Final Report

Effect of pre-harvest fasting and modifications to post-harvest handling on the quality of farmed southern bluefin tuna

by

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TABLE OF CONTENTS

NON-TECHNICAL SUMMARY

Tuna are red meat fish and, on the Japanese market, the red colour of the flesh is one of the factors which dictates the worth of the fish. The pigment responsible for the attractive red colour is oxymyoglobin. However, this pigment is readily oxidised during storage of the fish and forms a brown pigment metmyoglobin. The loss of red colour of the flesh reduces the value of the fish and can be followed by measuring the flesh colour with a colour analyser.

Another cause of colour change in tuna flesh is increased lactic acid levels. Lactic acid is produced from glycogen in tuna flesh when fish struggle during harvesting and when the fish enter rigor mortis. Upon completion of these processes, the flesh acidity stabilises at a 'terminal' level. The elevated lactic acid levels and the consequent fall in pH causes 'burning' of the tuna flesh, regarded as a strongly negative attribute on export markets.

This project work looked at the effect of pre-harvest treatments and post-harvest handling methods on colour quality of tuna flesh. Flesh pH and colour were measured during chilled storage of tuna which had been fasted for four days prior to harvest and compared to results obtained from fed tuna, harvested commercially. The effects of different immediate post-harvest handling methods (bleeding fish in ambient seawater and the standard use of ice slurry) were looked at for each pre-harvest treatment. The pH was recorded at 1 and 4 days post-mortem and colour of the flesh was monitored up to 11 days after slaughter.

Pre-harvest fasting, raises the pH of the flesh measured at 4 days and improves the flesh colour. However the rate of acidification, revealed by the pH measurement at 1 day post-mortem, was apparently influenced by the post-harvest bleed treatment. The differences in acidification may be related to other changes in the carcass which result in a subtle differences in flesh colour.

The factors that had most effect on the flesh colour of the tuna were the period of storage and the position in the tuna steak where the measurements were made. Redness varied throughout the steak , whereas unsurprisingly 'lightness' and 'yellowness' were recorded as highest in the area of the fat band.

A statistically significant, though slight, improvement in flesh colour was measured on *akami* (inner part of the carcass) of fasted tuna that were first bled in ambient seawater prior to slurrying on shore. The effect was subtle because the flesh colour deteriorates considerably during the 11 days of chilled storage. Further work is required to confirm the general efficacy and impact of fasting. This pre-conditioning step may help to influence and standardise flesh quality.

Results obtained from the research indicate that pre-harvest treatment, harvesting practices and post-harvest procedures used do affect the colour of tuna flesh and hence influence market quality.

Preliminary trials with a sedative applied during fish harvesting indicated that the reduced struggling of the fish resulted in less intensive rigor and a

reduction in the rate of pH change. These factors produced tuna with a higher quality flesh.

Degradative processes continue to occur throughout post-mortem storage, even with fish stored chilled, hence controlling the temperature of the fish during transport remains of prime importance for product to reach market in optimum quality. Further more, detailed investigation into the most appropriate accompanying harvest and post-harvest practices still needs to occur.

BACKGROUND

In 1994, more than 1500 tonnes of farmed Southern Bluefin tuna were exported to Japan. They achieved an average market value of Y2500/kg which equated to a total of AUD$50 million. With the current expansion of the industry, it has been estimated that tuna farming is likely to become Australia's largest seafood export earner in the near future.

Many of the handling principles and practices in this recently developed industry are based on methods used within the traditional wild-caught fishery. These practices include rapid processing and chilling immediately the fish are caught. Different from the wild fishery, farmed tuna can be caught and processed under controlled conditions however there is an absence of practices adapted specifically for this industry.

In most aquaculture operations a period of fasting prior to harvest is used to improve the quality of the product. It is suggested that such fasting may result in improved colour and flavour of fish flesh through depleted muscle glycogen reserves. Reduced muscle glycogen could lead to reduced lactic acid present and therefore higher muscle pH. This prevents colour deterioration which occurs under more acidic conditions. Excessive lactic acid formation results in poor flesh colour in tuna and when levels are severe "burning" (yake niku) of the flesh occurs rendering the flesh unsuitable for consumption and of little value.

Modifications to current post-harvest practices, as to be investigated in this proposal, may lead to advanced procedures being developed specifically for farmed tuna. The adoption of such practices will result in higher and controlled quality of farmed tuna providing greater return for the industry.

NEED

The Southern Bluefin tuna farming industry uses procedures for harvesting and post-harvest handling based on methods developed in the wild capture fishery. The only viable marketing option for these fish is to sell the product in the Japanese market. At various times poor quality of the fish, in particular the flesh colour, has been noted in the Japanese market and this has been directly reflected in very poor prices for these fish.

A study of the effects of alternative handling procedures on tuna flesh quality is needed. This will determine whether more appropriate protocols for pre-harvest preparation, harvesting methods and post-harvest procedures should be applied specifically for farmed tuna.

OBJECTIVES

1. To make preliminary assessment of pre-harvest fasting as a husbandry technique to improve tuna quality.

2. To examine the effect of modifications to post-harvest handling practices on tuna quality.

3. To examine the impact of seasonal variation (winter versus summer harvest) on tuna quality.

   This objective was not completed. Trials were conducted in August 1995 (winter period) but the planned summer months trials could not be carried out as no fish were available after the devastating mortalities which occurred in 1996. It was approved that the research be continued within a major subsequent project.

Objective 2 was extended in an approved variation (Variation to Project Agreement, dated 16 February 1996). The variation consisted of the objective:

4. To assess the merits of rested harvest of tuna through the use of AquiS

METHODS

Experimentation was split into two stages, one set of trials to occur in winter months with cool ambient seawater conditions and the other planned for warmer seawater conditions.

Fish used in trials were from identical cages and were previously fed and treated in a similar manner prior to conducting the experiments. Fish were subjected to pre-harvest treatments in combination with post-harvest treatments and flesh samples analysed for changes and differences in pH and colour.

For full details of methodology, refer to attached reports (Appendix 1 and Appendix 2).

RESULTS and DISCUSSION

These are presented in full within the reports attached as Appendix 1 and Appendix 2.

BENEFITS

The research work has provided base level knowledge for managing the quality of farmed tuna. Industry involvement in carrying out the project allowed immediate transfer of knowledge and information of results to the tuna farming industry. We are advised that much interest exists in the harvesting procedures and that some tuna farmers have adopted the alternative pre- and post-harvest techniques in their own trials.

The immediate and direct beneficiary of this knowledge is the Southern Bluefin tuna farming industry. Revenue increases of Y500-1500/kg are considered realistic if the colour of tuna flesh is improved.

However, while the tuna farmers in South Australia are the primary beneficiaries, all Australian tuna fisheries benefit from the understanding of factors affecting the myoglobin of tuna flesh. Hence, whether the fish are farmed or wild caught, modifications to handling practices based on knowledge gained will provide better quality management options. Additionally, the information gained during this work has wider implications for other aquacultured species.

FURTHER DEVELOPMENT

The results of this work show that fasting in combination with different post-harvest handling techniques does improve tuna flesh colour and hence, market quality. However after the promising initial results, work was halted by the tuna kill occurring in early 1996.

Given this circumstance, approval was sought from FRDC and the NSC to incorporate the further trials into a much larger project developed subsequent to this work. This research project is currently being undertaken in collaboration with Aquaculture CRC Ltd, South Australian Research and Development Institute (SARDI), Tuna Boat Owners Association of Australia (TBOAA) and Southern Bluefin Tuna Research Farm and is financially supported through the FRDC.

CONCLUSION

Results obtained from this research, although limited, give a clear indication that pre-harvest treatment, harvesting practices and post-harvest procedures used do affect the colour of tuna flesh and hence, market quality.

Preliminary assessment of pre-harvest fasting (Objective 1), showed that fasting fish for as little as four days prior to harvest raises the post mortem pH of the flesh and improves the flesh colour. However, it should be noted that the fasting effect of improved colour occurs for only a limited time, as the flesh pigment breaks down with post mortem storage of the fish.

Studies on the effect of post-harvest handling methods (Objective 2 and then the variation Objective 4), demonstrated that post mortem handling of the fish does also influence flesh colour. Degradative processes continue to occur throughout post mortem storage, even with fish stored chilled. The flesh colour and pH analysis results indicated that it was not the terminal pH level that was so important as the combination of final pH and the rate at which that pH was reached. The rate of pH fall is affected by fasting, harvest stress and temperature.

Controlling the temperature of the fish during transport remains of prime importance for product to reach market in optimum quality. Further more detailed investigation into the most appropriate accompanying harvest and post-harvest practices still needs to occur.

ACKNOWLEDGMENTS

Acknowledgments are noted on each report.

Appendix 1

Report on

The effects of harvest treatments on tuna flesh colour

relating to Objectives 1 and 2
of

Project No. 92/125.23

Report prepared for the NSC
as part of
Project No. 92/125.23

The Effects of Pre-Harvest Fasting and Modified Post-Harvest Handling on the Flesh Colour of Farmed Southern Bluefin Tuna.

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Centre for Food Technology
Department of Primary Industries
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INTRODUCTION

Tuna are a red meat fish. The pigment in tuna responsible for the attractive red colour of the sashimi is oxymyoglobin, the same pigment responsible for the colour of beef (MacDougall 1982). Unlike the situation in cultured salmonids, where the relatively stable pigment astaxanthin is present in the flesh, the red pigment of tuna is readily oxidised during chilled storage to form the brown pigment metmyoglobin (Chow *et al.*1988). This colour degradation can be followed conveniently, as in this study, by measuring the colour of the tuna flesh using a colour analyser (Ochiai *et al.* 1988).

Tristimulus colour meters are devices suited to process and quality control applications and are designed to measure light in a way equivalent to how the human eye perceives light. Using these devices, colour can be measured numerically by creating scales for lightness, hue and chromacity of the colour. A "colour space" is a term used for these dimensions or axes used to express the colour of an object or a light source. As the meters are designed for use on solid surfaces, results from semi-opaque or transparent
materials such as meat must be interpreted carefully to consider the effects of light trapping and scatter within the sample, (Little 1964).

The Commission Internationale de l'Eclairage (CIE) has developed a number of methods for expressing colour numerically. In 1976 CIE devised the L*a*b* colour space to provide more uniform colour differences in relation to discernable colour differences.
L*a*b* colour space is a commonly used method of measuring colour in many applications. L* represents lightness and is a measure of the brightness of the object based on total reflected light. The lightness of the object is independent of the perceived 'colour.' The values a* and b* are the chromaticity coordinates. a* and b* measure colour directions: +a* is in the red direction, -a* is in the green direction, +b* is in the yellow direction and -b* is in the blue direction.

The values of a* and b* can also be expressed as 'circular' statistics by calculating hue angle and chromacity. Hue angle (h) describes, in our case, the relative red/yellowness of the sample, the balance of wavelengths reflected off the sample. Chromacity (C*), the distance of the point from the origin of the graph, is the measure of how vivid or intense that hue is, and is independent of the hue.

Research is currently under way to develop manufactured feeds for southern bluefin tuna. In the first feeding trial, in 1994, the tuna took a number of weeks to wean onto the pelleted feeds and lost a considerable amount of condition. Yet despite this loss of fat content (or perhaps because of it), the post mortem pH of the tuna was elevated from expected levels and the flesh redness of these tuna was remarkably stable during post mortem storage. . How starved does a tuna have to be to 'improve' the redness of the flesh but without compromising the

animal's fat content? Fasting is actually a common husbandry practice in numerous aquaculture industries but has not been evaluated for tuna.

The post-harvest handling practices currently applied to farmed tuna have largely been carried across relatively unchanged from procedures used in the wild capture fishery, where rapid handling and chilling are used to try to prevent the occurrence of tuna burn (Jerrett 1984). Because farmed tuna can be harvested and handled under different circumstances to those applying at sea, this project seeks to assess the existing practices in order to evaluate alternative methods for handling captive tuna.

In this report we compare the flesh pH and colour during chilled storage in tuna that were fasted briefly before harvest with those treated normally as well as examining the possible effects of alternative post-harvest handling techniques. Further experimentation in this project was stopped by the fish kill in 1996 and this research is continued within a much larger project supported by FRDC.

MATERIALS AND METHODS

This experiment was the first of two proposed trials aimed at evaluating the potential benefits of fasting farmed tuna prior to harvest. The trial (part 1) was conducted on 24th August 1995 during cool ambient seawater conditions of 12.7°C. The project was divided into 2 parts because it was anticipated that during the cooler months (July to September) there may be little or no notable effect on quality of changes in the feeding or handling practices. The colour of the tuna meat at this time of year is normally better than later in the year. Unfortunately, the devastating fish kill in Boston Bay in 1996 prevented the repeat of the experiment during warmer water conditions. This study was then incorporated into a larger research proposal to FRDC (Project number 97/364).

Fish from 2 identical cages were used in this experiment. Both groups had been previously fed and otherwise treated in a similar manner. In the experiment, 4 groups of 9 fish were used (18 fed and 18 fasted). The fasting treatment involved fasting tuna for 4 days prior to capture.

Fish from both the fed and fasted treatments were subject to 2 methods of on-board handling. One treatment involved the use of standard chilling practices using an ice slurry into which fish were placed after killing and coring. The other group were allowed to bleed in seawater at an ambient temperature of approximately 13°C for 2 to 3 hours prior to chilling in the ice slurry.

Fish core temperatures were measured at harvest. Flesh pH was measured using a stab electrode, after 24 hours and again after 4 days using a surface electrode. Flesh colour was measured using a Hunter Labscan Colorimeter at 4, 8 and 11 days measuring CIE L* (lightness) a* (redness) and b* (yellowness). When reading colour, the freshly cut steaks were left on ice for about 30 min in a cold room to allow colour development. Colour measurements were then made at 7 points in each steak (Figure 1) using the Hunter meter.

Changes in colour during storage of flesh samples were analysed by analysis of variance using Genstat 5 Release 3.2 with three sub-plots to account for repeated measures.

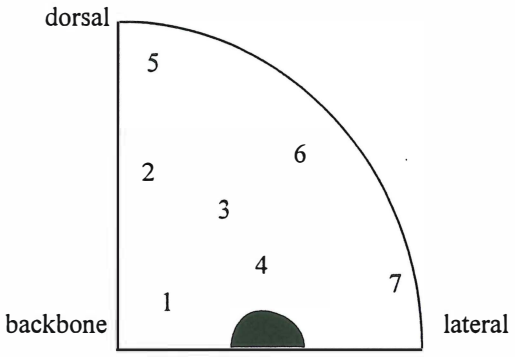

Figure 1. Steak of dorsal loin showing locations of sampling points. Dark meat indicated approximately by black shading. Akami positions 1-4 and toro positions 5-7.

RESULTS

Core Temperature

Tuna coming from the fasted cage tended to have a higher body temperature (mean 26.0°C) than those from the fed cage (mean 24.9°C).

Terminal pH

Flesh pH measured one day post-mortem was significantly effected by fasting and by post harvest bleeding treatment. Fasted tuna had lower pH (mean pH 6.35) than fed tuna (mean pH 6.49). Tuna allowed to bleed in ambient seawater following killing had significantly higher pH one day post mortem (mean pH 6.51) compared to slurried tuna (mean pH 6.33).

The terminal pH (measured 4 days post-mortem) was significantly higher in fasted tuna (mean pH 5.75) than in fed tuna (mean pH 5.67) (Figure 2). This was the reverse of the pattern seen one day post mortem.

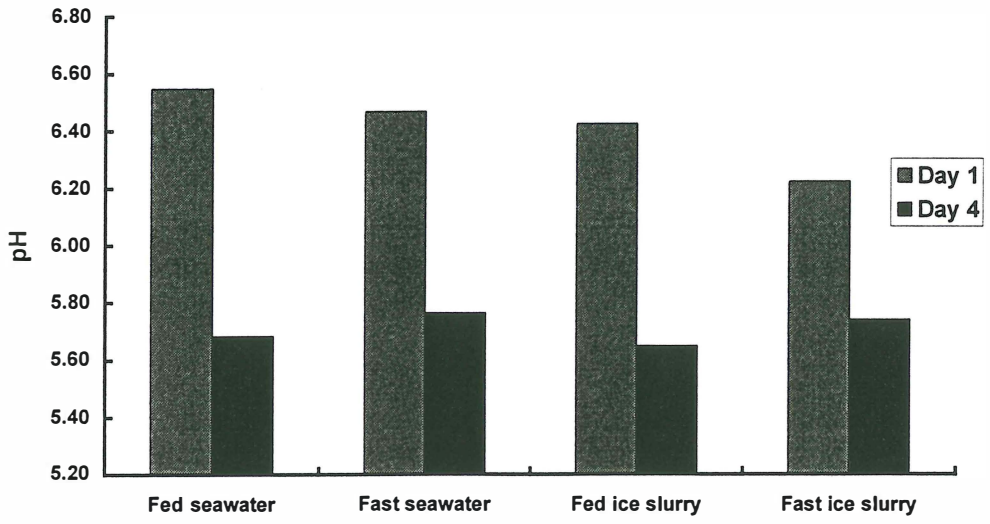

Figure 2. Differences in initial and 'terminal' pH in meat from tuna harvested in each treatment

Lightness

CIE lightness (L*) is an expression of the total amount of light reflected back at the sensor from the sample. The position in the steak and period of chilled storage significantly effected average lightness of the steaks sampled (Probability(P) of the samples coming from populations with the same average <0.001). Treatment had no significant effect on lightness (P=0.46).

Using the values of the mean square (MS) from the analysis of variance (ANOVA) as a guide to the impact of various effects, the factors that effect the lightness the most were, position on steak (MS= 3110.3) followed by days of chilled storage (MS= 1045.7). The L* of the flesh increased with distance away from the backbone (Figure 3), with positions 5,6, and 7 (in the fat band or toro) being highest and position 1 the lowest.

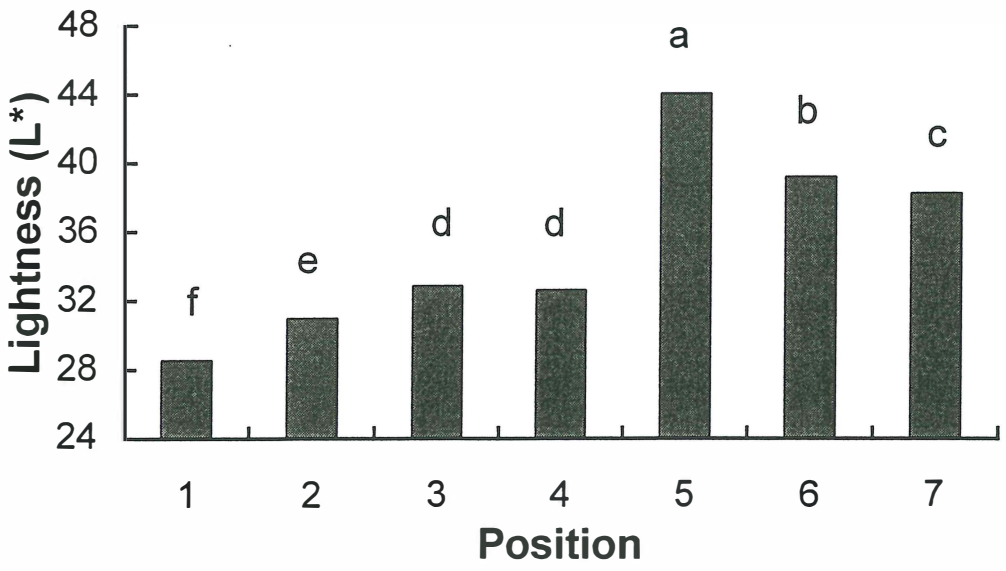

Figure 3. Effect of position in the steak on the measured lightness (L*) of the sample. Refer to figure 1 for explanation of positions. Bars with different letters above them are significantly different, that is, P<0.05)

The lightness also increased with storage time, rising from an average of 32.9 at 4 days to 36.9 after 11 days, though the greatest change occurring between 4 and 8 days (at 8 days, average L* was 35.9). There was a significant interaction between period of chilled storage and the position measurements were made on the steak ($P<0.001$) (Figure 4). Essentially, the change in L* with time is confined to the inner part of the carcass (*akami*). Lightness did not differ significantly with time in the fat-band (*toro*) positions 6 and 7. It differed significantly between each time in the *akami* positions 1 to 3, though the increase was largely shown in the step between 4 and 8 days storage. At positions 4 and 5, L* only differed significantly between 4 and 8 days, reaching a plateau thereafter.

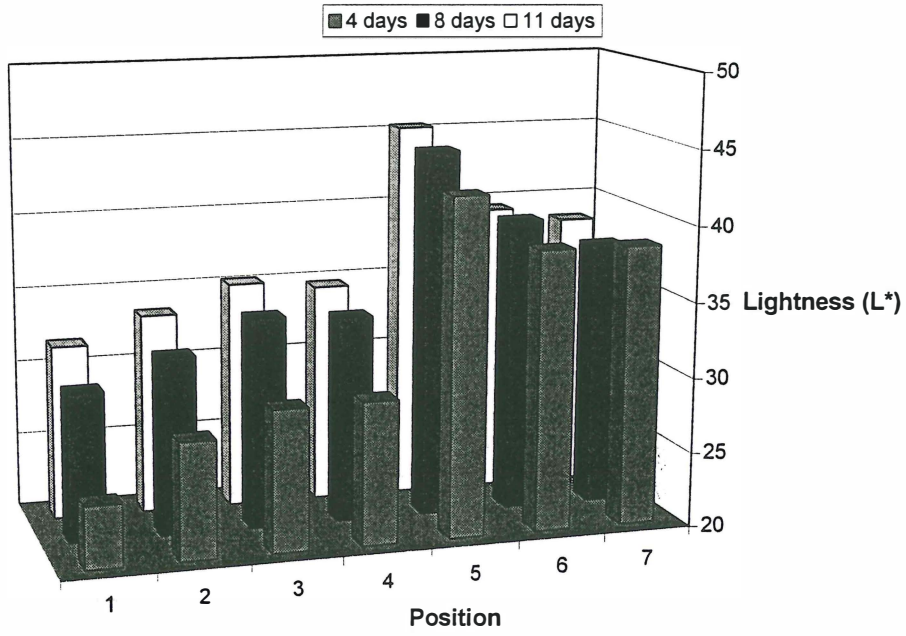

Figure 4. Interaction between position in the steak and period of storage. Note that the largest change in lightness occurs in the akami (positions 1 to 4). Refer to Figure 1 for diagram explaining positions.

There was a significant interaction between treatment and the position on the steak ($P<0.001$). The fasted, ice-slurried tuna in particular (Figure 5) have a higher lightness in the fat band positions. Otherwise, the only other difference worthy of note is that the fed/seawater bled tuna have a higher lightness at position 1.

There was no significant interaction between treatment and period of chilled storage ($P=0.095$).

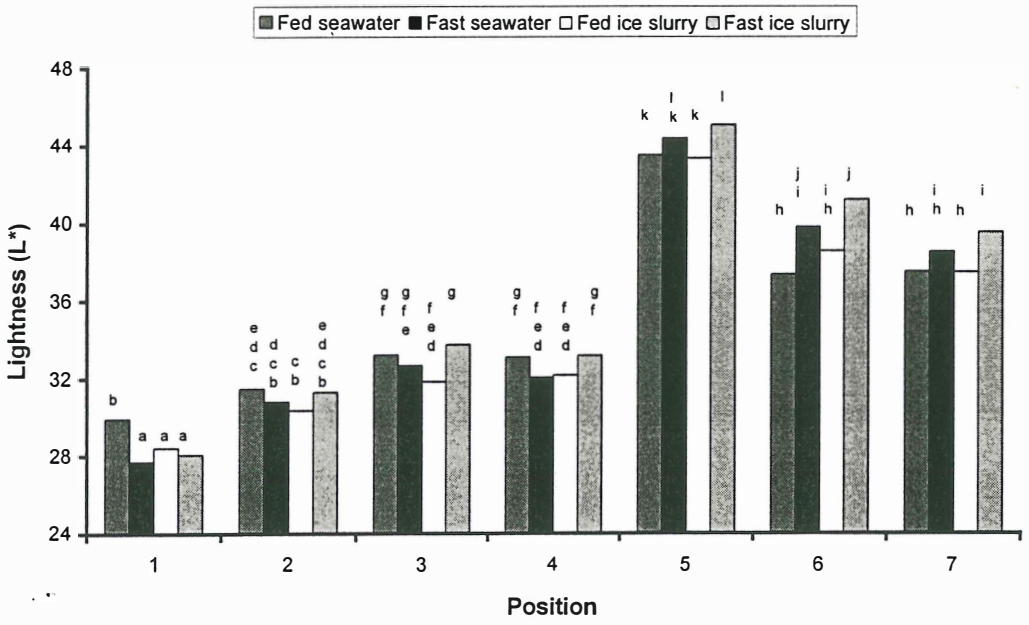

Figure 5. Interaction between treatment and position in the steak. Note in particular, the toro positions (5,6,7) of fasted tuna. Refer to Figure 1 for an explanation of positions of measurement. Bars with the same letter above them are not significantly different ($P>0.05$)

Cie A* ('Redness')

The coordinate a* describes the degree of red chromaticity or 'redness' of the flesh. The position in steak, and period of chilled storage on ice both significantly effected the average a* of the flesh ($P<0.001$). The factor that effected the a* the most was the period of chilled storage (MS=418.14) followed by the position in the steak (MS=62.7). Note that the order has swapped around from that seen for L*, lightness. Treatment itself did not effect the average a* of the flesh ($P=0.482$).

The value of a* fell during storage on ice, from an average of 6.19 at 4 days to reach 3.72 after 11 days. The fall mostly occurred in the interval between 4 and 8 days. Position 1 had the highest redness within the steak (Figure 6), followed (in descending order) by position 7, 4 and 5. The lowest a* values were seen in positions 3, 6 and 2. This pattern does not conform to the strict *toro/akami* division shown in the L* lightness data.

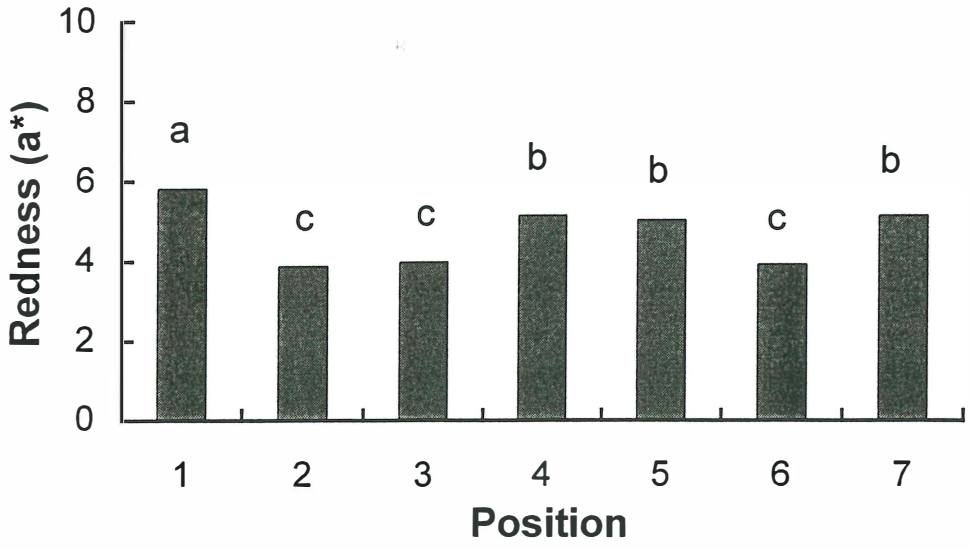

Figure 6. Effect of position of measurement within the steak on the CIE a* ('redness') of the steak. Refer to Figure 1 for an explanation of positions of measurement. Bars with the same letter above them are not significantly different ($P>0.05$)

There was a significant interaction between post-harvest treatment and position in the steak ($P<0.001$) (Figure 7). When fish were both fasted and seawater bled, they had significantly higher values of a* in the *akami* (positions 1 to 4) than that of *akami* of other tuna.

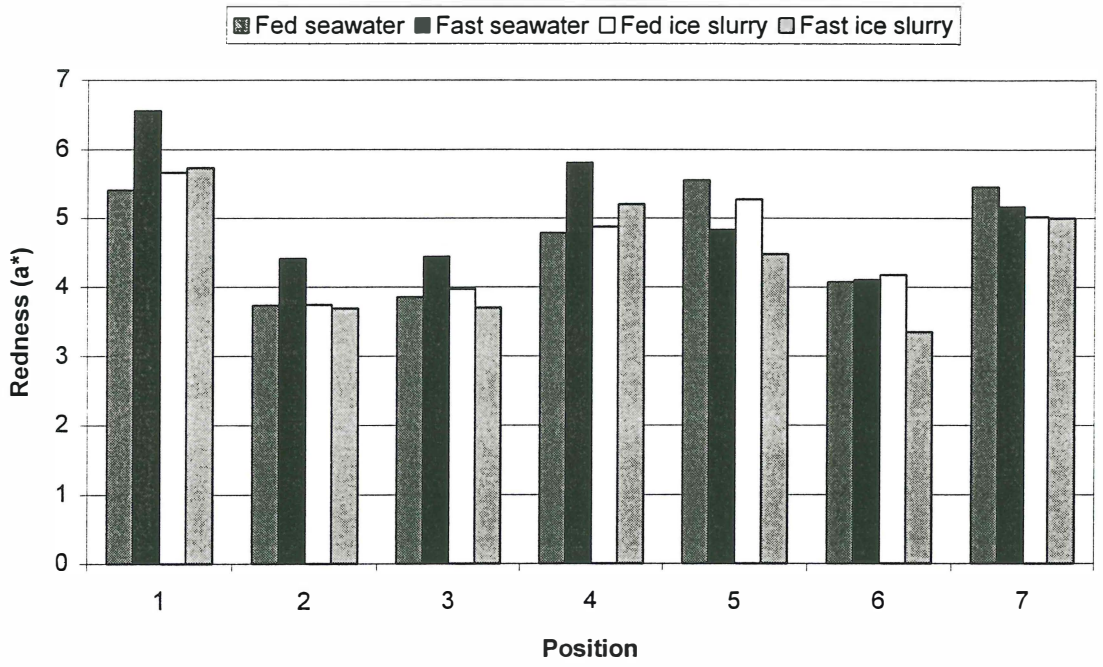

Figure 7. Effect of fasting/post-harvest handling treatment on the redness (a*) of the tuna steak, note in particular the results at akami positions where the fasted/seawater treatment is significantly different from that of the other treatments (1-4). The letter susperscripts of the multiple comparisons following the analysis of variance have been omitted here for simplicity.

There was a further significant interaction between period of chilled storage and position in the steak ($P<0.001$). While a* tended to fall with time, in many positions on the steak, the fall was confined to the interval between 4 and 8 days (Figure 8). However, after 8 days, in positions 3, 5 and 6, a* was still significantly different from the value at 11 days.

There was no significant interaction between treatment and period of chilled storage.

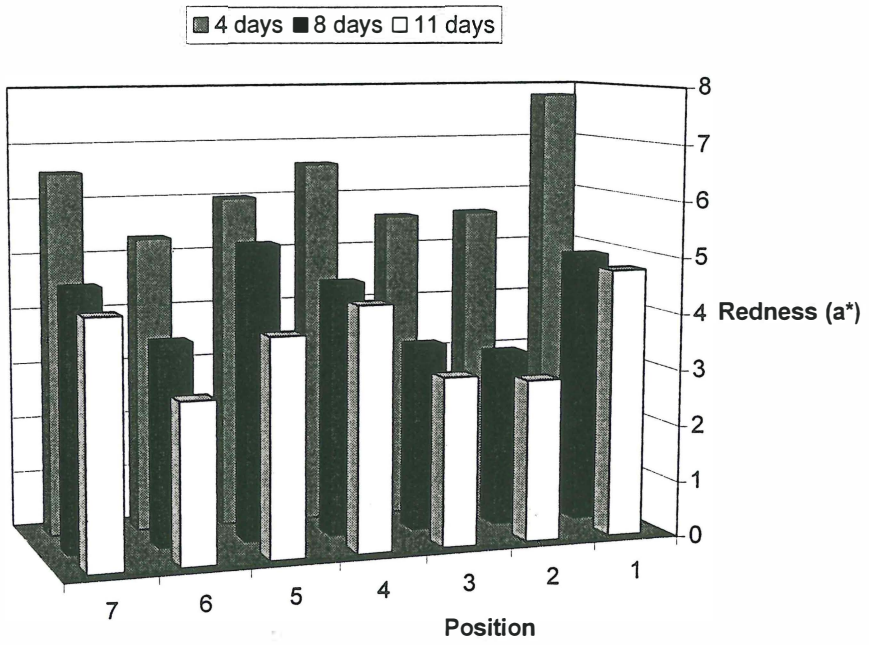

Figure 8. Interaction between period of chilled storage and position of measurement. Note in particular the marked change in redness (a*) in the first 8 days of storage.

Yellowness

The coordinate b* indicates the yellow chromaticity or 'yellowness' of the sample. As in the case of the other parameters of 'colour space' the position in steak and period of chilled storage on ice all significantly effected the yellowness of the flesh ($P<0.001$). The factors that effected the yellowness the most were position in the steak (MS= 174.6) and days of chilled storage (MS= 86.7). Treatment did not significantly effect the yellowness ($P=0.647$).

In many respects the position effects for yellowness reflect those for lightness (cf. Figure 3 and 9). Yellowness was highest in the positions corresponding to the *toro* or fat band (5,6 and 7). Yellowness fell from an average of 7.31 after 4 days chilled storage to 6.25 after 8 days and did not change significantly thereafter (being 6.34 at 11 days).

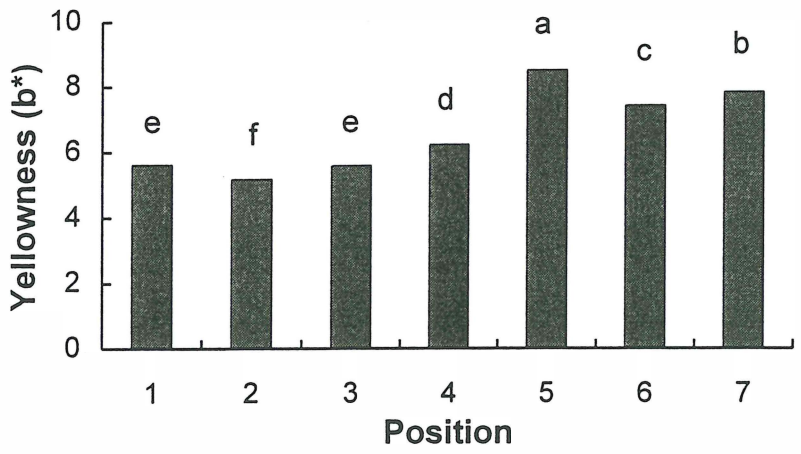

Figure 9. Effect of position of measurement within a steak cut in cross section from a loin on the values of yellowness (b*) Refer to Figure 1 for an explanation of positions of measurement. Bars with the same letter above them are not significantly different ($P>0.05$)

There was a significant interaction between position and period of chilled storage, with the fall in yellowness being most evident at all except positions 3 and 4 (Figure 10).

There was no significant interaction between treatment and either period of chilled storage (P=0.559) or position in the steak (P=0.059).

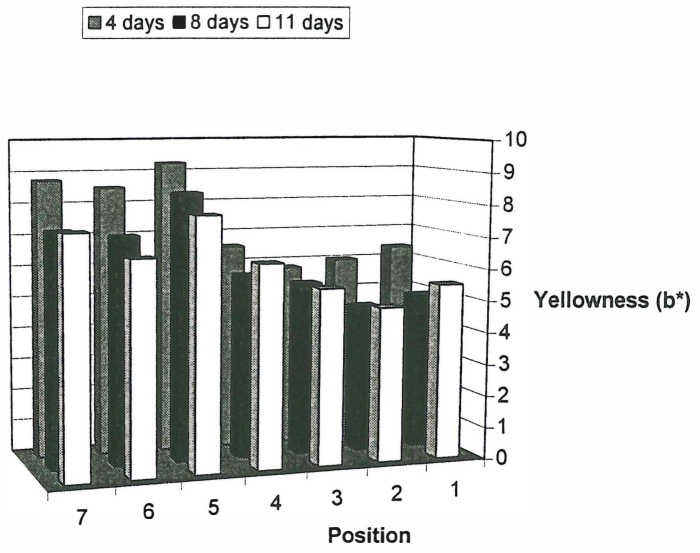

Figure 10. Interactions between position in the steak and the time of storage for redness (a*) and yellowness (b*) measurements

Hue Angle

Hue angle is calculated from the values of a* redness and b* yellowness, such that a low hue angle is more red than it is yellow (Figure 11). Close correspondence can thus be expected between values of hue angle and values of redness and yellowness presented above. Thus, the position in the steak and period of chilled storage on ice significantly effected the hue angle of the flesh ($P<0.001$), with the highest mean square applying from the period of chilled storage effect (MS= 6223.03 versus 3710.38 for position).

As this is simply a transformed method of presenting a* and b*, the results of the calculations won't be presented here.

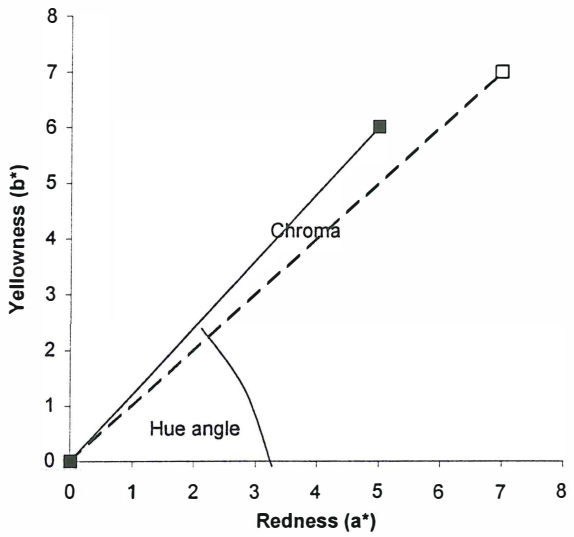

Figure 2. Relationship between the colour space coordinates a*, b* and values of hue angle and chroma.

The concept of hue angle can be appreciated by first considering simple plots of average a* and b* for each position in the steak (Figure 12). The hue angle is the angle from the redness axis of the line drawn from each point back to the origin (a*,b*=0). Where b* is greater than a*, the points lie closer to the b* axis. These points have a high hue angle.

Generally, hue angle was significantly higher (more yellow) in the fat band positions (5 to 7) than in the *akami* positions (1, 2, and 4). However, position 3 was not significantly different from position 7. The hue angle of position 1 was significantly lower (redder) than at all other positions.

Hue angle increased significantly with time. So, despite the fact that both a* and b* decreased over time, the greatest fall occurs in a*. There was also a

significant interaction between position and period of chilled storage. The overall rise in hue angle or 'yellowing' with time was greatest in the *akami* positions, particularly in the period between 4 and 8 days storage. This makes sense in that the *toro* positions have less scope for further yellowing because of the fat influence here. While the change was greatest initially (between 4 and 8 days), a significant rise in hue angle still occurred between 8 and 11 days storage in all positions but position 5.

There was also a significant interaction between position and post-harvest treatment, following on from that found with redness (a*). Flesh of tuna that were fasted and slow bled had the lowest hue value (i.e. were redder in hue) for all akami positions.

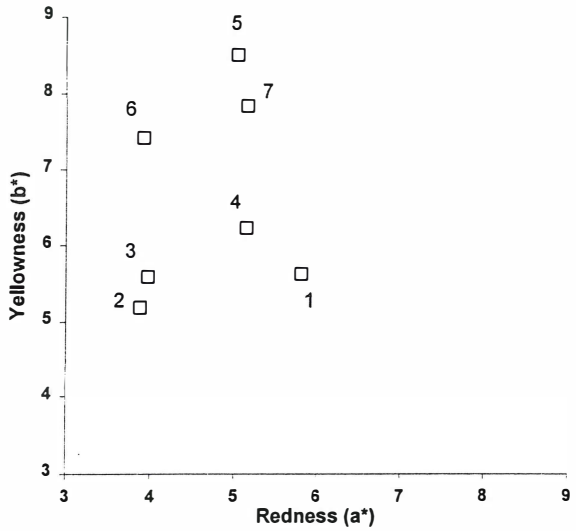

Figure 32. Average a* and b* for each position measured.

Chroma

Chroma is a measure of the intensity or saturation of the colour. The chroma is the length of the line drawn from a point in a*,b* space back to the origin (a*,b*=0) (Figure 11). The position in the steak and period of chilled storage on ice significantly effected the colour saturation or chroma of the flesh, with the highest mean square occurring with the chilled storage effect.

On average, chroma fell significantly between 4 and 8 days chilled storage, not changing significantly when recorded again at 11 days. This is a reflection of the

fact that both a* and b* fell with time. The points are moving closer to the origin or if you like 'loosing colour.' There was also a significant interaction between position and period of chilled storage, largely explained by a more regular fall in chroma at fat-band positions 5 and 6. Chroma at these positions (particularly so for position 5) was significantly different at each sampling time, rather than the fall being confined to a dramatic difference between the first two samples as in the case of the other positions (largely the akami).

There was also a significant interaction between position and post-harvest treatment. Chroma was higher in the akami positions in flesh of tuna in the treatment that was not fed and slow bled than in both slurried treatments , but these values were not significantly different from values at these positions for tuna that were fed and slow bled.

DISCUSSION

Colour analysis during this experiment shows that fasting for as little as four days raises the post mortem pH of the flesh and improves the flesh colour in the akami of southern bluefin tuna that have been bled in cool ambient seawater rather than ice-slurried immediately. The effect is subtle since there are other deteriorative processes at work when tuna flesh is stored chilled which have a dramatic effect upon its shelf life. The pigment involved breaks down during post-mortem storage. While the evidence is there to continue looking at fasting as a means of improving flesh characteristics in farmed tuna, there is also indications in this study that if the fish are fasted, then the post-mortem handling of the fish does influence the flesh colour.

It is a commonly held belief that if tuna are not killed quickly, their spinal cord destroyed and immediately put in an ice slurry then their flesh will burn (Jerrett 1984). This may well apply to tuna caught at sea, which struggle violently during harvest, but it didn't hold with the farmed fish used in this study. Tuna carcasses bled in ambient seawater showed flesh colour that was at the very least comparable to that of tuna placed in an ice slurry and of course in some cases it was marginally better. The reason why slurrying is not so crucial for farmed tuna may be that the handlined tuna used in this study arrive on the boat promptly and with very little opportunity to struggle. Net crowding of tuna, and any excessive struggling, may of course have a different outcome.

There is another conceptual reason why slurrying should not be necessary. Tuna are warm-blooded. Nobody puts cows in an ice slurry. It takes up to a day or so for an ice slurry to chill a tuna, so most of the biochemical changes happening in the immediate hours post-mortem (eg. rigor mortis) will happen as the carcass core temperature is dropping. It is hard to see that it would matter whether the carcass was in cool seawater or a brine ice slurry at this time. Yet, all the same it

is interesting that the flesh pH seems to be falling faster toward terminal pH in fasted tuna that were slurried than in carcasses from the same cage that were left to bleed in ambient seawater, or in carcasses of fed tuna handled either way (Figure 2). Perhaps it is not so much the level of terminal pH reached, but that level combined with the rate of pH fall that contributes to the colour improvement associated with the combined fasting and post-mortem bleed treatments. Close attention to this factor is required in future studies. Another point to watch in future studies is that if tuna are fasted, then slurrying them immediately may actually prove to be worse than bleeding them in ambient seawater, at least in terms of flesh colour (Figure 7).

It is important to emphasise that fasting only briefly delays the inevitable. One of the factors that consistently had a major impact on the colour of tuna flesh was the period of time that the samples spent in chilled storage. Tuna 'loses' its colour with time, so any alternative harvesting and handling practices adopted in the course of this research still need to be seen as one part of the process of getting the product to the market in prime condition. Controlling the temperature of the product during transport remains as important as it has always been.

The other factor that had a major and consistent impact upon flesh colour was the position that measurements were taken from in the steak itself. Different parts of the steak have characteristic colour properties. In this study we have simply averaged out this variation (along with the less notable variation in colour between fish) in order to detect the changes being brought about by our experimental treatments but all the same this diversity of colour within the *akami/toro* is real and deserves comment. At one level, the cause of the variation is clear, the locations in the fat band clearly have a higher lightness and yellowness. We expect fat accumulation to do this. The variation in redness is presently difficult to interpret. The problem with using colour analysis to try to study only one half of the colour 'equation' namely deterioration of oxymyoglobin is that colour also faithfully registers the fat content and perhaps other physical aspects of the meat that do not change during post-mortem storage.

So what does the high a* (and low Hue angle and higher chroma) in the akami positions following fasting and slow bleed treatment mean? Does this mean that the flesh was redder in colour or that the treatment has physically changed the meat in some other way that the meter registers as a change in colour. These physical differences are usually expressed in the Lightness parameters. Differences in fat content clearly alter the opacity and light absorbing properties of the steak (eg. Figure 3). Similarly, the rise in *akami* lightness with time (Figure 4) and fall in chroma can be simply explained as the flesh becoming more opaque with time and bouncing more white light straight back at the meter. But the differences in redness we saw in the *akami* (Figure 7) appears to be a real reflection of the colour, since the lightness of the samples did not differ significantly between treatments in the akami (Figure 5). Because of this, we conclude that the a* effect noted occurs because of greater absorbance by red

pigment in the sample, not because of opacity or differences in light scattering properties.

To conclude, the result to date indicate that fasting and ambient seawater bleeding slightly raises the redness of *akami* in tuna carcasses, and by implication, this should have a general reinforcing effect on the quality of the carcasses being shipped to market. Further experiments are clearly required to confirm this finding, to establish the efficacy of this practice under commercial conditions, and in particular to see what harvesting and post-harvest bleeding practices are most suited to accompany it.

Acknowledgements

This project was supported by the National Seafood Centre, an initiative of the Fisheries Research and Development Corporation and the Queensland Department of Primary Industries.

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Appendix 2

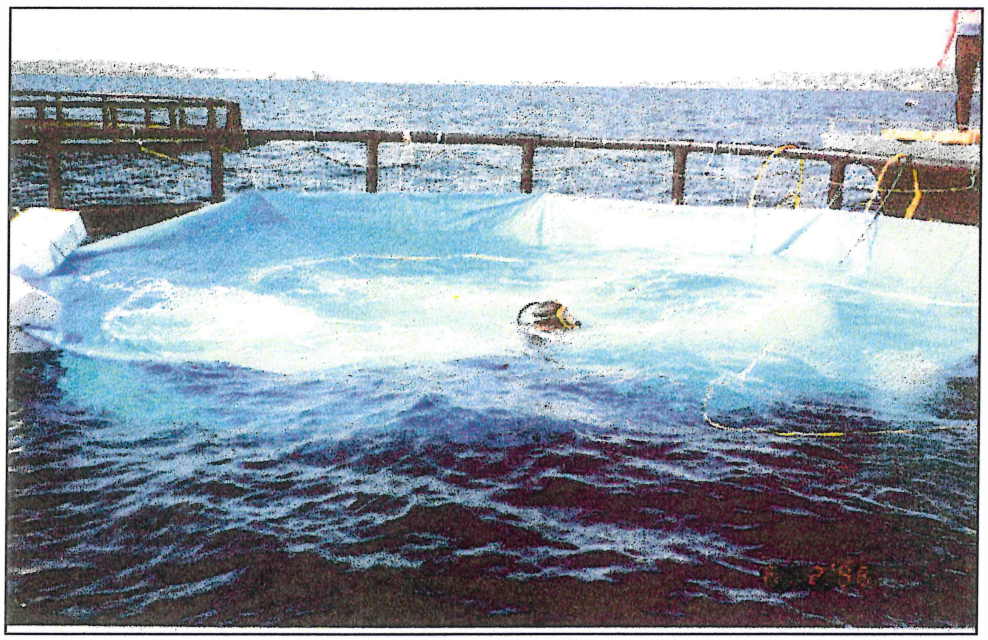
Report relating to

the approved variation for Objective 2

becoming Objective 4

Preliminary Trial Report on

The Effectiveness of AQUI-S™ as a Sedative for use in Research, Husbandry and Harvesting of Farmed Southern Bluefin Tuna



B Goodrick, P.Exley
International Food Institute of Queensland

J Holland
New Zealand Institute for Crop and Food Research

A Smart
South Australian Research and Development Institute

K Rough
Tuna Boat Owners Association of Australia

February 1996

Contents

Page

Executive Summary

This report considers a number of aspects of recent trials using a newly developed, food grade sedative (AQUI-S™) in the handling and harvesting of southern bluefin tuna. Separate individual reports are incorporated together.

This initial trial has shown that AQUI-S™ is effective as a sedative for southern bluefin tuna. Fish were lightly sedated by approximately 10 minutes after application of AQUI-S™ at a concentration of 17 ppm such that a commercial grading operation would be possible. After 20 to 30 minutes rested harvesting could be carried out.

Initial results indicated higher post-harvest quality as indicated by less intensive rigor and a reduction in the rate of pH change. However no conclusive result was obtained. The compromise in methodology aimed at reducing the cost of this exercise and the lack of any previous experience in such trials hindered the opportunity to obtain a fully rested harvest and the full subsequent benefits.

Revival trials were not conclusive but indications were that this will be possible with further development of the technique. A separate recovery operation should be investigated. There were no adverse reactions to the **AQUI-S™** by the fish or by divers exposed to this solution for periods exceeding 1 hour.

Improved methodology has been devised that will aim to achieve a true rested harvest and it is recommended that trials be repeated in the near future to gain a deeper insight into the full potential benefits to be achieved with this technology.

Development of new commercial technology for efficient harvesting, live fish handling and husbandry, using **AQUI-S™** as a tool should be planned as a combined research project as soon as practicable, preferably prior to registration of **AQUI-S™** for aquaculture use.

Acknowledgements

The financial support and the assistance of staff of the Tuna Boat Owners Association, and Tony's Tuna International is gratefully appreciated. We also gratefully acknowledge the Financial support provided through the National Seafood Centre.

SCOPE:

Improvements in Harvesting and Post-Harvest Quality through Rested Harvest with AQUI-S™

B Goodrick, Senior Seafood Technologist, **P Exley**, Technician, International Food Institute of Queensland; **J Holland**, Technician, New Zealand Institute for Crop and Food Research

BACKGROUND

Normal harvesting practices for tuna are at worst an extremely stressful and damaging practice for valuable sashimi grade tuna. At best fish are subject to a short but inevitable adrenalergic (escape response) reaction during capture which in itself can initiate quality deterioration after harvest. Improved harvesting efficiency and post-harvest quality will be achieved if fish are harvested while rested and hence the application of the concept of "rested harvest".

This technology involves careful consideration of the activities involved and the resultant effect on tuna during the overall harvesting operation. Increases in activity, or initiation of stress or escape responses need to be avoided.

The application of a suitable sedative, which is used as a stress and exhaustion prevention tool, combined with the development of other rested harvest practices will facilitate a rapid and stress free harvesting operation. The mode of action in improving post-harvest quality is to reduce the large amounts of energy expended by fish immediately before harvest. The resulting muscle cells have greater energy reserves after the oxygen supply stops.

Apart from the quality benefits, the fish are much more easily captured and handled.

EFFECTIVENESS/RESULTS

A video of the harvesting operation and use of AQUI-S™ to sedate the tuna clearly indicates the effectiveness of this sedation tool in calming and sedating the tuna and ultimately anaesthetising them. However, to be effective and beneficial as an aid in achieving quality benefits the fish need to be in an unexcited and unstressed state at the time of sedation. As this was a preliminary trial a number of compromises were made such that a fully rested sedation operation was not possible.

Many of the fish were repeatedly meshed during the crowding operation prior to sedation. The effect of transferring the fish to a separate cage less than a week before the trials is also unknown. The results achieved are therefore variable but do however indicate the potential for substantial improvements in harvesting efficiency and product quality.

We examined the core temperatures, muscle pH, flesh tension (as a measure of rigor state) and colour of both control and AQUI-S™ treated fish. Notes recorded during the trial by Jan Holland are attached as Appendix 2.

Temperature

Core temperatures on landing (Figure 1) indicate a substantial cooling trend for tuna in the Aqui-S treatment. Further trials are needed to determine the legitimacy of this observation. The core temperatures on landing of the 2nd Aqui-S group (2 fish) neither confirm nor refute this data.

pH

pH is a measure of the acidity of the muscle which has a relationship to the colour of the meat, and generally good colour and colour stability is associated with higher pH values. Figure 2 indicates the average pH changes of each group. A difference is notable with the pH falling more slowly for the AQUI-S™ treated fish. The level of variability between individual samples (fish) is however quite substantial and likely to be due to the handling of fish prior to treatment.

In order to try and gain a better insight into the potential quality benefits of true rested harvesting we compared the results for the 3 best and 3 worst cases for each treatment. This presumeed that a number of the fish may have been less stressed during the crowding/harvesting operation. Figure 3 shows this comparison of highest values which indicates a slower rate of post-harvest pH decline of AQUI-S™ treated fish which should be considered to be a very positive indicator of quality improvement potential. In the AQUI-S™ treated fish, the flesh pH is retained above pH 6.5 for approximately 13 hours after harvest whereas control fish were generally at or below pH 6.5 at the time of harvest. In a similar pattern the AQUI-S™ treated fish remained above pH 6.0 for approximately 5 hours longer.

Figure 4 shows the comparison for lowest pH values in each treatment. This figure shows significant variability but in all cases a rapid decline in pH, perhaps slightly slower in the AQUI-S™ treated fish. The pattern is consistent with fish that are exercised or exhausted prior to harvest

Colour/Appearance

Results for colour are inconclusive. No significant difference in colour was detected by machine colour analysis. (Refer Figure 5)

Whole G&G fish (2 treated and 2 controls) which were examined both whole and dressed, by myself Brian Leneve, (Kebachi seafood exports) and Hiro Tabata, (Nippon seafood) after 3 days on ice showed little if any difference on a group treatment basis. Any difference between

the treatments was less than the level of variation between fish again confirming the need to carefully plan and conduct the trial to ensure uniform rested harvest conditions are achieved.

Examination of the cut loins by B Leneve and H Tabata after 4 days post-harvest, also showed minimal difference in the flesh samples on a group basis. The general comments were that the tuna is not of particularly good quality at this time of year. The comments received for these fish are listed in Appendix 1.

Flesh Tension (as a measure of rigor state)

Figure 6 graphs the stiffness or muscle tension of the tuna from the time of harvesting as they stiffen and enter rigor mortis and then soften as the muscle again relaxes. Both duration and intensity of the rigor process have implications for the elasticity and strength of the tuna flesh which will in turn influence the texture and the integrity of the meat. This has a significant influence on the suitability of the flesh for sashimi and sushi where the flesh is sliced very thinly. Fish which enter rigor slowly are generally considered to have superior quality and will have firmer and more elastic flesh.

These results indicate that Aqui-S treated fish enter rigor more slowly and gently. This suggests that the anticipated benefits will be achievable. A delay in the onset of rigor of 5 to 10 hours is indicated by the data (Fig 6). A more significant result can be expected if the tuna are treated with AQUI-S™ while in a rested state.

CONCLUSIONS/RECOMMENDATIONS

The results of this preliminary trial show that the use of AQUI-S™ is effective in sedating and anaesthetising southern bluefin tuna. Positive indications of the benefits to be obtained in terms of harvesting and post harvest quality are promising but not conclusive as the methods used in this trial were a compromise of cost against quality of results.

This trial was indeed valuable as an opportunity to gain experience and foresight into suitable methods which could be employed to perform objective and controlled trials aimed at determining the full potential benefits to be gained through the application of rested harvest technology incorporating sedation with AQUI-S™.

The behavioural changes and "handleability" of AQUI-S™ treated tuna can be observed on the video footage of these trials. It should be noted however that the methods used in this trial need substantial development before further trials are conducted and that the scope and technology for maximum commercial benefit need to be investigated as a project in co-operation with commercial operators and the experimental farm.

It is recommended that further preliminary trials be conducted in the near future using improved techniques and equipment and that the scope of the trials be expanded to cover additional aspects of recovery, levels of sedation, harvesting methods and post-harvest handling.

SCOPE OF REPORT:

Application for Research Purposes

Alastair Smart - Research Scientist (Tuna Nutrition) on Tuna Research Farm (SARDI and TBOAA).

BACKGROUND

1. There is considerable potential for the use of anaesthesia in weight sampling fish to minimise stress and handling damage. The present system to weigh, measure, tag, and transfer fish involves hooking, or crowding and netting fish, and physically guiding them into a stretcher. If the fish were lightly anaesthetised en masse (i.e. 20-50 at a time) so that recovery was fast and individuals did not require hydroventilation, this would be of great benefit to nutrition trials. The effects of handling stress on growth are marked and the initial, interim, and final weights are the main parameters for detecting fish growth and therefore the viability of manufactured feeds being developed.

2. In addition, specific operations such as archival tag insertion into the gut cavity of fish, blood sampling, etc., is required for specialised research projects. The use of complete anaesthesia will be especially useful for operations such as these where the fish is out of the water for extended periods and acquiescence is required.

RESULTS FROM TRIAL

The Tues 6th Feb 1996 trial on SBT at the Tuna Research Farm demonstrated that SBT can be successfully anaesthetised with the fish anaesthetic, AQUI-S™. However, further work is necessary to develop the recovery process as this was inconclusive from the preliminary trial and it is fundamental to the above research operations.

CONCLUSIONS

The use of AQUI-S™ to minimise stress during fish harvesting showed obvious potential from the preliminary trial. This is an option the research farm and commercial operations can use and develop for market harvests pending a more thorough investigation of the logistics and benefits.

For research operations, once the recovery process is fully developed and is feasible, the above operations could be more effectively performed using anaesthesia.

SCOPE OF REPORT:

Aqui-S™ and its Application for Southern Bluefin Tuna Husbandry Purposes

Kirsten M. Rough, MAppSc, aquaculture (candidate) Research Scientist - aquaculture (fish health) Tuna Boat Owners Association of Australia Lot 124 South Quay Boulevard, PO Box 1146, Port Lincoln SA 5606.

BACKGROUND

The use of anaesthesia to non destructively examine or sample aquacultured species is vital to operations with most cultured species. To date all handling procedures in tuna culture (includes trial intra-peritoneal and intra-muscular vaccination, sampling blood, tagging, and minor surgical operations) have been conducted on live (non sedated) individuals. However, blood sampling has also been conducted on recently euthanased specimens where an individual's response with time is not essential. These can all be carried ut satisfactorily with non-sedated individuals provided that procedures are conducted very rapidly (tuna less than 1.5 minutes out of the water).

In the future it is highly likely that more substantial procedures requiring a longer out of water period will be conducted on these fish. With this in mind the use and effects of anaesthesia on tuna need to be investigated. My involvement in this trial was to:

1. assess the effects of Aqui-S™ anaesthetic on various haematological and clinical biochemistries (includes parameters indicative of stress and those currently used to assess fish health and condition), and
2. maintain a tuna under anaesthesia for a period of 120 minutes, revive it and assess longer term effects.

The procedure used to maintain a tuna under anaesthesia for 120 minutes was as follows:

- all fish to be treated were herded into a liner within the sea cage which had been pretreated with Aqui-S™
- fish observed within the liner whilst succumbing to the anaesthetic
- divers retrieving sedated fish after they had dropped to the bottom of the liner and showed no signs of movement or struggle when held
- at this point (20 minutes after initial induction) the individual to be maintained sedated was placed into a 250 L tank treated with Aqui-S™ on board the boat

- maintenance of sedation was achieved by constant irrigation of the gills with anaesthetic solution

- revival was carried out by irrigating gills with untreated sea water in the tank until the fish could swim unaided, then tuna was returned to sea cage.

RESULTS

1. Blood analyses pending.

2. Tuna could be successfully anaesthetised at a concentration of 17μL/L. Induction was fairly rapid, with loss of equilibrium being obtained in approximately 10 minutes.

Respiratory obstruction was clearly evident by agitated and avoidance swimming behaviour and 'coughing' when aeration was introduced by waterwick diffusion.

The plane of anaesthesia was not as readily determined visually in tuna as in other fish due to the fact they respire by ram-ventilation (rather than buccal pump). But heart rate could be readily monitored both audibly by stethoscope and tactility.

Upon the tuna's transfer from the treated liner within the sea cage to the stretcher and tank on board the boat, the heart rate was very erratic. The speed was 132 beats per minute, but the pattern varied from a sequence of 5-6 very rapid consecutive beats and 3-5 seconds of nothing, to 1-3 beats with rhythmic half second intervals and then back to the former 5-6 rapid beats.

After 30 minutes the heart rate had slowed to a rhythmic 56 beats per minute. Interestingly, at this speed it could be controlled (faster or slower) by water flow, although the latter was not specifically measured. Bradycardia was evident when tuna had been held in the 17 μL/L solution for 60 minutes, heart rate dropping below 24 beats per minute. At this point the tuna was partially revived to approximately 50 beats per minute.

Complete revival took in excess of 45 minutes, during which time extensive damage was done to the tuna's epithelium due to the stretcher. Upon return to the sea cage the tuna was swimming rapidly and compulsively and had trouble maintaining equilibrium. It could however, effectively navigate avoiding the nets and divers.

The tuna died overnight and was retrieved from the sea cage by a diver for autopsy. Necropsy demonstrated several necrotic lesions on the ventricle. Unfortunately, autolysis was too severe to contemplate histopathological analysis. The scenario of rapid autolysis occurs more frequently when farmed southern bluefin tuna have been exhaustively exercised prior to death.

CONCLUSIONS AND RECOMMENDATIONS

I believe that these tuna can be successfully sedated and revived. Although this fish did not survive more than 12 hours after revival, there were many factors that would have contributed to its demise. For the purpose of extended sedation I recommend that aspects such as induction and maintenance dosages, sedation time, temperature and aeration mechanisms should be considered/altered.

The respiratory obstruction with the introduction of fine air bubbles was to be expected as this previously has consistently resulted in 100% mortality of tunas in confined spaces. I recommend this be avoided by using liquid oxygen in any future trials.

APPENDIX 1:

Notes Recorded by Jan Holland
Aqui-S™ Tuna Trials 6.2.96

- Crowding and pursing operation took 20 minutes.

- AQUI-S™ was added at 11.10 am, at a nominal concentration of 17 ppm. Within 10 minutes the fish were noticeably slower, the odd cough was also present. (There was concern that this could be due to the fine bubbles from the oxygenation. Oxygen was turned off). At 12 minutes definite signs of equilibrium loss were noted with the fish running into the sides of the liner. At 14 minutes the fish were starting to surface and by 15 minutes there was no avoidance by the fish of obstacles placed in their path.

- The first fish were removed at 11.26 (16 minutes after the introduction of the anaesthetic). From the video footage this was a bit early as the first fish did struggle on removal from the water. The last fish in this run was removed from the water at 11.42 (32 minutes after the introduction of the anaesthetic). Fish at this stage were well anaesthetised, very definitely alive and bled well. General comments were that the fish were bleeding well and later when the control fish were harvested it was noted that the AQUI-S™ fish did have a better external appearance. (That is better eye colour and clarity, more iridescence and more blue and purple colours.)

- Due to the logistics of the bag size and number of fish involved two runs of anaesthetic treatment were needed. The second crowding operation took ¾ of an hour and the fish had actually escaped at one point during this time. Also some fish had been caught between the liner and the bottom of the net. This was very stressful for the fish. When the fish were put into the liner some more AQUI-S™ was added (¼ of the original amount). No oxygen was turned on during this time due to the concerns of oxygen bubbles on the gills. Dissolved oxygen readings were approximately 500% but there was some doubt as to whether the meter was giving a reliable reading.

- The second lot of AQUI-S™ was added at 12.25 and the first fish was removed at 12.42 (17 minutes after the introduction of AQUI-S™). In this exercise the fish did not seem to go out as well and the reaction was more erratic. This could be due to low oxygen levels and the fact that the oxygen was not on to help disperse the anaesthetic. (This method appeared to work very well the first time). Also the concentration of AQUI-S™ was by guess work only.

- Revival of the fish from anaesthetic.

- 1. One fish was removed from the anaesthetic after 10 minutes. At this stage it was sedated. The fish was brought up onto the deck and the deck hose was placed I its mouth to force water through the gills. After 3 minutes the fish had given several kicks and after

5 minutes was operculating on its own. The fish was placed back in the water after 7 minutes but kept on being caught in the net. Even though it was able to move and operculate on its own it did not have the ability to stay out of the net at this stage. A diver accompanied it for approximately ½ an hour but eventually put it back in the anaesthetic at 1.15 pm. (This revival could have been successful if the fish was placed in another liner for recovery. This would ensure the fish did not become exhausted whilst regaining complete equilibrium.)

- 2. The second fish was removed from the anaesthetic to a bin on the deck at 12.55 (30 minutes after the introduction of AQUI-S™). The fish was placed on a stretcher and the water contained a nominal concentration of 17 ppm AQUI-S™. Heart beat was able to be monitored with a stethoscope. (It was generally commented on that the heart beat was very fast but I am not sure what, if anything, this was being compared to.) This fish was kept in this state for 2 hours with partial revival been carried out three times during this exercise. Fish was then revived which appeared to be successful. However when it was left to carry on under its own steam it went straight to the bottom and died. Post mortem results show that it had died of a heart attack.

- 3. One fish had been left in the anaesthetic for 80 minutes before being removed and placed in a bin full of seawater on the deck. this fish appeared to be dead on removal from the water. When harvested this was proven to be the case and the darkness of the blood suggested that it may have died from lack of oxygen. The first fish also appeared to have expired but when this fish was harvested the blood gave no indication of this. Also the pithing and coring process elicited strong reactions not usually seen if the fish has died.

- Recovery operations were also compromised by the fact that these particular fish were stressed in the crowding operation.

GENERAL COMMENTS

- Fish showed no awareness to the introduction of AQUI-S™ and no adverse effects were noted. Some slight coughing and a slight initial increase in the speed of swimming were the first observable signs of any effects of the sedative.

APPENDIX 2:

Examination of G&G Tuna at Sydney
9th February 1996
B Goodrick, B Leneve, H Tabata

Comments

Generally not much difference in colour or condition.

Fish No	Comments
48 aqui-s	Fattiest, second for flavour, less bitter than 87
80 aqui-s	A little bitter , best colour, best flavour
87	Very bitter, belly OK, worst flavour
19	No fat third for flavour

Conclusion

It was generally difficult to pick much of a difference and I would be reluctant to conclude that any difference was detected in this trial.

RESULTS FROM KEBACHI SEAFOOD EXPORTS RE GENERAL ADVICE ON QUALITY INFO RECEIVED BY PHONE MON 12TH FEBRUARY 1996 - BRIAN LENEVE TO BG

Comments

Packaged loins examined at Kebachi by Hiro Tabata, General Manager, Nippon Seafood Sydney Fish Market and Brian Leneve Kebachi seafood Exports.

General

Some of the loins were slightly wet and this may have influenced the external colour? The fish were evaluated 4 days after packaging and the general comments were that the fish loins were in better condition than expected, as the farmed fish are not expected to have a good colour life especially at this time of year with water temperatures of approx 20C.

Fish No	Treatment	Comments
94	AQ	Colour starting to turn not too good
52	AQ	Fair
72	AQ	Light and changing a little
55	AQ	"
64	AQ	OK
58	Control	OK
62	"	OK best fish
77	"	OK good fat
47	"	OK
59	"	Fair colour good fat

Figure 1. **Landing Temperatures of Control & Sedated SBT**

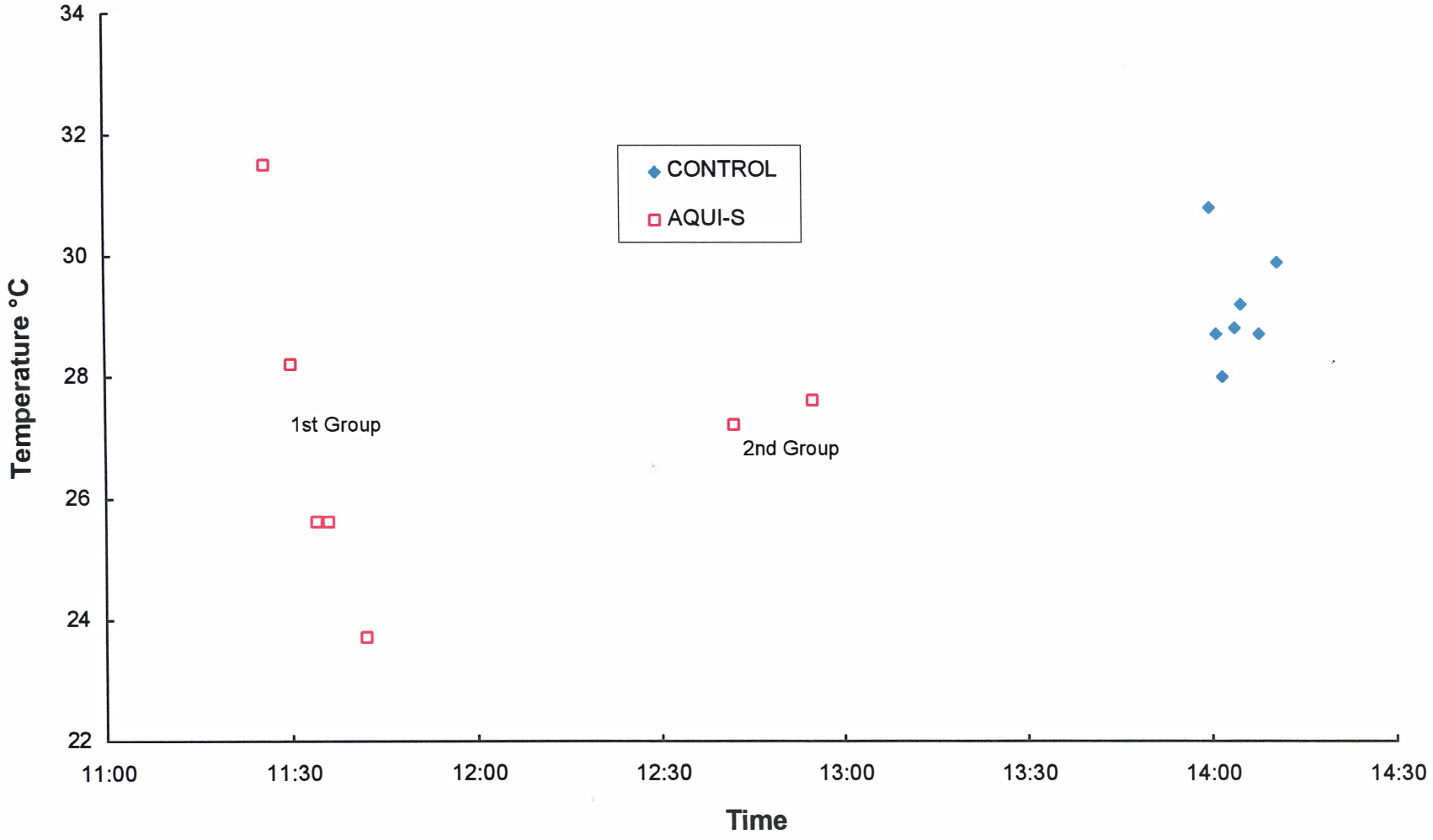
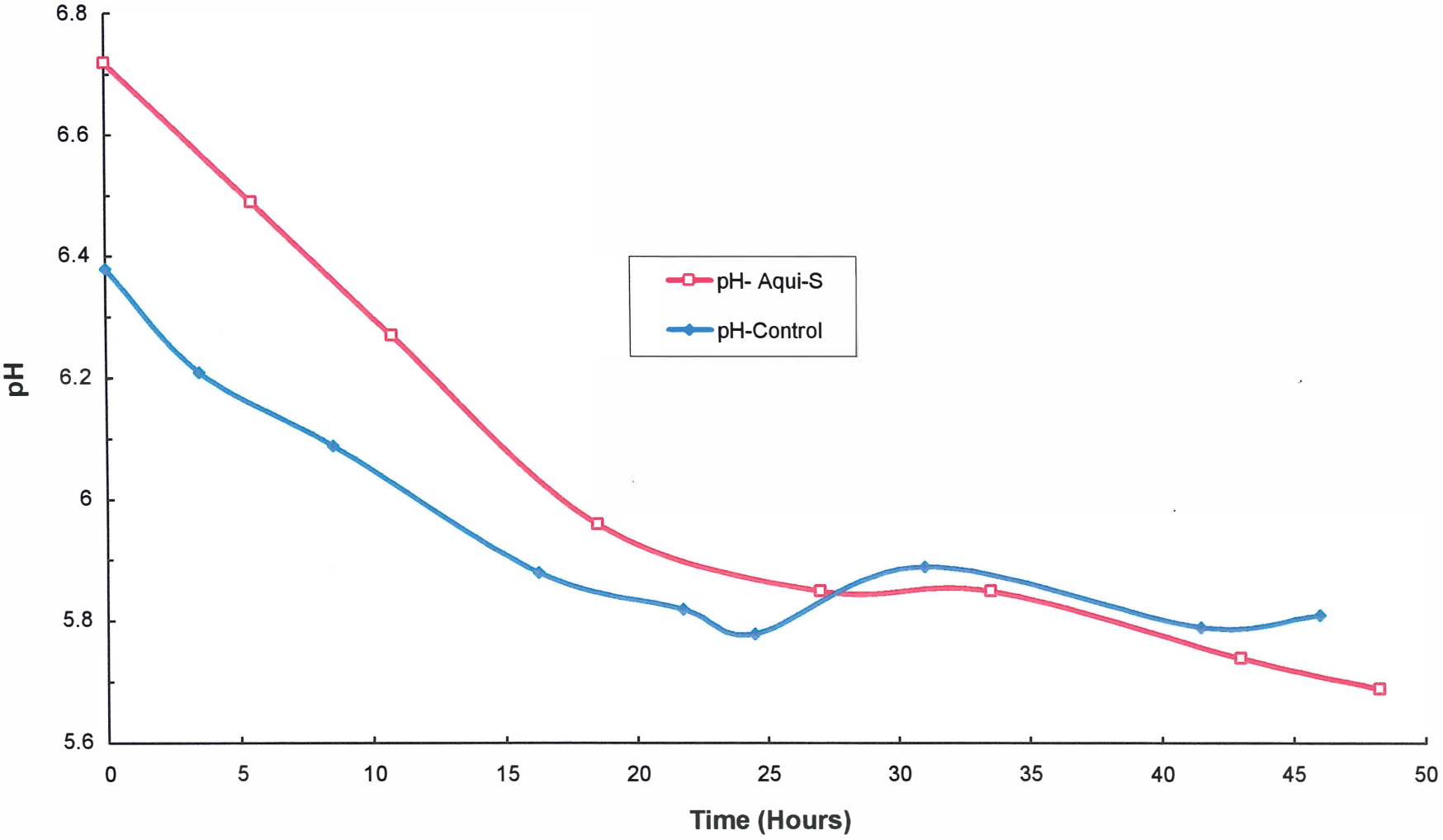
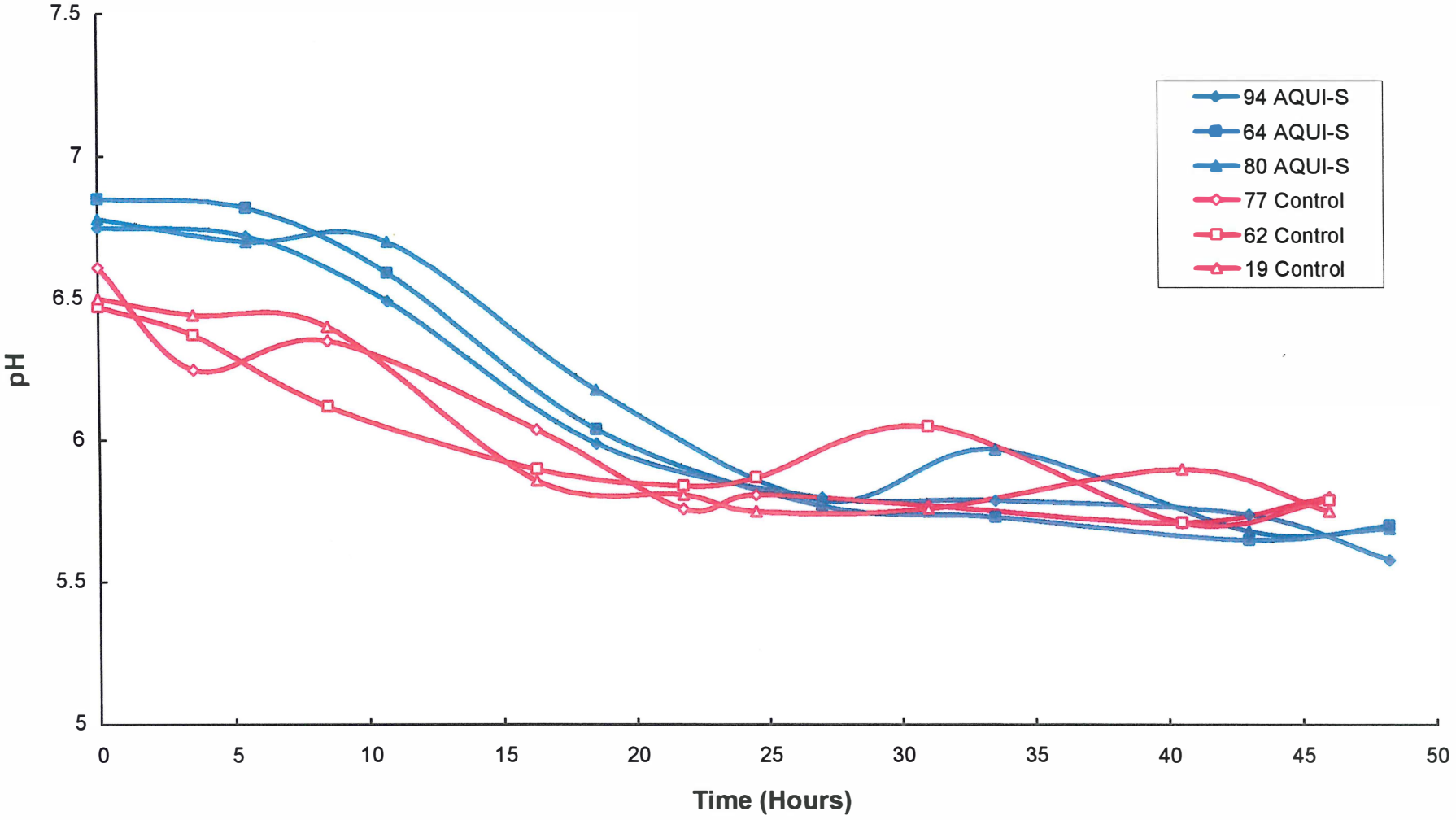


Figure 2. **Post-Mortem pH Changes in SBT sedated with AQUI-S™ Pre-Harvest**



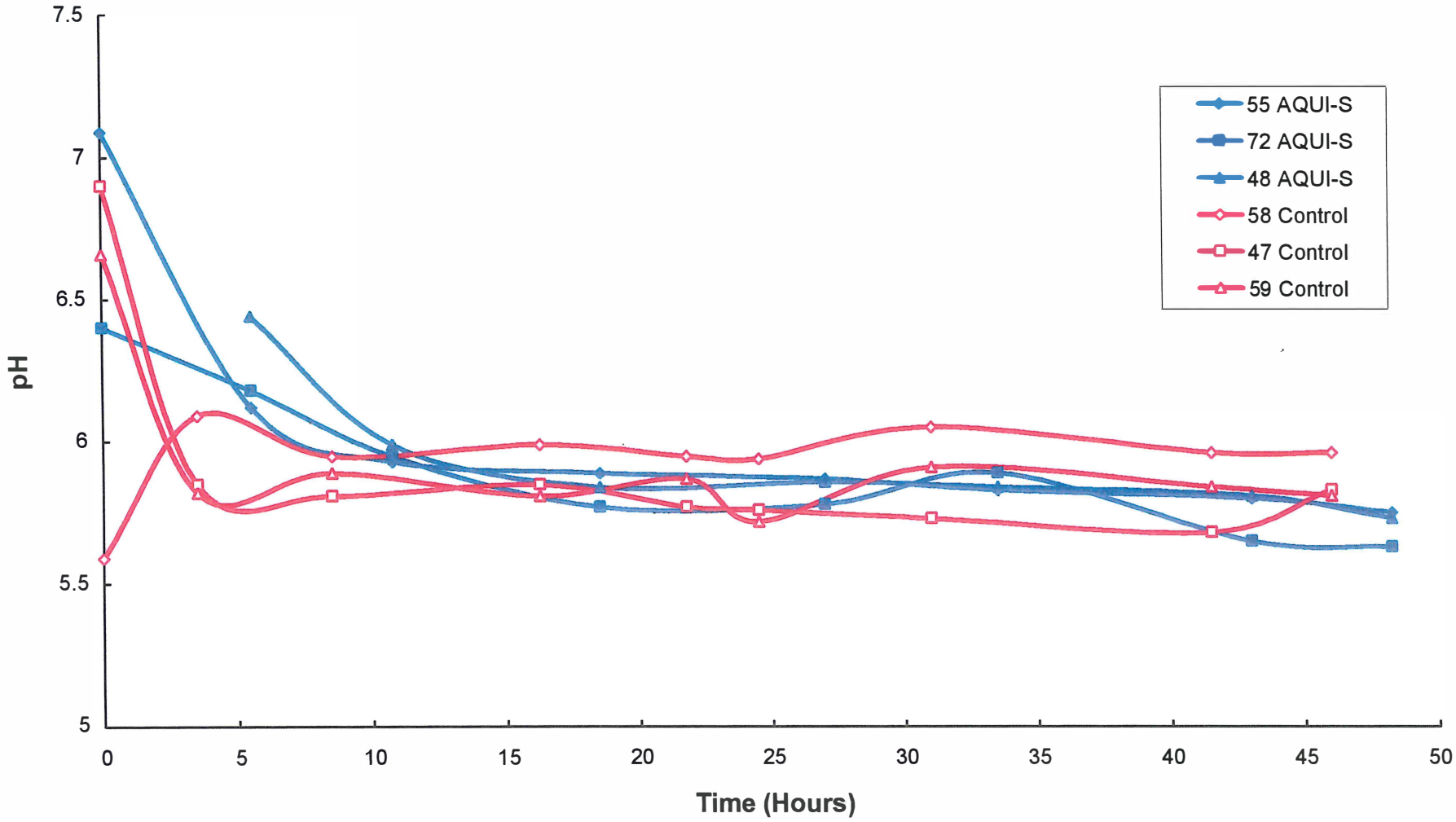
Goodrick B, Exley P, IFIQ Sea/Tuna AQUI-S/Tuna1.xls

Figure 3. **Comparison of highest post-mortem pH values in Control & AQUI-S treated SBT**



Goodrick B, Exley P, IFIQ Sea/Tuna AQUI-S/Tuna1.xls

Figure 4. **Comparison of Lowest Post-Mortem pH values in Control & AQUI-S treated SBT**



Goodrick B, Exley P, IFIQ Sea/Tuna/AQUI-S/Tuna1.xls

Figure 5. **Redness (a*) v Time for Control and Sedated SBT**

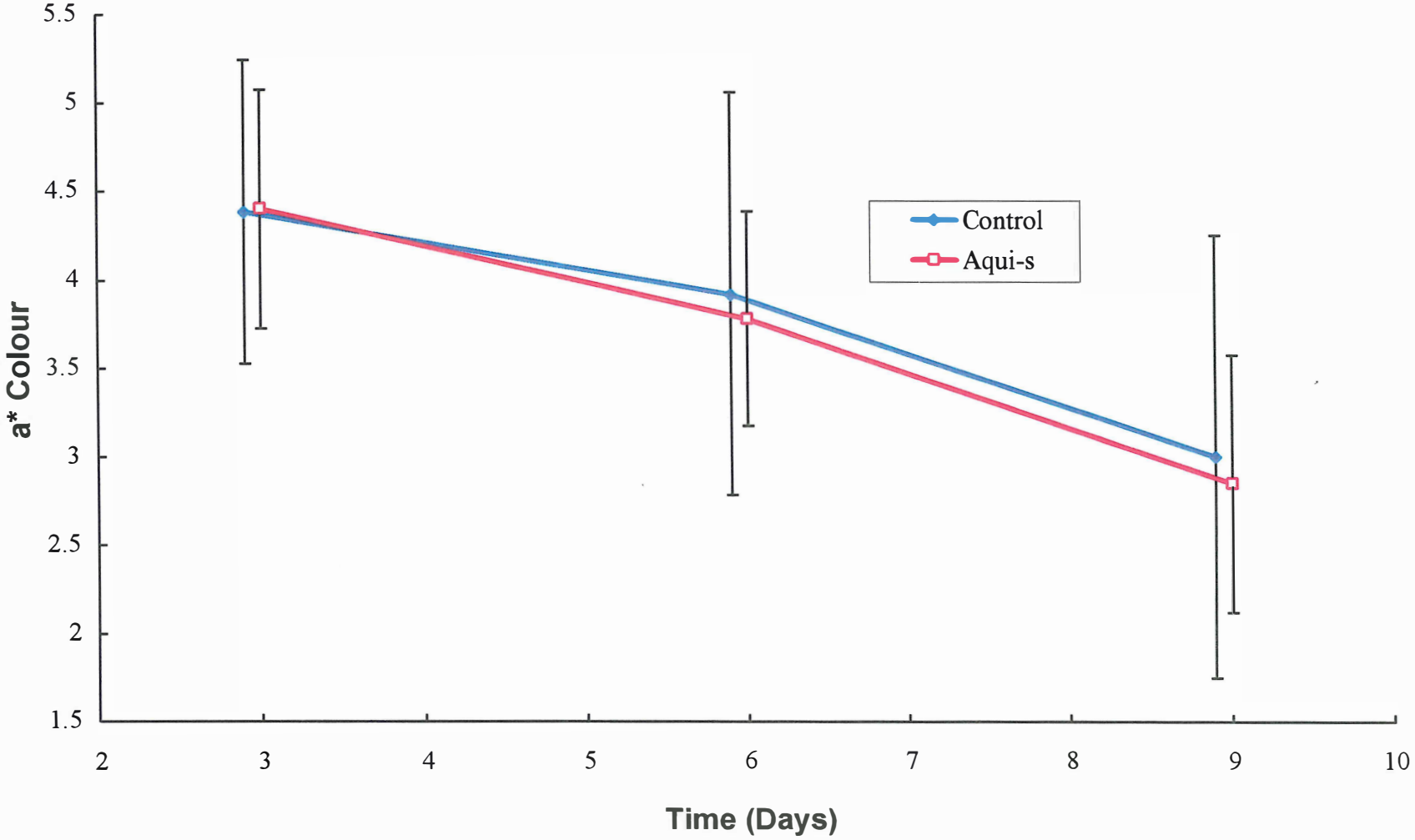


Figure 6. **Rigor Development as indicated by Deflection Presure v Time**