSI % (Mean ± sd)
1	Immature	0	-
2	Immature	22 (96%)	0.45 ± 0.07
3	Mature	1 (4%)	2.2
4	Mature	0	-
5	Mature	0	-

Table 2. Reproductive development stage, number of fish sampled and % of total, and mean gonadosomatic index (GSI % ± sd) of the male fish sampled in the 2 days of the experiment.

Stage	Maturity	Number (%)	GSI % (Mean ± sd)
1	Immature	2 (8%)	0.05
2	Immature	1 (4%)	0.45
3	Mature	0	-
4	Mature	16 (67%)	0.72 ± 0.68
5	Mature	5 (21%)	0.15 ± 0.08

Histology of the ovaries confirmed the macroscopic assessments in all but 2 fish – one female assessed as a Stage 3 was not found to be undergoing vitellogenesis so was reassigned to Stage 2, one Stage 2 male was found to be a Stage 2 female (human error rate of 4%). Stage 2 females had many primary oogonia and occasional oocytes which were undergoing cortical alveolar accumulation and endogenous, but not exogenous, vitellogenesis (Fig. 6). The one Stage 3 fish had many oocytes that were readily visible to the naked eye (~0.5mm diameter), unfortunately histology was not possible as the eggs became dehydrated and fell out of the slots in the cassette when placed in the fixative.

Stage 3 ovaries (106g) from fish G12. Scale: scalpel (blade and handle) is 18cm long.
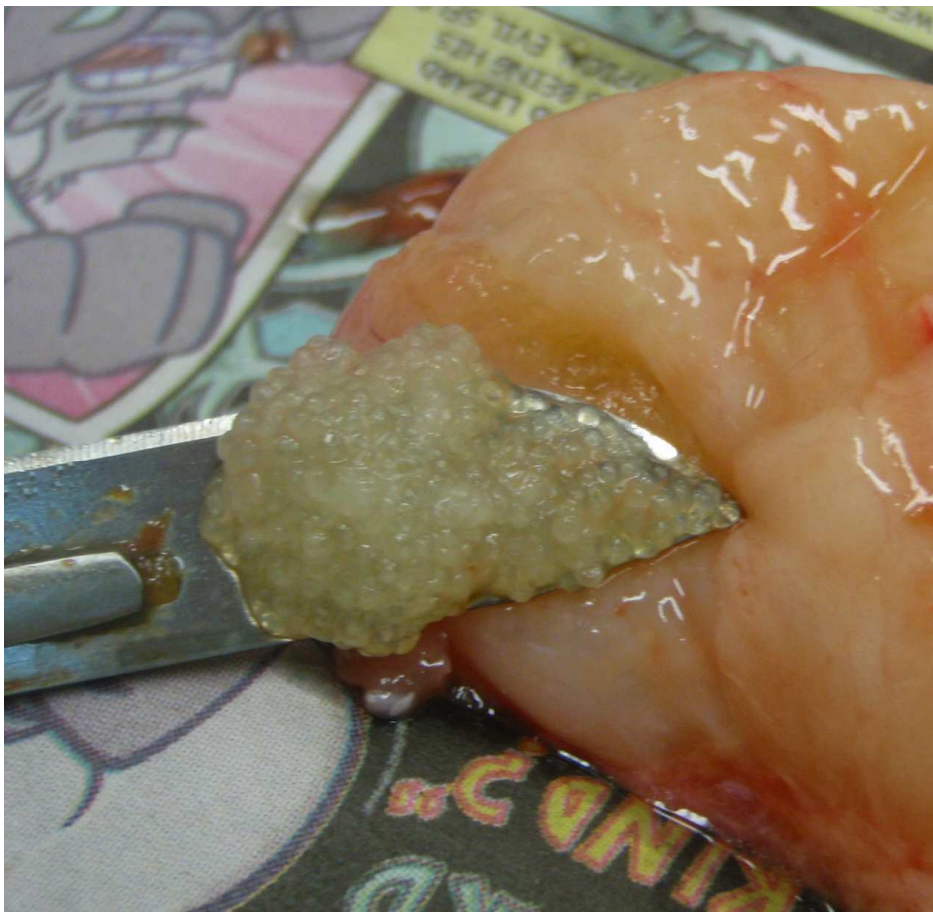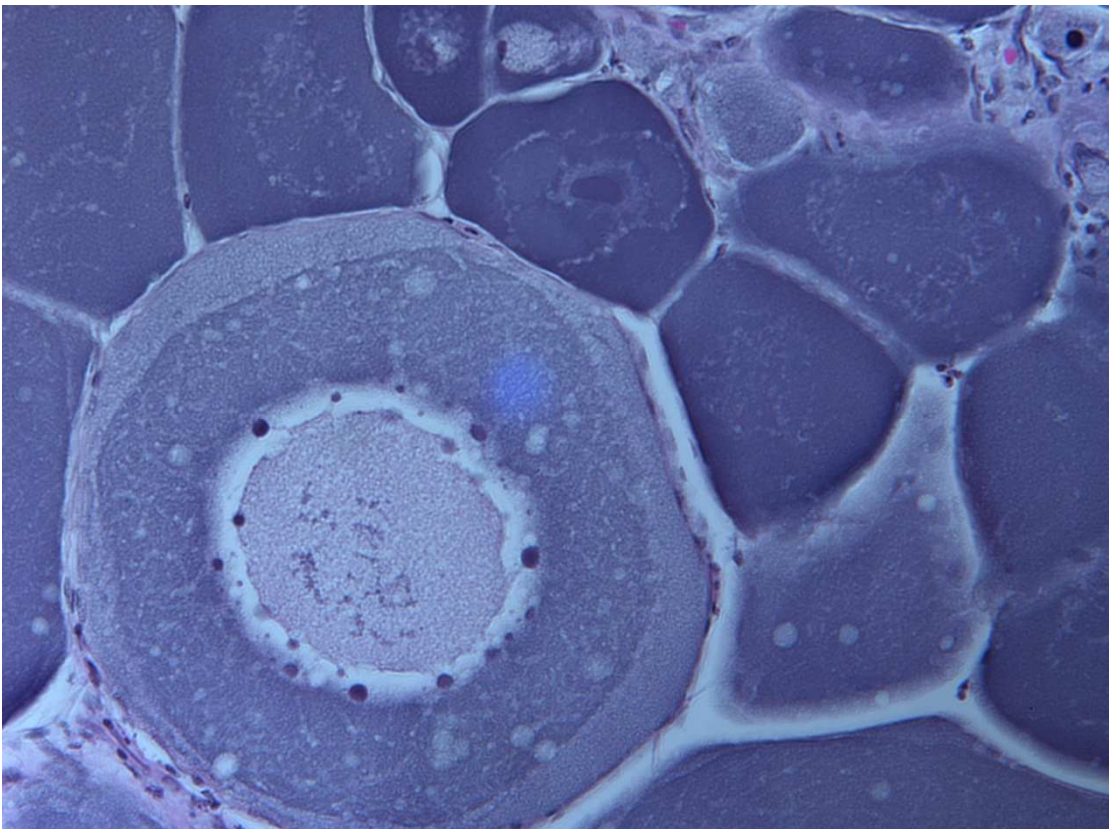

Close up of vitellogenic eggs removed from Stage 3 ovary. Scale: width of scapel blade is 12mm.

Figure 5. Female gonad.
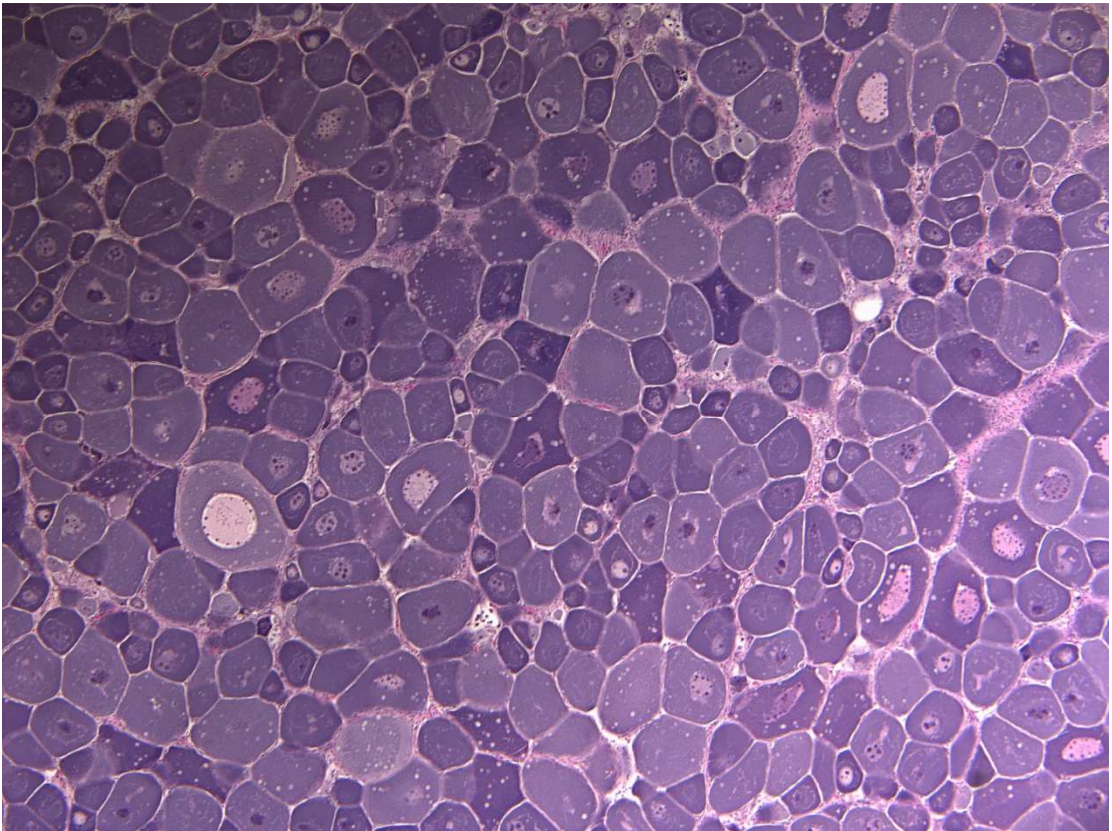
Fish Y16 – Stage 2 (x10)

Fish Y12 – Stage 2 (x60)

Figure 6. Female histology.

Male fish tended to be heavier (4.46 ± 0.09 vs 4.32 ± 0.09 kg) and have a higher condition factor than female fish (1.46 ± 0.03 vs 1.43 ± 0.02; Figures 7 & 8).
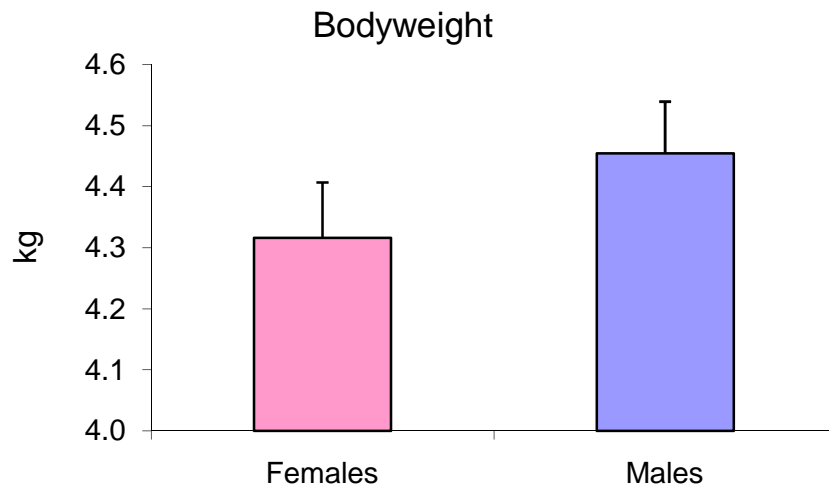

Figure 7. Bodyweight (kg; mean ± sem) of male (n=24) and female (n=23) fish sampled in this study. Difference P=0.280; not significant.
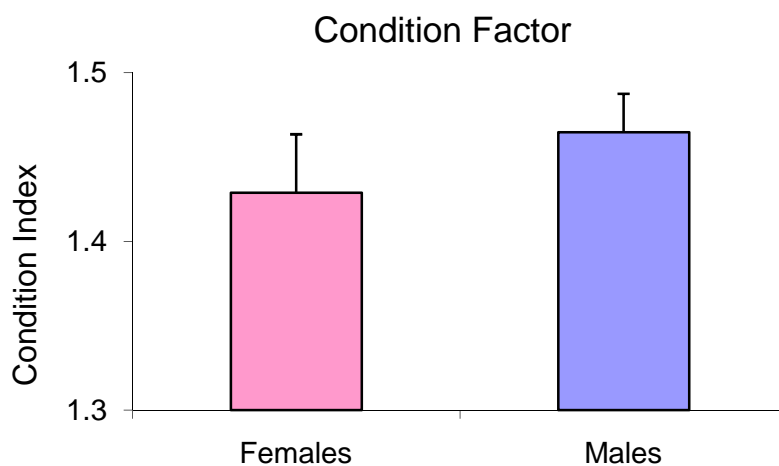
Figure 8. Condition factor (bodyweight (g)/fork length (mm)^3 x 100; mean ± sem) of male (n=24) and female (n=23) fish sampled in this study. Difference P=0.406; not significant.

Overall carcass composition (comprising Head and Frame, Fillet and Viscera components) for males and females is shown in Figures 10 and 11. Statistical comparisons of the data are made below.

Carcass Components (kg)
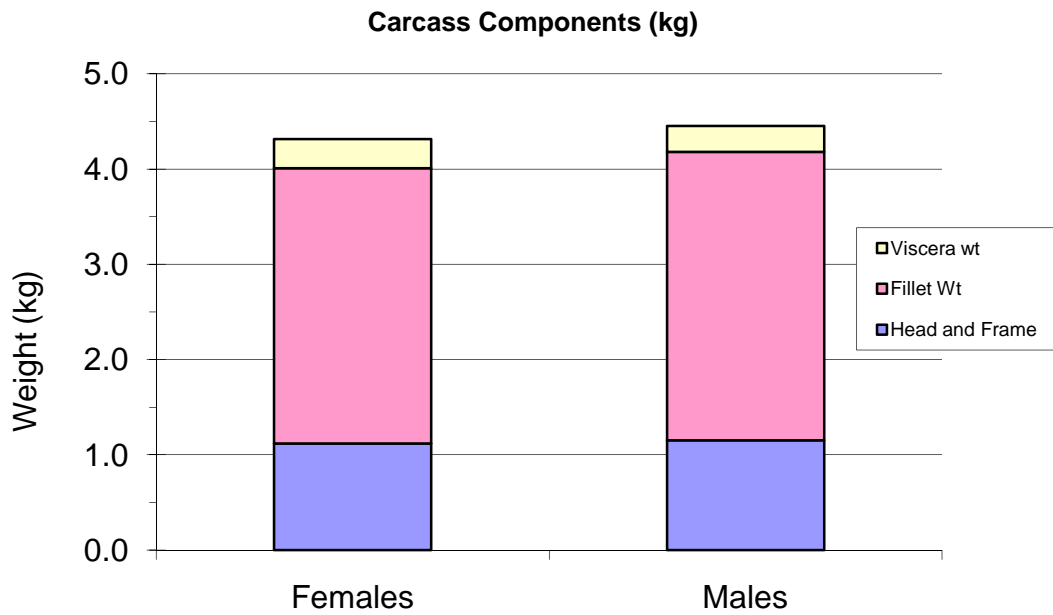
Figure 9. Carcass composition (weight kg) of male (n=24) and female (n=23) fish sampled in this study.
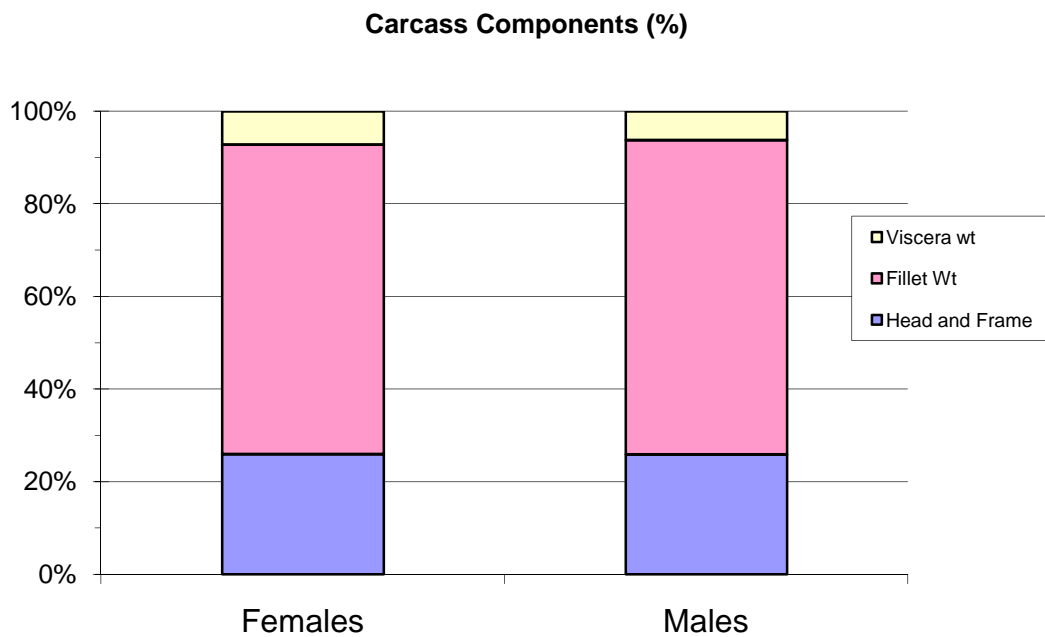
Carcass Components (%)

Figure 10. Carcass composition (as a % of bodyweight) of male (n=24) and female (n=23) fish sampled in this study.

There was no difference in Head and Frame weight or % between the two sexes, however there was a statistical significance in fillet yield with male fish yielding 3.03 ± 0.06 kg of fillet vs 2.89 ± 0.07 kg for the females (Figure 11). Fillet yield (skin on, bone-in) as a percentage of whole body weight was 67.9% for males and 66.8% for females.
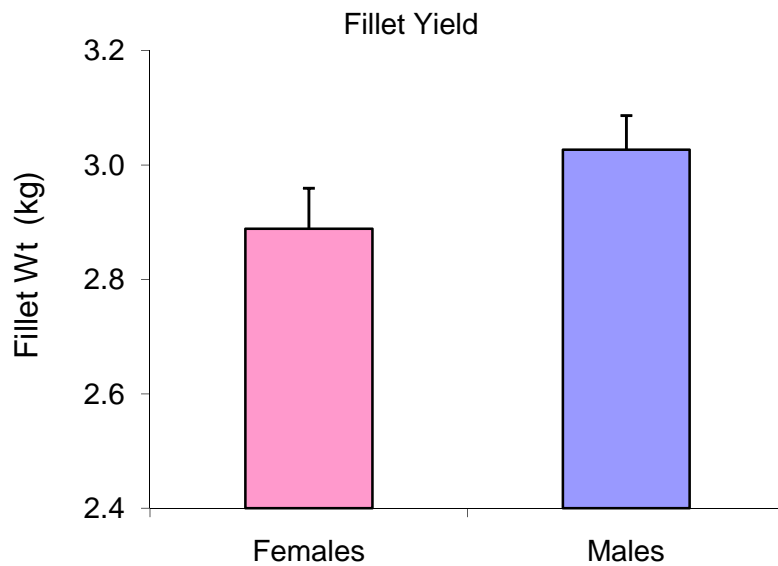
Figure 11. Fillet yield (kg; mean ± sem) of male (n=24) and female (n=23) fish sampled in this study. Difference **P=0.035**; statistically significant.

Males also had significantly less visceral mass (includes stomach, pyloric caecae, intestine, liver, gonad and visceral fat, but not heart, gills, swimbladder or kidney) than did females (Fig 12). This was mostly due to a significantly lesser amount of visceral fat (Fig 13), but there was no statistical differences in hepatosomatic index (HSI) or gonadosomatic index (GSI).
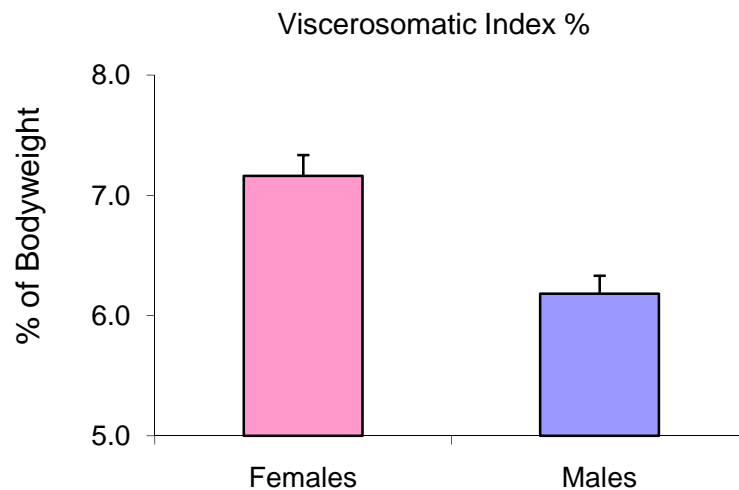
Figure 12. Viscera weight (as a % of whole bodyweight; mean ± sem) of male (n=24) and female (n=23) fish sampled in this study. Difference **P=0.0001**; statistically significant.
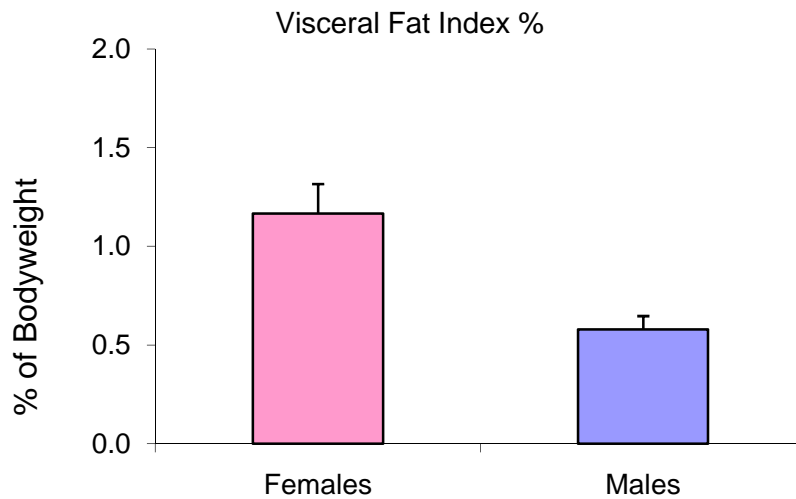
Figure 13. Visceral fat (as a % of whole bodyweight; mean ± sem) of male (n=24) and female (n=23) fish sampled in this study. Difference **P=0.001**; statistically significant.
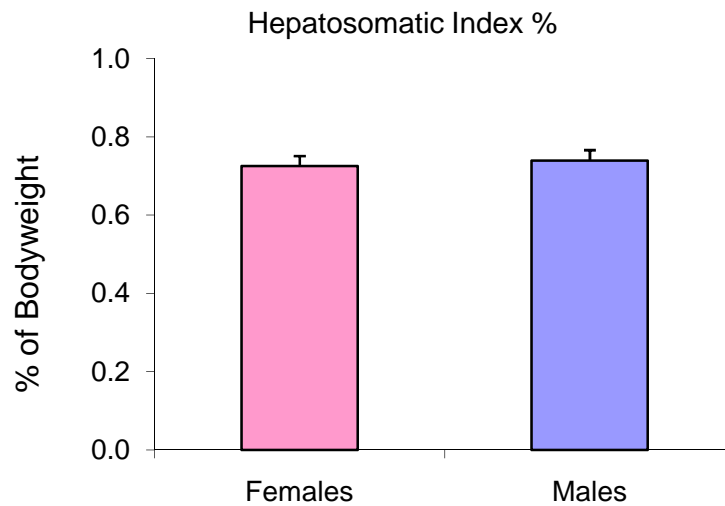

Figure 14. Liver weight (as a % of whole bodyweight - hepatosomatic index; mean ± sem) of male (n=24) and female (n=23) fish sampled in this study. Difference P=0.707; not significant.
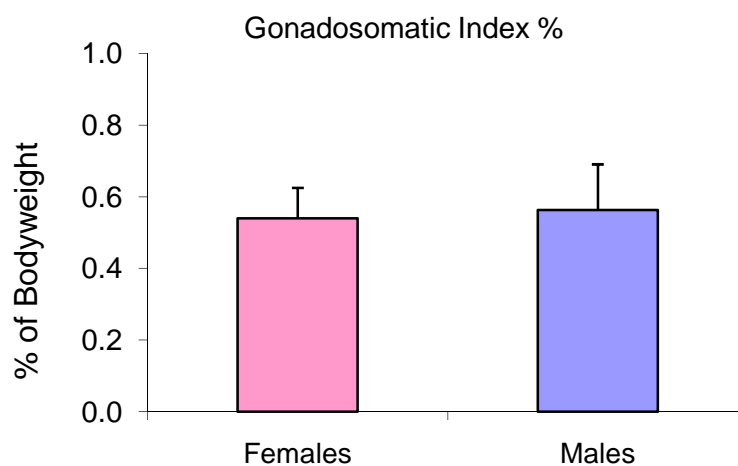

Figure 15. Gonad weight (as a % of whole bodyweight – gonadosomatic index; mean ± sem) of male (n=24) and female (n=23) fish sampled in this study. Difference P=0.989; not significant.

Similarly, there was no difference in the average levels of flesh moisture and crude fat between male and female fish (Figs 16 & 17). Flesh crude fat varied between 5 and 13% and was significantly inversely correlated to flesh moisture (Fig 18).
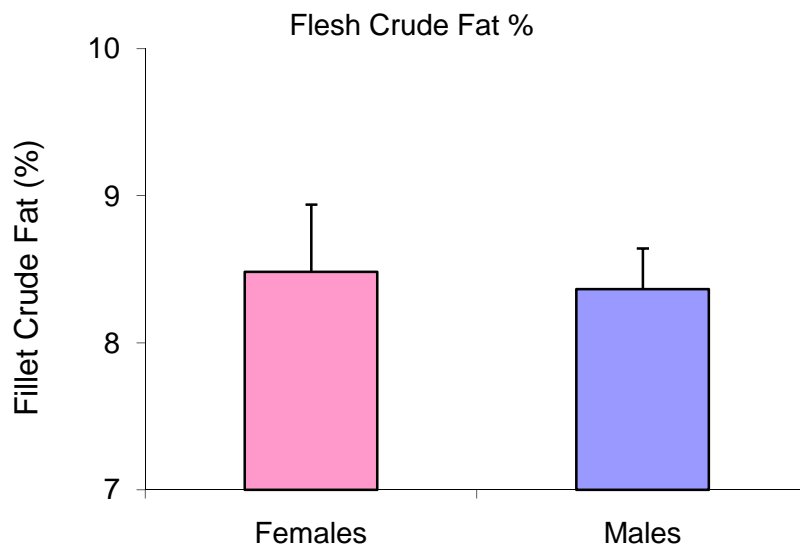

Figure 16. Flesh crude fat (as a % of fillet weight; mean ± sem) of male (n=24) and female (n=23) fish sampled in this study. Difference P=0.827; not significant.
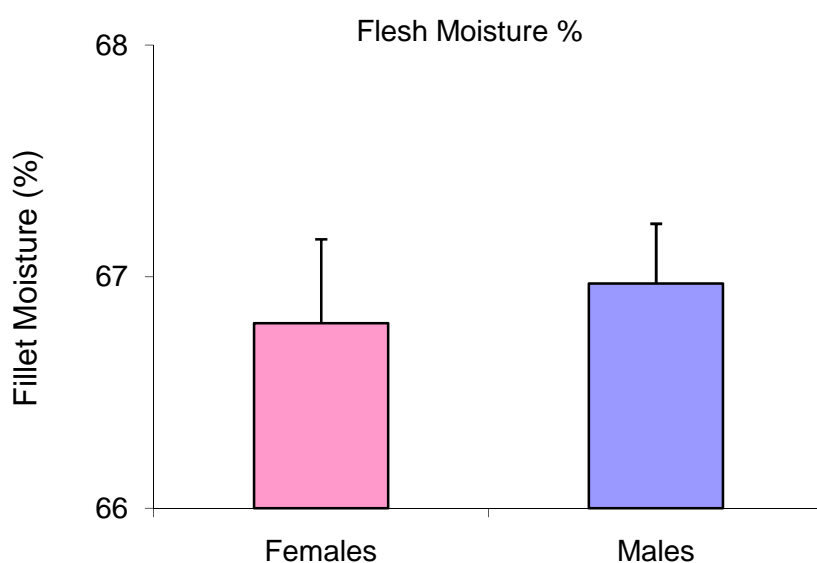

Figure 17. Flesh moisture (as a % of fillet weight; mean ± sem) of male (n=24) and female (n=23) fish sampled in this study. Difference P=0.703; not significant.
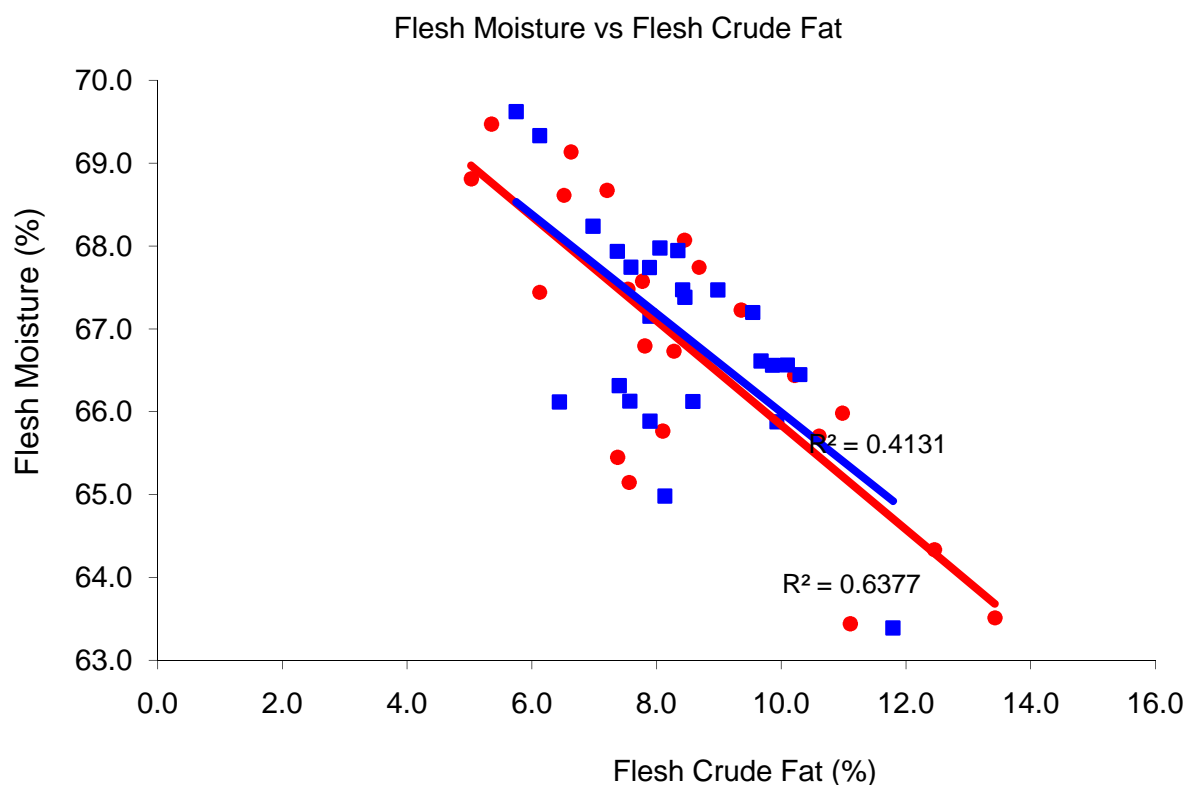
Figure 18. Regression between flesh crude fat (%) and moisture (%) for individual fish sampled during this study. Male (blue squares, blue line; n=24) and female (red diamonds, red line; n=23) relationships are both **P=0.0001**; statistically significant.

Sex Differences in Post-Mortem Characteristics

There was no difference between male and female Yellowtail Kingfish in most characteristics either at-harvest or post-mortem. The measurements of blood pH (at harvest, and 11 hr later), plasma cortisol and lactate, flesh pH (at harvest), flesh firmness (by penetrometer and Durofel), and drip loss % were not different between sexes (Appendix 3; Figs Sex 1-10). There was a trend that male fish went into a greater degree of rigor on Days 1 and 2 post-harvest (Appendix 3; FigsSex 8), and this may have been associated with a slightly higher flesh pH on Days 2 and 3 post-harvest (Appendix 3; Fig Sex 9).

Within the limitations of the daily sampling schedule the change in eye clarity between days was very obvious. All eyes were bright and clear on day 0 (with some showing signs of cloudiness by 6-8 hrs post-harvest at unloading), most eyes were scored as 1 or 2 on day 1, but by day 2 almost all eyes were scored as 0 again (see Fig 3). Eye clarity did not differ between males and females on day 1 (P=0.913; data not shown).

Similarly, there were no differences in the sensory attributes of the fillets of male and female fish on day 8/9 (data not shown).

Male Reproductive Development Stages

Of the 24 male fish sampled in this study only 2 (8%) were defined as Stage 1, and another 1 (4%) as a Stage 2. Sixteen (67%) and five (21%) were defined as Stages 4 (running ripe) and 5 (spent), respectively. No fish was classified as a Stage 3. Thus, 11% of sampled males were immature and 89% were mature. Some of the larger testes are shown in Figure 19.

Histology of the testes confirmed the macroscopic assessments. The Stage 1 and 2 males had only connective tissue (pink) and spermatogonia (light blue-purple) (Figs 20 & 21). Stage 4 fish had very obvious quantities of dark blue-black spermatozoa filling the lumen of the tubules, with groups of light blue-purple spermatogonia, spermatocysts and spermatids cells scattered along the walls of the tubules throughout the cross section of testes (Fig 21). Stage 5 males still had some residual spermatozoa within the lumen of the tubules towards the centre of the testis, but the periphery of the testis contained a lot of connective tissue and tubules containing a layer of spermatogonia but no spermatozoa within the lumen (Fig 22).

The GSI of the males in each Stage is shown in Figure 23. Stage 4 spermiating males had an average GSI of 0.72% (testes weight of 32g). The greatest male GSI recorded was 2.93% (testes weight 135g).
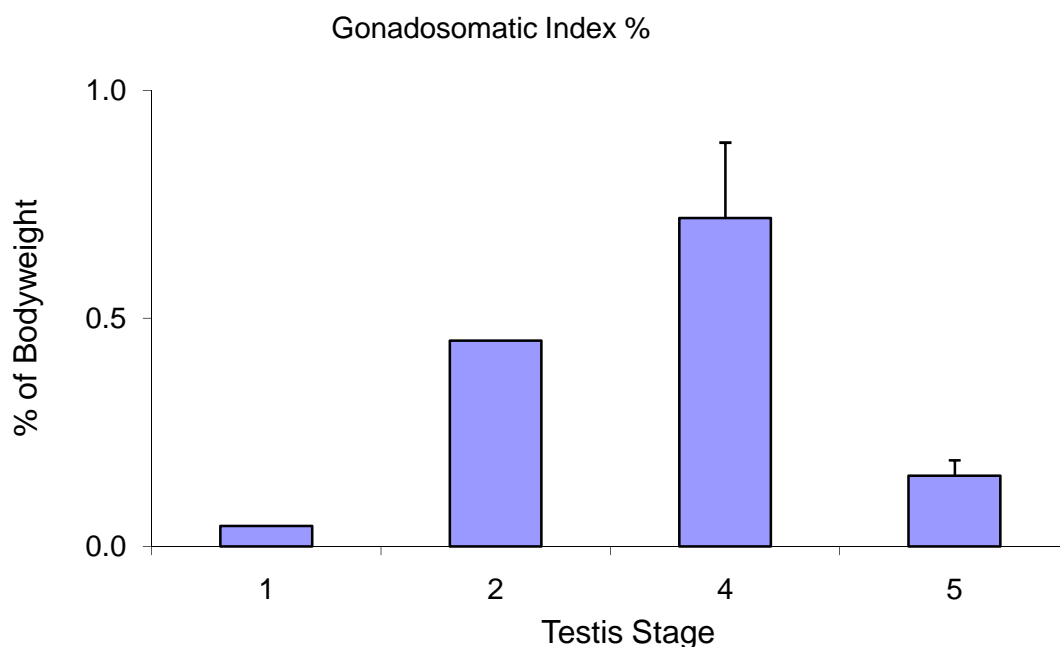

Figure 23. Testes weight (as a % of whole bodyweight – gonadosomatic index; mean ± sem) of males according to testes stage (n=2, 1, 16, 5 respectively) sampled in this study. Difference P=0.093 (stages 4 and 5 only); not significant.
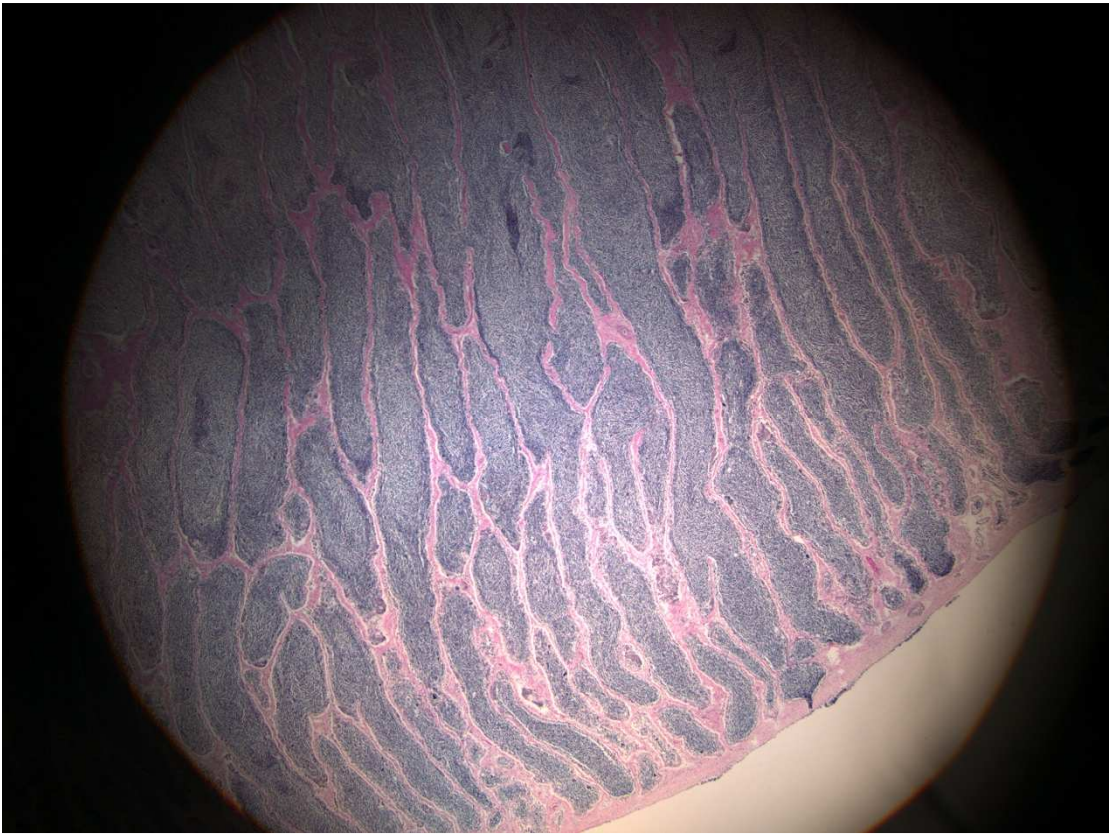
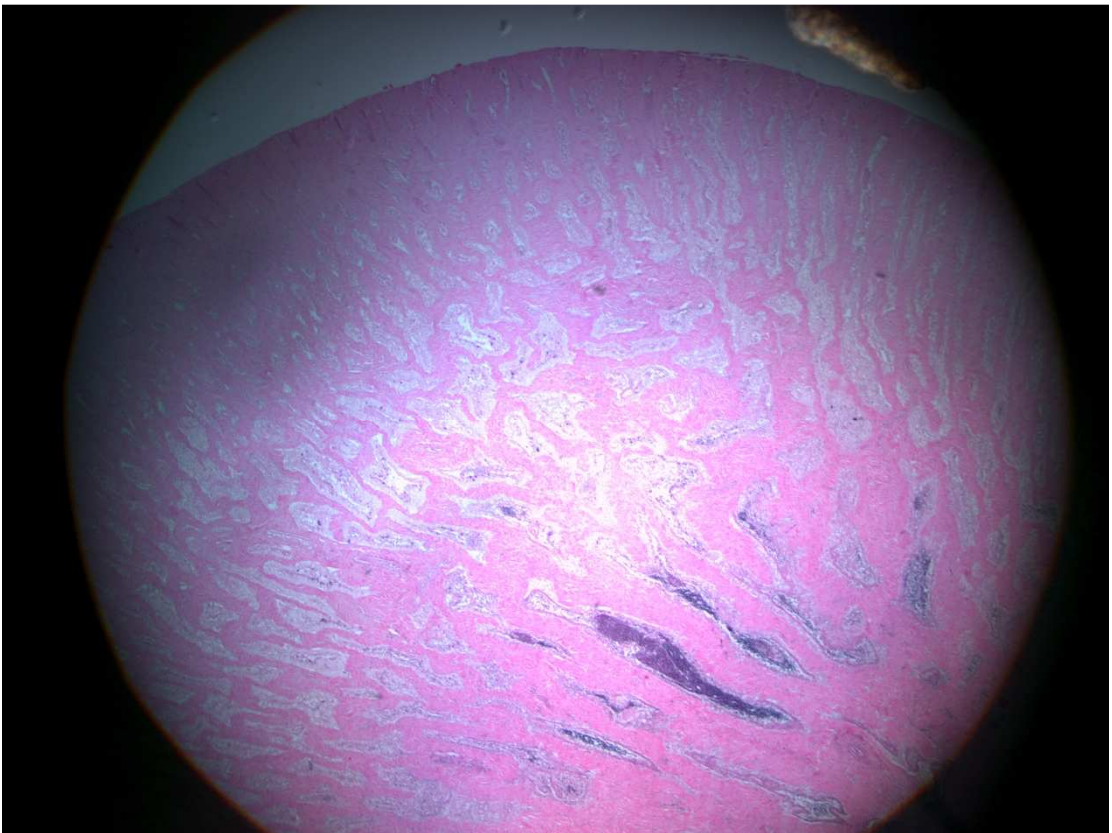Stage 4 testes (135g) from fish G15. Scale on ruler is in cm.

Stage 4 testes (46g) from fish Y11. Scale bar is 1cm.
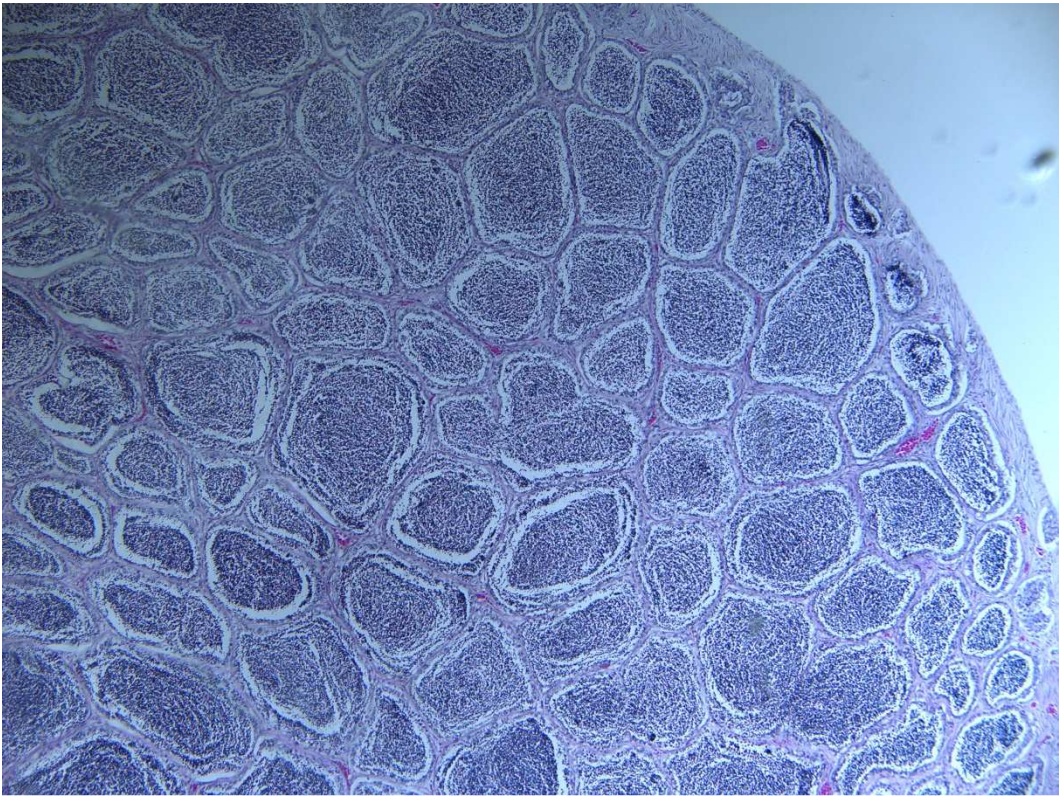
Figure 19. Male gonads.
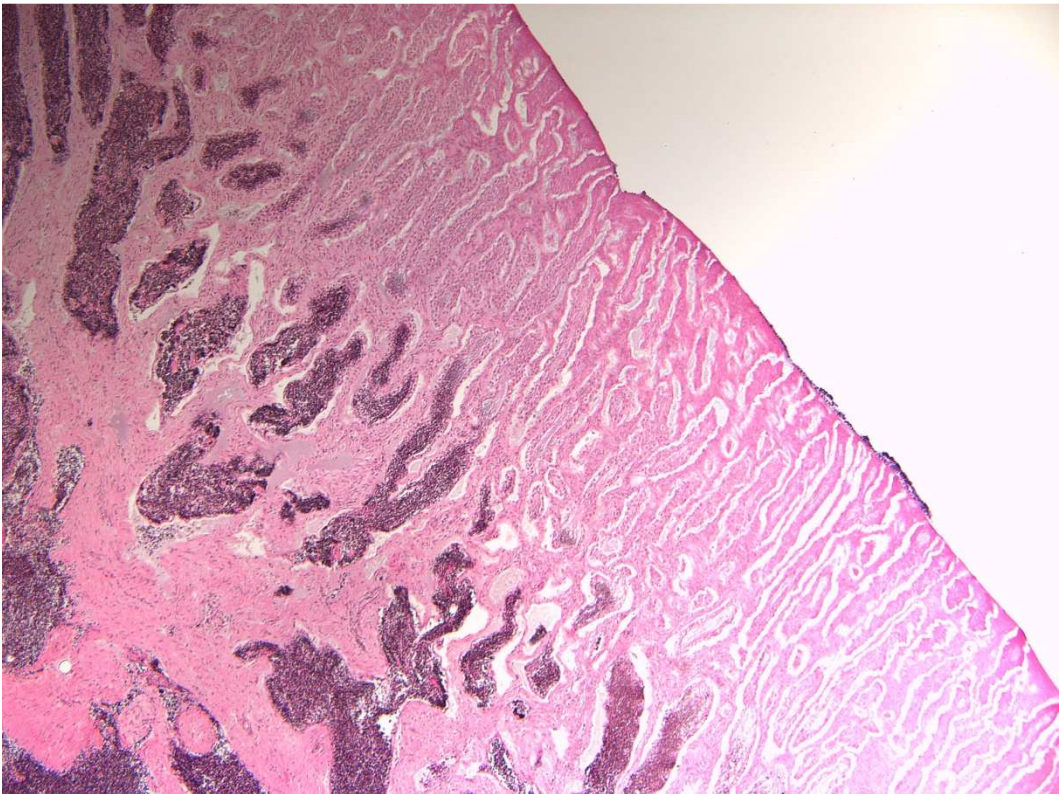
Fish B4 – Stage 4 (x4)

Fish B9 – Stage 5 (x4)

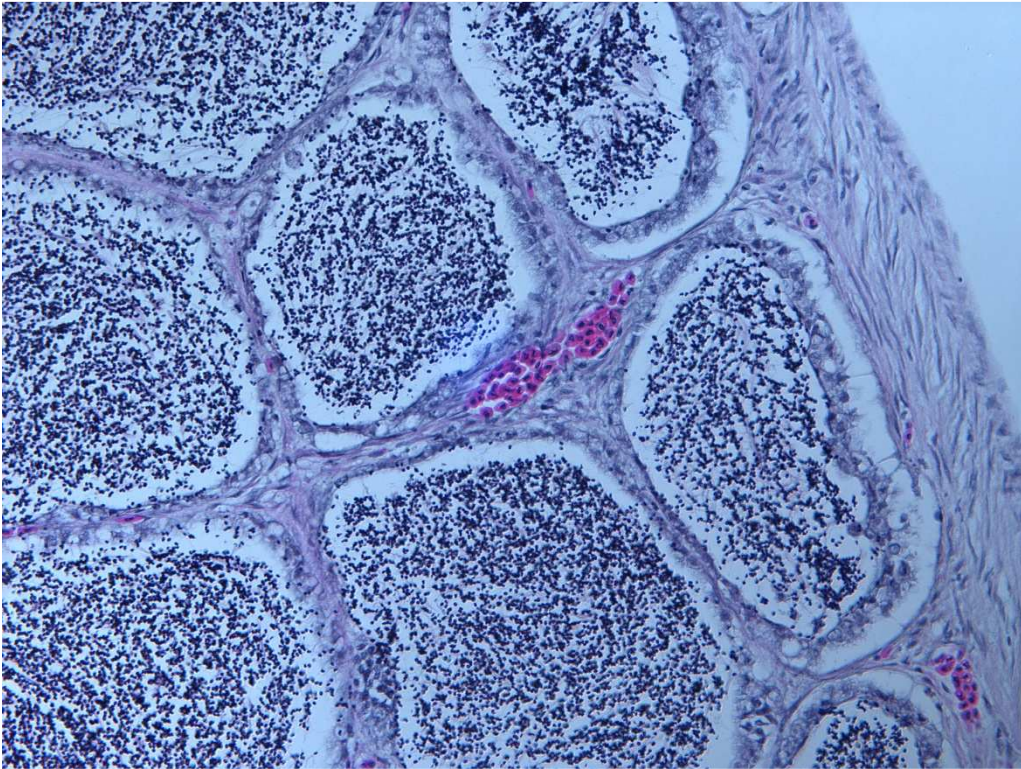**Figure 20.** Testis histology (low power magnification).
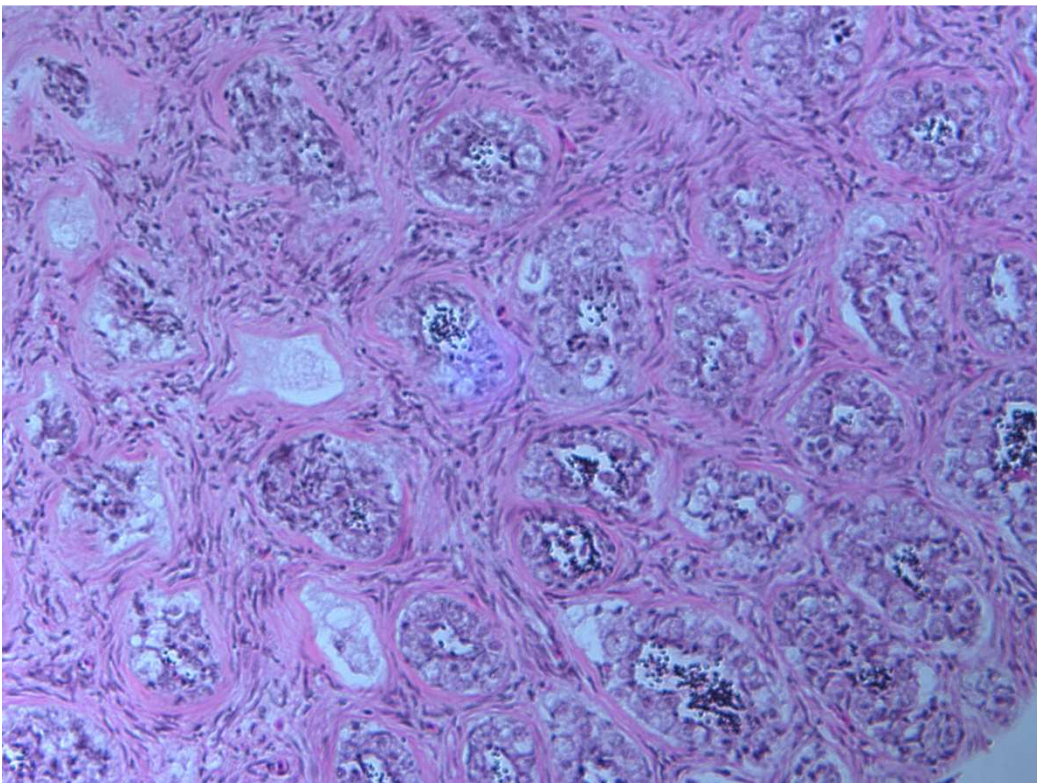
Fish B12 – Stage 4 (x10)

Fish G1 – Stage 5 (x10)

Figure 21. Testis histology (medium power magnification)

Fish B12 – Stage 4 (x60)

Fish Y4 – Stage 5 (x40)

Figure 22. Testis histology (high power magnification)

Stage 4 and 5 males had the same mean bodyweight (4.40 kg), but Stage 5 males had a significantly lower % fillet yield (66.7 vs 68.4%; or 2.93 vs 3.01 kg) and a significantly lower fillet crude fat % (with a correspondingly higher level of fillet moisture) than Stage 4 fish (Figs 24 & 25), there was no difference in the somatic (eg HSI, visceral fat %) or most carcass characteristics (eg flesh pH) of male fish that were at different reproductive development stages (Figs 26-31).

Figure 24. Fillet yield (as a % of whole bodyweight; mean ± sem) of males according to testes stage (n=2, 1, 16, 5 respectively) sampled in this study. Difference **P=0.011** (stages 4 and 5 only); significant.

Figure 25. Viscera weight (as a % of whole bodyweight; mean ± sem) of males according to testes stage (n=2, 1, 16, 5 respectively) sampled in this study. Difference P=0.980 (stages 4 and 5 only); not significant.

Figure 26. Liver weight (as a % of whole bodyweight – hepatosomatic index; mean ± sem) of males according to testes stage (n=2, 1, 16, 5 respectively) sampled in this study. Difference P=0.795 (stages 4 and 5 only); not significant.
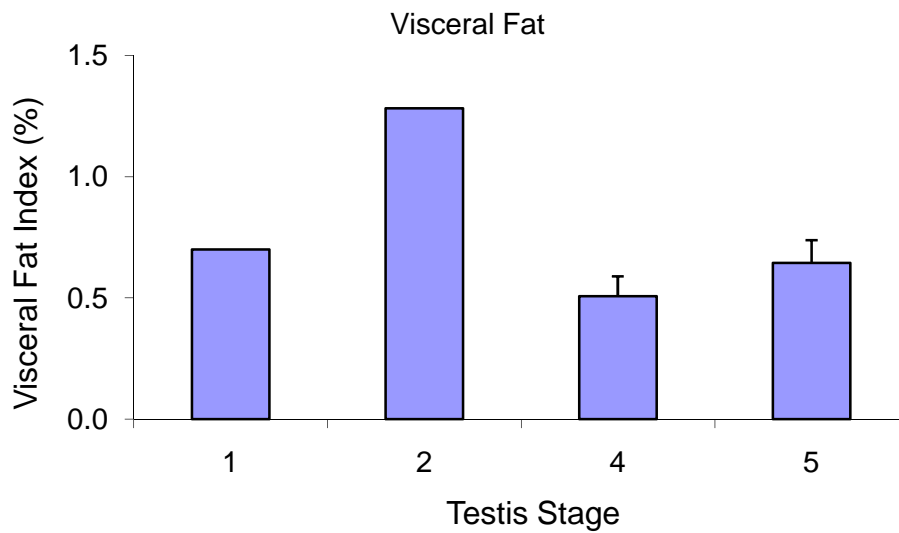
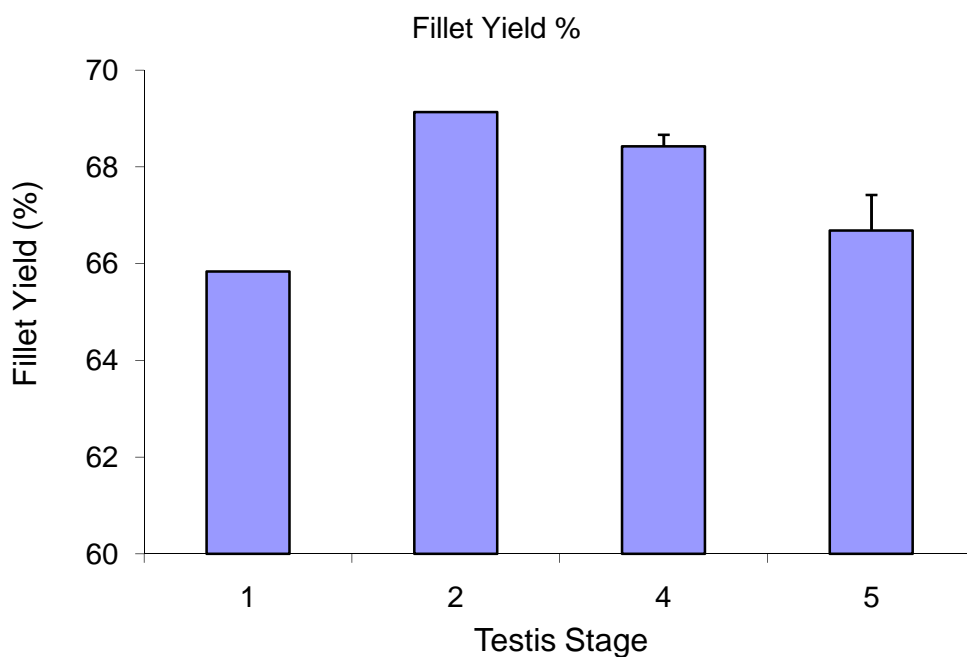**Figure 27.** Visceral fat weight (as a % of whole bodyweight; mean ± sem) of males according to testes stage (n=2, 1, 16, 5 respectively) sampled in this study. Difference P=0.391 (stages 4 and 5 only); not significant.
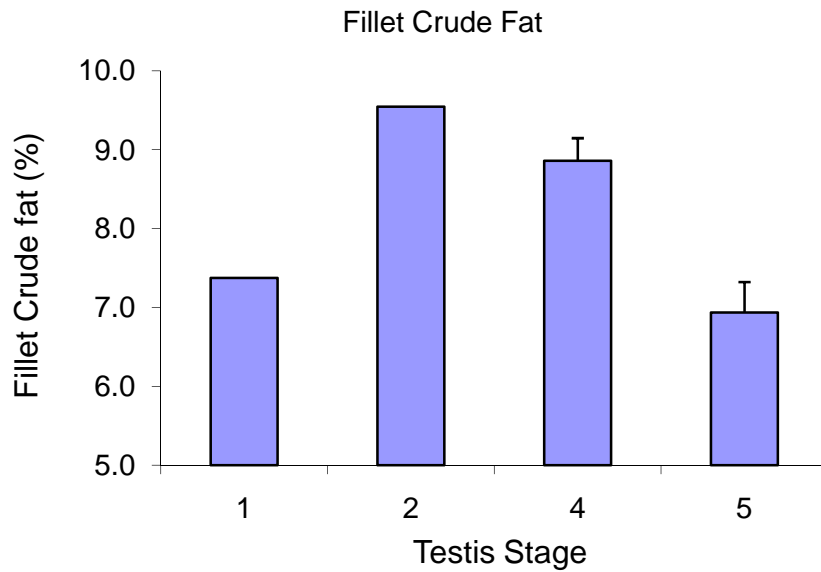
Figure 28. Flesh crude fat (as a % of fillet wet weight; mean ± sem) of males according to testes stage (n=2, 1, 16, 5 respectively) sampled in this study. Difference **P=0.003** (stages 4 and 5 only); significant.
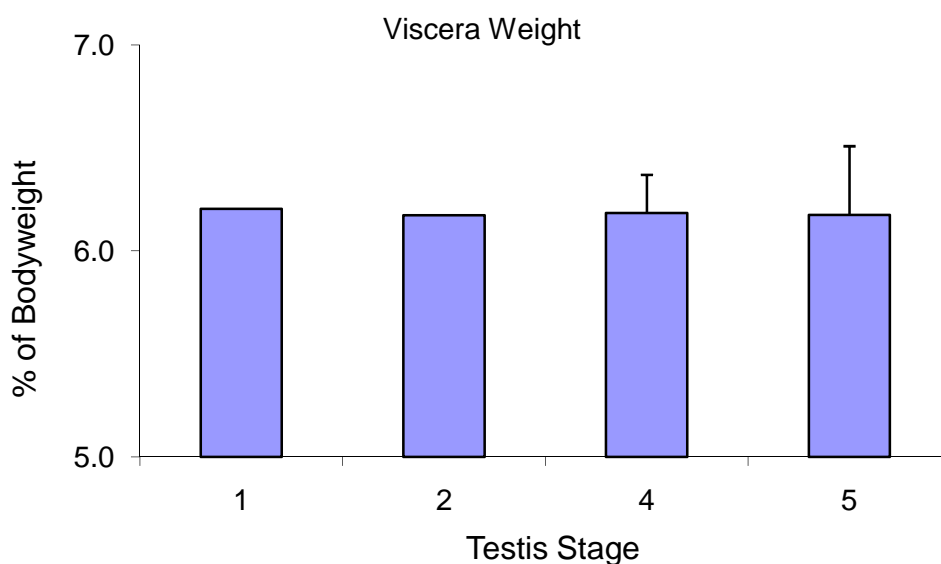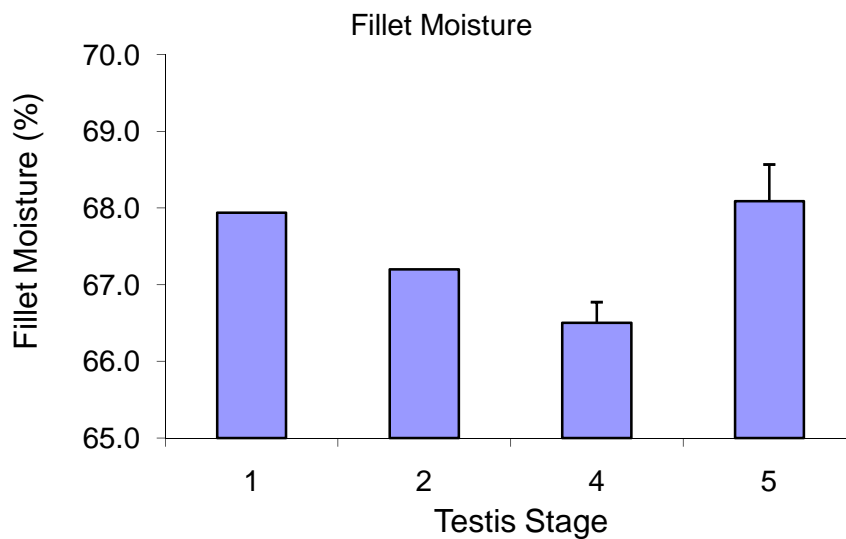

Figure 29. Flesh moisture (as a % of fillet wet weight; mean ± sem) of males according to testes stage (n=2, 1, 19, 5 respectively) sampled in this study. Difference **P=0.010** (stages 4 and 5 only); significant.
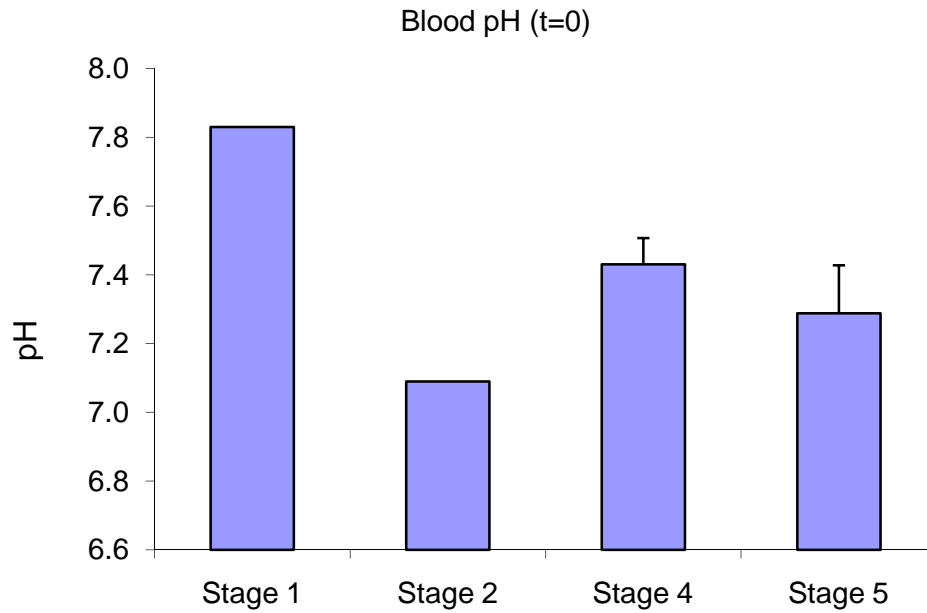
Figure 30. Flesh pH (mean ± sem) of males at time of harvest according to testes stage (n=2, 1, 19, 5 respectively) sampled in this study. Difference P=0.075 (stages 4 and 5 only); not significant.
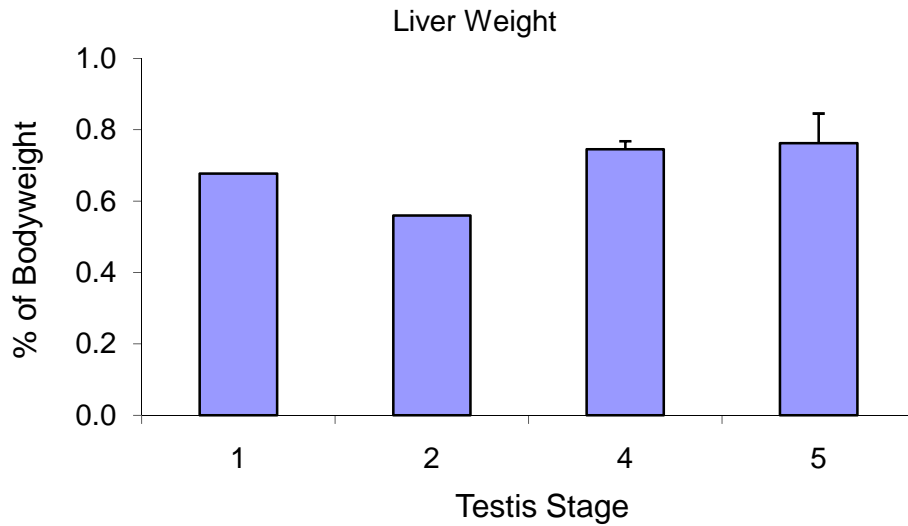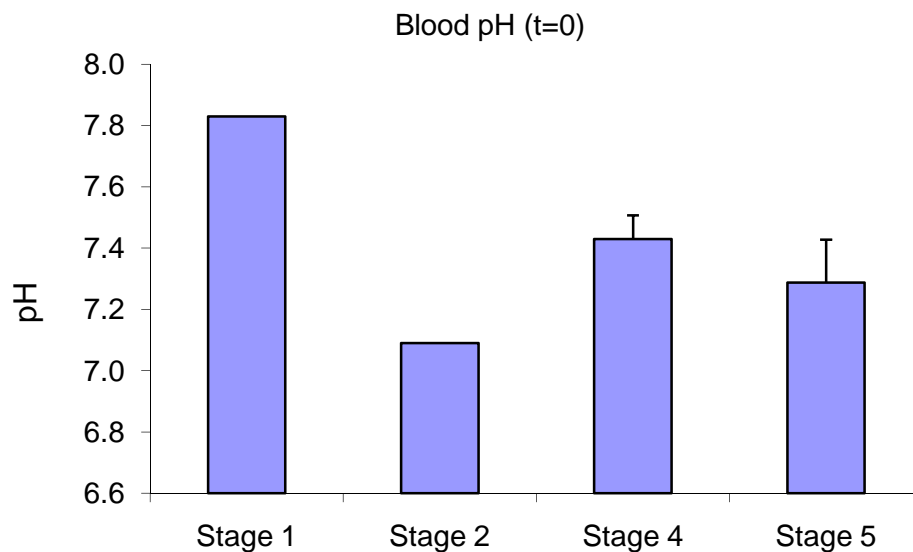

Figure 31. Blood pH (mean ± sem) of males at time of harvest according to testes stage (n=2, 1, 19, 5 respectively) sampled in this study. Difference P=0.427 (stages 4 and 5 only); not significant.

There was no significant impact of male maturation stage on post-mortem (day 1-9) flesh characteristics such as: flesh pH, rigor index, drip loss, penetrometer or Durofel readings, fillet gaping, fillet firmness and smell. Figures in Appendix 3.

In the present study the most obvious difference in fillet colour was due to time, with all fillets showing noticeable browning over the 5 days of storage (refer to Fig 2). It is considered unlikely that many of the fillets would have been marketable on day 8/9 based on their colour. The

37

difference in appearance of fillets ascribed to the different colour categories by the experienced assessor on day 8/9 was subtle compared to the considerable effect of time. Despite the subtleties of the colour scoring system, there was a greater proportion of the Stage 5 fish categorised as having brown flesh, compared to the Stage 4 fish (60 vs 15% respectively; Fig 32 & 35). The small number of Stage 5 fish (n=5) sampled should be noted, and the weighting given to this observation needs to be considered in this light. None of the other sensory attributes (firmness, smell) differed between male maturation stage (Figs 33 & 34) and neither were believed to be of any concern in terms of customer acceptability of product.

Figure 32. Fillet colour of Stage 4 and 5 males (n=19, 5 respectively) on day 8/9 post-harvest in this study. Proportion of fillets independently subjectively assessed as 'good', 'slightly brown' and 'brown' is shown.

Figure 33. Fillet firmness of Stage 4 and 5 males (n=19, 5 respectively) on day 8/9 post-harvest in this study. Proportion of fillets independently subjectively assessed as 'soft' and 'firm' is shown.
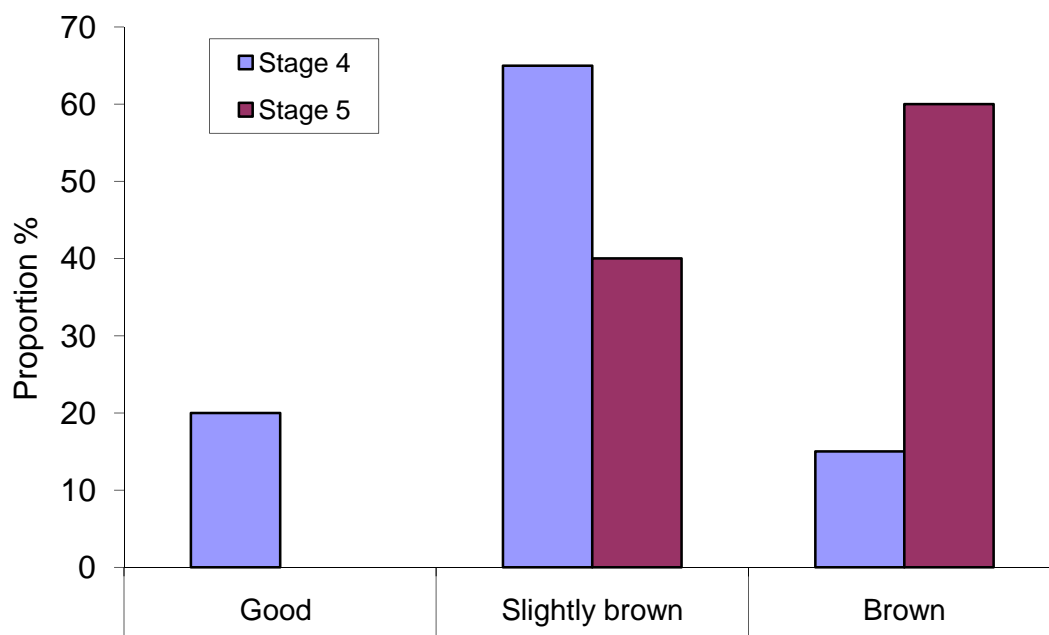
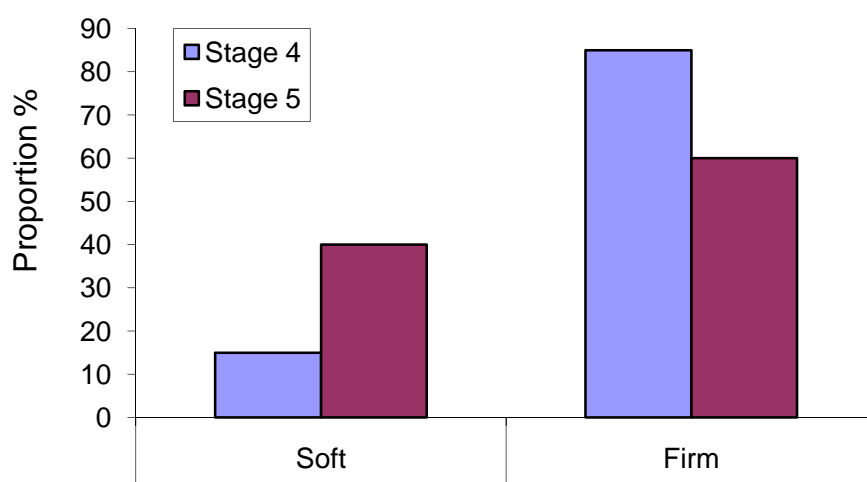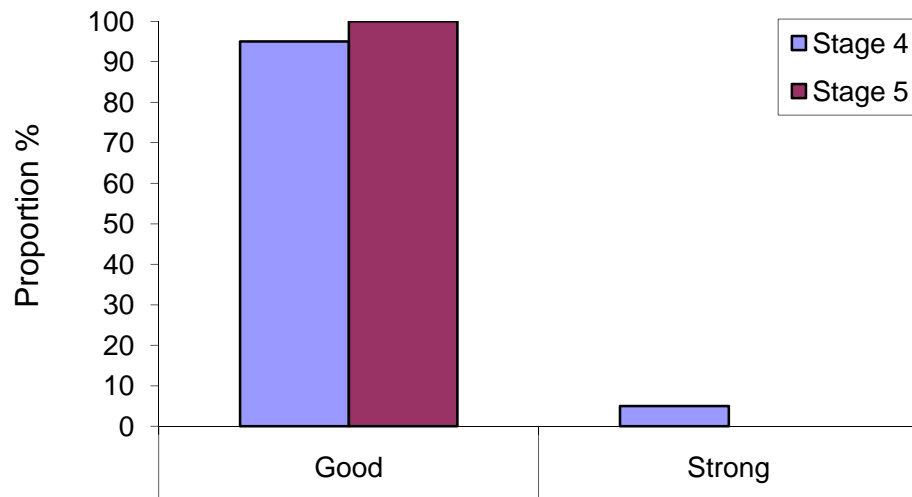**Figure 34.** Fillet smell of Stage 4 and 5 males (n=19, 5 respectively) on day 8/9 post-harvest in this study. Proportion of fillets independently subjectively assessed as 'good' and 'strong' is shown.

Harvest Method

The fish sampled over the 2 experimental days were separated into 4 categories. There were those taken by hook harvest (n=3 and 12 on days 1 and 2, respectively), the commercial harvest on day 1 (n=20), the last hour of the commercial harvest on day 2 (n=8), and the 'floaters' (n=4).

There were significant differences in some of the stress indicators and tissue parameters measured between these 4 categories. Plasma lactate was extraordinarily high in the 4 fish characterised as 'floaters' (Figure 36). Their value was 6 fold higher (40.1 ± 1.83 mmol/L) than the average for the rest of the commercially harvested fish (6.29 ± 0.46 mmol/L), which was, in turn, 2.5 times higher than the hook harvested fish (2.58 ± 0.33 mmol/L).

Plasma Lactate

Figure 36. Plasma lactate (mean ± sem) at time of harvest (males and females combined) according to harvest type (n=15, 20, 8 and 4, respectively). Difference **P=0.0001**; significant.

The trend shown for plasma cortisol levels was broadly similar to the plasma lactate levels (Fig 37). Floaters again had the highest values (47.3 ± 4.26 ng/ml), with the average for the commercially harvested fish having values about half of their level (28.2 ± 1.65 ng/ml), hook harvested fish (at 3.7 ± 0.80 ng/ml) had levels about 12 and 7 fold lower than the other two groups, respectively.

Figure 37. Plasma cortisol (mean ± sem) at time of harvest (males and females combined) according to harvest type (n=15, 20, 8 and 4, respectively). Difference **P=0.0001**; significant.

In contrast, and somewhat surprisingly, the blood pH and flesh pH data did not follow the same pattern (Figs 38, 39, 40, and 41). At time of harvest, the sequential commercial harvested fish had significantly higher blood pH and flesh pH values than all of the other groups, including the last hour commercial harvest fish and the floaters. Even if there was an error in calibrating the pH meter on Day 1 (when the sequential harvest fish were sampled), the values obtained for the remaining 3 types of fish (all but 3 hook harvested fish which were sampled on day 2), still do not fit with our understanding or expectations. This will be further explained in the Discussion.

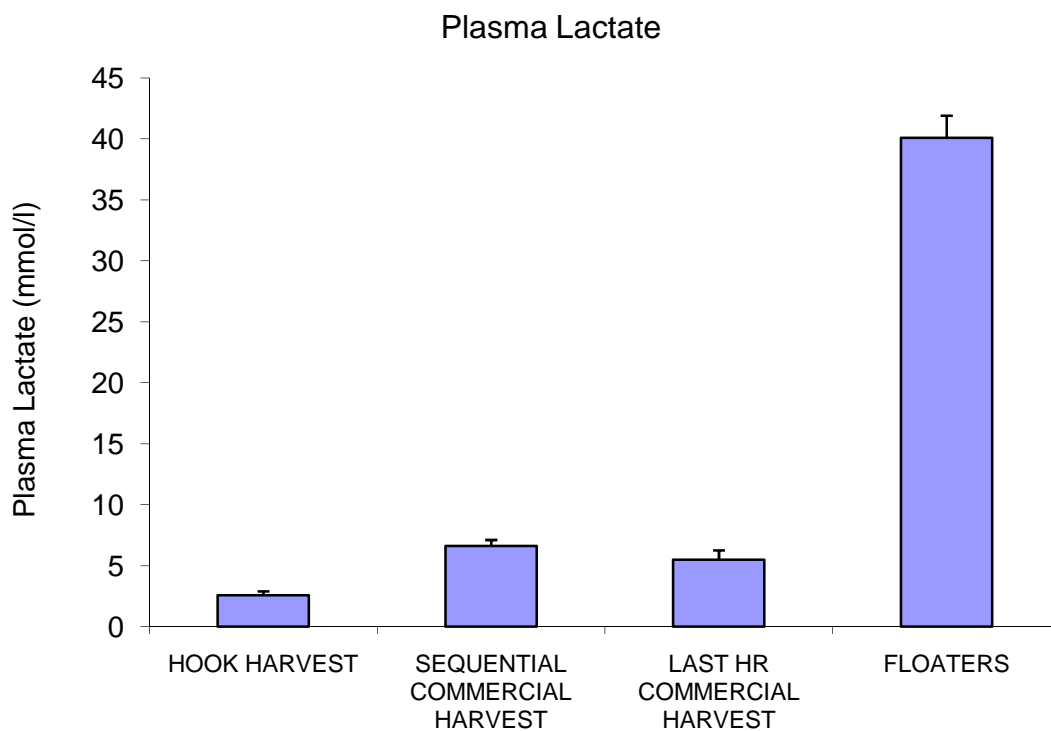**Figure 38.** Blood pH (mean ± sem) at time of harvest (males and females combined) according to harvest type (n=15, 20, 8 and 4, respectively). Difference **P=0.0001**; significant.
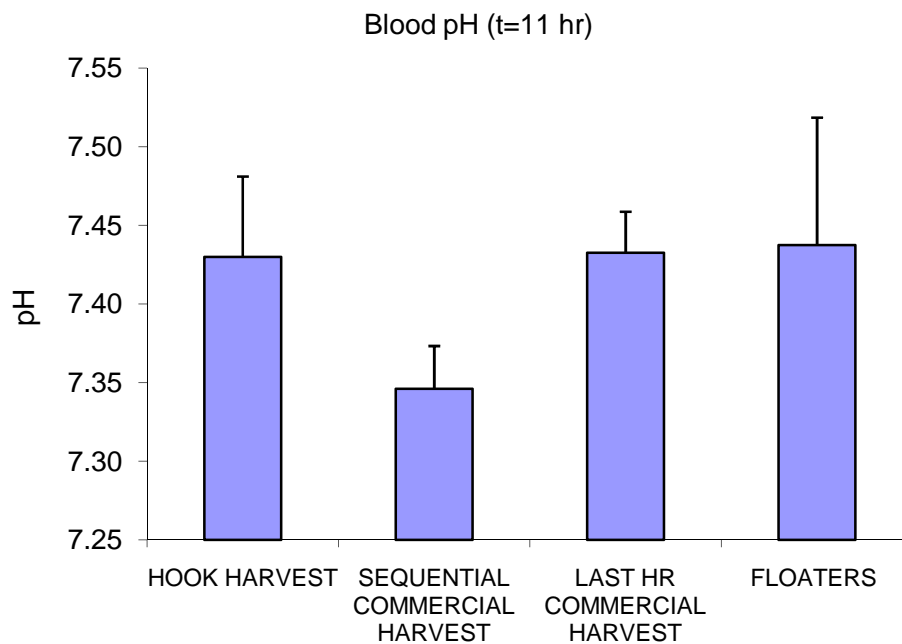
Figure 39. Blood pH (mean ± sem) remeasured 11hr post-harvest (males and females combined) according to harvest type (n=15, 20, 8 and 4, respectively). Difference P=0.331; not significant.
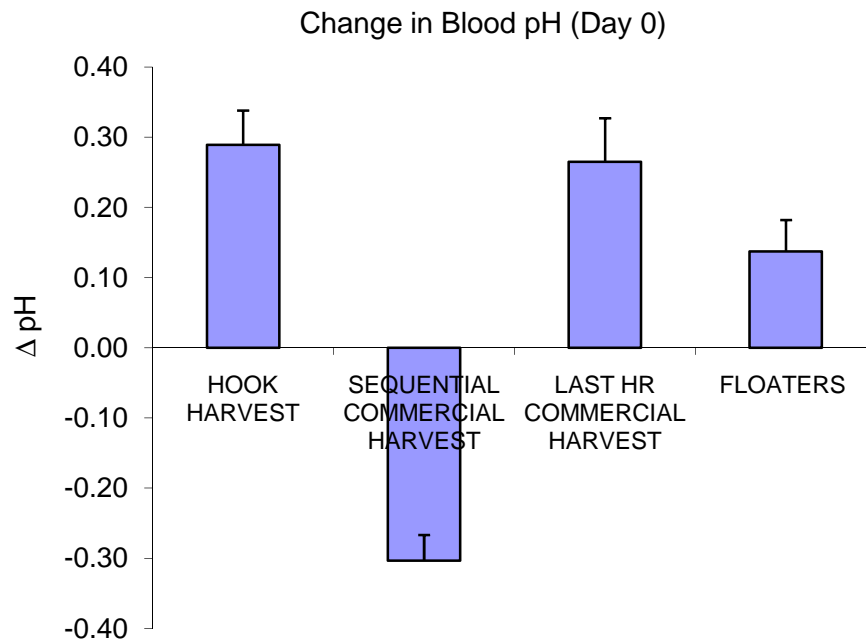
Figure 40. Change in blood pH (mean ± sem) between time of harvest and remeasurement 11 hr later (males and females combined) according to harvest type (n=15, 20, 8 and 4, respectively). Difference **P=0.0003**; significant.
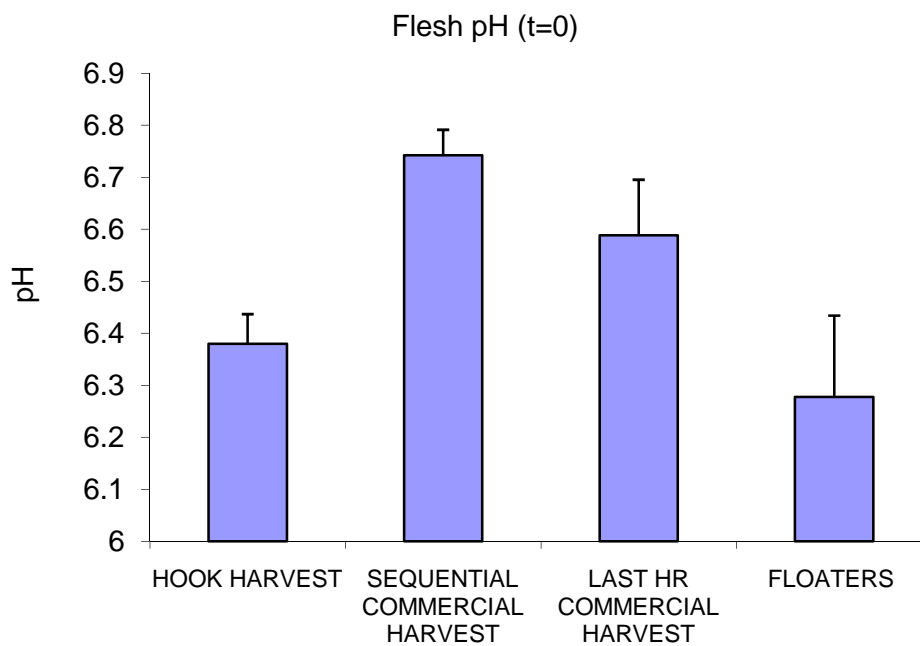
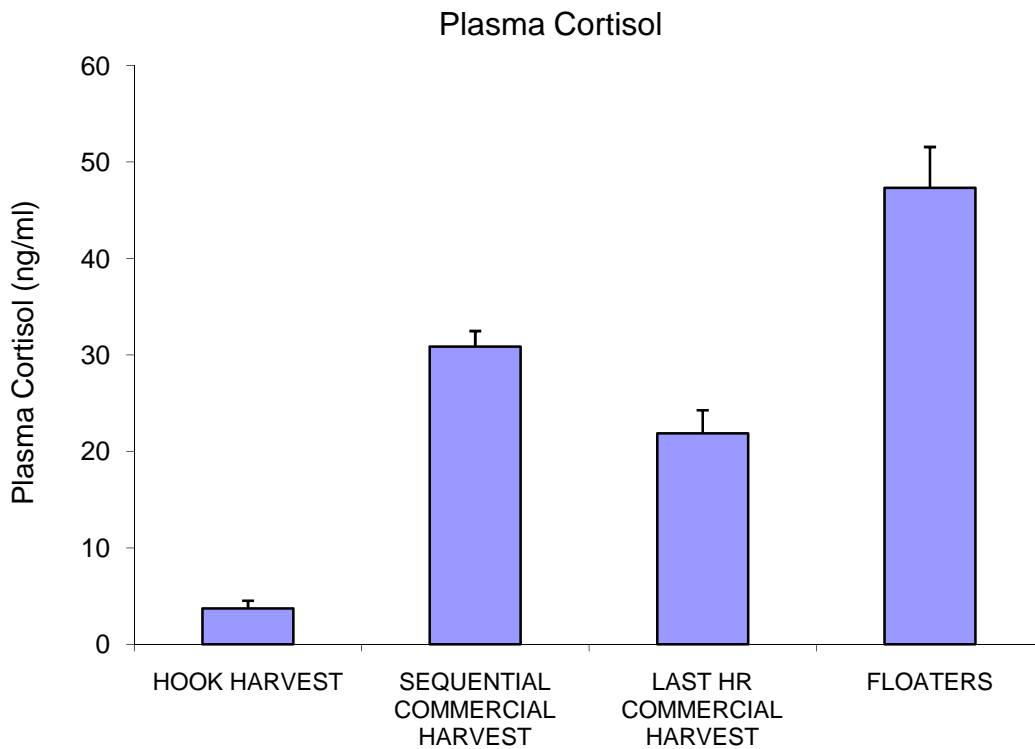**Figure 41.** Flesh pH (mean ± sem) at time of harvest (males and females combined) according to harvest type (n=15, 20, 8 and 4, respectively). Difference **P=0.0005**; significant.

Due to problems with mucus, scales and clotting blood, too few fish could be measured by the penetrometer on day 0 to allow statistical comparisons to be made on carcass firmness. The Durofel meter, however, was able to be used to collect data, and some significant differences were found (Fig 42). A lower Durofel score means the product is softer.
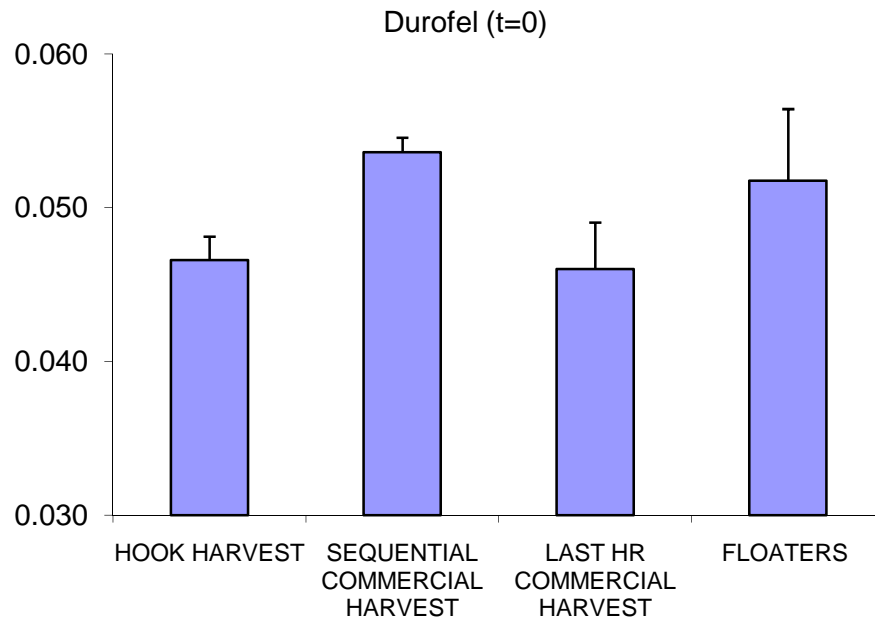
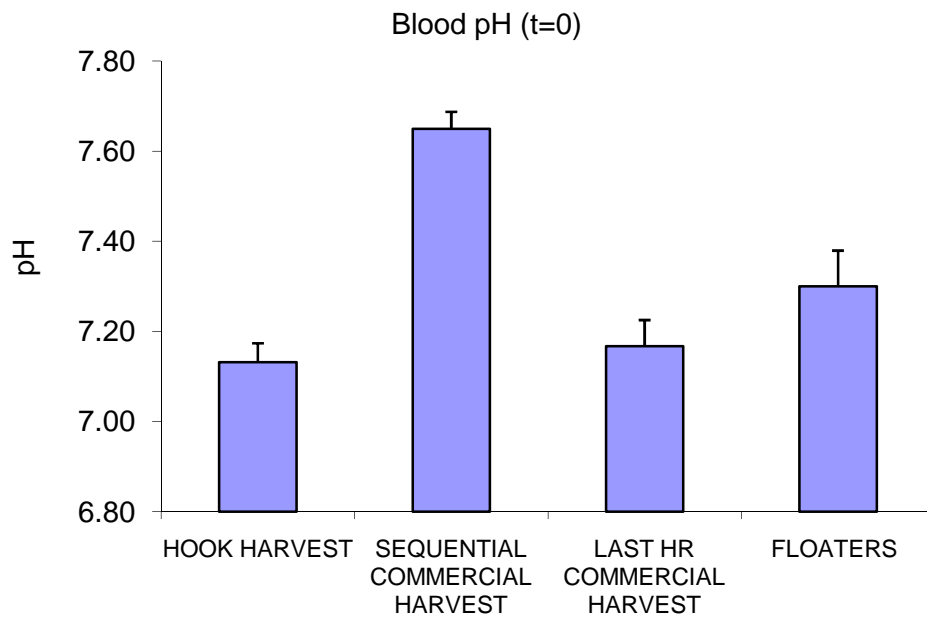**Figure 42.** Carcass firmness as measured by Durofel (mean ± sem) at time of harvest (males and females combined) according to harvest type (n=15, 20, 8 and 4, respectively). Difference **P=0.007**; significant.

Eye clarity - The limited data (ie single observation on each of days 1, 2 and 3) recorded from this trial cannot substantiate any difference in the <u>duration</u> of changes in eye clarity associated with harvest method or sex (data not shown). However, there is a suggestion that the <u>extent</u> of change in eye clarity was associated with harvest type (Fig 43). Whilst 'floaters' had the greatest eye score, followed by 'last hour commercial harvest', the moderate score for the 'hook harvested' fish relative to the 'sequential commercial harvest' fish means there is no consistent pattern that could be interpreted as being due to stress.
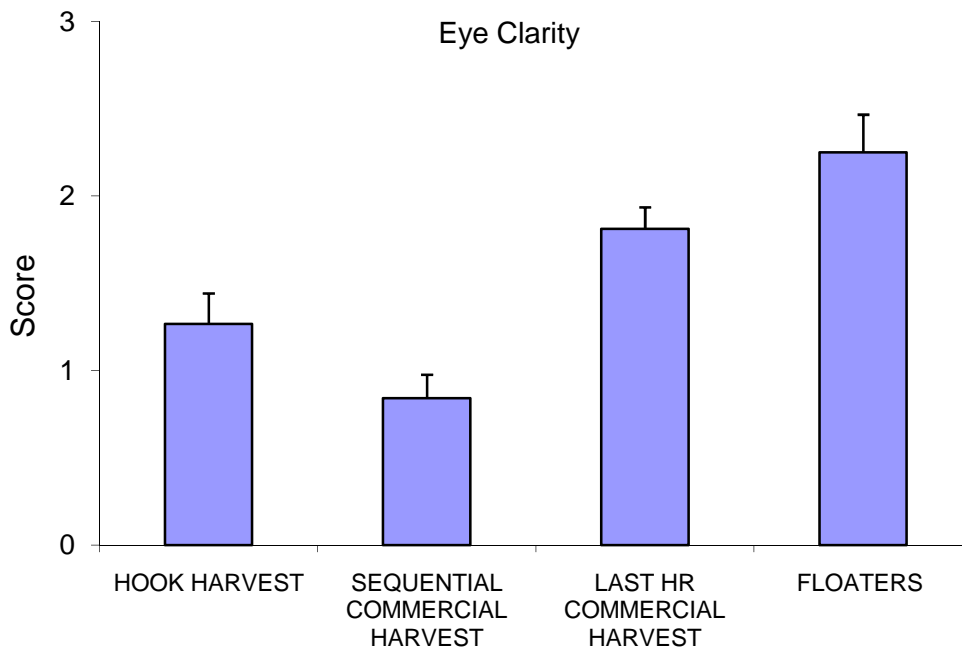
Figure 43. Eye clarity (mean ± sem) as determined subjectively on day 1 post-harvest according to harvest type (n=15, 19, 8 and 4, respectively). Scoring system: 0= none, 1=some, 2=moderate, 3=dense. Difference **P=0.0001**; significant

Harvest Types – Changes in carcass and fillet attributes with storage time

Penetrometer values were recorded for most fish on Day 1. The carcass firmness of sequential commercial harvest fish was less than values measured in the other 3 harvest types (Fig 44). There was little suggestion that the penetrometer readings on Day 1 (when the fish were in rigor) were substantially different from those taken on Day 0.

There were significant differences in carcass and fillet firmness as measured by Durofel meter but the trends were not always evident or consistent (Fig 45). Only values on Day 0 and 1, and Day 3 (fillet) and Day 8/9, are directly comparable as different sized Durofel heads were used to test whole fish and fillets. There was a suggestion that Durofel values increased slightly in hook harvested and last hour commercial harvest fish from Day 0 to 1 as the fish went into rigor, but there was a clear generalised decrease (softening) of the fillet from Day 3 to Day 8/9.
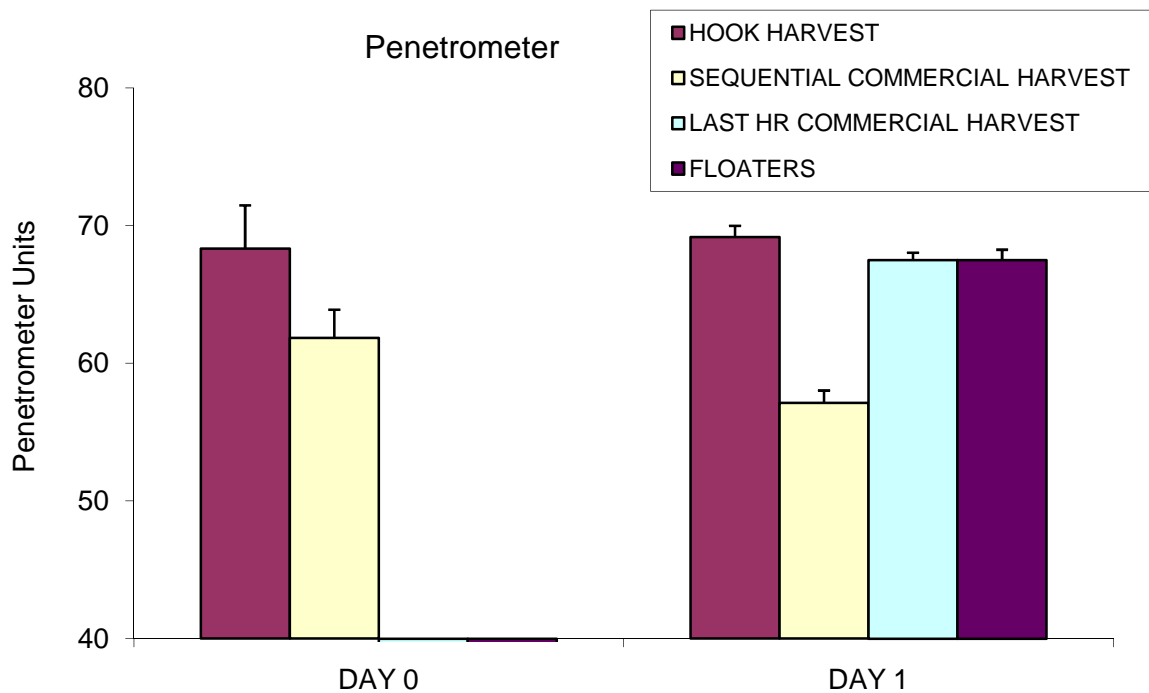
Figure 44. Carcass firmness as determined by penetrometer on day 0 (n=3, 19, 0 and 0) and day 1 (n=12, 8, 8 and 4) according to harvest type. Difference Day 0 not done, Day 1 **P=0.0001**; significant.
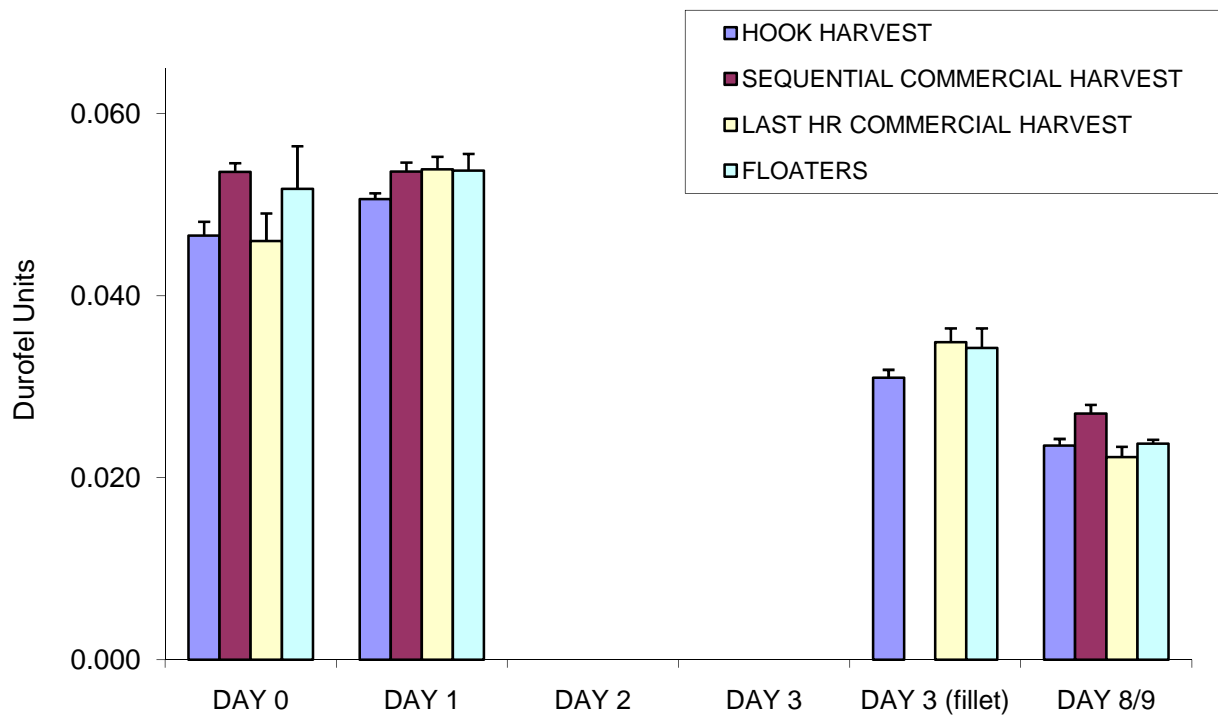
Figure 45. Carcass and fillet firmness as determined by Durofel on day 0 and 1 (n=15, 20, 8 and 4), and day 3 (n=12, 0, 8 and 4) and day 8/9 (n=15, 20, 8 and 4) according to harvest type. Differences **P=0.007**, P=0.097, P=0.099 and **P=0.007**, respectively; significant differences in bold.

Carcass rigor index (with 0 being full rigor and 100 being no rigor) data are shown in Figure 46. All fish are, by definition, scored 100 at time of harvest and by the next morning all were in advanced stages of rigor mortis. A negative rigor index indicates a measurement artefact that is found in hard rigor fish and related to the thickness of the fish's body, the curvature of the main body and the exaggeration caused by the extended horizontal from which the tail dangle is measured. The data did show some consistent trends with the rigor measured in 'last hour commercial harvest' and 'floater' harvest types being stronger than that observed in the 'sequential commercial harvest' fish (Fig 46). All fish in the hook harvest, last hour commercial harvest and floater harvest groups peaked in their rigor index on Day 1 whereas the 'sequential commercial harvest' group peaked on Day 2.
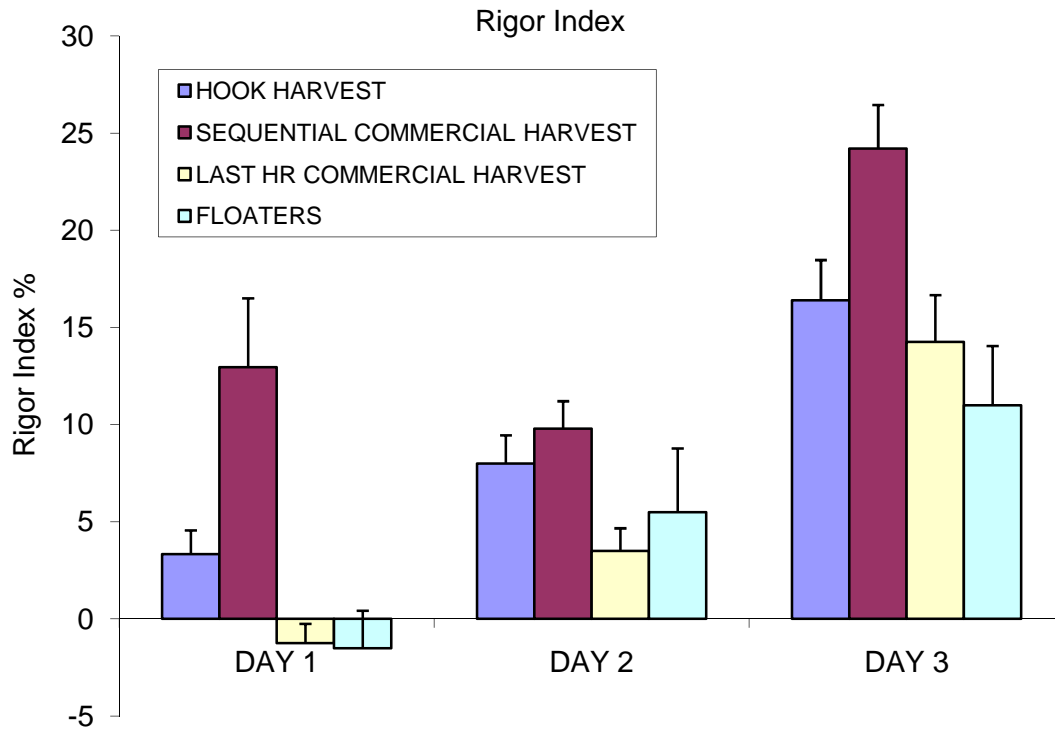
Figure 46. Carcass rigor index on day 1, 2 and 3 (n=15, 19, 8 and 4) according to harvest type. Differences **P=0.006**, P=0.085 and **P=0.008**, respectively; significant differences in bold.

Flesh pH decreased in carcass and fillets over time and differed significantly between harvest types but there were no clear trends (Fig 47). On one occasion (carcass and fillet on day 3) the value in the sequential commercial harvest group was significantly higher than the others, but on 2 other days (1 and 2) the pH was higher in the 'last hour commercial harvest' and 'floater' groups.
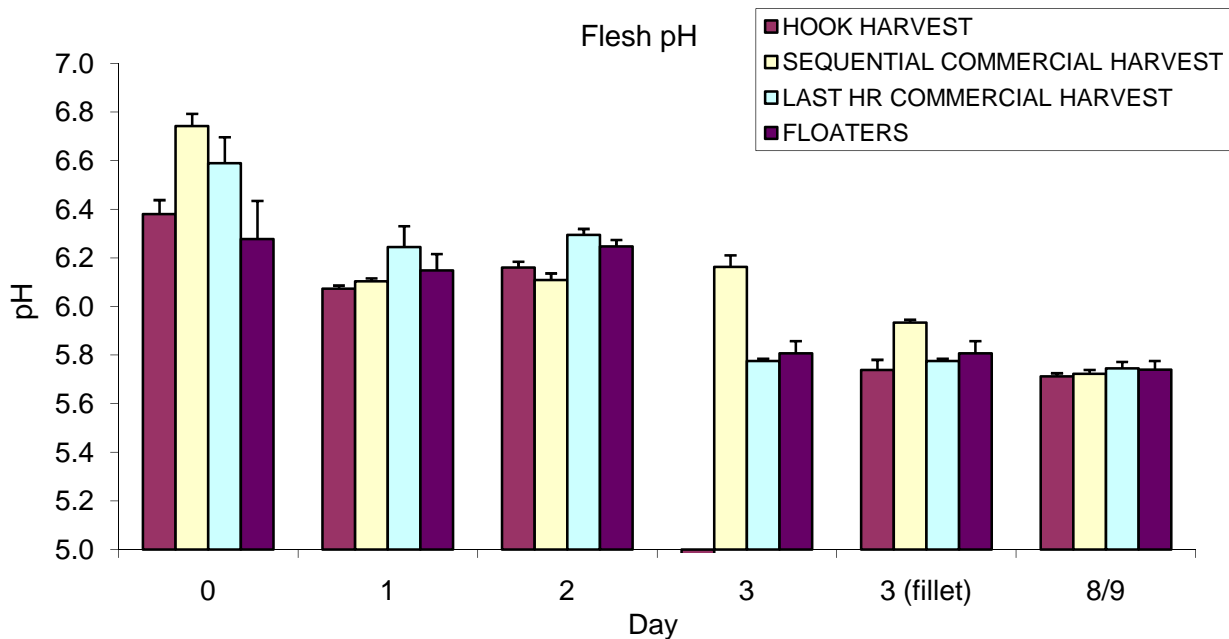
Figure 47. Flesh (carcass and fillet) pH on day 1, 2, 3 and 8/9 post-harvest according to harvest type (n=15, 20 or 19, 8 and 4 except on day 3 (carcass) when it was n=0, 4, 8, and 4). **P=0.0005, P=0.022, P=0.0008, P=0.0001, P=0.0002** and P=0.705, respectively; significant differences in bold.

Fillet Attributes

At the end of the trial (days 8/9 post-harvest, and day 5/6 post-filleting) here was a significantly greater drip loss from 'sequential commercial harvest' fillets than from fillets derived from fish in other harvest type groups (Fig 48). However, there was no difference in gaping score between groups, with few fish showing any gaping and most of that being assessed as only 'some evident' (Fig 49). None of the 'floater' fish showed any gaping.
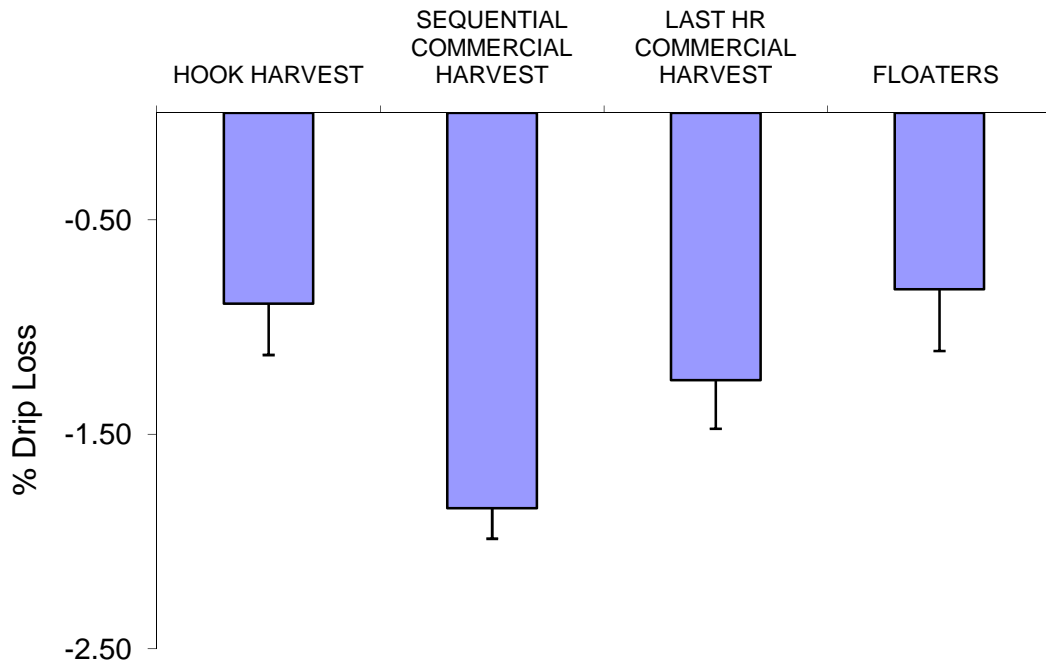
Figure 48. Drip loss (as a % of fillet wet weight; mean ± sem) from day 3 to day 8/9 post-harvest (males and females combined) according to harvest type (n=15, 20, 8 and 4, respectively). Difference **P=0.003**; significant.
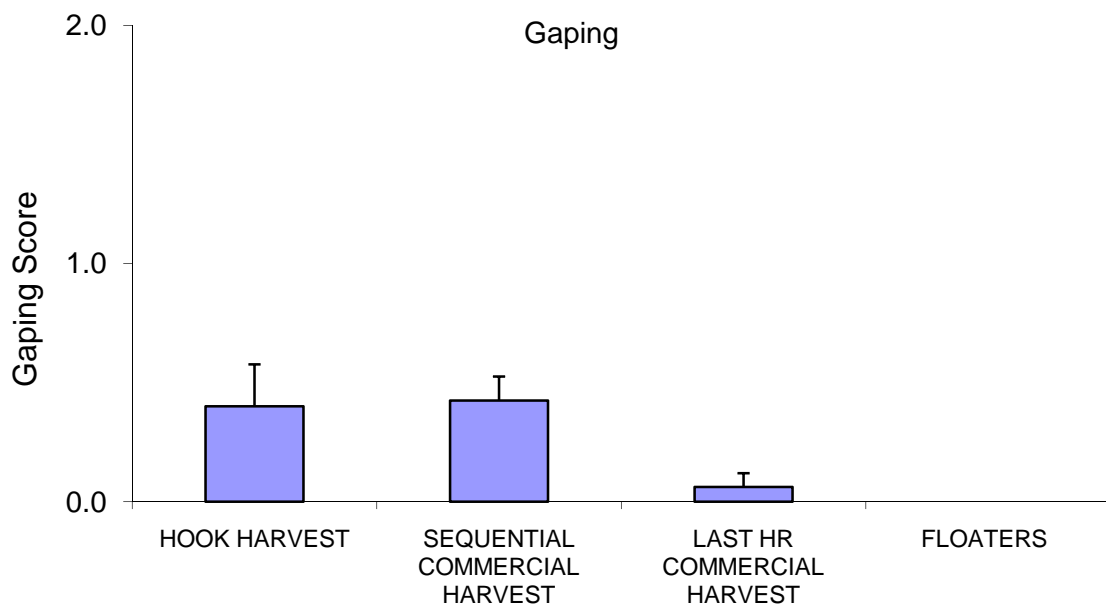
Figure 49. Fillet gaping (mean ± sem) as determined subjectively on day 8/9 post-harvest (males and females combined) according to harvest type (n=15, 20, 8 and 4, respectively). Scoring system: 0= none, 1=some evident, 2=obviously evident. Difference P=0.209, not significant.

The percentage of fillets showing a colour subjectively assessed as 'good', 'slightly brown' or 'brown' for each harvest type is shown in Figure 50. No statistical analysis was performed on the data. Three of the harvest types showed a distribution that peaked in the 'slightly brown' category, the exception was the 'hook harvested' group that had fewest observations in this category, and equal proportions in 'good' and 'brown'.
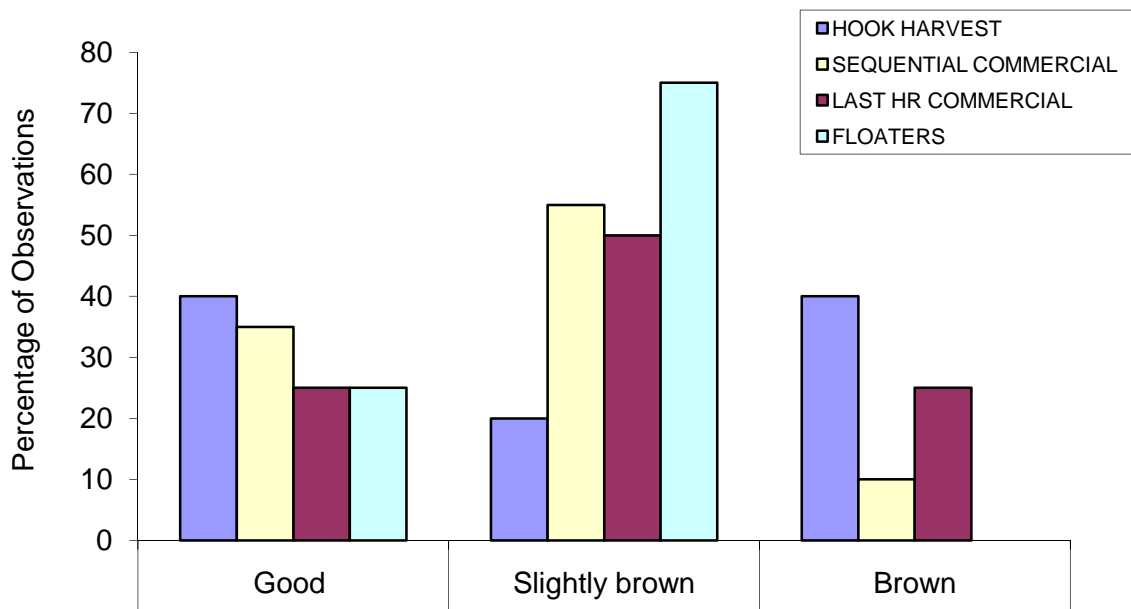
Figure 50. Fillet colour on day 8/9 as subjectively assessed by an independent fish processing expert for the different harvest types (n=15, 20, 8 and 4, respectively).

Fillet smell and firmness were also assessed on day 8/9. Only two categories of each attribute were scored. Most fish were assessed as falling into the more desirable categories (good and firm, respectively), and there was no obvious difference between the fillets from fish harvested by the different approaches (Figs 51 and 52).
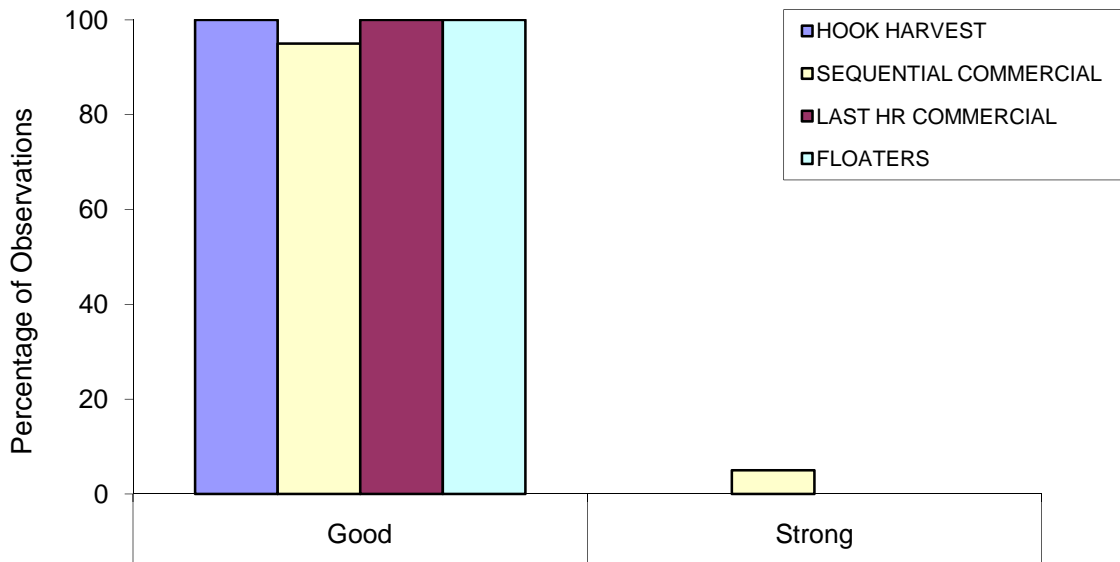
Figure 51. Fillet smell on day 8/9 as subjectively assessed by an independent fish processing expert for the different harvest types (n=15, 8, 20 and 4, respectively).
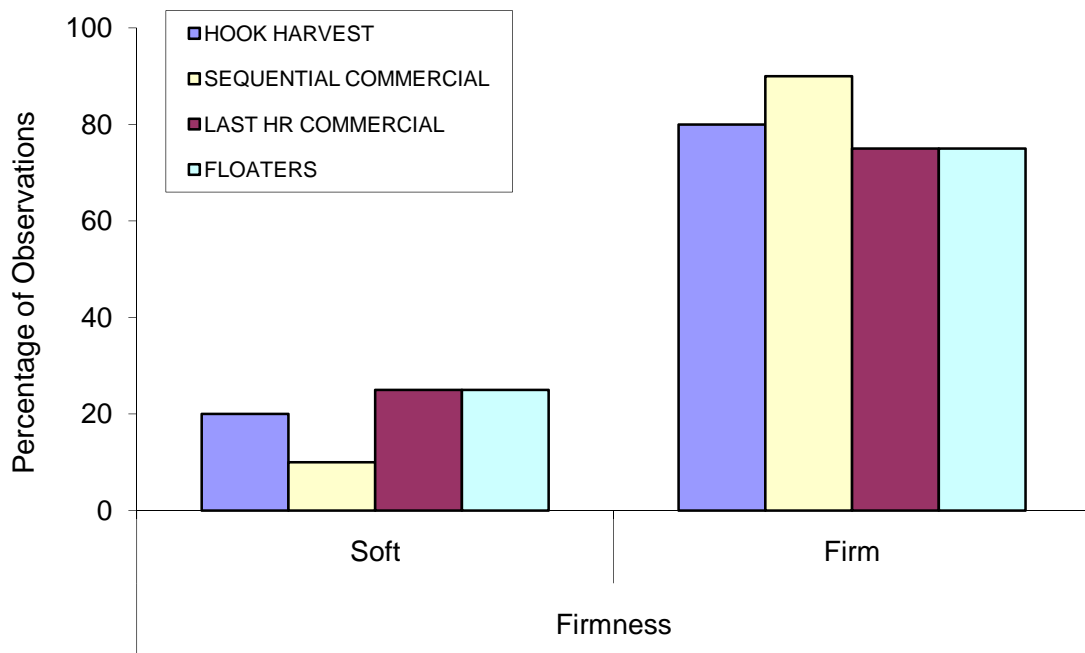
Figure 52. Fillet firmness on day 8/9 as subjectively assessed by an independent fish processing expert for the different harvest types (n=15, 8, 20 and 4, respectively).

Commercial Harvest – Sequential trends

It is reasonable to expect that the stress associated with metabolic exhaustion and/or poor water quality during net crowding might increase over time, therefore the fish euthanised early in the harvesting event might show lower stress responses than those euthanised late in the harvesting event. Data from the first day of sampling have been analysed to see if there is any evidence of this.

Figures 53 and 54 show the blood pH at time of harvest, and 11 hrs later (after CO_2 has degassed) of fish in harvest order. There was a non-significant trend for blood pH to decrease (ie the blood to become more acidic) with harvest sequence (Fig 53) as would be expected if stress was increasing during the harvest; however, the blood pH measured at 11 hrs post-harvest (which is mostly due to lactic acid) is basically similar in all the sampled fish, irrespective of sampling order (Fig 54).

Flesh pH measured at harvest also shows a trend to decrease with harvest sequence as might be expected if stress were increasing with confinement time (Fig 55). However, as with the blood pH (t=0) data, the trend was non-significant. Figure 56 shows the relationship between blood pH and flesh pH for the 20 sequentially harvested fish at t=0. Although there is a trend for blood and flesh pH to be correlated, it is not a strong trend, and the trend disappears completely when the effect of CO_2 on blood pH is subtracted (ie the blood pH at t=11 hr values; Fig 57).

Figure 53. Blood pH of individual fish at time of harvest in sampling order throughout a commercial harvest. Regression line and correlation co-efficient show the trend and goodness of fit of the fish to that relationship. Regression P=0.172; not significant.

Figure 54. Blood pH of individual fish remeasured 11hr post-harvest in sampling order throughout a commercial harvest. Regression line and correlation co-efficient show the trend and goodness of fit of the fish to that relationship Regression P=0.879; not significant.

Figure 55. Flesh pH of individual fish at time of harvest in sampling order throughout a commercial harvest. Regression line and correlation co-efficient show the trend and goodness of fit of the fish to that relationship Regression P=0.142; not significant.
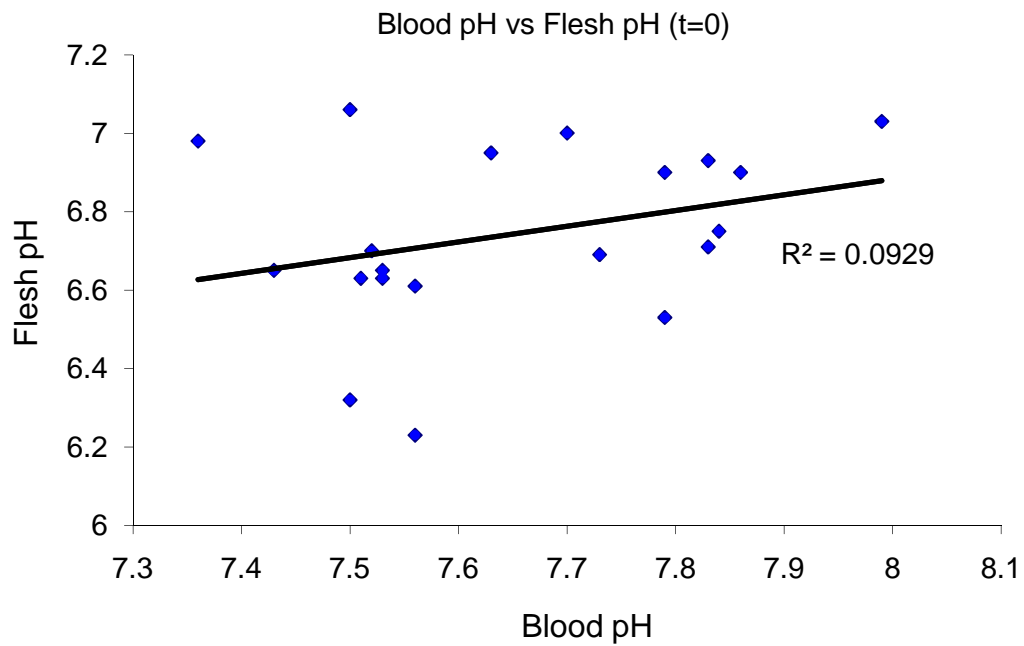
Figure 56. Blood and flesh pH of individual fish at time of harvest (t=0) from a commercial harvest. Regression line and correlation co-efficient show the trend and goodness of fit of the fish to that relationship. Regression P=0.191; not significant.

Figure 57. Blood (t=11hr) and flesh pH of individual fish at time of harvest (t=0) from a commercial harvest. Regression line and correlation co-efficient show the trend and goodness of fit of the fish to that relationship Regression P=0.616; not significant.

Other blood stress indicators are lactate and cortisol; the values of these parameters measured for each fish in harvest sequence are shown in Figures 58 and 59. Plasma lactate levels show no trend with harvest sequence, whereas plasma cortisol levels appear to show an increase from the beginning of the harvest process to fish Y5 (about 1 hr into the harvest event), after which the levels stay similar. The overall regression for plasma cortisol is not statistically significant.
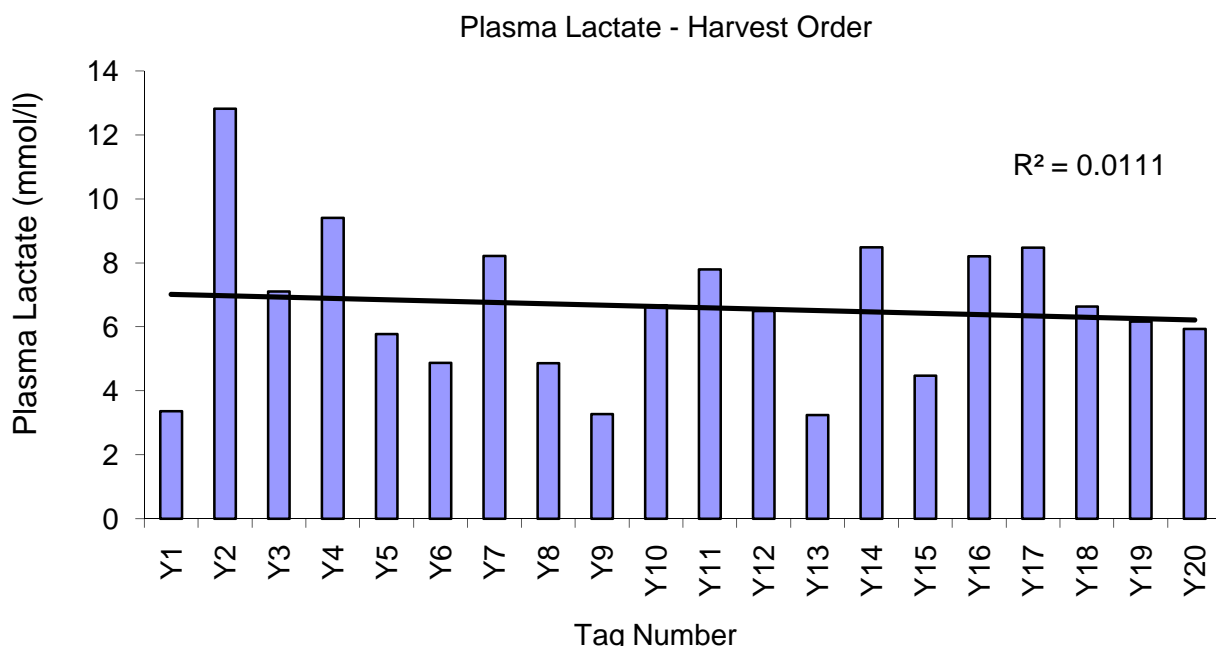
Figure 58. Plasma lactate concentrations of fish in sampling order through a commercial harvest. Regression line and correlation co-efficient show the trend and goodness of fit of the fish to that relationship. Regression P=0.658; not significant.
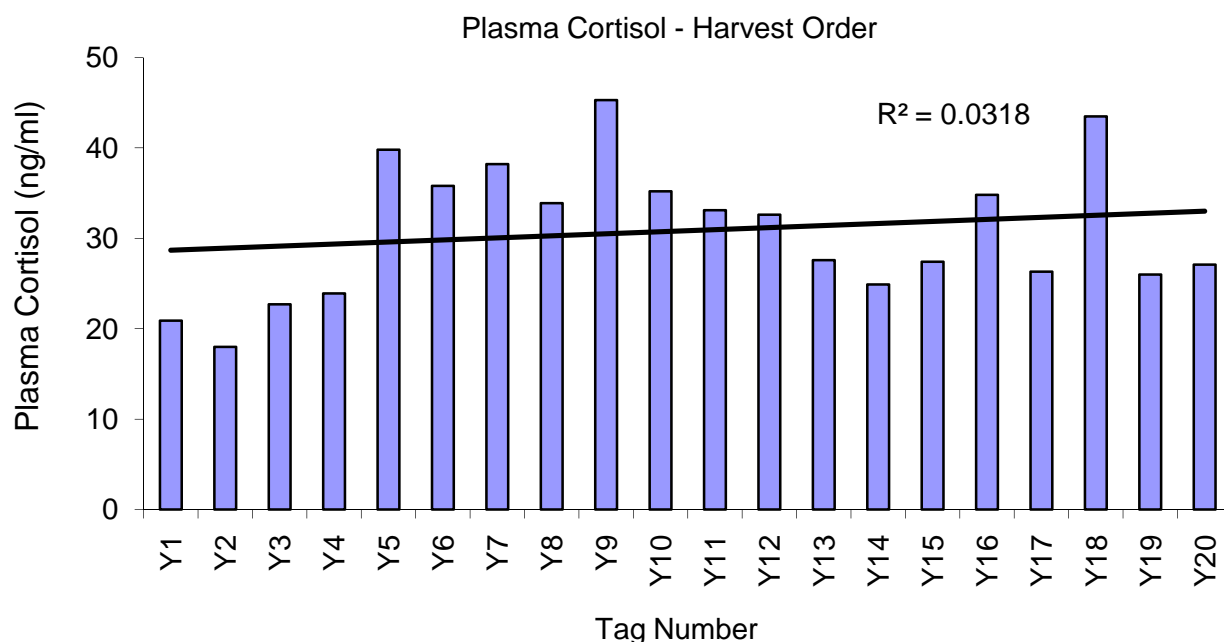
Figure 59. Plasma cortisol concentrations of fish in sampling order through a commercial harvest. Regression line and correlation co-efficient show the trend and goodness of fit of the fish to that relationship. Regression P=0.451; not significant.

As might be expected, plasma lactate values (largely lactic acid) are significantly correlated to blood pH values at t=0 and t=11hr (Figs 60 and 61).

Plasma Lactate vs Blood pH (t=0)

$R^2 = 0.335$
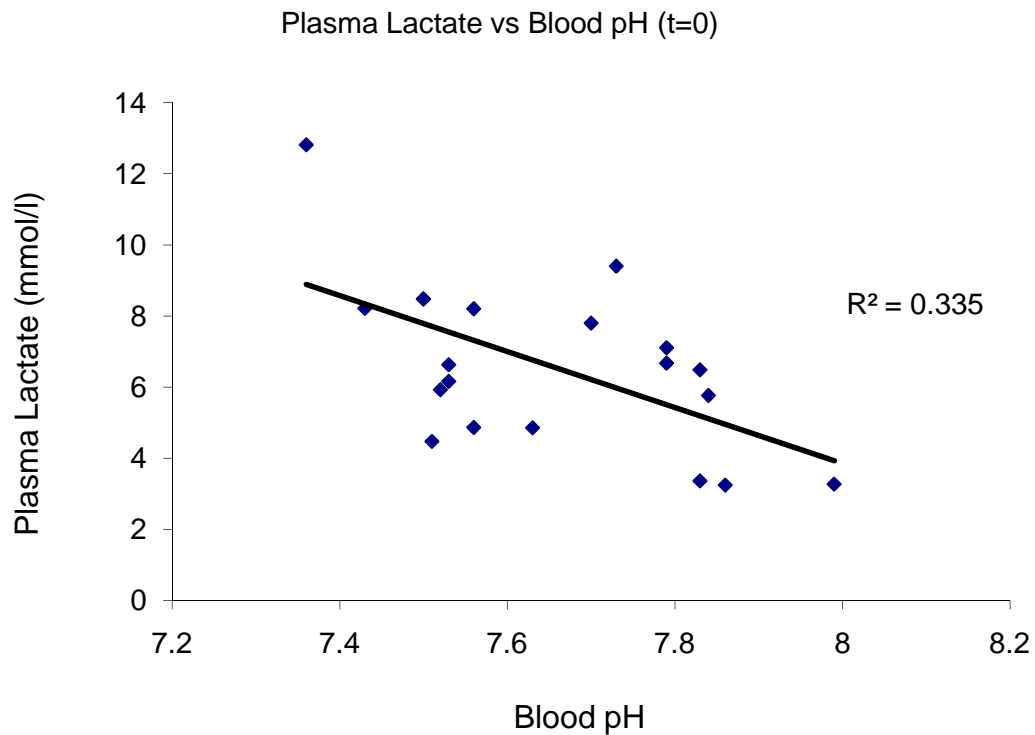
Figure 60. Blood pH levels (at t=0hr) vs plasma lactate concentrations of individual fish at time of harvest from a commercial harvest. Regression line and correlation co-efficient show the trend and goodness of fit of the fish to that relationship. Regression **P=0.004**; significant.

Plasma Lactate vs Blood pH (t=11hr)

$R^2 = 0.2789$
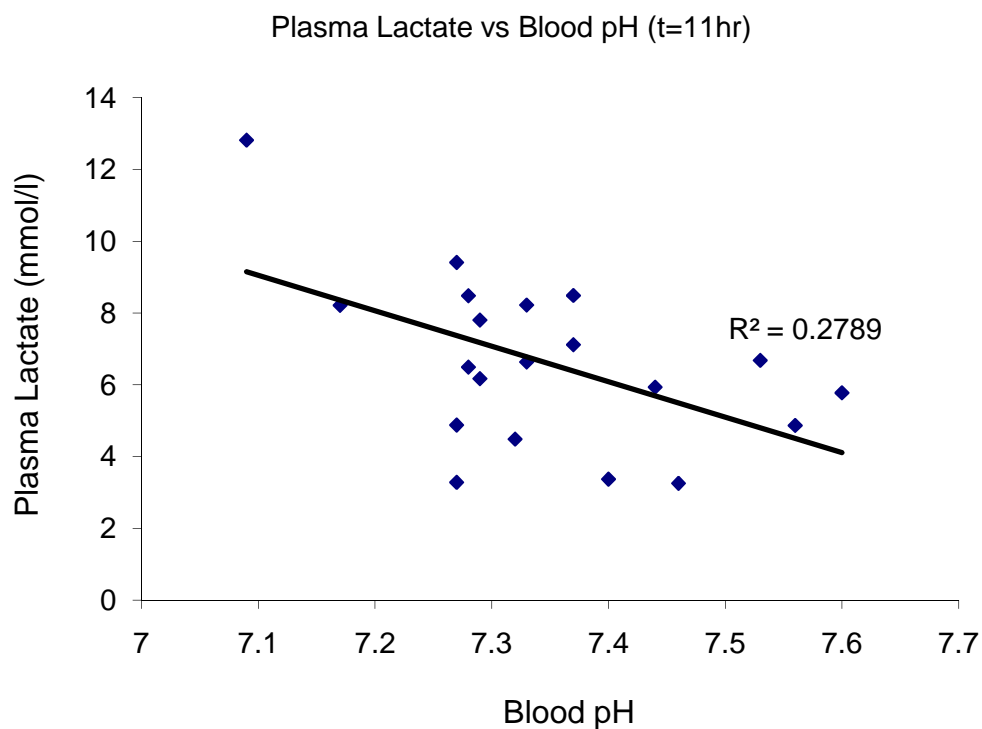
Figure 61. Blood pH levels (at t=11hr) vs plasma lactate concentrations at time of harvest for individual fish from a commercial harvest. Regression line and correlation co-efficient show the trend and goodness of fit of the fish to that relationship. Regression **P=0.0167**; significant.

There was no significant regression relationship or trend between harvest order and carcass firmness (Figs 62 and 63; lower scores indicate softer flesh), or eye clarity score (Fig 64).
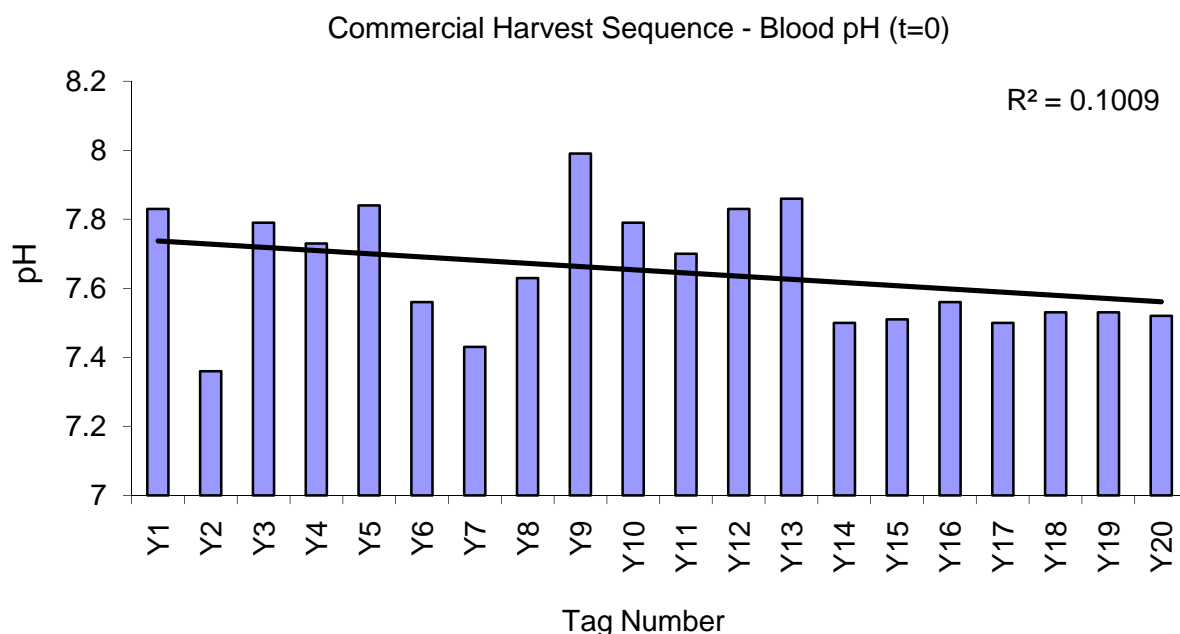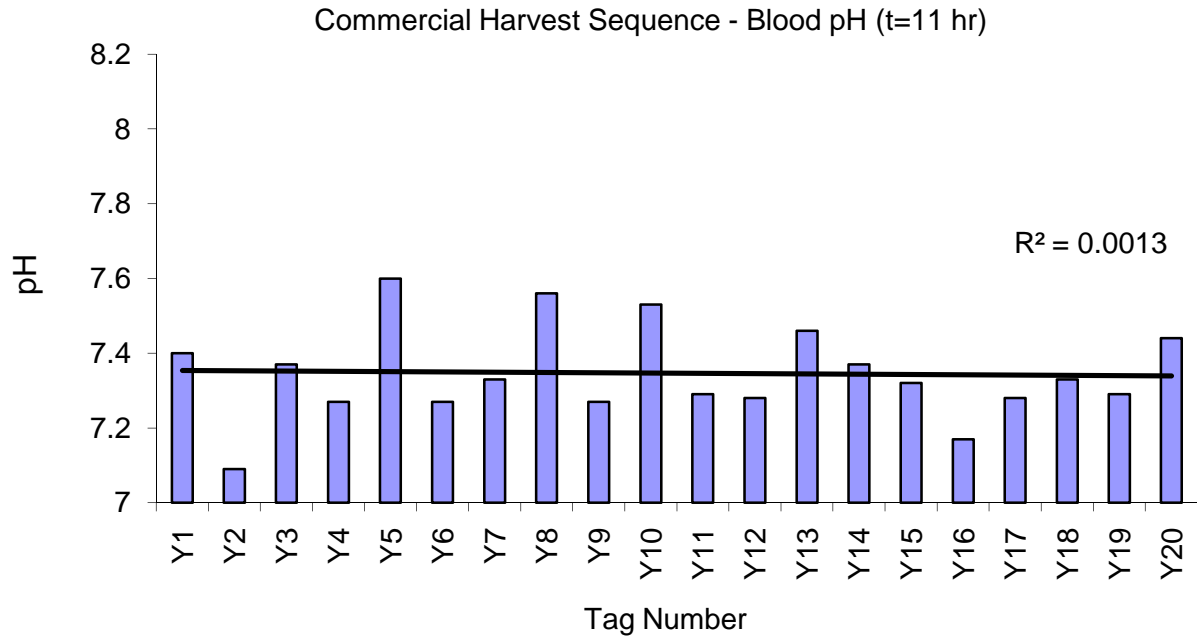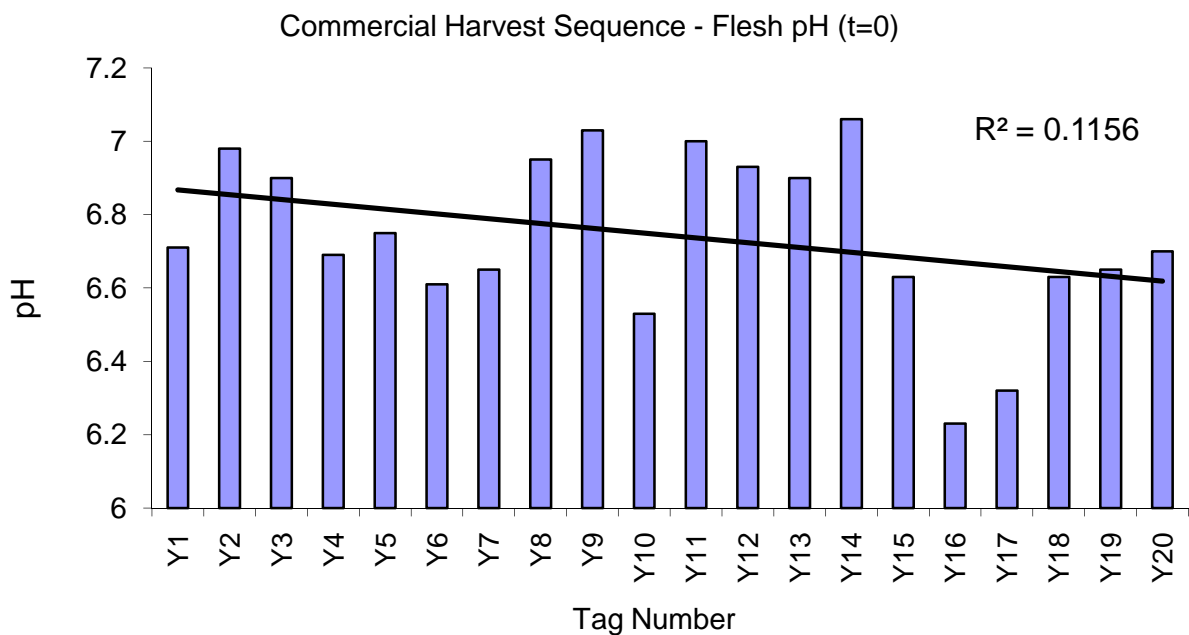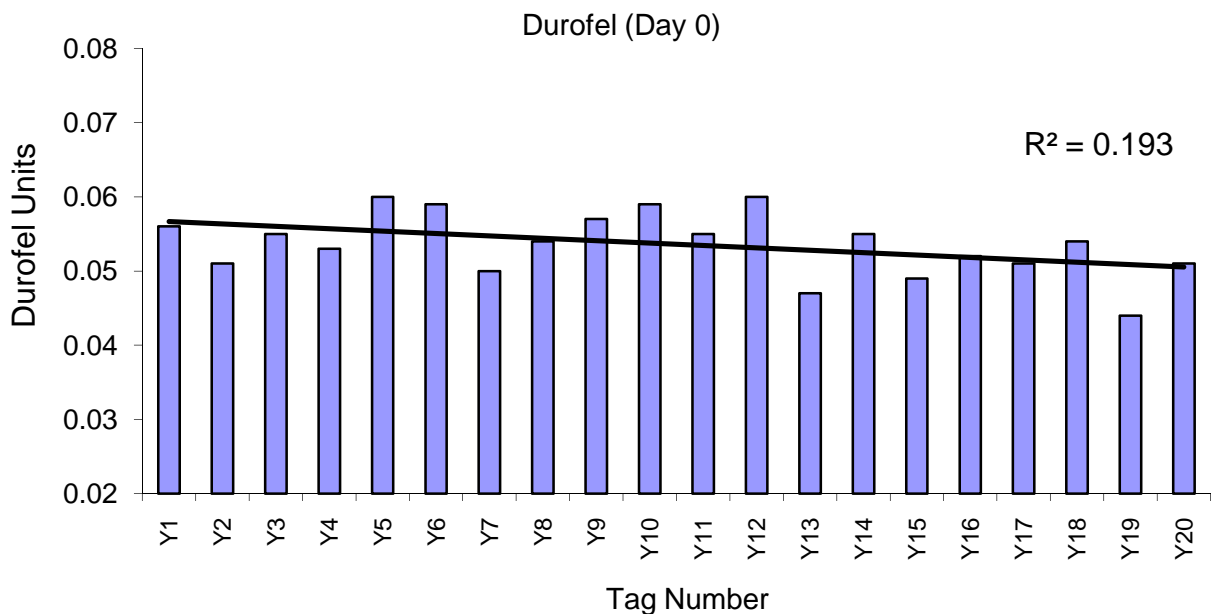

Figure 62. Carcass firmness as measured by Durofel of individual fish on day 1 in sampling order throughout a commercial harvest. Regression line and correlation co-efficient show the trend and goodness of fit of the fish to that relationship. Regression P=0.176; not significant.
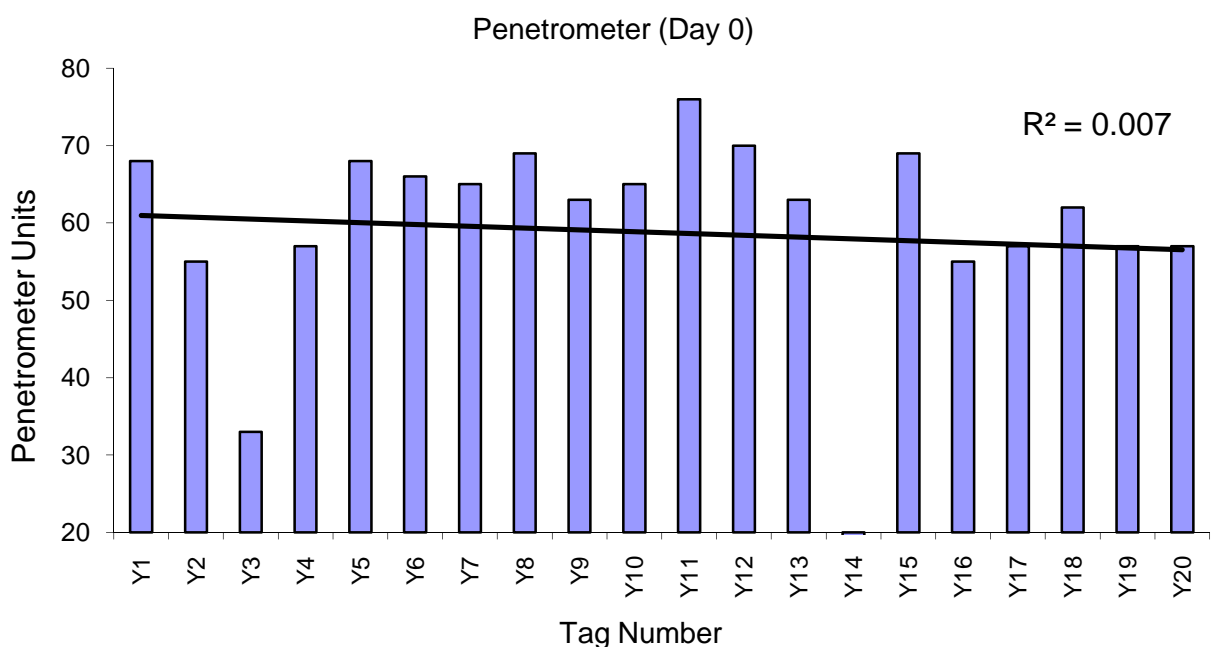

Figure 63. Carcass firmness as measured by Penetrometer of individual fish on day 1 in sampling order throughout a commercial harvest. Regression line and correlation co-efficient show the trend and goodness of fit of the fish to that relationship. Regression P=0.834; not significant.
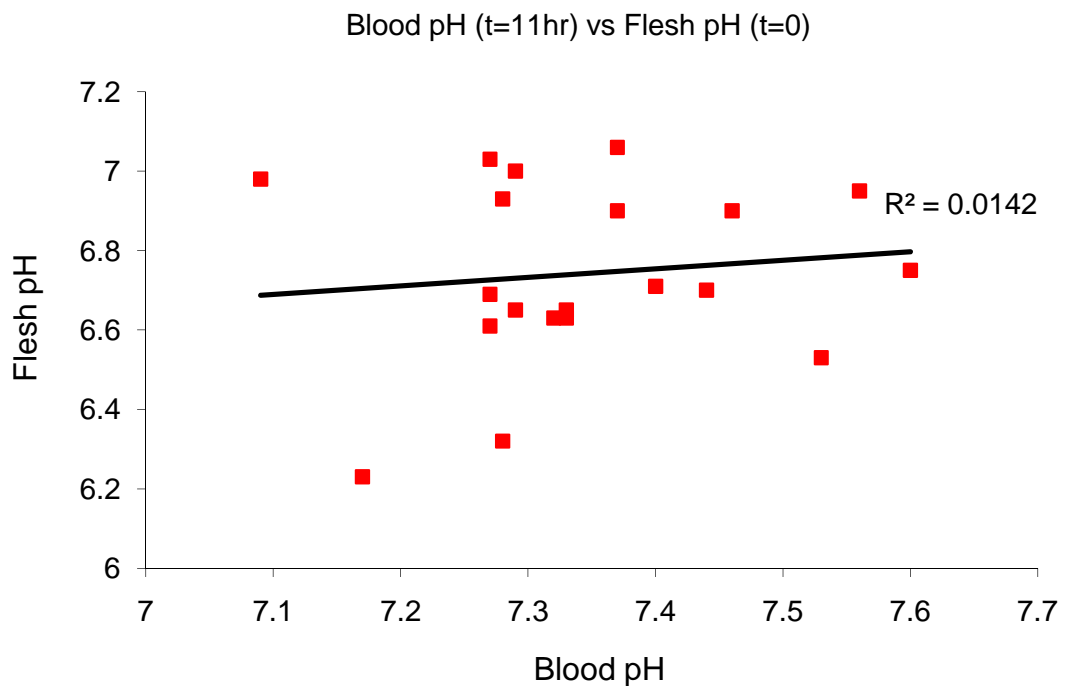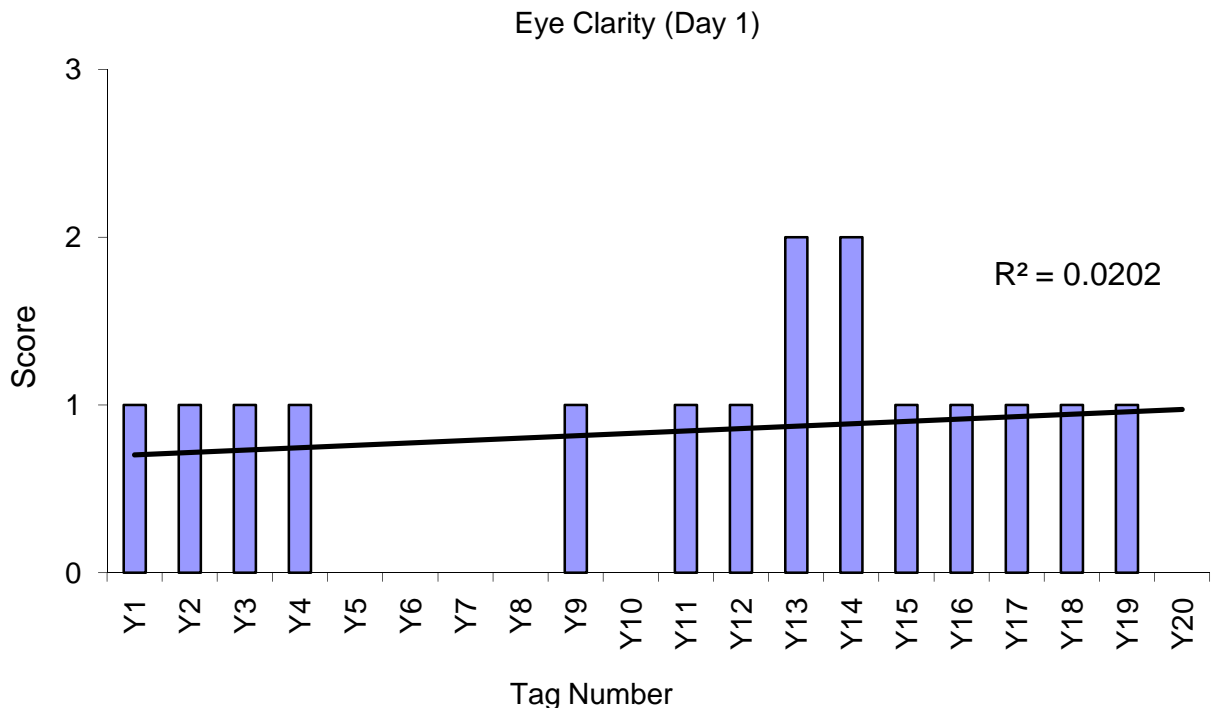
Figure 64. Eye clarity scores (subjective) of individual fish on day 1 in sampling order throughout a commercial harvest. Regression line and correlation co-efficient show the trend and goodness of fit of the fish to that relationship. Scoring system: 0=none, 1=some, 2=moderate, 3=severe. Regression P=0.562; not significant.

There was no indication of a significant impact of harvest sequence on any of the post-mortem (day 1-9) flesh characteristics such as flesh pH, rigor index, drip loss, penetrometer or Durofel readings, gaping, fillet colour, firmness or smell. Figures in Appendix 3.

Post-harvest fish handling

Temperature loggers placed in the on-deck holds and inserted into the stomach of harvested fish showed that the cooling systems on the boat were adequate to chill the water to approximately 5 degrees when the first fish were consigned, and then the water chilled further to 0 degrees at unloading (about 17:00-18:00 on day 1, and 18:30-19:30 on day 2). The short spikes in water temperature (eg evident around noon, 5.15 and 6.30pm in Fig 65) were due to activities that required the loggers to be temporarily moved (ie to allow harvested fish to be pushed to the back of the on-deck hold, and for unloading at the wharf). Until the temperature loggers were inserted into the fish stomach they were measuring air temperature (16 degrees on Day 1 and 25 degrees on Day 2), shortly after that time they showed 19-22 degrees reflecting the fish core temperature (largely a factor of sea water temperature and perhaps some post-exercise heat production). The core temperature of the harvested fish fell below 5 degrees within 1-3 hours of immersion (Figures Temp 65, 66 & 67). The longest time taken for a temperature logged fish to cool below 5 degrees was 3 hr for fish B1. This was one of the 'floater' fish so even after it had been attached to the line in the on-deck hold, it floated in the surface water at the hatch in full sunlight. This would not happen in a normal mesh bag containing 50 fish that is deliberately pushed towards the back of the on-deck hold. The fish remained between 0 and -2 degrees after transfer to the ice slurry tanks on the truck, and until processed in the factory the next day.

There is nothing in these data to suggest that the post-harvest chilling of the fish could be contributing to a poor quality or shelf life of the product.
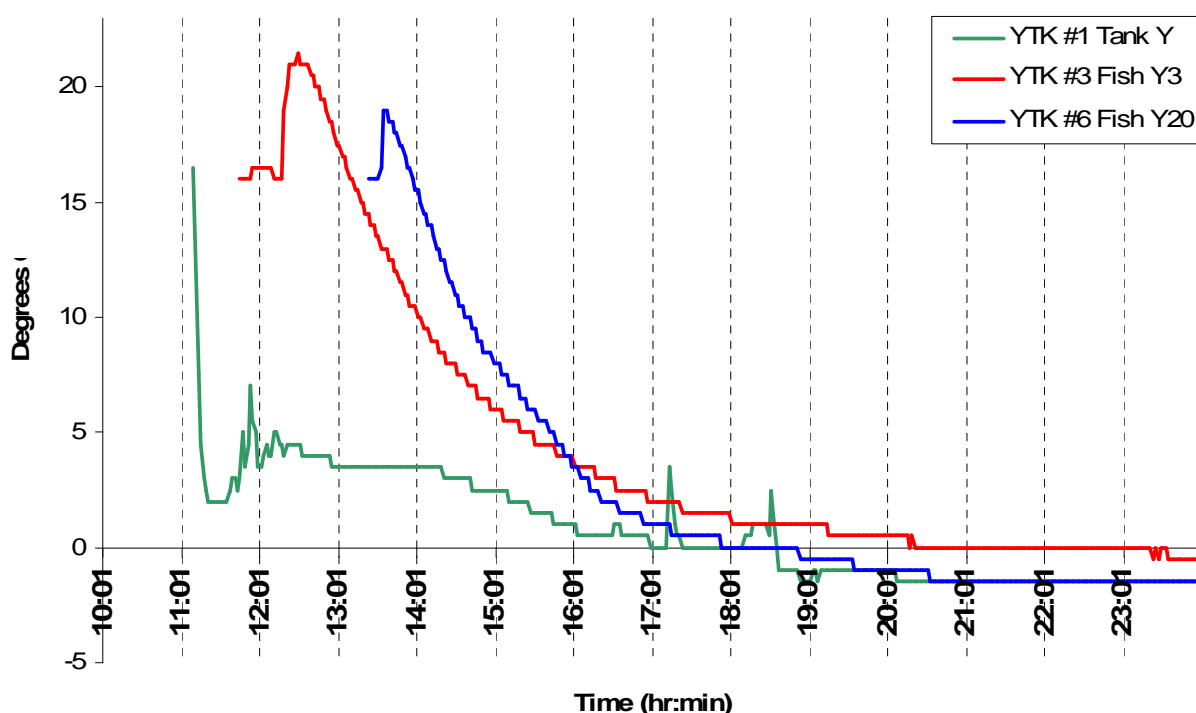
Figure 65. Core body temperature of sequential commercially harvested fish (day 1).
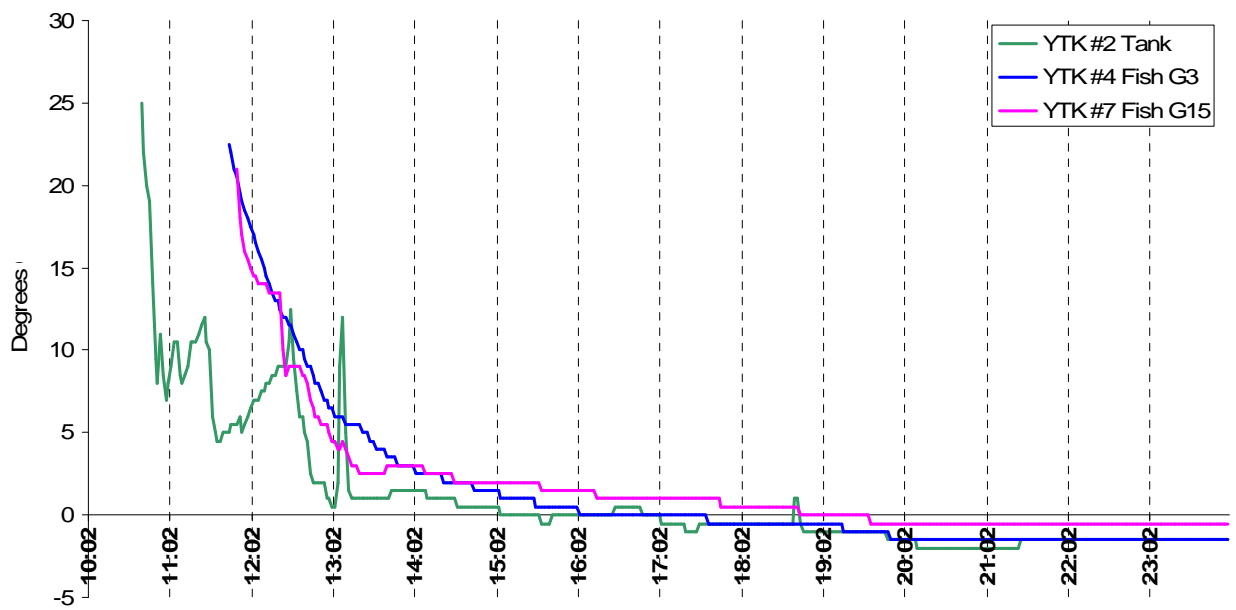
Figure 66. Core body temperature of hook harvested fish (day 2).
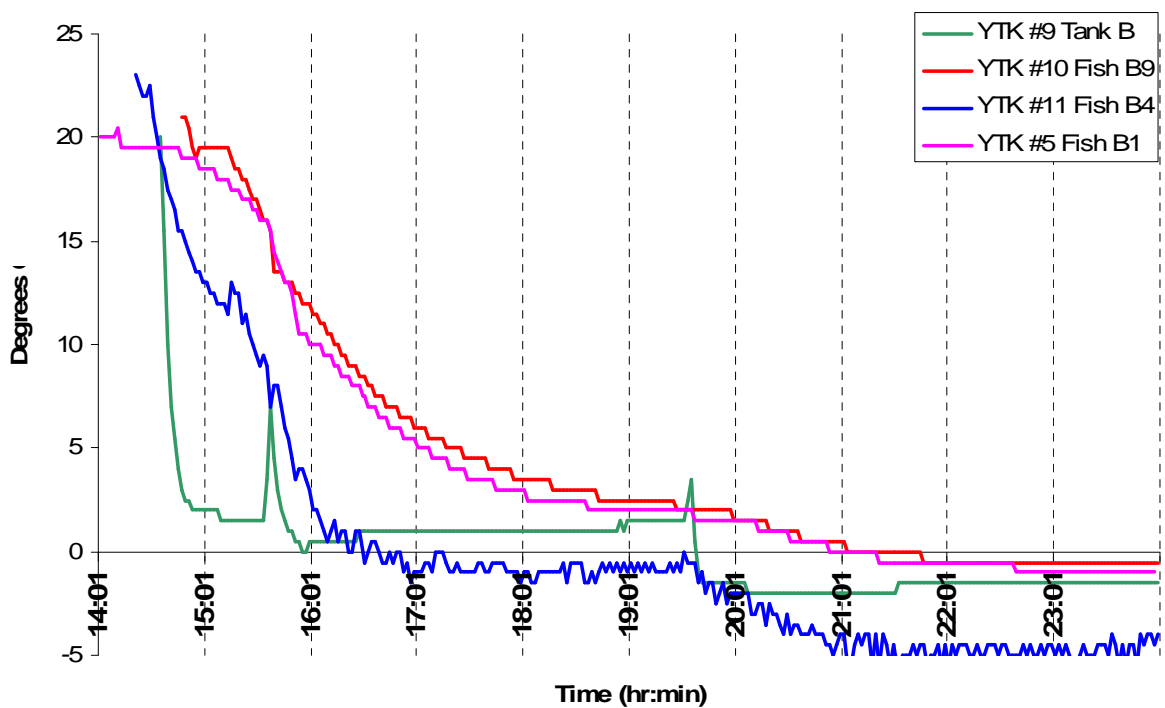
Figure 67. Core body temperature of last hour commercial harvest and floater fish (day 2).

Discussion

This preliminary study was carried out to try to determine if there was any significant effect of sexual maturation and/or harvest stress from a new 'dead haul' harvesting process on flesh quality attributes of 2+ YTK. The study was carried out on two consecutive days in mid-December 2008, and so the data are only representative of harvest practices and stages of maturity evident at that time.

Sex and Reproductive Maturity

The fish sampled in this study had a sex ratio approximately 1:1. This is expected for a broadcast spawning species when the larvae or fingerlings have not gone through a bottleneck with high levels of mortality or environmental extremes or perturbations. It also suggests that the two sexes do not show a pronounced difference in growth rate that would result in sex-biased populations/pontoons as a consequence of any size-grading regime carried out more than 6 months pre-harvest.

Only one (4%) of the females sampled was in an advanced reproductive condition. The oocytes which were released from the ovary on day 3 after filleting were visible to the naked eye and were estimated to be close to 1mm in diameter (see Photo gonads). Unfortunately, the sample of oocytes put aside for histology was lost as the cassette was moved between containers of fixative – presumably because of dehydration and shrinkage of the tissue. The histology may have clarified whether the oocytes had undergone final maturation and ovulation, as they appeared to be very loose in the ovary lumen. All fish were stripped at the time of harvest to identify any spermiating males, but no female released any ovulated eggs at this time suggesting that this female had not ovulated in the pontoon. The fact that fish were not filleted until the 4th day post-harvest gives more reason to believe that the loose appearance of the oocytes in the ovary may have been due to post-mortem mechanisms.

In contrast, 88% of male fish were found in advanced stages of reproductive maturity (either running ripe, or spent). It is not unusual for male fish to mature at an earlier age, or smaller size, than female fish of the same species. The pattern is well known and understood in salmonid aquaculture (Aksnes et al., 1986; Bilinski et al., 1984). Fish also commonly exhibit 'precocious maturation' where a proportion of the population (again, usually males) become sexually mature at a younger age and size than the rest of the cohort. Precocious maturation can be a problem in aquaculture of some species, with genetic, nutritional and environmental factors contributing to increase the proportion of a population exhibiting precocious maturity.

There is limited information available of the age or size at maturity of wild YTK in South Australia. There is some suggestion that maturity occurs at 4-5 years of age, and 74cm length (PIRSA Aquaculture). However, data from NSW suggests 50% of females mature at 84cm

whereas 50% of males are mature at 47cm, at an age of just 1 (Gillanders et al, 1999). New Zealand YTK were reported to be even larger at maturation (females 94cm, males 81cm)(Poortenaar et al, 2001). Hatchery reared aquacultured YTK were found to mature at 13 months, 3.5kg and 50cm (Kolkovski amd Sakakura, 2004). For these reasons, it is suggested that the maturation exhibited by these farmed YTK is not precocious.

Male maturity and spermiation

No Stage 3 males (an early phase in maturation, mainly characterised by small quantities of viscous, thick milt expressed at stripping and when testes are sectioned) were found during this work. This is probably because the study was undertaken near the end of the spawning window (as evidenced by the presence of some spent males) and so all the maturing 2+ fish had already passed through the Stage 3 phase to become Stage 4 or 5. Alternatively, it may have been because the gonads were not removed until day 4 post-mortem and the milt in the gonad may have undergone some hydration from tissue breakdown or infiltration from the surrounding ice slurry making the milt more copious and less viscous. A sampling protocol that assessed male maturity stages at monthly intervals throughout the year would resolve this question.

All male fish taken from the commercial harvest process that had gonads assessed macroscopically as Stage 4 (running ripe, or spermiating) were able to be stripped of sperm at the time of harvest. In contrast, the majority of Stage 4 males (assessed by sperm volume and consistency when the testes were sectioned) caught by hook harvest could not be stripped of milt when they were landed. This finding suggests there may be a degree of control over milt expression that is assisted by stress. However, it's unlikely that the control mechanism is purely nervous, as spermiation was not apparent immediately after hook harvested fish were spiked through the brain. Such information should be considered in future sampling trials as stripping of hook harvested fish could underestimate the number of running ripe males in a pontoon/population.

If there were a need to separate/differentiate spermiating from non-spermiating fish during the commercial harvest process (for product quality, shelf-life or yield reasons, see discussion below), it would be possible. The process worker that deals with the 'tail-gating' fish that do not get stunned or bled automatically by the pneumatic mechanism, could look for milt and easily direct spermiating and non-spermiating fish in different directions. Whilst the spermiating group would be all Stage 4 males, the non-spermiating group would contain females, and, depending on timing in the spawning window, Stage 1, 2, 3 and/or 5 males. If, as suggested below, Stage 5 males might have less desirable product quality attributes, this level of sorting would not help redirect those fish to a marketplace where those characteristics would not be apparent.

The presence of Stage 5 (spent) and no Stage 3 males in the sampled population suggests that this cohort had passed the peak of reproductive maturity by mid-December. Consequently, it

may be that in the timing of this study we have missed the maximal testes size (GSI), as the mean value for Stage 4 (spermiating) fish was 0.72% of bodyweight, with the highest individual value of 2.93%. Certainly, routine farm production sampling records have indicated individual fish GSIs with higher values (>6%), but with some variation between growout sites and months (Joe Ciura, pers. comm.). Ongoing sampling programs with larger numbers of fish being taken either on farm or in the processing area would refine the mixed picture we currently see.

The impact of maturation on somatic growth (in particular fillet yield) will be reflected by the duration and magnitude of the increase in GSI – with high GSIs (eg 15-20%) and/or prolonged periods of spermiation (>3 months) – being of greatest concern. What are the conclusions for this particular cohort of fish? With a single snapshot assessment it is not possible to say anything about the duration of increased GSI, but farm records do suggest males in a population can have elevated GSIs from October to December - 3 months. However, on the that basis and the relatively small maximal GSI values (<6%), it suggests that Yellowtail Kingfish are unlikely to suffer greatly in this regard.

Indeed, on the contrary, the fact that male fish were heavier, had a higher condition factor, and produced heavier fillets than female fish would strongly suggest that there could be a positive impact of maturation on product yield. On average mature male fish yielded 140g more fillet than immature female fish. At a wholesale price of $15/kg for fillets, a maturing male is worth an extra $2.10 per fish. However, yield is just one aspect of product quality, the others are appearance and shelf-life. Fortunately, unlike some other species of fish, there was no outward sign of maturation (eg changes in skin colouring, jaw shape, flesh firmness) of mature (spermiating/spent) male YTK sampled in this study. Thus, it's highly unlikely that the outward appearance of mature male fish could raise any issue with customers.

On the other hand, there was a suggestion that the fillets of spent male fish tended to either become more brown, or brown more quickly, than other male or female fish. The browning is probably due to lipid oxidation (Sohn et al., 2005). Unfortunately, there is no way of identifying spent male fish and partitioning them from the rest of the harvest without gutting the fish, and this does not necessarily fit with current post-harvest practice whereby whole chilled carcasses are dispatched to customers or markets. It may be that this is a step that needs to be taken from Oct-Jan when mature males are evident in the population. However, it is worth restating that the difference in fillet colour between Stage 4 and 5 males on day 8/9 post-harvest (or day 4/5 post-filleting) was quite subtle, so caution should be exercised when interpreting this observation.

In fact the factor having the greatest affect on changes in fillet colour was time – with all fillets showing considerable surface browning over the 4-5 day period of refrigerated storage. This browning could certainly be an issue for markets and customers that require a long fillet shelf-life. Additional work to control and minimise fillet browning over time is strongly endorsed.

Eye cloudiness scoring.

During the experiment it was observed that the eyes (particularly the lenses) of all the fish changed in opacity/translucency. At harvest the eyes were bright, clear and black, and no lens was visible. After the fish had been in chilled water for 6-8 hours (ie some of the first fish harvested at the time of unloading), the surface of the lens in one or both eyes had started to become opaque and ragged, almost as if there was a membrane sloughing off the outside of the lens. Both eyes on the same fish would not necessarily show the same level of opaqueness at the same time. By the time fish were given their rigor sampling assessment the next morning (~18 hours post harvest), almost all fish had cloudy or severely cloudy lenses, with 'floater' fish having more severe scores than other harvest types. However, 24 hours later (day 2 post-harvest), lens cloudiness was decreasing in almost all of the fish. Indeed, the next day (72 hours post-harvest), all of the lenses were clear and black again, but by now the cornea was starting to become slightly opaque.

Degenerative changes in fish eyes are commonly associated with time since harvest and storage temperature (thereby indicating post-harvest handling practices, and in some cases predicting shelf-life) and this is why eye appearance (colour/opacity/shape) is commonly used as an indicator of fish freshness and why it often appears as a parameter in Quality Index Methodology (QIM) approaches (Bremner 1985; Huss 1995; Hyldig and Green-Petersen, 2004). What isn't commonly known is that there can be a temporary change in lens opacity that resolves with time. This is called a "cold cataract" and happens when low temperature affects the outer protein layers on the cornea and lens and changes the structure, conformation and solubility of the proteins causing the opacity (Loewenstein and Bettelheim, 1979). A cold cataract, therefore, actually indicates an effective post-harvest chilling process is in place. In the present study the Yellowtail Kingfish heads were discarded on day 4 post-filleting, so it's not possible to say how the eyes changed beyond this timepoint. For the reason that it appears to be temporary or reversible, lens clarity/translucency is probably not a good indicator of fish quality in this species. On the other hand, changes in eye form, cornea cloudiness and iris colour are non reversible and have been used as a factor in a QIM for YTK (Boulter et al., 2009). Any market comments about eye or lens translucency being a problem for product acceptability should be addressed by this information.

Harvest Stress

Plasma cortisol and lactate levels measured in this study fit largely with expectations (the exception is for the 'floater' fish and they will be discussed separately). Hook harvested fish have low levels of both cortisol and lactate, suggesting they are relatively 'unstressed' in the harvest pontoon despite the 5 day a week harvest schedule. In contrast, the commercially harvested fish have elevated cortisol and lactate, indicating these fish have been exposed to a

63

period of physiological and metabolic stress. It is well known that plasma lactate and cortisol levels take several minutes to increase following onset of stress or activity (Erikson et al., 1997; Robb et al., 2000; Thomas et al., 1999), so the increases measured here probably reflect the 'stressfulness' of the crowding and pumping components of the harvesting process. How stressful is this process? Unfortunately, the fish that were put into one of the deck tanks for overnight confinement to provide the 'high stress' treatment died, so it's not possible to say. Comparison of absolute cortisol and lactate levels from YTK with those from other species is useful, but not without risk. Compared to captured and confined tuna and salmon, the YTK levels are low; however compared to captured and confined barramundi, they are high. Further studies would be needed to characterise the magnitude and duration of the YTK stress response to different acute and chronic stressors.

In contrast to the plasma lactate and cortisol data, the blood and flesh pH data are somewhat at odds to what would be expected. The blood and flesh pH results show that hook harvested fish have an unexpectedly low average pH value compared to the commercially harvested fish ('sequential' fish especially, but also the 'last hour' fish) suggesting that the hook harvested fish have a high blood acid loading. The results of the 11 hr re-measurement of blood pH reflect this with a substantial increase in pH from 7.13 to 7.43 units due to assumed CO_2 de-gassing. From these data it is reasonable to assume that the hook harvested fish were metabolically stressed. Hook harvested (but otherwise unstressed) fish should have low blood and flesh carbonic and lactic acid levels and a pH value of about 7.80. The 30 seconds or so that passed between hooking the fish, bringing it quickly to the side of the cage, netting and lifting it from the pontoon across to the harvest boat, to when it was spiked through the brain immediately prior to being blood sampled, would not be expected to result in such an accumulation of carbon dioxide or lactic acid.

The other aspect of the pH data that needs mention is the decrease in blood pH measured after 11 hr in the commercially harvested fish on day 1. This is very unexpected. Some species have red blood cells that have membrane-associated enzymes which can continue to metabolise and change apparent 'at harvest' values for certain parameters during storage, however all of these blood samples were immediately placed into ice which would have greatly inhibited any metabolism. Further, blood samples from hook harvest, commercial harvest and floaters were all handled in the same way, meaning any post-sampling changes due to metabolism should have affected all samples, which was not the case.

Can a pH meter calibration problem be ruled out? Not entirely. The sequential commercial harvest fish were sampled on day 1, whilst the hook harvested, last hour commercial harvest and 'floater' fish were sampled on day 2. Thus, there is little basis to suspect a calibration problem on day 2, however it is not as easy to rule out the possibility of a calibration problem on the boat on day 1, which was rectified before the blood pH was re-measured in laboratory 11 hr later. If this were the case, it would mean that the absolute 0hr (blood and flesh) pH and change

in blood pH values from 0 to 11hr would be invalid for the sequential commercial harvest fish. Whilst this may be the case, the relative differences in pH (blood and flesh) between sequential commercially harvested fish at 0hr are likely to be still valid.

When all fish data was pooled there was no significant correlation between blood pH (measured twice, first at time of harvest, or again 11 hrs later after CO_2 had degassed), and flesh pH measured at time of harvest. This suggests that the acid-base composition of the blood is largely independent of that of the white muscle (flesh). There was, however, as expected, a very strong significant relationship (P=0.004) between blood pH (at harvest) and plasma lactate concentrations. So, it appears that blood pH is a good, easy to measure, indicator of metabolic stress (ie anaerobic metabolism).

Floaters

Taking the plasma lactate and cortisol results together, it suggests that 'floaters' are severely metabolically challenged fish. Floaters are known by commercial operators to have poorly formed swimbladders and such fish often respond in stressful situations (eg net changes, parasitic bathing treatments, and harvests) by rising to the water surface and remaining there until after the stressor is resolved (hence their name). This fits with our understanding of swimbladder physiology and function. Fish can adjust the amount of gas (oxygen) in their swimbladder to modify their buoyancy when they cope with changes in water depth. Oxygen transfers into and out of the swimbladder via a special capillary bed called the rete mirabile and changes in the pH within the rete tissue allows oxygen to unload or load from or to the red blood cells. The observations made in this study suggest one of two things (1) that the stress which results from crowding of the fish causes the swimbladder to over-inflate with oxygen (either from a localised rete pH effect – since circulating blood pH was not affected), or (2) the shallowing up of the harvest net brings the fish with the deformed swimbladder to the water surface, but unlike the other fish they cannot efficiently remove the now expanded volume of gas from the swimbladder, therefore they remain positively buoyant.

Whatever the mechanism, neither relies on high plasma lactate or cortisol levels. Thus, it appears as though the high levels of lactate and cortisol observed in these fish are an indirect consequence of the swimbladder deformity. It may be that the ongoing disorientation (ie lying on the side) and/or prolonged metabolic activity of trying to swim down against the floatation created by the over-inflated swimbladder, cause the very high lactate and cortisol levels measured. If this is the case, it does suggest that the stress responses measured in the commercially harvested fish are not maximal.

If metabolic and/or stress responses are related to poor flesh quality attributes, it seems likely (and fortuitous) that these 'floater' fish would be an ideal sub-set of fish to study. So, was there any suggestion that floater fish have significantly worse flesh quality attributes or shelf life than

other fish? First, it has to be acknowledged that there were only 4 floater fish sampled in this study, so the conclusions have to be considered preliminary at best. There was some suggestion that floater fish went into a harder rigor that lasted longer than other harvest types, and they did have the highest eye clarity score (indicating a more severe lens cloudiness was observed); however, there was no suggestion that floater fish had worse flesh quality attributes (colour, smell, firmness, gape or drip loss) than hook harvested or commercially harvested fish. So, is there any reason to discard floater fish from a commercial harvest? Or is there any evidence to indicate that high levels of stress indicators have any deleterious affect on product quality? At this point, the limited evidence would suggest there isn't.

Commercial Harvest – Sequential trends

Fish stress responses often take 30 – 60 minutes to peak following imposition of a stressor. The process of crowding the fish prior to starting harvesting took at least 1 hour before the first fish were delivered to the stunning and bleeding machine. Three people working around the ring of the harvest pontoon, progressively shallowing the net and moving the fish towards the harvesting end, positioning the net to prevent fish bagging in the folds, crowding but not over-agitating the fish, positioning the pipe attached to the fish pump, all takes time and patience. Given the time and processes involved, it is not surprising that there was a measurable stress response detected in the commercially harvested fish. However, there was little indication that the magnitude of the fish stress responses increased during the harvest so the ongoing conditions (water quality, crowding, confinement) did not appear to be additionally stressful, however neither was there any signs of acclimation to the conditions. Similarly, there was no evidence of a detrimental trend in post-harvest carcass quality attributes with harvest duration.

Changes in Post Harvest Carcass Attributes

All of the results in rigor mortis and flesh/fillet pH changing through time of post-harvest storage are entirely consistent with expected patterns. Within the constraints of the daily sampling regime, the slow and steady pattern of onset and resolution of rigor mortis of YTK was typical for unstressed fish. The fact that there was no difference in the rate at which hook harvested, commercially harvested and even floater fish went into and out of rigor, would strongly suggest the fish crowding, confinement and delivery practices which comprise the commercial harvest process are not having a deleterious effect on carcass quality attributes. Stressed fish generally enter and come out of rigor mortis more quickly than unstressed fish (Jerrett et al., 1996; Lowe et al., 1993; Ostenfeld et al., 1995) due to the changes in muscle biochemistry which result from physiological stress responses and heightened levels of metabolic activity during the stressor/harvest. Flesh pH decreases post-mortem as lactate is generated as a result of ongoing anaerobic metabolism of stored glycogen in the tissues. Differences in the initial and

final (ultimate) pH levels measured in the flesh can indicate differences in the glycogen content of the flesh at the time of death. Given that there were no major differences in flesh and fillet pH post-mortem it suggests the fish were all in similar metabolic condition at the time of death, again indicating the commercial harvest process is not unduly impacting upon product quality attributes.

Harvest and Post-Harvest Procedures

Feedback on harvesting operations was given verbally to *Ulysses* crew during on both days of the trial and by telephone and email to Clean Seas Tuna staff immediately following the trial. The main points noted were:

- The crowding of the fish prior to harvest, the fouling on the net and the resulting water quality (particularly dissolved oxygen levels). Unfortunately it was not possible to measure or monitor dissolved oxygen levels in the crowded part of the net during the harvest. All of the activity associated with the crowd would undoubtedly increase the oxygen demands of the fish, but with the variables of tide flow and net fouling, would it always be high enough to not be limiting? A more effective fish pump (see next point) would reduce the crowding density and time of harvest, but it may be worthwhile monitoring dissolved oxygen and/or installing an air line from the boat to aerate the water for the harvested fish.

- The fish pump used to take the fish from the harvest pontoon to the whirlpool section of the stunner/bleeding component was not powerful enough to draw the 2+ fish into the harvest funnel. When the pump was running it was possible to see one or two YTK swimming and holding position in the flow within the translucent pipe between the pontoon and the pump. Those fish would have been swimming for a prolonged period prior to exhaustion and eventual transfer to the pump itself. A new larger capacity pump has since been purchased and will soon be installed (December 2009).

- The pneumatic stunning operation was not always executed satisfactorily even when a single fish went into the compartment. Examination of random fish for evidence of the position and extent of the strike showed that some fish were being hit too far forward (ie in front of the eyes) and so those fish were not stunned during the bleeding out. Some YTK heads were dissected in the processing factory on Day 4. The brain was exposed after removing a slice of skin, muscle and bone at least 15mm thick from above the eye line. The brain case starts at least 10mm from the posterior edge of the eye. The skull bone was 3-4mm thick and appeared to have a ridge of tough cartilage-like material that ran along the midline of the skull and extended anterior to it. Compared to most other fish the researchers have worked with, the YTK brain appears to be particularly strongly protected from external forces. This would suggest that it would be particularly important

to optimise the position and force required to stun these fish. Modifications to the pneumatic mechanism were made in early 2009 to increase the force delivered by the stunning bolt, since then the fish have been effectively stunned during gill cutting and bleed out.

- Post-harvest handling (chilling on the boat and transport to the factory) appears to be well controlled given some of the logical difficulties during unloading. A new chute has been fitted to the end of the bleed out conveyor that takes fish directly into a chilled water tank to further improve post-harvest temperature control.

Benefits and Adoption

Producers of Yellowtail Kingfish will benefit from this information as it helps them better understand their product so that they can better address customer questions, either by providing data (eg stress response parameters) or advice about differences in product specification that are seasonal/maturational in nature. A better understanding of the impact of the factors that can affect product quality and shelf-life will allow producers of Yellowtail Kingfish to focus their attention and future R&D funds on those issues that have the greatest impact in their own business and in the broader marketplace.

Further Development

Monthly sampling of 0+, 1+ and 2+ Yellowtail Kingfish is being undertaken as part of the Seafood CRC project "Understanding YTK" that started in June 2009. The greater numbers of fish (60 x 0+ and 30 x 1+ per month), and the greater frequency of sampling (monthly, plus additional processing factory sampling in November and December), will improve our understanding of the extent and duration of sexual maturation in growout fish. Determination of seasonal aspects of product quality (carcass composition) and shelf-life will further clarify the nature and extent of the suggested deleterious impact of male maturation on fillet shelf-life.

Changes made to the harvesting platform and process (eg a larger fish pump, better positioning and striking force from the pneumatic stunner) could be re-assessed by repeating the sampling and measuring stress indicators. Although there was no evidence of a deleterious impact of harvest stress on product quality or shelf-life in the present study, any improvement to make fish harvesting more welfare-friendly is to be welcomed.

Planned Outcomes

The main outcome from the project will be an improvement in product quality to the end customer, the simplest measure of which is the number and nature of customer complaints. It is

expected that if these changes to the harvest system are implicated quickly, then improvements in fish handling and on-board processes will be immediate.

Conclusion

This preliminary study was undertaken to determine the extent of any impact of harvest stress and/or sexual maturation of males on product quality and shelf life attributes of 2+ farmed Yellowtail Kingfish. At the time the study was carried out (mid-December) only 4% of female fish were showing signs of advanced sexual maturation, in contrast 67% of males were producing copious amounts of runny milt, and a further 21% were already spent. Thus 88% of the males were sexually mature. GSI's in most fish were less than 1% with a maximum of 2.9% recorded in this study. The relatively small size of the mature gonad would suggest there is unlikely to be a major energetic cost of maturation in 2+ fish that would detract from somatic growth.

Indeed, male fish tended to be slightly heavier and have a slightly greater condition factor than females. Fillet yield from males was slightly, but significantly, greater than females (67.9 vs 66.8%). This was probably because females had a significantly larger visceral index (7.16 vs 6.18%), with significantly more visceral fat (1.17 vs 0.58%) than males. There were no differences in liver size (0.73%), gonad size (0.56%), fillet crude fat (8.4%) or fillet moisture (66.8%), between the sexes. The difference in fish weight and fillet yield meant that male fish produced an average of 140g of additional fillet that would, at a wholesale price of $15/kg, be worth an additional $2.10 of saleable product per fish.

There were no significant differences between males and females in their physiological stress indicators at harvest, or the physical responses of the carcass post-harvest. Thus, sex, in itself, did not appear to detrimentally affect the flesh attributes or shelf life of product in this trial.

There were, however, significant differences in some product attributes related to the degree of maturation of the male fish, with spent fish having a 1% lower fillet yield, a 2% lower level of fillet crude fat with a 1.5% higher level of fillet moisture, and showing a tendency to produce proportionally more browner coloured fillets than running ripe male fish (ie 60% vs 15%). The low number of spent males (n=5) sampled in this trial means these results should be treated with some caution. Since 2+ males harvested from January onwards will be predominantly spent, the extent of these shelf life issues can be better assessed by carrying out additional sampling at this time.

Physiological stress indicators measured at the time of harvest (eg plasma lactate and cortisol) indicated that commercially harvested fish had higher stress levels than hook harvested fish. It is likely that factors associated with the pre-harvest crowd (high levels of activity, probably

limited dissolved oxygen, metabolic exhaustion due to fish swimming against the pump for some time) combine to cause this stress response. Interestingly, however, there were no signs that the stress of the crowd increased with crowding duration. This may be because the fish acclimated to the crowd (as suggested by the crew), but also because the crowd was not being constantly adjusted to compensate for the reduction in fish numbers (and thus density) due to harvesting. Adjustments in harvest net depth, water volume and fish density, are made infrequently if at all.

In contrast to the significant differences measured in stress response parameters, there were few significant differences in product quality attributes or shelf life between the different harvest treatment groups. Even the swimbladder deformed ("floater") fish that had the highest levels of stress indicators at time of harvest had flesh quality characteristics that were similar to hook harvested (low stress) fish.

Taken together, these data suggest that Yellowtail Kingfish is a robust species that, although stressed by harvest procedures, shows little, if any, deleterious impact on the quality and shelf life of the resulting chilled product. However, there is some suggestion that spent male fish may have a shorter product shelf life, with fillets tending to brown more, or faster, than fillets from female, immature or running ripe male fish.

References

Aksnes, A., Gjerde, B., Roald, S.O. 1986 Biological, chemical and organoleptic changes during maturation of farmed Atlantic salmon *Salmo salar*. Aquaculture,

Bilinski, E., Jonas, R.E.E., Peters, M.D., Choromanski, E.M. 1984. Effects of sexual maturation on the quality of coho salmon (*Oncorhynchus kisutch*) flesh. *Can Inst Food Sci Technol* J, 1984

Boulter, M., Poole, S. & Bremner, H.A. 2009. Australian Quality Index Manual: a tool for evaluating changes in chilled seafood. FRDC project 2003/237.

Bremner, HA. 1985. A convenient, easy to use system for estimating the quality of chilled seafoods. In Fish Processing Bulletin No. 7. Division of Horticulture and Processing, DSIR, Auckland, NZ: 59-70.

D'Antignana, T. 2007. The effect of pre- and post-harvest factors on the flesh quality characteristics of farmed southern bluefin tuna. PhD thesis, Flinders University.

Driedzic, W.R. & Hochachka, P.W. (1978) "Metabolism in Fish During Exercise" in *Fish Physiology (Vol. VII)* (Eds: W.S. Hoar & D.J. Randall), Academic Press, New York.

Erikson, U., Sigholt, S. & Seland, A. (1997) Handling stress and water quality during live transportation and slaughter of Atlantic salmon (*Salmo salar*). *Aquaculture*, **149**:243-252.

Gillanders B.M.; Ferrell D.J., Andrew N.L.1999. Size at maturity and seasonal changes in gonad activity of Yellowtail Kingfish (Seriola lalandi; Carangidae) in New South Wales, Australia. New Zealand Journal of Marine and Freshwater Research, 33: 457-468

Huss, H.H (1995) *Quality and Quality Changes in Fresh Fish*. FAO Fisheries Technical Paper 348, Food And Agriculture Organization of the United Nations, Rome.

Hyldig, G. & Green-Petersen, D.M.B. (2004) Quality index method – An objective tool for determination of sensory quality. *Journal of Aquatic Food Product Technology,* **13** (4):71-80.

Jerrett, A.E, Stevens, J. and Holland, A.J. (1996). Tensile properties of white muscle in rested and exhausted Chinook salmon (*Oncorhynchus tshawytscha*). Journal of Food Science. 61(3): 527-532.

Kolkovski, S. and Sakakura, Y. 2004. Yellowtail Kingfish from larvae to mature fish – problems and opportunities. In "Avances en Nutricion Acucola VII. Memorias del VII Simposium Internacional de Nutriciaon Acuicola. 16-19 Noviembre 2004". Cruz Suarez, LE et al. Hermosillo, Sonora, Mexico.

Lowe, T.E., Ryder, J.M., Carragher, J.F. and Wells, R.M.G. (1993). Flesh quality in snapper, *Pagrus auratus*, affected by capture stress. Journal of Food Science. 58: 770-773.

Loewenstein MA, Bettelheim FA. 1979. Cold cataract formation in fish lenses. Exp Eye Res 28:651-63.

Ostenfeld, T., Thomsen, S., Ingolfdottir, S., Ronsholdt, B. & McLean, E. 1995. Evaluation of the effect of live haulage on metabolites and fillet texture of rainbow trout (*Oncorhynchus mykiss*). *Water Science & Technology*, **31**(10):233-237.

Pankhurst, N.W., Sharples, D.F., 1992. Effects of capture and confinement on plasma cortisol concentrations in the Snapper, Pagrus auratus. Aust. J. Mar. Freshw. Res. 43, 345–356

Poortenaar, C.W., Hooker, S.H. and Sharp N. 2001. Assessment of Yellowtail Kingfish (*Seriola lalandi lalandi*) reproductive physiology, as a basis for aquaculture development. Aquaculture 2001, vol. 201, no3-4, pp. 271-286 (1 p.3/4)

Reid, RA, Durance, TD, Walker, DC, Reid, PE. 1993. Structural and chemical changes in the muscle of chum salmon (*Oncorhynchus keta*) during spawning maturation. *Food Res Int*, **26**, 1-9.

Roth, B., Veland, J.O., Moeller, D., Imsland, A. & Slinde, E. (2002) The effect of stunning methods on rigor mortis and texture properties of Atlantic salmon (*Salmo salar*). *Journal of Food Science*,**67**:1462-1466.

Roth, B., Slinde, E., Arildsen, J. 2006. Pre or post mortem muscle activity in Atlantic salmon (*Salmo salar*). The effect on rigor mortis and the physical properties of flesh *Aquaculture*, *257, 504-510*

Roth, B., Jenssen, M.D., Jonassen, T.M., Foss, A. & Imsland, A. Change in flesh quality associated with early maturation of Atlantic halibut (*Hippoglossus hippoglossus*). Aquaculture Research **38,** 757 – 763.

Robb, D.H.F, Kestin, S.C and Warriss, P.D. (2000). Muscle activity at slaughter: I. Changes in flesh colour and gaping in rainbow trout. *Aquaculture*. 182: 261-269.

Saunders, R.L., Henderson, E.B., Glebe, B.D. 1982. Precocious sexual maturation and smoltification in male Atlantic salmon (*Salmo salar*). *Aquaculture* 28, 211-229.

Sigholt T., Erikson, U., Rustad, T., Johansen, S., Nordtvedt, T.S. & Seland, A. 1997. Handling stress and storage temperature affect meat quality of farm-raised Atlantic salmon (*Salmo salar*). *Journal of Food Science*. **62**:898-905.

Skjervold, P.O., Fjaera, S.O., Ostby, P.B. & Einen O. 2001. Live-chilling and crowding stress before slaughter of Atlantic salmon (*Salmo salar*). *Aquaculture*. **192**(2):265-280.

Sohn, J.H., Taki, Y., Ushio, H., Kohata, T., Shioya, W. & Ohshima, T. (2005) Lipid oxidations in ordinary and dark muscles of fish. Influence on rancid off-odor development and color darkening of yellowtail flesh during ice storage. *Journal of Food Science,* **70**(7):490–496.

Tejada, M. & Huidobro, A. (2002) Quality of farmed gilthead seabream (*Sparus aurata*) during ice storage related to the slaughter method and gutting. *European Food Research and Technology*, **215**:1-7.

Thomas, P.M. (2008). Using contemporary grading technologies to maximise product quality of farmed tuna. Maximising the control of quality in farmed SBT. Southern Bluefin Tuna Aquaculture Subprogram, Aquafin CRC Project 2.2. FRDC Project No. 2001/248.

Thomas, PM, Pankhurst, NW & Bremner, HA. 1999. The effect of stress and exercise on post mortem biochemistry of Atlantic salmon and rainbow trout. *J. Fish Biol.* 54: 1177-1196.

Van de Vis, H., Kestin, S., Robb, D., Oehlenschlager, J., Lambooij, B., Munkner, W., Kuhlmann, H., Kloosterboer, K., Tejada, M., Huidobro, A., Ottera, H., Roth, B., Sorensen, N., Akse, L., Byrne, H. & Nesvadba, P. (2003) Is humane slaughter of fish possible for industry? A*quaculture Research*. **34**:211-220.

Appendix 1: Intellectual Property

No new intellectual property was raised by this project.

Appendix 2: Staff

- **John Carragher**   SARDI
- **Mark Thomas**   Flinders University
- **Ryan Wilkinson**   University of Tasmania
- **Mohan Raj**   SARDI
- **Mike Thomson**   Clean Seas Tuna
- **Joe Cuira**   Clean Seas Tuna
- **Tom Bayly**   Clean Seas Tuna
- **Terry, Liam, Sam and Alex**   Clean Seas Tuna

APPENDIX 3

Harvest and post-harvest data from Sex, Male Maturation and Harvest Sequence analyses that complete the presentation.

Sex comparison – harvest and post harvest parameters
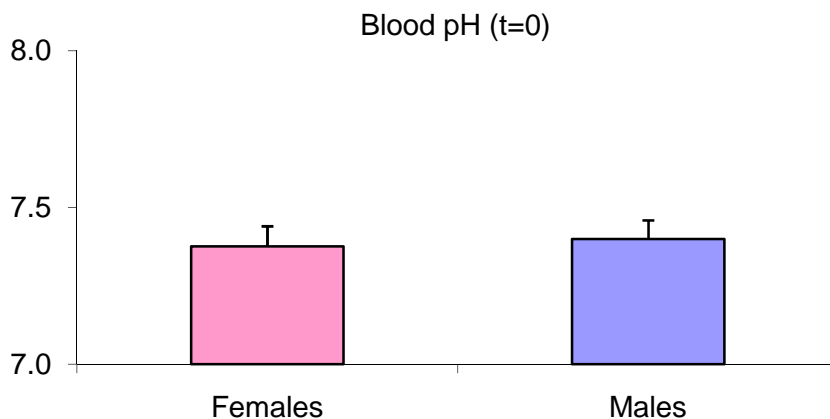
Fig Sex 1. Blood pH (mean ± sem) at time of harvest for male (n=24) and female (n=23) fish. Difference P=0.794; not significant.
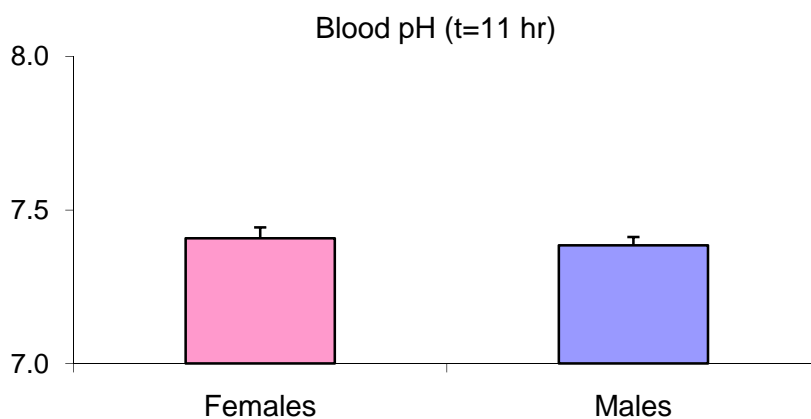
Fig Sex 2. Blood pH (mean ± sem) remeasured 11hr post-harvest for male (n=24) and female (n=23) fish. Difference P=0.615; not significant.
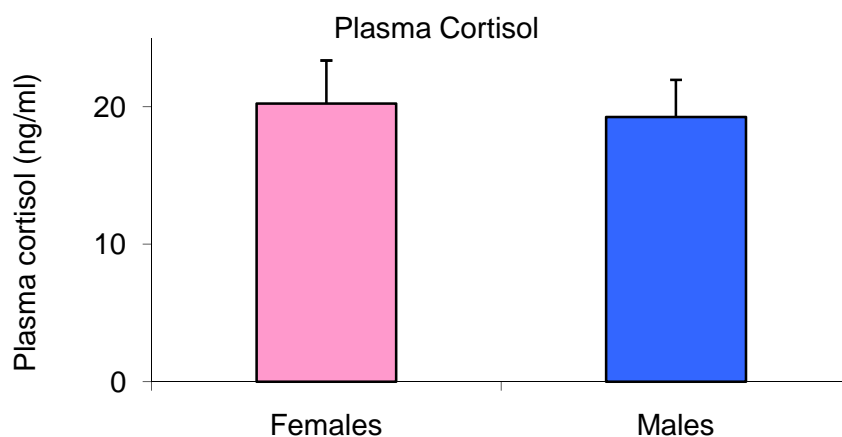
Fig Sex 3 Plasma cortisol (no 'floaters') (mean ± sem) at time of harvest for male (n=22) and female (n=21) fish. Difference P=0.643; not significant.
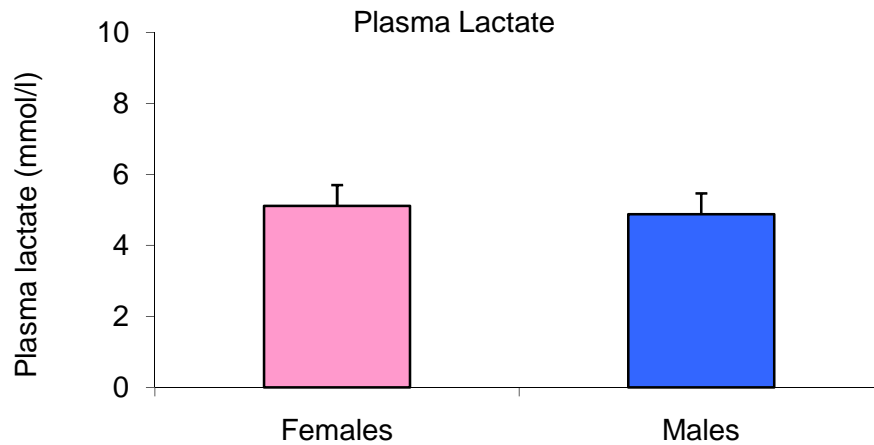
Fig Sex 4 Plasma lactate (no floaters) (mean ± sem) at time of harvest for male (n=22) and female (n=21) fish. Difference P=0.459; not significant.
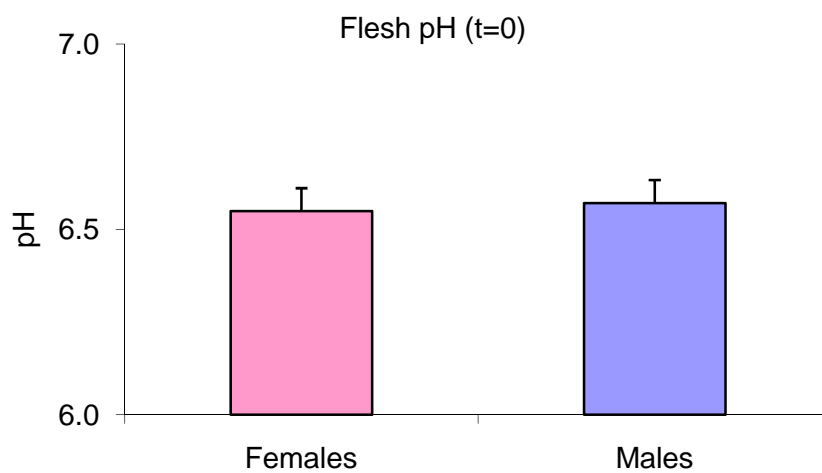
Fig Sex 5. Flesh pH (mean ± sem) at time of harvest for male (n=24) and female (n=23) fish. Difference P=0.819; not significant.
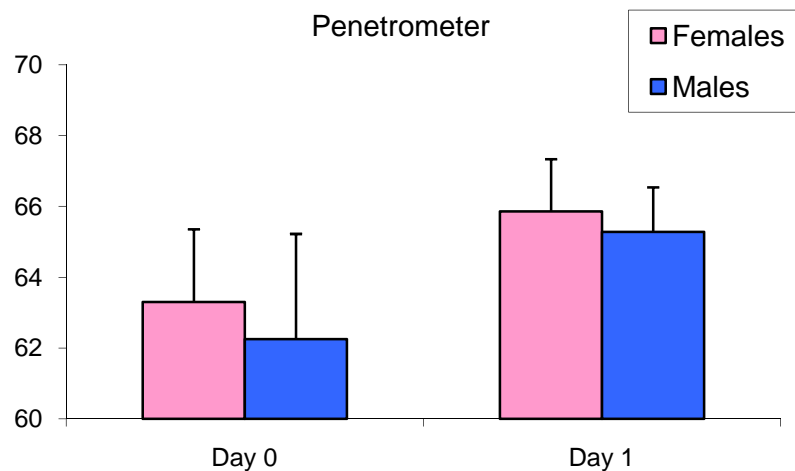
Fig Sex 6. Carcass firmness as measured by penetrometer (mean ± sem) at time of harvest (day 0) and on day 1 for male (n=24 & 18 each day respectively) and female (n=23 & 14 each day respectively) fish. Differences P=0.793 and P=0.774, respectively; not significant.
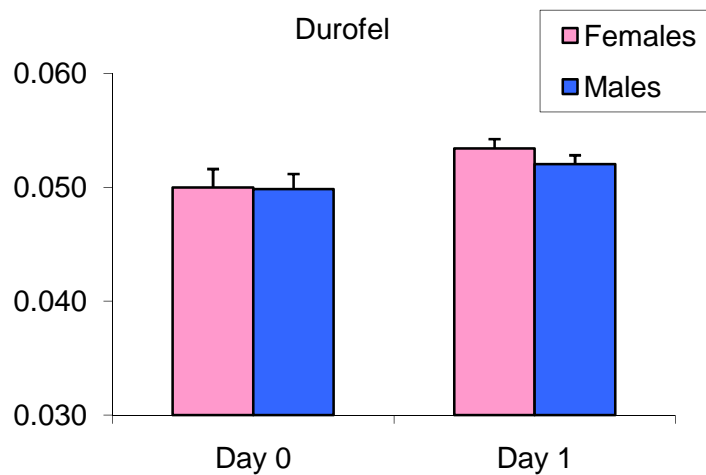
Fig Sex 7. Carcass firmness as measured by Durofel (mean ± sem) at time of harvest (day 0) and on day 1 for male (n=24 & 23 each day respectively) and female (n=23 both days) fish. Differences P=0.940 and P=0.248, respectively; not significant.
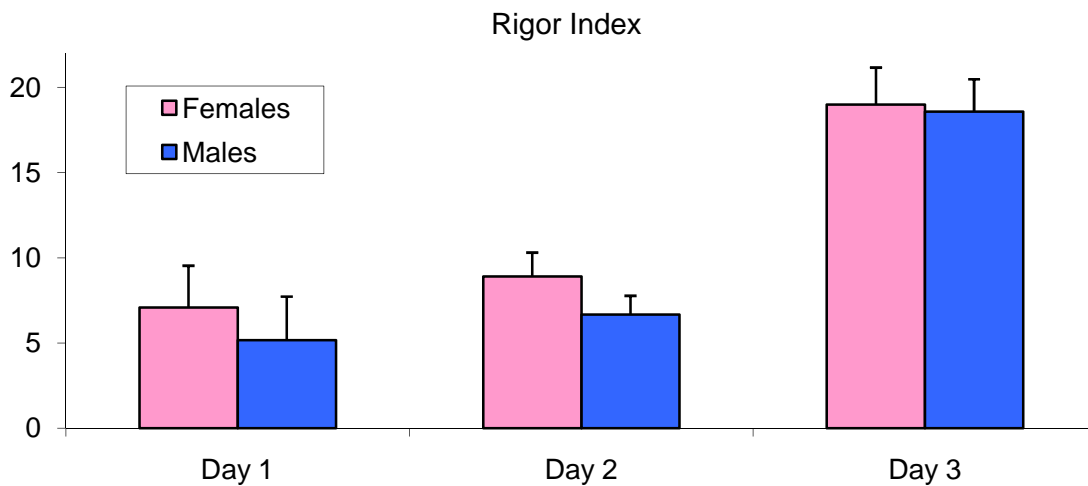
Fig Sex 8. Carcass rigor index (mean ± sem) on days 1, 2 and 3 post-harvest for male (n=23) and female (n=23) fish. Differences P=0.697, P=0.287 and P=0.888, respectively; not significant.
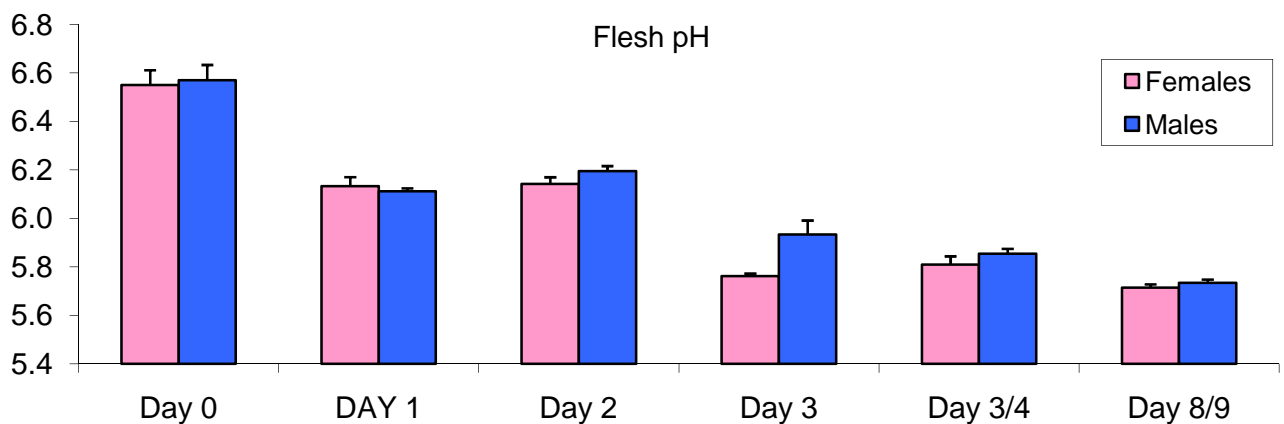
Fig Sex 9. Flesh pH (mean ± sem) at time of harvest (day 0) and on days 1, 2, 3 (carcass) and days 3 and 8/9 (fillet) post-harvest for male (n=22-23, except day 3 where n=11) and female (n=23, except day 3 where n=5) fish. Differences P=0.819, P=0.607, P=0.193, P=0.084, P=0.268 and P=0.337, respectively; not significant.
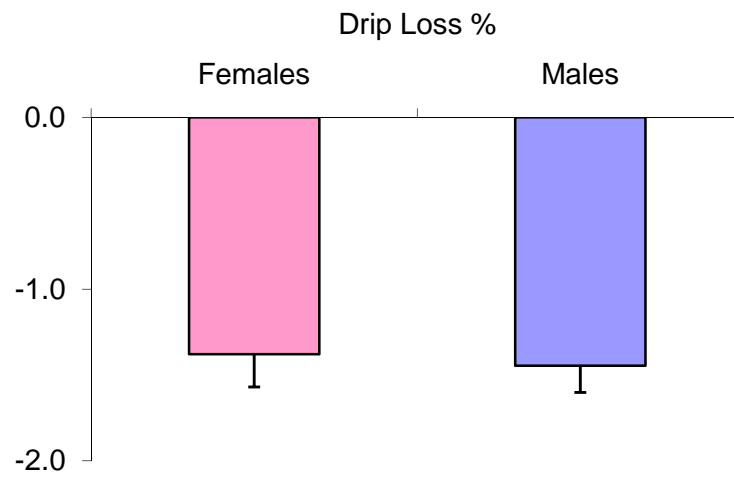
Fig Sex 10 Fillet drip loss (%; mean ± sem) from day 4 to day 8/9 post-harvest for male (n=24) and female (n=23) fish. Difference P=0.789; not significant.

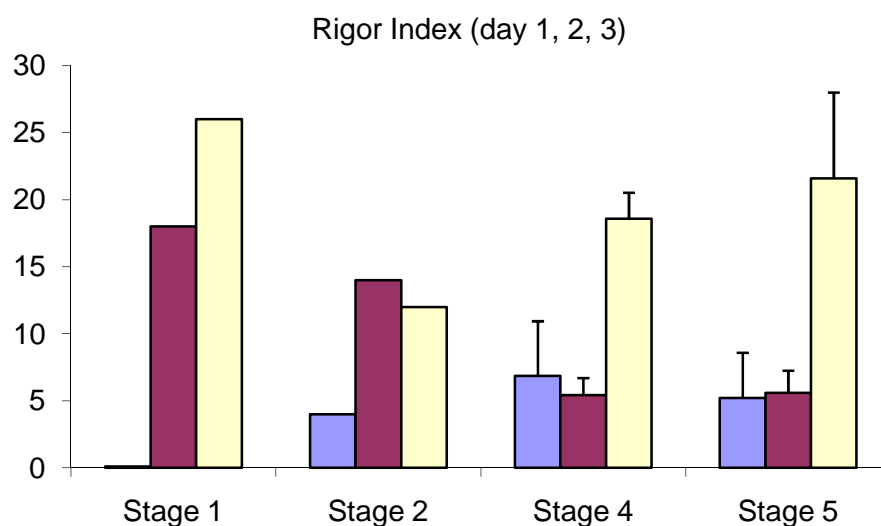# Male maturation comparison – harvest and post harvest parameters

Flesh pH (day 1, 2, 3)
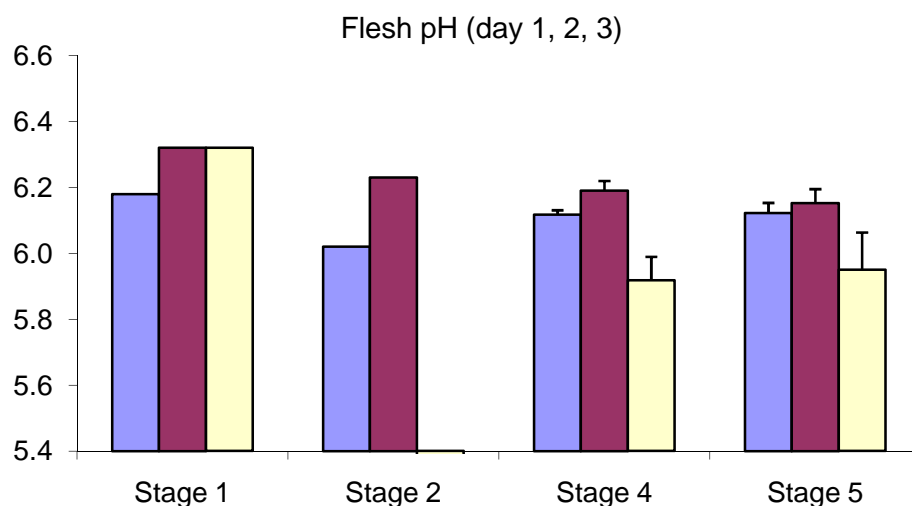
Figure Males A1. Flesh pH of males according to testes stage (n=2, 1, 16, 5 respectively) on days 1, 2 and 3 post-harvest. Differences between Stages 4 and 5 only P=0.893, P=0.515, and not analysed (n=2 for Stage 5) on each day, respectively; not significant.

Rigor Index (day 1, 2, 3)

Figure Males A2. Carcass rigor index of males according to testes stage (n=2, 1, 16, 5 respectively) on days 1, 2 and 3 post-harvest. Differences between Stages 4 and 5 only P=0.828, P=0.945 and P=0.575 on each day, respectively; not significant.

Flesh pH (day 8/9)
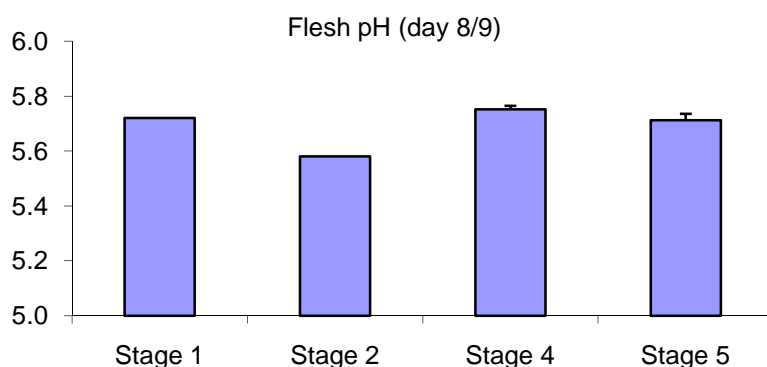
Figure Males A3. Fillet pH of males according to testes stage (n=2, 1, 16, 5 respectively) on day 8/9. Difference between Stages 4 and 5 only P=0.205; not significant.
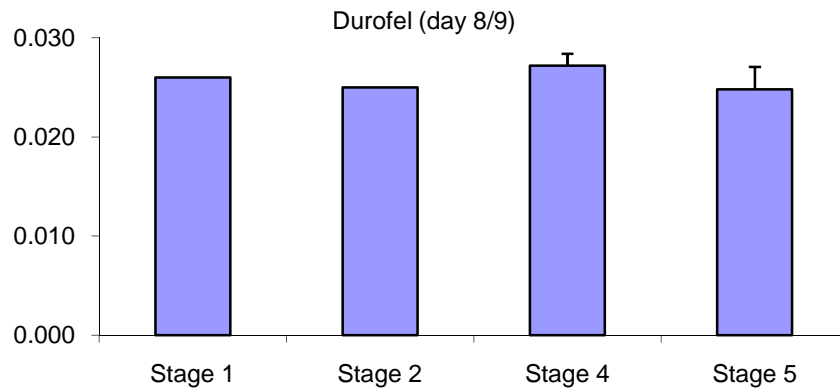
Figure Males A4. Fillet firmness as measured by Durofel of males according to testes stage (n=2, 1, 16, 5 respectively) on day 8/9. Difference between Stages 4 and 5 only P=0.364; not significant.
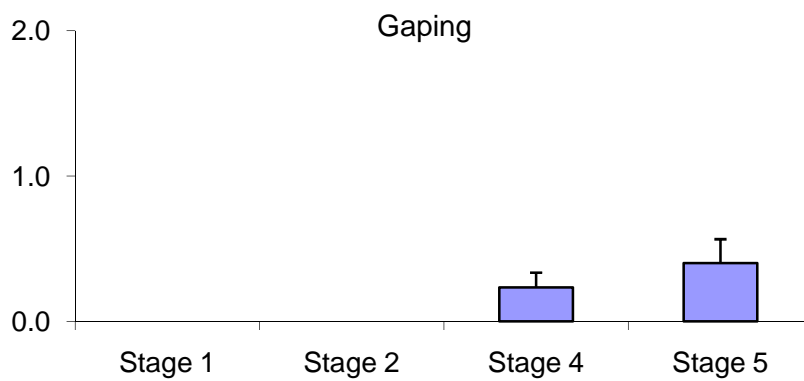

Figure Males A5. Fillet gaping (measured subjectively) of males according to testes stage (n=2, 1, 16, 5 respectively) on day 8/9. Difference between Stages 4 and 5 only P=0.449; not significant.
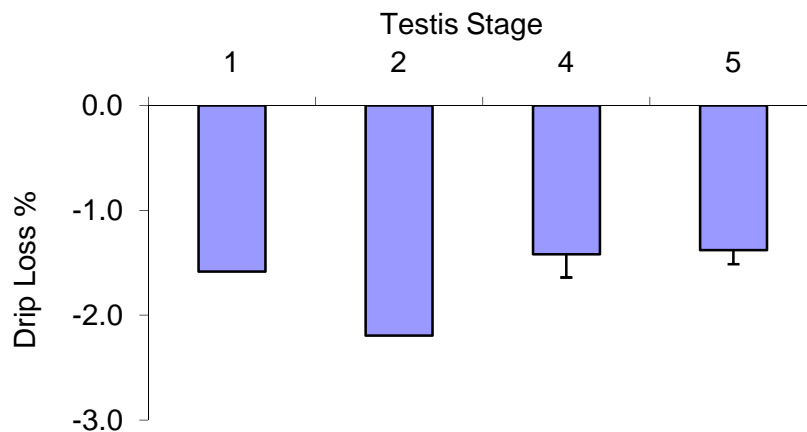

Figure Males A6. Drip loss (as a % of fillet wet weight; mean ± sem) from day 3 to day 8/9 post-harvest of males according to testes stage (n=2, 1, 16, 5 respectively) sampled in this study. Difference between Stages 4 and 5 only P=0.921; not significant.

Sequential harvest comparison – harvest and post harvest parameters

Commercial Harvest Sequence - Flesh pH (t=1 Day)
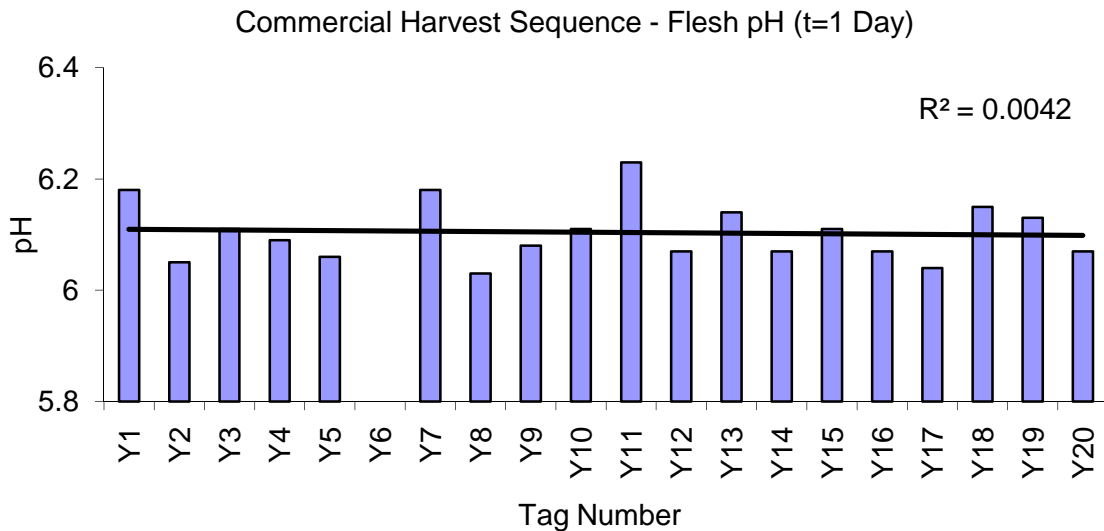
Fig Appendix Seq 1. Flesh pH on day 1 of individual fish in sampling order throughout a commercial harvest. Regression line and correlation co-efficient show the trend and goodness of fit of the fish to that relationship Regression P=0.793; not significant.
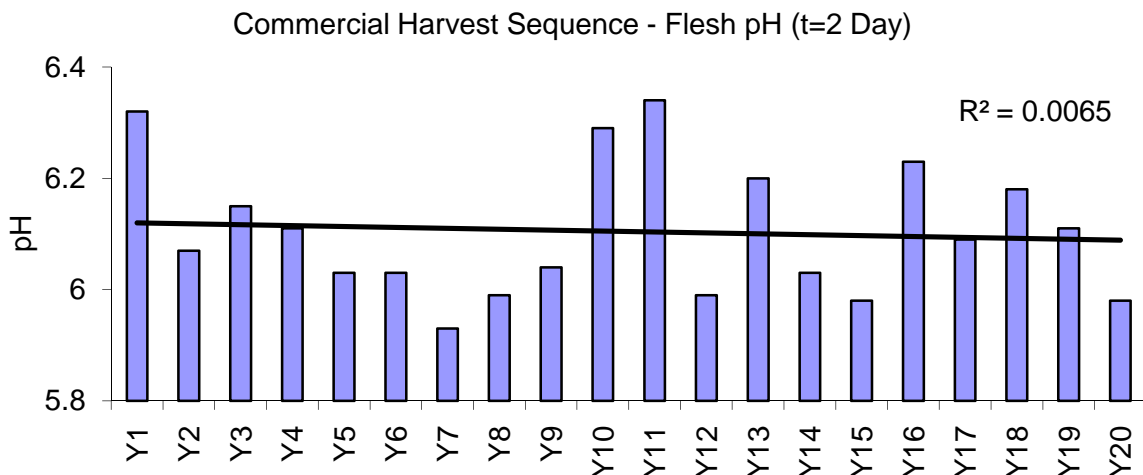
Commercial Harvest Sequence - Flesh pH (t=2 Day)

Fig Appendix Seq 2. Flesh pH on day 2 of individual fish in sampling order throughout a commercial harvest. Regression line and correlation co-efficient show the trend and goodness of fit of the fish to that relationship Regression P=0.736; not significant.
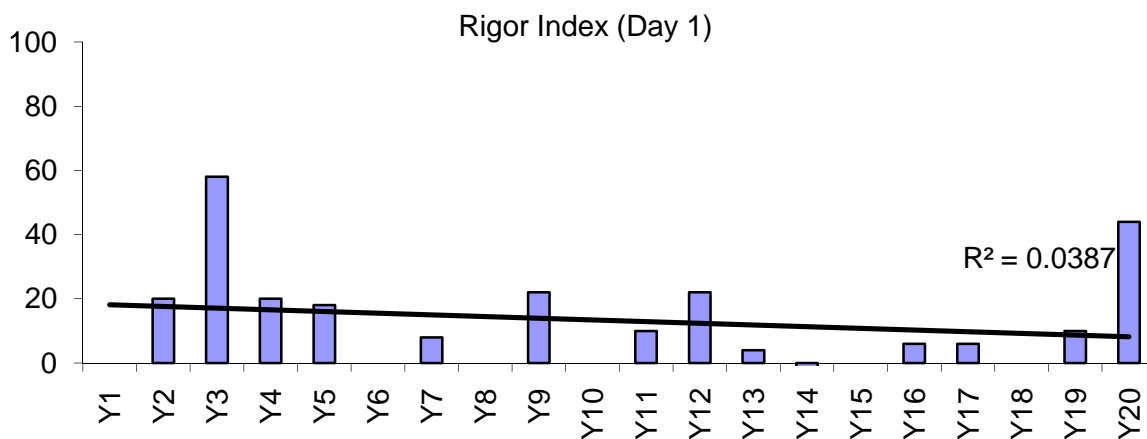
Rigor Index (Day 1)

Fig Appendix Seq 3. Carcass rigor index on day 1 of individual fish in sampling order throughout a commercial harvest. Regression line and correlation co-efficient show the trend and goodness of fit of the fish to that relationship Regression P=0.419; not significant.
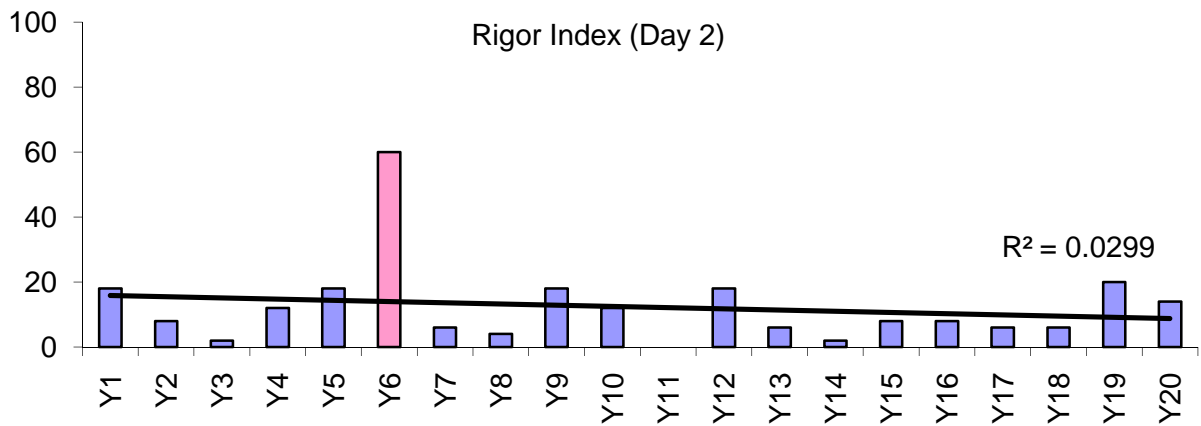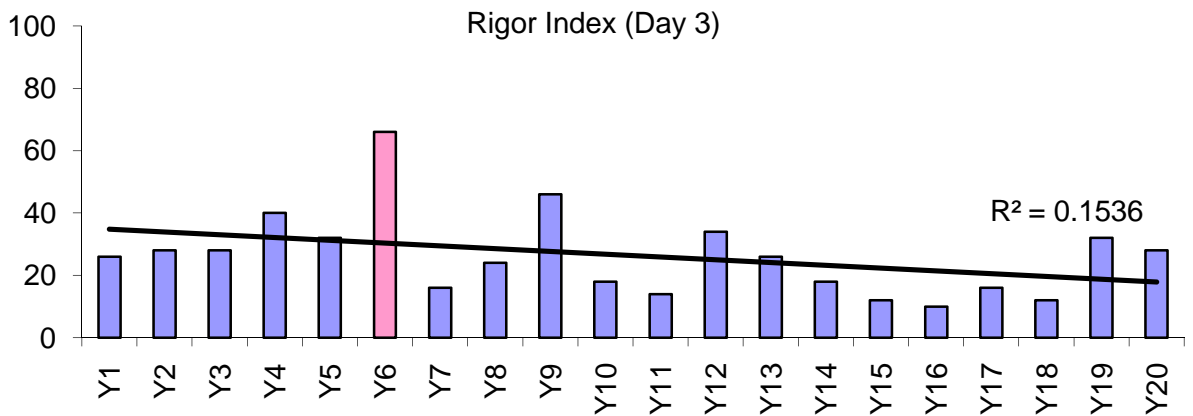
Fig Appendix Seq 4. Carcass rigor index on day 2 of individual fish in sampling order throughout a commercial harvest. The pink bar shows a fish that was misplaced for the measurement on day 1 but recovered for day 2 onwards. Regression line and correlation co-efficient show the trend and goodness of fit of the fish to that relationship Regression P=0.890; not significant.

Fig Appendix Seq 5. Carcass rigor index on day 3 of individual fish in sampling order throughout a commercial harvest. The pink bar shows a fish that was misplaced for the measurement on day 1 but recovered for day 2 onwards. Regression line and correlation co-efficient show the trend and goodness of fit of the fish to that relationship Regression P=0.111; not significant.
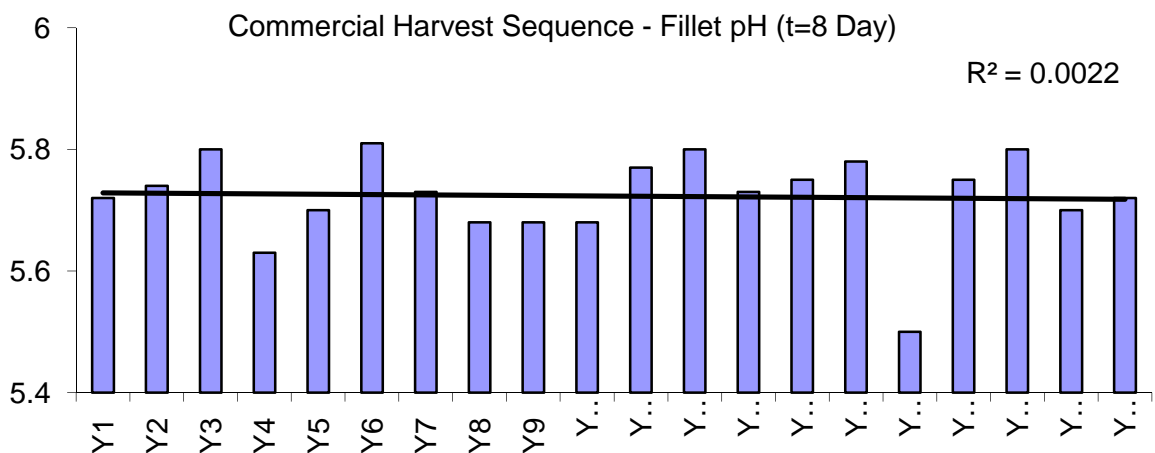

Fig Appendix Seq 6. Fillet pH on day 8/9 of individual fish in sampling order throughout a commercial harvest. Regression line and correlation co-efficient show the trend and goodness of fit of the fish to that relationship Regression P=0.845; not significant.
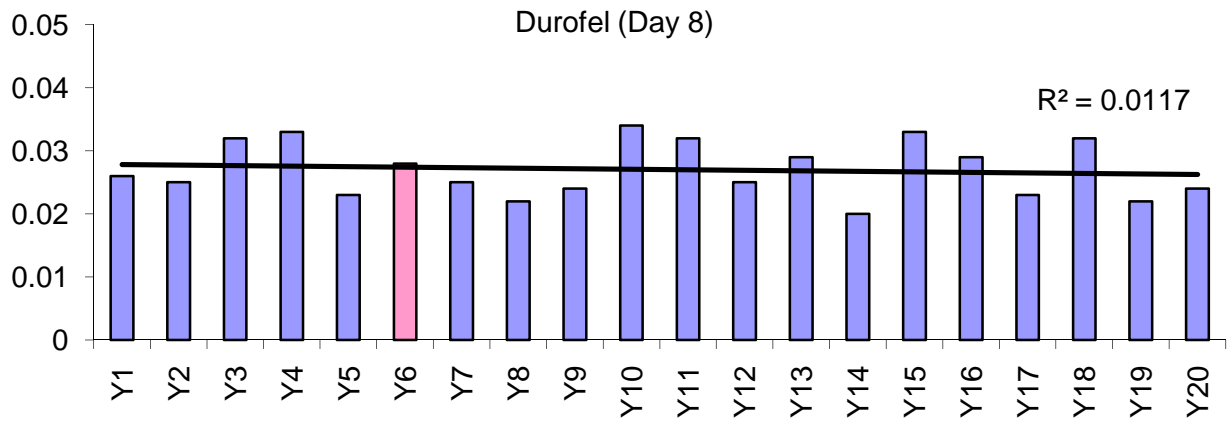
Fig Appendix Seq 7. Fillet firmness on day 8 of individual fish in sampling order throughout a commercial harvest. The pink bar shows a fish that was misplaced for the measurement on day 1 but recovered for day 2 onwards. Regression line and correlation co-efficient show the trend and goodness of fit of the fish to that relationship Regression P=0.649; not significant.
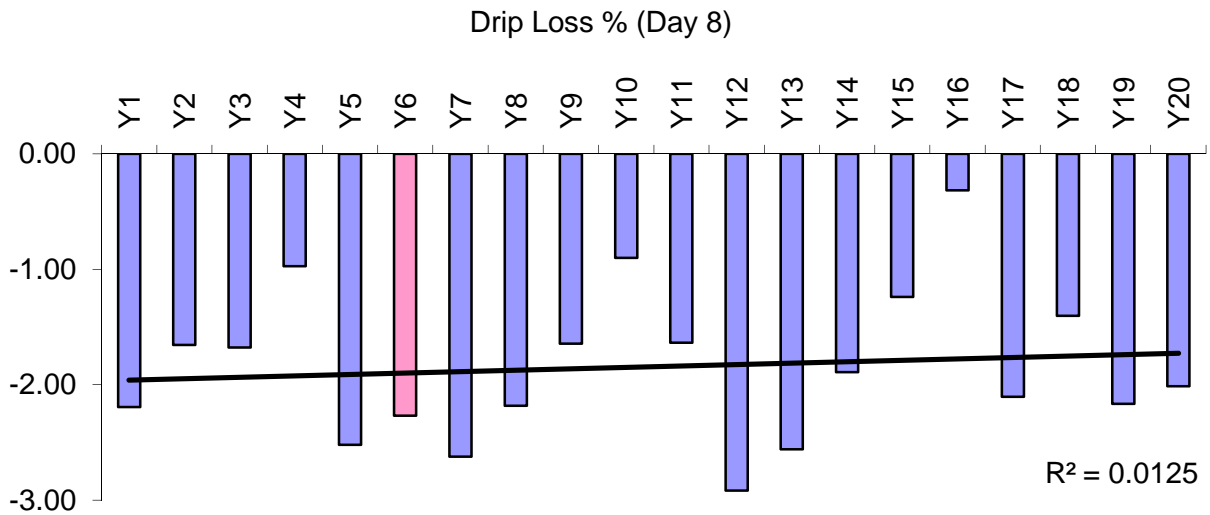

Fig Appendix Seq 8. Fillet drip loss between day 4 and day 8/9 for individual fish in sampling order throughout a commercial harvest. The pink bar shows a fish that was misplaced for the measurement on day 1 but recovered for day 2 onwards. Regression line and correlation co-efficient show the trend and goodness of fit of the fish to that relationship Regression P=0.638; not significant.
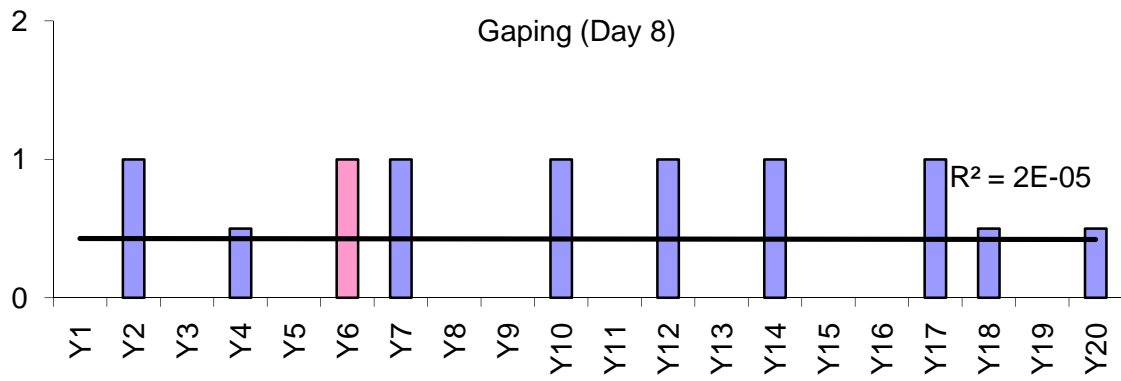
Fig Appendix Seq 9. Fillet gaping (subjective score) on day 8/9 for individual fish in sampling order throughout a commercial harvest. The pink bar shows a fish that was misplaced for the measurement on day 1 but recovered for day 2 onwards. Regression line and correlation co-efficient show the trend and goodness of fit of the fish to that relationship Regression P=0.980; not significant.