

**Management of ‘tough fish syndrome’
in tropical Saddletail snapper
(*Lutjanus malabaricus*) to re-instill
market confidence.**

***Andrew Forrest, Sue Poole, Paul Exley,
John Mayze, and Carl Paulo***



Queensland Government



Australian Government

**Fisheries Research and
Development Corporation**

Project 2010/207

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August 2012

Management of tough fish syndrome

ISBN: (978-0-7345-0428-9)

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Final report of project number 2010/207 submitted to the Fisheries Research and Development Corporation in August 2012.

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Acknowledgements

Many thanks to the stakeholders of the Northern Finfish Fishery for supplying of fish at their own cost, without which this work could not be possible. Particular thanks to our industry partners who contributed financially, namely **Bill Passey** (Australia Bay Seafoods), **Horst Fisher** (Northern Seafoods), **Steve Hinge** (Goldband Nominees Pty Ltd) and **David Caracciolo** (NT Fish Pty Ltd).

Thanks also the skippers of the vessels that participated in this project. In particular, **Grant Nicolias**, skipper of FV Starlight for giving me space on his vessel to perform our experiments, and taking the time to explain the ins and outs of the fishery ground and the fishing methods employed. And to **Raymond Passey** of FV Territory Leader for providing so much time and effort with his crew to enable the authors to complete this project.

Many thanks also to **Anita Sykes** and **Janet Stark** of CSIRO Food Science for completion of sarcomere analysis.

A very special thank you to **Katherine Sarneckis**, Chief Executive Officer of the Northern Territory Seafood Council for her tireless support and strict adherence to timelines.

The authors would also like to acknowledge the support and funding contribution of the **Fisheries Research and Development Corporation** without which this research would not be possible.

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OBJECTIVES:

1. To identify any post-capture practices that may influence the occurrence of tough fish syndrome (TFS).
2. To identify links between TFS and specific physiological factors in tropical Saddletail snapper
3. To establish variability of TFS in relation to season and capture location
4. To develop recommendations and strategies for industry stakeholders to minimise the impact of TFS
5. To communicate findings and recommendations to stakeholders and assist with the implementation of any changes to current fishing and handling practices

NON-TECHNICAL SUMMARY

OUTCOMES ACHIEVED TO DATE

A major achievement of this research is the confirmation of fish age being the primary driver of toughness in cooked Saddletail snapper flesh. Knowing the cause provides confidence to develop solutions and make business decisions for the reef fish fisheries.

The influence of other fish physiological factors was shown to have little impact with respect to fish flesh toughness. Additionally, there was no apparent connection between flesh toughness and seasonality nor year to year conditions. The possibility of toughness being engendered through inappropriate chilling immediately post capture was ruled out as a factor contributing to tough fish syndrome (TFS).

The outcome following on from identification of the cause of TFS surrounded developing procedures for reducing the incidence of tough fish where the cause was preventable. However, as post-harvest handling was not a contributor to toughness, implementing alternative handling procedures was irrelevant.

The focus of this outcome shifted to developing strategies to ensure fish at risk of exhibiting TFS did not enter the value chain. This was addressed by provision of currently available technology on non-invasive ultrasonic imaging and near infrared spectroscopy (NIRS) to the industry partners. Additionally, the authors proposed several alternative approaches to address minimising the impact of TFS. Each approach was considered fully with industry at a special stakeholder meeting (November 2011) and options reduced to the two most favoured by the key members.

Due to the obvious passion and commitment from industry towards resolving the TFS issue, and although beyond the scope of the project as stands, additional work was undertaken: an assessment of consumer perceptions of toughness in cooked Saddletail flesh and 'proof-of-concept' trials with enzyme treatment of tough fillets.

The current project was able to establish an accurate incidence of TFS in Saddletail snapper and was designed to substantiate the perceived correlation between fish age and flesh toughness, as well as assess the influence of other linked factors. A total of 504 Saddletail snapper, caught over 4 years from northern Australian waters, were subjected to flesh texture analysis. Of the total, the majority (71%) demonstrated a textural firmness in the range of 40-60 millijoules/gram (mJ/g) required to shear the flesh.

As previously reported the primary driver of toughness in cooked Saddletail snapper flesh appeared to be age of fish. Work in this current project confirmed this inference, with results demonstrating a highly significant correlation between fish age and flesh firmness. The previous work identified a potential seasonal influence for toughness. Additional data collected during this work allowed analysis four years of seasonal information. Analysis of the greater sample population demonstrated influence of seasonal effects was not significant over the four years examined.

The influence of sex, size and weight of fish was assessed with respect to cooked flesh texture. It was established that although male fish are significantly larger

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than female fish of equivalent age, no differences exist between the cooked flesh textures of fish of equivalent age. Stress is often attributed as affecting the textural quality of fish flesh and a ready measure of the stress experienced is final pH. Results showed differences in mean final pH values in fish were highly significant, however there is no obvious relationship connecting pH to cooked flesh toughness.

Cold shock syndrome can occur in tropical fish when their temperature is reduced abruptly from ambient and results in physical stiffening characteristics similar to rapid onset of rigor mortis. To establish whether this effect was influencing toughness of flesh from Saddletail snapper, trials were undertaken at different chilling rates immediately post-capture of fish. No significant differences in the rate of rigor mortis development were observed between three chilling temperatures. However, a clear pattern in the development of rigor was illustrated from the mean rigor values of fish from each chilling method. Fish subjected to the coldest chilling medium (ice slurry) showed a faster rate of rigor development than fish held at warmer temperatures. Fish remaining at ambient had delayed onset of rigor with subsequent slower development.

Our work has illustrated no cold shock in Saddletail when subjected to a sudden and large temperature differential beyond the normal rigor mortis development process. Nor was there any difference in sarcomere length observed between fish chilled at different rates. Both these findings support the conclusion that toughness in Saddletail snapper is not directly caused by the fish experiencing cold-shock syndrome.

Consumer perceptions of toughness

Texture analyses indicated and range of cooked flesh quality with respect to degree of toughness. It became critical to understand the consumer perception of toughness and 'how tough is too tough'. This information is required to establish at what level Saddletail snapper becomes unacceptable to the consuming public and therefore, what proportion of the catch is affected.

Results from a threshold of rejection consumer assessment showed a clear trend of reducing acceptability with increase in toughness. When this toughness level is applied to data collected from all Saddletail snapper over four years, 11.6% of all fish exceed this value of 72mJ/g in firmness. When the perceived toughness is reduced to 60mJ/g, the proportion of the catch exceeding that level increases to 25%. This illustrates that 1 in 4 within the catch may be perceived negatively at the consumer plate. Based on 2009-2010 fishery data such a proportion equates to \$13M revenue.

Strategies to minimise TFS impact in the marketplace

No simple assessment method is available for on-board use to measure a parameter that will predict the flesh texture of whole Saddletail snapper. From in-depth discussions with industry stakeholders to develop strategies to address this conundrum, three possible approaches were defined.

The aim is to differentiate fish with a higher probability of exhibiting TFS from the rest of the catch. As male Saddletail are much larger than female of equivalent age, length/age correlations are ineffective for Saddletail snapper without sexing the fish. So our focus turned to differentiating between males and females of mature age. A summary document was prepared on current applications of sensing and imaging technology.

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There are several processing technologies that may show promise with respect to mitigating the toughness of flesh when cooked. These have been reported here and communicated to the stakeholders. There is value in a simple strategy based on consumer information. Experience suggests that still only a small proportion of the population has knowledge of Saddletail snapper and its eating qualities. Education on the texture of Saddletail flesh and its suitability for specific cooking styles may remove the consumer surprise at the firmness of the flesh. Any such programme would need to highlight the positive qualities of Saddletail, of which there are many.

Consensus among the key industry stakeholders was for concept development of enzyme processing treatment for tough fish. Proof-of-concept trials undertaken demonstrated addition of a plant based protease prior to vacuum packaging supplied significant improvement in cooked fillet texture. This research has the potential to provide stakeholders with a value addition pathway for fish identified as high risk of developing TFS, and warrants further investigation within a commercial fish processing environment.

KEYWORDS:

Saddletail snapper, *Lutjanus malabaricus*, flesh toughness, fish texture, fish age, consumer perception,

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1. Background

Saddletail snapper constitutes a significant proportion of the catch in offshore finfish fisheries in northern Australia. A small but significant proportion of these fish become 'tough' at the point of cooking, resulting in an inedible product. Currently it is not possible to identify affected fish earlier than the consumer plate. The occurrence and the lack of any methodology to detect or manage Tough Fish Syndrome (TFS) is responsible for a strong consumer backlash which has resulted in a substantial reduction in price, cancelled orders and wasted product.

This project proposal is a direct result of primary findings from FRDC project 2008/208 *Improving profitability to Industry through the identification and management of 'tough fish syndrome' in tropical Saddletail snapper* (2010). The findings from FRDC 2008/208 evidence support of a correlation between season and flesh firmness, but it is insufficient to definitively identify the causative agents or formulate management options for the syndrome. There may also be other influencing factors. To eliminate unlinked influences and gain meaningful data on which to develop management strategies, it is proposed that similar methodology, as used in 2008/208, continue to be applied through this proposed project.

The project application for 2008/208 was developed at the specific urging of the reef fish value chain in the Northern Territory, Queensland and Western Australia. Industry stakeholders in all three jurisdictions are suffering large revenue losses caused by TFS. Outcomes from the research (both 2008/208 and this proposed project) directly address the profitability issues of reef fish fisheries and the local and export demand for reliably high quality Australian product.

The primary challenge is to positively identify the cause of the syndrome (which FRDC 2008/208 has, in part, achieved). This will facilitate development of management strategies to re-instill consumer confidence in the quality of Saddletail Snapper and reef fish in general.

1.1. *The problem*

TFS is exhibited by some tropical reef fish in which the texture of the flesh toughens severely after cooking, rendering the fish inedible. Such flesh toughness only manifests its self on the consumer plate. Prior to this point the fish is not visually different to any other fish, the fish fillet and the raw flesh has similar texture to that of non-tough fish, but upon cooking a 'tough' fish will have a texture that is described as "extremely rubbery", "car tyre-like". Market awareness of the risk is now widely entrenched with buyers refusing to handle specific reef fish. Queensland wholesalers (Cardinal Seafoods, Mackay Reef Fish Supplies) refuse to buy Grass Emperor off the boats as they consider the incidence of TFS to be unacceptably high. TFS is consequently causing significant revenue loss for the industry, as fully evidenced by the recent cancellation of large retail supply contracts for Saddletail Snapper.

1.2. *Current industry information*

TFS has been observed for several years, with increasing reported incidence over the last three to four years. As most of the available evidence is subjective, it is difficult to accurately state the real incidence. Rigorous data on the incidence of

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TFS within catches is needed to quantify the problem and determine the causative factors.

TFS has been reported across all Australian tropical reef fish fisheries and in fish caught by all methods of capture: dropline, trap and trawl. The problem appears to be pervasive and affects a significant proportion of several commercially significant species. Most tropical snappers are affected and specifically, species such as Saddletail snapper, Crimson snapper, Red emperor and Golden snapper are implicated. Reports indicate that occurrence is not consistent, with only a proportion of fish from any one catch affected. There are no obvious common factors denoting which fish will be 'tough' although colloquial evidence suggests that larger fish (>3kg) are more likely to exhibit this syndrome.

Communications with stakeholders reveal that Saddletail snapper is a dominant part of a multi-species fishery across the tropics. All fish landed must be marketed because returning lower-value species to the water is not an option. A large proportion of 'redfish' in a catch severely reduces the profit margin for any given trip, so much so that the species is often sold with a minimal or no margin simply to prevent wastage.

Round table discussions with major stakeholders in the fishery illicit the belief that TFS could also be influenced by cooking method or style. They suggest a similarity of situation as with different beef cuts – gravy beef and eye fillet steak are not appropriate for same cooking techniques. Industry was keen that this project proposal consider this aspect and as such, a cooking component has been incorporated in this application.

Dialogue with those involved in reef fisheries across northern Australia, indicates there are locational differences related to the incidence of TFS. It appears that large Saddletail Snapper (9-10kg) regularly caught off Agnes Waters (at the reef drop-off, lower Queensland east coast) do not exhibit TFS. Similarly, most Saddletail snapper caught in mid-latitude Western Australian waters are not 'tough'. The syndrome seems to only be prevalent in fish caught in truly tropical latitudes across northern Australia. Again, the industry is eager to understand whether this is, in fact, a reality and if it is the case – why?

1.3. Current science knowledge

Textural variation issues in fish muscle texture has previously been identified in many species including Atlantic halibut (Hagen *et al.* 2007), Atlantic Cod (Love 1979) and Japanese ayu (Ito *et al.* 1992). However, the majority of research has focused on issues surrounding the potential for tissue to soften or gape post-harvest rather than excessive firmness of the cooked muscle.

Toughness issues in terrestrial animal sources of meat have been broadly investigated and reported. Two extensive reviews in this area have been published by Shorthose and Harris (1991), and Harper (1999). What is clear from these reviews is the nature of the relationship between muscle structure, physiological function, phenotype, gene expression and levels of metabolic enzymes is so complex as to make determinations of cause and effect with respect to meat toughness extremely difficult (Harper 1999). Upon the death of an animal adenosine triphosphate (ATP) production ceases and the actinomyosin complex becomes more tightly associated. This is the process called *rigor mortis*. The rate at which *rigor mortis* commences is driven by the level of glycogen present within the muscle.

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However the relationship between muscle glycogen, ultimate muscle pH, sarcomere length and toughness is a complex relationship. In short, beef from a non-stressed animal rapidly achieves a pH<5.7 before the onset of *rigor mortis* (Tornberg 1996). However, pre-slaughter stress depletes the amounts of muscle glycogen and slows the rate of pH reduction post mortem. Devine and Crystal (1998) have demonstrated that muscle toughness will be maximised if the a final muscle pH falls in the range of 5.8-6.2.

Pre-slaughter stress has also been identified as playing a role in fish muscle quality. Glycogen depletion resulting from pre-slaughter stress reduces the time taken to go into *rigor mortis* and can also cause a stronger rigor mortis. Sigholt et al (1997) demonstrated 10 minutes of pre-slaughter stress in farm raised Atlantic salmon (*Salmo salar*) resulted in significantly softer fillets measured by texture analyser and descriptive sensory analysis.

The role of nutrition has been identified as being a significant factor in the quality of Atlantic cod (*Gadus morhua*) by Love (1979). More recently, starvation prior to slaughter has been demonstrated to significantly increase the hardness of raw fillets (Einen and Thomassen 1998). However this effect was not apparent after 12 days storage on ice, and a sensory panel found the starved fish (between 30 and 86 days) to have a significantly decreased fresh flavour. Eien and Thomassen concluded starvation prior to slaughter was a weak tool for changing fillet quality in Atlantic salmon.

Beef sarcomeres within the myofibrillar structure tend to shorten during *rigor mortis* (Dransfield 1992). However, the mechanism for shortening is not well understood. Careful temperature control during the first 24 hours post-slaughter has been identified as a key preventative measure to reducing the impact of sarcomere shortening (Koochmaraie *et al.* 1996). Beef muscles that are stretched under tension by attachment to immobile bones rarely demonstrate short sarcomeres (Harris and Shorthose 1988).

Very little research investigating the relationship between fish muscle sarcomere length and texture has been published. Yoon et al (1991) examined fish muscle sarcomere lengths during investigation into the relationship between trimethylamine oxide (TMAO) in freeze induced texture changes observed in frozen fish mince. Yoon concluded that TMAO had no role in the development of toughness in minced fish muscle. Sigurgisladottir et al (2001) investigated the differences in muscle microstructure and texture of fresh and smoked farmed Atlantic salmon (*Salmo salar*). Muscle fibres were found to shorten during the salting and drying process. However sarcomere lengths did not change.

In beef the two primary sources of textural variation are connective tissue and myofibrillar structure (Harper 1999). These structures are also considered by Harper to interact mechanically. Harris and Shorthose (1988) and others suggest myofibrillar structure contributes more than connective tissue with regard to overall toughness in beef. However Kuypers and Kirth (1995) determined that connective tissue did make a contribution towards toughness in beef, and this contribution was disproportionately greater than would be expected with respect to the weight of connective tissue in muscle.

Collagen content has previously been identified as having a direct influence on the texture of raw fish (Sato *et al.* 1986). More recent authors have achieved mixed results. Li et al (2005) demonstrated no relationship between fillet firmness and

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total collagen content and a weak relationship ($p=0.057$) between alkali insoluble collagen and fillet firmness. Whereas Hagen *et al* (2007) found a stronger relationship ($p<0.001$) between alkali insoluble collagen and fillet texture describing 24% of the observed variation.

Perhaps of more interest is the formation of mature intermolecular cross-links between collagen fibres; providing tensile strength to the collagen fibres. The formation of these cross-links has been determined to occur with age within the connective tissue matrix (Bailey 2001). Both Li (2005) and Hagen (2007) identified a strong relationship between the mature collagen cross-linking compound, hydroxylysyl pyridinoline (PYD), and raw texture in Atlantic Salmon (Li *et al.* 2005) and Atlantic Halibut (Hagen *et al.* 2007). The linear relationship identified by Hagen accounted for up to 64% of observed variation.

A further complication surrounds the role of cooking in determination of meat toughness. Several authors have identified low correlations between the toughness of cooked beef and the properties of raw meat (Devine and Chrystall 1998; Lepetit and Culioli 1994). Cooking has also been previously unsuitable for rheological testing of fish muscle (Dunajski 1980). Einen and Thomassen (1998) have also suggested that pre slaughter starvation effect raw and cooked fillets in different ways.

Many factors have an influence on the eating quality of flesh texture in fish. These factors include:

1. Physiological factors such as size, condition, age, gender and sexual maturity, season, muscle structure, collagen content, diet and capture location;
2. Capture methods including immediate post-capture handling, onboard chilling method and storage time;
3. Cooking practices including cooking method, cooking time; and
4. Phase of rigor mortis of the fish at cooking.

Under ideal handling conditions, tropical fish go into and through rigor mortis slower and more gently than temperate species (Curran *et al.* 1986; Poole 1991). It is also known that tropical fish can suffer from 'cold-shock' syndrome similar to that occurring in beef carcasses when chilled suddenly (Curran *et al.* 1986). Investigations using nucleotide analysis within the FRDC 2008/208 failed to reveal any significant cold shock event, however this cannot be completely discounted. Analysis of samples by electron microscopy (carried out as part of the work) revealed a trend that suggested a cold shock event may have occurred within fish exhibiting toughness.

Textural irregularities have been demonstrated to be seasonal in some fish species (Hagen *et al.* 2007). Specifically, differences in collagen types present, their role and degree of cross-linking of collagens is associated with textural variability in raw fillets. For example, Hagen demonstrated that aquacultured halibut harvested in spring would result in firmer texture than other seasons.

Results from the current study (FRDC 2008/208) being undertaken have illustrated a significant difference between the textural firmness of Saddletail snapper caught in late spring/early summer to those caught in autumn. Significant differences were also observed between male and female fish caught in early summer. However, these differences were less significant when fish were captured in autumn.

1.4. *This project*

This project built upon the results already obtained from the previous study (FRDC 2008/208) to obtain a clearer understanding of the role of seasonal effects. The role of 'cold shock' in the development of TFS has also been investigated. Stakeholders have also been provided with a review of non-invasive technologies that may assist in identifying fish with a high risk of developing TFS, as well as some product concepts that directly address the use of fish exhibiting TFS.

2. Need

Data obtained from field trips as part of the current project (FRDC 2008/208) indicate the development of TFS may not be due to inappropriate onboard handling practices. Textural issues appear to be due to a combination of biological, geographical and seasonal factors. Results from the current research suggest a significant influence of both sex and season on the toughness observed in Saddletail snapper. Current findings do not discount potential compounding influences such as 'cold shock' for example.

The need for stakeholders is to develop an understanding of these effects on the development of TFS within the Saddletail snapper resource. Once this has been achieved, strategies can be developed to best manage the issue. This may involve the use of non-invasive technology to identify fish at risk of exhibiting TFS and remove them from the supply chain.

Further work is also required to find suitable markets for fish identified as high risk of TFS. This may include novel product concepts not currently utilised with the value added seafood product market.

These outcomes will allow stakeholders to market their premium product with confidence, achieve an increase in price commensurate with premium quality reef fish and ensure the ongoing sustainable use of the resource. And also support the development of value added seafood products from those fish identified as high risk of developing TFS.

This proposed project addresses the focus of increasing profitability and optimum utilisation of fish identified by the NT, Qld and WA within their respective current fisheries research and development priority documents.

3. Objectives

1. To identify any post-capture practices that may influence the occurrence of TFS
2. To identify links between TFS and specific physiological factors in tropical Saddletail snapper
3. To establish variability of TFS in relation to season and capture location
4. To develop recommendations and strategies for industry stakeholders to minimise the impact of TFS
5. To communicate findings and recommendations to stakeholders and assist with the implementation of any changes to current fishing and handling practices

4. Methods

4.1. Planned field trips

Three major field trips were conducted over the course of the project. A summary of the field trips is presented in Table 1. The trial names used for this project are a continuation from those in the previous work (FRDC project 2008/208).

Table 1. Field trip and sample collection summary across project.

Project	Trial Name	Season/Year	Fishing/Transport	Total Fish (629)
2008/208	FT1	Oct 2008	Trap/Fresh	50
2008/208	FT2	Dec 2008	Trawl/Frozen	111
2008/208	FT3	Apr 2009	Trawl/Frozen	101
2008/208	FT4	June 2009	Trap/Frozen	31
2008/208	FT5	Oct 2009	Trawl/Frozen	109
2010/207	FT6	Oct 2010	Trap/Fresh	45
2010/207	FT7	Dec 2010	Trawl/Frozen	99
2010/207	FT7	Dec 2011	Trawl/Frozen	83

4.2. Transport and logistics

Fish landed fresh were packed into approved air freight Styrofoam boxes and sent to Brisbane by Australian Air Express the same day. Fish landed frozen were sent to Brisbane by frozen truck transport.

4.3. Sample processing (HFSP Coopers Plains QLD)

Fish were kept frozen at -29°C prior to processing. Fish were allowed to relax at 4°C for 24 hours and then immersed in ambient tap water for 2 hours prior to filleting and sample collection. Fork length, weight, sex, and ultimate pH of muscle were recorded at this time. Muscle pH was recorded using a TPS pH unit (Model number WP 80, Springwood, QLD).

4.4. Rigor assessment

The primary aim of this experiment was to determine whether 'cold shock' previously observed in tropical species (Curran *et al.* 1986; Parry *et al.* 1987) could be forced by standard commercial fish practices applying to Saddletail snapper. And if so, determine whether this 'cold shock' phenomenon causes a shortening of muscle fibre sarcomere length as is seen during 'cold shortening' in land based animals carcasses.

Live landed Saddletail were euthanased by brain spike prior to immersion in the chilling media. Three temperature profiles were utilised for the chilling process directly after euthanasia. These were ice slurry (0-1 °C) temperate seawater (12-

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15°C) and ambient seawater (29-30°C). A total of 15 fish were assessed per treatment. A summary of the treatments is presented in

Table 2. Summary of chilling treatments.

Treatment	Temperature	No of fish assessed
Ice Slurry	0-2°C	15
Temperate	12-15°C	15
Ambient	29-30°C	15

Sampled fish were assessed for rigor development prior to immersion and then every 30 minutes for 4 hours. After which, fish were transferred to the brine tank to continue chilling and storage as per the standard protocols of the vessel.

Rigor assessment was achieved by use of a five point category scale to describe the state of rigor. The five categories correspond approximately to the angle of flexibility observed in the fish. Fish were scored as presented in Table 3.

Table 3. Observed rigor stage and scoring system.

Presence of rigor	Observed bend in fish (° of bend from horizontal)	Rigor score
No rigor	~90°	1
Some rigor	~60-70°	2
Moderate rigor	~45°	3
Significant rigor	~20-30°	4
Full rigor	~0°	5

Sampled fish were placed right-hand side down on a nylon cutting board so that the tail would hang over the edge of the board. Each fish was positioned so that the end of the pelvic fins aligned with the edge of the board to allow for equivalent tail hang. Also, the fish was positioned so that a line from the end of the pelvic fins to the bottom jaw line was perpendicular to the edge of the board. This method is illustrated in Figure 1.

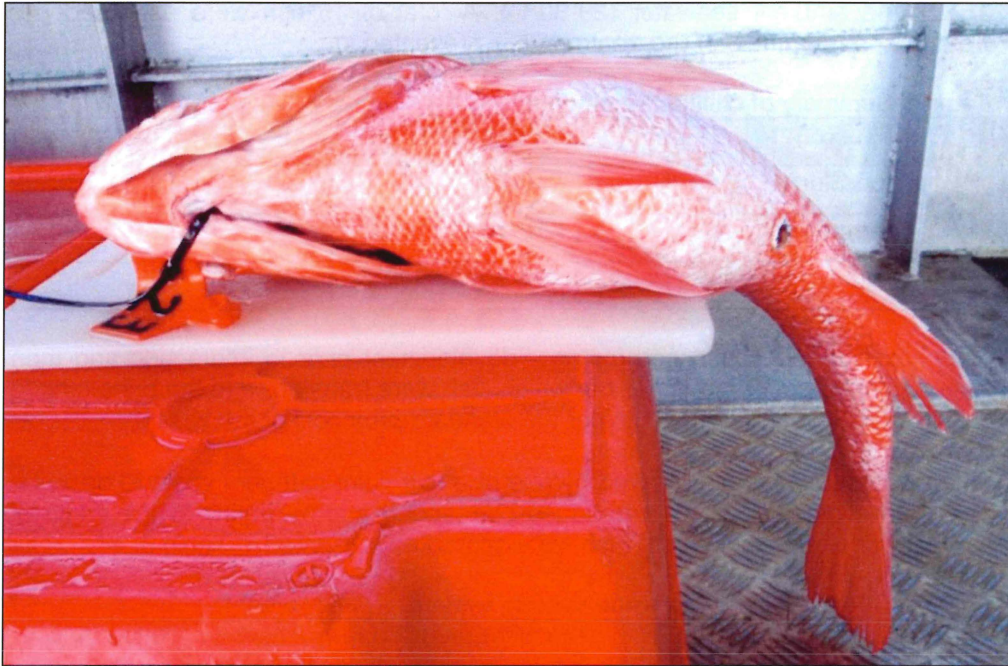


Figure 1. Rigor assessment by observed fish flexibility.

To confirm the presence of 'cold shortening', tissue samples were taken for laser diffraction analysis as described by (Bouton *et al.* 1973). Sarcomere lengths were measured using a helium-neon gas laser diffraction technique on unfixed portions taken from frozen (-20°C and -80°C) samples. The laser has a wavelength of 635nm, and was used as the light source to obtain diffraction patterns from muscle fibre samples held between glass microscope slides.

This process provides an accurate measure of sarcomere length and muscle fibres exhibiting cold-shortening will display significantly shorter sarcomere length than non-affected muscles. This work was completed by Anita Sykes and Janet Stark of CSIRO Food and Nutritional Sciences, Coopers Plains.

4.5. Temperature logging

Temperature logging of 2 whole fish, brine tank, and both fish holds, was conducted during FT6. using a Thermocron temperature logger (OnSolution, Baulkham Hills, NSW). Temperature values were taken every 15 minutes.

4.6. Texture analysis

Texture analysis was performed on cooked portions of Saddletail fillet. The left side of the fish was always used as the right side had tissue sample taken from it and results would have been compromised.

Filletts were vacuum-packed into plastic bags and steamed at approximately 95 °C for 20 minutes. The filletts were then allowed to return to room temperature (24 °C) prior to texture analysis. This would take approximately 2 hours.

Analysis was conducted on an Instron 5543 texture analyser (Instron Corporation, 825 University Avenue, Norwood MA, USA.) using a 500N load cell and a modified Kramer-Shear cell. The modification was to remove two of the five

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blades. This was decided after a fish identified as being tough in preliminary assessments gave values that were in excess of the load limit of the cell. Figure 2 shows the Instron in use with a sample of Saddletail in the modified Kramer-Shear cell.



Figure 2. Instron texture analyser with sample loaded into the Kramer-Shear cell.

Two samples per fillet were taken for assessment. These samples were taken longitudinally from the shoulder end of epaxial myotome. The samples were placed within the cell so that the blades were cutting across the direction of the muscle fibres. A photograph of a fillet being sampled is presented in Figure 3.

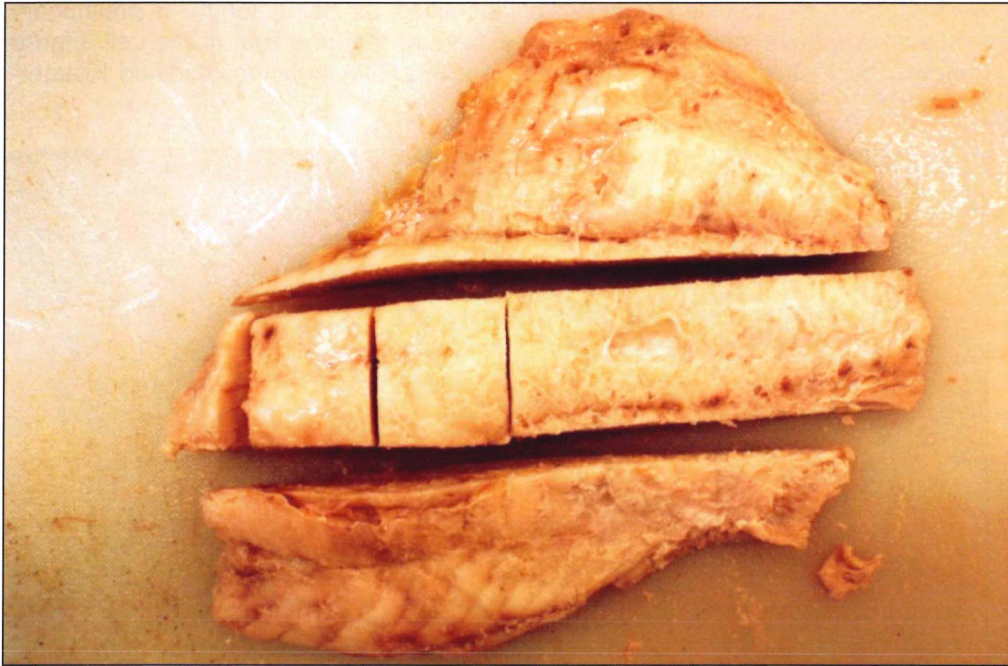


Figure 3. Photograph of sample preparation for texture assessment by Instron.

Two forms of data were collected during this analysis. The first is peak force of shearing and is expressed in newtons (N). This is the maximum force required to shear the cooked muscle. The second is total energy required to shear the cooked muscle and is expressed in millijoules (mJ). The results presented here are also divided by the weight of the sample (g) and expressed in millijoules per gram (mJ/g).

4.7. Sarcomere Length Determination

Sarcomere lengths were measured using a helium-neon gas laser diffraction technique on unfixed portions taken from frozen (-20°C and -80°C) samples. The laser has a wavelength of 635nm, and was used as the light source to obtain diffraction patterns from muscle fibre samples held between glass microscope slides. Sarcomere length was determined from the diffraction pattern displayed on a frosted screen (Bouton *et al.* 1973). Sarcomere length (μm) was calculated from the average distance (mm) of the inner and outer diffraction bands from the centre of the screen. The mean of 4 readings was taken per sample (not every sample had 4 readings). This work was completed by Anita Sykes and Janet Stark of CSIRO Food and Nutritional Sciences, Coopers Plains.

4.8. Estimation of fish age by otolith increment and predictive model based on otolith weight

Fish age data was sought after analysis of results from FT2 in 2008 (Forrest *et al.* 2010). During this work, a small but significant relationship was identified between fork length and work done. From this point on, otoliths were collected from all field trips. However, otoliths were not collected during FT2 and no age data exists for those fish sampled.

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Saddletail collected during field trips FT3 and FT5 were examined for age estimation

with the assistance of Queensland Fisheries staff from Southern Fisheries Centre, Deception Bay using the standard method of increment determination of otolith cross-sections (Fisheries-Queensland 2009).

During the course of this work our access to Queensland Fisheries became severely limited and another method of fish age estimation was sought. The age data collected from FT3 and FT5 was used to develop a predictive (linear) model that would assist in providing an estimate of fish age from otolith weight.

4.9. Consumer Assessment: Threshold of rejection

Samples of Saddletail snapper used for this assessment were duplicate fillets of the previous field trip used for texture determinations (field trip 8). Upon initial processing, the left side fillet is used for texture assessment, and the right side fillet is kept frozen for retention (-29°C). It is these right side fillets that were used for consumer assessments.

As these fish have all been assessed for cooked texture, we can establish categories of texture values for samples to be allocated to. For this assessment, four categories of texture were established. Texture values are expressed in millijoules per gram (mJ/g) of cooked fish sample. These are presented in Table 4.

Table 4. Categories of texture used for consumer assessment.

Texture category	Texture range (mJ/g)	Mean texture (mJ/g)
1	35.43 – 40.14	37.58 (±1.82)
2	48.01 – 51.05	49.66 (±0.99)
3	55.80 – 63.59	59.40 (±2.83)
4	68.33 – 76.48	71.98 (±2.51)

Consumer assessments are usually conducted via one of two basic methods. The first involves the use of category or line-scales to determine how much a consumer either likes or dislikes a product. Category scales involve using an odd number of categories; usually five, seven or nine categories. The premise of this style of assessment is to obtain a quantified value of likeness or dislikeness. This method is usually employed when comparing products against each other. The authors have previously been used category scale consumer assessment of new apple varieties in comparison to current commercial apple cultivars (Zeppa 2007).

However for the purpose this assessment a binary forced selection method was determined to be more applicable. In this method, consumers are forced to make a selection to accept or reject a sample on any given or suite of parameters. Similar assessments have previously been conducted a broad range of consumers products including rancidity in milk (Hough *et al.* 2004) and beef (Platter *et al.* 2003). This method is also used in non-food consumer applications like mobile multi-media image quality (Jumisko-Pyykk *et al.* 2008). For this assessment a level of rejection was deemed to be 50% of consumer responses. This is consistent with the work of Hough *et al.* (2004) and others.

Sixty (60) consumers of seafood were recruited from staff on site at 39 Kessels Road Coopers Plains (Queensland Government). Panellists were qualified as

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being those who consumed seafood at least once a month. Consumers were also asked demographic questions on seafood buying preferences and frequencies.

Filletts of Saddletail were prepared in the same manner as that used for the initial texture analysis (Forrest *et al.* 2010). Whole filletts were vacuum-packed in barrier bags and steamed in a Unox LineMiss™ steamer oven (Model XF135 Padova, Italy) at 95°C and 60% humidity for 20 minutes. Filletts were then allowed to rest for 5 minutes at ambient temperature (24°C) and then cut in sample portions of approximately 2cm x 2cm square. Samples were then placed in small foil trays and a layer of foil was wrapped over the tray. Samples were then returned to an oven set at 65°C until panellists were ready to receive samples for assessment.

Assessments were conducted in the sensory booths at Coopers Plains. The questionnaire was completed via computer using Compusense® *Five* (Guelph, Ontario, Canada). Panellists were presented the four samples in a randomised order.

Consumers were asked "*Please find sample XX and taste a portion of this sample. Is the texture of this fish acceptable to you?*" Answers available to consumer were either "*Yes, this texture is acceptable to me*" or "*No, this texture is unacceptable to me and I would reject this dish.*" Consumers were then asked to comment on any aspects of the samples that they liked or disliked.

A printed copy of the questionnaire in full can be found in Appendix 3.

4.10. Statistical analysis

Statistical analysis was conducted using GenStat Fourteenth Edition, version 14.1.0.5943 supplied by VSN International Ltd (www.vsnl.co.uk)

5. Results and discussion

5.1. Rigor mortis progression and 'cold shock' (FT6)

5.1.1. Rigor mortis progression

No significant differences in the speed of rigor development were identified between the three chilling media temperatures previously described (Table 2). However, a trend in the development of rigor is apparent from comparison of the mean values for fish from each of the chilling methods (Figure 4). Fish subjected to ice slurry progressed to rigor at a faster rate than fish held at warmer temperatures without exhibiting any instantaneous stiffening as is the case in 'cold shock.' The variability between fish within each chilling system is large though as indicated by the error bars in Figure 4.

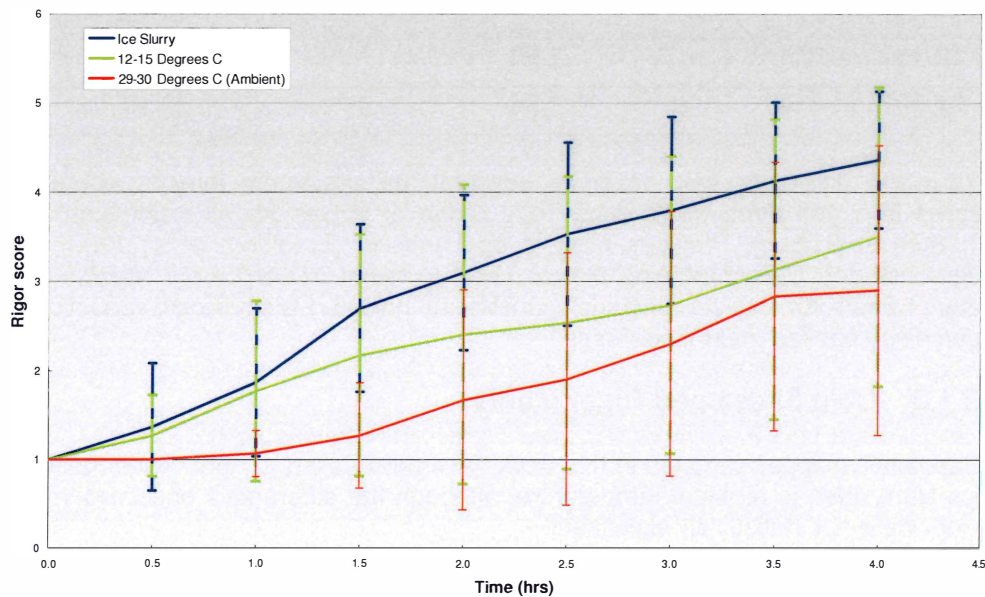


Figure 4. Mean rigor scores over 4 hours for Saddletail snapper in three media temperatures (standard error bars are shown).

Some of the variability between individual fish is explained by the fishing method. The fish are wild caught by trap and, as such, the amount of stress experienced cannot be controlled, quantified or avoided. The pre-existing physiological condition of the fish is also an unknown. This has strong bearing as stress experienced by the fish rapidly depletes the levels of ATP present in the muscle, the onset of rigor may be accelerated by the amount of time spent in the trap prior to harvest.

The suggestion that temperature has a direct influence upon the development of rigor is reasonable and consistent with the work of Jerrett et al (2002). Jerrett demonstrated that in Pink snapper (*Pargus auratus*) post-mortem metabolic rates sharply increased below 6° C and above 16° C. The lower threshold is also in agreement with the work of Iwamoto (1987) and Watabe (1989).

The upper limit identified by Jerrett is not consistent with the data represented in Figure 4. However, the value of 16° C as the upper threshold determined by Jerrett

is close to the mean annual sea temperature reported in their work (being 21 °C in summer and 10°C in winter). With sea temperature in the Arafura Sea being significantly higher than this range, and with far less seasonal variation, it may be reasonable to suggest that the upper threshold for Saddletail snapper will be much closer to ambient temperature (29-30 °C) than 16 °C as reported by Jerrett.

5.1.2. Sarcomere Length Analysis

No significant differences were observed between the sarcomere lengths of muscle sample from each of the treatment methods. A summary of these results are presented in Table 5.

Table 5. Summary of sarcomere length analysis.

Treatment	Mean Sarcomere Length (µm)	Standard Deviation
Ice slurry (n=14)	1.91	±0.06
12-15 °C (n=15)	1.92	±0.09
29-30 °C (n=15)	1.96	±0.10

Very little difference was observed between the sarcomere lengths of muscle fibres from the three treatments. Total range of values for all treatments was 1.78µm to 2.08µm. Previous work in beef muscle (Smulders *et al.* 1990) has demonstrated cold-shortening effects need to result in sarcomere lengths less than 1.65µm for trained consumer panellist to indicated a significant reduction in perceived cooked meat tenderness.

5.1.3. Cold Shock and Rigor mortis

Saddletail snapper sampled in this work have progressed to rigor mortis faster in ice slurry than in ambient temperature, although the differences observed in this work were not statistically significant.

However, when comparing sarcomere lengths from muscle fibre within the same treatments, there is very little difference between any of the results between any of the treatments. Therefore the mechanism observed here where colder temperature result in fast rigor development is unlikely to be a 'cold-shortening' event as previously seen in land based animal carcasses such as beef (Bouton *et al.* 1973).

An increase in the rate of post mortem ATP depletion would hasten the development of rigor mortis as its onset is determined by the complete exhaustion of free muscle ATP (Love 1980). This may be described as a 'cold-shock' as previously observed by Curran *et al.* (1986), however this mechanism appears to result in very little change in sarcomere length and therefore cannot be attributed to a 'cold-shortening' event.

5.1.4. Temperature logging

Of the six temperature loggers used to monitor several aspects of the chilling and storage process during this trial, only four were successfully retrieved. A summary of the location of these loggers is presented in Table 6.

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Table 6. Summary of temperature loggers.

Logger ID	Location	Data retrieved
#86	Brine tank (return inlet cage)	No (logger destroyed)
#16	Brine tank (side baffle support)	Yes
#26	Day 2 Saddletail (inside gill plate)	Yes
#92	Day 10 Saddletail (inside gill plate)	Yes
#70	Fish hold (starboard side)	Yes
#30	Fish hold (port side)	No (logger lost onboard)

Logger #16 was employed to monitor water temperature within the brine tank. The logger was attached to the baffle in the brine tank as seen in Figure 5.



Figure 5. Temperature logger in brine tank (red tag).

Temperatures were logged throughout the fishing trip and this data is presented in Figure 6.

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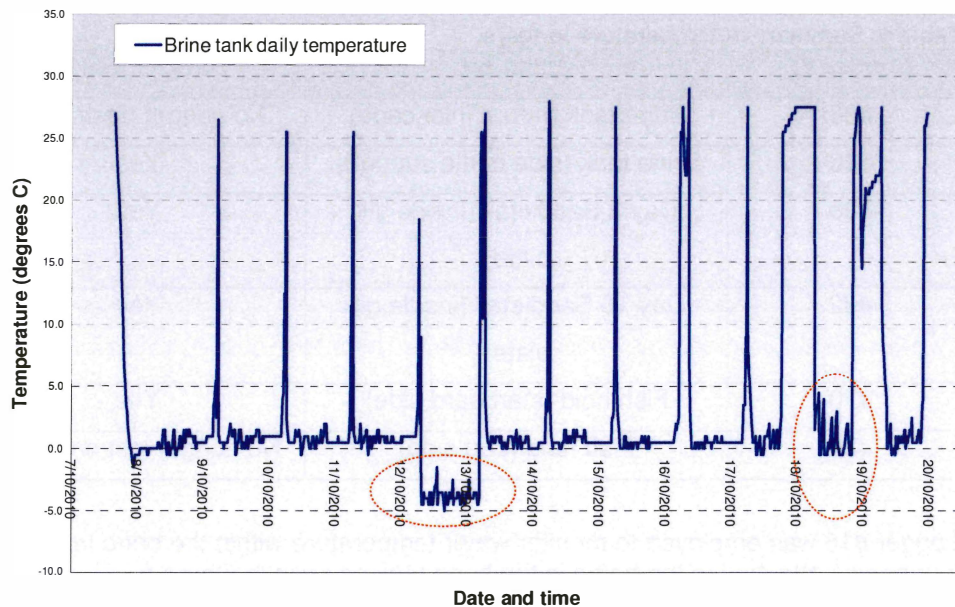


Figure 6. Daily temperature log of brine tank for all fishing day.

Very little variation in brine tank temperature was observed throughout the fishing trip. During fishing days brine temperatures rarely achieved temperatures outside the range of 0-3°C. However, two notable exceptions are apparent (see circled areas in Figure 6).

The first is the temperature achieved during fishing on the 12th of October. The temperature set point appears to have been lowered to around -4°C for this day, only to return to previous temperature ranges in the ensuing days of fishing.

The second event is on the fishing day of the 18th of October. The variation in temperature observed on this day is much greater than any other. This observed effect is consistent heavy temperature load from a large quantity of fish being loaded into the brine. However, the temperature does not exceed 5°C at any time; any chilling capacity appears to be coping with the load well as temperature spikes are quickly resolved.

Logger #26 and #92 were attached to a Saddletail snapper and positioned inside the gill cavity. The purpose of these loggers was to monitor the external temperature to which the fish were exposed during chilling and storage. So the temperature data captured with these loggers more accurately describe the conditions to which the fish were exposed rather than the internal temperature of the fish themselves.

Logger #26 was attached to a Saddletail on the second day of fishing (08/10/2010). This data is represented in Figure 7.

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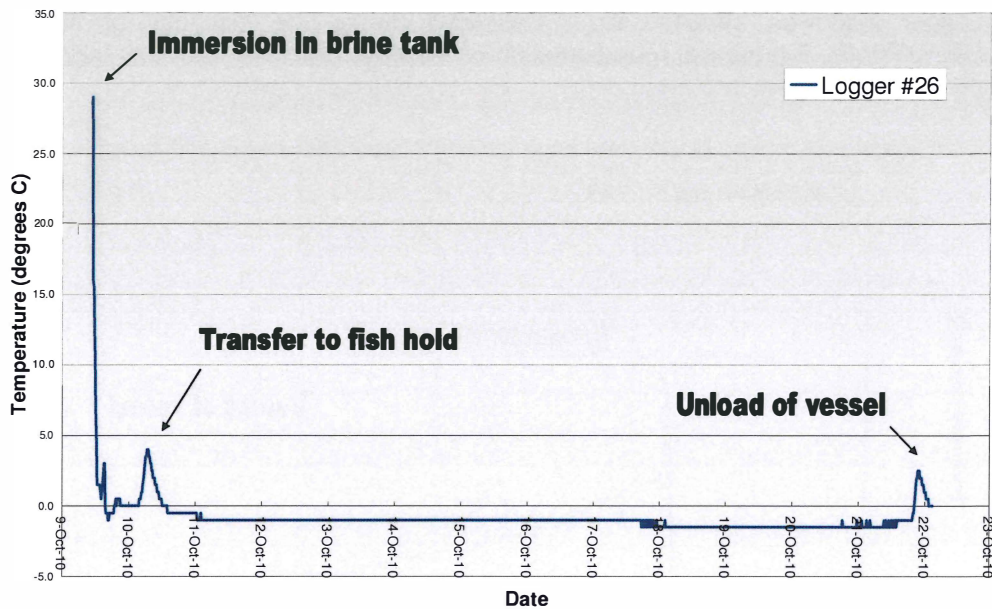


Figure 7. Temperature data for logger #26.

The data presented in Figure 7 shows that this fish has chilled quickly, and once transferred to the refrigerated brine tank, has maintained a temperature as low as -2°C until unload. A representation of the first 48 hours of chilling for logger #26 is presented in Figure 8.

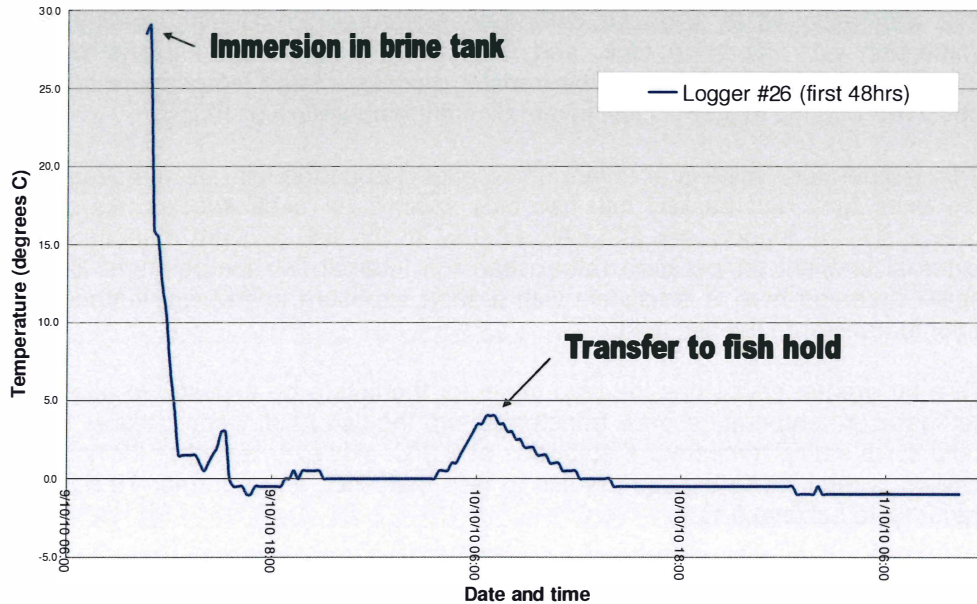


Figure 8. First 48 hours of chilling and storage for logger #26.

The Saddletail attached to logger #26 is very quickly exposed to temperatures below 2°C within the first two hours of chilling, and then achieves temperatures below zero. Early the next morning, the transfer of the fish into bins and then into the fish hold results in a small rise in exposed temperature. However, this quickly reduces back to sub-zero temperatures within six hours. This temperature profile is consistent with industry best practice.

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Logger #92 was attached to a Saddletail during the last day of fishing (19/10/2010). A graphical representation of the data collected from this logger is presented in Figure 9.

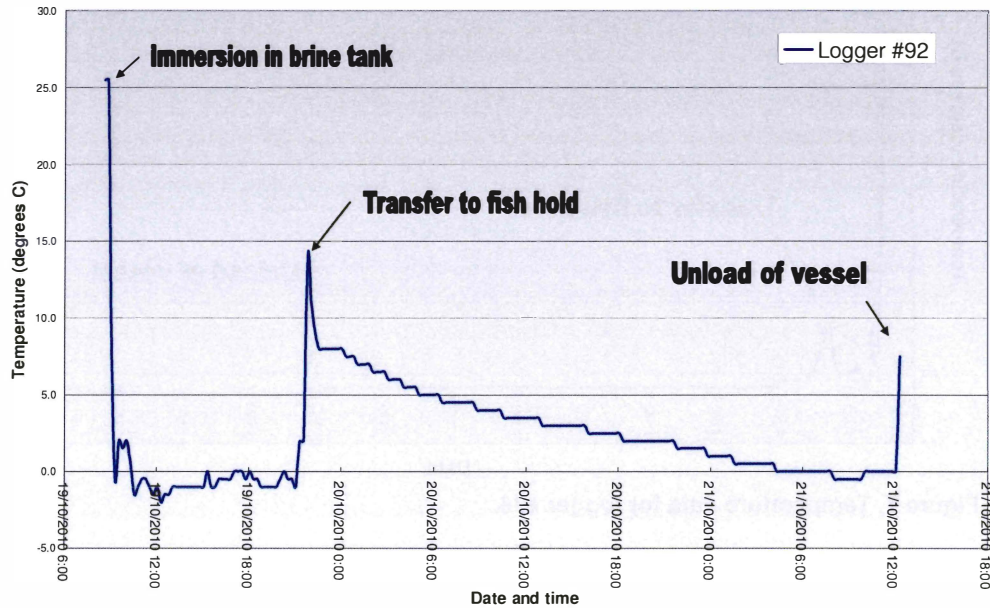


Figure 9. Temperature data for logger #92.

The temperature profile presented in Figure 9 clearly demonstrates that logger #92 was exposed to sub-zero chilling temperatures almost immediately after immersion with the brine tank, and this sub-zero temperature persisted until transfer to the fish hold. During the transfer process a large temperature spike is observed with the logger achieving a maximum temperature of 14.0 °C.

This temperature spike is an event not unexpected as the fish are removed from the brine tank and packed into fish bins specific for each species. As stated previously, the loggers are attached external to the fish and are measuring the external ambient temperature rather than the internal fish temperature. So the spike observed here is consistent with a short exposure to ambient temperature prior to transfer to the fish hold.

Of a far greater concern is the time taken for the fish to be exposed to adequate refrigeration temperature once transferred into the fish hold. Upon transfer to the hold surrounding temperature stabilises at 9.0 °C. However a further 12 hours of chilling is required to expose the fish to less than 4 °C, and a further 18 hours is required to achieve 0 °C.

Logger #70 was used to monitor hold temperature in the starboard fish hold. The logger was placed directly onto fish in a fish bin and transferred to the starboard fish hold. The data is represented in Figure 10.

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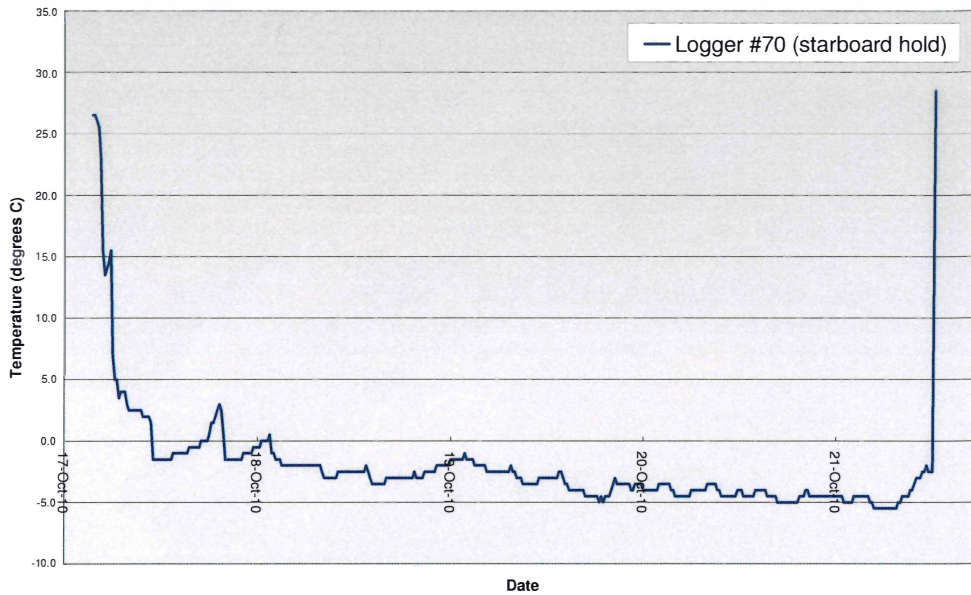


Figure 10. Temperature profile from starboard fish hold.

Logger #70 is exposed to temperature below 4°C within 3 hours in the fish hold. Despite a couple of small temperature spikes, slight sub-zero temperature is maintained for the duration of the trip. However, during the last 4 days at sea, the logger temperature approaches, and for a brief time exceeds -5.0 °C.

This occurrence is not desirable as fish will freeze at this temperature. Several bins of fish were observed as being either partly or fully frozen during unload of this vessel. The image below (Figure 11) shows a bin of Red Emperor (*Lutjanus sebae*) that were all frozen solid.



Figure 11. Bin of frozen red Emperor during unload.

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Figure 12 below also shows a bin of mixed Cod being emptied into a bin of ice. All fish were frozen solid.



Figure 12. Bin of frozen mixed Cod frozen together and emptied into ice bin.

These fish went into a mixed bin of fish for transport to a wholesaler. The rest of the bin was made up of mostly Saddletail snapper as can be seen in Figure 13 below.



Figure 13. Mixed bin of frozen Cod and Saddletail snapper.

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Almost all of the Saddletail observed in this bin were frozen solid. Figure 14 below is a close up of one of these Saddletail.

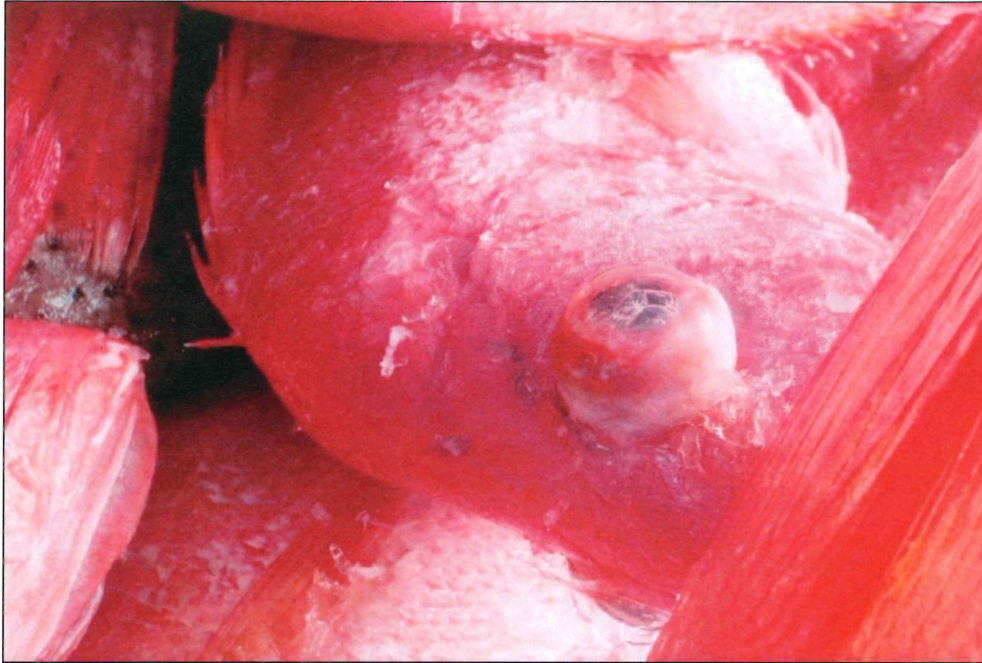


Figure 14. Frozen Saddletail in mixed bin.

Without internal temperature monitoring it is difficult to determine exactly what temperature these fish had achieved during their time in the fish hold. However, from the logger used during this field trip, a temperature of below -5°C is a distinct possibility.

Some fish transported to Brisbane also showed signs of being exposed to elevated temperatures likely to result in spoilage. Figure 15 below is of a Saddletail landed on the 3rd day of fishing (11/10/2010) showing signs of receiving temperature abuse at some stage.

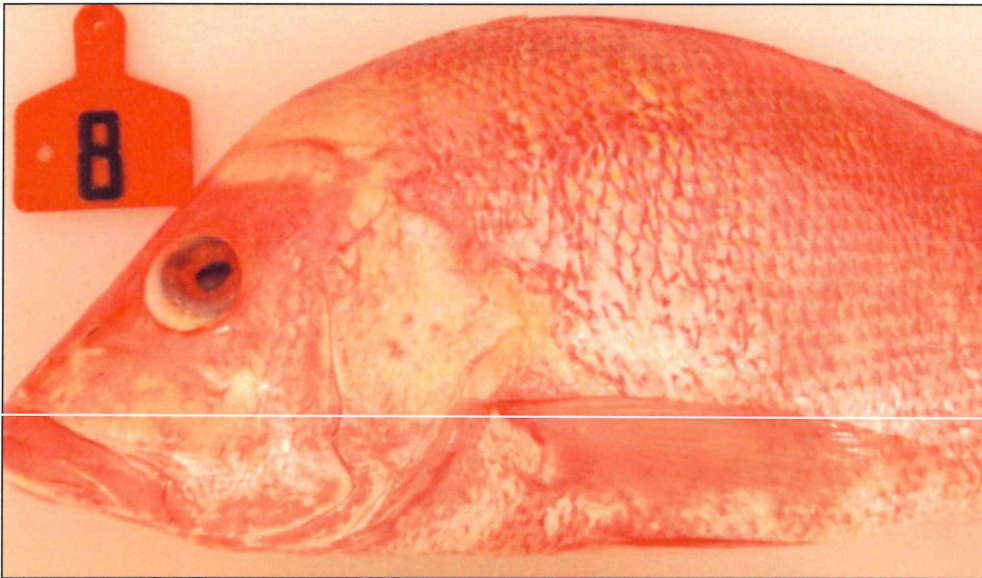


Figure 15. Saddletail snapper exhibiting temperature abuse symptoms.

Signs of temperature abuse include sinking of the eye ball into the socket and discolouration of the red colour in the skin. This discolouration is most notable on the dorsal region above the head, the gill plate, and skin of the belly cavity. Several of the fish transported to Brisbane displayed similar symptoms. This discolouration is caused by significant periods of elevated temperature above 4 °C. Given that this fish was packed in ice from the time it was unloaded from the vessel, the cause of this discolouration is most likely have been from a hot spot within the fish hold. A similar hot spot was detected by logger #92 (Figure 9).

5.1.5. Field Trip Summary (FT6)

Saddletail snapper go into rigor faster in ice slurry than in ambient temperature, although the differences observed in this work were not statistically significant. However, when comparing sarcomere lengths from muscle fibre within the same treatments, there is very little difference between any of the results between any of the treatments. Therefore the mechanism observed here where colder temperature resulted in fast rigor development is unlikely to be a 'cold-shortening' event as previously seen in land based animal carcasses like beef (Bouton *et al.* 1973).

The results achieved for this species appear to be consistent with those observed in the work of Jerrett *et al.* (2002) who demonstrated that post-mortem exposure of Pink snapper (*Pargus auratus*) muscle tissue to temperatures below 6 °C resulted in sharp increases in metabolic rate due to increased membrane permeability, and a rapid depletion of ATP (adenosine triphosphate).

An increase in the rate of post mortem ATP depletion would hasten the development of rigor mortis as its onset is determined by the complete exhaustion of free muscle ATP (Love 1980). This may be described as a 'cold-shock' as previously observed by Curran *et al.* (1986), however this mechanism appears to result in very little change in sarcomere length and therefore cannot be attributed to a 'cold-shortening' event.

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One problem associated with using commercial practice to supply fish for this type of experiment is not being able to determine how long a fish has been in a trap prior to harvest. Consequently, the amount of stress experienced by any given fish is neither known or controllable under these conditions. Some of the fish harvested in this work went into rigor within 2 hours of chilling, whereas others did not achieve full rigor within the 4 hours of assessment.

A significant result may have been achieved by using live and rested fish prior to euthanasia and immersion within the different chilling temperatures. This would minimise the potential for large variation observed within each of the treatments with regard to the time taken for the onset of rigor.

The results obtained from the temperature loggers have proved useful, even though two of the loggers were lost or destroyed during the field trip. Some of these results are encouraging and some are less than satisfactory.

The performance of the brine tank was an encouraging result. This vessel and this brine tank have been the subject of a previous field trip (October 2008). During this trip, temperature logging demonstrated the refrigeration system used in the brine tank would struggle to maintain temperature under heavy load from large numbers of fish.

During the field trip of October 2008, brine tank temperature rose to values over 5°C on six separate occasions of heavy load due to large numbers of fish being landed. During this trip, the highest temperature achieved under load of fish was 4°C and this was only achieved once.

Of greater concern was the large variation in chilling efficiency observed within the fish holds. The two fish logged during this work provide vastly different results.

Logger #26 (Figure 8) was attached to a Saddletail on the second day of fishing and the results presented in demonstrates chilling presented in the early days of fishing is of a standard equivalent to industry best practice. However, logger #92 (Figure 9) demonstrates that the chilling efficiency observed from logger#26 is not consistent throughout the fish holds. This may be due to ineffective insulation, ineffective chilled air circulation, or a combination of both these and other influences yet to be determined.

Logger #70 (Figure 10) also clearly shows that fish hold temperatures can descend below -5.0°C and provides clear evidence to explain why so many fish were unloaded from the vessel fully frozen. Freezing fish in this temperature range results in decreased shelf-life when thawed, increased drip loss when filleted and inferior product quality generally as the slow freezing produces large ice crystal within the fish muscle, maximising the damage to the muscle tissue. This is particularly relevant for high value species such as Red emperor (*Lutjanus sebae*) and Coral trout (*Plectropomus* spp).

5.2. Analysis of TFS across years and seasons

5.2.1. Sample Collection

Input was sought from stakeholders in all three fishing methods in the sector being trap, line and demersal trawl. It was envisaged samples would be sought from all 3 fishing methods. However, this proved problematic due to limited access to suitable onboard freezing capacity on several of the vessels supplying fish for this project.

Onboard-freezing of fish has been an essential part of the sample collection process due to results obtained after the initial field trip for FRDC project 2008/208 in November of 2009 (Forrest *et al.* 2010). The results from this work suggested post-mortem changes in muscle chemistry associated with endogenous enzyme activity during cold storage exhibit an effect on cooked muscle texture.

In short, muscle texture was directly affected by time spent cold storage. Endogenous protease and collagenase activity *in situ* continued during cold storage and greatly influenced texture analysis results. Freezing on fish onboard was thus deemed essential in obtaining samples that were subject to as little post-mortem enzymatic activity as possible.

With this issue in mind it was determined that only one vessel was capable of providing sufficient numbers of Saddletail at point of capture, and also had sufficient freezer capability to rapidly freeze and store whole frozen fish onboard. A summary of the field trips is presented in Table 7.

Table 7. Summary of field trips.

Trial name	Date of sample collection	Number of fish (n=504)
FT2	Nov/Dec 2008	111
FT3	Apr/May 2009	101
FT5	Nov 2009	109
FT7	Dec 2010	100
FT8	Apr/May 2011	83

5.2.2. Texture data for all trips

Texture data for all trips is best presented in a frequency histogram. This histogram is presented in Figure 16.

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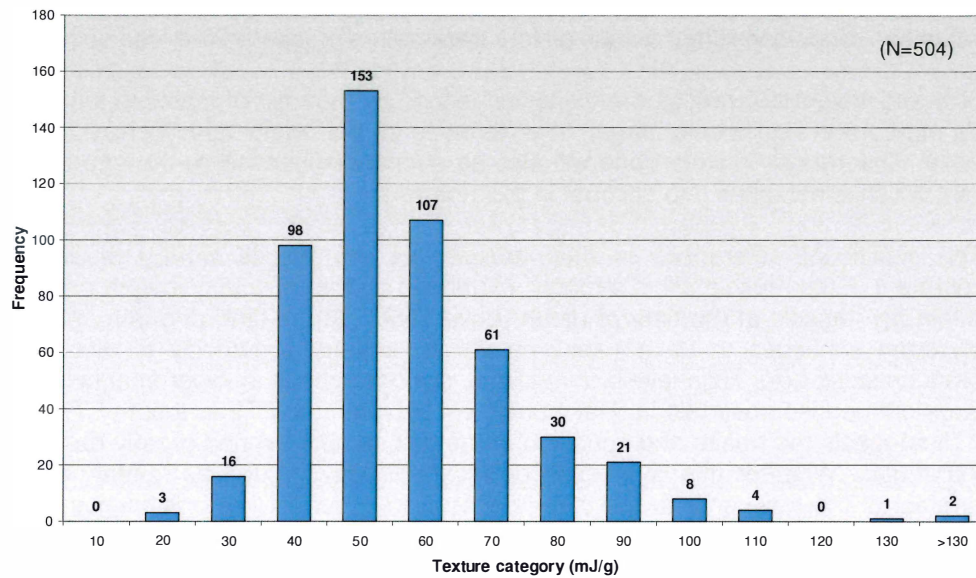


Figure 16. Frequency histogram of texture data for all fish (FT2 to FT8)..

From this histogram above, over 70% of all fish measured have a work done (WD) value between 30mJ/g and 60mJ/g. This texture range is acceptable by the majority of consumers assessed during a formal taste panel (Chapter 5.4). Fish presenting WD values above this threshold represent those most at risk of being rejected by consumers.

5.2.3. Analysis of results by field trip

Significant differences were found in almost all measured parameters across all field trips. A summary of results is presented in Table 8.

Table 8. Summary of mean results for both sexes of Saddletail snapper.

Parameter	Field trip					Sig.
	FT2 (n=111)	FT3 (n=101)	FT5 (n=109)	FT7 (n=99)	FT8 (n=83)	
WD (mJ/g)	53.13 ^b (1.494)	60.63 ^a (1.749)	46.52 ^c (1.508)	51.44 ^d (1.574)	54.67 ^d (1.728)	p<0.001
pH (ultimate)	6.38 ^c (0.011)	6.43 ^b (0.012)	6.56 ^a (0.011)	6.32 ^e (0.011)	6.35 ^d (0.013)	p<0.001
WT (g)	1886.7 ^c (36.65)	2000.4 ^b (42.91)	1530.7 ^d (36.99)	1842.5 ^c (38.61)	2318.9 ^a (42.39)	p<0.001
FL (cm)	50.28 ^c (0.34)	51.70 ^d (0.40)	47.05 ^e (0.35)	49.40 ^d (0.36)	54.46 ^a (0.40)	p<0.001

Means followed by a different letter are significantly different at the stated level. Standard errors are shown in brackets.

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Although significant differences exist between the mean values of the five sets of samples, trends consistent across all four parameters measured are less obvious.

Size and weight differences are highly influenced by the ratio of males to females, as males are significantly larger than females of the same age (Forrest *et al.* 2010). Differences in work done will also be strongly influenced by the age of the fish, which is not taken into account in this analysis.

The significant differences in final muscle pH are results worthy of further comment. Final muscle pH is strongly influenced by the muscle glycogen present within fish muscle at the time of death (Love 1980). At the time of death, muscle glycogen continues to be metabolised via an anaerobic pathway to ultimately produce lactic acid. High levels of available glycogen result in lower final pH. The large differences observed in final muscle pH between the FT5 and both FT7 and FT8 suggests the health and condition of the fish sampled varied greatly between individuals. Whether this is a result of seasonal activity such as rainfall, or an artefact of the trawl shot at the time is difficult to ascertain with confidence. FT5 also had the smallest fish of all trips and this may influence the pH result.

5.2.4. Analysis of results by trip and sex

Further understanding of the trends is gained upon examination of the trends across the field trip by sex. A summary of these results is presented in Table 9.

Table 9 Summary of field trip data for male and female Saddletail snapper

Parameter	Female					Male					Sig.
	FT2 n=57	FT3 n=42	FT5 n=56	FT7 n=64	FT8 n=25	FT2 n=54	FT3 n=39	FT5 n=53	FT7 n=36	FT8 n=58	
WD (mJ/g)	57.36 ^b (2.05)	58.29 ^{ab} (2.39)	47.46 ^c (2.07)	51.27 ^c (1.94)	62.76 ^a (3.10)	48.66 ^c (2.11)	63.14 ^a (2.48)	45.53 ^d (2.13)	51.73 ^c (2.58)	51.18 ^c (2.03)	p<0.001
pH (ultimate)	6.34 ^e (0.015)	6.41 ^d (0.017)	6.54 ^d (0.015)	6.31 ^f (0.014)	6.34 ^e (0.023)	6.41 ^d (0.015)	6.46 ^c (0.018)	6.57 ^a (0.015)	6.32 ^e (0.018)	6.35 ^e (0.015)	p<0.001
WT (g)	1806 ^d (41.7)	1699 ^e (48.58)	1401 ^f (42.07)	1612 ^e (39.35)	1965 ^c (62.97)	1971 ^c (42.84)	2324 ^b (50.41)	1667 ^e (43.25)	2250 ^b (52.47)	2471 ^a (41.34)	p<0.001
FL (cm)	49.39 ^e (0.39)	49.23 ^e (0.46)	45.43 ^d (0.40)	47.30 ^f (0.37)	51.48 ^d (0.60)	51.22 ^d (0.40)	54.37 ^b (0.48)	48.77 ^e (0.41)	53.14 ^c (0.50)	55.75 ^a (0.39)	p<0.001

Means followed by a different letter are significantly different at the stated level.
Standard errors are shown in brackets.

Significant differences exist in all parameters measured both between trips and between male and female Saddletail snapper. These differences are more apparent when the results are expressed graphically. This can be seen in Figure 17 through to Figure 20.

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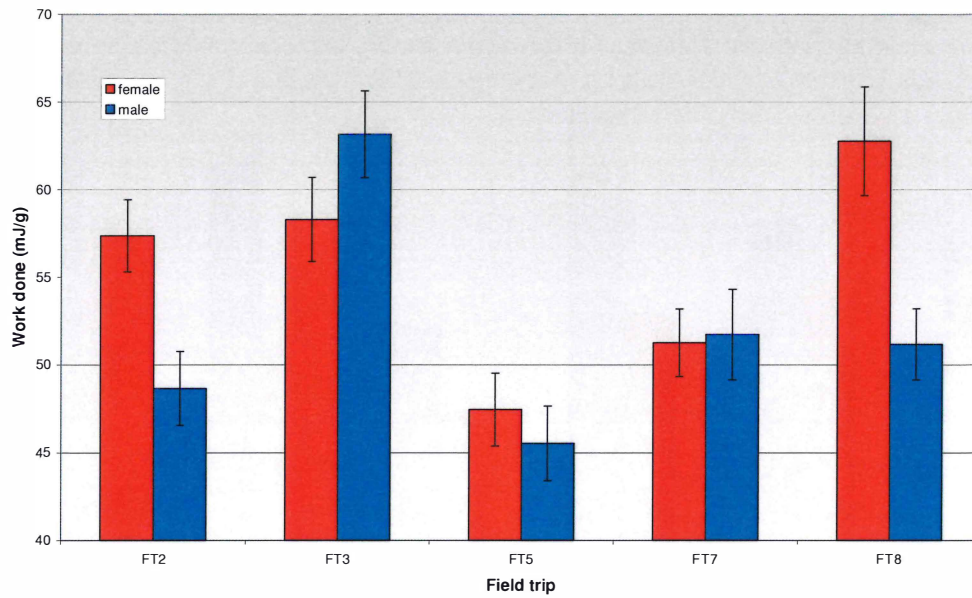


Figure 17. Mean work done values for male and female Saddletail snapper.
(Standard error bars are shown)

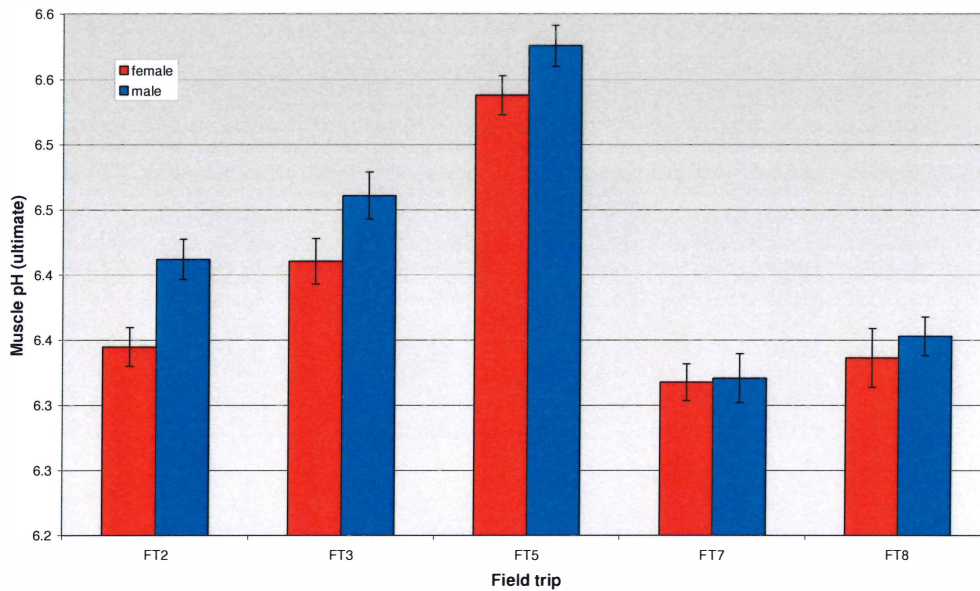


Figure 18. Mean muscle pH (ultimate) for male and female Saddletail snapper.
(Standard error bars are shown)

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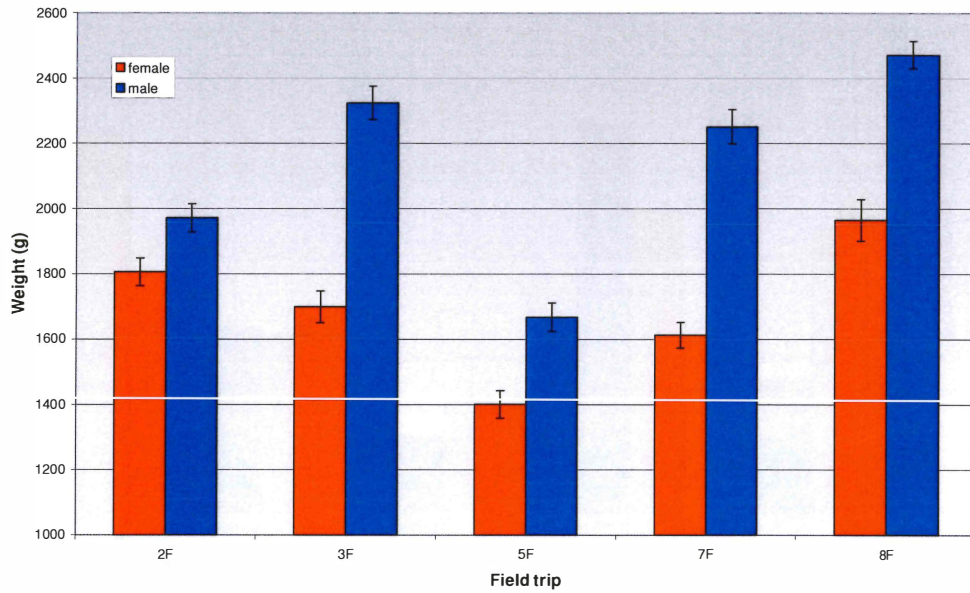


Figure 19. Mean fish weight for male and female Saddletail snapper.
(Standard error bars are shown)

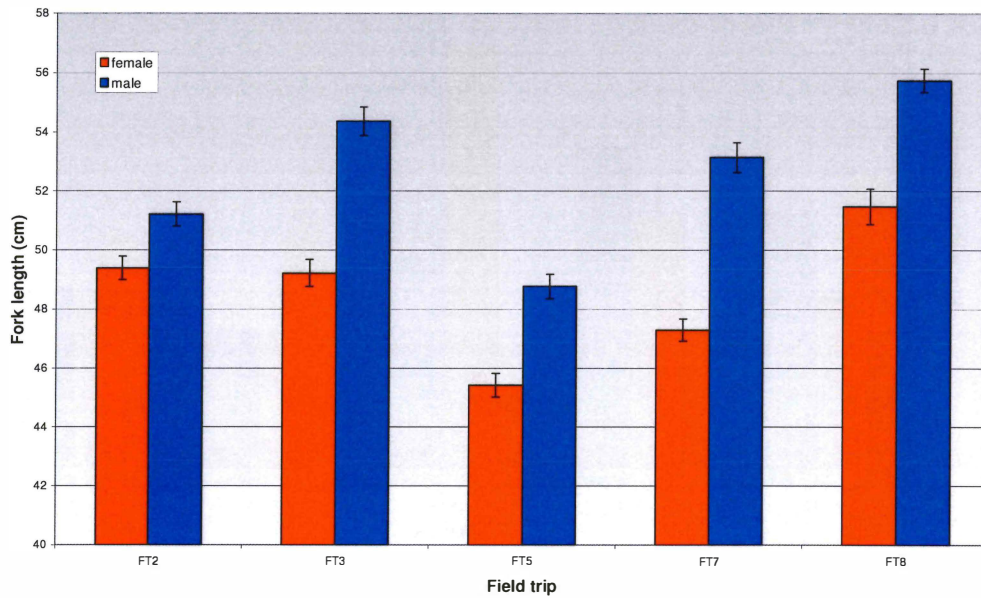


Figure 20. Mean fork length for male and female Saddletail snapper.
(Standard error bars are shown)

For female Saddletail, fish size correlates reasonably well to work done. The same cannot be said for male Saddletail. Differences in mean pH values are highly significant, however there is no obvious relationship to other parameters.

5.2.5. Analysis of results by estimation of fish age

Age of fish has previously been identified as a significant driver of toughness in cooked Saddletail snapper flesh (Forrest *et al.* 2010). Fish age estimation was completed by otolith section for Saddletail from FT3 and FT5 with the assistance of Fisheries Queensland (Fisheries-Queensland 2009). Further access to otolith section age determination became problematic due to time and budget constraints. As such, an alternative method of age determination was sought.

Age determination by otolith weight has been attempted on many species around the world (Cardinale *et al.* 2000; Fletcher 1991). Closer to home, otolith weight has been employed as a tool for age estimation on significant commercial species such as common coral trout (*Plectropomus leopardus*) by Lou *et al.* (2005).

The age data collected from FT3 and FT5 (n=210) was used to develop a predictive (linear) model that would assist in providing an estimate of fish age from otolith weight. The model is presented in Figure 21.

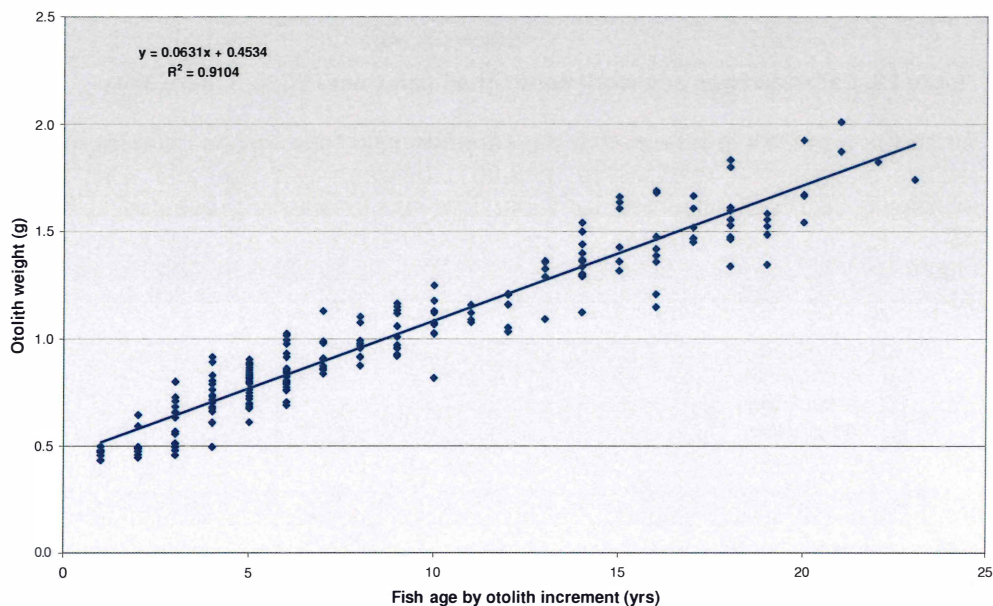


Figure 21. Relationship (linear) between fish age and otolith weight for all Saddletail snapper collected during FT3 and FT5 (n=210).

Fish age for Saddletail collected during FT7 and FT8 was estimated using formula presented in Figure 21. The mean weight of both left and right otoliths was used to achieve this. No otoliths were collected during FT2 so no age estimation of these fish was possible.

Using the predictive model age data and otolith section ages (n=373), a significant exponential relationship ($p < 0.001$) between estimated fish age and work done describing 45.5% of the observed variation. This relationship is presented in Figure 22.

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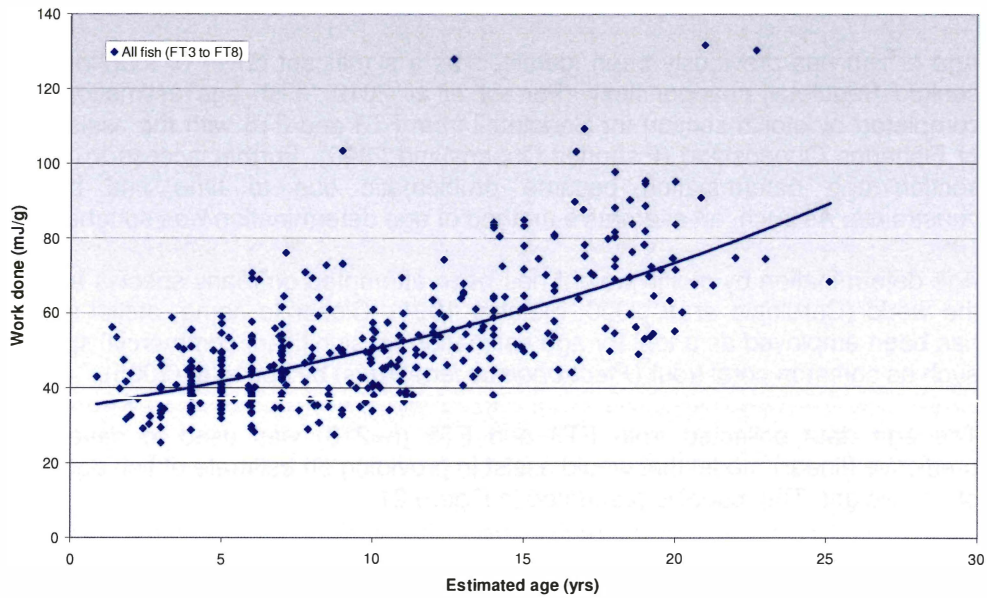


Figure 22. Estimated age and work done for all fish from FT3 to FT8 (n=373).

When the age data is broken into the separate field trips, exponential regression reveals a significant relationship ($p < 0.001$) between fish age and work done, explaining 53.1% of observed variation. This relationship is presented in Figure 23.

Figure
23

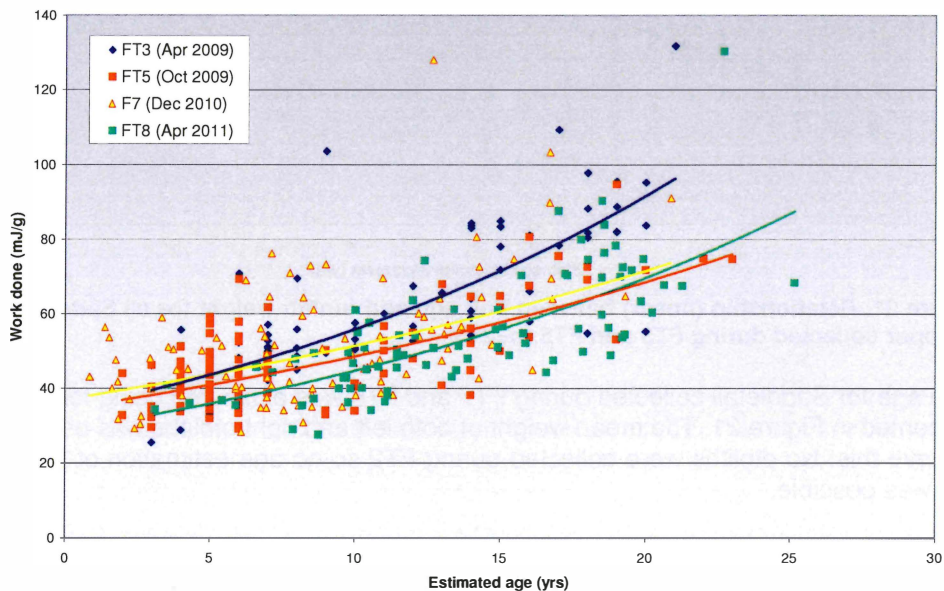


Figure 23. Estimated age and work done for FT3 ($r^2=0.65$), FT5 ($r^2=0.47$), FT7 ($r^2=0.27$) and FT8 ($r^2=0.57$).

Differences appear to exist between the trend lines of the trips. However these differences are not significant. The observed differences become less noticeable as the data is presented by sex as well as trip.

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Exponential regression with groups (sex and trip) reveals a significant relationship ($p < 0.001$) between fish age and work done explaining 57.1% of observed variation. This result is presented graphically in Figure 24 and a summary of the r^2 values from this analysis is presented in Table 10.

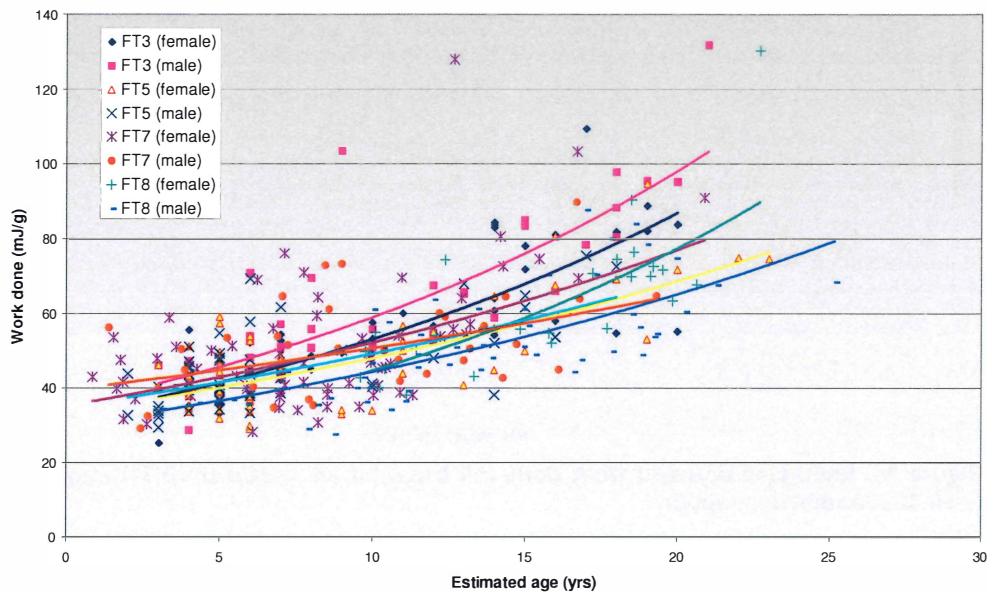


Figure 24. Estimated age and work done for all trips and both sexes.

Table 10. Summary of r^2 values from Figure 24.

Field trip	female r^2 values	male r^2 vales
FT3	0.67	0.68
FT5	0.50	0.42
FT7	0.30	0.22
FT8	0.64	0.52

Although some differences are apparent between the sexes and field trips, the differences are not significant. For example, the male fish from FT3 exhibit much more firmness than the males of FT8. However, the female Saddletail from FT3 and FT8 exhibit almost identical trend line with good r^2 values (being 0.67 and 0.64 respectively).

When the relationship between sex and texture is examined the data sets are even closer. Exponential regression with groups (sex) reveals a significant relationship ($P < 0.001$) between fish age and work done explaining 45.6% of observed variation. This relationship is presented in Figure 25.

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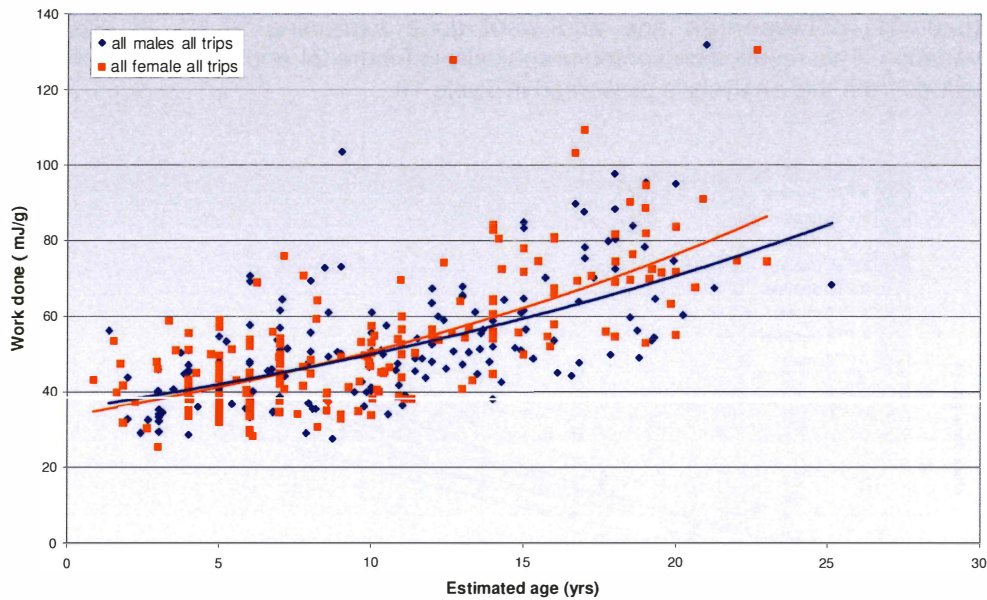


Figure 25. Estimated age and work done (all trips) for all female ($r^2=0.51$) and male ($r^2=0.42$) Saddletail snapper.

The similarity between the trend lines of male and female fish across all four field trips suggest other differences previously observed between trips may in fact be a trip effect. In other words, the data from any individual trip appears to be somewhat skewed by the group of fish sampled during that trip. The much larger number of fish represented by the data in Figure 25 (being 187 females and 186 males) provide a sounder basis for analysis as the trip effect is evened out over the four field trips.

When the results are examined by season a very similar trend is observed. Exponential regression with groups (season) reveals a significant relationship ($p<0.001$) between estimated age and work done explaining 45.3% of observed variation. This result is presented in Figure 26.

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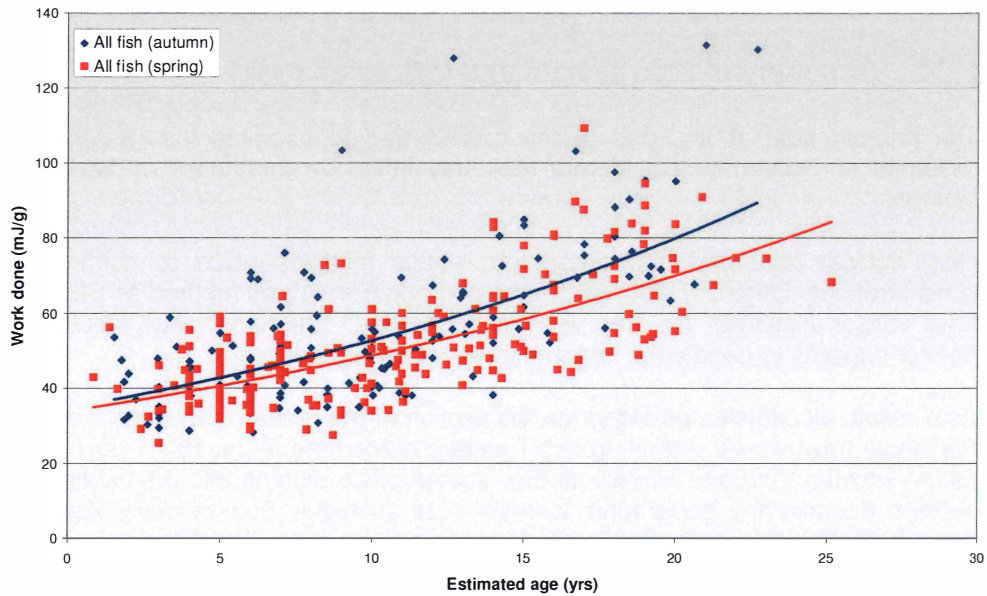


Figure 26. Estimated age and work done (all trips) for Saddletail snapper landed in autumn ($r^2=0.47$) and spring ($r^2=0.49$).

There is very little difference between the data sets presented in Figure 26. Exclusion of the outlying values above 100mJ/g results in near identical trend lines and the variation explained increases to 48.1%.

As with the previous examination of the data by sex, examining the data set by season alone appears to reduce the influence of individual trips to skew the results. From the result presented in Figure 26 there appears to be no seasonal influence on flesh texture at all.

5.2.6. Summary of age, season and texture analysis

The primary goal of this part of the project was to examine the influence of seasonal and geographical factors that may influence toughness in Saddletail snapper.

Geographical data was not presented in this milestone due to commercial considerations. Originally, fish were to be sourced from a trap method as well as a trawl vessel. However, the trap vessels operating in this fishery had insufficient freezer capacity to handle the large numbers of samples required.

As a result, all samples provided for this section of the research were provided by the single trawl vessel operating in NT waters at the time. As such, in view of the fishery moving to quota, as well as new stakeholders coming into the fishery, the authors deemed the geographic location data as highly commercially sensitive and not suitable for publication in this report.

Seasonal influences have been considered by other researchers as a possible explanation for variation in cooked texture (Hagen *et al.* 2007; Ito *et al.* 1992). Additional data collected during subsequent seasons has allowed us to compare four years of data. Analysis of this data has demonstrated that any seasonal effect previously observed, becomes less relevant over the four years examined.

However, as we have reported (Forrest *et al.* 2010), the primary driver of toughness in cooked Saddletail snapper is fish age. And although fish age has been estimated from otolith weight for two of the four trips rather than traditional otolith sectioning, the results presented here illustrate a highly significant correlation between fish age and flesh firmness.

5.3. Current applications of sensing or imaging technology in fish biology

This section is a summary of currently available technology suitable to assist stakeholders in removing Saddletail at risk of developing TFS from their catch.

Identifying fish age non-invasively would be the perfect application. However, this is not currently achievable with currently available technologies. The next best selection tool would be identifying the sex of Saddletail snapper non-invasively. This would at least supply stakeholders to use currently available fork length data for each gender, as male fish are significantly larger than female fish of the same age.

Summary of available technologies

Two forms of technology are currently being used for determination of fish sex. These are ultrasound sonography, and near infra-red spectroscopy (NIRS). They each have their own advantages and disadvantages.

Option 1: Ultrasound imaging:

Advantage: Machines are easily obtainable from several suppliers.

Disadvantage: Require significant levels of expertise to operate and identify sex organs.

Option 2: Near infra-red spectroscopy (NIRS):

Advantage: Requires minimal training once a model has been developed.

Disadvantage: Significant expertise is required to develop predictive model and machines are more expensive, and require significant understanding to setup.

A more detailed explanation of each of the technologies follows.

5.3.1. Ultrasound imaging

Ultrasound technology for medical applications emerged from the large investment in sonar technology during WWII. Researchers in Sweden, Scotland and the USA began developing medical applications during the late 1940's and early 1950's. Modern diagnostic ultrasound sonography operates between the frequencies of 2 to 18 megahertz (MHz).

Put simply, higher frequencies produce higher resolution images, however the higher the frequency the less penetration through tissue. Superficial structures close to the skin are scanned in the range of 7-16MHz. Deeper tissue such as kidney requires lower frequencies in the region of 1-6MHz.



Figure 27. The Hitachi Aloka SSD-500V portable convex sector/linear ultrasound scanner commonly used in small animal applications.

More recently, this technology has been trialled on several fish species for various purposes. The most common application is to non-invasively determine sex at sexual maturity for live fish. This technology has been successfully applied to Striped bass (Blythe *et al.* 1994), Atlantic cod (Davie *et al.* 2003; McEvoy *et al.* 2009), Atlantic halibut (Robichaud *et al.* 1998) and African catfish (Anchionye-Nzeh and Jimoh 2010). Ultrasound has also been utilised to identify Atlantic salmon suffering from previously undiagnosed cardiomyopathy (Sande and Poppe 1995). In Australia this technology has been applied to Murray cod (Newman *et al.* 2008).

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Central to the use of this technology, is the ability to observe cross-sectional views of internal organs. In most fish species, there is a significant difference in size and structure between male and female gonads.

However, using the scanner requires significant training to operate successfully, and understanding of not only the mechanism of the scanner, but the anatomy of the fish species being observed. This is the single most limiting factor preventing the use of this technology. Extensive knowledge of fish anatomy will be intrinsic to the success of implementing this kind of technology.

In Australia, suppliers of equipment who provide training as well are difficult to find. The most prominent supplier of equipment suitable for small animal applications is BCF Ultrasound Australia. They supply machines suitable for small animal use and conduct training seminars in Australia and New Zealand.

Pricing for an ultrasound scanner of this nature would be just under \$10K for a new machine, and somewhere around \$5K for a second hand unit.

Potential supplier details:

BCF Ultrasound Australasia Pty Ltd
Unit 10/56 Norcal Road
Nunawading 3131 Victoria
Australia

t: +61 3 9894 8980 f: +61 3 9894 8991
Sales: sales@bcfultrasound.com
Service: service@bcfultrasound.com
Accounts: marie@bcfultrasound.com

5.3.2. Near infra-red spectroscopy (NIRS)

Near infrared spectroscopy (NIRS) has been utilised to develop non-invasive methods of determining various parameters of food quality, particularly in horticulture produce. Non-invasive determination of fat content in avocado is a well studied methodology in Australia (Wedding 2009; Wedding *et al.* 2010).

However, unlike the ultrasound device that creates a cross sectioned image of the internals of the fish, NIRS relies upon the successful development of a predictive model to allow determinations to be made. The model is developed by scanning the object in question with a beam of NIR frequencies in the range of approximately 700nm to 1100nm and recording the levels to which these frequencies are reflected off the target and back to the sensor. It is these responses to the NIR frequency range and use of powerful statistical analysis software that permit the development of a predictive model.

This model can be made on as little as 100 replicates. However, greater accuracy requires many more replicates, and numbers approaching 1000 replicates are common attained when developing a model.

Once a model has been developed, the NIRS can be connected to a computer for recording of results and a simple yes/no (male/female) determination is provided by the computer. This technology greatly reduces the reliance subjective observations by the operator and thus greatly reducing the error rate, and speed at which determinations can be made. This technology also lends itself well to be integrated within automated processing systems.



Figure 28. An image from Davis *et al* (2006) showing the use of the SW-NIRS device on a Chinook salmon.

A recent undergraduate study at Oregon State University has demonstrated short wave NIRS (700-1100nm) can be used to identify the sex of mature Chinook salmon with a very high degree of accuracy (Davis *et al.* 2006). Although there is

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little in the way of further studies on other species, it is highly likely that this methodology will be applicable to most fish species of similar body size.

The use of this device also requires a reasonable amount of training to operate. And again, a sound knowledge of the anatomy of the fish is again essential.

Development of the predictive model is the most complicated stage of introducing this technology. Many hours of replicate sampling is required, as well as a thorough understanding of physics, mathematics, and statistical analysis tools. This is probably the single major drawback preventing

Costs of NIRS devices usually start around the \$10K mark for a hand-held device. Portable machines are available for remote use. However, model development is best done in the laboratory environment to create standard conditions.

5.3.3. Potential supplier details:

Brett Treacy
Bret-Tech / Brett's Electrical
10 Blackall Street,
Woombye Qld 4559

T (07) 5442 3766 Bret-Tech
T (07) 5442 3722 Brett's Electrical
F (07) 5442 3076
Email: brett@bret-tech.com.au
Web: www.bret-tech.com.au

5.4. Consumer assessment of Saddletail texture: Threshold of rejection with regard to texture.

5.4.1. Results

Complete assessments were obtained from 53 panellists. Prior to evaluation of the fish samples, panellists were asked some questions regarding their buying frequency as seafood preferences.

Question 1 asked the panellists to pick an age category that best described them. This question is asked to ensure the panellists represent a good cross-section of seafood purchasers. These results are presented in Figure 29.

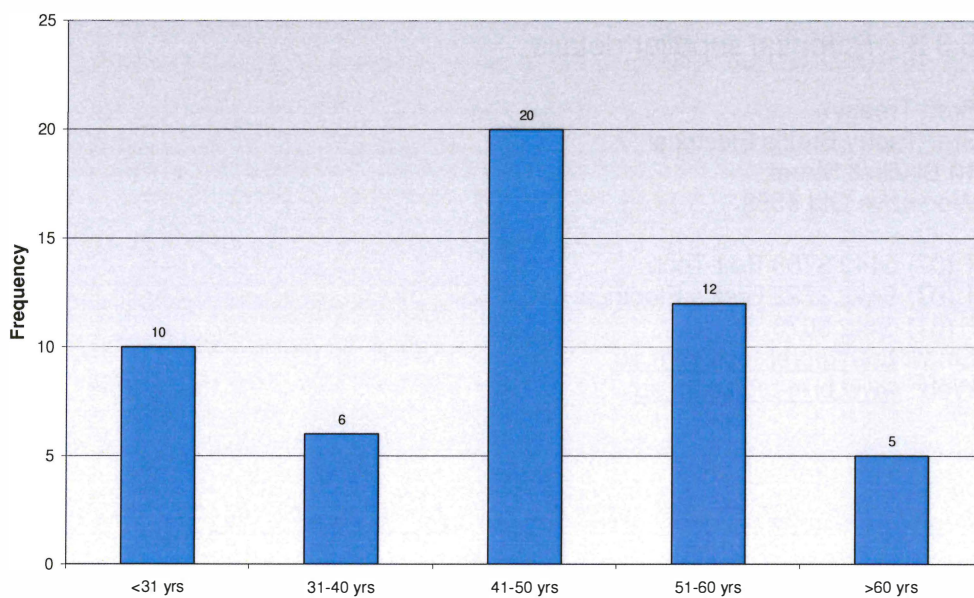


Figure 29. Age categories of consumer panellists.

The age distribution of panellists in Figure 29 is consistent with the distribution of seafood consumers in Australia (Ruello 2006). Almost 70% of panellists described themselves as age 41 years or greater. This age group contains the vast majority of seafood consumers in Australia.

Question 2 asked how often they purchased the groceries in the household. Panellists were asked how often they purchased the groceries in their household. These results are presented in Figure 30.

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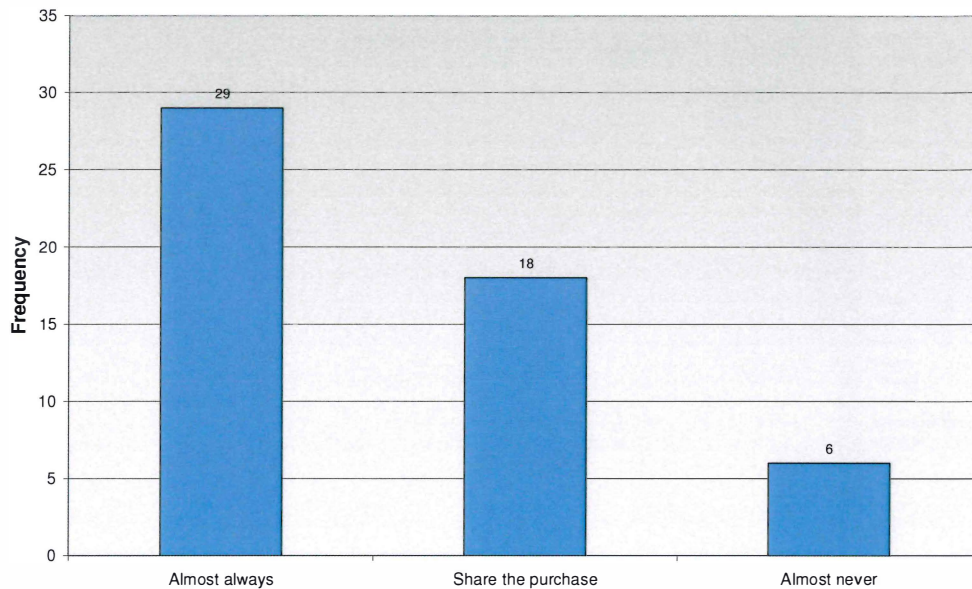


Figure 30. Seafood purchase frequency of panellists.

The results in Figure 30 demonstrate that the majority of panellists (47 of 53) were involved in grocery purchase. This result demonstrates our consumers are regularly involved in the purchase of food for their households.

Question 3 asked them how often they purchased fresh or frozen seafood. These results are presented in Figure 31.

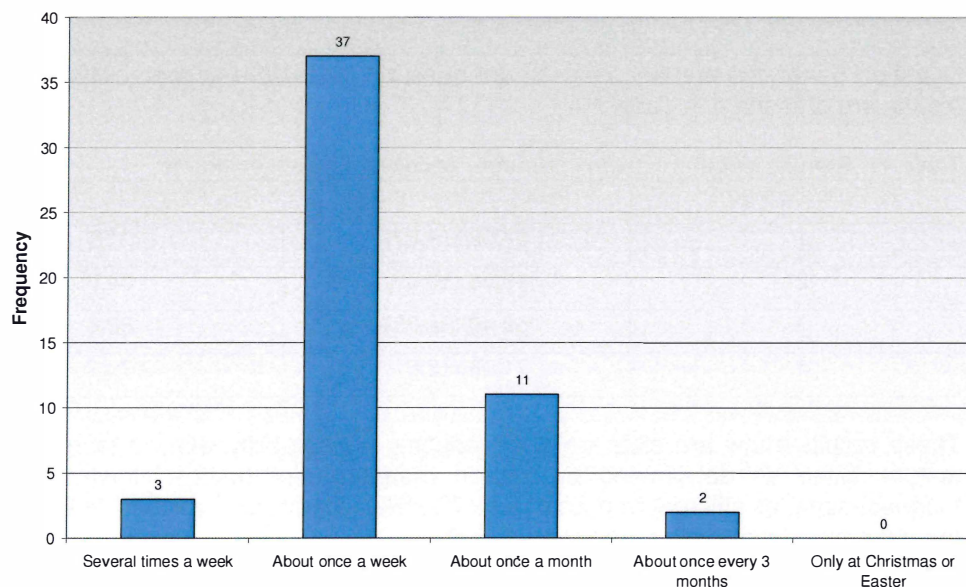


Figure 31. Seafood purchase frequency of panellists.

Question 4 asked panellists to name the three most commonly purchased seafood products. A total of 138 responses were received. These results are presented in

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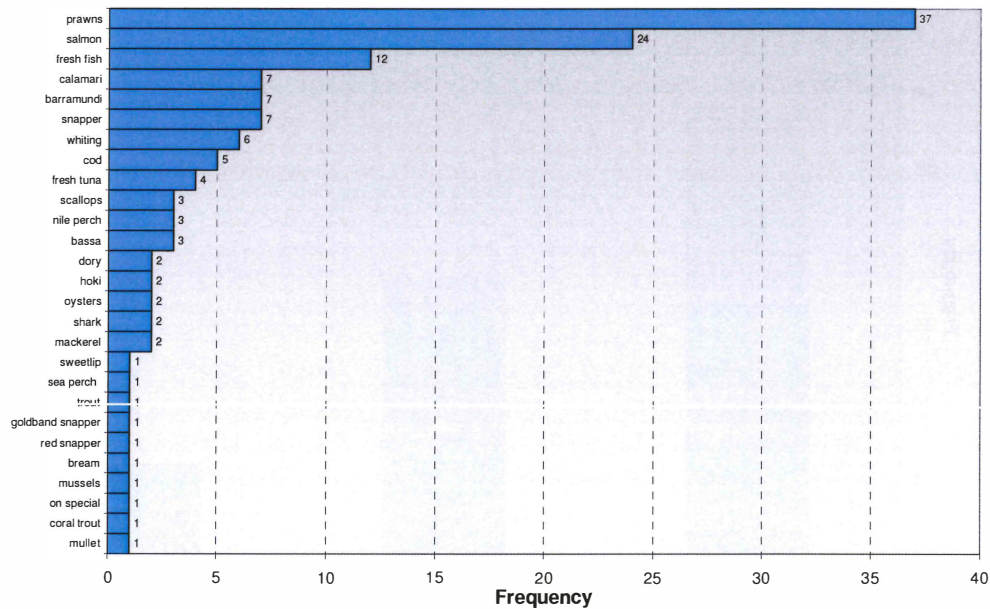


Figure 32. Commonly purchased seafood products for panellists (n=138).

Results from this question demonstrate a large variety of seafood products consumed by the panellists. However, the most commonly purchased products (prawns and salmon) represent almost half of all responses (44%). The next highest individual product named is *fresh fish*. All responses that could be included as fresh fish (including specifically named species) represent 60 responses or 43% of all responses. From this result we can confirm our panellists are representative of seafood consumers of the seafood category (fresh fish) that Saddletail snapper are most applicable to.

Question 5 was the primary assessment question for texture acceptability. These results are presented in Table 11.

Table 11. Results summary from consumer acceptability assessment.

Texture Category	Mean texture (mJ/g)	% Acceptability (n=53)
1	37.58 (± 1.82)	100
2	49.66 (± 0.99)	94.3
3	59.40 (± 2.83)	88.6
4	71.98 (± 2.51)	79.2

These results show a clear trend of reducing acceptability with an increase in texture value as determined by Instron measurement (mJ/g). However, the toughest samples still received an overall 79.25% acceptance; which is well above the determined threshold of rejection of 50%.

Question 6 asked panellists to provide comments on any particular aspect of the sample that they liked. Question 7 asked panellists to provide comment on any aspect of the sample that they disliked. These comments can be found in full in Appendix 2. Comments range from those about, flavour, texture, aftertaste, colour, odour, and comparisons with other seafood products. A large portion of these comments were reserved for texture, both like and dislike comments. A summary of the number of like comments can be found in Table 12.

Table 12. Summary of like comments relating to texture.

Texture category	Total like comments	Like comments for texture	% of total comments
1 (38mJ/g)	51	29	56.9
2 (49mJ/g)	49	29	59.2
3 (49mJ/g)	36	13	36.1
4 (72mJ/g)	43	10	23.3

The results from this question demonstrate a trend of decreasing 'like' comments regarding texture in accord with increasing texture value. The majority of responses for both category 1 and 2 comments related to texture. Interestingly, 10 of the 43 comments for category 4 fish samples were still describing aspects of the texture that were desirable.

Question 7 asked for responses to anything that was disliked in any of the samples. A summary of the results that related to texture are presented in Table 13.

Table 13. Summary of dislike comments relating to texture.

Texture category	Total dislike comments	Dislike comments for texture	% of total comments
1 (38mJ/g)	20	5	25.0
2 (49mJ/g)	25	6	24.0
3 (49mJ/g)	25	7	28.0
4 (72mJ/g)	36	24	66.7

The first observation from this data is dislike comments were reported in much fewer numbers than like comments. The trend of total dislike comments made is also the inverse of those seen for like comments.

However, the most important result from this question is the number of dislike comments relating to texture (24) as a proportion of the total number of dislike comments (36) for toughest samples (category 4). Over 66% of dislike response for this sample related to texture that was not desirable. This result suggests that the samples consumed by these panellists were very close to the point at which the texture could be deemed to be unacceptable.

5.4.2. Discussion

The results from the consumer acceptability assessment provided no clear threshold of consumer rejection of cooked Saddletail texture. However, with further examination of the dislike comments provided by panellists, two thirds of dislike comments for samples from category 4 (66.7%) mentioned texture as at component of what they didn't like about that sample. So although consumers would not reject the sample presented to them in the acceptability assessment, the firmness of texture was a factor that was perceived by the consumers and described by the majority of respondents as an attribute that they did not like.

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This result brings into question the choice of a forced binary assessment rather than the use of hedonic or category scales. However, untrained sensory panellists such as these are well-known to be hesitant in utilising the full range of selection available to them in such assessments. This behaviour is primarily driven by a fear of being wrong and being called to account. In this case, presenting a forced accept/reject question to consumers may have been somewhat intimidating to some panellists and they have avoided providing a rejection answer in case they are wrong.

This may also be a result of their understanding of the product itself. Of the 138 responses to fish names, only one response used the term *red snapper*. And no panellist used the term *Saddletail*. For many panellists this may have been the first time they have ever tasted Saddletail snapper. And when presented with new food products consumers can often feel confused as to what it is they are actually tasting. In such an environment, asking a consumer to reject the product may be asking too much as they are not confident they know enough about what they are eating.

Using a category or hedonic scale may have provided consumers with more confidence to provide slightly more negative feedback without going to the point of all out rejection. However, scaled measures are best employed when panellists can be trained to understand the limits of the scales they are using. Without such training, panellists invariably use very little of the scale making discrimination between samples sometimes difficult, and more often impossible.

Another factor which may have played a role is the size of the samples assessed by the consumers. Portions were cut into 2cm by 2cm squares, which can simply be described as bite size portions. This is done for two reasons. Firstly, to standardize the samples as much as possible. But also, to ensure panellists do not consume too much of the product as meat, and particularly fish, has a significant satiety effect. In other words, fish can make you feel 'full' even after consuming relatively small amounts. Asking panellists to taste food when they are 'full' is not good practice as their responses will be influenced by this feeling.

Presenting panellists with a whole cooked fillet of Saddletail may have produced quite different results. Asking panellists to cut a portion off a whole fillet may have provided them with a better understanding of how a fillet may appear when it presents with toughness. This primarily the method most consumers of beef would use to assess the texture of a steak of beef either at home or in a restaurant. However, this is not standard practice in the vast majority of meat assessment involving consumers within the literature. Also, the logistics and resources involved would be far greater than those available for this assessment.

However, analysis of comments received from the dislike question (question 7) provide reveal some clear preferences. The fact that 66.7% of respondents described texture as one of the attributes of category 4 that was disliked is a significant result. The overall trend of increasing numbers of dislike comments with increasing texture values also supports the notion that the tougher samples are being liked less.

Making a claim regarding the threshold of acceptance cannot be supported with rigorous statistical significance with this type of data. However, it is entirely reasonable to suggest from the comments, the threshold of rejection for Saddletail would be very close to this value of 72 millijoules per gram (mJ/g). Regardless of threshold of rejection, it is apparent from this data that toughness at this level

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(approx 72mJ/g) is clearly disliked by two thirds of this panel of regular seafood consumers.

When this threshold is applied to data collected from all Saddletail snapper in previous field trips (n=504), approximately 13% of all fish measured for texture, exceed this value of 72mJ/g in firmness. This can be applied to the entire catch provided the sample set that we have is representative of the whole catch. Further consultation with industry stakeholders will develop a more accurate estimation. Once this has been established, a value can be placed upon this portion of the catch, and appropriate measures can be considered to address the issue and prevent tough Saddletail from ending up in front of consumers.

5.5. Enzyme treatment of Saddletail snapper to improve cooked texture

As a direct result of stakeholder consultation, enzyme treatment was proposed by the authors as presenting an opportunity to add value to fish identified as high risk to developing TFS. The goal of this work was to develop a 'proof of concept' for an enzyme treated Saddletail fillet or portion. A review of commercially available food enzymes approved for use in Australia was conducted.

Enzymatic treatment has been widely investigated as an opportunity to improve the value of lesser quality cuts of beef (Whitehurst and van Oort 2010). This is achieved by degradation of the myofibrillar proteins (intracellular) or the connective tissue (primarily collagen). Almost all proteolytic enzymes have activity on both structure, however the degree of these activities can vary greatly.

Enzymes from common food sources include papain (papaya), bromelain (pineapple), zingibain (ginger), actinidin (kiwifruit) and ficin (figs) are available as refined enzymes for use as food ingredients. Many of these whole foods (e.g. ginger) have had centuries of use as ingredients in meat marinades designed to achieve more acceptable meat texture. More recently many enzymes from microbial sources have been granted *generally recognised as safe* (GRAS) status by both the USFDA (USA) and FSANZ (Australia and NZ) for use as processing aids in food manufacturing.

Incorporating one or more of these enzymes in a value-added fish product, either as purified enzymes (e.g. serine protease) or as whole food ingredients (e.g. ginger) may provide market opportunity for fish at risk of presenting with toughness when cooked.

Contact was made with an enzyme supplier in Australia. Advice was sought as to the most suitable enzyme for or requirements. The recommendation was to use a plant derived protease at first and evaluate efficacy.

This view is supported by other literature for the beef industry (Calkins and Sullivan 2007). This enzyme has demonstrates a preference for collagen prior to myofibrillar components of muscle tissue And has also has demonstrated activity at 0°C. Both of the attributes are essential for the application on Saddletail snapper in a value added product format.

5.5.1. Trial 1: Determination of effective dosage

This trial was simply to determine the amount of enzyme required to impart a noticeable effect on the cooked texture of the fish. Fish fillets used in these trials were left over B samples from field trip 8 (April 2011). These B samples had been stored at -29°C since processing back in May 2011. Theses sample also have Instron texture measurements from the A samples analysed at this time.

Enzyme solutions were prepared with pre-chilled (4°C) deionised water at various concentrations. Saddletail portions were approximately 40g each in mass, and 20ml of enzyme solution was applied per concentration and stored in Glad zip-lock bags, as can be seen in Figure 33.



Figure 33. Trial 1 samples after 24 hours storage at 4°C.

Treated samples were cooked on an induction cook top using a non-stick frypan and canola oil. Samples were cooked approximately 3-4 minutes per side or until deemed to be thoroughly cooked. Samples were allowed to rest prior to tasting for approximately 5 minutes. A summary of the treatments and the comments from an informal taste panel are presented in Table 14.

Table 14. Summary of trial 1 enzyme treatment.

Treatment(g/L)	Volume(mL)	Sample size(g)	Comments
0 (control)	20	39.84	Very firm
0.2	20	42.19	No difference
0.5	20	34.21	No difference
1.0	20	39.42	A very slight difference (softer)
2.0	20	41.86	More noticeable difference

Results from this trial demonstrated that although the enzyme had a noticeable effect, the dosage needed to be increased to understand the limits of activity better.

5.5.2. Trial 2: Increased dosage and efficacy over time

The second trial was to use higher concentration of enzyme to determine efficacy limits, and also determine the effect on texture over a longer time period (7 days).

For this trial, in view of the longer time frame in cold storage, the enzyme solution was adjusted with lactic acid to obtain a pH of 4.0. This pH will reduce the opportunity for microbiological growth, while not affecting the activity of the enzyme during the storage time. Ratio of fish portion to enzyme solution (2:1) was maintained as per trial 1. Samples were stored in barrier bags (250x150mm) and vacuum flushed at 95% vacuum as can be seen in Figure 34.

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Figure 34. Samples prior to assessment during Trial 2.

Fish portions were to be sampled at 1, 3, 5 and 7 days of cold storage. A summary of the samples treatments and their identification is presented in Table 15.

Table 15. Summary of Trial 2 treatments.

Fish ID	8026	8015	8009	8059
Enzyme (g/L)	Day 1	Day 3	Day 5	Day 7
0 (ctrl)	D1C0	D3C0	D5C0	D7C0
2	D1C2	D3C2	D5C2	D7C2
5	D1C5	D3C5	D5C5	D7C5
10	D1C10	D3C10	D5C10	D7C10

Samples were cooked as previously described, and tasted by an informal taste panel. A summary of day 1 results are presented in Table 16.

Table 16. Summary of day 1 results for trial 2.

Sample ID	Comments from taste panel
D1C0	Firm texture with noticeable connective tissue
D1C2	Minor level of softening compared to the control
D1C5	Significant difference in texture from control. Palatable sample.
D1C10	Large difference in texture from control. A little 'chalky' in mouth feel. Not very palatable.

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Best results were obtained from enzyme concentration of 5g per litre solution. This sample was significantly softer than the control and quite palatable to eat.

However, the most striking result was that of the 10g per litre enzyme solution. This sample had clearly experienced significant enzyme activity. However, even though the texture was much softer, the organoleptic qualities (or eating experience) were quite poor and not desirable. The samples did not really taste much like fish. The experience was more like eating a beef or lamb meat that had spent several hours in a slow cooker. Clearly there is a threshold of enzyme digest that needs to be considered in future formulations.

Day 3 results are presented in Table 17. At this point, to ensure the ongoing safety of the samples, pH values of the enzyme solutions were also taken.

Table 17 Summary of day 3 results for trial 2.

Sample ID	Solution pH	Comments from taste panel
D3C0	6.25	Firm but not overly tough
D3C2	6.21	Very little softening
D3C5	6.20	Noticeable softening and loss of connective tissue. Still quite palatable.
D3C10	6.16	Too far gone. Almost mushy.

Results from day 3 were very much in line with those of day 1. Enzyme activity appeared to be having continued no further than the samples from day 1. This was confirmed after discussing these results with the enzyme supplier (Enzyme Solutions Pty Ltd).

The supplier had previously advised the enzyme had the capability to utilise itself as substrate, and enzyme preparation should only take place immediately prior to addition to the substrate. So in this case, we can assume that all enzyme activity has ceased somewhere around the 24 hours post addition to substrate.

The pH of the enzyme solutions had also increased to an unsafe level. Interaction with the fish muscle proteins, either with or without assistance by the enzyme, had buffered the solutions to a pH range that was no longer safe to store at refrigerated temperatures. For this reason, and the cessation of further enzyme function, this trial was discontinued at this point.

5.5.3. Trial 3: Short soak time treatments.

The trial involved using the enzyme as a short soak step of 30 minutes. Similar treatments have been reported for soak times as short as 15 minutes (Quaglia *et al.* 1992). This style of treatment is also more applicable to a commercial processing operation.

For this assessment a relatively high level of enzyme concentration (100g/L) was used at various time intervals. Samples were kept refrigerated during the soak time (4°C) in zip lock bags. The individual fish used for this assessment (ID 8006) was also previously determined as being very tough (90mJ/g) and representative of the very toughest of fish observed. Samples were cooked immediately after the

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completion of the soak time and assessed by our informal taste panel. A summary of these results are presented in Table 18.

Table 18. Summary of results from trial 3.

Sample ID	Soak time	Taste panel comments
S1T00	0 (control)	Very firm. Noticeable connective tissue
S1T15	15 minutes	Very little difference if any from control
S1T30	30 minutes	Noticeably softer, but still quite palatable
S1T60	60 minutes	Significantly softer and unpalatable. Too soft, almost mushy in mouth feel

Soak time of 30 minutes provided the most palatable sample with significant reduction in toughness. However, the enzyme concentration used in this trial was far above what would be commercially cost effective. The extent to which further activity continued in cold storage post-soak is also unknown. The value of this trial is the clear demonstration of the efficacy of the enzyme at 4°C for commercially efficient timeframes, even if the enzyme concentration is excessive.

5.5.4. Trial 4 short soak time and overnight storage under vacuum.

Our previous trials had identified the first 24 hours of storage as being the likely limit of activity for the enzyme on Saddletail portions. This allows the enzyme to be treated as a processing aid with respect to the Food Standards Code (Standard 1.3.3, Table to clause 16) provided the enzyme has no further activity within the food. Under these conditions, enzyme treatment will be possible as a short soak and then transferred to vacuum or modified atmosphere packaging (MAP).

For this trial, three enzyme concentrations were used (10, 30 and 50g/L) under a soak time of 30 minutes. Samples were placed in barrier bags for the duration of the soak. Once the soak time had expired, the enzyme solution was drained from the bag and a vacuum seal (95% vacuum) was applied to the bag. Samples were then transferred to the cold room (4°C) for 24 hours prior to informal taste panel assessment. The fish used for this trial was previously determined as being very tough (88mJ/g). A summary of results and comments is presented in Table 19.

Table 19 Summary of results and comments for Trial 4.

Sample	Enzyme(g/L)	Taste panel comments
S2C00	0 (control)	Very tough and chewy.
S2C10	10	Slight reduction in toughness. Quite palatable
S2C30	30	Significantly softer. A little chalky but good flavour
S2C50	50	Very soft and chalky. Not palatable

The action of the enzyme is clearly visible in the following figures. Figure 35 shows a cross section of the control sample.

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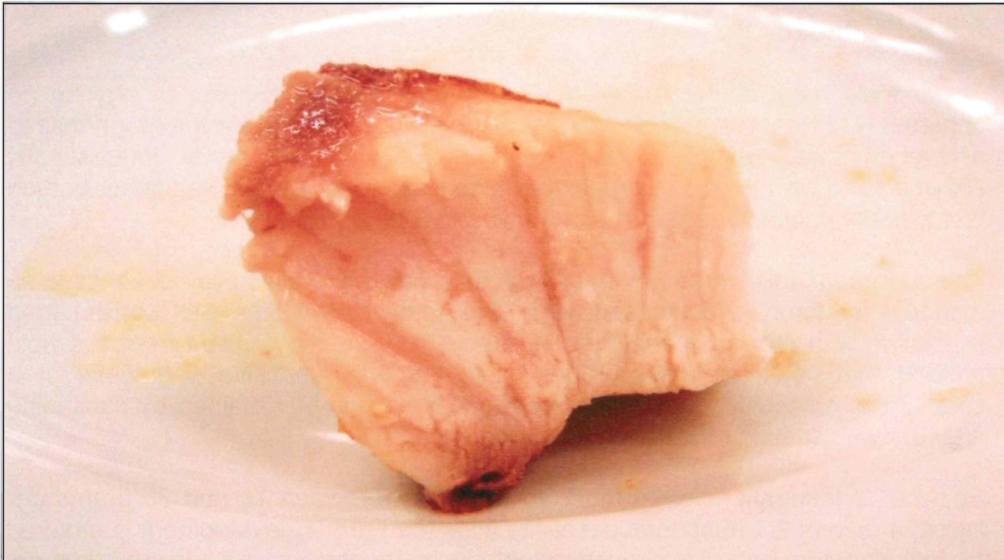


Figure 35. Cross section of control sample from Trial 4.

Individual muscle blocks are bound tightly together by the connective tissue between them. In most fish species this collagen melts readily upon cooking. However as the authors have previously reported the collagen within Saddletail snapper muscle becomes less soluble to heat as fish age (Forrest *et al.* 2010). It is these collagen matrices that are the target of the enzyme.



Figure 36. Cross section of 50g/L enzyme treated sample from trial 4.

Figure 36 demonstrates the efficacy of enzyme treatment at 50g/L. Although this sample was deemed beyond palatability by the taste panel, the action of the enzyme upon the collagen matrix is apparent.

5.5.5. Conclusions

These trials have demonstrated the ability of a purified enzyme to provide an opportunity to treat fish previously identified with TFS and reduce the toughness to a level that was deemed palatable by the informal taste panel. Interestingly, enzyme function beyond a certain point resulted in a cooked Saddletail portion that was not palatable regardless of the softness of the texture.

The short soak followed by overnight vacuum packaging also proved effective as a model for future product concepts. This method complies with current food legislation with regard to enzymes as processing aids, and also offers a simple method that could be incorporated within the time sensitive operations of a commercial processing facility. Greater shelf life would be obtained by packaging after enzyme soak in modified atmosphere pack.

Enzyme concentrations were found to work best between 10 and 30 grams per litre at a ratio of 2:1 (Fish to concentrate volume). Further development is required to optimise the enzyme concentration levels to achieve the desired texture. Optimisation is also required to best fit individual processing operations for processing time, labour costs and material cost sensitivities.

6. Benefits and adoption

Results from this research project have delivered critical sound information on the cause of tough fish syndrome affecting Saddletail snapper caught in northern waters. To date, lack of market confidence with respect to the eating quality of Saddletail snapper has been the key driver of under-valuation of this reef fish species within Australia. The new information that fish age is the main contributing factor to the textural qualities of cooked Saddletail provides a basis for sound commercial business decisions.

In light of the direct link between cooked flesh toughness and age of fish, the results from the research enables industry stakeholders to reconsider how best to market their product. Older fish that are more likely to exhibit toughness should be excluded from the high-end table fish market where a premium is attained for peak eating quality. Customers within the market where quality is paramount should only be supplied with younger fish and hence this will protect market confidence. Implementation of such a strategy will benefit stakeholders and participants throughout the supply chain, including consumers.

Work within the project also resulted in new and real information on consumer perceptions of toughness in fish flesh. Consumer reaction to different degrees of toughness in cooked Saddletail flesh provides the basis for understanding the extent to which industry needs to invest in solutions that will minimise the impact of tough fish syndrome.

Strategies to minimise impact of tough fish syndrome

Full realisation of benefits from this research can only occur through selection of practical TFS minimisation strategies. Removal of older fish that are more likely to be tough upon cooking will improve the reputation of the species as a premium table fish. The direct result of this will be an increase in consumer confidence and satisfaction of Saddletail snapper as a high quality eating fish.

Development of possible strategic options should a cause of toughness be identified, was a focus of this research project and to this end a specific stakeholder meeting was held to discuss the feasible options that could be applicable within commercial reef fish business operations. An agenda of suggested strategies was tabled at the meeting as a basis for discussion and selection of preferred pathway(s). The discussion paper is attached to this report in Appendix 5 along with the minuted discussion outcomes.

From in-depth discussion of the options proposed, industry stakeholders determined preference for two pathways forward: use of ultrasound to sex fish either on-board or on-shore and enzyme softening of fish fillets. Both these options were considered theoretically feasible within the commercial fisheries however, the direct applicability and effectiveness in reducing the impact of tough fish in the market needs to be determined prior to adoption by industry.

Pathways to adoption and implementation

An assessment of the effectiveness of ultrasound data with respect to differentiating fish sex in whole fish is necessary to justify industry investment in expensive technology. Industry project partners were keen for this to go ahead in the near future.

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Within this current project, we have provided additional results by way of a 'proof-of-concept' with respect to the applicability of enzyme technology for the softening of tough fish fillets. The plant derived protease, demonstrated positive benefit and it provides a marketing positive related to the source of the enzyme.

Of note, is the passion and commitment by the key industry stakeholders to progressing forward with a solution for TFS. This was strongly evidenced through expressed willingness to invest heavily in additional infrastructure and technology should this be required.

Communication with Stakeholders and Industry

Industry stakeholders were kept abreast of project work during the course of the project by way of regular updates through the NTSC quarterly newsletter. Several meetings were also held in Darwin with key stakeholders during the course of this project. The most significant of which (November 2011) resulted in additional outcomes being sought from the research in line with stakeholder expectations. One final meeting will take place in Darwin in November of this year to present final report findings and discuss future options.

During the course of the project, the profile of the work completed so far has resulted in the researchers being approached by several industry figures from around the country including Paul Richards (Director Cardinal Seafoods Pty Ltd), Chris Swallow (Purchasing and Marketing Administrator Kailis Bros), Matthew Vujica (Supafin Seafoods Pty Ltd) and John Susman (Fisheads Seafood Strategy).

7. Further development

7.1. Consumer threshold of rejection for cooked Saddletail snapper

Results to date have provided a trend with regard to the threshold of rejection for the texture of cooked Saddletail snapper. However, a greater level of significance would be obtained from a redesigned questionnaire and a greater number of consumers.

There are two areas of consideration for redesigning the questionnaire. Firstly, the size of the sample assessed by the consumer should be closer to the size of the portion that would be consumed in the home or restaurant. For the purposes of maintaining valid scientific method, portion size was uniform across all samples presented (2cm x 2cm). This is standard practice in consumer and sensory assessments.

However, in this case, the cutting into a larger portion, or whole fillet of this species would have provided consumers with more feedback with respect to the texture of the fish portion. In this situation, consumers would be able to draw upon previous experience with cutting cooked fish portions. This is consistent with our requirements for consumers to be qualified as regular consumers of seafood, and we are drawing directly upon this experience for our results.

Secondly, a much larger group of consumers is required to permit more rigorous analysis of the results obtained. Our assessment relied upon using Queensland Government staff available on site at Coopers Plains within the time available, and with limited resources. A group of at least 100 consumers would allow for more stringent questioning and analysis, and result in more rigorous statements with regard to significant results.

7.2. Determine efficacy of ultrasound imaging for determining the sex of Saddletail snapper

Determination of sex would certainly provide an opportunity to remove the majority of Saddletail snapper at risk of exhibiting TFS. Once the sex of any fish was determined, the measure of fork length could be used as an approximate for that fish. Fork length data captured during this project could be added to existing databases developed by Newman (2000; 1996) and others.

The group that is the highest risk is mature female. Mature male fish grow much larger than their female equivalents. Mature females may also present a reasonable straightforward sex determination by ultrasound due to their large gonads when mature. Removal of fish at risk of exhibiting TFS would provide stakeholders with a strong marketing opportunity.

7.3. Optimisation of enzyme treatment of Saddletail snapper within commercial protocols

Treatment of Saddletail snapper portions with a plant derived protease shows promise as a method of obtaining more value from fish at risk of exhibiting TFS.

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Further work would address issues surrounding the implementation of the process with a commercial operation. These would include;

1. Optimisation of the dosage level required to achieve significant results on a commercial scale;
2. Optimise the ratio of enzyme solution to fish material for commercial scale
3. Determine optimum MAP conditions for enzyme treated Saddletail snapper fillets and portions;
4. Establish the shelf life of enzyme treated Saddletail snapper portions (MAP, vacuum packed, and frozen) under best manufacturing practice conditions; and
5. Determine material cost efficiencies available for purchasing enzyme on commercial scale.

Addressing these issues would provide stakeholders with a sound basis to make investment decisions as to the viability of developing enzyme treatment of Saddletail snapper portions into value added products for wholesale or retail.

8. Planned outcomes

The major planned outcome from this research project was the determination and confirmation of the cause of toughness in Saddletail snapper. This was achieved convincingly with a direct link demonstrated between fish age and toughness of cooked fish flesh.

The influence of other physiological factors were shown to have little impact with respect to fish flesh toughness. Additionally, there was no apparent connection between flesh toughness and seasonality nor year to year conditions.

The possibility of toughness being engendered through inappropriate chilling immediately post-capture was ruled out as a factor contributing to tough fish syndrome.

The planned outcome following on from identification of the cause of TFS, surrounded developing procedures for reducing the incidence of tough fish syndrome where the cause was preventable. However, as post-harvest handling was not a contributor to toughness, implementing alternative handling procedures was not relevant.

Where the cause was identified as being less easily controlled, the planned outcome refocused on mechanisms for identifying tough fish, either on-board or during processing steps. This has been achieved through provision of current available technology information on non-invasive ultrasonics and imagery to the industry partners. Additionally, the authors proposed several alternative approaches to address minimising the impact of TFS. Each approach was considered fully with industry at a special stakeholder meeting (November 2011) and options reduced to the two most favoured by the key members.

Due to the obvious passion and commitment from industry towards resolving the TFS issue, and although beyond the scope of the project as stands, the authors agreed to undertaking additional research to provide some information needed next. The work included an assessment of consumer perceptions of toughness in Saddletail flesh and 'proof-of-concept' trials with enzyme treatment of tough fillets

9. Conclusions

9.1. Cold shortening and 'cold shock'

Cold shortening was not observed in Saddletail snapper. The development of rigor mortis in Saddletail occurs at faster rates in ice slurry than at ambient temperature. However, this is simply the product of exhaustion of available ATP within the muscle, and not a result of cold shortening.

9.2. Drivers of TFS in Saddletail snapper

The primary driver of TFS in Saddletail snapper is fish age. The collection of fish over four years of research has removed any previously held notions of seasonal effect within the data set. There is also little or no difference between the texture values obtained for male and female Saddletail snapper. Any anomalies within the results are the product of a trip effect, and are not significant when examined with the larger data set.

9.3. Available technology

Stakeholders requested a summary of available technology that may assist with identifying Saddletail at risk of exhibiting TFS. Two methods showing promise were identified. These are ultrasound imaging, and near infra-red spectroscopy. Both technologies are available within Australia in various forms. The most straightforward application would be the use of small animal ultra sound imaging available to most veterinarians in Australia. Details of suppliers of these technologies were supplied to stakeholders.

9.4. Consumer preferences

Our consumer threshold of rejection method did not provide statistically significant results. However, analysis of the comments reveal an overall trend that has identified a value of approximately 72mJ/g of work done as point at which the majority of consumers commented that the fish portion they tasted was too tough or firm.

However, even at this value at least half of the respondents would not have liked the sample of fish. So any attempt to remove these fish would apply a threshold of rejection much lower. A reasonable value for this task would be 60mJ/g of work done.

This represents approximately 25% of the catch observed during this research and significant proportion of the commercial catch. With this data in hand, stakeholders now have the ability to apply resources commensurate of the value of catch at risk of TFS.

9.5. Enzyme treatment

Enzyme treatment of Saddletail with TFS has proved to be worthy of further investigation. The enzyme displayed high efficacy and was relatively simple to apply within a value added fish product concept.

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Current food legislation requirements with regard to the use of plant derived protease material are such that an enzyme treated product could be produced and sold within Australia with little regulatory hindrance. There would also be no labelling requirements to list the enzyme as an ingredient, provided the enzyme can be demonstrated to have no activity at point of sale. This has yet to be tested clinically. However, advice from the enzyme supplier provides confidence that there would be no detectable activity remaining at point of sale.

Further work would require the optimisation of the enzyme within a commercial framework to develop efficiencies of process, cost and scale. This process would also involve the application of the enzyme to Saddletail portions within MAP and frozen product systems.

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11. Appendix 1: Intellectual Property

There are no intellectual property issues arising from this research project. All results, findings and developed methods have been extended to the stakeholders in the Northern Demersal and Northern Trawl Fisheries. All information belongs in the public domain.

12. Appendix 2: Project Staff

Principle Investigator:

Sue Poole Principal Seafood Scientist, IFT, DAFF (Qld)

Technical and Analytical staff:

Andrew Forrest Research Scientist (Food Technology), IFT, DAFF (Qld)

John Mayze, Principal Seafood Technician, IFT, DAFF (Qld)

Paul Exley, Senior Seafood Technician, IFT, DAFF (Qld)

Carl Paulo, Senior Seafood Technician, IFT, DAFF (Qld)

Sharon Pun, Analytical Technician, IFT, DAFF (Qld)

13. Appendix 3: Consumer panel questionnaire

The following is a print out of the actual questionnaire used for the assessment from the program used for the assessment (Compusense 5.0). The format is from the software design function and does not accurately represent how the questionnaire appeared on the computer screen.

Project: 2012 TFS V2 SESSION01

Welcome Text

**WELCOME to the tropical snapper taste session.
Please press 'continue' and the bottom of this page to commence the assessment.**

Instruction Text

Today you will be tasting four samples of steamed fish and be asked a simple question relating to the eating quality of each sample.

Please answer the questions as though you were eating this fish at home.

Please cleanse your palate with water between samples.

Thank You Text

**You have now completed the assessment.
Thank you very much for taking to time to participate.
Please be sure to collect a treat on the way out.**

Instruction Type: Pre-Question

This section of the assessment is a set of questions

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relating to your seafood buying and consumption.

Please click on the category that most represents your answer.

Question Text

Which of the following age brackets do you belong?

Choices: 5

Value

31 years or less 1

Age 31 - 40 years 2

Age 41 - 50years 3

Age 51 - 60 years 4

Age 61 years and above 5

Question Number: 2

Question Type: Multiple Choice

Question Title: Groceries

Blinding Codes: Standard

Question Type: Demographic

Placement: First Opportunity

Font Name: Arial

Size: 10

Instruction Type: None

Question Text

How often do you buy the groceries in your household?

Choices: 3

Value

Almost always 1

I share the purchase of groceries 2

almost never 3

Question Number: 3

Question Type: Multiple Choice

Question Title: purchase frequency

Blinding Codes: Standard

Question Type: Demographic

Placement: First Opportunity

Font Name: Arial

Size: 10

Instruction Type: None

Question Text

How often do you buy fresh or frozen seafood?

Choices: 5

Value

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- Several times a week 1
- About once a week 2
- About once a month 3
- about once every 3 months 4
- Only at Christmas or Easter 5

Question Number: 4

Question Type: Comment
Question Title: species most purchased
Blinding Codes: Standard
Question Type: Demographic
Placement: First Opportunity
Force Answer: No
Keyboard: No

Instruction Type: Post-Question

The next series of questions will relate to the samples in front of you.

If the sample you are requested to taste is not in front of you, please press the 'assistance' button in front of you on the right.

Please be aware that these are whole fish samples and despite the great care we have taken, you may encounter a bone or scale.

Question Text

Which are your 3 most commonly purchased seafood species?
(you can choose from any fish, crustacean or mollusc)

Question Number: 5

Question Type: Multiple Choice
Question Title: Acceptability question
Blinding Codes: Standard
Question Type: Sample Related
Font Name: Arial
Size: 12

Instruction Type: None

Question Text

Please find the fish sample %01 and taste a portion of the sample.
Is the texture of this fish acceptable to you?

Choices: 2

Value

Yes this texture is acceptable to me 1

No, this texture is unacceptable and I would reject this dish 2

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Question Number: 6

Question Type: Comment

Question Title: Like comment

Blinding Codes: Standard

Question Type: Sample Related

Force Answer: No

Keyboard: No

Instruction Type: None

Question Text

What if anything did you like about sample %01?

Question Number: 7

Question Type: Comment

Question Title: dislike comment

Blinding Codes: Standard

Question Type: Sample Related

Force Answer: No

Keyboard: No

Instruction Type: None

Question Text

What if anything did you dislike about sample %01?

Qld Dept of Primary Industries and Fisheries May 18, 2012 13:52 Page: 10
Compusense five 5.0 [2008/11/27] (c) Compusense Inc. 1986-2008

14. Appendix 4. Comments from consumers relating to question 6 and question 7 of the consumer assessment

Please note: Spelling and typographic errors are left in to provide exact responses by consumers.

14.1. Like comments

Category 1

Moist, melt in the mouth texture, colour good
Tender, sweet
Really nice and soft texture
Good texture, mouth feel and appearance. Liked most.
Moist tasty.
Very soft, easy to chew, very tasty, pleasant to eat.
Good flavour and soft flesh
Good texture, soft, smell fresh
Only just acceptable for firmness, taste OK
The texture is very nice, firm, almost no smell. I liked the taste
Tender, but meaty enough to bite into
Nothing really it was overall rather tasteless
Good flavour and flesh more flaky
Flaked off really well, colour and texture good
Nice flavour,
Much nicer, more palatable and more moisture
This meat is also solid and full and the taste and smell is better the previous sample
Sweet taste. Firm texture
Tasted fresh
Texture was soft and slightly falls apart but with a little chewiness to stop it being mushy flaked well in mouth.
Slightly better colour
Velvety, lovely favour and taste
Moist, had flavour, smooth texture
Firm but tender texture, moist, good colour
Reasonable texture and moisture
Nice light texture, more subtle flavour than previous two samples
Nice white flesh, great texture
The fact that it is a bit chicken like
Moist, subtle flavour
Nice and moist fish with not a strong sea smell
Fairly strong flavour even texture
Flavour and smell
Soft texture, colour, flavour
Reasonable flavour
Texture and flavour were fine for me.
Flavour
Flesh was a little flakier than the previous and drier or lest moist
Good flavour, not dry and it flaked apart nicely -
Was not too tough

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Easy to eat, melts in your mouth

Nice

The texture was good, a little chewer than the previous sample, nice fish taste, a stronger taste though than the previous samples but was quite pleasant.

The slight saltiness

Taste and flaky texture

Tastes better than 785, like the flakiness as opposed to the hardness of 758

Best texture because it was flakey and easy to cut with fork, mild flavour and smell

Good eating, good mouth feel

Flaked easily, fresh taste

Texture excellent, flavour enjoyable

Taste, lack of bones

Good texture

Slight oily/fish flavour

Easy to cut

Category 2

moist, good colour, good after taste

moist, white,

the sample has clean-fresh taste

Appearance is good

Soft, moist, easy to chew

Soft flesh

good texture, soft meat, smell fresh

firm, moist

I liked it, similar to the previous, with firm muscles,

low smell and nice taste.

meaty texture a bit like chicken

firm texture, no strong fishy smell

its firmness would be suitable for it to retain during cooking in eg a curry etc.

Retained flavour

firm texture white colour

nice smell, firm texture, good flavour

this fish is of a milder texture and flavour than 524,

The meat is solid and full. This can fill up with only a small amount

not a strong taste

best mouth feel, smooth and moist, segments fell apart while chewing

firmer than 524 and 122 but still highly acceptable

flake sections broke apart easily in mouth but more

resilience during chewing

fishy, moist, same taste as sample 525

smooth texture , nice flavour

juicy

moist but firm texture.

Mild to pleasant fish taste

I didn't particularly like it (would rank it 3rd of the 4 samples).

firm texture but not too firm

its tenderness

very moist

very soft texture and moist inside

GREAT taste for me , excellent flavour and texture!

flavour and smell

nice and moist

flavour

good texture - better than 124.

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good flavour.

A little more tender than the previous 2, Flavour similar. All good

Fleshy, moist

Meaty but not too tough, and not too dry

nice

less dense not as good as 526

texture was nice and fish taste was nice and mild

less so than the previous sample.

I liked the softness of this sample and it had a slight sweetness to it

taste and texture

tastes ok reasonable texture

very nice flavour, visually appealing (nice white flesh), good fishy taste without being too strong

firm, reasonably flavoursome

sweeter, nicer texture

Good flavour, firm texture.

firm texture

no bones

Good texture and flavour

It's quite juicy. the fish muscles separate well, so it's easy to cut with a fork.

Category 3

good after taste

tender

i really like the smooth texture

Good appearance and mouth feel

You left some scales in it, uncool.

moist

texture ok, taste good.

smell fresh, the texture is ok

firm, moist

Similar to the previous, but the texture seems harder, taste ok. I personally like fish with firm flesh

tender texture, but still 'meaty' enough to bite into, sweet-briney flavour

it had a stronger ocean taste and smell and overall

it was quite pleasant to eat

good flavour and still quite firm.

great texture, flavour less like bait more like a nice quality fish

even colour, nice smell

Tuna -like texture, firm and strong taste

I don't mind eating this sample but the smell is a bit strong when I open the sample but it tastes OK afterward

Firm. Not too strong a taste.

some what moist texture was excellent - flaked well with just right amount of resilience expected in large flaked fish flesh

Firm and easy to cut using fork.

fishy taste, cooked ok, moist

smooth texture ,nice taste, moist

colour is good, texture is fine

moist but firm texture

Slight flakiness of texture

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its saltiness
moderate texture
nice and soft
strong flavour
flavour and smell

nice and moist
white colour, moderately soft texture, flavour
better texture than 124.
good taste
Probably this sample (like the last one) was a little more tender than the first 2.
Flavour of all 4 was consistent.
Sweet flavour, juiciness
Fleshy and tasty not dry or flakey
Fish was meaty and juicy, not too dry and quite flavoursome, although not strong
fishy taste
Nice smooth taste, not too fishy, nice and flakey
Better than 124. Less dense and flavour good.
A really nice fish taste but no overpowering, texture
was softer than 757.
texture and taste
firm texture, nice ocean fish taste, not too strong,
low odour
ok, reasonably firm, bit chewy
oily, chewy
Not much - taste ok
no bones
texture
flavour OK
Quite hard to cut it. It seems really compact but in mouth it's not really disgusting.
It sticks a bit to my teeth, not really enjoyable.

Category 4

white colour
Good mouth feel, slight fishy odour
taste was ok
Tasted fresh
smell fresh,
taste is OK

I still like it, but I preferred the previous ones.
Similar characteristics with the previous
flavour nice
it had a little more flavour than the previous 343
the flavour was a little more oceanic than 343
firm, very lightly flavoured
firm, smelt nice, not too flakey
it is edible but not my choice of fish
the taste is similar to sample 122 .I find no problem with this sample also Good
Taste
good flavour, moist and good mouth feel
just border line ok - suitable for curries perhaps

A bit more flavour
dry but tasty

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smooth texture ,nice flavour
fish flavour, tender texture, nice colour
Not much.
nice white colour, firm texture but not too firm
soft flesh, not chewy
very moist, quite firm good for cooking curry
Has a nice fresh taste
nice appearance (flakier) good flavour and nice texture
flavour and smell

nice and moist
colour, moderately soft texture,
reasonable flavour.
All good again
Fleshy and fresh
Good flavour and not dry
not a muddy taste, not salty
less dense but funny taste
It has a nice mild fish taste and is not
overpowering, was quite enjoyable taste

white, well presented
nice firm texture, low odour
ok
tasted 'clean and fresh'
texture firm, flavour good
no bones taste
flavour Ok

14.2. Dislike Comments

Category 1

bland
No specific dislike
n/a
no
a bit too firm

nothing
a bit powdery texture at the finish
i found that it had a rather strong scent and the flesh was not as firm as the
previous 755 and 343
a little moister but still a tad dry
weird taste not fishy but more dirty....
not as tightly packed as others, somewhat uneven
in colour, slight sliminess between the flakes of fish
Nothing
Nothing. I like the smell taste and texture of the fish
Nothing
A bit dry in the mouth when chewing
nil

nil

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sample tasted fine

plain flavour

bland taste

I quite liked it, but would like to taste it with flavour enhancers (lemon, sauce or spices).

a bit floury taste

did not taste as fresh

metallic aftertaste

Maybe a little drier than I would prefer, but still fine.

Although texture was acceptable, it was slightly chewy

not as flavoursome as previous sample

Nothing it was very nice

nil

Maybe a little dense

No

A little chewy

nothing

nothing

nothing

nothing

Nothing

nothing

Nil

A little bit dry. It sticks a little bit to my teeth, but less than sample 527.

Category 2

slightly bland

Little bit fibery

bit dry

not very tasty

Less flavour compared to 754

no

a little too firm

nothing

It was a little bland

perhaps a little dry

a little too fish flavoured, I prefer a milder flavour

A slight 'sliminess' at first

Again this is tuna or shark -like flesh very wholesome but tasteless

The taste is bit strong and fishy

Not much taste at all

nothing

nil

Seem to a bit leathery

nil

nothing

inconsistent colour (some black dusts within tissue & brown surface), stringy texture

nil

Texture a bit too firm. Flavour quite strong – almost muddy.

a bit dry but it is the best for me so far

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does not taste fresh and didn't have the sea smell at all which made it tasteless

brown colour, firm texture

No problem

Slightly bland flavour

Different Fish taste didn't taste like ocean fish but was nice

Nothing

a little chewy

the flavour not as good as 526 maybe 345 a little

soggy

no

presentation, it looked a bit too brown

nothing

nothing

maybe bit too firm, didn't melt in the mouth

Nothing

nothing to report

Nil

Category 3

slightly dry

tender to eat but hard to pull apart

fishy backnote

woops, see previous.

A little dry.

Fish tasted old, chewy and lack of flavour

the meat is not soft enough

nothing

nothing

nothing really

this was fine but still a touch dry

slight dark colour on bottom.

more slimey in texture on the tongue

this type of fish needs more flavour

by itself not very tasty

I don't like the smell of the fish at first but it still

passes my test

Nothing

a bit dry and crumbly

nil

nil

nothing tasted fine

plain flavour, springy texture, not juicy

bland taste

Flavour wasn't overwhelmingly good

'Fishy' taste too strong, too tough

a bit chewy and a bit too hard

slight metallic flavour

a bit rubbery

strong bitter aftertaste

No problem

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N/A

I prefer fish that flakes away a little more but it wasn't unpleasant

Nil

that was pretty good

No it was great

A little chewy and a bit bland

nothing

bit rubbery

didn't look as nice as usual fish with brown lines through it. not as visually appealing

bit too firm & chewy

oops wrong comments in last box

oily and chewy and smell

Texture rubbery, flavour ok but bland

nothing to report

Rubbery texture

Too hard to cut with a fork. sticky to the teeth. And I prefer when fish kind of melts in my mouth.

Category 4

slightly rubbery in the mouth

sl dry

the texture of the sample is a bit chewy and rubbery

Odour

It seemed to be a little bit, but not unacceptably (to me), tough and perhaps a little dry too.

a bit dry

texture was rubbery, overcooked, hard to chew

A little Chewy

the texture is not very good, the meat is not soft

firm to the point of hard

The colour of the meat was not as white as the other samples. Taste is still ok, but I preferred the one immediately before.

too chewy, as though it was dried out but it was still

juicy?

it was perfectly fine

still a bit dry

Strange texture. Very firm and didn't seem to have the layers

would be disappointed if this fish texture was presented in restaurant

too dry

a bit dry and denser in texture

1 dry mouth feel

Rubbery texture

bland taste

Rubbery texture.

the strong remaining taste

not as soft

texture a little firm, but still acceptable

Maybe a little drier than I would have liked, but still OK

Texture was like rolled chicken loaf and dry

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n/a

A little bit 'chewy' or 'rubbery' - did not make it unpleasant but it definitely didn't flake away nicely the way I like my fish.

Not very flakey

taste not as good as 124

It was a bit chewy as if it was over cooked

Too chewy

texture a little bit rubbery

nothing

a bit chewy

bit too firm

little bit tough

Nothing

nothing to report

too dry

Really compact. It looks more like a chicken texture. It sticks a bit to my teeth. I wouldn't like it in a 'cooked fish dish' but why not if it was covered with a butter.

15. Appendix 5: Discussion material and stakeholder meeting minutes (November 2011).

Discussion points for decision on next actions

FRDC Project 2010-207: Toughness in Saddletail snapper flesh

Project objective 4:

To develop recommendations and strategies for industry stakeholders to minimise the impact of tough fish syndrome

Where we are at....

- cause of toughness identified – correlated to age of fish
- fish >15 yrs old have higher likelihood of being tough

so where to next ? – several approaches: (in no specific order)

Toughness – consumer perception

- need to establish the level of toughness that is unacceptable to consumers
- this will establish what portion of the catch affected consumer perception-wise

Methods to determine age

To differentiate tough fish from rest of catch – ideally need to age fish on back deck

- length (or weight) correlations
- sex determination
- otoliths (ear bones) – visual / near infrared (NIR)
- scales – NIR

Processing technology

- enzyme treatment – whole fish / fillet stage
 - papain (papaya), bromelain (pineapple), zingibain (ginger) etc
- high pressure processing – value-added products
- product concepts

Market placement repositioning

- consumer information
- major qualities of Saddletail

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TFS stakeholder meeting – 02 November 2011 (NTSC, Darwin)

Present: Bill Passey, Horst Fischer,
Katherine Sarneckis (NTSC), Rob Fish (NTSC)
Andrew Forrest (DAFF QLD), Sue Poole (DAFF QLD)
Julie Martin (NT Fisheries)

Main discussion outcomes:

1. Andrew provided the results and knowledge achieved to date within the project (summary attached - PowerPoint).
 - flesh toughness is correlated to fish age – the older the fish, the more likely it will exhibit toughness
 - Saddletail are difficult to age onboard as usual fish length correlation methods are not applicable for this species
2. Male fish are typically larger than female fish – however need to examine internally to determine sex
 - fish could be sexed at processing
 - discussion ranged into in-line technologies possible to sex fish in whole state
 - x-ray, ultrasound, imaging were mentioned

Action – desktop study undertaken to summarise possible technologies and any current application worldwide **AF/SP**

3. Need actual information on consumer perception of toughness – what level of toughness is unacceptable and hence what portion of the catch is involved

Action – carry out consumer acceptance/rejection assessment. Design, conduct and interpret **AF/SP**

4. Interest in enzyme use during processing to reduce flesh toughness
 - enzymes from natural sources available commercially - papain (papaya), bromelain (pineapple), zingibain (ginger)
 - successfully used in the meat industry
 - treatment cost /kg important factor

Action – design and conduct proof of concept trials to determine the success of enzyme treatment in reducing flesh toughness **AF/SP**