Optimising Harvest Practices of Yellowtail Kingfish

Trent D'Antignana, Erin Bubner and Maximiliano Canepa

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Authors: Trent D'Antignana, Erin Bubner and Maximiliano Canepa

Authors Affiliation: Lincoln Marine Science Centre, Flinders University, Port Lincoln, South Australia

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Office Mark Oliphant Building, Laffer Drive, Bedford Park SA 5042
Postal Box 26, Mark Oliphant Building, Laffer Drive, Bedford Park SA 5042
Tollfree 1300 732 213 Phone 08 8201 7650 Facsimile 08 8201 7659
Website www.seafoodcrc.com ABN 51 126 074 048

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Non-Technical Summary

Project 2010/778: Optimising Harvest Practices of Yellowtail Kingfish

PRINCIPAL INVESTIGATOR: Trent D'Antignana

ADDRESS: Lincoln Marine Science
Flinders University
PO Box 2023, Port Lincoln, South Australia 5606

PROJECT OBJECTIVES:

1. Improve and adapt existing automated stunning and bleeding technology for use on Yellowtail Kingfish (YTK) so that the stun/bleed success rate increases from 65 to 95%.
2. Improve on board bleeding practices – quantifying the differences between dry and wet bleeds in ambient and refrigerated seawater without sacrificing flesh quality attributes.
3. Determine if acute and chronic stress have an impact on rigor and flesh quality characteristics of YTK.
4. To minimise the post mortem formation of cold cataracts in YTK through modifying cold chain practices.
5. To develop Standard Operating Procedures for harvest practices that promote efficiency and maintain freshness in YTK.
6. Quantify the physiological, biochemical and haematological characteristics of wild YTK – what does a "normal" YTK look like?

OUTCOMES ACHIEVED

The project will directly benefit the Yellowtail Kingfish industry, in particular Clean Seas Tuna Ltd; however, it will have flow on effects to other finfish industries and seafood processing facilities.

The first planned outcome was: to prolong rigor onset and resolution and increase products shelf life to 14 days. This outcome was not achieved because the priority areas changed during the project due to decreasing fish volumes. However, significant progress was made in understanding the factors affecting rigor mortis and the effect of harvest processes on product quality in farmed YTK, including:

- This project evaluated the effect of harvest operations on flesh quality attributes of YTK .The results indicated that rigor onset time decreased when the fish were stressed prior to or during harvest
- YTK showed modest stress responses to handling procedures associated with harvesting (crowding, confinement, low water quality) and the level of stress and anaerobic metabolism increased with confinement/harvest duration resulting in significant differences in the physiological status (e.g. muscle glycogen, lactate and ATP levels) of the fish at harvest.
- Stressed and anaerobically challenged YTK entered rigor mortis slightly faster than fish that were not as challenged, however all YTK were in full rigor by 12 h post-harvest. Rigor resolved slowly in all fish and by 84 to 96 h post-harvest all YTK were still in 50% rigor.
- Differences in the physiological status of muscle tissue between stressed and unstressed fish were generally minor during post-harvest storage to 96 h.
- Despite these physiological differences in stressed and unstressed YTK at harvest, there were no significant differences in the objective and subjective indicators of carcass quality (quality index score, K value) up to 96 h post-harvest or fillet quality (flesh gaping, colour, smell or firmness) post-filleting.
- Although best practice harvest husbandry practices should always be adhered to, the findings imply that YTK quality attributes are resilient to stress suggesting that a consistent product should always be achievable even when husbandry practices are less than desirable

The second planned outcome was: to reduce the number of personnel required to harvest (from 7 FTE to 4 FTE) and reduce the at-sea time required to harvest the fish

by 20%. This outcome has been achieved.

- Harvest vessel crew numbers were reduced from seven to four, resulting in a saving of approximately $150,000 per annum.

The third planned outcome was: a reduction in the value of rejected product from approximately $280,000 per annum to $140,000 per annum.

- Due to CST's significant downsizing in YTK production since this target was set, the value of rejected product cited in this outcome is no longer appropriate. It is clear, however, that as a result of this project there has been a 40% reduction in the number of customer complaints relating to poor harvest practices; there have been no incidents of fish being dumped due to poor harvest practices (i.e. a 100% reduction) and there is now full utilisation of B-grade product and reject fish.
- Some of the quality issues that are still queried in the marketplace have been addressed within this project. For instance we have determined that eye cloudiness in YTK post-harvest is caused by chilling the fish in seawater. Doing so affects the osmolarity of the eye fluid which leads to the denaturation and precipitation of proteins onto the lens. Reducing the salinity of the ice slurry water to 15ppt or less prevents this osmotic perturbation allowing the eyes to stay clear.
- Diets deficient in taurine had a higher incidence of eye cloudiness suggesting that taurine supplementation feed may go some way to reducing the incidence of eye cloudiness of YTK in chilled seawater. It was proposed that it may act as an osmolyte and buffer the liquid in the eye.
- We demonstrated that exsanguination of harvested YTK was essential to reduce the incidence and severity of blood spotting in the fillet.
- There was no significant benefit of a specific bleed bin in the post-harvest process, low and similar levels of fillet blood spotting were observed in fish that bled out in the RSW tank.
- However, the issues around the disposal of larger volumes of bleed water after fish are unloaded, and the possible effect of greater concentrations of blood in the RSW tank on carcass hygiene and fillet quality still need to be addressed.

The fourth planned outcome was: an increase in stun/bleed efficacy from 65% to a minimum of 95% using automated harvesting technologies. This outcome has been achieved.

- We determined that the mid-brain of YTK of fork length 57 to 67 cm (2.3 to 3.6 kg

bodyweight) was positioned 81 to 105 mm from the tip of the snout and at a depth of approximately 25 mm below the surface of the head.

- This distance from the snout to the brain dictated what the trigger setting in the modified Seafood Innovations automated harvesting system should be set at for this size of fish. This setting ensured the percussive piston would strike the fish directly above the brain and produce an effective stun, and that the bleed knife would strike the area of the heart ensuring good exsanguination of the carcass.
- The modified Seafood Innovation automatic harvesting system worked most effectively for YTK of this size when the original flat ramp that directs the fish towards the trigger and piston/knife strike zone was replaced by a ramp that had a deep V channel along it. This, together with vertical guide plates that were properly adjusted to suit the width of the fish, ensured the fish were vertical when the trigger was activated and maximised the effectiveness of the stun or bleed action.
- All of these small improvements to the modified Seafood Innovations automatic harvesting system have increased the efficacy of the stun/bleed from 65% to > 95% and resulted in improvements in fish welfare and harvest efficiency.

The fifth planned outcome was: increase throughput by automated harvesting machinery from 10 fish per minute to 30 fish per minute. This outcome has been achieved.

- Harvest efficiencies have increased 3-fold to 30 fish per minute.
- This was largely achieved by minimising "pile ups" and blockages in the automatic harvester through modifying the entrance shape to the channels, changing the positions and settings for the guide plates in the stunner/ bleeder units, improving the trigger position and shape and inserting V-ramps. These improvements also reduced the number of fish requiring manual bleeding and stunning, minimising the need to pause the automated stunning machinery.

The sixth and final planned outcome was: to develop and have harvesting personnel apply standard operating procedures (SOPs) which promote best practice. This outcome has been achieved.

- A harvest manual was prepared and SOPs relating to harvesting were updated.
- A nominated Clean Seas Tuna Ltd staff member has participated in internal audit training to assess compliance of harvesting operations to SOPs.
- Two user manuals have been written to facilitate harvest crew use of the

operational software ("Stun Count Manager - quick instructions") and the setup and maintenance of the stunner and bleeder units ("LT 50 Flow-Through Stunner and Bleeder Manual").

The information generated by this project is being used by Clean Seas Tuna Ltd management to improve efficiency, flesh quality attributes and reduce variability of Yellowtail Kingfish products supplied to domestic and international markets.

LIST OF OUTPUTS PRODUCED

1. A generic SOP for harvesting YTK has been developed, but as the automated harvesting machinery is no longer in operation a SOP including its use has not been developed. However two user manuals pertaining to the automated harvesting machinery have been developed .These documents remain confidential to Clean Seas Tuna Ltd.
2. A reference database for a wide range of haematological, biochemical and flesh composition parameters has been established from wild YTK that were of a similar size range to harvested farmed fish. The aim of this was to help identify 'normal' from 'abnormal' results from farmed fish, and give some direction for further changes to aquaculture diets and health management to improve fish health and productivity in culture. However, the validity of the wild YTK sampled offshore of Sydney has been questioned by the high incidence of parasites and other disease conditions found in following histopathology.

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1. Introduction and Background

Yellowtail Kingfish (*Seriola lalandi*, Valenciennes, 1933) (YTK) is a temperate, carnivorous, marine fish species in the family Carangidae that is widely distributed throughout the Pacific and Atlantic oceans (Kolkovski and Sakakura, 2007; Nugroho et al, 2001). The farming of YTK was established in Australia in the late 1990's, predominately occurring in the Spencer Gulf of South Australia (Fernandes and Tanner, 2008; Hutson et al., 2007). YTK is a fast growing and economically valuable species and is therefore well suited to aquaculture (Fernandes and Tanner, 2008). Clean Seas Tuna Ltd (CST) is the largest producer of YTK in Australia, with production peaking at approximately 3500 tonnes in 2009. Despite a recent decline in production due to unforseen health related issues of the fish that has now been largely eliminated, an imminent increase in production with lower production costs is anticipated to satisfy economy of scale and the strong market demand. It is anticipated that CST will be profitably producing 1500 tonnes of YTK by 2015.

Farmed YTK is considered to be a premium quality fish, ideal for sashimi and sushi, dishes comprised of raw fish. To be acceptable for such delicacies the flesh has to be oily, firm and slightly translucent, with a fine creamy texture and a delicate flavour. The price received, and the reputation of the supplier, are largely dependent on the quality of the product produced. Therefore it is of great interest to optimise the production of high quality fish, especially in the increasingly competitive white fish market. However, despite the appealing flesh quality attributes of YTK, CST has received a considerable number of customer complaints reporting issues with product quality, including: blood spotting in the flesh, poor shelf life, 'old' appearance and soft and gaping flesh.

A considerable amount of research and development has been directed towards YTK production; however, little attention has been directed towards understanding how husbandry practices influence their flesh quality attributes and quantifying the costs of harvesting the product. The Australian Seafood CRC project 2008/901 conducted a preliminary examination on the effect of harvest stress on flesh quality attributes. The project was unable to draw any firm conclusions about the relative stressfulness of the commercial 'dead haul' harvesting procedure that was employed by CST at the time, but it did state that the species was robust and that harvest stress appeared to have no significant deleterious impact on the flesh quality attributes and shelf life of chilled products. It also noted that further studies are required to increase the

understanding of stress responses in this species and the effect that they have on flesh quality attributes.

To achieve a quality driven increase in the farm gate price of YTK, CST and any other producers must ensure that harvest husbandry practices consistently produce fish with optimal flesh quality attributes, whilst utilising cost effective and efficient techniques. Therefore, the overall objective of this project was to develop systems and techniques that consistently produce a premium quality product, whilst minimising costs associated with harvesting practices. In addition, the project also assisted the company in addressing a major unforeseen YTK health issue that occurred after the project commenced, shifting the company's primary focus from optimising harvest practices to simply ensuing fish were available for harvest and continuality of supply.

This project comprised of six activities, each with distinct aims:

1) develop systems that allowed efficient use of automated harvesting technology on YTK;
2) optimise on-board bleeding practices to maintain flesh whiteness;
3) investigate impact of acute and chronic stress on flesh quality;
4) investigate commercial harvest stress and determine its impact on flesh quality;
5) examine the factors influencing the post-harvest development of cold cataracts in YTK and;
6) quantify the physiological, biochemical and haematological characteristics of wild YTK.

1.1 Need

The production of farmed YTK needs to be a profitable enterprise. Improving the consistency and overall quality of the product to justify a quality driven increase in farm gate price and reducing the cost of production by improving harvest efficiencies is essential to achieving this. Understanding how the harvest husbandry technique influences the flesh quality attributes of YTK will allow CST to develop and apply best practice standard operating systems, reduce customer complaints and improve the company's position in the increasingly competitive market.

1.2 Objectives

1. To improve and adapt existing automated stunning and bleeding technology for use on YTK so that the stun/bleed success rate increases from 65 to 95%.
2. To improve on board bleeding practices – quantifying the differences between dry and wet bleeds in ambient and refrigerated seawater without sacrificing flesh quality attributes.
3. Determine if acute and chronic stress have an impact on rigor and flesh quality characteristics of YTK.
4. To understand and minimise the post mortem formation of cold cataracts in YTK.
5. To develop Standard Operating Procedures (SOPs) for harvest practices that promotes efficiency and maintains freshness in YTK.
6. Quantify the physiological, biochemical and haematological characteristics of wild YTK – what does a "normal" YTK look like?

2. Methods

Fish sampled in this study were managed according to standard commercial practice, unless otherwise indicated. Factors such as feeding regime, management of fish health and diet, pontoon location, grading and splitting of cages and the harvest schedule were outside the control of this project. However, the impact of these factors on project activities were minimised where possible.

2.1 Activity One: Develop systems that allow automated harvesting technology to be used on YTK so that the stun/bleed success rate increases from 65 to 95%.

2.1.1 Fish morphological parameters

To apply suitable modifications to the automated harvesting system (i.e. positioning of the triggers, guides and the placement of the fish in the vertical axis of the stunners and bleeders), morphological parameters were taken from commercially harvested fish of marketable size (i.e. fish fork length and whole wet weight were between 56 and 68 cm and 2.3 and 3.6 kg, respectively). Morphological parameters measured included: fish fork length; fish whole weight; the dorso-ventral position of the brain (the distance between the top of the head and the dorsal and ventral surfaces of the brain: Figure 1); the anterior-posterior positioning of the brain (the distance from the inferior lip to the anterior (telencephalon) or posterior (metencephalon) region of the brain: Figure 1) and; the lateral position of the brain (the width of the head in line with the edge of the operculum plate: Figure 2). The

mid-point of brain depth was determined using the average measurements from the four different coordinates described above.

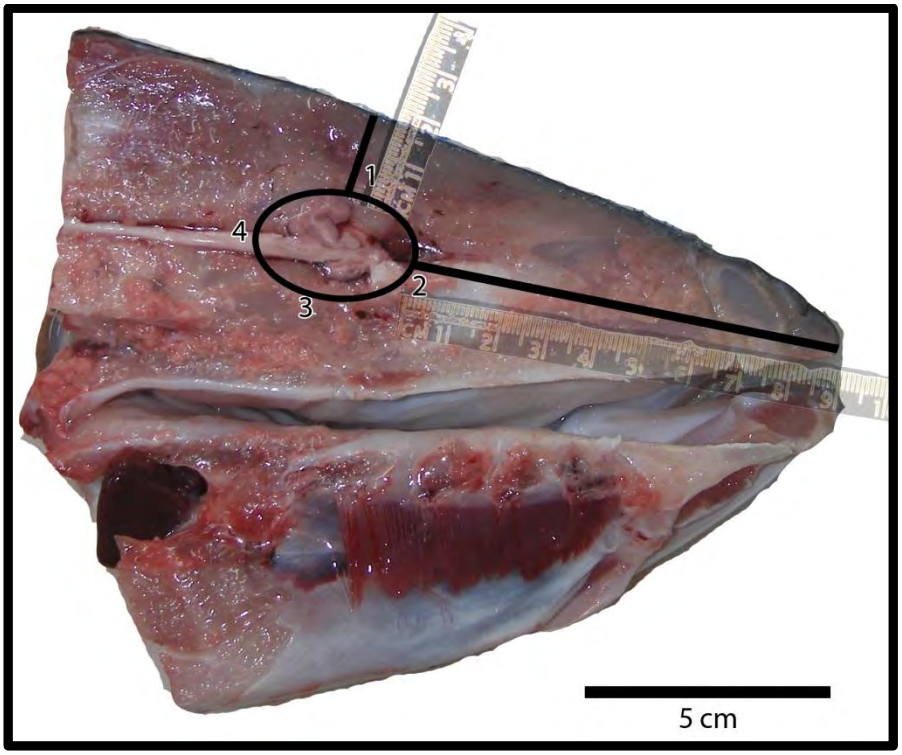

Figure 1. Dorso-ventral section through the midline of a Yellowtail Kingfish (*Seriola lalandi*) head. Depth of the brain was determined by measuring the distance measured between the top of the head and (#1) the dorsal surface and (#3) the ventral surface of the brain. Anterior-posterior positioning of the brain was determined by measuring the distance between the inferior lip of the fish and (#2) the anterior (telencephalon) and (#4) posterior (metencephalon) region of the brain.

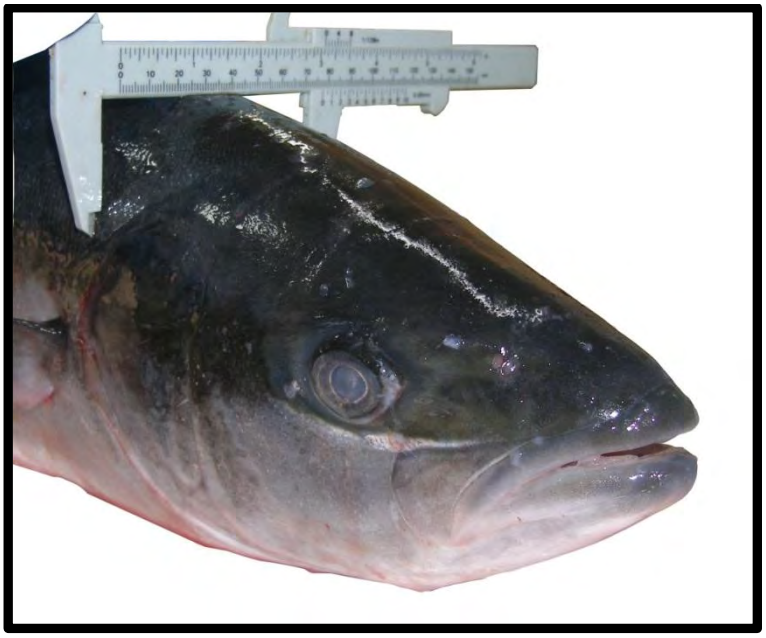

Figure 2. Width of the head of Yellowtail Kingfish (*Seriola lalandi*) measured using the edges of the opercula as reference points.

2.1.2 Automated harvesting technology

2.1.2.1 Standard commercial harvest practice

At the time of this activity, a 'dead haul' harvest method and automated harvesting technology were used to harvest 2+ year class YTK . The dead haul method involves crowding the fish by manually lifting a net inside the holding cage or designated harvest raceway to a depth of approximately one metre. The YTK are then drawn up from the crowd net by a fish pump and delivered to a whirlpool on the harvest vessel that leads to 3 channels on the automated harvesting system. Each channel has a set of control gates, a stunner unit, an inverter chute and a bleeder unit, after which the 3 channels join to deliver the fish to a single conveyor (Figure 3). In brief, once delivered to the whirlpool the fish have a choice of which of the 3 channels to swim into but entry of a fish into a channel is controlled by an automated entry gate. The gate closes immediately after a fish enters the channel, preventing multiple fish from entering it at one time. Once inside the channel, a fish quickly swims with the flow of water into the stunner unit. Immediately after the fish is struck it is released from the stunner unit and falls down a twisted chute that quickly inverts the fish, at the base of the inverter is the bleeder unit. The triggers to the stunners and bleeders in each channel are activated by the swimming momentum and presence of the fish, respectively. After the fish is released from the bleed unit it is deposited on a single conveyor and manually assessed for appropriate stunning and bleeding.

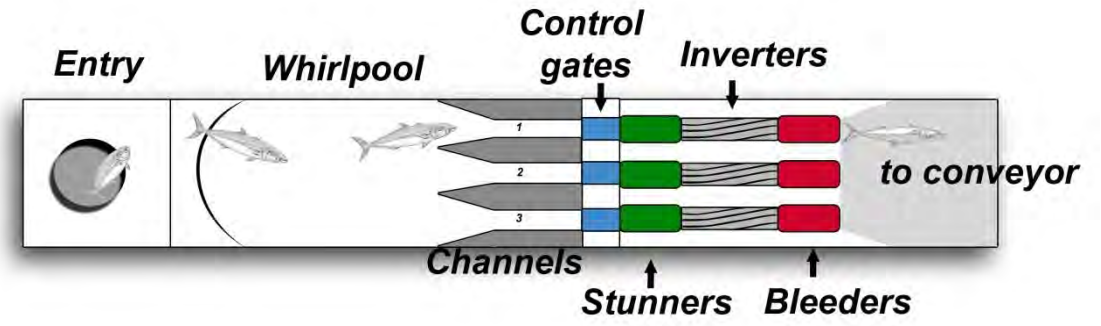

Figure 3. Schematic diagram of the whirlpool, channels, control gates, stunners, inverters, bleeders and conveyor in the automated harvesting system used for Yellowtail Kingfish (*Seriola lalandi*).

To describe the bleeders and stunners in more detail, they originally consisted of: 1) a flat stainless steel ramp that slides the fish upwards to deliver it to the trigger plate for stunning or bleeding, the ramp quickly drops away after the relevant blow has been struck allowing the fish to be released; 2) four adjustable position settings that modify the height of the pneumatic actuator (i.e. the stunner or bleeder mechanism) to accommodate fish of different sizes for appropriate stunning and bleeding (i.e. larger fish position A, smaller fish position D; see Figure 4); 3) two stainless steel guide plates at the sides of the ramp that hold the fish vertically for stunning and bleeding, it has six adjustable widths to cope with different sized fish (see Figure 4) and; 4) a stainless steel trigger plate with five different position settings to aid in accurately delivering the stunner and bleed knife blows. The height of the pneumatic actuator and width of the channel guides were adjusted according to the size of the fish, type of ramp used (see Section 2.1.2.2) and following assessment of the effectiveness and accuracy of bleed and stun blows. For marketable size fish of approximately 3 kg, the width of the channel guides were set at position "1" when using a 'V' ramp and "2" when using a 'semi-V' ramp; while the height of the pneumatic actuator were set at position "C" when using the 'V' ramp and "B" when using the 'semi-V' ramp.

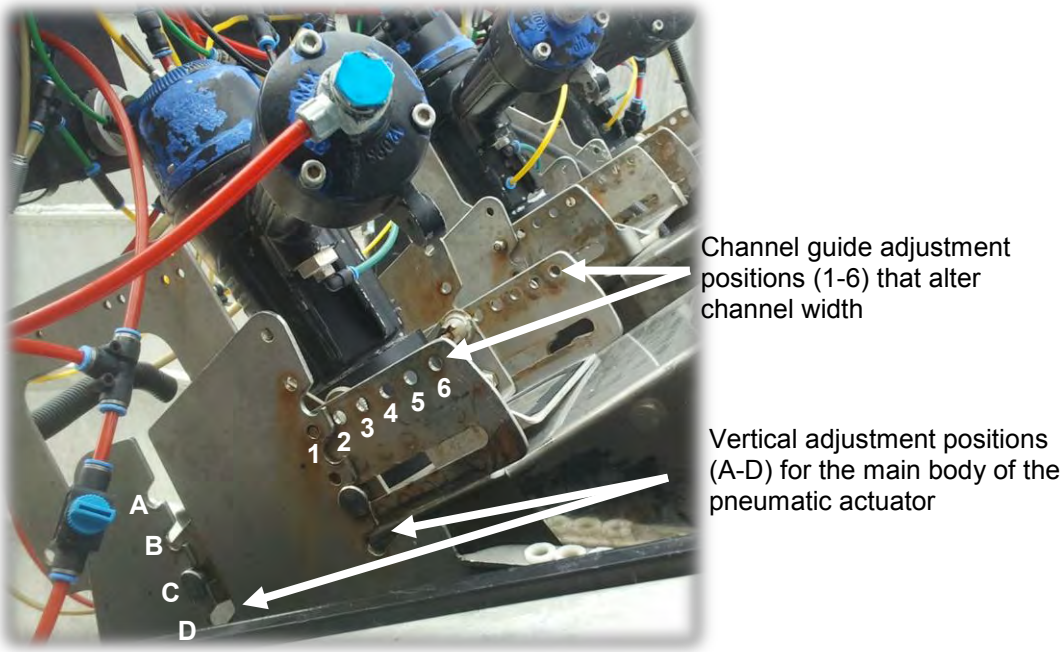

Figure 4. Adjustment positions for the stunner and bleeder component of the automatic harvesting system.

2.1.2.2 Modifications to the automated harvesting technology

As described above, the fish acquire a position underneath the stunner and bleeder units by sliding over a stainless steel ramp. In an attempt to increase the stun and bleed efficacy, three different ramp designs were examined: 1) the "original" flat design provided by Seafood Innovations (Figure 5A); 2) a "semi V ramp" design with the plate consisting of "V" sided channel with a flat base (Figure 5B) and; 3) a "full V ramp" design with the plate consisting of "V" shaped channel (Figure 5C).

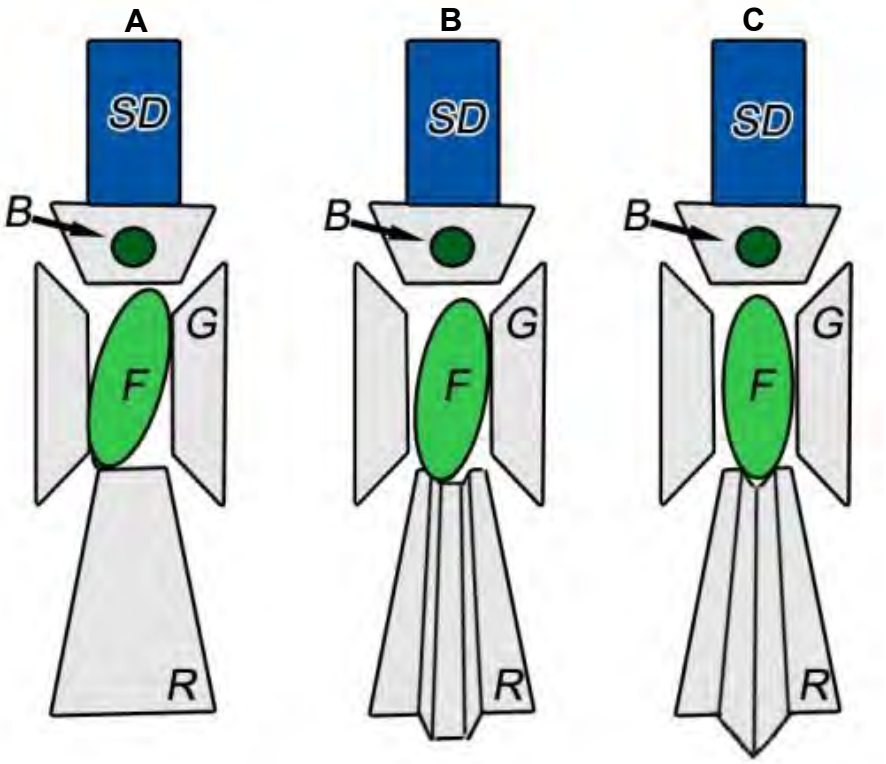

Figure 5. Modifications of the stainless steel ramps in the automatic harvesting system to improve accuracy in stun and bleed: A) original flat ramp; B) modified semi V ramp and; C) full V ramp. SD: stroke deliverer, B: blade or stunner piston, G: vertical guide plates, F: fish, R: ramp.

In addition, two different types of bleeder blades were also examined, they were: 1) an aluminium piston with a three-sided pyramidal shape at the end of it as shown in Figure 6A and; 2) a piston made of stainless steel that was of a similar three-sided pyramidal shape, but it had a retractable stainless steel blade on each of the three edges of the pyramid (Figure 6B).

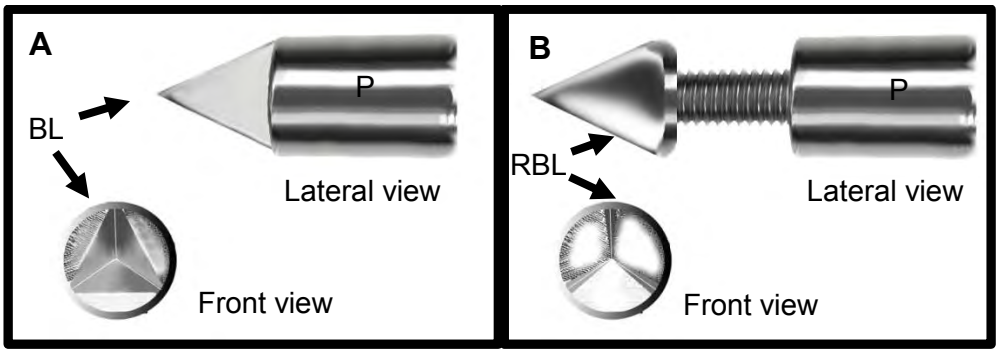

Figure 6. A) Aluminium piston with a three-sided pyramidal shape blade at the end to bleed Yellowtail Kingfish (*Seriola lalandi*) in the automated harvesting system. B) Stainless steel retractable blade with three razors on a three-sided pyramidal shape used to bleed Yellowtail Kingfish (*Seriola lalandi*) in the automated harvesting system. P: Piston, RBL: retractable blade, BL: blade.

2.1.2.3 Assessment of the modifications to the automated harvesting technology

To assess the accuracy of the stunners, a diagram was developed showing different potential stunning locations (Figure 7). This was done after a preliminary assessment of fish body movements post stunning, together with observations of the relative positioning of the brain (Section 2.1). Three areas were identified: (SS) successful stunning, (PS) partial stunning and (US) unsuccessful stunning, which was any area outside of the designed SS and PS areas (Figure 7). When the fish were stunned in the SS zone, the force of the blow was always 100% effective at achieving stunning.

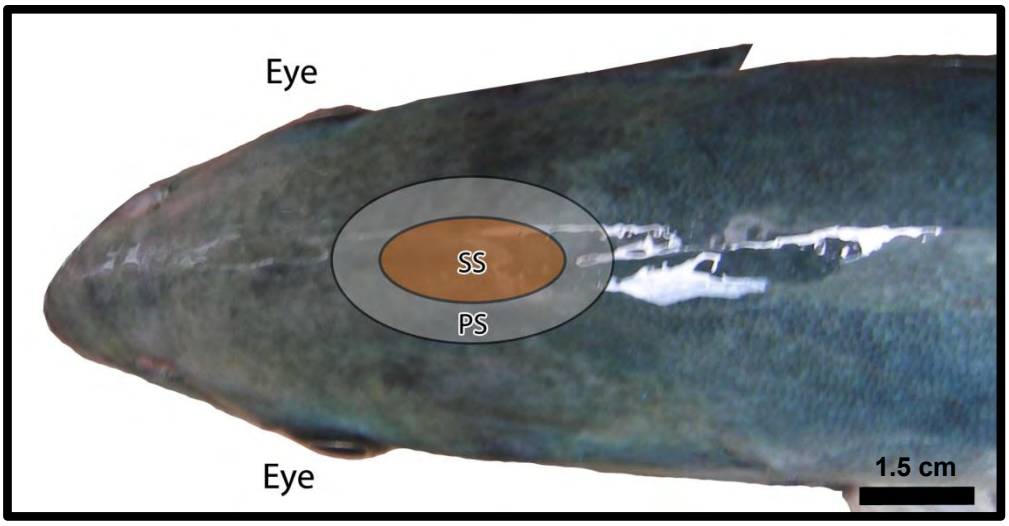

Figure 7. Target stunning zones on the dorsal region of the head of Yellowtail Kingfish (*Seriola lalandi*). SS: successful stunning, PS: Partial stunning.

To assess the accuracy of the bleeders, a diagram was developed showing appropriate bleed locations, using morphological areas of YTK that can be easily distinguished (Figure 8). Specifically, the ventral region of the YTK was divided in 11 target bleeding zones labelled from A to K. When the F zone was reached by the blade, the fish bleed was considered to be 100% effective. The bleeding zones in the left and right sides of the fish were scored separately to identify if the movement of the fish through the inverter chute tended to result in the fish being either under- or over-rotated when deposited in the bleeding unit.

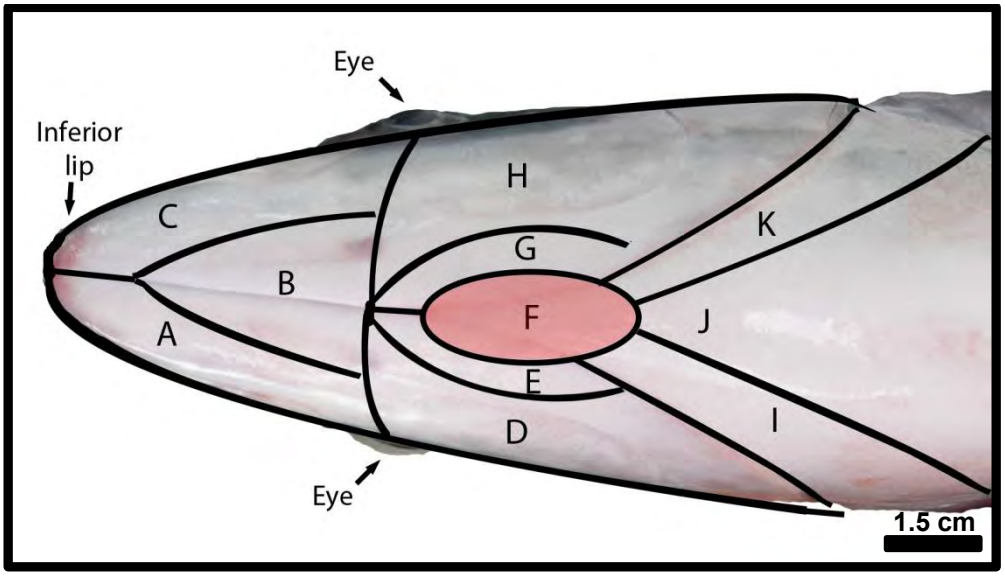

Figure 8. Target bleeding zones in the ventral region of the head of Yellowtail Kingfish (*Seriola lalandi*).

Assessments were conducted during commercial harvests of approximately 2500 fish from either grow-out cages or raceways offshore of Port Lincoln and >100 fish were evaluated in each assessment event. Bleed and stun accuracy were evaluated using various combinations of the different ramps and blades described in Sections 2.1.2.1 and 2.1.2.2, but it was not possible to collect data on all possible combinations of ramps and blades. In addition, research staff also observed:

- harvest start and finish time;
- total number of fish harvested;
- the number of fish harvested per channel, per minute (i.e. fish/channel/min);
- body movement after stunning and bleeding;
- whether the fish showed any characteristic eye movement after stunning and bleeding;
- whether the fish showed any characteristic gill/operculum movement after stunning and bleeding;

2.1.3 Statistical analysis

Statistical analysis was carried out using SPSS 20.0 for Windows (SPSS Inc.). Results were assessed using a one-way analysis of variance (ANOVA). Significance was set at $P < 0.05$. Data are presented as mean ± SE.

2.2 Activity Two: Optimising on-board bleeding practices whilst maintaining flesh whiteness

2.2.1 Experimental fish, sample collection and analysis

YTK (~3.5kg) investigated in this activity were harvested using the 'dead haul' method, as described in Section 2.1.2.1. Three groups of fish were examined:

1) fish automatically stunned and bled, that were held in a bleed bin containing ambient temperature seawater (13-22°C) for 10 minutes, then transferred to a refrigerated (-1 to 1.5°C) seawater (RSW) holding tank (bleed bin);
2) fish automatically stunned and bled and transferred directly to the RSW holding tank (no bleed bin) and;
3) fish automatically stunned, but not bled and placed directly in the RSW holding tank (unbled).

Group one represented the industry practice at the time of the activity, group two represented an alternative practice and group three was a positive control for blood spotting in the white muscle. To be representative of fish taken through the duration of the harvest, a total of nine YTK were randomly selected for each group from approximately the start, middle and end of the harvest. Each fish was numbered with a tail tag for identification purposes. Upon return to shore all fish were transferred to ice slurry and held overnight. The following day the fish were filleted, as per the "Japanese cut" (head and tail removed, gutted, left and right whole fillets produced with pectoral fins, ribs and pin bones left intact), then the individual fillets were vacuum packed. The packed fillets were kept chilled on ice and transported to the Lincoln Marine Science Centre for immediate visual assessment (for consistency the left fillet was assessed only). In a double blind randomised protocol a panel of five seafood professionals gave a subjective score to each fillet based on apparent blood in the white flesh. Scores ranged from 0 to 3, where 0 = no or minimal visible blood and 3 = maximal visible blood. Two extreme examples (0 and 3) of blood affected fillets were first presented to the panel as reference scores.

2.2.2 Statistical analysis

The scores from all panellists were pooled and the average score for each treatment group plotted as mean ± SE. Group means were analysed by one-way ANOVA in SPSS 20.0 for Windows (SPSS Inc.). Significance was set at $P < 0.05$.

2.3 Activity Three: Acute and chronic stress and its impact on YTK rigor and flesh quality

2.3.1 Experimental fish

YTK were transferred from offshore sea cages during the month of September 2012 to eight 1000 L tanks in an aerated flow through system at the Lincoln Marine Science Centre. The fish (*n* = 96) were 10 to 12 months of age, 865.17 ± 13.67 g (whole wet weight) and 41.06 ± 0.18 cm (fork length). The fish were acclimated for three weeks prior to the start of the experiment. During the acclimation period they were housed in ambient water temperature conditions, with a photoperiod of 14 h light and 10 h dark and fed to apparent satiation daily with the commercial feed.

2.3.2. Experimental design, sample collection and analysis

Treatments were randomly allocated to the tanks. The water level in four of the tanks was lowered to confine the fish for 90 mins each day for 5 days. On the sixth day 3 fish were dip netted out and sampled (i.e. the repeated disturbance and rested harvest group) from each of the 4 tanks, then the water level was lowered for 90 mins and then 3 more fish were sampled (i.e. the repeated disturbance and unrested harvest group). The fish in the other four tanks were left undisturbed until day six when 3 fish were dip netted out and sampled (i.e. undisturbed and rested harvest) from each of the 4 tanks, then the water level was lowered for 90 mins and then 3 more fish were sampled (i.e. the undisturbed and unrested harvest group).

Conditions during the experimental period simulated typical commercial practice when fish are harvested from a commercial raceway. Specifically, the fish were maintained under fasting conditions, the water temperature was ambient (16.8 ± 0.2^{o}C) and the photoperiod was maintained at 14 h light and 10 h dark. The shallow water confinement treatment consisted of turning the water flow off and draining the 1000 L tanks to a depth of 20 cm (a water volume of between 150 - 200L) for 90 min. During shallow water confinement period the swimming activity of the fish increased and they tended to cluster together more. Dissolved oxygen (D.O.) levels were monitored and recorded every 15 min and maintained above 3.0 mg/l by turning the water flow on and off periodically whilst still maintaining the 20 cm water depth.

Three fish were sampled per tank on each sampling occasion. The fish were individually removed from the tanks using a dip net, turned on their back and physically restrained in the net to obtain a blood sample from the caudal vein (using a 1 ½ inch, 19 G needle and 5 ml syringe) within 2 min of capture. The fish were then

killed by brain spike, bled and numbered with a tail tag. Blood samples were transferred into a 4 ml tube containing EDTA and stored at 4°C for analysis of haematocrit and lactate and glucose levels within 1 h after collection (see Sections 2.7.3 and 2.7.1.1, respectively). The remaining blood samples were centrifuged at 1500 g for 7 min and the plasma was then transferred to -80°C storage for analysis of cortisol at a later date (see Section 2.7.2). Measurements of weight, fork length and tail droop for rigor assessment were taken. A small tissue sample (5 to 6 g) was also taken from the dorsal right epaxial musculature proximal to the head, put into a labelled plastic bag, immediately frozen on dry ice and then transferred to -80°C storage for analysis of nucleotides, glycogen and lactate at a later date (see Sections 2.7.5 and 2.7.1.2). The fish were then placed in ice slurry and stored overnight in insulated boxes (58 x 37 x 44 cm, L W H) with the temperature of the slurry monitored and recorded every 5 min (0.4 ± 0.5^{o}C) (Pendant temp/light, HOBO).

At 24 h post-harvest, the fish were then transferred into insulated boxes (71 x 23 x 19 cm) containing two ice packs, as per standard industry practice, and held in a cool room (4^{o}C) until 96 h post-harvest. At 6, 12, 24, 48, 72 and 96 h post-harvest small tissue samples (5 to 6 g) were taken from the dorsal right epaxial musculature. Tail droop for rigor assessment and quality index were also assessed (see Sections 2.7.4 and 2.7.6.1, respectively). After the final assessment, each fish was filleted and the left fillet was put into a plastic bag and stored in a constant environment room at 4^{o}C. The fillet was assessed at time 0 and 96 h post-filleting for flesh quality attributes using the method described in Section 2.7.6.2. This protocol simulated cold chain and processing practices associated with exporting YTK product to the EU.

2.3.3. Statistical analysis

Statistical analysis was carried out using SPSS 20.0 for Windows (SPSS Inc.). Data for haematocrit, lactate and glucose levels in blood samples and quality assessment for fillets were analysed using a one-way ANOVA, and a repeated measures one-way ANOVA for glycogen and lactate in the flesh, rigor mortis and quality index scores for whole fish. When the time x treatment interaction term was significant, simple effects analyses were conducted for the repeated measures analysis. The significance was set at $P < 0.05$. Data are presented as mean ± SE.

2.4 Activity Four: Harvest stress and its impact on YTK product quality

2.4.1 Experimental fish, sample collection and analysis

YTK examined in this activity were between 20-24 months of age, 2.75 ± 0.12 kg (whole wet weight) and 57.64 ± 0.46 cm (fork length). The experiment was conducted in a commercial raceway offshore of Port Lincoln in April 2012. The water temperature during the experimental period was approximately 18°C. Two different harvesting methods were examined; 1) hook harvest (HH), as a control group and; 2) using commercial harvest procedures current at the time of the activity (CH).

The HH fish (n = 10) were harvested using hook and line. The fish were hooked and killed on a foam mat by cranial spike and bled within 30 s of capture. Blood samples were collected from the bleed point into a 10 ml tube containing heparin for analysis of lactate and glucose within 1 h after collection (see Section 2.7.1.1). The remainder of the blood sample was stored at 4°C until they were taken to the laboratory on shore, where they were centrifuged at 1500 g for 7 min and the blood plasma transferred to -80°C for analysis of cortisol at a later date (see Section 2.7.2). Small tissue samples (5 – 6 g) were taken from the dorsal right epaxial musculature, put into labelled plastic bags and frozen on dry ice before being transferred to -80°C storage until analysis of nucleotides, glycogen and lactate at a later date (see Sections 2.7.5 and 2.7.1.2, respectively). Measurements of total body weight, fork length and tail droop for rigor mortis assessments were also recorded (see Section 2.7.4 for description of rigor mortis assessment). Fish were sequentially tagged (1-10) with a tail tag.

The CH fish (n = 30) were obtained at three time points throughout the harvest: 0 (CH0), 60 (CH60) and 120 min (CH120) from the beginning of the commercial harvest. The commercial harvest consisted of a shot of 2,700 fish harvested in 2.5 h. The fish were harvested using a dead haul procedure, as described in Section 2.1.2.1. Briefly, the raceway net was lifted by hand to reduce the depth of the raceway to approximately one metre for the duration of the commercial harvest. Fish were lifted on-board the harvest vessel using a pump at a rate of approximately 15 fish per min. The experimental fish were removed from the processing line immediately after they had been stunned and bled in the automated harvesting system. Blood and tissue samples were obtained and morphological parameters (i.e. fork length, weight and tail droop) were measured as described for the HH group. CH fish were sequentially tagged (11-40).

Following sampling and tagging, HH and CH fish were placed in a mesh bag and stored in refrigerated sea water (1.5 ± 0.7^{o}C) until the harvest vessel returned to shore (~4 h after the start of HH fish harvesting). Upon return to shore all experimental fish were transferred into an ice and seawater slurry and held overnight (0.4 ± 0.5^{o}C). The following day, the experimental fish were transferred in insulated boxes (71 x 23 x 19 cm) containing two ice packs, as per industry standard, and held in a cool room (4^{o}C) until 96 h post-harvest. Small tissue samples (5 – 6 g) were taken at times 12, 24, 48, 72 and 84 h post-harvest and transferred to a -80^{o}C storage freezer until required for analysis of nucleotides, glycogen and lactate levels (see Sections 2.7.5 and 2.7.1.2, respectively). Measurements of tail droop for rigor mortis assessments and quality index were also recorded at these time-points (see Sections 2.7.4 and 2.7.6.1, respectively). After the final assessment, each fish was filleted and the left fillet assessed at time 0, 24, 48, 72 and 96 h post filleting for flesh quality attributes using the method described in Section 2.7.6.2. The left fillet was stored at 4^{o}C in a plastic bag during this time.

2.4.2 Statistical analysis

Statistical analysis was carried out using SPSS 20.0 for Windows (SPSS Inc.). Lactate, glucose and cortisol levels in blood were analysed using a one-way ANOVA and a LSD post-hoc test to identify differences between treatment groups. Glycogen and lactate levels in flesh, rigor mortis and quality index scores for whole fish and fillets were analysed by using repeated measures one-way ANOVAs. When the time x treatment interaction term was significant, simple effects analyses were conducted for the repeated measures analysis. The significance was set at $P < 0.05$. Data are presented as mean ± SE.

2.5 Activity Five: Investigate the development of eye cloudiness in YTK post-harvest

2.5.1 Effect of storage temperature on the development of eye cloudiness

YTK (*n* = 9) from the 2+ year class were obtained from a commercial harvest, transferred to insulated containers with cold seawater (4°C) and transported to the Lincoln Marine Science Centre. Once arriving at the Centre, three fish were randomly assigned to one of the follow groups: 1) ice slurry; 2) 1°C seawater and; 3) 4°C seawater. The fish were then stored in insulated containers (58 x 37 x 44, L W H) in the prescribed condition for up to 24 hours following harvest. The ice slurry was created and maintained according to commercial practice (i.e. 60% of the container volume was filled with ice and the remaining volume filled with fish and seawater), and the temperature of the seawater in groups two and three were stabilized by storing the insulated containers in temperature controlled rooms. The temperature in the insulated containers was monitored and recorded every minute (Pendant temp/light, HOBO). The development of eye cloudiness was assessed for each fish at 0, 2, 4, 6 and 24 h using a scoring system where 0 = no cloudiness, 1 = partial cloudiness and 2 = complete cloudiness.

2.5.2 Development of quantitative method to facilitate the analysis of eye cloudiness

Both eyes of each fish from a separate group of 3 to 4 kg YTK that were commercially harvested and kept in ice slurry for 24 h were photographed with a digital camera (CAMEDIA, C-7070, Olympus) attached to a tripod. The photographs were subsequently analysed using ImageJ 1.46r (National Institutes of Health, USA) software. Firstly, the area of the lens was measured in arbitrary units (Figure 9A) and then the area of the lens with no cloudiness was measured (Figure 9B). The following equation was then used to determine the percentage area of cloudiness (or cold cataract (CC)), where a = area measured in Figure 9A and b = area measured in Figure 9B:

$$CC\ (\%) = 100 - \left({}^{b}/_{a} * 100\right)$$

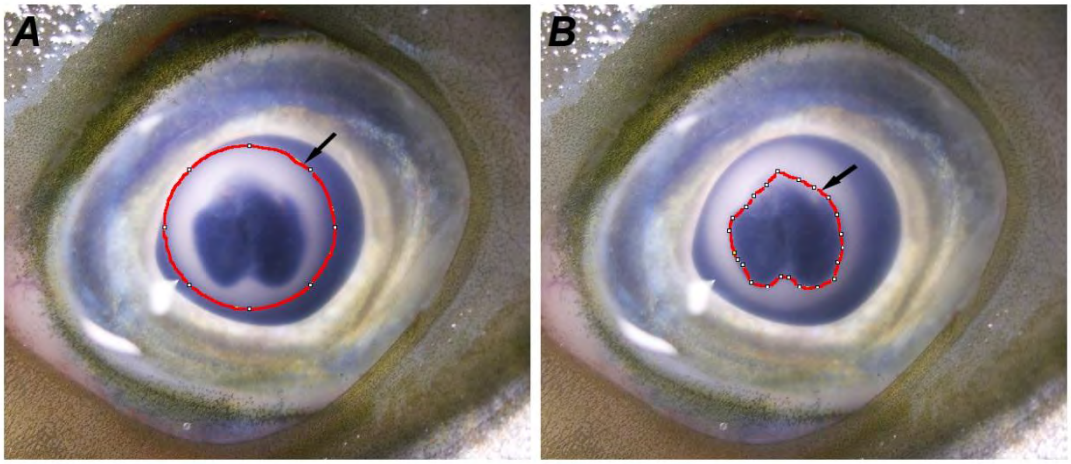

Figure 9. Photograph of Yellowtail Kingfish (*Seriola lalandi*) eye showing the area of the whole lens (A) and the area of the same lens without cloudiness (B), from which the percentage of eye cloudiness was calculated.

2.5.3 Effect of taurine supplementation in the diet on the development of post-harvest eye cloudiness during ice slurry storage

YTK (*n* = 30) were sourced opportunistically from a commercial trial that was examining the effect of five different diet formulations (i.e. three commercial diets, and two of these diets with taurine supplementation) on fish performance. The trial ran over a three month period and at the conclusion six fish per group were randomly harvested using a dip net, killed by brain spike, bled and tagged and immediately transferred into ice slurry. Eye cloudiness was then examined for each tagged fish at 10 and 24 h post-harvest using both the subjective scoring and quantitative methods described in Section 2.5.1 and 2.5.2, respectively.

2.5.4 Osmotic effects on the development of post-harvest eye cloudiness

2.5.4.1 Pilot trial

YTK (*n* = 10) from a 2+ year class were obtained from a commercial harvest. Whilst still on-board the harvest vessel, the eyes of each fish were surgically removed and randomly placed into four 5 L insulated containers (23.5 x 15.5 x 15.0 cm, L W H) containing about 3 L crushed ice. A total volume of 2 L of water (each with a different ratio of seawater and fresh water) was then added to the 5 L containers. The different water compositions were: 1) 25% seawater : 75% fresh water; 2) 50% seawater : 50% fresh water; 3) 75% seawater : 25% fresh water and; 4) 100% seawater : 0% fresh water. The eyes were transported to the Lincoln Marine Science Centre, where the salinity and temperature of the solutions in the containers was monitored at 0, 4, 8, 12, 24 h post-harvest and eye cloudiness was assessed at 8, 12, 24 h post-harvest using a pre-determined subjective scoring system (Table 1). The liquid

content of the eyeball (probably a combination of aqueous and vitreous humours) was collected at the end of the experimental period by syringe and needle (Figure 10A-C) and the salinity measured by refractometer and recorded.

Table 1. Qualitative scores, description and representative images of the development of eye cloudiness/cold cataracts in Yellowtail Kingfish (*Seriola lalandi*).

Score	Description	Representative image
0	No cataract.	
1	Some incipient cataract at the edge of the lens or the centre.	
2	Evident cataract but no more than 50% of the eye and no noticeable white cataract.	
3	More than 50% of the eye lens with cataract and/or matte white cataract present.	

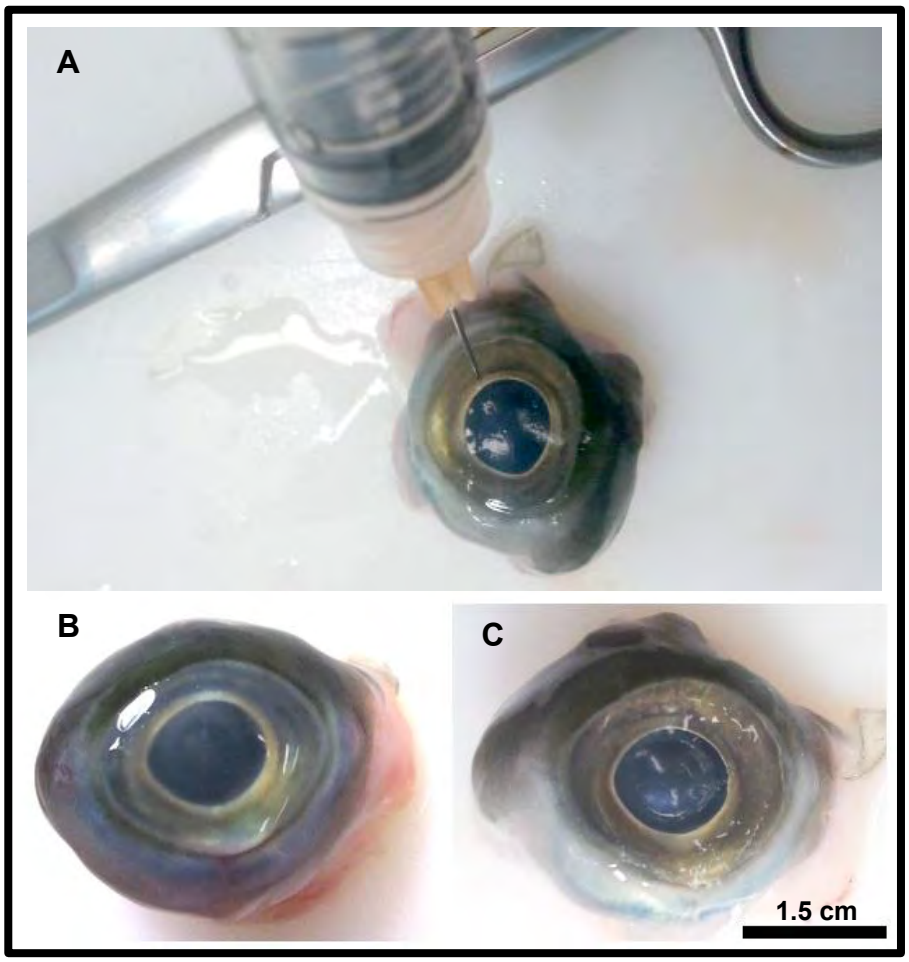

Figure 10. (A) Procedure for sampling the liquid content of the eyeball from Yellowtail Kingfish (*Seriola lalandi*): (B) eye before and (C) after liquid eyeball content was removed.

2.5.4.2 Full scale trial

YTK (*n* = 25) from a 2+ year class were collected from a commercial harvest and transferred into five 100 L insulated containers (58 x 37 x 44 cm, L W H) (5 fish per container) with 60 L ice and 40 L of: 1) 50% seawater, 50% fresh water; 2) 60% seawater, 40% fresh water; 3) 70% seawater, 30% fresh water; 4) 80% seawater, 20% fresh water and ; 5) 100% seawater, 0% fresh water. Salinity was measured and recorded for each container at times 0, 3, 6, 12, 24 h post-harvest. At the end of the experiment, three fish per treatment had their eyeball liquid contents removed by a syringe and needle, using the method described in Section 2.5.4.1, and the salinity of the fluid was measured and recorded. Both eyes were photographed and eye cloudiness assessed using the pre-determined scoring system and quantitative method described in Table 1 and Section 2.5.2, respectively. The temperature of the water/ice slurry in each container was monitored and recorded every 5 minutes (Pendant temp/light, HOBO).

2.5.5 Statistical analysis

Statistical analysis was carried out using SPSS 20.0 for Windows (SPSS Inc.). Data were analysed using one-way ANOVA. The significance was set a $P < 0.05$. Data are presented as mean ± SE.

2.6 Activity Six: Physiological, biochemical and haematological characteristics of wild YTK

2.6.1 Wild fish sample collection and analysis

Twenty three wild YTK caught by recreational fishers offshore of Sydney, New South Wales were sampled opportunistically during February 2013. The weight of the fish ranged between approximately 1.5 to 3 kg.

Blood samples were taken from the caudal vein using a 1 ½ inch, 19 G needle and 5 ml syringe immediately after the fish were caught. The fish were killed by brain spike, bled and numbered with a tail tag and put into an ice slurry. The blood samples were transferred into 1 ml tubes containing lithium heparin and stored at 4°C for transport to Idexx Laboratories Pty Ltd, Sydney, Australia for biochemical and haematological analysis within 12 h of collection. Detailed methodologies of these analyses are commercial in confidence. Blood samples were also transferred into 4 ml tubes containing EDTA and analysed by research staff for haematocrit and osmotic fragility (see Sections 2.7.3 and 2.7.7, respectively) within 12 h of collection. The remaining blood sample was then centrifuged at 1500 g for 7 minutes and the resultant plasma was split into two aliquots, frozen and transferred in dry ice for transport to the Lincoln Marine Science Centre. Once at the Lincoln Marine Science Centre the samples were transferred into -80°C storage until required for analyses of urea and cholesterol, which were determined according to the manufacturer's instructions using UREA N B kit (439-17501, Wako. Distributor 2012: Novachem) and T-CHO E kit (439-17501, Wako. Distributor 2012: Novachem), respectively.

To represent the average whole fillet a 'quality cut' cross section of the dorsal and ventral muscle was taken from the left side fillet, as illustrated in Figure 11. The whole liver was also collected and both were transferred onto dry ice for transport to the Lincoln Marine Science Centre. Once at the Lincoln Marine Science Centre they were transferred into -80°C storage prior to shipping to Assure Quality Ltd, Auckland, New Zealand for analysis of proximate composition (fat and protein), fatty acids, amino acids and cholesterol levels. Liver samples were pooled (fish 1 to 12 and 13 to

23) for analysis of proximate composition (n = 2). Detailed laboratory methodologies are commercial in confidence.

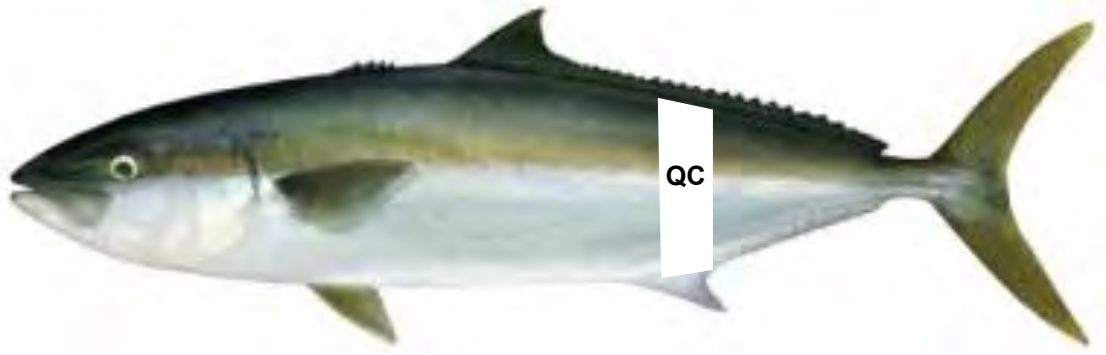

Figure 11. Approximation of wild Yellowtail Kingfish (*Seriola lalandi*) left fillet sampling site for the quality cut (QC), a cross section of both dorsal and ventral meat to represent whole fillet.

The day after the wild YTK samples had been collected in Sydney, a further 13 blood samples were collected from farmed YTK from Port Lincoln (these samples were taken using disodium EDTA as the anticoagulant) and sent to the Idexx laboratory in Sydney for haematological and blood biochemical analyses for comparison to the wild fish.

2.7 Sample analysis

2.7.1 Glucose, glycogen and lactate

2.7.1.1 Blood sample

Blood glucose and lactate levels were measured using an *Accutrend plus* dual glucose/lactate meter (Roche) with a detection limit of 0.8 mmol/L and 1.1 mmol/L for lactate and glucose respectively. When lactate values were below the detection limit, a value of 0.8 mmol/L was arbitrarily assigned.

2.7.1.2 Tissue sample

Muscle tissue samples collected in Activity 3 were sliced and a weighed amount (~1 g) added to a 50 ml centrifuge tube containing 3 ml of cold 0.6 M perchloric acid. The samples were homogenised for 1 min at 12000 RPM four times using an Omni Prep Multi-Sample Homogenizer (OMNI International, Part Number: 06-021), before a further 2 ml of cold 0.6 M perchloric acid was added and the tissue extract homogenised twice more for 1 min at 12000 RPM using the Omni-Prep. Muscle tissue samples collected in Activity 4 were crushed to a powder using a mortar and pestle on dry ice, and a weighed amount (~1 g) was added to a 10 ml centrifuge tube containing 5 ml of cold 0.6 M perchloric acid and vortexed for 15 seconds. Samples

from both Activity 3 and 4 were then allowed to extract on ice for 20 minutes before being centrifuged for 5 min at 3000 g. The supernatant was then filtered (0.45 µm) into 2 ml cryogenic vials for storage at -80°C until analysis. Extracted samples were removed from -80°C storage and thawed at 4°C in a cool room before commencing analysis.

Lactic acid levels were obtained using the UV-method for the determination of L-lactic acid (10139084035, L-lactic Acid 30, r-biopharm – Roche). Briefly, the perchloric acid extracted tissue sample was adjusted to pH 7.0 with 5M K_2CO_3 and then diluted with distilled water (1:10). The lactic acid content was assessed according to manufacturer instructions. The values were expressed as mmol/kg of muscle tissue.

Glycogen levels were obtained using a Glucose Assay kit (Sigma, GAGO-20) and amyloglucosidase (AMY) solution (Sigma, A1602), according to manufacturer instructions. The glycogen level was calculated by the difference between incubating the samples with and without AMY. Absolute values were obtained from incubation of known glycogen concentrations in the assay. The values are expressed as mg/g of extracted tissue.

2.7.2. Cortisol

Cortisol concentrations were determined in the blood plasma of the fish by radioimmunoassay, following standard ethyl acetate extraction, as described by Pankhurst and Sharples (1992). The minimum detectable limit of the assay was 600 pg/ml.

2.7.3. Haematocrit

Duplicate blood samples (~50µl) were loaded into standard heparinised capillary tubes (75mm), sealed with capillary tube sealant and spun in a micro-haematocrit centrifuge at 12,000 rpm for 5 min. The length of the red blood cells in the capillary tube and the total plasma and red blood cell length (i.e. total length) were then measured and haematocrit level was calculated as follows:

$$Haematocrit\ (\%) = \frac{Red\ blood\ cell\ length}{Total\ length} \times 100$$

2.7.4. Rigor index

Rigor index was determined using the 'droop' method (Iwamoto et al. 1987). Briefly, the intact fish were placed on a horizontal table with the posterior half of the body hanging off the edge of the table (Figure 12). The vertical height of the droop was measured on the left and right side of the fish and the two values were averaged (Figure 13). The index was then calculated as follows:

$$Rigor\ Index = \frac{(D_o - D_t)}{D_0} \times 100$$

where D represents the vertical height of the droop (i.e. distance between the "fork" of the caudal fin from the top of the table), D_0 represent the initial values obtained immediately after harvest and the D_t represents values obtained from the subsequent time points. A freshly harvested fish will have maximal droop (0%), a fish in full rigor will have no droop (i.e. 100%) and as rigor resolves the droop returns.

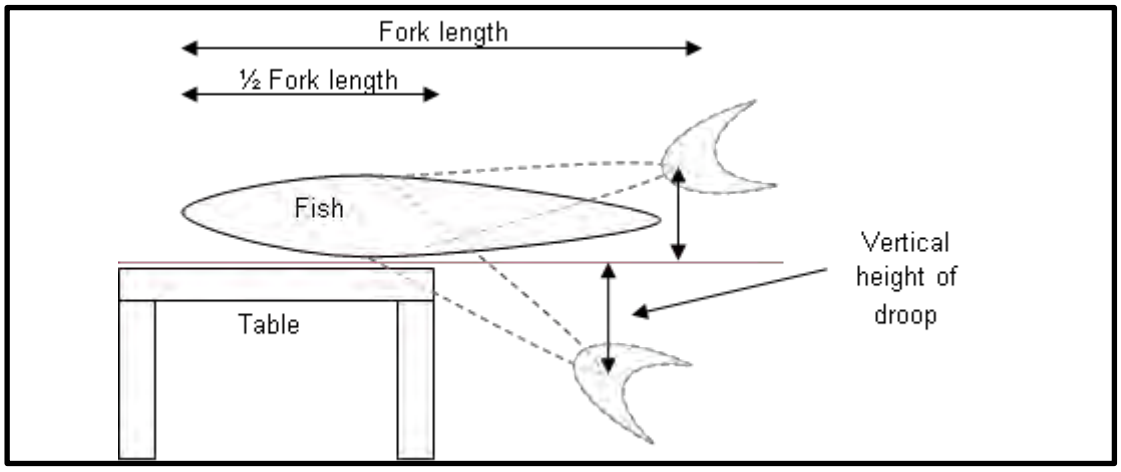

Figure 12. Determination of rigor index for finfish (modified from Iwamoto et al. (1987)).

2.7.5. Nucleotide analysis and K value

Nucleotides were extracted from muscle tissue in 0.6M perchloric acid, as described in Section 2.7.1.2. ATP and its breakdown products were then quantified by the HPLC methods of Ryder (1985), Van der Boon et al. (1992) and Ozugul et al. (2000). Specifically, filtered (0.45 µm) extracts were analysed by HPLC in a phosphate buffer / acetonitrile gradient mobile phase using the gradient profile outlined in Table 2. The injection volume was 5 µl and ATP and its breakdown products were eluted on a Prevail C18 5µm column (Waters, 150 mm x 4.6 mm) and identified by UV detector at 254nm. The total separation time was 5 min and the gradient run was for 8 min to ensure full separation.

Table 2. HPLC gradient profile for separation of ATP and its breakdown products

Time (min)	Flow (ml/min)	Mobile phase composition (%) at end of each period	
		Phosphate buffer	Acetonitrile
0	1.7	100	0
2	1.6	95	5
3.5	1.6	95	5
5	2.0	75	25
6	2.0	70	30
7	2.0	100	0
8	2.0	100	0

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

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

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

2.7.6. Quality index

2.7.6.1 Whole fish

Quality index was assessed using a modified version of the method outlined in the Australian Quality Index Manual for whole YTK (Table 3) (Boulter et al 2009).

2.7.6.2 Fish fillet

Fillet gaping, drip loss, colour and firmness were assessed using the quality index score system modified from Carragher et al. (2009) (Table 4).

Table 3. Quality Index scheme for whole Yellowtail Kingfish (S*eriola lalandi*) (modified from Boulter et al. (2009)).

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

Table 4. Quality Index scheme for Yellowtail Kingfish (S*eriola lalandi*) fillets (modified from Carragher et al. (2009))

Characteristic	Description	Score
Gaping	No gaping evident	1
	some gaping evident	2
	Obvious gaping evident	3
Colour	No browning	1
	Slight browning	2
	Very brown, particularly in the anterior – dorsal region	3
Smell	Fresh, seaweed smell	1
	Musty, strong fish smell	2
	Stale, rancid smell	3
Firmness	Very firm – flesh quickly spring back into position	1
	Firm – flesh slowly spring back into position	2
	Slightly soft – slight indent in flesh when pressed	3
	Soft – indent left in flesh when pressed	4
Total quality index score		**4 - 13**

2.7.7 Osmotic fragility

Osmotic fragility was determined using a slightly modified procedure from that outlined in Bektas and Ayik (2010). In brief, 10 µl of each blood sample was exposed to a series of 1 ml NaCl solutions ranging from 0.85% NaCl to 0.0% NaCl (distilled water). After an incubation period of 30 min on ice the solutions were centrifuged for 7 min at 1000 rcf. The optical density of the supernatant was then determined spectrophotometrically at 540 nm. The percentage haemolysis was expressed relative to the solution having the highest optical density. Plots of % haemolysis vs NaCl concentration were drawn for each fish and from these curves the %NaCl concentration corresponding to 50% haemolysis was determined. No statistical analysis was performed on the osmotic fragility data.

3. Results

3.1 Activity One: Develop systems that allow automated harvesting technology to be used on YTK so that the stun/bleed success rate increases from 65 to 95%

3.1.1 Morphological measurements influencing stun efficiency

In marketable size fish the average (± SD) depth to the mid-point of the brain measured from the top of the head was 25.05 ± 1.82 mm. The average (± SD) mid-point position of the brain on the horizontal axis measured from the inferior lip was 95.07 ± 5.20 mm. There was no significant correlation between fish fork length and the mid-point of brain depth (Figure 13). However, for fish between 2.3 and 3.6 kg there was a significant correlation between the mid-point position of the brain on the horizontal axis and fish fork length (Pearson coefficient= 0.748, P<0.01) (Figure 14).

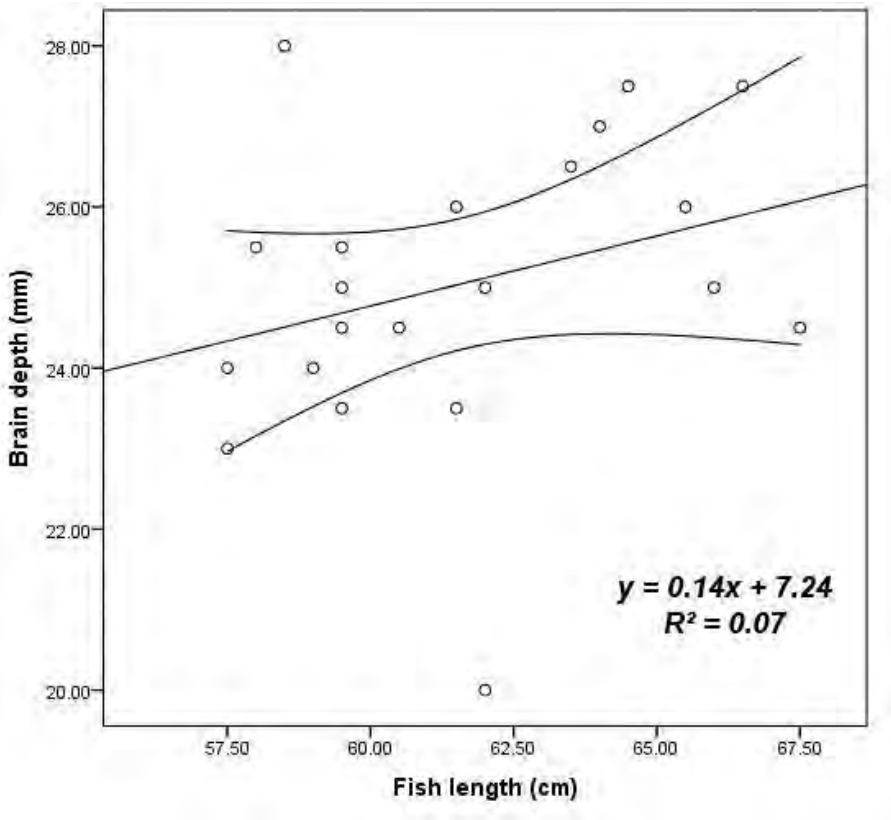

Figure 13. Correlation between the mid-point of brain depth measured from the top of the head and the fork length of Yellowtail Kingfish (*Seriola lalandi*). Confidence intervals (95%) of the mean are shown on each side of the trend line.

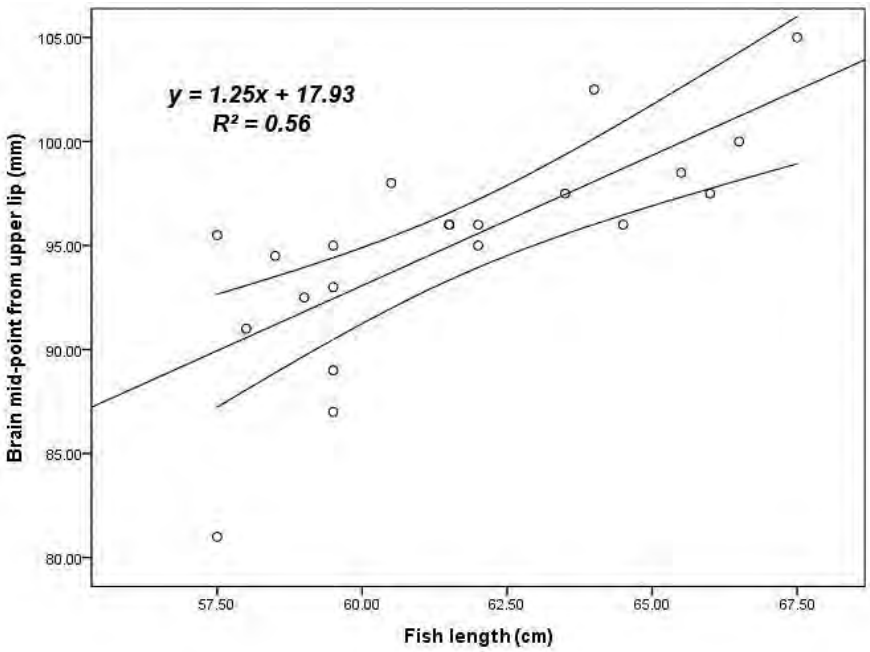

Figure 14. Correlation between the mid-point position of the brain measured from the inferior lip and fork length of Yellowtail Kingfish (*Seriola lalandi*) (Pearson coefficient= 0.748, P<0.01). Confidence intervals (95%) of the mean are shown on each side of the trend line.

3.1.2 Modifications to automatic harvesting technology

3.1.2.1 Ramp influences on stun accuracy

Stun accuracy (i.e. stun location in the SS zone) significantly increased by 2.5 times from 33.11 ± 4.23 to 80.89 ± 7.13 % when using the full V ramp, compared to the flat ramp (P = 0.001) (Figure 15). In addition, subsequent further modifications to the full V ramp in the stunner unit were made (i.e. the ramp was bent down at the top where the fishes head is located) to minimise damage to the lower part of the operculum that was occurring when the stunning blow was applied. During post-stun assessments, operculum and eye movement were deemed unreliable indicators of stun effectiveness, due to difficulties in accurately assessing them. Specifically, operculum movement appeared to occur sporadically after stunning making it difficult to determine if it has actually ceased or not and as the eyes do not protrude from the body of the fish it was difficult to clearly observe if they were moving or not.

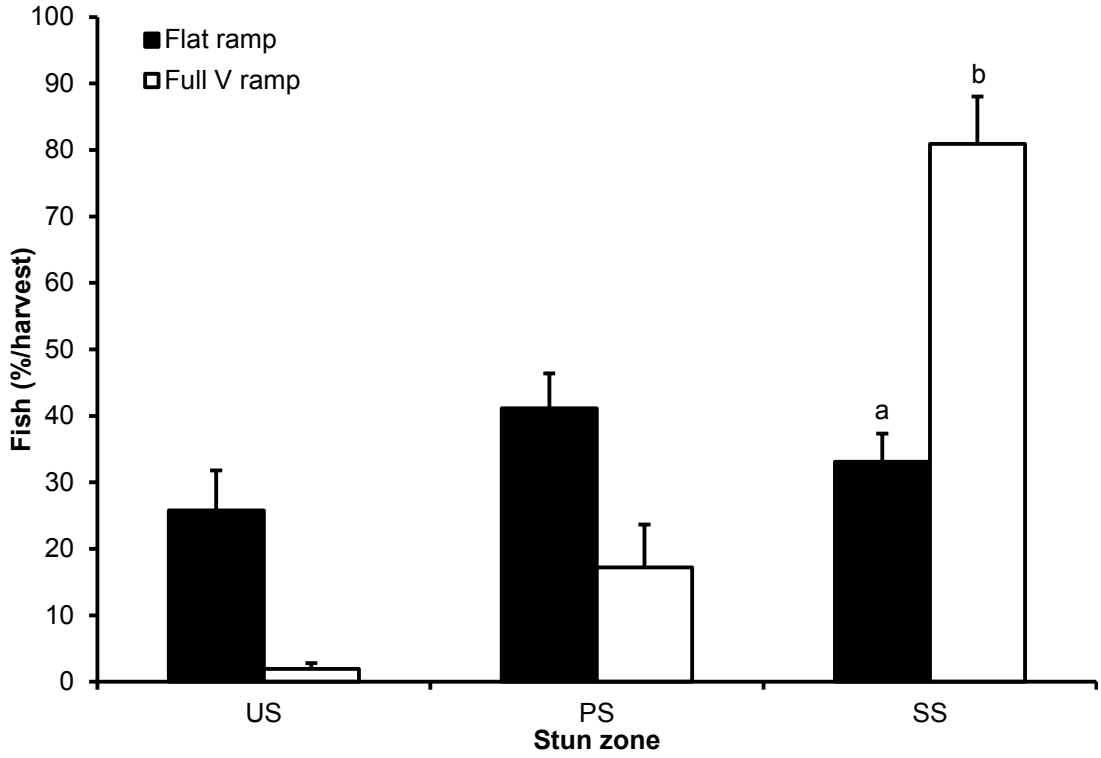

Figure 15. Mean (± SE) % of Yellowtail Kingfish (*Seriola lalandi*) per harvest event stunned in the target stun zones (US: unsuccessful stun, PS: partial stun and SS: successful stun) when using the flat ramp and full V ramp in the automatic harvest system. Different superscripts indicate significant differences within SS stun zone only ($P < 0.05$).

3.1.2.2 Ramp and blade influences on bleed accuracy

Bleed accuracy (i.e. bleed location in the F zone) increased significantly from 64.8 ± 7.65 % to 81.45 ± 13.45 % when using the full V ramp with the aluminium non-adjustable blade, compared to a semi V ramp with the aluminium non-adjustable blade ($P = 0.04$) (Figure 16). However, when the full V ramp was used together with the stainless steel adjustable blade, bleed accuracy increased further to 96.77 ± 3.16 % ($P = 0.001$) (Figure 16). Preliminary observations on the accuracy of the original flat ramp showed poor performance and was therefore not assessed here.

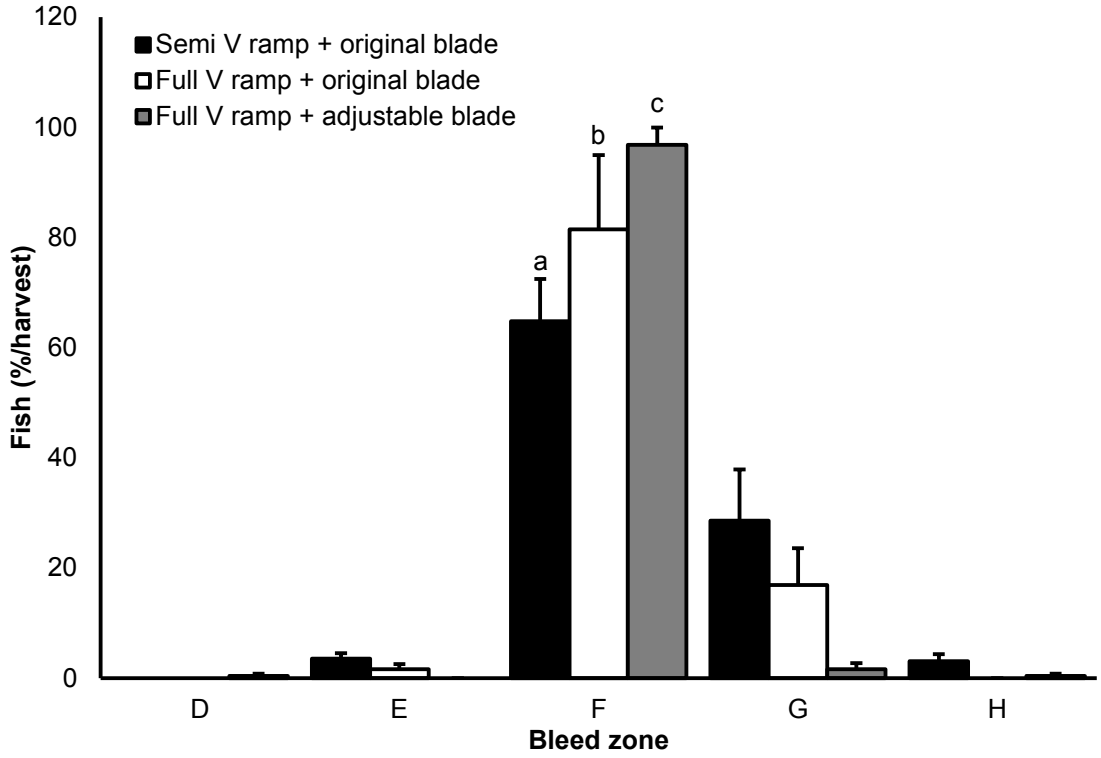

Figure 16. Mean (± SE) % of Yellowtail Kingfish (*Seriola lalandi*) per harvest event bled in the target bleed zones (D to H) when using the semi V ramp and full V ramp together with the original aluminium pyramidal blade and the alternative stainless steel version with retractable cutting blades in the automated harvesting system. Different superscripts indicate significant differences within bleed zone F only ($P < 0.05$).

3.2.2.3 Number of fish harvested per minute

Preliminary observations by industry personnel indicated an increase in the number of fish harvested from approximately 10 to 30 per minute (data not shown). The number of fish delivered from the whirlpool to the three channels on the automated harvesting system was uneven with a higher number of fish typically delivered to channel three and fewer fish to channel one (see Figure 17).

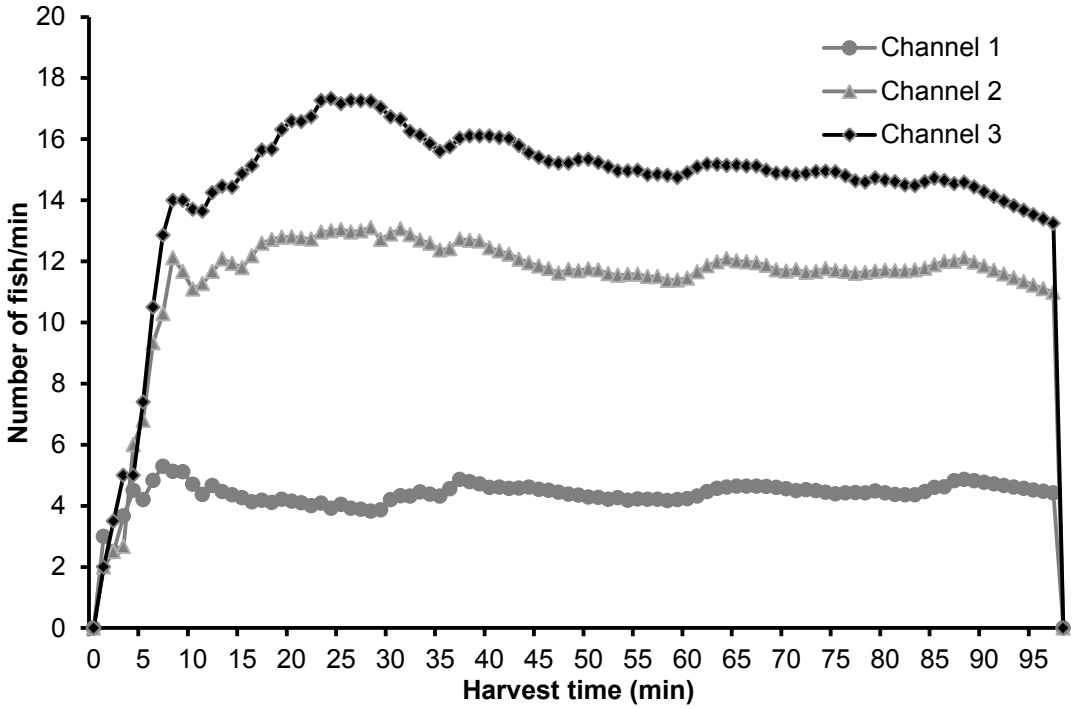

Figure 17. Number of Yellowtail Kingfish (*Seriola lalandi*) delivered from the whirlpool to the three channels on the automated harvesting system per minute during a typical harvest event.

3.2 Activity Two: Optimising on-board bleeding practices without reducing flesh whiteness

There was a significant effect of bleed method on the visual score of the YTK fillet ($P < 0.001$) (Figure 18). In post-hoc multiple comparisons, the visual score of the unbled group was significantly higher (i.e. more blood was evident) compared to both other groups ($P < 0.001$), but there was no significant difference between the fish exposed to the bleed bin and no bleed bin treatments ($P = 0.529$) (Figure 18).

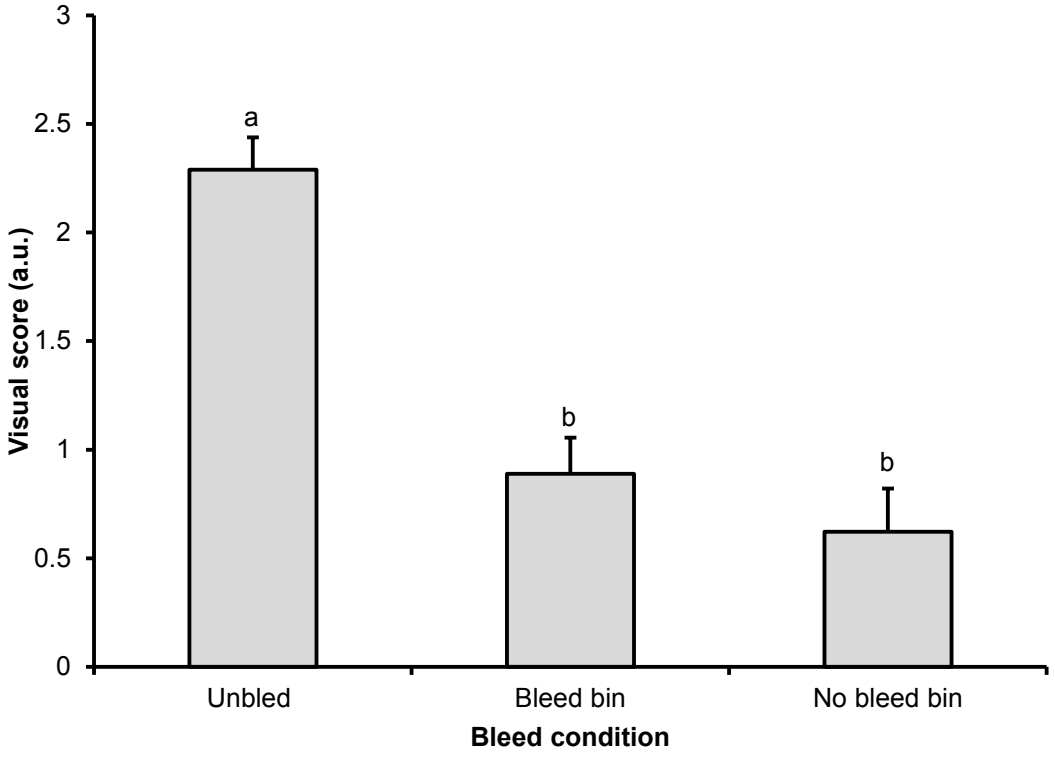

Figure 18. Mean (± SE) fillet blood scores in fillets from Yellowtail Kingfish (*Seriola lalandi*) harvested the previous day (Unbled: fish automatically stunned but not bled and placed directly into RSW holding tank; bleed bin: fish automatically stunned and bled, held in an ambient seawater bleed bin for up to 10 minutes, then transferred to the RSW holding tank and; no bleed bin: fish automatically stunned and bled and transferred directly into RSW holding tank) (n = 9). Subjective scoring scale went from 0 = no visible blood, to 3 = maximum visible blood. Significance indicated by different superscripts ($P < 0.05$).

3.3 Activity Three: Acute and chronic stress and its impact on YTK product quality

3.3.1 Blood haematology and biochemistry

Haematocrit, plasma glucose and plasma lactate levels were significantly lower in fish in treatment groups one and two (i.e. both rested harvest treatments) compared to fish in treatment groups three and four (i.e. both unrested harvest treatments) ($P < 0.05$) (Figure 19A-C). However, no significant differences were observed between the undisturbed and repeatedly disturbed groups in each pair ($P > 0.05$) (Figure 19A-C). Lactate levels of fish in treatment groups one and two (the rested harvest treatments) were close to, or less than, the detection limit of the assay (0.8 mmol/L).

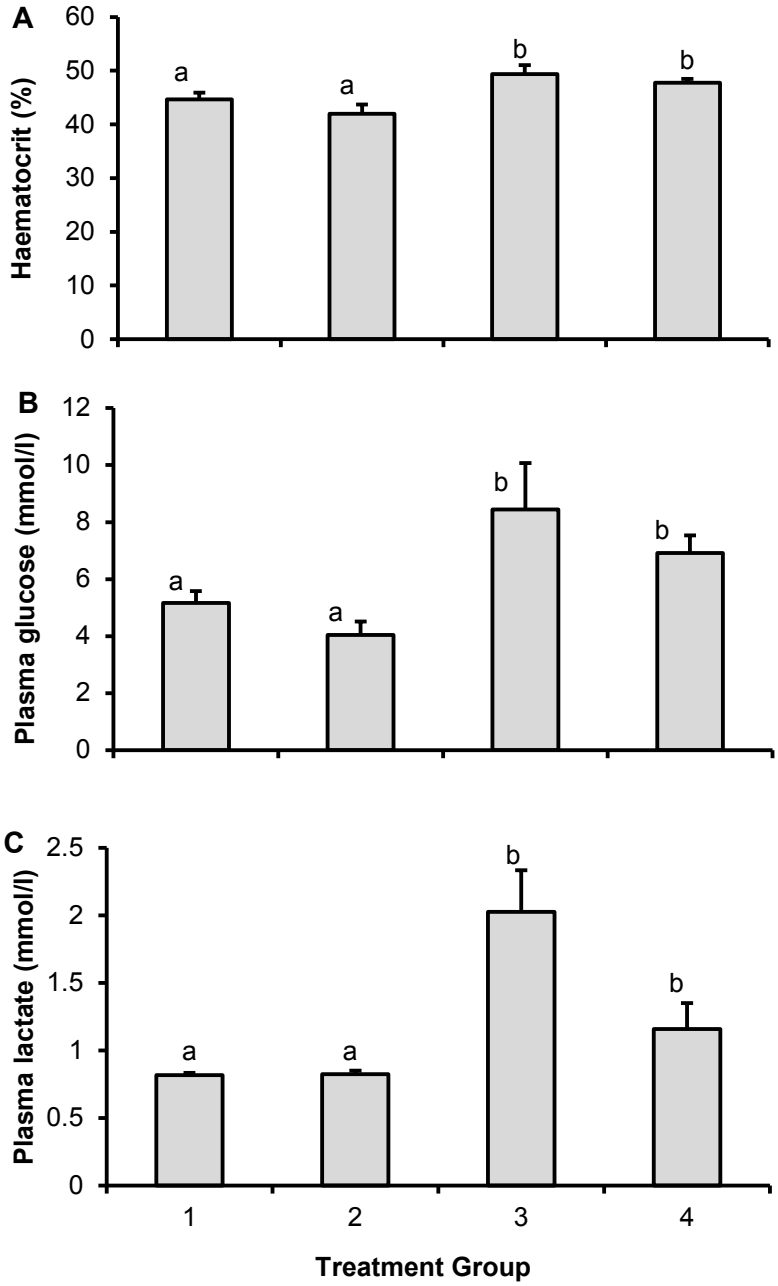

Figure 19. Mean (± SE) (A) haematocrit (%), plasma (B) lactate and (C) glucose (mmol/l) levels in Yellowtail Kingfish (*Seriola lalandi*) exposed to to different conditions prior to harvest: 1) undisturbed and rested harvest); 2) repeated disturbance and rested harvest; 3) undisturbed and unrested harvest and; 4) repeated disturbance and unrested harvest (n = 4). Significant differences indicated by different superscripts ($P < 0.05$).

3.3.2 Flesh biochemistry

Glycogen levels in the flesh were significantly higher in treatment group one compared to treatment groups two, three and four immediately post-harvest (i.e. 0 h post-harvest) ($P \leq 0.001$) (Figure 20A). Muscle glycogen levels sharply decreased at 6 h post-harvest in all treatment groups compared to the levels observed at 0 h post-harvest and thereafter remained fairly stable at all subsequent sampling points (Figure 20A). No significant differences were detected between the four treatment

groups at the subsequent sampling points, except at 24 h post-harvest where treatment group four was significantly less than treatment group one (P = 0.024) (Figure 20A). Lactate levels in the flesh generally increased for each treatment group with increasing time post-harvest (Figure 21B). Statistical differences were detected between some of the treatment groups at some of the time points evaluated, as shown on Figure 20B. Levels varied between the groups over time with no consistent trend evident at each time point; except for treatment group one, which was significantly higher than treatment group two at each time point, and treatment groups two and four which were significantly lower than groups one and three at 24 and 96 h (Figure 20B).

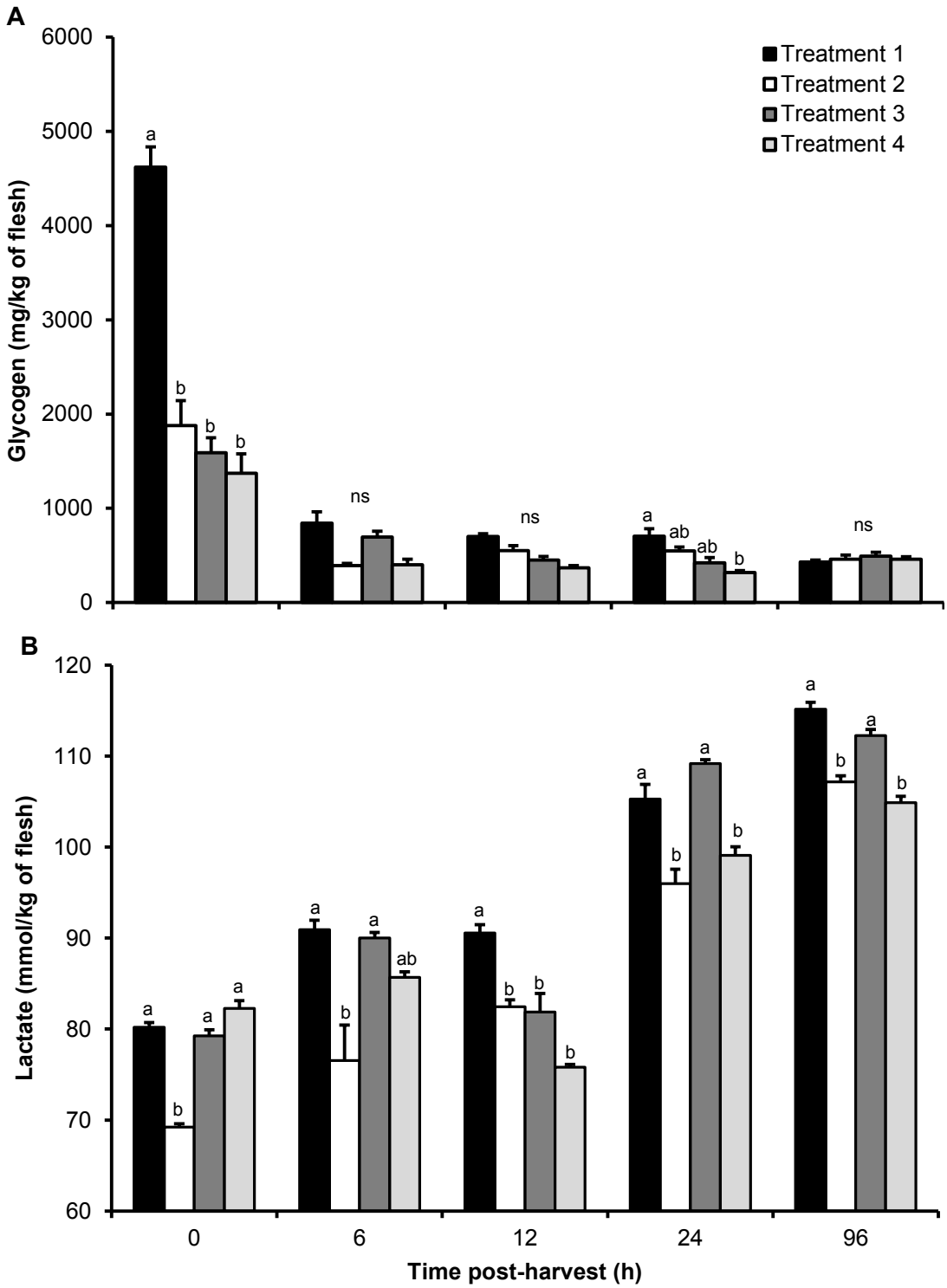

Figure 20. Mean (± SE) (A) glycogen (mg/kg of flesh) and (B) lactate (mmol/kg of flesh) levels in Yellowtail Kingfish (*Seriola lalandi*) post-harvest (n = 4). Fish were exposed to different conditions prior to harvest: 1) undisturbed and rested harvest; 2) repeated disturbance and rested harvest; 3) undisturbed and unrested harvest and; 4) repeated disturbance and unrested harvest. Different superscripts indicate significant differences at that time point ($P < 0.05$). ns: no significant differences within the time point analysed.

3.3.3 Rigor mortis, quality index, K value and ATP levels

All the fish progressed towards 100% rigor within 12 h post-harvest, with treatment group four reaching 100% rigor by 6 h post-harvest, followed by treatment groups one, two and three by 12 h post-harvest (Figure 21). At 6 h post-harvest rigor index was significantly lower in treatment group one compared to treatment groups three and four (P=0.001 and P=0.016, respectively) and significantly lower in treatment group two compared to treatment group four (P=0.004). The resolution of rigor commenced in all treatment groups after 24 h post-harvest (Figure 21). All treatment groups followed a similar pattern of resolution; however, treatment group four typically had a higher rigor index compared to all other groups at each time point examined (Figure 21). Rigor index was significantly lower in treatment group one compared to treatment group four at 72 h post-harvest (P=0.014); however, no significant differences were detected between the four treatment groups at 12, 24, 48 or 96 h post-harvest (P>0.05) (Figure 21).

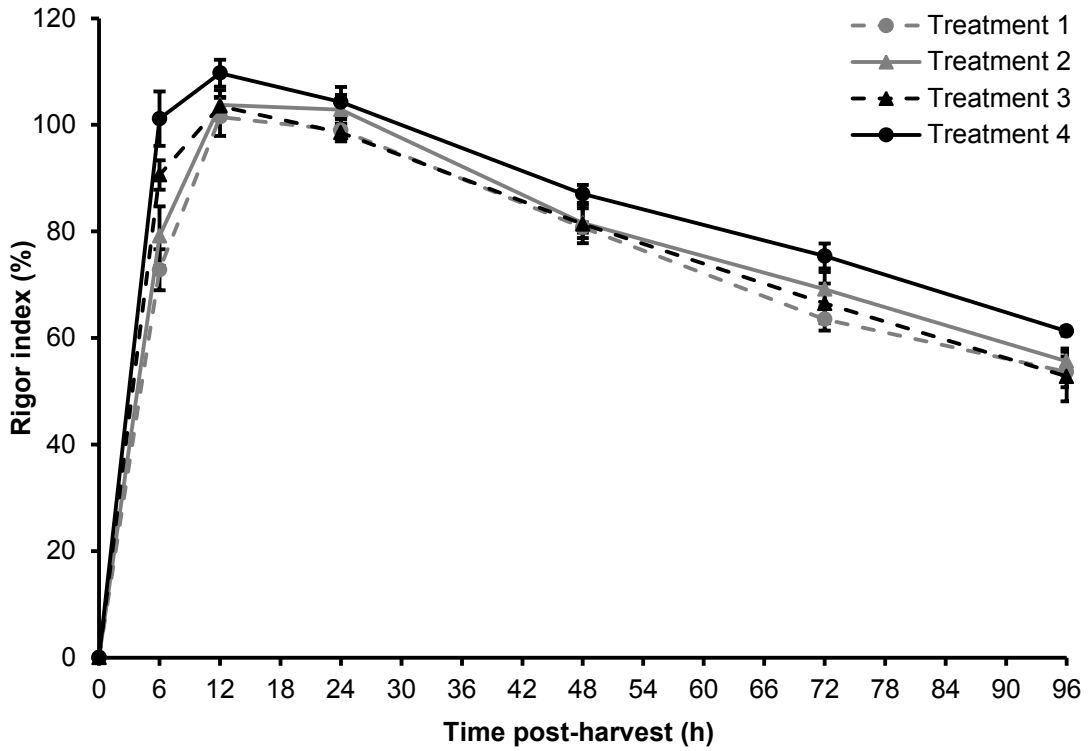

Figure 21. Mean (± SE) rigor index (%) of Yellowtail Kingfish (*Seriola lalandi*) up to 96 hours post harvest (n = 4). Fish were exposed to different conditions prior to harvest: 1) undisturbed and rested harvest); 2) repeated disturbance and rested harvest; 3) undisturbed and unrested harvest and; 4) repeated disturbance and unrested harvest. Post-harvest the fish were kept in an ice slurry until 24 h, and then moved to insulated boxes with two ice packs kept in a 4°C room.

There were no significant differences detected in quality index scores of whole fish between the treatment groups at any of the time points examined post-harvest

(Figure 22). In addition, the quality index scores for the fillet attributes gaping, colour, smell and firmness did not show any significant differences between the treatment groups at 96 h post-filleting (Figure 23A-D).

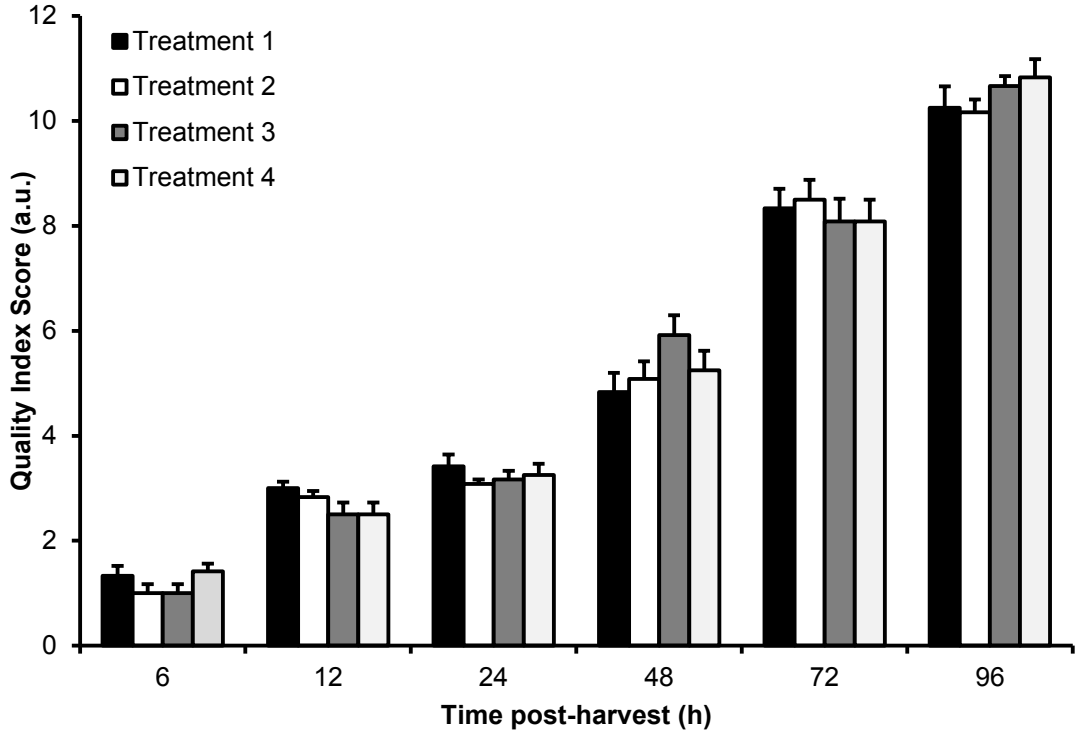

Figure 22. Mean (± SE) quality index score of whole, fresh Yellowtail Kingfish (*Seriola lalandi*) post-harvest (n = 3). Fish were exposed to different conditions prior to harvest: 1) undisturbed and rested harvest); 2) repeated disturbance and rested harvest; 3) undisturbed and unrested harvest and; 4) repeated disturbance and unrested harvest. Post-harvest the fish were kept in an ice slurry until 24 h, and then moved to insulated boxes with two ice packs kept in a 4°C room.

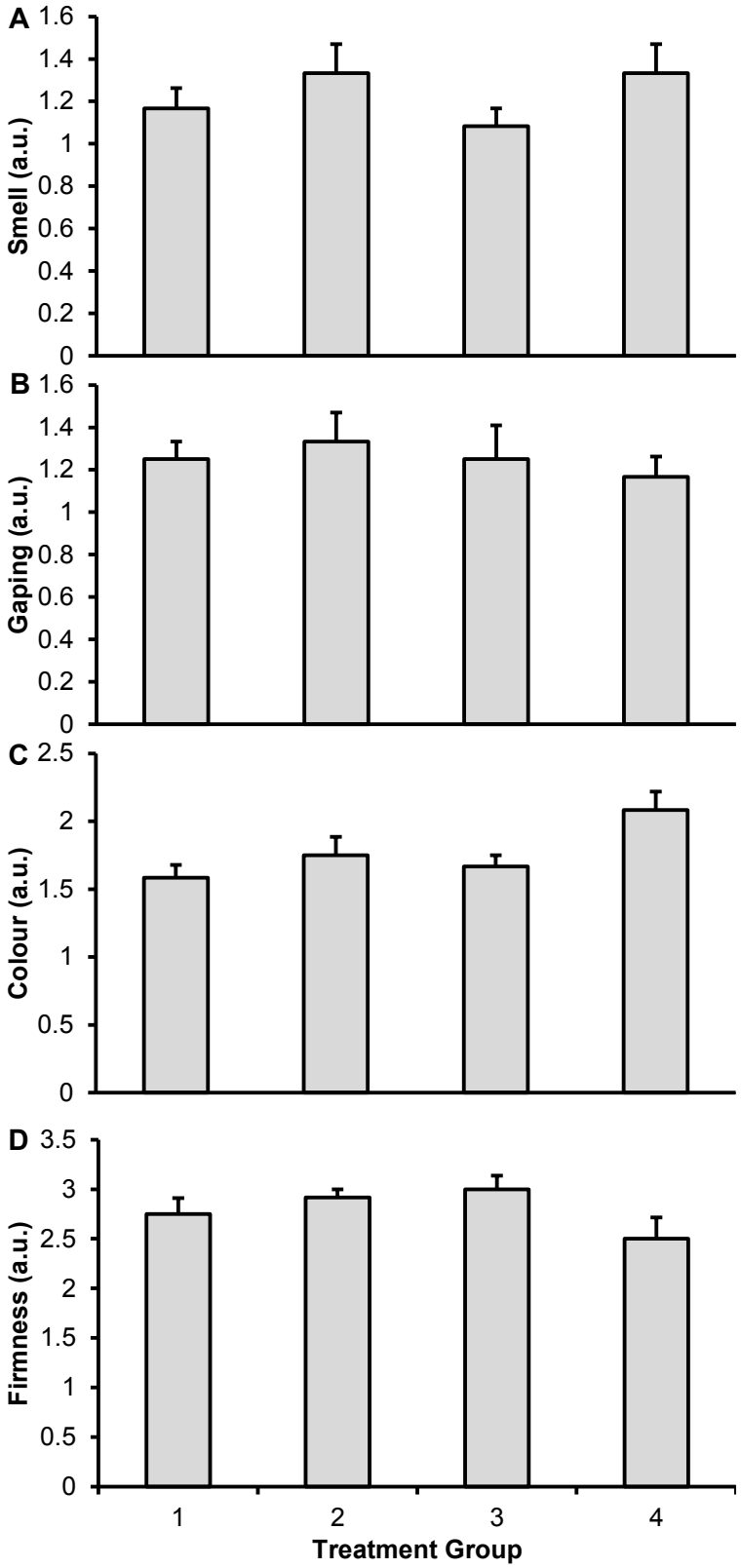

Figure 23. Mean (± SE) (A) smell, (B) gaping, (C) colour and (D) firmness score of fresh Yellowtail Kingfish (*Seriola lalandi*) fillets 96 hours post-filleting (n = 4). Fish were exposed to different conditions prior to harvest: 1) undisturbed and rested harvest); 2) repeated disturbance and rested harvest; 3) undisturbed and unrested harvest and; 4) repeated disturbance and unrested harvest. The scoring chart for each attribute is given in Table 4.

K value increased with increasing time post-harvest for each treatment group from approximately 4 to 20 % (Figure 24). K value varied between the groups over time with no consistent significant trend evident at each time point, however groups two and four tended to have the higher K values at most time-points after 12 h post-harvest, with some of these differences being statistically significant (Figure 24).

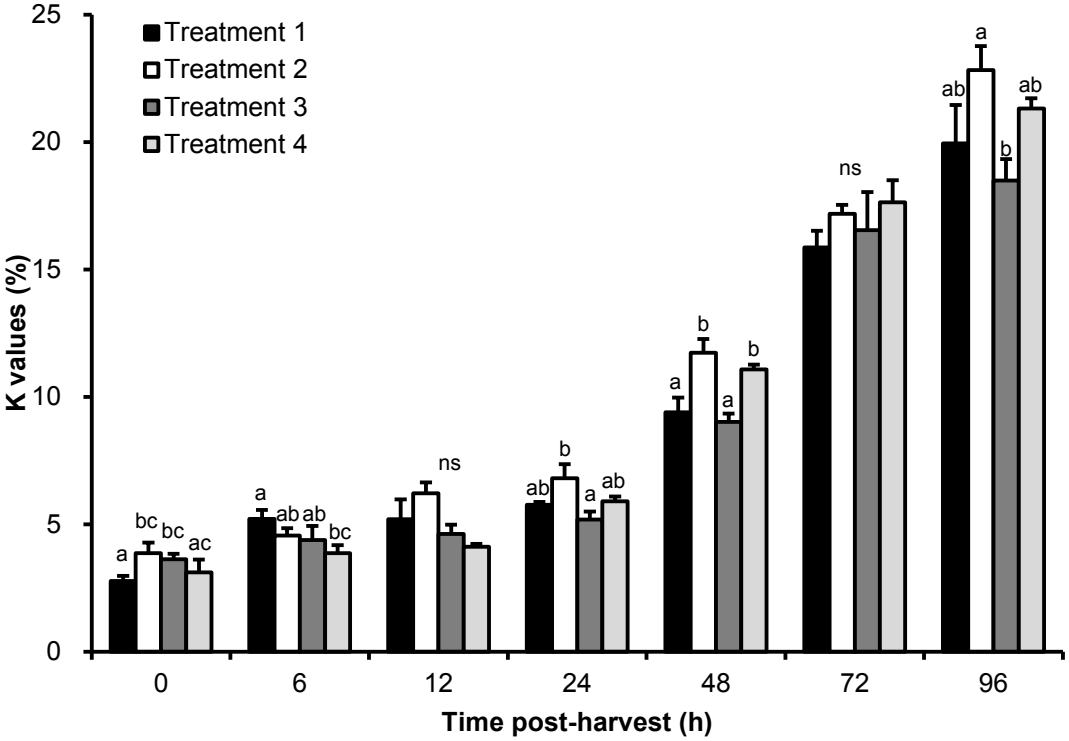

Figure 24. Mean (± SE) K value (%) of Yellowtail Kingfish (*Seriola lalandi*) flesh post-harvest (n = 3). Fish were exposed exposed to different conditions prior to harvest: 1) undisturbed and rested harvest); 2) repeated disturbance and rested harvest; 3) undisturbed and unrested harvest and; 4) repeated disturbance and unrested harvest. Post-harvest the fish were kept in an ice slurry until 24 h, and then moved to insulated boxes with two ice packs kept in a 4^{o}C room. Different superscripts indicate significant differences at that time point ($P < 0.05$) (ns: no significant differences within the time point analysed).

ATP level, evaluated as a percentage of total adenylates, was significantly higher in treatment groups one and two (about 24 %), compared to treatment groups three (14 %) and four (7.5 %) immediately post-harvest (i.e. 0 h post-harvest) ($P < 0.05$) (Figure 25). At 6 h post-harvest, ATP concentration decreased substantially in all treatment groups to approximately 2 % (Figure 25). ATP levels remained very low from this point in all treatment groups up to 96 h post-harvest, with no significant differences detected between the groups ($P>0.05$) (Figure 25).

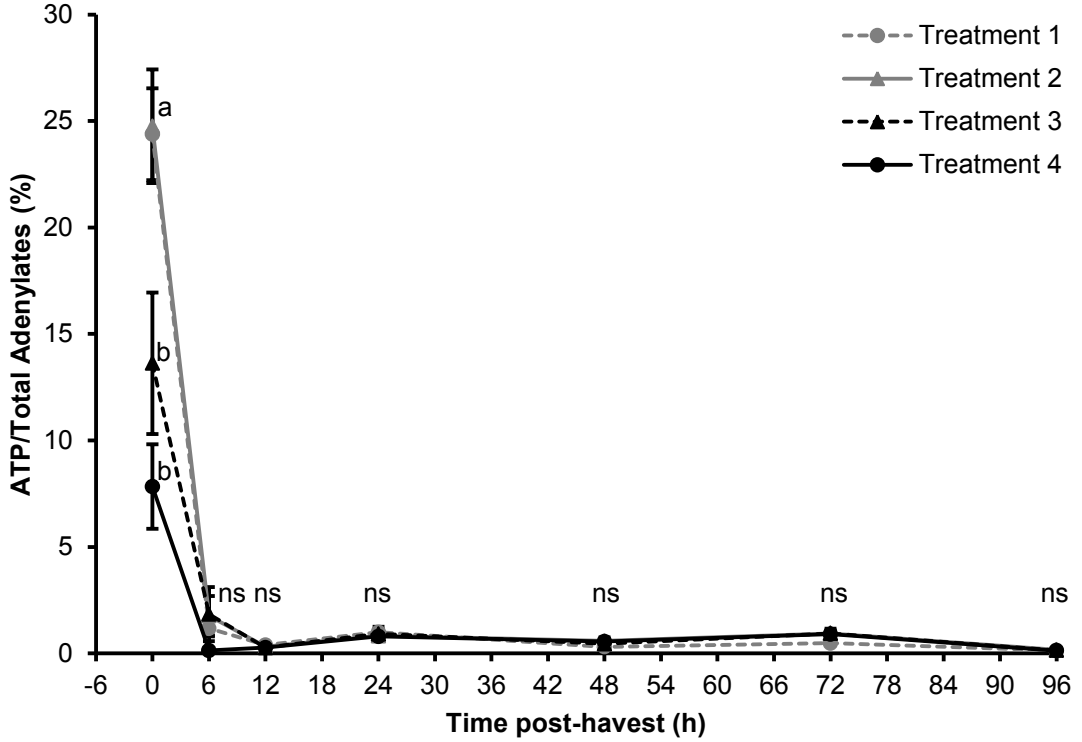

Figure 25. Mean (± SE) ATP content, as percentage of total adenylates, in the flesh of Yellowtail Kingfish (*Seriola lalandi*) post-harvest (n = 4). Fish were exposed to different conditions prior to harvest: 1) undisturbed and rested harvest); 2) repeated disturbance and rested harvest; 3) undisturbed and unrested harvest and; 4) repeated disturbance and unrested harvest (n = 3). Post-harvest the fish were kept in an ice slurry until 24 h, and then moved to insulated boxes with two ice packs kept in a 4^{o}C room. Different superscripts indicate significant differences at that time point (P < 0.05) (ns: no significant differences within the time point analysed).

3.4 Activity Four: Harvest stress and its impact on YTK quality

3.4.1 Blood biochemistry

Blood cortisol levels increased with increasing harvest crowding times, increasing from 51.03 ± 11.21 ng/ml to 150 ± 16.54 ng/ml in HH and CH120 fish, respectively (Figure 26A). Cortisol levels were significantly lower in HH, CH0 and CH60 fish compared to CH120 fish (P < 0.001, P < 0.01 and P < 0.001, respectively). In addition, levels were also significantly lower in HH fish compared to CH60 fish (P = 0.035) (Figure 26A). Similarly to blood cortisol levels, blood lactate levels increased with increasing harvest crowding times (Figure 26B). Lactate levels were significantly lower in HH fish compared to CH60 and CH120 fish (P = 0.009 and P < 0.001, respectively) and in CH0 fish compared to CH120 fish (P = 0.05) (Figure 26B). In contrast, there were no significant differences in blood glucose levels between the different treatment groups (Figure 26C).

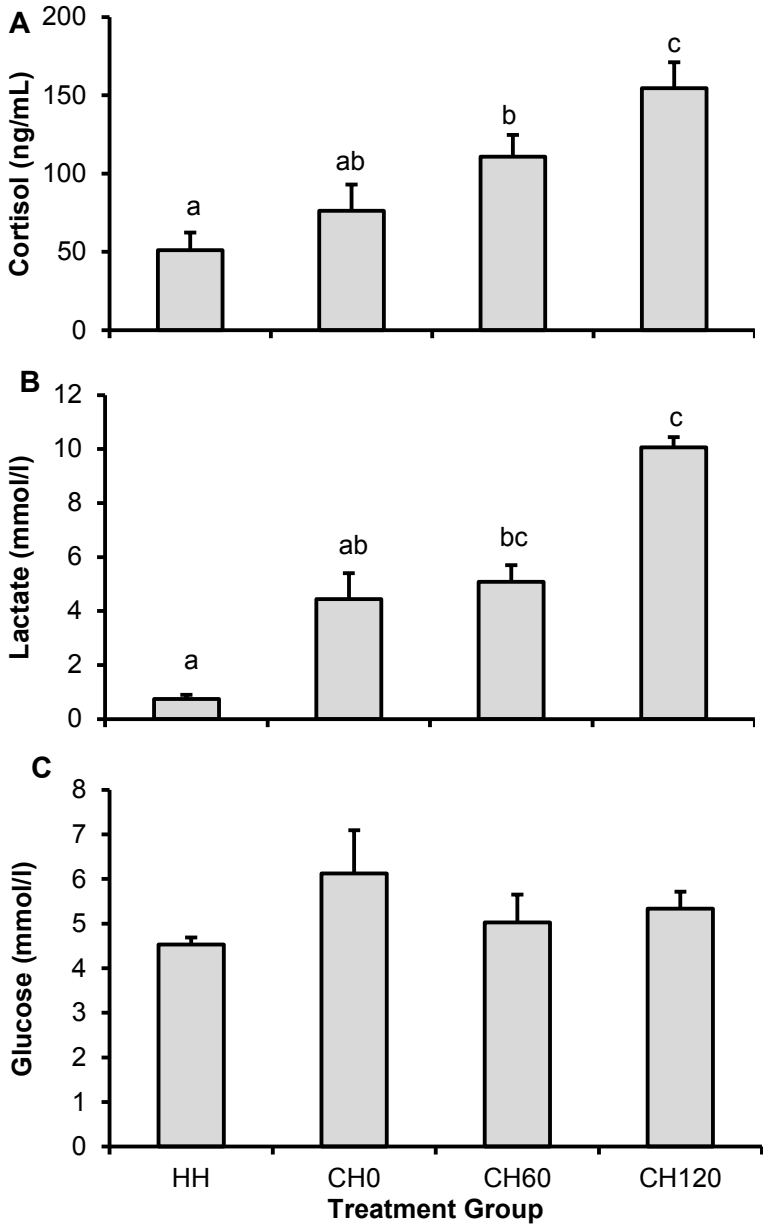

Figure 26. Mean (± SE) plasma (A) cortisol, (B) lactate and (C) glucose levels of Yellowtail Kingfish (*Seriola lalandi*) harvested under different conditions (HH: hook harvest fish and CH0, CH60 and CH120: commercial harvested fish sampled at 0, 60 and 120 min from the beginning of the harvest, respectively) (n = 10). Significant differences indicated by different superscripts ($P < 0.05$).

3.4.2 Flesh biochemistry

Glycogen levels in the flesh immediately post-harvest (i.e. 0 h post-harvest) were significantly higher in HH, CH0 and CH60 fish compared to CH120 fish ($P < 0.01$) (Figure 27A). Levels then sharply decreased at 12 h post-harvest in all treatment groups (Figure 27A). The levels remained fairly stable at all subsequent sampling points and no further significant differences were detected between treatment groups (Figure 27A). There was an inverse relationship between glycogen and muscle lactate, as lactate levels in the flesh immediately post-harvest (i.e. 0 h post-harvest) were significantly lower in HH, CH0 and CH60 fish compared to CH120 fish ($P = 0.01$) (Figure 27B). At 12 h and 24 h post-harvest lactate levels progressively increased for each treatment group and at 84 h post-harvest were similar to the levels observed at 24 h post-harvest (Figure 27B). No significant differences were detected between the treatment groups at any of these later sampling points (Figure 27B).

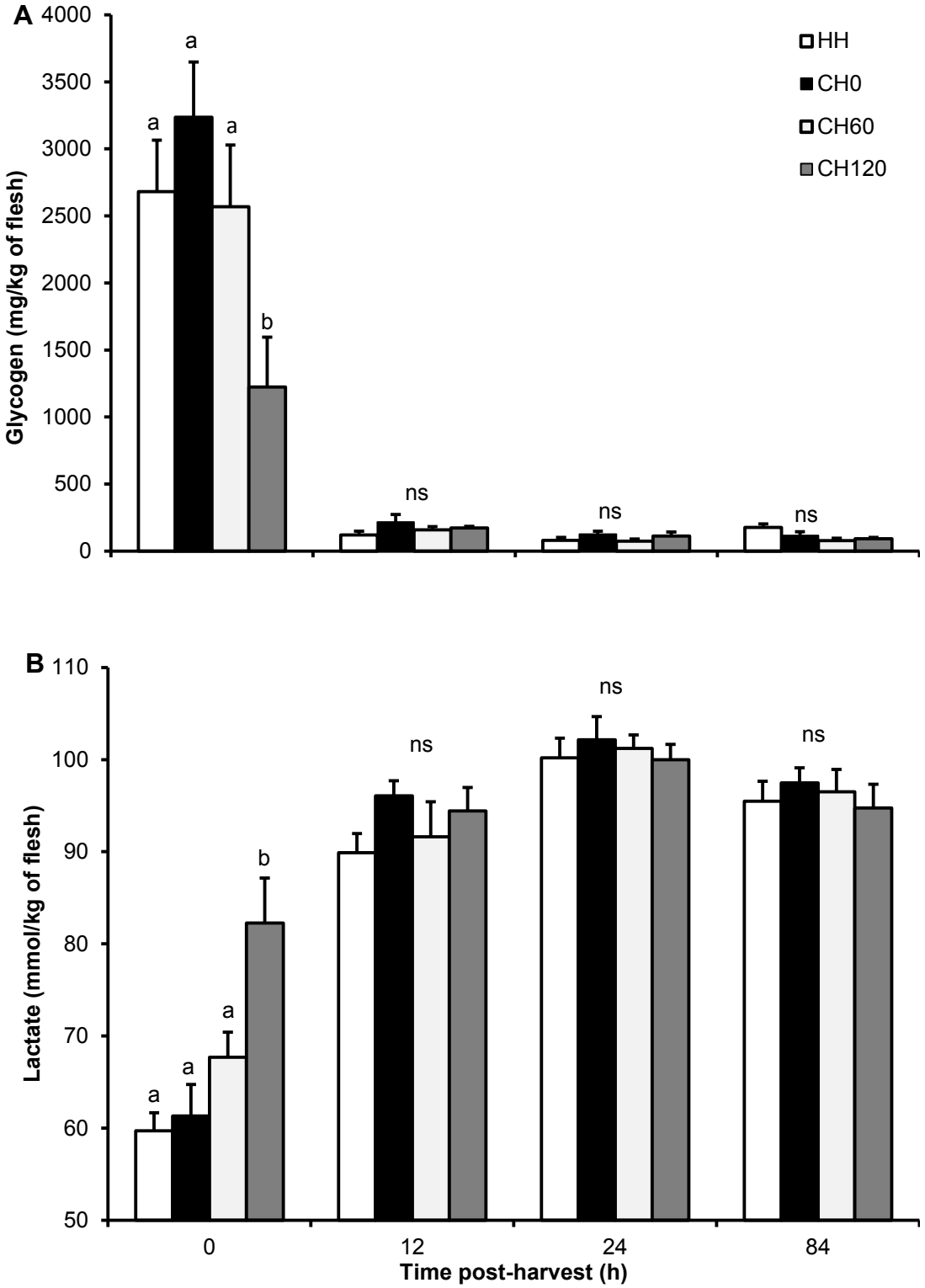

Figure 27. Mean (± SE) (A) glycogen (mg/kg of flesh) and (B) lactate (mmol/kg of flesh) levels in Yellowtail Kingfish (*Seriola lalandi*) harvested under different conditions (HH: hook harvest fish and CH0, CH60 and CH120: commercial harvested fish sampled at 0, 60 and 120 min from the beginning of the harvest, respectively) (n = 10). Significant differences indicated by different superscripts ($P < 0.05$).

3.4.3 Rigor mortis, quality index, K value and ATP levels

For each treatment group, the fish progressed towards 100% rigor within 12 h post-harvest (Figure 28). The resolution of rigor was then observed in each treatment group from 24 h post-harvest (Figure 28). No significant differences were observed between any the treatment groups at any of the time points examined post-harvest (Figure 28).

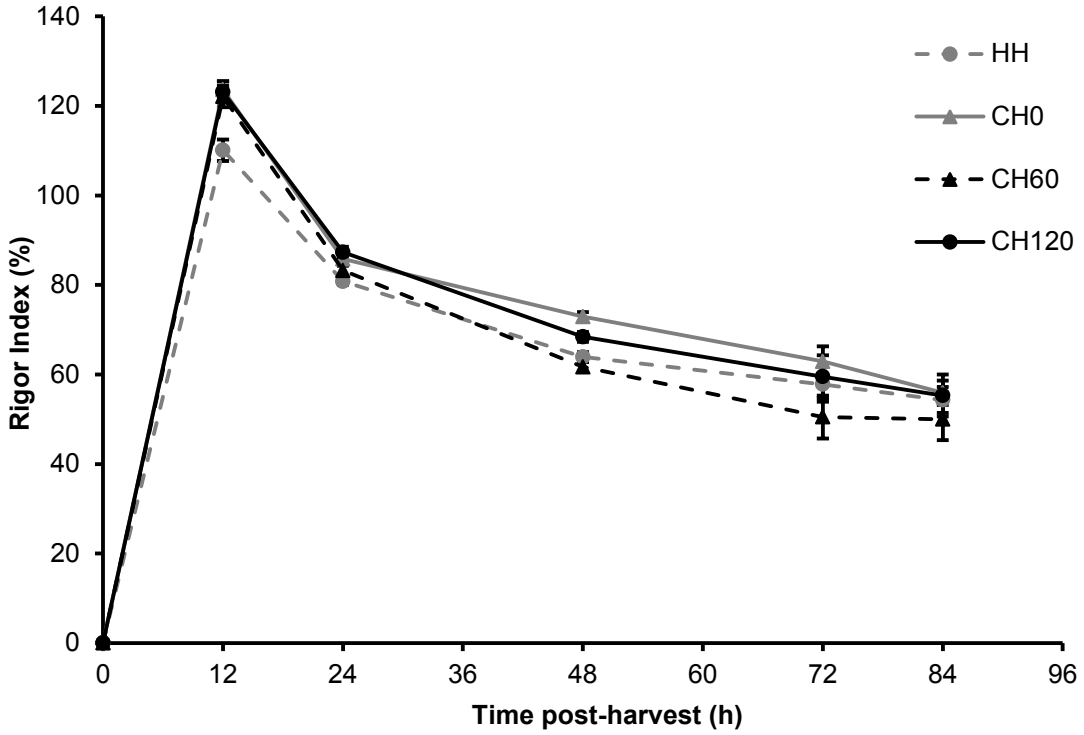

Figure 28. Mean (± SE) rigor index (%) in whole, fresh Yellowtail Kingfish (*Seriola lalandi*) harvested under different conditions (HH: hook harvest fish and CH0, CH60 and CH120: commercial harvested fish sampled at 0, 60 and 120 min from the beginning of the harvest, respectively) (n = 10).

Significant differences in the whole fish quality index score were observed at 12, 48 and 72 h post-harvest, but not at 24 and 84 h post-harvest (Figure 29). Specifically, at 12 h post-harvest the quality index score of HH and CH120 fish were significantly lower than CH0 and CH60 fish ($P < 0.05$, $P < 0.01$, respectively) (Figure 29). At 48 h post-harvest the quality index score of HH fish was significantly higher than CH0 fish ($P < 0.01$) and at 72 h post-harvest the quality index score of HH fish was significantly higher than CH0 and CH120 fish (both $P < 0.01$) (Figure 29). The quality index score of the fillet attributes gaping, smell and firmness did not show any significant differences between the treatment groups at any of the time points examined post-filleting (Figure 30A, B and D). However, significant differences were detected for the quality index score for colour at 24 and 96 hours post-filleting (Figure 30C). Colour score was significantly lower in CH60 fish compared to HH, CH0 and

CH120 fish at 24 h post-filleting ($P < 0.01$, $P < 0.01$ and $P < 0.05$, respectively) and HH fish compared to CH0 fish at 96 h post-filleting ($P < 0.05$) (Figure 30C).

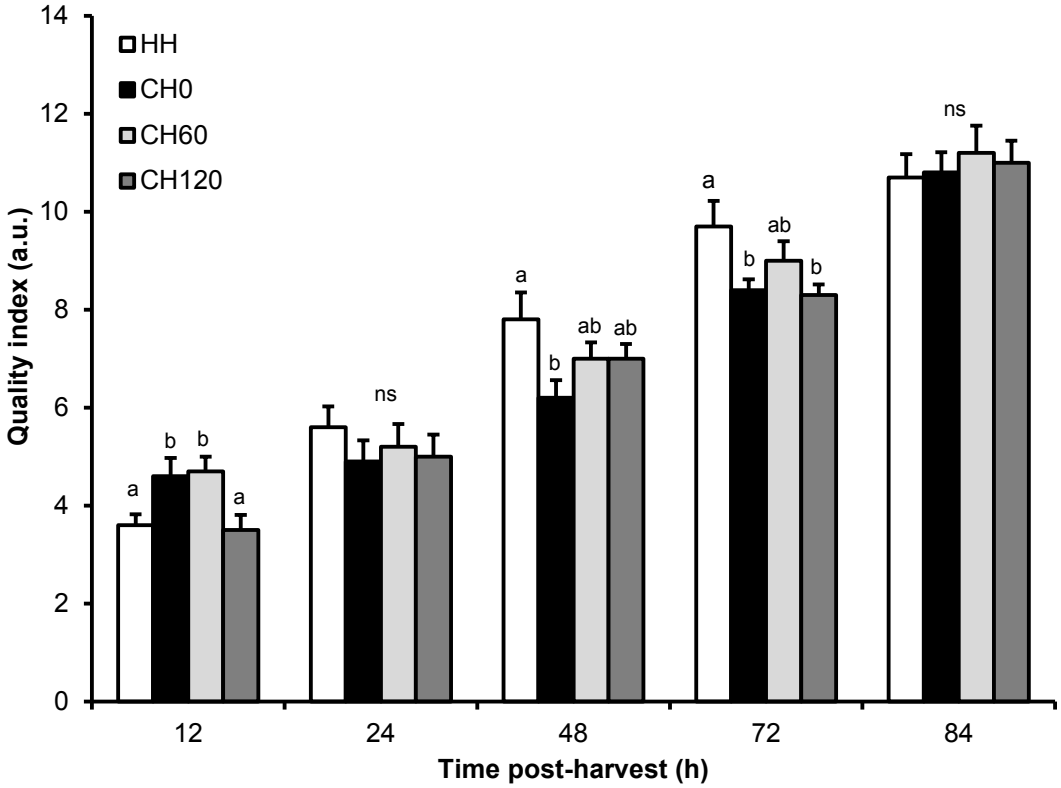

Figure 29. Mean (± SE) quality index score of whole, fresh Yellowtail Kingfish (*Seriola lalandi*) harvested under different conditions (HH: hook harvest fish and CH0, CH60 and CH120: commercial harvested fish sampled at 0, 60 and 120 min from the beginning of the harvest, respectively) (n = 4). Significant differences indicated by different superscripts ($P < 0.05$).

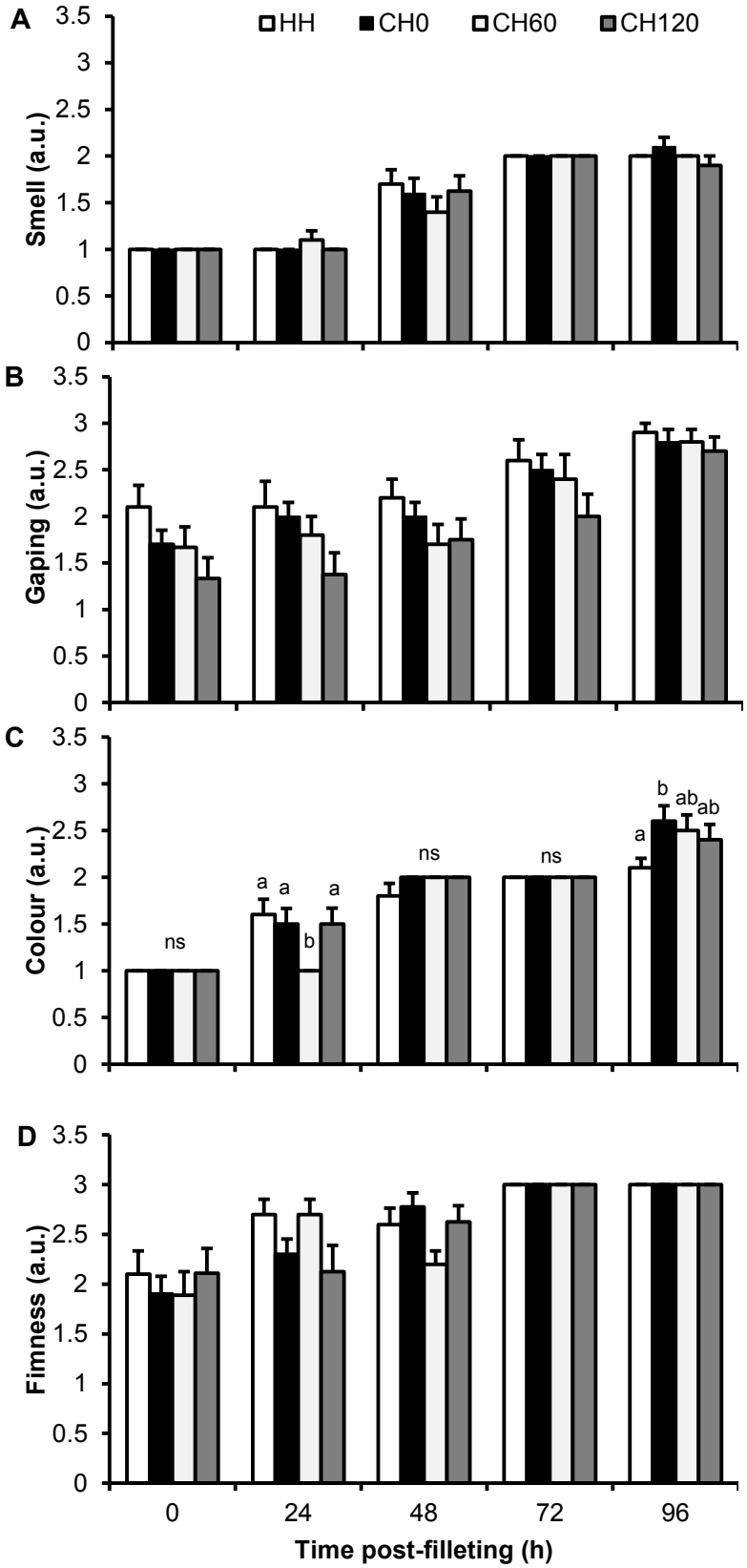

Figure 30. Mean (± SE) (A) smell, (B) gaping, (C) colour and (D) firmness score of Yellowtail Kingfish (*Seriola lalandi*) fillets at 0, 24, 48, 72 and 96 hours post-filleting (n = 4). Fish were harvested under different conditions (HH: hook harvest fish and CH0, CH60 and CH120: commercial harvested fish sampled at 0, 60 and 120 min from the beginning of the harvest, respectively). The subjective scoring scale is shown in Table 4. Different superscripts indicate significant differences at that time point ($P < 0.05$). ns: no significant differences within that time point.

K value increased with increasing time post-harvest for each treatment group from approximately 2 to 20 % (Figure 31). The K value for HH fish was always greater than the other three treatment groups, which were similar to each other, and the difference between them increased as time post-harvest increased. There was no significant interaction effect between time and treatment; however, there was an overall significant difference between treatment groups HH and CH0 ($P<0.05$).

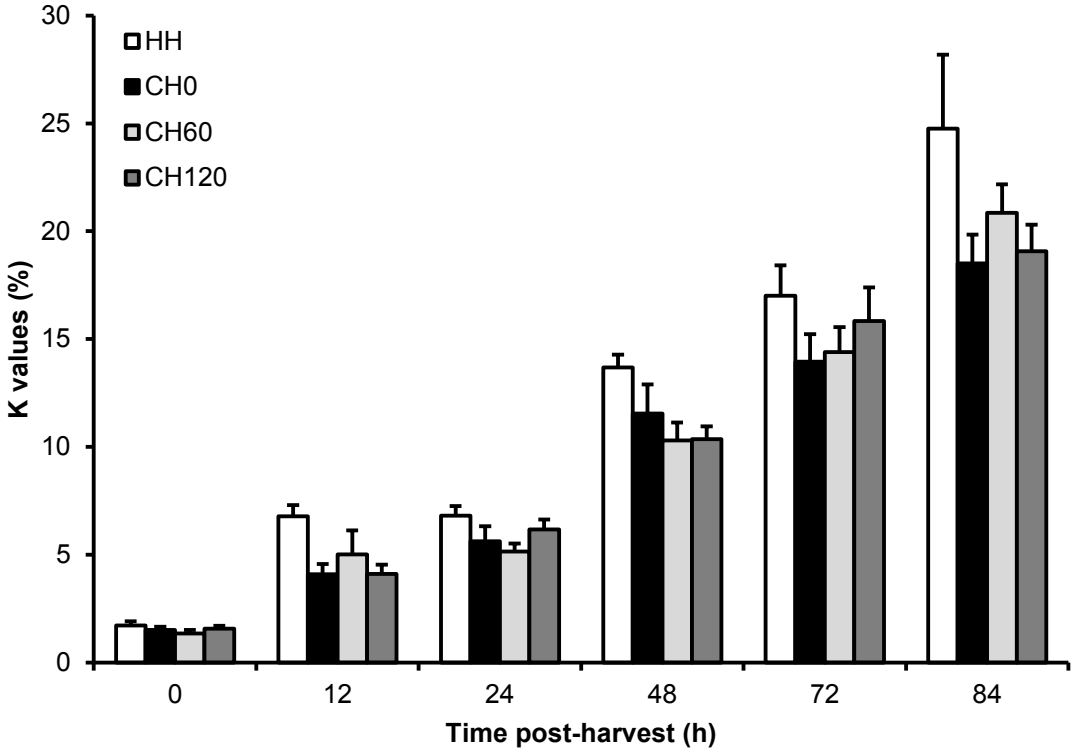

Figure 31. Mean (± SE) K value in the flesh of (*Seriola lalandi*) post-harvest from fish harvested under different conditions (HH: hook harvest fish and CH0, CH60 and CH120: commercial harvested fish sampled at 0, 60 and 120 min from the beginning of the harvest, respectively) (n = 10).

3.5 Activity Five: Investigate the development of eye cloudiness condition in YTK post-harvest.

3.5.1 Effect of storage temperature on the development of eye cloudiness

Eye cloudiness developed in each treatment group, regardless of the seawater temperature the fish were stored at (Table 5, Figure 32). Eye cloudiness reached a score of two in fish stored in an ice slurry at 4 h post-harvest, while for fish stored in seawater at 1 and 4°C a score of two was not achieved until 24 h post-harvest (Table 5).

Table 5. Mean (± SE) eye cloudiness score for Yellowtail Kingfish (*Seriola lalandi*) stored in an ice slurry and seawater at 1°C and 4°C at 0, 2, 4 , 6 and 24 hours post-harvest (n = 3).

Treatment	Hours post-harvest (h)				
	0	**2**	**4**	**6**	**24**
Ice slurry	0	1.3 ± 0.33	2	2	2
1°C seawater	0	0.7 ± 0.33	1.3 ± 0.33	1.3 ± 0.33	2
4°C seawater	0	1	1	1	2

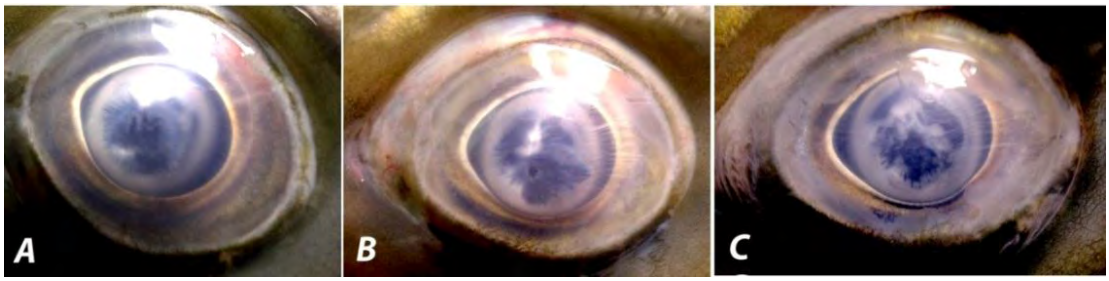

Figure 32. Photographs showing eye cloudiness development in Yellowtail Kingfish (*Seriola lalandi*) stored for 24 hours in seawater at (A) 1°C, (B) 4°C and (C) in an ice slurry.

3.5.2 Effect of taurine supplementation in the diet on the development of eye cloudiness in fish held in ice slurry

Eye cloudiness was observed at 10 and 24 h post-harvest in fish from each of the 5 diet treatment groups held in ice slurry (Figure 33). Fish fed commercial diet one with no taurine supplementation (D1) had the highest percentage of eye cloudiness at 10 and 24 h post-harvest (85 and 80%, respectively) (Figure 34). Fish fed commercial diet two with taurine supplementation (D5) had the lowest percentage of eye cloudiness at 10 and 24 h post-harvest (approximately 60 and 30%, respectively) (Figure 34). No significant differences in eye cloudiness were detected between fish fed the different diets at 10 h post-harvest ($P = 0.28$); however, at 24 h post-harvest eye cloudiness was significantly lower in fish fed commercial diet two with taurine supplementation (D5) compared to fish fed commercial diet one with no taurine supplementation (D1) ($P = 0.018$) (Figure 34).

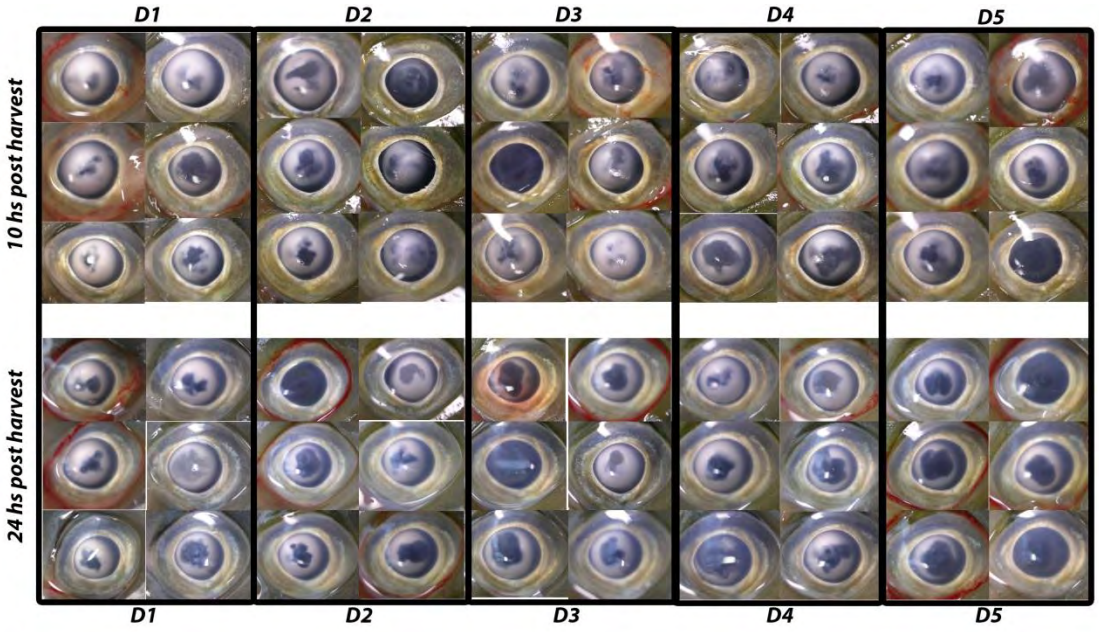

Figure 33. Eye cloudiness development in Yellowtail Kingfish (*Seriola lalandi*) fed five different diets at 10 and 24 hours post-harvest when held in ice slurry: D1 = commercial diet one with no taurine supplementation; D2 = commercial diet two with no taurine supplementation; D3 = commercial diet three with no taurine supplementation; D4 = commercial diet three with taurine supplementation; and D5 = commercial diet two with taurine supplementation.

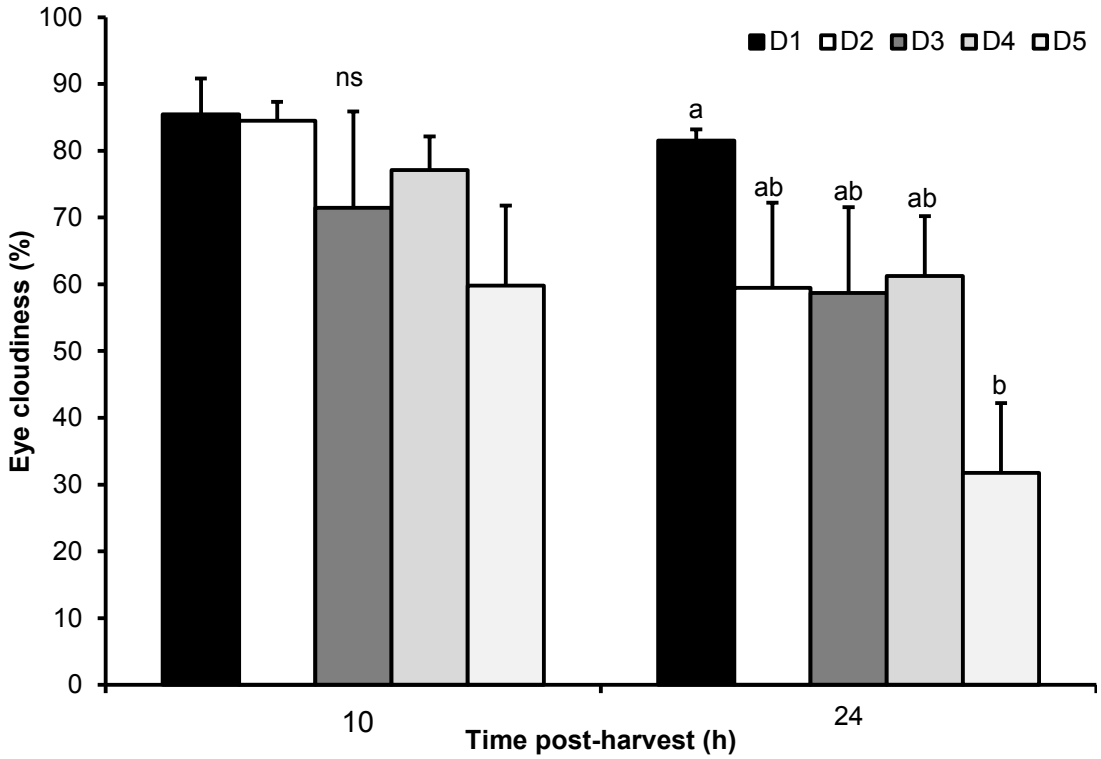

Figure 34. Mean (± SE) eye cloudiness (%) development in Yellowtail Kingfish (*Seriola lalandi)* fed five different diets at 10 and 24 hours post-harvest when held in an ice slurry: D1 = commercial diet one with no taurine supplementation; D2 = commercial diet two with no taurine supplementation; D3 = commercial diet three with no taurine supplementation; D4 = commercial diet three with taurine supplementation; and D5 = commercial diet two with taurine supplementation (n = 6). Different superscripts indicate significant differences within that time point ($P < 0.05$). ns: no significant differences within that time point.

2.5.3 Osmotic imbalance on the development of eye cloudiness

2.5.3.1 Pilot trial

The salinity of the water in the ice slurry changed substantially over the assessment period as the ice melted (Table 6). The greatest decrease in salinity was measured in the container with 100% seawater, where salinity fell from 31 to 20 ppt (a drop of 35%) and the lowest in the container with 25% seawater, ranging from 9 to 7 ppt (a drop of 22%)(Table 6). The greatest decrease in salinity was measured in the first 8 h of storage.

Table 6. Salinity (ppt) of the water in the ice slurry that the Yellowtail Kingfish (*Seriola lalandi*) eyes were stored in during the assessment period.

Seawater	Time post-harvest (h)				
	0	**4**	**8**	**12**	**24**
100%	31	23	24	21	20
75%	22	18	18	16	16
50%	15	13	13	11	10
25%	9	9	7	8	7

The development of eye cloudiness varied between the different treatment groups examined (Table 7). Specifically, eye cloudiness score was significantly higher in the eyes stored in 100 % seawater at 8, 12 and 24 h post-harvest compared to all other treatment groups, except at 8 h post-harvest when it was not significantly different to eyes stored in 75 % seawater (Table 7). Eye cloudiness was also significantly higher in eyes stored at 75 % seawater compared to eyes stored in 50 and 25 % seawater at 12 h post-harvest (Table 6). The salinity of the liquid content of the eyeball also significantly varied between the treatment groups, increasing with the increasing percentage of seawater in the ice slurry, as shown in Table 7.

Table 7. Mean (± SE) eye cloudiness score and salinity (ppt) of the liquid content of the eyeball (LCE) of Yellowtail Kingfish (*Seriola lalandi*) eyes stored in ice slurries containing 25, 50, 75 and 100 % seawater at 8, 12 and 24 hours post-harvest (n = 20).

Seawater	Eye cloudiness score			LCE salinity (ppt)
	Time post-harvest (h)			
	8	**12**	**24**	
100%	2.00 ± 0.00^{b}	2.80 ± 0.20^{c}	3.00 ± 0.00^{b}	15.2 ± 0.5^{c}
75%	1.00 ± 0.00^{ab}	1.60 ± 0.25^{b}	0.60 ± 0.25^{a}	13.8 ± 0.2^{b}
50%	0.00 ± 0.00^{a}	0.40 ± 0.25^{a}	0.20 ± 0.20^{a}	13.0 ± 0.0^{ab}
25%	0.00 ± 0.00^{a}	0.40 ± 0.25^{a}	0.20 ± 0.20^{a}	12.0 ± 0.0^{a}

Different superscripts indicate significant differences within a column ($P < 0.05$).

2.5.3.2. Full trial

Eye cloudiness had developed in treatment groups from 60 to 100 % seawater by 3 h post-harvest. Eyes stored in 50 % seawater did not develop eye cloudiness at all during the assessment period (see Figures 36, 37 and 38). Eye cloudiness score and relative area of eye cloudiness significantly differed between the treatment groups at the different time points assessed post-harvest, as indicated on Figures 38 and 39. The general trend was that, the higher the salinity of the slurry water, the faster the cloudiness developed and the greater the area of lens that was involved.

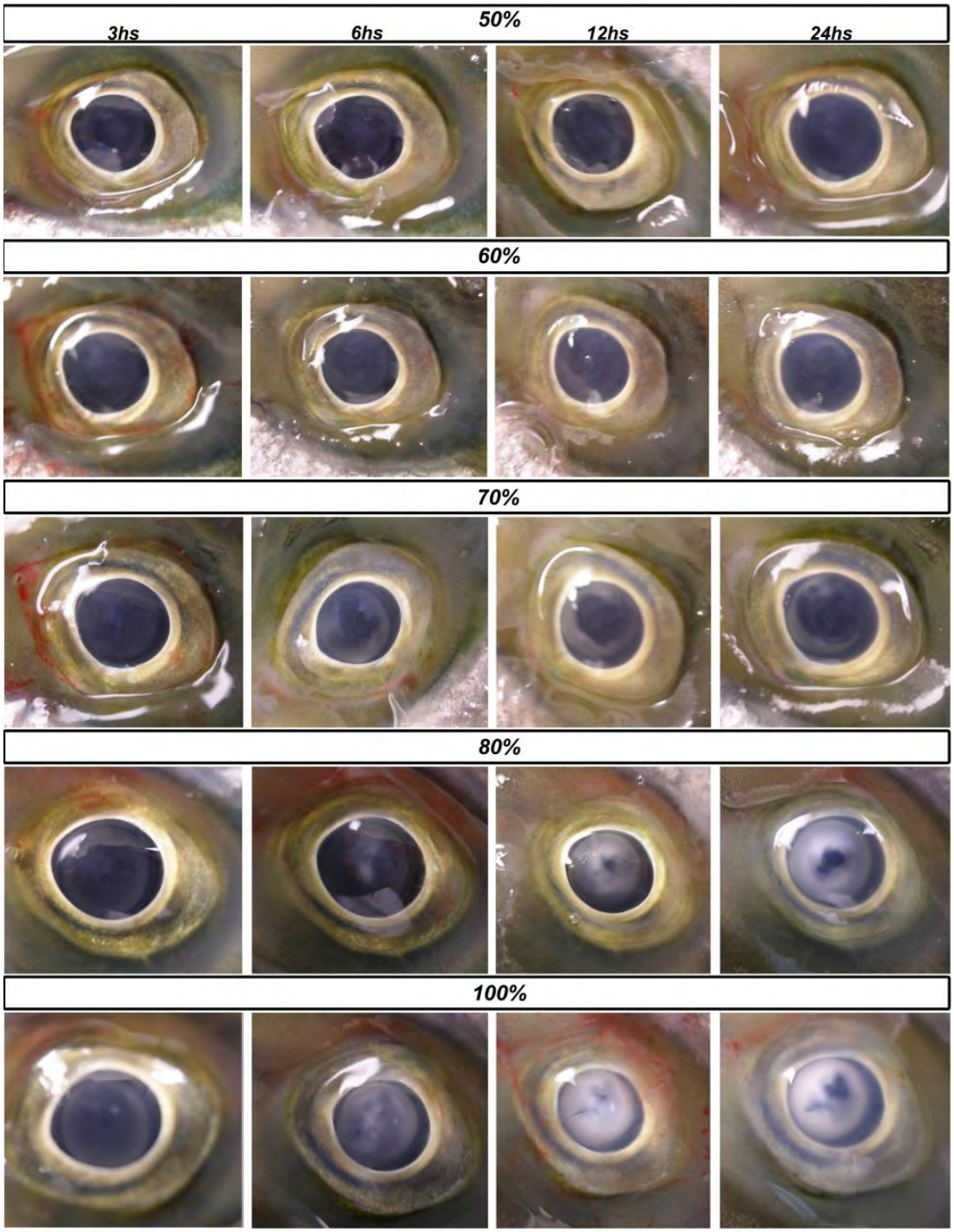

Figure 36. Eye cloudiness development in Yellowtail Kingfish (*Seriola lalandi*) eyes stored in ice slurry prepared with five different concentrations of seawater (50, 60, 70, 80 and 100 %) at 3, 6, 12 and 24 hours post-harvest. Photographs were taken of the same eye in each treatment group at each time point.

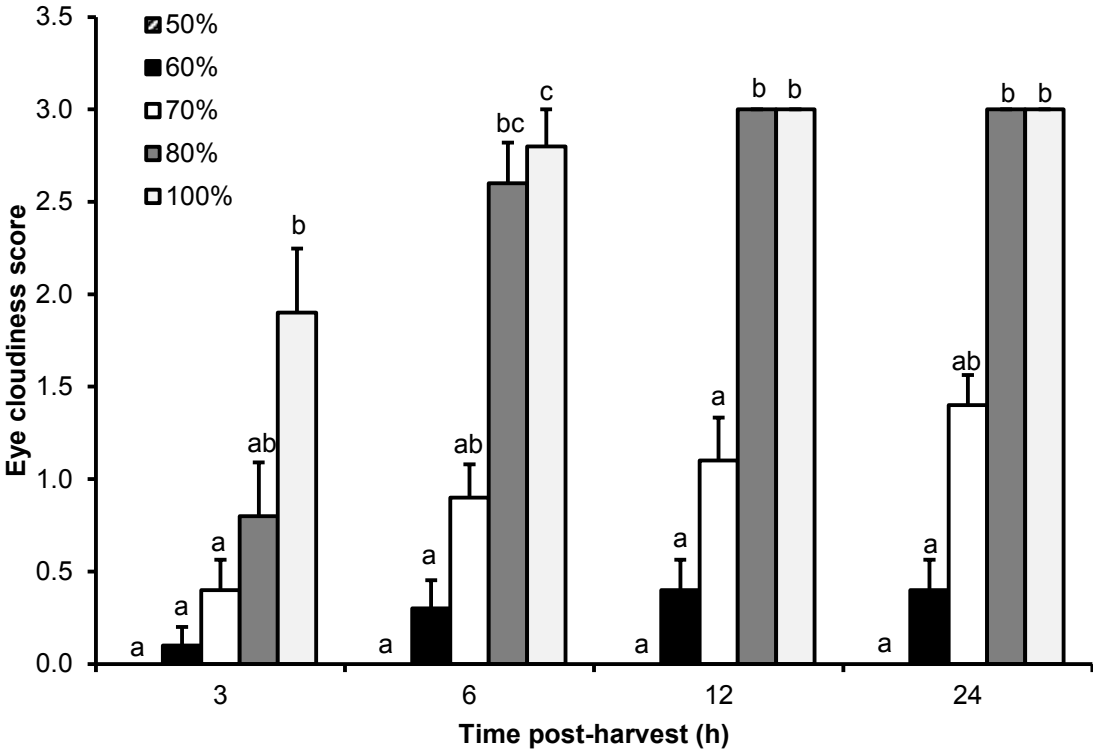

Figure 37. Mean (± SE) eye cloudiness score of Yellowtail Kingfish (*Seriola lalandi*) eyes stored in ice slurry prepared with five different concentrations of seawater (50, 60, 70, 80 and 100 %) at 3, 6, 12 and 24 hours post-harvest. Different superscripts indicate significant differences within each time point ($P < 0.05$) (n = 5).

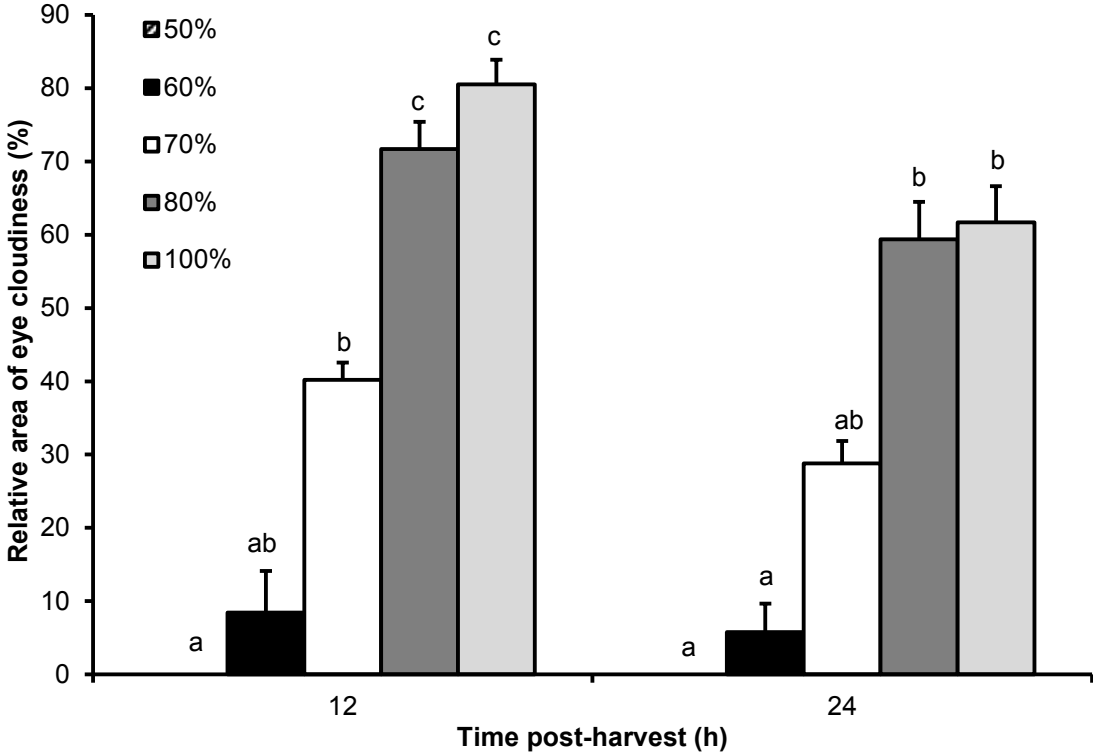

Figure 38. Mean (± SE) relative area (%) of eye cloudiness of Yellowtail Kingfish (*Seriola lalandi*) eyes stored in ice slurry prepared with five different concentrations of seawater (50, 60, 70, 80 and 100 %) at 3, 6, 12 and 24 hours post-harvest. Different superscripts indicate significant differences within each time point ($P < 0.05$) (n = 5).

At the completion of the 24 h assessment the salinity in the containers the eyes were stored in ranged between 14 ppt in the ice slurry prepared with 50 % seawater to 28 ppt in the ice slurry prepared with 100 % seawater (Table 8). Similarly, the salinity of the liquid content of the eyeball was lowest in eyes stored in ice slurry prepared with 50 % seawater and highest in the eyes stored in ice slurry prepared with 100 % seawater (Table 8). The temperature of the ice slurry also varied between treatment groups, with lower temperatures observed in ice slurry with increasing concentrations of seawater (Table 8).

Table 8. Mean (± SE) salinity (ppt) of five ice slurries and the liquid content of the eyeball (LCE) of Yellowtail Kingfish (*Seriola lalandi*) eyes at 24 hours post-harvest. The ice slurries were prepared with five different concentrations of seawater (50, 60, 70, 80 and 100%) (n = 3). The temperature range of each of the different ice slurries in the 24 hours is also shown.

Seawater	Salinity (ppt)		Temperature (°C)	
	Ice slurry	LCE	Min.	Max.
50%	14	13.3 ± 0.9[a]	-0.44	2.84
60%	16	13.7 ± 0.7[a]	-0.78	3.58
70%	18	14.0 ± 0.0[a]	-0.89	3.89
80%	21	15.0 ± 0.0[a]	NA	NA
100%	28	17.0 ± 0.6[b]	-1.46	2.09

NA: data not available as the temperature logger was damaged during the experiment
Different superscripts indicate significant differences (P < 0.05).

3.6 Activity Six: Physiological, biochemical and haematological characteristics of wild YTK

Haematological parameters and blood biochemistry of wild and farmed YTK analysed in this project are presented in Tables 9 and 10; and muscle and liver biochemistry of wild YTK in Tables 11, 12 and 13, respectively. A majority of the haematological and blood biochemistry parameters were significantly different between the wild and farmed fish, and a number of comments on these data were made by the Dr Martin Copeland, the Idexx chief pathologist, Glenside, Adelaide:

COMMENTS

Terminology: note that the term Granulocytes is not used as a collective term in these results but refers specifically to what others describe as neutrophils or heterophils.

Sample Quality: There is obvious artefact in these samples.

In the haematology smears all blood smears contained ruptured leucocytes referred to as basket cells. They are most evident in the Port Lincoln samples. Because of this differential counts from Sydney samples should be considered as reasonable but slightly imprecise estimates of leucocyte numbers whereas the extent of cell lysis in the Port Lincoln samples renders their differential counts imprecise and unreliable. In addition, it is worth noting that red cells can shrink and swell depending on the type of anticoagulant used and length of storage.

In the biochemistry results the marked variation in sodium/potassium values indicates cell membrane damage leading to a failure in the Na/K pump and aberrant sodium/potassium results. The variation between sites probably reflects differences in sample collection and handling procedure particularly the length of sample storage.

Clinical interpretation: Despite the presence of artefact there are significant variations between and within sites.

Haematology: Low PCV, MCV and MCH can occur with mineral deficiencies and chronic disease.

Biochemistry: Aside from impaired renal function, urea levels can increase during the post-prandial period, with variable protein intake and with fluctuations in protein metabolism. Creatinine varies with muscle mass. Calcium is transported bound to albumin and total calcium values will reflect albumin levels. Albumin often varies with hydration status in healthy animals. Globulin levels are typically low in young, immunologically naive animals. Cholesterol levels increase during the post-prandial period and like triglycerides can decrease in animals with reduced energy intake and absorption.

Multiple tissues and organs from the wild YTK were sent to Dr Michelle Dennis, Specialist Veterinary Pathologist, for histopathology. Dr Dennis' report was considered and summarised by CST's consulting veterinarian, Dr Matt Landos from Future Fisheries Veterinary Services. Extracts of his report are copied below:

The histopathology results highlighted a range of pathologies. In light of these changes in tissue, the haematology and biochemistry results may not be used as base-line, "healthy" reference data.

A range of pathological diagnoses were reported:

1. Mild to marked non-suppurative myocarditis +/- intralesional blood fluke: Inflammation of the heart muscle (18/23), 2 fish had evidence of adult blood fluke (1/18) and eggs (1/18).
2. Mild to marked non suppurative enteritis with coccidiosis: Inflammation of the intestines in varying degrees associated with coccidian parasites (18/23 fish affected). Coccidians have been observed in cultured kingfish, and associated with enteritis.
3. Mild splenic stromal fibrosis: There is a small change in the functional tissue of the spleen, with increases in fibrous tissue (11/23 fish). The cause of splenic fibrosis is not completely understood, but it is speculated to be associated with previous, generalised stress.
4. Mild non-suppurative hepatitis, aetiology unknown: Inflammation of the liver, with no infectious agents detected in the organ (11/23 fish).
5. Coelomic granulomas with intralesional pigmented fluke eggs (consistent with didymozidae): Areas of chronic inflammation (granulomas), associated with fluke eggs within the coelomic cavity (9/23 fish)
6. Mild granulomatous mural gastroenteritis with intralesional migrating nematode larvae: Chronic inflammatory responses within the gastrointestinal tract (stomach and intestines), caused by migrating worm larvae (8/23 fish).
7. Mild to moderate, mixed epicarditis, aetiology unknown: Inflammation of the outer layer of the heart (epicardium), with no infectious agents observed. There are a number of different inflammatory cells involved (8/23 fish). This is a lesion described in cultured fish. It was identified by Fran Stephens, WA, that a Kudoa-like parasite was associated with the inflammatory response. However, these parasites have not been reported in this instance.

8. Mild perivascular gastritis: Inflammation of the stomach, centred around blood vessels, with white blood cells infiltrating (5/23 fish).
9. Mild renal tubular proteinosis: There is protein accumulation within the tubes of the kidney (4/23). The significance of the renal proteinosis is not currently understood, however it has been seen occasionally in caged fish.
10. Mild to moderate intestinal distomiasis: Intestinal parasites present within the intestinal mucosa (4/23 fish)
11. Mild focal granulomatous nephritis, aetiology unknown: Chronic inflammatory spots in the kidney, with no infectious agents reported (2/23 fish)
12. Mild lamellar blood fluke eggs: Blood fluke eggs within the afferent artery of the gill filaments (2/23 fish)

[Dr Dennis] stated that "Overall, these fish had much higher levels of infectious disease than I have observed in the cultured kingfish accessions" (coccidiosis, didymozoidosis, distomiasis, and blood fluke).

~78% fish were infected with coccidian parasites (18/23 fish). This parasite has been observed in cultured fish, however its involvement with enteritis was questioned. Some mild inflammatory response was seen to the coccidian parasites in these wildfish. It is suspected that low infestations of coccidians in the gut may be related to immune function of the host. However, due to the difficulties in culture of this parasite, little research has been conducted. Unpublished DNA data reveals that coccidians are species specific. If further research is to be done, DNA analysis and typing is recommended (pers comm. K. Molnar 2013).

The relatively high levels of parasitism in these wildfish was unexpected. Possibilities may include location based water quality of Sydney Harbour, and potential contaminants in the local foodwebs. Sydney harbour has known levels of dioxins, and restrictions are in place for public health protection from consumption of seafood from this location. Dioxins are a group of chemically related compounds that are persistent within the environment, and are known to accumulate slowly in the food chain within the fatty tissues of animals. Dioxins are considered highly toxic, and can cause endocrine disruption and alterations in immune function.

As wildfish have an unexpected and relatively high level of disease, the confidence that we have true baseline haematology and biochemistry results is reduced. As these baselines are to be used as benchmarks for commercial production it is imperative that we can be confident in them.

To further study "normal" pathology and blood parameters in Yellowtail Kingfish, it is recommended that a scaled down repetition of this study be performed in a "pristine" body of water e.g. Montague Island, offshore from Bermagui (NSW). Small kingfish are routinely caught at this site.

CST currently uses blood parameters to assist in assessment of the health of farmed stock, evaluation of diets, and for disease investigation, accurate reference ranges are needed to be confident in our interpretation of the data collected. Thus far, we have limited confidence in results to evaluate the current diets due to a lack of confidence in our reference ranges. The individual differences reported by pathology, in location of lesion and lesion severity are reflected in the high standard deviations observed in haematology and biochemistry parameters.

Table 9. Haematological parameters of wild (n=22) and farmed (n=13) Yellowtail Kingfish (*Seriola lalandi*).

		Wild			Farmed		
Parameter	**Unit**	**Range**	**Mean**	**SD**	**Mean**	**SD**	**P**
RBC	x10^12/ml	1.45 - 4.47	3.08	0.80	3.71	0.28	***
HGB	g/l	77 - 174	118.05	24.40	101.23	15.70	*
MCHC	g/l	143 - 322	231.45	40.33	216.69	24.95	ns
PCV	l/l	0.35 - 0.60	0.50	0.06	0.47	0.04	*
MCV	Fl	121 - 314	176.82	49.16	126.00	9.73	***
MCH	pg	23 - 71	40.09	9.70	27.15	2.48	***
Platelets	x10^9/ml	14 - 64	31.77	13.72	28.15	21.29	ns
WBC	x10^9/ml	6.4 - 17.6	11.01	2.99	8.31	2.27	**
Granulocytes	%	25 - 63	45.00	10.23	20.69	9.79	***
Lymphocytes	%	30 - 67	44.18	11.27	73.54	8.17	***
Monocytes	%	0 - 9	4.14	1.91	5.62	5.08	ns
Eosinophils	%	0 - 22	6.45	5.85	0.15	0.38	***
Basophils	%	0 - 1	0.09	0.29	0.00	0.00	ns
Hct	%	33.33-54.10	43.30	5.46	nd	-	-
FT 50%	%	0.66 - 0.82	0.73	0.04	nd	-	-

RBC – red blood cells; HGB – haemoglobin; MCHC – mean corpuscular haemoglobin volume; PCV – packed cell volume; MCV – mean corpuscular volume; MCH – mean corpuscular haemoglobin; WBC – white blood cells; Hct – haematocrit; FT 50% - NaCl concentration for 50% hemolysis of red blood cells.
ns = not significant; * P<0.05; ** P<0.01; *** P<0.001.

Table 10. Blood biochemistry parameters of wild (n=22) and farmed (n=13) Yellowtail Kingfish (*Seriola lalandi*).

		Wild			Farmed		
Parameter	**Unit**	**Range**	**Mean**	**SD**	**Mean**	**SD**	**P**
Urea	mg/100ml		5.39	1.35	9.30	2.39	***
Cholesterol	mg/100ml		6.56	1.45	3.53	1.17	***
Bile Acids	umol/l	0.6 - 59.3	11.50	14.29	11.93	9.58	ns
Total Bilirubin	umol/l	0.2 - 6.4	0.94	1.31	0.48	0.29	ns
Sodium	mmol/l	186 - 264	219.32	26.80	180.69	11.42	***
Potassium	mmol/l	1.5 - 4.5	2.00	0.70	16.36	3.83	***
Creatinine	mmol/l	0.01 - 0.06	0.02	0.01	0.03	0.02	*
Calcium	mmol/l	3.2 - 5.46	4.04	0.71	2.99	0.45	***
Triglyceride	mmol/l	0.69 - 11.10	2.16	2.33	1.82	1.96	ns
Magnesium	mmol/l	1.95 - 6.77	3.60	1.46	1.38	0.43	***
Protein	g/l	33 - 53	43.59	5.64	31.85	6.91	***
Albumin	g/l	7 - 18	12.82	2.91	11.77	3.11	ns
Globulin	g/l	25 - 38	30.77	3.49	20.08	4.35	***
ALT	IU/l	2 - 83	20.00	17.27	51.08	26.42	***
ALP	IU/l	17 - 181	39.14	34.73	36.08	34.98	ns

ALT – alanine amino transferase; ALP – alkaline phosphatase
ns = not significant; * P<0.05; ** P<0.01; *** P<0.001.

Table 11. Muscle proximate, cholesterol and fatty acid levels of wild Yellowtail Kingfish (*Seriola lalandi*).

Parameter	Unit	Mean	SD	Range	n
Proximates					
Protein	% m/m	23.72	0.52	22.9-24.8	23
Fat	% m/m	1.12	0.70	0.60-3.00	23
Fatty Acids^					
Trans Fat content	% m/m	<0.1			23
Polyunsaturated Fat	% m/m	0.47	0.21	0.30-1.00	23
Monounsaturated Fat	% m/m	0.30	0.28	0.10-1.10	23
Saturated Fat	% m/m	0.35	0.22	0.20-0.90	23
Butyric C4:0	mg/100g	<10			23
Caproic C6:0	mg/100g	<10			23
Caprylic C8:0	mg/100g	17	35	10-180	23
Capric C10:0	mg/100g	18	38	10-190	23
Lauric C12:0	mg/100g	142	168	10-860	23
Trisdecanoic C13:0	mg/100g	<10			23
Myristic C14:0	mg/100g	1499	563	670-2540	23
Pentadecanoic C15:0	mg/100g	405	76	300-550	23
Palmitic C16:0	mg/100g	18921	710	17860-20380	23
Margaric C17:0	mg/100g	597	78	420-760	23
Stearic C18:0	mg/100g	9525	898	7720-10700	23
Arachidic C20:0	mg/100g	186	46	120-330	23
Docosanoic C22:0	mg/100g	81	23	10-130	23
Tetracosanoic C24:0	mg/100g	132	25	86-170	23
Total SFA	mg/100g	31523			
Decenoic C10:1	mg/100g	<10			23
Myristoleic C14:1	mg/100g	45	44	10-120	23
Pentadecenoic C15:1	mg/100g	18	16	10.0-61	23
Palmitoleic C16:1	mg/100g	1839	939	860-3700	23
Heptadecenoic C17:1	mg/100g	260	78	190-440	23
Octadecenoic C18:1n-6	mg/100g	60	26	10.0-93	23
Octadecenoic C18:1n-7	mg/100g	2683	241	2300-3250	23
Oleic C18:1n-9	mg/100g	12678	4865	7770-23510	23
Eicosenoic C20:1n-9	mg/100g	1260	687	440-2790	23
Eicosenoic C20:1n-11,13	mg/100g	145	82	10-340	23
Docosenoic C22:1n-9	mg/100g	183	69	100-330	23
Docosenoic C22:1n-11,13	mg/100g	<10			23
Tetracosenoic C24:1	mg/100g	891	157	540-1100	23
Total MUFA	mg/100g	18803			
Linoleic C18:2n-6	mg/100g	1301	196	1020-1850	23
Gamma Linolenic C18:3n-6	mg/100g	60	50	10-140	23
Steridonic C18:4n-3	mg/100g	257	139	80-520	23
Eicosadienoic C20:2n-6	mg/100g	210	36	150-280	23
Dihomo-gamma-linoleic C20:3n-6	mg/100g	115	17	88-150	23
Arachidonic C20:4n-6	mg/100g	1960	397	1080-2620	23
Docosatetraenoic C22:4n-6	mg/100g	1030	228	630-1510	23
Docosapentaenoic C22:5n-6	mg/100g	198	42	130-290	23
Total n-6 PUFA	mg/100g	3498			
Alpha Linolenic C18:3n-3	mg/100g	290	92	160-540	23
Eicosatrienoic C20:3n-3	mg/100g	99	22	67-170	23
Eicosatetraenoic C20:4n-3	mg/100g	328	186	84-790	23
Eicosapentanaeoic C20:5n-3 (EPA)	mg/100g	3539	763	2490-5700	23
Heneicosapentaenoic acid C21:5n-3	mg/100g	36	36	10-130	23
Docosapentaenoic C22:5n-3	mg/100g	2013	257	1630-2790	23
Docosahexaenoic C22:6n-3 (DHA)	mg/100g	32890	6338	20120-40570	23
Total n-3 PUFA	mg/100g	39194			
EPA+DHA	mg/100g	36429			
Total LC PUFA	mg/100g	38442			
n-3:n-6		11			

^ Fatty acid values are based on 100% fat content

Table 12. Muscle amino acid levels of wild Yellowtail Kingfish (*Seriola lalandi*).

Parameter	Unit	Mean	SD	Range	n
Aspartic acid	mg/100g	2236	265	1839-2873	23
Threonine	mg/100g	1252	123	1064-1468	23
Serine	mg/100g	908	82	770-1037	23
Glutamic acid	mg/100g	4012	350	3395-4650	23
Proline	mg/100g	489	107	294-678	23
Glycine	mg/100g	879	70	769-1007	23
Alanine	mg/100g	1453	78	1330-1574	23
Valine	mg/100g	1069	66	963-1171	23
Isoleucine	mg/100g	1174	72	1026-1308	23
Leucine	mg/100g	1918	87	1769-2083	23
Phenylalanine	mg/100g	791	57	633-882	23
Histidine	mg/100g	1446	119	1267-1651	23
Lysine	mg/100g	1755	97	1566-1941	23
Arginine	mg/100g	1422	72	1285-1527	23
Methionine	mg/100g	883	61	766-993	23
Tyrosine	mg/100g	821	50	748-924	23
Cystine	mg/100g	354	54	278-452	11
Tryptophan	mg/100g	259	21	207-283	11
Taurine	mg/100g	141	19	108-174	23

Table 13. Liver proximate, cholesterol and taurine levels of wild Yellowtail Kingfish (*Seriola lalandi*).

Parameter	Unit	Mean	SD	Range	n
Proximates					
Protein	% m/m	19	2	18-20	2*
Fat	% m/m	5	1	4-6	2*
Cholesterol	mg/100g	556	332	304-1647	23
Amino Acid					
Taurine	mg/100g	603	90	467-862	23

* Liver pooled from 12 fish for each sample

4. Discussion

This project sought to investigate several issues relating to commercial harvest practices for Yellowtail Kingfish, with the aim of modifying any aspect to improve product quality and/or harvesting efficiency. The issues examined included: improving the success rate of stunning and bleeding fish through the automatic harvesting machine; demonstrating the most effective approach for bleeding fish to avoid blood in the fillet; measuring, for the first time, the effect of acute and chronic stress on product quality parameters for up to 96 h post-harvest; identifying the factors related to the development of eye cloudiness in harvested YTK that are put into ice slurry; and developing a reference database of important biochemical, physiological and haematological parameters from wild YTK to compare to aquacultured fish in order to define what is 'normal' for this species.

4.1 Activity One: Develop systems that allow automated harvesting technology to be used on YTK so that the stun/bleed success rate increases from 65 to 95%

Effective harvesting, stunning and bleeding of fish are essential for producers of farmed fish. Poor harvesting, stunning and bleeding practices negatively affect perceptions of animal welfare, produce measureable physiological stress responses at harvest, change post-harvest characteristics of the carcass, and physical, biochemical and sensory attributes of the fillet during its shelf life (see reviews by Ashley, 2007; Borderias & Sanchez-Alonso, 2011; Lines & Spence, 2012; Ottera et al., 2001; Robb, 2001; Wall, 2001). It is widely accepted that the optimal conditions to protect welfare and reduce impacts on flesh quality occur when there the fish are not stressed or exhausted pre-slaughter, when the slaughter method is effective and rapid, and the fish is bled and chilled quickly post-harvest. Most reasonably large commercial fish farms have to use mechanised systems to harvest stock, so these systems need to function well with the species and size of fish being harvested in order to achieve these welfare and product quality goals. However, each farm will need to adapt and fine-tune its harvest and slaughter systems in order to maintain and improve upon product quality in a very competitive seafood marketplace.

Since 2009, CST has been using the dedicated harvesting vessel *Ulysses* that is equipped with a powerful fish pump that quickly delivers the fish to a 3 channel automatic percussive stunning and bleeding system. Past benchmarking projects (Carragher et al., 2009) and ongoing operator observations, have reported that

effective rates of stunning and/or bleeding of 3 - 4 kg YTK have been (~65%), less than what is expected when this and similar equipment is used on other species (> 95%). The effectiveness of this same brand of equipment when harvesting 5 - 6 kg Atlantic salmon is very high (Goodrick, pers. comm.; Smart, pers. comm.), so it is unlikely that the stunning and bleeding system *per se* is inadequate, but that the equipment needs to be fine-tuned in order to specifically suit YTK.

When appropriately delivered, percussive stunning has demonstrated to be highly efficient in a variety of species (e.g. Roth et al., 2007). To determine the appropriate site to deliver an accurate stun to YTK it was first necessary to investigate the position of the brain within the head, relative to external dimensions and structures, in particular the distance of the brain from the tip of the fish's snout. These data are essential to accurately position the trigger so that when the snout hits the activator trigger, the top of the head immediately covering the brain is against the opening of the tube that contains the percussive piston, in order to get an effective stun. The results obtained in this study showed that, for farmed YTK with a fork length between 57 and 68 cm, the mid-point of the brain was significantly correlated to fish fork length ($P < 0.01$) and was 81 to 105 mm from the tip of the snout. In contrast, there was no significant correlation between fish length and the depth of tissue from the top of the head to the mid-point of the brain, which was more consistent at 23 to 28 mm.

This variation for the brain distance from the snout could be explained by the variability observed in the head shape of farmed fish. In all the harvests of the farmed YTK some of the fish sampled had a tapered head shape that was also found in wild fish (Canepa & D'Antignana, personal observations), whilst other fish had a 'blunt' head shape.

The percussive piston in the stunner unit strikes a durable plastic hinged flap (30 mm long and 20 mm wide) which transfers the percussive force to the surface of the fish's head. This flap provides a stunning blow over the mid-point of the brain of the wide range of fork lengths for fish in this harvest class. The finding that the depth of the brain within the head was generally similar in fish of this size range is good, as it indicates that the same force of percussive blow can affect the brain of all fish more or less equally (i.e. striking force does not have to be adjusted). The use of excessive percussive force can lead to eye haemorrhage, burst or displacement which detracts from fish appearance in the marketplace (Roth et al., 2007).

In a similar way to determining the optimal trigger setting for the stunner, following trials with the various different settings of bleeder unit and observation of the position of the wound left from the bleed piston, a combination of settings was found where most fish were struck in the "F" zone (Figure 8), which overlies the heart, or in larger fish is where the aorta leaves the heart carrying blood to the gills and body. This position is ideal for a rapid and effective bleeding of the carcass, as long as the blood does not clot too quickly as has been reported for other species (e.g. Roth et al., 2009). The next most common site for the bleed piston to strike the fish was area "G" which is to the immediate right of the "F" zone. This suggests that some fish were entering the bleeder unit having being rotated less than 180^{o} through the inverter chute. As shown in Figure 15, this slight under-rotation could be corrected by changing either the depth profile of the ramp so that the dorsal surface of the fish was located centrally in the bleeding unit, and/or positioning the vertical guide plates closer together, putting the ventral surface perpendicular to the bleed piston.

Observations made following automatic stunning and bleeding in the correct areas suggested that eye, operculum or body movement were not reliable indicators of effective stunning in YTK. Body movement was ambiguous in that properly stunned fish and ineffectively stunned fish both exhibited flapping activity. Similarly, it was not possible to distinguish between effective and ineffective stunning based on observations of eye or operculum movement in YTK. This is in contrast, in salmon and other species, where these characteristics are useful assessment tools (Lines & Spence, 2012)

Modification to the profile of the ramp in the stunner and bleeder units was shown to give major improvements in the effectiveness of each of these automated processes. The original flat ramp supplied by the company obviously works well with salmon and trout in other situations, but the smaller size (3 – 4 kg YTK compared to 5 – 6 kg salmonids) and differences in the cross-sectional profile of YTK and salmonids (YTK probably being more tapered and laterally compressed, than the rounder and blunter salmonids) means that it is not a 'one ramp fits all' situation. The full V ramp modification used in this study clearly made a huge difference to the accuracy and effectiveness of the stun and bleed units, with the successful stun percentage increasing from 33 to 81%, and the proportion of unsuccessful stuns going from 26% down to 2%. This modification must be seen as a significant improvement in the welfare of the fish through the slaughter process, and a step which is likely to reduce some of the variability in YTK product quality too.

When the YTK automated harvesting system was working effectively at about 30 fish/min, it was noted that the 3 channels were not handling similar numbers of fish. Channel "3", which was the right hand channel, was taking at least 50% of the fish (or 15-17 fish/min), whereas channel "1" (on the left hand side) was only taking 13% (4 fish/min). Whilst this does not seem to be a major issue, it does have consequences: it suggests that there is the opportunity to increase the harvesting efficiency to 45-50 fish/min by having all 3 channels working at 15-17 fish/min; it will result in uneven wear and tear of the mechanical and pneumatic components in the different channels; blockages or missed stuns/bleeds due to two fish entering a channel at the same time are more likely when one channel is favoured over the others. Initial attempts to equalise the use of each channel by putting temporary dividers into the whirlpool to try to funnel fish away from channel 3 were not successful. At this stage, the causes of the uneven distribution of fish into the channels are unclear and further observations and potential modifications in the whirlpool should be tested.

As a result of the improvements made by this project, the automatic harvesting of YTK has attained the target of 30 fish/min with a success rate for effective stunning and bleeding of >95%.

4.2 Activity Two: Optimising on-board bleeding practises without sacrificing flesh whiteness

Blood retention in fillets can be a major reason for downgrading of product originally destined for high value markets (including sashimi and smoking), but is also undesirable in product destined for other end uses too. Blood retention in the fillet can be caused by pre-harvest or at-harvest factors (e.g. bruising caused by physical trauma of coming on board a boat; Borderias & Sanchez-Alonso, 2011), but is more commonly caused by incomplete exsanguination of the fish post-harvest (e.g. Roth et al., 2009). Some wild caught and most aquacultured finfish are bled shortly after capture to improve flesh quality and shelf life, as the presence of blood clots in the flesh is unattractive, can lead to wastage of product because of extra trimming, but they also acts as focal points for biochemical reactions involving the iron that is in the haemoglobin of the blood or myoglobin of the red muscle. These biochemical reactions include: promoting formation of free radicals that can lead to lipid oxidation and proteolysis of the muscle (Ando et al., 1991).

Most studies suggest that the earlier after harvest that bleeding is carried out, the better it is for product quality; similarly, most studies demonstrate that substantial blood loss is required to maximise the benefits (reviewed by Borderias & Sanchez-Alonso, 2011) and factors such as rapid clotting or inappropriate water temperature can inhibit bleeding. Based on the results of the present study there was a clear advantage in bleeding YTK to reduce the incidence of blood spotting in the flesh, but there was no additional benefit on this attribute of a bleed bin in the specific manner trialled. The outcome of this activity has significant implications for process flow on the harvest vessel, potentially reducing harvesting costs by eliminating two labour intensive steps. It also has the potential to increase harvest vessel on-board safety, as it could eliminate the movement of large bags of fish across the deck by HIAB.

However, the other consequences of eliminating the bleed bin stage need to be considered. If the fish bleed out in the RSW tanks, what are the implications for disposal of that larger volume of blood-contaminated water after unloading (compared to the smaller volumes of bleed bin water generated during harvest)? What effect could the higher concentration of iron in the RSW have on the carcasses during the several hours that they will be immersed in it? Will the higher concentrations of blood in the RSW affect the microbial load on the carcasses or increase the potential for blood parasite translocation? So, whilst it seems attractive and justified to change the harvest process based on these data, it would be prudent to be aware that there may be other, unintended, consequences of this decision, and further research would be necessary to quantify the various downstream impacts.

4.3 Activity Three: Acute and chronic stress and its impact on YTK product quality

In almost all the studies that have been carried out on pre- and at-harvest activities and fish product quality, the issue of 'stress' has been tested or cited (reviewed by Borderias & Sanchez-Alonso, 2011; Robb, 2001). But what is 'stress', how does it differ from activities such as increased swimming speed, increased fish density during crowding or confinement, poor water quality; and at what stage does it become an issue? This project has not attempted to address those higher order questions, but rather to look at whether there is evidence to suggest that the practical activities that occur during a commercial YTK harvest impact substantially on the quality of the carcasses and fillets produced.

The results from the blood (haematocrit, plasma glucose and lactate concentrations) suggested that all of these parameters were significantly affected (increased) by a 90 min period of shallow water stress and lowered dissolved oxygen concentrations immediately prior to sampling (Figure 19). However, there was no indication that 6 repeated daily instances of this same stressor had any significant effect on these same 3 parameters, suggesting that the fish recovered within the 24 h period and the stressor was not cumulative.

In contrast, the muscle glycogen and lactate levels did suggest persistent (> 24 h) or cumulative effects of the stressor. Thus, muscle glycogen levels at the time of sampling were highest in rested harvest fish (Figure 20A), and significantly lower (by 50%) in fish that were confined for 90 mins, and repeatedly exposed to the 90 min confinement stressor over 6 days. This decrease in muscle glycogen level is expected for fish that have been aerobically active pre-sampling. Although glycogen levels decrease substantially post-harvest in all the fish, the same trend is shown in the different treatment groups although the differences are not significant. With muscle lactate the results at 0 h are not so easy to explain because muscle lactate usually indicates the amount of anaerobic metabolism the fish/muscle has done (Robb, 2001), and the level in undisturbed and rested harvest fish (Treatment 1) is similar to the undisturbed and unrested harvest fish (Treatment 3) and repeatedly disturbed fish with a pre-sampling period of confinement (Treatment 4), and higher than the repeatedly disturbed but rested harvest fish (Treatment 2). It would be expected that the muscle lactate level would be lowest in the fish in Treatment 1, the reason for this finding is not clear. However, as the fillets are stored for a longer time a pattern does become clearer, and it is that repeatedly stressed fish accumulate lactate less quickly than previously unstressed fish. This suggests that repeatedly stressed fish have either (a) become compromised in their ability to undertake anaerobic metabolism as a result of the longer period without food combined with the repeated episodes of aerobic and anaerobic metabolism over the previous 5 days, or (b) are they acclimating to the stressor because it is no longer novel?

Glycogen and lactate metabolism are intrinsically linked to ATP levels in the tissue because ATP is the preferred nucleotide for providing energy for metabolic processes both pre- and post-mortem (Milligan, 1996). ATP levels in a tissue are depleted by intense physical activity of those tissues, and so the 0 h ATP gives us further information about the metabolic state of the fish from the four different Treatment conditions (Figure 25). As expected, the muscle ATP levels in the two

rested harvest Treatment groups (1 & 2) are significantly higher than the two unrested harvest groups; and of these the ATP level in the repeatedly disturbed group (Treatment 4) is lower (but not significantly so) than Treatment 3 fish that were only stressed once. These results generally agree with the glycogen and lactate data from the same fish.

Taken together, these results suggests that commercially harvested YTK may have a different muscle biochemistry depending on whether they are taken at the beginning or end of a single harvest event, or depending on if they taken in the first or last harvests when a cage/raceway repeated harvest approach is used. Whether these differences have a bearing on measureable attributes of the carcass and fillet quality will be discussed below.

The amount of ATP in the muscle tissue also largely dictates the development and onset of rigor mortis in the carcass, with low levels of ATP causing the muscle to contract and rigor to be established (Mørkøre et al., 2008). Within 6 h of slaughter, all of the fish in this study had very low levels of ATP (< 3% of total adenylates were as ATP; Figure 25), and rigor was well progressed (> 70% rigor in all groups) with the amount of rigor being inversely proportional to the amount of muscle ATP. Thus, the fish in Treatments 3 & 4 were further progressed into rigor by 6 h compared to fish in Treatments 1 & 2. This finding is consistent with other studies in the literature, which show that stressed fish are more likely to go into rigor earlier. However, whilst these differences in the rates of ATP decrease and rigor onset were observed between the various treatment groups, their magnitude and extent were very minor. All the fish were in 100% rigor by 12 h and by 96 h the rigor in all the fish was resolving but all were still 55-65% in rigor. This level of variation is not exploitable in a commercial setting, and therefore there is little reason to change harvest practices to manage or optimize the onset or resolution of rigor mortis.

K value (also interpreted as 'freshness') is calculated from the concentration of hypoxanthine and inosine that accumulates in tissue as a result of progressive anaerobic metabolism of successively lower energy nucleotides (ATP > ADP > AMP > IMP > IHx > HxR > Hx)(Huss, 1995). As tissues deteriorate with time and storage conditions the K value rises. At harvest (0 h) all of the flesh in the four Treatment groups had low K values (< 5%), with the undisturbed and rested group tending to be lower than the others (Figure 24). As post-harvest time increased, particularly after 24 h, the K values in all Treatment groups also increased with values in Treatments 2

& 4 tending to increase faster than Treatments 1 & 3. This suggests that the freshness of repeatedly disturbed fish is lost at a slightly faster rate than the fish that weren't repeatedly disturbed. However, the extent of this increased rate of deterioration in freshness by 96 h post-harvest was less than 24 h. A K value of 20% is sometime proposed as an upper limit for fish that can be used for sashimi or other high quality purposes (Ehira, 1976). On this basis, the fish in all treatment groups could have been used for this purpose within 72 h of harvest. This observation was made despite the less than ideal post-harvest conditions for promoting freshness – the fish were repeatedly removed from their ice slurry or insulated storage boxes and handled every 6, 12 or 24 h and they were stored in a chiller at 4^{o}C. These conditions are not representative of standard post-harvest storage practices for YTK, and as such the shelf-life of commercially harvested and processed fish would be considerably better than indicated by this experiment.

Objective and subjective assessment of the quality index of the whole fish (Figure 22) and the attributes of fillets at 96 h post-harvest (Figure 23), revealed all products were in good condition and there were no significant differences between any of the Treatment groups. These findings add to the extensive literature on the effects of pre-harvest stress on fish product quality attributes (Robb, 2001; Borderias & Sanchez-Alonso, 2011). In contrast to many of the findings on salmon, trout, seabass, seabream, cod, halibut etc., where pre-harvest stressors have had commercially significant effects on product quality, this study on farmed YTK (and the earlier preliminary study by Carragher et al., 2009) suggest that there is little, if any, significance of modest amounts of acute or chronic pre-harvest stress on commercial outcomes. Overall, this suggests that YTK is perhaps a more robust species than some of the others, and/or the harvest processes are less severe than in other aquaculture situations. Whichever is/are the case, the result can only be good for the YTK industry in positioning it's product at the premium end of the marketplace.

In summary, these findings suggest that although there were some significant differences in the biochemical properties of the fish that were exposed to a single (acute) confinement stress pre-harvest, and/or fish that were exposed to a repeated (chronic) series of confinement stressors, there was ultimately no discernible impact on the quality of the product out to 96 h post-harvest. It should be noted that this experiment was carried out in a highly controlled tank environment in the laboratory, and so the results may not be directly transferable to at-sea harvest conditions.

4.4 Activity Four: Harvest stress and its impact on YTK quality

Whereas the previous experiment was very useful, it was a tank-based laboratory study, and so there is doubt over how representative it was of an at-sea harvest operation. Consequently a further study was carried out to determine the stressfulness of a single at-sea harvest event, and whether the automated harvesting process and/or the duration of crowding prior to harvest, make significant differences to fish biochemistry and/or product quality attributes.

Two of the three blood/plasma stress indicators (cortisol and lactate) showed that hook harvested fish had significantly lower stress levels than the commercially harvested fish, with the levels of these stress indicators increasing as confinement time increased (Figure 26). Blood glucose levels, on the other hand, did not change significantly with harvest method or crowding duration. The increases in cortisol and lactate level are consistent with many other studies, and indicate that the YTK were anaerobically challenged when delivered to the automatic harvesting conveyor. This suggests that the YTK, either in the harvest net and/or in the immediate area where the inlet of the fish pump is located, are swimming at a rapid rate or for an extended duration, such that they are incurring an oxygen debt. Anaerobic metabolism of glucose (or breakdown of glycogen) will generate some ATP to power cellular processes but will also produce lactic acid (lactate), much of which will remain in the muscle and some will be transferred to the bloodstream to be excreted. The dissolved oxygen levels in the crowded raceway net should be monitored during harvest events and, if found to be low, supplemented with diffused oxygen as required.

The results of muscle tissue glycogen and lactate observations at 0 h match the patterns of stress indicators in the blood (Figure 27). Muscle glycogen levels were lowest in the fish sampled after 120 mins of confinement, whereas the levels in the other 3 Treatment groups were similar to each other. However, it is worth noting that at 2500-3300 mg/kg they were substantially less than the 4800 mg/kg which was measured in the undisturbed and rested tank-held fish in the previous study. Muscle lactate levels at time of harvest were low and similar to those measured in the previous tank study, and there was a significant increase in muscle lactate levels with increasing time of confinement. Muscle K value (calculated from the energy level of nucleotides) was very low (~2%) and similar in all the fish at 0 h.

The first opportunity to sample the carcasses of the YTK was after 12 h, and by this time the glycogen level in the muscle was very low and similar in all treatment groups; muscle lactate level was increased relative to 0 h, but again similar in all treatment groups; and K value was increased to ~5% and was significantly higher in hook harvested fish compared to the commercially harvested ones. Rigor index in all the fish was in excess of 100% by 12 h. During post-harvest storage to 84 h there were no differences between treatment groups in muscle glycogen, lactate, K value and rigor index, and the pattern of change in each parameter was consistent with what was observed in the tank-based stress experiments.

So far, the results of this study have been very similar to what was observed in the previous tank-based experiment. However, were the objective and subjective quality attributes different between the at-sea treatment groups? There were some significant differences between the hook harvested and commercially harvested fish in terms of their quality index scores at 48 and 72 h post-harvest (Figure 29); with all commercially harvested fish having lower scores (i.e. appearing better) than the hook harvested group. By 84 h post-harvest there was no difference in the quality index scores of all the fish. There were no differences in fillet smell, gaping or firmness between the different harvest treatments at any time out to 72 h post-harvest (Figure 30); however fillet colour was slightly (but significantly) better in hook harvested fish at 72 h.

In summary, the results of this at-sea harvest stress study suggest that although there were differences in some stress indicators and muscle biochemistry at the time of harvest, neither harvest method nor increasing harvest crowding duration significantly affected muscle biochemistry or the perceived quality of whole fish or fish fillets at times out to 84 h post-harvest. This conclusion is in agreement with that drawn from the results of the previous tank-based experiment, and strongly supports the notion that YTK is a robust species that does not show significant product quality consequences of modestly stressful situations associated with harvest.

4.5 Activity Five: Investigate the development of eye cloudiness in YTK post-harvest

The phenomenon of post-harvest eye cloudiness is also known in the literature as "cold cataract". The conventional understanding is that eye cloudiness is produced when the lens is cooled below a critical temperature which is species specific (Kiss et al., 2004). At that temperature, there is a phase separation of protein-water mixture in

the liquid of the eye (Petitt and Forciniti, 2000) and some of the soluble proteins denature and precipitate onto the lens. Other studies have shown that osmotic changes of the liquid in the eye may also be involved in cataract formation (Poston et al., 1978).

The results of a series of small experiments carried out in this study showed that water temperature is contributing to the formation of eye cloudiness in YTK; with the colder the water, the faster the cloudiness developed (Table 5). Two separate experiments tested the effect of salinity/osmolarity on the rate and extent of eye cloudiness formation in YTK eyes. In both trials the rate and severity of eye cloudiness was affected by the salinity of the water, with salinities of 15ppt (< 50% seawater) showing no, or little, eye cloudiness even at water temperatures < 2^{o}C (Figures 37 & 38).

Whilst getting to the bottom of the question of what causes eye cloudiness in YTK is scientifically interesting, it raises some industry issues that are both practical and about perception. Firstly, eye cloudiness happens because the fish are very cold. Cold fish are good for the producer and the customer in that they can be confident that the rate of post-mortem changes in flesh quality and spoilage are much slower at 0-2^{o}C than if the fish are at say 4^{o}C. However, the customer wants to buy fish that have black, translucent eyes because that is more commonly an attribute found in the freshest of fish, and cloudy eyes are most commonly found in old, poor quality fish. The results of this study have shown that it is possible to have very cold YTK with clear eyes, all it requires is for the fish to be harvested into and stored in 15ppt salinity water. However, on the west coast of South Australia large volumes of potable fresh water are not so readily available, and filling RSW tanks or fishbins with fresh water would take extra time and use additional fuel.

The somewhat opportunistic trial looking at the extent and rate of development of eye cloudiness in fish fed on different diets does offer some hope in this regard. The industry partner was carrying out a trial to examine the effect of additional dietary supplementation with the essential amino acid taurine on the health and performance of juvenile YTK. Taurine has many important physiological roles such as in osmoregulation, membrane stabilization, and conjugation with bile acids and bile pigments (Takagi et al., 2006). A literature review indicated that an imbalance of this amino acid content in the diet could also contribute to the development of eye cloudiness (i.e. cold cataract) because it has been implicated in stabilizing protein

structures against denaturation through its role as an osmolyte (Arakawa and Timasheff, 1985).

Five diets were trialled, with diets 4 & 5 containing 2% supplemental taurine. The results showed that the different diets had some effect on eye cloudiness formation in ice slurries made with 100% seawater, with diet 5 (one of those containing the additional taurine) reducing the extent of eye cloudiness (Figure 34). However, there was no significant effect seen in diet 4, the other formulation containing extra taurine, perhaps suggesting the taurine may be acting synergistically with another compound present in the diet. For example, in salmon, preliminary studies showed that a dietary deficiency in methionine correlates to cataract (Poston et al., 1978). Since inclusion of extra taurine in the diet has improved the health and performance of juvenile YTK, it is now included in all commercial diets to avoid those same problems. Given that extra taurine helped to reduce the incidence of eye cloudiness in 100% seawater ice slurries, it may be that the incidence of post-harvest eye cloudiness has decreased in these fish since the diet has been changed. However this will be difficult to quantify as the incidence and severity of eye cloudiness has never been a quality attribute actively measured by CST. Nonetheless this research has provided insight into the formation of cold cataracts and a method of mitigation should market acceptance of YTK due to eye cloudiness become a significant issue.

Activity Six: Quantify the physiological, biochemical and haematological characteristics of wild YTK – what does a "normal" YTK look like?

Currently, every aspect under study in farmed YTK is conducted within a context where the healthy/normal condition of the species is unknown. Consequently, data from the results of previous experiments on captive YTK, or even other fish species, are usually used as controls and this is neither satisfactory nor good practice. For this reason, it was decided to sample a reasonably large number of wild YTK that were of a similar size to the aquacultured fish, and to perform analyses for a wide spectrum of blood and muscle parameters. The wild fish were sampled off Sydney harbor (just offshore from the heads) to avoid the possibility of sampling potential escapees from the YTK farms on Eyre Peninsula. The general appearance of the wild YTK was a tapered body shape which differed from the shape commonly seen in the farmed fish. The wild fish were also brighter and showed more distinct green/yellow body color to the farmed fish.

Comments made by the IDEXX chief pathologist in the results clearly highlight the significant artefacts observed, particularly in relation to the various types of white blood cells observed in the samples. He also mentioned differences in several of the blood biochemistry parameters and how these differences may, at least in part, be due to two things – differences in the types of anticoagulant used between the Sydney and Port Lincoln sampling events and the length of time between when the samples were taken and when they were analysed. Clearly, one key conclusion from this work is that in order for the data to be of any use, the way in which the wild and farmed fish are sampled, and the time-temperature conditions between the sample site and site of analysis, both need to be carefully considered and as far as possible consistent. Despite the potential sampling artifacts, the pathologist suggested that a number of the results in the farmed fish are suggestive of chronic health problems as evidenced by low PCV, MCV and MCH. Health management is an ongoing issue in all aquaculture ventures, and the YTK operations at CST are no different. However, the report by Dr Dennis and the commentary by Dr Landos on the histopathology of the wild YTK sampled in this study cast a different light on the haematology and blood biochemistry results. Both veterinarians were somewhat surprised by the high incidence to which the wild fish carried infection by various parasites and showed characteristics of other disease conditions. Indeed, because of this Dr Landos argues that these wild fish are not suitable to use to provide 'normal' reference values for YTK in culture situations, and that another population of fish be sampled instead. This obviously puts into question the validity of any comparisons between wild and farmed fish made in this report, and only further sampling of wild fish can address it.

Muscle amino acid composition from wild and farmed YTK can be compared (Table 14). The general pattern of amino acids in the two sources of YTK is similar (i.e. the same amino acids are either high, medium or low), but the % differences can be large, particularly for those amino acids that are minor components.

Table 14. Comparison of muscle amino acid levels of wild Yellowtail Kingfish (*Seriola lalandi*) (n=22) and those of farmed YTK (n=13). Data from the farmed fish was collected and supplied by Clean Seas Pty Ltd.

	Wild YTK (% of protein)	SD (%)	Farmed YTK (% of protein)	SD (%)	% Difference in Farmed YTK
Alanine	6.1%	0.3%	5.4%	0.3%	-12.2
Arginine	6.0%	0.3%	6.0%	0.3%	-0.2
Aspartic acid	9.4%	1.1%	11.0%	1.0%	16.9
Glutamic acid	16.9%	1.5%	13.9%	2.1%	-17.7
Glycine	3.7%	0.3%	3.7%	0.5%	-0.7
Histidine	6.1%	0.5%	5.3%	0.5%	-13.1
Isoleucine	4.9%	0.3%	4.6%	0.1%	-6.2
L Cystine	1.5%	0.2%	1.1%	0.2%	-28.4
Leucine	8.1%	0.4%	6.8%	0.2%	-15.4
Lysine	7.4%	0.4%	7.0%	0.5%	-5.0
Methionine	3.7%	0.3%	2.6%	0.1%	-31.0
Phenylalanine	3.3%	0.2%	3.1%	0.1%	-6.7
Proline	2.1%	0.5%	3.2%	0.3%	53.8
Serine	3.8%	0.3%	2.7%	0.2%	-29.0
Taurine	0.6%	0.1%	0.8%	0.1%	34.1
Threonine	5.3%	0.5%	4.1%	0.2%	-21.7
Tryptophan	1.1%	0.1%	1.0%	0.1%	-8.7
Tyrosine	3.5%	0.2%	3.1%	0.1%	-9.2
Valine	4.5%	0.3%	4.1%	0.1%	-9.5

These same farmed Port Lincoln fish were not analysed for a complete set of muscle or liver composition and biochemical data, instead we have to compare the wild fish results to work done by D'Antignana et al. (2012)(Table 15).

Table 15. Comparison of muscle protein, crude fat, fatty acid class and n-3 long chain fatty acid levels of wild (this study) and farmed (D'Antignana et al., 2012) Yellowtail Kingfish (*Seriola lalandi*).

Parameter	Unit	This Study	D'Antignana et al, 2012*
Protein	%	23.72	22.7
Crude Fat	%	1.12	10.7
Trans Fat content	%	<0.1	0.1
Polyunsaturated Fat	%	0.47	3.45
Monounsaturated Fat	%	0.30	4.50
Saturated Fat	%	0.35	2.90
Total n-6 PUFA	%	0.04	1.12
Total n-3 PUFA	%	0.43	1.93
Eicosapentanaeoic C20:5n-3 (EPA)	%	0.04	0.77
Docosapentaenoic C22:5n-3 (DPA)	%	0.02	0.26
Docosahexaenoic C22:6n-3 (DHA)	%	0.37	0.63

* The average of dorsal and ventral loins in summer and winter fish

It can be seen from this comparison that although the wild and farmed YTK have similar muscle protein levels (22-24%), the crude fat levels are about 10-fold higher in the farmed fish. It could be argued that the D'Antignana et al (2012) value is the mean of the values for the dorsal and ventral loins in summer and winter fish, and these samples of muscle are not the same as the post-vent dorso-ventral quality cut taken in the present study. However, D'Antignana et al (2012) also measured 5-10% crude fat in a post-vent quality cut (no fatty acid class or n-3 long chain fatty acid analysis). Similarly, Carragher and Wilkinson (2012) took a similar post-vent dorso-ventral quality quality cut from farmed fish and found that crude fat levels varied between about 2 and 8%, depending on season and/or reproductive stage. These values are higher than the 1.12% fat value from wild fish in the present study.

With substantially different crude fat levels in wild and farmed fish (D'Antignana et al., 2012), it is not surprising that levels of all the fat classes and n-3 long chain fatty acids are also higher in the farmed fish. It suggests that care must be taken when comparing wild and farmed fish for any of the haematological, biochemical and compositional parameters reported in the present study, because differences in the nutritional status, size, age and season when the samples were collected may have a bearing on the conclusions.

5. Benefits and Adoption

This entire project was carried out in close consultation and collaboration with CST and its commercial processing partner Australian Bight Seafoods. Thus, the findings from the project have been fed back to CST in a timely way and have been used by senior management, the harvest manager and the harvesting team to:

- improve the effectiveness of the automatic stunning and bleeding technology on the harvest vessel to improve fish welfare, reduce stress, reduce variability in product quality and increase harvest productivity
- determine that a specific bleed tank stage in the post-harvest line is not necessary to ensure that blood spotting in the flesh is minimised. However, the consequences of allowing fish to bleed out in the RSW tank need to be further researched before this step should be eliminated

- demonstrate that YTK exhibit typical physiological stress responses when exposed to acute and repeated stressors in both simulated and actual commercial harvest situations
- demonstrate that, despite the resulting variability in the biochemical status of the muscle tissues at harvest (glycogen, lactate and ATP levels), the post-harvest attributes of the carcasses (rigor index, quality index score) and fillets (colour, firmness, gaping, smell) do not appear to be detrimentally affected at 72 to 96 h post-harvest
- demonstrate that the occurrence of eye cloudiness in many YTK post-harvest is largely due to the effective cooling of the fish in RSW tanks that affects the solubility of proteins in the eye
- demonstrate a method to reduce the incidence and extent of eye cloudiness by reducing the salinity of the cooling medium to 15ppt or less
- demonstrate that taurine-supplemented diets may reduce the incidence and/or severity of eye cloudiness in cold seawater possibly by osmotically buffering the liquid in the eye
- gather samples of blood and muscle tissue from wild YTK to have them analysed for a wide range of haematological, biochemical and compositional parameters with the aim of compiling a reference database for comparison with samples taken from cultured YTK. However, the validity of the wild YTK sampled offshore of Sydney has been questioned by the high incidence of parasites and other disease conditions found in following histopathology.

Overall, the results of this project have allowed CST to significantly streamline labour costs associated with YTK harvest, improve the management of the harvest and post-harvest cold chain, and minimise the amount of product rejected by the processor and customers. Good harvest practices have been developed that will help CST consistently deliver increasing volumes of high quality products.

6. Further Development

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

- how can the whirlpool and/or channels within the automatic stunner and bleeder system be better balanced such that approximately equal numbers of fish are processed through each of the 3 channels? This would allow fish to be harvested at close to 60/min, further increasing harvest efficiency
- what are the consequences of allowing YTK to bleed out in the RSW tanks instead of in a specific bleed bin? Are there issues with the disposal of large volumes of blood water after unloading, and what is the impact on carcass microbiology and spoilage?
- are there any longer-term (>7 days) differences in product quality attributes of YTK that experience different amounts of stress during the harvest process?
- how extensive is the incidence of eye cloudiness now that all fish are getting the taurine supplemented diet? Is there a need to see if other dietary osmolytes could be administered to reduce the incidence further? What are the practical issues for supplying potable freshwater to the RSW tanks to reduce the salinity/osmolarity to <15ppt?
- how do the haematological, biochemical and compositional values measured from cultured YTK differ from the values in the new wild YTK database? What aspects differ, and in what ways could they affect the performance and/or appearance of the cultured fish?

Despite these questions the research highlighted that YTK flesh quality attributes are particularly resilient to stress and harvest artefacts. Likewise the research clearly identified measures to reduce the incidence of eye cloudiness and characterised the issues with automates harvesting and went a considerable way to elevating the problems encountered. However such knowledge has yet to be wildly disseminated outside of CST. To promote extension of the research outside of CST the authors propose to present this research at the World aquaculture Conference in Adelaide, 2014. This event should prove to be the perfect event for further dissemination as a Seriola session will be held at the conference.

7. Planned Outcomes

This project will directly benefit the YTK industry, specifically CST, however it will also have flow on implications and benefits to other finfish industries and seafood processing facilities.

Public Benefit Outcomes

The planned outcomes that have public benefit were:

- To prolong rigor onset and resolution and increase the products shelf-life to 14 days
- An increase in stun/bleed efficacy from 5% to a minimum 95% using automated harvesting technology

Changing priorities due to declining numbers of fish attaining harvest size meant that the first outcome was not achieved; however, a much improved understanding was gained of the factors affecting the onset of rigor mortis and the effect of harvest stressors on product quality attributes of YTK. The second outcome was achieved with the overall result that fish welfare has been improved through the entire harvest operation. Faster harvest processing rates mean that fish are not confined in crowded harvest nets or raceways for long periods, and a >95% automatic stun rate means that far fewer fish are conscious during bleed out. The quality of the YTK product going to the marketplace is now more consistent and with higher specifications for quality attributes, this has resulted in an improvement in levels of customer satisfaction and enhances the consumer experience that all seafood producers can benefit from.

Private Benefit Outcomes

The planned outcomes that have benefit to CST were:

- To reduce the number of personnel required at harvest (from 7 FTE to 4 FTEs) and reduce sea time hours required to harvest the fish by 20%.
- A reduction in the value of rejected product from approx. 280K pa to $140K pa
- An increase in stun/bleed efficacy from 5% to a minimum 95% using automated harvesting technology
- Increase throughput by automated harvesting machinery from 10 fish per minute to 30 fish per minute
- To develop and have harvesting personnel apply an agreed SOP which promotes best practice

All of these outcomes were achieved with the overall result that CST now has systems and processes in place to monitor, audit, train and continuously improve on YTK harvest activities. This has reduced direct and indirect costs of harvest activities by reducing the number of staff needed to do the job, whilst significantly reducing the proportion of downgraded fish and wastage of product, all of which helps to improve the financial return to CST. The findings will ultimately be beneficial to other YTK producers in Australia.

Linkages with CRC Milestone Outcomes

This project has addressed Seafood CRC Milestones, including:

1.5.2 Milestone

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

2.8.6 Milestone

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

8. Conclusion

This study sought to increase our understanding about the harvest procedures that affect the quality attributes of farmed YTK products, with the overall aim of improving product quality and consistency to the marketplace. The activities in this project have done this by demonstrating:

- The mid-brain of YTK of fork length 57 to 67 cm (2.3 to 3.6 kg bodyweight) was positioned 81 to 105 mm from the tip of the snout and at a depth of approximately 25 mm below the surface of the head.
- This distance from the snout to the brain dictated what the trigger setting in the Seafood Innovations automated harvesting system should be set at for this size of fish. This setting ensured the percussive piston would strike the fish directly above the brain and produce an effective stun, and that the bleed knife would strike the area of the heart ensuring good exsanguination of the carcass.
- The Seafood Innovation automatic harvesting system worked most effectively for YTK of this size when the original flat ramp that directs the fish towards the trigger and the piston/knife strike zone was replaced by a ramp that had a deep V channel along it. This ensured the fish was vertical when the trigger was activated and maximised the effectiveness of the stun or bleed action.
- All of these small improvements to the Seafood Innovations automatic harvesting system have increased the efficacy of the stun/bleed from 65% to > 95% and resulted in improvements in fish welfare.
- The exsanguination of harvested YTK was demonstrated to be essential to reduce the incidence and severity of blood spotting in the fillet.
- There was no significant benefit of a specific bleed bin in the post-harvest process, low and similar levels of fillet blood spotting were observed in fish that

bled out in the RSW tank. Eliminating the bleed bin will improve process flow and FTE's at harvest

- The issues around the disposal of larger volumes of bleed water after fish are unloaded, and the possible effect of greater concentrations of blood in the RSW tank on carcass hygiene and fillet quality still need to be addressed.
- YTK showed modest stress responses to handling procedures associated with harvesting (crowding, confinement, low water quality) and the level of stress and anaerobic metabolism increased with confinement/harvest duration resulting in significant differences in the physiological status (e.g. muscle glycogen, lactate and ATP levels) of the fish at harvest.
- Stressed and anaerobically challenged YTK entered rigor mortis slightly faster than fish that were not as challenged, however all YTK were in full rigor by 12 h post-harvest. Rigor resolved slowly in all fish and by 84 to 96 h post-harvest all YTK were still in 50% rigor.
- Differences in the physiological status of muscle tissue between stressed and unstressed fish were generally minor during post-harvest storage to 96 h.
- Despite these differences, there were no significant differences in the objective and subjective indicators of carcass or fillet quality (quality index score, flesh gaping, colour, smell or firmness) up to 96 h post-harvest or post-filleting, respectively.
- These results suggest that YTK is a robust species that is not susceptible to the harvest stress-induced consequences for product quality that have been reported for other species.
- This finding suggests that farmed YTK should be able to meet the high product quality specification at the premium end of the marketplace.
- Eye cloudiness in YTK post-harvest is caused by chilling the fish in seawater that affects the osmolarity of the eye fluid which leads to the denaturation and precipitation of proteins onto the lens. Reducing the salinity of the ice slurry water to 15ppt or less prevents this osmotic perturbation, and the eyes stay clear.
- The practicality of using large volumes of potable fresh water in the RSW tanks on the harvest vessel needs to be resolved to address this marketplace issue. Likewise the impact of a <15ppt ice slurry on important flesh quality attributes should be examine prior to commercial adoption .
- There was a suggestion that the use of taurine supplemented feed may go some way to reducing the incidence of eye cloudiness of YTK in chilled seawater as it may act as an osmolyte and buffer the liquid in the eye.

- A reference database for a wide range of haematological, biochemical and flesh composition parameters has been established from wild YTK that were of a similar size range to harvested farmed fish. This should help to identify 'normal' from 'abnormal' results from farmed fish, and give some direction for further changes to aquaculture diets and health management to improve fish health and productivity in culture.

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10. Appendices

Appendix 1: Intellectual Property

There was no new intellectual property produced in this project.

The only items of intellectual property are the SOPs produced using the results of this study. These remain commercial in confidence to Clean Seas Tuna Pty Ltd.

Appendix 2: Staff

The staff engaged in this project were:

Flinders University

Dr Trent D'Antignana

Dr Erin Bubner

Dr Maximiliano Canepa