Harvest and slaughter methods for farmed Barramundi to minimise fish stress and achieve premium market quality and improved fish welfare outcomes

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Executive Summary

This year, the Australian Barramundi Farmer's Association (ABFA) has learned how harvest teams on farms can take simple preparatory steps to ensure the quality and consistency of Australian farmed Barramundi reaching the market. Seafood scientists from the Queensland Department of Agriculture and Fisheries (DAF) have worked with the farmers that have nurtured their Barramundi for the last two years through the grow-out phase and have demonstrated through on-farm trials that the harvest phase is just as crucial. Research undertaken with farmers has shown that it is important to keep water dissolved oxygen levels above 5.0 mg/L as the fish are concentrated together beside the pond bank for harvest, particularly at high water temperatures. The next step is to have the correct number of ice slurry bins on hand to receive the tonnage the farm plans to harvest, with the correct ratio of fish, brine and ice to ensure a rapid chill. The researchers put forth these recommendations after comparing post-mortem changes in key quality indicators in Barramundi harvested at three farms in North Queensland. These were compared to results in similar fish harvested under controlled conditions at a Barramundi farm (rested versus stressed). All data gained will serve as a baseline 'toolbox' to compare future harvest technologies that will be adopted to progress the pursuit of quality.

Background

Barramundi aquaculture is a significant industry within Australia fulfilling a constant demand for this iconic species. Stress during harvest and slaughter can have significant impacts on flesh quality. It is in the commercial interest of aquaculture operators to ensure that these practices are humane and the stress on fish is minimised, to maintain premium product quality.

With the support of the ABFA, DAF focused on measuring stress factors during current commercial harvest processes on several Barramundi farms to gain knowledge on critical factors that impact fish, including product quality and fish welfare. These findings were utilised to provide practical operational changes or observations to form a guide to on-farm best practices to minimise the stress imposed during harvesting.

Pre-slaughter and slaughter practices impose stress on fish with subsequent impacts on flesh quality. When fish experience stress, a sequence of hormonal and metabolic processes is triggered, the resulting compounds from which, combined with behavioural indicators, can be used as indices to determine stress levels in the fish. Under stressful conditions, there is greater muscular activity causing anaerobic glycolysis to occur, which results in increased lactate presence in the blood. Increased muscle activity also causes rapid degradation of Adenosine Triphosphate (ATP) and changes in flesh pH due to increased lactate levels. These responses influence the biochemical processes that occur in the fish post-mortem and hence flesh quality.

An essential first step for this research was to hold discussions with nine ABFA Barramundi farmers and profile current harvest practices commonly used, as well as to obtain industry information on what is practical and adoptable within different farm operations. The next step was to determine stress imposed by current practices for individual farms. With this baseline information established, relevant trial work was devised and discussed with the industry. All trial work was conducted in situ on farm and any protocol developed had to be cost effective and practical for ready implementation. This way, farms could immediately benefit by fine tuning current harvest protocols to reduce harvest and slaughter stresses and improve the welfare of farmed Barramundi across the industry.

Aims/objectives

The project had four main objectives:

1. Determine stress imposed on fish during harvest operations and develop methods for stress reduction.
2. Evaluate methods of slaughter that minimise stress to fish, incorporating animal welfare best practice.
3. Refine and adapt protocols on farm with industry to ensure practicality and cost-effectiveness.
4. Prepare guidance material on effective protocols for industry best practice in formats suitable for ready reference by industry.

Methodology

Baseline stress levels were undertaken on farm to simulate rested and stressed Barramundi harvests. To simulate rested conditions, AQUI-S was added to the tanks to render the fish unconscious with no response to external stimulus using the prick test and eye roll test. To simulate stressed conditions, fish were attempted to be captured using a dip net as the water level was dropped in the tank. This invoked avoidance stress on the fish as well as the stress of being removed from the water. Rigor and pH change were measured and recorded at 0, 1, 2, 4, 8, 12, 24, 30, 48 and 72 hours post-harvest when logistically possible. A sample was removed directly after harvest for chemical analysis of K-value, nucleotide content and lactic acid. The remainder of this fish was filleted and skinned before being vacuum packed and frozen for texture analysis. The second fillet was vacuum packed separately and frozen for sensory evaluation using a formal taste panel. None of the fish were bled prior to processing.

On farm commercial harvests with varying dissolved oxygen (DO) levels, water temperature and harvest volumes were monitored to assess which processes within the harvest were causing the greatest amount of stress to the fish. Stress in commercial harvest was also assessed by the onset and development of rigor mortis, flesh pH, nucleotide compounds and lactic acid presence in flesh samples. Relevant harvest data were also captured, including: pond water temperature, DO levels in the net and fish harvest volume. Additionally, ice slurry parameters were also collected, including: slurry composition (ice to water ratio, salinity), temperature, load of fish into the ice slurry bin and fish chilling profiles. The on-farm trials were then compared against the benchmark controlled rested and controlled stressed trials.

Results/key findings

Results showed a pH change of controlled rested harvest Barramundi from time of harvest up to 48 hours ranged from 7.09 – 7.39. Fish that are harvested with a minimal amount of injury or stress will have high energy reserves, a higher pH and firmer flesh. Controlled stressed harvest fish pH values ranged from 6.38 – 6.72. Fish that are allowed to struggle prior to death generally have lower energy reserves, a low pH and softer flesh. A statistical comparison of the pH values for rested and stressed fish at time 0 in this study showed that stressed fish have a significantly lower mean pH compared to rested fish ($F_{1,4} = 419.6$; $p < 0.001$). The trend in pH values post-harvest was also found to differ between the stressed and rested fish. The overall mean pH obtained for stressed and rested fish in the first 48 hours post-harvest was significantly lower in stressed fish ($F_{1,4} = 23.5$; $p = 0.007$).

Stressed Barramundi enter rigor quickly with firm contraction of rigor beginning to slow at approximately three hours from harvest. In contrast, rested Barramundi take longer to go into rigor, taking approximately nine hours from harvest. This is consistent with the flesh having more muscle glycogen present at the time of death and taking longer to metabolise to lactic acid. This delayed time to enter rigor effectively preserves quality and extends the shelf life of the fish. A statistical comparison of the rate of exponential increase confirmed that stressed fish in this study have a significantly shorter time to enter rigor compared to the rested fish ($F_{1,4} = 38.80$; $p = 0.003$).

The lactic acid values for the fish samples from stressed verses rested harvest trials ranged from 1.65 – 2.72 mg/g flesh weight (FW) for the rested fish and 1.69 – 2.80 mg/g FW for the stressed fish. A

statistical comparison of the mean lactic acid for rested fish and for stressed fish indicated no significant difference due to the harvest method ($F_{1,4} = 0.37$; $p = 0.577$). Further comparisons made against results from on-farm harvests showed significant differences between a harvest that was performed with low oxygen levels or rafting fish when compared to a rested harvest.

Change in pH and rigor form the cornerstones of a baseline toolbox for all further comparisons when evaluating how much stress Barramundi have been exposed to during the harvest procedure. While lactic acid results confirmed Barramundi had been exposed to extreme levels of stress, the ability to measure pH and rigor on farm allows for easier, quicker and more cost-effective testing to be conducted while still achieving results that are able to detect significant differences in harvest practices.

Cooked samples from the rested harvest fish all required more energy to cut through the sample than the stressed fish, indicating a firmer cooked texture for rested harvest fish and a softer texture for the stressed harvest fish. This can be attributed to the stressed fish having used up available energy during the struggle (high muscle activity) of the harvest and thus accelerating the rigor process. The fish then go into the onset of rigor much faster, leading to the potential to tear muscle fibres. As the fish flesh resolves and softens from the rigor process, the resulting flesh is softer and sometimes quite mushy.

The sensory assessment of controlled rested and stressed Barramundi highlighted that rested Barramundi samples were described by the trained panel as having a blue-white colour with a sweet mild oceanic flavour. The texture was assessed as having firm, plump, juicy fibres that slide apart in the mouth. The stressed Barramundi samples were described by the trained panel as having a yellow-white-grey appearance with a meaty baked potato flavour. There was a low bland umami flavour with a slight acidic, metallic, undesirable liver aftertaste. The texture was assessed as having a soft flesh that was mushy and non-structured. There was no significant difference in either flavour or texture between the stressed and rested samples when assessed by an untrained panel of regular consumers.

Using the baseline toolbox to compare commercial farm harvests showed that harvest performed with low dissolved oxygen levels (2.1 mg/L) during harvest causes a significant increase in the onset of rigor and increased lactic acid levels resulting in low flesh pH. This in turn causes quality defects, softening of texture and loss of shelf life. ***Low dissolved oxygen levels during harvest are inhumane and unacceptable in terms of animal welfare.***

When comparing the range of ice slurries used, it was noted that on hot days, extra ice was occasionally added to the slurry to combat elevated air and water temperatures. If too much ice was added and the mixture increased close to a 1:4 water to ice ratio, the slurry became too dry and allowed the fish to raft on top of the ice. Results compared against the baseline toolbox showed significant differences in all rigor, lactate and resulting pH measurements. This is a result of fish taking up to 45 minutes to die when left exposed to air due to acidental rafting. ***In terms of animal welfare, rafting fish is an unacceptable practice and inhumane.*** Reduced fish load in 1000 L bins to approximatly 350–400 kg is recomended to ensure that fish can remain below the water level in the ice slurry bin. This also allows room for mixing and topping up ice levels to maintain consistent temperatures. Monitoring the daily water and air temperature for each day's harvest will allow custom ice slurry ratios to be mixed prior to harvest.

Ice slurry bins monitored during on-farm trials showed insufficient mixing within each slurry bin. This resulted in temperature stratification up to 11 °C between the top and bottom and salinity stratification of up to 13.5 g/L. All ice slurry bins must be mixed immediately once filled and returned to the processing area to be hooked up to a continuous mixing system that is able to mix the slurry to a consistent low temperature for the entire chilling process.

Recommendations

- ***Regularly train staff on the importance of a stress-free harvest and refer to the summary booklet.***
- ***Maintain pond bottoms during the grow out season to avoid build-up of organic material consuming oxygen as it decomposes.***
- ***Assess the daily air and pond temperature prior to harvest.***
- ***Mix a suitable slurry that is not so dry that it allows fish to raft on top of the ice.*** *Ensure that salt is fully dissolved at ambient temperature prior to ice or chilled water being added.*
- ***Pond dissolved oxygen levels must be monitored and maintained above 5.0 mg/L during the crowd and harvest process.*** Crowding must not begin if the dissolved oxygen level is below 5.0 mg/L. To maintain high DO levels, different approaches are possible: add bottled oxygen to every harvest, use extra paddle wheels or alternatively, delay harvest until pond DO has increased.
- ***Harvest operations should minimise fish stress as much as practicable.***
- ***Reduce the volume of fish in each crowd when temperatures are high and DO is low.***
- ***Do not overload ice slurry bins. Only load 350 to 400 kg of fish in each 1000 L slurry bin at one time.*** This allows all the fish to sink below the waterline of the ice slurry reducing the chances of the fish accidentally sitting on top of the ice. Reduced fish volumes in the bin also gives the crew a better chance to manually mix the bins prior to being hooked up to an automated mixing system.
- ***Adopt methods to ensure sufficient ice slurry mixing to minimise temperature and brine stratification for the duration of the chilling process.***
- ***Ensure that there is sufficient space in the ice slurry bin to add more ice if needed, without having to drop water and concentrated salt out of the bottom of the bin first (350–400 kg should achieve this).***

These recommendations bring the Australian farmed Barramundi industry's use of ice slurry as a slaughter method to a consistently high standard.

We recommend using the current data learned from these trials as a benchmark toolbox to evaluate any new refinements or new technology to be trialled. We have shown that pH and rigor results can detect significant differences between the level of stress that fish are exposed to during the harvest process on farm. We recommend continuing to use this toolbox to enable ABFA members to visualise the gains made in reducing stress when new harvest systems are introduced and trialled on farm.

As a follow-on project, the Queensland Government's commitment to aquaculture "Transforming Aquaculture Initiative Project" has committed further funding for continuing aquaculture research in consultation with ABFA.

Keywords

Barramundi, Crowding, Harvest, Ice slurry, *Lates calcarifer*, Rested harvest, Rigor, Stress.

Introduction

Barramundi aquaculture is a significant industry within Australia fulfilling a constant demand for this iconic species. Current production is around 10,000 tonnes per annum, with an expected year-on-year increase forecast over the coming seasons that will generate an industry gross value of production of over $100M. Australian seafood consumers have demonstrated a willingness to pay a premium for farmed Barramundi sourced locally, but the quality of the Australian product must be consistently high to justify this (Harrison et al., 2013; Lawley, 2015). Stress during harvest and slaughter can have significant impacts on the flesh quality of fish (Robb & Kestin, 2002).

Over recent years, the ABFA has supported research to gain information on stress levels in Barramundi imposed by different handling methods (Wilkinson, 2012). This body of information provides a preliminary baseline from which development of refined harvest and slaughter practices can occur. Key factors imposing stress on fish within the harvesting operation are:

- crowding/herding of fish
- method of removal from water
- time out of water
- and slaughter method.

This project was driven by and undertaken in collaboration with ABFA members. An essential first step for this research was to hold discussions with ABFA Barramundi farmers and profile current harvest practices commonly used, as well as to obtain industry information on what is practical and adoptable within different farm operations. The next step was to determine stress imposed by current practices for individual farms. With this base information established, relevant trial work was devised and discussed with the industry. All trial work was conducted in situ on farm and any protocol developed had to be **cost effective** and practical for **ready implementation.** This way, farms could immediately benefit by fine tuning the current harvest protocols to reduce harvest and slaughter stresses and improve the welfare of farmed Barramundi across the industry whilst improving flesh quality.

It is well known that pre-slaughter and slaughter practices impose stress on fish and that elevated stress levels impact flesh quality (Lowe et al., 1993; Sigholt et al., 1997). Stress in fish triggers a sequence of hormonal and metabolic processes, and these chemical changes, along with any behavioural indicators, can be used as indices to quantify how much stress the fish experienced (Mazur & Iwama, 1993). The primary response to imposed stress is an immediate release of adrenaline which triggers the release of cortisol into the blood stream. Cortisol enables faster mobilisation of glucose to provide extra energy for the fish to avoid the deteriorating situation (Erikson, 1997).

Vigorous swimming or struggling is a common response of fish to netting and confinement. This muscular activity can be so intense that the fish cannot infuse its tissues with sufficient oxygen to fully metabolise the liberated glucose. Anaerobic glycolysis occurs in a bid to sustain enough muscle ‘energy’ (ATP) to support muscle contraction. Lactic acid accumulates in the muscles and drives down the muscle pH before the lactate and acidity spreads to the blood (Erikson, 1997). The muscle in an exhausted fish prematurely acquires key biochemical characteristics expected of muscle in a dead fish with no blood circulation. Killing that exhausted fish promotes detrimental post-mortem changes and quality loss (Sigholt et al., 1997), problems that do not arise if the fish is killed while at rest, i.e., the so-called ‘rested harvest.’

To determine baseline stress levels, trials were undertaken on farm to simulate rested and stressed Barramundi harvests in a controlled manner. This allowed sampling of fish from a zero stressed environment as well as a controlled stressed environment to set baseline results for our quality parameters. Samples were tested and compared for flesh pH, lactic acid and K-value (ATP and its degradation products) as well as rate of rigor mortis and sensory attributes. Rigor mortis development was used as a stress indicator with Atlantic salmon by Skjervold (2001) in a similar manner.

These baseline results were used to compare results from regular commercial harvests. This allowed the quality parameter results to be assessed against each harvest day protocols and conditions.

Objectives

1. Determine stress imposed on fish during harvest operations and develop methods for stress reduction.
2. Evaluate methods of slaughter that minimise stress to fish, incorporating animal welfare best practice
3. Refine and adapt protocols on farm with industry to ensure practicality and cost-effectiveness
4. Prepare guide material on effective protocols for industry best practice in formats suitable for ready reference by industry

The project objectives were all achieved. Information gathered from the project resulted in a modification of on-farm practices with success and a short guide detailing the key points produced as a reference for the industry.

Methods

All fish used in these trials were euthanised prior to sampling according to DAF Animal Ethics Committee. Animal Ethics permits SA-2019-11-718 and SA 2020/02/731.

Stress in post-mortem fish was assessed by several measures, including: the onset and development of rigor mortis, flesh pH, concentration of nucleotide compounds and lactic acid in flesh samples. Relevant harvest data was also captured, including: pond water temperature, dissolved oxygen (DO) levels in the net and fish harvest volume. Additionally, ice slurry parameters were also collected, including slurry composition (ice to water ratio, salinity), temperature, load of fish into the ice slurry bin and fish chilling profiles.

The various methods for measuring the stress levels are outlined below:

Flesh pH

Flesh pH was taken by inserting the glass tipped pH probe (TPS Probe part number EPIJNW-111225, pH meter model TPS WP81) into the fish flesh directly adjacent to the sample that was removed for laboratory testing. For pH monitoring, a small incision was made using a sharp knife and the pointed glass tipped pH probe gently inserted into the fish (approximately 25 mm) with the flesh closing back around the probe (Plate 1). Readings were recorded once the measurement had stabilised. The pH meter was calibrated with standard buffer solutions of pH 4 and 7 prior to each round of testing to avoid any chance of pH drift. Between each reading the probe was washed in mild detergent to remove any fat deposits before being rinsed in deionised water prior to each analysis.

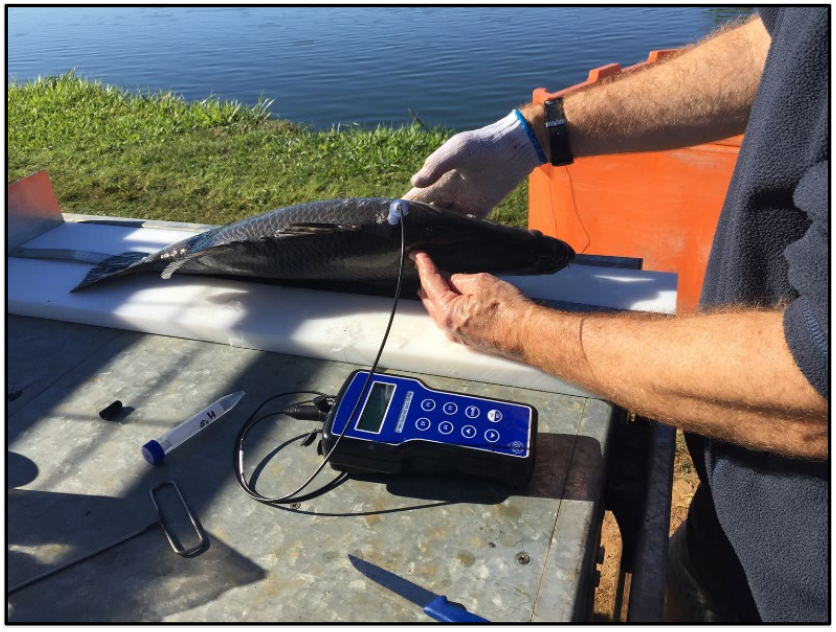

Plate 1 Photo showing position of the pH probe for monitoring change in pH during the rigor mortis process.

Penetrometer flesh tension (as a measure of rigor state)

The rigor state or flesh tension was measured using a modified fruit pressure tester (EFFEGI: model FT 011) fitted with a 19 mm plunger disk. Flesh tension was measured, as the pressure or force required over an area of 2.84 cm^2 to cause a maximum possible deflection of 6 mm in the fish surface. This field penetrometer is akin to a small portable version of the Instron texture meter used in a laboratory to measure force required for a 6 mm depression of the skin of a whole Barramundi. The original Messtorff

tool measured how deeply a blunt ended piston, under the constant pressure of a compression spring, could be pushed into the side of a fish (Amlacher, 1961). In this improvement, the plunger has a fixed depth of action, causing less interference to the sample from repeated use, and rather than record depth of penetration, the instrument dial records the force required at different stages of rigor. Goodrick et al. 1998 successfully trialled this method alongside tail droop method as described by Iwamoto et al. (1987).

Penetrometer measurements were performed on n=5 fish for each harvest treatment. All fish were tagged using individually numbered cattle ear tags zip tied through the lower jaw and gill for identification. Each fish was removed from the commercial farm ice slurry and laid horizontally on a flat board. Readings were taken from both sides of the fish in three defined locations, providing six readings per fish. These included:

1) On the lateral line, halfway between the pectoral and the front of dorsal fin.
2) On the lateral line, below the rear of the dorsal fin.
3) On the lateral line, directly above the anus (Figure 1).

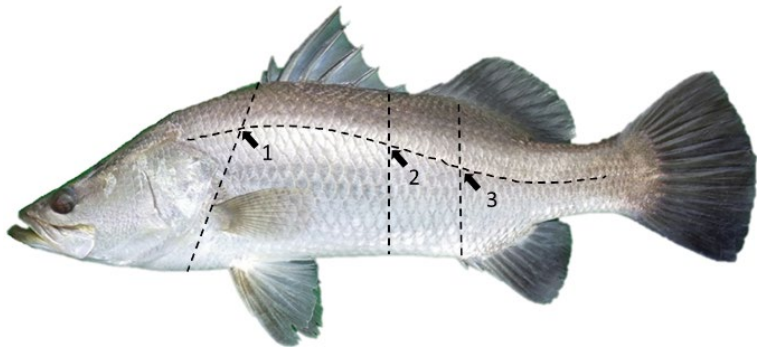

Figure 1. Penetrometer testing locations.

Repeated readings showed no evidence of damage to the Barramundi due to the large tough scales. Once readings were performed the fish was immediately returned to the ice slurry until the next time point for measurement was due**.** Readings were collected for each fish at time points (0, 1, 2, 4, 8, 12, 24, 30, 48 and 72 hours). The readings from left and right sides were combined before averaging. This method of measuring the state of rigor was used to eliminate problems as the fish progressed through the rigor process and potentially remained in a bent position while in a commercial ice slurry. Previous studies use the tail droop method (Iwamoto 1987), but this becomes problematic in commercial slurries when fish proceed into rigor mortis and remain bent.

Dissolved oxygen

Dissolved oxygen (DO) of the pond water was taken using a OxyGuard Handy Polaris 2, version 4.15. The portable probe was lowered into the water approximately 1 meter from the edge of the pond, within the confinement of the crowd net. The probe was gently raised and lowered 5–10 cm/sec as per instruction manual until reading was stable. Reading was manually recorded in three positions within the crowd and averaged.

Measurements were taken at the following three time points:

- before harvest began
- at the time the first brail was taken out of the crowd
- at the time the last brail was taken out of the crowd

Dissolved oxygen for the controlled rested and stressed trials was measured in the same way by placing the probe directly into the tank and raising and lowering the probe until the reading stabilised.

K-value and lactic acid analyses

Extraction of nucleotides and lactic acid

A 40g flesh sample was removed by cutting a 15 mm strip of flesh perpendicular to the lateral line and adjacent to the front edge of the dorsal fin (Figure 2). The skin and dark muscle were removed before the shoulder portion of the sample was wrapped in aluminium foil and immersed directly into liquid nitrogen. Samples were held frozen in liquid nitrogen during transportation to the Coopers Plains DAF research facility. On arrival, they were transferred to a -80°C freezer to await testing for K-value and lactic acid.

Samples for K-value and lactic acid extraction were removed from frozen storage at -80 °C. The aluminium foil was removed carefully within 5–10 min (samples were not allowed to fully thaw). The fillet was chopped into small pieces using a knife and milled for 1 min using a ball mill (Retch MM400) to obtain a consistent paste. Five (5) g of the resulting paste was accurately weighed into 50 mL centrifuge tubes in duplicate (labelled A and B) and 25 mL of cold 0.6 M PCA was added to each tube as quickly as possible. The tubes were vortexed for 2 min and centrifuged at 4 °C (4000 rpm) for 15 min. Twenty (20) mL of the resulting supernatant (extract) was pipetted into a 50 mL glass beaker containing a magnetic stirring bar. The pH of the extract was adjusted to 6.5–6.8 with the help of a calibrated pH meter by adding 1.0 M and/or 0.1 M KOH; then the stirring was stopped, and the stirring bar removed. The solution was allowed to settle (≈ 5–10 min) prior to being decanted into a 50 mL centrifuge tube. The tubes were kept at 4 °C overnight to ensure complete potassium perchlorate precipitation. The next day these tubes were centrifuged at 4 °C (4000 rpm) for 15 min and the supernatants were carefully transferred into other 50 mL centrifuge tubes. The solutions were made up to 40 mL using MilliQ water and then mixed well. The resulting solutions were divided into two sets of 10–12 mL and stored at -80 °C prior to analysis.

Perchloric acid (PCA) extraction, followed by neutralisation with potassium hydroxide, was used to separate the nucleotides and lactic acid from the fish muscle. The methodology used was a modified procedure of Ryder (1985). The PCA extract was used for both nucleotide and lactic acid analyses. This dual testing on the same extract was proposed and verified by Ogata et al. (2016).

HPLC analysis of nucleotides

The High-Performance Liquid Chromatography (HPLC) conditions utilised in this methodology were a modified version of those outlined in Aliani & Farmer, 2005.

Sample tubes stored at -80 °C were thawed and an aliquot of each was filtered through 0.45 µm, 33 mm, nylon syringe filters and analysed using a Shimadzu (Shimadzu Co., Kyoto, Japan) HPLC system consisting of a system controller (SCL-10Avp), degasser (DGU-12A), pump A (LC-10AD), pump B (LC-10AD), auto-sampler (SIL-20AC), column oven (CTO-10AC) and photo-diode array detector (SPD-M10A) linked to LabSolutions software.

A stock solution containing the six nucleotides (freshly prepared and stored at -80 °C as per Table 1) was used to prepare four working standards. Previously prepared individual standard solutions were also used to accurately identify their retention times.

Table 1. Six (6) nucleotide stock solutions prepared for standards

Barramundi K value: Standards Date 08 Sept 2020
Mix STD: (IMP+ ADP+ATP+ AMP + HxR + Hx): in 100mL MilliQ water
IMP: 36.03mg (99.4%) = 35.81382mg (358.1382 mg/L)
ADP: 1.57mg (98%) = 1.5386 mg (15.386 mg/L)
ATP: 2.03mg (99%) = 2.0097 mg (20.097 mg/L)
AMP: 1.96 mg (99%) = 1.9404 mg (19.404 mg/L)
I: 3.64 mg (100%) = 3.64 mg (36.4 mg/L)
HYP: 2.54mg (99.4%)= 2.52476 mg (25.2476 mg/L)

The response (peak area) generated by injecting these working standards permitted construction of a calibration curve. By applying to the peak areas of the identified nucleotides in the fish samples; the concentrations could be calculated after applying the appropriate dilution factor.

Determination of K-value

The nucleotide concentrations were expressed as µmoles/g of fish muscle.

The K-value could then be calculated using the following equation:

$$\text{K-value \%} = \frac{(HxR + Hx)}{(ATP + ADP + AMP + IMP + HxR + Hx) \times 100}$$

HPLC analysis of lactic acid

The filtered (0.45 µm, 33 mm, nylon) extracts were analysed using a Shimadzu HPLC system consisting of a system controller (SCL-10Avp), degasser (DGU-14A), low pressure gradient forming switching valve (FCV-10ALvp), pump (LC-10ATvp), auto-sampler (SIL-20ATHT), column oven (CTO-10Avp) and photodiode array detector (SPD-M10Avp) linked to LabSolutions software. Chromatographic separation was performed with an ion-exclusion column (BIO-RAD Aminex HPX-87H, 300 x 7.8 mm, Cat. No. 125-0140) at 50 °C.

The mobile phase consisted of 0.01 N (5 mM) H_2SO_4 in MilliQ water. Separation was achieved by isocratic elution at a flow rate of 0.6 mL/min over 20 min. The injection volume was 20 µL and detection was monitored at 210 nm.

A stock solution was prepared by accurately weighing 100 mg of lactic acid and diluting to 100 mL using MilliQ water. The stock solution was used to prepare three working standards ranging in concentration.

A calibration equation was determined from the correlation between peak area and standard lactic acid concentration. The lactic acid content of the fish flesh was calculated after applying the appropriate dilution factors and expressed as mg/g flesh weight (FW).

Controlled rested and stressed harvests trials

To determine baseline stress levels, trials were undertaken with fish held in tanks on-farm to simulate rested and stressed Barramundi harvests.

A total of 60 Barramundi (*Lates calcarifer*) with an average size of 4.0 kg (fork length 67.3cm) were harvested from three grow out ponds using commercial practices on a north Queensland commercial freshwater farm.

Twenty fish from the first pond were brailed from the crowd and placed directly into a 1000 L bin of oxygenated water on the back of a ute. They were immediately transported to a live holding facility with six (6) x 5000 L freshwater recirculating tanks. Ten (10) random fish were transferred from the ute using a dip net and placed in to the first 5000 L tank. The final ten (10) fish in the ute were transferred to the second 5000 L tank.

The harvest was set up on the second pond and the same process repeated, transferring two (2) lots of ten (10) fish into two (2) more 5000 L tanks.

The third pond was harvested using the same process as before, transferring the final two (2) groups of 10 fish into the final two (2) tanks.

The 60 fish (ten (10) in each of the six (6) tanks) were left to recover for two (2) days in oxygenated water at 6.9 mg/L at a temperature of 26.1 °C.

Controlled rested trials

To simulate rested conditions, AQUI-S was added at 17 mg/L to the first tank for 15 minutes to render the fish unconscious and insensible with no response to external stimulus. Fish noted as insensible display an absence of - opercular movement, eyes remain immobile, and there is an absence of response to painful stimuli, (Kestin et al., 2002).

Tank 1 fish (n=10) were transferred into a commercial ice slurry for 15 minutes to euthanise. Five (5) fish were removed, one at a time and brain spiked (Iki jime) to meet animal ethics requirements. A 40 g flesh sample was then removed by cutting a 15 mm strip of flesh perpendicular to the lateral line and adjacent to the front edge of the dorsal fin (Figure 2). The skin and dark muscle were removed before the shoulder portion of the sample was wrapped in aluminium foil and immersed directly into liquid nitrogen. Samples were held frozen in liquid nitrogen while being transported to the Coopers Plains DAF research facility. On arrival they were transferred to a -80 °C freezer, awaiting further testing for K-value and lactic acid.

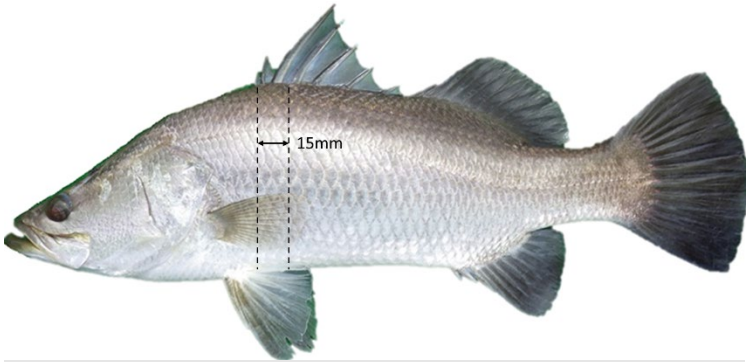

Figure 2. Position of sample taken for K-value and lactic acid.

Flesh pH was recorded at the time of sampling using a TPS WP81 pH meter. The remainder of the sampled fish were chilled in ice overnight before being filleted, skinned and frozen (-18 °C) to be transported via frozen transport to the Coopers Plains DAF research facility. On arrival they were held frozen at -30 °C before testing for texture and sensory attributes.

The remaining five (5) fish in the tank were iki jimmied (brain spiked) before being tested for change in pH and rigor at 0, 1, 2, 4, 8, 12, 24, 30, 48 and 72 hours.

The same procedure was repeated twice more to replicate rested conditions using fish initially harvested from the other two ponds. This provided a total of n=15 for onsite assessment of pH and rigor; plus, n=15 for K-value, lactic acid and sensory assessment.

Controlled stressed trials

To simulate stressed conditions, the water level in the holding tank was reduced to fully expose the fish (approximately 2cm deep). While the water level was reduced the fish were chased around the tank using a dip net. This invoked avoidance stress on the fish, as well as the stress of being removed from the water for euthanising. This process took 15 minutes, after which time all 10 fish were transferred using a dip net into a commercial ice slurry for 15 minutes. Five (5) fish were removed and iki jimed, prior to the same flesh sampling method described for the control rested trial samples and transport back to the DAF Coopers Plains research facility.

The remaining five (5) fish in the tank, were iki jimied before being tested for change in pH and rigor over the following 48 hours.

The same procedure was repeated twice more to replicate the stressed conditions using fish initially harvested from the other two ponds. This provided a total of n=15 for onsite assessment of pH and rigor; plus, n=15 for K-value, lactic acid and sensory assessment.

Controlled rested and stressed harvest – Texture analysis

Five (5) fish from each treatment were filleted and skinned. The portion of the fillet behind the dorsal fin of the left fillet was retained for texture analysis (as per Figure 3).

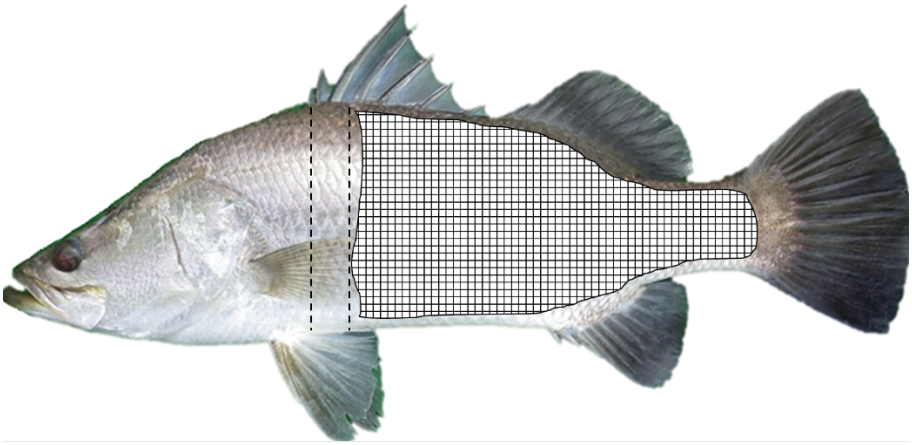

Figure 3. Diagram showing portion of the fillet used for texture analysis

Each fillet portion was vacuum sealed and frozen at -18 °C. The samples for texture analysis were subsequently transported frozen to the DAF Coopers Plains testing facility and held frozen at -30 °C until testing was performed. Samples were thawed overnight in a cold room at 4 °C. Vacuumed packaged fillet portions were steamed at 95 °C for 20 minutes using a steam oven model number UNOX XF135. Following cooking, samples were cooled to room temperature (2 hours) and prepared for testing by cutting the fillet sample into three loins, trimming out the red muscle. Three loin samples were accurately cut providing 25 x 35mm pieces, starting at the head end of the middle loin (Plate 2). The three samples from each fish were then subjected to mechanical texture assessment. This was performed using a Warner Bratzler cutting blade (Lloyd part # 01/3428) attached to a Model TA1 Lloyd universal texture testing machine that measured force/work required (Nmm) to cut through each sample as a measure of the firmness or texture. The blade was driven at a speed of 100 mm/min and force recorded using a 100N load cell. The sample of flesh was weighed before being orientated so the blade cut perpendicularly to the surface muscle fibres across the grain. The Lloyd unit was interfaced with Nexygen Plus 4 software to give the force/work required (Nmm) to cut through each sample. Three results for each fillet were downloaded into excel and converted to (N) energy required per gram of sample to adjust for the thickness of each sample.

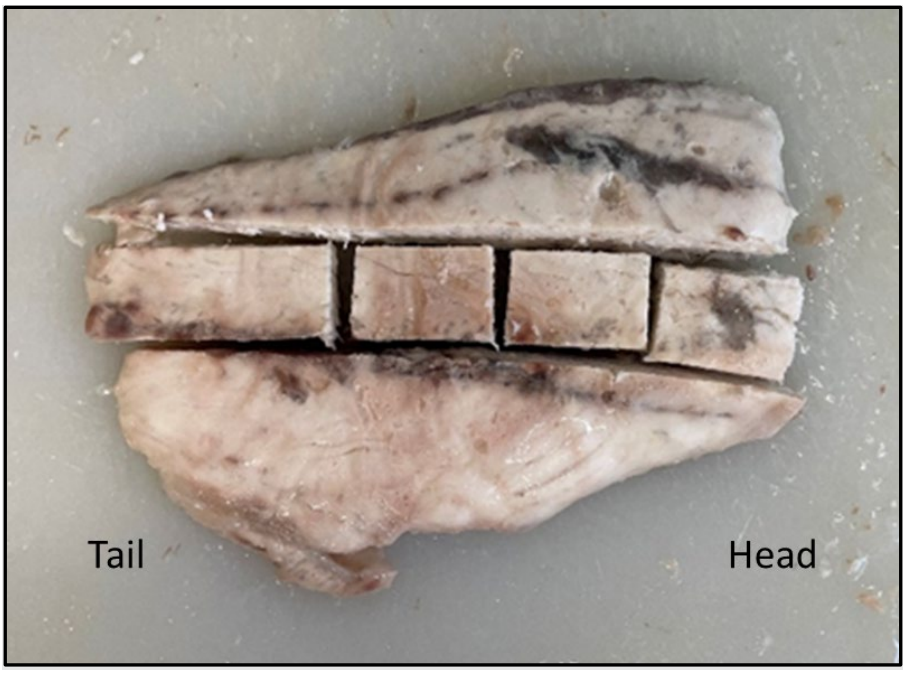

Plate 2. Location of three by 25mm x 35mm samples cut from the middle loin starting from the head end.

Controlled rested and stressed harvests – Sensory evaluation

Frozen fillets were freighted to the DAF research facility in Coopers Plains, Brisbane. On arrival at the research facility samples were held frozen at -30 °C until being thawed in a 2 °C cold room overnight the day before testing.

All samples were prepared on the day of the trial. Defrosted fillets were prepared for sensory assessment using a single standardised cook method. Only the middle loin section of each fillet was utilised, with the top dorsal and bottom belly sections discarded along with all red muscle. Medallion pieces measuring 15 mm thick were prepared from the middle loin. Cooking was conducted on one sample set at a time, as per the trial design. All samples were cooked in parallel on separate Roband grills pre-heated to 220 °C. Medallions were cooked on the cut surface, flipping to the opposite side after 1 min 50 sec or as soon as the middle portion turned from opaque to white. After flipping a thermocouple probe (Comark C26) was inserted into the visually thickest medallion to check that the sample was cooked to 70 °C. Cooked medallions were immediately plated into foil trays with aluminium foil lids and quickly served to panel participants.

Controlled rested and stressed harvests – Trial design difference test

A triangle test methodology was implemented for the trials conducted with fish from each of the three different ponds (Table 2). The International Organisation for Standardisation (ISO) describes the aim of a triangle test, to determine whether a perceptible sensory difference or similarity exists between samples (ISO 4120:2021). This method is a forced choice procedure and is applicable whether the difference exists in a single sensory attribute or several attributes.

Table 2. Triangle test trial matrix

Trial	Sample set	Samples being compared
1	Orange samples	Orange 6 vs Orange 26
		Orange 7 vs Orange 27
		Orange 8 vs Orange 28
		Orange 9 vs Orange 29
		Orange 10 vs Orange 30
2	Blue samples	Blue 6 vs Blue 16
		Blue 7 vs Blue 17
		Blue 8 vs Blue 18
		Blue 9 vs Blue 19
		Blue 10 vs Blue 20
3	Purple samples	Purple 6 vs Purple 16
		Purple 7 vs Purple 17
		Purple 8 vs Purple 18
		Purple 9 vs Purple 19
		Purple 10 vs Purple 20

Frequent fish consumers (n=33) took part in each trial, recruited from staff located at the Health and Food Sciences Precinct, Coopers Plains. Samples were cooked and placed in individual, blind coded foil tart tins. The trial took place at the DAF sensory facility, Coopers Plains, under controlled conditions. Red lighting was used to mask naturally occurring colour differences between samples and therefore made participants focus on the flavour and texture of the samples (Plate 3).

Three samples of each triad were presented simultaneously to the participants, following a structured design, on a line to be sampled always from left to right. Within the triad, assessors were able to make repeat evaluations of each sample as desired. Assessors were instructed to evaluate the samples in the order provided. They were informed that of the triad, two samples were identical and one different. Each assessor was then asked to indicate which of the three samples was different from the other two. Assessors were not given the option of reporting no difference, as this is a forced choice methodology. However, they were asked whether their answer was a guess. If the answer was not a guess, assessors were asked further questions to outline their reasons for this answer. Those that selected flavour and texture from the pre-determined answers as the reason for difference were asked to provide more detail using the keyboard as to what was different about the flavour/texture. Assessors were provided with water and crackers in order to cleanse their palates in between samples.

Plate 3. Sensory panellists evaluating samples under red lighting.

Controlled rested and stressed harvests – Trial design trained panel

A trained group of assessors (n=7) that regularly assess farmed Barramundi for shelf life and quality attributes took part in a qualitative taste panel to provide detailed descriptions for each of the fish samples tested during the triangle testing. The panel included DAF staff from specialist areas in seafood, food technology and sensory and consumer science. The tasting session was conducted at the sensory facility in Coopers Plains, under controlled conditions. Samples were blind coded to prevent any bias. Panellists tasted samples and recorded their key observations for appearance, aroma, flavour and texture using a keyboard to allow free typing. Following individual attribute generation, the panel leader led a group discussion to determine a consensus regarding the profiles of the samples.

On farm Barramundi harvest trials

An initial survey of industry was conducted to establish current commercial crowding, harvesting and chilling protocols. Survey results are presented in Appendix 2.

Conventional farm harvests assessing stress likely to be imposed on the fish during the crowding phase of harvest were carried out on three separate North Queensland (NQ) farms. Fish stress levels (flesh quality, pH and rigor) were determined for the very first fish removed from the crowd (short crowd) and the very last

fish from the crowd (long crowd) to gain information on the stress caused by crowd duration. Tests were carried out using the specific farms typical harvesting practices and hence these trials also provided data on stress currently imposed on commercially harvested Barramundi. All fish were euthanised prior to invasive testing as per animal ethics approval.

A conventional net and brail system was used by trained harvest teams for the five (5) harvests monitored (A, B, C, D and E) outlined below. Each harvest team used a similar approach. A harvest net with floats on the top edge and a weighted bottom edge was pulled slowly across the pond to partition the desired number of fish for the harvest. One end of the net was dragged around the edge of the pond to form a loop enclosing the fish until they were sufficiently crowded.

Crowding was monitored using a four-point stress scale.

1 – calm water no visible sign of fish struggling or coming to the surface

2 – fish breaking the surface occasionally jumping

3 – fish coming to the surface gasping

4 – fish floating upside down unable to maintain orientation

Approximately, 100–200 kg of fish were removed per batch at a single time using a brail net and lifted out of the pond. Fish were spilled into a 1000L bin containing an ice slurry by opening the cod end of the net once positioned over the bin. Fish were spilled into the bin until full (approximately 400 kg +).

After the final fish ice slurry bin from that the days harvest was filled, all bins were transported back to the processing area.

For the short crowd fish, groups of five (5) random Barramundi were taken from the slurry of the first bin filled, one group for flesh analysis and the other group for pH and penetrometer flesh tension. Before sampling, each removed fish was checked to confirm that it was unconscious and insensible showing an absence of: opercular movement, eye movement, response to painful stimuli via a prick to the tail using a needle and eyeroll test as described Kestin *et al* (2002). They were iki jimed to euthanise before being sampled for chemical analysis. A 40 g sample was removed from each of the 5 fish, by cutting a 15 mm strip of flesh perpendicular to the lateral line, adjacent to the front edge of the dorsal fin (Figure 2). The skin and dark muscle were removed before the shoulder portion of the sample was wrapped in aluminium foil and immersed directly into liquid nitrogen. Samples were held frozen in liquid nitrogen while being transported to the Coopers Plains DAF research facility. On arrival they were transferred to a -80 °C freezer and held frozen until testing for K-value and lactic acid.

A second batch of five fish were removed from the ice slurry of the first bin, then iki jimed to euthanise before being sampled. Fish were tagged with a number attached to a cable tie that was fastened around the operculum and tested for pH and rigor state before being placed back into the ice slurry. The pH and rigor were monitored at 0, 1, 2, 4, 8, 12, 24, 30, 48 and 72 hours.

The long crowd fish were sampled from the last bin filled. As before, fish were held in the ice slurry until there was nil response to external stimulus (approximately 20 minutes) and were also iki jimed as directed by the animal ethics approval. Five (5) fish were removed and processed following the same procedure as discussed above, to provide 40 g samples for future chemical testing. A further five (5) separate fish were tagged and tested for pH and rigor and returned to the ice slurry, for further post-mortem monitoring of pH and rigor over the following 48 to 72 hours.

Whole fish core temperatures

Core temperatures were taken of whole fish held chilled in the ice slurry by cutting a small incision with a sharp knife in the thickest part of the shoulder flesh of the Barramundi just in front of the dorsal fin to the left side of the centre line of the fish (Plate 4). A Thermocron TC temperature logger (Onsolution NSW

Australia) was inserted and pushed down into the incision until the probe rested against the backbone of the fish. The flesh of the fish closed back over the narrow incision, fully concealing the entry point of the logger. Temperatures were decoded using eTemperature software V9.06.

Plate 4. Showing the position of the entry point for the temperature logger to be inserted into farmed Barramundi.

Ice slurry temperatures

Ice slurry temperatures were recorded using a thermocron TC temperature logger (Onsolution, NSW Australia). Loggers were suspended from a string allowing the logger to record the ice slurry temperature at the desired position in the slurry (top or bottom of the slurry bin). Temperatures were decoded using eTemperature software V9.06.

Laboratory Ice slurry testing method

To identify ice to water ratio performance efficiencies and any stratified temperature differences, a trial of various ice slurry mixtures was tested.

A matrix of 16 ice slurry variables, covering four (4) ice to water ratios by four (4) different salt concentrations (Table 3) was determined and assessed based on temperature and stratification differences. The upper and lower limits of these ratios were identified in the Barramundi farmer survey.

Each of the 16 ice-water ratios verses. salt concentration slurry variables were measured and recorded as per the following method:

- The appropriate amount of Olsson's kiln dried fine pacific salt was weighed out to +/- 0.1 g and added to a 200 L non insulated Nally tub.
- 25L of potable town water at 22 °C was measured using a 25 L bucket with a calibrated fill line scribed in the side.
- The water and salt were mixed until completely dissolved.
- Initial salinity was measured using a TPS meter model WP-81 fitted with a TPS K10 (EC1003) conductivity probe for measuring salinity.
- The appropriate volume of ice was measured using the same calibrated bucket in multiples of 25 litres depending on the ice ratio required.
- The ice slurry mixture was continuously stirred for 5 minutes.
- At 5 minutes salinity was recorded again.

- At 5 minutes the slurry temperature was recorded using a Comark Instruments USA model C26 digital thermometer +/- 0.1 °C.
- A new slurry was made up in the same way for each of the 16 matrices.

Table 3. Combinations of water, ice and brine tested.

Brine (salt %)	**Water:ice** ratio
2	1:1
	1:2
	1:3
	1:4
3	1:1
	1:2
	1:3
	1:4
4	1:1
	1:2
	1:3
	1:4
5	1:1
	1:2
	1:3
	1:4

Data collection and analysis

The questionnaire used to collect data was designed using the specialist sensory software EyeQuestion® (version 5.0.4.4). The partner data analysis tool EyeOpenR® (version 5.0.4.4) was used to analyse the data obtained from the triangle tests. If the number of correct responses is greater than or equal to the number given in the correct statistical table (which corresponds to the number of assessors), it can be concluded that a perceptible difference exists between the samples.

Single time point data collected from the replicated rested and stressed harvest trials were analysed using analysis of variance (ANOVA). All significance testing was performed at the 0.05 level. Confidence intervals (CI) about the sample means were calculated for the un-replicated on farm trials. Payton et al. (2003) showed that when 84% CI do not overlap, the type one error is approximately 0.05 and therefore the samples can be considered significantly different. Analyses were performed using Genstat for Windows, 21st Edition. Sample number of fish were taken into account and reduced to minimum numbers possible for animal ethics requirements.

Results and Discussion

Rested and stressed harvests trials

Flesh pH

Measurements of pH change of individual Barramundi (n=5 per replicate by 3 replicates) from time of harvest up to 48 hours were averaged and are presented in Figure 4. In this study, the mean pH values at time 0 for stressed fish (6.59 ± 0.11) was significantly lower ($F_{1,4} = 419.6$; $p < 0.001$) compared to rested fish, (7.25 ± 0.12). The trend in pH values post-harvest was also found to differ between the stressed and rested fish. The overall mean pH obtained for stressed and rested fish in the first 48 hours post-harvest was significantly lower in stressed fish ($F_{1,4} = 23.5$; $p = 0.007$). Rapid increases in post-mortem pH were observed in repetitively sampled Barramundi from both treatments. Even though a fresh cut was made each time the pH was taken beside the last reading, acid may have been diluted out as Barramundi were re-submerged in the slurry. While this artefact is unfortunate, repetitive sampling minimises the number of unsaleable fish generated by on-farm observations. This initial hump in flesh pH is not seen when Barramundi are filleted and the flesh bagged and chill-stored (DAF, Unpublished data, Percival, 1999, Poole et al., 2000, Wilkinson et al., 2008).

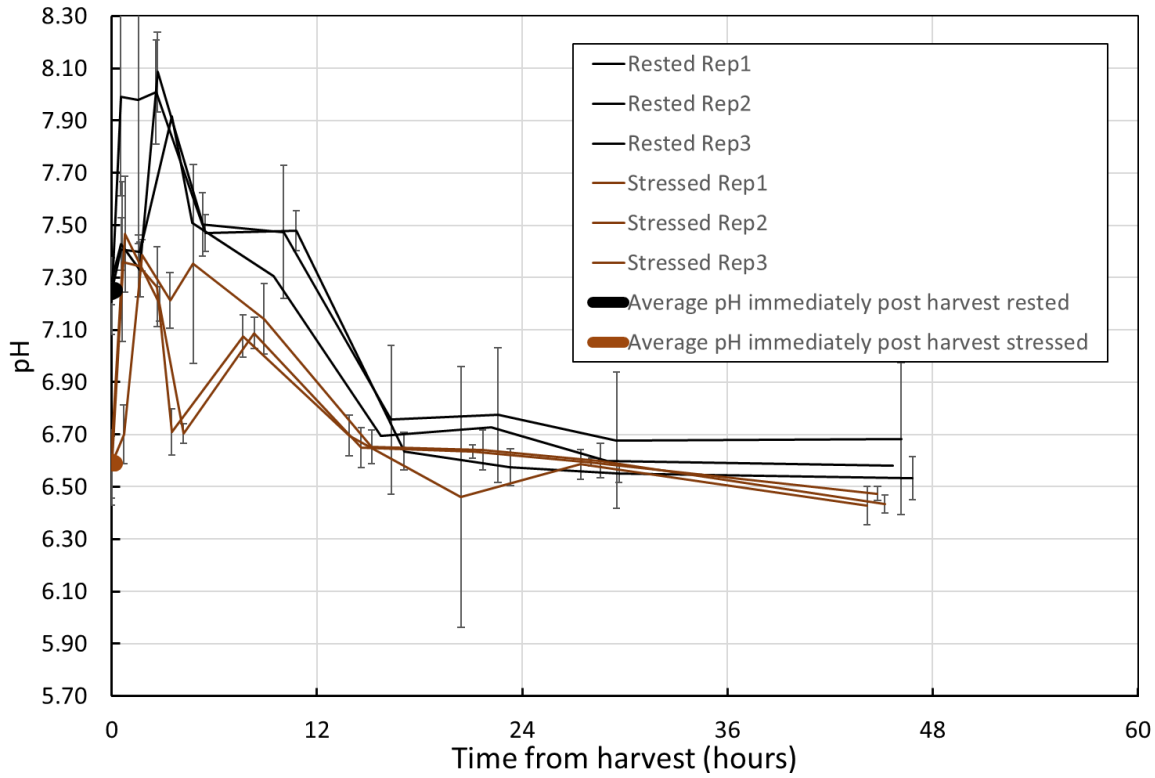

Figure 4. Average pH of controlled stressed versus rested harvest trials with Barramundi.

Typically, a living fish has a flesh pH more than pH 7.0 upon capture. For example, previous research on Barramundi reported by Wilkinson et al. (2008) identified pH of 7.12 ± 0.06 for rested Barramundi, similar to the results of a parallel study by Howieson et al. (2013). This is also within the range of values found previously in European sea bass (*Dicentrarchus labrax*), carp (*Cyprinus carpio*), and several salmonid species (pH ranged from 7.00 to 7.63) (Ogata et al., 2016). In contrast, a study by Robb et al. (2000) identified that rainbow trout (*Oncorhynchus mykiss*) typically have a resting pH of between 7.6 and 7.8. Wilkinson et al. (2008) suggested that the lower pH value exhibited by Barramundi may be characteristic of this species or

could also be due to a small degree of activity and lactate production during the harvest and slaughter method. An earlier study by Poole et al. (2000) showed Barramundi given a lethal dose of Aqui-S (60 ppm) or gradually sedated with CO_2 returned a flesh pH of around 6.9 when sampled immediately, not significantly different from ice-slurry killed individuals in that study (pH 7.01). When Barramundi were placed in freshwater super-saturated with CO_2, recreating a technique originally applied to Atlantic salmon, their aversive reaction to that medium saw lower flesh pH (pH ~6.82) (Poole et al., 2000). This is similar to levels observed when un-sedated Barramundi were scoop-netted and killed by percussive stunning in air, (Percival, 1999).

Simulated stressed harvest of Barramundi at time 0, returned a flesh pH value like that observed in the study of Wilkinson et al., (2008) study i.e., 6.35 ± 0.09, a finding corroborated from Barramundi harvested from sea-cages by Howieson et al. (2013). This general trend for handled and stressed fish to record a low pH (i.e., below 7.0) immediately after death was further confirmed by observations in other fish species utilising a range of harvest methods considered to be stressful (Einen & Thomassen, 1998; Robb et al., 2000). Wilkinson et al. (2008) reported that flesh pH of rested fish was initially significantly higher and it also remained elevated until 18 h post-harvest. Similarly, this study found that rested Barramundi had a higher muscle pH until approximately 16 h post-harvest before decreasing to around the 6.5–6.8 range.

Stressed Barramundi are starting post-harvest storage at a lower pH, potentially leading to softer flesh and possible muscle gaping over time. Fish that are harvested with a minimum amount of injury or stress will have high energy reserves, a higher pH and firmer flesh (Lowe et al., 1993). Fish that are allowed to struggle prior to death generally have lower energy reserves, a low pH and softer flesh (Sigholt et al., 1997; Mazur et al., 1993). After death muscle glycogen is broken down via glycolysis to pyruvic acid and then lactic acid. As this happens, the flesh becomes more acidic. In a range of fish species, increased activity and stress prior to death, and the subsequent endocrine response, have been found to result in a rapid drop in muscle pH due to increased lactic acid from white muscle anaerobic metabolism (Azam et al., 1989; Berg et al., 1997; Clements et al., 2002; Poli et al., 2003; Poli et al., 2005; Stien et al., 2005; Erikson et al., 2006). A decreased muscle pH results in a rapid onset of rigor mortis and may also result in reduced shelf-life, increased muscle gaping and blood spotting, flesh texture alterations and reduced water holding capacity of the muscle (Jerrett et al., 1996; Robb and Kestin, 2002). A low post-rigor pH is often associated with soft flesh in well-nourished fish with good nutritional status and high glycogen stores (Skjervold et al., 2001).

Rigor

Rigor mortis is a condition that causes muscles to stiffen/contract and is set in motion soon after a fish dies. The degree of muscle contraction is dependent upon the energy reserves remaining in the fish and the temperature at which the fish enters rigor. Thus, fish held at room temperature proceed through rigor faster than those stored in ice. The changes characteristic of rigor mortis include a drop in pH, a conversion of glycogen to lactic acid, a loss of protein buffering capacity, a decrease in creatine phosphate, and a breakdown of ATP (Szczesniak & Torgeson, 1965).

Rigor mortis is the first post-mortem process that has a major influence on the appearance and structure of the fish muscle (Berg et al., 1997), particularly if fish are processed in rigor which may result in reduced fillet yield, texture alteration and increased gaping (Einen et al., 2002; Jerrett et al., 1998; Özogul and Özogul, 2004).

Rigor or stiffness of the muscle is strongly related to flesh quality. When severe, rigor processes can result in quality deterioration in the form of 'muscle gape', or in fillets, shortening of the flesh. When rigor processes are delayed the flesh quality is generally improved, and this is usually accompanied by an extension in shelf-life (Einen et al., 2002; Jerrett et al., 1998).

Many changes in the quality and textural attributes of Barramundi can be brought about because of the harvesting and processing conditions used after capture. Shear force values for the flesh of very fresh fish are high because rigor mortis develops so quickly that contraction cannot be avoided. As rigor progresses, the values drop and become more stable.

The degree of struggling upon capture (muscle activity) can also influence the final texture of the fish. Fish that are harvested with a minimum amount of activity, injury or stress will have high energy reserves, a higher pH and firmer flesh. However, fish that are allowed to struggle (having high muscle activity) prior to death, generally have lower energy reserves, low muscle pH and softer flesh.

Results from this trial show that stressed Barramundi enter rigor quickly (Figure 5), with a firm contraction of approximately 65 penetrometer units, beginning to slow at approximately three hours from harvest. In contrast, the rested Barramundi take approximately nine hours to go into rigor from harvest (Figure 5). This is consistent with the flesh having more muscle glycogen present at the time of death and taking longer to metabolise to lactic acid. Similar results were found in CRC salmon trials by Goodrick et al. (1998), which showed that stressed salmon entered rigor more quickly than rested salmon, albeit much softer in peak stiffness at around 30 units. Goodrick et al. also demonstrated the same effect of stressed salmon entering rigor faster using both the penetrometer method or tail droop method as used by Iwamoto et al. (1987). This is in agreement with the findings of Howieson et al. (2013) and Wilkinson et al. (2012), where both studies used the conventional tail-droop method. In the current study, the peak stiffness of around 70 units agreed with that reported previously for the technique in Barramundi (Poole et al 2000). These findings were similar to those reported by Wilkinson et al., 2008.

This delayed time to enter rigor effectively preserves quality and extends the shelf life of the fish. A statistical comparison of the rate of exponential increase confirms that stressed fish in this study take a significantly shorter time to enter rigor compared to the rested fish ($F_{1,4}$ = 38.80; p = 0.003).

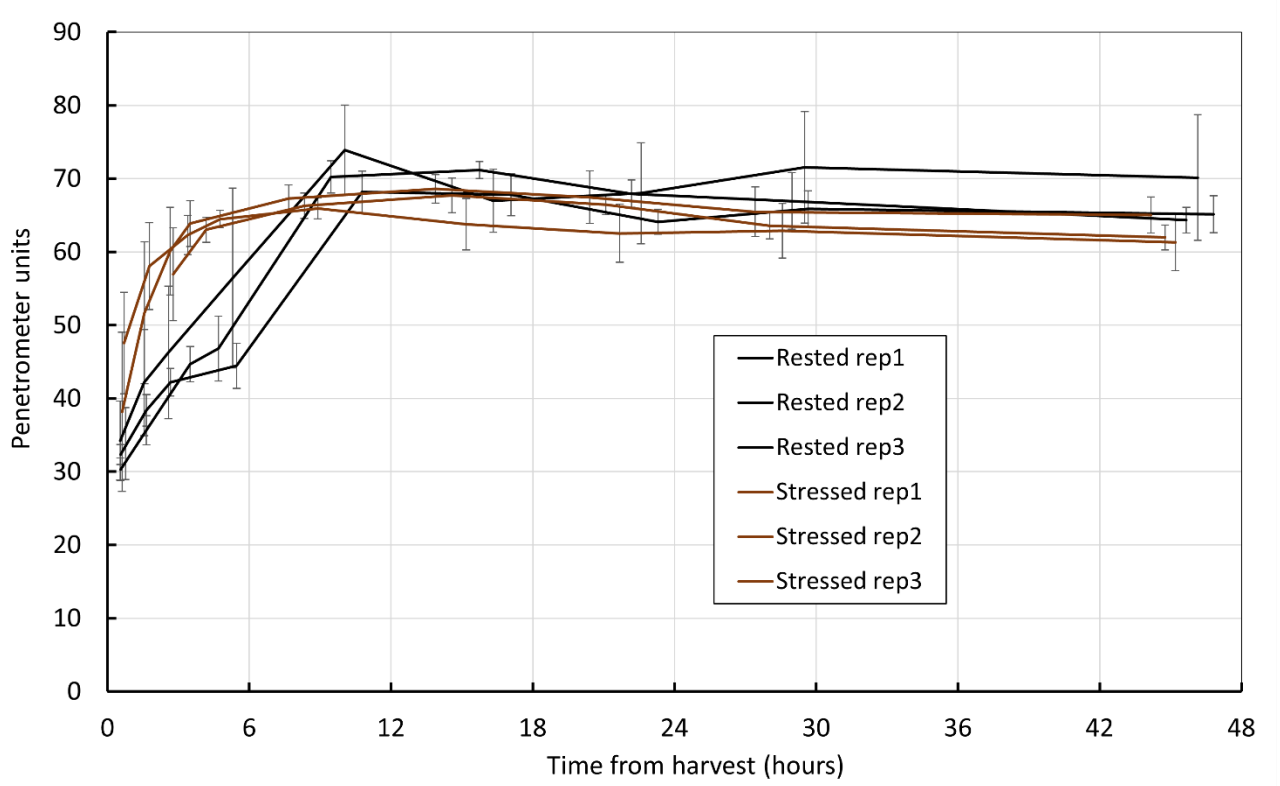

Figure 5. Average penetrometer results controlled rested versus stressed harvest trials with Barramundi. Penetrometer units increase as fish become stiff through the changes associated with rigor mortis.

Wilkinson et al. (2012) reported that pre-rigor filleting allows muscle fibres to contract freely from the vertebrae. A consequence of this is that there is lower tension between muscle fibres and myotomes, which results in less flesh gaping (Einen et al., 2002). Furthermore, pre-rigor processing has been shown to

lead to considerably firmer texture (Jerrett et al., 1998) and superior colour (Skjervold et al., 2001). It is recommended that once rigor starts in the fish or fillets, further handling and processing should be avoided until the rigor resolves (Erikson, 2001). Rigor resolution in salmon has been reported to take up to two to four days (Robb, 2000). This current study measured rigor for up to forty-eight hours and noted that rigor had not fully resolved in that time frame. The project team has previously performed quality assessments on farmed Barramundi with fish shipped from North Queensland to Brisbane via road freight taking more than nine days to fully resolve. (Figure 6).

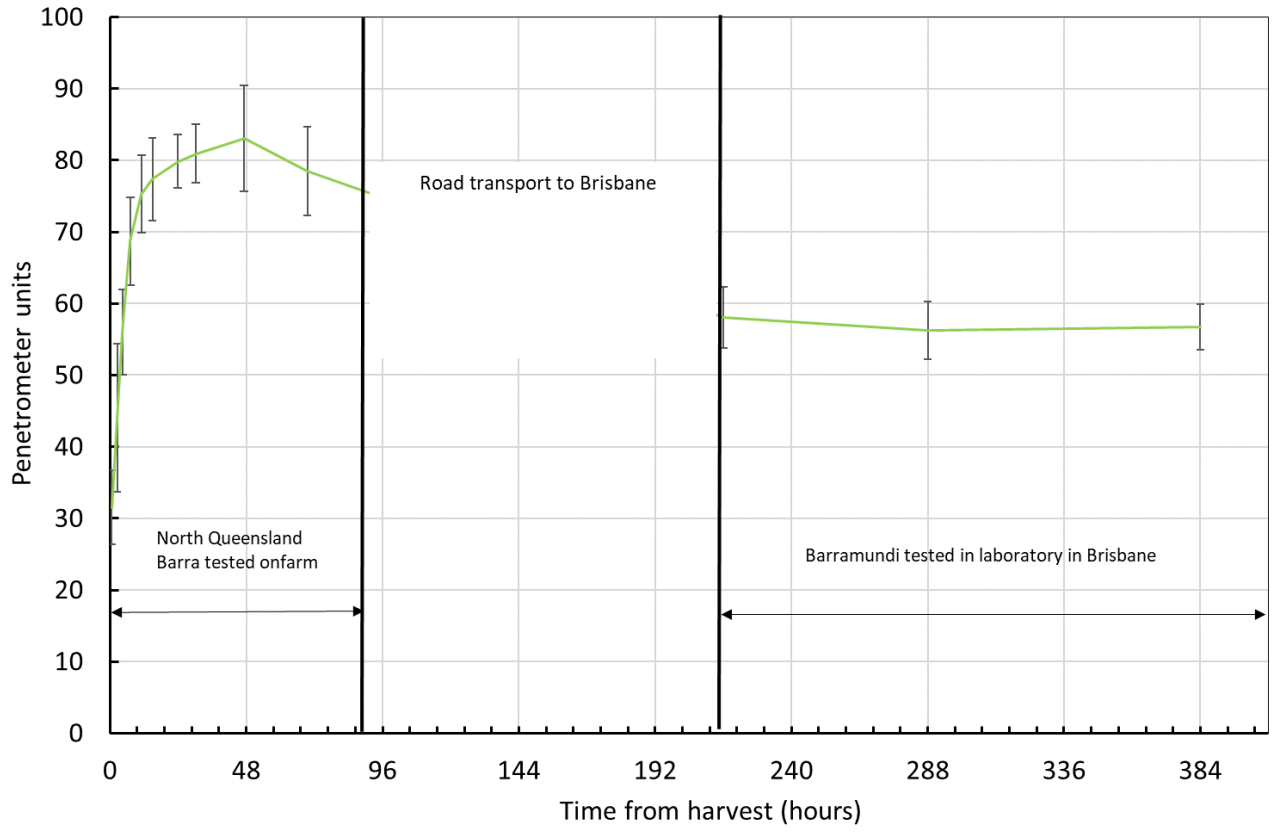

Figure 6. Penetrometer results tested on farm and again post road freight to Brisbane.

Texture of cooked fillets

It is well accepted that stress resulting in significant muscle activity before slaughter can affect several quality characteristics of fish flesh (Azam et al., 1989; Erikson et al., 1997; Sigholt et al., 1997; Skjervold et al., 2001), specifically fillet texture and gaping (Lavety et al., 1988; Andersen et al., 1994;). Even short 10-minute crowding stress was found to cause rapid onset of rigor mortis, resulting in softer flesh texture (Sigholt et al., 1997).

Cheng et al. (2014) conducted a review on how different handling methods play a significant role in fish texture measurements. Sigholt et al. (1997) studied the effect of handling stress on the texture of farm-raised Atlantic salmon and reported that the handling stress had an influence ($P< 0.001$) on the firmness of salmon fillet. In order to investigate suitable methods for handling the fish prior to slaughter, Roth et al. (2002) made a preliminary exploration on the texture changes of Atlantic salmon by reviewing three diverse handling methods, including stunned by electricity, stunned with carbon dioxide (CO_2), and percussion prior to slaughter. Compared with the other two stunning methods, stunning with CO_2 method played an obvious role in the fish texture, resulting in an earlier onset and resolution of rigor mortis, thus accelerating the softening of the muscle tissue.

Sensory analysis of Barramundi has occurred previously in nutrition and off-flavour research (Glencross, 2006). At the same time some consumer reports have identified 'mushy' flesh in marketed Barramundi, but sensory research in Queensland and Western Australia has not previously confirmed a link to harvest

practices, though data from commercial harvests was limited (Calogeras, 2015). Flesh texture has however firmed up significantly in response to preharvest fasting (Howieson et al., 2013) and in response to experimental diets (Glencross, 2006), both circumstances where it is conceivable that stores of muscle glycogen fall, potentially altering the extent of post-mortem acidification. This hypothesis is consistent with earlier reports that Barramundi had firmer flesh when growing poorly on an inadequate experimental diet, (Williams & Barlow, 1999).

In the current study, the cooked samples from the rested harvest fish from each replicate all required significantly more energy to cut through the sample than the stressed fish ($F_{1,4}$ = 7.81; p = 0.049) (Figure 7). The overall average energy required to cut the rested fish was 16.7 Nmm/g compared to 9.0 Nmm/g for the stressed fish. This indicates a firmer cooked texture for rested harvest fish and a softer texture for the stressed harvest fish. The stressed fish have used up glycogen during the struggle (high muscle activity) of the harvest and this has accelerated the rigor process. The fish go into the onset of rigor much faster, leading to the potential to tear muscle fibres. As the fish flesh resolves and softens from the rigor process, the resulting flesh is softer as seen in these results and are sometimes quite mushy.

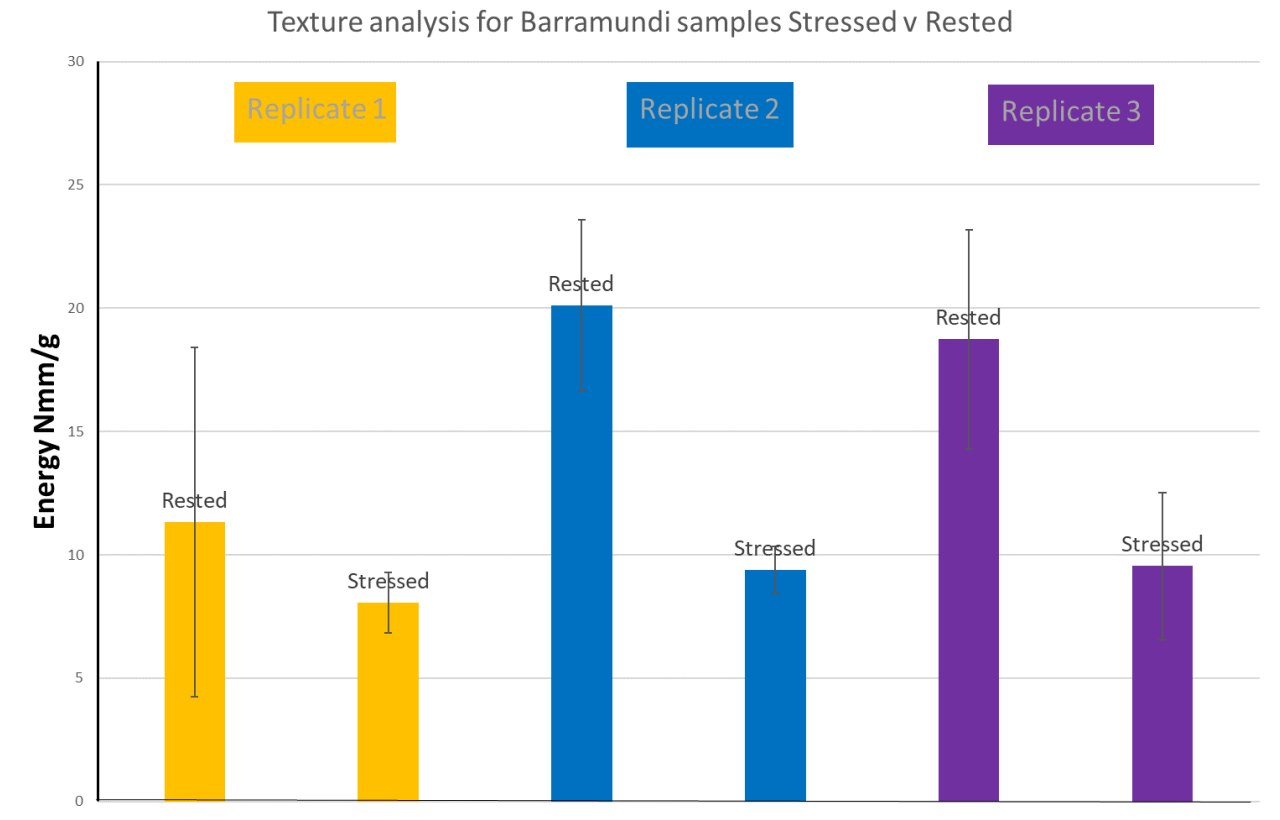

Figure 7. Average texture (force/energy (Nmm) required to cut through sample) of flesh samples of farmed Barramundi from controlled stressed versus rested harvest trials.

K-value and lactic acid analyses

The nucleotide and lactic acid concentrations of Barramundi subjected to either harvest treatment (mean ± SD, n=15) are compared to the initial muscle pH in Table 4. Despite the stark difference in initial muscle pH and rigor mortis rate, the two treatments did not differ significantly (P>0.05) in any of the biochemical quality/welfare indicators examined, though high variation was observed in the ATP concentration of rested harvest fish. Raw data is presented in Appendix 6 and 7.

Table 4: Combined pH, nucleotide (µmoles/g FW) and lactic acid (mg/g FW) concentration means, standard deviations (SD) and ranges for fish subjected to controlled stressed and controlled rested harvest trials.

Treatment	Results	pH	ATP µmoles/g	IMP µmoles/g	Hx µmoles/g	K value %	Total Nucleotide µmoles/g	Lactic Acid (mg/g FW)
Unstressed AQUI-S 16/06/2021 Samples (77 - 91)	Av.	7.25	0.23	11.08	0.08	0.62	11.71	2.24
	SD	0.12	0.13	1.04	0.03	0.18	1.06	0.23
	Range	7.09 - 7.39	0.115 - 0.815	8.238 - 13.304	0.044 - 0.173	0.24 - 0.85	8.6 - 13.9	1.65 - 2.75
Stressed 16/06/2021 Samples (92 - 106)	Av.	6.59	0.18	12.01	0.11	0.62	12.60	2.32
	SD	0.11	0.03	0.82	0.05	0.26	0.86	0.26
	Range	6.38 - 6.72	0.078 - 0.220	9.542 - 13.413	0.046 - 0.194	0.22 - 1.32	9.9 - 14.0	1.69 - 2.80

Nucleotide content and K-value

Negligible ATP, elevated IMP levels and low K-values observed in Table 4 are typical of fresh-frozen Barramundi fillets, as adenylate nucleotides (ATP, ADP and AMP) are rapidly degraded, and IMP accumulates rapidly when the Barramundi dies (Saito et al., 1959, Williams et al., 1993). This result is remarkable: these t=0 samples were in liquid nitrogen until processed for analysis. The negligible accumulation of hypoxanthine (Hx) attests that the samples remained fresh and did not thaw and spoil in transit. ATP content of the spotted mackerel killed by neck breaking was determined to be around 7.8 µmoles/g immediately after death, while the fish killed by struggled suffocation possessed ATP levels that started decreasing rapidly and diminished to trace amounts 2 hours after death, (Ogata et al., 2016). The authors reported the ATP concentration of the neck broken fish remained around 6.3 to 9.1 µmoles/g for 2 hours after death and then decreased gradually to around 3.2 µmoles/g at 8 hours.

Post-mortem nucleotide degradation has not been measured previously in Barramundi; however a similar study was conducted a decade ago with farmed Atlantic cod. When sampled immediately, percussive and rested harvest treatments delivered high muscle ATP levels, unlike levels seen in cod chased around a tank, (Digre et al., 2011). The ATP levels observed in these ice-slurried Barramundi are similar to those returned by 15 min air asphyxiation of rainbow trout, yet in that study the less stressed electro- and CO_2-stunned fish were sampled immediately after application of the stun and not slurried (Concollato et al., 2016). Sectors like Atlantic salmon process the killed fish almost immediately so published t=0 samples in those represent the fish immediately post-treatment rather than post-chill. However, in commercial conditions, farmed Barramundi may be chilled in a slurry for as much as a day before they are packed and shipped. It is important to understand whether ice-slurrying the living muscle of Barramundi can interfere with the benefits of prior harvest treatments. This point will be picked up in the discussion later.

Lactic acid content

The lactic acid values for the muscle samples from stressed verses rested harvest showed no significant difference due to the harvest method ($F_{1,4}$ = 0.37; p = 0.577). At equilibrium, the muscle lactic acid concentration should be causally correlated with muscle pH, but a correlation did not occur in this t=0 data (r = -0.131; p = 0.491) (Appendix 5). Perhaps at this one time point soon after death, treatment may determine the availability of H^+ ions on a cut fillet surface, even though sample homogenisation and extraction quantifies uniform amounts of lactate inside the muscle. Studies of longer post-mortem periods have reported a significant linear relationship between muscle pH and lactic acid content (Fukuda et al., 1984; Ogata et al 2016). In the former study, by 8 hours after death of chub mackerel samples the lactic acid reached 9.04 mg/g and pH decreased to ~ pH 5.8.

The averages of ~2.5 mg/g FW in this experiment using these ice-slurry killed Barramundi (Table 5) are similar to those obtained in Barramundi sampled during on farm trials: Trial 1 (1.78 – 2.74 mg/g FW), Trial 2 (2.38 – 3.48 mg/g FW) and Trial 3 (2.35 – 3.28 mg/g FW). Additionally, the results are also comparable to the initial lactate concentrations of spotted mackerel (*Scomber australasicus*) killed instantaneously by neck breaking, measured to be around 2.21 mg/g FW (Ogata et al., 2016). However, all are elevated compared to experimental results for average levels of muscle lactic acid of rested-harvest Channel catfish at slaughter: 0.81 – 0.90 mg/g FW (Bosworth et al 2007). Those catfish were harvested with Aqui-S sedation, immediately iki-jimed and samples taken.

Few outside of our laboratory have measured total muscle lactic acid content in Barramundi. Barramundi that are Aqui-S sedated, iki-jimed and sampled without ice-slurrying returned a post-mortem lactic acid level in muscle of 0.7 mg/g FW (DAF unpublished data). Muscle lactate is a baseline parameter worth measuring as fish generally keep this stress metabolite inside the muscle, there is a delay before the indicator enters the blood, (Noble et al., 2018). Percival (1999) found that plasma lactate levels in Barramundi had little utility as an indicator of shallow water stress or exercise, but the parameter reliably rose in cases of severe hypoxia (<10% saturation) but harvest crowds will elevate blood lactate concentration (Howieson et al., 2013). Yet it was 60 min after simulated conventional harvest before plasma lactate level increased significantly in Barramundi (Wilkinson et al., 2008),

This is not the first time that baseline and stressed Barramundi have returned the same muscle lactic levels. The act of catching and killing Barramundi for sampling purposes is reported to exert a strong equalising effect on their muscle biochemistry despite prior exposure of some to severe stressors (Percival 1999). If the lactic acid does not accumulate before death, it can still be produced during and after death. That author found no significant effect of prior stress, even severe hypoxia (1h at 5–10% of saturation at 26 $^{\circ}$C), on lactic acid and pH in the muscle immediately post-mortem (Percival, 1999). Initial post-mortem lactic acid levels in those Barramundi were 1.2 – 1.6 mg/g FW and muscle pH was 6.6 to 6.9 regardless of treatment, (Percival, 1999). However, those Barramundi were scoop-netted and immediately killed using a manual percussive strike to the head. That author recommended that sedation was probably needed to capture baseline levels of muscle lactate. However, the rested Barramundi in the current study were sedated- where the two studies differ is that our fish were placed in an ice slurry for 15 min before the fish were retrieved and the muscle sampled.

Muscle lactic acid levels exceeding 1 mg/g FW in fish have been associated with anaerobic glycolysis resulting from either hypoxia or exhausting exercise (Speers-Roesch et al., 2013). Woo and Chiu (1997) reported an even higher baseline level of muscle lactic acid content in Barramundi, at ~3 mg/g FW. Those authors did not describe sedation or euthanasia method in the sampling methods and their result exceeds some levels reported when other fish species are highly stressed by capture by anglers 1.0 – 4.2 mg/g FW (Arlinghaus et al., 2009, Davidson et al., 1997, Lowe et al., 1993, Killen et al., 2003).

The rested and chased Barramundi compared in Table 4 were all removed apparently dead from a slurry and death was assured by iki-jime before cutting the fish. Ice-slurry killing of fish is often described as asphyxiation, but apart from our own unpublished work, which underpinned a previous DAF-ABFA study (Poole et al., 1999), the literature search produced few measurements that might help in understanding what cold-aversion does to lactic acid levels in the muscle of farmed Barramundi. Ice-slurry killed grass

carp have lower muscle lactate levels, and higher ATP, than corresponding carp that were manually percussively stunned and not slurried (Qin et al., 2016). Different lactate levels persisted there despite the slurry step. More muscle glycogen is consumed when conscious rainbow trout enter ice-water versus unconscious electro-stunned individuals entering a slurry, presumably due to the aversive behaviour often described when conscious fish enter ice-cold water, (Bermejo-Poza et al., 2021). But that study showed that lower glycogen level was associated as expected with lower muscle pH. On the contrary, the current results show that the simple field measure of muscle pH at the time of death is a more reliable indicator of changes in post-mortem quality of harvested Barramundi than laboratory measures of muscle lactic acid content, the two are observed to be decoupled here by an unknown process.

Summary

K-value and lactic acid results highlighted the following points:

- In this study, harvest method was found to have a significant impact on the flesh pH of Barramundi. ***Fish harvested using AQUI-S (controlled rested trial) exhibited a higher flesh pH in comparison to fish harvested using a simulated more stressful conventional method.***
- When comparing Barramundi handled in different ways immediately after ice slurry slaughter, measures of flesh pH and rigor mortis appear to be more reliable as quality indicators than chemical measures of muscle lactic acid and ATP levels undertaken later in the laboratory.

Triangle and trained sensory panel testing

Demographics of untrained sensory panellists

Figure 8 shows the demographic information of participants (n = 33) that took part in this research. The cohort represented a cross section of age and included more females (73%) than males (27%). The majority were either the main household shopper (67%) or share this responsibility with someone equally (24%). Woolworths (70%), Coles (58%) and Aldi (52%) are the most popular stores for the main grocery shop. Sixty-four percent purchase their seafood from supermarkets and 45% from seafood markets. Forty-eight percent consume Barramundi at least once a month and the remainder consume it less frequently.

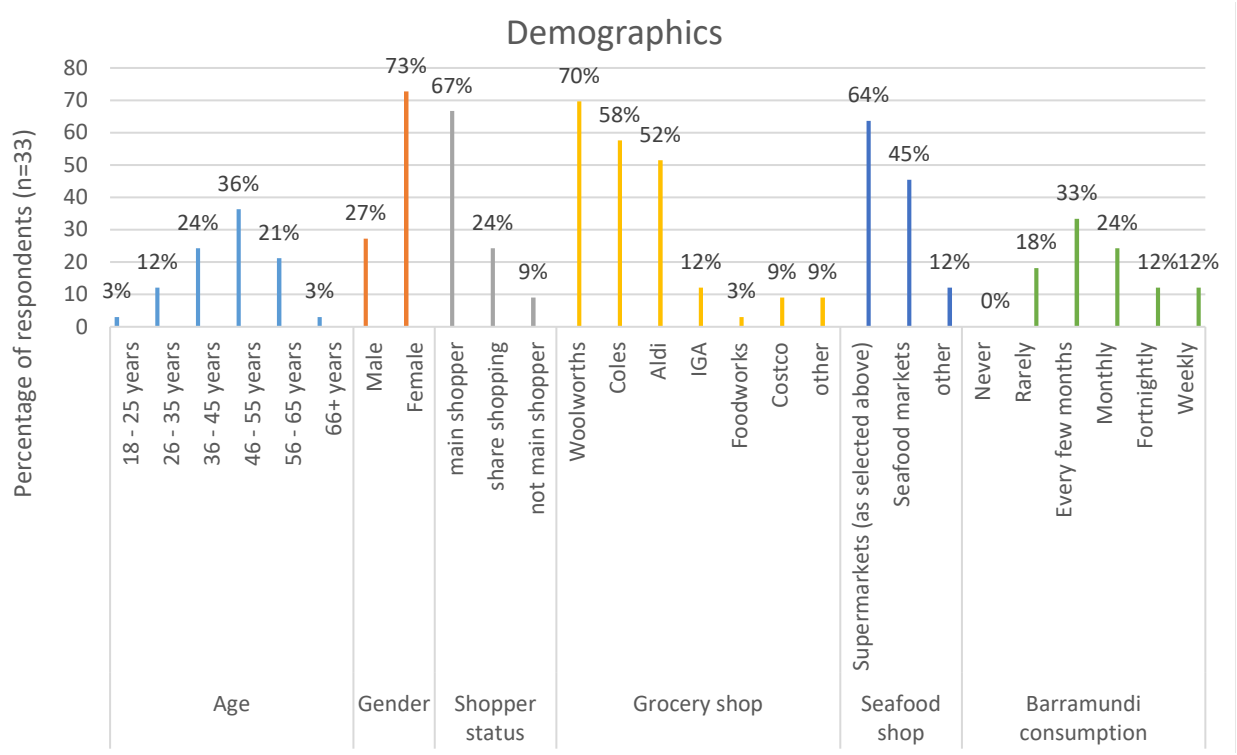

Figure 8. Demographic information of untrained triangle test participants (age, gender, shopper status, where they do their main grocery shop, where they purchase seafood and Barramundi consumption frequency)

Triangle test

Positive identification of the 'odd' sample in the triangle tests are depicted in Table 5. As per ISO method 4120:2021, results presented show the number of correct responses (i.e., those that correctly identified the different sample) out of 33 panellists. Results highlighted in red show the number of panellists able to differentiate between rested and stressed samples to a statistical significance level.

Table 5. Triangle test results

Sample sets	No. of correct responses
Orange	25
Blue	16
Purple	14

*Significant at α=0.001

Note:

- *For the result to be significant at a 95% confidence level, a minimum of 17 respondents would need to get the correct answer.*
- *For the result to be significant at a 99% confidence level, a minimum of 18 respondents would need to get the correct answer.*
- *For the result to be significant at a 99.9% confidence level, a minimum of 21 respondents would need to get the correct answer.*

In the orange sample set, 25 out of 33 respondents selected the correct sample as the odd one out. This means that the difference is detectable by consumers and the result is significant at a 99.9% confidence level. In the blue and purple sample sets, 16 and 14 out of 33 respondents respectively selected the correct sample as the odd one out. Overall, these results were not statistically significant at a 95% confidence level.

Was your answer a guess?

After completing the triangle test, respondents were asked if their answer was a guess or if they had a reason for their choice. Figure 9 shows that 91%, 70% and 82% of respondents had reason to choose their answer for the orange, blue and purple sample sets, respectively.

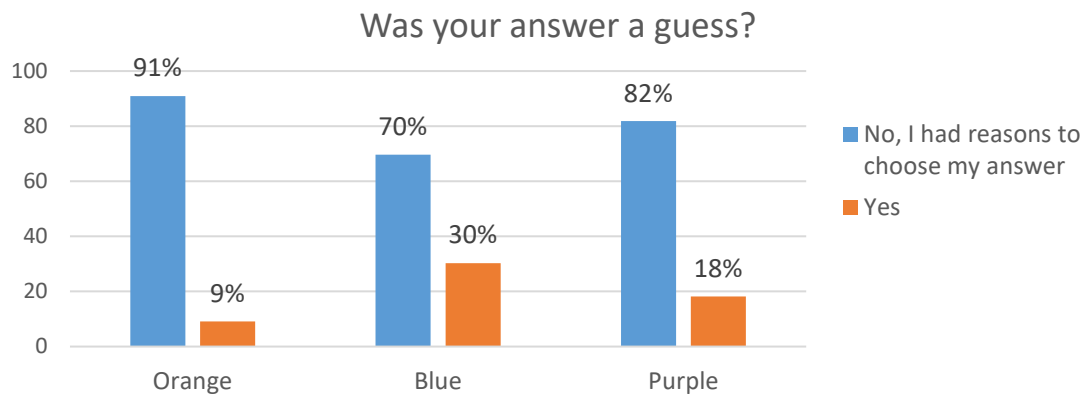

Figure 9. Percentage of responses that were guessed

Reasons for difference between samples

Respondents that said they had a reason for providing their answer were asked whether this reason was based on appearance, aroma, flavour or texture. Respondents were able to select multiple attributes from this list. Figure 10 shows that flavour and texture were selected by the majority of respondents across all three sample sets. When asked to explain this further, differences in sweetness and overall strength of flavour or fishy flavour were the most common flavour differences explained by respondents when allowed to give full responses via the keyboard. This was similar across all three sample sets. For texture, the most common explanations provided by respondents were differences in moisture, softness/flakiness, and firmness of the samples. Again, this was similar across all three sample sets.

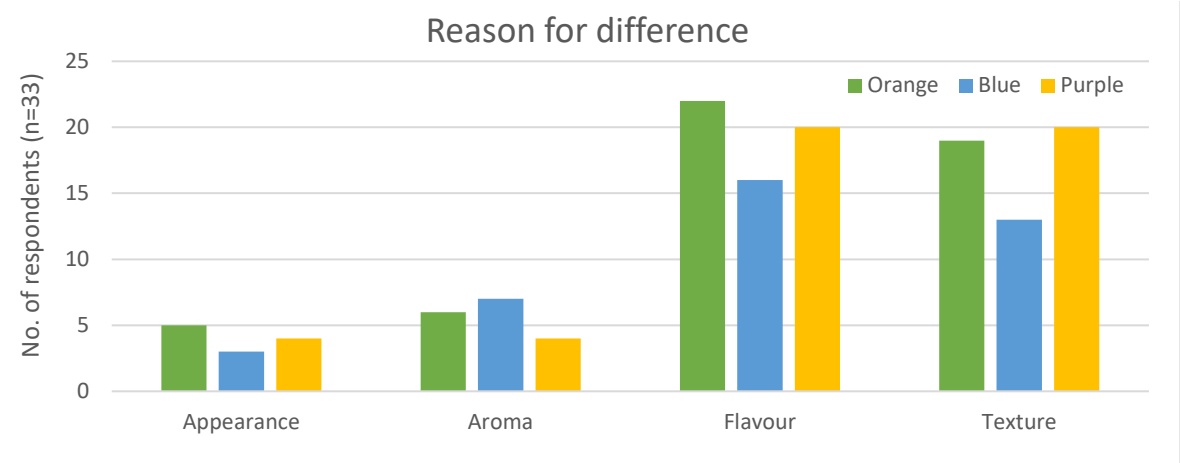

Figure 10. Reasons provided for difference

Preferred samples

After explaining their reasons for selecting the odd sample in each triangle test, respondents were asked which sample codes they preferred. Tables 6 and 7 show the number of respondents that preferred each

sample code based on flavour and texture respectively. The majority of respondents preferred the flavour of the stressed samples in the orange and purple sample sets. In the blue sample set, equal numbers of respondents said that they prefer both the stressed and rested sample. For texture, the majority of respondents preferred the stressed samples in the orange and purple sample sets and the rested sample in the blue sample set.

Table 6. Preferred sample codes based on flavour

Sample names	Sample codes	No. of respondents preferred	Total no. of respondents preferred (codes combined)
Orange rested	381	5	9
	637	3	
Orange Stressed	235	10	**24**
	451	14	
Blue Rested	706	4	11
	547	7	
Blue Stressed	934	7	12
	758	5	
Purple Rested	432	1	4
	108	3	
Purple Stressed	872	10	**23**
	261	13	

Note: Red numbers indicate a sample that was preferred by the majority of respondents

Table 7. Preferred sample codes based on texture

Sample names	Sample codes	No. of respondents preferred	Total no. of respondents preferred (codes combined)
Orange Rested	381	4	9
	637	5	
Orange Stressed	235	6	**15**
	451	9	
Blue Rested	706	7	**12**
	547	5	
Blue Stressed	934	4	6
	758	2	
Purple Rested	432	5	8
	108	3	
Purple Stressed	872	7	**18**
	261	11	

Note: Red numbers indicate a sample that was preferred by the majority of respondents

Trained panel and discussion

During attribute generation, ***the rested Barramundi samples were described by the trained panel as having a blue-white colour with a sweet mild oceanic flavour. The texture was assessed in the blind panel as having firm, plump, juicy fibres that slide apart in the mouth***. The trained panellists were able to pick up AQUI-S residual as an unknown taint or unusual after taste. Those who were familiar with AQUI-S were able to describe it as clove taint although it was only subtle flavour against the sweet mild umami flavour of the Barramundi (refer to Table 8).

Table 8Table 9. Sensory attribute generation by trained panel from controlled stressed versus rested harvest trials

Sample code	Appearance	Aroma	Flavour	Texture
527 Rested	This sample was a **white/blue grey** colour with **moist segments** visible.	This sample was characterised by a **sweet /mild /oceanic** aroma with **low clove/cinnamon** notes.	This sample was characterised by a **meaty /sweet/ umami** flavour with hints of **low salt/seawater cooked potato and slight taint.**	This sample was **firm/ plump/ soft/ tender/ flaky muscle fibres** with a **moist oily mouthfeel**.
780 Rested	This sample was a **white/blue grey** colour with **moist pale greyish lines visible.**	This sample was characterised by a **sweet/seawater/mild fishy** aroma with **unusual background** notes. Some panellists picked up the background aroma of AQUI-S but were not familiar with this taint in fish and had trouble describing it	This sample was characterised by a **meaty/ umami/ sweet Barra flavour** with **hints of seawater and low clove taint.**	This sample was soft /flaky and moist with bouncy/rubbery firm muscles bundles.
926 rested	This sample was a **pale white** with a **grey strands**.	This sample was characterised by a **mild fishy/oceanic** aroma with **low baked potato** notes. Some form of taint was noted.	This sample was characterised by a **meaty slightly bland flavour** with **low salt** and a **low clove taint**.	This texture was the **best** out of all the samples with a **flaky /juicy soft tender texture white muscle bundles sliding apart in the mouth.**
103 Stressed	This sample was **white/ grey colour** with metallic sheen.	This sample was characterised by a **meaty/hot potato** aroma with low **oceanic** *note.*	This sample was characterised by a **bland meaty baked potato** flavour with **low umami and salt**.	This sample was a **flaky/ soft smooth mouthfeel** that was tooth packing slightly dry.
372 Stressed	This sample was **white /yellow/grey colour** with a **glossy look.**	This sample was characterised by a **meaty/ hot potato** aroma with a **mild fishy finish**.	This sample was characterised by a **bland low umami flavour** with an **old /acidic/ metallic finish**.	This sample had a dry fibrous texture with a tooth-packing chalky texture.
618 Stressed	This sample was a **white/ grey/yellow colour** with a **smooth juicy** appearance.	This sample was characterised by a **strong meaty /hot potato** aroma with seawater notes.	This sample was characterised by **low meaty/potato/umami/sweet** flavours with **slight metallic liver** notes.	This sample had a **mushy melt in the mouth texture** with a **dry tooth-packing finish.**

In contrast, the stressed Barramundi samples had a yellow white-grey appearance with a meaty baked potato flavour. There was a low bland umami flavour with a slight acidic, metallic, liver after taste. This metallic-liver flavour is understandable given the uncooked samples of the stressed flesh had a pink hue prior to cooking due to excess blood cells present in the flesh. This gives a similar flavour taint to eating the red or dark meat muscle band in a fish, where it has an enriched concentration of blood cells to facilitate aerobic activity used in exercise. The acidic taste could come from slightly elevated levels of lactic acid post stress and exercise.

When the panellists were able to compare the samples side by side in an open discussion, it was more obvious to detect the differences and feel the plump fibres in the rested fish. The muscle bundle slid apart when squashed in the mouth, whereas the stressed fish flesh was soft but the texture was mushy and non-structured. This could be attributed to the engorged blood cells bursting when cooked and breaking down the cell walls contributing to a mushy texture. The flavour difference was also notable when treatments were compared side by side. The rested fish retained sweet umami salty flavour, whereas there was an acidic, old, metallic flavour in the stressed fish as previously discussed.

Triangle and trained sensory panel discussion

This study has shown that in two out of three sample sets, untrained consumers could not discern the difference between Barramundi samples treated with AQUI-S and untreated samples.

Although untrained consumers could not always discern the difference between samples, the main areas where they thought samples differed, were flavour and texture. Respondents felt that samples varied in their sweet taste, strength of overall flavour, strength of fishy flavour, softness/firmness and moisture/juiciness.

In the orange and purple sample sets, the majority of untrained panellists preferred the flavour and texture of the stressed samples. Whilst in the blue sample set there were an equal number of respondents saying

that they preferred the flavour of AQUI-S treated sample than the stressed sample. The texture of the AQUI-S treated sample was preferred by the majority of untrained consumers in the blue sample set.

These results show that there is not a significant difference in either flavour or texture between the rested and stressed samples when assessed by an untrained panel of regular consumers.

The triangle taste panel was not able to significantly decern between the two treatments and it is understandable as the differences that the trained panellists were able to focus on were very subtle. This also means that everyday consumers were not able to statistically pick up that there was a flavour taint from the use of AQUI-S as a rested harvest tool even though the trained panel were able to.

While it was not the intent of the research, further focused sensory investigations would need to be conducted before AQUI-S could be considered for use by ABFA as a harvesting tool. AQUI-S was only used in these trials as a tool to determine base line differences between stressed and rested fish.

Flesh colour

While the project did not specifically test the colour differences in the fish flesh, it was noted prior to cooking during the sensory trials, the colour of the flesh in the stressed fish visually showed a raw flesh colour had a distinct red hue (Plate 5). In contrast, the rested fish showed a raw flesh colour being more clear or opaque (Plate 5). Future studies could scrutinise level of residual blood in Barramundi fillets undergoing pre-harvest and harvest treatments. Previously, Howieson et al. (2013) used colour analysis in an investigation of skin and fillet melanisation. Svalheim et al. (2020) also showed this pink colour in stressed Atlantic cod as a quality parameter. Barramundi are not bled at harvest so the difference must be explained by blood re-distribution within the fish. There is already some corroborating evidence of this. Percival (1999) reported that release of extra red blood cells from the spleen was a common feature of the Barramundi stress response.

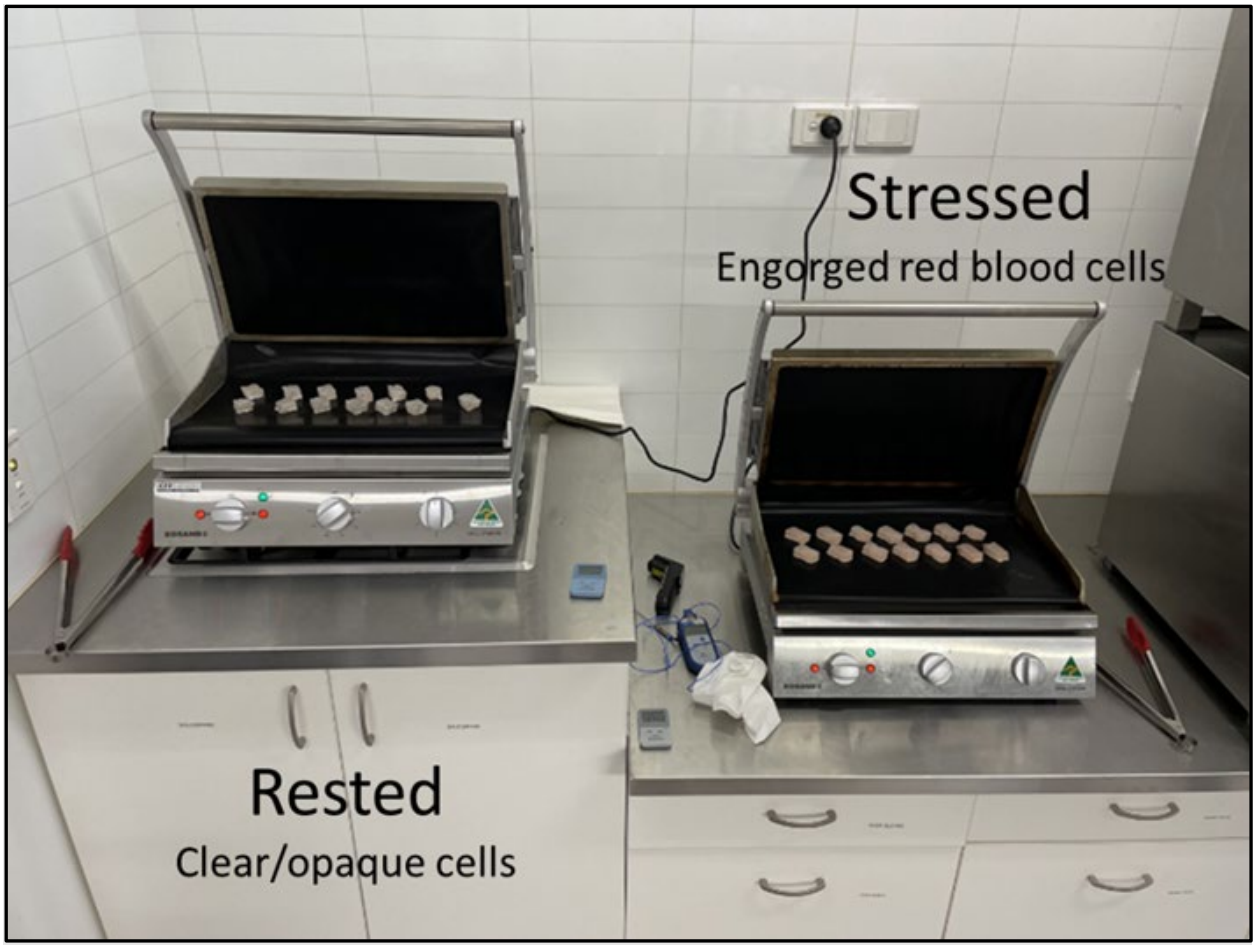

Plate 5. Raw flesh samples of the controlled rested (left) and controlled stressed (right) Barramundi.

When the controlled stressed fish samples are cooked the flesh turns from a red/pink to a yellow/white colour (Plate 6). This could possibly be attributed to the engorged blood cells bursting when cooked and releasing cellular fluid and moisture contributing to a mushy texture. In contrast, the controlled rested cooked fish changed from a clear/opaque to blue/white colour (Plate 6), retaining moisture and plump fibres resulting in a firm white flesh.

Plate 6. Cooked flesh samples of the controlled rested (left) and controlled stressed (right) of Barramundi.

Summary

The sensory assessment conducted by trained and untrained panellists highlighted the following points:

- The rested Barramundi samples were described by the trained panel as having a blue-white colour with a sweet mild oceanic flavour. The texture was assessed as having firm, plump, juicy fibres that slide apart in the mouth.
- The stressed Barramundi samples were described by the trained panel as having a yellow white-grey appearance with a meaty baked potato flavour. There was a low bland umami flavour with a slight acidic, metallic, undesirable liver after taste. The texture was assessed as having a soft flesh that was mushy and non-structured.
- There was not a significant difference in either flavour or texture between the stressed and rested samples when assessed by an untrained panel of regular consumers.
- When stressed fish samples are cooked the flesh turns from a red/pink to a yellow/white.
- When rested harvest fish are cooked the flesh turns from a clear/opaque to blue/white.

Farm Trials

Five on farm harvests (A – E) were monitored to assess which processes within the harvest were causing the largest amount of stress to the fish. The on-farm harvests were then compared against the baseline controlled rested and controlled stressed trials.

Harvest A:

Harvest A was a 4-ton harvest with the average size of the fish being 3.1 kg and 61.8 cm in length. At the time of harvest the pond water temperature was 28.2 °C. Dissolved oxygen measured prior to harvest was 5.5 mg/L. By the time the fish were sufficiently crowded to begin harvesting the dissolved oxygen in the pond had dropped to 3.7 mg/L at 40 minutes from the start of crowding. By the time the last brail was removed from the crowd the dissolved oxygen had dropped to 2.1 mg/L at 1 hour 50 minutes from the start of harvest.

As the oxygen levels dropped below 3.7 mg/L, it was observed that the fish were reacting with high activity (i.e., jumping and splashing stress level 2), trying to avoid the net as the holding area was slowly reduced. The fish quickly became visually stressed (level 3) with dorsal fins showing above the water level, gasping for breath. By the time the dissolved oxygen level reached 2.1 mg/L the fish were unable to maintain an upright swimming position and were exhausted (stress level 4) (Plate 7).

Plate 7. Exhausted Trial A fish due to low pond dissolved oxygen levels during harvest.

pH

The flesh pH results from Harvest A, show that the fish in a low dissolved oxygen environment (red line) have a lower terminal pH with an average of 6.34 for short harvest and an average of 6.37 for long harvest at 49 hours post-harvest. The corresponding 84% CI about the means are 6.28–6.40 (n = 5) for the short harvest and 6.32–6.43 (n = 5) for the long harvest. In comparison to the controlled rested and stressed trial, fish having an average terminal flesh pH of 6.60 (84% CI: 6.53–6.67, n = 15) and 6.45 (6.43–6.47, n = 15), respectively at 46 hours (Figure 11.).

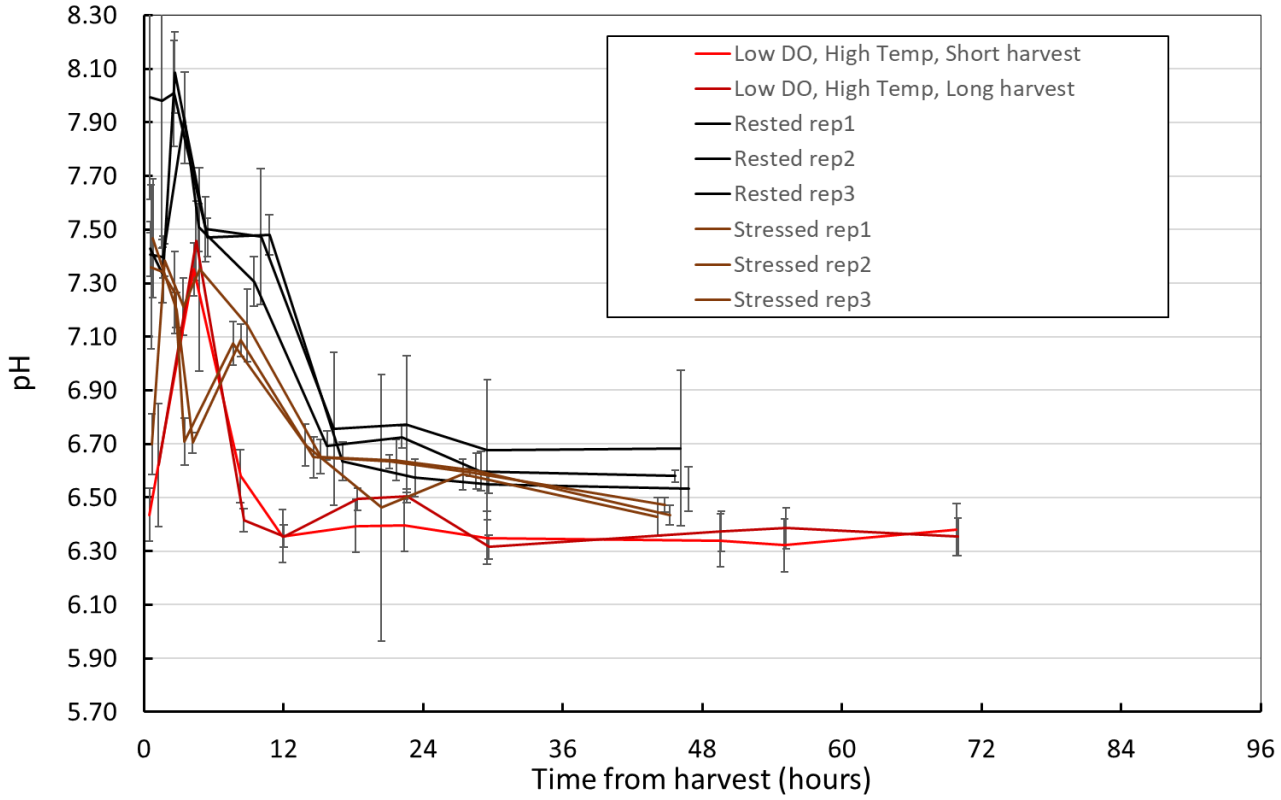

Figure 11. Average pH from Harvest A fish in low dissolved oxygen harvest (red line) compared with the controlled stressed (brown line) and the controlled rested harvest (black line).

As previously discussed, this is consistent with the fish stress response reported in literature. The non-overlap of the 84% CI for the low dissolved oxygen harvests with the 84% CI for the rested harvest, suggests there is a significant difference between these samples. These results show a consistent trend that fish obtaining a lower flesh pH, correlates with increased stress (muscle activity) that the Barramundi has been exposed to prior to slaughter. The pH results obtained in Harvest A (red line short harvest), reflects that the fish appear to have been more stressed than the controlled stress group (brown line).

Rigor

Rigor results based on penetrometer assessment for Barramundi from Harvest A are presented in Figure 12. The rate of rigor onset displayed by both the fish from the low pond dissolved oxygen harvest (red line) and the controlled deliberately stressed fish (brown line) exhibit a similar trend. Both have a steep rigor onset shown as a sharp increase in penetrometer units (force), which then slows down in speed and intensity at approximately three hours post-mortem. In both cases, the energy reserves have been depleted as the glycolysis pathway converts remaining energy to lactic acid. As the energy is finally converted, the fish begins to soften and eventually rigor mortis resolves, and the fish will go limp. This is in contrast to the controlled rested treatment (black line), where the intensity of rigor does not begin to slow until approximately nine hours post-mortem. This illustrates the extended time it takes for the higher energy reserves of the rested fish to be broken down, in comparison to the stressed fish with little energy reserves. The final penetrometer resolution results show that the fish harvested under low dissolved oxygen conditions (Harvest A) are trending softer when compared to the controlled rested or controlled stress fish at 48 hours. The corresponding 84% CI are 57.2–62.8 and 56.9–59.2 for low dissolved oxygen short and long harvest respectively (n = 5), and 64.0–68.0 and 61.6–64.0 for the rested and stressed trials respectively (n = 15). The 84% CI for the low dissolved oxygen conditions do not overlap with the rested CI suggesting there is a significant difference in the final penetrometer results. Softer flesh in fish that have been exposed to stress events (high muscle activity) in Harvests A, is consistent with the texture and penetrometer results obtained from the controlled stress group.

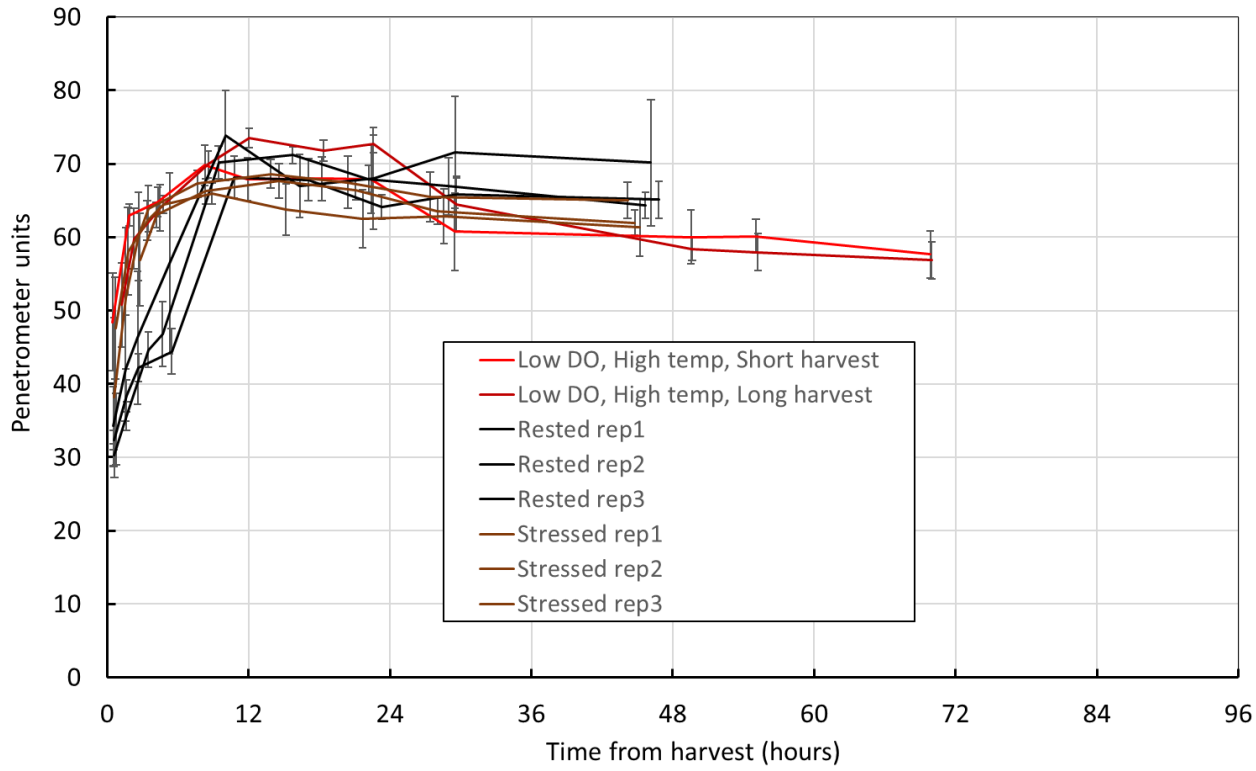

Figure 12. Average penetrometer results from the Harvest A fish in low dissolved oxygen (2.1 mg/L) harvest (red line) at the start and end (plus 1 hr 50 mins) of the harvest compared with the controlled stressed (brown line) and the controlled rested harvest (black line). Penetrometer units increase as fish become stiff through the changes associated with rigor mortis.

Lactic acid

Lactic acid results (Figure 13) confirm the trend that the fish from Harvest A have been exposed to more stress at low oxygen levels than the controlled stressed and controlled rested trials. The mean lactic acid for the low dissolved oxygen short and long harvests is 2.87 (84% CI: 2.81–2.92, n = 7) and 2.95 (2.81–3.10, n = 7) respectively. These are significantly higher than the lactic acid observed in the controlled stressed (2.23–2.42, n = 15) and rested groups (2.13–2.34, n = 15). We would expect these low oxygen fish to exhibit the same traits of softer texture, metallic flavour and yellow cooked colour as the controlled stressed and rested group.

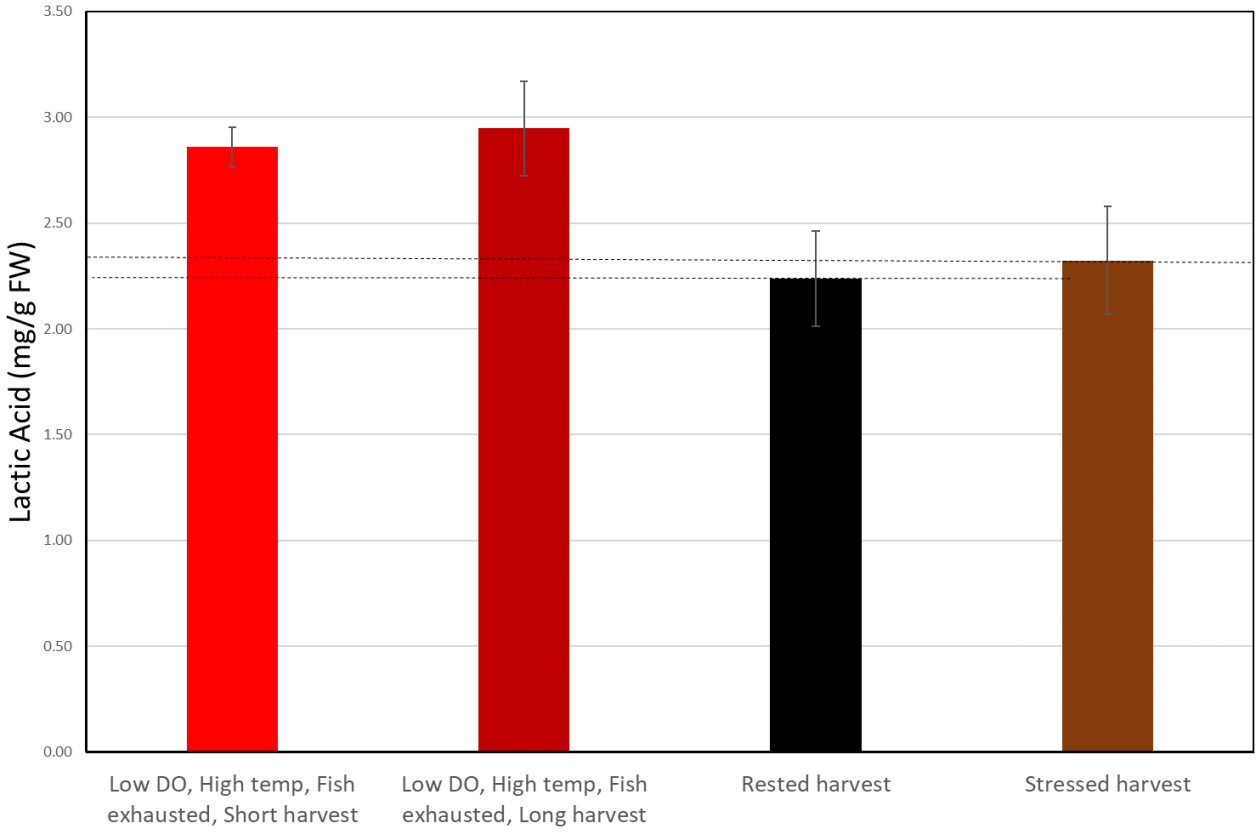

Figure 13. Average lactic acid results from Harvest A fish in low dissolved oxygen (2.1 mg/L) harvest (red columns) at the start and end (plus 1 hr 50 mins) of the harvest compared with the controlled stressed (brown line) and the controlled rested harvest (black line).

Summary

All harvests monitored during the harvests showed that the fish were consuming oxygen during the harvest, with Harvest A having the largest drop in dissolved oxygen. In harvest A, the dissolved oxygen levels had dropped to a point where the fish quickly became exhausted.

The minimum recommended DO levels for Barramundi grow-out are > 4 mg/L (Rimmer and Russell, 1998, Ayson et al., 2013). Generally, a concentration of 5 mg/L dissolved oxygen is required for optimal health in a high-density pond culture (Boys et al., 2021; Boyd and Hanson, 2010). Some fish species can tolerate low dissolved oxygen better than others, and smaller fish typically tolerate it better than larger fish (McNeil and Closs, 2007; Gilmore et al., 2018). Most fish will become distressed once dissolved oxygen falls below 4 mg/L and will die at concentrations less than 2 mg/L (Gehrke, 1988; Small et al., 2014). Studies to date show that Barramundi at rest in an experimental chamber are not stressed by moderate, short-term falls in DO, (Percival, 1999, Collins et al., 2013). They ventilate harder to sustain respiration down to a critical oxygen level of around 2mg/L, below which gill ventilation cannot deliver enough oxygen, (Butler et al., 2007). Agitated fish in a harvest crowd are not at rest. Active Barramundi may well experience difficulties before DO levels reach 2mg/L.

The results from Harvest A demonstrates that low dissolved oxygen during the harvest process can cause elevated lactic acid levels and reduced pH levels, as a result of crowding stress driving increased oxygen demand. Increased muscular activity, caused by stressing conditions at slaughter is a great concern from both the point of fish welfare and the direct impact that stress has on the flesh quality.

Dissolved oxygen levels should be monitored and maintained above 5mg/L during the entire crowd and harvest process by adding supplementary aeration or bottled oxygen as required. Crowding must not begin if the dissolved oxygen level is below 5mg/L.

This is particularly important once water temperatures begin to warm up above 25 °C limiting the amount of oxygen that is able to be absorbed into the water column. Oxygen is considered poorly soluble in water and its solubility is related to pressure and temperature. Water at 30 °C has a dissolved oxygen limit of 7.5 mg/L, whereas water at 20 °C has a dissolved oxygen limit of 9 mg/L. This makes it important to monitor dissolved oxygen levels when water temperatures are high, as there is a much lower oxygen solubility. It is important to understand the relationship between % saturation, temperature and mg/L solubility to avoid low oxygen levels during harvest.

Delaying harvest start times until later in the morning allowing dissolved oxygen to rise to suitable levels will help. Manipulating the algal blooms in the pond prior to harvest days can also help reduce oxygen demand for early morning harvests.

Reducing the volume of the crowd will also reduce the oxygen demand during the crowding and harvesting process to help maintain suitable dissolved oxygen levels. Reducing the volume of the crowd will also allow fish to be harvested in a timely manner and avoid excessive holding in a confined space. It was observed that farms are only taking an estimated guess at how many fish they will capture with each pass of the harvest net. This can become problematic when more fish are caught than are required for the days harvest. It may be worth undertaking future investigations to see if there are any under water technologies that can measure the volume of fish in the harvest to provide real-time feedback to harvest crews. Trials to date give an indication that smaller harvest volumes provide less stress on fish during harvest. However, farmers have no way to accurately measure this and rely on experience and best guess on the day to judge how many fish they capture with each shot of the net.

Fish in the crowd must be monitored to see if they are showing signs of stress. Using a simple stress level system as described in the methods to allocate the visual stress attributes can help assess your harvest. Assigning a number to each of the level stress responses such as gasping and excessive gill rate, dorsal fins protruding above the water line and inability to maintain upright position can help with immediate action to rectify the problem taken at each level.

Harvest B:

A higher oxygen harvest was observed at a similar temperature, on the same farm as harvest A. The harvest was conducted by the same team, in the same manner as harvest A. There was no capacity to test extra samples after observing this extra harvest.

On this occasion only one (1) ton of fish was harvested in a different pond to the previous days harvest. The harvest also occurred later in the morning. Pond water temperature at time of harvest was 27.8°C. Dissolved oxygen measured approximately one meter from the edge of the pond within the harvesting area prior to harvest was 6.16mg/L. By the time the fish were sufficiently crowded to begin harvesting the dissolved oxygen in the pond had dropped to 6.06mg/L at 30 minutes from the start of crowding. The reduced fish mass had less impact on dissolved oxygen demand than observed in harvest A. The Harvest B fish at harvest were a lot calmer assessed at level 2 stress, (depicted in Plate 8), than the fish in harvest A, with very few fish jumping and trying to avoid the harvest net. Harvest B harvest had no fish at level four as in previous days harvest A. Some fish displaying enough energy to jump/splash in the final crowd of the net. Visually these fish appeared to have plenty of energy remaining when finally crowded in the brail. In contrast, Harvest A had many fish in the net that were too exhausted to swim at level 4 stress.

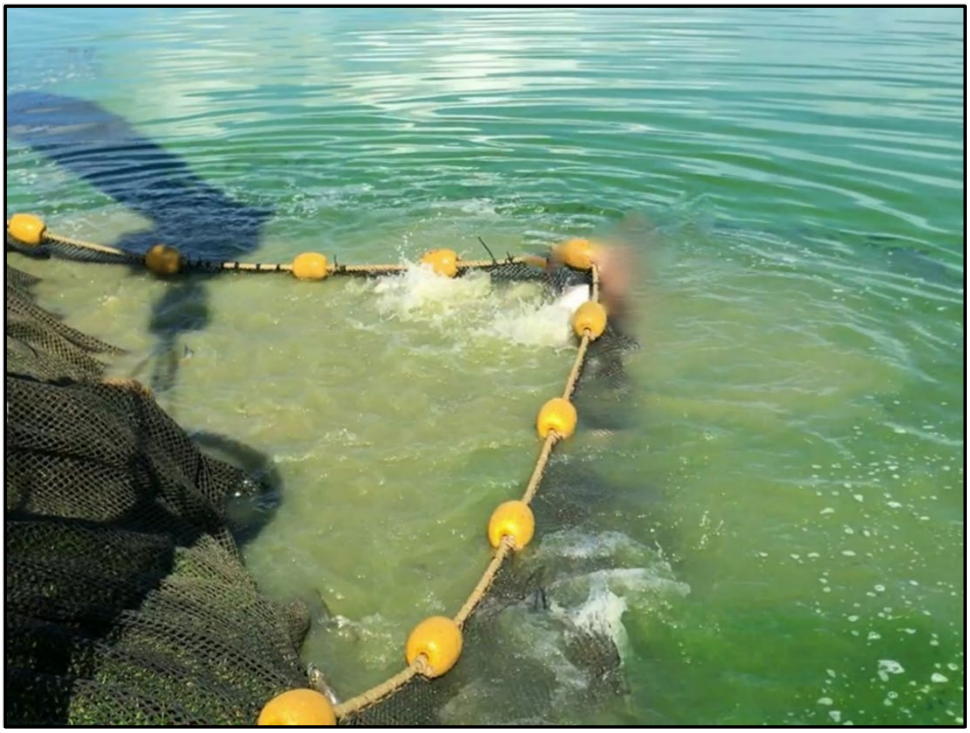

Plate 8. Harvest B – modified harvest, with high dissolved oxygen levels (>6mg/L) and low fish numbers (1 ton), resulting in fish having energy to splash in the net. There were no visible signs of exhausted fish in this trial.

Harvest C:

A total of 6.2 ton of fish with an average size of 4.5 kg (fork length 64.7cm) were harvested. Pond water temperature was 28.5 °C at the time of harvest. Dissolved oxygen measured approximately one meter from the edge of the pond within the harvesting area prior to harvest was 4.7 mg/L. By the time the fish were sufficiently crowded to begin harvesting the dissolved oxygen in the pond had dropped to 4.1 mg/L at 45 minutes from the start of crowding. By the time the last brail was removed from the crowd the dissolved oxygen had remained at 4.1 mg/L at 1 hour 56 minutes from the start of harvest.

The fish appeared to be overall quite calm at the start of the crowd (level 1 stress), with some fish jumping and franticly swimming (level 2 stress) but settled quite quickly. At the point of maximum crowding, the fish were very calm (level 1 stress) as they had been given enough time to settle. The fish visually appeared to have plenty of energy reserves, jumping once in the brail. Up to this point this looked like a very calm and controlled harvest.

Fish were brailed into a 1000 L bin containing an ice slurry. However, the ice slurry in these bins were too stiff/firm, with too much ice and not enough water to create a fluid slurry consistency. Many fish remained on the surface of the ice.

This is a considerable problem, particularly in the hot months when farmers are trying to combat high ambient air and water temperatures by adding extra ice into the ice slurries. This allows the last of the fish in the brail to sit on top of the stiff ice slurry mix when the bins are filled to capacity (Plate 9). When this occurs, the fish that are above the water level in the ice slurry or on top of the ice, are exposed to ambient air temperatures and take considerably longer to die. During this harvest, the fish took 45 minutes before they had nil reaction to external stimulus. When bins are filled to capacity, this makes it very difficult to push the fish down under the water level and near impossible when the ice slurry mixture is too dry.

Fish were held in the ice slurry at the processing area until there was nil response to external stimulus (this took longer than expected, approximately 45 minutes, probably due to the fish rafting on the ice). Fish for short and long crowds were removed from the top ice layer, above the water level, in the first and last bins respectively. This was done to assess whether rafting fish was a point of stress. The fish were euthanised as previously described.

Plate 9. Fish sitting on top of the ice slurry when the ice slurry mixture is too stiff. Bins have been filled to capacity.

pH

The flesh pH results show that Harvest C fish had a pH of 6.37 (84% CI: 6.35–6.38, n = 5) for the short harvest and a pH of 6.26 (6.25–6.28, n = 5) for long harvest at 48 hours post-mortem (Figure 14). This is significantly lower than the controlled stressed group that had a pH of 6.45 (6.43–6.47, n = 15). The time difference between the short harvest sample and the long harvest sample was 1 hour and 11 minutes. While the fish appeared to be calm for this harvest and there were moderate levels of dissolved oxygen in the final harvest crowd (4.1 mg/L), these fish were sampled from the group of fish remaining on top of the ice slurry bin, as per Plate 9. The resulting pH levels show that the Harvest C fish have been exposed to a higher level of stress than the controlled stressed group. These results also indicate that the fish have been exposed to greater stress than the fish monitored in the low oxygen harvest A. This stress is a direct result of the fish taking 40 min to die. In terms of animal welfare this is an unacceptable practice and was found to be the largest stress point encountered across all harvest practices.

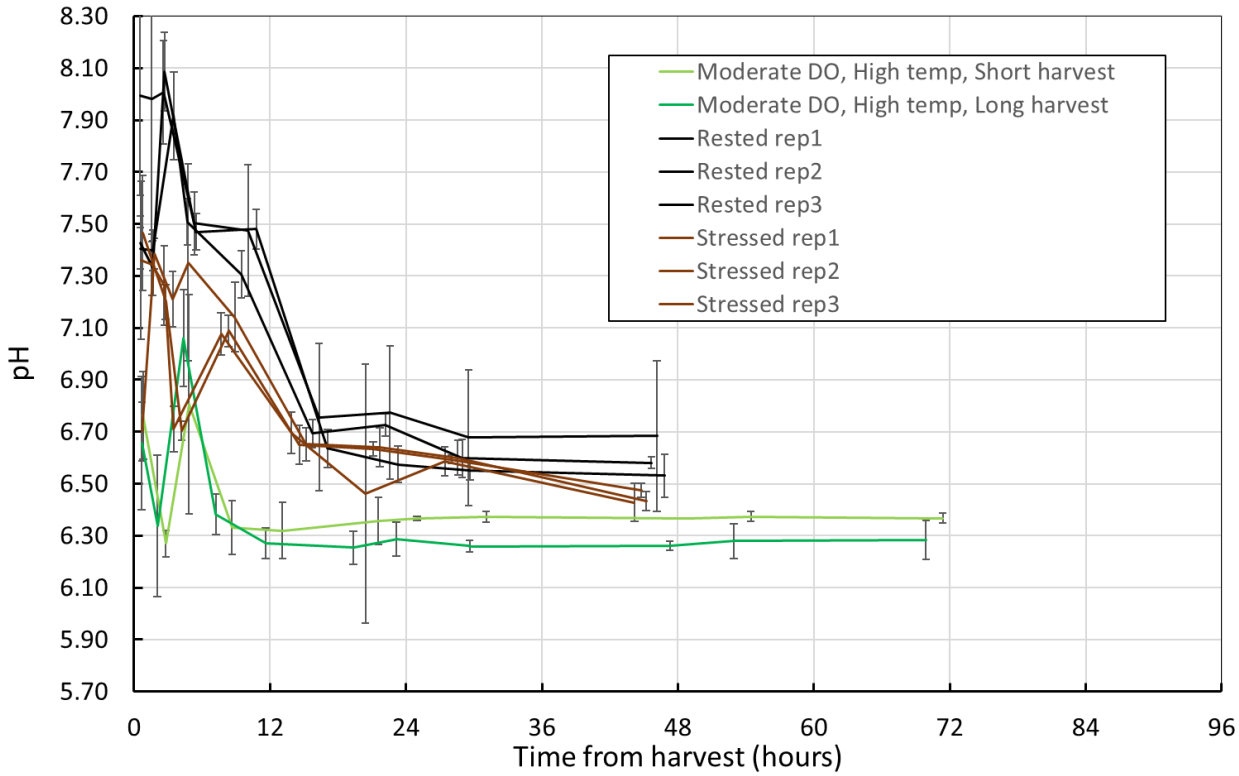

Figure 14. Average flesh pH results from the Harvest C fish in moderate dissolved oxygen (4.1 mg/L) harvest, rafting on top of ice (green line) at the start and end (plus 1 hr 59 mins) of the harvest compared with the controlled stressed (brown line) and the controlled rested harvest (black line).

Rigor

The rigor results from Harvest C are presented in Figure 15. The fish from the controlled deliberately stressed fish (brown line) and fish sampled from the top of the ice slurry mixture from the moderate oxygen harvest from Harvest C (green line), display a similar trend. Both had a rapid rate of rigor onset before beginning to soften at approximately three hours post-harvest. This is in contrast to the controlled rested group (black line), beginning to soften at approximately nine hours post-harvest.

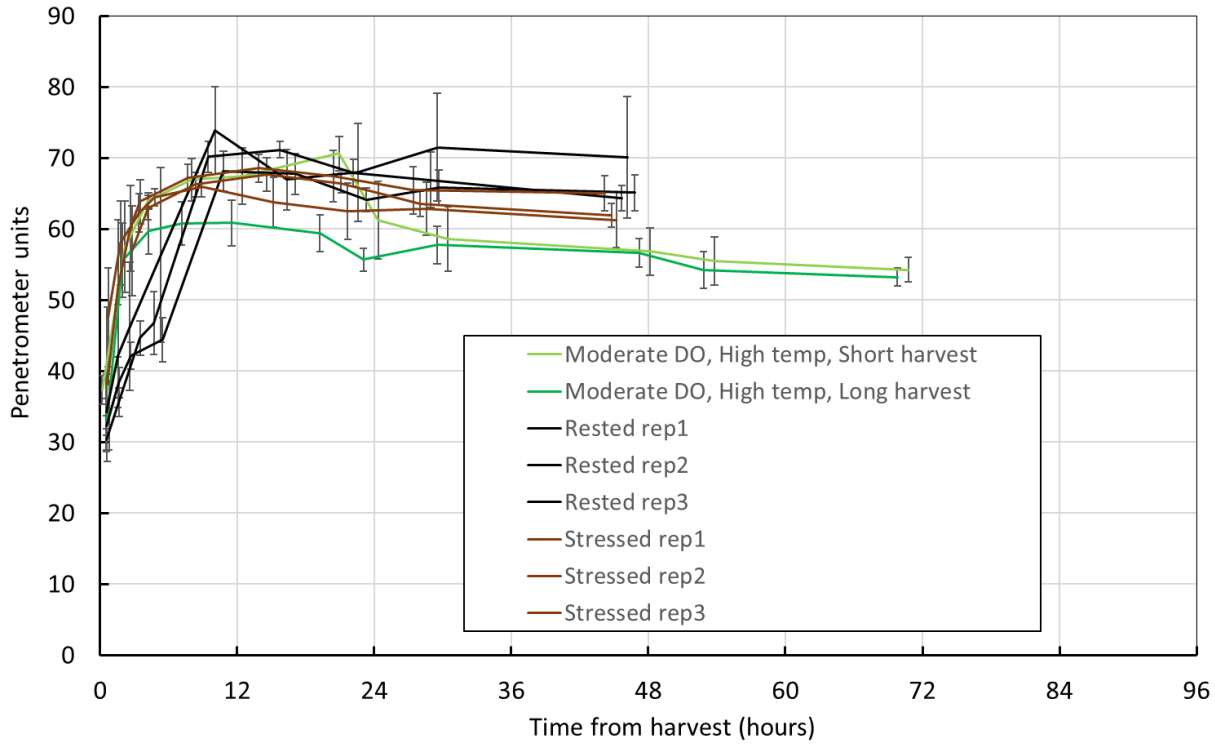

Figure 15. Average penetrometer results from Harvest C fish in the moderate dissolved oxygen (4.1 mg/L) harvest, rafting on top of ice (green line) at the start and end (plus 1 hr 59 mins) of the harvest compared with the fish from the controlled stressed (brown line) and the controlled rested harvest (black line). Penetrometer units increase as fish become stiff through the changes associated with rigor mortis.

The final resolution of the penetrometer results at 48 hours, show that the Harvest C fish harvested and left on top of the ice slurry (Plate 9) are significantly softer than the controlled rested or stress fish being approximately 56.9 (84% CI: 54.3–59.4, n = 5) for the short harvest, 56.3 (54.7–57.8, n = 5) for the long harvest. Softer flesh in fish that have been exposed to stress events is consistent with the texture and penetrometer results from the controlled stressed group. The terminal penetrometer reading for Harvest C being lower than the low dissolved oxygen Harvest A, indicates that the fish in the Harvest C harvest were exposed to more stress than the low dissolved oxygen harvest (Harvest A). This again demonstrates that leaving fish on-top of the ice and exposed to the air is not acceptable.

Lactic Acid

The lactic acid results complete the story for Harvest C, with the short harvest averaging 3.63 mg/g (84% CI: 3.52 – 3.73, n = 5) and long harvest averaging 3.44 mg/g (3.25–3.63, n = 5) (Figure 16). These results are significantly higher than the controlled stressed group obtaining an average lactic acid level of 2.32mg/g (2.23 – 2.42, n = 15). Barramundi held in the crowd for longer did not accumulate more lactic acid than the fish harvested earlier in the crowd. In balance, two interpretations can be weighed. Firstly, it is reasonable to expect that air asphyxiation of fish maximises production of lactic acid in the fillet regardless of time spent in a calm well-oxygenated harvest crowd. These mean muscle lactic acid Levels of 3–4 mg/g FW are in the range expected of recreationally caught fish, (Arlinghaus et al., 2009, Davidson et al., 1997, Lowe et al., 1993, Killen et al., 2003). Secondly, perhaps lactic acid accumulation in these asphyxiated fish has reached its maximum possible level or ‘equalised’ in the same manner that it did in the previous rested versus stressed harvest experiment. The average glycogen concentration, the precursor of the lactic acid, is likely to be the same in two groups of Barramundi taken at random from one pond.

With lactic acid being an indicator of stress, this demonstrates that leaving fish on top of the ice slurry exposes them to higher levels of stress. Again, this is a practice that is easily addressed or avoided, by reducing the volume of fish placed in the ice slurry bins and modifying the ice to water ratio in the ice slurry, as discussed in sections on “Overloaded ice slurry bins” and “Ice slurry composition and fish loading”.

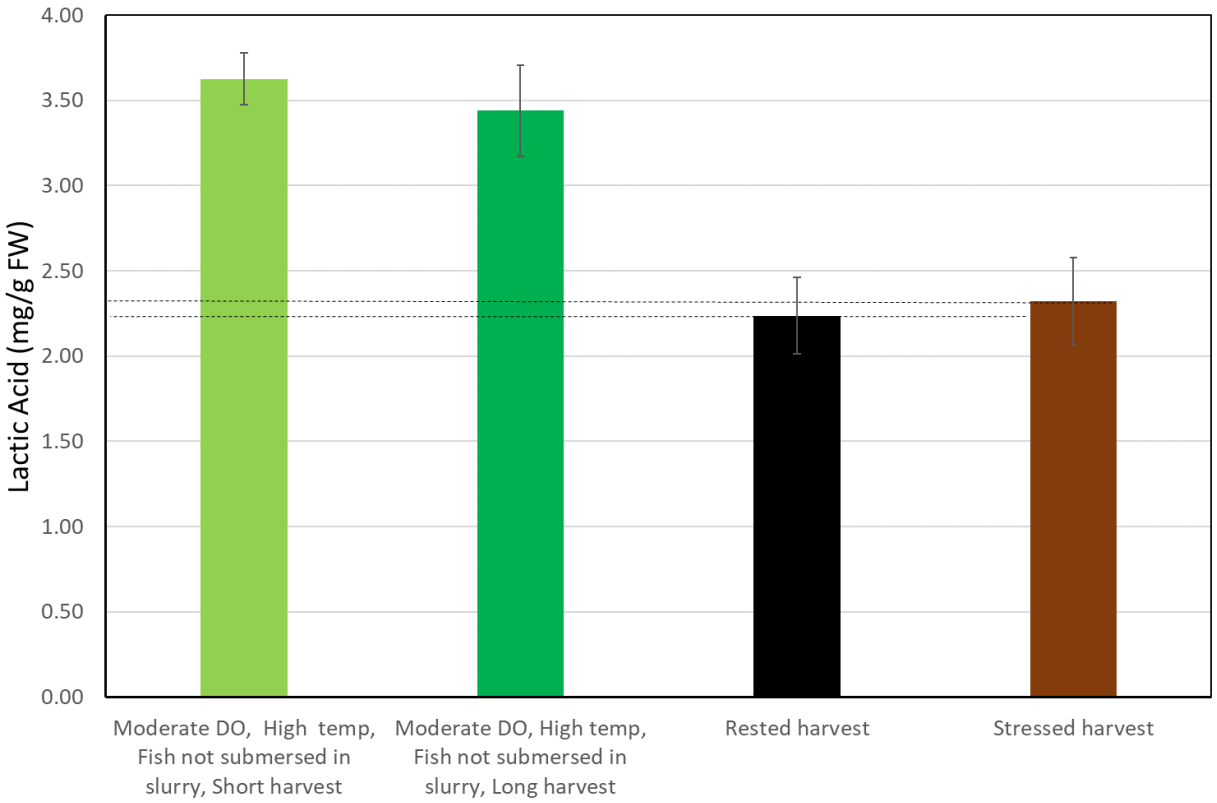

Figure 16. Average lactic acid results from Harvest C fish in moderate dissolved oxygen (4.1 mg/L) harvest, rafting on top of ice (green columns) at the start and end (plus 1 hr 59 mins) of the harvest compared with the controlled stressed (brown line) and the controlled rested harvest (black line).

Harvest D:

A total of 1.2 ton of fish with an average size of 4.1 kg (fork length 66.1 cm) were harvested. At the time of harvest the pond water temperature was 21.1 °C. Dissolved oxygen prior to harvest was 5.5 mg/L. From the start of crowding to the time the fish were sufficiently crowded (20 minutes) to begin harvesting (short crowd) the dissolved oxygen in the pond had dropped to 5.1 mg/L. Due to the small number of fish in the crowd (1.2 ton), the brailing after filling the first bin was delayed for one hour to induce a stress effect, similar to that of a larger crowd of fish that would take longer to process. The fish remained very calm (stress level 1) in the crowd and the dissolved oxygen remained at 5.1 mg/L until the last brail was removed from the crowd (long crowd) at 1 hour 20 minutes from the start of harvest. The time difference between the short harvest sample and the long harvest sample was 1 hour.

pH

The flesh pH results presented in Figure 17 show that the fish from Harvest D in a high dissolved oxygen environment (blue line), have a higher terminal average pH of 6.80 for the short harvest and an average of 6.73 for the long harvest at 48 hours. The corresponding 84% CI's about the means are 6.72–6.87 (n = 5) for the short harvest and 6.69 – 6.76 (n = 5) for the long harvest. In comparison to the fish from the controlled rested and controlled stressed trials having an average terminal pH of 6.60 (84% CI: 6.53–6.67, n = 15) and 6.45 (6.43–6.47, n = 15) at 46 hours, respectively. The Harvest D 84% CI do not overlap with the rested or stressed 84% CI, which follows the trend that the Harvest D fish have been exposed to less stress than the controlled rested group. This may be due to the pond conditions in Harvest D having a water temperature of 21.1 °C and a dissolved oxygen level of 5.1 mg/L. Whereas, the pond water temperature in the controlled rested trial was 26.1 °C and dissolved oxygen level of 6.9 mg/L.

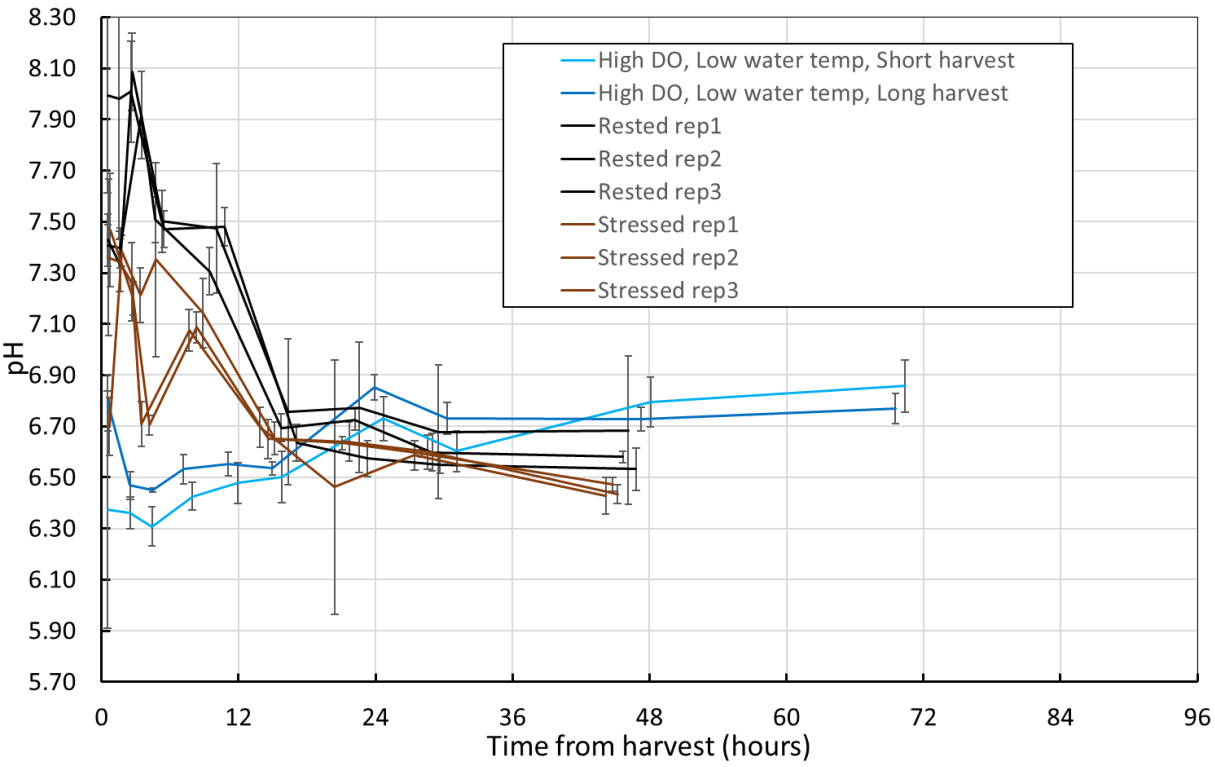

Figure 17. Average flesh pH from Harvest D fish in the high dissolved oxygen (5.1 mg/L) harvest (blue lines) at the start and end (plus 1 hr 20 mins) of the harvest compared with fish in the controlled stressed (brown line) and the controlled rested harvest (black line).

Rigor

The rigor results from Harvest D, as a high dissolved oxygen harvest (blue lines) falls between the controlled stressed (brown line) and the controlled rested (black line) at the onset of rigor, approximately up to eight to nine hours post-harvest. Harvest D fish (blue line) progress into rigor before beginning to soften after approximately eight to nine hours post-harvest (Figure 18). This is similar to the controlled rested group (black line) beginning to soften at about nine hours.

The final resolution of the penetrometer results for Harvest D fish, harvested from cool pond water with a temperature of 21.1 °C and a dissolved oxygen level over 5 mg/L, have softened less than both the controlled stressed and controlled rested with rigor readings at 48 hours post-harvest. The corresponding 84% CI are 81.3–86.5 and 77.6–88.5 for low dissolved oxygen short and long harvest respectively (n = 5), and 64.0 – 68.0 and 61.6 – 64.0 for rested and stressed respectively (n = 15). The 84% CI for the high dissolved oxygen conditions do not overlap with either the rested or stressed CI suggesting there is a significant difference in the final penetrometer results. These higher rigor readings are in keeping with rested or lower stressed fish showing a delay in the onset of rigor mortis and a firmer terminal texture when exposed to short term stress such as harvesting. This extension of rigor process is associated with a better flesh quality and extended shelf life of the product.

These results are promising and show that when care is taken during the harvest process, ensuring dissolved oxygen levels are kept above 5 mg/L and fish are kept an appropriate ice slurry (i.e., not exposed to ambient air or kept on top of the ice), then a low stress harvest can be achieved. These low stress harvest penetrometer results are closer to a true rested harvest, where the fish were fully anesthetised throughout the entire process.

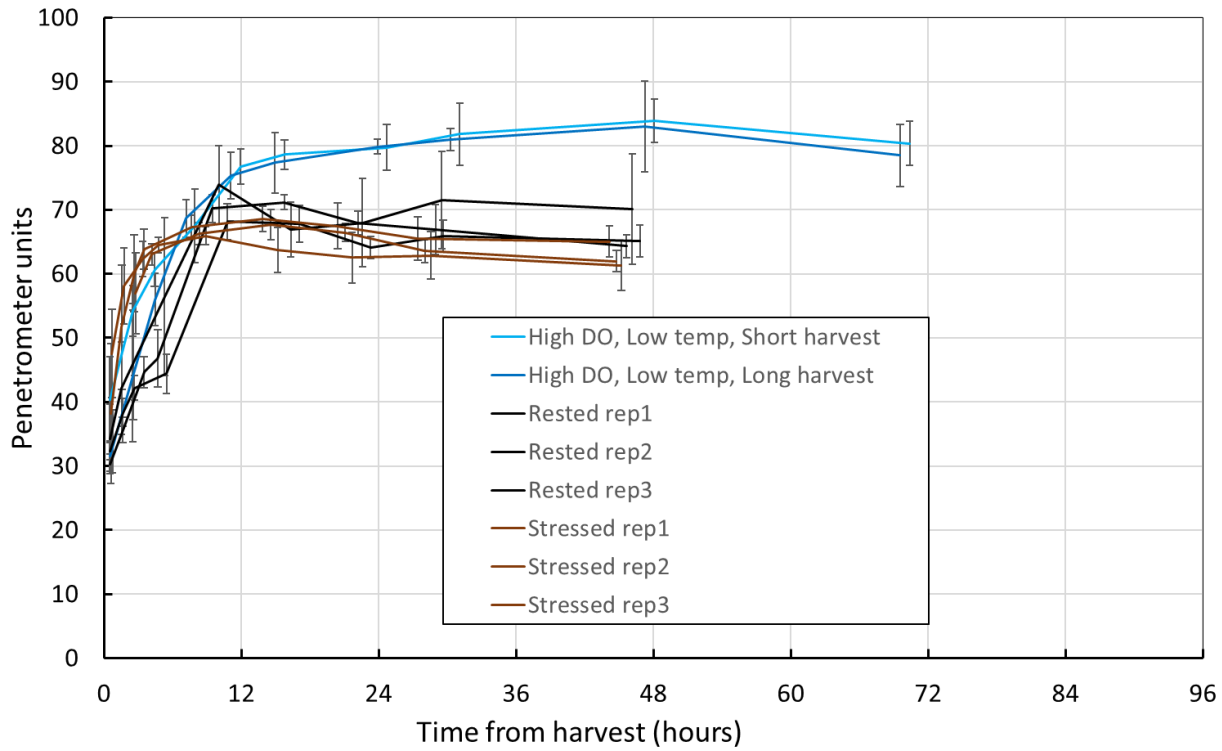

Figure 18. Average penetrometer results from Harvest D fish in high dissolved oxygen (5.1 mg/L) harvest (blue lines) at the start and end (plus 1 hr 20 mins) of the harvest compared with fish from the controlled stressed (brown line) and the controlled rested harvest (black line).

Lactic acid

The Harvest D lactic acid results agree with the pH and rigor results from the harvest, with the short harvest averaging 2.25mg/g (84% CI: 2.06–2.44, n = 7) and the long harvest averaging 2.16 mg/g (1.97–2.35, n = 7) (Figure 19). These results show no significant statistical difference when compared against the controlled

stressed or controlled rested group with lactic acid values of 2.32mg/g (2.23–2.42, n = 15) and 2.24 mg/L (2.13–2.34, n = 15), respectively. To pick up from the discussion of Harvest C, observations show lower levels of lactic acid but once more the short and long crowd samples are not significantly different. Reading lactic acid as an indicator of stress, the results from Harvest D demonstrates that it is possible to harvest fish under conditions that do not expose the fish to excessive stress, i.e., low volumes in the crowd and high dissolved oxygen levels. Temperature may potentially have another role here, but to explore this would require measures of the lactic acid content at 48h post-mortem. At cooler times of year farmed Barramundi may have less muscle glycogen and hence a higher flesh pH at 48h post-mortem.

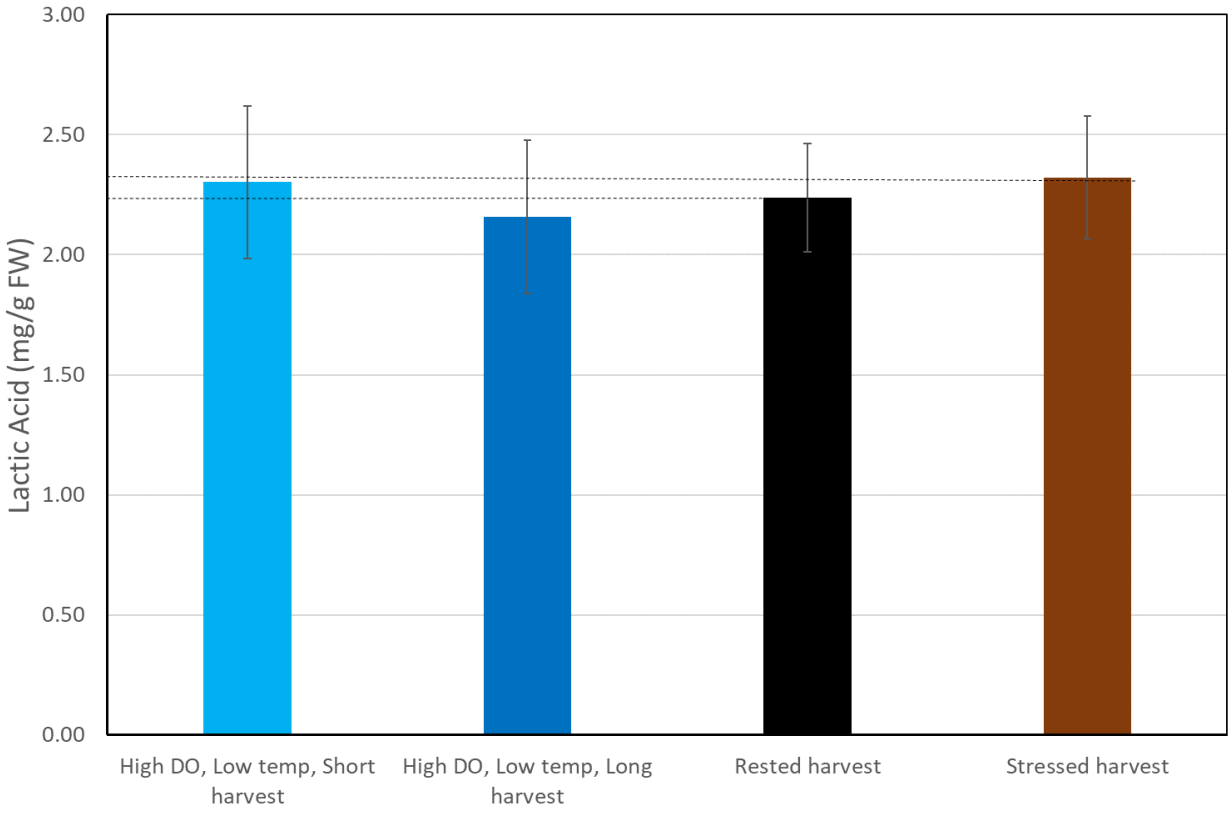

Figure 19. Average lactic acid results from Harvest D fish in high dissolved oxygen (5.1 mg/L) harvest (blue columns) at the start and end (plus 1 hr 20 mins) of the harvest compared with the controlled stressed (brown line) and the controlled rested harvest (black line).

Harvest E:

Harvest E is a repeat of Harvest D with very similar harvest conditions. Harvest E was performed in the same pond, by the same harvest crew as Harvest D on the following day. Harvest E was a low volume harvest of 1.3 ton, with the average size and length of the fish being 4.1 kg and 66.5 cm, respectively. The pond water temperature was 21.4 °C. Dissolved oxygen measured prior to harvest was 5.4 mg/L. By the time the fish were sufficiently crowded to begin harvesting, at 21 minutes from the start of crowding (short harvest), the dissolved oxygen in the pond had dropped to 4.7 mg/L. Similar to Harvest D, due to the small number of fish in the crowd (1.3 ton), the next brails were delayed for one hour. This was employed to induce a stress effect, similar to a larger crowd of fish that would take longer to process. The fish remained very calm in the crowd (level 1 stress) and the pond dissolved oxygen level remained stable at 4.7 mg/L at 1 hour 21 minutes from the start of harvest. The time difference between the short crowd sample and the long crowd sample was 1 hour.

pH

The flesh pH results presented in Figure 20, show that the fish from Harvest E in a high dissolved oxygen environment (yellow lines) have a terminal average pH of 6.72 (84% CI: 6.65–6.78, n = 5) for the short harvest and an average pH of 6.73 (6.65–6.81, n = 5) for the long harvest at 48 hours. This is slightly higher than the controlled rested and controlled stressed trials, with the terminal flesh pH being an average of 6.58 and 6.43 at 46 hours, respectively. The 84% CI for the Harvest E harvests marginally overlap with the controlled rested group indicating they have been exposed to a similar level of stress. These results follow the same trend as Harvest D from the previous days harvest (blue results in Figure 17), where the fish have a higher terminal flesh pH than the controlled rested group indicating that they have been exposed to less stress. This could be due to the cooler water temperature of 21.4 °C and 21.1 °C for Harvest E and D, respectively. Even though the pond dissolved oxygen level was lower at 4.7 mg/L in Harvest E, compared to 5.1 mg/L for Harvest D. In contrast, the pond water temperature and dissolved oxygen levels for the controlled rested trial was 26.1 °C and 6.9 mg/L, respectively. Given the overlap of the flesh pH 84% CI for Harvest D and E, there are no significant differences between the two trials.

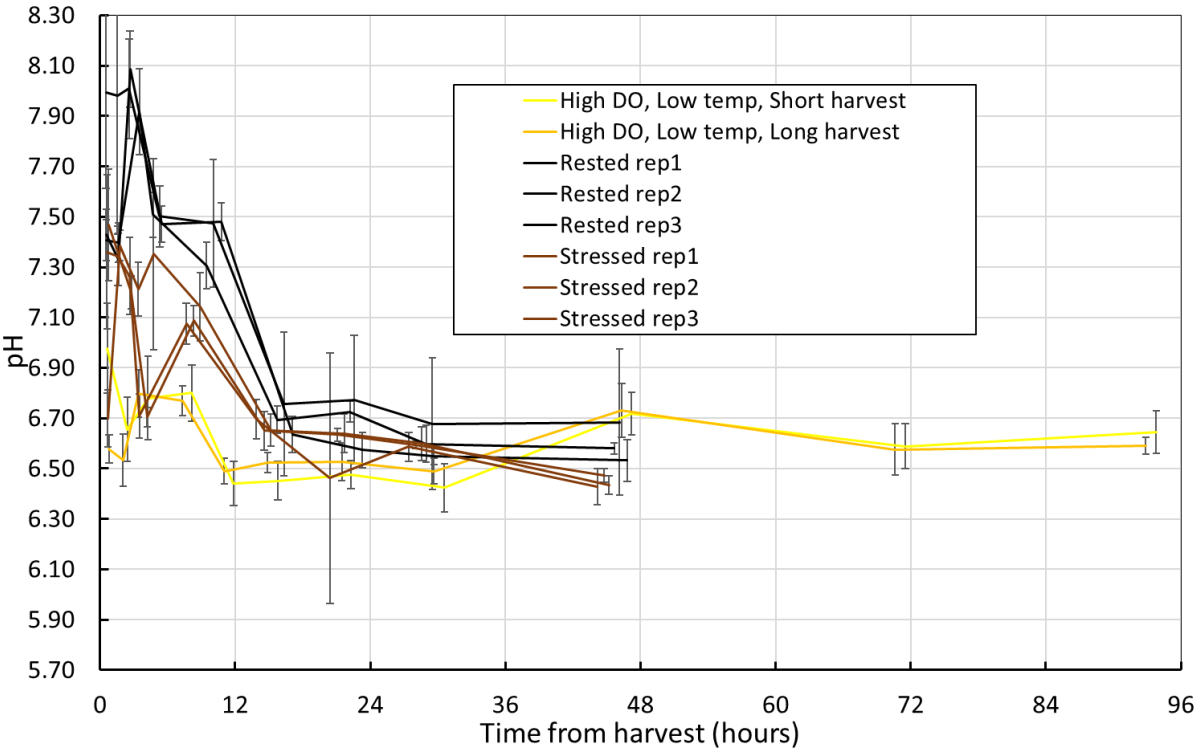

Figure 20. Average flesh pH from Harvest E fish in high dissolved oxygen harvest (yellow line) compared with the controlled stressed (brown line) and the controlled rested harvest (black line).

Rigor

The rigor results from Harvest E as a high dissolved oxygen harvest (yellow lines) are presented in Figure 21. These results follow a similar trend as the controlled stress group (brown line). This indicates that the fish in Harvest E have been exposed to similar stress when compared to the controlled stressed group. Fish from Harvest E progress into rigor before beginning to soften at about nine hours post-harvest. This rigor result is not quite as good Harvest D's previous days harvest, where there was a delay in the onset of rigor of seven to nine hours.

The final resolution of the penetrometer results show that the Harvest E fish harvested from a cool pond water temperature of 21.4 °C and dissolved oxygen level of 4.7 mg/L, have remained firmer with a mean rigor reading of 79 (84% CI: 74.6–83.7, n = 5) and 78 (72.2–84.0, n = 5) penetrometer units at 48 hours post-harvest for short and long harvest respectively. When compared to both the controlled stress and controlled rested fish with 63 (61.6–64.0, n = 15) and 66 (64.01–68.0, n = 15) penetrometer units, respectively. Harvest E obtained similar rigor results as Harvest D (83 units at 48 hours) from the previous days harvests.

The only observed difference between Harvest D and Harvest E was a slightly lower pond dissolved oxygen level for Harvest E, having 4.7 mg/L compared to 5.1 mg/L for Harvest D at the initial point of harvest. These results agree with literature where it is reported that a concentration of 5 mg/L dissolved oxygen is required for optimal fish health and quality in a high-density pond culture (CSIRO PUBLISHING Marine and Freshwater Research 2021; Boyd and Hanson 2010). This may explain why Harvest E rigor results were slightly worse that Harvest D.

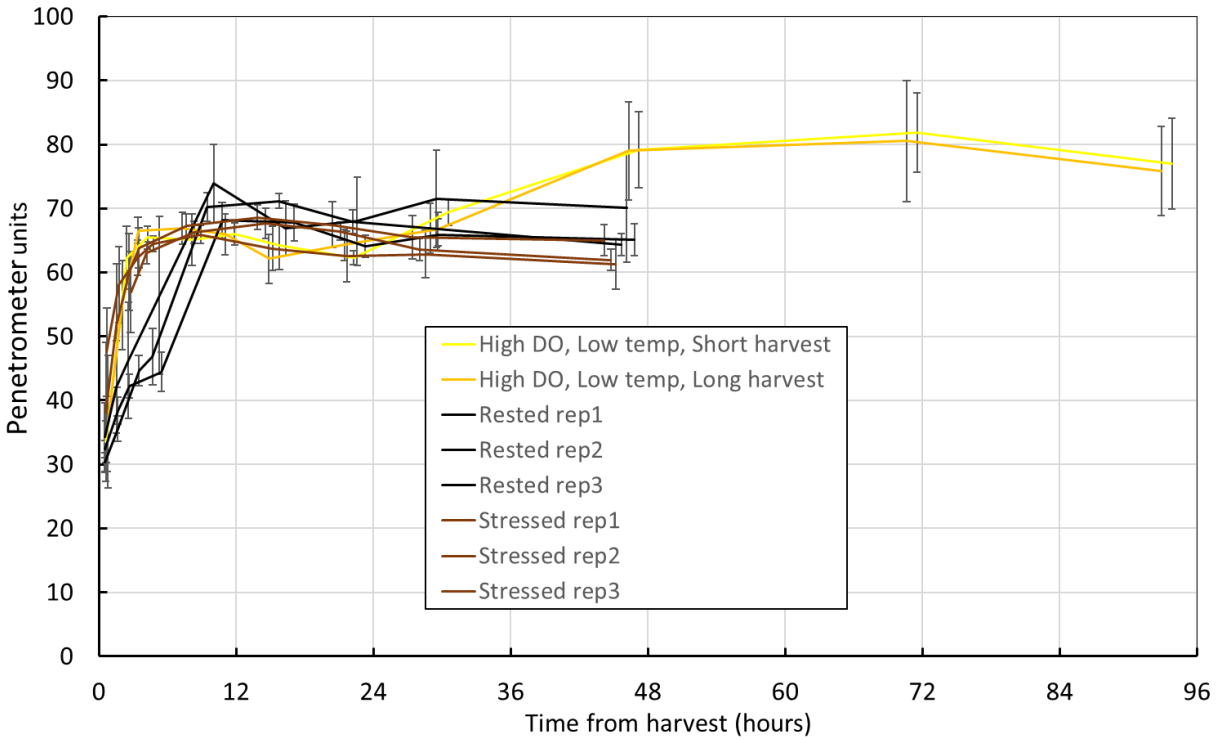

Figure 21. Average penetrometer results from Harvest E fish in a high dissolved oxygen harvest (yellow line) compared with the controlled stressed (brown line) and controlled rested harvest (black line).

Lactic acid

Harvest E lactic acid results are similar to the previous days Harvest D results, with the short harvest E averaging 2.35mg/g (84% CI: 2.19–2.52, n = 7) and long harvest averaging 2.20mg/g (2.00–2.40, n = 7) (Figure 22). Harvest E results show no significant statistical difference when compared to the controlled stressed or controlled rested group of 2.32 mg/g and 2.24 mg/L, respectively. This indicates that Harvest E

fish were not exposed to a significant amount of stress. It is also consistent with the prediction that in the absence of stress, Barramundi from the same pond will have a similar capacity on average to generate lactic acid in the fillet. There was no significant difference in the lactic acid results between Harvest D and Harvest E. This was a harvest stress study- to answer the question of whether post-mortem factors in the slurry that can rapidly bring lactic acid concentration in the fillet to a point typical of the Barramundi's nutritional status, or time of year, would need further observations.

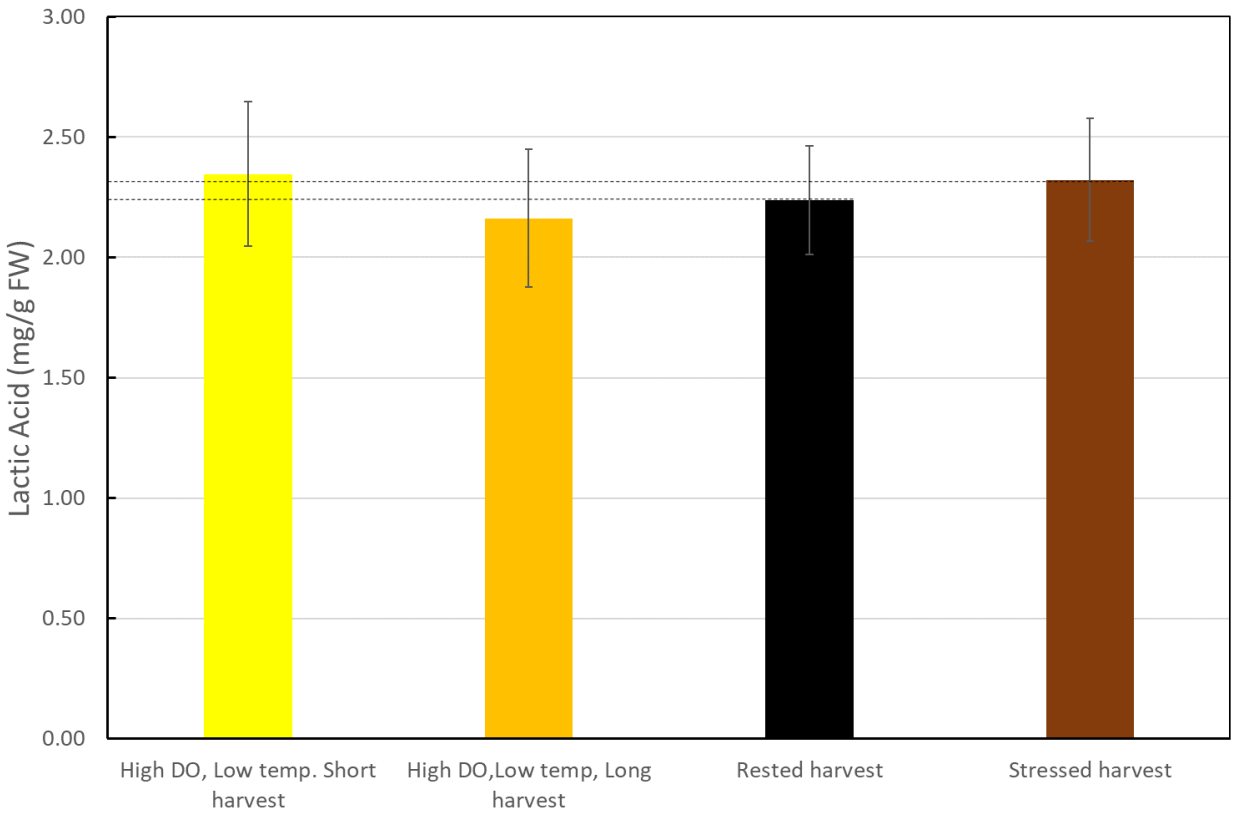

Figure 22. Average lactic acid results from Harvest E fish in high dissolved oxygen harvest (yellow bars) compared with the controlled stressed (brown bars) and the controlled rested harvest (black).

Comparison of Farm Harvests with Rested vs Stress Baseline

The pH results have been discussed individually for each trial in previous sections. Figure 23 shows the pH results for all trials overlayed together to visually compare the differences. The harvests with high oxygen represented by the yellow and blue lines show resolution of flesh pH higher than harvests that exposed the fish to excessive stress represented by the green and red lines. With no overlap between the 83% confidence intervals, this demonstrates that the fish from the high oxygen harvests above 5.0 mg/L are significantly less stressed than fish that have been harvested in low oxygen conditions or left rafting on top of the ice when in the slurry bins.

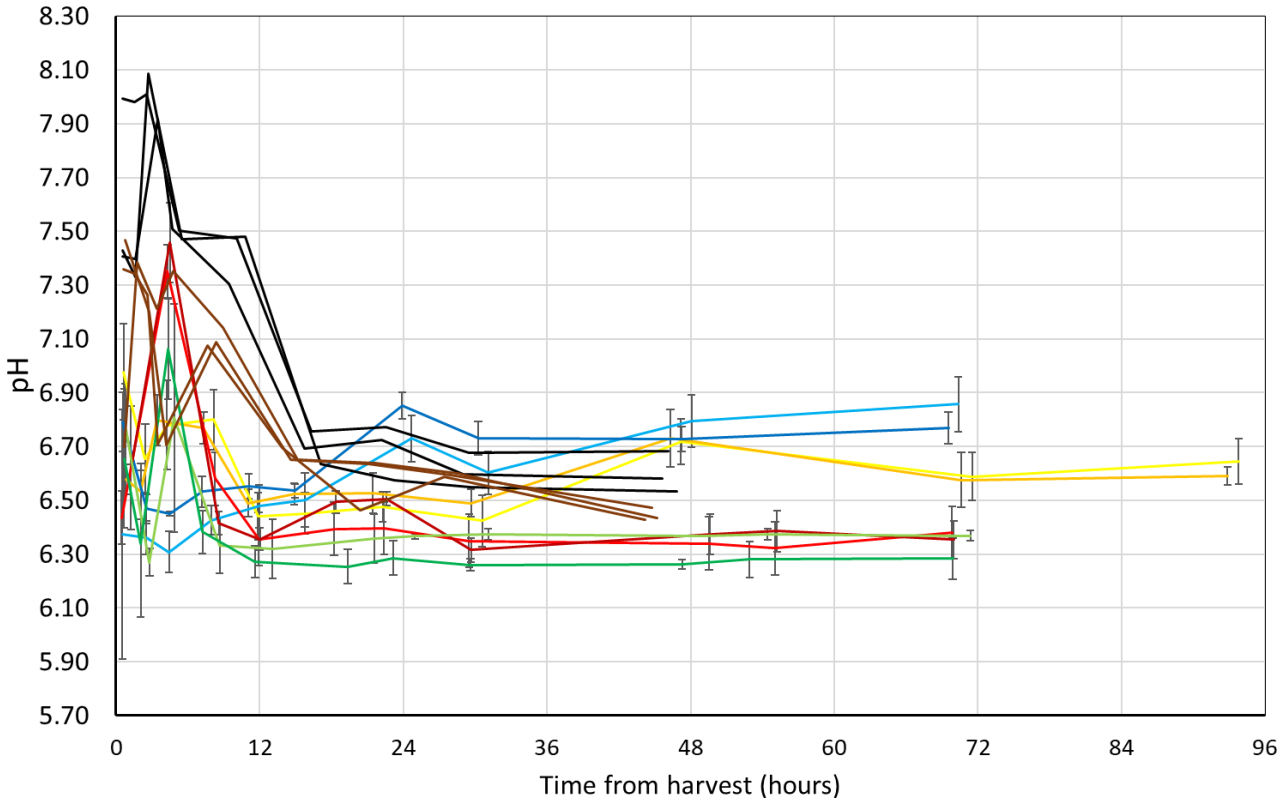

Figure 23. pH results from all harvests.

Legend

- Controlled rested trials (black lines) average terminal pH of 6.60 (84% CI: 6.53–6.67, n = 15).
- Controlled stressed trials (brown lines) average terminal pH of 6.45 (84% CI: 6.43–6.47, n = 15).
- Trial A (red line) low dissolved oxygen environment an average pH 6.34 (84% CI: 6.28–6.40, n = 5) for short harvest and an average of 6.37 (84% CI: 6.32–6.43, n = 5) for the long harvest.
- Trial C (green line) fish had an average pH of 6.37 (84% CI: 6.35–6.38, n = 5) for the short harvest and an average pH of 6.26 (84% CI: 6.25 – 6.28, n = 5) for long harvest.
- Trial D (blue line) fish had an average pH of 6.80 (84% CI: 6.72–6.87, n = 5) for the short harvest and an average of 6.73 (84% CI: 6.69–6.76, n = 5) for the long harvest.
- Trial E (yellow line) fish had an average pH of 6.72 (84% CI: 6.65–6.78, n = 5) for the short harvest and an average pH of 6.73 (6.65–6.81, n = 5) for the long harvest.

Rigor

The rigor results have been discussed individually for each trial in previous sections. Figure 24 shows rigor results for all trials overlayed together to visually compare the differences. The harvests with high oxygen represented by the yellow and blue lines show that the fish are resolving from rigor significantly firmer than harvests that exposed the fish to excessive stress represented by the green and red lines. With no overlap between the 83% confidence intervals, this demonstrates the harvests monitored with high oxygen are significantly less stressed than fish that have been harvested in low oxygen conditions or left rafting on top of the ice when in the slurry bins. This confirms the same trend in results from the pH results.

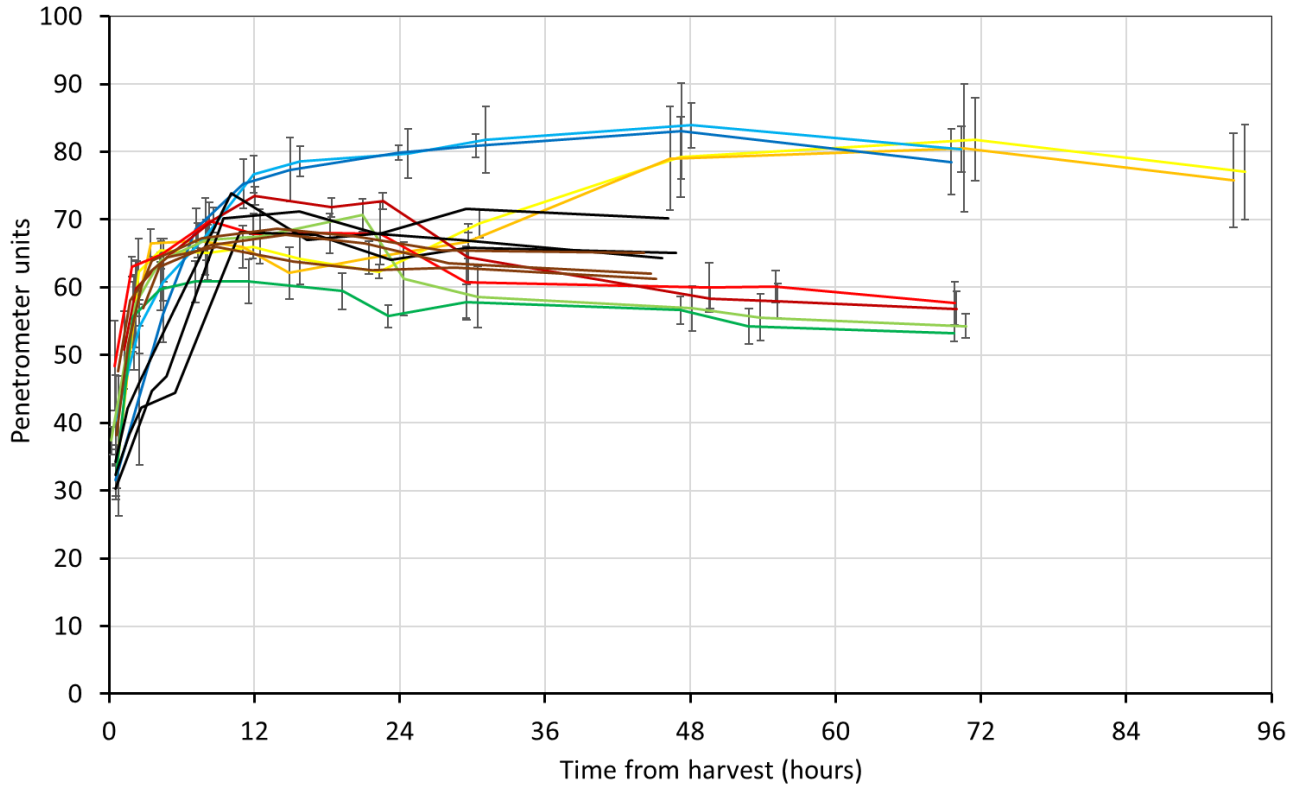

Figure 24. Penetrometer results from all trials.

Legend

- Controlled rested trials (black lines) penetrometer confidence interval (84% CI: 64.0–68.0, n = 15).
- Controlled stressed trials (brown lines) penetrometer confidence interval (84% CI: 61.6–64.0, n = 15).
- Trial A (red line) low dissolved oxygen environment penetrometer confidence interval (84% CI: 57.2–62.8, n = 5) for short harvest and (84% CI: 56.9–59.2, n = 5) for the long harvest.
- Trial C (green line) moderate DO fish not submersed in slurry penetrometer confidence interval (84% CI: 54.3–59.4, n = 5) for the short harvest and (84% CI: 54.7–57.8, n = 5) for long harvest.
- Trial D (blue line) high dissolved oxygen environment penetrometer confidence interval (84% CI: 81.3–86.5, n = 5) for the short harvest and (84% CI: 77.6–88.5, n = 5) for the long harvest.
- Trial E (yellow line) high dissolved oxygen environment penetrometer confidence interval (84% CI: 74.6–83.7, n = 5) for the short harvest and (84% CI: 72.2–84.0, n = 5) for the long harvest.

Lactic Acid

The lactic acid results confirm the comparison of stress results. Figure 25 shows lactic acid results for all trials overlayed together to visually compare the differences. Again, the harvests with high oxygen represented by the yellow and blue bars show that the fish have been exposed to significantly less stress than harvests that exposed the fish to low oxygen or rafting on top of the slurry represented by the red and green lines respectively. With no overlap between the 83% confidence intervals, this demonstrates that the fish from the high oxygen harvests are significantly less stressed than fish that have been harvested in low oxygen conditions or left rafting on top of the ice when in the slurry bins. This confirms the same trend in pH and penetrometer results.

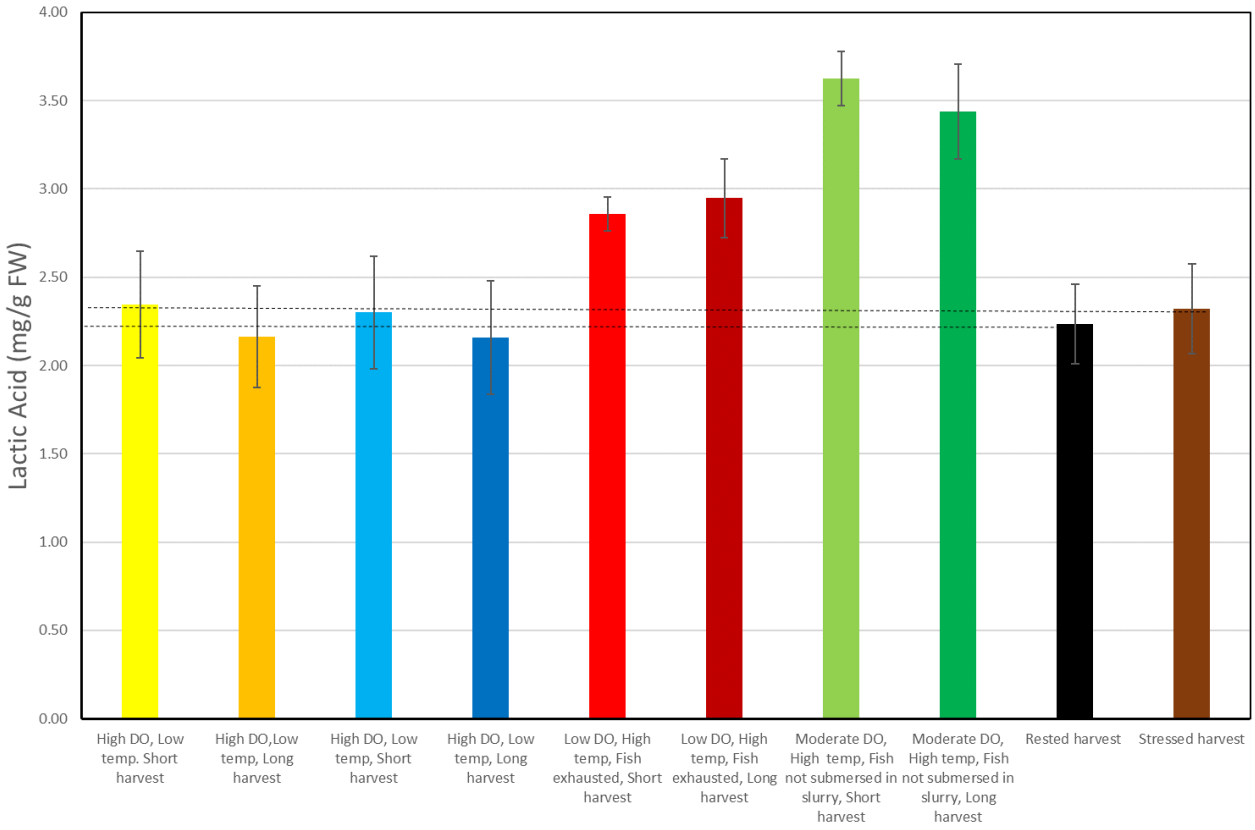

Figure 25. Barramundi lactic acid levels immediately post-harvest.

Legend

- Controlled rested trials (black bar) lactic acid confidence interval (84% CI: 2.13–2.34, n = 15).
- Controlled stressed trials (orange bar) penetrometer confidence interval (84% CI: 2.23–2.42, n = 15). Trial A (red line) low dissolved oxygen environment lactic acid confidence interval (84% CI: 2.81–2.92, n = 7) for short harvest and (84% CI: 2.81–3.10, n = 7) for the long harvest.
- Trial C (two green bars) moderate DO fish not submersed in slurry, lactic acid confidence interval (84% CI: 3.52–3.73mg/g, n = 5) for the short harvest and (84% CI: 3.25–3.63mg/g, n = 5) for long harvest.
- Trial D (blue bars) high dissolved oxygen environment penetrometer confidence interval (84% CI: 2.06–2.44mg/g, n = 7) for the short harvest and (84% CI: 1.97–2.35mg/g, n = 7) for the long harvest.
- Trial E (yellow bars high dissolved oxygen environment, lactic acid confidence interval (84% CI: 2.19–2.52mg/g, n = 5) for the short harvest and (84% CI: 2.00–2.40mg/g, n = 5) for the long harvest.

Interpretation of harvest and post-harvest findings

Fish crowding

Fish crowding for the purpose of harvesting was observed on farms as slow and calm to avoid stress to the fish. Barramundi will naturally start to stress as they become aware of the harvest net enclosing and becoming an obstacle or barrier in the pond. The net must be introduced in a slow and steady manner in the pond to reduce the risk of sudden avoidance reaction by the fish which will result in stress. The reaction to the introduction of the net will not be as severe if the fish are given space and time to adjust.

The person controlling the harvest must be able to easily communicate the need to adjust or slow the Harvest down with all team members. The team must communicate and manoeuvre the net in such a way as to avoid pockets or folds that may trap fish. The fish should only be crowded in such a manner that they are still able to swim in a deep enough body of water to limit stress, while awaiting the brail operation or fish pumping to be conducted. Brail must be of suitable mesh gauge for the size of fish to be harvested and maintained in good working order to avoid physical damage to the fish. Reducing the volume of each brail pickup to approximately 100 kg will reduce the weight on the fish within the brail, avoiding excessive stress and damage.

Monitoring dissolved oxygen levels in the net at the time of harvest gives harvest crews an indication on what is happening with the fish and if they are rapidly consuming the available oxygen. One harvest team member should be appointed to watch for visual signs of stress in the fish as well as monitoring real time dissolved oxygen levels. If the fish are showing signs of stress, such as dorsal fins above the surface of the water (level 2 stress), gasping, excessive gill rate (level 3 stress) and inability to maintain upright position (level 4 stress), then immediate action should be taken to rectify the situation (Plate 10). The pond dissolved oxygen level is thus, a critical control measure on how the harvest is progressing and if action is required to modify the harvest to ensure minimal fish stress.

Plate 10. Stressed fish with dorsal fin above the surface of the water, excessive gill rate and inability to maintain upright position.

Pond dissolved oxygen levels must be monitored and maintained above 5.0 mg/L during the crowd and harvest process. This is particularly important as water temperatures begin to increase above 25 °C limiting the amount of oxygen that is able to be absorbed into the water column. ***Crowding must not begin if the dissolved oxygen level is below 5.0 mg/L. Dissolved oxygen levels must be maintained above 5.0 mg/L for the entire harvest operation by adding extra aeration or bottled oxygen as required.*** Even delaying

harvest start times until the morning dissolved oxygen has risen to suitable levels will help. Manipulating the algal blooms in the pond prior to harvest days can also help reduce oxygen demand for early morning harvests. Another problem that may occur is hydrogen sulphide poisoning when oxygen levels are low. (Refer to Appendix 7 for further explanation).

Reducing the volume of the crowd will also reduce the oxygen demand during the crowding and harvesting process to help maintain suitable dissolved oxygen levels. Reducing the volume of the crowd will also allow fish to be harvested in a timely manner and avoid excessive holding in a confined space, thus limiting the length of time exposed to crowding stress. Reducing the volume in the crowd can be used as a mitigation strategy.

It was also noted that farms are only taking an estimated guess at how many fish they will capture with each pass of the harvest net. This can become problematic when more fish are caught than are required for the days harvest. Trials to date give an indication that smaller harvest volumes provide less stress on fish during harvest, however farmers have no way to accurately measure this and rely on experience and best guess on the day. It may be worth undertaking future investigations to see if there are any under water technologies that can measure the volume of fish in the crowd enclosure to give real-time feedback to the harvest crews.

Overloaded ice slurry bins

On farm observations revealed that ice slurry bins are occasionally overloaded resulting in some fish remaining on top of the ice slurry or only being partially buried in the ice. The two main reasons this is occurring include:

1. Farms are adding too many fish in each bin and there is physically not enough room for the fish to remain submerged under the water level of the ice slurry (Figure 30, zone 3). This is not always intentional; however, it happens when there are a few extra fish in the brail that are dropped on top rather than being put into a next ice slurry bin.
2. The ice slurry ratio being too stiff or dry causing the fish to raft on top of the ice (Plate 11). This was evident on harvest days that were hot (30 °C+), where farms were adding extra ice to combat high water, fish volumes and high ambient temperatures.

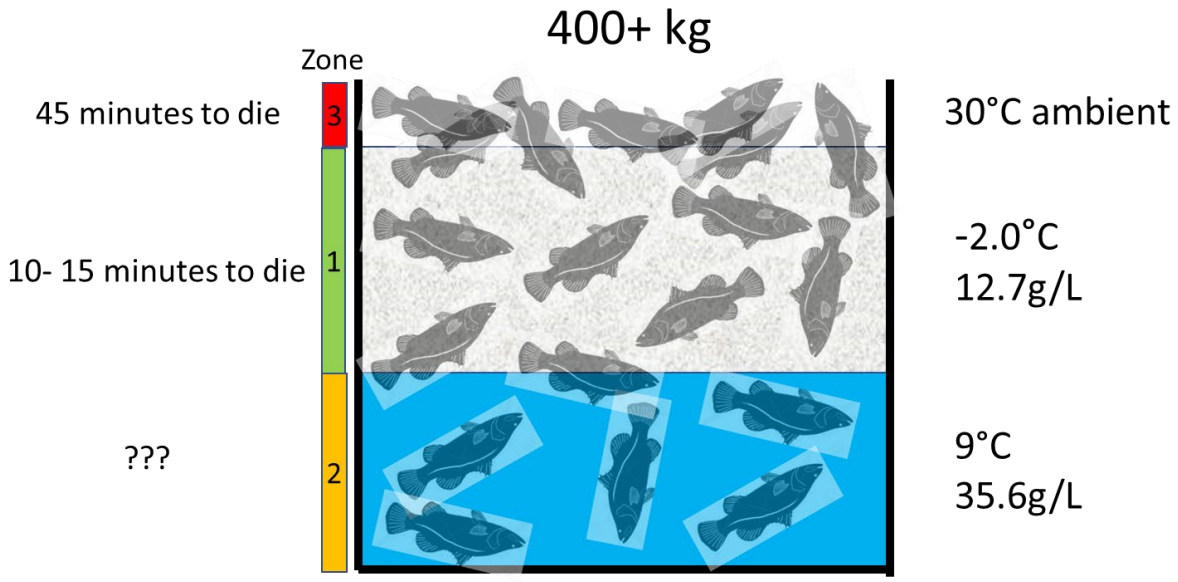

Figure 26. Diagram of commercial slurry bins depicting 3 distinct zone when bins are not continuously mixed.

Plate 11. Example of an unintentionally overloaded bin.

Unfortunately, in some instances the fish remaining on the top of the ice are taking up to 45 minutes before they die. Rigor, pH and Lactic Acid results all demonstrate elevated stress levels (Figure 14, 15 and 16).

Whole Barramundi core temperatures in commercial ice slurry.

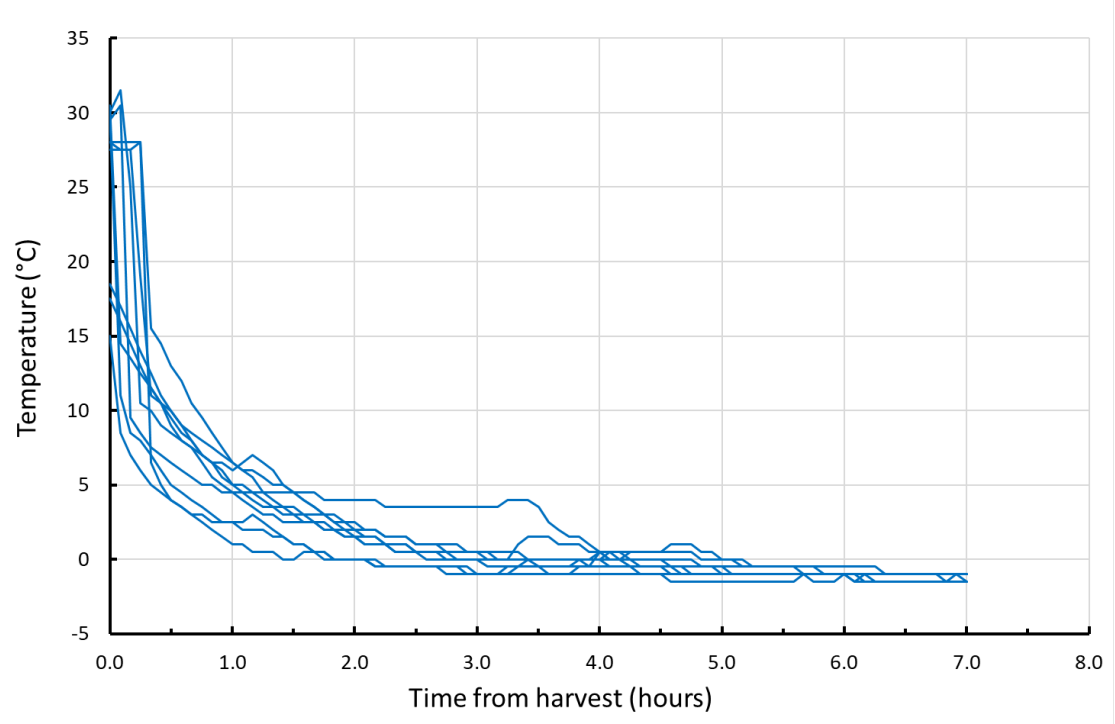

Figure 27. Individual core temperatures of whole farmed Barramundi in commercial ice slurry

The core temperatures of fish were monitored from unmixed bins to gauge how efficient these were at chilling the fish post-harvest. On-farm measurement of chilling effect of a 1:1 water/ice ratio in a 2% brine slurry is illustrated in Figure 31. Fish were obtained from pond water at a temperature of 28 °C and data was continuously recorded at 5-minute intervals over a seven (7) hour period.

Core temperatures were monitored from fish taken just below the slurry water line level in the coldest section of the ice slurry bin. These results show that the whole fish are chilled to below 4°C in the first 2 hours. Fish located on the bottom, in the warmest part of the bin were not monitored for core temperature. If fish numbers were reduced in each bin along with consistent mixing, then fish may cool faster and more consistently than current methods used.

Ice slurry composition and fish loading

The project investigated ice slurry composition and monitored the effect of fish load on slurry parameters over time. Table 10 provides salinity and temperature data of freshly created ice slurries made with different water/ice ratios and incorporating different salt levels. These ice slurry compositions were simulated at DAF's Coopers Plains facility, based on typical commercial slurries used on Australian Barramundi farms.

When comparing the range of ice slurries used on commercial farms, it was noted that on hot days, farms were adding extra ice to the slurry to combat ellevated air and water temperatures. If too much ice was added and the mixture increased close to a 1:4 water to ice ratio, the slurry became too dry and allowed the fish to raft ontop of the ice (Plate 13).

Table 10. Salinity and temperature of different ice slurry compositions trialled.

Brine (salt %)	**Water: ice** ratio	**Slurry salinity** (ppt)	**Slurry temperature** (˚C)
2	1:1	nt	-0.9
	1:2	nt	-0.8
	1:3	13.4	-0.8
	1:4	11.2	-0.8
3	1:1	28.7	-1.2
	1:2	27.5	-1.2
	1:3	25.2	-1.2
	1:4	25.7	-1.2
4	1:1	38.3	-1.7
	1:2	35.5	-1.6
	1:3	27.1	-1.5
	1:4	27.7	-2.1
5	1:1	45.6	-2.1
	1:2	44.5	-2.1
	1:3	43.5	-2.1
	1:4	40.9	-2.0

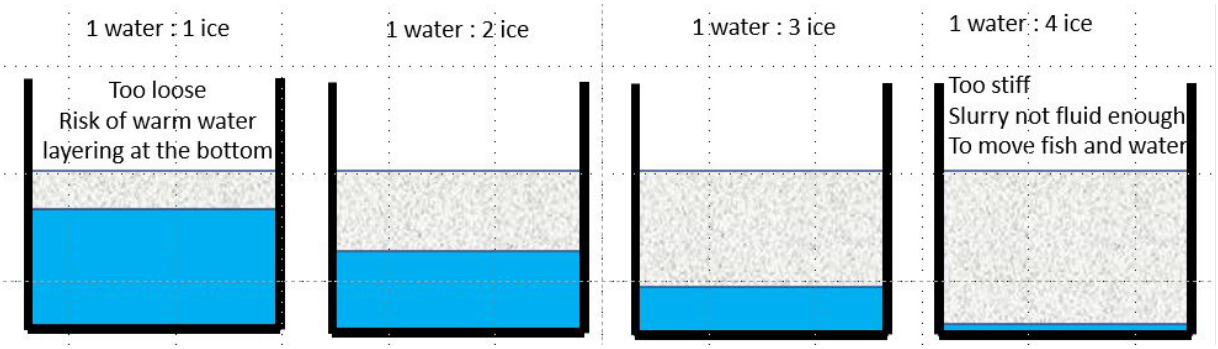

Figure 28. Diagram representing depth of ice in slurry at various ice to water ratios. Blue section representing water only with the grey section being the floating ice once it has come to equilibrium post mixing.

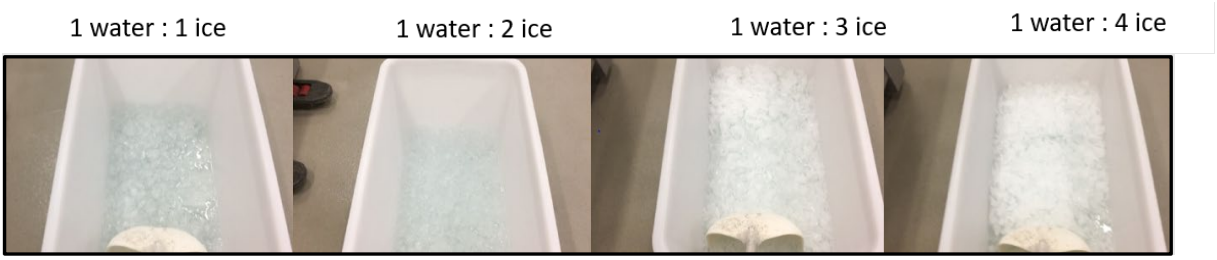

Plate 12. Photos of ice slurry ratios ranging from 1:1 to 1:4 ice to water.

Plate 13. Example of fish rafting on top of ice slurry that is too stiff.

This problem is compounded if the bin is filled to capacity with fish (400 kg+), as it is not possible to push the fish below the ice due to limited room remaining in the bin to manovour the fish.

Each farm has a slightly different method of making their ice slurries due to farm logistics and ice making capacity. We recommend that daily discissions be made about the water and ambient temperatures, then adjust the ice slurry ratio to make a suitable slurry mix for the harvest day conditions on each farm. Keeping in mind that 1: 4 water to ice is too stiff. We cannot recommend a particular ice water ratio that will be ideal on every farm, as each ice machine makes different size and thickness flakes of ice which alter the melting properties and each day the daily temperature of the water added is different (particularly summer to winter). These variables effect the final slurry ratio, once the mix has equilibrated. A broad starting guide is to ensure enough water in the bin to allow the raft of ice to float, so the bottom ¼ of the depth of slurry is just water and ¾ of the slurry depth is floating ice at the start of harvest as depicted (Figure 33).

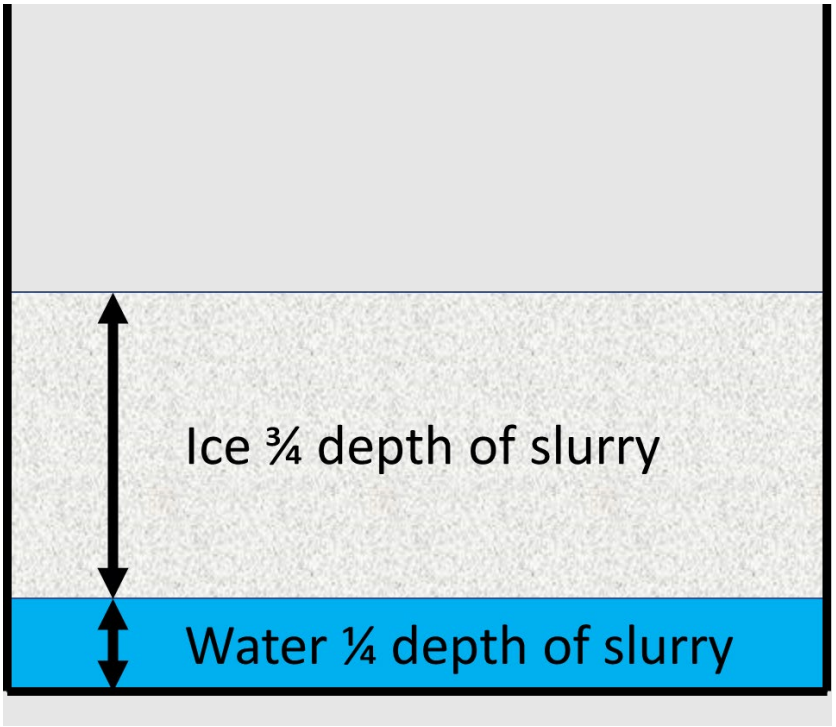

Figure 29. Diagram representing a starting point (guide) for the ice slurry mixture ratio, just prior to harvest.

When adding salt to the ice slurry bins, it is essential to ensure that the ***salt is fully dissolved at ambient temperature before adding to the ice***. It is difficult to dissolve salt at low temperatures and incidents have been observed with salt not being mixed prior to addition of harvested fish (Plate 14).

Plate 14. Salt not dissolved prior to adding to ice

Mixing ice slurry bins

Once the bins are loaded with fish, they are transported from the pond side back to the processing area with varying degrees of urgency, depending on the farm and established protocols. All farms mix the bins manually and re-ice differently. It was observed that these activities are not seen as equal priority on all farms, with the longest delay till re-icing recorded, being four hours from pond side and the shortest delay being two hours. To add to this problem, when fish remain in the ice slurry bin without effective mixing, salinity and temperature stratification occurs. This results in large differences between the top and the bottom of the bin, for both the salt concentration and temperature. For example, insufficient mixing results in the salt sinking, creating a high salt concentration at the bottom of the bin and a low salt concentration at the top.

Figure 34 reveals a temperature difference of up to 11 °C between the top and bottom of the same ice slurry bin. In this instance, fish remained in the stratified ice slurry for up to four hours before any mixing commenced. Even when mixing commenced, it was not sufficiently effective to mix the ice slurry to obtain a uniform temperature throughout the bin. Alongside the temperature stratification, there is a difference in salinity levels ranging from 12.7 g/L at the top of the slurry to 35.6 g/L at the bottom of the slurry (Figure 34).

All commercial Barramundi farms that participated as part of this project utilised some form of mixing of the ice slurry. However, all farms were unable to maintain a uniform ice slurry temperature and salinity level over the entire chilling process prior to pack out.

We recommend that each farm investigates their on-farm slurry bin mixing protocols to ensure adequate flow within each bin is sufficient to create a single low temperature in the bin with no stratification over the entire chilling process.

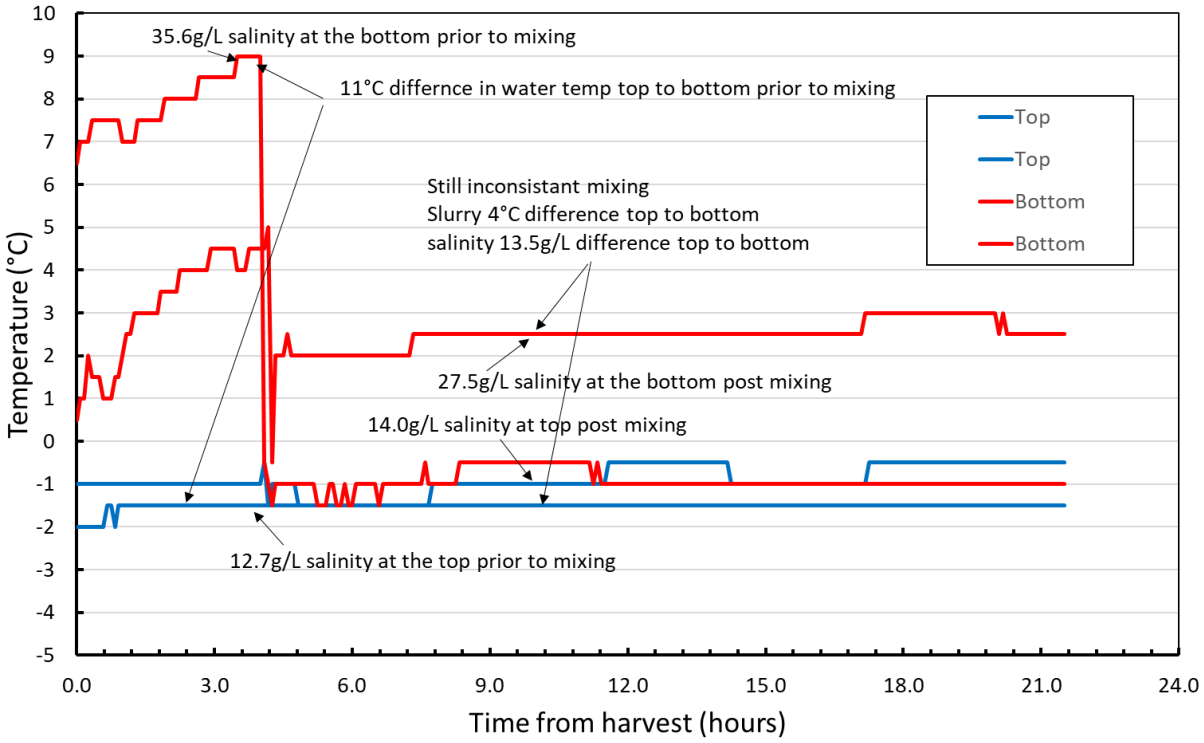

Figure 30. Example of ice slurry temperature variations prior to mixing (approximately 420kg Barramundi in 1000L).

Discussion

Currently the industry does a great job of focusing on the quality and health of the fish while maximising the Feed Conversion Ratio (FCR) through the grow-out process. When it comes to harvesting, the current focus appeared to be based on a calm efficient harvest. The most effective way of getting the fish out of the water and into the ice slurry. This is an understandable goal as the fish are being removed from warm water temperatures and are exposed to hot ambient temperatures. Therefore, it makes sense to get the fish into ice slurry as quick as possible.

Determine stress imposed on fish during harvest operations and develop methods for stress reduction.

Measuring and reducing stress in harvest crowds

Hand-held instrumental measures, initial flesh pH and the rate of rigor mortis for several hours, showed greater utility as descriptors of stress in experiment and on farm than laboratory assays of initial concentrations of muscle metabolites in harvested Barramundi. This is the first time that harvest stress has been associated with significant post-mortem softening of flesh in Barramundi though the untrained consumer panel was inclined to prefer the texture and flavour of the stressed fish. A lesson to draw is that not all variation in quality is bad, but the variation may still need to be understood in a controlled panel environment. Intriguingly, during sample preparation, the flesh from the different treatments seemed to differ in appearance - a parameter we had not planned to measure electronically.

Very high initial fillet lactate concentrations are associated with oxygen starvation, either in dense harvest crowds or in fish asphyxiating on top of an ice slurry. A causal link between these observations is reasonable to draw. What is harder to explain is why initial lactic acid levels in fillets in the rested harvest v stressed experiment and the lower temperature farm trials suggest a rapid breakdown of muscle glycogen into lactic acid occurred in rested harvested fish. Despite this evidence of metabolic activity, muscle ATP content could not be sustained.

The lowest post-mortem lactic acid levels in this study were seen in Barramundi harvested from the coolest water, though for Barramundi loss of muscle energy reserves are more likely at pond temperatures below 20 °C (Rodgers et al., 1993a, 1993b). Glycogen concentration is seldom studied in Barramundi and these are only t=0 measurements, we do not know where the lactic acid levels peaked afterwards. Nutritional research on this species focuses upon nutrient assimilation and growth, not on muscle function, and sensory panels have not generally reported quality problems following nutrition trials, (Glencross, 2006). The one caveat on this is that the insulin-like growth factor IGF-1 is likely to modulate glycogen reserves in Barramundi muscle (Drakenberg et al., 1997, Degger et al., 2000). Channel catfish are probably exposed to a wider seasonal temperature change than Barramundi, but they demonstrate this principle: post-mortem acidification was sharply attenuated in winter-acclimated catfish (Bosworth et al., 2007). However, operational indicators like rigor and flesh pH successfully explain the quality variation observed, so there seems to be no case for biochemical analysis of other muscle parameters.

Activity of Barramundi in the crowd is relatively easy to judge by eye and salmonid codes of practice also prioritise well planned and gentle harvest crowds. Nevertheless, this project has identified that dissolved oxygen level in some circumstances the crowd can reach critical levels. In this case, the method would be to monitor the oxygen level during harvest and balancing this by adding oxygen and reducing the number of fish in each crowd. Flesh pH and rigor results from these current trials suggest there is a trend that the longer the fish are in the crowd net, then the more stressed they will become. Reducing the oxygen demand during the harvest process would enable DO levels above 5 mg/L to be maintained, ultimately reducing stress. Adding oxygen and/or extra aeration to every harvest maximises the dissolved oxygen in the water and reduces the stress that Barramundi are exposed to.

Lessons for future research

Standard research and industry methods should be documented that actively score the behaviour of Barramundi in harvest crowds. Barramundi's tolerance of low DO is based upon a compensatory rise in opercular ventilation rate (Butler et al., 2007) but poor water transparency may hide this specific response from the harvest team.

Season and fish condition should be considered in future research to establish the extent to which muscle energy status at different times of growth determine the post-mortem pH of Barramundi, which in turn is an important driver of product quality outcomes.

An unexpected difference in sample appearance and colour emerged during sensory analysis. A variety of hand-held instruments can measure flesh colour and transparency in excised samples. Panel members liked the appearance of the cooked flesh of stressed fish, yet this is a source of quality variation worth following up in future harvest research. Some methods are readily accessible. For example, colour meters were used in a study of barramundi fillet greyness, (Howieson et al., 2013). The pink hue to the uncooked flesh points to the presence of blood in the fillet, a pigment that is also accessible to visible/near-infra red hyperspectral techniques (Svalheim et al., 2019 and 2020).

Methods of slaughter that minimise stress and incorporate animal welfare best practice.

Best practice use of ice slurries

Ice slurries have for many years been the fastest and hence most humane method available for tropical fish farmers to kill their fish. But as technology capable of immediately stunning fish becomes more widely available the sector is under pressure to defend continued use of the practice, (OIE, 2019). The methods available to the Barramundi industry to optimise the effectiveness of slurries is to control the volume of fish in each slurry bin and control the ice-water ratio of the slurry. This allows for more efficient mixing of ice-cold slurry water around each fish. This also reduces the time taken for the fish to die in the iced slurry. Less fish in each bin and optimum slurry ratios also prevents fish rafting on top of the ice so that fish die as quickly as possible. Careful attention to slurry use will remain important. Once new stunning techniques are available, it is likely that Barramundi will be ice-slurried immediately afterwards to ensure that the unconscious fish do not revive, (OIE, 2019).

Killing fish in an ice-slurry is sometimes called asphyxiation but a literature search returned no studies that weigh the impact of reduced tissue metabolic rate in the cold against the aversive exercise and respiratory arrest that accompany thermal shock. The symptoms at death may be complicated by differences between species, acclimation temperature and experimental design. Grass carp killed in ice-slurry for 20 min emerged with a muscle ATP concentration of ~2.5 umol/g, whereas in that same study, electro-stunning by itself exhausted the fish further than the slurry did- most adenylate nucleotides had already converted to IMP (Scherer et al 2005). An experiment using rainbow trout emphasised the long delay before fish stopped moving in the slurry- the authors didn't report ATP levels but reported that K-value immediately post-mortem that were already higher in ice-slurry killed fish than in manual percussive stunned individuals, (Özogul and Özogul, 2004). That is tantalising evidence that ATP stocks were consumed in the slurry. Yet in a tilapia experiment, dead fish sampled after 20 minutes in an ice-slurry showed moderate albeit diminished ATP levels when compared to samples from tilapia sedated using carbon dioxide in water (Oliveira-Filho et al 2015). In contrast, ice-slurry killed Barramundi have almost no ATP in their muscles.

Cold shortening remains a possibility here. Rapidly chilling tropical fish exposes ATP degrading enzymes in muscle to elevated Ca^{2+} concentrations, this enzymatically exhausts the ATP present –muscle fibres contract in the absence of ATP, and the fish rapidly stiffens, (Lee et al., 1998). Aversive struggling and cold shortening during the 15 minutes in the slurry could feasibly deliver Barramundi muscle samples to the liquid nitrogen with almost no ATP remaining. Tropical fish plunged into ice-water frequently show a faster onset of rigor than typical of cold-water fish, but regardless in each case stress during harvest primes rigor

to begin sooner after death, (Jerrett et al., 2008). This is apparently why the current study showed faster rigor mortis in the stressed harvest treatment even though another process in the chilled muscle that is independent of exercise may have later depleted ATP level in the rested treatment

Lessons for future research

Knowing that cold shortening is possible in Barramundi does not provide a strategy for responding to it. At this stage, alternative cooling regimes to address this phenomenon in isolation have not proved beneficial for Barramundi quality (Poole et al., 2000). In contrast, harvest stress has a well demonstrated impact on flesh pH and rigor mortis rate and it is a source of variation that is largely under the farmer's control (Jerrett et al., 1998).

On farm Protocols that ensure practicality and cost-effectiveness

Crowd dissolved oxygen may be a critical control point, and we propose a value of greater than 5mg/L is maintained through harvest particularly at high water temperatures. Cost effective strategies to immediately modify current harvest protocols have been successfully trialled on farm with industry. These protocols are both practical and cost-effective. If they have not already, harvest teams may need to change their focus slightly to think about harvesting their fish as though it was a transfer rather than a harvest. This keeps each fish alive right up to the slaughter process, without physical damage and with maximum amounts of oxygen available during the crowd and harvest.

Harvest volume and slurry composition at harvest will also be critical control points for harvest teams to monitor.

Farms should prioritise, if they have not already, techniques for managing harvest volume and slurry composition and also developing a method for reliable and continuous mixing within each slurry bin to minimise temperature and brine stratification for the duration of the chilling process.

These straightforward steps will optimise the current industry use of ice slurry as a slaughter method. This greatly reduces the harvest-slaughter stress that Barramundi are exposed to while new methods to address more humane harvesting are investigated.

Guide material on effective protocols for Industry best practice

An A4 booklet has been produced and printed to be distributed to all ABFA farms. This booklet is a summary of the critical activities that affect the stress on Barramundi during the harvest and slaughter process and recommendations for the best way to address these problems.

Conclusions

This project determined through farm trials that during the harvest process the critical activities that caused the most amount of stress were low dissolved oxygen levels and large amounts of fish in the crowd, adding pressure on oxygen demand. Recommendations have been made for farms to always monitor levels throughout the harvest and maintain dissolved oxygen above 5 mg/L by adding oxygen or some form of aeration. The volume of fish crowded each time should be determined by the oxygen demand in each pond. Reducing volumes of fish in the crowd will assist in maintaining high dissolved oxygen levels and also reduce the time fish are held in confined space prior to slaughter.

When evaluating the methods of slaughter to minimise the effects of stress, the use of ice slurries that were too stiff was a major issue, allowing the fish to raft on top of the ice. Fish exposed to air during this process took too long to die. Overloading of bins also led to inadequate cooling of fish. There was inadequate mixing of the slurry in the bins which meant a consistent, uniform low temperature could not be achieved. All of these factors individually are cause for concern when addressing animal welfare issues and are detrimental to quality. Recommendations have been made to address each problem.

Recommendations

The pursuit for quality and consistency of Australian farmed Barramundi is a continuous journey. This project was expected to make practical and cost-effective recommendations. The following recommendations are considered to be easily implemented and can have a dramatic effect on the welfare and ultimate quality of farmed Barramundi.

We recommend:

- ***Regularly train staff on the importance of a stress-free harvest and refer to the summary booklet.***
- ***Maintain pond bottoms during the grow out season to avoid build-up of organic material consuming oxygen as it decomposes.***
- ***Assess the daily air and pond temperature prior to harvest.***
- ***Mix a suitable slurry that is not so dry that it allows fish to raft on top of the ice.*** *Ensure that salt is fully dissolved at ambient temperature prior to ice or chilled water being added.*
- ***Pond dissolved oxygen levels must be monitored and maintained above 5.0mg/L during the crowd and harvest process.*** Crowding must not begin if the dissolved oxygen level is below 5.0 mg/L. Maintain high DO levels. Different approaches are possible: add bottled oxygen to every harvest, use extra paddle wheels or alternatively, delay harvest till pond DO has increased.
- ***Harvest calm and slow (treat every harvest like a fish transfer)***
- ***Reduce the volume of fish in each crowd when temperatures are high and dissolved oxygen levels are low.***
- ***Do not overload ice slurry bins. Only load 350 to 400 kg of fish in each 1000 L slurry bin at one time.*** This allows all the fish to sink below the waterline of the ice slurry reducing the chances of the fish accidentally sitting on top of the ice. Reduced fish volumes in the bin also gives the crew a better chance to manually mix the bins prior to being hooked up to an automated mixing system.
- ***Need to adopt methods to ensure sufficient ice slurry mixing to minimise temperature and brine stratification for the duration of the chilling process.***
- ***Need to ensure that there is sufficient space in the ice slurry bin to add more ice if needed, without having to drop water and concentrated salt out of the bottom of the bin first (350–400kg will achieve this).***

These recommendations bring the Australian Farmed Barramundi industry's current use of ice slurry as a slaughter method to a consistent high standard. Our data has shown that when performed using all of our recommendations, it is possible to harvest your fish in a low stress manner.

During the course of this project an unprecedented global push began to remove ice slurry as an acceptable method for the euthanasia of farmed fish. This is part of increased pressure to further improve the welfare of animals at slaughter. There is therefore an urgent need to revisit the crowding, harvesting and slaughtering of Barramundi.

In the pursuit of high quality and welfare, the industry will need to balance what can be done to change existing practices alongside the capital investment needed for new technology. The project FRDC 2021-051 "Preliminary evaluation of electro stunning technology for farmed Barramundi" has concluded. This provides valuable insight for the best way for the industry to proceed.

We recommend that the new practices and technology can be benchmarked using the existing 'toolbox' of flesh quality parameters used in these trials. We have shown that pH and rigor index can detect significant differences between the level of stress that fish are exposed to during the harvest process on farm. This will enable ABFA members to quickly visualise the gains made in reducing stress when harvest systems are refined, or new systems trialled on farm.

Extension and Adoption

Individual farm trial results have been discussed with each farm manager personally to maintain anonymity over results. This has allowed maximum extension of any problems noted on individual farms to be fully discussed and changes to protocols made. Disclosure of individual results has been able to be shared across the ABFA by de-identifying any data prior to reporting.

The final report will be made available to ABFA members along with a copy of an A4 printed summary booklet as a handy reference tool.

A final presentation delivering a summary of results will be presented at a ABFA workshop/conference.

Reduction to loading densities in slurry bins and effective mixing protocols have been trialled on-farm with instant improvements shown. These new protocols have been adopted on some farms immediately (In-house result from on-farm changes not released as part of this report).

Reduction of volume of fish in the crowd to maintain dissolved oxygen levels above 5.0mg/L has been demonstrated on farm. This combined with a delay in harvest time allowing the daily dissolved oxygen levels to increase prior to harvesting resulted in fish that were no longer physically exhausted.

Project materials developed

The final report will be made available to ABFA members along with a copy of an A4 printed summary booklet as a handy reference tool.

An extension booklet for Barramundi farmers is attached in Appendix 3.

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Appendix 2. Summary of ABFA survey

Harvesting Practices documented

It was important to gain specific understanding of individual farm operations to develop an operational research plan that would be relevant across all Barramundi farms. Australian Barramundi Farmers Association (ABFA) members agreed information collection would be most effectively achieved on-line and a questionnaire was developed using Survey Monkey platform.

Information sought was limited to practices that are known to impose stress on fish through the harvest and slaughter process and did not consider further supply chain phases. Particular attention was paid to question clarity and that each question related to a specific action within the harvesting process to illicit only one piece of information. Wherever possible, responses were designed as selective choice by listing or use of a sliding scale for numerical responses to minimise time for response. The draft survey was run by key ABFA members to check for relevant Industry language and ease of response.

There was an excellent response from ABFA members. Response information is taken directly from survey responses, with some conversion to common measure units where needed, as summarised following.

Production systems

In Australia, Barramundi is most commonly aquacultured in open-air ponds and these are located in tropical regions. The ponds are earth-based, with one farm using lined ponds. There are three farms operating recirculating aquaculture systems and currently only one sea-based cage production system. Table 9 provides indication of size of different operations.

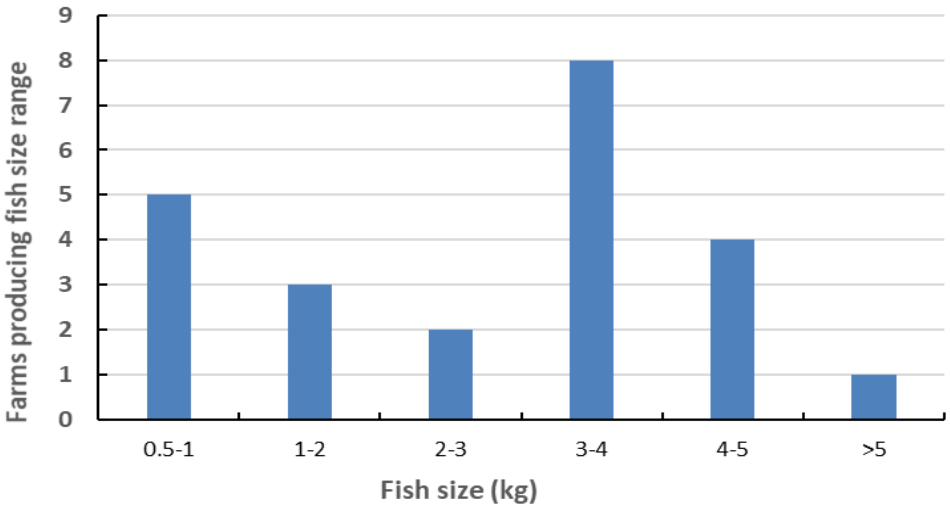
Table 11. Productions systems

System		No. of ponds/tanks	Grow-out system size	
			(Hectares)	(Megalitres)
Outdoor ponds	6	24 - 58	0.1 – 0.7	
Outdoor lined ponds	1	24		1.0
Indoor recirculation tanks	1 *	21		6.0
Ocean cages	1	36		50

*Only 1 of 3 of this production type responded to survey

The number of ponds/tanks/cages within a production system varies between 13 and 58, with four farms having <25 grow out units and five farms having between 35 and 60 grow out units. Most farms produce multiple size ranges of fish for market (Figure 31). Five of nine farms produce ~1kg size fish, however many farms provide larger size fish: seven farms providing 3–4 kg fish and four of those farms also provide 4–5 kg fish.

Figure 31. Size ranges of Barramundi produced by farms



Harvest practices

All farms withhold feed prior to harvesting and the common duration is 1 day. One response indicated 1–2 days dependent on water temperature, another farm typically withheld feed for 2 days and a further one uses a 5-day withholding period. Most farms do not include a fish purging step immediately prior to harvest. For the two farms that do, purging is for 4–7 days and 3 weeks duration respectively. The volume of fish harvested from one pond is different across farms, ranging from 1 tonne on smaller farms to 35 tonnes on a large farm (Table 2).

Table 12. Crowding parameters on individual farms.

Total harvest volume from one pond/tank/cage (individual farm)	**Time for to harvest volume from one pond/tank/cage** (hours)	**Number of crowds to achieve total harvest volume**	**Volume of fish in separate crowd** (tonnes)	**Typical duration of separate crowd** (minutes)
1	1	1	1	30
23	5	3	7	90
8	3	3	3	60
2	1	1	2	30
35	7	2	15	70
15 *	5	n/a	n/a	n/a
7	2	1	7	30
16	3	4	6	40
8	3	1	8	30

* RAS producer does not crowd fish for harvest

Correspondingly, the duration of harvest is different farm to farm, varying from 1 hour to 7 hours. Again, dependent upon the size of harvest, crowding of fish for harvest occurs once or through several individual crowds ranging in number from 1–4 and the maximum time fish remain in a crowded state is 90 minutes for the larger crowd volumes. Of note is that duration of crowds is less than 70 minutes, with the exception of the sea-cage production where fish may be crowded for 90 minutes. Comments proffered in survey

responses mentioned the reason to break a required large harvest volume into smaller separate crowd events was to reduce and minimise stress to the fish.

As part of production management practices, farm managers monitor stocking density of fish in ponds throughout grow-out phase of production. However, survey responses indicated that for any one crowding operation at harvest time, it can only be an educated guess as to exactly the number of fish that will be in each crowd. Where insufficient fish are obtained, further crowds are carried out. Contrastingly, where a greater volume of fish than expected are gained in one crowd, the additional fish are released back into the open pond. One farm does not ever return surplus fish back into the grow-out system.

Five farms measure oxygen levels of the water at the time the fish are fully crowded at each of pond with common reports of oxygen levels of 3–10 ppm. These farms typically add air or oxygen to the crowd waters. Six of the nine farms surveyed monitor fish behaviour through the duration of the crowd period.

For harvest of the crowded fish, two farms use a fish pump. However, the majority use a form of brailing from the crowd into the holding bin. Brailing methods include wet brail, dry brail, harvest net with crane or by hand using crates.

Slaughter practices

All open pond farms transfer fish from the crowd into a 1000L bin of ice slurry, with the RAS production system using a slurry bin of 760L or 10,000 L. The slurry composition varies farm to farm with seawater, potable water or bore water used for the aqueous component (Table 12). The ratio of water to ice is commonly a 1:1 ratio but other consistencies are also used.

Table 13. Composition of ice slurries used by individual farms.

Water type	Water : ice ratio
potable water	1 : 3
seawater	1 : 1
seawater	1 : 4
potable water	1 : 1
salt water	1 : 1.5
bore water	1 : 1
bore water	1 : 1
salt water	1 : 1
bore water	thick slush consistency

Whatever the water type used to make the slurry, many farms also add salt (sodium chloride). Two farms, one with potable water use and the other using seawater, add no supplementary salt. Table 13 provides the salt amount added and the volume of fish typically loaded into the slurry. The addition of salt has an influence in reducing the temperature of the slurry and therefore may permit a lesser amount of ice required, especially when loading larger volumes of fish.

Table 14. Salt added to slurry and fish volume typically loaded.

Added salt to slurry (kg)	**Slurry temperature** (°C)	**Fish weight loaded** (kg)
0	unknown	300
2-3	0-1°	500
0	0 °	450
5-6	-5 °	350
10	≥ -8° *	500
8	0 °	300
7.5	-2°	470
9	2	500
12.5	unknown	400

*starts at -8°C and rises as fish loaded in

All farms use the ice slurry step as the slaughter method and time fish spend in the slurry ranges from 4 hours to 30 hours depending on farm operation. The majority of farms (6 of 9) retain the fish in the slurry for 18-20 hours. The time of death of fish in the slurry is usually assessed by core temperature of fish (5 of 9 farms). Two farms determine point of death of the fish based on a specific time and one slaughter is adjudged as when body or gill movement of the fish is not evident.

Additional comments received

Several farm managers responded to the opportunity of suggesting investigation focus on specific aspects of harvest practice that would be beneficial information for their operation:

- benefit gained from addition of oxygen to the crowd for reducing fish stress
- application of a stunning process for reduction of fish stress
- effect of bottom disturbance the weeks before dragging harvest net

and an additional consideration, not directly related to reduction of stress:

Appendix 3. Copy of A4 booklet for ABFA members

Barramundi - Harvest and slaught

Appendix 4. List of Project staff

Contracted Project Team

Sue Poole, Paul Exley, Carl Paulo, Simone' Moller, Ishita Pramanik

Additional Team investment

Brett Wedding, Steve Grauf, Luke Pavich, Jenson George, David Edwards, Sharon Pun, Dianna Liu,

David Williams, Brian Paterson, Carole Wright.

Appendix 5. Linear relationship between the stress indicators of pH and lactic acid

Several investigations have stated that there is a significant linear relationship between the stress indicators of pH and lactic acid content (Fukuda et al., 1984; Ogata et al 2016). In the former study, the lactic acid increased to about 100.3 µmoles/g and pH decreased to around 5.8 at 8 hours after death of chub mackerel samples.

To investigate whether the values obtained in the current study provide evidence in support of this statement is graphically presented in Figure 32.

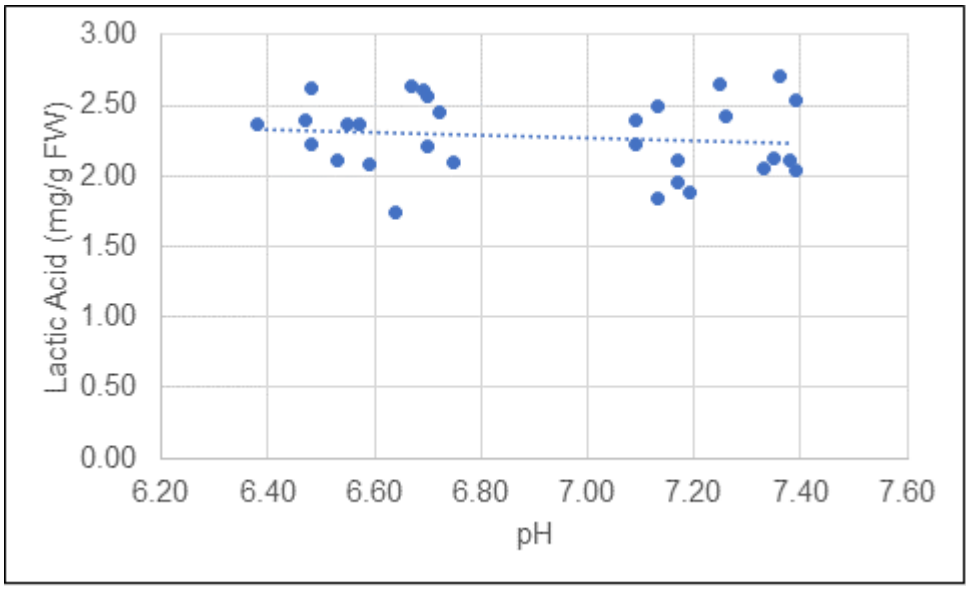


Figure 32: Changes in the lactate content (mg/g FW) and flesh pH for fish collected from both treatments during controlled stressed verses rested harvest trials. Data are the mean of duplicate analysis.

There is no evidence of a significant linear correlation between lactate content and pH measured on the Barramundi samples collected in Stressed verses Rested harvest trials (r = -0.131; p = 0.491).

Ogata et al. (2016) further stated that lactate gradually accumulated in accordance with ATP degradation in the muscle of spotted mackerel with a correlation existing between lactate and ATP contents. To determine whether a similar statement could be made about Barramundi, a graph of the two parameters from the current trial was constructed (Figure 33).

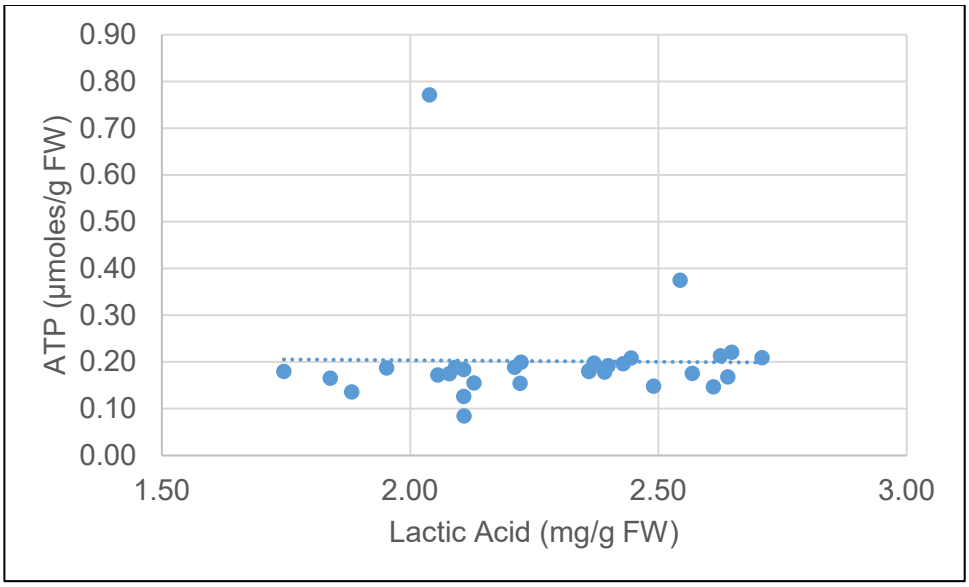


Figure 33: Changes in the ATP (µmoles/g FW) and lactic acid (mg/g FW) contents for fish collected from both treatments during controlled stressed verses rested harvest trials. Data are the mean of duplicate analysis.

The results from stressed verses rested harvest trials found no evidence to support Ogata's conclusion that there is a linear correlation between lactate and ATP contents ($r = -0.015$; $p = 0.936$). However if the two higher ATP concentrations are excluded, the correlation is marginally significant ($r=0.369$; $p=0.053$). Initial examination provided no evidence to exclude these results.

One of the aims of the current project was to obtain and collate information for the maintenance of fish product quality. The above correlations provide scientific evidence in deciding which attributes to monitor in the selection of post-harvest treatments that have the ability to defer rigor mortis with the subsequent maintenance of ATP content and high pH.

Appendix 6. Raw data ATP nucleotides and lactic acid results

Table 15. Levels of the six ATP breakdown products and the total nucleotides (µmoles/g FW) plus the calculated K-value (%) for fish collected during controlled stressed verses rested harvest trials.

Treatment	Sample	Lab Rep	ATP µmoles/g	ADP µmoles/g	AMP µmoles/g	IMP µmoles/g	Hx µmoles/g	HxR µmoles/g	K value %	Total Nucleotide µmoles/g
Orange AQUI-S	77	A	0.186	0.147	0.179	11.736	0.098	0.000	0.80	12.3
16/06/2021		B	0.182	0.143	0.175	11.217	0.097	0.003	0.85	11.8
	78	A	0.213	0.170	0.187	12.695	0.078	0.000	0.59	13.3
		B	0.206	0.187	0.181	12.625	0.062	0.000	0.47	13.3
	79	A	0.253	0.183	0.216	12.239	0.105	0.005	0.85	13.0
		B	0.496	0.252	0.216	11.246	0.099	0.002	0.82	12.3
	80	A	0.198	0.162	0.165	12.527	0.090	0.003	0.71	13.1
		B	0.194	0.158	0.156	11.849	0.082	0.004	0.69	12.4
	81	A	0.211	0.183	0.186	11.741	0.044	0.002	0.37	12.4
		B	0.230	0.181	0.193	11.954	0.052	0.005	0.45	12.6
Orange Stress	92	A	0.206	0.160	0.183	12.061	0.064	0.005	0.55	12.7
16/06/2021		B	0.220	0.161	0.198	12.801	0.060	0.004	0.47	13.4
	93	A	0.175	0.147	0.153	12.118	0.095	0.000	0.75	12.7
		B	0.176	0.149	0.154	12.341	0.098	0.004	0.79	12.9
	94	A	0.092	0.158	0.099	9.542	0.059	0.000	0.59	9.9
		B	0.078	0.232	0.078	10.650	0.057	0.000	0.52	11.1
	95	A	0.188	0.028	0.149	12.249	0.187	0.000	0.22	12.8
		B	0.189	0.029	0.149	12.141	0.186	0.000	0.23	12.7
	96	A	0.159	0.074	0.184	12.065	0.149	0.004	0.61	12.6
		B	0.198	0.080	0.142	12.318	0.177	0.000	0.62	12.9
Blue AQUI-S	82	A	0.168	0.185	0.163	11.539	0.053	0.002	0.45	12.1
16/06/2021		B	0.128	0.221	0.127	11.282	0.049	0.000	0.42	11.8
	83	A	0.151	0.125	0.127	10.094	0.081	0.003	0.80	10.6
		B	0.159	0.136	0.135	10.573	0.078	0.003	0.73	11.1
	84	A	0.149	0.194	0.146	10.713	0.047	0.002	0.44	11.3
		B	0.195	0.189	0.157	10.950	0.057	0.000	0.50	11.5
	85	A	0.205	0.164	0.184	11.969	0.061	0.002	0.50	12.6
		B	0.178	0.136	0.158	10.388	0.048	0.005	0.48	10.9
	86	A	0.189	0.148	0.184	11.165	0.094	0.000	0.80	11.8
		B	0.142	0.111	0.141	8.796	0.073	0.000	0.79	9.3
Blue Stress	97	A	0.123	0.283	0.108	12.255	0.047	0.000	0.37	12.8
16/06/2021		B	0.171	0.187	0.162	11.786	0.046	0.000	0.37	12.4
	98	A	0.185	0.175	0.176	12.210	0.058	0.000	0.45	12.8
		B	0.164	0.170	0.157	11.543	0.057	0.000	0.47	12.1
	99	A	0.182	0.135	0.176	11.619	0.115	0.003	0.96	12.2
		B	0.154	0.120	0.143	10.120	0.104	0.000	0.98	10.6
	100	A	0.214	0.182	0.205	12.991	0.061	0.050	0.81	13.7
		B	0.185	0.143	0.179	11.529	0.061	0.033	0.77	12.1
	101	A	0.200	0.155	0.193	12.538	0.116	0.039	1.17	13.2
		B	0.162	0.144	0.166	11.194	0.119	0.038	1.32	11.8
Purple AQUI-S	87	A	0.185	0.031	0.150	12.601	0.156	0.000	0.24	13.1
16/06/2021		B	0.190	0.038	0.163	13.304	0.161	0.000	0.27	13.9
	88	A	0.157	0.124	0.135	10.698	0.073	0.000	0.65	11.2
		B	0.115	0.103	0.098	8.386	0.068	0.000	0.78	8.8
	89	A	0.124	0.041	0.280	11.114	0.173	0.054	0.81	11.8
		B	0.184	0.140	0.146	11.413	0.079	0.000	0.66	12.0
	90	A	0.120	0.095	0.118	8.238	0.054	0.014	0.79	8.6
		B	0.133	0.102	0.133	8.870	0.061	0.011	0.77	9.3
	91	A	0.727	0.324	0.254	10.257	0.048	0.015	0.54	11.6
		B	0.815	0.303	0.194	10.189	0.052	0.006	0.51	11.6
Purple Stress	102	A	0.182	0.144	0.178	11.554	0.088	0.000	0.73	12.1
16/06/2021		B	0.177	0.138	0.149	11.753	0.089	0.000	0.72	12.3
	103	A	0.188	0.054	0.154	13.413	0.194	0.000	0.39	14.0
		B	0.188	0.057	0.150	13.047	0.185	0.000	0.42	13.6
	104	A	0.205	0.030	0.150	12.815	0.193	0.000	0.22	13.4
		B	0.190	0.032	0.146	12.372	0.181	0.000	0.25	12.9
	105	A	0.172	0.176	0.163	11.797	0.061	0.009	0.57	12.4
		B	0.187	0.149	0.179	11.751	0.079	0.005	0.68	12.4
	106	A	0.216	0.164	0.194	13.232	0.107	0.011	0.85	13.9
		B	0.201	0.153	0.185	12.629	0.112	0.003	0.87	13.3

Table 16.Lactic acid content (mg/g FW) and flesh pH for fish collected during controlled stressed verses controlled rested harvest trials.

Treatment	Sample	Lab Rep	Lactic Acid (mg/g FW)	pH
Orange Rested 16/06/2021	77	A	2.04	7.17
		B	2.17	
	78	A	2.75	7.36
		B	2.67	
	79	A	2.63	7.39
		B	2.46	
	80	A	2.45	7.26
		B	2.40	
	81	A	2.72	7.25
		B	2.57	
		Av.	2.49	7.29
		SD	0.24	0.09
Orange Stress 16/06/2021	92	A	2.53	6.48
		B	2.72	
	93	A	2.57	6.70
		B	2.57	
	94	A	2.03	6.53
		B	2.19	
	95	A	2.10	6.75
		B	2.09	
	96	A	2.40	6.47
		B	2.39	
		Av.	2.36	6.59
		SD	0.25	0.13
Blue Rested 16/06/2021	82	A	2.45	7.13
		B	2.53	
	83	A	2.06	7.35
		B	2.20	
	84	A	1.94	7.33
		B	2.17	
	85	A	2.48	7.09
		B	2.32	
	86	A	2.01	7.13
		B	1.67	
		Av.	2.18	7.21
		SD	0.26	0.12
Blue Stress 16/06/2021	97	A	2.60	6.69
		B	2.62	
	98	A	1.98	6.59
		B	2.17	
	99	A	2.80	6.67
		B	2.48	
	100	A	2.29	6.48
		B	2.16	
	101	A	2.44	6.38
		B	2.28	
		Av.	2.38	6.56
		SD	0.24	0.13
Purple Rested 16/06/2021	87	A	1.97	7.17
		B	1.93	
	88	A	2.12	7.19
		B	1.65	
	89	A	2.20	7.09
		B	2.24	
	90	A	2.18	7.38
		B	2.04	
	91	A	2.08	7.39
		B	2.00	
		Av.	2.04	7.24
		SD	0.13	0.13
Purple Stress 16/06/2021	102	A	2.33	6.55
		B	2.39	
	103	A	2.22	6.70
		B	2.20	
	104	A	2.48	6.57
		B	2.26	
	105	A	1.69	6.64
		B	1.80	
	106	A	2.42	6.72
		B	2.47	
		Av.	2.23	6.64
		SD	0.28	0.08

Appendix 7. K-value

The breakdown of Adenosine triphosphate (ATP) nucleotides are well documented as an indicator of fish quality (Saito et al., 1959). While nucleotide results from these harvests do not give a direct reflection of stress that the Barramundi have been exposed to, they do reflect that they are all below 20% ATP umoles/g indicating that all fish tested are all of high quality (Saito et al., 1959).

The degradation of ATP (Figure 26) leads to the formation of adenosine diphosphate (ADP) (Figure 27) and rapidly degrades to the accumulation of adenosine monophosphate (AMP) (Figure 28) and inosine monophosphate (IMP) (Figure 29).

While not significant, observations show harvests that exposed the fish to elevated levels of stress (low oxygen harvest A red columns and fish rafting on ice harvest C green columns) broke down slightly faster from ATP (Figure 26) accumulating elevated results in ADP (Figure 27).

Harvests that exposed the fish to reduced stress (high oxygen harvests D blue lines and E yellow lines) broke down slower from ATP (Figure 26) accumulating lower results in ADP (Figure 27).

Further breakdown of nucleotides to AMP (Figure 28) and IMP (Figure 29) show no significant trends. Given the high quality of the fish sampled, this is not surprising that there was no discernible increase to later breakdown products.

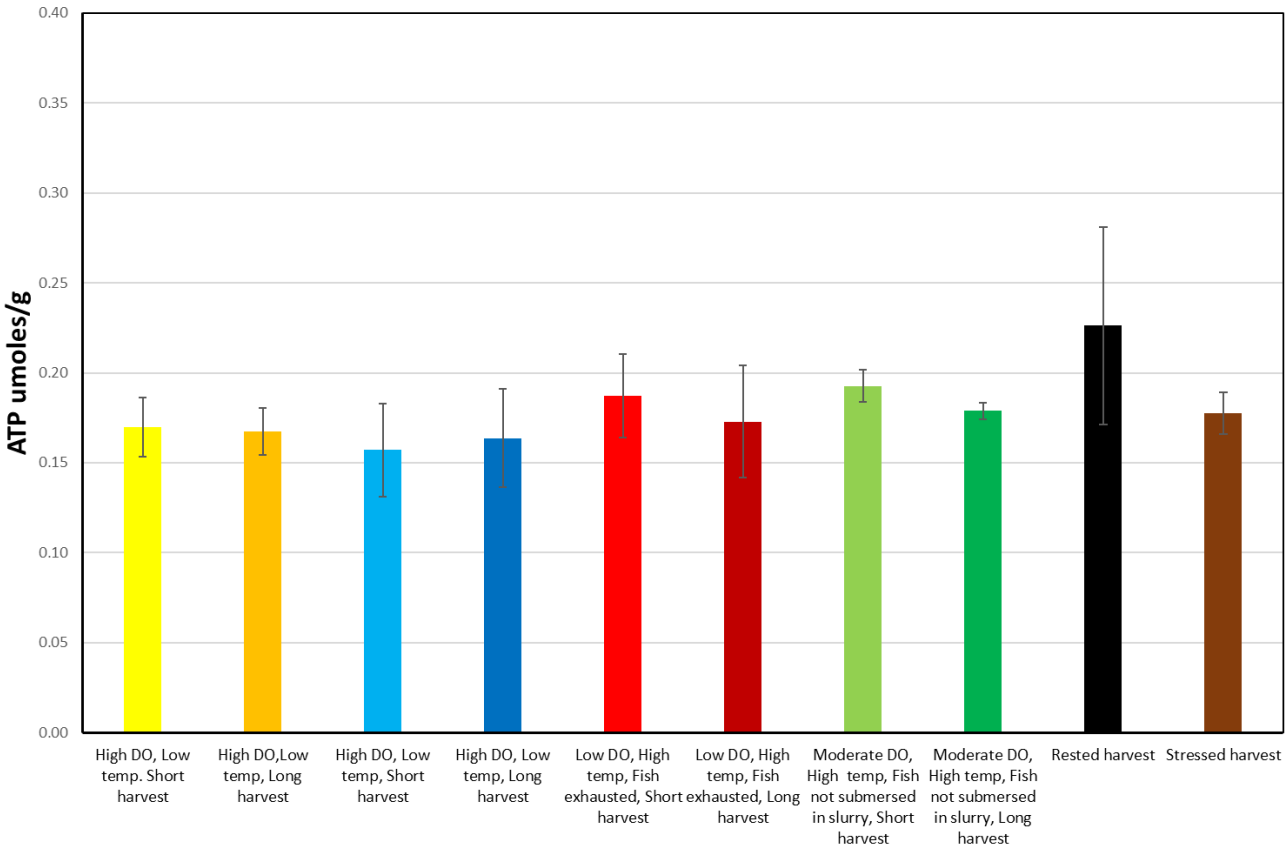


Figure 34. ATP levels in Barramundi immediately post-harvest under different harvest conditions.

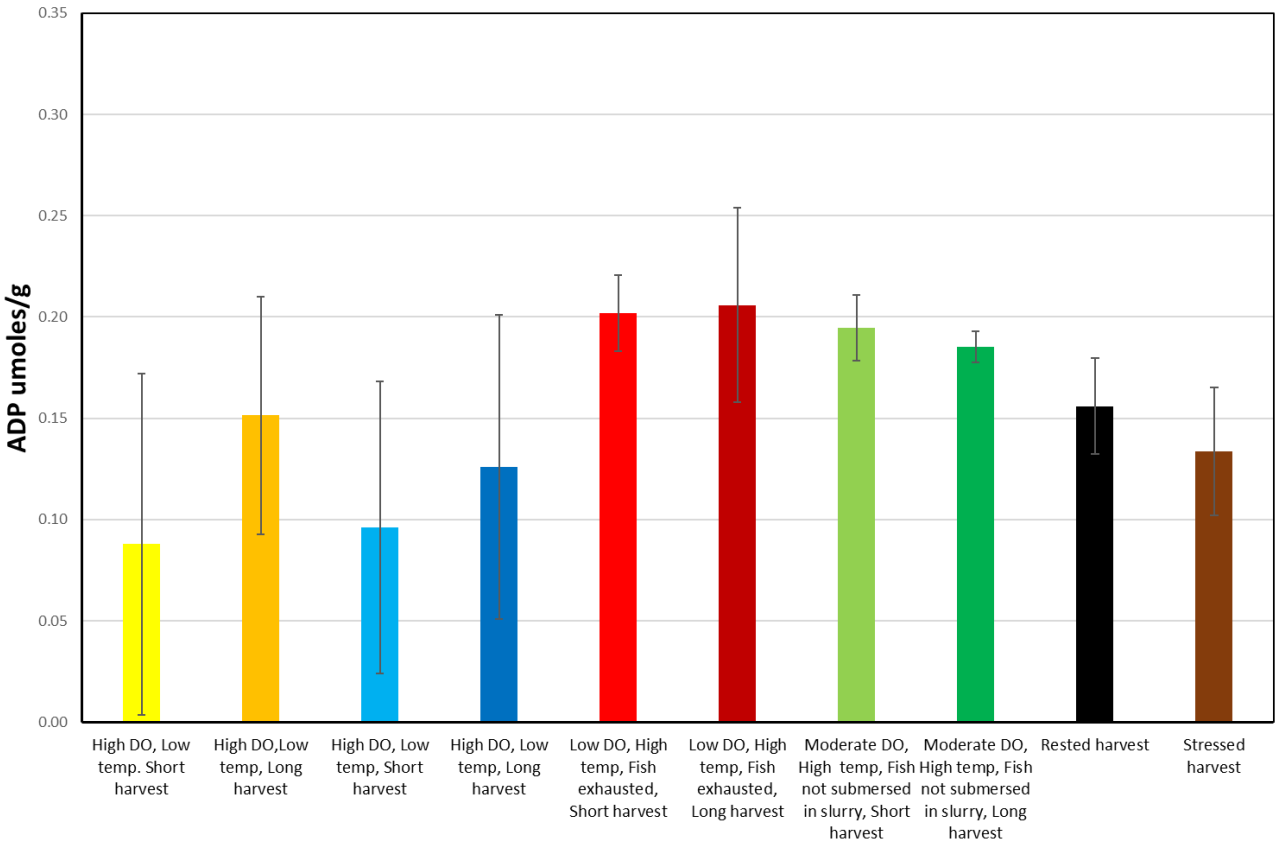


Figure 35. ADP levels in Barramundi immediately post-harvest under different harvest conditions

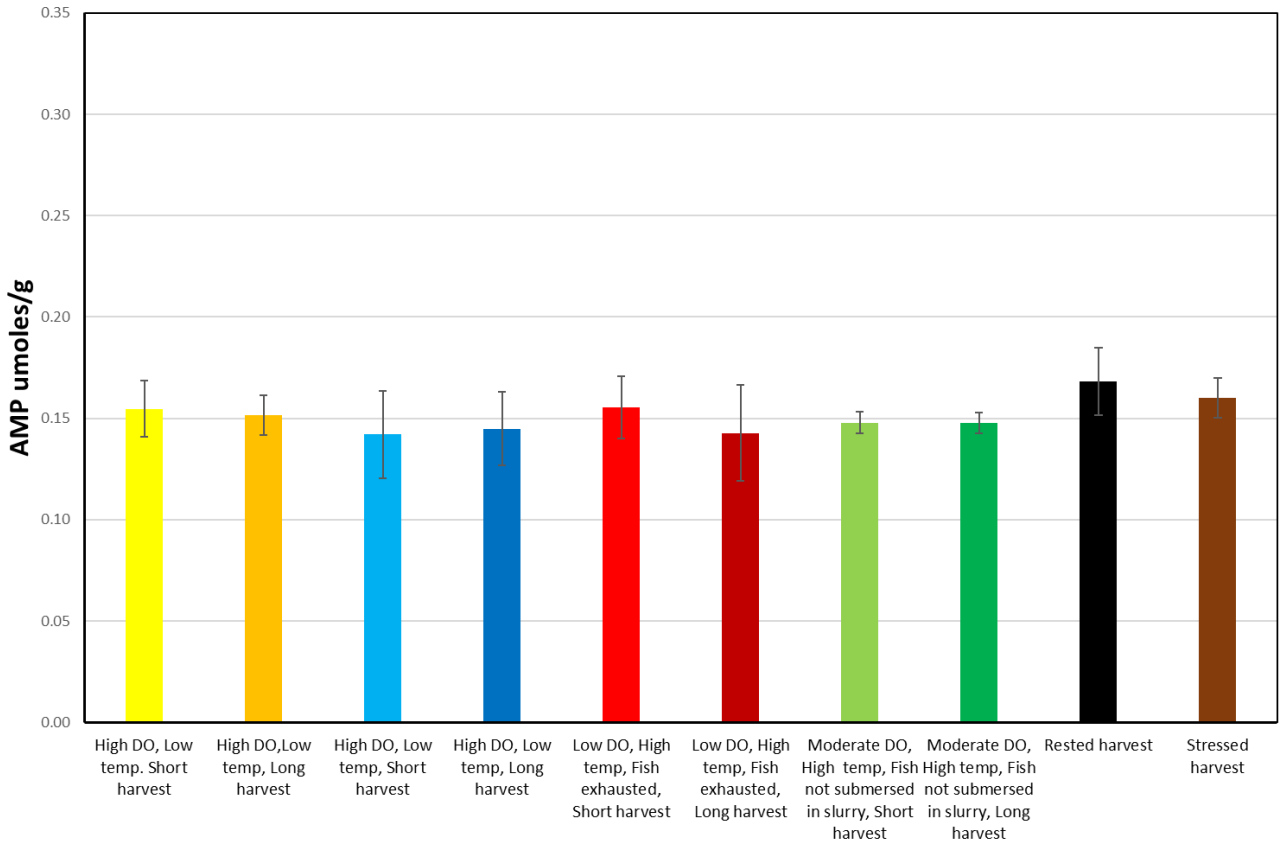


Figure 36. AMP levels in Barramundi immediately post-harvest under different harvest conditions

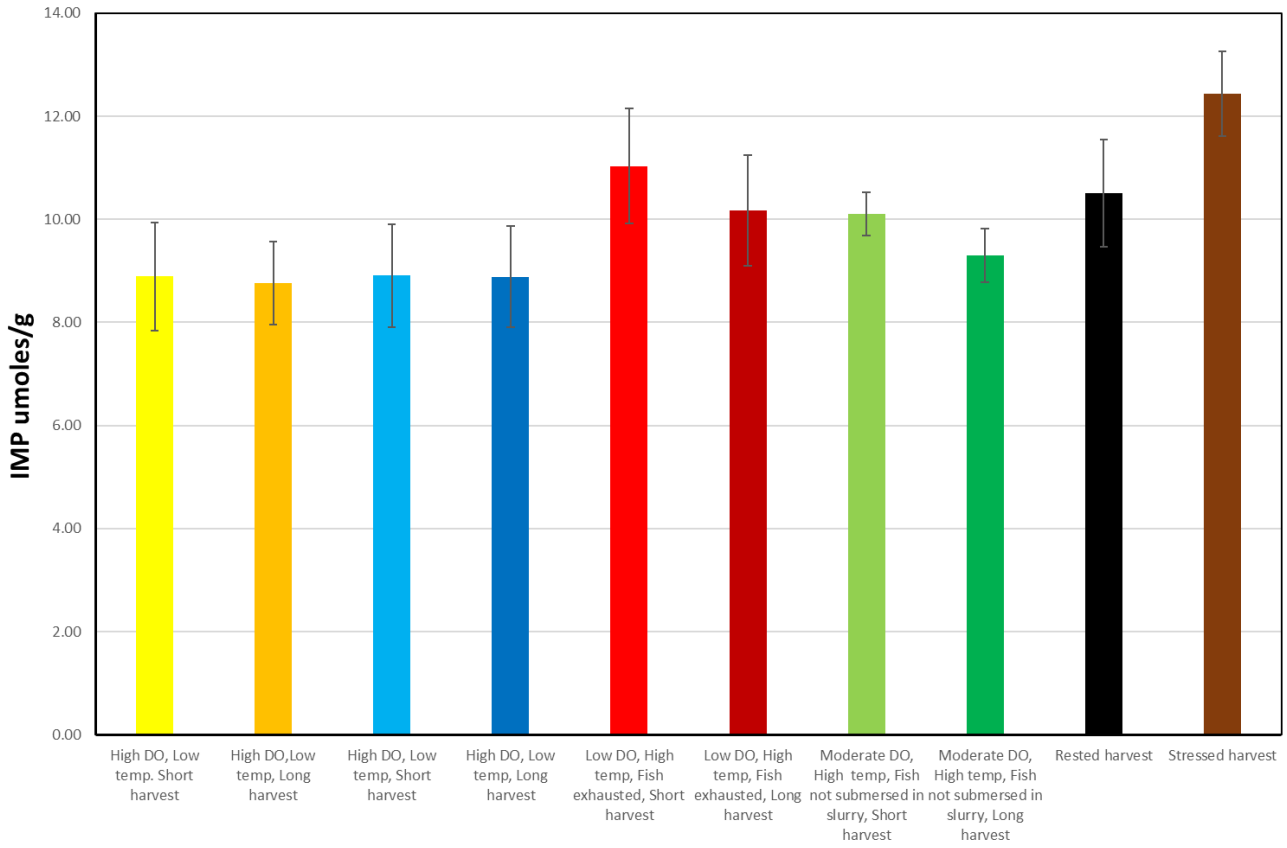


Figure 37. IMP levels in Barramundi immediately post-harvest under different harvest conditions

Appendix 8. Hydrogen sulphide poisoning

While DAF did not monitor hydrogen sulphide levels in any ponds it is worth farms checking for if they are experiencing unexplained fish behaviours and low dissolved oxygen events during harvest.

Hydrogen sulphide (H_2S) is a gas that is produced during the anaerobic breakdown of organic matter by bacteria. It is commonly known as 'rotten egg gas' due to its strong, distinctive and pungent smell. There have been several cases of high fish mortalities in aquaculture ponds, where H_2S poisoning has been suspected. H_2S can cause rapid death with few, if any, diagnostic signs. Problems have occurred in poorly aerated ponds following the disturbance of bottom sediments causing sudden oxygen deprivation during harvest procedures.

H_2S interferes with fish respiratory mechanisms causing hypoxia. It is Important to look for erratic swimming around pond, rapid breathing, redness in fins and tails, loss of equilibrium and possible death. If there are signs of rotten egg smell when experiencing these symptoms, then it would be worth having water samples tested by a laboratory for conformation.

The addition of extra aerators that provide oxygen and reduce still areas within the pond, reducing excess feeding in high water temperatures that may cause sediment build-up may help reduce the problem. The use of bioactive products to actively break down organic material and maintain pond bottom health may also help. Even dragging a chain across the bottom separate to the harvest event may help reduce the effect of hydrogen sulphide poisoning. Fully drying out ponds to oxidise organic detritus and properly preparing between each grow out season is essential (Boyd, 2014).