Developing large rocklobsters, *Jasus edwardsii*, as a premium value-added product: Key sensory and biochemical characteristics of the flesh.

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ABSTRACT

The Southern Rocklobster, *Jasus edwardsii*, supports a commercial fishing industry worth $180 million AUD per annum, the majority of which is exported live to Asia. The current market demands for smaller rocklobsters can sometimes result in discounting of the larger individuals, a significant financial loss for the industry. Value adding of large rocklobster into processed product may help combat this loss; however, there is financial risk associated with the development of new products for new markets without first understanding the product variability. The aims of this thesis were to quantify raw product flesh characteristics using physical, biochemical and sensory approaches, determining the extent of variation in those characteristics, and finally to investigate the potential biological and post-harvest sources of that variation.

One of the initial requirements was the establishment of previously undefined key descriptors of sensory properties for raw rocklobster flesh, which were texture (chewiness and crunch), flavour (metallic, lobster and sweetness) and appearance (pinkness and translucency) (Chapter 2). These were tested using a combination of triangle tests and a hybrid descriptive test using a trained sensory panel. The trained panel found no significant difference in the texture, flavour or appearance of raw flesh between large and small rocklobster (Chapter 4). However, differences in the sensory descriptors of flesh translucency, pinkness and lobster flavour were significantly influenced by frozen storage of the product and the section of tail from which a sample was sourced (Chapter 4). Biochemically, these differences were largely associated with

variation in flesh adenylates, with AEC, IMP load, total adenylate pool and K value being identified as the key contributors.

Of all the potential sources contributing to variation in flesh biochemical properties, post-harvest factors such as 'batch' (i.e. rocklobsters processed on a single day) had a dominant influence (Chapter 3). The difference detected in flesh characteristics between batches was greater than any seasonal pattern such as moult stage. Biological variables such as rocklobster condition and shell colour had no significant influence on flesh properties (Chapters 3 & 4). White rocklobsters are currently discounted in the live export trade; however this does not appear to be necessary for value added product owing to the lack of significant differences to red rocklobsters across a range of biochemical parameters (Chapter 3). Rocklobster physical condition (which has previously been associated with prior stress) was not shown to affect flesh biochemistry or sensory properties (Chapter 4). This result was not expected and may reflect the potential recovery of rocklobsters sampled in this study prior to processing. These findings suggest that commercial rocklobsters, which have had similar recovery, are unlikely to show reduced sensory properties.

Recent commercial interest has focussed on holding rocklobster in tanks to provide year-round supply. As a result, the impacts of tank-holding and feeding on rocklobster flesh sensory properties were investigated (Chapter 5). Rocklobsters that were tank-held and fed for up to four months produced flesh with similar physical, biochemical and sensory properties to freshly caught rocklobster. Tank-holding therefore offers a viable solution

to operators wanting a year-round supply of fresh product from a resource which is subjected to a restricted fishing season.

A Japanese consumer panel was established to assess the greatest differences in flesh properties as detected by the trained sensory panel. The Japanese consumer panel assessed raw flesh from fresh, short and long-term frozen storage treatments (Chapter 4). This consumer panel detected similar differences in taste, texture and flavour as the trained panel, and whilst no significant overall preference was detected, half of the panellists showed a preference for rocklobster product that had been stored frozen for 18 months.

The findings from this research are useful for the commercial industry as they indicate that raw rocklobster flesh has little variation associated with discounting factors such as size and shell colour. Although the greatest variation in flesh biochemistry was seen with frozen storage, even long term storage produced rocklobster flesh properties which were favourable for some panellists. The commercially caught Southern Rocklobster appears to have raw flesh properties well suited for a value added product.

DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Michael Roberts

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CHAPTER ONE

General Introduction

The Southern Rocklobster, *Jasus edwardsii* supports a fishing industry in Australia with a net annual value of ~ $180m (Australian Southern Rocklobster Limited 2006). Almost 95% of the fishery's export is the live trade of whole rocklobsters. Large rocklobsters (above 1.5 kg) comprise approximately ~17% of the commercial fishery (calculated from Prescott et al. (1997)), and are often discounted by approximately $6 per kilogram to sell through the live trade market to Asia (Ferguson. A, *pers. comm.*). This discounting, below the price paid per kg for small rocklobster, equates to $4.9 million AUD lost annually for the combined fisheries of South Australia, Tasmania and Victoria. A new market direction of processed portions (value-adding) of large rocklobster may offer a solution to combat the required discounting. For value-adding of large rocklobster to be successful, there is a need to quantify any perceived variation in the flesh characteristics of the portioned product. The aims of this thesis were to quantifying product flesh characteristics using physical, biochemical and sensory approaches, determining the extent of variation in those characteristics, and finally to investigate the potential sources of that variation.

Biochemical indicators of flesh characteristics

Biochemical properties of flesh are routinely used to monitor changes in flesh characteristics associated with rigor mortis and tissue degradation during storage (Bremner 2003). Muscle nucleotides are of particular interest, as they have been associated with describing changes in rocklobster flesh characteristics post-mortem (Yamanaka and Shimada 1996). It was shown that with storage time, adenosine triphosphate (ATP) broke down into adenosine diphosphate (ADP), and then adenosine monophosphate (AMP). The further breakdown results in the production of inosine monophosphate (IMP), inosine and hypoxanthine, which are used to calculate a ratio called K value (Valle *et al.* 1996).

Yamanaka and Shimada (1996) identified K value as a useful indicator of freshness in rocklobster flesh.

Although this research will mainly focus on fresh product as appose to stored, it is important to establish the levels of ATP and related adenylate compounds, as these can vary in fresh rocklobster tissue and have been shown to change with prior stress (Speed *et al.* 2001). Specifically, abdominal muscle was sensitive to periods of emersion resulting in increased levels of muscle metabolites (lactate, glucose, ADP and AMP). There was also a difference between captive and wild rocklobster (muscle lactate and Arginine phosphate levels) possibly indicating energy usage related to stress in captive rocklobster.

Importantly, in addition to possible differences between wild and captive rocklobsters the variation within the wild populations remains unquantified. Flesh glycogen, moisture content and percent lipid have been used to characterise the nutritional condition of *J. edwardsii* from known areas of high and low shell growth (Musgrove 2001) and with the affects of starvation (McLeod *et al.* 2004). It is not known at what levels changes in these properties result in significant changes in sensory properties.

Sensory analysis

Recent rocklobster postharvest research has used flesh biochemical properties to investigate improved methods of post-harvest handling (Morris and Oliver 1999; Paterson *et al.* 2001; Paterson *et al.* 1997; Paterson *et al.* 2005). Changes in biochemical flesh properties are likely to be important for sensory characteristics of crustaceans. For example, Glutamic acid shows a synergistic effect with IMP or AMP

(as cited by Yamanaka and Shimada (1996)) to generate "umami" (a taste sensation of high importance to the Japanese consumer). In addition, Bremner (1988a) tested the sensory properties of Scampi, *Metanephrops* spp., with changes in adenylate flesh compounds and found that sensory panel acceptability did not significantly change over 8 days storage at 4°C. Despite significant nucleotide degradation with eight days storage, it was concluded that the strong positive scampi flavour was possibly enhanced by high flesh IMP levels. In addition, the flesh most likely had insufficient hypoxanthine to detract from overall acceptability.

Only 9 studies since 1978 have investigated both biochemical flesh properties and sensory characteristics for crustaceans (Table 1.1). With the exception of Bremner & Vieth (1980) and Bremner (1988b) who found no difference in sensory acceptability with frozen storage of rocklobster and scampi respectively, most of these studies have documented a loss of sensory acceptability with storage. In particular, ice-stored Scampi lost flavour acceptability after 13 days storage, which coincided with an increase in pH above 7.5 (Bremner 1985). Decreased sensory perception of odour and appearance has also been correlated with specific species of bacteria and conditions where they were linked to adverse odour characteristics of the tropical prawn (Chinivasagam *et al.* 1998). Zeng (2005) has since established correlations with decreased sensory perception of odour and appearance with total viable microbial counts, total volatile basic nitrogen, trimethylamine, and electronic nose results.

With the exception of Nelson *et al.* (2005), all previous studies have focussed on storage effects post processing. Nelson found no significant sensory differences between wild and cultured rocklobster, however, pre-processing practices may alter sensory properties. For example, stress prior to processing

alters adenylate level degradation responses with subsequent storage of finfish flesh (Thomas *et al.* 1999). Similarly in rocklobster, ATP,ADP and AEC have been shown to change in rocklobster flesh according to post harvest processes (Tod and Spanoghe 1997). Adenylates are reported to influence sensory properties of crustacea, for example, Scampi frozen stored for 12 months had less flavour, poorer texture and less overall acceptability than those stored 1-6 months. This corresponded with a decrease in total nucleotide pool after 6 months, characterised by IMP decrease and K value increase (Bremner 1988b). A similar increase in K-value and changes in IMP have been recorded for rocklobster flesh, although not in conjunction with sensory analysis (Yamanaka and Shimada 1996). It is therefore important to establish the link between adenylate levels experienced in rocklobster flesh with current commercial post-harvest practices and possible influences on the sensory properties of the flesh.

While the measurement of lobster biochemistry is important for detecting physiological changes in rocklobsters, evaluation of the processed product will ultimately depend on the sensory perception of the consumer market. Sensory perception of a product is based on a combination of flavour, texture, smell and conditioning. These perceptions are highly variable depending on individual taster's sensory sensitivity and personal preferences. So, the sensory properties of any product are dependent on both the product characteristics and the sensitivity and preferences of the taster. For this reason, sensory analysis is divided into two distinct methods. These are; (a) Descriptive Analysis, which focuses on sensory properties of the product in question; and (b) Consumer Analysis, which focuses on evaluating consumer responses to the product in question (Lawless and Heymann 1999). Consumer analysis is useful for locating or targeting a particular market demographic for a product. These analyses usually entail a simple survey, asking for a preference between samples, to identify the sensory properties the

taster liked and disliked. In contrast, a descriptive panel is often used to characterise a product based on sensory descriptive properties (Lawless and Heymann 1999). In essence a consumer panel gathers information mostly about the consumer preference, whereas the descriptive panel is focussed on the sample's properties. The descriptive panels are trained to use specific scales and compare two samples using a pre-determined set of indicators. Results obtained in descriptive panels are repeatable using other sufficiently trained panels and as such form a useful first step in finding differences for subsequent consumer panels to asses particular markets. Consumer tests, in contrast, are only relevant to the groups the panel represent.

Training a descriptive panel involves panellists learning to recognise specific intensities of a known standard for each sensory descriptor (e.g. lobster flavour). However, in the case of rocklobster, there are no samples known to differ in sensory description and therefore no standard product with which to train a panel.

Table 1.1 Summary of published literature on crustaceans combining biochemistry and sensory analysis of the flesh.

Reference	**Species**	**Treatment comparisons**	**Biochemistry**	**Cooked v Raw flesh**	**Panel composition**	**Sensory analysis used**
Bremner & Vieth (1980)	*Jasus edwardsii (previously called J. novaehollandiae)*	Live tailing v tailing after slush ice storage 1-48hrs, up to 40 weeks frozen storage.	Flesh pH, protein, potassium, driploss	cooked	18 Familiarity trials ran.	9 point Hedonic scale, colour, aroma, lobster flavour, off flavour, toughness, moisture, acceptibility
Bremner (1985)	Scampi: *Metanephrops andamanicus*	Storage of 17 days on ice	Flesh pH	cooked	12 untrained	Hedonic scales mandatory and free choice descriptive, Odour & Flavour
Bremner (1988b)	Scampi (Genus *Metanephrops): M. andamanicus, M. boschmai, M.australiensis*	Frozen storage (2, 6, 12mo) Whole scampi & tail section	Protein, wet weight, nucleotides	cooked	9 -16 untrained	Free choice hedonic scale, odour and flavour profiles
Bremner (1988a)	Scampi (Genus *Metanephrops): M. andamanicus,M. boschmai, M.australiensis*	Chilled storage (0, 4 & 8 days 4°C) Tail flesh	Protein, wet weight, nucleotides	cooked	16 untrained	Free choice hedonic scale, odour and flavour profiles

Yamanaka & Shimada (1996)	Japanese Spiny Lobster, *Panilurus japonicus*	Storage (0°C, 5°C, 20°C)	Nucleotides, amino acids	raw	15 panellists	Hedonic odour categories based on acceptable initial decomposition and advanced decomposition
Chini Vasagam et al (1998)	Tropical prawn, (Genus: *Penaeus): P. plebejus, P. erguiensis, P. esculentus/ semisulcatus, Metapenaeus bennettae*	Storage ice or ice slurry. 2 and 8 days.	Headspace volatiles.	raw	1 (experienced)	Hedonic odour categories for intensity for sulphidity and fruity.
Zeng *et al.* (2005)	Shrimp, *Pandalus borealis*	Storage, ice treatments	Proximate analysis, pH, water holding capacity, Total volatile basic nitrogen and trimethylamine	raw	6-9 trained	Appearance and smell combined in acceptability hedonic scale.
Nelson *et al.* (2005)	*Jasus edwardsii*	Tank-held (wet and dry feed) v wild caught	Fatty acids, Lipid	cooked	14 panellists (untrained)	Triangle tests
Roberts (2009), this study.	*Jasus edwardsii*	Tail section, rocklobster size, prior stress, frozen storage (times), tank-held v. wild caught.	Nucleotides, lipid content, moisture content, glycogen, lactate	raw	15-17 panellists trained and 16 consumer	Triangle test, descriptive hybrid test, hedonic preference (choice) test

The sensory attributes of rocklobster have not been defined. This presents some difficulty for the valid use of sensory analysis for this research. It is sometimes possible to train a panel on products other than those being tested, called reference samples (Lawless and Heymann 1999). For example, training a panel on the intensity of "crunch" may utilize a product such as celery as an end-point. However, the limitation of such training is the assumption that the variation in "crunch" within rocklobster flesh would rate on a scale that utilizes celery as an 'end point'. Determining an end point for a descriptive property, without knowing the variation within the product to be tested, may ultimately limit the panel's ability to detect a difference. Despite these recognized limitations, I have adapted sensory analysis methods (detailed in Chapter 2) to meet the need of investigating the variation in flesh characteristics that may be associated with production of a value-added product.

Rocklobster postharvest processing

Prior to the establishment of live trade, the global rocklobster industry was almost exclusively the export of frozen rocklobster tails (Montgomery and Sidhu 1972). The sensory properties of these products were studied and focused on the degradation of a frozen stored product, with limited research in Australia (*J. edwardsii:* formerly *J. novae-hollandiae:* Bremner and Veith 1980; Sidhu et al. 1974) and more extensively in South Africa (South coast Rocklobster *Panulirus gilchristi:* Coetzee and Simmonds 1988; Matta 1992; Nachenius et al. 1978; Wessels and Rudd 1976; Wessels et al. 1979). The latter work was key in establishing a reduction in rocklobster flavour with frozen storage (Matta 1992; Simmonds *et al.* 1992; Wessels *et al.* 1979). However the product

was always cooked. The cooking regime substantially changes flesh characteristics of rocklobster flesh, where over cooking was shown to relate to moisture loss (Coetzee and Simmonds 1988) and affect flesh texture "softness" (Simmonds *et al.* 1992).

Since the transition from tailing to live rocklobster export, very little research on sensory properties has occurred, with the exception of Norwegian trawled lobster species, *Nephrops norvegicus* (Gomez-Guillen *et al.* 2007; Lopez-Caballero *et al.* 2006). These papers assessed the ice-chilled storage life of raw flesh following different treatments, aimed at reducing melanosis. As a result, sensory analysis focused on the visual appearance and odour of flesh samples and did not assess flavour (Gomez-Guillen *et al.* 2007). These properties were rated to a scale based on 5 (very fresh) to 0 (very spoiled) and are not able to provide descriptive properties of raw crustacean flesh or the effects of ice chilled storage on flavour.

Factors affecting rocklobster flesh characteristics

There are a large number of potential sources of variation in flesh characteristics and ultimately sensory properties of fresh flesh. These can be categorised as either (a) biological (e.g. size or moult stage of rocklobster) or (b) post-harvest (e.g. stress, handling, storage and commercial diet). Biological variation is known to influence finfish flesh, where Atlantic salmon fillet fat content increased 12-13% during specific months (Morkore and Rorvik 2001). This may also be the case for rocklobster, as research shows that moult stage, which is seasonal in large rocklobster Ziegler et al. (2004), directly relates to flesh characteristics of Crustacea. Musgrove (2001) showed that the moisture content of rocklobster tail flesh decreases as moult stage progresses.

Further, supporting a possible interaction of moult stage with flesh properties, Wang et al. (2003) noted adenylate energy charge ratios change through moult stages of fresh water prawn *Macrobrachium nipponense*. It was thought that the adenylate ratio AEC may be a direct indicator of energy metabolic activity during the moult cycle (Wang *et al.* 2003). The adenylate energy values of Atlantic Salmon have also been shown to change with post-harvest stress (Thomas *et al.* 1999).

Stress events are measureable for rocklobster (Paterson and Spanoghe 1997). For example, stress is reflected with changes in haemolymph properties (Roberts 2001; Spanoghe 1996). Prior stress of rocklobster was also shown to influence flesh characteristics, where flesh from poor condition rocklobsters deteriorated quicker than from good condition rocklobsters (Boyd and Sumner 1973). This research indicates the likelihood of a causative link between the distinct biochemical changes within flesh associated with stress, and resulting sensory characteristics for rocklobster flesh.

Current industry practice for exporting live rocklobster is to hold them in recirculating tanks without feeding for up to two weeks. It is known that starved rocklobster use energy reserves during storage that can result in a reduction in lipid and glycogen within the flesh (McLeod *et al.* 2004). Diet during tank storage of rocklobsters may also influence flesh. Industry concerns also include the possibility that specific diet during tank-storage may taint the flavour of rocklobster flesh. It is the culmination of such industry concerns and the paucity of quantitative analysis of flesh changes within rocklobster that is the basis for this research.

Most recently, substantial industry effort has focused on the potential aquaculture of rocklobster, and the assessment of flesh characteristics likely to be produced by these methods (Nelson et al, 2005). In this case, a non-trained but experienced industry sensory panel was used to compare wild caught and tank-held (fed) rocklobster. Importantly (and in contrast to previous studies), sensory analysis was based on the properties of fresh product between treatments, rather than product sensory shelf life. The panel consensus resulted in no significant difference between treatments. However, voluntary comments provided a good starting point for establishing the key descriptors of fresh rocklobster flesh.

This study presents the unique approach of comparing biochemical differences in flesh due to biological and post-harvest handling, with the addition of sensory analysis. Characterising the product and comparing different biological and post-harvest treatments is important for addressing relevant industry concerns and identifying the potential product quality of a value added product. In this manner, the use of a descriptive sensory panel is therefore necessary to quantify differences in flesh parameters, as opposed to simply the acceptability of a product (which would be the outcome of using only a consumer panel). In order to analyse the sensory properties of flesh in this study (and in the absence of appropriate standards for descriptive analysis as described above), it was decided to develop a hybrid descriptive method to compliment standard triangle test methods (British Standard BS ISO 4120:2004). This was done in consultation with established food scientists at Regency Institute of TAFE SA (Chapter 2).

Finally, to maintain the relevance of this research to the commercial processing company, and off-set the costs of sourcing rocklobster, it was decided to process samples as they came through a private processing factory. As such, all samples processed were therefore subjected to variability of unknown industry practices pre-harvest and importantly reflect flesh quality expected in a commercial situation.

Research aims

The aim of this thesis was to quantify product flesh characteristics using physical, biochemical and sensory approaches, determining the extent of variation in those characteristics, and finally to investigate the potential sources of that variation. Each chapter follows a progression of ideas to assess possible biochemical and sensory variations in flesh of commercially harvested rocklobsters. Detailed chapter outlines are presented below.

Chapter 2

This chapter presents detailed methods for biochemical and sensory analysis of flesh samples that pertains to each chapter thereafter. Individual chapters contain only those methods specific to each experiment. A substantial amount of this chapter includes reviewing of established techniques for physiological, biochemical and sensory analysis and composition of a refined method. This includes;

- Development of a summarised table of existing definitions of moult staging (Table 1), along with photographic aids.

- Revised methods for glycogen and lactate analysis, driploss, and total lipid content
- The establishment of key sensory descriptors for rocklobster flesh
- Sensory panel selection process
- Justification for choosing appropriate sensory methods
- Summary of threshold tests for sensory panel
- Results from sensory panel training

Chapter 3

Within this chapter, I assess the biochemical variation of commercially harvested rocklobster over a period of two years. It was important to test a combination of processing and biological factors that could potentially influence biochemical properties of flesh. Specifically, this includes time within harvest season, moult stage, shell colour, and batch (individual processing day).

Chapter 4

Here, I present a comprehensive analysis of a number of potential sources of variation of sensory and biochemical properties of commercially processed *J. edwardsii* flesh. In addition, and of particular relevance to the rocklobster industry, was how these may translate to differences in consumer preferences. This chapter specifically addresses four

sources of variation using biochemical and sensory analysis that are of primary concern to rocklobster processors:

1. Variation in flesh characteristics within a rocklobster tail
2. Variation between rocklobster
    a. Rocklobster size
    b. Rocklobster prior stress
3. Stability of rocklobster flesh with frozen storage

The most significant variations detected in rocklobster flesh (frozen storage) were also assessed using a Japanese consumer panel.

Chapter 5

In order to match year-round supply demands of Southern Rocklobster (*J. edwardsii*) with the limitations of a six month fishing season, processors have started to hold rocklobster through the closed period of the commercial fishing season. The affect on both the biochemistry and sensory characteristics of flesh from these tank-held rocklobsters currently remains unknown. This chapter addresses the effects of tank-holding (both feeding and not-feeding) on biochemical properties of flesh and further investigates the resulting sensory properties of rocklobster that had been tank-held for four months (fed) vs. wild caught rocklobsters from the commencement of the following fishing season.

Notes on chapter style

Each research chapter in this thesis (Chapter 3 -5) presents original data and can be read as a separate, discrete study. Each chapter is preceded by a preamble that briefly describes the content of the chapter. Tables and figures are embedded within the text and all references are compiled at the end of the thesis, rather than at the conclusion of each chapter.

CHAPTER TWO

Flesh analysis: methods and justifications.

Physical and Biochemical properties

Table 2.1 lists each of the physical and biochemical parameters measured for rocklobster flesh, and an indication of what each of these show. A visual estimation of the condition of rocklobsters was used to select subjects for experiment 2b in Chapter 4. The condition categories in Table 2.2 have been shown to be good indicators of prior stress in commercially harvested *J. edwardsii* (Roberts 2001).

Flesh sample preparation

Approximately 40 minutes post drowning commencing, the lobster carapace was separated from the tail and 30 grams of flesh immediately removed from muscle group anterior oblique 1 Paterson (1968) of the abdomen. These muscle groups are located under the carapace and, once tail and carapace are separated, form the largest muscle bundles in the flesh protruding from the tail. One gram sub-samples of flesh were placed in plastic bags and wrapped in alfoil. These samples were stored at -70^{o}C in liquid nitrogen for future extraction of adenylates, glycogen and lactate. A second sub-sample (approx. 25g) was placed in a plastic bag and stored on ice for drip loss, moisture and fat analysis. Drip loss and moisture were analysed the day of the processing. The remaining flesh (>5g) was frozen at –20^{o}C for subsequent fat analysis.

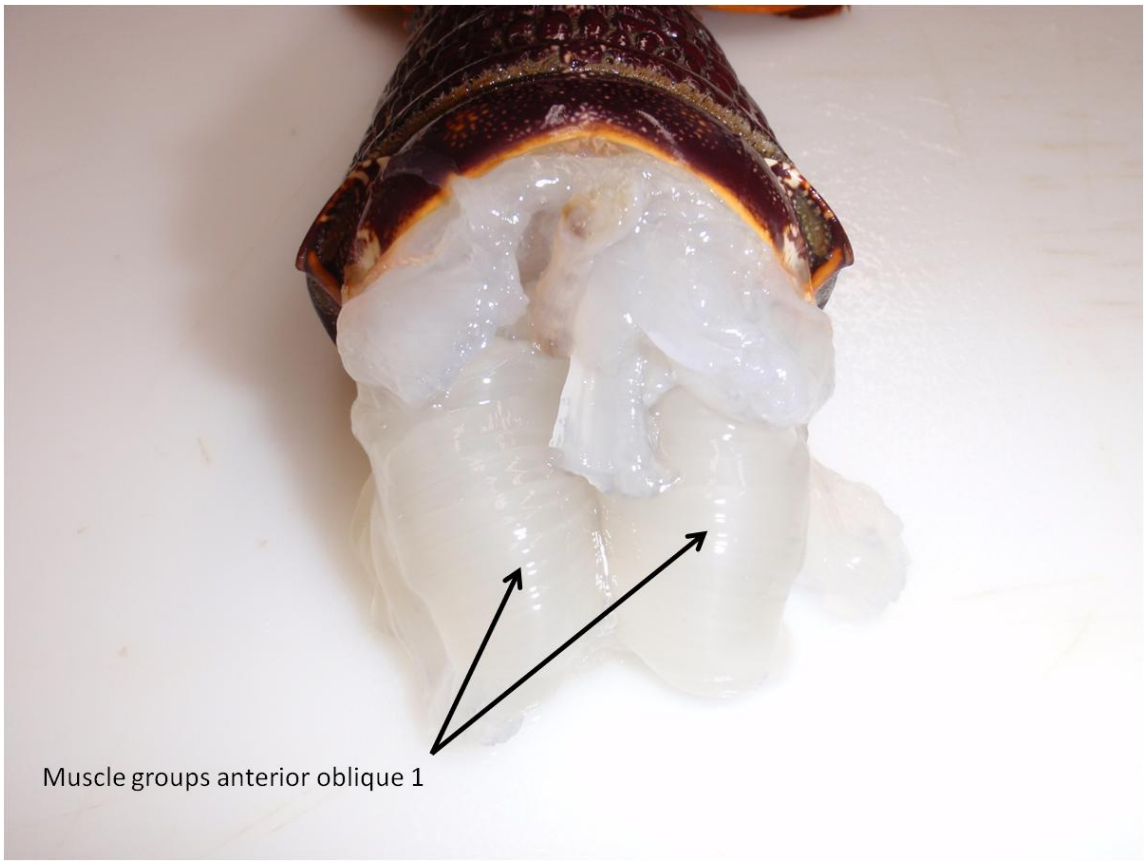


Figure 2.1 Location of muscle groups anterior oblique 1

Table 2.1 Physical and biochemical properties of rocklobster flesh.

This table was adapted from reviews (Bremner 2003; Paterson and Spanoghe 1997; Rodriguez-Jerez *et al.* 2000).

Indicator	**What it shows**
Moisture Content	Percent of flesh that is water
Driploss	Proportion of moisture lost due to time and storage treatment
pH	Acidic < 7 units > Alkaline reduces with decomposition affects taste
Physical condition category	The higher the category numbers the stronger and less stressed the rocklobster.
Lactate	Higher levels = lower pH
Glycogen	Higher levels indicate less decomposition and possibly sweeter taste
ATP, ADP, AMP	Instant energy reserves, freshness and condition of flesh
IMP	Higher levels with decomposition. Affects taste.
K value	A value of decomposition. Higher levels mean more decomposition
Hypoxanthine	Increasing values indicate an advanced state of biochemical breakdown, accompanied by "off" smell, even in the absence of bacterial contamination.
Total POOL	How much energy the flesh has or the condition of flesh
AEC	Energy level of the flesh

Table 2.2 Visual grading system for rocklobsters physical condition

This table is reproduced from the honours thesis Roberts (2001).

Physical condition category	Descriptor	Description of behaviour, posture
5	Aggressive	Tail held out straight and actively rasping hands with horns and flapping tail.
4	Lively	Flapping tail, antennae held up
3	Ok	Antennae help up, tail held out straight. No gap between carapace and tail.
2	Poor	Tail hanging low, small gap between tail and carapace, little movement unless shaken by hand.
1	Moribund	Gap between tail and carapace. Vigorous movement fails to bring response.

Moult staging

Moult stage was assessed by the rigidity of the rocklobsters exoskeleton (cephalothorax integument) combined with light microscopy of pleopods removed from underneath the tail. Table 2.3 presents a summary of moult staging according to Musgrove (2000), adapted from Aiken (1973) for *J. edwardsii*.

Assessing Shell Hardness

Shell hardness states were recorded as either soft over the whole cephalothorax, soft on the lower portion of the cephalothorax or hard exoskeleton all over, according to Musgrove (2000). The shell hardness is useful in determining the difference between early and late intermoult. Rocklobsters in early intermoult (C3) are characterized by soft lower portion of the cephalothorax. Rocklobsters in late intermoult (C4) have hard carapaces all over. Rocklobsters in premoult (stages D0' – D3') also have hard carapaces and can be identified by the formation of the new shell by examining the pleopod under light microscopy (Table 2.3).

Collection and assessment of Pleopod development

The second right pleopod (ventral view) from the tail cephalothorax joint was taken. Each pleopod was placed in a separate labelled plastic bag and frozen at -20^{o}C. Pleopods were thawed at room temperature for 12hrs preceding analysis and viewed under 40x and 100x magnification on a light microscope. Phase contrast was adjusted, where appropriate, for maximum picture clarity and penetration into the pleopod. Viewing

above 100x magnification was not deemed suitable as insufficient light was able to penetrate the pleopod. All pleopods were digitally photographed at 40X and 100X, and moult stage allocated (Fig. 2.2).

Extraction of Haemolymph

Haemolymph was removed from the pericardinal sinus of each individual rocklobster, using a sterile 22 gauge needle, inserted into the space between the top of the tail and the dorsal carapace (at an angle of approximately 15 degrees below horizontal). The needle was inserted 20-30 mm into the rocklobster and 1.5ml of haemolymph carefully extracted.

Haemolymph colour and Refractive index

Each syringe was placed over a light box within 30 seconds of extraction and haemolymph pigment stage recorded. Pigment colours were matched to the 'Southern Rocklobster Blood Colour Reference Card' from Musgrove and Babidge (2003). Haemolymph samples were immediately analysed for refractive index using a refractomometer with Refractive index accuracy of 0.001 (calibrated with distilled water). The period between extraction of haemolymph from the rocklobster to analysis of refractive index remained brief (<1min) to avoid the haemolymph clotting before analysis. Post analysis, the stage was cleaned with distilled water and wiped dry with a fresh tissue. The remaining haemolymph was frozen stored (-20ºC) for subsequent lactate analysis.

Table 2.3 Moult staging characteristics.

This table summarises information from Aiken (1973) and Musgrove (2000) on changes in shell state and setal development of pleopods for *Jasus edwardsii.*

	Stage	Shell State	Setal and cuticle development
Post Moult	A	Extreme flaccidity	Absence of cuticular thickening in the pleopods and setae
	B	Soft over whole carapace, parchment like	Setal bases more defined and walls thicker
Intermoult	C1	Soft over whole carapace parchment like	Increased cuticular thickening
	C2	Rigid around the horns, but soft further back and on the sides	Increased cuticular thickening
	C3	Rigid everywhere but the posterior lateral margins of the carapace	Increased cuticular thickening
	C4	Rigid everywhere	Increased cuticular thickening, can be completely occluded
Premoult	D0’	Rigid everywhere	Epidermal retraction (leaving fluid filled amber zone), no setal development
	D1’	Rigid everywhere	Tips of new setae visible as erect cones between epidermis and cuticle. Invagination papillae visible at base of cones - no setal vagination
	D1’’	Rigid everywhere	Setal invagination begins
	D1’’’	Rigid everywhere	Barbules appear along setal axis
	D2’	Rigid everywhere	Epicuticle deposition and setal bifurcation
	D3’	Rigid everywhere – crunch becomes detectable	Epicuticle folds softening of exoskeleton
Ecdysis	E	Shedding	

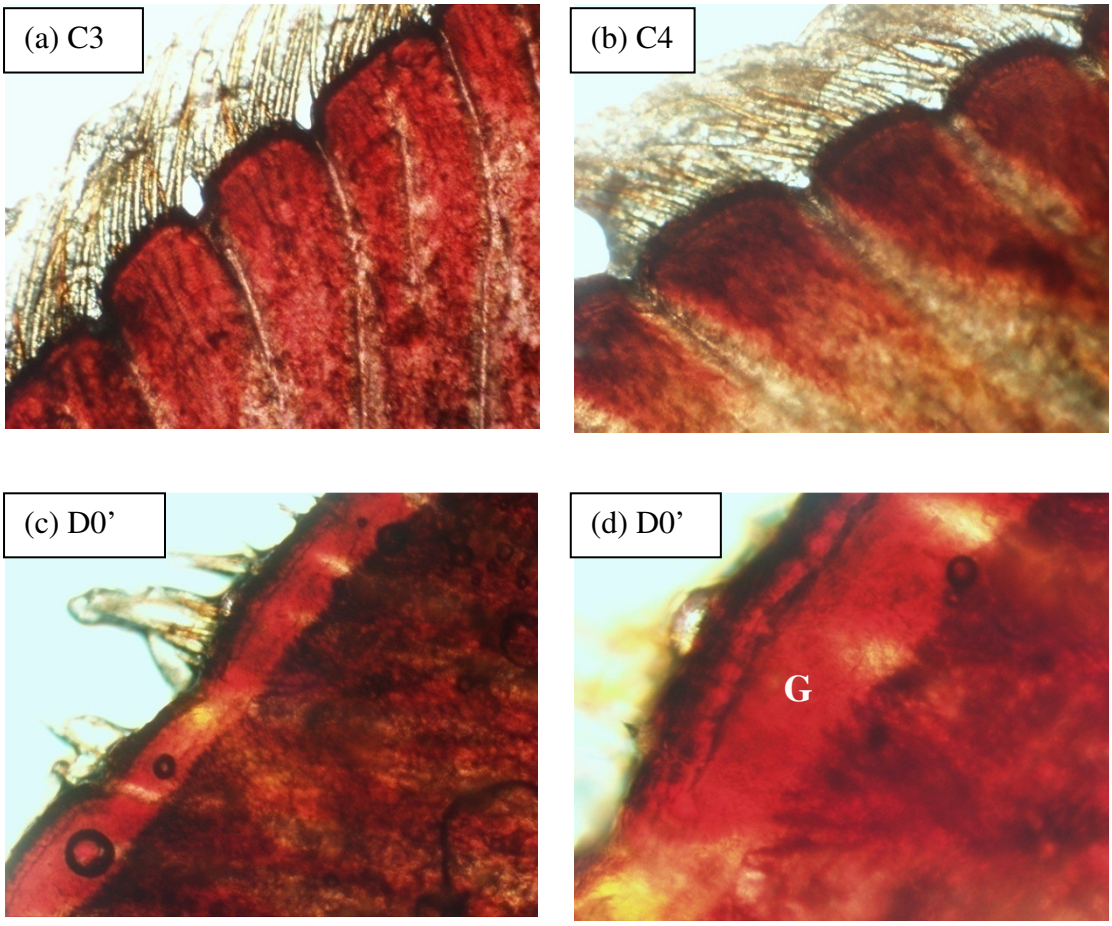


Figure 2.2 Pleopod images for the three moult stages encountered.

(a) C3 (b) C4 and (c) D0' (40x magnification) and (d) (100x magnification of same pleopod as (c)). G = gap left from epidermal retraction. No tips of new setae visible.

Drip Loss Analysis

Driploss analysis commenced the same day that the rocklobster was processed; this was typically after less than 6 hours storage on ice. Driploss recorded the amount of weight lost from the flesh sample stored at $4^{o}C$ over approximately 6 days. To facilitate the removal of water that dripped off the flesh, two layers of mesh were used underneath the flesh in a Petri dish with lid. The first layer was thick (1mm) Nylon fly wire with $1cm^2$ squares. The second layer was fine nylon fly mesh with $1mm^2$ squares. The fine nylon fly wire was weighed (to 0.1mg) then 10g of flesh sample was added and the fly wire reweighed. The fly wire and flesh was then replaced inside the Petri dish, covered and stored in a refrigerator at $4^{o}C$ for 6-8 days. Weight was measured after each 24h period from day 5 until constant. Driploss was calculated from the constant weight reading (between days 6 and 8) as a percent of initial weight of flesh. As some moisture was left on the fine fly wire, a constant reading over the two days ensured that this moisture had sufficiently dripped off.

Moisture

Moisture content analysis commenced the same day that the rocklobster was processed; this was typically less than 6 hours storage on ice. Moisture content recorded the percent of moisture in the flesh sample of total initial weight. This was done by weighing a 5 gram flesh sample (to 0.1mg) and drying in a vented oven at $60^{o}C$ until constant dry flesh weight was reached. This usually occurred between 4-8 days. A labelled $5cm^2$ piece of alfoil was weighed to 0.1mg, the flesh was then added and the total weight

recorded. The flesh and alfoil were weighed every 24h from day 4 until constant weight reached. The weight of water lost was calculated as a percentage of initial flesh weight.

Total Lipid

The total lipid concentration of rocklobster flesh was measured using protocols based on the NSF(1994) methods. For each sample, 12g of wet flesh was homogenized and 5g measured in a tarred 50ml beaker. 20g of anhydrous sodium sulphate was added and the combined contents transferred to a mortar and ground until dry and uniform in appearance. The resulting flesh was added to 40ml ethyl acetate in the original beaker and agitated for 1h at 160 rpm on a flat bed rotational shaker. Post-settlement, the supernatant (lipid solution) was pipetted into a drying beaker of known weight. A second extraction of the sample was conducted, adding 20ml of ethyl acetate to the original tared beaker and further agitated for 1h. The final supernatant was pipetted into the same drying beaker. An evaporation control was prepared with 40ml of ethyl acetate pipetted into a new drying beaker of known weight. Both drying beakers were placed in fume cabinet and left until all liquid solvent had evaporated. Beakers were then placed in an oven (40˚C) for 10 min and weighed (to 0.1 mg). The final weight of extracted lipid was expressed as a percent of the initial flesh weight.

pH

The pH meter was first calibrated with standard buffer solutions pH 4, 7 and 9. The flat ended probe was rinsed with distilled water and gently wiped with a dampened tissue. Three consecutive pH readings were recorded (moving the probe each time) for each

lobster sample (muscle groups anterior oblique 1 and 2 of the abdomen). Calibration was re-checked following measurement of three flesh samples.

Analytical sample preparation

A gram of ground frozen flesh was added to 5ml of 0.6M Perchloric Acid (10.40 ml 70% PCA diluted with 200 ml distilled H_2O). The flesh was ground in a mortar and pestle kept chilled by bedding in dry ice. Samples were stirred, left on ice for 10 minutes and centrifuged at 4500 r.p.m for 10 minutes. One ml of the supernatant was removed for glycogen analysis. The remaining supernatant was buffered with 5M Potassium carbonate buffer (6.91g of KCO_3 in 10ml distilled H_2O) up to a pH of 8, and sub-samples of 1 ml set aside for adenylate analysis and 0.5ml for lactate analysis. All samples were stored at -70^{o}C to prevent degradation. Frozen haemolymph samples were then thawed and any clots broken up with a stainless steel probe. Each sample was centrifuged at 13,000 r.p.m for ten minutes, and the serum removed. For lactate, 100 µl of serum was added to 200 µl 0.6 M Perchloric Acid and 5 µl of 5 M Potassium carbonate buffer.

Lactate (Flesh & Haemolymph)

The protocol for lactate analysis was followed from an L-Lactic Acid enzymatic UV method test kit (Boehringer Mannheim. *L-Lactic acid UV method*). Lactate control solutions (0, 0.05, 0.1, 0.2, 0.4 & 0.6mg/ml) were prepared with distilled H_2O.

Lactate was measured using the following protocol: An aliquot of 40µl of a) buffered flesh solution (flesh lactate) or b) haemolymph serum solution (haemolymph lactate) was added to the following solutions by pipetting into a micro cuvette (total volume 0.896ml):

Solution 1 (Glycylglycine buffer)	0.400ml
Solution 2 (NAD, lyophilizate)	0.080ml
Suspension 3 (Glutamate-pryruvate)	0.008ml
Distilled H_2O	0.360ml
Solution 4 (L-lactate dehydrogenase)	0.008ml*

*Note: Reaction Activated with addition of solution 4.

Each cuvette was capped with parafilm and gently mixed (avoiding bubbles). Colour change was noted, and if sample colour was stronger than controls, a half dilution of the sample with PCA was prepared in a new Microcuvette and reagents added. Samples were left at room temperature (20°C) for 4 minutes and absorbance recorded on a bench-top spectrophotometer (Metertech UV/VIS SP 800) set to 340nm.

Glycogen

Glycogen analysis followed a protocol adapted from Krisman (1962). Iodine reagent (0.26 g I_2 and 2.6 g KI dissolved in 10ml distilled water) and saturated $CaCl_2$ solution (227.5g analytical grade $CaCl_2$ dissolved in 500 ml distilled water) were prepared. 1.92 ml I_2KI and 500 ml saturated $CaCl_2$ solution were mixed to create a final reagent and

stored in dark glass bottle. For flesh samples that had been prior extracted in PCA, 200 µl was added to 1.3 ml reagent in a microcuvette, capped with parafilm and gently mixed. Colour change was noted and if sample colour was stronger than controls, a half dilution of the sample with PCA was prepared in a new microcuvette and reagent added. Samples were incubated at room temperature (20˚C) for 40 minutes and absorbance recorded on a bench-top spectrophotometer (Metertech UV/VIS SP 800) set at 460nm. Control solutions (0, 0.1, 0.4, 0.7, 1.0 & 1.3 mg/ml) were made with oyster glycogen from Sigma Aldrich and dilute PCA. The PCA solution consisted of 10.40 ml 70% PCA added to 200 ml distilled H_2O.

Adenylates

Adenylates were analysed with High Performance Liquid Chromatography (HPLC) by technicians at the Lincoln Marine Science Centre according to the methods outlined in Thomas et al. (1999). Immediately prior to analysis, the samples were slowly defrosted on ice and filtered using a 0.45 micron filter into (HPLC) total recovery glass vials. The samples were then placed into a Waters, Alliance 2695 separations HPLC system for the adenylate runs that took approximately 1 hour per sample. An Alltima C18 5 µm, 250 mm x 4.6 mm column was used with an isocratic mobile phase of 0.02 M KH2PO4 + 0.03 M K2HPO4 at a flow rate of 1.5 ml min-1 with an injection volume of 10 µl. Peak area and retention times were monitored using a Waters 486 UV VIS detector, at 254 nm. Standards were purchased from the Sigma Chemical Company.

Statistical analysis of biochemical changes

Biochemical data is often recorded in mixed measurement scales such as % or mmolL often with zero responses. These data sets often fail to meet the assumptions of normality required for standard univariate analysis, which limits the comparison of treatments. Biochemical analysis of rocklobster flesh within this study was used as a potential proxy for indicating the likelihood of detecting subsequent sensory findings. From this perspective it was important to be able to have some form of ranking or overall comparison between treatments establishing how different they are based on biochemical variation. The statistical package PRIMER was originally designed for dealing with large environmental datasets (Clarke and Warwick 1994). However, by transforming the data to standardize between the different parameters, this technique can be used effectively on biochemical datasets to compare the overall proximate composition between different flesh samples (see also Woodcock and Benkendorff 2008). An advantage of this method is that the analysis of similarity does not require normally distributed data (Clarke and Gorley 2001), and can be used for uneven experimental design. Briefly, non-metric MDS provides a two dimensional representation of multiple data parameters generated from a similarity matrix subjected to 1000 computer permutations. The dissimilarity between two samples is shown on a two-dimensional "map", where the greater the distance between the samples, the greater the dissimilarity. The use of a stress coefficient reflects the degree to which the "map" is a true representation of dissimilarity matrix, and therefore, the ordination can be a simple visual tool to represent the "closeness" of the composition of any two samples (Clarke and Gorley 2001). Importantly each dot on an MDS graph represents the combined biochemistry from an individual rocklobster (not the treatment mean) and

allows the visual assessment of variation within a treatment vs. between treatments. Multivariate analysis of the data is then possible using ANOSIM, which will identify significance between treatment groups. Perhaps the most useful contribution of multivariate analysis is that once a significant difference between treatments has been established, SIMPER analysis can be used to identify which biochemical variable or factor contributes most to the variation detected. This allows the correlation of biochemical responses to sensory results.

Sensory Analysis

This thesis focused on answering the following three questions by sensory analysis

- Is there a sensory difference in rocklobster flesh between treatments (e.g. frozen *vs.* fresh)?

- If there is a difference, which sensory attributes are most pronounced?

- If the panel detected a difference, was there a preference?

Panel set up

Choosing the sensory panel

For logistical reasons it was important to establish a descriptive panel in Adelaide where the lobster processing factory was based. A suitable sensory analysis lab and expertise was available in the Regency Institute of TAFE. The sensory analysis lab consisted of 15 cubicles with controlled lighting and slide panels for providing and removing samples. The Regency Institute of TAFE is a specialised vocational tertiary institute that provides training and qualifications for Chef, Butchery, Bakery and other food professionals. As such, this institute provided a large pool of food professionals available for participation in long term panels. To co-ordinate these panellists and provide sensory analysis guidance, Dr Jim Ralph was contracted to this project.

The recruiting

Contacting the potential panellists was conducted by Jim Ralph. Nineteen panellists were identified for the initial evaluations (7 female, 12 male), ranging in age from 23 – 65 years. Although these panellists had not previously been involved in sensory descriptive panels for seafood, all had experience in previous sensory trials. All panellists liked lobster, with 17 of the 19 panellist's regularly eating seafood; 20% of the panel had previously eaten raw lobster (Table 2.4). Unfortunately not all the panellists were able to attend all panel evaluations resulting in less than 19 panellists for some evaluations.

Table 2.4 Trained sensory panel demographic.

Number of panellists	Demographic
19	Australian by "birth" or long term residents
19	Previous sensory panel experience
19	Like eating seafood
17	Regular consumers of seafood (at least once per month)
16	Eat sashimi once per year
4	Previously eaten raw lobster

Protocol used for determining descriptors

Variability independent of rocklobster cooking

It was decided that sampling raw samples would provide the most standardised method for comparing rocklobster samples for use with a descriptive panel. The use of raw samples avoided the potential variation in sensory properties associated with cooking rocklobster (Coetzee and Simmonds 1988; Dagbjartsson and Solberg 1971). The separate issue of effects of cooking on rocklobster may be addressed in the future once this research has identified significant variations in sensory properties in the raw rocklobster flesh.

Processing of rocklobster flesh for sensory sampling

All rocklobster were killed using standard industry practice of drowning in freshwater (Musgrove. R, *pers. comm.*). Approximately 40 minutes post drowning commencing; the rocklobster carapace was separated from the tail. Flesh from muscle groups anterior oblique 1 and 2 (Paterson 1968) of the abdomen was kept for sensory analysis. These muscle groups are located under the carapace; however, once tail and carapace are separated they form the largest muscle bundles in the flesh protruding from the tail (Fig. 2.1). Each oblique muscle group produced 4 sensory samples (10-15g) (Fig. 2.3) for a total of 8 per rocklobster, so samples within a treatment were pooled and randomly allocated to panellists. At the time of processing these samples were dissected to be of similar size and appearance and then individually vacuum packed in air and blast frozen to -80^{o}C. All sample treatments were labelled with a three digit random number code so panellists were unaware of which treatment they were assessing. Once blast frozen,

samples were stored at -20°C. Twelve hours prior to sensory analysis, samples were thawed at 4°C. All defrosted samples were removed from the refrigerator placed on an ice bath and immediately transferred to individual sensory booths for analysis (or group table for characterization trials). Each sample was presented on a plastic plate. Each panellist had a plastic fork to manipulate the sample with and a pair of scissors to open vacuum packed sample.

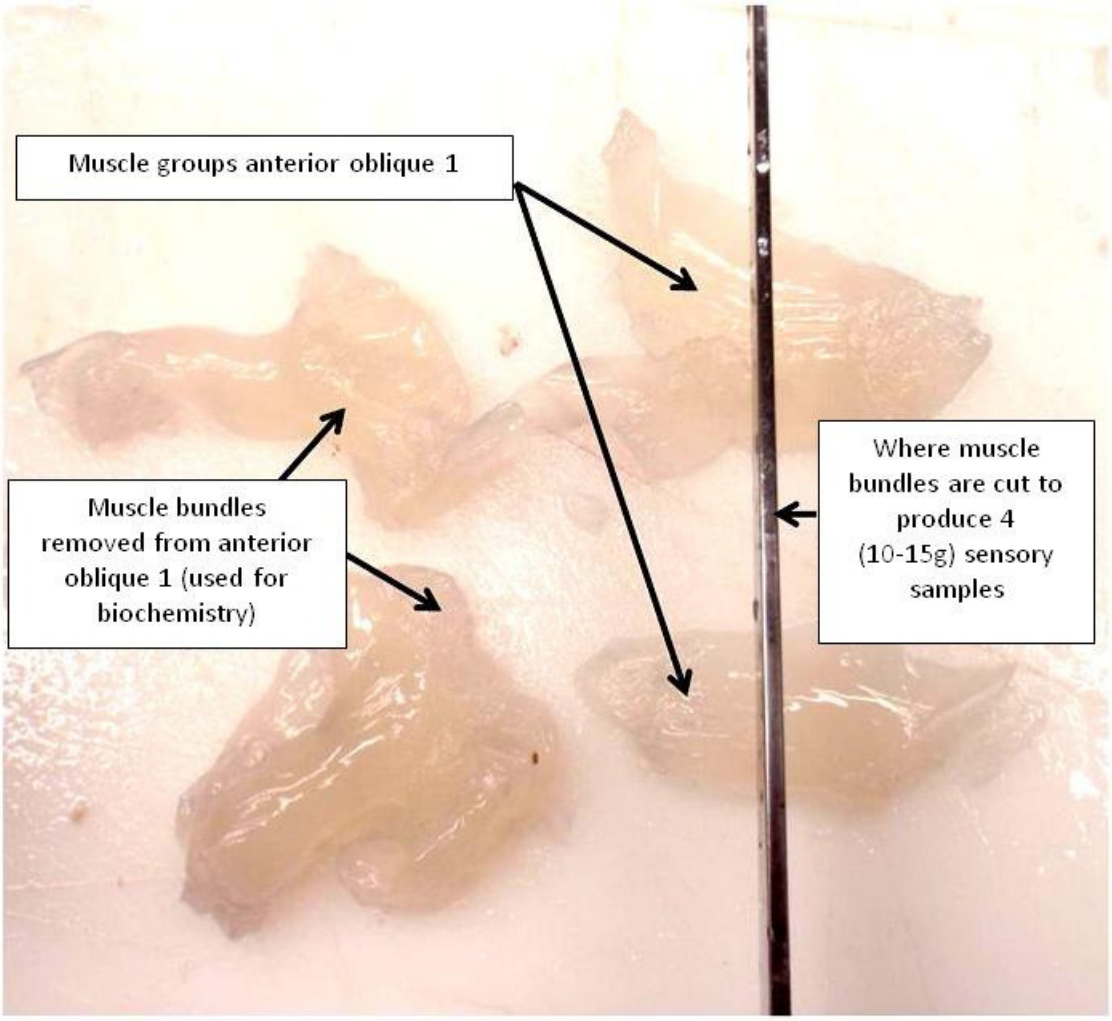


Figure 2.3 Illustration of cutting anterior oblique 1 for sensory analysis samples.

Characterisation of key descriptors for raw rocklobster flesh

The first step towards establishing a descriptive panel was to characterise the product and the language used to describe these characteristics. A literature survey revealed few sensory studies that conducted sensory descriptive analysis on rocklobster and only two that had conducted sensory analysis on the same species being investigated here, *J. edwardsii*. Only three previous sensory studies of lobster used descriptive properties for evaluation (Bremner and Veith 1980; Gomez-Guillen *et al.* 2007; Perez-Won *et al.* 2006). Gomez-Guillen et al. (2007) evaluated the Norway lobster for odour and colour using number scales, based on "very fresh" to "very spoiled". Perez-Won et al. (2006) evaluated the textural properties of Blue squat lobster for the textural characteristics; hardness, cohesiveness, elasticity and chewiness. Bremner & Veith (1980) investigated the acceptability of frozen stored Southern Rocklobster, (*J. edwardsii*), as used in this study. Their panel was trained to assess off-flavour, flesh colour, toughness, and moisture to give an indication of acceptability. These studies were focussed on assessing the degradation of lobster flesh with storage. As the focus of my research was to assess the difference between treatments using fresh samples, including but not limited to storage, a full characterisation of rocklobster flesh was conducted.

Finally, although not a descriptive panel, Nelson et al. (2005) used an experienced consumer panel made up of fishing industry representatives to assess wild and tank held rocklobster using simple triangle tests. The panellists were encouraged to provide additional comments on distinguishing sensory properties of particular samples. These distinguishing sensory attributes are presented in Table 2.5, and form the best basis for

determining suitable descriptors of sensory attributes of Southern Rocklobster despite none of these studies having analysed raw rocklobster flesh.

Table 2.5 Rocklobster sensory characteristics reported in literature

Summary of characteristics reported in Nelson et al. (2005).

Appearance	Flavour	Texture
Off white	Sweet	Firm
White	Metallic	Moist
Pink	Crayfish flavour	Melt in mouth
Red	Sour	Chewy
	Acid	Stringy
		Sticky

Tasting procedure

The characterisation of rocklobster flesh was achieved via a round table discussion (Lawless and Heymann, 1999). 17 panellists were divided into two sittings based on availability. Vacuum packed samples were stored in an ice bucket until needed. The packaging was removed and placed on each panellist's plate as appropriate. Panellists were encouraged to taste portions and note descriptors. Each panellist described the characteristics of flavour and texture. Where difficulty was experienced in adequately describing characteristics, suitable descriptors from Table 2.5 (Nelson *et al.* 2005) were suggested as panellists described similar properties. Following the initial tasting, and using a list of all sensory descriptors detected by the panel, a subsequent tasting was used to order these descriptors in terms of strongest attribute. The variation between samples detected during these tastings was tempered to take into account those characteristics most often seen in flesh samples.

Results

The panel commented on a wide range of descriptive sensory attributes of raw rocklobster flesh. The most consistent and pronounced attributes detected in rocklobster flesh are presented in Table 2.6. For each descriptor a word label has been provided for the upper and lower limits expected in rocklobster flesh.

Table 2.6 Key sensory descriptors of raw rocklobster flesh.

A full definition of each descriptor including word limits for high and low values.

Sensory attribute	Definition	Low	High
Appearance			
• **Translucent**	Ability to see through flesh sample	Not translucent	Extremely translucent
• **Pink Flesh**	The amount of pink colour exhibited	White	Extremely pink
Flavour			
• **Overall lobster flavour**	Flavour that is distinctly associated with lobster as opposed to crustacean or seafood like flavours.	None	Strong lobster flavour
• **Sweetness**	The strength of sweetness exhibited when tasting flesh.	Not sweet	Extremely sweet
• **Metallic**	A bitter metallic flavour as expected if tasting metal.	No metallic flavour	Extreme metallic flavour
Texture			
• **Crunch**	The sudden removal of resistance when first bitten into with front teeth.	No crunch	Extremely crunchy
• **Chewy**	The amount of effort required to break up flesh samples into smaller pieces.	Not chewy	Extremely chewy

Threshold testing

Qualifying instrument – sensitivity panel

The main flesh characteristics of raw *J. edwardsii* have been identified in Table 2.6. Unfortunately suitable reference samples for these characteristics were not available from which to train the descriptive panel. This posed a problem for comparing results from this panel to any future sensory evaluation of rocklobster flesh. It was therefore necessary to establish this specific panel's sensory sensitivity.

Methods

To establish the sensitivity of the descriptive panel used in this research, panellists were asked to taste sugar, salt and citric acid solutions (Fig. 2.4) of increasing (log scale) concentration. This ranged from 8-0.0125 g/100ml sugar, 0.64 – 0.01 g/100ml salt and 0.128 – 0.002 g/100ml citric acid. All solutions were made with bottled water stored at 4^{o}C until served. These solutions were prepared by Jim Ralph, Regency TAFE of South Australia. Panellists were asked to assess three solutions for each concentration. Two of the solutions were pure bottled water, with only one being the solution of known value. The lowest concentration detected for each panellist was taken as the last concentration where the panellist correctly detected the solution from the two bottled water controls. Panellists were told that some comparisons may contain three bottled water samples, and an 'all water' response was available so panellists were not obliged to guess if they could not detect a response (Fig. 2.4).

Results

All panellists detected each of the three constituents of taste to some level. Fifteen panellists successfully detected the full range of salt and acid samples. The panel average for lowest detected concentrations was 0.500g/100ml for sugar, 0.042g/100ml for salt and 0.008 g/100ml for acid.

Threshold Levels of Detection of Taste Compounds

Date:_____

Enter your personal code number here:_____

You are asked to sample dilute solutions of a compound (salt, sugar and acid) and identify the lowest concentration at which you can taste the compound.

In **Part 1** of the evaluation you will sample solutions of **sugar**.
In **Part 2** of the evaluation you will sample solutions of **salt**.
In **Part 3** of the evaluation you will sample solutions of **citric acid**.

Procedure

You will be given 21 containers of solution. These are grouped in 7 levels with 3 samples per level. Each level is labeled with a letter from A to G in Part 1, H to N in Part 2 and O to U in Part 3. Each of the 3 samples within a level is labeled with a number from 1 to 3. Thus, the solutions are arranged for each part of the trial as shown below:

Part 1: Sugar			Part 2: Salt			Part 3: Acid		
A1	A2	A3	H1	H2	H3	O1	O2	O3
B1	B2	B3	I1	I2	I3	P1	P2	P3
C1	C2	C3	J1	J2	J3	Q1	Q2	Q3
D1	D2	D3	K1	K2	K3	R1	R2	R3
E1	E2	E3	L1	L2	L3	S1	S2	S3
F1	F2	F3	M1	M2	M3	T1	T2	T3
G1	G2	G3	N1	N2	N3	U1	U2	U3

Two of the 3 samples in every level contain water. The remaining sample contains either water or the chemical under investigation (sugar, salt or acid).

Transfer a small amount of each of samples A1, A2 and A3 to 3 sample cups and replace the lids on the containers. ***Care: do not confuse the identity of the samples in the 3 cups.*** Taste each of the 3 solutions and record your result using the layout on the reverse side of this sheet. If you believe that one of the solutions contains sugar then circle its code number on the record sheet. If you believe that all of the samples are water then record this result. Discard the used tasting cups in the bin.

Repeat the procedure for all 7 levels of sugar.

Figure 2.4 Threshold questionnaire

RESPONSE SHEET

If you can taste the compound in one of the 3 samples then circle the number of this sample. If you cannot taste the compound then circle "all water".
Remember – all 3 samples may be water. Two of the samples are water in every case.

Part 1: Sugar

A1	**A2**	**A3**	**all water**
B1	**B2**	**B3**	**all water**
C1	**C2**	**C3**	**all water**
D1	**D2**	**D3**	**all water**
E1	**E2**	**E3**	**all water**
F1	**F2**	**F3**	**all water**
G1	**G2**	**G3**	**all water**

Part 2: Salt

H1	**H2**	**H3**	**all water**
I1	**I2**	**I3**	**all water**
J1	**J2**	**J3**	**all water**
K1	**K2**	**K3**	**all water**
L1	**L2**	**L3**	**all water**
M1	**M2**	**M3**	**all water**
N1	**N2**	**N3**	**all water**

Part 3: Acid

O1	**O2**	**O3**	**all water**
P1	**P2**	**P3**	**all water**
Q1	**Q2**	**Q3**	**all water**
R1	**R2**	**R3**	**all water**
S1	**S2**	**S3**	**all water**
T1	**T2**	**T3**	**all water**

Figure 2.4 Threshold questionnaire (continued)

Sensory Methods

Discriminative (Triangle test)

The identification of a significant sensory difference between rocklobster flesh from different treatments was the primary objective of this research. To achieve this, a standard sensory analysis method for triangle tests (British Standard BS ISO 4120:2004) was followed. Briefly, sensory panellists are presented with three samples, two from the same treatment and one from a different treatment. Panellists were asked to identify the 'odd one out'. Two triangle tests were conducted for each sample, (1) based solely on appearance and (2) a combination of texture and flavour. The first test was necessary so the visual appearance of samples could be separated from any texture or flavour differences. A significant finding was found when panellists were able to correctly identify samples with a probability of more than 95% (i.e. a less than 5% chance that the result could have been based on random selection). The Triangle test outcomes (i.e. whether the "odd" sample was correctly or incorrectly identified) were analysed using Binomial Distributions and One-tailed significance tables according to the methods (British Standard BS ISO 4120:2004). Each panellist analysed the control and sample from a treatment only once, thus the number of responses equalled the number of panellists for each comparison ($n \geq 15$). The triangle test method has a low risk type 1 error (i.e. false detection of a difference) so that it clearly established if there was a difference between treatments. The identification of a significant difference assisted in placing value in the actual textural or flavour assessment of treatments identified using the Hybrid descriptive test (described below). Triangle tests were not replicated owing to constraints of availability of volunteer panellists; and limited numbers and expense of

rocklobster samples. Replicating the same test was not viable without reducing the number of comparisons.

Descriptive (Hybrid)

The aim of this method was simply to detect which sensory attribute or attributes were likely to be the most pronounced between different samples, where difference was established by the more robust triangle test. The inclusion of a descriptive test was necessary to provide additional classification of a treatment for possible correlation with the biological or biochemical properties primarily investigated. This additional information was also of use to the rocklobster company, by further characterising their product.

A review of previous literature had failed to identify any Quantitative Descriptive Analysis (QDA) of rocklobster. As specific sensory attributes of rocklobster flesh had not been previously identified, and given the other requirements of this research project, the establishment of a full QDA trained panel was not feasible. For these reasons a hybrid descriptive test was established. The developed hybrid test consisted of a "relative to reference" unstructured line scale with verbal endpoints (Lawless and Heymann 1999). A reference sample (control) was provided for comparison with each sample to be tested. This was marked as the centre of the line for each attribute scale. Each panellist compared a second sample to the control, and recorded a mark on the line scale to correspond with either a higher or lower response compared to the control. No detectable difference could be recorded by marking the same point as allocated to the

control, which is labelled A in Figure 2.5. The attributes chosen and the anchors for the extremes on the scale were as identified in Table 2.6. The distance (mm) from the left of the scale to the control mark was compared to the distance from the left of the scale to the mark corresponding to sample intensity. Significant differences were established using an independent samples T test with the significance level set at $P < 0.05$ (SPSS statistical package, version 12). This method provided the direction of the change, more or less intense than control, combined with numerical statistical analysis.

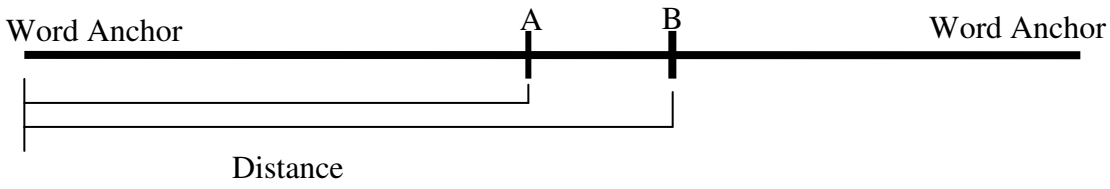


Figure 2.5 Unstructured Hybrid descriptive test line scale.

This line scale was used by sensory panellists to mark the intensity of flesh samples. The distance is measured for the reference sample A and sample B from the left of the unstructured line scale.

Hedonic (preference or choice)

Lawless and Heymann, (1999) have recommended that preference tests should not be tacked onto descriptive tests. This is based on the reasoning that:

- Descriptive panellists do not represent a consumer group.
- Panellists are in an analytical frame of mind and may not view the sample as a whole product as expected with consumer tests.
- Finally, even if data is used only from those panellists that were able to discriminate between samples, some of them are most likely guessing (correct by chance).

If a significant difference was detected using triangle and hybrid descriptive tests there was no way of knowing if either treatment was acceptable, which was of additional interest to the commercial industry. While acknowledging that reference responses from the trained panel are unlikely to represent market consumers; a panel, and or individual, preference between treatments was of interest as it means that treatment was still acceptable. A non-forced paired preference test was conducted at the end of the descriptive hybrid test. It was left until last to limit any potential influence the hedonic preference question may have on the trained panel. A non-forced option was included so panellists that had not found a difference, with triangle or descriptive tests, were not forced to choose a sample. A non preference selection was also of interest as it indicated that both samples were of similar acceptability level. Statistical analysis of paired preference tests does not allow for the inclusion of a no preference option, so all no preference responses were removed from analysis as recommended (Lawless and Heymann 1999). Preferences were analysed using binomial Paired-Preference test probability table (two-tailed $P<0.05$) (Lawless and Heymann 1999). Once no preference

selections were removed, the number of preferences was often low, which reduced the power of the test. As this was a result of a large number of panellists having no preference it was felt that this was appropriate in avoiding type 1 error (Quinn and Keough 2002).

Sensory panel questionnaire and procedures

The sensory questionnaire (Fig. 2.6a) asked panellists to conduct the Descriptive hybrid tests for each descriptor, followed by the preference question and finishing with the two triangle tests.

Descriptive Test (Fig. 2.6 b, c & d)

The panellists were not informed what the treatment was, the reference control was labelled "A" and the sample to be analysed "B". They were instructed that one of the samples is the "A" sample, and that they should assess this one first followed by the "B" sample. Only one sample of A and B was provided for all the descriptive tests and also the subsequent preference test. The descriptors were structured such that they followed in a natural progression of eating, thus allowing one sample for all descriptors. The panellists were asked to quantify both the direction and magnitude of the difference for each attribute between the "A" and "B" samples by marking a position on a horizontal line, where the "A" sample was set as the centre point.

Preference (Fig. 2.6 d)

The panellist's sheet also invites them to express a preference for either the "A" or "B" sample (or neither). The tray with any uneaten flesh samples and the response sheet is then returned to the researchers via a hatch.

Triangle Test (Fig. 2.6e)

Three samples of rocklobster flesh were presented with individual three digit numeric codes. The panellists first had to pick the odd sample out based on appearance, then finally based on flavour and texture combined. The same samples were used for both triangle tests; however the first test was visual and thus did not affect the flesh sample. The panellist's sheet also invited them to make comments; for example; were there any other sensory attributes detected, but which were not assessed?

Sensory Evaluation of Sashimi-Style Lobster

Date:_____

Enter your personal code number here:_____

You are asked to compare and evaluate samples of lobster.

In Part (1) of the evaluation you will:
Allocate scores on a line scale for various visual, flavour and textural attributes for lobster sample B when compared to lobster sample A.

In Part (2) of the evaluation you will:
Allocate scores on a line scale for various visual, flavour and textural attributes for a lobster sample D when compared to lobster sample C.

In Part (3) of the evaluation you will:
Perform a triangle test where you will select the odd sample from a set of three samples of lobster. This test will not start until all of Part (2) has been completed.

If you have any questions at any time, please ask the server

Figure 2.6a Sensory questionnaire (pg. 1)

PART 1 EVALUATION OF PAIRED SAMPLES OF LOBSTER

Scale explanation

You will be asked to compare Sample B to Sample A. Sample A corresponds to the centre point of your scale. After you have analysed both samples for the descriptor you will be asked to place a mark where you determine the intensity of B would be on the scale in relation to Sample A.

Note: Samples A and B may be from the same lobster treatment or from different treatments.

Experimental Procedure

Check that you have been supplied with two samples of lobster flesh. One should be labelled A while the other labelled B.

Please rinse your mouth with water and a cracker biscuit before you begin the evaluation.

Remove the samples from the ice and plastic wrapping.

Assess sample A then B for each of the descriptors as they appear on the data sheet. Make sure you record a value for B sample before assessing the next descriptor.

When you have assessed all the descriptors on the sheet you will be able to record any further differences between flesh samples that may not have been covered by the previous descriptors.

When done pass the tray through the window in cubicle.

Figure 2.6b Sensory questionnaire (pg. 2)

EVALUATION OF THE FIRST PAIR OF SAMPLES OF LOBSTER

For each sample do not taste the lobster until you reach question 3
Place a fine vertical line on the scale to represent sample B.

1. How TRANSLUCENT is the lobster?

not translucent	A	extremely translucent

2. How PINK the lobster?

white	A	extremely pink

3. How much CRUNCH does the lobster have?

no crunch	A	extremely crunchy

4. How SWEET is the lobster?

not sweet	A	extremely sweet

5. How intense is the OVERALL LOBSTER FLAVOUR?

no flavour	A	strong flavour

Figure 2.6c Sensory questionnaire (pg. 3)

6. How much METALLIC flavour does the lobster have?

no flavour	A	strong flavour

7. How CHEWY is the lobster flesh?

not chewy	A	extremely chewy

Additional comments

Sample (A) ALSO differed from sample (B) in that it was:

Did you have a preference for A or B?

Circle the one you preferred

A B No Preference

Please check you have completed all the scales.

Alert the server that you have completed the evaluation

REPEATED FOR SAMPLES "C" and "D"

Figure 2.6d Sensory questionnaire (pg. 4)

PART (3) LOBSTER TRIANGLE TEST

Please rinse your mouth with water and a cracker biscuit before you begin the evaluation.

Do not taste the product until you have read the instructions and evaluated the samples for visual differences

You will be given three coded samples. Two of these samples are the same and one is different. Do not change the order in which they are presented.

Appearance

Please look at the samples and circle the number below that corresponds to the sample that looks different from the other two. You must make a choice.

673 059 348

Disregard the appearance of samples and continue.

Texture & Flavour

Asses each of the samples in the order presented from left to right. Circle the number below that corresponds to the sample that is different from the other two.

You must make a choice.

673 059 348

Additional comments

_____

_____

_____

_____

_____

_____

_____

_____

_____

Please alert the server that you have completed the evaluation

Thank you for participating in this sensory evaluation

Figure 2.6e Sensory questionnaire (pg. 5)

Training the panel

The primary objective of training the panel was to reach a point where all panellists were sufficiently trained to use the sensory questionnaire, and importantly, the descriptive scale described above. In the case of the descriptive scale this required that a difference between control and sample was represented similarly for all panellists on the scale.

Familiarisation

This research was started with no previous complete classification of rocklobster sensory properties and with no known treatments that would alter these to provide adequate scale points. This left little option but for panellists to taste a substantial amount of raw rocklobster so as to appreciate and establish the range of sensory variation in raw rocklobster flesh. This was done through a discussion panel described above, to establish key attributes, and initial practice runs of the sensory methods using control samples "reference tests" (described below). The "reference tests" involved comparing two samples of rocklobster flesh which were either the same treatment or control. This method was useful in familiarising the panellists with the sensory methods and calibrating them to the sensory scales.

Error for descriptive test

These training methods reduced the chance of Type 1 error; namely finding a significant difference between treatment and control that does not exist. As the panel is used as an instrument for descriptive analysis, it was important to have minimal variation in

responses between panellists for each attribute. Each panellist (i.e. the instrument) was calibrated through training to give similar values on the intensity scale to the panel consensus.

Firstly, a series of comparisons using samples homogenously mixed from rocklobster 'controls' were compared without the panellists' knowledge. These reference trials were periodically conducted throughout subsequent analysis to check the consistency of the panel (described in detail below). Where panellists in some instances detected a difference between control samples, they were instructed that these differences were due to variations between samples and the descriptive scale was designed to detect significant differences between treatments. It was explained that although they were able to detect a difference between both samples, the magnitude of this was actually much smaller than they had recorded on the intensity scale. As such, any differences detected between treatments are likely to be more definite. It is possible that this method of training may result in an increased chance of a Type 2 error (Quinn and Keough 2002); where real differences in the key descriptors between control and treatment samples are not detected. This may occur if the group consensus is not sensitive enough and truly sensitive panellists have been trained not to report a difference. However, determining which of the key sensory descriptors were associated with significant differences between control and treatment (as detected by the triangle tests) was of greater importance than detecting all possible differences. The point at which the panel was fully trained was based on objective determination of when panellists did not detect a

significant difference between control samples. This approach ensures a greater chance that any differences detected are significant.

Reference trials

The most common rocklobster specimen encountered at the processing facility was a large red male rocklobster. Specimens meeting the criteria of male, red hard carapace and weight 2-2.5kg were selected as control samples. All the rocklobster selected as controls were in either Intermoult (C4) or early Premoult (DO). Control samples from a single days processing were then randomly allocated as "A" or "B" samples and the panel asked to evaluate using the sensory questionnaire (Fig. 2.5). These control *vs*. control trials were conducted until there was no significant difference for any test.

Results

Three reference trials were completed. The triangle tests produced no difference in any of the three trials. However the hybrid descriptive test detected a difference in the first comparison with colour (t = -2.296, p = 0.036) and crunch (t=3.256, p = 0.005). For the second reference trial only one comparison in the hybrid descriptor test, crunch (t = 2.263, p = 0.038) was significantly different, all other descriptors and triangle tests were not significantly different. The third trial had no significant differences. Thus, following three reference trials, panellists would not find a difference unless significant, thereby being sufficiently trained for this analysis.

Removal of panellists

Sensory protocol suggests that panellists that produce extreme responses with descriptive analysis should be removed from the panel following training (Lawless and Heymann 1999). However, in this instance, no panellist was consistently extreme in their responses, therefore it was decided that no exclusions from further testing or statistical analysis took place.

Sensory session program

A total of 6 sessions were run over three months with the trained volunteer panel (Table 2.7). A final session used a Japanese consumer panel just prior to the conclusion of all research for this project. Control rocklobsters were selected as per the criteria specified for the reference trials. A large pool of anterior flesh samples was used for session 1 and 2. Following this, 6 suitable rocklobster were used for each treatment. Session 4 established that there was no sensory difference between the middle tail sections and anterior flesh. This enabled session 5 to use the same 6 rocklobster for all fresh samples and session 6 to only use 6 small rocklobster to supply the required number of samples. In both of these instances the first tail section (closest to anterior flesh) was used to ensure that samples were as close as possible to anterior flesh. Session 7 did not require samples for preference tests and only 5 rocklobster were used to establish to pool for the fresh samples.

Table 2.7 Summary of sensory panel sessions

Session number	Date	Description of tests
Session 1	June 2005	Panel meeting, discussion of what is required, taste raw rocklobster. The participating panellists tasted raw rocklobster again and establish key descriptors. Anterior samples used.
Session 2	7/7/05	Control (anterior) vs. control (anterior) Control (anterior) vs. control (anterior)
Session 3	28/7/05	Threshold testing of panel
Session 4	11/8/05	Control (anterior) vs. control (anterior) Anterior flesh vs. Posterior tail sections Anterior flesh vs. Middle tail sections (data not presented , no significant difference detected)
Session 5	23/8/05	10 month frozen (anterior) vs. fresh (anterior) Fed (anterior) vs. wild (anterior) Short term (anterior) vs. fresh (1st tail section)* *Note all fresh samples came from same six rocklobster to avoid batch effects necessitating the use of the 1st tail section.
Session 6	6/9/05	Small (anterior & first medallion)* vs. Large (anterior) Weak tail strength (anterior) vs. Lively (anterior) *Too supply sufficient sensory samples flesh from the 1st medallion had to be used on the small rocklobster.
Session 7	24/11/05	Fresh (anterior) vs. 1 month frozen (anterior) 2 week frozen (anterior) vs. 18 month frozen (anterior)

CHAPTER THREE

Variation in the flesh of the commercially harvested Southern Rocklobster, *Jasus edwardsii*.

Abstract

Since the introduction of catch quotas the value of the Southern Rocklobster fishery has been largely influenced by rocklobster price. Current consumer demand for red, plate-sized individuals has resulted in less value per kilogram for landed rocklobsters that are large and white. Adding value by creating new products would allow processing companies to obtain better returns from that proportion of the catch not desired for live Asian export markets. As yet the variability in flesh characteristics of commercially caught rocklobster is unknown and may have implications for ensuring a consistent high quality value added product. To address this, twenty biochemical and proximate parameters of flesh were tested in rocklobsters caught from different batches (i.e. day of processing), years, moult stage and shell colours. Importantly, no significant variation was detected between lobsters of differing shell colours, which supports the use of these individuals as a value added product. The majority of variation in flesh biochemistry was primarily attributed to the factor of batch. This has implications for post-harvest practices that may be responsible for this variation. In order to gauge if these biochemical variations are sufficient to adversely affect flesh quality for the consumer, future research should include sensory analysis.

Introduction

The commercial fishery for the Southern Rocklobster, *J. edwardsii,* in South Australia contributes approximately $80 million annually to the local economy (EconSearch 2005). The highest proportion of this catch is from the Southern Zone Fishery, which has recorded stable catch levels since the 1993/1994 season (EconSearch 2005). Despite recent small increases and decreases in catch quota, the value of the fishery is largely influenced by rocklobster price (EconSearch 2005). Future increases in the value of the rocklobster industry are likely to be dependent on successfully adding value to existing catch quota. In the 2003/2004 season approximately 78.24% of the total South Australian catch was exported live (calculated from EconSearch (2005)). Although paying premium prices, the live markets can discount when rocklobster are larger than the preferred plate size which results in less value per kg for larger rocklobster (Ferguson Australia, *pers. comm.*). To capitalize on a current gap in the market, a private company Ferguson Australia Pty Ltd has started processing these large rocklobsters into value added products (e.g. medallions of tail flesh). As value added product sells for a higher price per kilogram, there is a need to quantify the variability in flesh characteristics of wild caught rocklobster; and therefore its suitability for premium product lines.

Variation in flesh characteristics has been reported for several other fisheries. For examples, variation in the Pacific Oyster (*Crassostrea gigas*) has been linked to time of season (Linehan *et al.* 1999) and as seen with finfish, is most likely related to changes associated with spawning (Li *et al.* 2009; Nielsen *et al.* 2005) and periods of high

growth (Morkore and Rorvik 2001). Specific to Crustacea, seasonal variation may also occur due to moult stage, which varies across season in large rocklobster (Ziegler *et al.* 2004). Changes in flesh characteristics with moult stage include decreased moisture content across later moult stages (Musgrove 2001), and increased adenylate energy charge ratios (thought to be a direct indicator of energy metabolic activity) leading up to and post moulting (Wang *et al.* 2003).

Other sources of variation in flesh characteristics are likely to be linked to prior stress events. Significant stress responses associated with biochemical changes from anaerobic metabolism and energy usage have been recorded in haemolymph of *J. edwardsii* throughout the commercial post harvest chain (Roberts 2001), and with simulated stress events that altered muscle nucleotides (Morris and Oliver 1999). Biochemical analysis of muscle nucleotides has been used for the determination of flesh freshness in the Japanese Spiny Rocklobster (Yamanaka and Shimada 1996) and physical condition or stress in *J. edwardsii* (Morris and Oliver 1999; Speed *et al.* 2001). These same indicators of stress have been linked with changes in flesh characteristics with finfish (Thomas *et al.* 1999). Despite this, changes in rocklobster nucleotides due to prior stress are yet to be linked directly to changes in flesh sensory properties, but are expected.

The sensory perceptions of rocklobster flesh are related to its compositional, physiological and biochemical properties (Bremner 2003). The proximate composition (~ 73% water, ~ 23% protein, ~ 2.3 % lipid, ~ 1.7 % ash; McLeod (2004)) and fatty acid profiles (Nelson *et al.* 2005; Nichols *et al.* 1998) for *J. edwardsii* have been established.

The associated sensory characteristics, along with lipid and fatty acid profiles of *J. edwardsii*, were recently reported with lobsters caught from the wild compared to tank held and fed lobsters (Nelson *et al.* 2005). These studies have provided fundamental knowledge towards understanding the important properties of rocklobster flesh that can be used to improve product marketing. However, conclusions have been based on lobsters sampled from one point in time (Nelson *et al.* 2005) or from lobsters that had spent time in captivity (McLeod *et al.* 2004), which does not address the possible variation in the industry with commercially caught rocklobster. As such, the consistency of flesh characteristics remains largely unknown; 1) as they vary across seasons; and 2) as experienced by commercial processors. Given this, the aims of this study were two-fold, 1) to investigate possible sources of variation in flesh quality of commercially harvested lobsters and 2) to establish the extent of this variation.

Methods

Lobster sampling

To address the variability of commercial catch, lobsters were required to be compared across different days, seasons and years. While limited in the numbers of rocklobster able to be sampled it was felt that the 58 rocklobster sampled should allow sufficient scope for the detection of variation. Sampling rocklobster on different days limited the scope of this study to biochemical and proximate analysis, as we were not able to control for storage variation to allow sensory analysis.

Lobsters were sampled as they were being processed by a commercial lobster processing company based in Adelaide, South Australia, between July 2003 and June 2005 (n = 58, Table 3.1). The months and days chosen for sampling lobsters were on an opportunistic basis coinciding with when the factory was processing and the availability of time to process samples. An effort was made to ensure that rocklobster were sampled on months that covered a full fishing season over several years. Lobsters were chosen haphazardly from those being processed on the day, selecting a rocklobster from different areas of the bin or bins prior to drowning, until the required number had been reached. Rocklobster were tagged prior to processing to enable identification throughout biochemical data analysis. All rocklobster selected were classed as good condition (at least category 3, refer to Chapter 2) and were deemed suitable for processing by the factory. Lobsters had a mean weight of 2.5 kg and carapace lengths between 164 mm and 217 mm. All rocklobster were killed as per standard industry practices of drowning in fresh water (Musgrove. R, *pers. comm.*).

Haemolymph analysis

Haemolymph colour was measured according to the pigment stage method of Musgrove and Babidge (2003). Moult staging was conducted using shell rigidity and light microscope analysis of the second right (dorsal view) pleopod for developing cuticle and setae (Musgrove 2000) (refer to Chapter 2 for details).

Flesh properties and biochemistry

Lactate and glycogen analysis was completed with frozen stored flesh (-70^{o}C) as per the protocols in Chapter 2. Adenylate samples were analysed using HPLC methods described in Thomas et al. (1999) at Lincoln Marine Science Centre, Pt Lincoln, South Australia. Flesh samples were dissected and prepared for driploss, moisture and lipid content analysis as per Chapter 2.

Table 3.1 Batch matrix for lobsters used for biochemical analysis for chapter 3.

Batch	**Year**	**2003**	**2003**	**2004**	**2004**	**2004**	**2005**	**2005**	**2005**
	Month	**Jul**	**Oct**	**Mar**	**May**	**Oct**	**Jan**	**Jun**	**Oct**
Sex	Male	6	6	6	14	7	7	6	6
	Female	-	-	-	-	-	-	-	-
Moult stage	C3	-	-	-	1	-	-	-	1
	C4	6	5	6	13	1	7	5	5
	D0’	-	1	-	-	6	-	1	-
Haemolymph	1	-	-	4	5	2	2	3	3
Colour index	1.5	6	3	2	6	3	5	3	2
	2	-	3	-	2	2	-	-	-
	2.5	-	-	-	1	-	-	-	-
	3	-	-	-	-	-	-	-	-
	3.5	-	-	-	-	-	-	-	1
	4.0	-	-	-	-	-	-	-	-
	4.5	-	-	-	-	-	-	-	-
Shell Colour	Red	1	6	6	7	2	4	6	6
	Red Speckly	-	-	-	-	3	-	-	-
	Speckly	5	-	-	-	2	3	-	-
	White	-	-	-	7	-	-	-	-
Shell	Hard	6	6	6	13	6	4	6	5
Hardness	Soft bottom	-	-	-	1	1	3	-	1

Statistical analysis

Non parametric multi-dimensional scaling (MDS) and analysis of similarities (ANOSIM) on PRIMER v 5 tested for differences in flesh biochemistry between possible factors influencing variation (batch, year, moult stage, shell colour, haemolymph colour index, and shell hardness). ANOSIM data was generated with normalised euclidean distance transformation to give each indicator an equal weighting for comparison between groups. Only the following indicators were used for the MDS and ANOSIM analysis; pH, moisture, driploss, total lipid, lactate, glycogen, total adenylate pool, K value, IMP load and AEC. This was necessary to avoid individual adenylates being represented by themselves and again in Adenylate ratios. Global R values were used to determine the contribution of each factor to the total flesh variation.

One-way ANOVA using SPSS v 12 tested the difference in biochemical parameters of flesh between factors, where untransformed data conformed to the assumption of normality (Kolmogorov - Smirnov). Non parametric Kruskal Wallis tests were used where data did not meet this assumption. A comparison of C4 and D0' moult stage was conducted using Independent T-test where the assumptions of normality were met and Mann-Whitney U Test for violations of this assumption. All adenylates were included for comparison between factors.

Results

This research was conducted as an experimental survey of variability in commercially processed lobsters. As a consequence of this many of the factors tested were confounded. Thus for example, it was not possible to isolate the effects of moult stage or shell colour from possible batch effects (Table 3.1).

Factors affecting flesh characteristics - Batch

Batch was the main factor associated with different metabolic flesh characteristics (ANOSIM, Global R = 0.634, Table 3.2). Year, moult stage and shell colour were also associated with changes in flesh characteristics, however the Global R values for these were much less than recorded for Batch (Table 3.2). The differences in biochemistry between batches are greater than the variation within an individual batch, as evidenced by lobsters from the same batch being close together in the MDS plot, which shows clusters with clearly defined batches (Fig. 3.1). All the biochemical variables measured in lobster flesh significantly varied between batches (Table 3.3). Maximum ATP value (~30µmol/g) was higher than previously reported and will be addressed in the discussion. Substantial changes between batches occurred with driploss, lactate, total adenylate pool and K value. Mean drip loss between batches varied from 4 to 12% (Fig. 3.2). The lowest mean values were recorded in March, May and October, spanning a full season. Muscle lactate also varied between batches (Fig. 3.3), ranging between 0.7 µmol/g and 2 µmol/g.

Table 3.2 Multivariate analysis of rocklobster flesh biochemistry:

This table presents (a) ANOSIM results for the difference between comparison groups and (b) pairwise comparisons for significant differences within the groups year, moult stage and shell colour.

(a) Comparison Groups	**Global R**	**Sample statistic**
Batch	0.634	0.1%
Year	0.308	0.1%
Moult stage	0.284	0.1%
Shell colour	0.200	0.3%
Haemolymph Colour Index	0.020	33%
Shell Hardness	-0.085	78.6%
(b) Year	**Pairwise R value**	**Significance level**
2003 *vs*. 2005	0.724	0.1%
2004 *vs.* 2005	0.316	0.1%
2003 *vs.* 2004	0.066	19.7%
Moult Stage		
C4 *vs.* D0’	0.346	0.1%
C3 *vs.* D0’	-0.036	44.8%
C4 *vs.* C3	-0.001	45.1%
Shell Colour		
Red *vs.* Red Speckly	0.558	0.1%
White *vs.* Red Speckly	0.825	0.8%
Red *vs.* Speckly	0.144	3%
Red Speckly *vs.* Speckly	0.392	3.1%
White *vs.* Speckly	0.172	4.2%
White *vs.* Red	0.054	28.3%

Sample statistic 5% = $P \leq 0.05$. Global R gives indication of the strength of difference, where 1= completely different, 0 = completely the same. Pairwise R values > 0.75 = well separated, R > 0.5 = clearly different, R < 0.5 = barely separate.

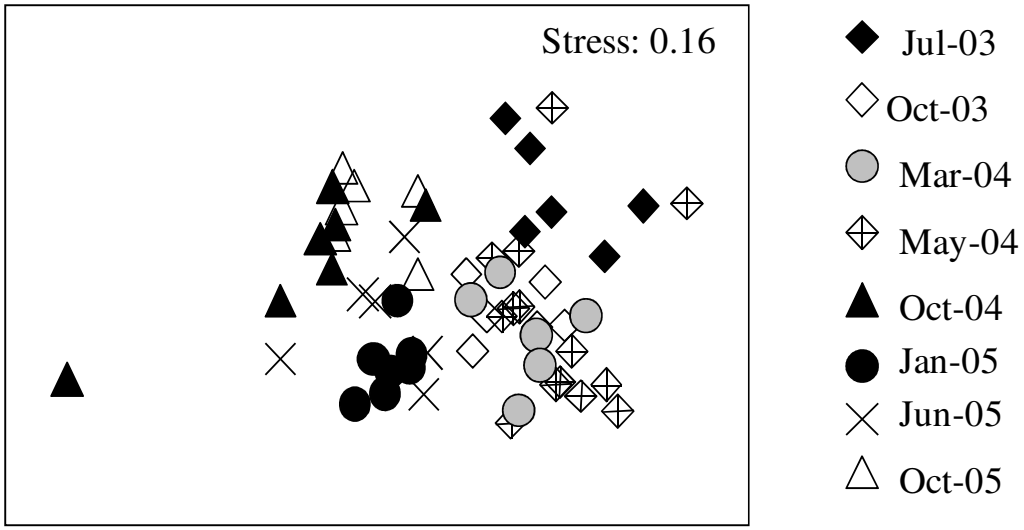


Figure 3.1 MDS of rocklobster variation with batch.

Non-parametric multi-dimensional scaling analyses shows associations of biochemical flesh characteristics for rocklobsters between batches. Resemblance used for permutations was normalised Euclidean distance.

Table 3.3 Variation of rocklobster flesh biochemistry with batch.

One way ANOVA's testing for differences in flesh biochemistry of rocklobsters between batches. Minimum and maximum values for all rocklobster are noted for comparison. Test statistic: F for parametric ANOVA; H for non-parametric Kruskal – Wallis.

Biochemical indicator	**df**	**Test Statistic**	**P**	**Minimum**	**Maximum**
Total Lipid (%)	7	F = 48	**	0.14	0.55
Flesh pH	7	F = 7	**	6.38	7.33
Moisture (%)	7	H = 36	**	70.05	76.67
Driploss (%)	7	H = 38	**	1.59	19.30
Glycogen (µmol/g)	7	F = 4	**	0.11	2.55
Lactate (µmol/g)	7	H = 15	*	0.09	4.54
ATP (µmol/g)	7	H = 45	**	6.18	31.28
ADP (µmol/g)	7	H = 38	**	0.05	5.24
AMP (µmol/g)	7	H = 32	**	0.00	1.99
Hypoxanthine (µmol/g)	7	F = 5	**	0.00	1.43
K value	7	F = 9	**	0.00	10.23
Inosine (µmol/g)	7	F = 36	**	0.00	2.29
IMP Load (µmol/g)	7	F = 38	**	0.00	1.07
Total Adenylate Pool (µmol/g)	7	F = 7	**	10.61	35.19
AEC	7	F = 26	**	0.80	1.00

* $P < 0.05$, ** $P < 0.01$

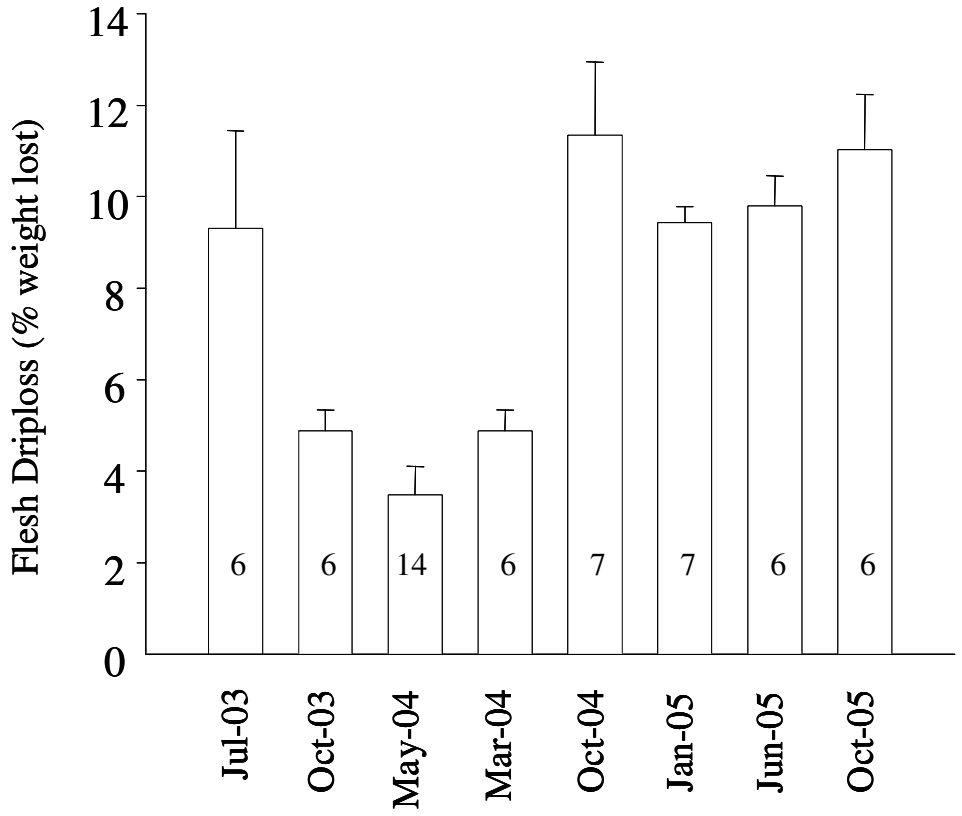


Figure 3.2 Flesh driploss between batches.

Mean (± SE) muscle drip loss (% wet weight lost) of flesh from rocklobsters of different batches.(n = numbers in columns).

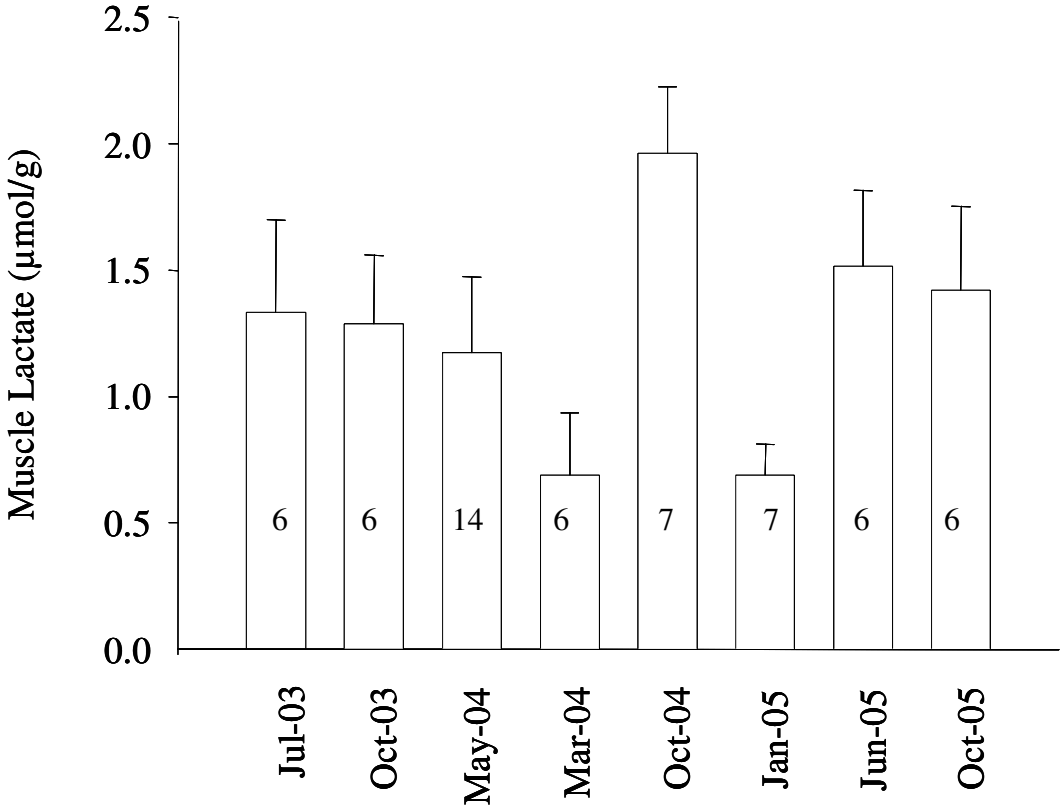


Figure 3.3 Flesh muscle lactate between batches.

Mean (± SE) muscle lactate of flesh from rocklobsters of different batches (n = numbers in columns).

Factors affecting flesh characteristics - Year

Year of sampling was the second most significant factor attributable to change in biochemical properties of processed lobster flesh (ANOSIM: Global R = 0.308, Table 3.2). Pairwise comparisons indicate that 2005 samples were significantly different to all other years sampled (Table 3.2). There was no difference between samples from 2003 and 2004 (Table 3.2, Fig. 3.4).

Factors affecting flesh characteristics - Moult Stage

Biochemistry of C4 and D0' stage rocklobsters differed significantly (Table 3.2). The inability to detect a significant difference in early intermoult (C3) categorized rocklobsters would be limited by the low incidence of this moult stage (n=2). In the MDS plot, these C3 lobsters also appeared within the same region as the D0' and C4 lobsters (Fig. 3.5). Statistical analysis revealed that most of the flesh indicators (with the exception of Moisture content, AMP, K value and AEC) were significantly different between D0' and C4 lobsters, pooled across years (Table 3.4). In particular, driploss increased two-fold from C4 to D0' moult stage (Table 3.4).

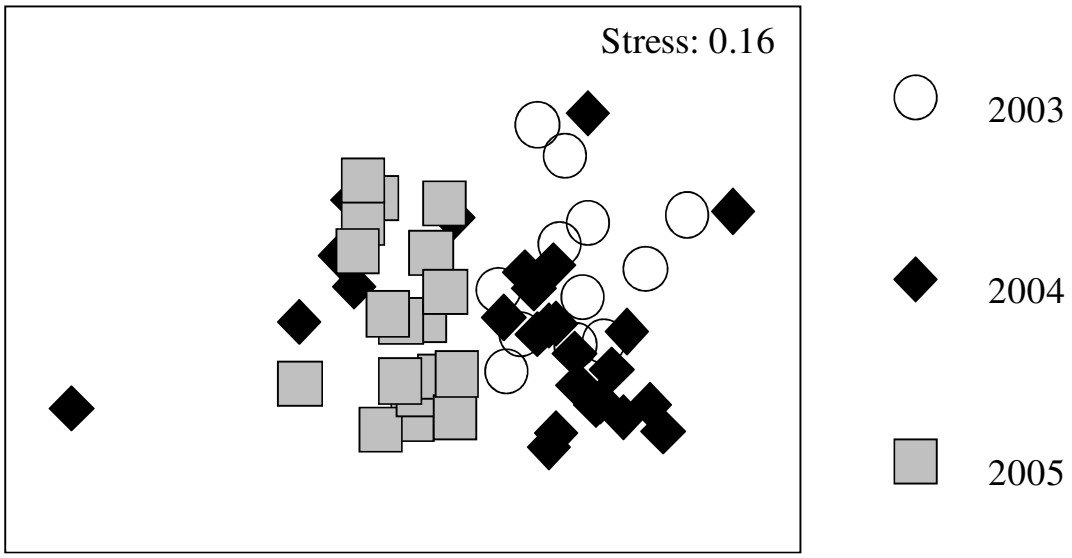


Figure 3.4 MDS of rocklobster variation with year.

Non-parametric multi-dimensional scaling analyses shows associations of biochemical flesh characteristics for lobsters between years (batches pooled for each year). Resemblance used for permutations was normalised Euclidean distance.

Factors affecting flesh characteristics - Shell colour

Multivariate analysis revealed that shell colour was also associated with changes in biochemistry (Table 3.2, Global R = 0.2). However, pairwise tests indicated that the flesh biochemistry of the two extremes of shell colour (Red vs. White) were not significantly different (Table 3.2). Therefore, shell colour is most likely confounded by batch (see Table 3.1). To further investigate this, red and white rocklobster processed on the same day (May 2004, Figure 3.6, hollow diamonds & hashed squares) were analysed separately using univariate analyses. However, there was no significant difference for any biochemical parameters measured and is therefore not presented. Not finding a significant difference between the two extremes of shell colour, despite shell colour being identified as a significant factor by multivariate analysis, would suggest that the confounding factor of batch has a greater influence on flesh biochemistry than shell colour . This supports the results presented in Table 3.2 which identified batch having a higher global R than shell colour (R = 0.63 vs. 0.2).

It is interesting to note that despite no difference in the two extremes of shell colours, the red speckly rocklobsters (speckly but predominately red) differed to all other shell colours (Table 3.2) and appeared to separate towards the left side of the MDS plot (Fig. 3.6). Biochemical differences between speckly rocklobsters and rocklobsters with all white carapaces were also detected in the ANOSIM (Table 3.2), despite some apparent overlap between these groups in the two dimensional representation of this data (Fig. 3.6).

Factors affecting flesh characteristics - Haemolymph pigment category and Shell Hardness

No difference was found between categories of haemolymph pigment (Table 3.2), where the majority of lobsters sampled were classed as either 1 or 1.5 pigment category (Table 3.1). Despite the difference in biochemistry associated with C4 and D0' moult stage, the low Global R value (-0.085) suggests there is no evidence to support a difference in flesh biochemistry for lobsters with different states of shell hardness.

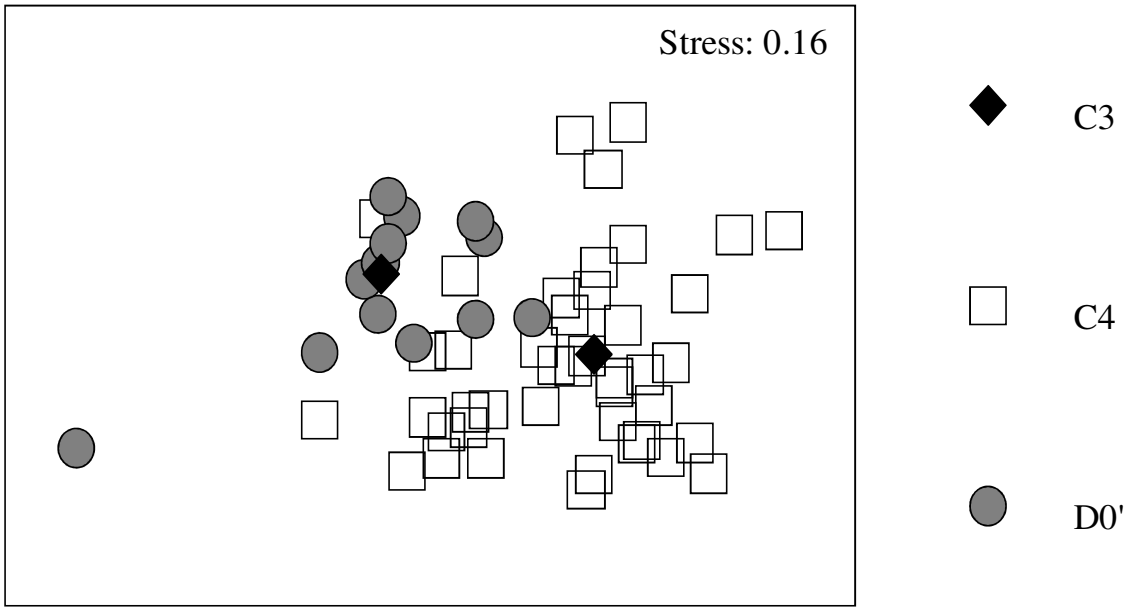


Figure 3.5 MDS of rocklobster variation with moult stage.

Non-parametric multi-dimensional scaling analyses shows associations of biochemical flesh characteristics for rocklobsters between different moult stages pooled across all years. Resemblance used for permutations was normalised Euclidean distance.

Table 3.4 Variation in rocklobster flesh biochemistry with moult stage.

Differences in flesh biochemistry between rocklobsters from moult stages C4 and D0'. Test statistic: t for Independent T-Test (parametric); Z for Mann-Whitney U test (non-parametric). Means ± SE presented for each moult stage.

Biochemical indicator	**Test Statistic**	**P**	**C4 (n=48)**	**D0' (n=8)**
Total Lipid (%)	Z = -4	**	0.26 ± 0.01	0.41 ± 0.03
Flesh pH	t = 2	*	6.88 ± 0.03	6.73 ± 0.04
Moisture (%)	t = -1	ns	73.44 ± 0.25	74.02 ± 0.25
Driploss (%)	Z = 3	*	6.55 ± 0.56	10.52 ± 1.13
Glycogen (µmol/g)	Z = -3	**	0.89 ± 0.09	0.37 ± 0.04
Lactate (µmol/g)	Z = -3	**	1.09 ± 0.13	1.72 ± 0.21
ATP (µmol/g)	t = 48	**	17.21 ± 0.73	24.24 ± 1.55
ADP (µmol/g)	Z = -2	*	1.90 ± 0.22	2.34 ± 0.29
AMP (µmol/g)	Z = -1	ns	0.12 ± 0.03	0.30 ± 0.16
Hypoxanthine (µmol/g)	Z = -3	*	0.12 ± 0.05	0.28 ± 0.12
K value	Z = -2	*	0.55 ± 0.18	2.44 ± 0.91
Inosine (µmol/g)	Z = -1	*	0.04 ± 0.02	0.38 ± 0.20
IMP Load (µmol/g)	Z = -4	*	0.40 ± 0.04	0.09 ± 0.05
Total Adenylate Pool (µmol/g)	Z = -2	**	25.89 ± 0.77	29.32 ± 0.89
AEC	Z = 0	ns	0.95 ± 0.00	0.94 ± 0.01

* $P < 0.05$, ** $P < 0.01$, ns = not significant

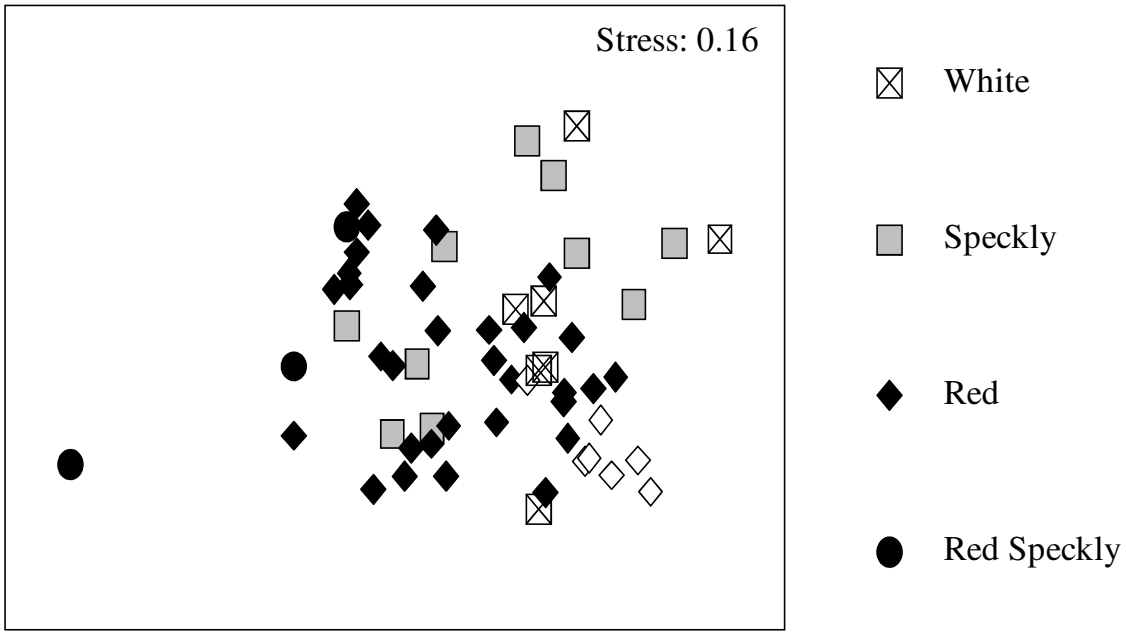


Figure 3.6 MDS of rocklobster variation with shell colour.

Non-parametric multi-dimensional scaling analyses shows associations of biochemical flesh characteristics for rocklobsters with different shell colours (pooled batches). Resemblance used for permutations was normalised Euclidean distance. Note, hollow diamonds ◇ refer to Red rocklobsters that were sampled in May 2004 to allow for direct comparison with white rocklobster (also sampled in May 2004) in order to assess biochemical differences associated with shell colour, without the confounding factor of batch.

Discussion

Batch (i.e. rocklobsters sampled on a particular day) had the greatest effect on rocklobster flesh biochemistry compared to all other potential sources of variation measured in this study. This study successfully established the amount of biochemical variation expected in processed rocklobster destined for value added product. Subsequent experiments will establish if this biochemical variation is of a scale likely to influence sensory attributes. It is acknowledged that to adequately determine where the difference in batch originates, detailed tracking of rocklobster from the pot through the post harvest chain to processing should be undertaken and greater numbers of rocklobster will be required. Other factors such as year, moult stage and shell colour did not contribute to the overall variation in flesh biochemistry to the same degree. The differences detected for moult stage and shell colour could also be driven by the confounding effects of batch in this study (Table 3.1).

Changes in flesh biochemistry with batch

There exists a wide range of potential post-harvest factors that can be attributed to the differences detected between batches. It was not feasible within this project to establish what potential post-harvest factors may influence the variation detected with batch above those factors recorded when processing. However it is of interest to speculate why this variation exists and the potential factors influencing the key sources of biochemical variation in fresh rocklobster flesh. All lobsters sampled in this project were sourced from across South Australia's fishing range. A batch that was selected by the factory could be a combination of multiple fishing vessels, and therefore trips. Lobsters may

have been caught in different areas. Musgrove (2001) reported differences in percentages of abdominal tissue and wet weight in lobsters harvested from areas of known fast and slow growth (as identified by Prescott et al. (1997)). In addition, fishing area has been shown to influence haemolymph lactate and tissue nucleotide levels in rocklobsters (Spanoghe 1996). These effects were generally correlated with greater stress levels in rocklobsters, according to the distance they were transported.

Capture and transport of lobsters on different vessels, and in different weather conditions has also been shown to significantly alter a number of parameters used to indicate the condition and prior stress of Western Rocklobsters (Paterson *et al.* 2001). It was suggested that factors such as boat design, experience of the fishers and distance of grounds from the factory, could all potentially influence the variation in rocklobster condition at time of sampling. This may explain the variation in flesh properties detected in this study. The transport of rocklobsters to the holding facilities required road transportation that would have varied in duration, multiple handling, re-tanking and variable water quality. During this time, or prior to, rocklobsters may have relied on anaerobic metabolism, resulting in build-up of lactate, as suggested by the maximum value of 4.54 µmol/g recorded in flesh. Other documented changes occurring with anaerobic metabolism include alterations in nucleotide levels, in both *J. edwardsii;* (Morris and Oliver 1999) and terrestrial red crabs (Morris and Adamczewska 2002). Previously recorded ATP values for stressed rocklobster were much lower (8.66µmol/g, Speed et al., 2001) than in this study (maximum value = 31.28µmol/g). The values in this study seem unusually high, however, there was no analytical reason for their

exclusion (i.e. they did not all belong to the same sampling analysis run and majority of all samples were within the ranges previously reported). Despite this, the minimum and maximum values of AEC in this study (which is a ratio of the adenylate values) (0.8-1.0, Table 3.3) are comparable to the variation with *Panulirus cygnus* in the West Australian fishery with mean recorded AEC ranging from 0.7-0.93 (Spanoghe 1996; Tod and Spanoghe 1997). These values were recoded from fresh samples of commercially caught rocklobster, as with this study, and therefore provide a useful basis for comparison between studies.

Changes in flesh biochemistry with season

No discerning patterns in flesh biochemistry over consecutive seasons were detected, despite significant variation across all flesh biochemistry indicators measured over the three years (Fig.s 5 & 6). For example, lobsters processed in 2003 recorded driploss values of ~ 3%, compared to ~11% for 2004 and 2005. However, the lowest mean values were recorded in March, May and October spanning nearly a full season, further supporting the lack of season changes in flesh characteristics. The biochemical composition of *J. lalandii* abdominal muscle over four years (using standardized harvest and post-harvest methods) has previously shown no consistent seasonal variation or trend for tail mass, moisture, lipid, protein and ash content (Cockcroft 1997). In contrast, biochemical differences reported in the Western Rocklobster, *Panulirus cygnus* during the warmer months have been associated with seasonal influences (Tod and Spanoghe 1997).

Changes of flesh biochemistry with moult stage

Biochemistry differed between late intermoult (C4) and early pre-moult (D0') rocklobsters. Unfortunately, having only two lobsters recorded as early intermoult stage (C3) precluded useful analysis of any comparison beyond late intermoult to early pre-moult. Rocklobsters in early premoult had higher ATP, ADP and AMP values when compared to lobsters in the intermoult stage. Significant differences in ATP between moult stages has been previously documented for the fresh water prawn *Macrobrachium nipponense*, where premoult individuals had flesh with twice as much ATP compared to intermoult (Wang *et al.* 2003). The lack of such large differences in the current study may be the result of not having any late-stage premoult rocklobsters to sample. The moult stages used in this study represent those likely to be encountered in lobsters graded and selected for processing. Future research may specifically target the full range of moult stages to address changes in flesh biochemistry between moult stages.

Changes in flesh biochemistry of rocklobsters with different Shell colours

Shell colour of rocklobsters was recorded to address the perceived difference between rocklobsters with red and white carapaces. Large white rocklobsters are sometimes worth less than comparable red rocklobsters to the consumer market, and are subsequently not currently targeted in the fishery, however are often caught in the same pots. White rocklobsters of the Western Australian *Panilurus cygnus* fishery are documented to be consistently weaker than the dark shelled animals identified as 'red rocklobsters', possibly associated with a large migration (Spanoghe and Bourne 1997). In the current study, red and white shelled rocklobsters had similar flesh biochemistry

(Table 3.2). The results from this study indicate there is no reason to discount large white rocklobsters based on flesh properties.

In conclusion, flesh biochemistry was shown to vary in commercially harvested lobsters selected as being suitable for processing. This variation was primarily attributed to the confounding factor of batch, over and above biological influences, such as moult stage and shell colour. This has implications for post-harvest practices, which were not controlled for in this study. It is possible that varying post-harvest practices between batches are responsible for the bulk of flesh biochemistry changes observed. Finally, the change in these biochemical properties needs to be correlated with results from sensory analysis to gauge if biochemical variations are sufficient to adversely affect flesh quality for the consumer.

CHAPTER FOUR

Sensory and Biochemical properties of commercially harvested rocklobster *Jasus edwardsii*.

Abstract

Sources of variation in rocklobster flesh quality include both operational (e.g. post-harvest handling techniques) and biological (e.g. variation within rocklobster itself) effects. This research addresses four key sources of variation of interest to rocklobster processors; 1) variation within rocklobster tail sections, 2) between rocklobster of different a) size and b) stress prior to processing; and 3) stability with frozen storage. Samples of flesh obtained from rocklobster were tested for variation in twenty biochemical parameters, and put to a trained sensory descriptive panel to test for differences in appearance, texture and flavour. In addition, assessment of sensory properties with frozen storage was undertaken using a Japanese consumer panel. The greatest sensory differences were detected with frozen storage and then between different tail sections. Sensory descriptors of flesh translucency, pinkness, and lobster flavour were the most significantly influenced across treatments (identified through hybrid descriptive tests), and were associated with the most pronounced biochemical differences, largely changes in adenylate ratios. Despite expectations, differences in sensory properties did not translate to a preference for fresh rather than frozen flesh, for either the trained descriptive panel or consumer panel. This indicates that despite different appearance, texture or flavour, frozen stored samples can be suitable as value added product.

Introduction

Sensory analysis on rocklobster to date has focussed on acceptability of flesh following a period of frozen or chilled storage (Bremner and Veith 1980; Coetzee and Simmonds 1988; Gomez-Guillen *et al.* 2007; Lopez-Caballero *et al.* 2006; Wessels *et al.* 1979). Key findings were the establishment of reduced "acceptability" of flesh sourced from poor condition rocklobsters prior to processing (Boyd and Sumner 1973) , and the establishment of a reduction in lobster flavour with frozen storage (Wessels *et al.* 1979). However this was based on a cooked product. Cooking regime has been shown to substantially change flesh quality (Coetzee and Simmonds 1988) and affect texture (Simmonds *et al.* 1992) of rocklobster flesh. The samples used for the research detailed in this chapter were raw to avoid any influence of cooking on resultant sensory evaluation. Although the above studies were instrumental in establishing research on sensory characteristics of rocklobster flesh, none have conducted descriptive analysis. Therefore, quantification of the intensity of texture and flavour is yet to be clearly defined.

Recently, Nelson et al. (2005) investigated the sensory properties of flesh from wild-caught and tank-held rocklobster, independent of storage. Although this used cooked flesh, and did not employ a descriptive panel, the experienced industry panellists were able to record key sensory descriptors of flesh. These were used to establish the key descriptors for this study, as outlined in Chapter 2. To build on this work, the next step was to use a trained descriptive panel looking at possible sources of variation prior to

storage and to further classify the changes in rocklobster flesh with biochemical analysis.

This chapter presents a comprehensive analysis of sensory and biochemical properties of commercially processed *J. edwardsii* flesh. Variation in flesh properties may be related to post-harvest handling and storage of the product, or possible biological influences such as animal size. Of relevance to the rocklobster industry is how biochemical variation may translate to differences in key sensory characteristics. This research addresses four sources of variation that are of primary concern to rocklobster processors. The following specific objectives are addressed:

1. Variation in flesh characteristics within a rocklobster tail

The aim of this experiment was two-fold (a) to determine if biochemical flesh characteristics vary between different sections of a rocklobster tail and (b) how much of the tail can be considered consistent for use as a sensory sample.

2a. Variation between rocklobster - Size

The aim of this experiment was to determine if flesh characteristics and sensory properties vary between large and small rocklobster. The perception that small rocklobster have sweeter and firmer flesh compared to large rocklobster is widespread and exists with recreational, as well as industry fishermen (*pers. obs*.). When supply meets demand, current market practice is to discount the price paid for large rocklobster

(above 'plate sized') on a per kilogram basis. Thus it is important to ascertain if large rocklobsters do have different sensory properties to small rocklobster. This may affect the ability to refer to value added products from large rocklobster as a 'premium product' over products from small rocklobster, if sensory differences are detected between different sized rocklobster.

2b. Variation between rocklobster - Stress

The aim of this experiment was to determine if rocklobster in poor condition have different flesh biochemical or sensory properties when compared to rocklobster in good condition. For the purpose of this investigation good condition was established as being in the condition category 4 (lively) Table 2.2. Roberts (2001) has established significant stress responses in commercially caught *J. edwardsii* that corresponded directly to condition category with lower condition categories exhibiting higher stress levels. Most of the commercially caught rocklobster survive subsequent live export; however the affect on sensory properties remains a key question and a future area of research. Considerable loss of lobster flavour in *J.* edwardsii has been associated with stress prior to death (Boyd and Sumner 1973), and this study represents an extension of this research, with the addition of descriptive sensory analysis to provide further insight into the specific changes.

3. Stability of rocklobster flesh with frozen storage

The aim of this experiment was to determine the possible effects of frozen storage (both short and long-term) on the biochemical and sensory properties of rocklobster flesh. It

was of interest to Ferguson Australia to ascertain the affect of (a) short-term freezing (i.e. weeks) (b) longer term storage (months) on flesh properties.

Evaluation of rocklobster flesh by a Japanese consumer panel

The aim of this experiment was to determine if the significant differences detected by the trained sensory panel for frozen stored flesh were likely to be detected by a consumer panel. Asian markets consume the majority of exported *J. edwardsii* from the Southern Australian fishery and the Japanese often pay premium prices for rocklobster sashimi (EconSearch 2005). For this reason, it was decided to use a Japanese consumer panel to assess raw rocklobster.

Methods

All sample preparations and practices for biochemical and sensory analysis followed standard methods outlined in Chapter 2. The experimental protocol for each of the four objectives is addressed below.

1. Variation in flesh characteristics within a rocklobster tail

Sensory analysis was conducted using samples from different rocklobsters than those used for biochemical analysis, as the amount of flesh available in each section prevented taking sensory analysis samples. Sensory analysis was conducted on similar rocklobster at a later date. In both experiments the treatment effects were standardised within a batch and no direct correlation was made between the biochemical and sensory data. Samples

were sourced from the same type of rocklobsters (large males of both shell colours). All rocklobster were in intermoult (C4) or early pre-moult (D0'). Biochemical samples were sourced from July 2003 and sensory from September 2005. For each lobster (n = 6 per treatment), the tail was separated from the remainder of the shell and sectioned into 15mm wide cutlets (medallions). Samples of muscle were collected from three regions of each tail. These were Anterior (meat from under carapace), Middle (a cutlet from halfway along the tail) and the Tail (the last three posterior cutlets closest to the telson) (Fig. 4.1). Sensory samples (10-15g) were cut from the main muscle sections of each medallion (as per Chapter 2), then blast frozen and stored at -20ºC for approximately 2 weeks before sensory analysis.

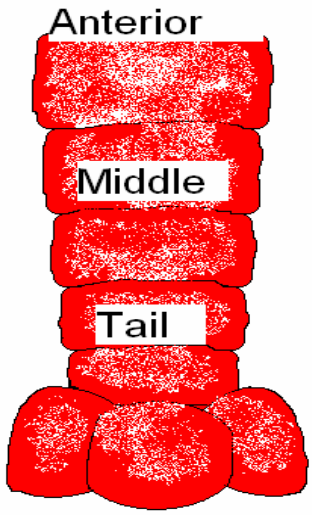


Figure 4.1 Schematic of rocklobster tail sections.

These are the sections sampled for biochemical and sensory analysis.

2a. Variation seen between rocklobster – Size

Biochemical and sensory properties of flesh were compared between legal size red male small (weighing 600 g, $n = 6$) and large (weighing greater than 2.5 kg, $n = 6$) rocklobsters (moult stage C4) (Fig. 4.2). All individuals were sourced by the commercial fishing company and thus represent what will be expected of commercial product. All rocklobster and samples were processed as per standard practice (Chapter 2) and the flesh from the middle section of the tail used for comparison. The middle section consisted of the second and third tail segments and had to be used as the small rocklobster had insufficient flesh in the anterior region for sensory analysis. Biochemical samples were taken as the rocklobsters were processed. Sensory samples were blast frozen and stored at -20^{o}C for two weeks prior to sensory analysis.

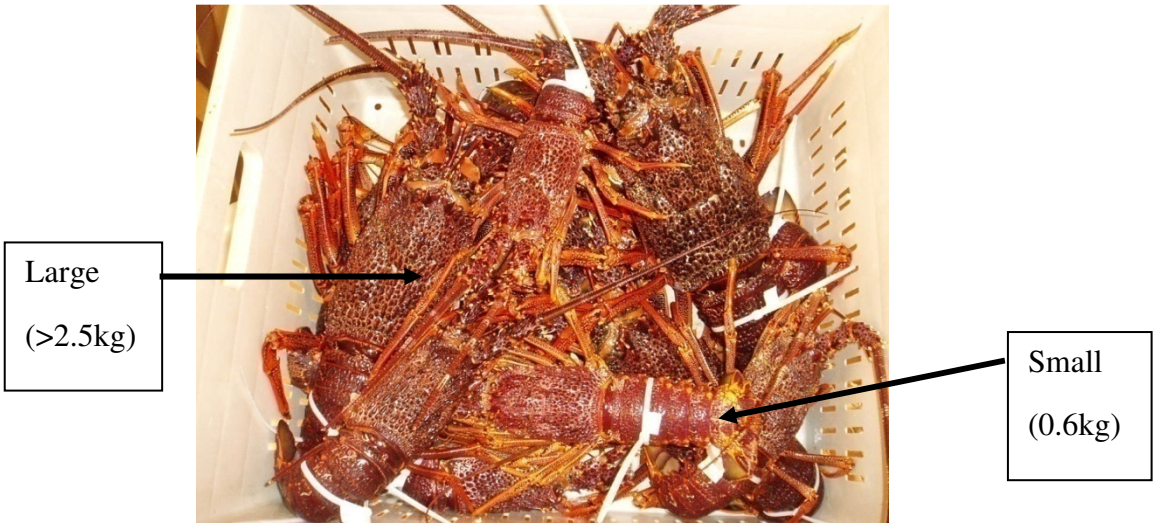


Figure 4.2 Picture of small and large rocklobster

2b. Variation seen between rocklobster- Stress

Commercially harvested rocklobsters were sourced from a single boat (red, male, 1-3 kg). Twelve rocklobsters (n = 6 each of poor and lively condition category scores, Chapter 2) were selected from the holding depot. The rocklobsters in poor condition were classified by the tail hanging low and a small gap between the tail and cephalothorax. There was little movement in the poor rocklobster unless shaken. The lively condition rocklobster (moult stage: flapped their tail when held and had their antennae held up in a defensive posture (Table 2.2). Each treatment had the same proportion of rocklobster moult stages (5 x DO' and 1 x C3). All rocklobster were subsequently transported at 8^{o}C and approximately 90% humidity to holding tanks (3.5h). All rocklobster were processed the following day using standard practices as outlined in Chapter 2. In addition to the standard biochemical analysis, haemolymph metabolites were recorded prior to processing to establish stress responses. These haemolymph properties were; pH, refractive index and lactate (Chapter 2).

3. Stability of rocklobster flesh with frozen storage

It was not possible to compare rocklobster flesh from the same batch across treatments. Sensory analysis required that both samples (short and long-term frozen) were compared with a fresh reference sample. For this reason flesh from different batches of rocklobster was used for the "fresh" sample at each time treatment. However, every effort was taken to standardise, with source rocklobster being large red males.

Experimental comparisons were:

(a) Short-term storage effects, where fresh samples of rocklobster flesh were compared to samples that had been frozen for two months (n = 6), and (b) long-term storage effects, where fresh samples were compared to samples that had been frozen for 10 months (n = 6). The fresh samples were processed the day prior to sensory analysis. Both the fresh and frozen sensory samples were kept at 4^{o}C over night to allow the frozen samples to defrost and both samples to reach the same temperature. Samples for biochemical analysis were taken on the day of sensory analysis to ensure biochemical properties represented those of samples analysed by the sensory panel.

Evaluation of rocklobster flesh by a Japanese consumer panel

The panel was set up using 16 Japanese immigrants to Australia. All the participants enjoyed eating raw seafood and were very eager to participate. This panel was used to test fresh vs. frozen stored rocklobster anterior samples (raw rocklobster as per the trained sensory panel). Presentation and processing of samples was outlined in Chapter 2. Two experimental comparisons were evaluated: (a) fresh vs. short-term (1 month) frozen flesh and (b) short-term (2 weeks) vs. long-term (18 months) frozen flesh. Test (a) represents a response where we know sensory differences exist. Test (b) was to present an extreme value as the trained panel had previously tested 10 month frozen storage. Currently commercial samples are frozen before sale, therefore for maximum relevance to the industry it was decided to compare the 18 month frozen samples to a very short frozen storage period, rather than fresh chilled.

The Japanese consumer panel was not trained in descriptive analysis, so only triangle and preference tests were conducted. To keep the questionnaire and the interpretation of it simple, preference tests were conducted on the samples provided for the triangle test. This translated to two of the three samples being the same treatment for preference testing. As this treatment had a two out of three chance of being selected (as opposed to the 50% chance when only 2 samples), preference test data was analysed using probability tables for triangle tests (British Standard BS ISO 4120:2004). A "no preference" option was also provided both for informative purposes and to avoid forcing a response. These responses were excluded from analysis, and the number of panellists adjusted accordingly for analysis as described above.

Panellists had an average age of 41yrs (21-70yrs) and had been in Australia for an average of 6 yrs (<0.5-16yrs). There were 6 males and 10 females. All the panellists ate raw seafood at least once every year, with 7 panellists eating raw seafood at least once a month and 5 panellists at least once a week. 5 of the sixteen panellists eat raw rocklobster at least once a year with the remainder eating raw rocklobster less than once a year on average.

Statistical analysis

Statistical analysis employed was the same across all experiments. Multivariate analysis was conducted using the software package Primer version 5. Euclidean distance was used to transform the data to standardize the measureable units for the different biochemical parameters tested. Multivariate biochemical composition of flesh samples was compared using multi-dimensional scaling (MDS) plots and analysis of similarities

(ANOSIM). Where ANOSIM detected significant differences between treatments, SIMPER analysis was used to compare the contribution of each parameter to the differences detected. Univariate statistics were conducted using SPSS version 12. Differences in individual biochemical parameters between treatments were tested using parametric ANOVA (for three or more treatments) or Independent T-test (for experiments with only two treatments) and non-parametric Kruskal Wallis (for three treatments) or Mann-Whitney U tests (for two treatments) when assumptions of normality were violated.

Results

1. Variation in flesh characteristics within a rocklobster tail

Biochemical Analysis

Biochemical composition of flesh samples differed between sections of rocklobster tail (Fig. 4.3, Table 4.1a; ANOSIM: Global $R = 0.263$, $p < 0.03$), where anterior and middle section flesh was significantly different from tail (Table 4.1b pair-wise comparisons; anterior *v*. tail: $p < 0.05$, middle *v*. tail: $p < 0.05$). Almost half of the variation between flesh from anterior and tail sections was attributed to the adenylate ratios AEC, IMP load and K value (Table 4.2; SIMPER: accumulated contribution to the dissimilarity = 47.21%). Individual adenylate concentrations were excluded from SIMPER analysis to avoid increased weighting of any single adenylate that is already covered in ratio calculations. However Table 4.3 shows lower levels of ATP and higher levels of AMP and IMP in the tail section compared to the anterior which affect AEC, IMP load and K

value. Moisture content was the only other contributing factor that explained over 10% of the variation (Table 4.2), however, total lipid content had a dissimilarity ratio greater than one, which equates to a more reliable indicator.

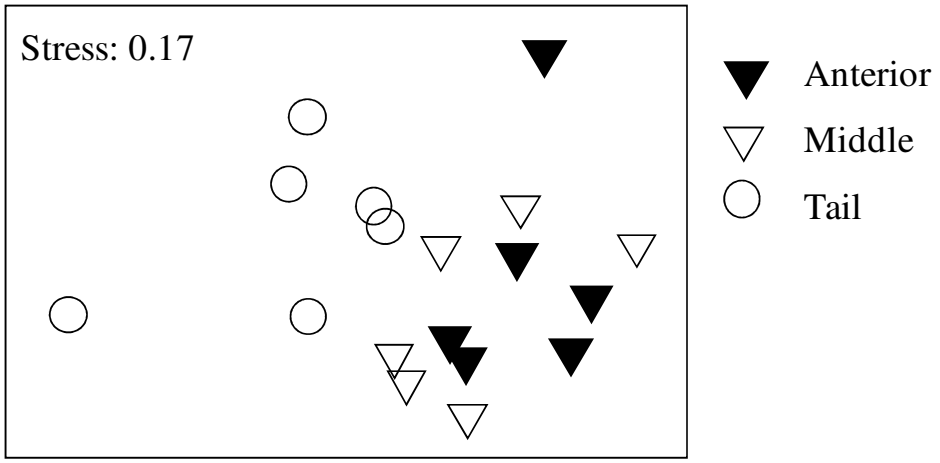


Figure 4.3 MDS of rocklobster variation with tail section.

Non-parametric multi-dimensional scaling analyses shows associations of biochemical flesh characteristics for rocklobsters between Anterior, Middle and Tail sections. Resemblance used for permutations was normalised Euclidean distance.

Table 4.1 Multivariate analysis of rocklobster tail section biochemistry.

This table presents results of (a) ANOSIM dissimilarity results testing the biochemical difference between anterior, middle and tail sections of flesh and (b) pair wise comparisons.

(a) Comparison Groups	**Global R**	**Sample statistic**
Sections	0.263	0.3%

(b) Pair-wise comparisons	**R value**	**Significance level**
Anterior *vs*. Middle	-0.126	87.7%
Anterior *vs*. Tail	0.437	0.4%
Middle *vs.* Tail	0.28	0.4%

Sample statistic 5%= $P \leq 0.05$. Global R gives indication of the strength of difference, where 1= completely different, 0 = completely the same. Pairwise R values > 0.75 = well separated, R > 0.5 = clearly different, R < 0.5 = barely separate.

Table 4.2 Key biochemical indicators for variation between tail sections.

This table presents the results of SIMPER analysis indicating the percentage contribution of each biochemical indicator to differences detected between anterior and tail sections of flesh. Average dissimilarity between treatments was 23.11%.

Biochemical indicator	**Dissimilarity ratio**	**Contribution %**	**Cumulative %**
AEC	1.37	18.43	18.43
IMP Load	1.07	15.11	33.54
K value	0.47	13.68	47.21
Moisture content	0.86	11.79	59.00
Total Lipid	1.02	9.14	68.15
Total Adenylate Pool	0.74	8.91	77.06
Flesh pH	0.82	8.87	85.93
Glycogen	0.91	7.24	93.17

Biochemical indicators are listed in order of decreasing contribution to the average dissimilarity (contribution %) between anterior and tail flesh up to 95% of accumulated dissimilarity (cumulative %). Dissimilarity ratio shows the dissimilarity between groups divided by the dissimilarity within groups. Dissimilarity ratio >1 = reliable indicator, <1= not reliable. Data was analysed using normailsed Euclidean distances.

Table 4.3 Variation in rocklobster flesh biochemistry with tail section.

Mean value (± SE, $n = 6$) for all biochemical descriptors of flesh from each of anterior, middle and tail sections of rocklobster. Test statistic: F value for parametric ANOVA, χ^2 for non-parametric Kruskal Wallis test.

Indicator	**Anterior**	**Middle**	**Tail**	**Test statistic**	**P**
Flesh pH (fresh)	7.15 ± 0.05	7.11 ± 0.05	7.07 ± 0.03	$F_{2,16} = 0.778$	ns
Moisture content (%)	73.67 ± 0.72	73.05 ± 0.22	75.30 ± 0.56	$F_{2,17} = 4.616$	*
Driploss (%)	7.60 ± 0.88	6.31 ± 0.53	7.12 ± 0.66	$F_{2,17} = 0.849$	ns
Total lipid (%)	0.38 ± 0.02	0.37 ± 0.02	0.36 ± 0.03	$F_{2,16} = 0.307$	ns
Lactate (µmol/g)	1.48 ± 0.37	1.55 ± 0.36	1.38 ± 0.28	$F_{2,15} = 0.067$	ns
Glycogen (µmol/g)	0.08 ± 0.00	0.08 ± 0.01	0.07 ± 0.01	$\chi^2 = 0.705$	ns
IMP (µmol/g)	7.43 ± 1.12	7.39 ± 0.69	8.18 ± 0.81	$F_{2,16} = 0.229$	ns
ATP (µmol/g)	11.39 ± 0.74	11.20 ± 1.17	7.48 ± 0.31	$F_{2,16} = 6.526$	*
ADP (µmol/g)	0.68 ± 0.06	0.91 ± 0.13	0.87 ± 0.08	$F_{2,16} = 1.517$	ns
AMP (µmol/g)	0.03 ± 0.01	0.10 ± 0.02	0.17 ± 0.02	$F_{2,16} = 16.06$	***
Hypoxanthine (µmol/g)	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	$\chi^2 = 0.975$	ns
Inosine (µmol/g)	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.03	$\chi^2 = 1.233$	ns
Total Adenylate Pool (µmol/g)	19.54 ± 1.79	19.60 ± 1.59	16.73 ± 0.95	$F_{2,16} = 1.048$	ns
K value	0.02 ± 0.01	0.03 ± 0.01	0.22 ± 0.17	$\chi^2 = 3.53$	ns
IMP load (µmol/g)	0.60 ± 0.06	0.61 ± 0.05	0.96 ± 0.09	$F_{2,16} = 8.61$	**
AEC	0.97 ± 0.00	0.95 ± 0.01	0.93 ± 0.01	$F_{2,16} = 12.724$	**

*P < 0.05, **P < 0.01, ***P < 0.001, ns = not significant

Despite contributing 14% of the dissimilarity between anterior and tail sections in the multivariate analysis, K value was not significantly different between sections in the ANOVA (Table 4.3, $p > 0.05$). This is likely a result of the greater variation within the tail samples (SE = ± 0.17) compared to anterior samples (SE = ± 0.01), as univariate analyses are more sensitive to heterogeneity in the data leading to type 2 errors (Quinn and Keough 2002). Despite being identified as a reliable indicator (Table 4.2), total lipid was responsible for less than 10% of the variation and did not differ significantly between all sections (Table 4.3). Muscle AMP increased in concentration toward the posterior end of the tail section, being the only descriptor to show significant differences between all three sections (Table 4.3: $p < 0.001$). Conversely, ATP concentration was lower in the tail than anterior (Table 4.3). These two descriptors likely contributed to the difference in adenylate ratios detected between anterior and tail flesh.

Sensory Analysis

As the major biochemical difference in flesh was between anterior and tail sections, these were used in sensory analysis. Despite this, no difference was detected between samples for texture and flavour using triangle tests (Table 4.4). However, 13 of the 17 panellists significantly identified the odd sample based on appearance alone (Table 4.4). This difference in appearance is evidenced by the identification of pinker tail flesh, as detected using the hybrid descriptive test (Fig. 4.4). The only other key sensory property detected as significantly different using the hybrid descriptive test was less lobster flavour in the tail flesh (Fig. 4.4). Seven panellists did not have a preference for either sample; however, 9 of the remaining 10 panellists preferred anterior flesh, which was statistically significant (Table 4.4).

Table 4.4 Sensory results for rocklobster tail section

This table presents three way triangle tests performed with the sensory panel comparing appearance, texture and flavours and preference tests for flesh from anterior and tail sections of rocklobster.

Three way Triangle Test
- Appearance 13 / 17 (significant)
- Texture and Flavour 8/17 (ns)
Preference Tests
-"No preference" chosen 7 times out of 17
-"Anterior" chosen 9/10 times (significant)
-"Tail" chosen 1/10 times (ns)

Significant $P < 0.05$ and ns = not significant $P > 0.05$. Triangle test significance from binomial distribution tables (British Standard BS ISO 4120:2004). Preference test significance from two tailed preference test probability tables, after excluding no preference responses (Lawless and Heymann 1999).

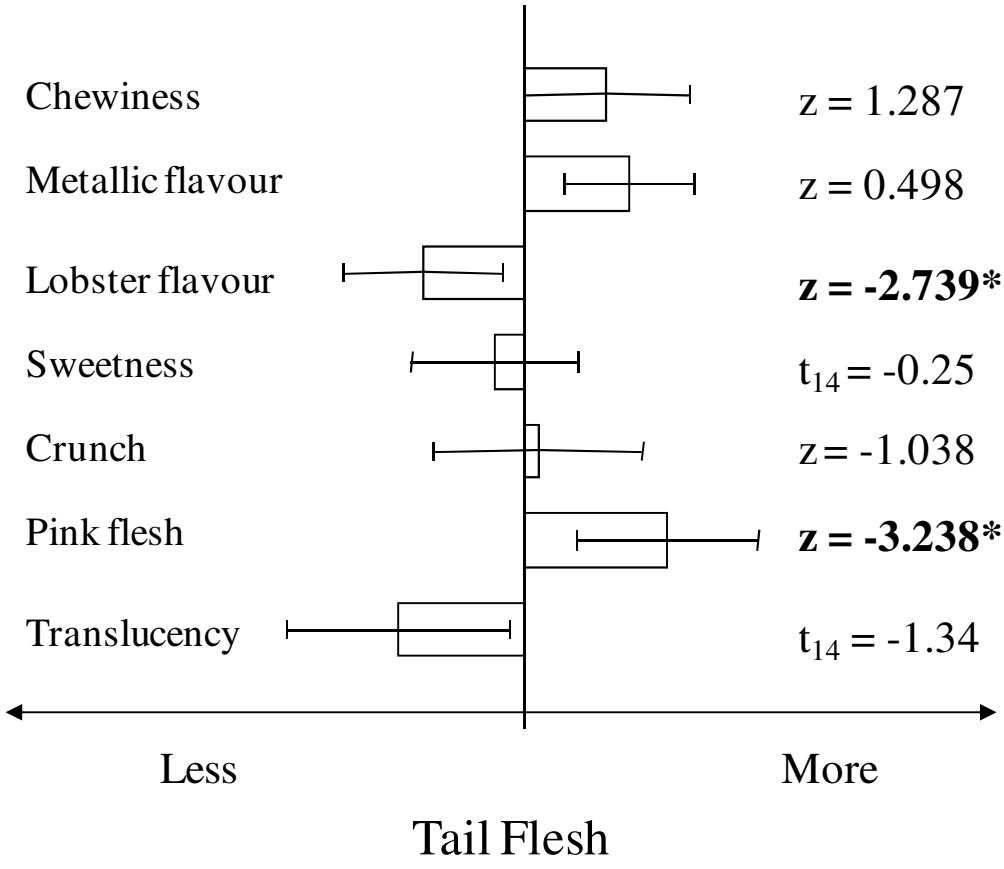


Figure 4.4 Sensory descriptive properties for tail section.

Mean (± SE) distance (mm) of tail responses marked on the unstructured line scale when compared to anterior reference (central line). Significance illustrated in **bold***, where $p < 0.05$. Statistical tests performed between treatments were Paired samples t-test for parametric data (denoted as *t*) or Wilcoxon signed-rank test (denoted as *z*) for non-parametric data.

2a. Variation seen between rocklobster – Size

Biochemical & sensory analysis

Overall, biochemical composition did not differ between flesh samples from large and small rocklobster (Fig. 4.5, ANOSIM: Global R = 0.22, sample statistic 36.1%), although small rocklobster flesh exhibited higher moisture content (Table 4.5: t-test, $p < 0.05$; by approximately 1%) and driploss (by approximately 3%) properties. No difference in either appearance or texture and flavour was detected by the sensory panel between large and small rocklobster samples (Table 4.6: triangle test). None of the seven sensory descriptors were significantly different, although there was an indication that small rocklobster flesh may be chewier (Fig. 4.6). Mean sweetness between samples was exactly the same. The lack of difference in sensory descriptors was reflected, with no significant preference for either large or small rocklobster flesh (Table 4.6: Preference tests).

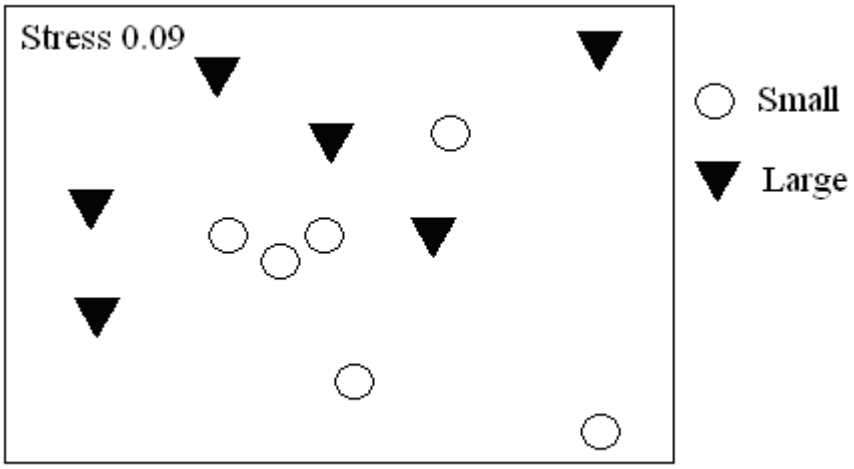


Figure 4.5 MDS of rocklobster variation with size

Non-parametric multi-dimensional scaling analyses shows associations of biochemical flesh characteristics for rocklobsters between small (open symbol) and large (filled symbol) rocklobster flesh. Resemblance used for permutations was normalised Euclidean distance.

Table 4.5 Variation in flesh biochemistry with rocklobster size.

Mean value (± SE, *n* = 6) for all biochemical descriptors of flesh from large and small rocklobsters. Test statistic: Independent samples T-test for parametric data (denoted as t) and Mann-Whitney U test for non-parametric data (denoted as z).

Indicator	**Large**	**Small**	**Test statistic**	**P**
Haemolymph colour	1.25 ± 0.11	1.50 ± 0.00	z = -1.92	ns
Flesh pH	6.73 ± 0.10	6.67 ± 0.07	t_{10} = 0.49	ns
Moisture (%)	73.12 ± 0.21	74.54 ± 0.58	t_{10} = -2.31	*
Driploss (%)	9.79 ± 0.67	12.25 ± 0.68	z = -2.08	*
Total lipid (%)	0.21 ± 0.02	0.24 ± 0.03	z = -0.17	ns
Lactate (µmol/g)	1.52 ± 0.30	1.54 ± 0.07	$t_{5.53}$ = -0.09	ns
Glycogen (µmol/g)	0.42 ± 0.08	0.27 ± 0.03	t_{10} = 1.66	ns
IMP (µmol/g)	1.86 ± 0.70	1.05 ± 0.40	z = -0.48	ns
ATP (µmol/g)	24.58 ± 1.03	23.14 ± 1.30	t_{10} = 0.87	ns
ADP (µmol/g)	3.18 ± 0.46	2.97 ± 0.13	t_{10} = 0.44	ns
AMP (µmol/g)	0.29 ± 0.13	0.24 ± 0.06	t_{10} = 0.36	ns
Hypoxanthine (µmol/g)	0.57 ± 0.26	0.33 ± 0.20	z = -0.64	ns
Inosine (µmol/g)	0.03 ± 0.03	0.31 ± 0.29	z = -0.05	ns
Total Adenylate Pool	30.50 ± 0.86	28.04 ± 1.31	t_{10} = 1.57	ns
K value	1.94 ± 0.89	2.38 ± 1.18	t_{10} = -0.29	ns
IMP load (µmol/g)	0.07 ± 0.03	0.04 ± 0.01	z = -0.48	ns
AEC	0.93 ± 0.01	0.93 ± 0.01	t_{10} = -0.09	ns

Subscript values denote *df* for t-test. *p < 0.05, ns = not significant

Table 4.6 Sensory results for rocklobster size

This table presents results from the three way triangle tests performed with the sensory panel comparing appearance, texture and flavours and preference tests of flesh from large and small rocklobsters.

Three way Triangle Test
- Appearance 7/16 (ns)
- Texture and Flavour 8/16 (ns)
Preference Tests
-"No preference" chosen 6 times out of 16
-"Large" chosen 4/10 times (ns)
-"Small" chosen 6/10 times (ns)

Significant $P < 0.05$ and ns = not significant $P > 0.05$. Triangle test significance from binomial distribution tables (British Standard BS ISO 4120:2004). Preference test significance from two tailed preference test probability tables, after excluding no preference responses (Lawless and Heymann 1999).

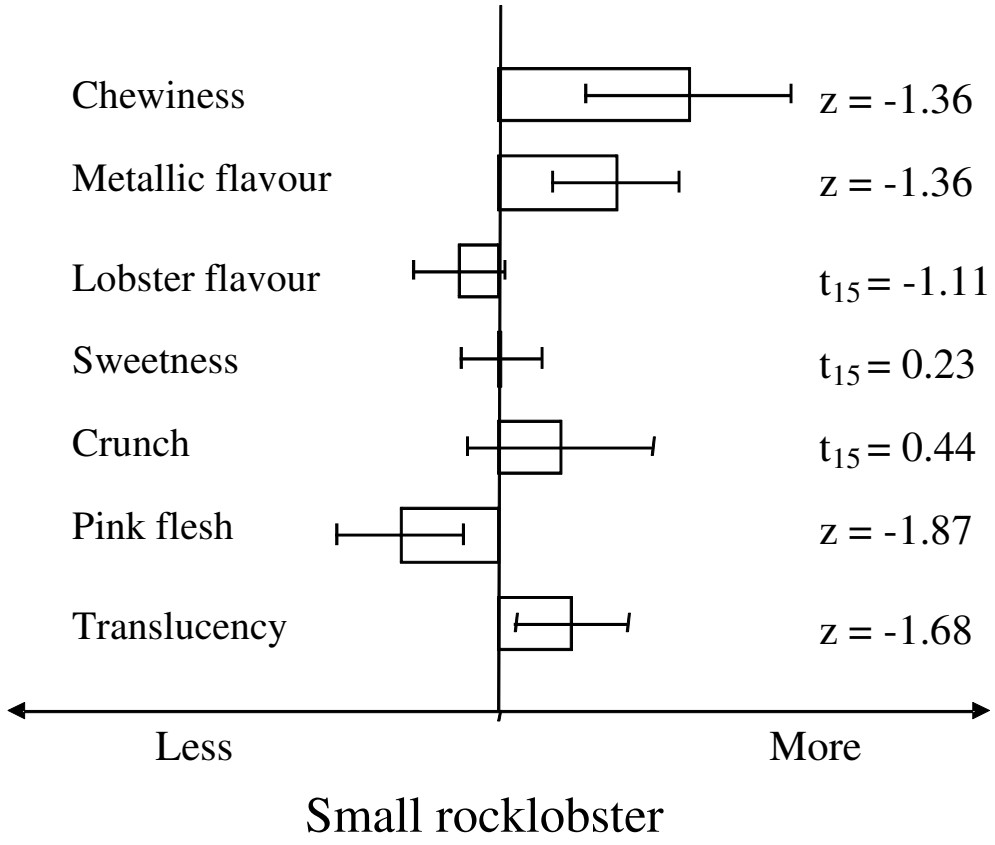


Figure 4.6 Sensory descriptive properties for small vs. large rocklobster.

Mean (±SE) distance (mm) of responses to small rocklobster flesh marked on the unstructured line scale when compared to large rocklobster reference (central line). Statistical tests performed between treatments were Paired samples t-test for parametric data (denoted as t) or Wilcoxon signed-rank test (denoted as z) for non-parametric data. All values not significantly different.

2b. Variation seen between rocklobster- Stress

Biochemical analysis

With the exception of haemolymph refractive index, none of the haemolymph properties, which would have indicated prior stress, differed between lively and poor rocklobsters (Table 4.7). It was noted that the rocklobster previously selected as poor condition were in 'lively' or 'ok' condition prior to processing in the factory. The only significant difference was that lively condition rocklobster had a higher and less variable refractive index than poor rocklobsters (Table 4.7: t-test, $p < 0.05$). Condition of rocklobster did not alter the biochemical composition of flesh (Table 4.7, Fig. 4.7; ANOSIM: Global R = -0.107, sample statistic =79.4%).

Sensory analysis

No difference in either appearance nor texture and flavour were detected for lobsters in lively *vs.* poor condition with triangle tests (Table 4.8). Very few panellists were able to correctly identify the odd sample for both appearance (Table 4.8: 2/16 panellists) and texture and flavour (3/16 panellists). Subsequently, sensory descriptors for both poor and lively flesh were not significantly different between lively and poor rocklobsters (Fig. 4.8: Hybrid descriptive test), and the panel did not preferentially choose flesh from either treatment (Table 4.8: "no preference" selected 9/16 times).

Table 4.7 Variation in flesh biochemistry with rocklobster physical condition.

Mean value (± SE) for all biochemical descriptors of flesh from poor condition (n = 5; stressed) and lively condition (n = 6; not stressed) rocklobsters. Test statistic: Independent samples T-test for parametric data (denoted as t) and Mann-Whitney U test for non-parametric data (denoted as z).

Indicator	**Poor condition**	**Lively condition**	**Test statistic**	**P**
Haemolymph pH	7.21 ± 0.07	7.13 ± 0.04	z = -0.82	ns
Refractive index	1.350 ± 0.002	1.354 ± 0.000	t_9 = -2.45	*
Haemolymph lactate (mmol/L)	0.70 ± 0.11	1.03 ± 0.32	t_9 = -0.941	ns
Flesh pH	6.74 ± 0.10	6.76 ± 0.06	t_9 = -0.18	ns
Moisture (%)	75.27 ± 0.60	74.64 ± 0.34	z = -0.913	ns
Driploss (%)	12.73 ± 1.28	11.03 ± 1.21	t_9 = -0.96	ns
Total lipid (%)	0.44 ± 0.01	0.42 ± 0.02	t_9 = -0.55	ns
Muscle lactate (µmol/g)	0.66 ± 0.17	1.42 ± 0.33	$t_{7.34}$ = -0.203	ns
Muscle glycogen (µmol/g)	0.22 ± 0.03	0.24 ± 0.05	t_9 = -0.32	ns
ATP (µmol/g)	29.00 ± 2.02	29.34 ± 0.78	t_9 = -0.17	ns
ADP (µmol/g)	2.03 ± 0.03	2.12 ± 0.13	z = -0.91	ns
AMP (µmol/g)	0.03 ± 0.01	0.03 ± 0.01	t_9 = 0.24	ns
Hypoxanthine (µmol/g)	0.02 ± 0.01	0.03 ± 0.02	z = -0.73	ns
Inosine (µmol/g)	0.05 ± 0.03	0.13 ± 0.11	z = -0.21	ns
IMP (µmol/g)	0.98 ± 0.39	0.30 ± 0.20	t_9 = 1.65	ns
Total Adenylate Pool (µmol/g)	32.11 ± 2.30	31.95 ± 0.92	t_9 = 0.07	ns
K value	0.24 ± 0.10	0.50 ± 0.40	z = -1.10	ns
IMP load (µmol/g)	0.03 ± 0.01	0.01 ± 0.01	t_9 = 1.68	ns
AEC	0.97 ± 0.00	0.97 ± 0.00	t_9 = 0.05	ns

Subscript values denote *df* for t-test with equal variances assumed (whole number) or violated (2 decimal places). *p<0.05, ns = not significant

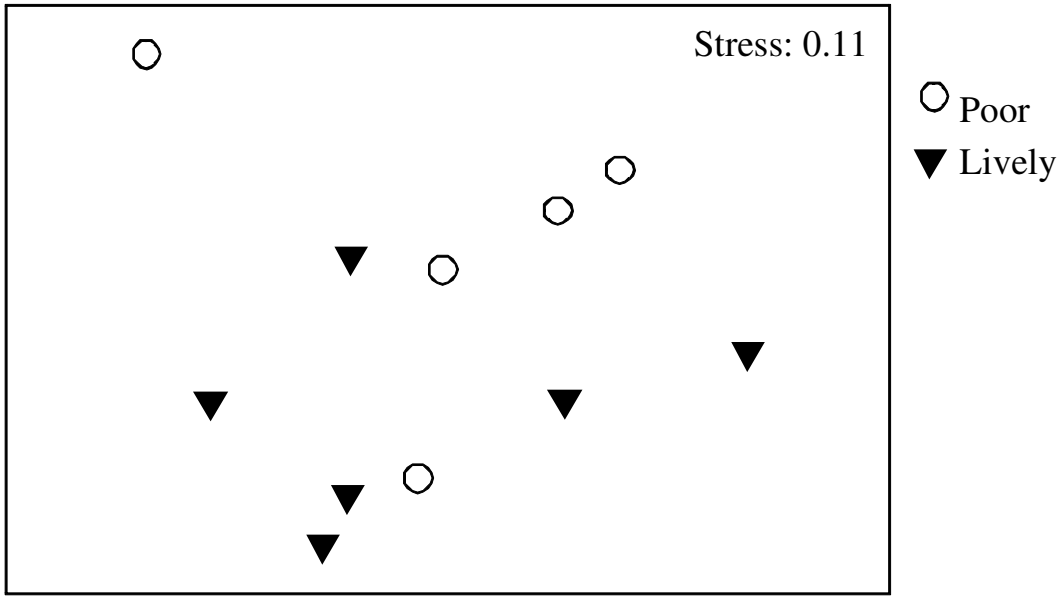


Figure 4.7 MDS of rocklobster variation with physical condition

Non-parametric multi-dimensional scaling analyses shows associations of biochemical flesh characteristics for rocklobsters between poor (open symbol) and lively condition (filled symbol). Resemblance used for permutations was normalised Euclidean distance.

Table 4.8 Sensory results for rocklobster physical condition

This table presents results from the three way triangle tests performed with the sensory panel comparing appearance, texture and flavours and preference tests of flesh from poor and lively condition rocklobsters.

Three way Triangle Test
- Appearance 2/16 (ns)
- Texture and Flavour 3/16 (ns)
Preference Tests
-"No preference" chosen 9 times out of 16
-"Poor" chosen 2/7 times (ns)
-"Lively" chosen 5/7 times (ns)

Significant $P < 0.05$ and ns = not significant $P > 0.05$. Triangle test significance from binomial distribution tables (British Standard BS ISO 4120:2004). Preference test significance from two tailed preference test probability tables, after excluding no preference responses (Lawless and Heymann 1999).

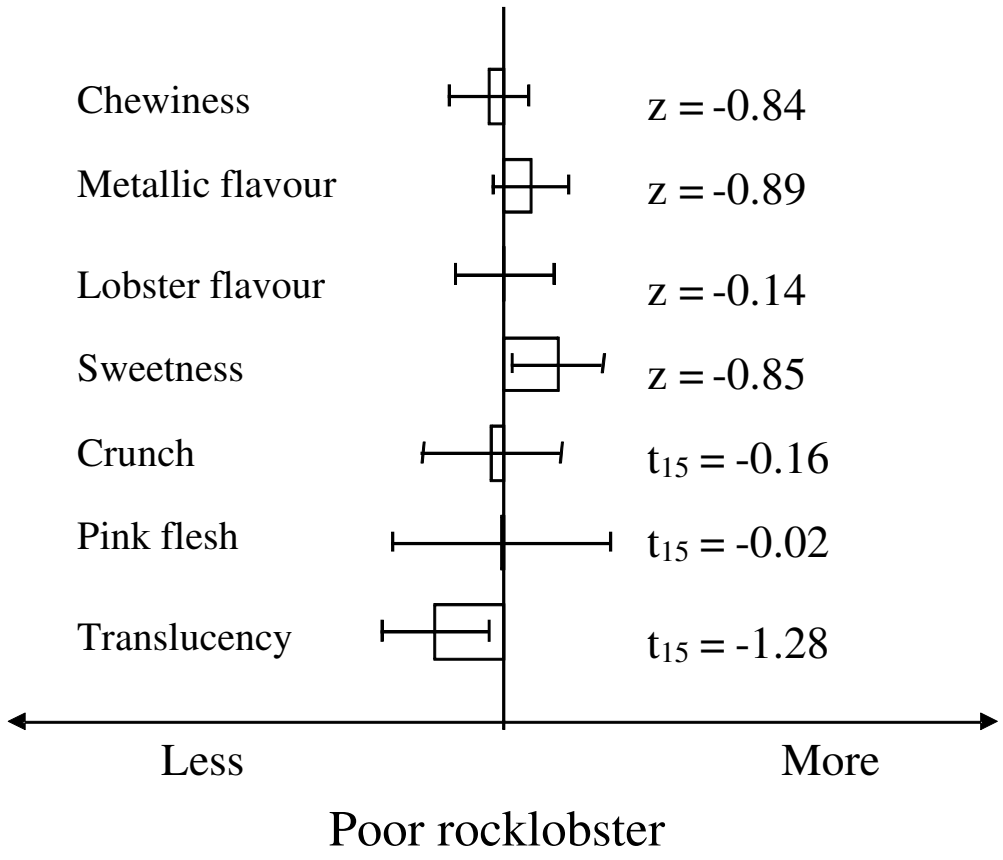


Figure 4.8 Sensory descriptive properties for poor vs. lively rocklobster.

The above figure depicts the mean (±SE) distance (mm) of "Poor" conditioned rocklobster flesh responses marked on the unstructured line scale when compared to "Lively" reference (central line). Positive values indicate greater intensity of descriptors, and vice versa. Statistical tests performed between treatments were Paired samples t-test for parametric data (denoted as t) or Wilcoxon signed-rank test (denoted as z) for non-parametric data. All values are not significant.

3. Stability of rocklobster flesh with frozen storage

Biochemical analysis

Multivariate analysis associated frozen storage with significantly altered biochemical composition of rocklobster flesh samples (Fig. 4.9, Table 4.9: ANOSIM, Global R = 0.835, $p < 0.01$). Regardless of length of storage, composition was different between control (fresh) and stored flesh (Table 4.9: Pair wise comparisons; Short-term *vs*. fresh: R value = 0.936, long-term *vs*. fresh: R value = 0.925). In addition, there was a difference between long term and short term storage, which was attributed the largest R value of 0.984 (Table 4.9). Finally there was a difference between the two sources of fresh samples as evidenced in Figure 4.9. The R value between fresh treatments was only 0.469 which shows that the difference between fresh samples in the two experiments is less extreme than that between the frozen treatments (Table 4.9).

Average dissimilarity between short-term frozen and fresh samples was ~16% (Table 4.10), which increased to ~ 35% between long-term and fresh samples (Table 4.11). The contribution of individual biochemical parameters to the dissimilarly observed between fresh and frozen stored samples was different depending on short and long-term treatments. Changes in adenylates were most important for short-term frozen flesh, where total adenylate pool and adenylate ratios (AEC, and K value) contributed to more than 60% of the dissimilarly between fresh and frozen samples (Table 4.10, SIMPER). A similar level of accumulated dissimilarity between fresh and long-term frozen samples included contributions from other indicators, such as muscle glycogen, lactate and total lipid (Table 4.11, SIMPER: Cumulative % = 62.81).

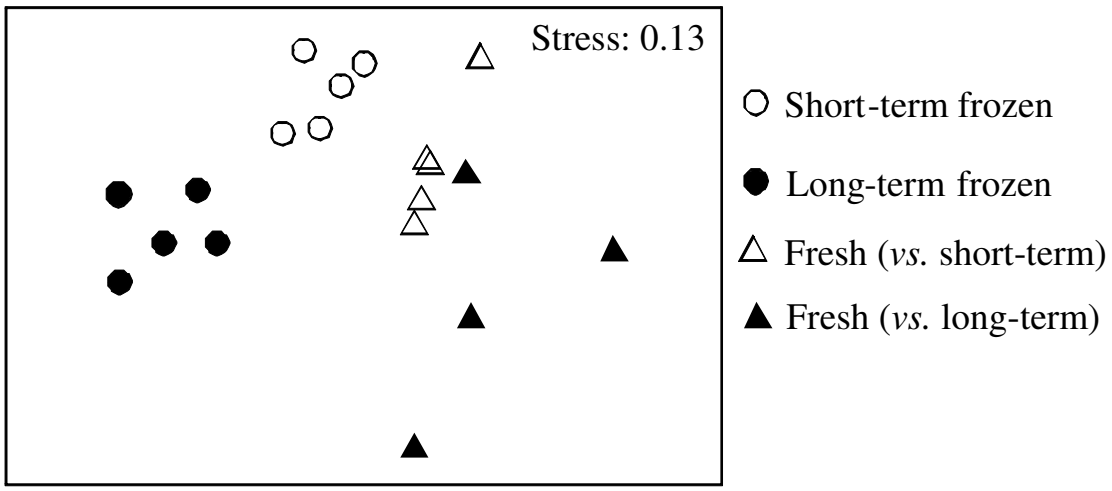


Figure 4.9 MDS of rocklobster variation with frozen storage.

Non-parametric multi-dimensional scaling analyses shows associations of biochemical flesh characteristics for lobsters between short (open circle) and long term (filled circle) stored flesh. Fresh flesh (control) samples are represented by corresponding open or filled triangles for each treatment. Resemblance used for permutations was normalised Euclidean distance.

Table 4.9 Multivariate analysis flesh biochemistry with frozen storage

This table presents (a) ANOSIM dissimilarity results testing the biochemical difference between short-term and long-term frozen flesh *vs*. fresh flesh and (b) pair wise comparisons

(a) Comparison Groups	**Global R**	**Sample statistic**
Storage	0.835	0.1%
(b) Pair-wise comparisons	**R value**	**Significance level**
Short-term vs. fresh	0.936	0.8%
Long-term vs. fresh	0.925	0.8%
Short-term vs. long-term	0.984	0.8%
Fresh (long-term comparison) vs. fresh (short-term comparison)	0.469	0.8%

Sample statistic 5% = $P < 0.05$. Global R gives indication of the strength of difference, where 1= completely different, 0 = completely the same. Pairwise R values > 0.75 = well separated, R > 0.5 = clearly separate, R < 0.5 = barely separate.

Table 4.10 Key biochemical indicators of variation with short-term frozen storage.

This table presents the results of SIMPER analysis indicating the percentage contribution of each biochemical indicator to differences detected between treatments of short-term frozen *vs*. fresh flesh. Average dissimilarity between treatments = 15.95%.

Biochemical indicator	**Dissimilarity ratio**	**Contribution %**	**Cumulative %**
AEC	2.13	31.35	31.35
Total Adenylate Pool	1.69	17.44	48.79
K value	4.71	12.53	61.32
Moisture content	0.87	10.01	71.36
Driploss	0.66	9.81	81.16
Flesh pH	1.04	8.46	89.63
Lactate	1.08	4.92	94.55

Biochemical indicators are listed in order of decreasing contribution to the average dissimilarity (contribution %) between fresh and frozen stored flesh up to 95% of accumulated dissimilarity (cumulative %). Dissimilarity ratio shows the dissimilarity between groups divided by the dissimilarity within groups. Dissimilarity ratio >1 = reliable indicator, <1= not reliable. Data was analysed using normalised Euclidean distances.

Table 4.11 Key biochemical indicators of variation with long-term frozen storage

This table presents the results of SIMPER analysis indicating the percentage contribution of each biochemical indicator to differences detected between treatments of long-term frozen *vs.* fresh flesh. Average dissimilarity between treatments = 35.25%

Biochemical indicator	**Dissimilarity ratio**	**Contribution %**	**Cumulative %**
K value	3.64	14.45	14.45
Glycogen	0.64	12.99	27.44
IMP load	1.46	12.36	39.79
Lactate	1.41	11.58	51.37
Total lipid	0.88	11.44	62.81
Flesh pH	1.27	11.00	73.81
Driploss	0.98	9.06	82.88
Moisture	1.44	6.51	89.39
Total Adenylate Pool	1.36	6.50	95.89

Biochemical indicators are listed in order of decreasing contribution to the average dissimilarity (contribution %) between fresh and frozen stored flesh up to 95% of accumulated dissimilarity (cumulative %). Dissimilarity ratio shows the dissimilarity between groups divided by the dissimilarity within groups. Dissimilarity ratio >1 = reliable indicator, <1= not reliable. Data was analysed using normalised Euclidean distances.

Univariate analysis revealed that only four of the 16 parameters were not altered with long-term storage (Total lipid, Glycogen, Hypoxanthine, Total Adenylate Pool; Table 4.12). For both treatments, all adenylate values (except hypoxanthine) were significantly different between stored and fresh samples, translating to differences between adenylate ratios (Table 4.12). K value appears to be a good indicator of rocklobster freshness, where fresh samples were below 0.5 units, but significantly increased in short term (Table 4.12: K value = 25) and long-term frozen (K value =39) samples. AEC level was lower in short-term than long-term frozen samples. Flesh pH was variable during storage, increasing significantly over short-term (Table 4.12, pH = 6.8) but significantly decreasing over long-term (pH = 6.46) relative to fresh samples. Long-term frozen samples contained twice as much lactate as fresh and short-term frozen samples (Table 4.12).

Table 4.12 Variation in flesh biochemistry with rocklobster frozen storage.

Mean value (± SE, *n* = 5) for all biochemical descriptors of flesh of short-term (3 month) and long-term (10 month) frozen stored samples of rocklobster. Test statistic: Independent samples T-test for parametric data (denoted as t) and Mann-Whitney U test for non-parametric data (denoted as z). Test statistic values in bold are significant, all others not significant. Subscript values denote *df* for t-test. *p<0.05, **p<0.01 ***p<0.001.

	Short term storage			Long term storage		
Indicator	Fresh	Frozen	Test statistic	Fresh	Frozen	Test statistic
Flesh pH	6.64 ± 0.02	6.80 ± 0.05	**t_8 = 2.88***	6.75 ± 0.06	6.46 ± 0.09	**t_5 = -2.74***
Moisture (%)	74.87 ± 0.74	75.23 ± 0.49	t_8 = 0.41	74.83 ± 0.26	72.90 ± 0.43	**t_7 = -3.61****
Dripless (%)	22.63 ± 2.84	18.11 ± 0.78	t_8 = -1.54	11.29 ± 0.62	20.37 ± 2.69	**t_7 = 2.93***
Total lipid (%)	0.41 ± 0.02	0.37 ± 0.04	t_8 = -0.75	0.69 ± 1.80	0.45 ± 0.02	$t_{3.1}$ = -1.34
Lactate (µmol/g)	2.20 ± 0.21	2.26 ± 0.47	t_8 = 0.12	2.04 ± 0.28	4.21 ± 0.36	**t_7 = 4.57****
Glycogen (µmol/g)	0.11 ± 0.06	0.01 ± 0.01	z = -1.702	0.37 ± 0.20	0.04 ± 0.02	$t_{3.04}$ = -1.68
ATP (µmol/g)	16.64 ± 0.57	9.00 ± 1.40	**$t_{5.29}$ = -5.05****	20.96 ± 3.57	8.75 ± 1.08	**z = -2.45***
ADP (µmol/g)	2.62 ± 0.10	1.18 ± 0.05	**$t_{5.85}$ = -13.42*****	2.66 ± 0.20	1.21 ± 0.03	**$t_{3.12}$ = -7.18****
AMP (µmol/g)	0.17 ± 0.01	9.29 ± 0.96	**z = -2.611****	0.12 ± 0.03	3.48 ± 0.83	**z = -2.49***
Hypoxanthine (µmol/g)	0.05 ± 0.00	0.09 ± 0.02	z = -1.392	0.02 ± 0.01	0.09 ± 0.04	t_7= 1.57
Inosine (µmol/g)	0.01 ± 0.01	7.06 ± 0.43	**z = -2.69****	0.00 ± 0.00	11.01 ± 0.60	**z = -2.56***
IMP (µmol/g)	1.33 ± 0.04	1.82 ± 0.18	**$t_{4.44}$ = 2.67***	1.98 ± 0.14	3.74 ± 0.32	**t_7 = 4.59****
Total Adenylate Pool (µmol/g)	20.81 ± 0.59	28.42 ± 1.32	**t_8 = 5.27*****	25.73 ± 3.79	28.28 ± 1.12	z = -1.23
K value	0.27 ± 0.05	25.20 ± 1.29	**$t_{4.01}$= 19.26*****	0.10 ± 0.04	39.64 ± 3.01	**z = -2.45***
IMP load (µmol/g)	0.07 ± 0.00	0.09 ± 0.01	**z = -2.35***	0.09 ± 0.01	0.30 ± 0.05	**t_7= 3.95****
AEC	0.92 ± 0.00	0.49 ± 0.05	**z= -2.65****	0.94 ± 0.01	0.71 ± 0.04	**$t_{4.15}$ = -6.47****

Sensory analysis

The biochemical differences between treatments were translated to significantly different sensory properties of fresh vs. frozen stored samples. Short-term frozen samples differed from fresh controls both in appearance (Table 4.13a, triangle tests: 12/15 panellists identifying the odd sample) and texture and flavour (11/15 panellists identifying the odd sample). The short term frozen samples were also identified as having significantly less lobster flavour and sweetness, and being pinker and more translucent than fresh samples (Fig. 4.10). Panellists were only able to identify a difference in texture and flavour of long-term vs. fresh samples (Table 4.13b, 11/15 panellists identified the odd sample), where frozen samples had significantly less lobster flavour (Fig. 4.10). Despite these significant differences in sensory attributes, no preference was indicated for fresh or frozen samples for both short and long-term frozen treatments (Table 4.13: preference tests).

Table 4.13 Sensory results for rocklobster frozen storage.

This table presents results from three way triangle tests performed with the sensory panel comparing appearance, texture and flavour and preference tests of fresh flesh relative to either (a) short-term frozen storage (3 months) or (b) long-term frozen storage (10 months).

(a) Short-term frozen	**(b) Long-term frozen**
Three way Triangle Test	
- Appearance 12/15 (p = 0.01)	- Appearance 8/15 (ns)
- Texture and Flavour 11/15 (p = 0.01)	- Texture and Flavour 11/15 (p = 0.01)
Preference Tests	
-"No preference" chosen 3/15	-"No preference" chosen 2/15
-"Fresh" chosen 5/12 times (ns)	-"Fresh" chosen 7/12 times (ns)
-"Frozen" chosen 7/12 times (ns)	-"Frozen" chosen 6/12 times (ns)

Significant $P < 0.05$ and ns = not significant $P > 0.05$. Triangle test significance from binomial distribution tables (British Standard BS ISO 4120:2004). Preference test significance from two tailed preference test probability tables, after excluding no preference responses (Lawless and Heymann 1999).

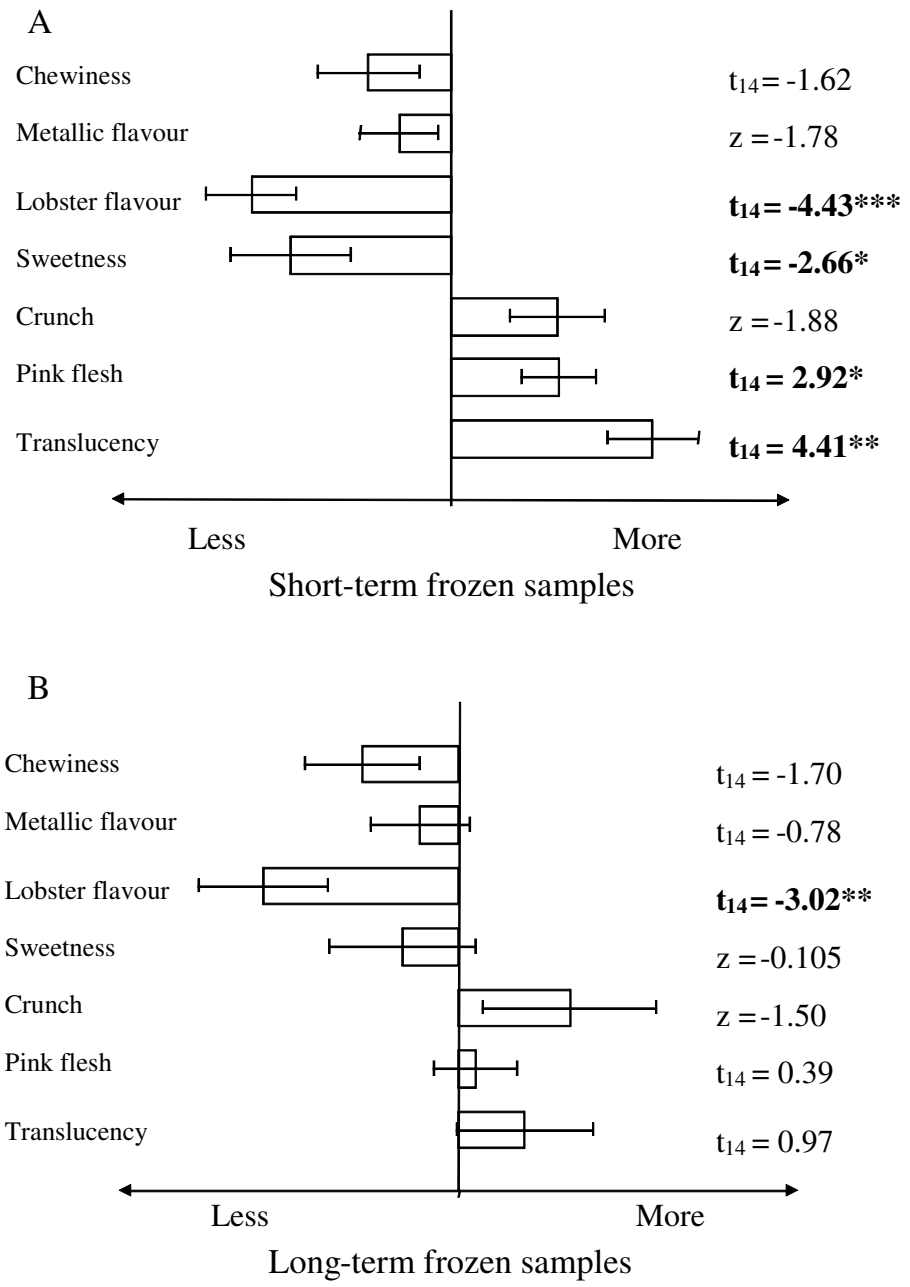


Figure 4.10 Sensory descriptive properties for frozen rocklobster flesh.

The above figure depicts the mean (±SE) distance (mm) of (a) short-term and (b) long-term frozen responses marked on the unstructured line scale when compared to Fresh reference (central line). Statistical tests performed between treatments were Paired samples t-test for parametric data (denoted as t) or Wilcoxon signed-rank test (denoted as z) for non-parametric data. Significance illustrated in bold, where *p<0.05, **p<0.01 ***p<0.001.

Evaluation of rocklobster flesh by a Japanese consumer panel

The Japanese consumer panel were mostly able to distinguish between fresh and short-term frozen samples based on texture and flavour (Table 4.14a: 9/16 panellists identified the odd sample), but not on appearance. Frozen storage did not result in a consumer preference for fresh flesh (Table 4.14). Alternatively, a significant preference for short-term frozen rocklobster over fresh samples was detected using the triangle test probability tables (9/15). Of the 9 panellists who had successfully identified the odd sample in the prior triangle test, only 3 preferred the fresh sample. The consumer panel also detected a significant difference between short-term (2 weeks) and long-term (18 months) frozen samples, based on texture and flavour, but not appearance (Table 4.14b). This did not translate to a preference for either flesh sample. Further, of the 9 that successfully identified the odd sample based on texture and flavour, only 4 preferred the short-term frozen sample.

Although no descriptors of rocklobster sensory properties were given, the descriptions recorded by the Japanese consumers were very close to those used for the trained panel. There were conflicting responses given for reasons of preference, with the descriptors "sweetness" and "flavour" being used as reasons for choosing both the short and long storage samples. However, "lobster flavour" was only used to describe the fresher samples. Despite this, some panellists commented that the fresh samples had no flavour at all. Of the panellists that preferred the 18 month frozen samples, they did so because of more flavour and taste, which was described as "seaweed", "seawater" and "fishy". Alternatively fresh samples were also preferred by some consumers because they were less fishy.

Table 4.14 Sensory results for Japanese consumer panel.

This table presents results from three way triangle tests performed with the Japanese consumer panel comparing appearance, texture and flavour and preference tests for (a) fresh flesh *vs*. short-term (1 month) frozen storage and (b) ultra short term (2 weeks) *vs*. ultra long-term (18 months) frozen storage.

(a) Fresh *vs*. Short-term	**(b) short-term *vs*. ultra long-term**
Three way Triangle Test	
- Appearance 6/16 (ns)	- Appearance 7/16 (ns)
- Texture and Flavour 9/16 (significant)	- Texture and Flavour 9/16 (significant)
Preference Tests	
-"No preference" chosen 1/16 times	-"No preference" chosen 1/16 times
-"Fresh" chosen 3/15 times (ns)	-"short" chosen 7/15 times (ns)
-"Short- term Frozen" chosen 3/15 times (ns)	-"long" chosen 2/15 times (ns)
-"Short-term Frozen" chosen 9/15 times (significant)	-"long" chosen 6/15 times (ns)

ns = not significant $\alpha > 0.05$, significant $\alpha < 0.05$ using one-tailed binomial distribution tables for three samples (British Standard BS ISO 4120:2004). For preference tests the "no preference" responses were excluded from analysis, as preference tests were conducted using triangle test samples the short term frozen and ultra-long term treatments were not pooled and data analysed using the triangle tests tables mentioned above.

Discussion

Despite investigating many potential sources of variation, very few resulted in significant differences in sensory properties of rocklobster flesh. Of all the potential biological and post-harvest processes that may have been found to influence flesh quality, the most significant differences in sensory properties resulted from post-processing storage. This association is not surprising, having previously been detected with crustaceans both on ice (Bremner 1985; Gomez-Guillen *et al.* 2007) and with frozen storage (Dagbjartsson and Solberg 1971). Irrespective of the length of storage, freezing rocklobster flesh had significant impacts on the sensory properties. The combined evaluation of texture and flavour (triangle tests) showed significant differences between fresh samples and short and long term frozen stored samples (Table 4.13). This is potentially due to frozen samples losing lobster flavour, as indicated by the hybrid descriptive tests (Fig. 4.10). In addition, the hybrid test detected the short-term frozen flesh as more translucent and pinker, corresponding to a significant difference in appearance with the triangle test.

Interestingly, chewiness did not increase in frozen stored lobster flesh, consistent with the previous findings of Dagbjartsson and Solberg (1971). However, their study utilised pre-cooked samples rather than raw product used in this study. It was also interesting to note the same directional trends were observed for all parameters with short and long term freezing (Fig. 4.10). Specifically, a reduction in sweetness and more translucency and pinkness in frozen samples; however these effects were only significant in the short term storage experiment. Importantly the significant sensory differences associated with

frozen storage did not translate to a preference for fresh flesh in either case. Therefore, whilst there are apparent differences in texture, flavour and appearance between fresh and frozen stored flesh, these are not great enough to evoke a panel preference. This has considerable implications for the industry where value-added product may successfully be frozen stored and exported, maintaining characteristics suitable for a premium product for up to ten months.

Test power

The non significant results for descriptive sensory properties in the long term storage treatment may simply be associated with the greater variation in biochemical attributes of the reference (fresh) samples compared to those used in the short-term comparison (Fig. 4.9). Such variation in the reference control samples may have subsequently reduced the ability of panellists to detect significant descriptive sensory differences in the long term frozen experiment. Given that statistical power would be quite low as a result of the high standard error, it could be asked if a larger panel size would have found descriptors such as chewiness and crunch as significantly different. Nevertheless, the significant sensory findings detected in frozen storage will facilitate the provision of samples with known differences in sensory properties for future panel training. This would enable the rejection of panellists who were not able to detect known differences, thereby reducing panel variation without the additional costs and logistical constraints of increasing panel size.

Variation in flesh characteristics within a rocklobster tail

Along with variation with frozen storage, the only other significant sensory results were associated with different sections of the rocklobster tail. Biochemically, the greatest variation between tail sections was attributed to adenylates (Table 4.2). Adenylate energy levels are known to vary within different muscles of *J. edwardsii* (Speed *et al.* 2001) and this is thought to reflect the energy demand and expected use of these muscle groups. This research showed no difference in total adenylate pool along the length of the tail with all samples recording high AEC levels indicating very little nucleotide degradation. However, levels of AMP and ATP varied across tail sections (Table 4.3), where ATP was lowest in the posterior end corresponding with a slight increase in AMP. This finding is consistent with differential energy demand across different tail sections and suggests that, despite being low, nucleotide degradation may occur later in the anterior samples than further down the tail. It cannot be ruled out that the size differences and location of segments may have resulted in differential temperatures between death and sampling, which would be expected to influence nucleotide breakdown.

Previous research has documented no significant differences in texture between segments of the lobster tail (Dagbjartsson and Solberg 1971), and whilst this study supports this finding, both appearance (pinkness) and flavour (lobster flavour) were found to be significantly different in posterior samples (Fig. 4.4). However, as sensory analysis was conducted on different samples than used for the biochemical analysis (owing to availability of tail section flesh), the link between adenylates and decreased

sensory descriptors cannot be conclusive. Decreased lobster flavour was shown above in samples with a greater degree of adenylate breakdown from ATP (Section 3; frozen stored flesh). Given the interesting biochemical results detected between tail sections and the significant sensory results detected, this question would benefit from further research.

Variation seen between rocklobster – Size

One of the most common perceptions of varying flesh texture and flavour is that related to size of rocklobster. Small rocklobsters are reported to have sweeter, firmer and moister flesh than their large counterparts. This perception extends from fishers through to industry processors and exporters. However, this study has shown that very little difference in flesh characteristics exists between large and small rocklobster. Biochemically, flesh from smaller rocklobster was moister (Table 4.5), but there was no definitive difference in sensory descriptors. Specifically, panellists recorded zero difference in sweetness of flesh from large and small rocklobster (Fig. 4.6), which is particularly interesting given the above perceptions. It should be noted that this sensory analysis was conducted on raw samples of rocklobster flesh. This was important to avoid any possible influence of variation in cooking time on the sensory properties of each treatment. It is therefore possible that the strongly held belief that small rocklobster have firmer and sweeter flesh may be associated with variation in cooking time used by commercial and recreational fishermen. Rocklobsters are typically boiled before consumption, and as such, cooking time for smaller rocklobster is much less. In this manner, larger rocklobster may be more prone to over-cooking. Over cooked flesh often

results in a drier, tougher product (Coetzee and Simmonds 1988; Dagbjartsson and Solberg 1971) and may have lead to the misconception that lobster size influences taste.

Variation seen between rocklobster- Stress

The sensory properties of flesh from rocklobster landed in poor condition were assessed with the use of a descriptive panel to quantify any apparent deleterious effect on flesh quality, as has been previously documented (Boyd and Sumner 1973). Despite these *a-priori* expectations, no significant difference in flesh characteristics between poor and lively condition rocklobster was found (Table 4.8). The main difference between these two studies may have been reflected in a difference in starting condition of the poor and lively rocklobsters used. For example, at the time of processing, individuals previously allocated to the "poor" condition category had haemolymph, lactate and pH levels that would indicate recovery from prior stress events (Roberts 2001). It is likely that the lobsters recovered upon being placed in holding tanks over night prior to processing, and therefore may not be reflective of "poor" rocklobster at the time of processing. The lack of discernable difference in sensory properties between "lively" and "poor" flesh should therefore be interpreted with caution. Regardless, current industry practice includes overnight storage of live rocklobster in good quality water. If the above recovery holds true, these results would be a good representation of what would be expected from commercial catch. Whilst prior stress did not conclusively affect sensory properties in this study, it can be speculated that 'poor' individuals that subsequently recover prior to processing are as equally suitable for value added product.

Sensory-biochemical correlations

One of the initial objectives of this research was to ascertain which biochemical properties of flesh may be associated with significant sensory results. Given that detectable sensory differences in triangle tests resulted only from frozen storage (texture and flavour) and different sections of tail (appearance), this is difficult. However, in the cases where a significant sensory response occurred, the use of multivariate biochemical dissimilarity analysis enabled the identification of which biochemical parameters varied most between treatments. Adenylate ratios; AEC and K value (frozen storage) and IMP load (tail sections), contributed greatest variation between treatments (12.36% - 31.35%). It is likely that changes in these parameters specifically may indicate significant differences in sensory properties. This is further evidenced by the lack of detectable differences in sensory properties between treatments where adenylate ratios did not differ (e.g. size and prior stress of rocklobsters). K value has been shown to be a good indicator of freshness (assessed by sensory acceptance) in rocklobster flesh (Yamanaka and Shimada 1996), further validated by the large changes (an order of magnitude) associated with frozen storage and associated sensory responses in this study. In addition, K value recorded the highest dissimilarity ratios with simper analysis (Tables 4.10 and 4.11) indicating K value to be the most reliable indicator of changes with frozen storage. It follows that K value may be a good indicator of potential sensory differences with storage.

Appearance of flesh was characteristically different between treatments in both the tail sections and short-term frozen experiments (triangle tests). The hybrid descriptive tests

identified flesh pinkness as a potential correlate with significant implications in both comparisons. The incidence of pink flesh has been previously reported for *J. edwardsii* (Nelson *et al.* 2005) and is likely to be a result of high astaxanthin pigment levels. Astaxanthin is an important carotenoid in crustaceans for physiology (Linan-Cabello *et al.* 2002) and is also known to be deposited in the flesh of finfish from dietary sources (Bjerkeng *et al.* 1999). Further Musgrove (2001) reported increases of Astaxanthin in rocklobster haemolymph towards the later stages of intermoult to early post-moult, as sampled in this study. It is likely that pink flesh will be seen in processed rocklobster product. Based on conversations with the Japanese panel the characteristic of pink flesh has the potential to be desired by some consumers.

Evaluation of rocklobster flesh by a Japanese consumer panel

Results of the Japanese consumer panel complemented those of the trained descriptive panel. Significant texture and flavour differences between fresh and frozen stored samples were detected by the panel (9/16 consumers), regardless of length of storage. The only significant panel preference was for short term frozen samples over fresh samples. Interestingly, there were eight panel members that preferred long-term frozen samples over short term frozen samples. These panellists reported a “fishy” and “seaweed” flavour, with one panellist concluding that it was the fresher sample despite being stored for 18 months. Some of these consumer panel members (not all could speak English) described this unique flavour as being similar to that of “umami”; a flavour component used to classify seafood by the Japanese (Lawless and Heymann 1999). It has been suggested that higher levels of glutamic acid and accumulation of IMP and/ or

AMP with storage of rocklobster flesh may result in higher levels of “umami” (Yamanaka and Shimada 1996). Frozen storage trials above showed a similar increase in IMP and AMP (Table 4.12), which may explain the individual preference of long-term flesh in the Japanese panel.

In conclusion, the greatest sensory differences in rocklobster flesh were detected with frozen storage and then between different regions of tail muscle. These differences were associated with the most pronounced biochemical differences, largely reflected by changes in adenylate ratios. Despite expectations, differences in sensory properties did not translate to a preference for the fresher flesh. This was further supported by the Japanese consumer panel. Although both panels correctly identified different flesh treatments via triangle testing, the lack of preference indicates that despite different appearance, texture or flavour, both treatments were acceptable to some panellists.

CHAPTER FIVE

Tank holding of Southern Rocklobster, *Jasus edwardsii*: effects on flesh biochemistry and sensory attributes.

Abstract

In order to match year-round demands of Southern Rocklobster (*J. edwardsii*) with the limitations of a seven month fishing season some processors have started to hold rocklobster through the closed period of the commercial fishing season. It is critical for industry that effects of tank holding on the biochemistry and sensory characteristics are determined. Whereas previous holding experiments with *J. edwardsii* have utilised sea-cages and focused on lobster growth, survival and condition, I used land based recirculating systems to assess impacts on flesh characteristics. The land based tanks are comparable to those tanks currently used for holding rocklobster commercially prior to live export. Rocklobsters were tank-held for a short-term period (1 month) and biochemical comparisons made between fed and not-fed treatments. Further, flesh from long-term (4 month) tank-held and fed individuals was compared to that of fresh wild-caught rocklobsters, using biochemistry and sensory analysis. Short-term tank-held rocklobsters did not differ in any of the twenty biochemical flesh characteristics tested, regardless of feeding regime. Compared to freshly caught wild rocklobster long-term tank-held fed rocklobsters had significantly lower levels of hypoxanthine and inosine, contributing to a lower K value (normally associated with fresher flesh). Despite these differences, no significant sensory differences were detected between long-term tank-held (fed) and wild caught rocklobster by a trained sensory panel. The lack of detectable sensory difference between treatments has positive implications for the industry,

supporting the viability of holding rocklobster across the closed part of the season. Thus, similar flesh charactersitics can be expected from rocklobsters held in tanks for up to four months, compared to commercially caught rocklobster.

Introduction

The commercial fishery for the Sothern Rocklobster *J. edwardsii* is a large fishery in South Australia, contributing approximately $80 million annually to the local economy (EconSearch 2005). This fishery is subject to a closed season that extends to roughly seven months of the year, limiting the scope for year round supply to South Australian rocklobster processors. In order to meet the demands of a fluctuating market throughout the fishing season, processors store rocklobsters in land based re-circulating tanks for periods of up to two weeks without feed (*pers. obs.*). There exists the potential to use these existing systems to hold rocklobster for the duration of the closed part of the season. While the majority of South Australia's rocklobster catch is currently exported live, there is growing commercial interest in the processing of rocklobsters into value-added portions and packaged products. This has resulted in interest focused towards the use of existing land-based recirculating holding systems to hold lobster, in good condition, through the extended closed season, thus facilitating consistent supply. However, it is not presently known how long rocklobster can be held without feed and maintain good flesh quality. Previous studies have shown small changes in abdominal moisture content and lipid content of flesh associated with starving in *J. edwardsii* (Bryars and Geddes 2005; McLeod *et al.* 2004). However, it has not been established at

what stage these changes become significant in terms of sensory properties, and requires further investigation.

To date, experiments on feeding and holding *J. edwardsii* have focussed on rocklobster grow out utilising sea-cages to assess rocklobster growth and survival (Bryars and Geddes 2005; Hooker *et al.* 1997; Jeffs and James 2001). However, the concept of value-adding the South Australian catch by enabling strategic marketing and product enhancement during the closed season (via live holding) was addressed and recommended by Bryars and Geddes (2005). Four diets were trialled and all were successful in keeping lobsters alive, promoting growth at moult and maintaining or improving condition. Despite this apparent adaptation to diets in captivity and suitability of *J. edwardsii* for extended holding, routine feeding has not been commercially adopted. The advent of new value-added products may see this change in the future.

The majority of the current published literature using sensory analysis on rocklobster has focused on the effects of storage temperature and preservative treatments on flesh characteristics (Bremner and Veith 1980; Gomez-Guillen *et al.* 2007; Perez-Won *et al.* 2006; Yamanaka and Shimada 1996). These studies have shown that rocklobster sensory and biochemical properties are dynamic with many changes during storage (discussed in Chapter 4). However, little research has been directed at comparing rocklobster sensory properties independent of storage effects. Only one research paper has looked at the sensory and flesh properties of lobster, comparing wild caught samples to captive rocklobsters that had been fed for 120 days (Nelson *et al.* 2005). Despite finding small

biochemical differences in lipid and fatty acid composition, no significant differences were detected by the sensory panel between wild and tank held rocklobster on these diets. However, that study may have been limited by the use of an untrained sensory panel.

In this study, I assessed the biochemical composition of rocklobster flesh, from short-term (1 month) and long-term (4 months) tank-held rocklobsters. In addition, I have used robust and comprehensive sensory analysis techniques with a trained panel (see Chapter 2) to investigate the potential effects on the sensory flesh characteristics of rocklobster.

Methods

Rocklobster sampling

Rocklobsters were held in re-circulating holding tanks in June 2004, end of 2003/2004 season, and maintained at 14^{o}C at SARDI Aquatic Sciences, West Beach, Adelaide SA. Individually tagged (cable tie around base of antennae) rocklobsters were weighed at Ferguson Ltd the start of the experiment and split into two treatments, either fed or not fed (n=28). Of most importance was to maintain product quality with minimal input costs, and as a result, octopus flesh caught by the rocklobster boats was identified as a cheap and readily available dietary source (Ferguson. A, *pers. comm.*). Rocklobsters in the fed treatment received 1-2% of their biomass in 2cm skinned octopus tentacle twice weekly. Each treatment was placed in divided 2 x10 x1m tanks maintained at 14^{o}C.

After 1 month, eight rocklobsters from each treatment (fed and not-fed) were processed for biochemical analysis. Due to increasing rates of cannibalism in the unfed treatment, the unfed treatment was discontinued after 2 months. After four months the fed treatment was compared to fresh wild samples from the start of the following fishing season. These fresh rocklobsters had been transported under the same protocols as the tank-held individuals prior to sampling.

Body weight and length was recorded from all lobsters at the time of processing (at Ferguson Australia Pty Ltd). Analysis of pH and refractive index, as well as moult stage was undertaken on each rocklobster before drowning. Haemolymph was collected from the pericardinal sinus by 2ml syringe inserted posterior of the cephalothorax. A separate needle and syringe was used for each rocklobster. Refractive index was measured by placing the fresh haemolymph on a refractometer (refer Chapter 2 for detail). Moult staging was conducted using shell rigidity and light microscope analysis of the second right (dorsal view) pleopod for developing cuticle and setae (Musgrove 2000).To avoid possible biochemical and sensory differences associated with cooking between individual samples and because current value-adding processes in this industry are focussed on raw uncooked packaged lobster, it was decided to conduct sensory and biochemical analysis on raw product.

Biochemical Analysis

A further seventeen biochemical parameters were measured for flesh from each rocklobster following methods outlined in Chapter 2. These were; flesh pH (fresh and frozen stored), moisture content, driploss, total lipid, muscle lactate and glycogen, an array of adenylates (ATP, ADP, AMP, IMP, hypoxanthine, inosine, total adenylate pool) and adenylate ratios (K value, IMP load and AEC).

Sensory analysis

A trained sensory panel of 15 members was established in the Food Science Division of Regency TAFE, Adelaide South Australia. Panellist training and selection involved comparing standard samples of rocklobster (usually large, red males), with complex methodology, as detailed in Chapter 2. Once training was complete, the panel was presented with samples from each of the experimental treatments; in this case comparing wild caught (October 2004) large red males with equivalent rocklobster that had been held and fed in tanks for 4 months. The sensory questionnaire and methods are detailed in Chapter 2.

Statistical analysis

Independent Samples T-Test (where data conformed to the assumption of normality: Kolmogorov – Smirnov) or non-parametric Mann-Whitney U test was used to compare biochemical parameters between treatments (short term fed *vs*. not-fed and tank held (fed) *vs*. wild caught).

Sensory descriptive analysis was assessed using paired-samples T test. For each of the seven descriptive scales, the mean panel results for the reference 'A' sample (wild caught) were compared to the mean for the second 'B' sample (tank held). Where the assumptions of normality were not met a Wilcoxon Signed Rank test was used. Sensory Triangle tests were analysed using probability tables (British Standard BS ISO 4120:2004).

RESULTS

Survival and growth of tank held rocklobster

Rocklobsters for all treatments were between 2 and 3kg mean weights (Fig. 5.1a). Rocklobsters in the feeding treatment were fed using an industry recommended rate of 1-2% of tank biomass fed twice weekly. Despite this feeding ration, all tank held treatments lost mean weight compared to initial weights recorded at the start of this experiment (Fig. 5.1b). During the first month in holding tanks both the fed and not fed treatments lost one rocklobster each to cannibalism. No cannibalism or mortality was recorded in the fed treatment between months 1 and 2. However, the non fed treatment recorded four mortalities, all with evidence of cannibalism. For this reason, the non-fed treatment was terminated at two months. Of all rocklobsters sampled, six of wild caught and two of tank-held fed rocklobsters were in D0' premoult stage, and the remaining in C4 intermoult.

Tank held rocklobster for 1 month, fed vs not fed

Biochemical flesh properties did not significantly differ between fed and not-fed rocklobsters held for one month (Table 5.1, Fig. 5.1). Haemolymph refractive index was higher in fed rocklobsters, although not significant (Table 5.1; $t = 1.896$). Total lipid content (i.e. fat content) was lower with the fed treatment (Table 5.1; t = -2.21).

Tank held fed 4 months vs. wild caught rocklobster

Despite losing an average of 2.34% weight over four months (Fig. 5.1b), tank-held (fed) rocklobsters had very similar biochemical properties to the wild caught rocklobsters at the start of the following season (Table 5.2). Of the twenty characteristics of flesh investigated, only four were significantly different between treatments (Table 5.2). Thawed flesh pH was significantly higher in wild caught than tank-held (fed) rocklobsters (Fig. 5.2f, Table 5.2; $t = -5.7$, $p < 0.001$). Wild caught rocklobster maintained flesh pH around 6.7 (Table 5.2) for both the fresh and thawed flesh samples, whereas pH decreased for tank-held (fed) individuals after frozen storage (Fig. 5.2a). Muscle hypoxanthine, inosine and K value were significantly different between wild caught and tank-held (fed) rocklobsters (Table 5.2 and Fig. 5.3), with wild caught rocklobster showing higher levels of hypoxanthine and inosine, despite having similar levels of ATP, ADP and AMP (Table 5.3). Rocklobster flesh appears consistent across all other characteristics. Total lipid content of tank-held (fed)individuals did not differ from wild rocklobster (Table 5.2). Similarly, total driploss remained at approximately 10% of initial flesh weight lost for both treatments (Fig. 5.2h).

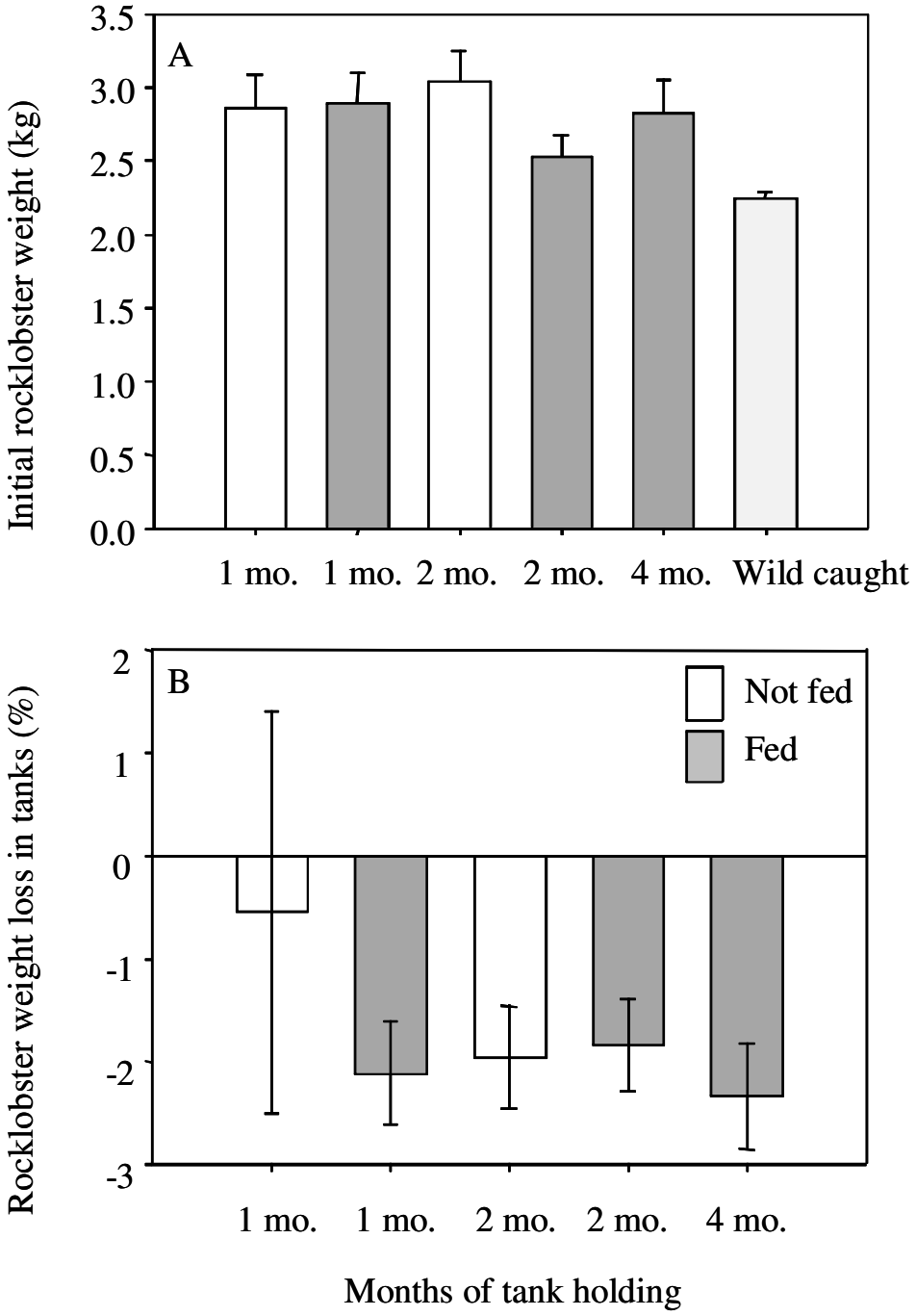


Figure 5.1 Tank held rocklobster weights

(A) Mean weight of rocklobsters (n = 8) for each treatment and (B) mean weight loss from being placed in tanks to time of processing for each treatment. Effects on the weight of rocklobsters after holding in land-based tanks with feed (filled bars) and without feed (unfilled bars) for up to four months (denoted as mo. on x-axis).

Table 5.1: Variation in flesh biochemistry with Fed vs. Not fed rocklobster.

Mean (± SE) values for biochemical descriptors of flesh from Fed *vs.* Not-fed (n = 8) rocklobsters after one month of tank holding. Statistical tests performed between treatments were Independent samples T-test for parametric data (denoted as t) or Mann-Whitney U test (denoted as z) for non-parametric data. ns = not significant where P>0.05.

Indicator	**Not Fed** **Mean ± SE**	**Fed** **Mean ± SE**	**Statistic**	**Significance**
Percent weight change %	-0.56 ± 1.95	-2.11 ± 0.50	z = -0.16	ns
Haemolymph colour	1.42 ± 0.15	1.5 ± 0.00	z = -0.63	ns
Refractive Index	1.352 ± 0.001	1.355 ± 0.001	t = 1.90	ns
Haemolymph pH	7.24 ± 0.02	7.24 ± 0.02	t = 0.14	ns
Flesh pH (fresh)	6.83 ± 0.02	6.77 ± 0.07	t = -0.59	ns
Flesh pH (thawed)	6.91 ± 0.03	6.78 ± 0.08	t = -1.42	ns
Moisture %	75.15 ± 0.66	74.77 ± 0.23	t = -0.55	ns
Driploss %	9.92 ± 0.62	10.92 ± 0.92	t = 0.90	ns
Total lipid %	0.20 ± 0.02	0.15 ± 0.01	t = -2.21	ns
Muscle Lactate (µmol/g)	2.39 ± 0.18	3.22 ± 0.39	t = 1.95	ns
Muscle Glycogen (µmol/g)	0.59 ± 0.10	0.55 ± 0.08	z = -0.24	ns

Table 5.2. Variation in flesh biochemistry with Tank-held vs. Wild rocklobster.

Mean (± SE) values for all biochemical descriptors of flesh from tank-held (fed) *vs.* wild caught (n = 8) rocklobsters. Test statistics between treatments were Independent samples T-test for parametric data (denoted as t) or Mann-Whitney U test (denoted as z) for non-parametric data.

Indicator	**Tank-held Mean ± SEM**	**Wild caught Mean ± SEM**	**Statistic**	**df**	**Sig.**
Refractive Index	1.359 ± 0.00	1.356 ± 0.00	t = 1.892	12	ns
Haemolymph pH	7.33 ± 0.03	7.28 ± 0.04	t = 1.149	12	ns
Flesh pH (fresh)	6.54 ± 0.05	6.71 ± 0.06	z = -1.791		ns
Flesh pH (thawed)	6.34 ± 0.04	6.73 ± 0.06	t = -5.654	12	***
Moisture %	73.96 ± 0.33	73.86 ± 0.32	t = 0.216	12	ns
Driploss %	10.73 ± 0.90	10.37 ± 0.76	t = 0.312	12	ns
Total lipid %	0.42 ± 0.01	0.47 ± 0.02	t = -1.901	8.32	ns
Muscle Lactate (µmol/g)	2.02 ± 0.21	1.96 ± 0.27	z = -0.384		ns
Muscle Glycogen (µmol/g)	1.09 ± 0.20	0.73 ± 0.18	t = 1.354	12	ns
IMP (µmol/g)	1.56 ± 0.27	1.52 ± 0.45	t = 0.078	12	ns
ATP (µmol/g)	21.93 ± 0.62	21.52 ± 1.45	z = -0.447		ns
ADP (µmol/g)	2.19 ± 0.19	2.37 ± 0.54	t = -0.322	7.52	ns
AMP (µmol/g)	0.25 ± 0.09	0.53 ± 0.27	z = -0.705		ns
Hypoxanthine (µmol/g)	0.17 ± 0.09	0.52 ± 0.18	z = -2.24		*
Inosine (µmol/g)	0.00 ± 0.00	0.70 ± 0.34	z = -2.241		*
Total Adenylate Pool (µmol/g)	26.11 ± 0.39	27.16 ± 0.94	t = -1.033	12	ns
K value	0.68 ± 0.34	4.56 ± 1.24	z = -2.62		**
IMP Load (µmol/g)	0.06 ± 0.01	0.06 ± 0.02	t = 0.000	12	ns
AEC	0.94 ± 0.01	0.93 ± 0.03	z = -0.196		ns

*$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = not significant,

Figure 5.2 Biochemistry of wild caught and tank-held (fed) rocklobster

Mean (± SE) values for biochemical flesh characteristics of wild-caught (*n* = 8, hatched bars) and tank-held (fed) rocklobsters fed for four months (*n* =8, filled bars). A) =Haemolymph colour, B) = Haemolymph refractive index C) = Haemolymph pH, D) = Flesh total lipid, E) = Flesh pH (fresh), F) = Flesh pH (thawed), G) = Flesh moisture content, H) = Flesh driploss, I) = Muscle lactate and J) = Muscle glycogen. Lower case letters denote significant differences.

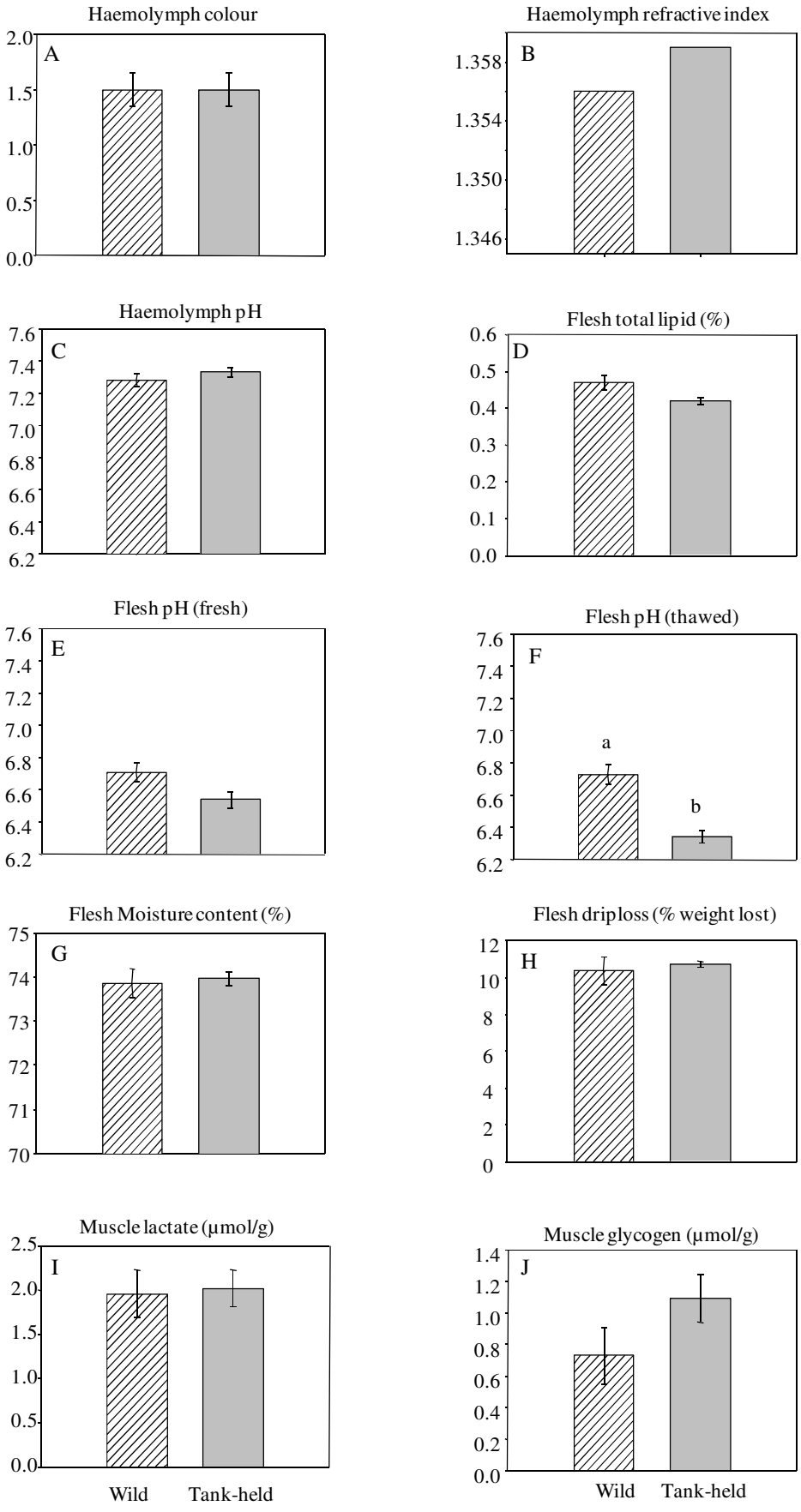
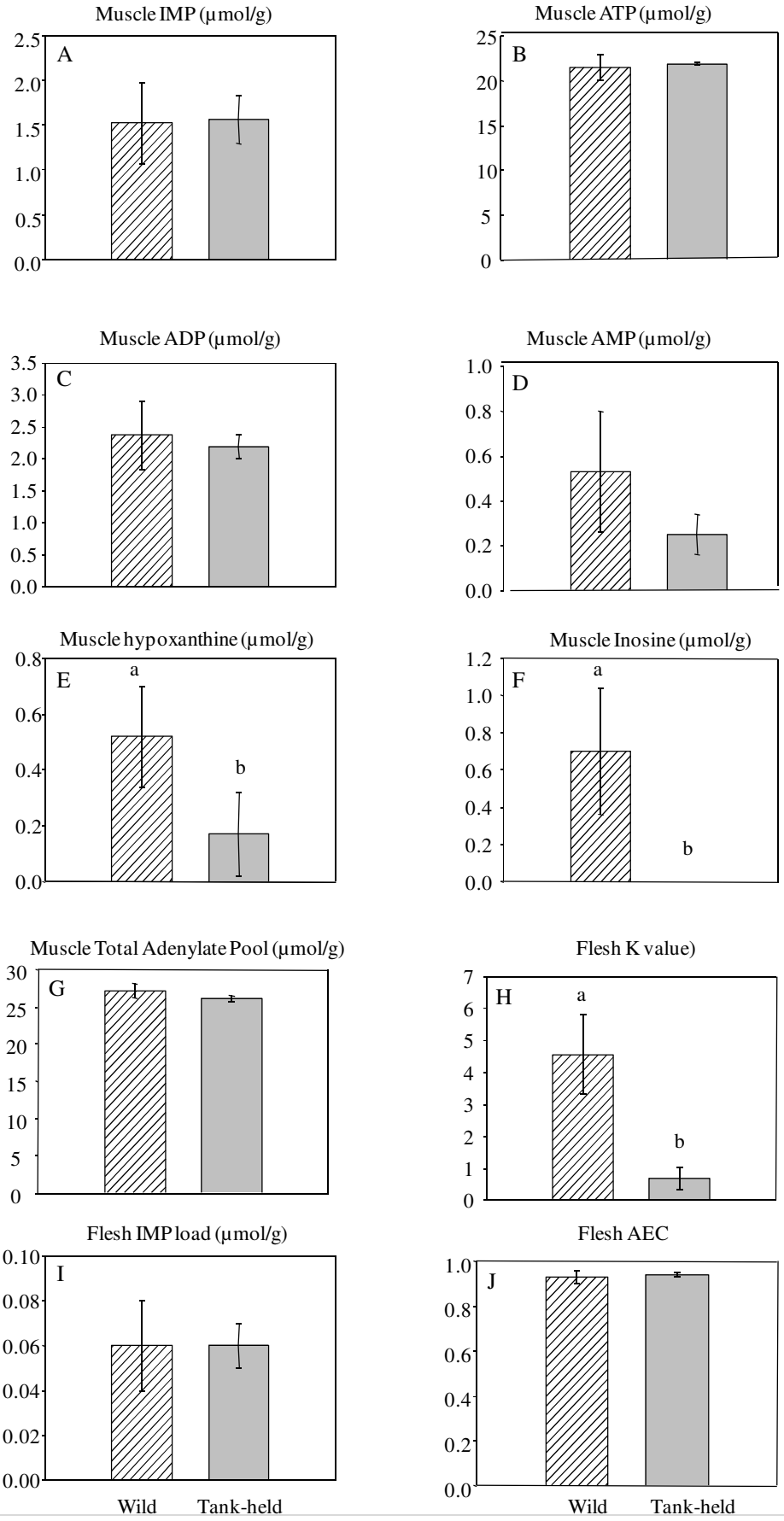

Haemolymph colour
A
2.0
1.5
1.0
0.5
0.0
Haemolymph refractive index
B
1.358
1.354
1.350
1.346
Haemolymph pH
C
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
Flesh total lipid (%)
D
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Flesh pH (fresh)
E
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
Flesh pH (thawed)
F
a
b
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
Flesh Moisture content (%)
G
75
74
73
72
71
70
Flesh drip loss (% weight lost)
H
12
10
8
6
4
2
0
Muscle lactate (µmol/g)
I
2.5
2.0
1.5
1.0
0.5
0.0
Wild
Tank-held
Muscle glycogen (µmol/g)
J
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Wild
Tank-held

Figure 5.3 Biochemistry of wild caught and tank-held (fed) rocklobster (cont.).

Mean (± SE) values for adenylate flesh characteristics of wild-caught (n = 8) and tank-held (fed) rocklobsters (n =8). A) = Muscle IMP, B) = Muscle ATP, C) = Muscle ADP, D) = Muscle AMP, E) = Muscle hypoxanthine, F) = Muscle Inosine, G) = Muscle total adenylate pool, H) = Muscle K value, I) = Muscle IMP load and J) = Muscle AEC. Lower-case letters denote significant differences.


Muscle IMP (µmol/g)
A
Muscle ATP (µmol/g)
B
Muscle ADP (µmol/g)
C
Muscle AMP (µmol/g)
D
Muscle hypoxanthine (µmol/g)
E
a
b
Muscle Inosine (µmol/g)
F
a
b
Muscle Total Adenylate Pool (µmol/g)
G
Flesh K value)
H
a
b
Flesh IMP load (µmol/g)
I
Flesh AEC
J
Wild
Tank-held
Wild
Tank-held

Sensory analysis

Both rocklobster treatments (wild caught *vs.* tank-held) were processed and stored in exactly the same manner prior to presentation to the sensory panel. The high value of the AEC (i.e. close to 1.0) and low K values indicate that all samples were still fresh (Table 5.2). Based on appearance of flesh samples, the three way triangle test resulted in 8 out of the 15 panellists detecting which of the three samples was different (Table 5.3). This was not found to be significant using an α-risk of 5% ($P<0.05$), which would indicate a moderate difference between samples (British Standard BS ISO 4120:2004). Based on the flavour or texture properties, seven of the 15 panellists correctly identified the different sample, which was also not significant for indicating a difference between treatments. Five of the 15 panellists had no preference between samples. Eight panellists preferred the wild caught over the tank held option. Only two panellists preferred the flesh from tank-held lobsters. Given the high number of panellists that had no preference, the panel had no overall significant preference for either sample using binomial probability calculations for detecting a significant preference (Lawless and Heymann 1999). The minimum value for a significant preference was 9 out of the 10 panellists (Lawless and Heymann 1999).No significant difference was detected by the panellists for any of the sensory descriptors using the descriptive scale (Fig. 5.4). A non significant trend indicated flesh sweetness and lobster flavour were rated higher for the tank-held (fed) treatment. The wild treatment had less metallic flavour on average, but this was also not found to be significant.

Table 5.3 Sensory results for rocklobster tank-held (fed) rocklobster.

This table presents results from three way triangle tests performed with the sensory panel comparing appearance, texture and flavour of flesh and preference tests of flesh from tank-held (fed) and wild caught rocklobsters.

Three way Triangle Test
- Appearance 8/15 (ns)
- Texture and Flavour 7/15 (ns)
Preference Tests
-"No preference" chosen 5 times out of 15
-"Wild" chosen 8/10 times (ns)
-"Tank held" chosen 2/10 times (ns)

Significant $P < 0.05$ and ns = not significant $P > 0.05$. Triangle test significance from binomial distribution tables (British Standard BS ISO 4120:2004). Preference test significance from two tailed preference test probability tables, after excluding no preference responses (Lawless and Heymann 1999).

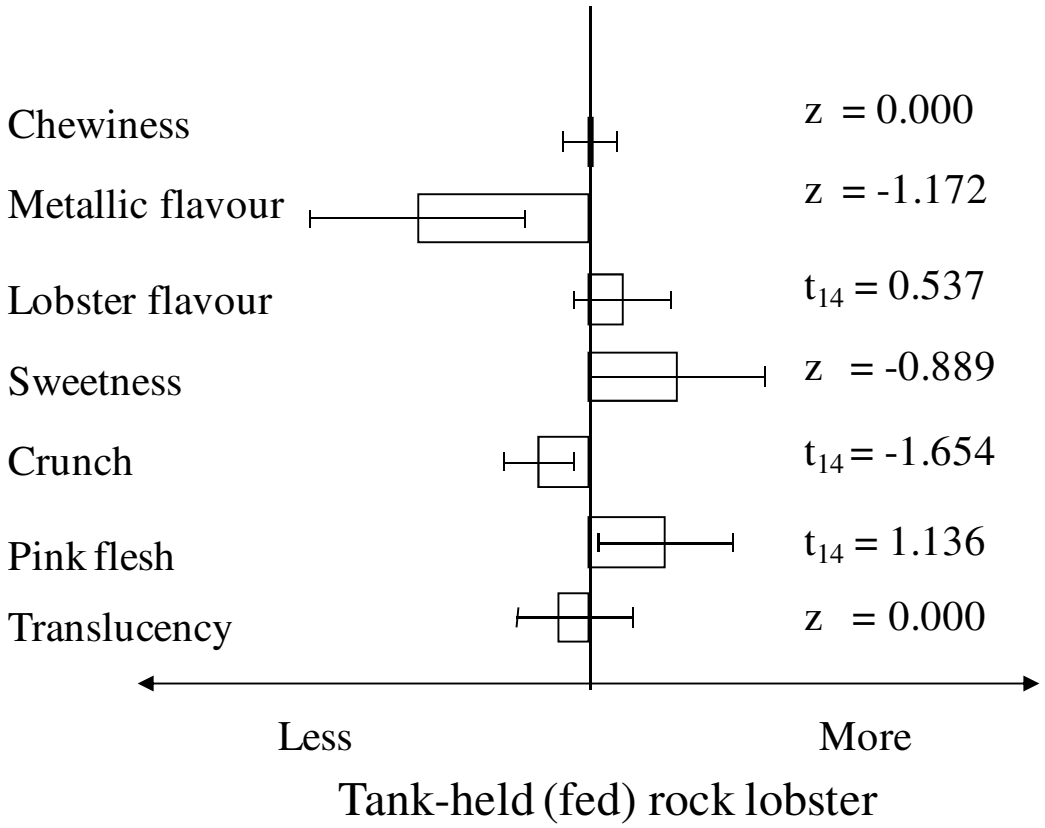


Figure 5.4 Sensory descriptive properties for wild *vs* tank held rocklobster.

The above figure depicts the mean (±SE) distance (mm) of tank-held (fed), marked on the unstructured line scale, when compared to wild caught rocklobster reference (central line). No significance difference for all tests, $p > 0.05$. Statistical tests performed between treatments were Paired samples t-test for parametric data (denoted as *t*) or Wilcoxon signed-rank test (denoted as *z*) for non-parametric data.

DISCUSSION

This study supports the option for holding rocklobsters in tanks for up to four months, with feeding on cheap octopus by-product, to facilitate out of season market supply. Holding rocklobster through the closed part of the season will require feeding. Although there was an incidence of cannibalism in both the fed and not fed treatment in the first month, the subsequent month showed a dramatic increase in cannibalism in the non fed treatment. It is therefore unlikely that not feeding tank-held rocklobster for two months or longer would be commercially viable. Analysis of flesh samples from fed, not fed and wild caught rocklobster, revealed little difference in biochemical characteristics between all these treatments.

Dall (1974a) reported an increase in tail flesh moisture content for the western rocklobster *Panulirus cygnus* (previously *P. longipes*), from 74.3% in a fed treatment, to 76.6% in a non fed treatment over 1 month. In contrast, no significant difference was detected in tail flesh moisture content between fed and not fed treatments in this study. A similar result was reported for fed and starved *J. edwardsii* over 28 days (McLeod *et al.* 2004), where tail flesh of both treatments had approximately 74% moisture content. Importantly there was no incident of cannibalism as they kept rocklobster separated (McLeod *et al.* 2004). Cannibalism has been shown to contribute sufficient nutritional value in *J. edwardsii*, such that a low ration treatment with higher levels of cannibalism out performed a higher ration treatment with a higher final mean weight (Thomas *et al.* 2003).

Rocklobster flesh is characterized by having low levels of total lipid. The low levels of lipid detected in this study may indicate that either lipid is not the main storage form of energy, or, more likely, the tail flesh of rocklobster is not an important site of energy storage in *J. edwardsii*. Previous research has identified the hepatopancreas of the rocklobster as an important storage organ (Cockcroft 1997; Musgrove 2001). It is likely that any difference in rocklobster energy reserves seen between fed and not fed and wild caught rocklobster may have only been evident in the hepatopancreas. Cockcroft (1997) determined that hepatopancreas lipid content was a better indicator for detecting high and low growth fishing sites than any tail flesh indicators of energy or water content in the South African rocklobster species *J. lalandii*. Musgrove (2001) reported a similar finding in *J. edwardsii*, where tail flesh was a poor predictor of rocklobster nutritional condition, whereas in comparison, differences in hepatopancreas dry weight were associated with differences between fishing sites of high and low growth. Therefore, the affects of the various treatments used in this study may also have only been evident in the rocklobster hepatopancreas. It is also possible that the tail flesh may be buffered by changes in the hepatopancreas.

Cellular energy levels were similar between tank-held (fed) and wild caught lobsters with only the adenylate values inosine, hypoxanthine and K value being significantly different. It is not known why the wild caught rocklobster would have higher values of inosine and hypoxanthine. These values are usually indicative of flesh break down during storage. However, all samples were processed on the same day, using the same protocol and stored together. As wild caught lobsters had recently been through the

postharvest commercial handling chain, it is possible that the formation of hypoxanthine and inosine may be associated with a response to prior stress. Regardless of the apparent differences between treatments, these values are still all relatively minor when compared to storage effects (e.g. Chapter 4). For rocklobster flesh, K value may typically range from 25-40 units after frozen storage (Chapter 3). The slightly higher values seen for the wild caught samples in this study were only 4 units, which therefore is still low in comparison with what is detected with storage. The difference in K value between the wild and tank held rocklobsters is due to the differences in both Inosine and Hypoxanthine (used to calculate the K value). The significant difference may indicate that the wild rocklobster flesh was more prone to adenylate break down. Nevertheless, the similarity in Total Adenylate Pool and AEC value indicate that both wild caught and tank-held flesh had very similar overall adenylate composition.

The levels of ATP measured in the wild caught and 4 month fed rocklobsters (~21 µmol/g) were much higher in anterior tail flesh than previously recorded for this species. Speed et al (2001) reported values of 8.66µmol/g from wild SCUBA caught rocklobsters that had spent a night in on-board holding tanks, and values of 8.6 $\mu molg^{-1}$ were reported for Japanese Spiny rocklobster (Yamanaka and Shimada 1996). Additionally, Speed et al (2001) were able to demonstrate that the levels of flesh ATP declined with stress events with a minimum value of 3.22 μmol^{-1}. Further, *J. edwardsii* that had been fed and acclimatized to holding tanks for 14 days had ATP tail flesh levels as low as 4.65 μmol^{-1} (Morris and Oliver 1999).

The high levels of ATP in this study may be indicative of the difference in post-harvest handling or methods of analysis. Morris and Oliver, 1999, used a test kit for ATP and NADH assay for ADP and AMP. While Speed et al (2001) and Yamanaka and Shimada (1996) used similar HPLC methods as this study. Further flesh lactate levels in Speed et al (2001) were approximately three times higher than recorded in this study, despite use of a similar method for analysis. This may indicate a difference in prior stress between rocklobsters between the different studies.

Moult stage may also influence weight gain. Rocklobsters used in this study were in late intermoult to early pre-moult stage. These individuals were selected at the end of the previous fishing season, so the moult stages present are likely to represent those actually selected for holding over the closed season. As rocklobster progress into the later stages of post moult and early pre-moult, their feeding decreases (Dall 1974b). These rocklobster may have simply had reduced appetites, resulting in a small amount of weight loss. In contrast, other research using this species of rocklobster and a similar size class attempted cage culture with feeding and recorded growth rates of 14.8-18.49% over 7 months (Bryars and Geddes 2005). Some cannibalism was recorded with between 83 and 95% of fed rocklobster surviving the 7 months, although only 65% survival was recorded for a not-fed treatment (potentially influenced by moulting during captivity) (Bryars and Geddes 2005).

Despite reducing cannibalism, the octopus diet at the ration level used in this study was not sufficient to maintain or increase rocklobster weight. This could be an issue considering that rocklobster are valued on a $/kg basis. However, this loss may be offset by the marketing advantages of year round supply. Octopus was used in this study due to its low cost and ease of availability for a company commercially fishing rocklobster. Supplementation with alternative product or manufactured diet may promote growth in these circumstances; however the affect on flesh characteristics would require further investigation.

Some weight loss was recorded for rocklobsters in this study, however, similar flesh characteristics were observed between fed and wild caught rocklobster. The lack of detectable difference in biochemical properties between treatments was reflected in the sensory analysis results, where panellists could not detect any significant difference between flesh samples from tank-held and wild caught rocklobster. The industry was initially concerned as to whether rocklobster flesh would pick up any taints associated with the octopus diet for tank held individuals. If an octopus flavour had been present, the panel would have detected the odd sample of flesh from tank-held fed individuals with the triangle test. Only seven of the 15 panellists were able to do so, based on flavour and texture, which was not statistically significant. Six panellists (of the seven that picked the right sample) had a preference, and all of these preferred the wild caught. Despite these six panellists picking the difference between fed and wild and then showing a preference, these values were not significant in terms of the overall panel response and must be interpreted accordingly.

A similar outcome was found with the descriptive tests, where no difference was detected in any of the sensory descriptors tested. The question of whether or not the panel was sensitive enough to detect a difference cannot be ruled out in cases where no significant response is detected. However, this panel had been trained extensively on the use of the sensory scale and methods, which also meant testing high volumes of raw rocklobster samples. Without the ability to provide samples of rocklobster flesh with known levels for each descriptor, there is little more that can be done. Furthermore, to interpret these results, it is useful to compare this non significant finding to significant findings attributable to frozen storage (Chapter 4). Any potential undetected differences between tank held (fed) rocklobsters and wild caught would be negligible compared to differences in frozen storage time. This is not surprising given the similarity in biochemical properties.

Combining the proximate, biochemical and sensory results shows that feeding octopus to satiation at a ration of approximately 2% (twice) per week does not have any adverse effects on rocklobster tail flesh characteristics. Importantly for the industry, rocklobster can be held through the off season, providing year round product that has the same flesh characteristics of rocklobster caught at the commencement of the new fishing season. In addition this feeding will prevent, or at least limit the incidence of cannibalism in tank-held rocklobster. This research therefore supports the suitability of holding rocklobster across the closed part of the season.

CHAPTER SIX

General Discussion

This thesis investigated key sensory and biochemical characteristics of Southern Rocklobster flesh, using a combination of objective (biochemical analysis and trained sensory panel) and subjective (Japanese consumer panel) methods. Individual rocklobster traits that currently reduce the value of the live export trade were found to have little influence on the characteristics of raw flesh. These biochemical and sensory findings support the use of these undervalued large rocklobster as a processed product, and provides an alternative to discounting through the live export market. In addition, the holding of rocklobsters in tanks and the provision of a year-round supply of consistent fresh product without jeopardising sensory characteristics is possible using existing industry facilities and practices. Finally, this research provides an important step between previous triangle tests and the development of future trained descriptive sensory panels for rocklobster flesh. The key sensory descriptors were not only identified, but significant changes in these descriptors have been established and observed to coincide with biochemical changes within flesh.

Implications for the rocklobster industry

The most overwhelming biochemical and sensory differences detected in rocklobster flesh in this study were due to variations in frozen storage of the portioned product (Chapter 4). Despite this, a Japanese consumer panel found long-term frozen stored rocklobster to be an equally suitable value-added product as short-term frozen samples. This presents a positive outcome for the commercial industry, where frozen rocklobster flesh remains acceptable, even with long-term (18 month) storage.

Additionally, in terms of a fresh product, feeding tank-held rocklobsters through the off-season resulted in flesh characteristics similar to that of the following fishing season (Chapter 5). Year round supply of both fresh and frozen product will have significant benefits for the industry (e.g. purchasing in bulk when prices are low, and keeping production facilities operational year-round). Conversely, the consumer panels acceptance of long-term frozen product; opens the possibility of only processing rocklobster during the commercial season, avoiding the need to hold rocklobster in tanks throughout the off season.

Despite perceptions within the industry that larger rocklobsters have less desirable sensory characteristics, rocklobster size did not significantly alter either flesh biochemistry or sensory properties (Chapter 4). Specifically, smaller rocklobsters are reportedly sweeter; however, neither glycogen levels nor sweetness differed between large and small individuals in this study. Secondly, white shelled rocklobsters are currently discounted for the live trade markets. However, this study showed no biochemical difference between dark red shelled and white rocklobster at the point of processing (Chapter 3), so no sensory analysis was conducted. Therefore, the processing of white rocklobster into value added product may be a practical alternative to discounting.

The effects of batch (day of processing) were substantially greater than the biological factors of shell colour, moult stage and season (Chapter 3), as well as size and poor prior condition (Chapter 4). The potential sources of batch variation could be due to different

post-harvest practices, such as prior stress and origin of capture. For example, stress has been identified to influence both biochemical flesh properties (Speed *et al.* 2001) and physical condition of rocklobster (Roberts 2001). Condition also alters rocklobster flesh sensory properties (Boyd and Sumner 1973). Although poor physical condition (as assessed prior to a brief recovery period) resulted in no difference in either biochemical or sensory properties in this study, the severity of stress is likely to play a part in the alteration of sensory properties. It was evident that the rocklobsters at the time of processing were not exhibiting levels of haemolymph stress indicators (Chapter 4) that have previously been recorded from stressed rocklobster in this fishery (Roberts, 2001). This is likely due to recovery in holding tanks overnight; therefore potential effects on sensory properties from stress could be negated if rocklobster are allowed to recover in good quality water prior to processing (Chapter 4). Prior stress may not be the only factor influencing the variation detected between batches. For example, origin of capture is known to influence rocklobster growth rates (Prescott *et al.* 1997) and flesh composition (Cockcroft 1997; Musgrove 2001). Further research into the effects of batch will need to control postharvest factors in order to distinguish the effects from natural temporal and spatial variation.

Identification of biochemical variation in rocklobster flesh

Despite the commercial interest in flesh quality, the term ‘quality’ is too subjective for research purposes as its ultimate evaluation only comes from consumers of the end product. Their assessment is reliant on subjective perceptions such as expectation, value and preference. These perceptions can be established, but rely on evaluating consumers,

rather than flesh. Therefore, in order to effectively interpret consumer preferences, the starting point for assessment of rocklobster flesh characteristics must be to establish the variability in flesh properties using objective, quantifiable measures. To address this, the variability in processed rocklobster flesh was evaluated using flesh biochemical properties. By following this with the establishment of a trained sensory panel, potential indicators for rocklobster flesh quality have been identified. This study has identified which biochemical changes are associated with sensory differences in flavour and texture, and further, that these same biochemical indicators can vary substantially with factors prior to processing. That is, factors such as batch can have an equally important influence on the flesh biochemistry as factors post-processing (e.g. storage).

To my knowledge, this study is the first application of multivariate analysis program Primer to examine biochemical differences in rocklobster flesh. A multivariate approach allows not only the ability to establish biochemical differences between treatments, but can also determine the specific properties that contribute most to the variability or differences between samples (Clarke and Gorley 2001). The dissimilarity ratio from SIMPER analysis identified the most reliable biochemical indicators for differences between treatments. In this study, adenylates, flesh pH and moisture content were the only biochemical parameters with the dissimilarity ratios greater than one. However it was found that whenever significant biochemical differences in flesh occurred, adenylates were always ranked as the major contributors to the variation. Specifically, K value was a good indicator for detecting differences between fresh and frozen stored product (Chapter 4). This was not unexpected, as K value has been previously identified

as an indicator of storage time in rocklobster flesh (Yamanaka and Shimada 1996). Conversely for fresh comparisons, adenylate energy charge (AEC) was a good indicator of biochemical differences between treatments, specifically suggesting a difference in tail sections which was also detected with sensory analysis (Chapter 4). AEC was also one of the key indicators associated with significant differences in flesh biochemistry between different batches of rocklobster (Chapter 3).

By coupling data from biochemical analysis and the hybrid descriptive tests used in this study, it is possible to speculate about the biochemical properties that were most likely to contribute to sensory perceptions of rocklobster flesh. A decrease in lobster flavour was observed with a decrease in AEC level (Chapter 4). Whilst it is not possible to directly correlate reduced AEC to a reduction in lobster flavour, this finding progresses the level of understanding of rocklobster sensory properties and the possible implications of changes in flesh adenylates in Crustacea. Future studies could investigate a correlation between lobster flavour and adenylate levels by simultaneously analysing these parameters across a range of rocklobster flesh samples. There were no other noticeable links between biochemical and sensory data detected in this study. For example, there was no statistical difference in glycogen levels in short-term *vs.* fresh samples where there was a difference detected for sweetness by the trained sensory panel (Chapter 4).

Analysis of sensory properties of rocklobster flesh

One of the unique aspects of this research was the use of sensory analysis to support in-depth biochemical analysis of flesh characteristics. Whilst we are not yet at the stage of using quantitative descriptive analysis, the hybrid descriptive test developed in this study enabled interpretation of specific descriptive properties that lead to differences in taste, texture and flavour. This test lends itself for development in future investigations, adding strength to the standard triangle tests used in most previous studies; which only detects a difference between samples and cannot establish the key sensory property characterising the difference. In addition, this study has established a difference associated with rocklobster storage and future studies could include this as a method of excluding panellists that are unable to detect this extreme difference between rocklobster flesh samples. The exclusion of panellists in this manner would increase the sensitivity of the panel to detect differences with batch. In instances where resources (number of panel sittings, number of panellists and flesh samples) are not as limited, it would be recommended to use a more standard approach, in particular the paired comparison test (Standard IS0:5495, 2005), which can account for the variability that may exist between control reference samples. Importantly, this standard test compares treatments on a line scale similar that that used in this study (descriptive hybrid test), but follows up with a subsequent comparison of control samples at the same sitting. This allows a correction or allowance for variation within control samples combined with bias within an individual panellist for each comparison. Adoption of this method should increase the sensitivity of the analysis, but doubles the amount of sittings and requires three times the amount of control samples.

Nevertheless, the results from this research indicate that any significant sensory differences not detected by this panel are less pronounced than the differences that were detected with frozen storage (Chapter 4). For example, there was no detected sensory difference with factors such as rocklobster size, and this was backed up by a lack of significant differences in the biochemical properties of the flesh (Chapter 4). Therefore the influence of size is clearly less than the difference evident with even short durations of frozen storage. Using this example, there would be little industry relevance in further investigating a factor such as size using raw product, especially if sold as a frozen product.

Future directions of this research

Three areas of future research to expand on the current study would be to investigate the impact of stress on flesh characteristics, the flesh properties of aquacultured product, and the influence of cooking on alteration of sensory properties of rocklobster. Physical condition (i.e. stress) has been previously reported to influence rocklobster flesh sensory properties (Boyd and Sumner, 1973). This study found no difference in biochemical or sensory properties associated with poor or lively physical condition prior to processing. However, due to the apparent overnight recovery of the lobsters, the question still remains as to the effect of stress at the time of processing. Following on from this, it would be beneficial to establish if the variation detected with batch was a result of prior stress or due to origin of capture.

The finding that tank-held rocklobster can have similar flesh characteristics to their wild-caught counterparts is important for future rocklobster aquaculture. It is not known if the similarity between tank-held and wild caught rocklobster in this study was due to a short-duration of holding or whether the octopus diet was able to produce similar flesh characteristics. Future research should establish if aquacultured product is similar to wild-caught, and if a finishing diet is required.

Cooking of rocklobster has been shown to have substantial affects on sensory properties, where over cooked flesh is drier and has less flavour (Coetzee and Simmonds 1988). The industry perception of smaller rocklobster having sweeter and moister flesh is more than likely linked to variation in cooking between size ranges. Although it was established that size had no influence on flesh characteristics (Chapter 4), to validate the industry perception any future sensory research would benefit from incorporating cooking protocol with the evaluation of size.

Finally, one of the key points of interest from this study is the indication that frozen storage may be associated with improving the flesh quality perception of rocklobster flesh. This was evidenced with a preference for short term frozen product over fresh samples by the Japanese consumer panel. In the long-term storage comparison, half of the preferences were for the sample that had been stored over 18 months (Chapter 4). It has been suggested previously that the break-down of adenylates may improve the flavour of fish (Bremner *et al.* 1988) and produce different flavour properties in rocklobster, including ‘umami’ (Yamanaka and Shimada 1996). Although not tested in

this study, it may be possible that the preferences for the long-term stored rocklobster samples were related to the development of “umami” tasting compounds during storage. Thus the characterization of “umami” should be incorporated into future descriptive tests assessing rocklobster flesh.

Conclusion

Despite numerous potential sources of variation, from biological and post-harvest factors, few sensory differences were detected in commercially processed rocklobster flesh. Importantly, where sensory differences were detected, key biochemical descriptors of rocklobster flesh were also shown to discriminate between samples. By following on from this research with the establishment of a Japanese consumer panel, it was shown that despite a loss of flavour and distinct changes in sensory properties with storage, frozen stored rocklobster may exhibit a positive sensory trait for further marketing. This research is also important for increasing our understanding of flesh biochemistry and possible links with sensory properties of rocklobster flesh. In particular, adenylates appear to be important biochemical indicators of changes in raw rocklobster sensory properties. Finally, this research has addressed some of the commonly held misconceptions, such as poorer flesh quality of large rocklobster, detrimental effects of tank-holding and possible significance of prior stress on flesh quality. The large and white undervalued rocklobsters of Southern Australia appear to be well suited for value added products.

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