2002/238 Rock lobster Post-Harvest Subprogram: quantification of shell hardness in southern rock lobster

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1. Objectives

- 1. To calibrate the rate of change in shell hardness before and after the moult of southern rock lobsters relative to lobster size, sex, region and temperature.
- 2. To identify the region of the exoskeleton that is most suited for measuring hardness.
- 3. To develop a prototype gauge that can be used by industry for measuring shell hardness of lobsters.

2. Non-Technical Summary

OUTCOMES ACHIEVED

This study developed functions to model the moult of southern rock lobster based on durometer readings. A single, rapid durometer measurement can thus be used for predicting the days before or after moult in catch-sampling research applications.

Durometer readings from ten different locations on the shell were tracked through the moult cycle to determine the optimal site for collecting data. We found that the optimal locations were two points along the side of the carapace; readings from these locations were consistent between different operators yet underwent large changes in hardness through the moult cycle. Change in shell hardness through the moult cycle was also calibrated for lobster size, sex, region and temperature although these appeared to have little effect, except at extremes of lobster size. Estimates of confidence limits were produced, which allow uncertainty or risk to be incorporated into analyses that contribute to management of the resource.

Although durometers are used to grade clawed lobsters in North America, we found that they were of limited value for grading individual rock lobsters in processing facilities or on-board fishing vessels. Problems included the sensitivity of metal components to salt water, sampling error from shell irregularity and limits to the range of shell hardness that could be measured. To facilitate the uptake of shell-hardness gauges by industry, the project was extended to include the development of an electronic gauge. This measured compression across the base of the tail and was successful in measuring shell hardness to the precision required by industry. The "squeezometer" was suited for use in processing facilities or on vessels due to its robust construction and resistance to salt water. Lobsters can be graded rapidly so that softer lobsters can be excluded from export consignments, thus improving quality and market reputation. The squeezometer is now being tested on vessels and options for manufacture are being explored.

The hardness of lobster shells changes through the moult cycle and this can affect market price; softer shelled lobsters tend to have lower meat yields and are also less able to cope with longer distance airfreighting, which is required for export to premium Asian markets. Consequently, the elimination of softer shelled lobsters from export product is desirable for maintaining market demand and higher prices. In quota managed fisheries there is also a resource benefit by reducing the landings of softer shelled lobsters as these animals have lower average individual weight, which implies that more individuals are harvested for each unit of quota.

Most lobster fisheries are managed or operated with consideration of the problem of soft-shelled animals, however these systems are currently sub-optimal. Examples of operational efforts to overcome the problem include the use of closed seasons to prevent harvesting when a large proportion of lobsters are soft, and hand grading by processors. Processors are readily able to separate extremely hard- or soft-shelled lobsters by hand but cannot repeatedly grade lobsters that are only slightly soft. People have their own subjective opinion about the boundary between hard- and soft-shelled, which means that it's not possible to develop agreed standards even within a single processing facility. Ideally, fishers, processors and buyers could use a defined and consistent standard so that product could be delivered to an agreed quality, and unacceptably soft animals could be left in the sea until they harden into premium product.

The shell hardness of lobsters can have a profound affect on analyses of data collected for fisheries research and ultimately on the size of commercial catches. Examples of analyses that are influenced by moult stage data include the quantification of growth in crustaceans and exploitation estimates. For this reason, shell-state, as a proxy for moult stage, is usually recorded in research or catch sampling of commercial crustaceans in conjunction with other standard information such as size, sex, or tagging details. Although this data is collected routinely, it tends to be variable and subjective due to regional variation in shell fouling organisms, and most importantly, by variation in how different observers classify shells. What one person calls newly moulted, another will call hard-shelled, even with the use of "standard" guides.

The difficulty in standardising measurement of moult stage has recently had a direct impact on the management of the Tasmanian rock lobster resource. Research to assess the effect of an extended open season in September was intended to provide a measure of the proportion of "soft" lobsters harvested. Results were inconclusive due to differences in opinion between individual skippers and processors of what constituted a "soft" lobster. Similar problems have been encountered in using research data to estimate exploitation or growth – in all cases it would be valuable to be able to objectively measure aspects of the shell related to moulting.

Devices termed durometers have been designed for measuring hardness of materials such as sheet plastics and have been adapted for quantifying shell hardness in other crustaceans. For example, they are used in North America by processors for grading clawed lobsters and by researchers working on snow crab. We tested durometers here for use with southern rock lobsters.

Objectives of the project were directed to the development of routine procedures for the use of durometers by researchers and industry. Comparison of durometer results from different regions on the exoskeleton demonstrated that the best region for taking readings was along the lateral suture on the side of the carapace. Readings from this area were most responsive to the moult cycle and also produced the most consistent results when multiple readings were taken from the same lobsters.

The effect of several factors on durometer readings was also examined; these were lobster size, sex, region and temperature. None of these factors had a quantifiable effect on durometer readings with the exception of lobster size; skewed readings were obtained from very large (>165 mm CL) and very small lobsters (< 90 mm CL). Lobsters of these extreme sizes typically lie outside the ranges sampled in southern rock lobster fisheries research programs.

The change in shell hardness through the moult cycle was quantified by durometer readings and used to develop a calibration curve. This curve provided an estimate of the time before or after the moult of southern rock lobsters based on a durometer reading. In addition, estimates of confidence around these values were also calculated to facilitate uptake of this procedure in catch sampling programs.

Although durometer readings could be collected rapidly and appear of value for catch sampling programs that provide fisheries management information, durometers appeared unsuitable for uptake by industry. This was because individual lobsters could not be graded with high precision – although durometer readings were objective, there was too much variation for perfect grading of individual lobsters. This meant that the current situation on fishing vessels and in processing sheds was repeated, as some lobsters were classed as too soft in one instance but adequately hard when retested. Another problem was that the gauges tended to be susceptible to salt water unless handled carefully.

Whilst recognising that durometers provided a valuable step forward in quantification of shell hardness, we considered that improvements could be made in the design of a gauge for measuring shell hardness. The process of developing an improved gauge initially proceeded by construction of a crude gauge to test the concept of measuring shell hardness by quantifying flex of the tail segments. When viewed in cross-section, the exoskeletons of segments of lobster tails have an arched form that should behave like a spring when flexed. We reasoned that changes in calcification of the exoskeleton through the moult cycle would alter the strength of this spring. One of the advantages of this approach was that a large area of the exoskeleton influenced the reading of shell hardness, which reduced random error. In contrast, durometer readings are measured by deflection of a small, nail-like rod that is pressed against the shell of the lobster. The small surface area contacted by the durometer rod can introduce errors as the surface of lobster shells are uneven and of variable thickness. Results from this initial trial were promising and further development of a gauge was undertaken.

An electronic gauge was subsequently developed that was robust and suitable for use by industry and has been termed a "squeezometer". Lobsters were held against the device, which activated a switch. This caused a small piston to be driven out to the surface of the lobster tail. The piston then moved forward a set distance further and the force applied back by the lobster tail was measured.

The squeezometer collects a series of rapid readings so that it can detect if the shell hardness reading is being affected by movement of the lobster, which could happen if the operator is aboard a rolling vessel. If movement is detected, the reading is extended until a stable reading can be obtained. For research purposes, readings of shell hardness can be relayed and downloaded to computer. For industry applications, the force readings were calibrated and used to illuminate a series of LEDs – the more LEDs illuminated the harder the lobster. This tool has considerable promise in improving the quality of product delivered to market and could also be used for high grading of catch at sea.

Further testing of the squeezometer at sea is underway and we are also investigating options for manufacture.

KEYWORDS: rock lobster, *Jasus edwardsii*, closed seasons, shell hardness, durometer, quality control, squeezometer.

3. Acknowledgments

This project was completed with the help of Debbie Gardner, Josephine Walker and Arron Strawbridge, who conducted data collection and maintenance of animals. Advice and assistance with evaluation of the equipment in a processor environment was provided by Ken Smith of A.J. Garths Seafood and Andrew Lawrie of Sky Seafoods, Robe, SA. Bob Hodgson designed and constructed our first prototype gauge.

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4. Background

The moult stage of commercial crustaceans can have a profound affect on analyses of data collected for fisheries research as it is used in analyses that affect stock assessments and management. Examples of analyses that are influenced by moult stage data include the quantification of growth in crustaceans, and exploitation estimates from techniques such as change-in-ratio. Growth is a particularly important input data for lobster fisheries management as it has a profound effect on assessment modelling outcomes and there is substantial scope for improvement of estimates in all Australian lobster fisheries. Processes such as growth that are associated with moulting are analysed with improved precision where information on moult stage is available. This issue of estimating moult stage affects numerous crustacean fisheries, both nationally and overseas. For this reason, shell state, as a proxy for moult stage, is usually recorded in research or catch-sampling of commercial crustaceans in conjunction with other standard information such as size, sex, or tagging details.

Although moult stage information is collected routinely, its value is often reduced due to subjective error. This is especially a problem in rock lobster and giant crab catch sampling where moult stage is estimated in the field by the degree of shell wear or amount of fouling organisms on the shell. Errors are introduced by regional variation in the organisms creating shell fouling, and most importantly, by variation in how different observers classify shells – what one person calls newly moulted, another will call hard-shelled, even with the use of "standard" guides.

The difficulty in standardising measurement of moult stage has recently had a direct impact on the management of the Tasmanian rock lobster resource. Research to assess the effect of an extended open season in September was intended to provide a measure of the proportion of "soft" lobsters harvested. Results were inconclusive due to differences in classification of what constituted a "soft" lobster – one fisher may consider half of the lobsters captured to be soft, another may consider almost none were "soft", while a processor may consider almost all the catch to be too soft for export. Similar problems have been encountered in using research data to estimate exploitation or growth – in both cases it is often useful to have a measure of whether animals have moulted into legal size in the current season, or previously.

An implication of these measurement errors in estimating moult stage is that the value of expenditure on catch-sampling research is reduced or even wasted. This in turn reduces the ability of managers and industry to make informed decisions on how to regulate harvests – which can have direct financial consequences for the industry.

The ability to quantify shell hardness of lobsters may also have direct application in the fishing industry. In both South Australia and Tasmania, processors/exporters are concerned about the high proportion of recently moulted lobsters coming to their facilities at certain times of the season (up to 30%). These recently moulted lobsters are usually unsuitable for export as they are weak and easily damaged, which can reduce beach price by up to \$10/kg. In addition, cannibalism of softer lobsters within holding tanks can be a significant source of loss. To overcome these problems, processors

request that fishers only retain lobsters of a certain degree of hardness. However, this is currently difficult given the subjectivity of the measurement of shell hardness.

A new, simple and quick solution to the ambiguity of current measures of shell wear is to quantify shell hardness with a durometer, which is a small, hand-held device originally designed to measure the flexibility of plastic sheeting (Figure 1; Hicks and Johnson, 1999). This method of measuring shell hardness is non-destructive and simply involves pressing the gauge against the surface of the shell. A spring mechanism measures resistance and readings are displayed on a dial. The process is very rapid and can be completed within seconds.

This system of standardisation of shell hardness has recently been introduced in Alaskan Dungeness crab fisheries and is also in use for grading of shipments of American lobsters in Nova Scotia, Canada (Pers. Comm. John Garland, Senior Biologist, Clearwater Seafoods, Nova Scotia). Before these devices can be used for Australian rock lobster applications, a series of short experiments were needed to calibrate hardness reading to the moult cycle. These experiments are described here.



Figure 1. Durometer models. Key differences between models are the size of the foot (4.8 mm or 11 mm) and the display system (either vernier or dial). Durometers come in different grades, depending on the hardness of the material to be measured; most crustacean applications are with A or C grade durometers. The two gauges tested in this project are at left, the Rex 1700^{TM} (narrow foot) and Rex 1500^{TM} (broad foot).

5. Need

A simple and quick solution to the ambiguity of current measures of shell wear is to quantify shell hardness with a durometer, which is a small, hand-held device originally

designed to measure the flexibility of plastic sheeting (Hicks and Johnson, 1999). This system of standardisation was recently introduced in Alaskan crab fisheries. Before these devices could be introduced for Australian rock lobster stock-assessment, a series of experiments were required to calibrate hardness readings to the moult cycle.

The calibration was needed to allow durometers to be used for Tasmanian research on the effect of September and November harvests on the mortality of discarded lobsters. In addition, processors in Tasmania and South Australia have expressed interest in developing a tool for use in grading of lobsters for export either at-sea or after animals have been landed. That is, fishers will be able to establish a quantitative shell hardness grade that processor will accept prior to landing of the catch. This would eliminate the current problem of the landing of lobsters that are ambiguously classed as "hard" by a fisher but "soft" by a processor – with resultant negative impacts on economic yield and markets.

6. Evaluation of alternative locations on lobster shells for collecting durometer measurements

6.1 Introduction

This section addresses objective 1: to identify the region of the exoskeleton that is most suited for measuring hardness.

In selecting a location on the exoskeleton to collect sample data, there is a need to balance sensitivity against random variation. That is, highly variable readings collected from a particular location of the exoskeleton of lobsters may be caused by either response to changes in shell hardness through the moult cycle, or simply random error. We sought a location that underwent large changes in shell hardness through the moult cycle and was also possible to measure with minimal sampling error. Possible sources of sampling error included difficulty in precisely defining the sample location, movement of the animal being measured, and irregularity of the shell.

6.2 Methods

We evaluated 10 different locations on the exoskeleton, chosen because they were either relatively flat, which is desirable for collection of durometer data, or because they were areas known to become softer in the lead up to moulting (Figure 2). Locations 4 and 5, which are along the lateral suture on the side of the carapace, become softer in the weeks leading up to moulting. Processors often use this area when hand-grading lobsters for shell hardness (Figure 3).

Each of these 10 locations were evaluated for sampling error, for both repeat measurements by a single operator (three runs), and also single measurements by four different operators. The same lobsters were repeatedly measured in each case using two

different A-scale durometer models (Rex 1700TM and Rex 1500TM). The key difference between these two models is the size of the footplate, which is 4.8 mm in the Rex 1700TM and 11 mm in the Rex 1500TM. Individual lobsters were identified by T-bar tags inserted ventrally into the abdomen and were randomised between samples by an assistant.

Sampling error was measured as the coefficient of variation for repeat measurements of the same animal (Sokal and Rohlf, 1995). This measure scales variation (as standard deviation) to the mean durometer reading so that soft and hard locations could be compared directly. The durometer scale ranges between 0 and 100 with hard samples that are not compressed by the durometer scoring the highest reading of 100. This upper limit presented a right-hand boundary to measurements so that variation was negatively correlated with the mean, which biases the use of coefficient of variation (Kadaba et al., 1989). The solution to this was to convert durometer readings to "durometer displacement", which was calculated as displacement = 100-durometer reading.

This initial experiment was used to refine sampling to 5 locations on the shell that were further evaluated in subsequent experiments involving: (i) sampling of large numbers of animals on a single occasion at a processing factory; and (ii) tracking individual animals through a moult cycle. The 5 locations selected for subsequent experiments were locations 2 and 3 on the abdomen or tail, locations 4 and 5 on the side, and location 7 on the sternum (Figure 2).

Single measurements were collected from animals held in tanks at a commercial processing facility in Robe, South Australia. Sampling was conducted during December, a time when we expected considerable variation in shell hardness between individual lobsters due to the timing of the annual moult. The aim was to evaluate the ability of durometers to detect the variation in shell hardness in these samples of lobsters. Readings were taken at each of the 5 locations on the exoskeleton from at least 200 lobsters and evaluated for variation between individuals. It was considered that locations that tended to have similar durometer readings between different animals would be of less use value for testing shell hardness. Although this experiment quantified variation in durometer readings at each shell location, it was not clear if this reading was due to actual changes in shell hardness, or merely greater sampling error at some locations. This distinction was addressed by the third experiment.

The third stage in selecting locations on the shell for measurement of hardness examined sensitivity to changes in shell hardness through the moult cycle. Five of the original 10 locations (locations 2, 3, 4, 5 and 7; Figure 2) were selected for regular monitoring in a group of animals maintained for several months either side of the main moulting period. A total of 106 male lobsters were successfully maintained through the moult for this component. Locations suitable for measuring hardness would ideally undergo large, consistent change in durometer readings through the moult.

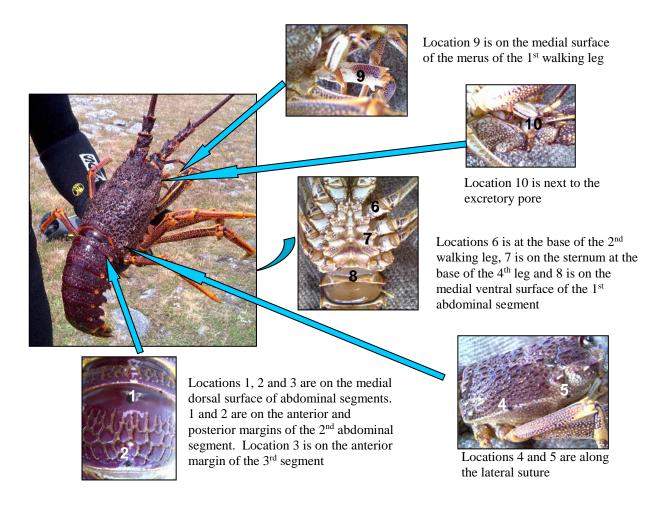


Figure 2. Locations on the exoskeleton of southern rock lobsters *Jasus edwardsii* tested for suitability of collection of durometer data.



Figure 3. Processor testing for shell hardness by squeezing along the lateral suture which runs down the side of the carapace. This region of the carapace was targeted in durometer trials (locations 4 and 5).

6.3 Results and discussion

Estimates of coefficient of variation for repeated measurements of the same animals by either the same or different operators indicated that variation was lowest at locations 4 and 5 (Figure 4). Sampling error with the narrow foot durometer was consistently lower for the single operator test but often higher than the broad foot durometer for the between operator test. All operators stated that they felt the narrow foot durometer gave improved results. The cause of the observed lower sample error from the broad foot durometer at some locations was multiple durometer readings of 100. This exercise also highlighted the need for training of operators in the use of durometers, ideally performed by comparing results from the same series of lobsters.

Sampling of large numbers of individual lobsters at the processing facility indicated that durometer readings from locations 2, 3 and 7 tended to approach the maximum value of 100, which suggests that changes in shell hardness would go undetected unless the shell was quite soft (Figure 5). A greater spread of readings was recorded from locations 4 and 5 which suggested either greater sensitivity to shell hardness (desirable) or greater sampling error (undesirable).

The selection of appropriate shell location was refined further by long-term, repeatedsampling of animals maintained in tanks through the moult cycle. This long-term tracking of shell hardness was conducted for 5 of the original 10 locations: locations 2, 3, 4, 5 and 7. Of these, locations 4 and 5 appeared to have greatest sensitivity to changes in the moult cycle (Figure 6).

Locations 4 and 5 thus appeared to be most suited to monitoring changes in shell hardness. Sample error was lowest at these locations yet durometer readings underwent largest changes before and after the moult. We conclude that the mean of readings from both of these locations should be used for quantifying southern rock lobster shell hardness by durometer.

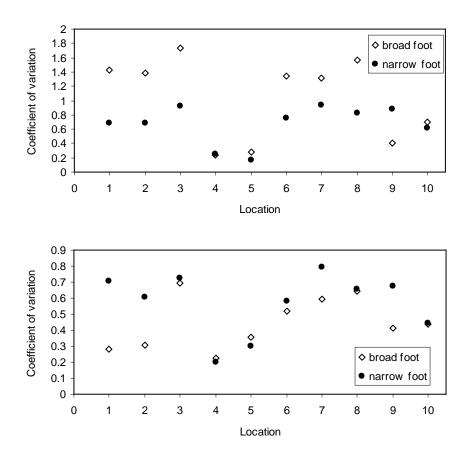


Figure 4. Variation between measurements taken either by the same operator on 3 repeated samples (upper) or by 4 different operators (lower) from different locations on the exoskeleton. Variation is measured as the mean coefficient of variation from 10 or 13 animals (upper and lower respectively) for displacement of durometer readings (displacement = 100 - durometer reading). Measurements were collected with 2 different durometers, one with a 4.8 mm base (narrow), and the other with a 11mm base. A lower value is desirable as it implies reduced sampling error. Note that absolute value cannot be compared between plots due to the difference in number of runs used to estimate variation.

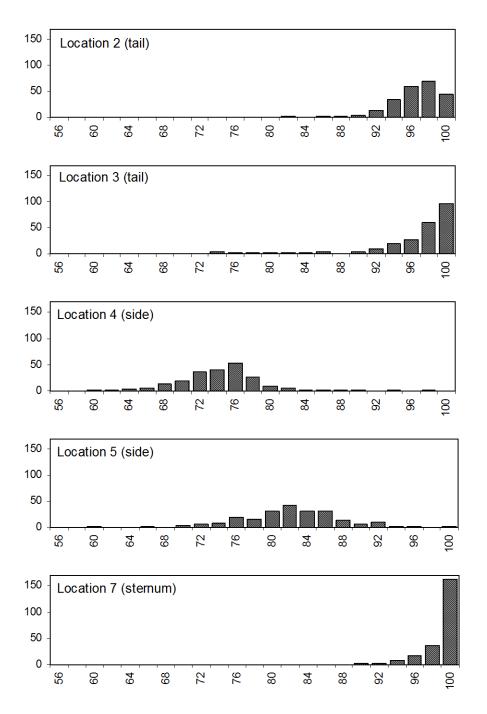
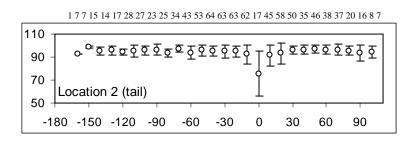
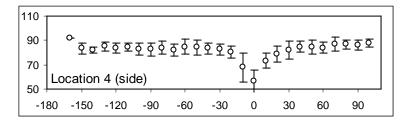
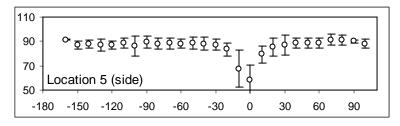


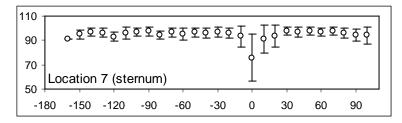
Figure 5. Frequency of durometer readings from samples of least 200 animals measured in December at Robe, South Australia. Greater spread in measurements at locations 4 and 5 indicates either greater sensitivity to shell hardness or greater sampling error.



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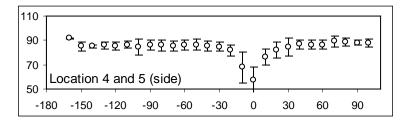


Figure 6. Durometer readings (+/- s.d.) in relation to time before and after moult from each of 5 locations tested on the exoskeleton of southern rock lobsters *Jasus edwardsii*. Number of measurements for each group is shown at top. Data are for repeated measurements of 106 individual animals pooled.

7. Effect of sex, site, size and temperature on shell hardness

7.1 Introduction

This section addresses the second objective of the project: to calibrate the rate of change in shell hardness before and after the moult of southern rock lobsters relative to lobster size, sex, region and temperature. Establishing the bias of calibration for each of these factors is important for applications where the timing of moulting is estimated from durometer readings. This is required where durometers are used to define periods of moulting, such as for setting of closed seasons.

Closed seasons are used as a management tool throughout the range of the fishery for southern rock lobsters although the timing varies across different states and zones. The roles of these closures have included several different management objectives such as reduction of effort and shift of effort from males to females. However, the main fishery closure in late spring is intended to reduce the capture of soft-shelled lobsters (Gardner and Frusher, 2000). Catching soft-shelled lobsters results in (1) reduced meat yield (Tegelberg, 1972), (2) increased discard mortality of sub-legal animals (Dufour et al., 1997), (3) increased handling mortality especially during air freighting, and (4) greater number of lobsters removed from the resource per unit of quota.

Setting of closed seasons to reduce catch of soft-shelled lobsters has historically been based on advice from fishers and processors about the times of year when soft-shelled lobsters would be expected. While this would appear a simple process, and one that would be established after more than 100 years of commercial fishing, the historical pattern of repeated change in timing of closed seasons indicates that consensus on biological patterns has been hard to reach. Thirteen changes have been made to the duration of the late spring closed season since its introduction in 1926 (Gardner and Frusher, 2000).

Among the difficulties in setting closed seasons is spatial variation in timing of moulting so that timing of closed seasons for one area may be unsuitable for another. Timing of moulting also varies from year to year. Compounding these issues is the subjectivity in classification of lobster as soft-shelled when grading by hand. Research that aims to assist in setting of closed seasons to reduce the catch of soft-shelled lobster would thus need to be applicable across a range of areas and for lobsters of different sizes and sex. The influence of these factors on durometer readings is examined in this chapter.

7.2 Methods

Experimental animals were maintained in tanks at two sites, the Marine Research Laboratories of the Tasmanian Aquaculture and Fisheries Institute and the Aquatic Sciences Centre of the South Australian Research and Development Institute. In both cases aquarium facilities were flow-through. In both facilities, lobsters were fed every second day with whole mussels, cockles, chopped squid or prawn pellets. Tanks were covered with black plastic to reduce light and provided with concrete blocks for shelter. Moulting of rock lobsters in Tasmania generally occurs at two times in the year, a male moult in spring (around September) and a female moult in autumn (around May). Experimentation in Tasmania was conducted around each of these periods to track the moult of each sex. In South Australia, moulting occurs in both summer and winter in both sexes so experiments were conducted during the fishing season between November and May. A failure of the thermostat in the South Australian system resulted in most of the animals being lost before moulting occurred, so results presented here rely mainly on Tasmanian data.

Animals of each sex were sourced from different sites (Maatsuyker Island, Bruny Island, Crayfish Point, King Island and Robe) and across a range of sizes (Figure 7). Growth rates varied dramatically between regions so that it was not possible to produce a factorial design with a similar size range of lobsters from each site. For instance, at Maatsuyker Island few females ever reach the legal size of 105 mm CL while at King Island much of the catch of females is above 140 mm CL. Given this constraint, we maintained at least 24 lobsters of each sex from each site broadly distributed across size ranges available from each region.

The effect of temperature was examined by maintaining females in either ambient flowthrough or in water heated by approximately 3°C with immersion heaters. The heated system was also flow-through to ensure that water conditions were equivalent between the two treatments. Ten lobsters were maintained in each treatment.

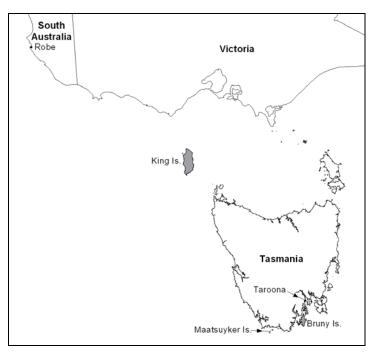


Figure 7. Location of sites where lobsters were collected prior to moulting. The Crayfish Point site was located at Taroona.

Durometer readings were taken at fortnightly intervals and averaged across locations 4 and 5 (Figure 2). Animals were inspected daily to determine day of moulting.

We examined the non-linear fit of durometer reading (Y) as a function of the time before or after moult (X; days) using a range of models including exponential, polynomial, quadratic, cumulative logistic, and hyperbolic. These provided poor fits with the exception of the cumulative logistic $Y = \beta_0 / 1 + e^{\left(-\beta_1 \left(-x - \beta_2\right)\right)}$ or

 $Y = \beta_0 / 1 + e^{\left(-\beta_1 (x - \beta_2)\right)}, \text{ for fits to pre and post moult data respectively, and the hyperbolic } Y = X / \left(\beta_0 + \beta_1 X\right) \text{ (as observed by Hicks and Johnson, 1999 in Dungeness crab).}$

Data were fitted using non-linear least squares in JMPTM and the influence of each of the factors of sex, site, size and temperature examined. 95% confidence limits for model fits were derived from estimates of the 95% confidence limits of each estimated parameter. In the case of the cumulative logistic, where 3 parameters were measured, the lowest model was constructed from the lower 95% confidence limit estimate for parameters β_0 and β_1 and the upper 95% confidence limit for parameter β_2 . This procedure was reversed to estimate the upper 95% confidence limit for the whole model.

7.3 Results and Discussion

7.3.1 Model selection

The logistic model provided the best fit to combined durometer reading data for samples prior to the moult whereas the residuals for the hyperbolic fit tended to be positive in the last 60 days before moulting occurred (Figure 8). Results were less clear in the period after the moult with both models appearing to provide similarly good fits. The logistic model was used for subsequent analyses given the superior fit to data collected from pre-moult animals.

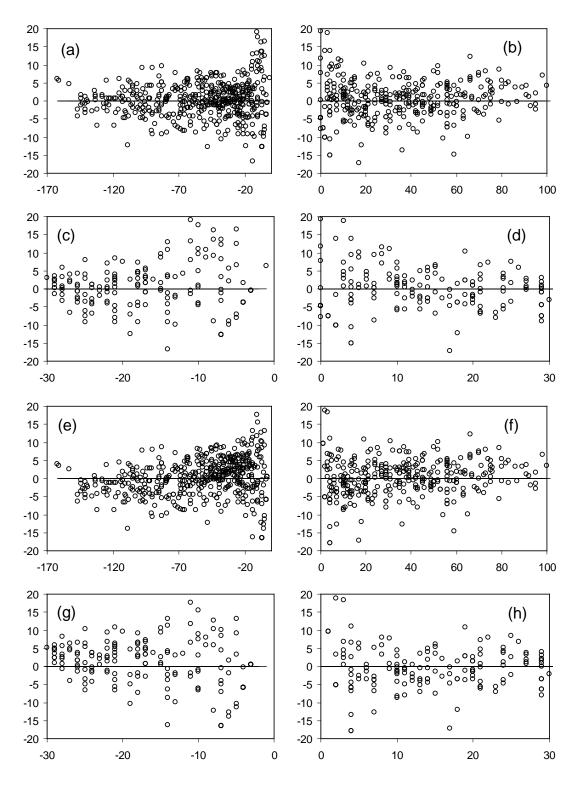


Figure 8. Residuals from the fit of durometer reading (Y) as a function of days since moult (X) of southern rock lobster *Jasus edwardsii*. Plots (a), (c), (e) and (g) are for fits prior to the moult (n=503), while plots (b), (d), (f) and (h) are for fits after the moult (n=354). Plots (a) to (d) are from fits to the logistic function $Y = \beta_0 / 1 + e^{\left(-\beta_1 \left(-x - \beta_2\right)\right)}$ **Error! Bookmark not defined.**(with the X term made positive for fits after the moult) and plots (e) to (h) from fits to the hyperbolic model $Y = X / (\beta_0 + \beta_1 X)$.

7.3.2 Effect of sex of lobster on shell hardness

The pattern of change of shell hardness in male and female lobsters was similar with large overlap in confidence limits (Figure 9). There was a weak suggestion that males harden more rapidly after moulting than females. Most females in this experiment moulted in June 2003, which is later than the normal moulting period of May in animals at the Crayfish Point site (Ziegler et al., 2002). Water temperatures were cooler during June 2003 than in November 2002 (13°C compared with 16°C) when most males moulted, which may account for the weak suggestion of slower rate of hardening by females. The direction of energy towards egg extrusion may also delay shell hardening in females. Neither of these possible influences on the rate of shell hardening had a significant impact on shell hardening and it appears that the durometer readings provide a similar measure of the time before and after moult for each sex. Consequently, the calibration described in Section 7.3.6 did not include the factor of sex of lobster.

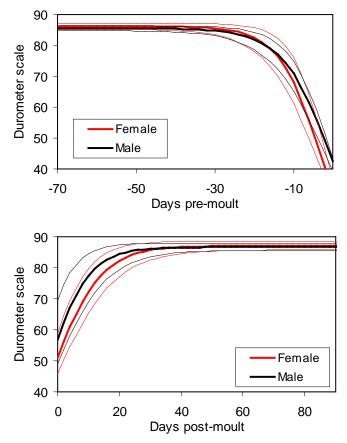


Figure 9. Logistic model fits (+/- 95% confidence limits) to durometer hardness measurements from male and female lobsters collected before (upper) and after (lower) the moult. Pre-moult fits are based on 171 and 341 samples from females and males respectively, and post-moult fits are based on 119 and 235 samples from females and males respectively.

7.3.3 Effect of temperature on shell hardness

Low temperatures are thought to slow calcium uptake and prolong shell hardening (Taylor et al., 1989) so we anticipated that temperature would influence the calibration between durometer reading and timing of moulting. The observed pattern in change in shell hardness for the ambient temperature and 3°C elevated treatments were similar (Figure 10). However, both pre- and post-moult curves indicated that rate of change in shell hardness was more rapid for lobsters in the elevated treatments. While our data are consistent with more rapid change in shell hardness at higher temperature, the confidence limits in the trend were too broad to distinguish the different curves. As a result, we were unable to account for the effect of temperature in the combined calibration curve presented in section 7.3.6.

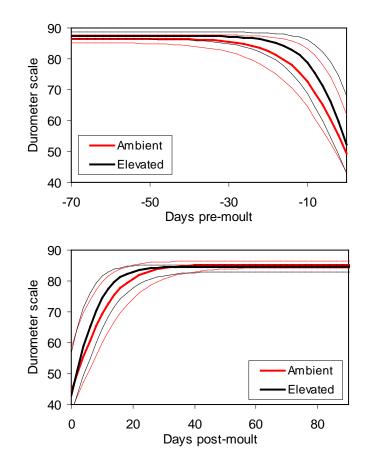


Figure 10. Logistic model fits (+/- 95% confidence limits) to durometer hardness measurements from lobsters held at ambient and elevated (3°C) before (upper) and after (lower) the moult.

7.3.4 Effect of site of origin of lobster on shell hardness

Lobsters were collected from several regions, which were selected due to their differing growth rates. The logistic model could not converge for the pre-moult sample from South Australia and the post-moult sample from King Island, so no fits are presented. Where fits were obtained, there was no evidence of an effect of site of origin on shell softening / hardening rate, with the exception of the post moult sample from South Australia. The fit to that sample produced an asymptote of around 75 on the durometer scale, which represented a soft- shelled lobster from other sites. That is, the durometer readings from South Australia indicated that lobsters failed to reharden after the moult. This is clearly not a natural situation and suggests that either: (a) the animals were stressed by some factor in the tank system that resulted in failure to harden normally after the moult; or (b) there was a difference in operation of durometers by samplers in Tasmania and South Australia.

Putting aside the anomalous result from South Australia, there was no indication of an effect of site of origin on hardening rate of lobsters. This suggests that site per-se does not need to be considered when interpreting durometer reading from field studies. This is not to say that factors such as temperature and lobster size that may differ between sites are unimportant.

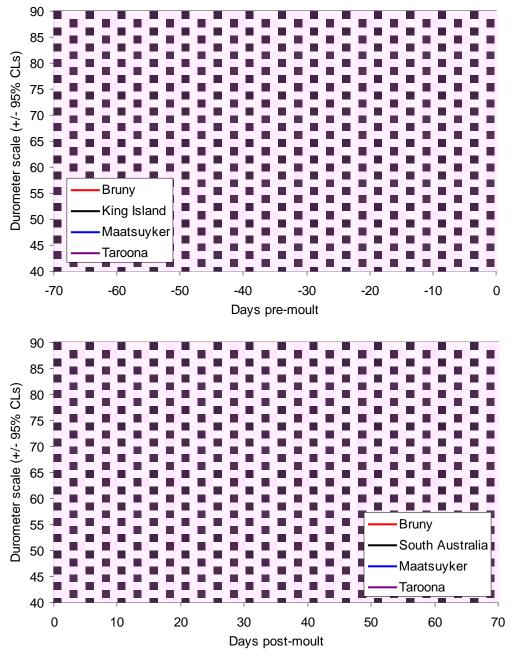


Figure 11. Logistic model fits (+/- 95% confidence limits) to durometer hardness measurements from lobsters from different sites collected before (upper) and after (lower) the moult.

7.3.5 Effect of lobster size on shell hardness

Lobster were sampled across a range of sizes, from 69 mm to 178 mm CL, to test the effect of size on shell hardness readings. Lobsters were grouped into 15 mm size bins, commencing at 60 mm CL. The logistic model converged for 7 of the size bins spanning the range 75-180 mm CL (Figure 12).

Model fits for most size bins were similar for both pre- and post-moult samples, with the exception of the smallest and largest bins (75-90 mm and 165-180 mm respectively). We interpret this as evidence that the spring of A-grade durometers

produced increased deflection in smaller lobsters, possibly even providing sufficient force to produce some shell flexion in fully hardened, smaller lobsters. The pattern in larger lobsters of 165-180 mm CL was atypical with less curvature and harder readings around the time of the moult. Larger animals were more difficult to sample due to difficulty in handling, and also the magnified effect of irregularities in the surface of the exoskeleton in larger animals. We believe that this atypical model fit is produced by these sampling difficulties, rather than an actual difference in the process of shell hardening in these larger animals.

In conclusion, size of the lobster does not appear to influence shell hardness measurements collected by durometers with the exception of the extreme in sizes, that is, below 90 mm and above 165 mm CL.

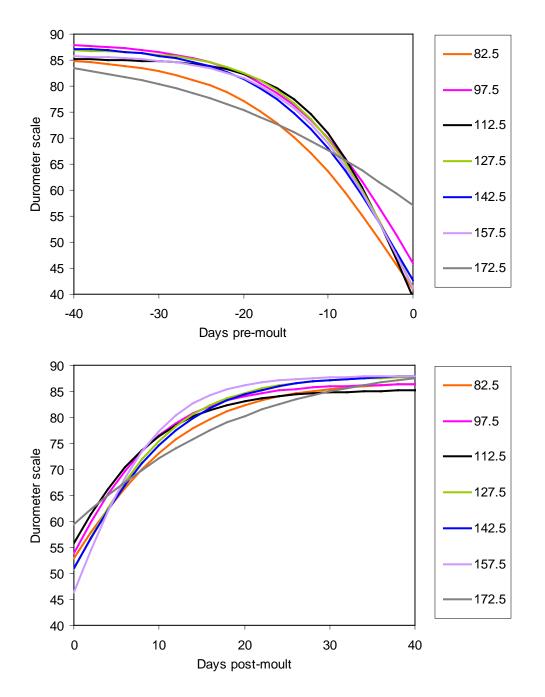


Figure 12. Logistic model fits to durometer hardness measurements from lobsters of different sizes sampled before (upper) and after (lower) the moult. Size categories are centred on 15 mm bins of carapace length.

7.3.6 Summary of the application of durometer readings for estimating the timing of moulting

The results of analyses on the effect of lobster size, site of origin, temperature and sex implied that a single logistic model could be used for most animals to predict days before or after moulting. This model was based on data from both sexes, from all Tasmanian sites, all temperature regimes, and animals in the size range between 90 and 165 mm CL. Data were restricted to this size range as fits to larger and smaller animals

displayed quite different trajectories, particularly for pre-moult (Figure 12). Model fits to this combined data set and associated parameter estimates are given in Figure 13 and Table 1.

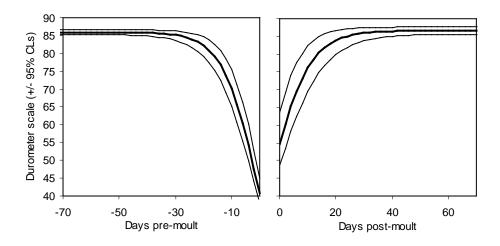


Figure 13. "Best" models relating moult timing to reading from narrow –foot, A-grade durometer. Models have been fitted to data from both sexes and all Tasmanian sites, excluding animals smaller than 90 mm CL and greater than 165 mm CL.

| Parameter | Estimate | Approximate Standard Error | Lower 95% CL | Upper 95% CL |
|------------|----------|-------------------------------|--------------|--------------|
| Pre-moult | | | | |
| ßo | 85.927 | 0.3505 | 85.247 | 86.616 |
| β_1 | 0.1596 | 0.0114 | 0.1391 | 0.1832 |
| β_2 | 0.6378 | 0.4901 | -0.4293 | 1.5165 |
| Post-moult | | | | |
| ßo | 86.615 | 0.4845 | 85.634 | 87.637 |
| β_1 | 0.1445 | 0.0127 | 0.1175 | 0.1771 |
| β_2 | -3.7137 | 0.7019 | -5.4901 | -2.3853 |

 Table 1. Parameter estimates for "best" model relating days before and after moult to shell hardness.

Model fits to the combined data shown in Figure 13 and Table 1 predict durometer reading from days before or after the moult. For application to fisheries research, we require the reverse situation, that is, to be able to predict the day before or after the moult from a durometer reading.

In order to predict days before or after moult from the logistic function, the function was converted to the following equation:

$$X = \frac{\ln\left(\frac{\beta_0}{Y} - 1\right)}{\beta_1} - \beta_2$$
 to provide estimates of days prior to moult (X) from

durometer readings (Y); and

$$X = \beta_2 - \frac{\ln\left(\frac{\beta_0}{Y} - 1\right)}{\beta_1}$$

to provide estimates of days after moult (X) from

durometer readings (Y).

These functions were used to model the time before or after moult based on durometer readings (Figure 14). These models represent the key applied outcome of the study for the use of durometers in southern rock lobster fisheries as they provide a means of predicting the days before or after moult from a single, rapid measurement. Estimates of confidence limits are also produced, which are important in incorporating uncertainty or risk into analyses used for management purposes.

Although of apparent value for data collection in research surveys, two issues prevent the application of these models to grading of individual animals in processing facilities. First, the confidence limits are too broad for providing precise information on specific animals. Secondly, we were unable to obtain precise grading by processors of lobsters into "soft" and "hard" categories; animals classed "hard" by one processor were considered "soft" by another. Most processors expressed the opinion that they were able to grade lobsters well by hand but this did not appear to be the case with poor repeatability of grading. That is, animals classed by hand by processors as borderline "hard" were often classified as "soft" when retested. Consequently there has been no attempt to mathematically define the bounds of durometer readings that indicate that specific animals are too soft for export. However, as a broad guide, most samples (>90%) of lobsters classed by processors as being too soft for export tended to have durometer readings less than 85. This bound may serve as conservative guide for research sampling of large numbers of lobsters where the aim is to limit the landing of soft-shelled lobsters of lower marketability.

The ranges of useful durometer readings from the A-grade narrow-foot durometer were restricted to between 50 and 85, which equates to a time period of approximately 20 days on either side of the moult. When only durometer data are collected, it is not possible to determine if lobsters are pre-moult or post-moult, however, classification of lobsters into either of these categories is typically quite simple and should be recorded at the same time as durometer readings. Features that indicate a pre-moult state include shell fouling, worn setae, presence of melanised areas of previous damage, darker colour on the membranes on the underside of abdominal segments and dull shell colour. If it is not possible to classify lobsters as pre- or post-moult on the basis of these features, more objective tests based on haemolymph pigmentation and protein can be applied as discussed in the following chapter.

A Microsoft Excel worksheet is attached to this report on disc with formulae and parameter estimates. This provides estimates of days before or after the moult from durometer readings based on the models in Figure 14.

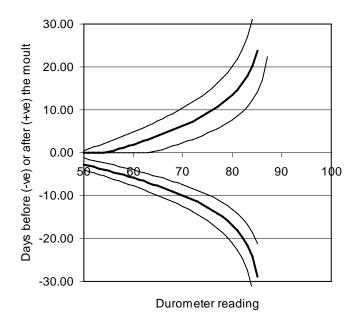


Figure 14. Effective ranges of measurements for estimating days before and after the moult from durometer readings. Bold line is the mean estimate and is banded by 95% confidence limits.

8. Comparison and integration of durometer readings with other indices of moult stage

8.1 Introduction

The process of moulting has a profound effect on a range of physiological processes in crustaceans, which implies that a range of different approaches could be used to track temporal changes in relation to moulting. Measurement of changes in the exoskeleton has been undertaken through moult-staging of the cuticle (Longmuir, 1983; Turnbull, 1989; Musgrove, 2000a), degree of carapace fouling (Levings et al., 2001; Cockcroft and Goosen, 1995), changes in radiometric isotope composition (Gardner et al., 2002; Nevissi et al., 1996) or shell hardness (Dufour et al., 1997; Foyle et al., 1989).

Many of these analyses are too time consuming for sampling large numbers of lobsters in catch sampling programs or in processing facilities so here we focused on three data sources that can be collected within a few seconds per lobster. Haemolymph protein and haemolymph pigmentation were measured in previous research as a guide to condition of lobsters (Musgrove 2000b; Musgrove 2001) and were sampled here to evaluate their use as a predictor of moult time.

In addition to haemolymph composition, we examined the use of pleopod trimming and subsequent regeneration after the moult as a source of additional data about timing of moulting. Pleopods are trimmed routinely in Tasmanian catch sampling where animals are tagged and released. Winstanley (1976) introduced this component of catch sampling as an alternative means of marking lobsters to dart tags but it has been retained since the introduction of T-bar tags. This is because the regeneration of pleopods indicates that lobsters have moulted, which cannot be established by change in

size for much of the State where moult increment is frequently less than 1 mm per year. Since pleopod clipping was introduced, over 40,000 tagged lobsters have been recaptured so there is a considerable database that could be used to refine information on timing of moulting. Before this can be used, improved information is required on the time period before moulting that pleopod clipping would need to occur if regeneration is to be observed after the moult. Lobsters held for durometer trials provided an opportunity to measure this process simultaneously.

8.2 Methods

Experimental animals were the same as those described in Chapter 7, page 10.

Pleopod regeneration was examined by trimming the distal half of the exopodite process from one pleopod on each lobster using scissors. This is the same process conducted in routine catch-sampling of southern rock lobsters in Australia to test if moulting has occurred between recapture events. Animals were checked daily to determine the time of moulting and this date was used to back-calculate days prior to moult that pleopod trimming occurred. Following moulting, the pleopods that had been trimmed were examined for signs of regeneration.

Post-moult pleopods were placed into one of three categories. The pleopods of animals with "obvious regeneration" had regrown so that it was clear that a moult had occurred. Any animal in this category would be detected by a brief glance from an observer during catch-sampling. In many cases regeneration was very slight with setae only visible along the trimmed margin, indicating that new exoskeleton had been deposited. These were classed as "slight regeneration", would only be apparent in catch sampling by detailed inspection and could easily be missed. The final category was "no regeneration" where the post-moult pleopods had not even regenerated setae along the trimmed margin.

Haemolymph pigmentation and haemolymph protein were measured according to the method of Musgrove (2001). Haemolymph samples were collected by pericardial puncture with a 1 ml syringe and 22-gauge needle. Syringes were then placed on a light box and the colour graded according to the pigment chart presented in Musgrove (2001).

Haemolymph protein was measured by placing a portion of the sample, around 50 μ l, in a hand held refractometer to measure the refractive index as developed for *Homarus americanus* (Leavitt and Bayer, 1977). Haemolymph refractive index (HRI) was converted to serum refractive index (SRI) according to the equation: SRI= (0.9121*HRI)+0.1172. The resultant estimate of SRI was then converted to serum protein (SP; g/l) by the equation: SP=(4936*SRI)-6609.3 according to regressions developed by Musgrove (2001) for *Jasus edwardsii*.

8.3 Results

8.3.1 Pleopod regeneration

The extent of regeneration of pleopods increased with the time before moulting that trimming occurred (Figure 15). Both sex and site appeared to influence the rate of redevelopment of pleopods, although measurement of the effect of sex is compounded by the time of year the moulting occurs and associated differences in water temperature. Site of origin of lobsters had a pronounced impact on regeneration even though lobsters were maintained in the same tanks for several months after capture. For both males and females, pleopod regeneration was most rapid in lobsters from Crayfish Point and slowest in lobsters from King Island. Almost all pleopods trimmed longer than 60 days before the moult in lobsters from Crayfish Point showed signs of regeneration, although the pleopods of some King Island animals failed to regenerate after 100 days. Obvious regeneration was apparent in most animals only after around 120 days, although this point was reached for Crayfish Point males at around 90 days.

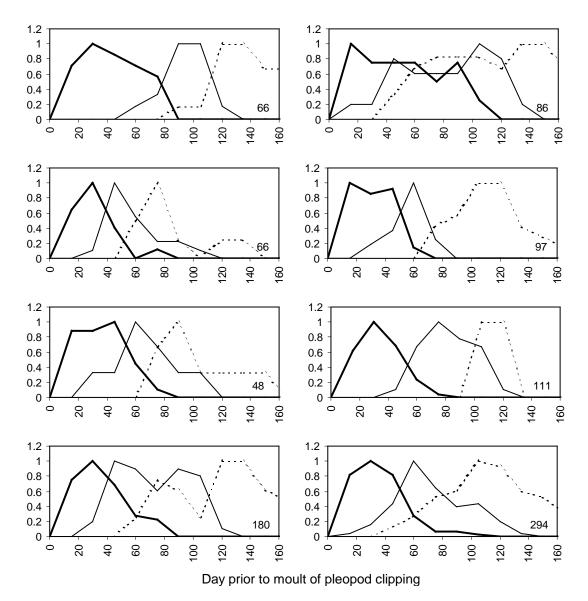


Figure 15. The extent of regeneration of pleopods from female (left column) and male (right column) lobsters. Pleopods were clipped at varying times prior to the moult and the extent of regeneration after moulting classified as: no regeneration (bold lines); slight regeneration (standard lines); or obvious regeneration (dashed lines). Upper pair of plots is for King Island, second row is Crayfish Point, third row is South Bruny Island, and bottom row is all regions combined. Total sample size is given in bottom right. Time categories are in bins of 15 days and frequencies have been scaled to 1. Haemolymph protein was variable between individuals but exhibited a clear pattern of increase prior to the moult and sharp decline in samples taken after the moult. Patterns between sexes and across sites were variable and no clear trend was apparent.

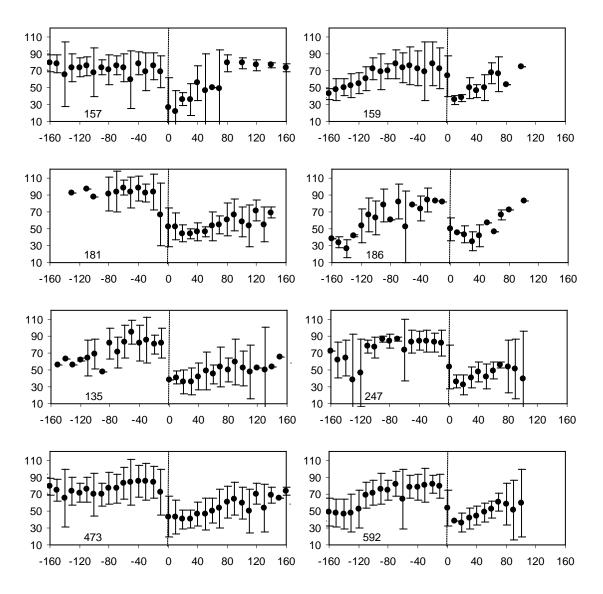


Figure 16. Haemolymph protein concentration (g/l) in relation to moulting (at day 0 on X-axis). Results are split for female (left column) and male (right column) lobsters. Upper pair of plots is for King Island, second row is Crayfish Point, third row is South Bruny Island, and bottom row is all regions combined. Total sample size is given at bottom left.

Haemolymph pigmentation followed a similar trend to that of haemolymph protein with increasing values prior to the moult and a sharp decline in samples collected after the moult. There was a clear difference between sexes with highly variable results from females. Many females had quite low (pale) pigment scores prior to the moult while others retained high pigment scores (orange) after the moult. In contrast, data for males was uniform and haemolymph pigmentation scores were quite distinct between pre- and post- moult samples. There was no clear trend between sites for either sex.

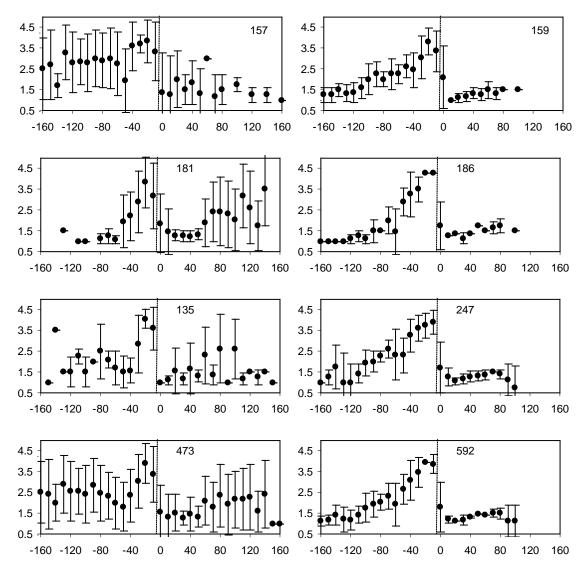


Figure 17. Haemolymph pigmentation index in relation to moulting (at day 0 on X-axis). Results are split for female (left column) and male (right column) lobsters. Upper pair of plots is for King Island, second row is Crayfish Point, third row is South Bruny Island, and bottom row is all regions combined. Total sample size is given at top of each plot.

8.4 Discussion

The regeneration of pleopods trimmed during catch-sampling clearly indicates that moulting has occurred since the previous release. However, our results show that the apparent lack of regeneration does not demonstrate that no moulting has occurred. This is because pleopods that are trimmed at a time close to the moult do not regenerate to any detectable level. Even very fine scale regeneration of setae along the trimmed margin may not be detectable on pleopods trimmed almost 3 months before the moult. These results imply that pleopod trimming is only a reliable method for gauging if moulting has occurred in recaptured animals if the time-at-large exceeds 3 months where observers carefully scrutinise trimmed margins for setae. Where regenerated pleopods are only detected when regeneration is more obvious, then this time period would be longer, around 4 months.

The apparent effect of site of origin of lobsters is interesting given that animals were maintained in the same conditions for several months prior to the moult. The general trend was one of most rapid regeneration in lobsters from Crayfish Point, followed by southern Bruny Island, and slowest regeneration in lobsters from King Island. This pattern doesn't follow the actual cline in latitude and growth rates between the 3 sites. One explanation is that the process of capture and transport to laboratory tanks impacted on the general health of animals so that animals transported greatest distance (King Island, around 400 km) were more affected than animals transported shorter distances (Crayfish Point, around 10 km). If the health of animals in tanks was compromised, then the estimates presented here of time for pleopod regeneration to occur are likely to be overestimates of those in natural conditions.

Both haemolymph pigment and haemolymph protein followed a similar pattern with a steady increase prior to the moult and a sharp drop at the time of the moult. Both these measures provide a method of discriminating pre-moult lobsters from post-moult animals. This is normally apparent from shell fouling and wear but can be difficult, especially for lobsters from deeper water. The calibration of durometer readings to days before or after moult described in chapter 7.3.6 was unable to differentiate pre- and post-moult animals so these techniques provide a solution if necessary.

Of the two methods, haemolymph pigmentation is the more rapid to measure and most suited for sampling at sea, however readings were highly variable for female lobsters. Many females failed to develop pigmented haemolymph prior to the moult although all males had a consistent pattern. This difference between the sexes may be due to utilisation of pigments during ovarian development in the lead up to moulting by females (Lee and Puppione, 1988, Dall et al., 1995). Regardless of the reason, sampling to track timing of moulting by females from haemolymph pigment appears less reliable for females than for males possibly necessitating clipping of pleopods for later determination of moult stage as suggested by Musgrove (2001).

9. Extension of the project and improved gauge design

Durometers are designed for measuring hardness of inert materials in industrial situations, rather than biological samples. Although some companies are able to modify the gauges to make them more suitable for application with crustaceans, these adjustments are generally constrained to changes in the strength of the pressurising spring and the size of the foot. We observed several shortfalls in the design of durometers for measuring lobster shell hardness through the course of normal sampling. These problems include sensitivity to salt water, sampling error introduced by the irregular texture of the exoskeleton, spatially small area of exoskeleton that is sampled so that small irregularities can affect results, and an inability of the durometer to measure across the full range of changes in shell hardness that occurs through the moult cycle (note the high incidence of durometer scores approaching 100; Figure 6, page 9). We attempted to overcome these problems with alternative designs that measured compression across the body of the lobster or across the tail. First, a crude prototype was constructed and used to measure compression across the tail on the 2nd abdominal segment. That gauge was a test of concept for the use of compression of abdominal segments. Following promising results from that gauge a refined electronic version was constructed that was easier to use and had higher precision.

9.1 Prototype compression gauge

The prototype compression gauge was constructed at low cost and utilised spring compression to indent the exoskeleton in the same manner as durometers (Figure 18). The key difference here was that compression occurred across the entire arch of the tail, rather than just across a small area as with the durometer (4 mm diameter). To operate, the gauge was first adjusted to the width of the tail and then locked. The spring-loaded piston was then activated to apply a constant force and the extent of compression of the tail was recorded with a dial gauge.

Results from this prototype compression gauge were compared with results from the Rex 1700TM durometer (Figure 1, page 2). The data derived form his gauge appear to respond to changes in shell hardness after moulting (Figure 19) and had similar variation, except when the shell was very hard (Figure 20).

Results from this prototype gauge were encouraging and suggested further development effort was warranted. This is because the gauge produced comparable results to those from the durometer yet was constructed with materials costing less than 20% of a durometer and with greater resilience to corrosion. Greater variation in measurements of lobsters with hard shells was encountered with the compression gauge although this was due to crude construction of the main brace, which could easily be refined.

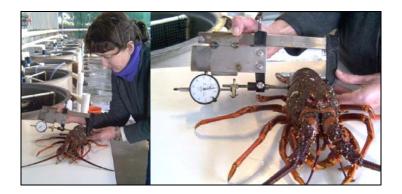


Figure 18. Prototype compression gauge being used to test shell compression across the tail (on the 2nd abdominal segment).

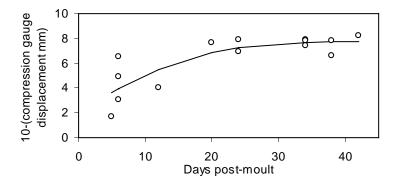


Figure 19. Cumulative logistic function fitted to compression gauge data from post-moult female lobsters. Data was transformed by subtracting from 10 to convert the logistic to the same trend as that from the durometer.

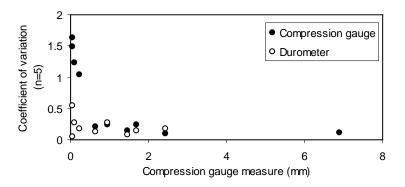


Figure 20. Comparison of sample variation between durometers. Each lobster was measured with both the prototype compression gauge and a durometer. A lower compression gauge score indicates that the lobster was more resilient to compression – that is, its shell was harder.

9.2 Electronic "squeezometer"

Research effort on the development of a gauge for measuring shell hardness was continued based on trials with the prototype lobster compression gauge described above. This development involved additional participants in the project and utilised an existing exchange program between the Department of Mechanical Engineering at the University of Adelaide and the University of Applied Sciences in Osnabrueck (Germany). This engineering research was conducted by Joerg Harmeling, Andre Kluge, Ian Brown and Dr Frank Wornle.

9.2.1 Squeezometer design

Plans and components of the electronic "squeezometer" gauge are shown in Figure 21, Figure 22 and Table 2.

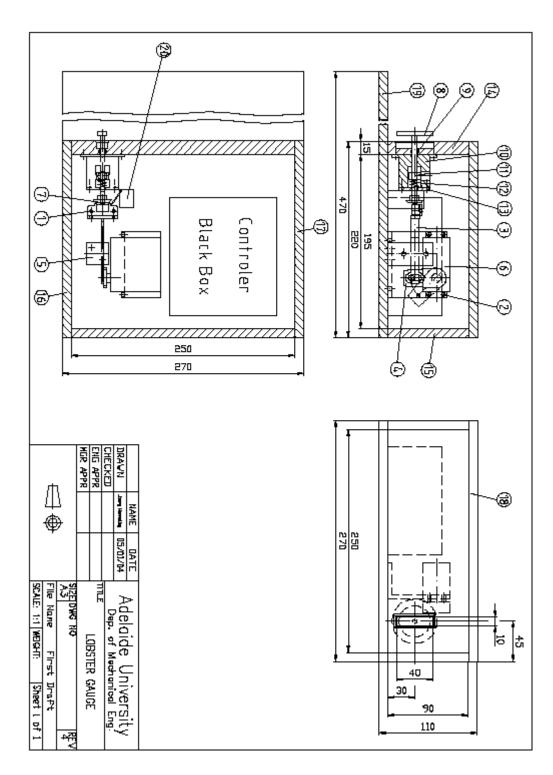


Figure 21. Design of lobster "squeezometer" gauge. Components are listed in Table 2.

| Comp. | Quantity | Description | Code designation / Drawing No. | Material | Remark |
|-------|----------|----------------------------|--------------------------------|------------|--|
| 1 | 1 unit | Force Sensor, with bracket | FS Series | | Oper.Temp40 to 85 °C; Max.overforce 5.5kg |
| 2 | 1 unit | Engine bracket | | 6060 | |
| 3 | 1 unit | Guiding rod | | 6060 | |
| 4 | 1 unit | Rocker arm levers | | plastic | |
| 5 | 1 unit | Guide piece | | plastic | |
| 6 | 1 unit | Sail servo engine | | | Speed:0.27sec/60° at 4.8V; Torque: 11.5kg*cm |
| 7 | 1 unit | Tappet connecting pin | | 6060 | |
| 8 | 1 unit | Plunger | | stainless | |
| 9 | 1 unit | Rubber sealing | | | |
| 10 | 1 unit | Cylinder liner | | plastic | |
| 11 | 1 unit | Adjusting collar | | 6060 | |
| 12 | 1 unit | Spring | | stainless | |
| 13 | 1 unit | Cylinder liner cap | | plastic | |
| 14 | 1 unit | Plunger plate | | 6060 | |
| 15 | 1 unit | Rear plate | | 6060 | |
| 16 | 1 unit | Side plate | | 6060 | |
| 17 | 1 unit | Side plate | | 6060 | |
| 18 | 1 unit | Top plate | | plexiglass | |
| 19 | 1 unit | Bottom plate | | 6060 | |
| 20 | 1 unit | Proximity switch | | | |

 Table 2. Component list for lobster gauge (note part number if linked to Figure 21).

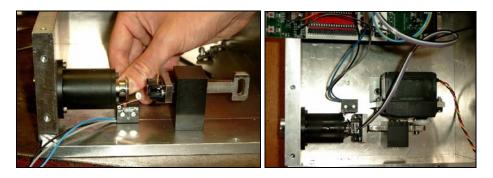


Figure 22. Detail of force sensor (left) and indentor rod with engine (right). The controller has been shifted out of field to top of picture.

9.2.2 Operation and positioning of lobster

The lobster was first positioned on the bottom plate (19) and pressed against the plunger (8). It was important that the measurement was made at the correct area of the lobster's body. During the measurement, the lobster was held firmly and constantly against the plunger plate (14). Once the lobster was positioned correctly, measurement was initiated (Figure 23).

The spring-loaded plunger remained outside of the case in the default initial position. When the lobster was pressed against this plunger, the proximity switch (20) was released by the tapped connecting pin (7), which triggered the sail servo engine (6). The sail servo engine, with the aid of the rocker arm levers (4), drove the guiding rod (3) back towards the lobster. The guiding rod, which carries the force sensor (1), pushed the plunger against the shell on the side of the lobster tail.

After activation of the sail servo engine, the measuring unit of the force sensor came in touch with the plunger. Where force was exerted onto the sensor, values could be determined.

The micro-controller regulated the entire measurement procedure and continuously received the current values of the force sensor. In order to avoid incorrect measurements, the sail servo engine was only activated when the force was greater than 10N. If the actuator was released unintentionally, the sail servo engine returned to its initial position. In order to avoid injuring the animal, the peak value with 40 N was also determined. When the 40N value was obtained, the engine stoped and returned to its initial position. The force on the sensor was detected as a voltage and amplified to the ADC (Analog – Digital Controler). An LED lit up depending on amplitude of the electric voltage. There were 8 LEDs, which were activated depending on hardness of the lobster shell. If no LEDs were lit up, the shell was too soft. If all 8 LEDs were lit the shell was very hard. Consequently, different hardness levels are displayed.

After removing the lobster from the measuring instrument, the plunger moved back to the initial position due to its spring loaded mechanism and a new measurement could be taken.



Figure 23. Prototype gauge to measure lobster shell hardness in development. A piston moves out until it touches the surface of the lobster and then moves a further 5 mm. The force that is required to produce this flex in the tail is measured – softer lobsters require less force. Readings are stored in memory for research applications. For industry application, LEDs are illuminated with illumination of all LEDs indicating the lobster is fully hardened.

10. Benefits and adoption

The project has met both objectives relating to development of protocols for the use of durometers: optimal sites on the carapace have been identified and calibrated for estimation of days pre- or post-moult from durometer readings. This thus provides a tool to meet the high-priority research need identified by the Tasmanian Crustacean Research Advisory Group: "The use of techniques to determine the proportion of lobsters moulting".

Two gauges are now available for quantifying the shell hardness state of lobsters. Narrow-foot A-class durometers have been calibrated to enable the estimation of days before or after moult, and a new custom built gauge can be used to categorise lobsters into hard or soft shell groups. These gauges provide slightly different options for research and industry application.

Measurements of shell hardness will now be routinely collected as part of TAFI catch sampling exercises undertaken around peak periods for moulting. Shell hardness gauges will be employed for future surveys specifically aimed to assess the impact of harvesting during periods when moulting is common. These surveys were conducted in 2001 and 2002 but were inconclusive due to the absence of the gauges developed here.

We can only speculate on future uptake of the shell hardness gauge by industry at this stage because the device was only designed and constructed immediately prior to completion of this report. Several fishers have expressed interest for grading catch at sea. Fishers anticipate higher spot prices from processors where catch can be guaranteed free of soft shelled lobsters to an agreed standard. We expect that the most

probable method of broad uptake by industry is through processors where gauges could be used for grading of lobsters prior to export to reduce losses in transit.

Quantification of benefits is naturally difficult and includes the effect of improved management of closed seasons on discard mortality and the number of lobsters removed per unit quota. Dufour et al. (1997) estimated that discard mortality of soft-shelled snow crabs (*Chionoecetes opilio*) was 14.3%, if losses in rock lobster are likewise substantial then the economic loss in yield could be substantial.

Satisfaction of markets with exported product can deteriorate if losses in transit are high due to the inclusion of weak, soft-shelled lobsters in shipments. Local processors report rapid and severe response by markets when there is a perception that the shipments contain weak lobsters. This can translate to declines in beach price of over \$10/kg as processors also need to recoup the loss of rock lobsters that die in transit. It is anticipated that the gauges developed here may prevent the shipment of lobsters that are likely to arrive at markets dead, which will assist in maintaining higher customer satisfaction and beach price.

11. Further development

Application of the research to improved management of harvesting around periods when moulting occurs requires that additional surveys be conducted during this period. The important issue of possible increased discard mortality of soft-shelled lobsters should be tested through capture-recapture tagging research. Previous attempts to use this approach have failed because lobsters could not be adequately classed as soft- or hard-shelled and there was complete reliance on industry for recaptures. A survey targeting this issue could now expect to gain good estimates of survival of soft-shelled discards from four x 1-week trapping surveys.

Promotion of the use by industry of the new electronic shell hardness gauges developed through this project will require several steps. Units will need to be constructed and we have located a small equipment manufacturer able to take on this task. We also anticipate that the need for small design modifications will arise as the units are used in processing facilities or on-board vessels. We anticipate that the manufacturer could undertake any further modifications.

12. Planned outcomes

The planned outcomes of the project have been met. The main planned outcome was the development of a calibration scale relating shell hardness to the time before or after moulting in southern rock lobsters. The specific application of provision of improved information on seasonal closures can now be pursued in future surveys. More general applications of improved ability to measure growth and exploitation rates will also be pursued. Substantial growth opportunities exist across the range of southern rock lobster for increased harvests through improved management; this is demonstrated in part by the rapid rates of stock rebuilding in Tasmania and the South Australian southern zone (Gardner et al., 2002; McGarvey et al., 1998). For revision of management improved basic biological data is required (Gardner et al., 2002). For instance in Tasmania harvest strategy projections from only 5 of the 8 stock assessment areas are based on actual tag-recapture data. Shell hardness data will assist in interpretation of tag-recapture data to improve growth estimates and thus our ability to evaluate alternative harvest strategies.

The development of the electronic shell hardness gauge met the planned outcome of provision to industry of a tool that could be used to objectively separate soft- and hard-shelled lobsters. This has the potential to lead to increase in the market price of southern rock lobsters and differentiation of product on the basis of quality.

13. Conclusion

Objectives of the project were directed to developing routine procedures for the use of durometers by researchers and industry. Comparison of durometer results from different regions on the exoskeleton demonstrated that the best region for taking readings was along the lateral suture on the side of the carapace. Readings from this area were most responsive to the moult cycle and were the most repeatable when multiple readings were taken from the same lobsters. The effect of several factors on durometer readings were also examined, these were lobster size, sex, region and temperature. None of these factors had a quantifiable effect on durometer readings with the exception of lobster size; skewed readings were obtained from very large (>165 mm CL) and very small lobsters (< 90 mm CL). Lobsters of these extreme sizes typically lie outside the ranges sampled in southern rock lobster fisheries research programs.

The change in shell hardness through the moult cycle was quantified by durometer readings and used to develop a calibration curve. This curve provides an estimate of the time before or after the moult of southern rock lobsters based on a durometer reading. In addition, estimates of confidence around these values are also calculated to enable uptake of this procedure in catch sampling programs.

Although durometer readings can be collected rapidly as part of catch sampling programs and appear to provide valuable fisheries management information for analysis of changes in populations, this technique appeared unsuitable for uptake by industry. This is because individual lobsters could not be graded with high precision – although durometer readings are objective, there is too much variation in repeated measures for perfect grading. This means that the current situation on fishing vessels and in processing sheds is repeated as some lobsters can be graded as too soft in one instance but adequately hard when retested. Another problem was that the gauges tended to be susceptible to salt water unless handled carefully.

Whilst recognising that durometers provided a valuable step forward in quantification of shell hardness, we considered that improvements could be made in the design of a gauge for measuring shell hardness. The process of developing an improved gauge initially proceeded by construction of a crude gauge to test the concept of measuring shell hardness by quantifying flex of the tail segments. When viewed in cross-section, the exoskeletons of segments of lobster tails have a similar form to arch springs. We reasoned that changes in calcification of the exoskeleton through the moult cycle would alter the strength of this spring. One of the advantages of this approach was that a large area of the exoskeleton influenced the reading of shell hardness, which reduced random error. Durometer readings are measured by deflection of a small, nail-like rod that is pressed against the shell of the lobster. The small surface area contacted by this rod can introduce errors as the surface of lobster shells are uneven and of variable thickness. Results from this initial trial were promising and further development of a gauge was undertaken.

The gauge finally developed is robust and suitable for use by industry. Lobsters are held against the device and a switch is triggered. This causes a small piston to be driven out to the surface of the lobster tail. The piston then moves forward a set distance further and the force applied back by the lobster tail is measured. For research purposes, readings of this force can be relayed and downloaded to computer. For industry applications the force readings are calibrated and used to illuminate a series of LEDs – the more LEDs illuminated, the harder the lobster. This tool has considerable promise in improving the quality of product delivered to market and could also be used for high grading of catch at sea.

Aside from investigating quantification of lobster shell hardness, several other factors that can be measured within a few seconds were tracked through the moult cycle. The aim of this research was to provide additional tools relating to moult stage for researchers conducting catch-sampling projects. These additional factors were haemolymph or "blood" pigment, haemolymph protein and regeneration of the swimmerets or pleopods beneath the tail. Haemolymph pigment and protein varied through the moult cycle and this pattern is described for both male and female lobsters. A single pleopod is routinely clipped in half in catch sampling projects to indicate if a recaptured lobster has moulted since being tagged and released. Where a regeneration of the pleopod has occurred it is inferred that the lobster has moulted. Results presented here indicated that no regeneration should be assumed for at least 60 days after release.

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15. Appendix 1: Intellectual Property

No compelling reason was identified to restrict distribution of results so these have been made publicly available with no protection or confidentiality. There is some potential for commercial development of the electronic gauge for measurement of shell hardness. The principle motivation for all parties is the development of the commercial fishery so progress in manufacture of units at this stage has proceeded without commercial objectives. Nonetheless, all eligible parties retain intellectual property and associated rights.

16. Appendix 2: Staff

People involved in the project were:

Scientific staff involved with biological experimentation:

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