

Improving profitability to Industry through the identification and management of 'tough' fish syndrome in tropical Saddletail snapper (*Lutjanus malabaricus*)

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



Project 2008/208

**Improving profitability to Industry
through the identification and
management of 'tough' fish syndrome
in tropical Saddletail snapper
(*Lutjanus malabaricus*)**

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

Project 2008/208

February 2010

TFS in Saddletail snapper

ISBN 978 0 7345 0412 8

Improving profitability to Industry through the identification and management of 'tough' fish syndrome in tropical Saddletail snapper (*Lutjanus malabaricus*)

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

Final report of project number 2008/208 submitted to the Fisheries Research and Development Corporation in February 2010.

Copyright Fisheries Research and Development Corporation and Department of Employment, Economic Development and Innovation, Queensland. 2010.

This work is copyright. Except as permitted under the Copyright Act 1968 (Cth), no part of this publication may be reproduced by any process, electronic or otherwise, without the specific written permission of the copyright owners. Information may not be stored electronically in any form whatsoever without such permission.

Disclaimer

The authors do not warrant that the information in this document is free from errors or omissions. The authors do not accept any form of liability, be it contractual, tortious, or otherwise, for the contents of this document or for any consequences arising from its use or any reliance placed upon it. The information, opinions and advice contained in this document may not relate, or be relevant, to a reader's particular circumstance. Opinions expressed by the authors are the individual opinions expressed by those persons and are not necessarily those of the publisher, research provider or the FRDC.

The Fisheries Research and Development Corporation plans, invests in and manages fisheries research and development throughout Australia. It is a statutory authority within the portfolio of the federal Minister for Agriculture, Fisheries and Forestry, jointly funded by the Australian Government and the fishing industry.

Information in this report is provided as general advice only.

For application to specific circumstances, professional advice should be sought.

The Department of Employment, Economic Development and Innovation, Queensland, has taken all reasonable steps to ensure that the information contained in this publication is accurate at the time of publication. Readers should ensure they make appropriate inquiries to determine whether new information is available on the particular subject matter

The Fisheries Research and Development Corporation plans, invests in and manages fisheries research and development throughout Australia. It is a statutory authority within the portfolio of the federal Minister for Agriculture, Fisheries and Forestry, jointly funded by the Australian Government and the fishing industry.

For enquiries or further copies of this report, contact

Innovative Food Technologies
Department of Employment, Economic Development and Innovation
19 Hercules St
HAMILTON QLD 4007 AUSTRALIA

Or

Fisheries Research and Development Corporation
P.O. Box 222
DEAKIN WEST ACT 2600 AUSTRALIA

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, Horst Fisher, Steve Hinge and David Caricciolo.

Thanks also the skippers of the vessels that participated in this project. In particular, Colin Goddard skipper of FV Starlight for 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.

A very special thank you to Katherine Sarneckis of the Northern Territory Seafood Council for her tireless support and strict adherence to timelines. Special thanks also to John MacCartie of Northern Territory Fisheries for assistance with field trips, arranging fish transport logistics and generally keeping his ear to the ground of the happenings to the fishery operators.

Many thanks to Ron Tume, Anita Sykes and Joanne Mountford of CSIRO Food Science for completion of sarcomere analysis, collagen determinations and supply of a standard of hydroxylysyl pyridinoline. A special thanks also to Robin Shorthose of CSIRO Food Science for making himself available to discuss scientific data in his personal time away from work.

The authors would sincerely like to thank Deception Bay staff who happily allowed our team access to their specialist equipment, including training and overseeing our results. They allowed our research to fit in around their own work and made time to train and explain methodologies. The two fields of food research and fish aging do not normally cross paths yet the collaboration between the two departments was pivotal in producing a crucial outcome for this research. We would especially like to thank Dr Ian Brown, Mark McLennan, Darren Smallwood, Michelle Sellin, and Stephen Wesche for their individual input in this project.

Thanks also to Dr Deborah Stenzel from the Centre for Microscopy and Microanalysis at Queensland University of Technology Gardens Point for light and electron microscopy services, and for providing input into understanding the microstructure of Saddletail muscle tissue.

Many thanks to David Williams for completing HPLC analysis of nucleotide content. Also for assisting with method development and completion of pyridinoline analysis.

2008/208	Improving profitability to Industry through the identification and management of 'tough' fish syndrome in tropical Saddletail snapper (<i>Lutjanus malabaricus</i>)
-----------------	--

PRINCIPAL INVESTIGATOR: Sue Poole
ADDRESS: Innovative Food Technologies
Department of Employment, Economic
Development and Innovation
19 Hercules Street
Hamilton QLD 4007
Tel: 07 3406 8689 Fax: 07 3406 8698

OBJECTIVES:

1. To determine whether incomplete rigor mortis resolution and 'cold shock' play a role in development of tough fish syndrome (TFS) in tropical Saddletail snapper.
2. To identify links between TFS and specific physiological factors in tropical Saddletail snapper.
3. Communicate findings and recommendations to stakeholders and assist with implementation of any changes to fishing or handling practices required.

NON-TECHNICAL SUMMARY

OUTCOMES ACHIEVED TO DATE

Saddletail snapper has been an under-valued table species in Australia due to consumer perceptions of toughness and inconsistent quality. The factors influencing cooked muscle toughness were investigated by instrumental texture, chemical analyses, electron microscopy and fish aging techniques. The major outcome of this project was identification of age as a significant driver of increased toughness in cooked Saddletail. Seasonal influences were also shown to have an influence on texture variation in cooked Saddletail muscle.

Onboard handling and chilling practices do not appear to be a strong contributing factor to toughness of Saddletail muscle. However, synergistic effects of fish age and chilling abuse have not been investigated to date.

Size differences between male and female Saddletail snapper were observed. Male Saddletail are significantly larger than females of the same age, and fish size did not correlate directly to age for either males or females. This is a complicating factor with regard to employing a simple size to age formula for eliminating older and therefore tougher fish entering the supply chain.

Saddletail snapper represent a major resource within the offshore fisheries of northern Australia. Current catch rates are well below triggers for management reviews. However, more licences are unlikely to be issued to new participants in any of the fisheries. This fact ensures that current stakeholders have little opportunity to expand their operations to maintain economic viability against ever increasing costs by increasing effort. Ongoing economic viability will only be assured through increasing the value of the resource through improved product quality.

Suppliers of red snappers (both Crimson and Saddletail) have endured a crisis of confidence in recent years. Ongoing complaints of toughness of cooked fillets have resulted in both species becoming highly distrusted by food service and restaurant operators alike. This has resulted in suppliers of both species being unable to command suitable pricing and undervaluation of the resource.

This project has investigated the possible causes of toughness issues in Saddletail snapper. Both direct handling and biological causes have been investigated during the course of this work. Handling practices were observed directly onboard vessels. Data collected included temperature profiles of fish and the chilling tanks, assessment of rigor mortis progression, as well as collection of biological data and tissue samples.

Toughness was measured on cooked fillets using an Instron texture analyser, incorporating a standardised cooking protocol. Chemical analyses relating to muscle firmness were conducted to provide a better understanding of the physiochemical microstructure of the Saddletail muscle tissue. Electron microscopy methods were also employed to assist in developing this understanding.

Another method that became critical to the success of this project was the extraction of fish otoliths for the determination of age. Age was found to be a fundamental driver in the development of toughness in Saddletail.

Although males are much larger than females of the same age, the increase in cooked muscle toughness increases with age at almost the same rate. This research also

revealed that both males and females grow very slowly after 7 to 10 years of age, so that fish of this age are not very different in size from those of 20 years of age.

Seasonal influences were also determined to influence the texture of cooked Saddletail. These influences are more difficult to explain with certainty. However, the most likely causes are either sexual maturity or food availability, or even a combination of both.

Onboard handling practices were not found to directly contribute to toughness issues in Saddletail. Chilling practices observed were found to vary greatly in efficacy. However, the potential exists for a synergistic effect of older and tougher fish becoming tougher due to 'cold shortening' through chilling abuse. This potential is certainly worth investigating further.

The information from this research provides stakeholders with a clear understanding of the contributing factors of tough fish syndrome in Saddletail snapper. More work is required to develop simplified tools that allow fishery operators to quickly and efficiently remove fish that pose a significant risk. The development of these tools will require regular stakeholder consultation due to their highly commercial nature. Successful implementation of such measures would increase market confidence in the species and provide an opportunity to develop value added products with fish deemed unsuitable for premium table fish markets.

KEYWORDS:

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

TABLE OF CONTENTS

1	Background	1
2	Need.....	3
3	Objectives.....	4
4	Methods.....	5
4.1	Planned field trips	5
4.2	Transport and logistics.....	5
4.3	Sample processing (IFT Hamilton QLD)	5
4.4	Rigor assessment	5
4.5	Temperature logging.....	6
4.6	Texture Analysis	7
4.7	Sarcomere Length Determination	9
4.8	Chemical Analyses	9
4.8.1	Nucleotide determination	9
4.8.2	Total collagen determination	9
4.8.3	Heat soluble collagen determination.....	9
4.8.4	Collagen cross-linking determination (Hydroxylysyl pyridinoline)	9
4.9	Microscopy.....	9
4.10	Estimation of fish age by otolith increment	9
5	Results/Discussion	10
5.1	Field trip 1 (Oct/Nov 2008).....	10
5.1.1	Temperature logging.....	10
5.1.2	Texture assessment.....	12
5.1.3	Rigor assessments.....	14
5.1.4	Nucleotide determination	15
5.1.5	Muscle pH.....	17
5.1.6	Analysis of last days catch.....	17
5.1.7	Analysis of catch by sex.....	18
5.1.8	Preliminary collagen determination.....	18
5.1.9	Sarcomere analysis.....	20
5.1.10	The effect of barotrauma on fish quality.....	21
5.1.11	Trip summary	25
5.2	Field trip 2 (Dec 2008)	26
5.2.1	Biological data and Instron results	26
5.2.2	Nucleotide Analysis.....	28
5.2.3	Collagen Analysis.....	28
5.2.4	Summary.....	30
5.3	Field Trip 3 (Apr/May 2009)	31
5.3.1	Biological data and Instron results	31
5.3.2	Collagen results	34
5.3.3	Summary.....	35
5.4	Field trip 4 (Jun 2009)	36
5.4.1	Biological and Instron data.....	36
5.5	Field trip 5 (Oct 2009)	37
5.5.1	Biological and Instron data.....	37
5.5.2	Summary.....	39
5.6	All field trip summaries.....	40
5.6.1	Field trip 1	40
5.6.2	Field trip 2	40
5.6.3	Field trip 3	41
5.6.4	Field trip 4	41
5.6.5	Field trip 5	41
5.7	Otolith examination for Saddletail age approximation.....	42
5.7.1	Age approximation of Saddletail from Field Trip 3 (April 2009).....	42

5.7.2	Age approximation of Saddletail from Field Trip 5 (October 2009)	44
5.7.3	Age and fork length data for FT3 and FT5	46
5.7.4	Summary of ageing results	46
5.8	Collagen cross-linking determination (pyridinoline)	47
5.8.1	Background	47
5.8.2	Results	47
5.8.3	Summary	49
5.9	Electron Microscopy	50
5.9.1	Background	50
5.9.2	Results	51
6	Benefits and adoption	54
7	Further Development	55
7.1	Segregation of fish with high risk of 'toughness'	55
7.2	What is 'acceptable' firmness?	55
7.3	Simplified identification of sex	55
7.4	Influence of season on texture	55
7.5	Synergistic effects of age and inappropriate chilling	55
8	Planned Outcomes	57
9	Conclusions	58
9.1	On-board deck practices	58
9.2	Biological factors influencing Saddletail texture.	58
9.2.1	The effect of age on cooked muscle texture	58
9.2.2	Seasonal variation in texture	59
10	References	60
11	Appendix 1 Intellectual Property	62
12	Appendix 2 Project Staff	62

LIST OF TABLES

Table 1. Field trip and sample collection summary..... 5

Table 2. Observed rigor stage and scoring system 5

Table 3. Comparison (ANOVA) of parameters for last day to remaining catch 18

Table 4. Mean size of male and female fish sampled..... 18

Table 5. Sarcomere measurements (μm) for 31 Saddletail snapper samples, taken immediately on capture and after full rigor had developed during chilling in refrigerated sea water 20

Table 6. Significant difference of means of measured parameter by sex..... 26

Table 7. Means of measured parameter by sex on field trip 3..... 31

Table 8. Significant difference between sexes and seasons excluding fish < 1kg (FT3).. 32

Table 9. Means of measured parameter by sex on field trip 4..... 36

Table 10. Means of measured parameters by sex on field trip 5..... 37

Table 11. Means of parameters by sex across field trips 2, 3 and 5 (fish > 1KG) 37

Table 12. Biological data and sarcomere length as determined by TEM (FT2 and FT3) . 51

LIST OF FIGURES

Figure 1 Rigor assessment by observed fish flexibility 6

Figure 2 Saddletail snapper showing temperature logger inside cavity from pre-rigor tissue and second tissue sample being taken upon full rigor. 7

Figure 3 Instron texture analyser with sample loaded into the Kramer-Shear cell..... 8

Figure 4 Photograph of sample preparation for texture assessment by Instron 8

Figure 5 Photographs of brine tank being filled and placement of temperature logger on return cage. 10

Figure 6 Daily temperature profiles for brine tank on FV Starlight and the total mass of fish placed in the tank during the haul (in brackets)..... 11

Figure 7 Temperature profiles during chilling of 31 Saddletail snapper sampled on FV Starlight 12

Figure 8 Mean total energy and fork length 13

Figure 9 mean peak force versus fork length..... 13

Figure 10 Comparison of Mean peak force and mean total energy for individual fish 14

Figure 11 Mean penetrometer readings of first 15 Saddletail sampled on FV Starlight..... 14

Figure 12 Nucleotide analyses of samples at landing..... 15

Figure 13 Nucleotide analyses of samples at full rigor 15

Figure 14 Final muscle pH and peak force versus total days stored on ice..... 17

Figure 15 Scatter plot of peak force and collagen content..... 19

Figure 16 Scatter plot of total energy and collagen content..... 19

Figure 17 Saddletail showing signs of barotrauma (eye and belly cavity). 21

Figure 18 Saddletail showing signs of barotrauma (lateral tissue). 22

Figure 19 Internal bleeding staining Saddletail fillet..... 23

Figure 20 internal bleeding in both fillets 23

Figure 21 Close of bleeding in belly cavity and backbone 24

Figure 22 Scatter plot of peak force versus fork length for both sexes..... 27

Figure 23. Scatter plot of peak force versus ultimate muscle pH for both sexes. 27

Figure 24 Scatter plot of ultimate pH and fork length for both sexes. 28

Figure 25 Comparison of peak force and total collagen results 29

Figure 26 Peak force (N/g) versus Total collagen (mg Hyp/g) by sex..... 29

Figure 27 Scatter plot of total energy versus fork length for males ($R^2=0.46$) and females ($R^2=0.39$) 31

Figure 28 Mean peak force values for male and female fish >1kg across season 33

Figure 29 Total energy values for male and female fish >1kg across seasons 33

Figure 30 Scatter plot of total energy and percentage insoluble collagen for male ($R^2=0.50$) and female ($R^2=0.47$) Saddletail snapper.....	34
Figure 31 Scatter plot of percentage insoluble collagen and fork length for male ($R^2=0.52$) and female ($R^2=0.53$) Saddletail snapper	35
Figure 32 Linear regression (with groups) of total energy and fork length for all males from FT2, FT3 and FT5 explaining 38.4% of variation.....	38
Figure 33 Linear regression (with groups) of total energy and fork length for all females from FT2, FT3 and FT5 explaining 30.5% of variation.....	38
Figure 34 Fork length and age (otolith increment) for males and females from FT3.....	42
Figure 35 Total energy and age (otolith increment) for males ($R^2=0.75$) and females ($R^2=0.71$) from FT3	43
Figure 36 Heat insoluble collagen and age (otolith increment) for males ($R^2=0.72$) and females ($R^2=0.62$) from FT3 (log scale trendlines)	43
Figure 37 Age and fork length data for male and female Saddletail from FT5	44
Figure 38 Total energy and age (otolith increment) for males ($R^2=0.44$) and females ($R^2=0.55$) from FT5	45
Figure 39 Age and fork length data for males and females from FT3 and FT5.	46
Figure 40 Longitudinal and transverse sections of connective tissue from Saddletail snapper showing helical collagen fibres at 15K, 30K and 60K times magnification respectively (size bars = 500/200/100nm respectively)	47
Figure 41 Total energy and PYD for male ($r^2=0.56$) and female ($r^2=0.49$) Saddletail from FT3.....	48
Figure 42 Heat insoluble collagen and PYD for male ($r^2=0.63$) and female ($r^2=0.36$) Saddletail from FT3.....	48
Figure 43 PYD content and age data for male ($r^2=0.73$) and female ($r^2=0.59$) Saddletail from FT3	49
Figure 44 TEM Micrograph of Saddletail muscle fibre at 30K times magnification (Size bar denotes 200nm).	50
Figure 45 Sarcomere length and total energy for Saddletail from FT2 and FT3 ($r^2=0.34$)	52
Figure 46 TEM micrograph of Saddletail muscle tissue at 5K times magnification (size bar denotes 1 μ m)	53

1 Background

This project application was developed at the specific urging of the reef fish supply chain in both Queensland and the Northern Territory. The Western Australian northern demersal fishers also strongly support the project. All jurisdictions are suffering large revenue losses caused by Tough Fish Syndrome (TFS). Outcomes from this research will directly address the profitability of reef fish fisheries and the demand for high quality Australian premium product.

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 on the consumer plate. Prior to this point the fish is not visually different to any other. 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, pers. comm.) refuse to buy Grassy snapper off the boats as they consider the risk of toughness occurring to be too high. TFS is consequently causing significant revenue loss for the industry, none more greatly evidenced than by the recent cancellation of large retail supply contracts.

TFS has been observed for several years, with increasing reported incidence over the last 3-4 years. As most of the available evidence is subjective, it is difficult to accurately state the real incidence. Rigorous data on the incidence of TFS within catches is needed to illustrate the factors that cause the toughness. This data can be very expensive to obtain due to the extremely large sample populations required, but by working cooperatively with Industry costs can be minimised.

TFS is reported across all reef 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. Industry reports up to 30% of catches for tropical snappers are affected. Species such as Saddletail snapper, Crimson snapper, Red emperor and Golden snapper are implicated (NT Seafood Council and NT Fish, pers. comm.). Reports also 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 there are indications that larger fish (>3KG) are more likely to exhibit this syndrome.

Innovative Food Technologies (QPI&F) has undertaken an extensive literature search on the issue of tough flesh in fish, however very little literature exists on textural issues in tropical fish species. Similar work has been completed on temperate species, but the issues with these species are generally a decrease in fillet firmness rather than increase. Relevant references have been included in the application. 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, muscle fibre number and density, collagen content and types, diet, and geographical location.
2. Capture methods including immediate post-capture handling, onboard chilling method and storage time.
3. Cooking practices including cooking method, cooking time and rigor mortis phase of the fish at cooking.

Under ideal handling conditions, tropical fish go into and through rigor mortis more gently and slower than temperate species (Curran et al. 1986; Poole 1991). It is also known that tropical fish can suffer cold-shock syndrome similar to that occurring in beef carcasses when chilled too rapidly (Curran et al. 1986).

Textural irregularities have been demonstrated to be seasonal in some species (Hagen et al. 2007). Specifically, the role of collagen types and cross-linking of collagens in textural variability observed in raw fillets. Hagen demonstrated that aquaculture Halibut harvested in spring would result in firmer texture than other seasons. Significant differences do exist between the post-mortem behaviour of tropical and temperate fish species. However, an investigation of the influence of collagen content and types in Saddletail snapper would provide a greater understanding of the textural qualities of commercially significant Australian tropical fish species.

A preliminary study was initiated by the trawl sector of the NT reef fishery (Bill Passey, Australia Bay Seafoods) who contributed \$25,000 in cash plus in kind towards an investigation into the syndrome. This study was undertaken by Innovative Food Technologies (QDPI&F). Results obtained were not conclusive due to a lack of fish displaying TFS.

The focus of this study was to identify the factors that cause development of TFS. Of all the possible influencing factors that may contribute to the syndrome, we will focus on those whose direct influence is quantifiable by the methods outlined in this proposal. These include pre-capture factors related to the fish biology and specific muscle physiology, and post-harvest factors influencing rigor mortis resolution.

Successful identification of these factors and their influence upon TFS will empower stakeholders to make an informed decision as to how to best utilise the resource and ensure improved profitability of the fishery into the future.

2 Need

The biomass of tropical red snapper in northern Australian waters has been estimated at 24,000t. A conservative management trigger point has set annual harvest levels at 2,400t. Current catches are well below this level. The majority of red snapper is caught by trawl, but there is also a potential to target them in trap and dropline fisheries.

Tough fish from these fisheries are identified on occasion at the point of cooking. Currently it is not possible to identify this syndrome at the point of capture or wholesale. There is an urgent need to identify the cause of TFS to minimise impact of the syndrome on the value of the resource and enable appropriate handling methods to be implemented where applicable.

TFS is causing a huge loss of revenue from the reef fish fishery due to strong negative reaction from the end-supply chain sectors with this phenomenon reducing the overall value of this, and other species in the fishery. The magnitude of such losses was made apparent recently when one of Australia's largest retailers cancelled a very large supply contract from a major fishery operator. Another major stakeholder in the fishery has had export orders rescinded.

Industry believes that if TFS in red snapper could be managed the current price of around \$4.50/KG could be increased up to \$8.00/KG, in line with other tropical snappers. This would lead to estimated additional \$3.0M/year revenue from this species under current catch levels. If the value of this species increased, there is potential to significantly increase sustainable catch levels and subsequent return to the community.

3 Objectives

1. To determine whether incomplete rigor mortis resolution and 'cold shock' play a role in development of tough fish syndrome (TFS) in tropical Saddletail snapper.
2. To identify links between TFS and specific physiological factors in tropical Saddletail snapper.
3. Communicate findings and recommendations to stakeholders and assist with implementation of any changes to fishing or handling practices required.

4 Methods

4.1 Planned field trips

Two major field trips (including a small preliminary trip) were planned for during the course of this project. However, five field sets of fish were collected during the project. This schedule of trips is outlined in Table 1.

Table 1. Field trip and sample collection summary

Trial Name	Season	Fishing Method/Transport	Total (402)
FT1	Oct/Nov 2008	Trap/Fresh	50
FT2	Dec 2008	Trawl/Frozen	111
FT3	Apr/May 2009	Trawl/Frozen	101
FT4	June 2009	Trap/Frozen	31
FT5	Oct 2009	Trawl/Frozen	109

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 (IFT Hamilton 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

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 follows:

Table 2. Observed rigor stage and scoring system

Presence of rigor	Observed bend in fish	Rigor score
no rigor	~90°	1
some rigor	~60-70°	2
moderate rigor	~45°	3
significant rigor	~20-30°	4
full rigor	~0°	5

The fish was placed on a nylon cutting board lying on its right side so that the tail would hang over the edge of the board. Each fish was positioned so that the end of the pelvic

fins would align with the edge of the board to allow for 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 presented in Figure 1 along with the penetrometer used in this experiment.



Figure 1. Rigor assessment by observed fish flexibility

Muscle tension was also assessed by use of a penetrometer to provide a quantitative measure of the rigor development observed. The penetrometer is a modified fruit pressure tester (EFFEGI: model FT 011) fitted with a 19mm plunger disc. Muscle tension is measured as the pressure required over an area of 2.84cm² to cause a maximum possible deflection of 6mm in the fish surface. Readings were taken only from the left side as the removal of tissue samples from the right side may have influenced the result from that side.

Three readings were taken from defined points along the fish. These were;

1. On the lateral line between the pectoral fin and the first dorsal fin.
2. On the lateral line between the rear of the pelvic fin and the dorsal fin.
3. On the lateral line between the front of the anal fin and the second dorsal fin.

4.5 Temperature logging

All fish that were sampled had a Thermocron temperature logger (OnSolution, Baulkham Hills, NSW) inserted into the cavity from which the tissue sample was taken (see Figure 2) Temperature values were taken every 15 minutes and loggers were removed from the fish when filleted in Brisbane.



Figure 2. Saddletail snapper showing temperature logger inside cavity from pre-rigor tissue and second tissue sample being taken upon full rigor

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.

Fillets were vacuum-packed into plastic bags and steamed at approximately 95°C for 20 minutes. The fillets 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 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 3 shows the Instron in use with a sample of Saddletail in the modified Kramer-Shear cell.



Figure 3. 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 4.

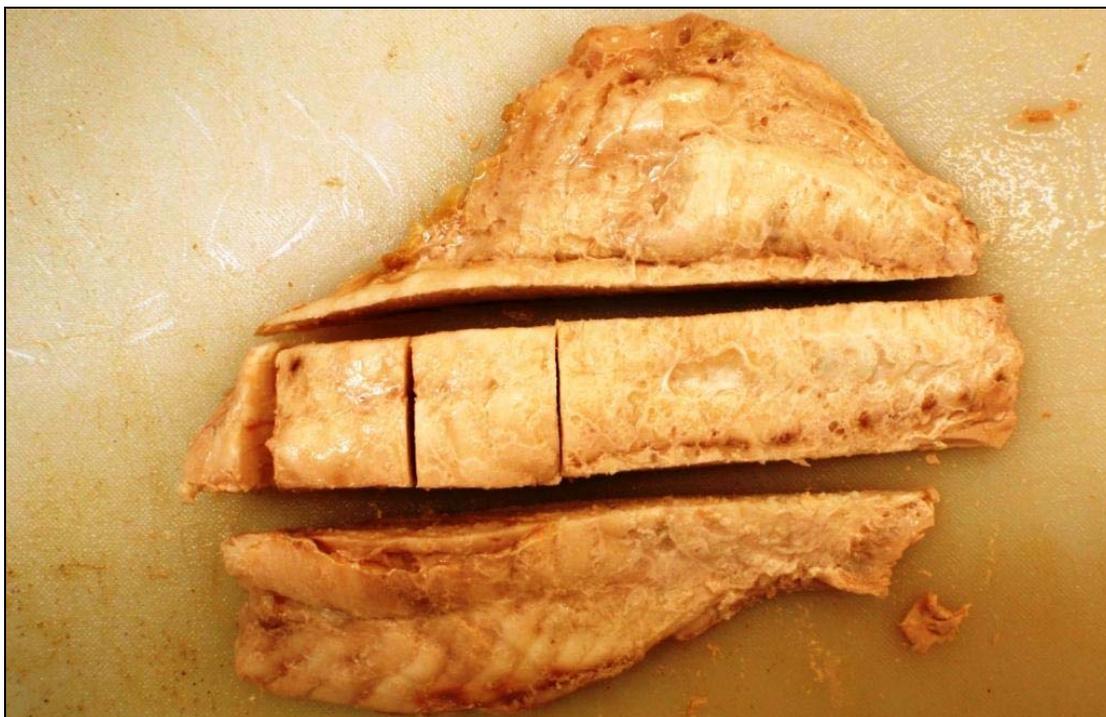


Figure 4. Photograph of sample preparation for texture assessment by Instron

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 joules (J). The results presented here are also divided by the weight of the sample (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 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).

4.8 Chemical Analyses

4.8.1 Nucleotide determination

Tissue samples were assessed by HPLC for nucleotide content to allow k-value calculations as determined by Saito (1959)

4.8.2 Total collagen determination

Total collagen content was determined by measuring the hydroxyproline content of lyophilised muscle tissue (in duplicate) according to the International Standard (ISO) method (1994), and is expressed as mg hydroxyproline (Hyp)/g dry weight muscle.

4.8.3 Heat soluble collagen determination

The heat solubility of the collagen in the muscle samples was determined using a modification of the method described by Hill (1966). Approximately 250mg of freeze-dried, ground muscle tissue was heated in $\frac{1}{4}$ strength Ringer's solution for 30 minutes at 65°C, reflecting the lower thermal stability of fish collagen compared to mammalian collagen. The hydroxyproline content of the soluble fraction was measured using the ISO method (1994) as described above for total collagen content. The heat-soluble collagen was expressed as a percentage of the total collagen.

4.8.4 Collagen cross-linking determination (Hydroxylysyl pyridinoline)

Amounts of hydroxylysyl pyridinoline cross-linking in collagen was determined using the method described by Li et al (2005).

4.9 Microscopy

Tissue samples of selected fish from field trips 2 and 3 were supplied to Dr Deborah Stenzel at the Centre for Microscopy and Microanalysis at Queensland University of Technology Gardens Point. A small number of samples will be imaged using Transmission Electron Microscopy (TEM) and light microscopy to attempt to identify any artefacts of 'cold shock' and accurately determine sarcomere length.

4.10 Estimation of fish age by otolith increment

Selected fish 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).

5 Results/Discussion

5.1 Field trip 1 (October/November 2008)

A total of 50 fish were collected during this trip. 31 of these were sampled during the first 9 days of fishing and were assessed according to the methods outlined previously. Another 19 fish were collected on the last day of fishing. No tissue samples were taken, or rigor assessments completed on these 19 fish. The vessel was unloaded at Darwin Harbour on Thursday 2nd October.

All 50 fish were packed into certified seafood air freight Styrofoam boxes at Darwin Fish Market and transport to Brisbane later that day by Australian Air Express. Fish were collected from Brisbane Airport Friday morning and repacked into ice storage. Fish were processed on Monday 6th and Tuesday 7th October. Texture assessments were conducted on both days.

A complete set of the raw data can be found in accompanying data CD.

5.1.1 Temperature logging

Brine temperatures were also logged during fishing. The logger was attached to the steel grill that surrounded the water return inlet to the refrigeration plant. The brine tank is displayed in Figure 5 and the temperature logger is visible in the right hand photo with a blue tag.



Figure 5. Photographs of brine tank being filled and placement of temperature logger on return cage

The daily temperatures profiles of the brine tank are presented in Figure 6.

TFS in Saddletail snapper

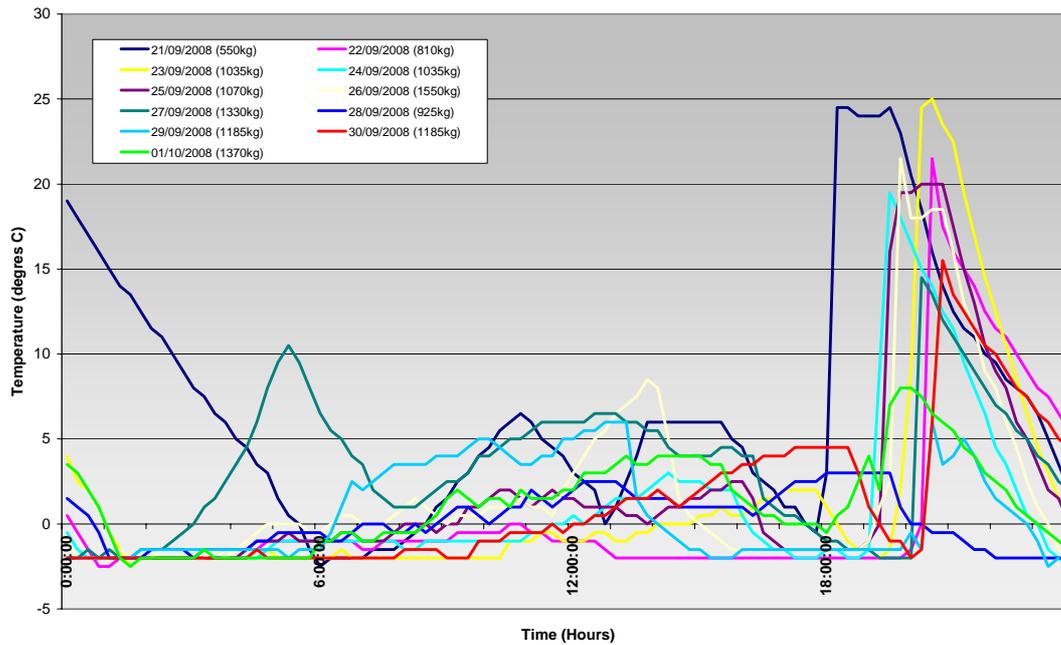


Figure 6. Daily temperature profiles for brine tank on FV Starlight and the total mass of fish placed in the tank during the haul (in brackets)

Daily fluctuations in temperature of the brine water are most likely due to load on the refrigeration system. However, total load in the tank per day does not accurately translate to fluctuations in water temperature. For example, the largest day's catch was 1550kg caught during 26th September and the brine water temperature never exceeded 2°C. Every other day (with the exception of 22nd September) brine water exceeded this temperature.

These observed fluctuations are more likely to be due to individual lines or even traps containing large numbers of fish being placed in the tank during a short amount of time. At the cessation of fishing, temperatures quickly return to at or below 0°C which would suggest that cooling capacity is adequate. Temperature profiles were obtained for the 31 Saddletail sampled (Figure 7).

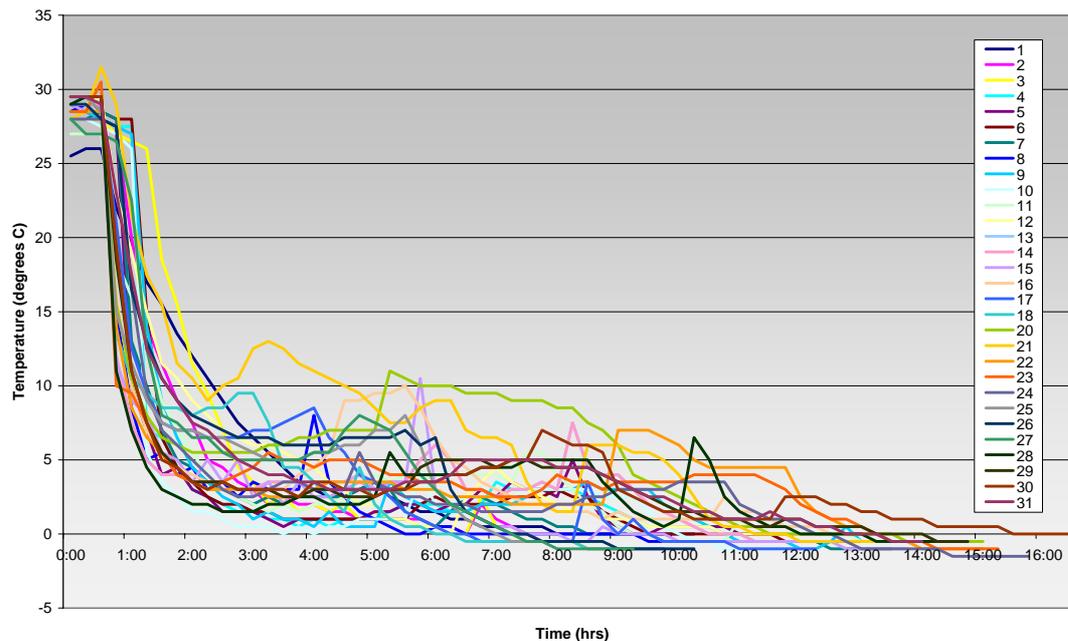


Figure 7. Temperature profiles during chilling of 31 Saddletail snapper sampled on FV Starlight

All fish logged achieve a core temperature below 10°C within 3 hours of chilling. However, from this point, core temperatures can fluctuate greatly. For example, fish 21 (landed 27th September 2008) has a core temperature of 31.5°C at landing and chills to 9.0°C within 2 hours and 30 minutes. From that point the core temperature increases to 13°C during the next hour, before core temperature falls to 1.5°C at the 8 hour mark. At this point, the core temperature rises to 6°C by the 9 hour mark, before falling below 5°C after 10 hours. This trend continues until a subzero core temperature is achieved at the 12 hour mark.

From Figure 6 the temperature of the water in the brine tank can be seen to be increasing from approximately 8:15am. Fish 21 was landed at approximately 9:00am when the brine water was approximately 2.5°C. Figure 6 also shows the brine tank water temperature increasing to 6.5°C during the next 3 hours.

However, the increase in core temperature observed in fish 21 is much higher than possible from the water alone, suggesting that freshly landed fish are in physical contact within the tank and transferring the heat within them to fish 21. This suggests that both the brine water volume and the current circulation within the brine tank are inadequate to maintain best practice chilling times. Inefficient chilling will result in fish of poorer quality at market and a decrease in effective shelf life.

5.1.2 Texture assessment

Texture analysis was conducted on all 50 fish collected during this field trip. Results presented in Figure 8 and Figure 9 present mean total energy and mean peak force versus fork length of Saddletail collected on this field trip.

TFS in Saddletail snapper

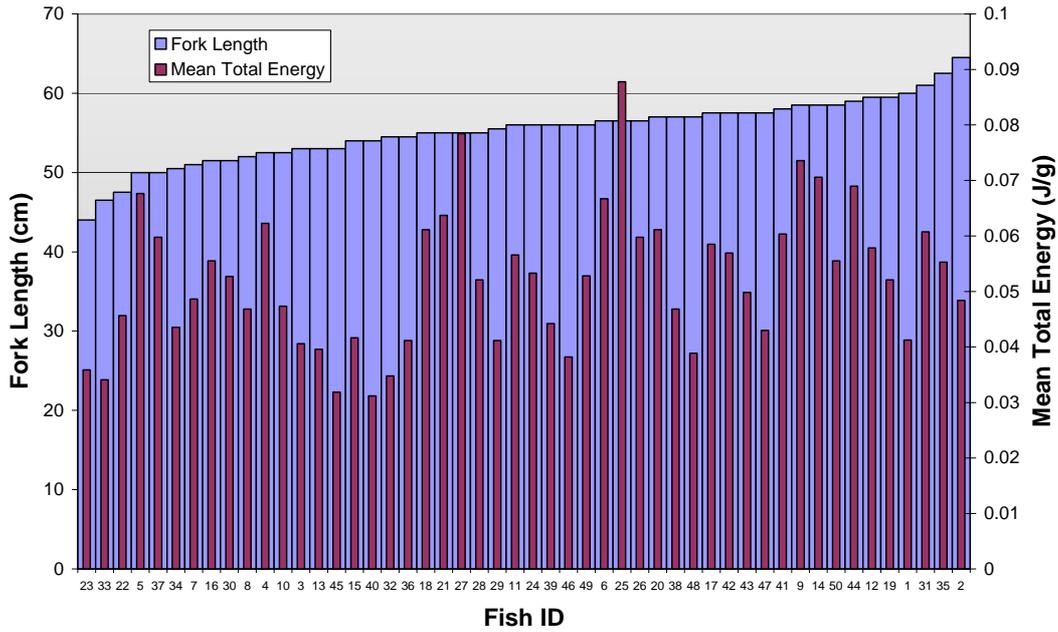


Figure 8. Mean total energy and fork length

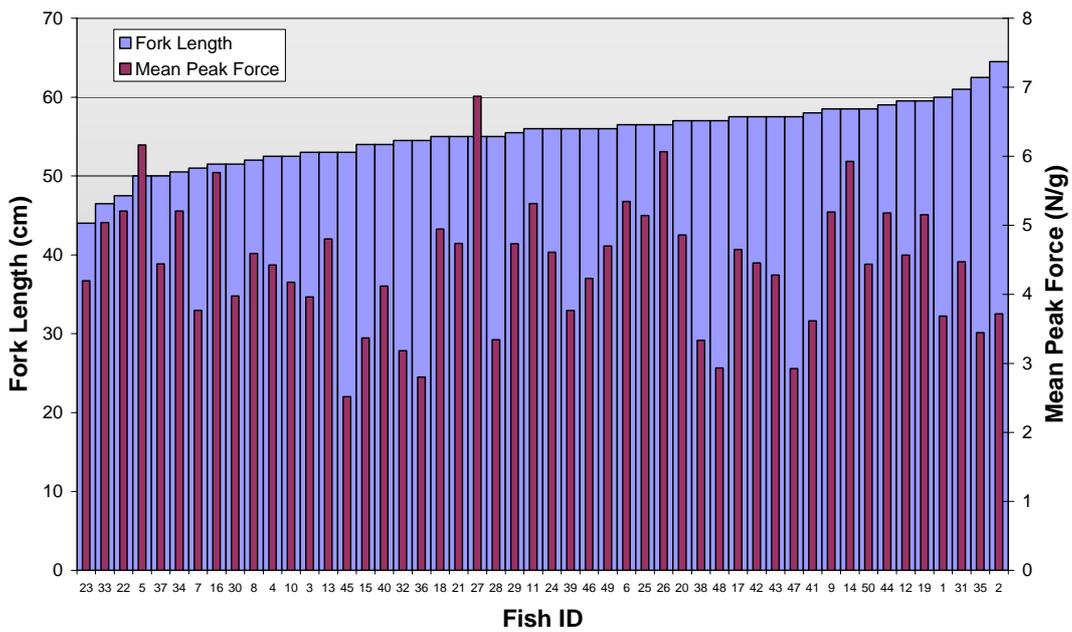


Figure 9. mean peak force versus fork length

Figure 10 presents both data for mean peak force and mean total energy for each fish sampled.

TFS in Saddletail snapper

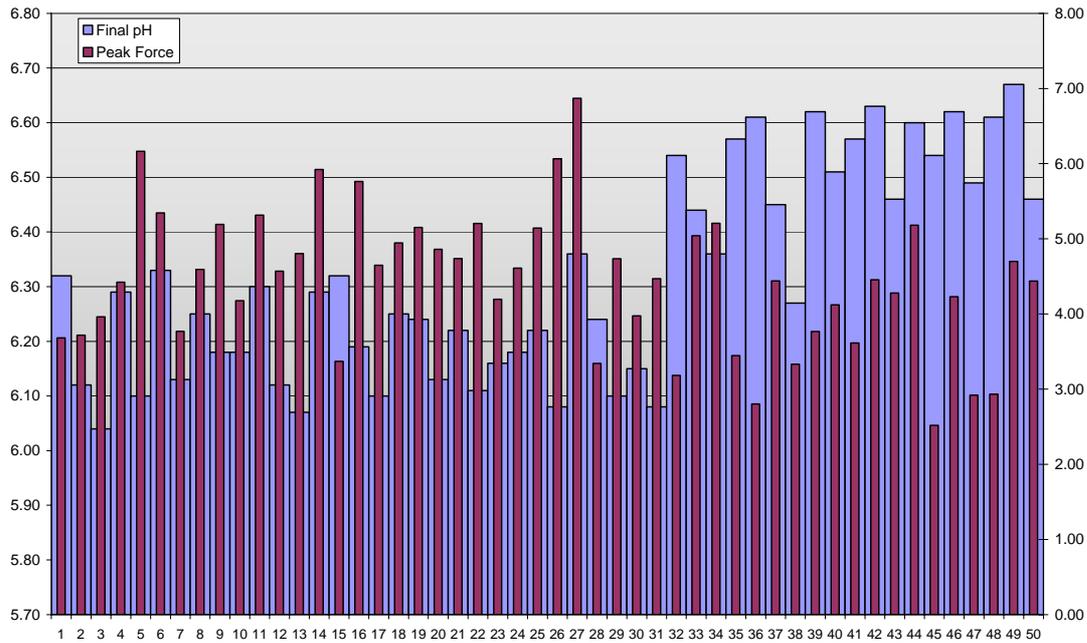


Figure 10. Comparison of Mean peak force and mean total energy for individual fish

The results obtained from the texture analysis provide no clear trends in the relationship between fish size and storage time to cooked fillet texture.

5.1.3 Rigor assessments

Saddletail required between 2 and 7 hours in the brine to establish full rigor. Penetrometer readings were taken on 15 of the 31 fish sampled. No further readings were taken due to instrument failure. These results are presented in Figure 11 and results will be discussed in conjunction with the nucleotide determination in the next section.

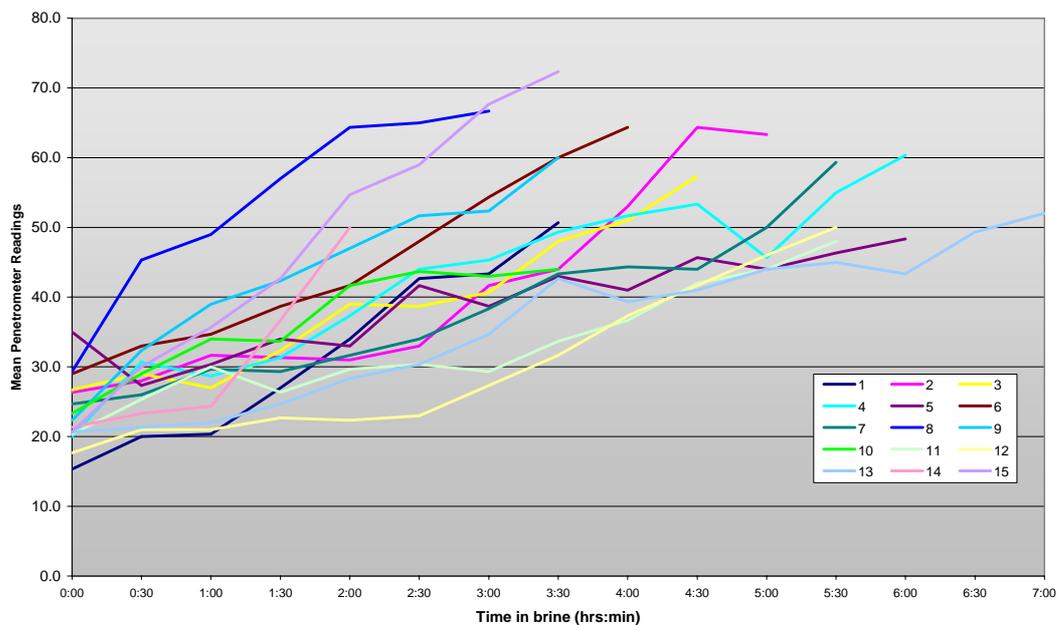


Figure 11. Mean penetrometer readings of first 15 Saddletail sampled on FV Starlight

5.1.4 Nucleotide determination

Nucleotide analysis was conducted on samples collected pre rigor and samples collected after the development of full-rigor as per the method described previously. Results of these analyses are presented in Figure 12 and Figure 13.

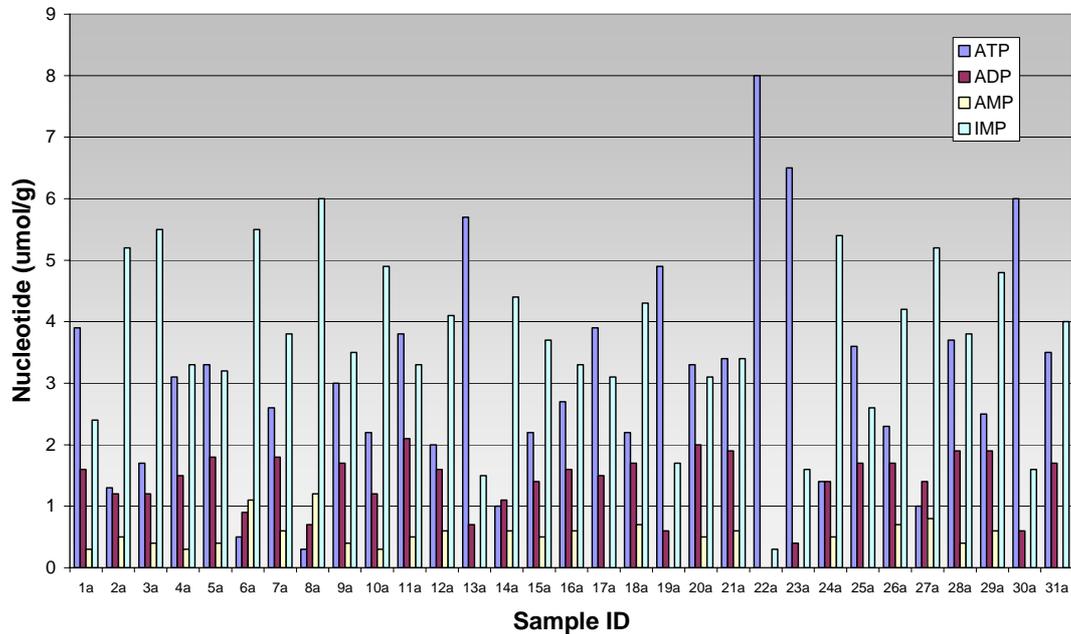


Figure 12. Nucleotide analyses of samples at landing

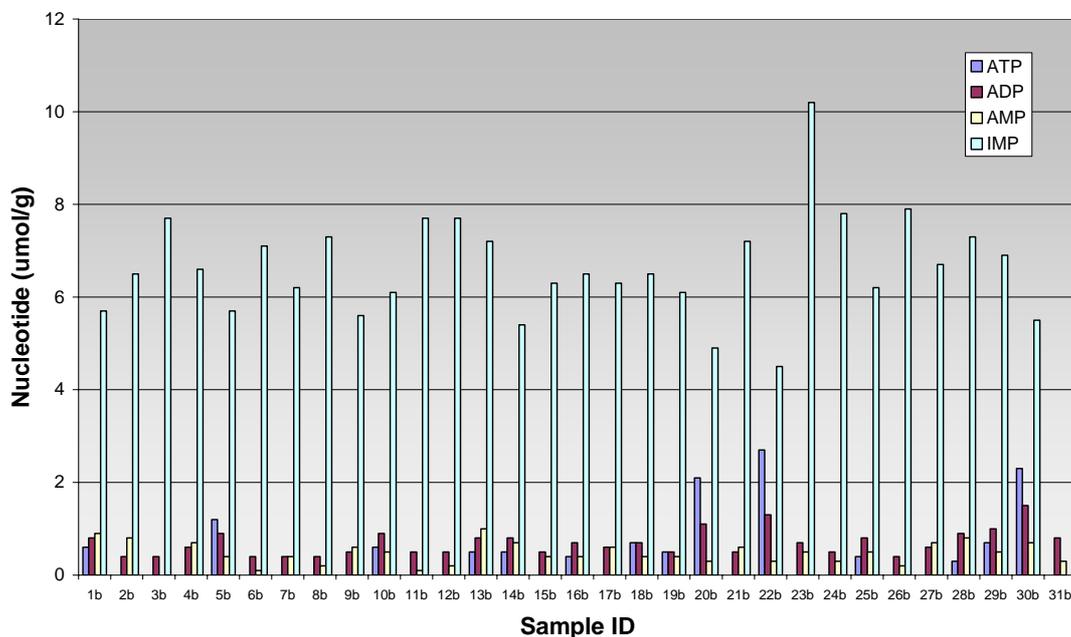


Figure 13. Nucleotide analyses of samples at full rigor

Quantities of ATP present in muscle tissue at landing vary greatly as can be seen in Figure 12. This may be explained by variation in the time individual fish have been in a trap. Any stress experienced during time in the trap will affect levels of muscle ATP.

There was a significant correlation between the ATP at landing and the time taken for full rigor to develop ($p < 0.001$) explaining 41.2% of the total variation. This result is consistent with previous published work of Love (1980) and others who have demonstrated that rigor mortis in fish commences when available ATP decreases to less than 5mmol concentration.

This exhaustion of ATP is confirmed by the analysis of a second tissue sample taken at the development of full rigor (Figure 13). Some exceptions exist, but they are not significantly different in texture.

The significance of this result is that the rigor or 'stiffness' observed in the fish sampled is most likely to be caused by normal ATP depletion and not a 'cold-shock' reaction to the sub-zero temperature of the chilling media as has been reported previously (Curran et al. 1986).

A weak but significant correlation ($p = 0.017$) exists between time to full rigor and ultimate pH. And although time to rigor is dependant on available ATP, no significant relationship exists between the ATP at landing and ultimate muscle pH. Further work involving larger numbers of fish sampled may provide a more conclusive link between ATP at landing and cooked fillet texture.

5.1.5 Muscle pH

Muscle pH data was collected when fish were landed, the onset of full rigor, and at filleting prior to texture assessment. Analysis of pH data revealed no significant trends with the exception of pH (at filleting) and texture which displayed a weak but significant inverse correlation ($p=0.02$). The changes in muscle pH are consistent with the work of others (Curran et al. 1986). These results are presented in Figure 14.

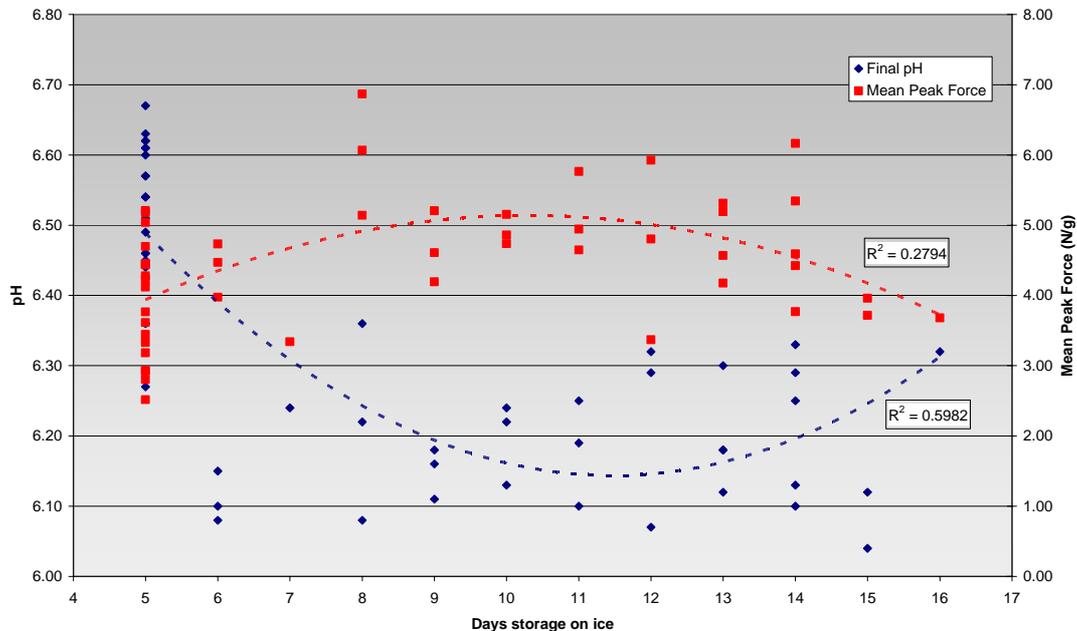


Figure 14. Final muscle pH and peak force versus total days stored on ice

5.1.6 Analysis of last days catch

From the results presented in Figure 14, observations of muscle pH and peak force taken from fish landed on the last day of fishing appear to be different from the rest of the catch. When one way analysis of variance (ANOVA) statistical analysis is conducted to compare the data from the last days catch, several significant differences emerge (Table 3).

Table 3. Comparison (ANOVA) of parameters for last day to remaining catch

Parameter	Last day (5 days on ice) (n=19)	All other days (6-16 days on ice) (n=31)	Significance
final muscle pH	6.53 ^a (±0.0217)	6.19 ^b (±0.0170)	p<0.001
Peak Force (N/g)	3.93 ^b (±0.19)	4.76 ^a (±0.15)	p<0.001
Total Energy (mJ/g)	46.7 ^b (±2.7)	55.8 ^a (±2.1)	p=0.011

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

Final pH of muscle tissue decreases after 5 days of storage on ice. This is consistent with the previously mentioned work of Curran et al (1986). However peak force, and to a lesser extent total energy, are significantly lower at 5 days on ice than fish stored up to 16 days.

The exact nature of the relationship between muscle texture and pH is not entirely clear. However the trend appears to be a reduction in final pH corresponds to an increase in muscle texture (see Figure 14). This relationship has been described previously (Bremner 2002) however the mechanism is not fully understood.

5.1.7 Analysis of catch by sex

No significant differences were found between the quality parameters assessed for male and female fish. However, the male fish sampled were significantly longer in fork length than females (p=0.002). These results are presented in Table 4.

Table 4. Mean size of male and female fish sampled

Parameter	Male (n=28)	Female (n=11)	Significance
Fork Length (cm)	56.214 ^a (±0.664)	51.727 ^b (±1.060)	p=0.002

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

5.1.8 Preliminary collagen determination

Total collagen analysis of 10 samples was conducted by Food Science Australia (CSIRO) Cannon Hill to assess the viability of conducting further analyses with comparison to texture data obtained from the Instron technique (Figure 15 and Figure 16).

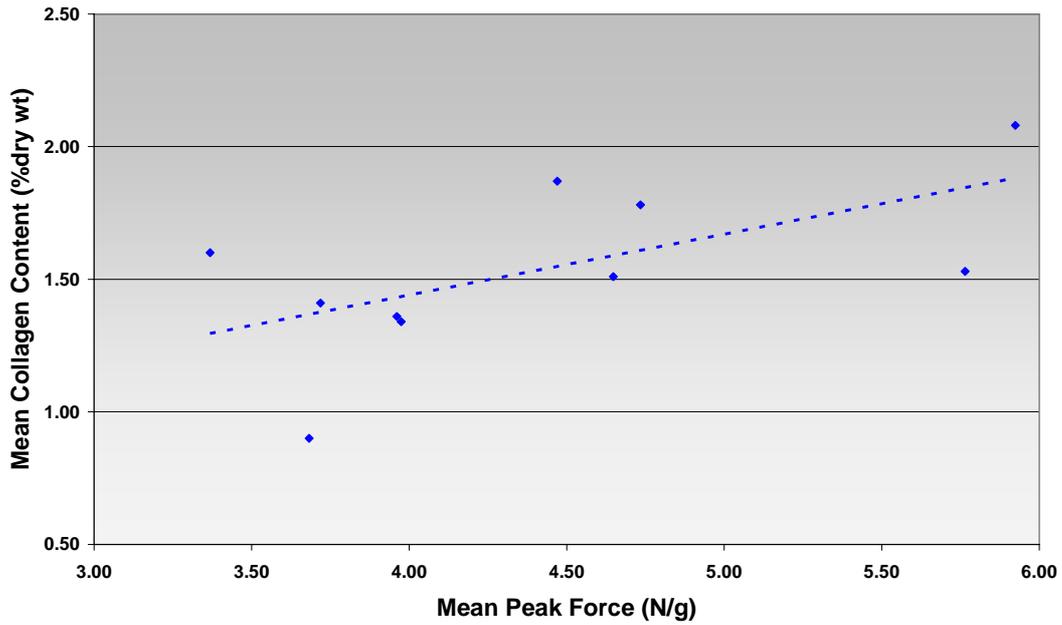


Figure 15. Scatter plot of peak force and collagen content

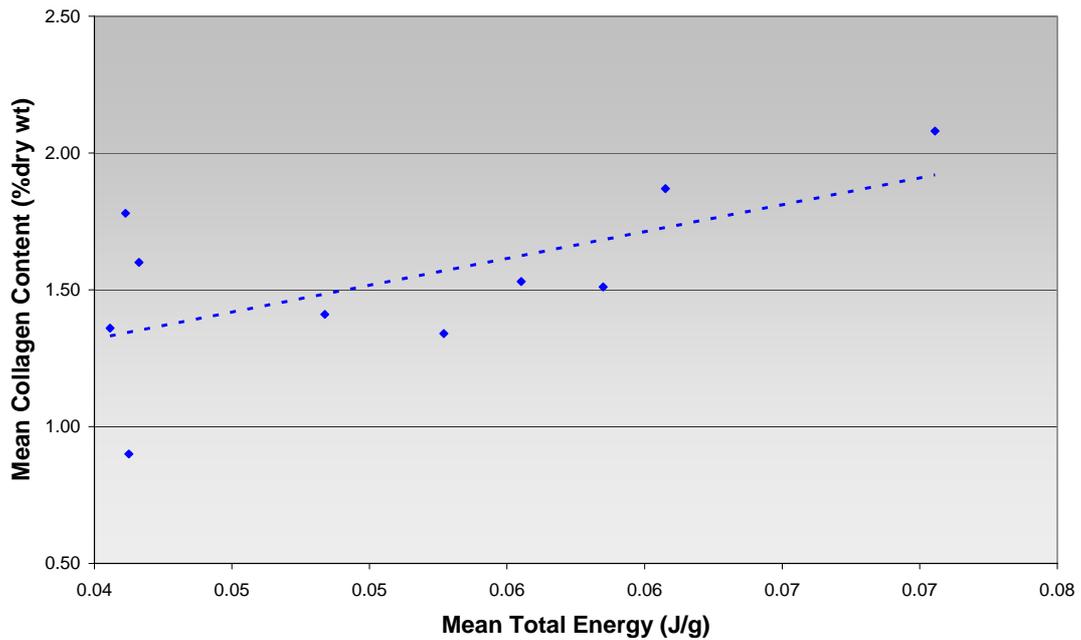


Figure 16. Scatter plot of total energy and collagen content

Good correlations were obtained for peak force ($r^2=0.61$) and total energy ($r^2=.62$). However, neither was significant due to the low number of data points. This result does suggest that further analyses may yield useful results.

5.1.9 Sarcomere analysis

Portions of thirty-one (31) Saddletail snapper fillets, collected at point of capture and after full rigor had developed during chilling in refrigerated sea water. Samples were frozen and stored onboard in liquid nitrogen. Upon return to Brisbane, samples were transferred to FSA Cannon Hill and were stored at -20°C until required for analysis.

The size of some of the samples received were below the minimum size required to obtain a reasonable sample for measurement for sarcomeres (50mm x 20mm x 5mm), and therefore the samples were difficult to section due to rapid thawing that occurred. To negate this effect, these samples were frozen at -80°C (not -20°C) prior to sectioning. The sarcomere lengths of the samples taken at capture and after full rigor had developed are presented in Table 5.

Table 5. Sarcomere measurements (μm) for 31 Saddletail snapper samples, taken immediately on capture and after full rigor had developed during chilling in refrigerated sea water

Sample ID	On capture	After full rigor development
1	1.47	1.08
2	1.82	1.97
3	1.08	1.80
4	-	1.32
5	-	1.10
6	1.08	1.83
7	1.08	1.92
8	1.08	1.82
9	1.21	1.79
10	1.08	1.90
11	1.08	1.86
12	1.74	1.89
13	-	1.09
14	1.66	1.81
15	1.08	1.90
16	-	1.65
17	1.15	1.90
18	1.82	1.83
19	-	1.08
20	1.08	1.22
21	1.09	1.80
22	-	1.08
23	1.69	1.75
24	1.54	1.87
25	1.08	1.12
26	1.10	1.08

Sample ID	On capture	After full rigor development
27	1.18	1.86
28	1.08	1.80
29	-	1.79
30	-	1.13
31	-	1.08

Blank cells (-) indicate no reading was possible for these samples.
 Low values (e.g. 1.08) indicate limits of detection for instrument (i.e. very short sarcomeres).

No significant relationship was found between sarcomere length and either of the cooked muscle texture parameters measured by the Instron. Muscle pH and fish size also displayed no significant relationship to sarcomere length.

5.1.10 The effect of barotrauma on fish quality

Almost all Saddletail landed during this field trip were exhibiting severe symptoms of barotrauma. These included eye bulging (exophthalmia), swollen abdomen, inverted stomach, and rippling of the skin ventral to the lateral line. Figure 17 below is of a Saddletail with a bulging eye and swollen belly cavity.



Figure 17. Saddletail showing signs of barotrauma (eye and belly cavity)

Barotrauma presents another symptom in this species at the lateral areas of muscle. Some rippling of the skin occurs caused by what appears to be gas bubbles forming between the skin and muscle in the area of skin below the lateral line to the area adjacent to the pectoral fin joint (Figure 18).



Figure 18. Saddletail showing signs of barotrauma (lateral tissue)

This effect is most likely to be the result of Saddletail physiology being incapable of adapting to the rapid change in pressure experienced during hauling of the fish traps. This is an effect not observed within Goldband snapper (*Pristipomoides multidens*) caught in the same traps. Goldband exhibit very little in the way of barotrauma symptoms. Barometric acclimatisation rates have been shown to vary greatly between near related species (Parker et al. 2006).

The source of the gas is most likely to be the air bladder within the dorsal region of the belly cavity. The signs of belly bloating suggest this air bag has no physiological mechanism for dispersing excess air quickly. The rapid increase in pressure within the air bladder will eventually cause a breach and air will find its way into surrounding tissue.

Our observations suggest this barotrauma is also causing a direct effect on fillet quality. Many of the fish processed during this and other field trips, exhibit signs of internal bleeding. Figure 19 is an example of some extensive bleeding observed.



Figure 19. Internal bleeding staining Saddletail fillet

At first observation these blood spots were considered to be a result of bruising from physical contact between fish and the trap during hauling. However, bruising is superficial and these marks do not extend to the skin. These areas of bleeding are observed in the tissue surrounding the backbone. Figure 20 shows both fillets and the other side of the previous fish (Figure 19).



Figure 20. Internal bleeding in both fillets

Bleeding to a lesser extent is observed in the left fillet. However, from this image the blood appears to have been forced from the belly cavity and from the blood vessels adjacent to the backbone. This suggests the swim bladder may be forcing blood back through the vessels and into the surrounding muscle. Figure 21 is close up image of the same fish.



Figure 21. Close of bleeding in belly cavity and backbone

From this image a large amount of blood is visible at the posterior end of the belly cavity. Blood can also be seen emerging from vessels adjacent to the backbone. Embolism has been reported as a result of barotrauma in other Australian species (Longbottom 2000). This blood pooling may be the result of pressure exerted by the swim bladder as it expands without any mechanism to depressurise.

These issues were discussed with Kjell Midling (Senior Scientist with Nofima Marine based in Tromso, Norway) on a recent visit to IFT Hamilton. Kjell has had extensive experience with the Atlantic halibut (*Hippoglossus hippoglossus*) and cod (*Gadus morhua*) fisheries of the North Sea. Kjell suggested that the symptoms of barotrauma exhibited by Saddletail were extreme, and consistent with a species that has no mechanism of alleviating this issue quickly during fish trap hauling.

A current industry solution suggested by Kjell was to slow the hauling of the trap during the last ten metres to the fishing vessel, taking at least 30 seconds to travel the last ten metres. This allows the fish to attempt to partially equilibrate their swim bladders at the shallower depth.

Such a method may greatly reduce the incidence of this internal bleeding which devalues the fish and the reputation of the species. However, these changes have the potential to slow fishing operations and any change in practice must take into account commercial considerations.

5.1.11 Trip summary

This field trip was designed more as a 'shakedown trip' than a complete sample collection trip. However, some valuable data has been collected and some significant findings have been made.

- Brine tank water temperature increased to almost 10°C under heavy load. However, this may be more likely to be an artefact of this fishing technique being employed than being due to inadequate capacity in the heat exchanger.
- Fish chilling rates varied greatly. This may also be in part due to the nature of the fishing. However the extent of the variation in chilling rates is large. This suggests the volume of chilling media (brine) is insufficient, and circulation of the media inadequate to provide industry best practice for fresh chilled products.
- Size or sex of fish was not a significant influence on texture of cooked samples.
- Storage time on ice is a significant influence on cooked fillet texture. Ultimate muscle pH is also intrinsically linked into this relationship. However, the mechanism for this relationship is not clear.
- Rigor development within this sample set of Saddletail snapper occurred primarily at the point of exhaustion of available ATP within the white muscle tissue. Therefore it is difficult to suggest that any 'cold shock' or cold shortening' was being experienced by the fish during the first hours of chilling.
- Time taken for rigor to commence correlates to ultimate pH, but muscle ATP was not a significant influence in this study. This would appear to be a contradictory statement. However, a larger number of sampled fish may provide a more conclusive result and will be the aim of further field trips on fresh chilled vessels.
- Saddletail experience severe symptoms of barotrauma including extensive internal haemorrhaging, which can greatly reduce fillet quality and market price. Methods to reduce this incidence should be trialled to allow any improvement in quality to be quantified and permit stakeholders to make sound commercial decisions on trap hauling practices.
- The current method of bleeding these fish onboard this vessel have limited efficacy with preventing blood spotting in Saddletail muscle.

5.2 Field trip 2 (December 2008)

The Second set of samples collected for this project was from the trawling vessel FV Territory Leader. 111 whole fish were frozen onboard and sent to IFT Hamilton for analysis. Samples arrived in Brisbane on 16th December 2008, but were unable to be processed due to staff and equipment availability until 6th January 2009.

Upon return from the Christmas break the Instron texture analyser broke down and required repair before processing could progress. This was achieved after much anxiety on 23rd February 2009. Sample processing commenced the next day.

5.2.1 Biological data and Instron results

Analysis of the raw data provided no significant correlations within the complete set of fish samples. Analysis of the differences (ANOVA) between the results by sex provided significant differences (Table 6).

Table 6. Significant difference of means of measured parameter by sex

Parameter	Male (n=54)	Female (n=57)	Significance
Weight (g)	1971.54 ^a (±39.75)	1806.33 ^b (±38.69)	p=0.004
Fork Length (cm)	51.22 ^a (±0.37)	49.39 ^b (±0.36)	p<0.001
pH (ultimate)	6.41 ^a (±0.0187)	6.34 ^b (±0.0182)	p=0.012
Peak Force (N/g)	4.75 ^b (±0.19)	5.79 ^a (±0.19)	p<0.001
Total Energy (mJ/g)	48.66 ^b (±1.8)	57.37 ^a (±1.8)	p<0.001

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

The male fish sampled were larger than female fish and achieved a higher ultimate muscle pH. Cooked muscle of female fish was significantly firmer than males by peak force and total energy measurements. The relationship between peak force and fork length for both sexes is presented in Figure 22.

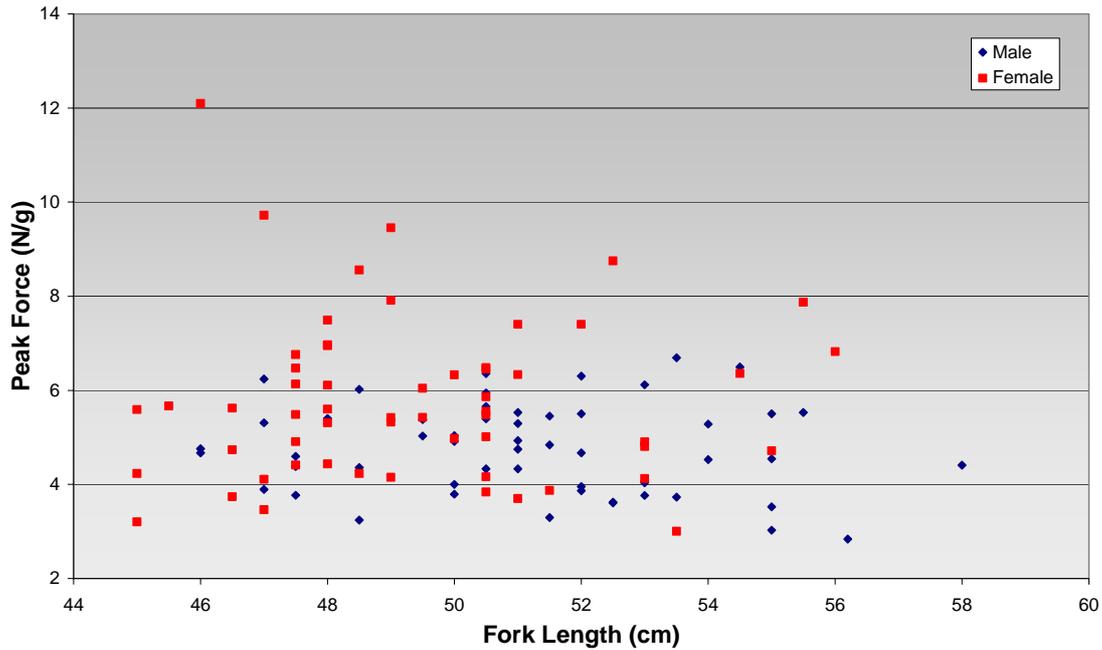


Figure 22. Scatter plot of peak force versus fork length for both sexes

The relationship between muscle texture and ultimate pH has been described previously (Curran et al. 1986) where muscle texture increased with a decrease in ultimate muscle pH. Such a trend would appear to exist in this data; however, statistical analysis provides no significant correlation (Figure 23).

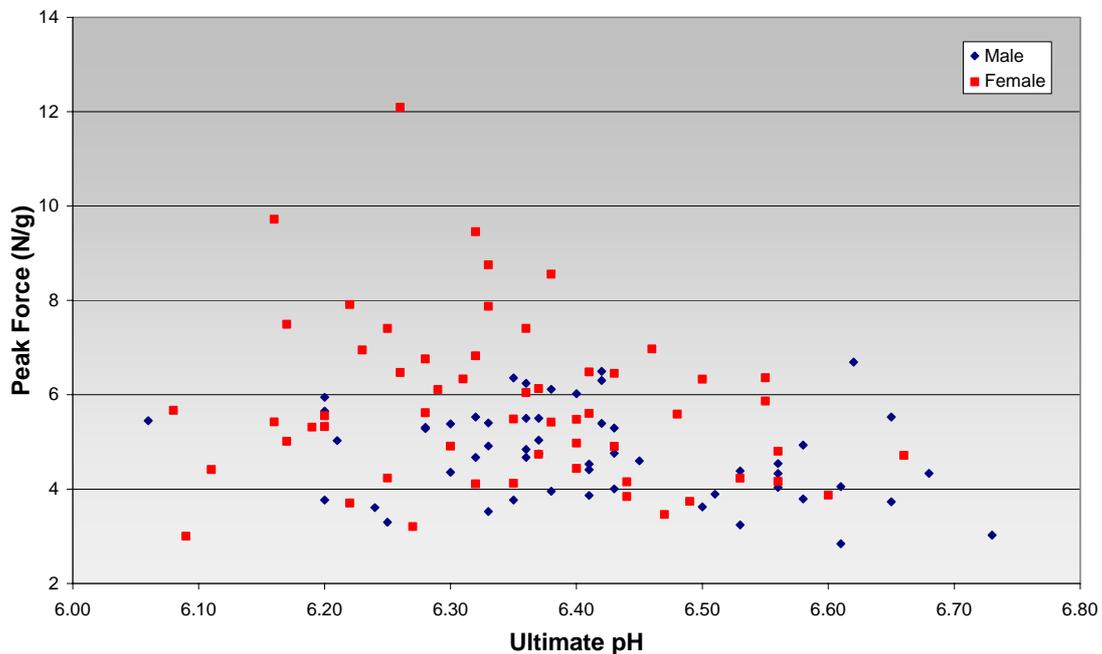


Figure 23. Scatter plot of peak force versus ultimate muscle pH for both sexes

Comparison of ultimate pH and fork length provide significant differences between the sexes. However no significant correlation exists between fork length and ultimate pH. This result is consistent for both sexes (Figure 24).

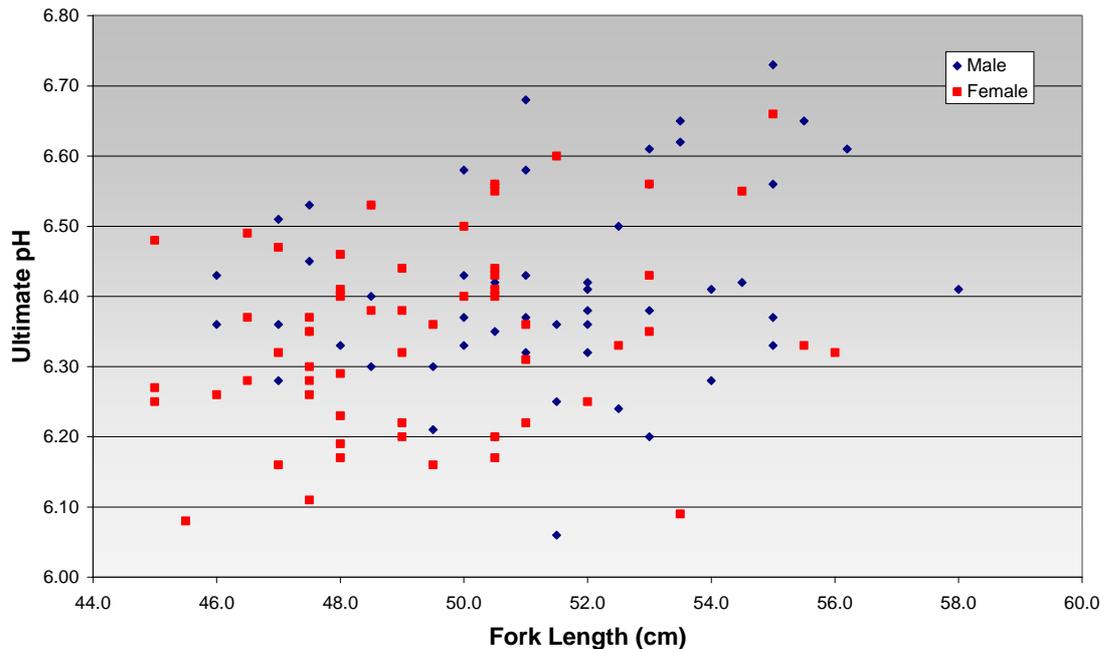


Figure 24. Scatter plot of ultimate pH and fork length for both sexes

5.2.2 Nucleotide Analysis

Analysis of nucleotide content was conducted on muscle tissue samples to investigate the possibility of a 'thaw rigor' event in the fish sampled. Thaw rigor results from thawing of fish frozen pre-rigor, or prior to the depletion of ATP significantly below the 5mM concentration (Bremner 2002). Upon thawing, a rapid degradation of ATP can cause an uncontrolled contraction of the muscle (Hiltz et al. 1974). This can result in excessive liquid loss, gaping, and shortening of fillets cut pre-rigor.

The fish collected during this field trip were all frozen onboard after 2-3 hours of chilling. To ensure that no thaw rigor influence is taking place, nucleotide analysis was employed to determine if any residual ATP could have influenced the texture of the fish.

Twenty fish were assessed for nucleotide content. These included fish belonging to the ten highest and the ten lowest peak force values obtained by the Instron texture analysis. No significant differences were found between individual nucleotides, total nucleotides or K-Values between the fish belonging to high or low peak force values. A full table of the results can be found in accompanying data CD.

5.2.3 Collagen Analysis

Determinations of total collagen were performed on fish possessing the 10 highest texture reading (peak force) and another 40 fish spread evenly across the range of texture values. These results are shown in Figure 25. Collagen results are expressed as milligrams of hydroxyproline (Hyp) per gram of muscle dry weight.

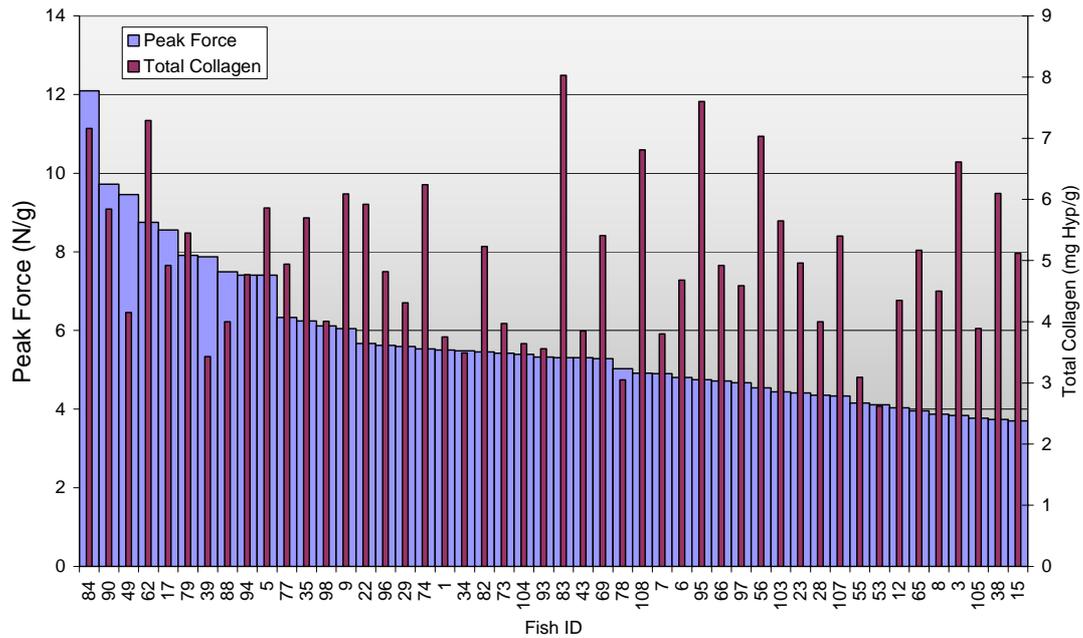


Figure 25. Comparison of peak force and total collagen results

These results illustrate no obvious relationship between peak force and the total collagen present in the samples. However, these results do not differentiate by sex; which has previously been demonstrated to be an influence affecting the outcome of the texture assessment (Figure 26).

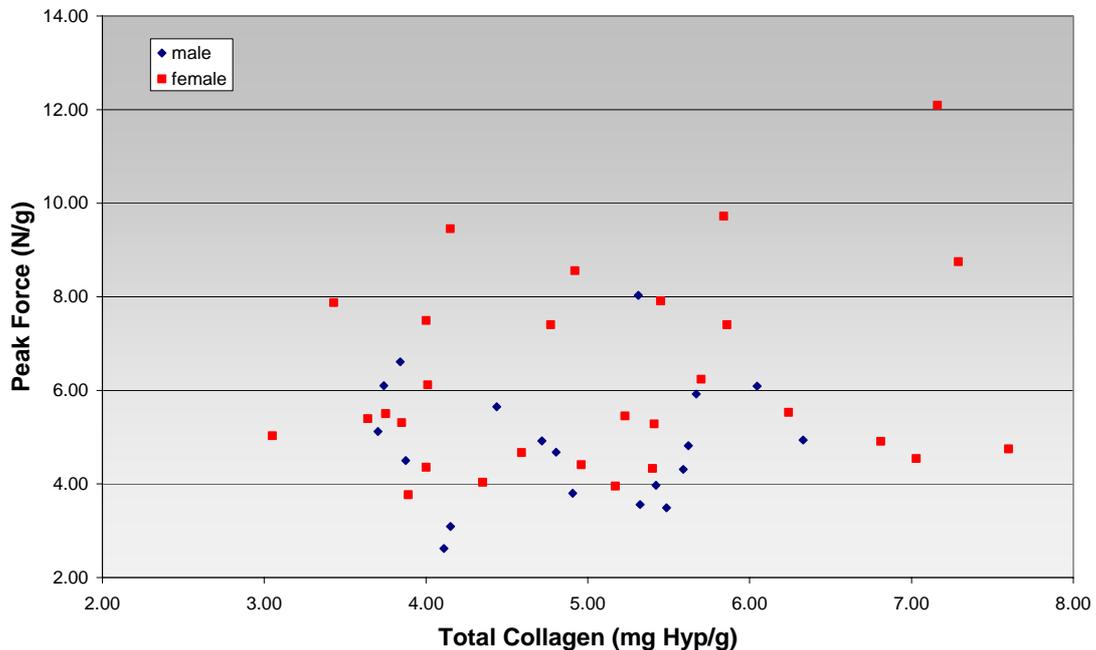


Figure 26. Peak force (N/g) versus Total collagen (mg Hyp/g) by sex.

No significant differences exist between the levels of total collagen between male and female fish (ANOVA). However, when the relationship between peak force and total collagen is analysed by linear regression using groups (sex) a weak but significant relationship was obtained ($p=0.031$). This result suggests that within the female population, the amount of total collagen present in the muscle may be an influence in the development of tough cooked fillet texture.

5.2.4 Summary

Several significant results have emerged from this group of fish sampled.

- Female fish were significantly tougher than male fish and significantly lower in ultimate pH.
- Female fish were significantly smaller (both fork length and mass) than male fish.
- Some females were found to contain higher levels of collagen than the males. However, this does not necessarily translate directly to a higher peak force value obtained from the cooked fish sample.

The difference in size is consistent with the work of Newman (2002) who conducted growth rate, age determination, natural mortality and production potential of the species from the Pilbara region of Western Australia between 1997 and 1999 .

However, the differences between the sexes in both texture and pH suggest an influence of maturity and seasonality. Female fish were easy to identify when filleting as almost all females had well developed ovaries. This would suggest the species was in the process of spawning. Food availability may also influence muscle pH as higher glycogen levels within muscle at harvest can result in high levels lactic acid.

Salini et al (2006) examined fish between Australia and Indonesia in 1999 and 2000 suggest the species is highly fecund, and are serial spawners in open water between October and February. The fish from this field trip were collected in early December 2008, and are highly likely to have been spawning at this time. The influence of spawning on fish muscle quality has been widely reported in many species in both wild harvest and aquaculture environments.

5.3 Field Trip 3 (April/May 2009)

5.3.1 Biological data and Instron results

Significant differences in weight and fork length were observed between male and female fish, consistent with previous results and published data (McPherson and Squire 1992; Newman 2002). However, significant differences in peak force and total energy were not observed between the sexes (Table 7).

Table 7. Means of measured parameter by sex on field trip 3

Parameter	Male (n=51)	Female (n=50)	Significance
Weight (g)	1976.7 ^a (±76.6)	1564.6 ^b (±77.3)	p<0.001
Fork Length (cm)	50.46 ^a (±0.87)	47.43 ^b (±0.88)	p=0.016
pH (ultimate)	6.47 ^a (±0.016)	6.42 ^b (±0.016)	p=0.026
Peak Force (N/g)	6.00 (±0.28)	6.54 (±0.29)	N.S.
Total Energy (mJ/g)	55.72 (±3.05)	54.08 (±3.07)	N.S.

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

Linear regression analysis revealed a small but significant correlation between fork length and total energy (p<0.001) explaining 40.1% of the total variation (Figure 27).

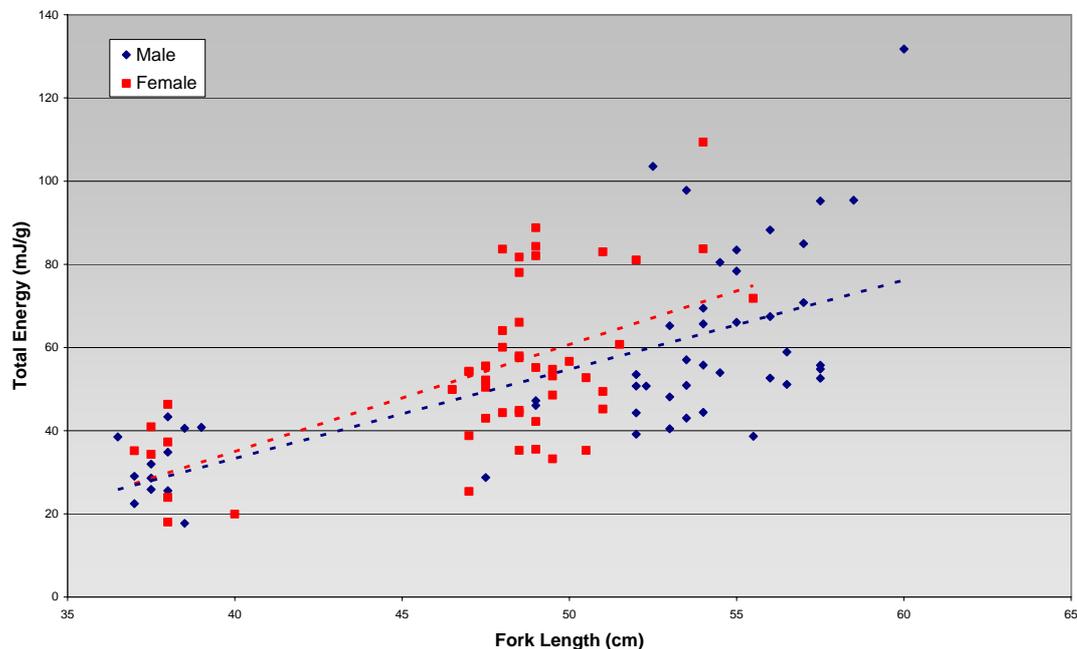


Figure 27. Scatter plot of total energy versus fork length for males ($R^2=0.46$) and females ($R^2=0.39$)

Figure 27 illustrates a trend of increasing firmness of cooked muscle in males and females with increasing fork length. This may suggest an effect of increasing firmness with increasing age. However this trend was not observed in the fish from December 2008 (FT2).

Figure 27 also reveals a subpopulation of significantly smaller fish exists within the sample set, and these fish displayed less firmness than most of the larger samples. The net effect will result in a skewing of the correlation trend in favour of a higher coefficient, suggesting the trend is stronger than would necessarily be the case. However, the texture values will also be affected and the mean texture values for both males and females will be reduced.

Analysis of variance was repeated on these results, excluding the smaller group and including fish obtained in early December 2008 (Table 8).

Table 8. Significant difference between sexes and seasons excluding fish < 1kg (FT3)

Parameter	Males		Females		Significance
	Dec 08 (n=54)	Apr 09 (n=39)	Dec 08 (n=57)	Apr 09 (n=42)	
Weight (g)	1971.54 ^b (±58.85)	2324.26 ^a (±43.78)	1806.33 ^c (±57.28)	1699.64 ^c (±42.19)	p<0.001
Fork Length (cm)	51.23 ^b (±0.64)	54.37 ^a (±0.41)	49.39 ^c (±0.62)	49.23 ^c (±0.39)	p<0.001
pH (ultimate)	6.41 ^a (±0.017)	6.46 ^a (±0.020)	6.34 ^b (±0.017)	6.41 ^a (±0.020)	p<0.001
Peak Force (N/g)	4.75 ^c (±0.24)	6.18 ^{ab} (±0.28)	5.79 ^b (±0.23)	6.67 ^a (±0.27)	p<0.001
Total Energy (mJ/g)	48.66 ^b (±2.25)	63.14 ^a (±2.65)	57.37 ^a (±2.19)	58.29 ^a (±2.55)	p<0.001
Total collagen (mg HYP/g)	4.98 ^a (±0.24) n=19	3.94 ^b (±0.23) n=21	5.00 ^a (±0.19) n=29	4.27 ^b (±0.19) n=29	p<0.001

Means followed by a different letter are significantly different at the stated level.

Standard errors are shown in brackets. HYP = hydroxyproline equivalents

Total collagen levels for both males and females were significantly higher in December than April. However, this trend is inconsistent with the trends observed in peak force or total energy.

No significant differences in size were evident in female fish across the seasons. The only significant differences were in muscle pH and peak force. Female fish displayed significantly higher peak force values in April than in December. However, total energy values were not significantly different.

Almost all parameters in male fish exhibited significant differences, with muscle pH being the only exception. Differences in values for peak force and total energy were highly significant (Figure 28 and Figure 29).

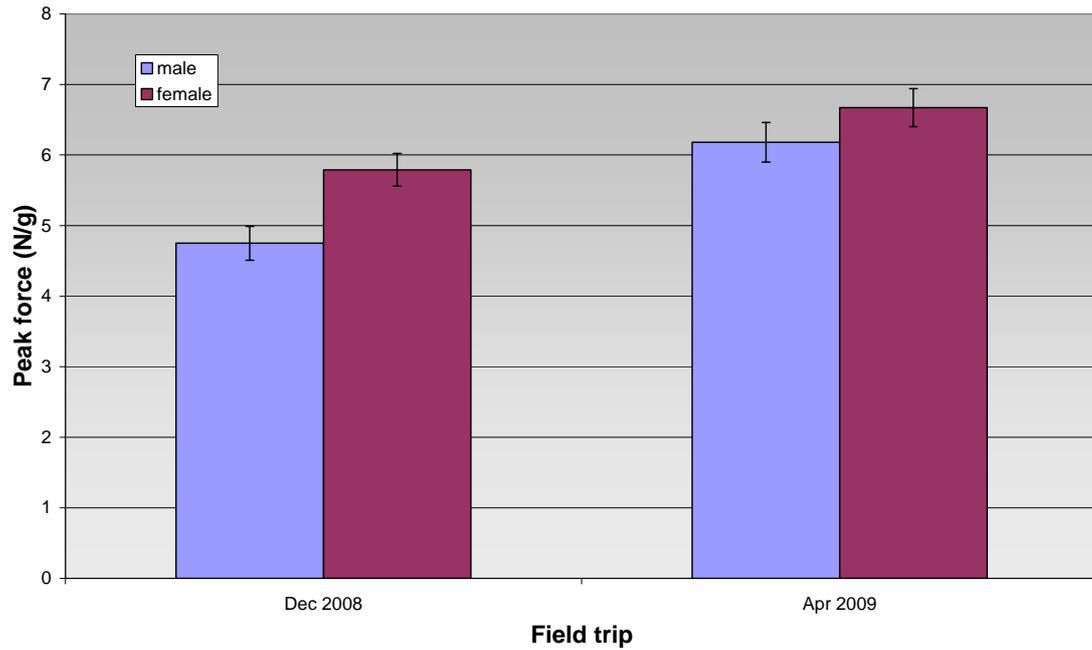


Figure 28. Mean peak force values for male and female fish >1kg across season
(Standard errors bars are shown)

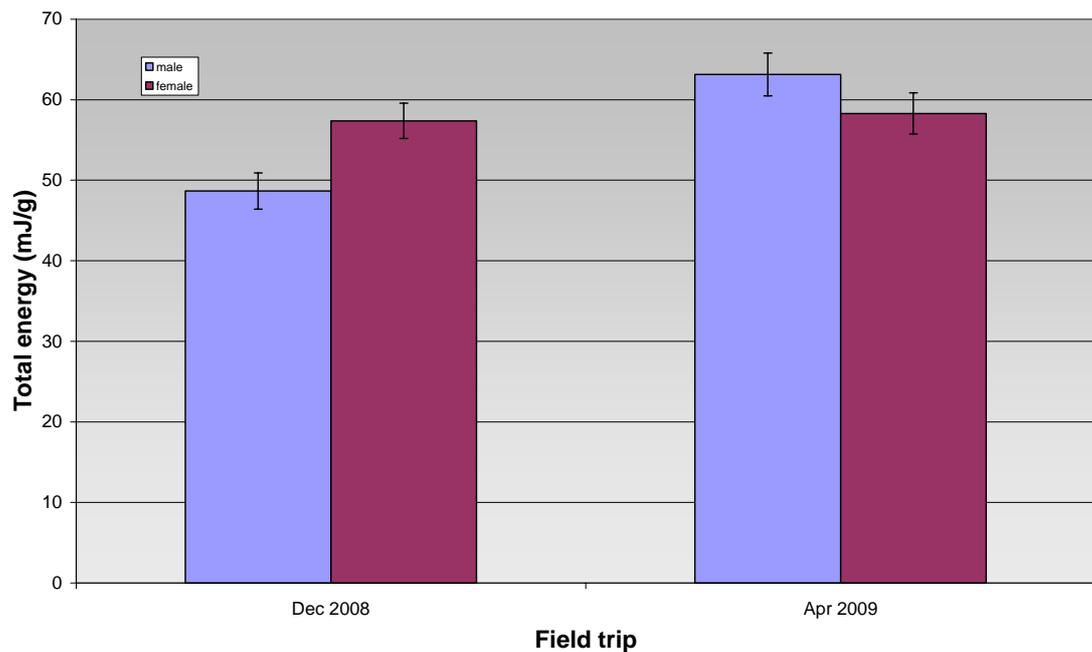


Figure 29. Total energy values for male and female fish >1kg across seasons
(Standard errors bars are shown)

Large differences in results for male fish are difficult to explain easily. Although male fish from April 2009 were significantly larger than those of December 2008, female fish were not significantly different in size across the two field trips.

The influence upon texture from the differences in size is also difficult to quantify. A small difference in total energy values between the male fish of each field trip might be expected from that data presented in Figure 27. However, such a large difference in the two texture measures of male Saddletail suggests a seasonal effect.

5.3.2 Collagen results

Of the 101 fish sampled a subset of 51 samples from the range of texture values was sent to Food Science Australia, Cannon Hill for total and heat soluble collagen content determination (hydroxyproline equivalents).

Determination of the heat soluble fraction of the total collagen present allows for a calculation of the heat insoluble fraction which is that fraction of the total collagen exercising influence over the cooked portion of fish muscle.

Previous attempts with this method had provided little in the way of significant data, hence a modification to the method was considered for these analyses. This involved reducing the temperature of solubilisation from 90°C to 65°C, and the exposure time from 60 to 30 minutes.

Analysis of the results yielded a weak but significant correlation between total energy and percentage of heat insoluble collagen ($p < 0.001$) explaining 43.3% of the total variation (Figure 30).

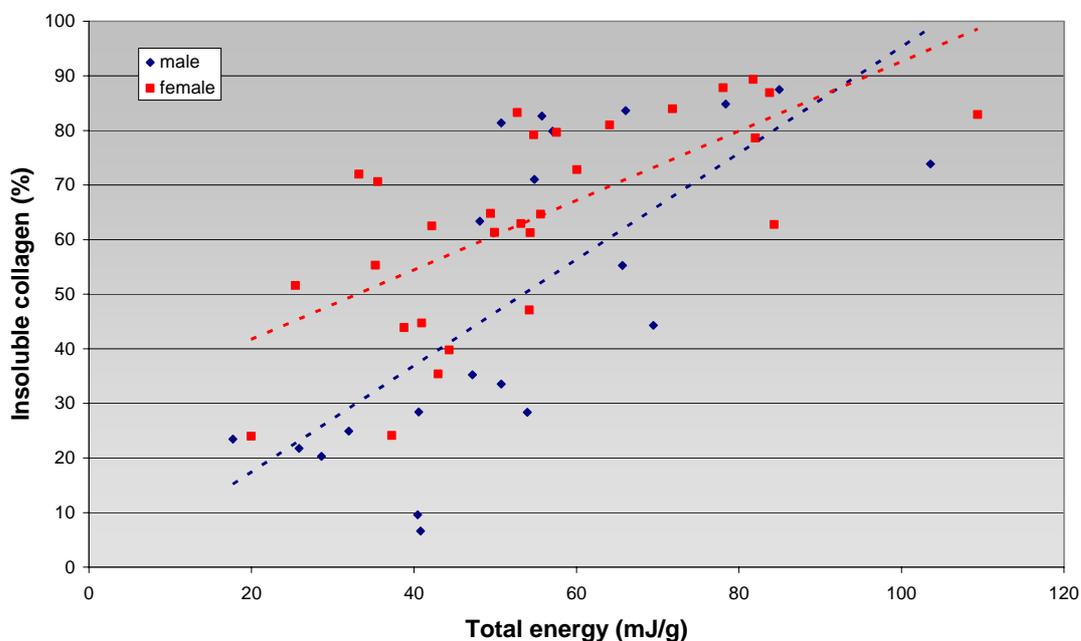


Figure 30. Scatter plot of total energy and percentage insoluble collagen for male ($R^2=0.50$) and female ($R^2=0.47$) Saddletail snapper

Further linear regression analysis identified another significant correlation ($p < 0.001$) between insoluble collagen and fork length for both male and female Saddletail; accounting for 54.0% of the variance observed (Figure 31).

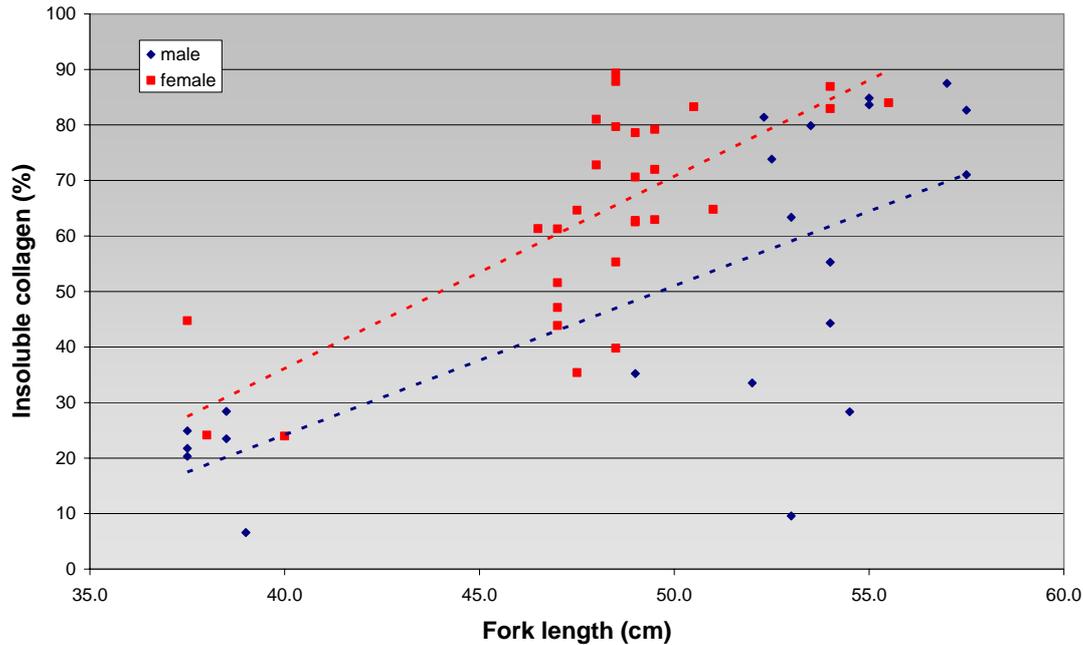


Figure 31. Scatter plot of percentage insoluble collagen and fork length for male ($R^2=0.52$) and female ($R^2=0.53$) Saddletail snapper

This finding is consistent with the results correlating fork length and total energy required to shear cooked muscle (Figure 27). Both these results support the trend of firmness with increasing fork length within males and females. The relationship between heat insoluble collagen, total energy and fork length was not observed in the collagen analysis from December 2008.

The modification to the collagen method appears to have provided greater fidelity in the data set. However, this does not explain why the clear relationship between total energy and fork length (Figure 27) has not been previously identified in the data collected from Saddletail in December 2008.

5.3.3 Summary

Field trip 3 (April 2009) has provided results enabling the identification of two significant trends within the full data set of the project to date.

- Significant difference in texture measurements exist between Saddletail from early summer (early December 2008) and late autumn (late April 2009). This trend was more distinct in male fish than females. Although fish size may play a role in this difference, seasonal variation due to sexual maturity, spawning season or food availability cannot be ruled out.
- A significant correlation between fork length, textural firmness (total energy) and heat insoluble collagen has been identified. This result suggests fish age may be an important contributing factor in the firmness of cooked Saddletail fillets.

5.4 Field trip 4 (June 2009)

5.4.1 Biological and Instron data

Data from this set of fish yielded little in the way of significant results. No differences were found between the male and female fish for any observed parameter and no correlations were found between any set of parameters (Table 9).

Table 9. Means of measured parameter by sex on field trip 4

Parameter	Male (n=20)	Female (n=10)	Significance
Weight (g)	2255.4 (±68.2)	2095.5 (±96.5)	N.S.
Fork Length (cm)	53.65 (±0.75)	51.60 (±1.05)	N.S.
pH (ultimate)	6.52 (±0.020)	6.48 (±0.028)	N.S.
Peak Force (N/g)	4.14 (±0.19)	4.27 (±0.27)	N.S.
Total Energy (mJ/g)	39.20 (±1.81)	41.56 (±2.55)	N.S.

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

The fish obtained from this field trip were obtained from a trap boat producing whole chilled fish. The fish samples were frozen onboard the vessel in the freezer hold used for bait storage. This method was designed to negate the impact of post-mortem enzymatic activity that would normally be associated with fresh chilled storage of fish; the usual practice of trap boats in the fishery (the trawl vessel used for previous field trips has a designated blast freezer specifically designed for quickly freezing fish at sea).

Unfortunately, the freezer used in this field trip appears to have struggled to freeze the fish quickly. Slow freezing results in the formation of large ice crystals within the muscle blocks causing damage to muscle blocks as they form. This would appear to be what has happened to these fish. Upon arrival at IFT, Brisbane for processing these fish looked in poor condition, being covered in large amounts of sheet ice and discolouration. The texture results obtained are significantly softer than those obtained from April 2009 and no trends exist within the data set.

5.5 Field trip 5 (October 2009)

5.5.1 Biological and Instron data

Significant size differences were identified between males and females. However, differences in texture and muscle pH were not present or only slightly significant (Table 10).

Table 10. Means of measured parameters by sex on field trip 5

Parameter	Male (n=53)	Female (n=56)	Significance
Weight (g)	1667.7 ^a (±32.75)	1401.0 ^b (±31.86)	p<0.001
Fork Length (cm)	48.77 ^a (±0.37)	45.43 ^b (±0.37)	p<0.001
pH (ultimate)	6.58 ^a (±0.013)	6.53 ^b (±0.013)	p=0.041
Peak Force (N/g)	5.24 ^b (±0.201)	5.87 ^a (±0.196)	p=0.028
Total Energy (mJ/g)	45.53 (±1.73)	47.46 (±1.68)	N.S.

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

Unlike previous trips (FT2 and FT3), significant differences were not found between the mean total energy values of male and female Saddletail.

Significant differences were also found when comparing males and females from previous field trips from the trawl vessel FV Territory Leader. These results are summarised in Table 11. As with the analysis of results from field trip 3, fish less than 1KG were omitted.

Table 11. Means of parameters by sex across field trips 2, 3 and 5 (fish > 1KG)

Par.	Males				Females			
	Dec 08 (n=54)	Apr 09 (n=39)	Dec 09 (n=53)	sig.	Dec 08 (n=57)	Apr 09 (n=42)	Dec 09 (n=56)	sig.
Weight (g)	1971.54 ^a (±51.42)	2324.26 ^a (±43.65)	1667.68 ^b (±51.90)	p<0.001	1806.33 ^a (±50.05)	1699.64 ^b (±38.59)	1401.05 ^c (±50.49)	p<0.001
Fork (cm)	51.23 ^b (±0.56)	54.37 ^a (±0.42)	48.77 ^c (±0.57)	p<0.001	49.39 ^a (±0.55)	49.23 ^a (±0.40)	45.43 ^b (±0.55)	p<0.001
pH	6.41 ^c (±0.02)	6.46 ^b (±0.02)	6.58 ^a (±0.02)	p<0.001	6.34 ^c (±0.02)	6.41 ^b (±0.02)	6.54 ^a (±0.02)	p<0.001
Peak force (N/g)	4.75 ^b (±0.23)	6.18 ^a (±0.23)	5.24 ^b (±0.23)	p<0.001	5.79 ^b (±0.22)	6.67 ^a (±0.28)	5.87 ^{ab} (±0.22)	p=0.041
Total energy (mJ/g)	48.66 ^{ab} (±2.19)	63.14 ^a (±2.35)	45.53 ^b (±2.21)	p<0.001	57.37 ^a (±2.13)	58.29 ^a (±2.42)	47.46 ^b (±2.15)	p<0.001

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

Significant differences were found in both texture parameters, but particularly in total energy. These differences appear to mirror those obtained from correlations with fork length data.

Linear regression reveals a small but significant correlation between total energy and fork length in both males and females (Figure 32 and Figure 33). This relationship is consistent with those seen previously in this project.

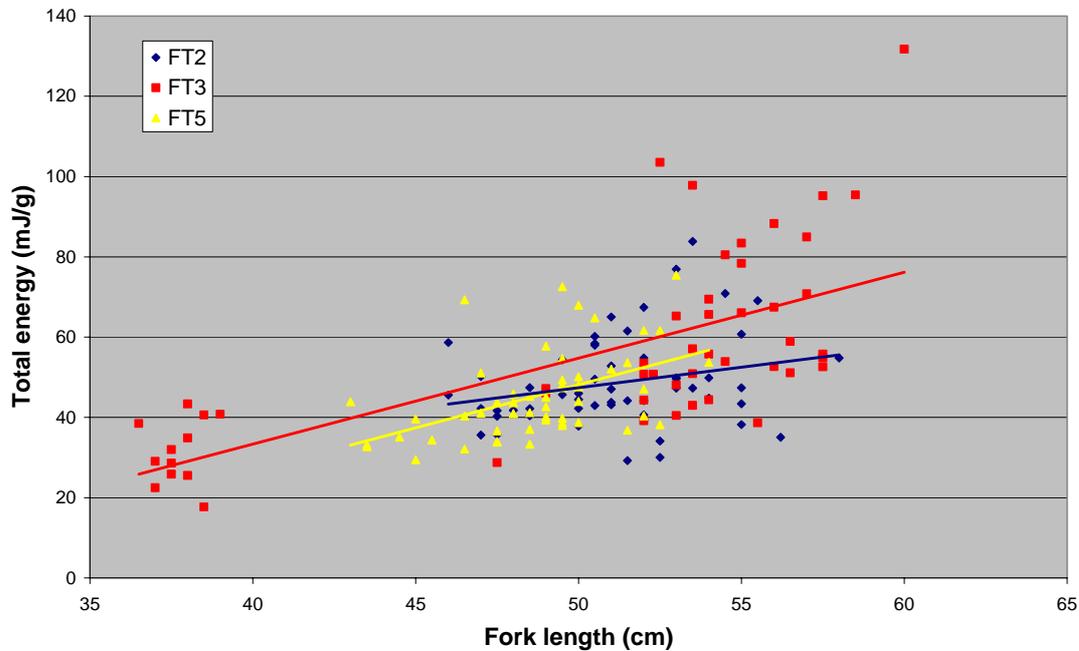


Figure 32. Linear regression (with groups) of total energy and fork length for all males from FT2, FT3 and FT5 explaining 38.4% of variation

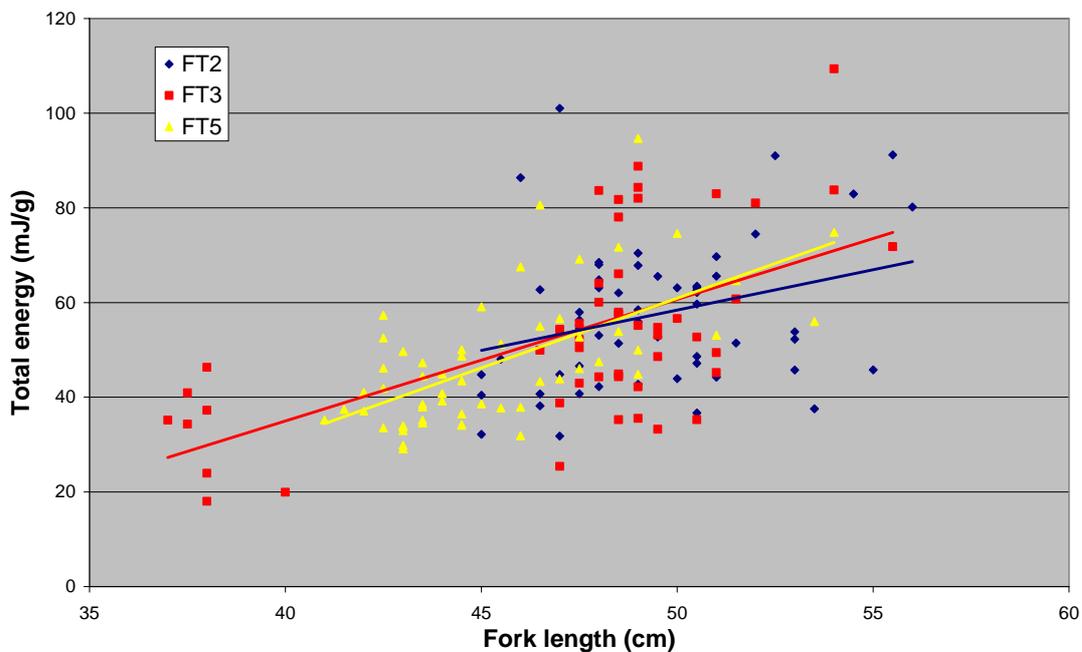


Figure 33. Linear regression (with groups) of total energy and fork length for all females from FT2, FT3 and FT5 explaining 30.5% of variation

5.5.2 *Summary*

- Significant seasonal differences in texture previously identified were less significant with the inclusion of this data set (FT5). The influence of fish size on the previously identified seasonal differences cannot be ruled out.
- A small but significant correlation between fork length and texture (total energy) has been consistently observed across 3 field trips and 2 seasons.
- This trend suggests that fish age may be a contributing factor in cooked muscle texture of Saddletail.

5.6 All field trip summaries

5.6.1 Field trip 1

- Brine tank water temperature increased to almost 10°C under heavy load. However, this may be more likely to be an artefact of this fishing technique being employed than being due to inadequate capacity in the heat exchanger.
- Fish chilling rates varied greatly. This may also be in part due to the nature of the fishing. However the extent of the variation in chilling rates is large. This suggests the volume of chilling media (brine) is insufficient, and circulation of the media inadequate to provide industry best practice for fresh chilled products.
- Size or sex of fish was not a significant influence on texture of cooked samples.
- Storage time on ice is a significant influence on cooked fillet texture. Ultimate muscle pH is also intrinsically linked into this relationship. However, the mechanism for this relationship is not clear.
- Rigor development within this sample set of Saddletail snapper occurred primarily at the point of exhaustion of available ATP within the white muscle tissue. Therefore it is difficult to suggest that any 'cold shock' or cold shortening' was being experienced by the fish during the first hours of chilling.
- Time taken for rigor to commence correlates to ultimate pH, but muscle ATP was not a significant influence in this study. This would appear to be a contradictory statement. However, a larger number of sampled fish may provide a more conclusive result and will be the aim of further field trips on fresh chilled vessels.
- Saddletail experience severe symptoms of barotrauma including extensive internal haemorrhaging, which can greatly reduce fillet quality and market price. Methods to reduce this incidence should be trialled to allow any improvement in quality to be quantified and permit stakeholders to make sound commercial decisions on trap hauling practices.
- The current method of bleeding these fish onboard this vessel have limited efficacy with preventing blood spotting in Saddletail muscle.

5.6.2 Field trip 2

Several significant results have emerged from this group of fish sampled.

- Female fish were significantly tougher than male fish and significantly lower in ultimate pH.
- Female fish were significantly smaller (both fork length and mass) than male fish.
- Some females were found to contain higher levels of collagen than the males. However, this does not necessarily translate directly to a higher peak force value obtained from the cooked fish sample.

5.6.3 *Field trip 3*

Field trip 3 (April 2009) has provided results enabling the identification of two significant trends within the full data set of the project to date.

- Significant difference in texture measurements exist between Saddletail from early summer (early December 2008) and late autumn (late April 2009). This trend was more distinct in male fish than females. Although fish size may play a role in this difference, seasonal variation due to sexual maturity, spawning season or food availability cannot be ruled out.
- A significant correlation between fork length, textural firmness (total energy) and heat insoluble collagen has been identified. This result suggests fish age may be an important contributing factor in the firmness of cooked Saddletail fillets.

5.6.4 *Field trip 4*

- Data from this set of fish yielded little in the way of significant results. No differences were found between the male and female fish for any observed parameter and no correlations were found between any set of parameters

5.6.5 *Field trip 5*

- Significant seasonal differences in texture previously identified were less significant with the inclusion of this data set (FT5). The influence of fish size on the previously identified seasonal differences cannot be ruled out.
- A small but significant correlation between fork length and texture (total energy) has been consistently observed across 3 field trips and 2 seasons.
- This trend suggests that fish age may be a contributing factor in cooked muscle texture of Saddletail.

5.7 Otolith examination for Saddletail age approximation

5.7.1 Age approximation of Saddletail from Field Trip 3 (April 2009)

All 101 fish from this field trip had both otoliths removed for determination of age by otolith banding increment. A summary of the age and fork length data is presented in Figure 34.

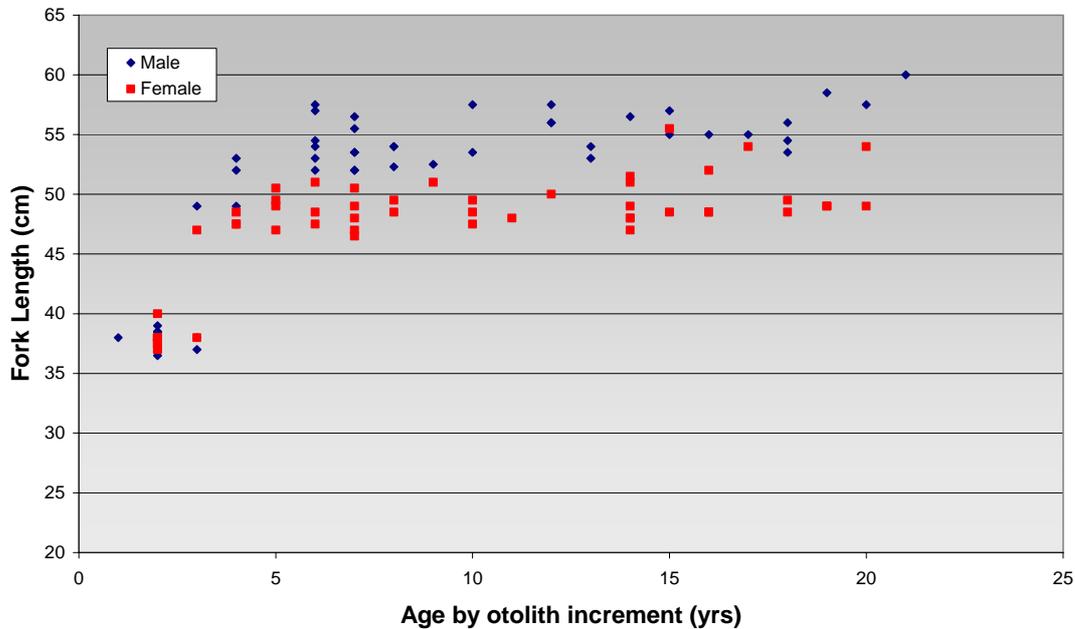


Figure 34. Fork length and age (otolith increment) for males and females from FT3

There was no significant difference between the ages of males and females from this field trip. The mean age by otolith increment of all male Saddletail was 8.39 (± 5.75 SD) and females were 9.52 (± 5.79 SD). Ages for male Saddletail range from 1 to 21 and females range from 2 to 20.

Linear regression (with groups) revealed a significant correlation ($p < 0.001$) between total energy and age accounting for 72.8% of observed variation (Figure 35).

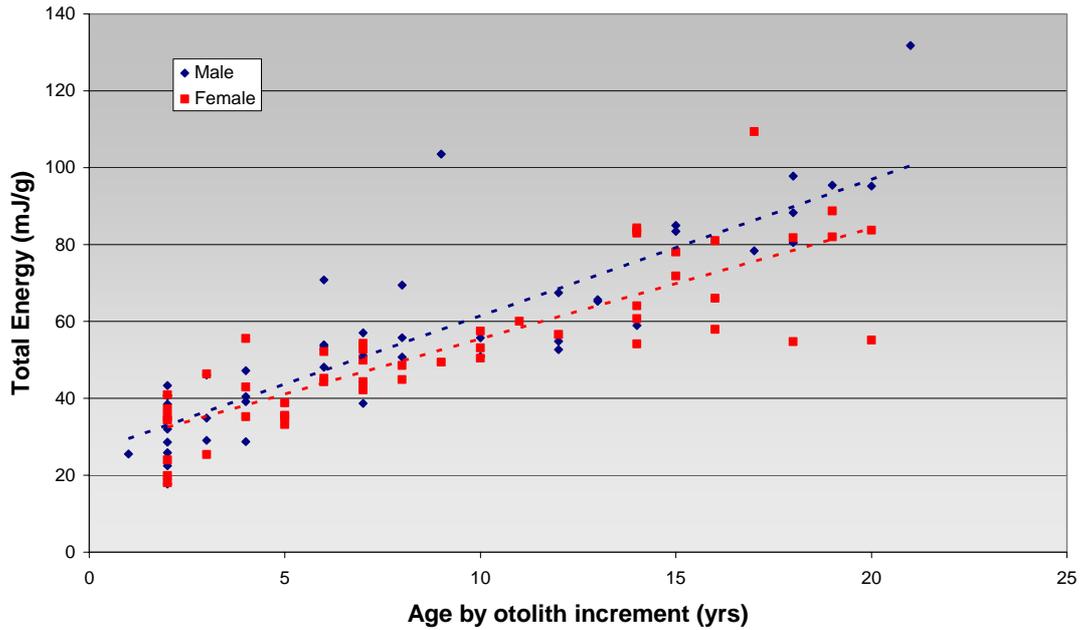


Figure 35. Total energy and age (otolith increment) for males ($R^2=0.75$) and females ($R^2=0.71$) from FT3

This result demonstrates that fish age has a significant effect on the cooked texture of Saddletail. The other important finding (Figure 35) is that this correlation is consistent for both males and females of a similar age, even though males are significantly larger than females of the same age. Figure 36 demonstrates the relationship between heat insoluble collagen and fish age from samples collected from FT3.

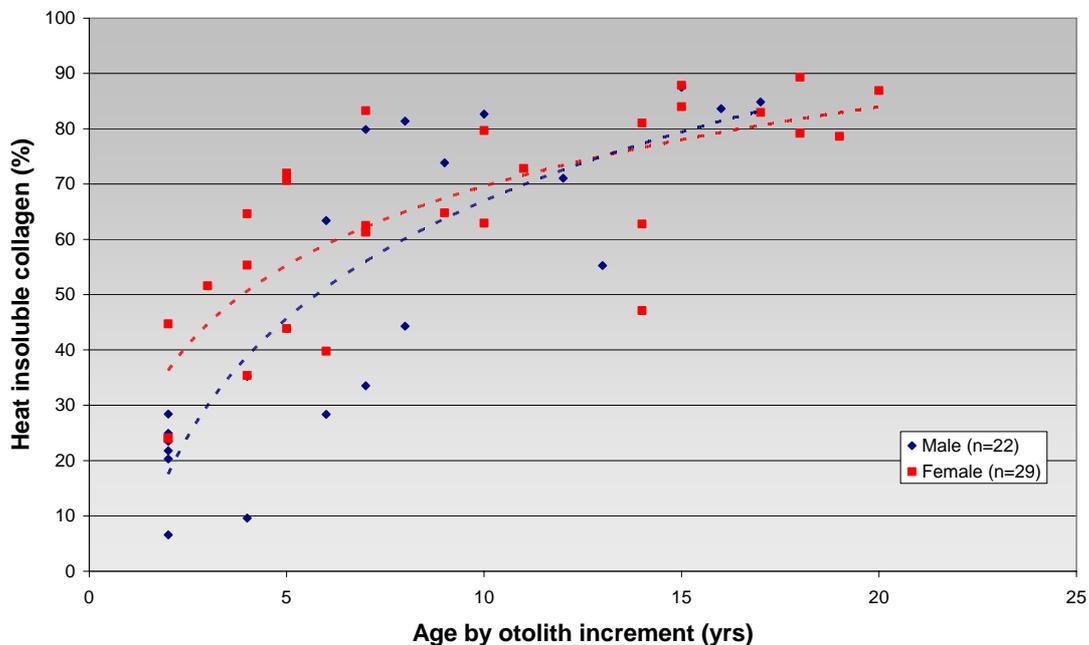


Figure 36. Heat insoluble collagen and age (otolith increment) for males ($R^2=0.72$) and females ($R^2=0.62$) from FT3 (log scale trendlines)

Previous analysis has identified a weak but significant relationship between cooked muscle texture (total energy) and heat insoluble collagen content of the muscle. The relationship presented in Figure 36 shows that insoluble collagen increases with age. This

suggests a possible mechanism to explain the increase of cooked muscle texture with age observed in Saddletail snapper.

5.7.2 Age approximation of Saddletail from Field Trip 5 (October 2009)

Otoliths were removed from all 109 Saddletail obtained from FT5. Otoliths were sectioned and imaged as outlined in the methods at Fisheries South, Deception Bay (DEEDI). Age and fork length data are presented in Figure 37.

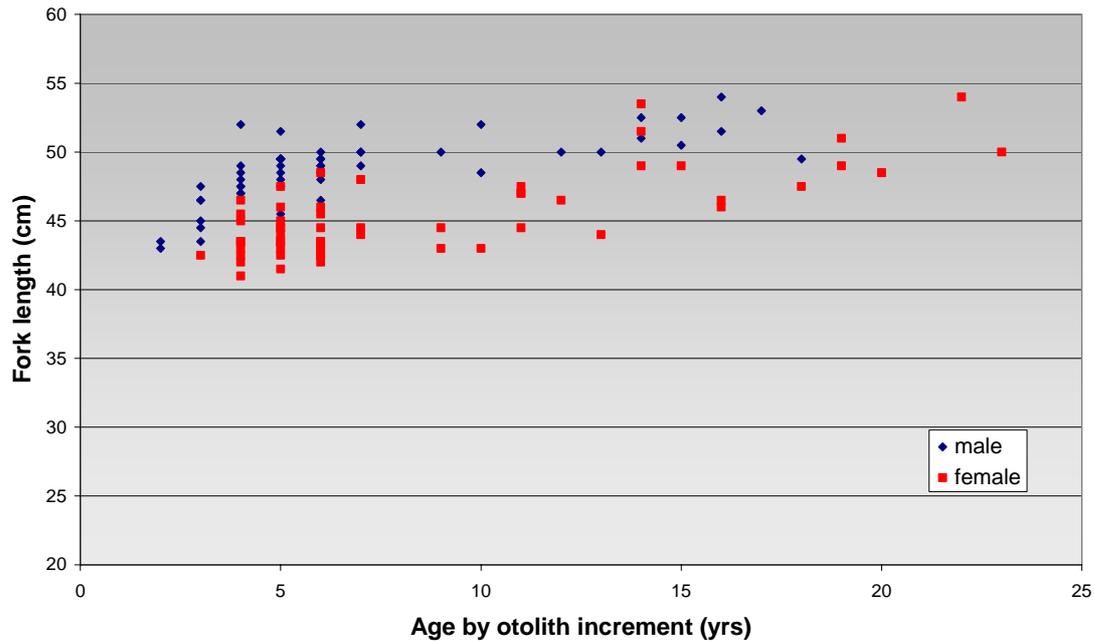


Figure 37. Age and fork length data for male and female Saddletail from FT5

Mean ages obtained from otoliths were 6.96 (± 4.31 SD) years for males and 8.71 (± 5.33 SD) years for females. Male Saddletail ranged in age from 1 to 19 years and females from 2 to 24 years.

Linear regression (with groups) revealed a significant correlation ($p < 0.001$) between total energy and age accounting for 49.8% of observed variation (Figure 38).

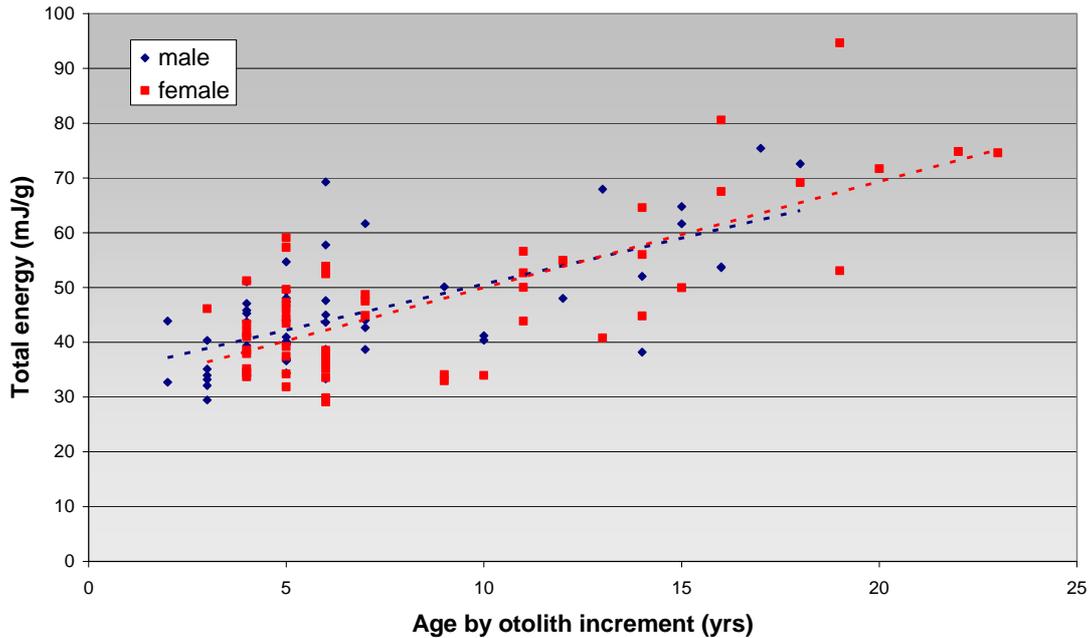


Figure 38. Total energy and age (otolith increment) for males ($R^2=0.44$) and females ($R^2=0.55$) from FT5

As previously identified from FT3, fish age is a significant driver in the firmness of cooked muscle texture of Saddletail. These results are consistent for males and females of the same age, as was also observed from FT3.

However, fish age accounts for less than half of the variation observed in total energy from FT5, whereas age accounted for almost 73% of observed variation in total energy in Saddletail from FT3 (see Figure 35). Another seasonal influence appears to be exercising an effect upon texture quality. This effect may be the result of sexual maturity or seasonal food availability.

Sexual activity at this time of year has been previously reported in Saddletail (Salini et al. 2006) and sexual activity has also been associated with textural issues in other species (Ito et al. 1992). However food availability and feeding habits have also been associated with seasonal variation in fish quality (Love 1979).

5.7.3 Age and fork length data for FT3 and FT5

Figure 39 Presents age and fork length data for both FT3 and FT5.

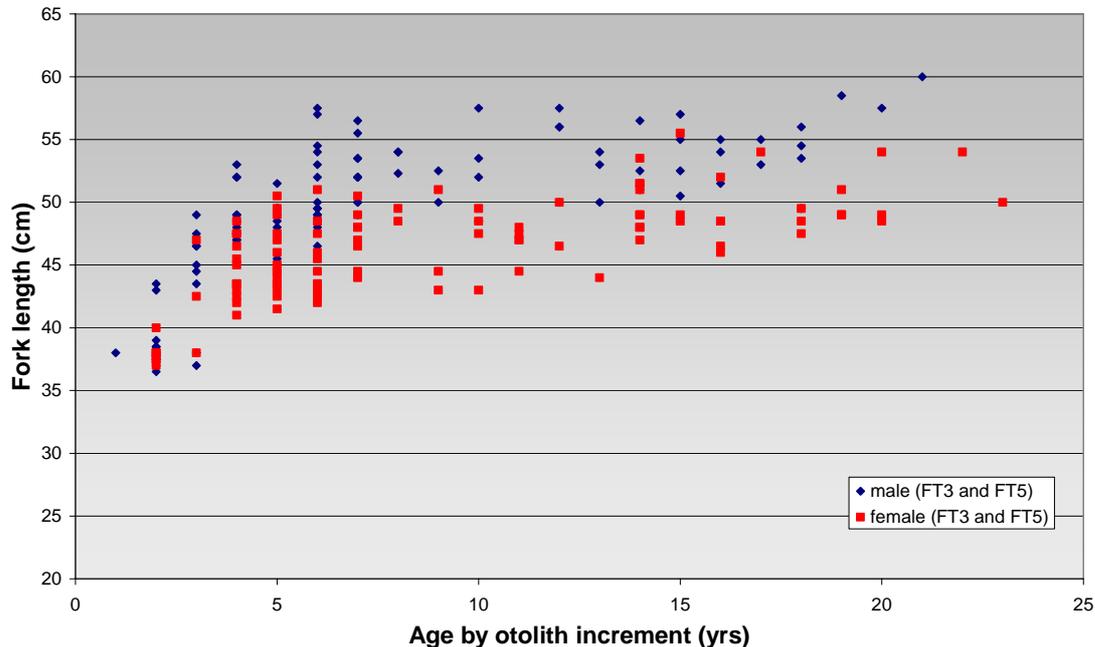


Figure 39. Age and fork length data for males and females from FT3 and FT5.

The most significant trend from this data is that both males and females grow very little in fork length from approximately 7 years of age. This trend demonstrates that fork length is a poor indicator of age for this species.

5.7.4 Summary of ageing results

- Fish age has emerged as the single most significant single factor influencing the firmness of cooked Saddletail snapper. Males and females are significantly different sizes at similar ages, but their cooked texture is not significantly different.
- The trend of increasing toughness with age corresponds with an increase in heat insoluble collagen content, without any significant increase in total collagen. This suggests that as fish age some of the heat soluble collagen in the muscle is becoming insoluble to heat, resulting in tougher cooked muscle texture.
- This trend is not consistent across the seasons. Saddletail from December presented a far greater inconsistency in texture, with respect to age, than those obtained during April/May. A seasonal influence relating to sexual activity or feeding activity appears to be at work.
- Fork length is a poor indicator of age for this species.

5.8 Collagen cross-linking determination (pyridinoline)

5.8.1 Background

Hydroxyllysyl pyridinoline (PYD) is used in clinical biochemistry as a biomarker for bone tissue turnover and human collagen density determination (Tan et al. 2003). PYD has also been identified as influencing the texture of fresh and smoked Atlantic salmon (Li et al. 2005). PYD can form strong covalent bonds between the telopeptide and helical portion of the collagen molecule (Kuboki et al. 1993). Ando (2006) has also demonstrated that PYD is found in acid insoluble collagen at levels up to 200 times those found in acid soluble collagen. The helical structure of collagen can be seen in the electron micrographs (TEM) of Saddletail inter-muscular connective tissue below in Figure 40.

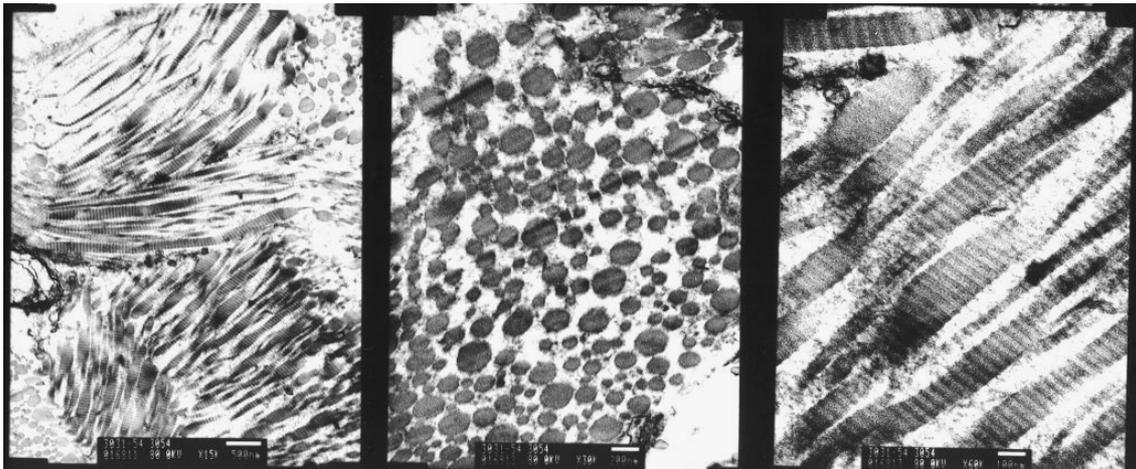


Figure 40. Longitudinal and transverse sections of connective tissue from Saddletail snapper showing helical collagen fibres at 15K, 30K and 60K times magnification respectively (size bars = 500/200/100nm respectively)

5.8.2 Results

51 Saddletail previously assessed for collagen determination from FT3 had a additional tissue sample assessed for PYD using the modified method outlined by Li (2005). Linear regression identified a significant correlation ($p < 0.001$) between PYD and total energy explaining 48.2% of observed variation (Figure 41).

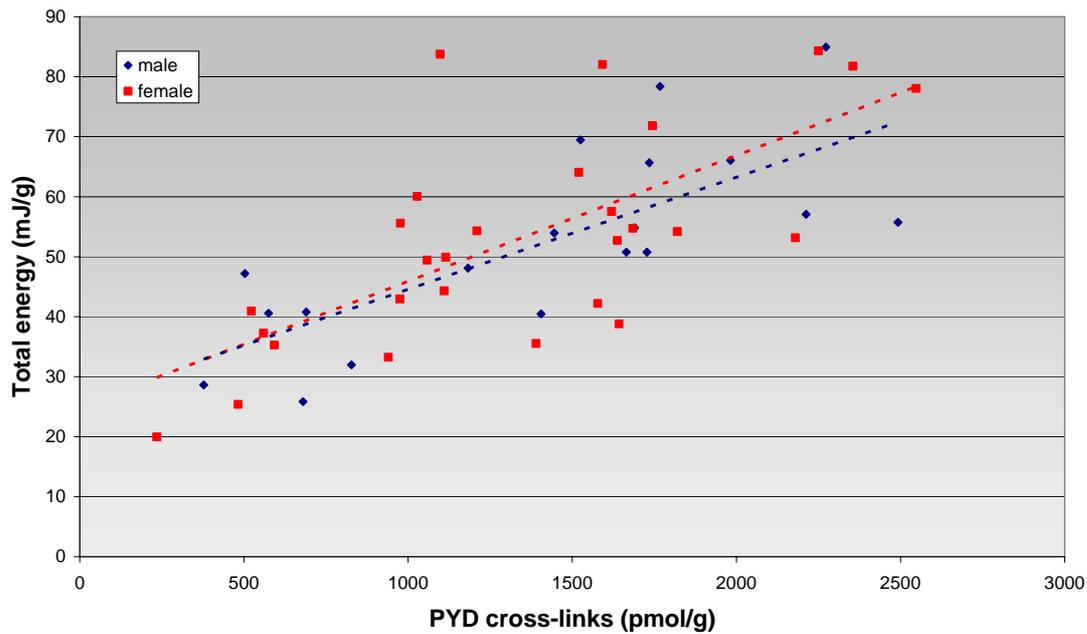


Figure 41. Total energy and PYD for male ($r^2=0.56$) and female ($r^2=0.49$) Saddletail from FT3

This result demonstrates the direct relationship of PYD cross-links to the cooked muscle firmness of Saddletail. However, PYD does not account for all variation observed and other influences are occurring.

A similarly significant correlation ($p<0.001$) was found between the percentage of total collagen being heat insoluble with PYD content; explaining 53.1% of observed variation (Figure 42).

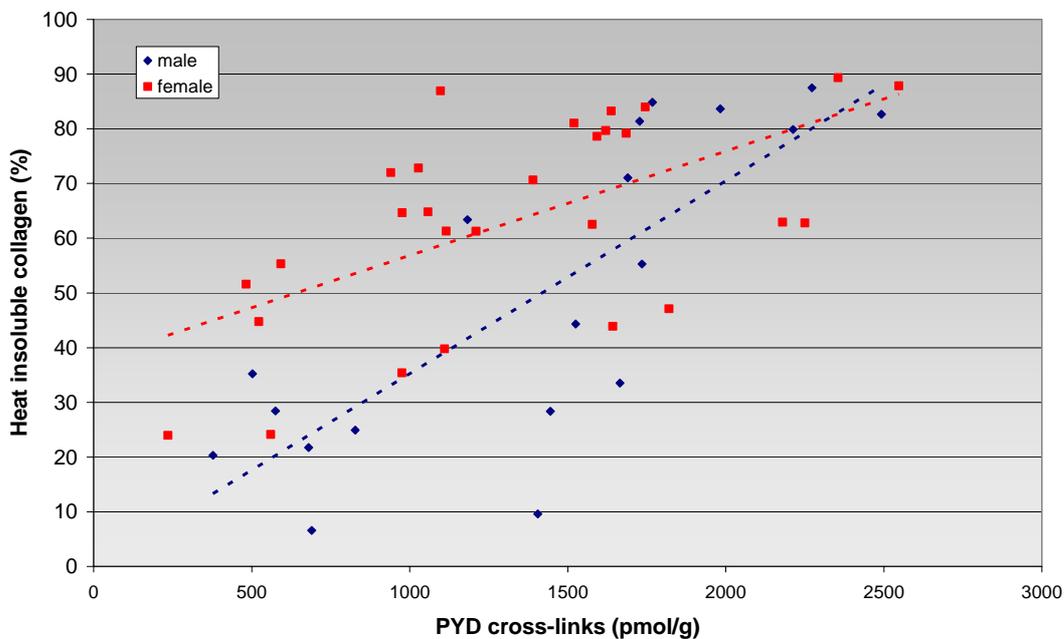


Figure 42. Heat insoluble collagen and PYD for male ($r^2=0.63$) and female ($r^2=0.36$) Saddletail from FT3

This demonstrates a role for PYD in the formation of heat insoluble collagen in Saddletail muscle. However, the relationship only describes a little over half the variation observed, suggesting other mechanisms at work beyond the scope of this analysis.

Linear regression also revealed a significant correlation ($p < 0.001$) between fish age and PYD content describing 49.5% of observed variation. Use of log curves on the graphed data produces more accurate trendlines consistent with biological ageing trendlines (Figure 43).

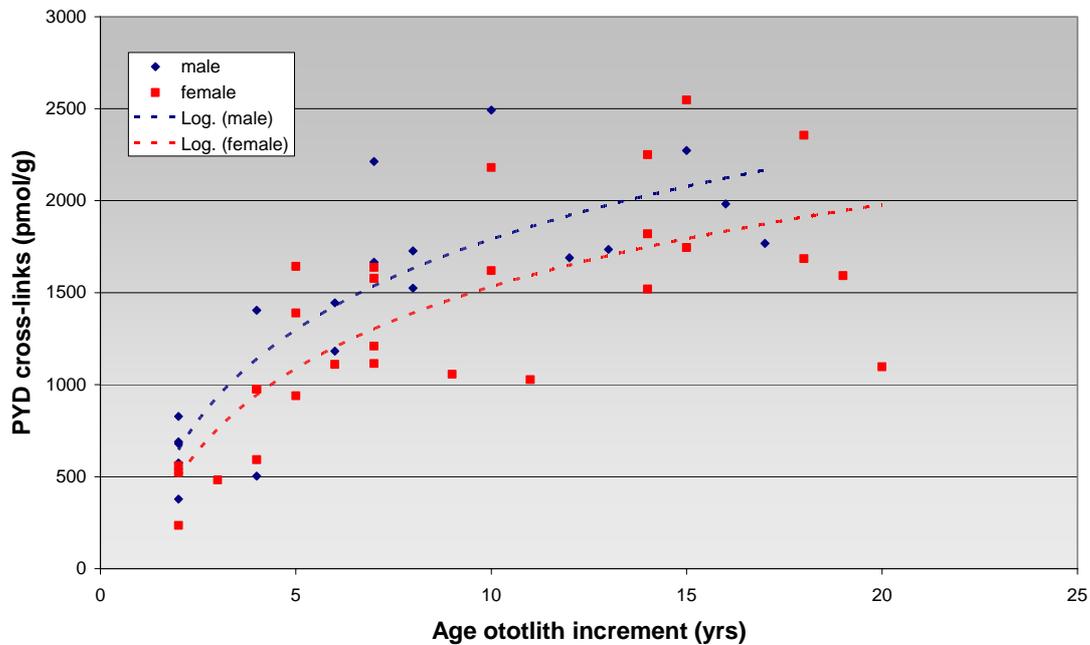


Figure 43. PYD content and age data for male ($r^2=0.73$) and female ($r^2=0.59$) Saddletail from FT3

5.8.3 Summary

- These results demonstrate a clear relationship between age and cooked muscle firmness in Saddletail snapper. These results also suggest a mechanism for this to occur.
- As Saddletail age more of the collagen in the muscle tissue becomes cross-linked. This cross-linking renders the collagen insoluble to heat and prevents melting of collagen under normal cooking conditions. As the collagen remains intact after cooking, the integrity of the intra-muscle connective tissue remains intact and the cooked tissue is difficult to break apart during mastication. The net result is the sensation of toughness when eating.

5.9 Electron Microscopy

5.9.1 Background

Electron microscopy was employed in an attempt to obtain more conclusive understanding of the role of sarcomere length and investigate the possibility of 'cold shortening' of Saddletail muscle. Light and scanning electron microscopy (SEM) have previously been employed to investigate the role of sarcomere length in fresh and smoked Atlantic salmon (Sigurgisladdottir et al. 2001).

Tissue samples 6 Saddletail from FT2 and 8 from FT3 were sent Dr Deborah Stenzel at Queensland University of Technology (QUT) for analysis by transmission electron microscopy (TEM). Tissue samples were taken from the dorsal epaxial myotome and frozen at 29°C and transferred to QUT. Sarcomere length was measured the distance between z-bands TEM micrograph prints (see Figure 44).

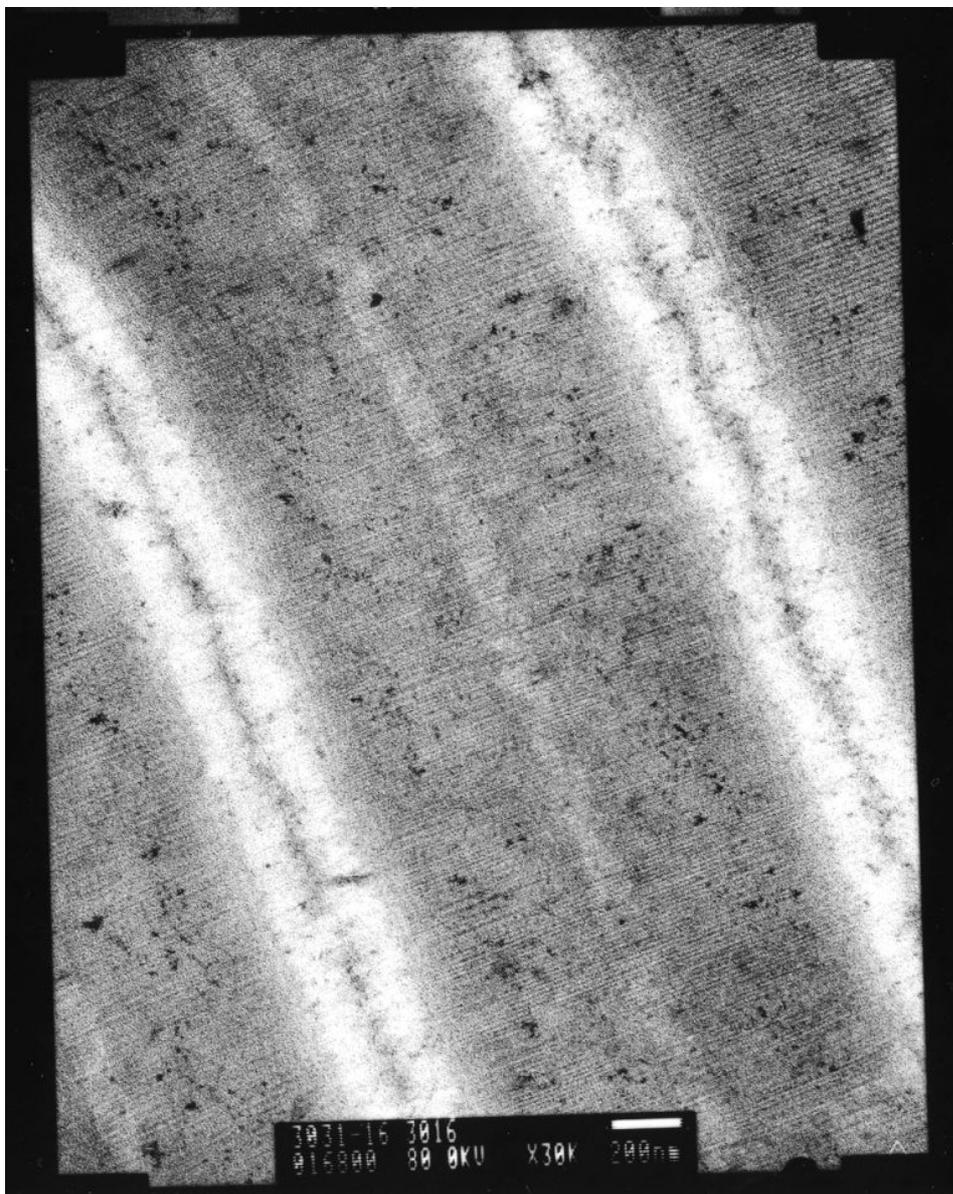


Figure 44. TEM Micrograph of Saddletail muscle fibre at 30K times magnification (Size bar denotes 200nm)

5.9.2 Results

A summary of biological data and sarcomere length is presented in Table 12.

Table 12. Biological data and sarcomere length as determined by TEM (FT2 and FT3)

ID	Wt (g)	fork length (cm)	pH (final)	sex (M1 F2)	Peak Force (N/g)	Total energy (mJ/g)	Sarc (μm)
2026	2432	56.20	6.61	1	2.84	35.07	1.36
2041	1684	48.50	6.53	1	3.24	40.44	1.66
2049	1689	49.00	6.32	2	9.45	67.81	1.50
2062	1905	52.50	6.33	2	8.75	91.02	1.13
2084	1472	46.00	6.26	2	12.09	86.37	1.20
2106	2351	52.50	6.24	1	3.61	34.11	2.05
3016	821	37.00	6.43	1	5.59	29.04	1.60
3024	912	38.50	6.62	1	3.11	17.71	1.47
3041	913	39.00	6.39	1	8.42	40.80	1.37
3054	1547	47.00	6.41	2	3.32	25.41	1.57
3055	1709	49.50	6.43	2	3.12	33.23	1.50
3071	2152	54.00	6.35	2	12.67	109.37	1.23
3073	2173	52.50	6.34	1	11.28	103.56	1.27
3096	1746	48.50	6.58	2	3.66	35.26	1.23

The mean sarcomere length for all fish was $1.44\mu\text{m}$ (± 0.24 SD). This is shorter than most commercial species. Atlantic salmon (*Salmo salar*) has been shown to have an average sarcomere length of $2.2\mu\text{m}$ (Sigurgisladottir et al. 2001), Mackerel (*Scomber japonicus*) has been reported at $1.9\mu\text{m}$ (Shindo et al. 1986). And Atlantic halibut (*Hippoglossus hippoglossus*) has been reported at $1.6\mu\text{m}$ (Olsson et al. 2003).

Linear regression analysis reveals a weak but significant relationship ($p=0.028$) between sarcomere length and total energy explaining 28.6% of observed variation. This relationship is graphically represented in Figure 45.

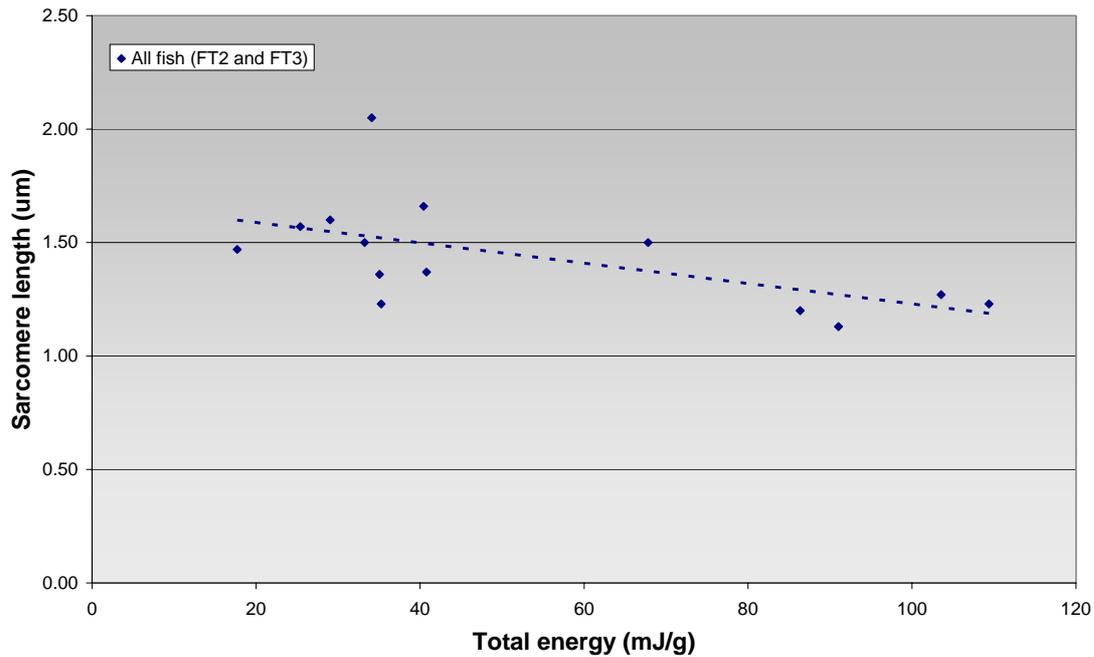


Figure 45. Sarcomere length and total energy for Saddletail from FT2 and FT3 ($r^2=0.34$)

Only a small number of samples were possible for this analysis as the process is cost inhibitive for large scale sarcomere determinations. Hence, little weight can be placed on the significance of the statistical analysis presented here.

However enough of a trend exists to warrant further investigation, particularly in view of the inefficiencies already identified in chilling systems onboard FV Starlight used for FT2. Repeating temperature profiling of fish chilling rates with best industry practice and further sarcomere determinations would be worthwhile in building a more complete understanding of the potential for this species to experience 'cold shock'.

An observation made by Dr Stenzel was that the tissue itself was highly resilient to the trauma of freezing. This fish tissue used for microscopy was frozen once onboard as a whole fish, thawed prior to processing, and refrozen for storage and transport to QUT. An image of the tissue on lower magnification is presented in Figure 46, and shows integrity of muscle structure with minimal degradation.

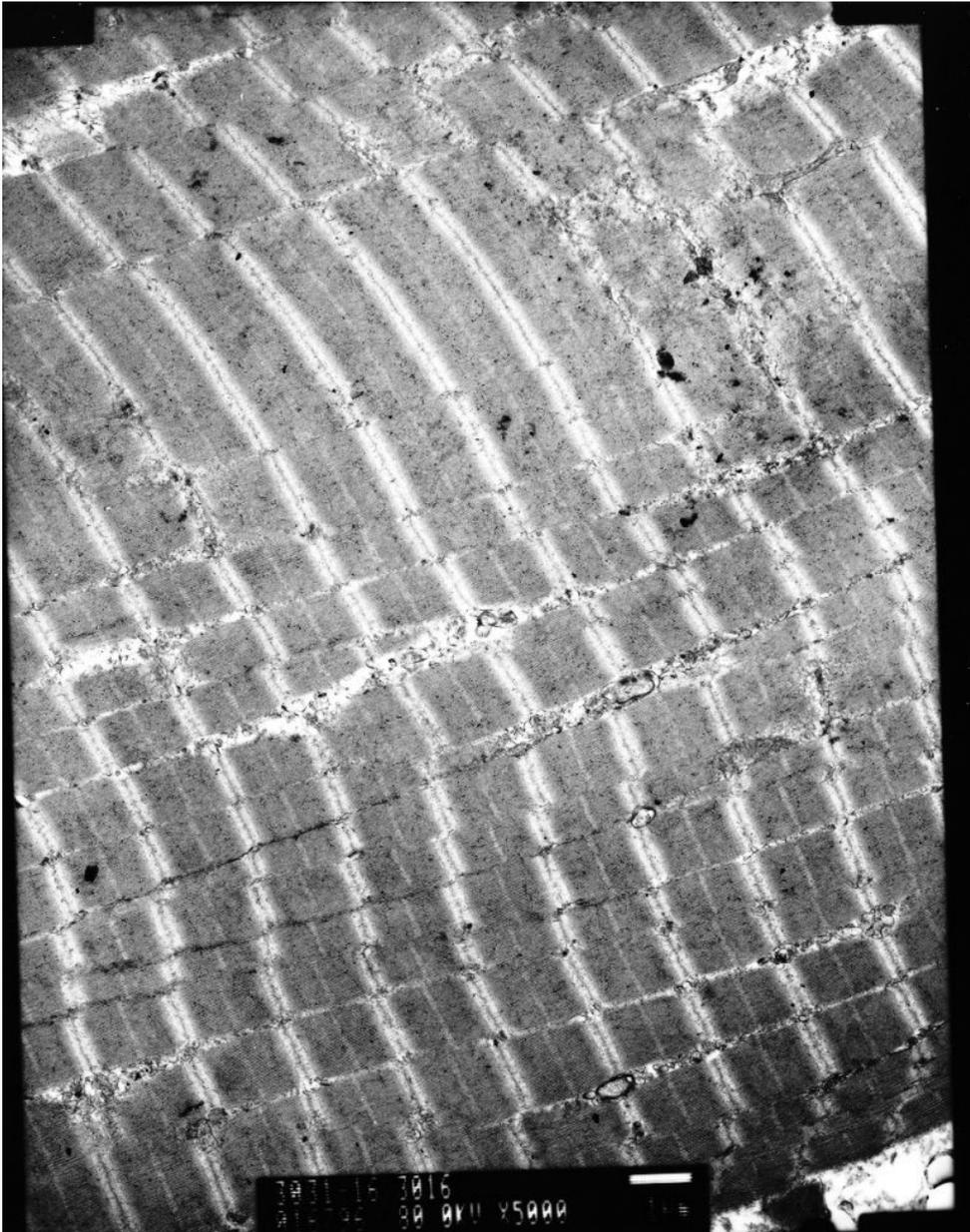


Figure 46. TEM micrograph of Saddletail muscle tissue at 5K times magnification (size bar denotes 1 μ m)

Dr Stenzel suggested the level of integrity of the muscle after multiple freezing events was very unusual and most unlike mammalian tissue. Most mammalian tissue is highly disrupted by freezing. However, as can be seen in Figure 46 individual muscle cells are intact and very little separation between cells has occurred.

This observation suggests intercellular connective tissue is difficult to disrupt with freezing. The muscle cells themselves contain significant microstructure capable of withstanding multiple freezing events and little evidence of autolytic degradation, as has been observed in other species (Bremner and Hallett 1985).

The role of this microstructure in the eating quality or perceived toughness of cooked muscle is difficult to extrapolate without undertaking trained taste panel assessments. However, the muscle tissue observed here displays a high level of structural integrity that may flow on to exercise an influence upon cooked muscle texture of this species.

6 Benefits and adoption

Depressed market confidence due to perceived issues of toughness has been the primary issue driving the undervaluation of this species throughout Australia. The findings from this research have provided previously unknown information on the likely cause of TFS. This will benefit all stakeholders throughout the value chain. Key changes in onboard handling protocols cannot yet be developed as further seasonal data needs to be obtained to confirm the current understanding.

Key Industry stakeholders were co-investigators on this project and were actively involved with the planning and undertaking of the research. Stakeholder meetings held in May and November of 2009 kept participants updated on results and provided opportunity to discuss future research direction. Further communications to summarise all findings will be conducted in person to the co-investigators at finalisation of this project.

The new information that fish age is a significant contributing factor to the textural qualities of cooked Saddletail provides a basis for sound commercial business decisions. However, significant seasonal influences have also been identified and variation between fish toughness cannot be wholly attributed to fish age.

Fishing vessel deck practices, particularly fish chilling practices have been identified as varying greatly in efficacy, but were found to not contribute to increased cooked muscle firmness. Through industry meetings, the issues with onboard chilling have been conveyed to fishing vessel operators and methods of overcoming these difficulties have been discussed. Improvements in chilling rates of fish onboard will improve product shelf-life throughout the value chain of the species.

The results obtained so far enable stakeholders to reconsider how best to market their product with respect to age. Larger fish that may be more likely to exhibit toughness should not be marketed to high value food service suppliers where a premium is received for high eating quality. These customers should only be supplied with fish of a smaller size where quality is paramount to protect market confidence.

Removal of larger and older fish that are more likely to be tougher upon cooking will improve the reputation of the species as a premium table fish. The direct result of this research will be an increase in product confidence.

During the time of conducting this project one industry stakeholder advised that a significant shift in market demand has already taken place for this species. Major supermarket chains are once again placing orders for the species, resulting in the species becoming a significant product (by volume) throughout the two dominant supermarket chains in Queensland, New South Wales and Victoria. This increase in demand has resulted in an increase in value by 15%. Increased product confidence will allow fishery operators and wholesalers to demand a higher premium for this quality Australian table fish.

7 Further Development

Further developments of the findings from this research are centred on five key areas:

7.1 Segregation of fish with high risk of 'toughness'

Tools and strategies are required for identifying a size or age of Saddletail where the risk of supplying potentially 'tough' product to any given market is reduced to an acceptable level.

7.2 What is 'acceptable' firmness?

The development of a quantified acceptable firmness is beyond the scope of this current project. However such a concept could be obtained by use of an appropriately designed consumer acceptability test involving a minimum of 96 consumers. The result of which would provide significant information as to the consumer preferences for cooked fish texture and a point at which cooked fish becomes unacceptable to be reasonably defined.

7.3 Simplified identification of sex

We have so far identified a potential firmness level (80mJ/g total energy) past which risks of tough texture are high. Consumer preference determination would also provide greater weight to this concept.

However, the application of size limits is complicated by the fact that males are much larger fish than females. As a result, the size limit for male would be 55cm and females would be 50cm.

The successful application of these or any other consumer defined limits would require the development of a simple, non-invasive, and easy to use method of identifying the sex of a whole Saddletail. At the writing of this the authors are unaware of such a method.

Consultation with the three fisheries departments involved with the management of this species (WA, NT and QLD) will be required for the successful development of such a method. Whatever technology is employed will need to be robust, affordable and simple enough for use onboard all types of fishing vessel involved in this fishery.

7.4 Influence of season on texture

Seasonality appeared to exert a significant influence on cooked muscle texture. Male Saddletail landed in December (2008 and 2009) were significantly softer than those landed in April (2009). These differences were not significant for females and variation in fish size is also a complicating factor. Total collagen levels for males and females were also significant across the seasons (December 2008/April 2009).

Further sampling across the seasons is required to establish the presence or otherwise of seasonal influences. Understanding the mechanism of this variability will enable increased accuracy when applying size limits to certain markets as these may become more or less important during periods of peak seasonal variation.

7.5 Synergistic effects of age and inappropriate chilling

Chilling practices observed during this research were found to vary greatly in efficacy. This has made identification of the incidence of a genuine and repeatable 'cold shock' event very difficult.

TFS in Saddletail snapper

Investigation of this phenomenon using industry best practice chilling equipment and empirical methods is still required. This is particularly important to the possible compounding of 'cold shortening' in older fish (greater than 10 years) that are already at high risk of exhibiting cooked muscle toughness.

8 Planned Outcomes

The planned outcome from this research work was the identification of factors, both on-board handling and biological which influenced the development of Tough Fish Syndrome.

Handling practices onboard the fishing vessels observed during the course of this work proved not to significantly contribute to the development of TFS in Saddletail snapper. These findings have been presented and discussed with co-investigators and other industry stakeholders at meetings in May and November of 2009.

Examinations of biological factors have identified 2 significant causes of firmness in cooked Saddletail snapper. Fish age has been identified as being the primary driver of cooked muscle firmness in Saddletail snapper. This fact is complicated by the significant size difference between male and female Saddletail. For this species age does not correlate with fork length after the first 5 years for females, and 7 years for males.

Preventing the older fish most at risk of developing excessive toughness from entering the premium supply chain is intrinsic to the restoration of confidence in this species. This may also provide opportunities to develop value-added products with older fish that may be redirected to more suitable markets in Australia or overseas. Industry partners are currently being consulted in how best to implement these measures.

Successful implementation of these measures will restore consumer confidence in the species as a quality table fish. This will result in improved returns to stakeholders, an increase in the value of the fishery, and improving sustainability of the fishery and its operators.

9 Conclusions

9.1 *On-board deck practices*

Handling practices onboard fishing vessels, particularly chilling methods have been identified as being inconsistent and varying greatly in efficacy. Chilling rates of Saddletail snapper varied greatly and refrigeration systems struggled to maintain brine water temperatures below 5°C under heavy load. Greater efficiency in chilling would result in longer shelf-life of whole fish within the entire catch.

However, these practices did not lead to observation of any direct influence over to cooked muscle texture of the species. The development of 'cold shock' or 'cold shortening' to a level that may influence cooked muscle texture has not been identified within the course of this work.

Observed stiffening of Saddletail during chilling coincided with the exhaustion of available ATP with the fast twitch muscle. However, the primary driver of increasing firmness identified in this research was not a product of excessively cold chilling practices.

9.2 *Biological factors influencing Saddletail texture.*

9.2.1 *The effect of age on cooked muscle texture*

This work has identified fish age as being the primary driver of increased firmness of cooked muscle texture in Saddletail snapper. As part of the aging process, hydroxylysyl-pyridinoline cross-links are formed between collagen fibres. The formation of these cross-links provides collagen fibres in the muscle fibre bundles with a greatly increased resistance to melting under heat conditions.

The rate of collagen cross-link formation during the lifespan of Saddletail appears to be consistent for both males and females. So at first glance, the simplest commercial solution would be to establish a size limit to minimise the risk of older fish entering the supply chain, and implementing this limit throughout the fishery.

Male Saddletail grow significantly larger than females. From the data we have collected, a reasonable size limit for female Saddletail that would reduce the risk of tough muscle texture would be 50cm fork length. Female Saddletail of 50cm fork length could be anything from 5 to 20 years of age. The same limit for male fish would exclude up to 70% of the male catch; as the majority of male fish have attained 50cm in fork length within 5 years.

Any size limits would be required to be sex specific. The identification of fish sexes either onboard, or during unload and processing will present a new set of challenges and complexities to fishing operations.

Implementing any size limits on fish to particular markets or wholesalers is also a commercial decision beyond the realms of this study. However consultation with industry stakeholders has commenced with the aim of achieving consensus with the industry to approach this issues.

9.2.2 *Seasonal variation in texture*

Significant seasonal variation in cooked muscle texture was identified in Saddletail snapper. Male fish were more significantly different than female fish between the seasons. However, male fish were more consistent within a season, and female fish were highly variable regardless of season.

Collagen content was also significantly different across the seasons. And as with texture, the male fish displayed greater differences across the season, but less variability within the season, than did females.

Further work is required in this field to establish exactly the drivers for this variation. Changes in cooked muscle firmness could be the result of issues of food availability or gonadotrophic development. Such variation has significant consequences for any self-imposed size limits of fish to specific premium markets. This is especially important for male fish due to their large size, and large observed variation in cooked muscle texture across the seasons.

10 References

- Ando, M., Y. Nakagishi, K. Yoshida, M. Nakao, T. Nakagawa, Y. Makinodan, Y. Tsukamasa and K.-i. Kawasaki (2006). "Pyridinoline concentrations in muscular and skin collagen of fish and relationship between collagen solubility and pyridinoline concentration in fish muscular collagen." Fisheries Science **72**(5): 1104-1108.
- Bouton, P. E., Fisher, Anne L., Harris, P.V. and Baxter, R.I. (1973). "A comparison of the effects of some post-slaughter treatments on the tenderness of beef." Journal of Food Technology **8**(1): 39-49.
- Bremner, H. A., (Ed) (2002). Safety and Quality Issues in Fish Processing. Cambridge, Woodhead Publishing Limited.
- Bremner, H. A. and I. C. Hallett (1985). "Muscle fiber - connective tissue junctions in the fish blue grenadier (*Macruronus novaezelandiae*). A scanning electron microscope study." Journal of Food Science: 50 (4) 975-980 **50**(4): 975-980.
- Curran, C. A., R. G. Poulter, A. Brueton, N. R. Jones and N. S. D. Jones (1986). "Effect of handling treatment on fillet yields and quality of tropical fish." International Journal for Food Science and Technology **21**(3): 301-310.
- Fisheries-Queensland (2009). Fisheries Long Term Monitoring Program Sampling Protocol – Fish Ageing: Section 2: Snapper. E. D. a. I. Department of Employment, Brisbane, Australia.
- Hagen, Ø., C. Solberg, E. Sirnes and I. A. Johnston (2007). "Biochemical and structural factors contributing to seasonal variation in the texture of farmed Atlantic halibut (*Hippoglossus hippoglossus* L.) flesh." Journal of Agricultural and Food Chemistry **55**(14): 5803-5808.
- Hill, F. (1966). "The solubility of intramuscular collagen in meat animals of various ages." Journal of Food Science **31**: 161-166.
- Hiltz, D. F., L. J. Bishop and W. J. Dyer (1974). "Accelerated nucleotide degradation and glycolysis during warming to and subsequent storage at -5degreesC of prerigor, quick-frozen adductor muscle of the sea scallop (*Placopecten magellanicus*)." Journal of the Fisheries Research Board of Canada: 31 (7) 1181-1187 **31**(7): 1181-1187.
- ISO (1994). Meat and meat products – determination of hydroxyproline content (International Organization for Standardization).
- Ito, K., H. Toyohara, M. Ando and M. Sakaguchi (1992). "Disintegration of the pericellular connective tissue of ayu muscle in the spawning season relevant to softening." Bulletin of the Japanese Society of Scientific Fisheries **58**(8): 1553 p.
- Kuboki, Y., M. Okuguchi, H. Takita, M. Kimura, M. Tsuzaki, A. Takakura, S. Tsunazawa, F. Sakiyama and H. Hirano (1993). "Amino-Terminal Location of Pyridinoline in Dentin Collagen." Connective Tissue Research **29**(2): 99-110.
- Li, X., R. Bickerdike, E. Lindsay, P. Campbell, D. Nickell, A. Dingwall and I. A. Johnston (2005). "Hydroxylysyl pyridinoline cross-link concentration affects the textural properties of fresh and smoked atlantic salmon (*Salmo salar* L.) flesh." Journal of Agricultural and Food Chemistry **53**(17): 6844-6850.
- Longbottom, S. (2000). The effect of capture depth on potential broodstock snapper (*Pagrus auratus*). . Muresk Institute of Agriculture. Perth, Western Australia, Australia., Curtin University of Technology. **Master's thesis**
- Love, R. M. (1979). "The post-mortem pH of cod and haddock muscle and its seasonal variation." Journal of the Science of Food and Agriculture **30**(4): 433-438.
- Love, R. M. (1980). " The chemical biology of fishes. Vol. 2: Advances 1968-1977, with a supplementary key to the chemical literature." Publ. by: Academic Press; London (UK).. 1980., 943 p.

- McPherson, G. R. and L. Squire (1992). "Age and growth of three dominant *Lutjanus* species of the Great Barrier Reef Inter-Reef Fishery." Asian Fisheries Science **5**((1992)): 25-36.
- Newman, S. J. (2002). "Growth rate, age determination, natural mortality and production potential of the scarlet seaperch, *Lutjanus malabaricus* Schneider 1801, off the Pilbara coast of north-western Australia." Fisheries Research **58**(2): 215-225.
- Olsson, G. B., R. L. Olsen and R. Ofstad (2003). "Post-mortem structural characteristics and water-holding capacity in Atlantic halibut muscle." Lebensmittel-Wissenschaft und-Technologie **36**(1): 125-133.
- Parker, S. J., H. I. McElderry, P. S. Rankin and R. W. Hannah (2006). "Buoyancy Regulation and Barotrauma in Two Species of Nearshore Rockfish." Transactions of the American Fisheries Society **135**(5): 1213-1223.
- Poole, S. (1991). Development of the tropical reef fish fishery: Assessment of specific handling methods for production of high quality chilled fish, Fisheries Research and Development Corporation (Commonwealth of Australia).
- Salini, J. P., J. R. Ovenden, R. Street, R. Pendrey, Haryanti and Ngurah (2006). "Genetic population structure of red snappers (*Lutjanus malabaricus* Bloch & Schneider, 1801 and *Lutjanus erythropterus* Bloch, 1790) in central and eastern Indonesia and northern Australia." Journal of Fish Biology **68**(SB): 217-234.
- Shindo, K., T. Tsuchiya and J. J. Matsumoto (1986). "Histological study on white and dark muscles of various fishes." Bulletin of the Japanese Society of Scientific Fisheries **52**: 1377-1399.
- Sigurgisladottir, S., M. S. Sigurdardottir, H. Ingvarsdottir, O. J. Torrissen and H. Hafsteinsson (2001). "Microstructure and texture of fresh and smoked Atlantic salmon (*Salmo salar*) fillets from fish reared and slaughtered under different conditions." Aquaculture Research **32**(1): 1-10.
- Tan, C. I., G. N. Kent, A. G. Randall, S. J. Edmondston and K. P. Singer (2003). "Age-related changes in collagen, pyridinoline, and deoxypyridinoline in normal human thoracic intervertebral discs." Journals of Gerontology Series a-Biological Sciences and Medical Sciences **58**(5): 387-393.

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, DEEDI

Technical and Analytical staff:

Andrew Forrest Research Scientist (Food Technology), IFT, DEEDI
John Mayze, Principal Seafood Technician, IFT, DEEDI
Paul Exley, Senior Seafood Technician, IFT, DEEDI
Carl Paulo, Senior Seafood Technician, IFT, DEEDI
Sharon Pun, Analytical Technician, IFT, DEEDI