SBT AQUACULTURE SUBPROGRAM

Project 4:

Effect of husbandry and handling techniques on the post-harvest quality of farmed southern bluefin tuna.

G.B. Goodrick, P.T. Thomas
B.D. Paterson, and A. Smart

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For enquiries, or further copies of this report, contact

Centre for Food Technology
Queensland Department of Primary Industries
19 Hercules St
Hamilton QLD 4007 AUSTRALIA

Or

Fisheries Research and Development Corporation
PO Box 222
DEAKIN WEST ACT 2600 AUSTRALIA
www.frdc.com.au

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1. NON-TECHNICAL SUMMARY

97/364 Southern Bluefin Tuna Aquaculture Sub-program Project 4: Effect of husbandry and handling techniques on the post-harvest quality of farmed southern bluefin tuna.

Principal Investigator:
G.B. Goodrick[1]/B.D. Paterson
Aquaculture CRC Ltd/Centre for Food Technology
19 Hercules St Hamilton Q 4007 Australia

Objectives
1. Develop and validate non-destructive flesh sampling techniques for product quality evaluation
2. If suitable non-destructive methods cannot be developed for research purposes in the first year of the study, or in any case in the second and third year, conduct pilot exports of dressed and packaged SBT to maximise the return to industry from the sampled fish, as well as establish a new product in the market place.
3. Study the effect of pre-harvest fasting, harvesting and post-harvest handling on the flesh colour and post-mortem biochemistry of farmed SBT
4. Develop practical preparatory, harvesting and post-harvest handling techniques to optimise desirable colour and flesh characteristics of farmed SBT
5. Communicate these techniques to the industry and monitor the impact on the product quality of fish marketed from commercial farms

Swimming activity during harvest of southern bluefin tuna (SBT) should be reduced as much as is practical. This study of the effects of husbandry and handling techniques on flesh characteristics could not demonstrate any significant changes in the flesh colour arising from the treatments applied, but it did show a clear relationship between harvest activity and body temperature in SBT on landing. The more that SBT struggle on capture, the hotter they become. If the objective is to chill the tuna as soon as possible after harvest, then reducing the initial temperature on the tuna is a good strategy. In this study, SBT were successfully sedated to achieve "rested harvest" using a purpose-built floating enclosure and anaesthetics such as carbon dioxide and Aqui-S. Of course, just because this study couldn't show an effect of harvest and other alternative practices on flesh colour doesn't necessarily mean that there isn't any effect. Tuna showing high levels of activity on harvest are landed with physiological and biochemical symptoms consistent with stress, and it is a general axiom that stress leads to poor flesh quality in fish and terrestrial livestock. It could simply be that existing methods of flesh colour measurement cannot meet the challenge posed by sampling fish in the narrow window of opportunity before the freshly-killed carcasses leave Port Lincoln. Another approach is probably required, perhaps by collecting data from carcasses exported to Japan.

The SBT farming industry at Port Lincoln in South Australia uses procedures for harvesting and post-harvest handling based on methods developed in the wild capture fishery. At various times, problems with the quality and, in particular, the flesh colour have resulted in poor prices on the Japanese market: the only viable marketing option for these fish. Some of these problems may relate to the feeding, harvesting and post-harvest handling of the farmed SBT.

These tuna are valuable as sushi and sashimi because of their bright red flesh and high fat content, but the flesh colour, flavour and shelf-life may all be seriously influenced by the harvest and handling practices. The body temperature of tuna can increase rapidly during activity and the flesh can become acidic if fish are intensively exercised or asphyxiated in the harvesting operation. As a result of such activity the flesh colour may be altered or colour stability reduced, shortening the shelf-life for use as products such as sushi. Reducing the level of hyperactivity in the harvest process has been a major aim of this research as well as examining the combination effects of pre-harvest fasting and changes to the on-board handling practices.

Tuna are very valuable, so methods and tools for inconspicuously sampling carcasses were developed before beginning an experimental sampling program (Objective 1). This meant that it wasn't necessary to destructively sample the carcasses and commence the alternative strategy, that of marketing loined and further portioned sushi (Objective 2). A tuna flesh colour reference set was also developed in conjunction with the new sampling tool, because of the ambiguous results being returned

[1] Present address: Seafood Innovations, 76 Campbell St, Sheldon.

by the electronic colour meter. This colour set is now undergoing evaluation by industry with a view to developing a working set of tuna flesh colours for routine husbandry use.

Two approaches were used here to assessing the impact of various factors on flesh characteristics in SBT (Objective 3). Firstly, a detailed study was undertaken into the effects of harvest stress on the post-mortem biochemistry of the tuna. Secondly, larger scale trials were conducted that sought to eliminate harvest stress altogether by attempting to anaesthetise SBT in bulk, in trials that also introduced other pre- and post-harvest treatments such as fasting and chilling methods respectively.

The biochemical studies showed that when harvested SBT show evidence of a stress response at death. When tuna struggle on harvest, they warm up and their blood becomes more acidic. Some glycogen in the muscle is converted to lactic acid, which then appears in the blood. However, the acid accumulated in the blood during harvest does not seem to influence the final pH of the flesh. That is probably determined by continued conversion of glycogen to lactic acid during rigor mortis. Data in this study raise the prospect that captive SBT may sometimes build up unusually high levels of glycogen, a carbohydrate used for storing glucose in muscle tissue. Exercise during harvest may actually be beneficial if it reduces the amount of glycogen remaining in the muscle of the dead tuna and in turn reduces the extent of further acidification during rigor mortis. This point will need further research, as unusually high glycogen levels on slaughter may explain occurrences of unusually low post-mortem pH and short shelf life sometimes observed in slaughtered tuna (cf. PSE pork).

In order to harvest SBT in large numbers without any struggling or exercise, a floating PVC enclosure was used in harvesting trials but with mixed results. Groups of SBT were successfully sedated using carbon dioxide and Aqui-S, but the logistical difficulties in corralling tuna from a crowd into this enclosure mean that they were probably not completely "rested" upon slaughter. In future, the sedation step needs to be better incorporated into a modified corralling procedure. No results were obtained that were compelling enough to require immediate changes in harvest practices. However, it was found that sedated tuna were cooler than non-sedated tuna, in line with the biochemical work, and that the blood of immobile tuna tended to be more acidic upon slaughter, though again no difference was evident in the terminal flesh pH. There was preliminary evidence that sedated SBT went into rigor more slowly than non-sedated SBT, both in terms of post-mortem flesh pH and relative contraction of excised muscle blocks. Post-harvest chilling of the carcasses influenced flesh characteristics, but these results were difficult to interpret except to say that the warm bleeding techniques tested here are not recommended. Fasting or withholding food from tuna for a week before harvest significantly elevated post-mortem flesh pH- but not in all cases, and the mechanism for this should be examined in more detail (particularly given to the impact of fasting on muscle glycogen levels).

Generally it is accepted that stress-free, or perhaps more strictly “exercise-free,” harvest will improve flesh quality by ensuring that the fish are not physiologically exhausted during harvest. This holds for other fish where exhaustion brings on the stiffening of rigor mortis early and accelerates the biochemical deterioration of the carcass. Perhaps we have had trouble demonstrating post-harvest changes here because of difficulties quantifying the colour of SBT flesh and interpreting the results obtained by colour meters. The colour chips developed in this project will hopefully solve this problem in future research. It could also be that we are sampling the tuna too early, at Port Lincoln, and not several days later when they are on the market floor. But finally it could also be because tuna are generally handled differently from other fish. Unlikely many other farmed fish, SBT are so big and so valuable that they are handled individually when slaughtered, lifting the bar in terms of the flesh quality expected from circumstances associated with bulk-harvested fish. Tuna are also active swimmers and frenzied exercise rather than being "stressful" may simply be how they feed. Indeed, some of the results obtained here suggest that it may sometimes be necessary to exercise captive tuna during harvest to burn up energy stores (eg. glycogen) that might otherwise damage the slaughtered carcass!

It had been hoped to recommend alternative husbandry practices from these experiments (Objectives 4&5) but this is not possible at this stage. Because of the practical difficulties with herding the tuna into the enclosure, future work could examine strategies for harvest of sedated tuna under commercial conditions, using a protocol encompassing the entire crowding and harvest process and a simplified enclosure. Notwithstanding the difficulty in demonstrating a benefit of these methods on flesh quality, an easily quantifiable objective here would simply be to limit rise in body temperature in the harvested tuna.

Keywords: tuna, harvesting, processing, rigor mortis, flesh colour

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3. BACKGROUND

Tuna farming in Australia really began around 1990 in response to the need to make best use of the stocks of southern bluefin tuna (SBT), which were considered to vulnerable to intense fishing pressure.

The need to receive higher returns and reduce catch rates helped focus the attention of fishers away from volume driven cannery demands to the more lucrative market for tuna sashimi in Japan. In the process, a change in the entire approach to husbandry was required to achieve the move from hunting to farming tuna.

In moving to farming operations, practices have been developed to care for the tuna being farmed, however the practices adopted for harvesting were derived directly from the experiences of the "wild" fishery. To a large extent these practices did not consider the effects of the methods used on the quality or welfare of the fish being slaughtered, but sought to compensate where possible through rapid chilling and on-board handling once the fish were on board.

The focus of the Aquaculture CRC's Product Technology-Aquaculture Practices project was to evaluate the effects of the existing practices and devise and test alternative harvesting and handling methods which could help to maximise the value of the fish harvested.

Southern Bluefin tuna are valuable as sushi and sashimi because of their bright red flesh and high fat content, but the flesh colour, flavour and shelf-life call be seriously influenced by the harvest and handling practices. The body temperature of tuna can increase rapidly during activity and the flesh can become acidic if fish are intensively exercised or asphyxiated in the harvesting operation. As a result of such activity the flesh colour may be altered or colour stability reduced, shortening the shelf-life for use as products such as sushi.

Reducing the level of hyperactivity in the harvest process has been a major aim of this research as well as examining the combination effects of pre-harvest fasting and changes to the on-board handling practices

4. NEED

The SBT farming industry uses procedures for harvesting and post-harvest handling based on methods developed in the wild capture fishery. At various times, problems with the quality and, in particular, the flesh colour have resulted in poor prices on the Japanese market: the only viable marketing option for these fish. Some of these problems may relate to the feeding, harvesting and post-harvest handling of the farmed SBT.

Further processing of whole fish into loins for export has been proposed, but this requires good stability of the attractive red flesh colour during storage and transport to market. Research is already under way to see if introducing manufactured feeds has any consequence for the quality of the farmed SBT flesh. To complement this ongoing work, and because of the importance of flesh colour and texture to the tuna market, a study of the effect of alternative handling conditions or procedures on SBT flesh quality in whole or dressed fish is needed to develop a new system of pre-harvest preparation, harvesting methods and post-harvest procedures, specifically for farmed SBT.

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5. OBJECTIVES

1. Develop and validate non-destructive flesh sampling techniques for product quality evaluation
2. If suitable non-destructive methods cannot be developed for research purposes in the first year of the study, or in any case in the second and third year, conduct pilot exports of dressed and packaged SBT to maximise the return to industry from the sampled fish, as well as establish a new product in the market place.
3. Study the effect of pre-harvest fasting, harvesting and post-harvest handling on the flesh colour and post-mortem biochemistry of farmed SBT
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6. METHODS

6.1. Objective 1: Development and validation of the inconspicuous sampling device

6.1.1 Development of the sampling device

The aim here was to devise a method to sample the commercially important part of the carcass rather than assessing quality based on the "tail-cut." To date during tuna feeding trials, sampling the commercial cuts of the tuna had to be done using methods that were conspicuous and detrimental to the marketability of the carcass. A big priority was to measure the colour of the carcass, so we immediately saw difficulties with existing sampling tools that take a very thin core from the fish. The fragility and small size of the sample would make it difficult to use with many of the instruments available such as colour and pH meters. But conversely, a large coring device providing sufficient sample for a diversity of analyses and uses would noticeably mark the carcass.

The solution was to build a device that could remove a thin *slice* of muscle from the tunas back, adjacent to the neural spines.

Sampling this region of the meat has a number of advantages…

- After sectioning of the carcass, this location represents the exposed meat surface of a tuna loin.
- The sample can also be withdrawn through an incision cut in the natural groove around the base of the dorsal fin, so the fact that the fish was sampled is not obvious without very close scrutiny.
- A flat sample means that the meat either side of the fissure will tend to fall back into place rather than stand open like the hole left by a conventional coring device.
- The sampler's track through the meat is discarded when the wholesaler trims the carcass into loins and smaller portions.

In order to increase the likelihood that a successful device would be produced we chose two starting concepts for development.

- Flattened corer.
- Double-knifed "biopsy" device

The ability of these prototypes to extract intact samples of uniform thickness were assessed on carcasses of tuna. The results of the these tests highlighted the strengths and weaknesses of the two approaches and this information was then used in the redesign and construction of the final working prototypes of the "Boston Bay-onet."

Specific validation trials were undertaken. One problem with any sampling regime for tuna is that the fish must be sampled soon after harvest before changes in flesh quality become apparent. It is helpful to know if changes in the chill-stored bayonet sample reflect changes in the carcass bound for Japan.

These trials compared the changes in pH and colour characteristics of the 'bayonet' samples to parallel changes in the intact carcass. The sampler was also used routinely in flesh sampling for the remainder of the study. Since the sampling tool breaches the skin of the tuna and penetrates the meat of this product, good manufacturing processes including strict hygiene procedures were followed when using the tool

6.1.2 Developing a tuna flesh colour reference set based on the sample

6.1.2.1 Development of draft colour set

For the initial screening to identify tuna-like colours, bayonet samples were obtained from tuna harvested during feed development trials at Port Lincoln. The samples were laid out on white tiles, covered in a gas permeable film and stored on ice until observations were made.

The colour of the samples was assessed by three observers under standardised conditions using a light box (Figure 1) with the aid of reference colour swatches. Colours used for this work were sourced from the Y80R Y90R and R portion of the NCS (Natural Colour System) set.

Lighting was standardised for colour swatch and sample observation and photography using a custom built light box and "photocrescenta" bulbs to give 'natural' light. The light box was constructed from white polystyrene seafood boxes and could be opened to add the tile and sample insertion and removal and view ports in the top opened to reveal the sample for observation and photography. When taking photographs in the light box a Kodak colour reference card was included in the field of view to allow true colour correction during processing, (Figure 2).

6.1.2.2 Manufacture of the draft chip set

A plastic chip format, with a matt surface, was chosen as the most appropriate to resemble a piece of flesh. The plastic had another advantage in that the colour could not fade easily as the dye was located right through the chip. Nevertheless, in the interests of colour stability, such chips should be stored in darkness when not in use.

Dyed plastic chips were then fabricated by a plastics company using a general test die. In simple terms, the ‘tuna-like’ swatches were scanned using a spectrophotometer. The required spectra for the chips were further altered to limit the effects of metamerism. This is the effect where different kinds of lighting conditions alter the perceived colour of an object.

Using this colour as a reference point, lighter and darker versions of the colour were made by adding white and black respectively to produce a draft set of plastic chips. This set of colours was shown to participants in the industry to get feedback. . The initial colours were effectively a range of colours which had some similar hues to tuna but which lacked any familiar texture, shape or translucent characteristics, so it was difficult if not near impossible to get definitive direction from the responses. The chips were also examined using a CIE colour meter and further validated in tests using real tuna flesh to see whether the target had been hit before manufacturing the first prototype tuna colour chips. The spacing or intervals between the respective chips were examined using the Minolta Chromometer to see if slight changes were required in the colour of individual chips.

Figure 1: Using the light-box to photograph a sample

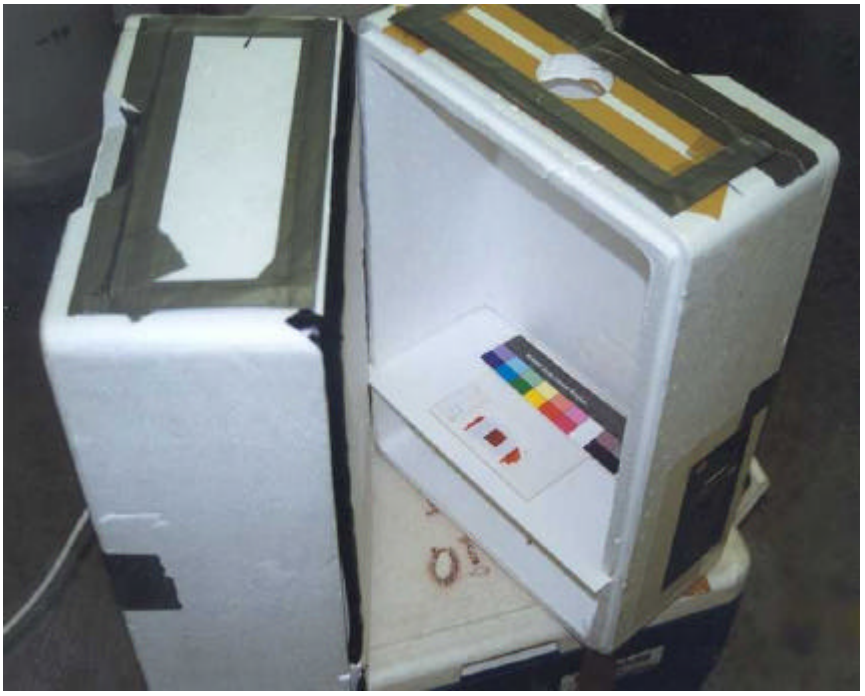

Figure 2. Detail of the light-box when opened to replace samples. Note the hole in the viewing port for mounting the camera.

6.1.2.3 Examination of draft colours using the CIE meter.

A specific die was designed and manufactured to allow direct comparison with a bayonet flesh sample or thin strip of tuna flesh (eg. Sushi). The die also incorporated a step in thickness to allow users to judge how variations in the sample depth may influence the apparent colour. A surface texture was also selected as a best compromise between the flesh of the tuna and causing minimal influence to the visual appearance of the colour.

At this stage of the process, we adopted the AUSMEAT meat grading torch to standardise lighting.

Using the new die, a short-listed colour range (discarding unrealistic colours) from the original draft set were then developed further by varying the kind of polymers used as well as the pigment concentration used in manufacture.

Further samples of tuna flesh were then assessed using…
- the new chips,
- the original group of NCS swatches as well as
- a series of Pantone colour swatches

The Pantone colour set was brought in at this stage because it had a wider range of apparently suitable red colours. It was hoped that the results of this work would widen the "colour space" required to represent tuna flesh colour, as well as provide the additional swatches required to manufacture additional chips. Aside from direct by-eye comparisons of the flesh, chips and swatches, the colour space was plotted out by comparing Minolta CIE colour readings of the draft chips to CIE-lab readings of the tuna-like NCS and Pantone swatches.

Once refinements of the colour space had been completed a test batch of three colour sets were produced for initial testing and evaluation.

6.2. Objective 2: Pilot exports of dressed and packaged SBT

Successful development of the sampling tool meant that it was possible to sample carcasses with minimal damage. For this reason it was not necessary to further dress and process the sampled carcasses and export these as dressed and packaged sashimi.

6.3. Objective 3: Effects of pre-harvest, harvest and post-harvest handling on flesh quality of tuna

Two approaches were used here to assessing the impact of various factors on flesh characteristics in SBT. Firstly, a detailed study was undertaken into the effects of harvest stress on the post-mortem biochemistry of the tuna. Secondly, larger scale trials were conducted that sought to eliminate harvest stress altogether by attempting to anaesthetise SBT in bulk, in trials that also introduced other pre- and post-harvest treatments.

6.3.1 The effect of harvest stress on physiological response and post-mortem biochemistry

6.3.1.1 Poled Wild-Fish

Poled wild-fish (PW) were line caught in February 1999, on fishing grounds located approximately 100 nautical miles off-shore from Streaky Bay, South Australia, using the traditional poling method. This method involved visually locating a surface feeding school of fish and encouraging them into a 'feeding frenzy' through the use of surface water spray and constant feeding with small fish. The tuna were caught on an artificial lure or baited hook attached to a short line and fibreglass pole. Once hooked, fish were hoisted on-board (ie. poled) and immediately killed by inserting a sharp, 13mm ?, stainless steel rod into the brain (*ike-jime*). Following this a cable was briefly inserted down the spinal cord. Time taken from hooking to slaughter was approximately 15 seconds. Fish were then bleed, by cutting the cutaneous lateral artery opposite the pectoral fin, and blood was collected from the wound using a 10 ml syringe treated with heparin. A muscle sample was then taken, using a 17mm ? stainless steel coring tool inserted into the bleed wound, and immediately frozen in liquid nitrogen. Muscle pH and temperature was measured by inserting a glass pH probe into the flesh. Fish were then tail tagged and placed onto a rope loop prior to storage at 0^0 C in seawater. During storage, muscle samples, pH and temperature were taken at approximately 3-4 and 24h post-mortem. Fish mean weight ± SE was 27.0 ± 1.4 kg.

6.3.1.2 Towed Line-Caught and Towed Line-Stressed Fish

These fish were captured in January 1999, off the Great Australian Bight in South Australia, by professional fishermen using purse-seine fishing technology. The fish were transferred, at sea, to a Polar Circkle type towing pontoon, where they remained for three weeks while the towing pontoon was transported to South Australian Research and Development Institute (SARDI) experimental farm site, near Pt Lincoln (34^0 43'S, 135^0 52' E). On arrival at Pt Lincoln the tuna were swum into a Polar Cirkle type 32 m ? by 15m depth sea – cage. Five days after transfer to the sea cage 10 fish were line-caught (Towed line caught group (TLC)) and immediately killed prior to blood and tissue collection. In order to simulate harvest stress, another 10 fish (Towed line-stressed group (TLS)) were subject to 5 min of struggling prior to being landed and immediately killed. On-board time taken to slaughter, slaughter method, and sample collection were the same here as for the PW, however in this experiment, fish carcasses were stored in an ice-slurry. Fish mean weight ± SE was 15.9 ± 0.87 and 14.4 ± 0.85kg for the TLC and TLS groups respectively.

6.3.1.3 Farmed Line-Caught and Farmed Net-Caught Fish

On the 13^{th} of April 1999 10 fish (Farmed line-caught (FLC)) were caught using a hook and line, from the SARDI experimental holding cages at Pt Lincoln. On the 11^{th} of May 1999 10 fish (Farmed net-caught (FNC)) were harvested, from the same cage as the FLC fish, by first corralling them in a purse seine net, and using divers to physically catch and remove the fish from the water. On-board time taken to slaughter, slaughter method, and sample collection were the same here as for the PW, TLC and TLS fish. Carcasses were stored in an ice-slurry. The mean weight ± SE of the fish was 22.7 ± 1.8 kg and 32.8 ± 1.7 kg for the FLC and FLS group respectively.

6.3.1.4 Analytical Procedures

Plasma cortisol was determined by radioimmunoassay (RIA) using a method based on the Pantex 125I radioimmunoassay kit (Immunodiagnostics, NSW, Australia) (Carragher et al. In progress).

Muscle samples were acid extracted, following the method described by Thomas et al. (1999)

Muscle levels of adenosine 5'-triphosphate (ATP) and its catabolites, adenosine 5'diphosphate (ADP), adenosine 5'-monophosphate (AMP), inosine 5'-monophosphate (IMP), inosine (HxR), and hypoxanthine (Hx)were measured using a method by Ryder (1985) and following Thomas et al. (2000).

K-values were calculated following (Saito et al. 1959) as:

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

IMP load following Van der Boon et al. (1992) was calculated as:

$$IL = \left(\frac{[IMP]}{[ATP] + [ADP] + [AMP]} \right)$$

Adenylate energy charge (AEC) was calculated as:

$$AEC = \left(\frac{[ATP] + 0.5 \times [ADP]}{[ATP] + [ADP] + [AMP]} \right)$$

Following the method described in (Thomas et al. 1999) plasma pH was measured using a glass spear pH probe (Activon 6 x 65mm) and plasma and muscle lactate was analysed enzymatically using a Sigma 826-UV kit. Plasma glucose and muscle glycogen was determined enzymatically using a Roche MPR3 124036 kit. Muscle glycogen determination was based on the method of Chan and Exton (1976).

6.3.2 Development and testing of a floating liner for applying harvest treatments to farmed SBT

6.3.2.1 Assembly of the enclosure

The basic concept of the harvest system is to allow fish to be introduced into an enclosure with minimal adverse activity, from where a process of sedation could be applied to facilitate rapid and effective killing. A portable enclosure system was developed for research purposes to test the prospects for an integrated enclosure and crowding system that was suited for commercial operations.

The enclosure (Figure 3) was designed to contain tuna in a volume approximately 12m in diameter and 7m deep. The structure was intended to be used in or outside of a tuna cage and allow tuna in through an entry door and to be deployable from a small vessel. Other design features were incorporated to ensure buoyancy and shape maintenance during tidal movements.

The desireable characteristics specified for the enclosure are indicated below.

- Readily deployed in approximately 45 minutes (± 10 minutes)
- Maintains integrity in currents (Design features were incorporated to ensure buoyancy and shape during tidal movements).
- Causes no behavioural change in the tuna (ie. stress, fish activity)
- Allows application of treatments/sedatives such as AQUI-S^{TM} and CO_2 to the enclosed fish.
- Easily removed in approximately 30 minutes (± 10 minutes).
- Facilitates free movement of the fish into the liner through zipped doorway.
- Buoyancy, durability, and effective in preventing tuna from escaping.
- Ability to adjust liner size and volume of water enclosed.

Prior to sea trials at Port Lincoln, the enclosure underwent a wet assembly trial at the diving pool at the Chandler Aquatic complex in Brisbane. Deployment and rested harvest trials at Port Lincoln were then successfully undertaken.

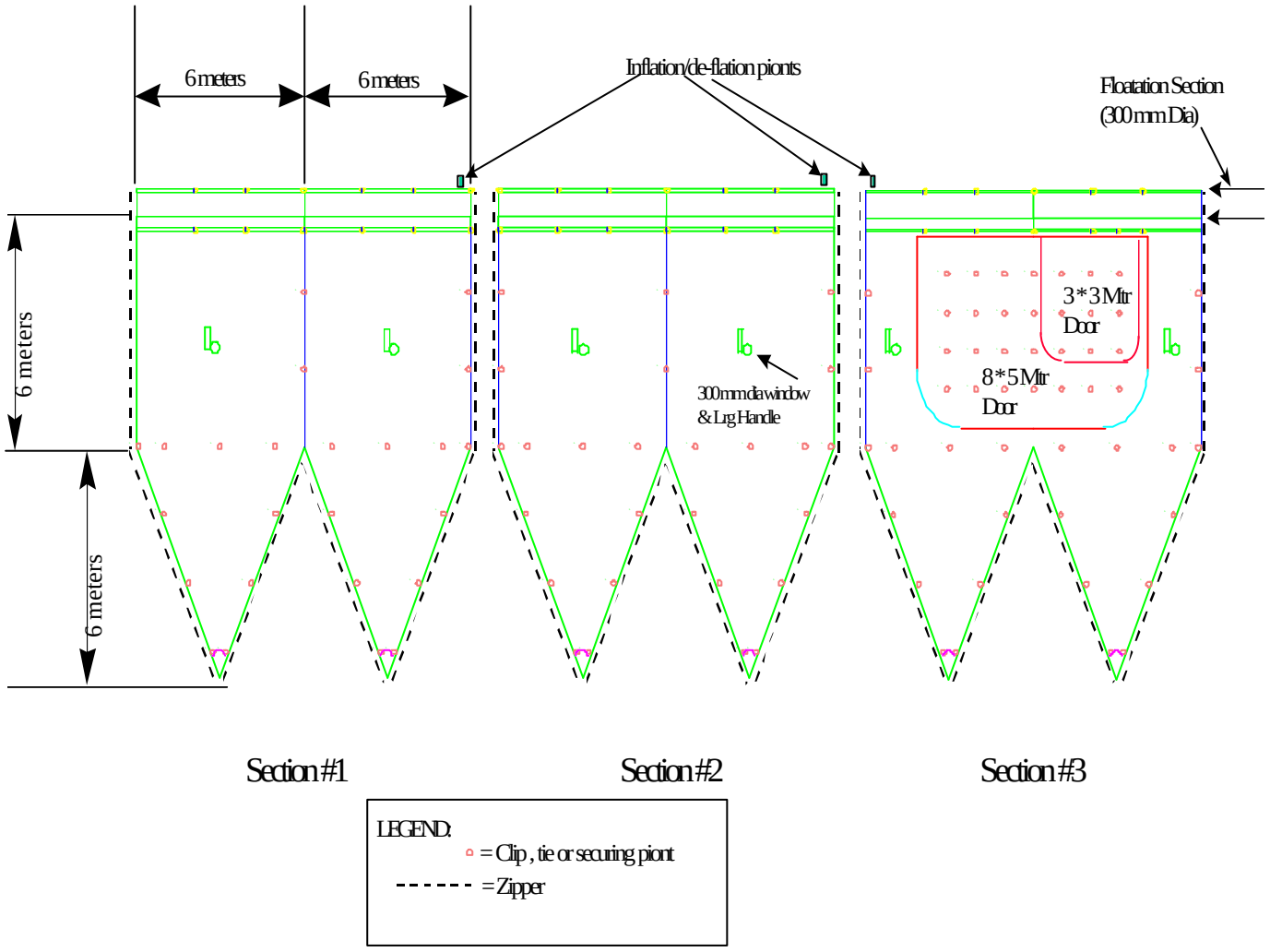

Figure 3. Schematic showing the components of the tuna harvest enclosure.

6.3.3 Harvest of tuna sedated using carbon dioxide

This trial involved herding the tuna into the enclosure and dosing the enclosed body of water with CO_2 gas, as an alternative to AQUI-S™ The objective was to sedate the fish (preventing stress and activity prior to and during harvest). Tuna harvested in this manner were then tested/compared with fish from normal harvest practices (involving net confinement) in regard to rigor development and flesh quality. To remove possible effects from the condition of fish in different cages, replicates of the trial were planned to be conducted on different cages.

6.3.3.1 Experimental regime

The effect of pre-harvest condition was studied by conducting a fasting treatment. Samples were taken by normal and rested harvesting operations from two production cages. Tuna harvested from each cage were analysed and their flesh quality parameters measured to 3 days postmortem. Food was withheld from the cages for 5 days and then fish were harvested (normal and rested) again from each cage and their flesh quality parameters measured as before.

The effect of alternative post-harvest handling methods on SBT was gauged by comparing postmortem flesh characteristics of fish harvested using one of two methods:

1. Immediate placement in ice slurry
2. Extended bleeding on board the vessel (bleeding in cool seawater) before slurrying fish back on shore

The experiment was divided into 2 trials, 5 days apart, to incorporate the fasting treatment as outlined below:

Trial 1 – Not Fasted

Rested harvest, v.slow cooling (10 tuna)
Rested harvest, ice slurry (10 tuna)
Control harvest, v.slow cooling (10 tuna)
Control harvest, ice slurry (10 tuna)

Trial 2 – Fasted (5 days later – no feeding)

Rested harvest, slow cooling (10 tuna)
Rested harvest, ice slurry (10 tuna)
Control harvest, slow cooling (10 tuna)
Control harvest, ice slurry (10 tuna)

6.3.3.2 Rested harvest

Rested harvest was achieved by placing the enclosure inside the 32m ? holding cages, net crowding the fish, and transferring the tuna via net corral through the 3m x 3m liner door. The 20 captured tuna were subsequently anaesthetised by the addition of CO_2 gas through a microporous membrane tube. From a practical viewpoint, CO_2 gas was more attractive an anaesthetic for use in 'rested harvest' with a cost of ~ $100 per trial compared to the $1,500 per trial cost of using Aqui-S. The control harvest treatment refers to fish being captured by typical commercial net crowding and diver recovery harvest practices.

6.3.3.3 Slow cooling

The alternative chilling practices (c/f. traditional ice slurries) examined was initially to place the slaughtered fish in a bin of seawater (14?C) followed by air chilling. However, this proved unsatisfactory. After completing trial 1 [non-fasted fish] the method was changed for Trial 2 to a less extreme method involving placement of slaughtered fish in a bin of 10?C cooled seawater followed by transfer into a traditional ice slurry 10 hours later.

6.3.3.4 Fasting

The treatments were repeated after fasting fish in both cages for 5 days

6.3.3.5 Flesh characteristics measured

6.3.3.5.1 Core Temperature

A hand-held digital thermometer with a narrow stainless steel temperature probe was used to record core temperature from bleed cuts for each fish harvested (taken just after slaughter) to determine the occurrence of any differences in body temperature between treatments. In addition, for some tuna in each treatment, thermocouple probes attached to small dataloggers in waterproof housings were implanted in the fish and used to obtain data on the flesh temperature changes during handling after harvest.

6.3.3.5.2 Rigor

Rigor was measured with a penetrometer immediate post-slaughter, and every 4-6 hrs in six positions on each fish (3 identical positions each side) (Figure 4) to investigate the effects of the treatments on the cycle of rigor mortis in the tuna.

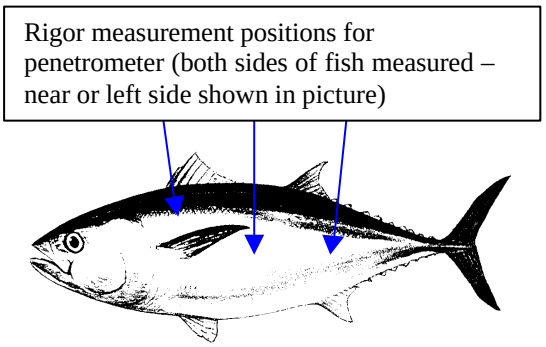

Figure 4 . Positions of rigor measurement using penetrometer

6.3.3.5.3 Inconspicuous sampling and measurement of colour and flesh pH

A handheld pH meter and spear sensor were used to record flesh pH through the bleed cut in each tuna immediate post-slaughter.

Flesh bayonet samples were taken at 8-10 hrs post-harvest using the inconspicuous sampling device (see section 6.1.1)and held chilled and covered with a plastic barrier film to prevent moisture loss but allowing oxygen transfer. The samples were measured at 12/24 hr intervals using a Minolta Chromometer (CR 100) and pH (using a surface pH sensor) to give an indication of flesh colour change and stability.

Flesh colour degradation was determined from the bayonet samples using the colour meter. This instrument analysed the colour using the CIE Lab scale (which categorises the colour into three axes, L= luminosity, a= red/green, and b= yellow/blue). The bayonet samples were divided into 3 equivalent portions and stacked on a white tile with the outermost (and most fatty) portion placed on the bottom. This stacking ensured the colour meter would not record through to the tile.

6.3.3.6 Note on interpretation of the results

In analysis of the results from this trial it is important to be aware that changes to the original experimental structure occurred as follows:

- the 2 production cages were fed different diets;
- trial 1 slow-cooling treatment was labelled very slow cooling to indicate the fact that fish were initially cooled in 14 C seawater followed by air chilling whereas trial 2 slow-cooled fish were initially cooled in 10 C cooled seawater followed by transfer into a traditional ice slurry 10 hours later;
- bayonet samples from trial 1 fish were inadvertently frozen in the chiller room
- the fish from the rested harvest treatment in trial 1 were harvested much faster and killed immediately as opposed to later samples that were handled about 1 min apart (as in trial 2)

6.3.4 Harvest of tuna sedated using Aqui-S

Following the anaesthesia of tuna using carbon dioxide, a trial Aqui-S harvest was undertaken in cooperation with a commercial tuna farm at Port Lincoln.
This was after Aqui-S was tested on two tuna in a preliminary trial to test the dosage and harvest procedure. The tuna harvested during the preliminary trial were included in the 22 tuna used for the validation of the bayonet sampler (section 7.1.2).

The aim of this larger harvest trial was to see if any differences in flesh characteristics could be identified in the tuna harvested in different ways.
Using the inflatable enclosure modified to 4 metre depth (by folding 2 metres at the top of the walls) and raiseable base, tuna in the commercial cage were crowded, with the assistance of farm personnel, through the doorway and dosed with a pre-diluted AQUI-S™ solution added via a 25mm ID hose and submersible pump to achieve a concentration of 18ppm.

After sedation and loss of sensibility the Aqui-S treatment tuna were killed by spiking and transferred to the boat by diver. Other tuna were taken from the crowd by divers as non-sedated controls. For repeat use trials the enclosure door was re-opened and a second group of fish crowded into the interior retaining the previous AQUI-S™ solution. Once sedated, these fish were similarly processed and sampled as below.

6.3.4.1 Core temperature and initial pH

Core temperature and pH were measured through the bleed cut following spiking and coring.

6.3.4.2 Flesh colour and post-mortem pH

Flesh was sampled using the “Bayonet sampling” technique at approximately 4 hours after harvest by sampling through the left side of the dorsal fin groove and then again after approximately 19 hours (the time of packing) by sampling through the right side of the dorsal fin groove. The “bayonet” samples were laid out on white ceramic tiles and tested for pH by surface electrode and then covered with permeable film and allowed to “Bloom” for 1hour under refrigerated conditions before colours were assessed using the recently developed draft tuna colour set.

6.3.4.3 Measuring of fillet shrinkage as an indicator of the rigor process.

Due to the difficulty experienced when using a penetrometer to measure rigor stiffening in tuna in the earlier carbon dioxide trial an alternative approach was used here. Part of the problem may be that the light and dark tuna muscle enters rigor at different rates, complicating the stiffening process in the trunk. One way to isolate this is to cut the muscles free of the skeleton and allow rigor to shrink the muscle.

To measure the shrinkage of tuna loins, selected fish (3 Aqui-S and 3 Controls) were filleted (loined) immediately after harvest and the dark muscle component separated.

The fish were cut and separated into polystyrene seafood boxes as shown in Figure 5. The left sides were covered by ice bags to simulate ice chilling (i.e. conduction) while the right side loins were chilled at a slower rate by separating the coolants from the loins being chilled so that only convection of chilled air bathed the samples. Temperature data loggers were inserted into the flesh to monitor the rate of cooling.

The initial lengths and changes in the lengths of each loin were recorded at regular intervals for 48 hours post-capture using toothpicks pushed into the polystyrene base of the seafood boxes.

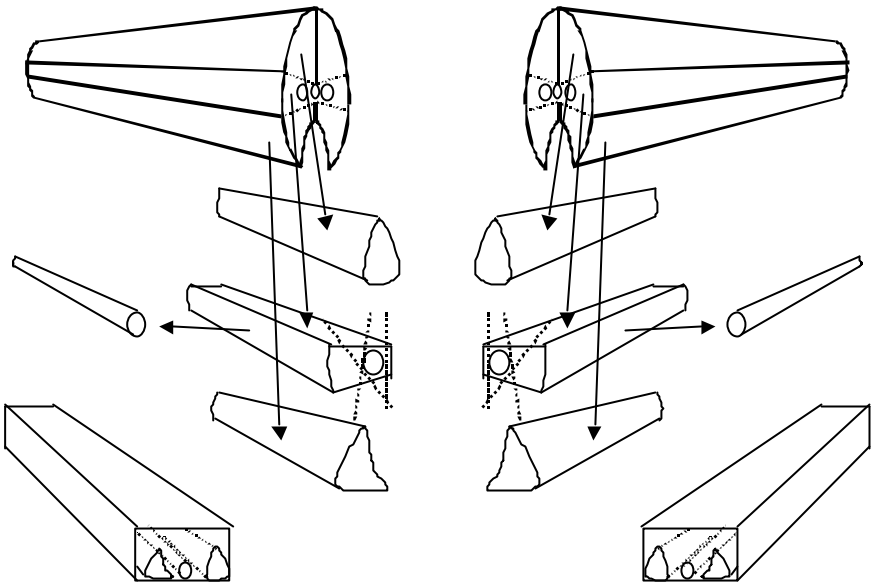

Figure 5. The method used to dress the tuna trunks to isolate light muscle and red muscle for rigor measurements

6.4. Objective 4: Develop techniques to optimise desireable colour and flesh characteristics of SBT

The aim here was to recommend, on the basis of the experiments conducted, new methods of pre-harvest, harvest and post-harvest handling. Then apply these new techniques in trial harvests and assess the impact on quality.

6.5. Objective 5: Communicate techniques to industry and monitor impact on the product quality of SBT

The findings of the project will be presented to regular tuna farming sub-program workshops.

If new husbandry techniques are developed in order to manipulate flesh quality, then present these to industry in specific workshops and initiate a program of monitoring the effectiveness of uptake and the outcome for product quality.

7. DETAILED RESULTS

7.1. Objective 1. Development and validation of the inconspicuous sampling device

Summary

An inconspicuous sampling procedure was developed that could be used to withdraw a thin slice of meat without any visible marking of the carcass. It was important to be able to sample carcasses following experiments in order to try to measure changes in flesh characteristics but this cannot continue to be done destructively because of the high cost associated with destroying fish. Most tuna leave Australia relatively intact; head on, gilled and gutted. Under these circumstances, tuna can be sampled using "tail cuts" but the tail will not necessarily show the same effects as the marketable part of the carcass. An inconspicuous sampling method solves this problem especially because of the likely sensitivity of the market any use of a sampling tool. Further details of the development of the sampling device are given in Section 7.1.1.

The successful completion of this objective meant that it was not necessary to initiate loining and processing of tuna carcasses at Port Lincoln in order to obtain destructive samples from fish (Objective 2). Loining may well become an attractive option for tuna processors in future, but until then, it will be necessary to undertake quality observations using samples obtained by devices such as the Boston Bayonet or traditional coring tools inserted into the bleed cut, while the carcasses are accessible in Port Lincoln. This in itself raises the issue of extrapolating from observations made at Port Lincoln, soon after harvest, to the characteristics of the carcass when presented to the market 4 to 5 days later. Being able to reliably predict the outcome for a carcass based upon measurements made soon after harvest were beyond the scope of this study. This would probably require a program of tracing and re-sampling identified carcasses in Japan.

Samples acquired in this way can be used to measure a number of important quality parameters. These include colour, acidity and chemical and biochemical parameters. The preliminary data available indicate that physical and chemical changes in the sample are accelerated with respect to changes in the entire carcass. Nevertheless, in respect to one important quality parameter, flesh pH, the terminal pH of the bayonet sample appears to adequately describe the post-mortem pH reached in the stored carcass (section 7.1.2).

Colour is perhaps the most important characteristic to measure in a tuna flesh sample, though in our experience it is the hardest to quantify. Because of this, we have developed a set of tuna flesh reference colours that can be used in conjunction with the bayonet sampler. The colours are used with a special light source to assign colour scores to particular samples and a workshop manual describing their use forms part of this report (Appendix 3).

The reference tuna flesh colours, in the form of 36 plastic chips, were developed by comparing actual flesh samples from a number of tuna species to standard colours, such as those used by the commercial printing industry. Further details of the development of the reference colour set are given in Section 7.1.3.

7.1.1 The Boston Bayonet

The “Boston Bayonet’ developed by this project was successfully used to sample a strip of flesh close to the backbone and from the skin to the spine, without showing any external marking. The sampler action uses two specially shaped knives that enclose and isolate the muscle sample allowing it to be withdrawn easily from the carcass. In this way, it operates analogously to some medical biopsy samplers. Of course, this device is only used on dead tuna so it is strictly a “necropsy” device.

During the trials the sampling technique was refined and applied in a manner which resulted in no external evidence of the sampling operation. This greatly enhances the scope of the research as samples can be taken from fish which are to be marketed without degrading the value and hence allows greater access to a range of fish and experimental treatments as the fish value is maintained. The sample is also suitable for electronic colour testing by layering in a prescribed pattern.

Use of the bayonet sampling device is explained in the accompanying workshop manual.

Figure 6. Photograph of the Boston Bayonet tuna sampler.

Figure 7. Inserting the sampler into the base of the dorsal fin

7.1.2 Validation of Bayonet sample against whole carcass

This trial involved drawing bayonet samples from 22 fish from differing feed and harvest treatments. was conducted to compare colour (L,a,b) readings in addition to providing colour information for the preliminary colour chip set.

Bayonet samples were taken at 24h post-mortem and stored refrigerated for 96 hours. The final pH reached by the stored bayonet sample (pH 5.66±0.08) (Figure 8) was similar to that obtained from the second, freshly drawn sample from the intact carcass (pH 5.65±0.14). One tuna carcass returned an unusually high pH when sampled the second time (pH 6.1), unlike the more typical value obtained from the stored bayonet sample (pH 5.59). This discrepancy is hard to understand.

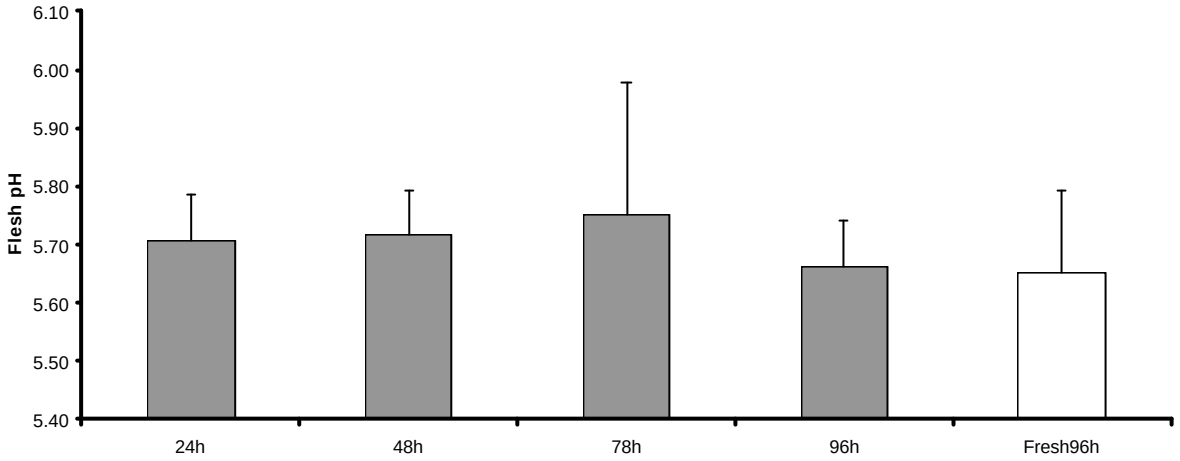

Figure 8. Flesh pH measurements (average± SD) of bayonet samples drawn from 22 tuna at 24h post-mortem and monitored again at 48, 78 and 96 hours post-mortem. Measurements from a subsequent sample taken freshly from the carcass at 96h post-mortem shown by the light bar.

Colour meter measurements of the stored bayonet samples show a number of general trends associated with discoloration of the sample (Figure 9). The average value lightness value of the samples rises. This probably means that all samples and the carcass itself is becoming more opaque with time and reflects more of the meter's light pulse back at its sensors. At the same time that apparent lightness rises, there is a trend for "redness" to fall in the exposed bayonet sample, but not in the carcass. Yellowness showed no definite trend.

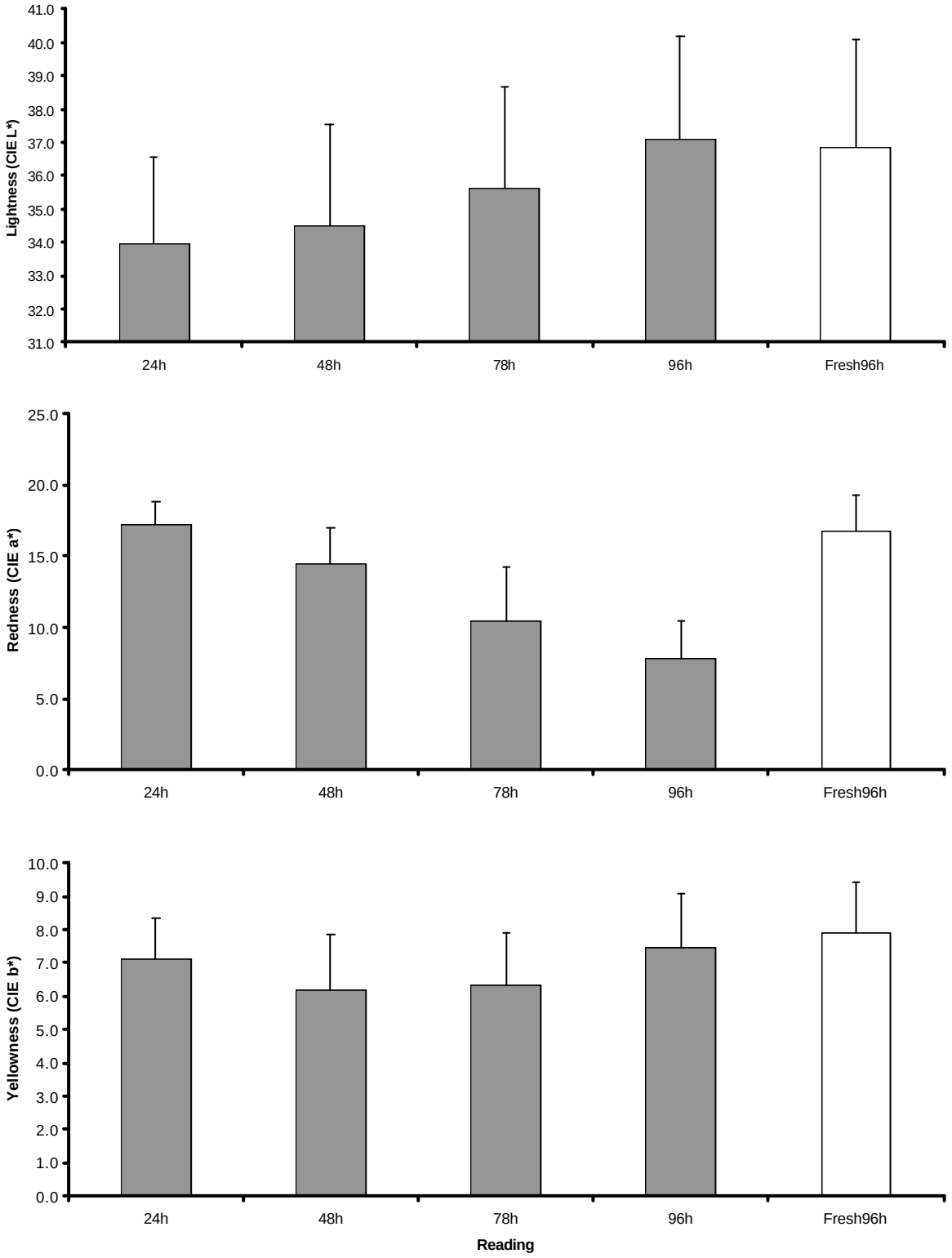

Figure 9. Colour meter measurements (average± SD) of bayonet samples drawn from 22 tuna at 24h post-mortem and monitored again at 48, 78 and 96 hours post-mortem. Measurements from a subsequent sample taken freshly from the carcass at 96h post-mortem shown by the light bar.

Table 1 shows correlations between measurements of pH and CIE colour readings from a bayonet sample taken from each fish 24h after slaughter and readings of a second bayonet sample taken at 96h post-mortem. The outlying pH 6.1 value has been excluded from this analysis.

Table 1. Correlations between pH and colour meter readings of a bayonet sample drawn from a carcass after 96h chilled storage and measurements obtained from an earlier bayonet sample measured and stored for the same period.

First bayonet sample		Carcass at 96 hours			
		CIE L*	CIE a*	CIE b*	pH
24 h	CIE L*	0.578	-0.111	0.126	0.128
	CIE a*	0.425	0.383	0.430	-0.279
	CIE b*	0.386	0.375	0.486	-0.371
	pH	-0.261	-0.682	-0.616	0.811
48 h	CIE L*	0.634	0.119	0.329	-0.032
	CIE a*	0.056	0.399	0.312	-0.403
	CIE b*	0.255	0.547	0.555	-0.600
	pH	-0.252	-0.813	-0.734	0.678
78 h	CIE L*	0.648	0.112	0.356	-0.001
	CIE a*	-0.031	0.087	0.020	-0.191
	CIE b*	0.370	0.475	0.578	-0.399
	pH	-0.097	0.096	0.139	0.218
96 h	CIE L*	0.688	0.253	0.497	-0.116
	CIE a*	-0.147	-0.339	-0.402	0.344
	CIE b*	0.461	0.563	0.684	-0.314
	pH	-0.190	-0.668	-0.644	0.785

Three aspects of the results stand out.

1. That flesh pH in the initial bayonet sample is reasonably well correlated (r=0.811) with the pH taken from a new carcass sample at 96h.
2. The overall lightness (L*) values of the two bayonet samples are also correlated (r=0.5 to 0.7).
3. Flesh pH of the 24h bayonet sample was inversely correlated (r=-0.6-0.7) to the redness (a*) and yellowness (b*) of the 96h re-sampling of the carcass.

While it is encouraging that samples taken early post-mortem from the carcass show some scope for predicting later flesh characteristics this needs to be further confirmed using sensory data. It is interesting that tuna with low flesh pH tend to show higher colour values (a* and b*). Ordinarily, this would be explained by a relationship between pH and reflectance, but here the 96h lightness L* is not well correlated with pH.

The successful development of the sampling tools meant that it was not necessary to proceed with the alternative loining program, objective 2.

7.1.3 The flesh colour reference set

7.1.3.1 Development of the draft colour set

Ideally a method of quantifying the colour of tuna flesh should be simple enough to give immediate and sensible, visual results, and of course, not unduly damage the carcass. There are a number of methods of assessing flesh colour available but these fall down in various ways. The sensory panel approach is able to quantify colour, but it has to be pared down to the bare minimum in order to be practical- it has to be as versatile as grading. In sensory analysis, a number of human observers, under standard conditions, answer questions about the appearance of the sample and other characteristics. Experienced panellists bring rigour and discipline to the work. The down-side is the amount of sample required, too much meat for routine assessment of carcasses.

The colour meter has similar weaknesses. They often require a fairly large sample to be representative- though the devices can be portable and they record results in a standard colour notation. Where the colour meter really falls short is that it is not designed for transparent meat samples. A colour meter works by flashing a pulse of bright light at a surface and interpreting the wavelengths of reflected light mathematically in terms of colour. This is expressed as an overall lightness value or “L” and a pair of colour coordinates, “a” and “b.”

To the machine, a semi transparent piece of meat appears to be dark and non-reflective. The colour meter parameters “L,” “a” and”b” are still useful in interpreting differences or changes in the appearance or composition of different tuna samples. But the major objection to the colour meter is that the parameters can’t be related easily to what humans experience as the colour of a piece of sashimi. For routine assessment of colour, say following a feeding trial, what is needed is a scheme whereby a single human observer can score the colour of a piece of meat in a standard manner. This uses the same approach as grading, except that what is required is a universal colour benchmark or system to describe the colour.

The tuna studied during the sampler validation work were also assessed to identify "tuna-like" colours that could form the basis of a colour measurement system. Prior to the trial, a range of reference colours were selected from the relevant colour spectrum of the NCS (Natural Colour System) to compare with actual tuna flesh colours obtained from “bayonet “samples. In addition to the fish used in the experimental trials, additional fish were examined from a number of commercial harvests. The range and frequency of colours were examined from the experimental trial samples at 24 and 96 hours using the selected NCS colour swatches. Fig 9, shows the distribution and frequency of the selected colour swatches at both 24 and 96 hours. The yellow boxes represent one Aqui-S harvested fish which did not vary in colour through the 96 hour period.

The selected colours were then scanned using a spectrophotometer, the spectral curves modified and a series of plastics produced as a draft set incorporating new colours to fill in the gaps (Figure 10).

7.1.3.2 Examination of manufactured draft colours using the colour meter.

The original colours (original chips 5 to 10) had been based largely on modifications to a commonly observed tuna flesh colour by adjusting lightness by adding white or black pigment. This was not particularly representative of the variations in tuna flesh colour, because of the limited samples used to establish the first chip set.

Through liaison with the chip manufacturer and representatives of Ausmeat Pty Ltd we attempted to establish a “colour space” for tuna, which would be based on a relationship between the red green and blue colour components of scans (rgb values) of the tuna flesh from a range of tuna samples. But the digital colour scanner did not perform satisfactorily. Instead, using improved reference lighting we found the closest match to the each sample using NCS (Natural Colour System) and Pantone colours. We then plotted colour meter readings of the colour swatches in CIE L*a*b* colour space. This showed that the existing colour set was not red enough for many samples of meat.

Having established several colours in the initial range that were close to and based on a close visual match to tuna flesh, several processes were explored to enhance the appearance of the chips to achieve a more tuna-like” appearance.

To establish the actual colour range (hue) large numbers of flesh samples were obtained from both yellowfin and bigeye tunas. Using the more advanced lighting and comparison system and the more refined pantone colour series, it became clear that we didn't have a colour space based upon a single flesh hue. Using the pantone colours matched to the samples, we saw that an additional red and a blue-red or maroon hue, which spaced reasonably well in CIE Lab colour space, were required.

The situation was that we now had 3 base colours or hues. The 2 additional colours were generated from the selected pantone references. A variation to each basic colour was also added on a subjective basis on the anecdotal and subjective presence of "dull" colours in tuna. The presence of these chips in the long term will be subject to review, however it appears so far that these darkened hues fit neatly into the colour matrix and are in-fact representative of some fish, perhaps due to the presence of low concentrations of metmyoglobin in the flesh.

Each of these 6 basic chips was then varied by change to pigment concentration from full strength to 10% of the original concentration selected in previous trials as mentioned above (6 chips from each basic form). Our matrix is thus 3 x 2 x 6 = set of 36.

This is the basis of the draft chipset (Figure 11). The "DRAFT 1" chips are identified by their Hue (C1 – C3) the type (light (L) or Dark (D)) and the pigment concentration 10-100. For example a common chip representing richly coloured SBT is identified by C2L80.

The chips are now subject to evaluation but are expected to be limited in application to bayonet samples from the trunk of the fish or comparison to sushi pieces or cuts of tuna. The colour reference set cannot be used on thickly cut 'tail-cuts' where the colours are darkened further by the sample thickness. They could possibly be used on thin tail cuts - but why sample the tail when sampling of the trunk itself is now possible?

Instructions for using the colour reference set are included in the workshop manual (Appendix 3).

With these standard chips as a benchmark we can start to look for differences in flesh colour between different factors such as…

1. feeds, for example to track the impact of vitamin supplements
2. husbandry/management practices
3. the effect of farm location/time of year

The reference set is by nature complicated- it goes in a box. We are seeking to simplify it to a working set of chips- something that fits in your pocket.

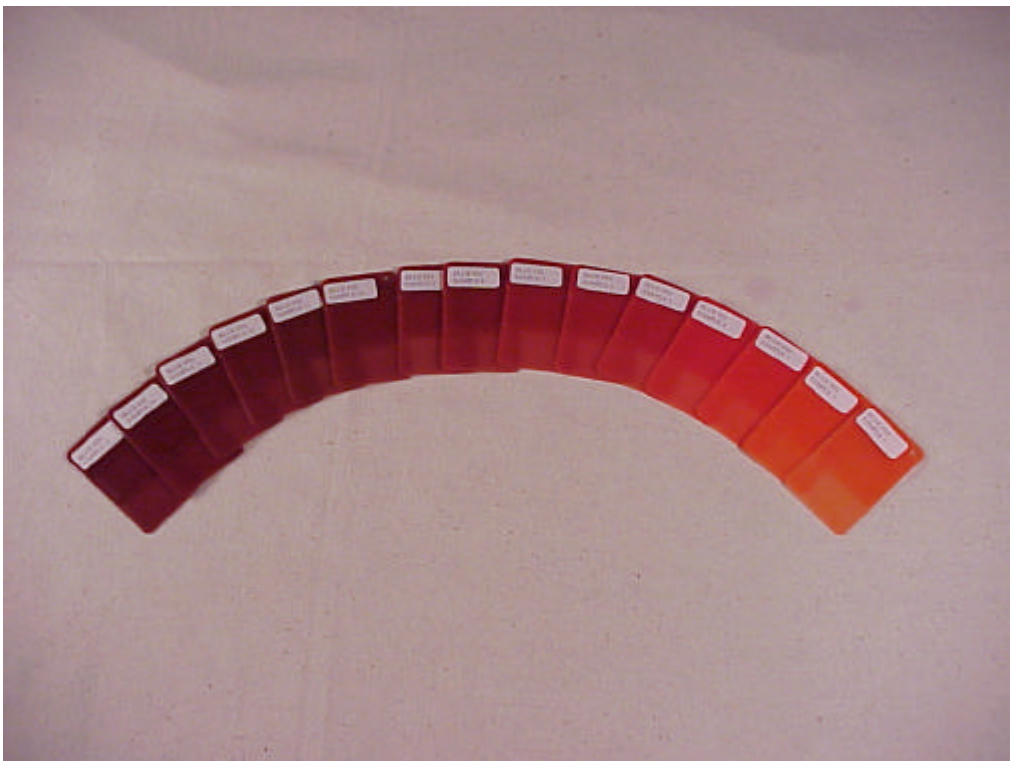

Figure 10. Draft tuna colours derived from the NCS colour swatches.

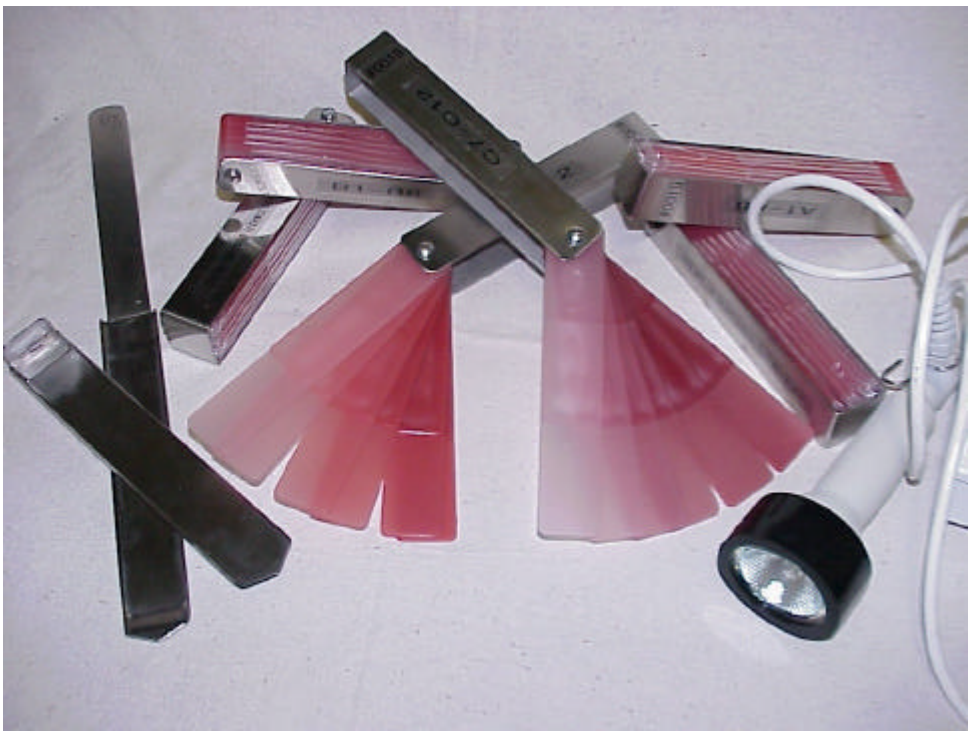

Figure 11. The tuna flesh colour reference set photographed alongside other elements of the assessment kit.

The question to ask here is what are the most important aspects of colour variation to record. The real strength of the chip approach is that rather than just giving results in averages, letters and numerals, you can also see what the colour looks like. This makes the results more accessible and understandable.

Measuring the colour of tuna flesh is not the same thing as grading it. The colour of harvested tuna probably needs to a measured as part of the overall management of the farming process. Tuna of course will still have to be graded. Grading remains an important benchmark of its own. Fish from feeding trials ought to be marketed to see what the outcome is. But the strength of pinning down and quantifying the colour alone is that it allows people to look for patterns and trends. A number of factors in the husbandry environment are almost certainly changing the flesh colour of farmed tuna. We understand so little about them because until now we've lacked a method to collect colour information in large amounts.

7.2. Objective 3: Effects of pre-harvest, harvest and post-harvest handling on flesh colour and post-mortem biochemistry of farmed SBT

Summary

This study showed that harvest could have major effects on certain flesh characteristics of SBT, and while some (such as flesh pH and temperature) were of concern with regard to further quality changes downstream, there was no data pointing clearly to certain harvest practices returning improved flesh quality.

Generally it is accepted that stress- or exercise-free harvest will improve flesh quality by ensuring that the fish are not exhausted during harvest. Perhaps we have trouble demonstrating post-harvest changes because we are sampling the tuna too early, at Port Lincoln, and not several days later then they are on the market floor. The problem could also be because tuna are handled differently from other fish. Unlikely many other farmed fish, they are so big and so valuable that they are handled individually when slaughtered, lifting the bar in terms of flesh quality. They are also active swimmers and frenzied exercise rather than being "stressful" may simply how they feed. Indeed, some of the results obtained here suggest that it may sometimes be necessary to exercise captive tuna during harvest to burn up energy stores that might otherwise damage the carcass!

Two approaches were used here to assessing the impact of various factors on flesh characteristics in SBT. Firstly, a detailed study was undertaken into the effects of harvest stress on the post-mortem biochemistry of the tuna. Secondly, larger scale trials were conducted that sought to eliminate harvest stress altogether by attempting to anaesthetise SBT in bulk, in trials that also introduced other pre- and post-harvest treatments such as fasting and chilling methods respectively.

Harvested SBT show evidence of a stress response at death, according to the biochemical and physiological studies of SBT harvested in different ways. When tuna struggle on harvest, they also warm up and their blood becomes more acidic. Some glycogen in the muscle is converted to lactic acid, which then appears in the blood. However, the acid accumulated in the blood during harvest does not seem to influence the final pH of the flesh. That is probably determined by continued conversion of glycogen to lactic acid during rigor mortis. Data in this study raise the prospect that captive SBT may sometimes build up unusually high levels of glycogen. Exercise during harvest may actually be beneficial if it reduces the amount of glycogen remaining in the muscle of the dead tuna and in turn reduces the extent of further acidification during rigor mortis. This point will need further research.

In order to harvest SBT in large numbers without any struggling or exercise, a floating PVC enclosure was used in harvesting trials but with mixed results. Groups of SBT were successfully sedated using carbon dioxide and Aqui-S, but the logistical difficulties in corralling tuna from a crowd into this enclosure mean that they probably were not entirely "rested" upon slaughter. In future, the sedation step needs to be better married with the wider harvest procedure. No results were obtained that were compelling enough to require immediate changes in harvest practices. However, it was found that sedated tuna were cooler than non-sedated tuna, in line with the biochemical work, and that the blood of immobile tuna tended to be more acidic upon slaughter, though again no difference was evident in the terminal flesh pH. There was preliminary evidence that sedated SBT went into rigor more slowly than non-sedated SBT, both in terms of post-mortem flesh pH and relative contraction of excised muscle blocks. Post-harvest chilling of the carcasses influenced flesh characteristics, but these results were difficult to interpret except to say that the warm bleeding techniques tested here are not recommended. Fasting or withholding food from tuna for a week before harvest significantly elevated their post-mortem flesh pH- but not in all cases, and the mechanism for this should be examined in more detail.

7.2.1 The effect of harvest stress on physiological responses and post-mortem biochemistry of SBT

The results showed that tuna exercising or struggling during harvest, including those harvested at a commercial farm, showed evidence of a "stress" response at death, in terms of elevated cortisol levels and accumulation of other stress indicators such as glucose and lactic acid. In addition, their body temperature was warmer and their flesh showed signs of muscle energy depletion. While the blood of the "stressed" tuna tended to be more acidic on landing, the preceding stress did not significantly influence the acidity of the muscle in the 24h dead carcass- indicating that harvest activity was not influencing post-mortem acidification in the tuna carcasses. Much of this is probably attributed to lactic acid produced from stores of glycogen in the flesh at death. Some evidence has emerged here to back a long held theory that farmed SBT may be accumulating unusually high glycogen levels under some circumstances. Whether or not activity or stress at harvest has any commercial consequences remains to be seen, but the findings in relation to temperature and energy depletion may have consequences for the future shelf life of the marketed flesh. These concerns are however tempered with the finding that harvest activity, rather than necessarily being a problem, may be a mechanism for ridding farmed tuna of excess glycogen prior to slaughter.

The potential impact of harvest stress on the physiology and post-mortem biochemistry of SBT was examined in two experiments. The detailed results and discussion are presented below. In order to remind the reader of the methodology, a brief outline is given here, but the details are located in section 6.3.1. One experiment compared SBT that were rapidly hand-lined (TLC) to those hooked but played on the line (TLS) and a second at a commercial farm, compared SBT caught using a hand line (FLC) to those harvested using net crowding (FNC). For comparative purposes, pole-caught SBT were also sampled at sea (PW). Blood and muscle samples taken from the tuna were analysed for a number of important physiological and biochemical parameters. Plasma pH, lactate, glucose and cortisol levels were determined from the blood samples. Muscle samples were analysed largely for indicators of the status of cellular "energy" levels. Glycogen and lactic acid were measured as a major source and end product respectively of anaerobic metabolism in the muscle. The energy available in the form of adenylate nucleotides was summarised by calculation of adenylate energy charge (AEC) from measured levels of individual compounds and the degree to which the pool of adenylates was temporarily depleted by calculation of the Inosine monophosphate load or IL. Since inosine monophosphate (IMP) is also the precursor of inosine and hypoxanthine, the breakdown product of the adenylate pool associated with loss of "freshness," the accumulation of these products were also expressed in terms of overall pool of adenylates and IMP as the K-value.

7.2.1.1 Detailed results of blood parameters

The plasma cortisol level of the FNC group was much higher than in fish that had been subject to any other capture method. Fish from the other groups had the same plasma cortisol levels. Plasma glucose level was higher in the FNC fish than the FLC or the PW fish (Table 2). Generally, groups of fish that had high plasma glucose also had elevated plasma cortisol.

Plasma lactate was higher in the FNC fish than fish from any other group (Table 2). The TLS group had higher plasma lactate than the remaining groups of fish, all of which had not been subject to any stress treatment during their capture. Plasma pH followed a similar pattern between fish groups as plasma lactate levels. The FNC group had a higher plasma pH than the farm fish that were not subject to capture stress treatment. However TLS and PW fish had the same plasma pH as the FNC fish that were subject to capture stress treatment.

Generally, tuna that had been subject to stressors that included netting, confinement and/or prolonged capture, had elevated plasma cortisol, glucose and lactate levels, and had lower plasma pH than fish that had been line caught and immediately slaughtered. In addition, fish that had been subject to net confinement and diver capture (ie the FNC group) had higher plasma cortisol and lactate levels than fish from any other group. Elevated plasma cortisol and glucose is a recognised pattern of events in fish that have been subject to stress, and is a result of an endocrine driven response to a perceived threat. Elevated plasma glucose levels are the result of the action of circulating catecholamines and cortisol which mobilise glycogen stores and providing fish with energy reserves, which help them deal with the prevailing stressors.

It has been noted that in tuna species lactate and H^+ ions, which accumulate in the muscle following hyperactivity, are very quickly seen in the blood (Bushnell and Jones 1994) and therefore directly reflect the anaerobic metabolic activity of the muscle. Therefore, the higher plasma lactate level and low plasma pH of the fish subject to harvest stress reflects higher anaerobic metabolism in the muscle of these fish compared to those from the other groups.

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Table 2. Plasma level of cortisol, glucose and lactate and pH, for fish subject to different harvest methods

Parameter	PW	TLC	TLS	FLC	FNC
Cortisol	8.2 ± 3.7a	1.4 ± 0.4a	8.6 ± 2.0a	3.5 ± 1.2a	108.4 ± 8.6b
Glucose	4.6 ± 0.2ab	4.9 ± 0.3bc	5.6 ± 0.6bc	3.6 ± 0.3a	6.8 ± 0.7cd
Lactate	3.1 ± 0.6a	1.1 ± 0.3b	6.1 ± 0.3c	2.2 ± 0.3a	11.5 ± 1.0d
pH	8.05 ± 0.04ab	8.18 ± 0.02a	8.00 ± 0.03b	8.22 ± 0.04a	7.95 ± 0.04b

Groups include: Pole caught wild-fish (PW); Towed line caught (TLC); Towed line stressed (TLS); Farmed line caught (FLC);and Farmed net caught fish (FNC). Values are mean + SE (*n* = 10). Values that are not significantly different (*P* >0.05) share common superscripts.

7.2.1.2 Detailed results of muscle parameters

7.2.1.2.1 Adenylate energy charge, IMP Load and K value

The Adenylate energy charge (AEC) values for the fish subject to capture stress treatment during harvest were not significantly different to fish from those groups which were not, with the exception of the TLS fish which had lower AEC values than the PW fish (Table 3). However, the IMP load (IL) was significantly higher in the TLS and FNC fish than the groups not subject to capture stress treatment (Table 3). Following 24h ice-slurry storage the mean (± SE) K-value for the TLS fish was higher (at 7.2 ± 0.5) than any other group (Table 3). The PW fish had the lowest K-value but this was not significantly different to the FLC fish.

Table 3. Muscle levels of IMP Load (IL), Adenylate Energy Charge (AEC) at death, and muscle K-values following 24h ice slurry storage for fish subject to different harvest methods.

Ratio	PW	TLC	TLS	FLC	FNC
IL	0.04 ± 0.0a	0.13 ± 0.0a	1.24 ± 0.2b	0.13 ± 0.1a	0.75 ± 0.4b
AEC	0.97 ± 0.0a	0.92 ± 0.0ab	0.84 ± 0.0b	0.96 ± 0.0ac	0.82 ± 0.1bc
K	1.20 ± 0.2a	2.73 ± 0.1b	7.22 ± 0.5c	1.94 ± 0.5ab	3.85 ± 0.7b

Groups identified as in Table1. Values are mean + SE (n = 10). Values that are not significantly different (P >0.05) share common superscripts

The AEC values measured in the tuna muscle at death, were at the high end of the range considered physiologically normal. These AEC levels were a result of a natural mechanism that functions to protect the continuing metabolic activity of the muscle. That the IL was much higher in the fish subject to stressors during harvest, than those that were not, indicates stressors associated with tuna harvesting initiate metabolic responses normally associated with excessive exercise or muscle hyperactivity and which precedes changes to AEC values.

After 24h storage, the K-value of the muscle in all groups was low and in the range of values expected for fresh fish. That there were slightly higher values found in the fish subject to capture stress treatment, may indicate an increase in the rate of post-mortem biochemical change in these fish, but without further sampling of the carcase during storage this is impossible to quantify.

7.2.1.2.2 Temperature

At death, muscle temperature was highest in the TLS and FNC fish but these temperatures were only significantly different from the TLC fish (Table 4). After 3-4h ice-slurry storage the PW and FNC fish muscle temperatures were higher than 19^0C, which was significantly higher than fsh from the other groups. Following 24h storage, fish from all groups had muscle temperatures under 3^0C. The difference between the sea temperature and the fish muscle temperature at death was 10.6 ^{0}C for the FNC group and this was significantly higher than any other group. The difference between the sea temperature at harvest and the fish muscle temperature at death was lowest in fish from the TLC group but this was not different from the PW fish.

Table 4. Muscle temperature (^{0}C) at death and during storage (mean $\pm$ SE, n = 10), sea temperature at time of harvest and muscle temperature at death minus sea temperature at harvest.

Harvest method	At death	3-4h Storage	24h Storage	Sea	At death - sea
PW	25.4 $\pm$ 0.4 ^{ab}	19.1 $\pm$ 0.9 ^{a}	2.2 $\pm$ 0.3 ^{acd}	21.4	4.0 $\pm$ 0.4 ^{ac}
TLC	24.6 $\pm$ 0.4 ^{b}	7.0 $\pm$ 0.4 ^{b}	1.0 $\pm$ 0.2 ^{bc}	22.2	2.4 $\pm$ 0.4 ^{a}
TLS	27.1 $\pm$ 0.4 ^{a}	7.0 $\pm$ 0.9 ^{b}	0.6 $\pm$ 0.3 ^{b}	22.2	4.9 $\pm$ 0.4 ^{bc}
FLC	26.5 $\pm$ 0.4 ^{ab}	10.4 $\pm$ 1.0 ^{b}	2.6 $\pm$ 0.4 ^{d}	19.4	7.1 $\pm$ 0.4 ^{b}
FNC	27.2 $\pm$ 0.9 ^{a}	19.6 $\pm$ 1.4 ^{a}	1.8 $\pm$ 0.4 ^{bd}	16.6	10.6 $\pm$ 0.9 ^{d}

Between harvest method means (within time) that are different ($P<0.05$) have different superscript letters. Groups identified as in Table 1

Muscle temperature at death and during post-mortem is important because rate of post-mortem change in animal flesh has been shown to be time/temperature dependent. That is the higher the muscle temperature the faster the rate of post-mortem biochemical change in any given time period. Elevated muscle temperature in SBT is associated with feeding, food digestion and muscle hyperactivity. That the TLS fish had a significantly higher muscle temperature at death than the line caught fish (TLC) indicates that activity during harvest will result in a rise in muscle temperature. However, in the farmed fish it is more difficult to determine the reason for the relatively high muscle temperature of the FNC fish, compared to the line caught group (FLC). The higher muscle temperature of the FNC fish could have been a natural compensation for lower sea temperature at the time of harvest, or temperature rise due to capture treatment induced muscle activity. Never the less, and with the exception of the PW fish, higher muscle temperature in the FNC fish at death, resulted in higher muscle temperature, following 3-4h ice-slurry storage, than other group. As the PW fish were not gilled and gutted prior to storage their high muscle temperature (following 3-4h storage) was not unexpected.

7.2.1.2.3 Lactate and pH

There was a rise in muscle lactate levels in fish from all groups over the 24h ice -slurry storage period. At death muscle lactate level was the same in fish subject to capture stress treatment (TLS and FNC groups), and in the TLC fish which were not subject to stress treatment (Table 5). Muscle lactate levels were the same for the PW and FLC fish, and these levels were lower than in the fish from groups that were subject to capture stress treatment. After 3-4h ice-slurry storage, muscle lactate levels were higher in the TLS fish than fish from any other group and lowest in the PW and FLC fish. After 24h ice-slurry storage, muscle lactate levels were the same and highest in the TLC, TLS, and FNC fish. Fish from the other two groups (PW and FLC) that were not subject to stress treatment, had the same muscle lactate levels, and these were significantly lower than all the other groups. At death the range of the group mean muscle pH for all the capture methods was 7.25 to 6.51 (Table 5). The PW fish had a significantly lower muscle pH at death (pH 6.51) than fish from any other group, with the exception of the TLS group. Following 3-4h ice storage, the pH of muscle of the TLC and TLS fish was the same. No other pH figures are available for this storage time. Following 24h ice slurry storage the range of the group mean muscle pH for all the capture methods was 6.03 to 5.76. However after 24h ice storage, the PW, TLC, and TLS fish had the same muscle pH which was significantly lower than the FLC and FNC fish.

Muscle lactate levels at death were higher in fish subjected to stressors during harvest, but lactate levels were also as high in fish from one of the groups that were not subject to known stressors. The significance of the elevated muscle lactate in the line caught group (ie TLC) is not known. Muscle lactate levels in exercised tuna have been noted at greater than 100 mmol.l^{-1} (Korsmyer et al. 1996). The levels recorded in this current work were well below those reported elsewhere, and this may be a reflection comparatively low level of muscular activity, prior to death, associated with the all the capture methods investigated here. Muscle pH at death was similar across all groups, and this indicates that the capture methods had little impact on the acidification of the muscle at death. This apparent stability of pH is probably the result of the high buffering capacity of the muscle in these fish. Atlantic salmon subject to stress treatment (involving high levels of activity prior to death), show a large and significant drop in muscle pH, compared to unstressed fish, which may be sustained during post-mortem storage (Thomas et al. 1999). In the current work, the lack of treatment effect on SBT muscle pH at death, indicates that SBT appear to have the capacity to compensate for the muscle acidification normally associated with harvest hyperactivity in fish. The reason for the lower muscle pH of the PW fish at death is unknown.

7.2.1.2.4 Glycogen

At death, the range of the mean ($\pm$ SE) muscle glycogen levels, for fish from all the capture methods was 30.1 $\pm$ 7.6 to 539.3 $\pm$ 158.3 mg.100g (Table 5). At death, the muscle glycogen level of fish from all groups was the same, with the exception of the FLC fish, which had significantly higher levels. After 3-4h ice slurry storage, the TLS fish had lower muscle glycogen levels than the PW and TLC fish but were the same as fish from the FLC and FNC groups. Following 24h ice-slurry storage, the muscle glycogen level was the same for fish from all groups with the exception of the FLC group, which had significantly higher levels.

At death muscle glycogen levels were highest in the FLC fish. These fish had been farmed for several months and then subject to line harvest prior to slaughter. This treatment would have allowed glycogen to build up in the muscle of these fish during the farming period, while the fast, low activity method of harvest would have avoided glycogen depletion prior to death. With the exception of the FLC group, muscle glycogen is almost totally depleted, in all groups of fish, following 24h ice-slurry storage. This indicates that at 24h post-mortem glycogen utilisation is still possible in the muscle of fish from the FLC group but is unlikely to continue in the muscle of fish from any other group. Post-mortem muscle glycogen level and utilisation is important because it has a large bearing the ultimate pH (ie the lowest pH post-mortem). The ultimate pH is known to have an affect on flesh quality characteristics of fish and terrestrial animals. In the current work, the ultimate muscle pH may have been reached in fish from those groups where glycogen was almost totally depleted after 24h storage. However, in the FLC and it would be expected that muscle pH would continue to drop during storage. In terrestrial animals the effect of post-mortem pH change on flesh quality is understood, to the extent that electrical muscle stimulation of beef carcases is regularly used to manipulate post-mortem pH and reveal ultimate pH. Ultimate pH value is then used to indicate flesh quality and a grading tool (Thompson pers comm.). Further investigation into the flesh quality of SBT may reveal that similar techniques can be applied to these fish.

Table 5. **Muscle levels of lactate pH and glycogen at death and following 3-4h and 24h ice storage for fish subject to different harvest methods.**

Parameter		PW	TLC	TLS	FLC	FNC
0h	Lactate	10.07 ± 1.75a	29.12 ± 4.43b	34.43 ± 1.40b	12.05 ± 0.97a	23.27 ± 1.86b
3-4h	Lactate	9.16 ± 0.90a	20.67 ± 1.51b	39.26 ± 1.96c	11.66 ± 0.71a	25.08 ± 2.32b
24h	Lactate	25.66 ± 2.62a	46.01 ± 3.60b	48.80 ± 1.84b	23.40 ± 3.57a	44.14 ± 1.26b
0h	pH	6.52 ± 0.1a	7.25 ± 0.05bc	6.81 ± 0.05ac	6.95 ± 0.09bc	7.04 ± 0.06bc
3-4h	pH	4.82 ± 0.16	6.01 ± 0.07a	5.89 ± 0.08a		5.84 ± 0.15
24h	pH	5.76 ± 0.07a	5.77 ± 0.05ab	5.79 ± 0.05ab	6.03 ± 0.05b	6.03 ± 0.06b
0h	Glycogen	167.18 ± 24.98a	151.69 ± 29.16a	30.146 ± 7.61a	539.26 ± 158.25b	53.54 ± 28.99a
3-4h	Glycogen	273.45 ± 23.47a	252.89 ± 49.43a	17.29 ± 5.43b	180.04 ± 64.87ab	133.53 ± 41.69ab
24h	Glycogen	30.18 ± 7.05a	2.39 ± 2.39a	7.15 ± 7.15a	238.40 ± 84.20b	39.348 ± 21.19a

Values are mean + SE (n = 10). Values that are not significantly different (P >0.05) share common superscripts. Groups identified as in Table 1

7.2.2 Harvest sedation of tuna

Ideally, when testing the effect of harvesting stress or activity on the flesh quality of tuna, it would be useful to have recourse to samples of tuna that have not been harvested or which have been disturbed as little as possible, to establish a baseline for parameters measured. In the biochemical studies, rapidly hand-lined fish were used as the practical benchmark for unstressed fish. The next and more difficult step is to anaesthetised tuna for harvest, both to produce unstressed fish for experimental purposes and also to examine the possible benefits and feasibility of using anaesthesia for tuna handling and harvesting. This was the aim of the second aspect of this work.

A floating PVC enclosure was designed and manufactured for the purpose of corralling and treating tuna with aquatic anaesthetics such as Aqui-S^{tm}. Considerable forethought went into making the construction of the enclosure as versatile as possible, giving it a closable gate and allowing it to be assembled entire or as a cut-down version with reduced volume. Before launching the enclosure at sea, it was wet tested in a diving pool to practice the procedure of assembly and breakdown and to get a foretaste of any snags prior to sea trials at Port Lincoln. The enclosure was then shipped to Port Lincoln and deployed in the large 30m holding cages at the experimental farm in May 1998, where it was used to successfully anaesthetise tuna using carbon dioxide gas for a series of harvesting trials.

That experience saw further refinements to simply the deployment process, the depth was reduced and a raisable floating platform and lining net was constructed to fit inside the enclosure to reduce the need for hookah divers to collect the sedated tuna. In this configuration it was used in a preliminary trial in 1999 where tuna were sedated using Aqui-S^{tm}. It was noted that the disoriented tuna could foul in folds of the inner net if it was not kept taut while raising the platform and the procedure was reviewed as a consequence.

The final trial with the enclosure was in June 2000 where it was used to repeatedly sedate batches of tuna using Aqui-S, in cooperation with a commercial tuna company. The enclosure was assembled as it would be for a commercial harvest and the platform was deployed into the liner and raised for the first time by crane. Unfortunately, crowding the tuna and guiding them into the enclosure did not proceed smoothly. The platform and net could not be raised evenly due to rolling of the vessel and the sedated tuna became entangled. In addition, the heavy duty zips used to assemble the enclosure began to fail, apparently due to simple wear and tear.

The prototype enclosure was found too complicated for routine commercial use, largely due to its age and prototype design. In future, enclosures for treatment or sedation of tuna will not need to be this complicated but their use needs to be better incorporated into the entire crowding operation, probably on a farm by farm basis. It is further recommended that a combination of ropes (strops) and velcro rather than zips be used to fasten any gates and joins in the fabric of the enclosure.

While tuna were successfully sedated in the enclosure using carbon dioxide and Aqui-S, the consequences of this for flesh quality could not be unambiguously demonstrated. In large part this was because of the logistical difficulties in harvesting the numbers of tuna required for ambitious multi-factor experimental designs. Also, crowding the tuna in order to guide them into the enclosure was not as successful as it would need to be for a stress-free harvest.

One result stands out however, namely that sedated tuna were cooler than control fish, with a suggestion that over time sedation cools the tuna down, which is an interesting corollary of the tendency for activity to make tuna hotter.

Sedated tuna were landed with a low blood pH but sedation had no impact on the terminal pH of the muscle probably because of the different metabolic factors driving post-mortem acidification in the muscle . Tuna are ram ventilators, and would be expected to show impaired respiration and develop low blood pH if they slow down or stop swimming. Carbon dioxide anaesthesia reinforces the tendency for the blood to acidify by also making the water bathing the tuna itself acidic!

The Aqui-S trial, even when there were doubts that the tuna were truly rested, nevertheless showed some tantalising evidence of the hypothesized slowing in the rate of rigor mortis, with a flesh pH higher than controls at 4h post-mortem, and preliminary excised muscle data suggesting less shrinkage in samples from Aqui-S fish but more data is needed to be sure. This is a technical outcome alone at this stage, because no significant difference in flesh colour could be demonstrated despite using the newly developed flesh colour chips.

Concerning effects of other factors on flesh quality, there is no doubt that post-harvest handling treatments influencing the appearance of the flesh in the earlier carbon dioxide trial but the results were not consistent and being colour meter results were difficult to interpret. There was also evidence confirming earlier findings that fasting these late season pilchard-fed tuna could significantly elevate the terminal pH. In line with the biochemical findings regarding glycogen level, more research is required into the factors effecting glycogen levels in the muscle and the implications for flesh quality.

7.2.2.1 Testing and deploying the enclosure

This tool for applying anaesthetics or other treatments has been developed and tested with the involvement of the commercial industry and tuna research scientists as well as vinyl fabrication specialists. It is also worth mentioning that the liner has potential for other SBT husbandry practices such as; therapeutic bathing should a disease condition arise in SBT treatable by this methodology, and, it has the capability to grade sedated non-market fish from market fish allowing them to be returned to the holding pontoon for finishing.

Prior to sea trials at Port Lincoln, the enclosure underwent a wet assembly trial at the diving pool at the Chandler Aquatic complex in Brisbane.

Rested harvest trials were successfully undertaken using the liner in May 1998, using the enclosure deployed in the ”full” (36M) circumference set-up (Figure 12, Figure 13) and the “part” (24M) deployment, (Figure 14).

7.2.2.1.1 Indoor pool trial

The pool trial demonstrated the ease with which the liner can be assembled and tested different protocols for deployment and retrieval. The chief limitation of the pool trial was the shallow depth preventing a perfect trial run. However, it did demonstrate the way a solid liner behaves in water and the difficulties associated with performing certain operations such as:

- increasing the volume of the liner once zipped up;
- retrieving the liner with even a slight amount of water bagged on top;
- pulling the gate straight up from the bottom;
- dropping the gate rapidly;
- inserting the hex-ring with liner all zipped without guide ropes and marked corners;
- divers performing all zipping - snorkellers can provide considerable assistance and this should be enhanced with the diver-surface communications in Port Lincoln.

The positives from the trial were as follows:

- inflatable sections easily inflated with SCUBA bottle
- panels easily manoeuvred and clipped/zipped together
- walls unfold and zip up well
- panels easily removed and roughly stowed by 2-3 people

With the additional modifications to design and protocols as a result of the pool trial, it was concluded that the enclosure should provide a suitable environment for demonstrating the effects of rested harvest on flesh quality of SBT.

7.2.2.1.2 Deploying the enclosure at sea for the carbon dioxide trial.

The use, need and benefits of this device and method for containing farmed tuna has been previously discussed. While the liner may be used to provide the facility for achieving rested harvest through sedation, it may also have applications in other research and commercial practices. As a tool, it was successfully demonstrated and easily assembled and disassembled from a small dinghy using 3 competent snorkel divers (Figure 12). The process of transferring tuna in and out of the liner was

shown to be simple and effective using a crowd net. Refinement of the actual fish retrieval and methods of anaesthesia were applied in later experiments, however the tool itself needs little major modification prior to future applications.

7.2.2.1.3 Testing the raiseable base- preliminary Aqui-S trial

Modified enclosure system using raiseable base tested and prepared for full trial and alternate system of enclosure deployment tested. Figure 15 shows the folding base in place in the enclosure in the raised position. This is the position which is used to facilitate harvest after the tuna have been sedated. In raising the base an efficient but sedate harvest is facilitated, as divers no longer need to hunt fish such that time and activity are both minimised.

Figure 12. Deployment of the enclosure (in its 3panel 36 metre configuration) and gas ring (being positioned by divers) from the "Toro."

Figure 13. Full liner deployed.

Figure 14. The enclosure in its reduced, 24 metre, 2-panel configuration

Figure 15. Divers installing the floating base and net into the enclosure to allow rested harvest operations.

7.2.2.1.4 Harvesting using a reduced enclosure and the raiseable base – Aqui-S trial.

The basic concept of the enclosure system is to allow fish to be introduced into the enclosure with minimal hyper or adverse activity, from where a process of sedation can be applied to facilitate rapid and effective killing. The use of the portable enclosure system for research purposes should provide an insight into the possibilities for an integrated enclosure and crowding system that is suited to commercial operations.

With the modifications to the enclosure and with correct deployment procedures used, the set-up was reasonably efficient. Problems were encountered through zipper failure resulting from broken teeth however while this was alleviated for the trial by wiring the zip in the damaged areas, the system would need to be secured in an alternative fashion for future use.

The speed of the sedation was however, a problem as we were unable to effectively use the raiseable base as intended to provide a stable working platform, and furthermore were unable to avoid creating folds in the net as the base was lifted by crane to expedite harvest.

7.2.2.1.4.1 Modifications to enclosure

For the June 2000 trials with a commercial tuna farm, the enclosure system was modified in several ways to improve operational ease. The depth was reduced to approximately 4 metres by creating a fold in the top of the side panels and holding in place with Velcro stripping. The base was also reduced to a 1 metre taper by incorporating a second set of internal zips. (This was necessary as the original design was much larger with 3 panels and the existing zip position resulted in a centre depth of some 5 metres below the wall depth due to the geometry when using only 2 panels. This re-configuration left the options available to use the enclosure in the full form in future if necessary.

The raiseable base and side nets were also modified to include a net door adjacent to the enclosure door and resizing to allow the top of the side nets to fit neatly over the enclosure inflation band. Horizontal ropes were also added to allow the sides to be lifted and secured at the inflation band to provide straight and tight net walls and a stable working platform.

7.2.2.1.4.2 Crowding techniques and transfer to enclosure

The enclosure and base were set up prior to beginning the harvest crowd. After deploying the modified enclosure, the raiseable base was lowered into place using a vessel-mounted crane (Figure 16). The side nets on the base were secured into place on the float ring of the enclosure (Figure 17) prior to fully lowering the base.

Existing crowding techniques involve the deployment of a fine net with a weighted bottom rope and float rope at the top. The net is deployed to separate a selected number of fish, usually in excess of the days requirements and then condense these into an area convenient to the vessel and relatively close to the surface. The "Crowd" may then be separated into a "deep" and "shallow" side, which is used for harvesting by diver. In this trial the shallow side was connected to the door of the enclosure by attaching a portion of the float rope around the doorframe (Figure 18). Tuna swimming inside the enclosure are shown in Figure 19.

Compromises were necessary to combine the normal harvest practice with the enclosure system. The main difficulty was that the doorway could not be located at the "end" of a net tunnel but rather in the long side of the shallow section of the harvest net. This meant that fish had to be forced by severe crowding to enter the enclosure. The situation was exacerbated to some degree by the size of the door which was reduced to a depth of 2.3 metres due to the fold in the walls. To resolve these issues an enclosure system needs to have a crowding and transfer system that is clearly compatible and that creates the clear opportunity for fish to enter the enclosure voluntarily. Some of the concepts used in fish trap design could be a guide here.

7.2.2.1.4.3 Enclosure and base operation

When in place and set up the enclosure was well shaped and of suitable volume and clear of internal obstacles that have added some complexity in the past. Assembly of the enclosure at the surface by maintaining the vertical sides folded and base ring holes taped, reduced the need for diving in the assembly process but reduced the size of the entry door. The modifications to the raiseable base side nets allowed the system to be raised and locked in place during testing but the rapidity of the sedation process prevented this process from being applied for the trial itself leading to difficulties with net folds and an unstable work platform mentioned previously. Both structures were easily transported, deployed and retrieved.

The enclosure structure, however, has been used numerous times and transported folded and handled extensively. As a result we encountered some difficulties due to damaged zips. Damage and missing teeth will render zips unusable and can cause loss of the fish or the AQUI-S™ solution from the enclosure if the zip gives way. Problems on the trial day were resolved by clamping the damaged zips in place however it is now suggested that alternate means of joining the side walls would need to be employed and that in all other areas additional security be incorporated into the fastenings.

Figure 16. Using a vessel-mounted crane to place the raiseable base inside the enclosure

Figure 17. The raiseable base in position within the enclosure and ready to be lowered. Note the coiled hose of the diffusion ring in the centre of the platform.

Figure 18. Shallow section of crowd net adjoining harvest enclosure. The open door is the lighter area in the enclosure wall (lower left).

Figure 19. Tuna in enclosure showing open entry door, The raiseable base (and associated ropes and hoses) is visible resting on the floor of the enclosure.

7.2.2.2 Harvest of tuna sedated using carbon dioxide

7.2.2.2.1 Anaesthesia, fasting and core temperature

'Sedation' was achieved after around 17-20 minutes exposure, with tuna exhibiting abnormal behaviour and either swimming to the surface and acquiescing to capture by divers or falling to the bottom if left unattended.

Analysis of variance reports that all factors; pontoon of origin, whether or not the fish were fasted, harvest method and chilling method, all significantly influence the core temperature of the fish. The role of the chilling method applied after the temperature was taken is difficult to comprehend unless there is an order of harvest effect in operation. While there appeared to be differences between the two pontoons when fed, these differences are less pronounced following the period corresponding to fasting.

Anaesthetised tuna had a lower core temperature when they reach the boat (Figure 20). The implication here is that sedated tuna (which do not swim actively during crowding and capture) do not exercise as much and generate less heat. The possible benefit of this in terms of post-harvest cooling should be considered in future research.

Counter to the pacifying effects of the anaesthesia, the apparently fasted tuna had a higher core temperature when harvested. It is difficult to tell from the data whether this is because fasted tuna are warmer to begin with or because they are more excitable during harvest operations. Curiously, the response is clearer in the pontoon fed with frozen pilchards supplemented with vitamins. The ANOVA indicated that there was a significant interaction between pontoon and fasting. Note that the tuna in the 'supplemented' west pontoon appeared to be quite cooler. Is this a sampling artefact, caused by a change in post-harvest handling? Core temperature likely depends greatly on the previous activity of the tuna and how long it has been out of the water when readings are made. The initial plan, because of concerns about sedated fish dying on the bottom of the enclosure was to capture and process the 'rested' fish rapidly, in bulk, but this soon became impractical on the boat used. After harvesting the supplement fed CO_2 treatment fish, the method had to be changed so that fish were handled and killed individually. This must be kept in mind when interpreting the pontoon*fasting interaction. The difference disappeared following fasting, when methods were presumably operating more smoothly.

7.2.2.2.2 Other measurements taken on landing

7.2.2.2.2.1 Blood pH

Interestingly, the anaesthetised tuna were more acidic on landing. This is of some concern. The idea is that rested fish generally have a higher flesh pH than fish that struggle during harvest. This normally happens because fish produce lactic acid as they struggle, lowering their muscle and blood pH. In this initial trial, we did not measure lactic acid levels in the tuna, but this point is picked up in section 7.2.1, where the propensity of tuna to acidify during harvest was examined.

The carbon dioxide anaesthesia may be directly responsible for the acidity found here in the blood/extra cellular fluid upon landing. Carbon dioxide lowers the pH of water, and this may compound the breathing difficulties encountered when a ram-ventilator like a tuna is disabled. The divergence in acidity was only transitory and we have no indication of what was actually happening in the muscles- remembering that tunas have evolved to handle acidity and their light muscle has a high buffering capacity.

7.2.2.2.2.2 Penetrometer readings (Rigor?)

The rigor “curves” were difficult to interpret along the same lines of the more classical curves obtained from fish such as salmonids. Repeated penetrometer readings were quite variable from one measurement to another. One reason for the variability might be that different parts of the carcass are going into rigor at the same rates. In particular, red muscle in tuna could complicate carcass stiffening because of the large amounts are present in the trunk compared with other fish varieties.

7.2.2.2.3 Necropsy samples

7.2.2.2.3.1 Flesh pH

The carbon dioxide treatment did not change the terminal pH. Once the dead tuna entered rigor, their flesh pH fell to similar ‘terminal’ pH levels (Figure 22).

Overall, the terminal pH for all treatments in this late Autumn trial was acceptable compared with previous experience with pilchard-fed tuna and literature values. Perhaps the initial acidity can be discounted as a symptom of the anaesthesia used, but the way to check that would be to knock tuna out using carbon dioxide and a more benign anaesthetic (eg. Aqui-S) and compare the outcomes.

There was no general improvement in terminal pH brought about by fasting the fish. A preliminary trial using a commercial pontoon in Month of 1995 indicated that withholding food for 4 days significantly raised the terminal pH of the tuna. The hypothesis proposed at that time, yet to be fully tested, is that fasting lowers the propensity of the flesh to acidify following slaughter- perhaps by reducing the amount of glycogen present before harvest. However, the results in this trial are less encouraging. The tuna in the pilchard fed pontoon responded as predicted with quite a marked amelioration in terminal pH after a week of fasting (Figure 24), but the tuna in the pontoon receiving the pilchard/supplement showed no change. Further work may explain the dynamics of fasting in this context. One possibility to consider is that tuna from the different pontoons differed in their initial glycogen “load” and this determined the scope of the response.

Figure 22. Flesh pH change during chilled storage for pilchard-fed SBT originally placed in an ice slurry immediately after capture.

Figure 23. Flesh pH change during chilled storage for pilchard-fed SBT originally chilled slowly after capture .

Figure 24. Flesh pH measured from bayonet samples at 3day post mortem taken from fed and fasted tuna harvest from each pontoon.

7.2.2.2.3.2 Colour meter

This was the first time we'd analysed large numbers of bayonet samples together, and the results are quite revealling, even if they raise more questions than they answer.

The chilled samples, stored under gas permeable film, changed appearance rapidly during the first 24 hours, according to the colour meter, in parallel with the fall in pH. The dramatic rise in L* or lightness in the first day of chilled storage is readily interpreted as a rapid onset of cloudiness or opaqueness in the sample (Figure 25, Figure 26).

The other CIE parameters a* and b* are also rising together in concert with this change in the light reflecting/ scattering properties of the sample (Figure 27, Figure 28). As both a* and b* are rising together, this is essentially a rise in the strength of the colour (i.e. the "chroma" increases), will little change in the hue, the balance of red and yellow. The colour meter is simply better able to resolve the colour as the sample becomes more opaque.

However, when L* value begins to stabilise after about 1 day post-mortem, the behaviour of a* and b* changes, with the samples starting to move toward the b* axis during post-harvest storage. This represents a rise in hue angle, and may represent what we classically think of as browning during discolouration of tuna meat.

The figures give the appearance that the CIE data is changing at different rates in fed and fasted fish. But the initial sampling times were different, and you'll recall, generally too early in pre-rigor in the first trial (fed fish) and this may mislead. It may be that the opacity change begins with the act of sampling the fish, rather than death per se. Still, this change in opacity is something to watch in future. In one respect it is a sampling artefact – but it may be that it also gives important information about the characteristics of the flesh.

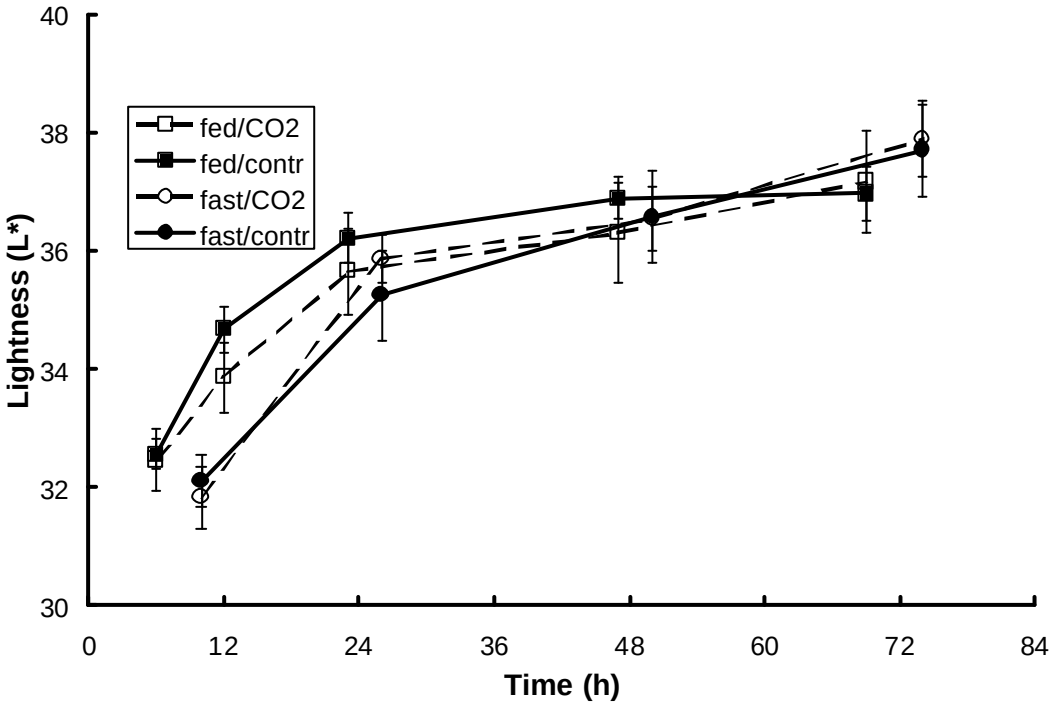


Figure 25. Colour meter results (CIE L*) for bayonet samples taken from ice-slurry chilled, fed and fasted, pilchard-fed SBT and chill stored for around 72h.

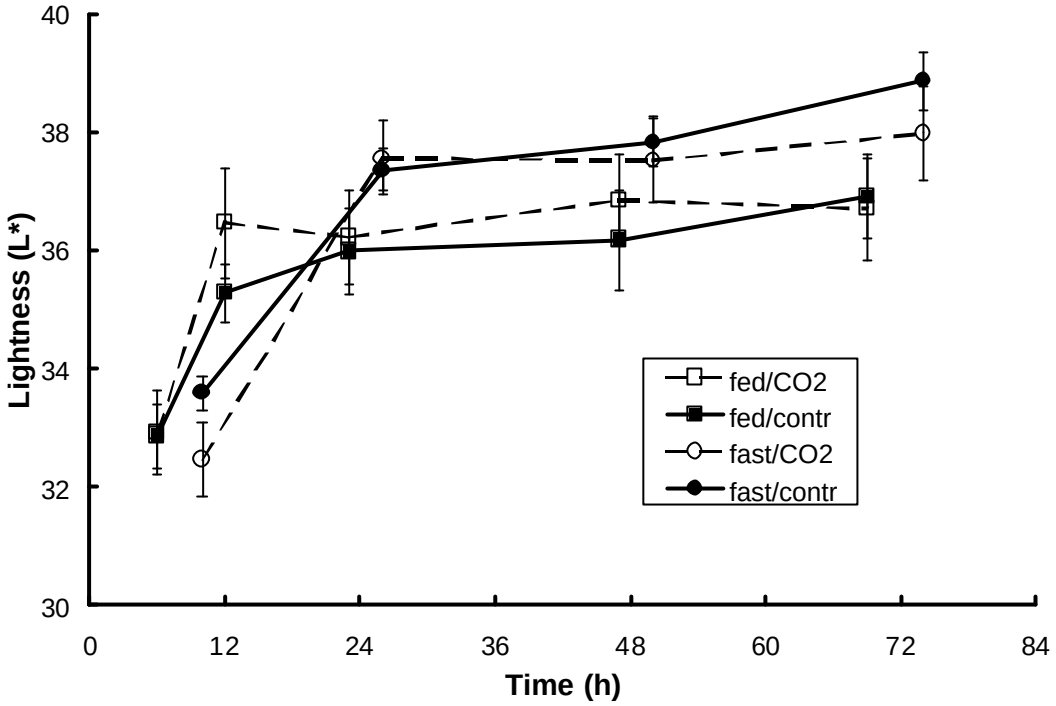


Figure 26. Colour meter results (CIE L*) for bayonet samples taken from SLOW chilled, fed and fasted, pilchard-fed SBT and chill stored for around 72h.

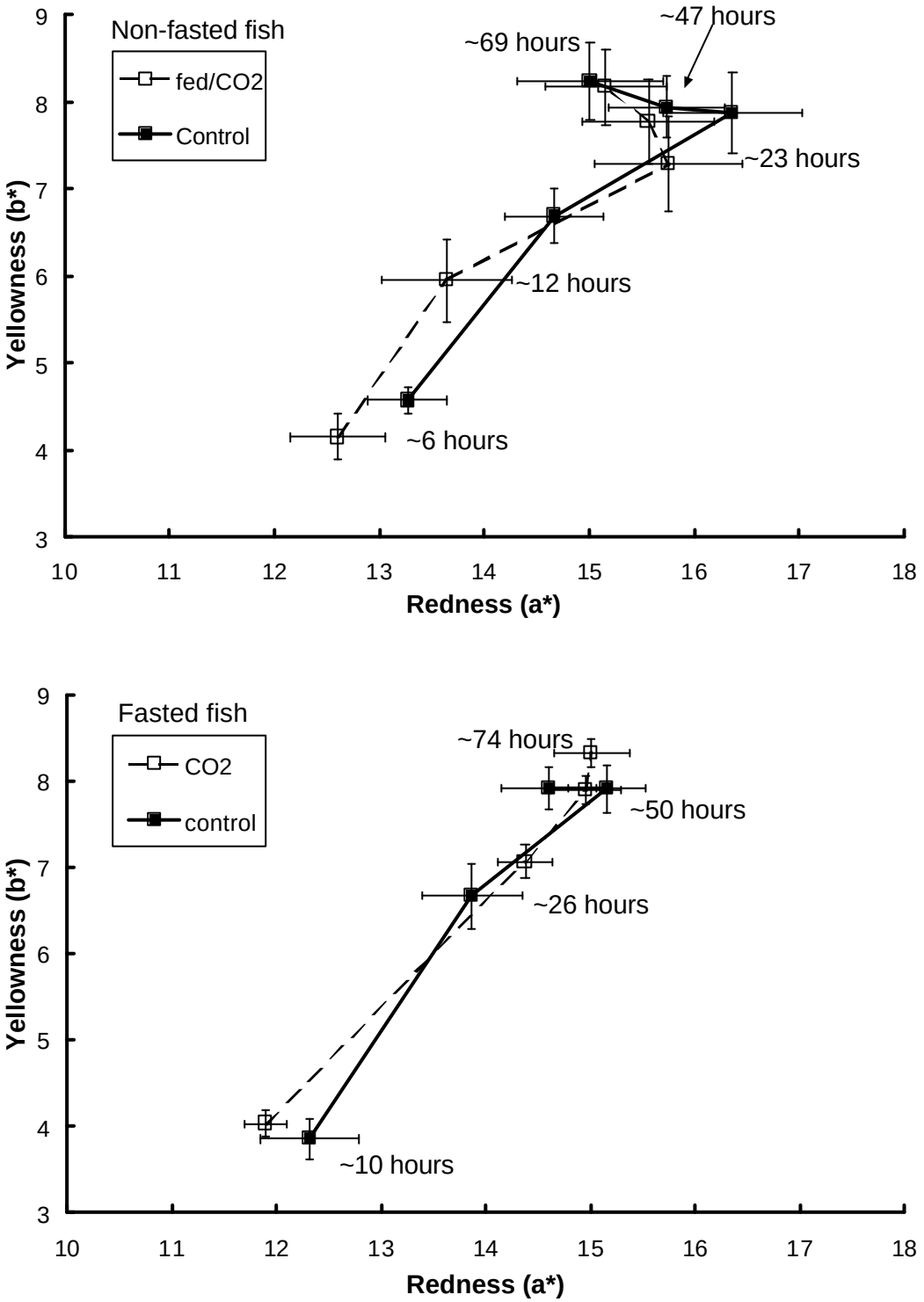
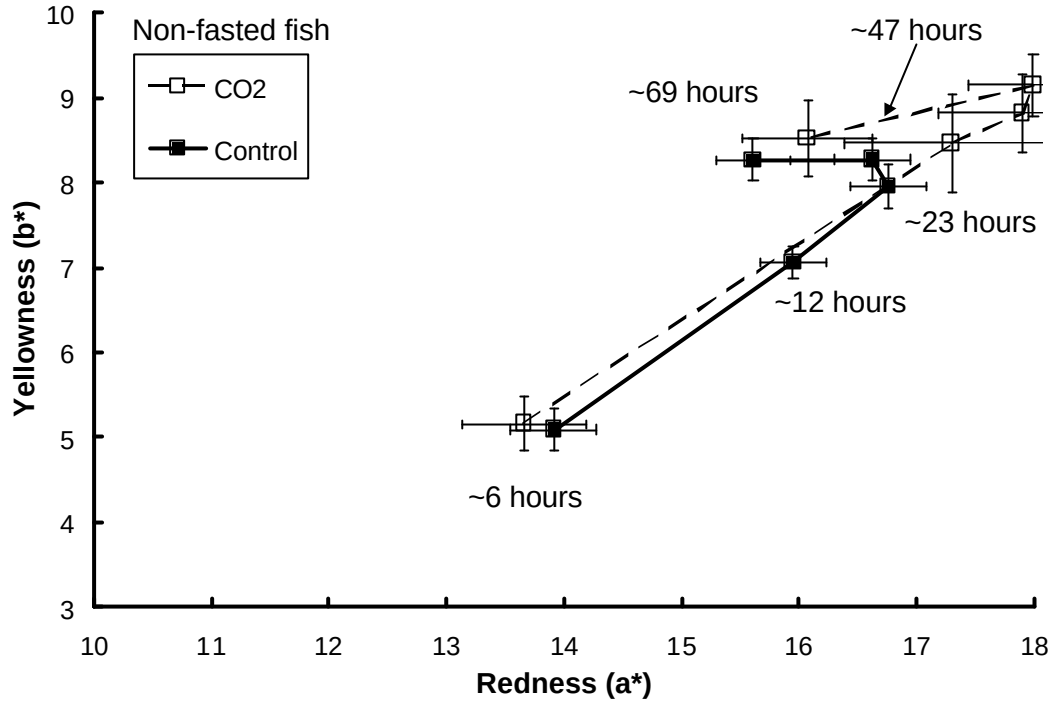


Figure 27. Colour meter results (CIE a* and b*) for bayonet samples taken from ice-slurry chille, fed and fasted, pilchard-fed SBT and chill stored for around 72h. The movement of the samples is plotted through one plane of CIE colour space.



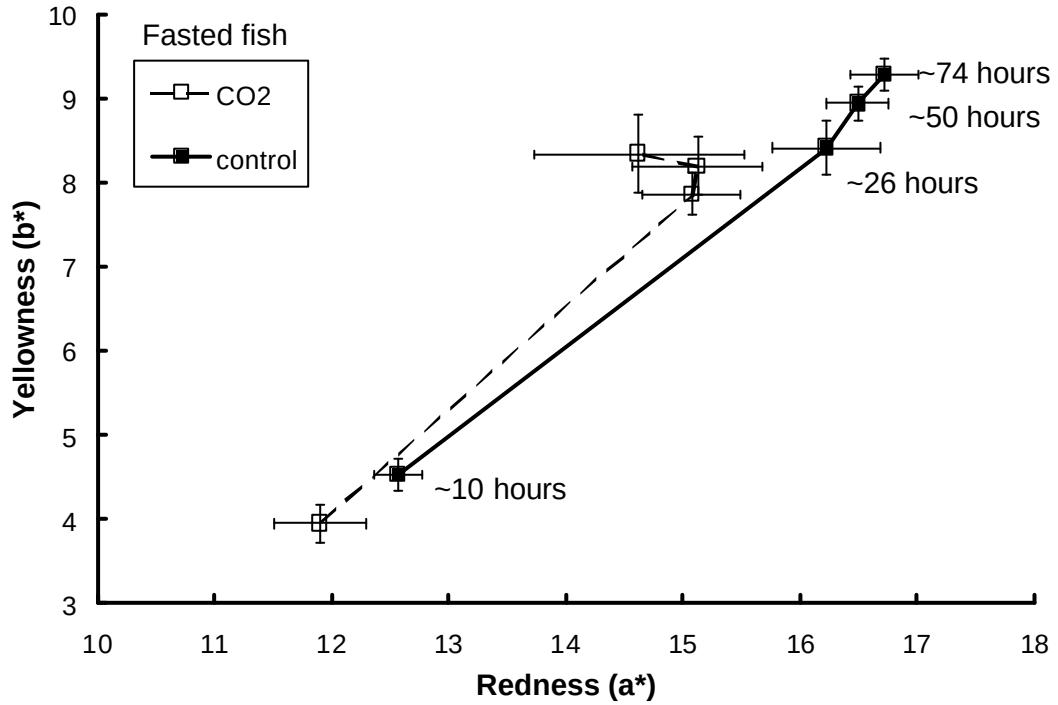


Figure 28. Colour meter results (CIE a* and b*) for bayonet samples taken from SLOW chilled, fed and fasted, pilchard-fed SBT and chill stored for around 72h. The movement of the samples is plotted through one plane of CIE colour space.

Beyond this characteristic change, the colour meter results showed no clear patterns when the day 3 post-mortem data is analysed. It was not possible to exploit the full replication of the experimental design because of problems encountered carrying out the plan at sea in the first. Improvements in the on-board handling of anaesthetised fish and a revision of the slow-chilling treatment was required after the first trial (the fed fish) and before conducting the harvest of fasted fish. Therefore, a straight-forward pontoon by pontoon analysis was carried out, comparing the fish from each pontoon to look for effects of harvest method and chilling method (Table 6).

Table 6. Summary of the ANOVA's for the fasting, CO2 harvest and chilling method trial. Where a significant effect was identified, the comparisons of means is represented by lowest\highest.

	Main effects		Interaction
Treatment	Harvest	Chilling	Harvest*chilling
Pilchard/fed	pH – n.s.	pH –n.s.	
	L* - n.s.	L* - n.s.	
	a* - n.s.	a* - n.s.	
	b* - n.s.	b* - n.s.	
	Hue - n.s.	Hue - n.s.	
	Chroma - n.s.	Chroma - n.s.	
Pilchard/fasted	pH - n.s.	pH - n.s.	
	L* - n.s.	L* - n.s.	
	a* - n.s.	a* - n.s.	a*
	b* - n.s.	b* slurry\slow	b*
	Hue - n.s.	Hue - n.s.	
	Chroma - n.s.	Chroma - n.s.	Chroma
Pilch.+Suppl./ fed	pH - n.s.	pH - n.s.	
	L* - n.s.	L* slurry\slow	
	a* control\rested	a* slow\slorry	
	b* - n.s.	b* - n.s.	
	Hue - n.s.	Hue slurry\slow	
	Chroma - n.s.	Chroma slow\slurry	
Pilch.+Suppl./ fasted	pH control\rested	pH slurry\slow	pH
	L* - n.s.	L* slow\slurry	
	a* - n.s.	a* slow\slurry	
	b* - n.s.	b* slow\slurry	
	Hue - n.s.	Hue - n.s.	
	Chroma - n.s.	Chroma slow\slurry	

The tuna taken from the pilchard-fed pontoon in trial 1 showed no significant difference in CIE Lab readings according to harvest method or chilling method. The same largely applied a week later when the fasted fish were sampled, though with one exception- the tuna chilled in an ice slurry had a significantly lower b* value than the slow chilled tuna. On the face of it, the lower b* implies less deterioration or browning of the colour when tuna were slurried. However the results from the supplemented pilchard fed pontoon contradicted this.

The tuna taken from the supplement-fed pontoon showed significant differences between chilling method for just about every parameter measured. In trial 1, the slurried tuna showed significantly lower L* and higher a* (and consequently, a lower hue and higher chroma). The second trial showed that the ice-slurried fish had the lower post-mortem pH, a higher L* now, higher a* and b* and consequently the higher chroma than the slow chilled fish.

It is hard to give a simple interpretation to these results, except to note that strictly a low L* usually represents a more transparent, and hence a fresher, meat sample. To some extent the values of a* and b* are probably not independent of changes in transparency (changes in L*) early in storage, so for the moment, the parameter to concentrate on is probably L*.

On balance, different slow chilling methods were used on the fed and fasted pontoons, so that might explain why the chilling effect was not consistent from trial to trial. However, it isn't clear why the pilchard-fed tuna showed no significant treatment effects, but the tuna from the other pontoon did.

Of course, just because the meter shows differences, we cannot take the step from there to establish what this means to the buyers. Furthermore, perhaps there were differences that the colour meter

could not detect because of compounding problems from sample integrity, physical factors (transparency, fat content) and variation between fish. Compared to the variation in the samples and the consistent changes in the bayonet samples themselves as they aged during storage, any putative treatment influences were apparently minor.

7.2.2.2.4 Conclusions

It is becoming clear that the colour meter is not the best tool that could be used on semi-transparent samples. It provides information about the gross differences or changes in appearance of the samples which are related to physical properties of the sample but it cannot answer a quite reasonable question in a way that the farmer's need answered. Did the flesh colour change? The best way to answer that is probably something like the colour chips: using colour to describe colour (section 7.1.3). The ultimate aim is to develop an understanding of the rate of change in the bayonet samples under a set of standard conditions in comparison with the whole fish, so we can anticipate the colour seen by the Japanese market from samples taken earlier in Port Lincoln.

At this stage, there is no real evidence that harvest of anaesthetised tuna has a significant impact on flesh quality. This may be because the tuna were stressed during harvest- or because we don't yet have the tools to detect the change. It would be surprising if excessive struggling by harvested tuna had no impact on flesh quality – but that is the extreme case. Perhaps the tuna were not sedated adequately. Alternatively, perhaps the 'control' harvest used here, along with the rapid handling and killing of individual fish, is already a significant improvement from many practices in the wild.

It is recommended that a trial using Aqui-S be conducted to endeavour to get truly 'rested' fish. This trial should also ideally be conducted on a commercial farm, so that realistic levels of activity can be produced with the control harvest. In contrast to the ambiguous result for harvest method, the chilling treatments did have a significant but not consistent effect on the flesh in a way that was apparent to the colour meter. However, neither of the slow chill treatments tested here are recommended for further practical trials. It isn't surprising that post-mortem chilling regimes would influence the appearance of the flesh, given the known role of temperature in biochemical deterioration in a carcass that is initially relatively warm.

7.2.2.3 Harvest of tuna sedated using Aqui-S

7.2.2.3.1 Effectiveness of AQUI-S™ for sedation

Once the AQUI-S™ solution was pumped into the enclosure and circulated quickly using oxygen via a 1 metre diameter air diffusion ring (Figure 17), the tuna started to loose orientation and sensibility after about 5 minutes and were immobilised after approximately 7 minutes. The concentration used was calculated on the basis of 18ppm AQUI-S™ to achieve a rapid "knock-out" and facilitate efficient harvesting, however lower concentrations may allow more time to apply the procedures necessary to raise the base platform and remove the fish on a commercial basis. In this case the platform was raised by crane (Figure 16) due to the rapid knock out and this resulted in an unstable working platform as the base was not held at a set height by the side nets. This is an important consideration in developing an enclosure based harvest system both to ensure stability of the raiseable platform and avoid forming pockets or folds in the side nets.

The initial dose of AQUI-S™ was used for a second time by simply opening the enclosure and net doors and crowding the fish into the enclosure. Once retained inside the enclosure the sedation was again rapid and effective. The same comments apply regarding the lack of time to carefully raise and lock the raiseable base in position. Additionally, divers who are familiar with the approach of holding fish by the isthmus caused fish to bleed into the enclosure during the first harvest, reducing visibility and adversely affecting the behaviour of the fish entering the enclosure causing most to immediately exit through the entry point and resulting in a need to crowd heavily in order to force fish into the enclosure. These are issues, which are resolvable through design, training and possibly using a lower AQUI-S™ concentration.

The recovery of sedated tuna could not be attempted due to the rapid sedation and inability to control the working environment under these circumstances. Some fish were caught in the side nets because the base was raised by crane and these fish could not be segregated.

7.2.2.3.2 Landed condition- Temperature

The landed core temperatures of 7 AQUI-S™ treated fish and 10 control fish are shown in Figure 29.

The data shows an average temperature of the control fish to be approximately 3°C higher than the fish harvested with AQUI-S™. This is despite the crowding effort applied to force the fish into the enclosure and must be viewed as beneficial.

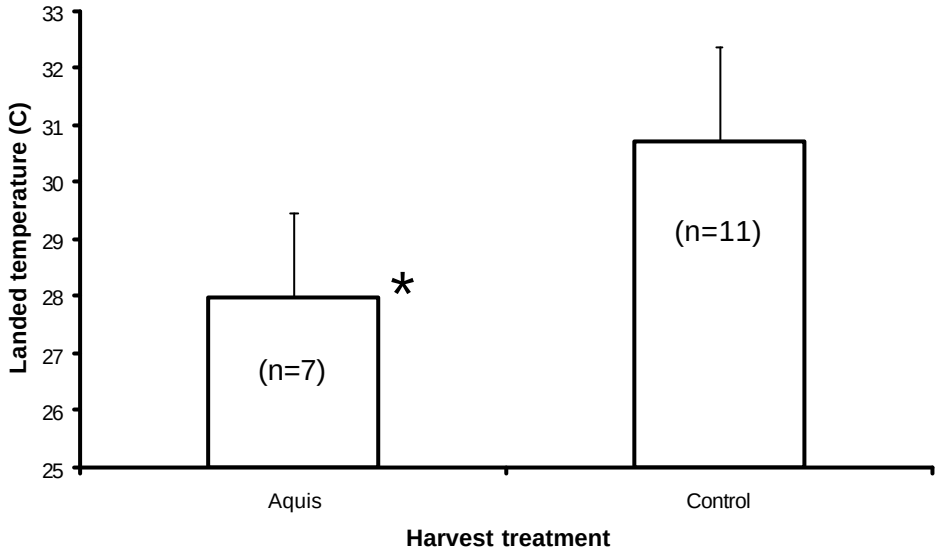


Figure 29. Landed temperature of tuna from the Aqui-S harvest trial

7.2.2.3.3 Flesh samples

Data for this trial is tabulated in Table 7 of this report. Bayonet samples taken at 4 and 19 hours represented the best opportunities to sample fish within the commercial time frame. Samples taken from the left side after approximately 4 hours were measured for surface pH and colour by visual comparison to the draft tuna colour reference system with the closest match noted. In order to aid interpretation of the colour results, these have been plotted (Figure 30) according to the chip series (The hues C1, C2, and C3) and the pigment level in the plastic (100, 80, 60…etc) where intermediate values were obtained using the half-thickness end of the chip. When first sampled, and even after "blooming" the samples to allow them to colour up in air, there was a surprisingly large percentage of the maroon colour C3 series- corresponding to un-oxygenated myoglobin. Samples are typically assigned to the red C2 series when the carcasses were re-sampled at 19h. Interestingly, in the interim, there has been an apparent increase in the strength or "saturation" of the colour. This continued when the bayonet samples were chill stored for longer, to the point that a large number of samples were rated as 100 on the existing scale. It could be argued that the existing colour set should be extended beyond the existing upper limit of 100, judging by the large spike of samples assigned to the category at the upper threshold which perhaps could have been assigned to chips with even higher pigment levels. In all, the variation in colour "saturation" between samples was considerable by this time.

Table 7. Raw colour and pH data obtained from bayonet samples of SBT harvested during the Aqui-S harvest trial.

Side of backbone	Left side samples taken 9:20 PM TUES13-6-00			Right side samples taken at 11:30AM wed 14-6-00			
measured at	9:20PM TUES13-6-00		12:20PM WED14-6-00	11:30AM wed 14-6-00		9:00AM Thu15-6-00	
Sample #	**pH**	**Colour chip**	**pH**	**pH**	**Colour**	**pH**	**Colour**
Aqui-S fish							
1	6.5	C3L20-30	6.11	6.26	C2L90-100A	6.05	C1L80
3	6.42	C3L40	6.11	5.9	C2L50A	5.9	C2L50-70B
4	6.54	C2/3L20	6.22	6.13	C3L60	5.95	C2L40-60
5	6.6	C3L40	6.1	6.12	C2L80A	5.93	C2L70A
6	6.73	C3L40	6.17	5.95	C2L100A	5.94	C2L100A
7	6.37	C3L30 - C3D20	6.17	5.93	C2L60-80B	5.83	C2L50-6-A
8	6.58	C3L60	6.07	5.97	C3L100A	5.81	C2L100A
9	6.39	C3L60	6.16	5.93	C2L90-100A	5.99	C2L100B
10	6.33	C3L30	6.03	5.92	C2L80-100A	5.95	C2L80B
11	6.28	C3L30	6.28	5.84	C1L60-80	5.88	C1L60
12	6.53	C3L60	6.14	6.09	C3L40	5.9	C2L50A
13	6.44	C2D	6.26	6.17	C2L90-100A	6.03	C2L100A
Control fish							
26	6.23	C3L40	6.2	6.01	C2L80A	5.96	C2L100B
27	6.54	C3L40	6.23	6.07	C2L80-100A	6.05	C2L100A
28	5.92	C2L100B	6.11	6.2	C2L80A	6.09	C2L100A
29	6.64	C3L40	6.25	5.87	C2L60-80A	5.85	C2L50A
30	6.1	C3L40	5.99	6.18	C2L60-80B	6.04	C2L40A
32	6.09	C2100B	6.21	5.94	C2L80-100B	5.88	C2L80-100B
33	6	C2L100-80B	6.16	6.09	C2L50A	5.95	C2L100A
34	6.02	C2L100	5.9	6.16	C2L100A	6	C2L70-80
36	5.89	C2L100-80A	5.99	5.84	C2L100B	5.93	C2L100A
37	6.03	C2L100B	6.04	5.82	C2L100B	5.83	C2L100A
A	6.3	C3L40	6.25	6.08	C3L60-80	5.97	C2L100B
B	6.16	C3L40-A	6.09	6.14	C2L60-80A	6.01	C2L50A

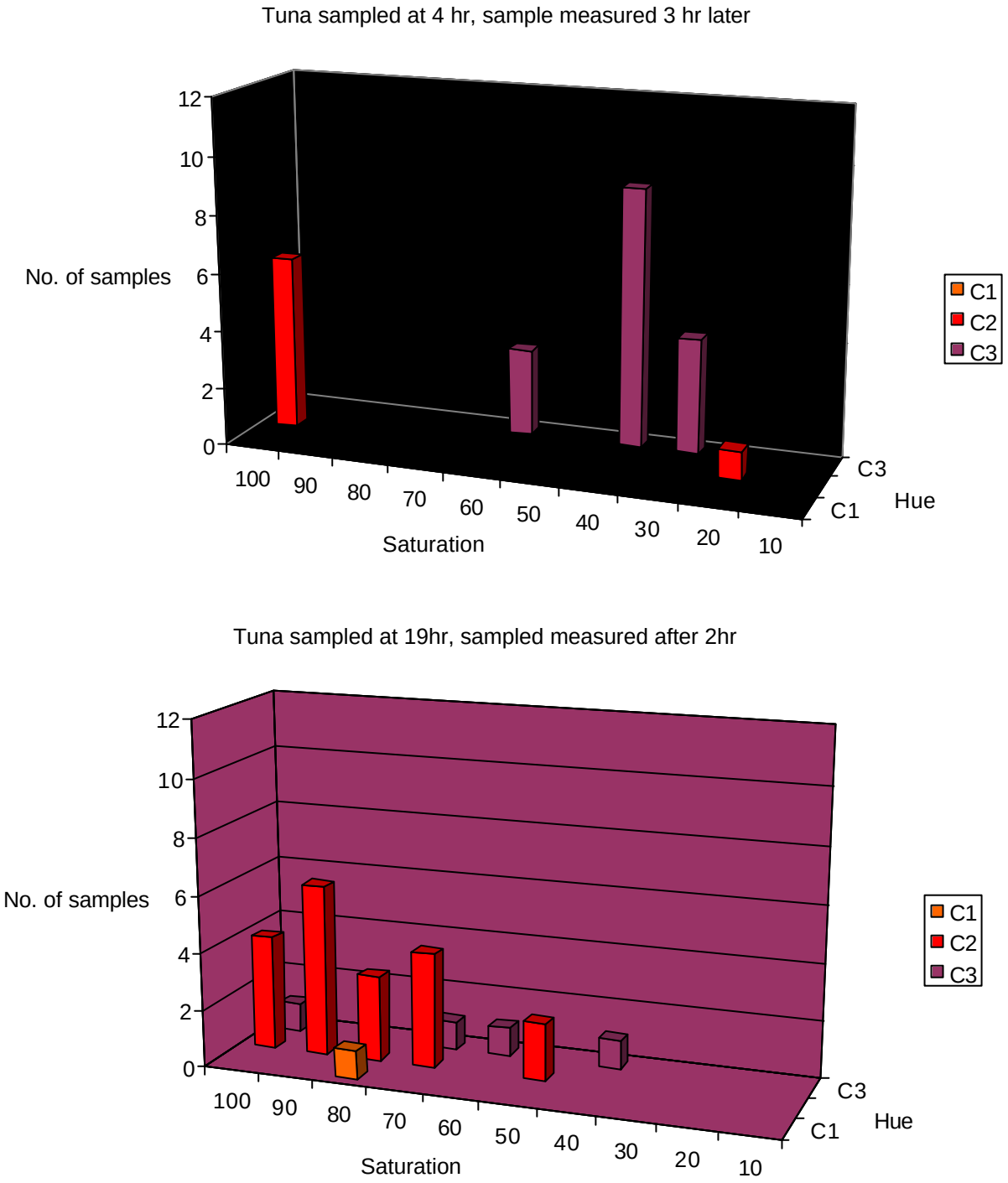


Figure 30. Frequency distribution of tuna samples sampled at 4 and 19 h post-mortem and assigned to particular hues (C1, C2 and C3) and different "saturation" or pigment loadings using the tuna flesh colour reference set.

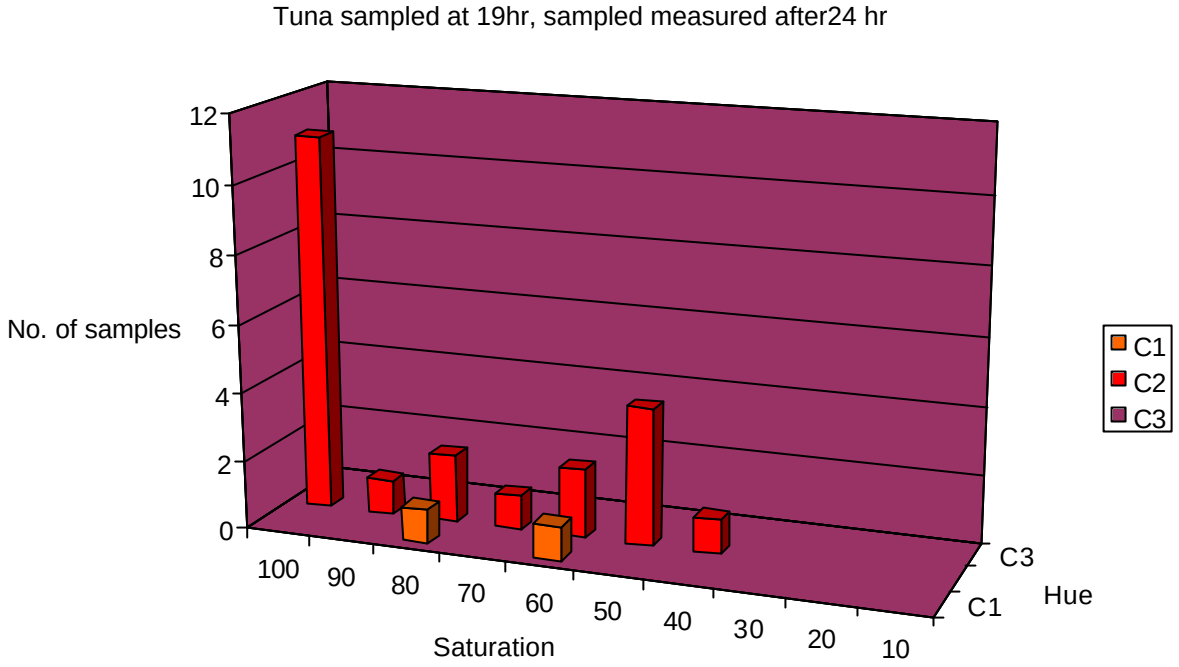


Figure 31. Frequency distribution of tuna samples sampled at 19 h post-mortem and chill stored for 24h before being assigned to particular hues (C1, C2 and C3) and different "saturation" or pigment loadings using the tuna flesh colour reference set.

The pH for left side samples at 4 and 19 hours post-mortem and right side samples taken at approximately 19 hours and re-measured at approx 36 hours are shown in Table 8. This data shows a significant difference at 4 hours for the averages but no significant differences at 19 hours or beyond. Whether the crowding pressure required to force the fish into the enclosure or the effect of some fish being caught in the loose net has affected this result is not known. It is however interesting to note the generally high flesh pH levels and good colour stability in the flesh of these fish over all.

Table 8. Average flesh pH of bayonet samples from tuna harvested using Aqui-S versus controls, from either side of the trunk, measured from 4 to 36 hours post mortem. Asterisk indicates averages that are significantly different at 5%.

	Flesh pH			
Treatment	**Left side, measured at**		**Right side, measured at**	
	4hrs	19hrs	19hrs	36hrs
CONTROL	6.16	6.12	6.03	5.96
AQUI-S	6.48*	6.15	6.02	5.93

7.2.2.3.4 Effect of AQUI-S and chilling rate on the rigour process

Three control fish and 2 AQUI-S harvested fish were subjected to the experimental treatments (Figure 32). While the data is not significantly different due to the individual fish variation the trends shown are interesting. The data shows that the dark muscle contracts more quickly and perhaps explains some of our earlier work which indicated complex rigour curves when measured by changes in the external compressive resistance of specific positions on the surface of the carcass.
The data also indicates that light muscle of fish treated with Aqui-S did not contract while it did appear to become firm during the post-mortem storage period. We also noted that bayonet samples were difficult to obtain from the Aqui-S fish at approx 22 hours compared to the normally harvested fish and this usually suggests that rigour has not fully developed.

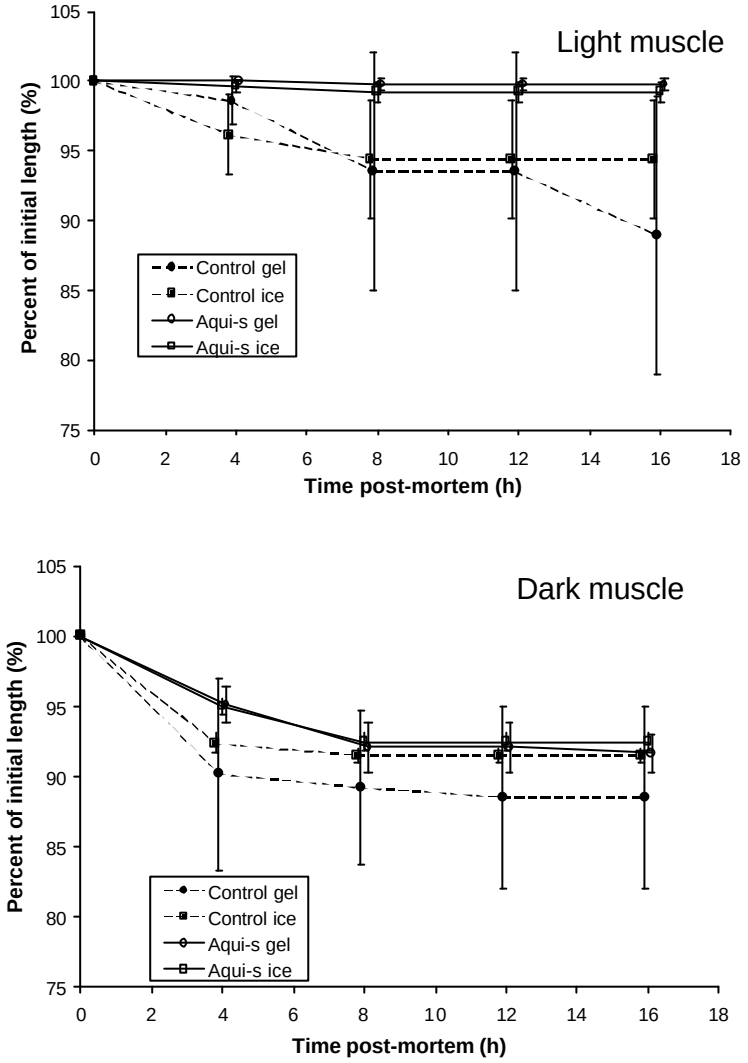
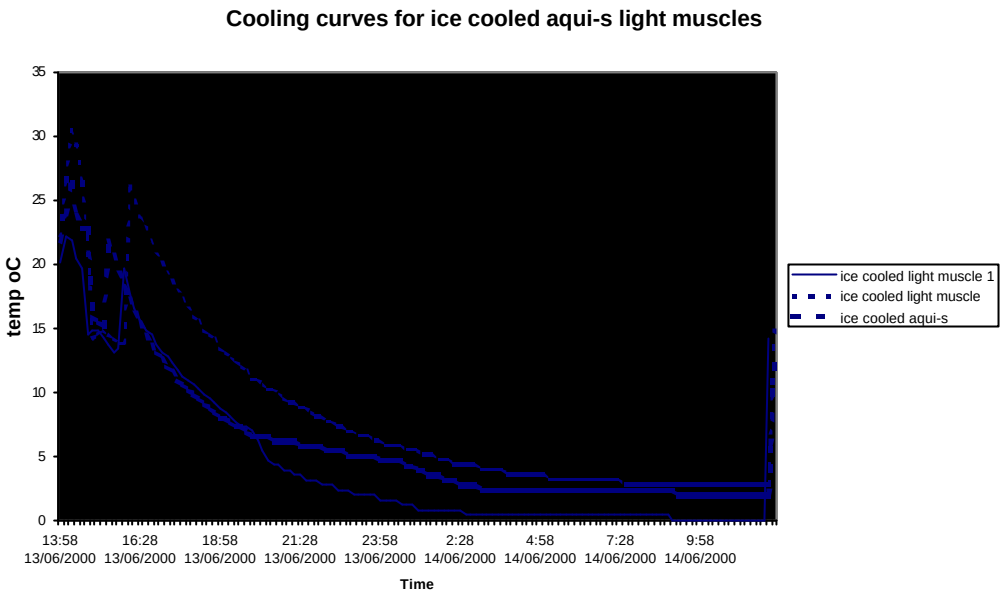


Figure 32. Shrinkage of light and dark muscle from tuna harvested in different ways and subjected to different chilling treatments.

7.2.2.3.5 Temperature data

Some samples in the slowly cooled muscles (gel (ice-pack) cooled) of the normal harvest continued shrinkage up to 15 hours. The cooling graphs show the chilling rates logged for various samples (Figure 33). Substantial variation is noted in both the commercial and experimental cooling operations due to the size of the fish and loins and the practices used. (The slurry was not agitated on this occasion and not all parts of the stored loin were in contact with the ice bags.) The dark muscle which shrank more quickly were from normally harvested fish, especially the slow cooled. This is however based on only a few fish, but may be worth investigating further. The result suggests that further investigation and refinement of methods of rigor measurement could provide a useful commercial tool for quality monitoring, especially in relation to harvesting.



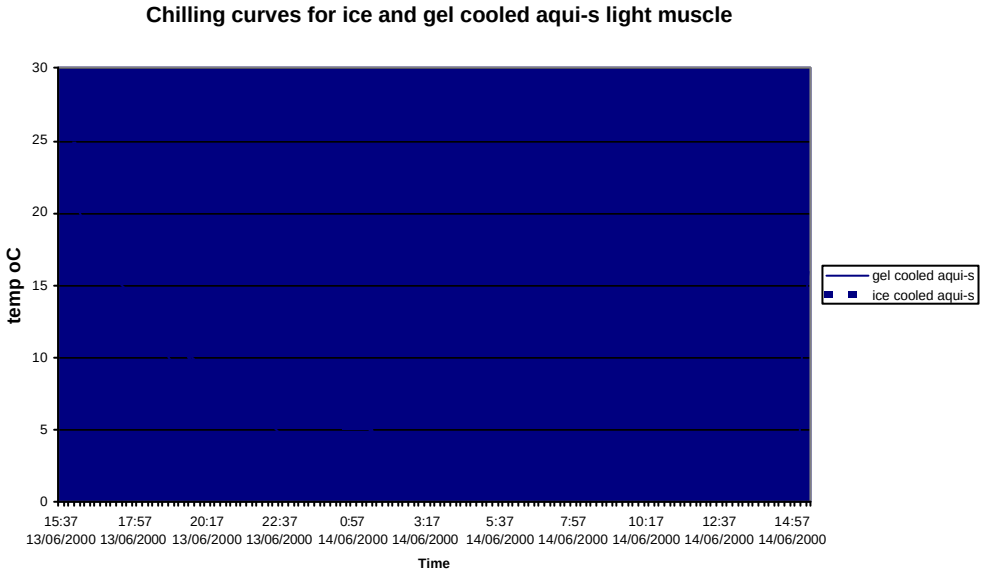


Figure 33. Cooling curves for tuna muscle samples used in the rigor experiment

7.3. Objective 4: Develop techniques to optimise desireable colour and flesh characteristics of SBT

No clear recommendations emerged from the work conducted for objective 3 for alternative methods of pre-harvest, harvest and post-harvest handling of SBT that optimise the flesh colour and other characteristics of the tuna.

Probably the most important quality related outcome that can be identified is the effect of activity in raising the body temperature of the tuna, and conversely, the ability of sedation to begin the carcass chilling process while the tuna is still alive.

7.4. Communicate techniques to industry and monitor impact on the product quality of SBT

Findings of the project were periodically discussed at workshops attended by industry. An industry workshop was planned to demonstrate the colour reference set and the inconspicuous sampling device to begin an industry evaluation process. At this workshop, participants would receive colours sets, light sources and sampling tools for trailing and use. The manual for this workshop is included as Appendix 3. These tools provide a capability of monitoring the impact of further research in this area, as well as trials undertaken by individual companies.

8. BENEFIT

The farmed SBT industry based at Port Lincoln in South Australia will benefit from this research through the availability of the inconspicuous sampling device and flesh colour reference set (Objective 1) and the findings and lessons learned from the pre-harvest, harvest and post-harvest experiments.

The inconspicuous sampling methods developed in this project will continue to be applied to research undertaken at Port Lincoln. This is allow the maximum information to be gained from tuna prior to them leaving Port Lincoln, yet without harming the market data also collected from these tuna.

The colour reference set will allow objective information to be collected about the flesh colour of farmed tuna so that farm management can use this information gathered within and between seasons in the formulation of research and marketing plans.

Tuna can be corralled in bulk and anaesthesia applied using carbon dioxide or Aqui-S (Objective 3) but as yet sedated tuna have not been resuscitated. Alternative harvest methods had no discernable effect on flesh colour. The rested harvest work was carried out using salmon. Unlike salmonids, tuna tend to be harvested and slaughtered individually rather than en masse. Perhaps this means that the current handling practices are already a step up from bulk crowding and scooping of salmonids with consequently less scope for improvement.

This is not to encourage a blasé attitude to harvesting and handling of SBT. This study identifies at the very least symptoms of exercise and stress developing during harvesting operations which have the potential to impact on flesh quality, involving as they do both exhaustion of muscle energy reserves and accumulation of lactic acid. Still, if any effect exists, it is not causing a dramatic change in flesh quality when samples are taken at Port Lincoln within a day of slaughter.

9. FURTHER DEVELOPMENT

It had been hoped to recommend husbandry practices from these experiments but this is not possible at this stage. Because of the practical difficulties with herding the tuna into the enclosure, future work should examine strategies for harvest of sedated tuna under commercial conditions, using a protocol encompassing the entire crowding and harvest process and a simplified enclosure. Notwithstanding the difficulty in demonstrating a benefit of these methods on flesh quality, an easily quantifiable objective here would simply be to limit rise in body temperature in the harvested tuna.

At the same time, more attention needs to be given to possible benefits of alternative harvest practices due to intervening factors that may have complicated these trials. One problem here is of course the practical measurement of colour. Development of the colour reference set was not completed in time to use it in anything but a preliminary basis for assessment of tuna from alternative harvest treatments. Instrumental methods of colour measurement are not well suited to use with transparent samples like tuna meat. They respond to the physical characteristics of the sample and allow some comparative conclusions to be drawn, for example the change in sample properties during storage however these results are difficult to interpret in terms of "colour."

Another problem is the apparent variability in both the flesh colour and colour stability in chilled storage observed in tuna within the same treatment. This variation may be explained by variation in pigment content and rate of oxidation of the pigments, and these aspects may need to be considered more carefully in future research.

This ambivalent finding regarding harvest method is of course based on samples taken from tuna within a day of slaughter at Port Lincoln. Failure to detect any effect of the treatments on flesh colour within only a day of slaughter does not mean that changes cannot develop after this time. However, to assess the impact of harvest-related treatments on the long term colour outcome for individual tuna will require sampling of tuna several days after slaughter. If this is not to involve keeping experimental fish aside in Port Lincoln, this will have to involve sampling carcasses in Japan.

This study confirms an earlier finding that fasting SBT under some circumstance prior to slaughter can reduce the fall in post-mortem flesh pH. Elevated levels of muscle glycogen in farmed tuna - how exercise during harvest influences this needs to be considered.

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Appendix 1. Intellectual property.

Following a period of industry evaluation, commercialisation of the colour chips developed by the project will be undertaken by QDPI in conjunction with FRDC and ATBOA. At this stage it is not anticipated that any form of IP protection will be sought for the colour chips and associated tools, and their manufacture and sale can be arranged with the companies concerned.

Appendix 2. Staff.

The following staff participated in this project

Mr Bruce Goodrick, Principal Seafood Technologist,
Dr Philip Thomas, Post-Doctoral Fellow, Flinders University of South Australia
Mr Paul Exley, Seafood Technician, Centre for Food Technology
Mr Darren Leighton, Seafood Technician, Centre for Food Technology
Dr Brian Paterson, Senior Seafood Physiologist, Centre for Food Technology

Appendix 3. Other Material

The colour workshop manual is attached.