Translocation of the southern rock lobster, *Jasus edwardsii*, to improve yield and marketability

PhD thesis by

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Photo: Shane Fava

Jasus edwardsii (Hutton,1875)

"It is not the strongest of the species that survive, not the most intelligent, but those who are the most adaptive to change."

— Charles Darwin —

Declarations

Statement of originality

This thesis contains no material that has been accepted for a degree or diploma by the University or any other institution. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due acknowledgment is made in the text.

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Abstract

The Tasmanian southern rock lobster (*Jasus edwardsii*) fishery has a single Tasmania-wide management system despite large spatial variations in the biology (growth) and market traits (shell colour, body shape and live transport condition). This has created uneven distribution of harvest rates around the State where red, fast-growing, shallow-water lobsters are heavily targeted by fishers due to their high market demand, while pale, slow-growing, deep-water rock lobsters have a much lower rate of exploitation. In an attempt to improve yield, marketability and value of deep-water southern rock lobsters, translocation of lobsters between regions was examined as a supplementary management strategy for the Tasmanian rock lobster fishery. Adult deep-water lobsters were moved inshore to shallow-water reefs where changes to growth, market traits and body condition were monitored through recapture surveys.

Red pigmentation in shell colour decreased with depth across southern Australia. Most of the catch is sold into Asian live markets where there is a preference for red lobsters. This market preference has lead to the price discounting of lobsters,

which was estimated at a total of AUS$6.67 million / year for the Tasmanian Rock Lobster Fishery. Morphological market traits of leg length and abdomen shape were also different between deep and shallow-water *J. edwardsii* populations and between the sexes in each population. Nutritional indicators of condition did not differ among adult deep-water, shallow-water and translocated male lobsters, however fatty acid profiles indicated dietary differences between deep and shallow-water lobsters. Haemolymph condition indices detected significant differences in the post-harvest condition between deep-water and shallow-water lobsters.

Translocating small, pale adult lobsters into a shallow water habitat resulted in a number of changes important to the yield and value of the fishery. Growth rates of translocated adult lobsters increased at their first moult in their new habitat, exceeding that of resident deep-water lobsters from the original site. Growth of translocated females exceeded resident shallow-water females in the first year post-release. Translocation changed the pale colouration of deep-water lobsters into the bright red grade most sought after by the Asian market, however changes in morphology were only partial and may require several moults for a complete change in shape. Dietary fatty-acid

profiles of translocated lobsters matched those of the resident lobsters, while significant levels of essential omega-3 fatty acids in the muscle tissue of translocated lobsters suggest enhanced nutritional condition after translocation. The post-harvest condition of resident shallow-water lobsters and translocated lobsters were similar when recaptured 12 months later.

Translocation could be an effective management tool to add value to the less marketable deep-water southern rock lobsters. These results on the magnitude and timing of improvements in market traits will now contribute towards the economic and biological evaluation of the feasibility of translocation as a fisheries enhancement strategy for the Tasmanian Rock Lobster Fishery.

Statement of co-authorship

Chapters 2-6 of this thesis have been prepared as scientific manuscripts as identified on the title page for each chapter. In all cases sample design, data analyses, interpretation of results and manuscript preparation were the primary responsibility of the candidate, but were carried out in consultation with supervisors, and with the assistance of co-authors whose contributions are outlined below:

Dr Caleb Gardner

Dr Gardner (Tasmanian Aquaculture and Fisheries Institute (TAFI), University of Tasmania (UTAS)) provided the role of a principal supervisor for this PhD program. He provided the initial funding and financial support for all aspects of this investigation and his research team at TAFI provided technical support for all related field work. He provided advice relating to experimental design, lobster biology and fishery management, and on statistical analyses of results on all chapters of this thesis.

Dr Bridget S Green

Dr Green is a research fellow (TAFI, UTAS) and co-investigator on the translocation project and provided the role of a co-supervisor for this PhD program. She provided advice on the statistical analyses of results, manuscript preparation for Chapters 3, 4, 5, 6 and the overall structure of thesis.

Dr Adrian Linnane

Dr Linnane leads the Rock Lobster Sub-Program at the South Australian Research and Development Institute (SARDI) and was responsible for the co-ordination of the translocation field trials in South Australian waters. He provided shell colour data from South Australian commercial lobster catches for analyses in Chapter 2 and also provided lobster samples and digital image data for morphometric analyses in Chapter 3.

Mr David Hobday

Mr Hobday led the Invertebrate Section at Fisheries Victoria at the Department of Primary Industries, and was responsible for the co-ordination of the translocation field trials in Victorian waters. He provided shell colour data from Victorian commercial lobster catches for analyses in Chapter 2 and also provided lobster samples and digital image data for morphometric analyses in Chapter 3.

Dr Michaela A Guest

Dr Guest was a post-doctoral fellow affiliated with UTAS, TAFI and CSIRO. She provided advice on the subject matter of diet as it relates to lipid profiles of invertebrates, statistical analyses and had the role of research supervisor for this component of the thesis.

Dr Peter D Nichols

Dr Nichols leads the Food Futures Flagship program at CSIRO (Commonwealth Scientific and Industrial Research Organisation) and provided laboratory space, equipment and instrumentation for the lipid and fatty acid analyses in Chapter 4. His specialised knowledge of lipid biochemistry and analytical techniques assisted in the interpretation of the data.

We the undersigned on behalf of all co-authors agree with the above statement of co-authorship for each of the published or submitted peer-reviewed manuscripts contributing to this thesis:

Signed:……Date:……

Dr Caleb Gardner – principal supervisor (TAFI/UTAS)

Signed:……Date:……

Dr Bridget S Green – co-supervisor (TAFI/UTAS)

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Finally, I dedicate this thesis to my family, Appa, Amma and Thuvara, whose own journey across the seas made this endeavour possible. Thank you for your patience, love and understanding and instilling in me strong roots and wings so I can achieve all that I can.

~Arani~

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Chapter 1

General Introduction

1.1 Fisheries enhancement systems

A decreasing trend in global catch rates and landings from capture fisheries have raised concerns about the capacity of wild harvest to supply the growing demand for fishery products (Garcia and Grainger 2005; Pauly et al. 2005). In response, capture fisheries and aquaculture sectors are expanding, restoring depleted stocks, and establishing enhancement programs to improve production and management of fishery resources (Caddy and Defeo 2003). Among the diverse strategies that are being implemented to achieve these objectives, there is an emerging field of fisheries enhancement systems, which combines the practice of releasing cultured organisms with harvest under the principals of wild fisheries (Lorenzen 2005).

Enhancement initiatives are highly diverse and viewed to be intermediate between aquaculture and fisheries in terms of both technical and human control. The three widely adopted enhancement systems defined by Lorenzen (2008) and Bell et al (2008) are;

- *Restocking* – the release of cultured juveniles into a wild population to restore severely depleted spawning biomass to provide regular, substantial yields.
- *Stock enhancement* – augments the natural supply of juveniles to overcome recruitment limitation.
- *Sea-ranching* – the release of cultured juveniles into unenclosed coastal environments for harvest at a larger size in "put, grow and take" operations. There is no intention of allowing released juveniles to augment spawning biomass, as in restocking, or to strengthen year classes, as in stock enhancement. However, where the animals reach maturity before the desired harvest size, they will contribute to egg production.

Within each of these broad enhancement systems, the designs of the operations vary to suit the biological or fishery characteristics of the target species. For instance, under specific circumstances the "put, grow and take" approach that defines sea ranching is slightly modified for enhancement operations where released animals are sourced from adults within the stock instead of hatchery reared juveniles. The use of adults instead of juveniles in enhancement fisheries systems is not uniquely defined, but falls under the broad category of translocation.

Translocation is the mediated movement of wild individuals or populations from one area with free release in another, in an attempt to establish, re-establish, or augment a population (IUCN 1987). Its applications in terrestrial systems range from conservation to pest management (Griffith et al. 1989; Fischer and Lindenmayer 2000) and for the aquatic systems it has aided restocking, stock enhancement and sea ranching operations (Bell et al. 2005). Most recently, a similar term "managed relocation" (aka 'assisted colonisation' and 'assisted migration') has been coined to describe the translocation of species to areas beyond their natural range to prevent their extinction from the effects of climate change (Sax et al. 2009; Schlaepfer et al. 2009). Thus the concept of translocation has many applications.

Among the common themes across all applications of translocation, is the debate of their appropriate and responsible use. Translocation associated risks often parallel those for the release of cultured juveniles, and stem from the uncertainty surrounding the interaction between wild and released populations (e.g. genetic mixing, resource competition), the behaviour of released individuals in the new environment (e.g.

survival, movement) and the ecological impact from population manipulation (e.g. spread of disease, trophic interaction). From a conservation perspective, enhancement strategies are sometimes opted as a last resort (Griffith et al. 1989) and in the case of managed relocation, the risk of species extinction is too large to disregard the option altogether (Sax et al. 2009). From a fisheries management perspective, stakeholders may only favour the fisheries enhancement option if it outperforms alternative management measures or provide a wider range of benefits and economically feasible (Lorenzen 2008). A common agreement in all instances is that the decision to establish enhancement systems requires rigorous evaluation supported by comprehensive research.

1.2 Background to management of the Tasmanian Rock Lobster Fishery

Management of the southern rock lobster resource in Tasmania began with gear restrictions, minimum legal size limits and a prohibition on harvest of soft-shelled or ovigerous lobsters in the late 1880's. Gear restrictions were later modified in the early 1900's to allow the use of pots (traps), plus limited entry, restrictions on the total number of pots in use and closed seasons (Gardner et al. 2004). In 1998 an individual transferable

quota (ITQ) management system was introduced in response to declining catch rates. Under an ITQ system, licence-holders were given a defined access to a share of the resource. An annual finite catch limit provided better management and monitoring of the resource, and as a result, catch rates steadily increased and lobster stocks began rebuilding around the State. Ten years after the introduction of ITQ's, the status of the fishery has significantly improved with an increase in state-wide egg production, legal biomass, catch rates and commercial revenue (Haddon and Gardner 2009) (Fig 1).

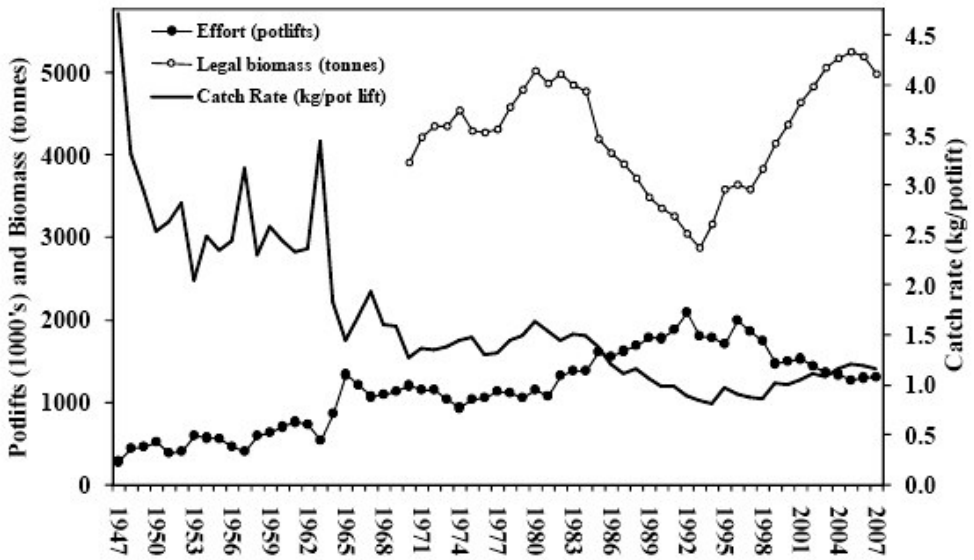

Fig 1. Historical trends in estimated fishing effort (pot-lifts), estimated catch-rate (kg/pot-lift) and estimated legal-sized biomass. Since 1998 a steady increase in legal biomass with recovering catch rates. (Haddon and Gardner 2009)

The main impact of capping catch through ITQ's was a shift in focus from maximising catch to optimising the value of the catch (Bradshaw et al. 2001). Fishers shifted fishing effort from offshore areas where the catch rate was high but the unit value was low, into inshore areas where the value of the catch was higher although catch rates were low (Frusher et al. 2003). Increased commercial fishing effort inshore is a product of market demand as fishers operating inshore harvest a higher valued product and have lower operational costs compared to fishers working on offshore grounds.

1.3 Characteristics of *Jasus edwardsii* Biology, Market and Fishery

The southern rock lobster, *Jasus edwardsii* has a wide distribution across southern Australasia in a range of depths (1 – 200 m) and habitats (Booth 2006), although reportedly no genetic differentiations among spatial stocks (Ovenden et al. 1992) due to its long larval phase (Bruce et al. 2000). In Australia *J. edwardsii* is the basis of 3 major commercial fisheries, in the states of South Australia, Victoria and Tasmania, with a gross value of exported product of AUS$134 million in 2006 (Wood et al. 2007). Biological characteristics of *J.*

edwardsii are variable throughout its' geographic range, particularly between deep and shallow-water populations. Two biological traits that significantly impact management of the commercial industry are growth rates (McGarvey et al. 1999) and size at onset of female sexual maturity (Gardner et al. 2006).

In Tasmania, growth of *J. edwardsii* is faster in shallow water and in more northern latitudes (Fig 2). Deep-water females are slow-growing and in some areas they never reach the legal minimum length (LML) for harvesting, but since they reach sexual maturity at a smaller size they contribute significantly to the state-wide egg production (Punt and Kennedy 1997). Conversely in the north of the State, growth rates are so fast that females are often removed by the fishery before they reach sexual maturity and do not contribute to egg production. The use of a single size limit throughout Tasmania where the target species exhibits high spatial variation in demographic traits appears as an inefficient harvest strategy of the available resource.

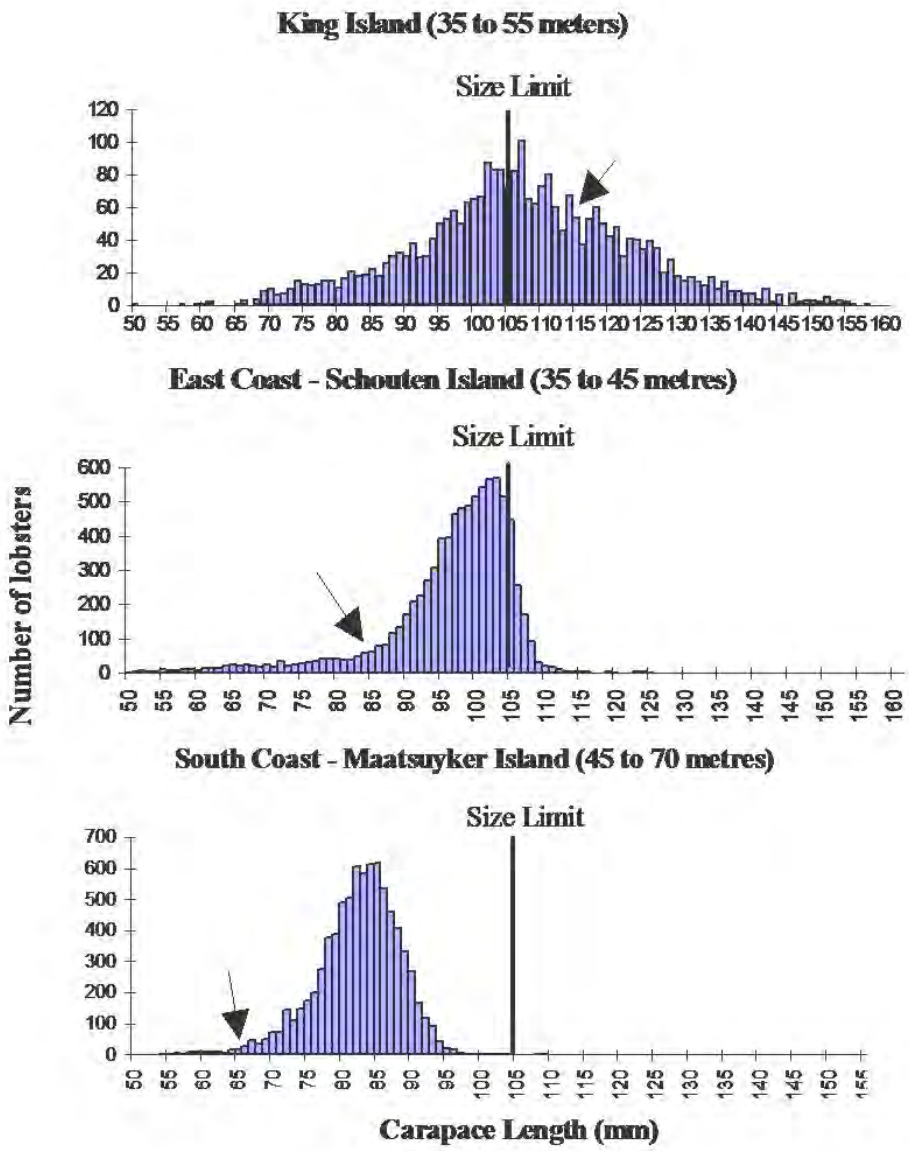

Fig 2. Size (CL) frequency distribution of female *J. edwardsii* sampled from north (King Island), east (Schouten Island) and south (Maatsuyker Island) coast of Tasmania. The solid vertical line indicates the legal minimum size and arrows indicate the size at which 50% of the females are mature.

The majority of the southern rock lobster catch is exported live to China where it is popular among the sushi restaurant trade and during festivals and special occasions (Roberts 1994). The red shell colouration of southern rock lobsters is a highly regarded market trait by Asian consumers due to the cultural association of the colour red with luck, prosperity and happiness (Konosu and Yamaguchi 1994). However, *J. edwardsii* harvested from depths greater than 30 m have very pale shell colouration (McGarvey et al. 1999). In addition to shell colouration, body-shape (leg length and abdomen shape), vitality and condition of lobster during live transport are also key market traits. The Asian market pays a higher premium for lobsters in a narrow size range (800 – 1500 g) without limb and carapace damage and which exhibit high vitality on arrival. Pale coloured, deep-water lobsters are generally sold at discounted prices or alternatively sold as cooked product on the domestic market. The price differential between deep-water pale lobsters and shallow-water red lobsters is variable and lowest during winter months when catch rates of all lobsters are low and market demand is high. So, in order to maximise their profits, some fishers adopted a fishing strategy which targets inshore areas until catch rates decline, then target offshore regions in winter to take advantage of higher market prices for pale lobsters (Frusher et al. 2003).

The interplay between the biological, market and fishery characteristics of *J. edwardsii* in Tasmania presents several management challenges, but also opportunities for improving yield, value and sustainability of the industry.

1.4 Translocation as a fisheries enhancement strategy

In response to the management issues surrounding spatial differences in egg production, ecosystems effects, marketing and yield, several options for spatial management are under consideration. For example, different regional minimum legal size limits may alter average harvest sizes so that they are better matched with regional growth rates and female size at maturity. This aims to increase egg production in northern regions and increase state-wide catch. But under this strategy, catches of extremely small and large lobsters and pale coloured lobsters would still fail to satisfy the Asian market demands and be sold at discounted prices, and so this option might improve the yield and sustainability but not maximise the value of the fishery. Other options include: increased quota allocation to direct effort to offshore deep-water regions, spatial closures, maximum size limits, and new market destinations. Some of these options

provide increased economic yield while others would increase egg production levels, however none could improve the value of the product. Thus these options have their own strengths and weaknesses as they effectively target one or a few objectives for improving a spatially heterogeneous fishery but fail to address others.

Translocation is one option that when used as an enhancement strategy could deliver improvements in yield, value and sustainability. Sea ranching through multiple translocations would be an exclusively field-based operation where large numbers of adult deep-water lobsters would be removed from sites of low productivity in deep-water regions of the State and be released into areas of high productivity inshore. The success of this strategy depends on translocated deep-water lobsters surviving, and adopting the biological characteristics of the resident shallow-water lobsters. Translocating deep-water lobsters aims to enhance the inshore stocks by increasing productivity, catch rates and quality of yield. In achieving these objectives, the Tasmanian Rock Lobster Fishery may use translocation as a supplementary strategy in conjunction with existing ITQ controls, provided it relieves the spatial pressures addressed above and meets the following constraints:

management of risk of disease transfer and genetic mixing to acceptable levels; high survival rates of released animals; low immigration rates of released animals back to deep water habitats; translocated animals adopt the market traits of shallow-water lobsters; a neutral or positive impact of additions and removal of animals on the ecosystem; and economic feasibility.

Invertebrate resources world-wide are coming under pressure as rising demand and prices for these generally high-value species leads to their overexploitation (Caddy and Defeo 2003). For Australian fishery resources in particular, there is a large gap in their management performance between their current use and their best use (Colquhoun and Archbold 2009) as illustrated by the Tasmanian southern rock lobster resource. Thus novel management initiatives are sometimes necessary to progress towards a better fishery performance. This thesis describes a 2-year experimental stage of a large-scale fishery enhancement experiment, with the specific objective of improving marketability and yield for the Tasmanian rock lobster industry.

1.5 Study Objectives and thesis structure

The specific study aims of this thesis research were to:

1) Quantify the spatial variation in the key market traits of shell colour, morphology, growth, physiological and nutritional condition between deep and shallow-water populations; and

2) Evaluate changes to traits influencing yield and marketability of deep-water lobsters after translocation.

Despite its commercial importance, the change in lobster colour with depth and throughout its range in the three commercial fishing states of South Australia, Victoria and Tasmania has not been comprehensively documented. In **Chapter 2** I investigated the relationship between lobster colour and depth and how this varies between regions. This information was then incorporated with catch rate records in the years before and after the quota management change to estimate the economic impact of colour discounting on the Tasmanian Rock Lobster Industry.

While the key market traits of shell colour and body-shape are widely accepted to vary throughout its distribution, this information is largely anecdotal. In **Chapter 3**, quantitative measurements of shell colour and body-shape traits were taken from deep and shallow-water lobster populations from Tasmanian, South Australian and Victorian fishing grounds. This provided baseline information for comparisons of these traits in deep-water lobsters after translocation to determine whether translocated lobsters had adopted the traits of the resident shallow-water lobsters in Tasmania.

Differences in market traits have been presumed to be related to nutritional differences between deep and shallow-water populations. In **Chapter 4**, using lipid and fatty acids analyses I investigated the nutritional condition of shallow and deep-water populations and whether this altered after translocation.

Differences in live transport condition between deep and shallow-water lobsters suggest differences in their physiological condition in response to stress. In **Chapter 5**, I assessed the post-capture condition of deep-water lobsters before and after translocation in comparison to shallow-water lobsters using

several haemolymph indices to determine how translocation may have altered the post-harvest condition of deep-water lobsters.

Increase in yield through translocation requires translocated lobsters to increase their growth and adopt the growth rate of the resident population. **Chapter 6** provides preliminary results of the short-term growth changes of deep-water adult male and female lobsters translocated from a slow-growth to a high-growth region.

Practical application and research serves a key intermediate role for quantitative assessment of outcomes from fisheries enhancement strategies. Stakeholders evaluating the overall feasibility of translocation require quantitative data to measure success and model predictive scenarios. This thesis provides a detailed quantitative assessment of the morphological and physiological differences between regions of high and low lobster productivity and how these differences respond to population manipulation. This research is part of a broader investigation evaluating translocation aided sea-ranching as a suitable fisheries enhancement strategy to improve the yield, value and sustainability of the southern rock lobster fishery (**Chapter7**).

Chapter 2

Colour variation in the southern rock lobster *Jasus edwardsii*, and its economic impact on the commercial industry

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Abstract

Southern rock lobster *Jasus edwardsii* varies in shell coloration throughout southern Australia. Predominantly exported as a live product to Asian markets, price is influenced by colour with prices higher for dark red than paler coloured lobsters. This paper explores spatial and depth variation in shell colour for the Tasmanian, South Australian and Victorian Fisheries using catch sampling data. The proportion of red lobsters decreased with depth and prevalent in depths less than 30 m, whereas paler coloured lobsters dominated the deeper depth ranges. The depth of transition where 50% of lobsters were classified as red showed a weak trend of increase with latitude from southern Tasmania to northern South Australia. Under quota management, lobster colour was a significant driver of fleet dynamics as fishers target areas of high price per unit. Consequently, catches of pale lobsters from deeper depths remain low despite the high catch rates in these areas. The colour price differential varies seasonally (higher in summer) so fishers increase supply of pale lobsters during winter. Discounting on colour equates to AUS$6.67 million/year for the Tasmanian Fishery alone, which indicates value from management or marketing research to reduce discounting.

2.1 Introduction

Southern rock lobster *Jasus edwardsii* (Hutton, 1985) are found throughout southern Australia and New Zealand and are fished across their distribution (Kailola et al. 1993). In Australia, the state-managed fisheries of Tasmania, South Australia and Victoria are managed using quota systems for the commercial sector with a gross value of exported product of AUS$134 million dollars in 2006 (Wood et al. 2007). These fisheries exploit a large depth range with catch taken from 1 to 250 m (main fishing range 1-140 m) (Booth 2006). Inshore populations of *J. edwardsii* are of a bright red colour and receive greater fishing effort by both the commercial and recreational sectors than lobsters caught from deeper depths that are of a paler coloration (Bryars and Geddes 2005). Commercially classed as "pale/brindle" and "white" lobsters, the exoskeletons of deep-water lobsters are mosaics of red, orange, and yellow tones. Colour variation is one of the many spatial differences in the biology of this species, and market grading of lobsters according to colour is a common practice in lobster processing facilities in Australia (Bryars and Geddes 2005).

Red coloured lobsters are in high demand throughout the year, driven by the Chinese market that receives 90% of Australia's exports (Wood et al. 2007). High demand is due to the cultural association of the colour red with happiness, luck and prosperity (Konosu and Yamaguchi 1994). Lobsters are displayed alive in restaurants so appearance and condition are also critical for this live market. In Australia, lobsters lacking uniform red coloration are often sold at discounted prices or sold into the local market for cooked product (Harrison 2004). Therefore, improving the price of pale coloured lobsters has been identified by the commercial industry as an opportunity for expanding value of the industry (Gardner and van Putten 2008a, b). Yet despite its commercial importance, information on the spatial distribution of colour categories and how they change and relate to depth has not been explored to date. This information is important in understanding seasonal fishing patterns and fishing effort particularly as it relates to the change in management system in 1998 when a shift in emphasis to quality of product occurred (Ford 2001). Thus data from fisheries independent surveys and commercial catch sampling were examined in this study to determine spatial and depth patterns in lobster colour. The beach price differential that exists owing to colour grading was examined seasonally and the economic

impact of colour differences was estimated both before and after management change for the Tasmanian rock lobster industry.

2.2 Materials and Methods

2.2.1 Catch effort and colour data

The Tasmanian Rock Lobster Fishery is managed as a single zone but because of demographic variation the fishery is divided into eight areas for stock assessment purposes (Semmens et al. 2006). The South Australian Rock Lobster Fishery is separated into two discrete fishing zones, Northern and Southern, and the Victorian Fishery is divided into Western and Eastern zones (Fig. 1) (Department of Primary Industries 2003; Linnane et al. 2006). Colour, depth at capture, effort and location data were obtained through fisheries independent research catch sampling surveys and commercial catch sampling conducted by all three state fisheries for the period 1993–2006. Observers conducting these surveys in each of these regions recorded colour according to the three categories used by lobster processors: red, pale, and white, based on the intensity and distribution of the red pigmentation in the exoskeleton (Fig. 2).

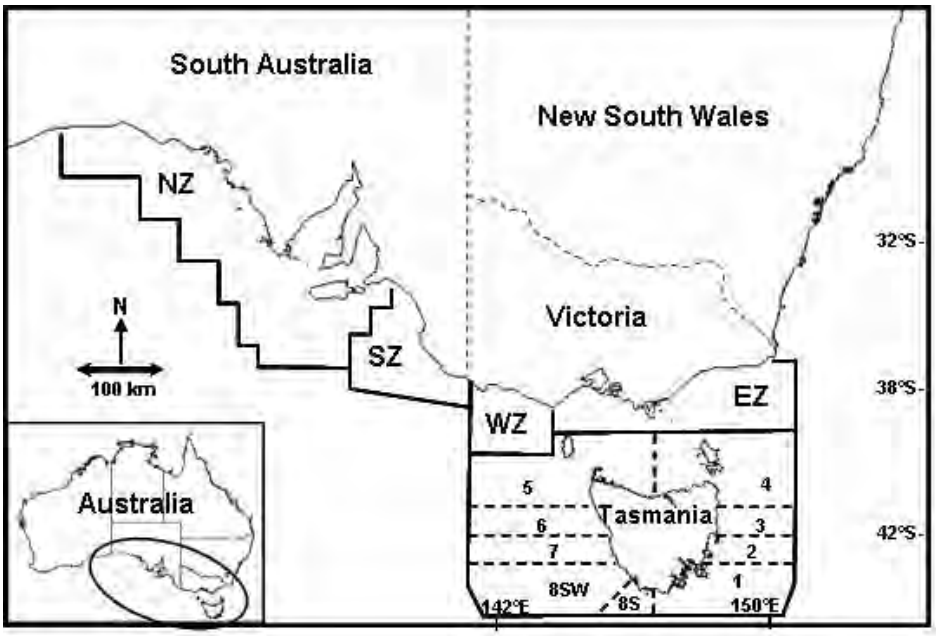


Fig. 1 South-east region of Australia showing the fishing zone boundaries for the three fisheries: Northern Zone (NZ) and Southern Zone (SZ) of the South Australian Fishery, Western Zone (WZ) and Eastern Zone (EZ) of the Victorian Fishery, and the 8 stock assessment areas of the Tasmanian Fishery (Area 8 spilt further into south and southwest regions).

Colour varies in a continuum so this qualitative grading includes an unknown degree of observer variation. Using these data, the proportional change in the three lobster colour categories with depth was evaluated for each of the three state fisheries. Commercial fishers report that they preferentially direct effort into shallower water to increase the proportion of the catch in premium colour grades. Differences in effort distribution would be expected to create a gradient of increasing lobster density with increasing depth. We examined this pattern in the Tasmanian dataset using catch rate as a proxy for lobster

density for time periods before (1992–94 and 1995–97) and after (2000–02 and 2003–05) the quota management change.

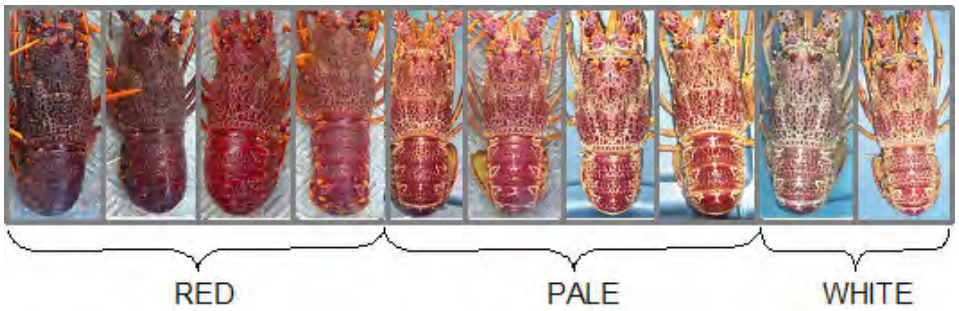


Fig. 2 Natural colour variation in *Jasus edwardsii* from a deep red colour (far left) to pale cream colour (far right), categorised by the three commercial grades of red, pale, and white.

2.2.2 Data analyses

Sample sizes in each region from all three states were variable and ranged between 843 and 13 780 lobsters. Data were analysed from all fishing zones and from the original eight stock assessment areas of Tasmania. Area 8 in Tasmania was split into two zones (south and southwest) because of the large number of lobsters collected from this area. The relationship between the proportion of lobsters that were classed as pale (P) and depth (x) was modelled for each region with a logistic function of the form: $P = e^{(a+bx)} / \left(1 + e^{(a+bx)}\right)$

where the coefficients a and b were estimated by maximising the log likelihood derived from using the log transformation (Neter et

al. 1990). These models were then used to estimate the depth at which 50% and 95% of the population were classed as pale, thus providing an estimate of the depth at which 50% and 5% of the population were classed as red (D50% and D5%). Estimation of uncertainty around these estimates followed the method of Turner et al. (2002) which determined 95% confidence limits around model fits from 500 simulations for each area in a bootstrapping routine where data were randomly sampled with replacement from each of the depth bins (Haddon 2001). The middle 95% of the bootstrap replicates constituted the confidence interval. Confidence limits derived by this method reflect the uneven distribution of certainty around estimates of the transition of colour with depth.

2.2.3 Economic impact

Economic implications of colour variation were examined for the Tasmanian fishery which has been managed through individual transferable quota since 1998 (Bradshaw 2001). A result of this management regime was that market revenue after 1998 could only vary through changes in beach price rather than through number of lobster landings. Since beach price is influenced by lobster colour, changes to revenue after the shift to

quota management is partially a function of the colour composition of catches landed. The economic impact of colour of landings was explored for three time series: before quota (1992–95); immediately before and at management transition (1996–99); and during an extended period after quota introduction (2000–05). Each time series is reviewed by summer (November–March) and winter (April–September) periods owing to seasonal changes in fleet dynamics. Historical monthly price of lobsters was obtained in Australian dollars from sales records of an exporting lobster processor (provided by Ian Hawthorn, Red Lobsters Pty Ltd). For the summer period of 2006 the average seasonal beach prices (over 5 months) were AUS$25.50/kg for red, AUS$20.20/kg for pale, and AUS$18.60/kg for white lobsters. For the winter period the average seasonal beach prices (over 6 months) increased to AUS$36.00/kg for red, AUS$32.17 for pale and AUS$31.17/kg for white lobsters. Prices for historical data were adjusted for inflation using national consumer price index with 2005 as the base year.

Although commercial fishers record all catch and effort in compulsory logbooks, this data series does not include colour of catch. Consequently, the proportion of catch in each colour grade was estimated on the basis of the average depth at which

pots were deployed as recorded by fishers. Gross revenue was then estimated from catch, estimated proportions of catch in each colour grade and the beach price for each colour grade. The estimated gross revenue for the colour graded (discounted) catch was then contrasted with estimated gross revenue that would occur in the absence of price discounting by colour by using only the beach price of red coloured lobsters. This approach provided an estimate of the loss in revenue because of differentiation based on colour only, but still inclusive of the seasonal price differential.

2.3 Results

A strong relationship between depth of capture and lobster colour was observed in all three states (Fig. 3). Red lobsters comprised greater than 50% of the catch in depths less than 33 m in Tasmania, 43 m in Victoria, and 64 m in South Australia. Significantly reduced proportions of red lobsters were encountered in depths greater than 60 m but did not disappear from the depth profile. The proportion of pale lobsters in depths down to 100 m was higher in Tasmania and Victoria than in South Australia and white lobsters were a minor component of the colour composition in all states except in Tasmania where it was 50% of the catch at 100 m depth (Fig. 3).

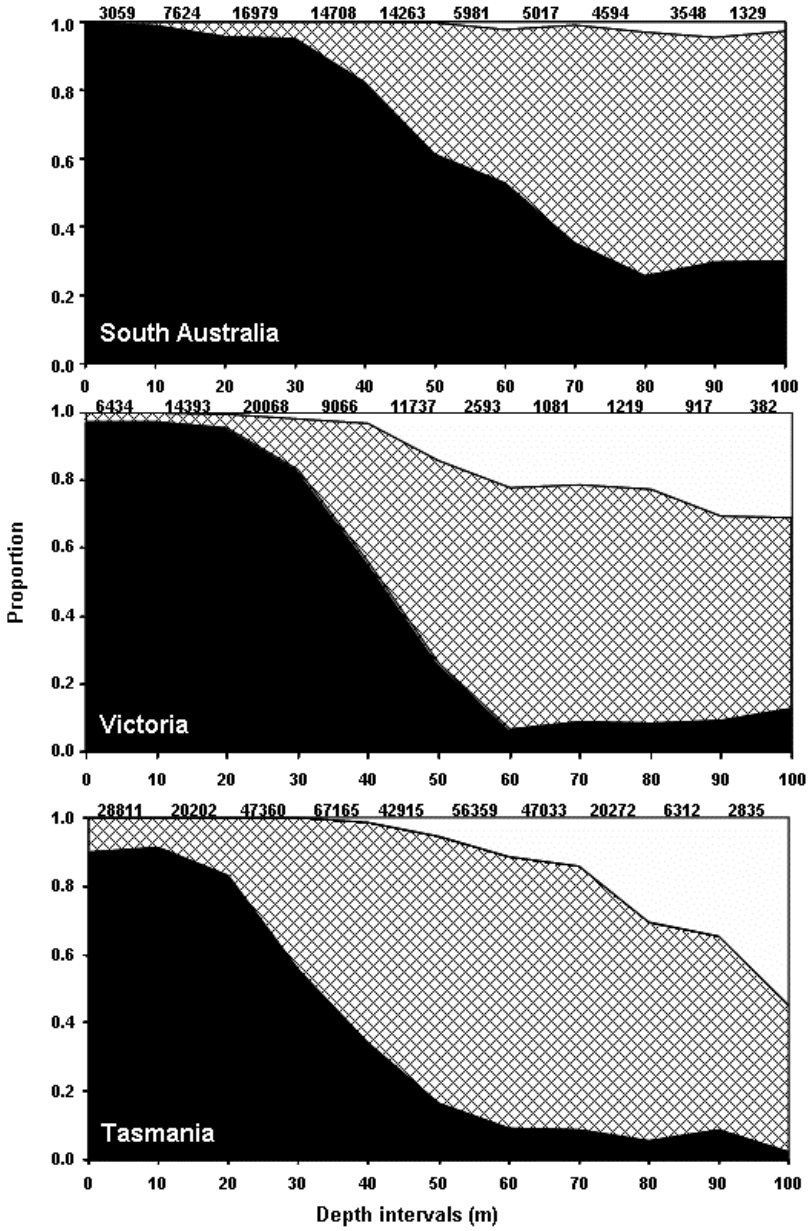


Fig. 3 Profile of colour change with depth of all lobsters sampled from each state: South Australia (1993–2005: n = 77, 102); Victoria (1993–2006: n = 68, 787); Tasmania (1993–2003: n = 340, 224). Numbers on the top of plots show the sample size for each depth interval. (Black area = red lobsters; Hatched area = pale lobsters; White area = white lobsters).

The depth at which 50% of lobsters were red (D50%) varied between regions with a weak trend from the northwest of South Australia to the southeast waters of Tasmania. The shallowest D50% was observed in southern Tasmania with a range of 32–39 m whereas the deepest D50% was observed in the Northern Zone of South Australia at 90 m (Fig. 4). This spatial trend was considered weak because variation between locations was high, for example, a significantly higher D50% value was estimated from Area 6 than from either of the adjacent areas (Areas 5 and 7; Fig. 4). Model estimates of D5% relative to D50% for each region indicate the rapidity of the transition from red to pale colour with depth: where D5% is close to D50% the transition is very sharp and occurs over a small depth range. The difference between D5% and D50% was greatest for Area 8SW, however, this appears to be a spurious result given the much smaller ranges for adjacent areas (Areas 8S and 1). This atypical pattern in Area 8SW appeared to result because the biological pattern was not correctly specified by a logistic function in this single case so the model fit was poor at the extremity of D5%. Overall the model estimates from the bootstrapping analyses were similar to the observed median depths for colour transition except for two sites, the Western

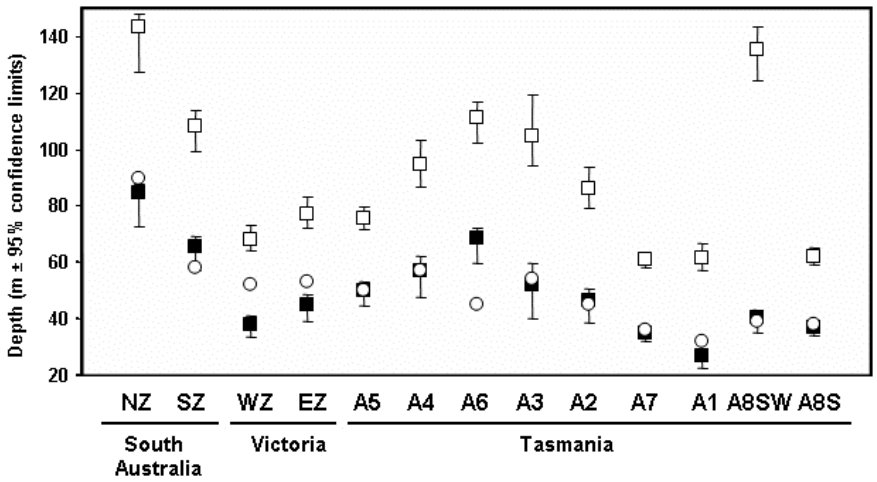


Fig. 4 Observed median estimates of the depth at which 50% of sampled lobsters were red colour (open circle) from the 13 regions across the three fisheries relative to the logistic model estimates of the depth at which 50% of sampled lobsters were red colour (filled square) and depth at which 5% of sampled lobsters were red colour (open square). Error bars indicate confidence limits around each point estimate.

Zone of Victoria and Area 6 of Tasmania (Fig. 4). In both these instances the transition in colour with depth was not symmetrical and thus neither the median value nor bootstrap estimate of D50% was ideal.

Overall, catch rates increased whereas the quantity of lobsters harvested decreased with depth, although catch rate trends were highly variable in depths greater than 100 m owing to limited data (Fig. 5). In all regions, catch rates inshore (<30 m depth) showed little difference before and after quota

introduction but there was marked increase in the tonnages of lobsters harvested. At greater depths, in the years post quota, catch rates were at their highest whereas the average annual catch remained unaffected in Areas 1 to 4 and dropped slightly in Areas 5 to 8. In the years after quota introduction, there was an increased shift in catch rate trends at all depths greater than 30 m in the southwest region of Tasmania (Areas 7 and 8), while catch remained unaffected by management change.

The price differential between summer and winter months increased more than two fold post quota owing to the discounting value being less in winter (Table 1). Discounting of catch on the basis of colour had a greater impact on gross value of product (GVP) in summer than in winter owing to both greater discounting in summer (when supply is greater) and also increased supply of non-red coloured lobsters.

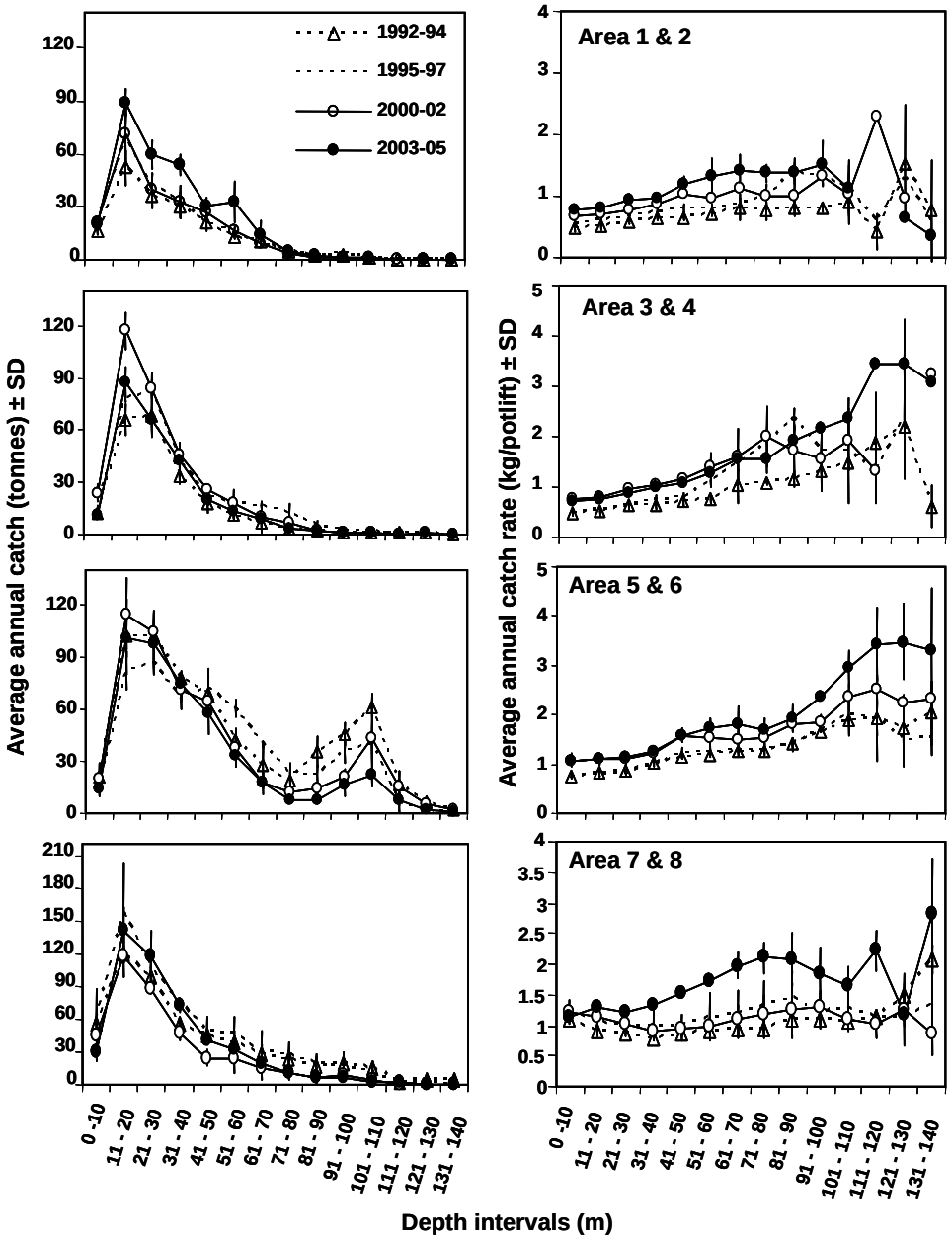


Fig. 5 Comparison between total numbers of lobsters harvested with depth (left column) and change in catch rates with depth observed (right column)(mean ± SD) for the four time periods for the four condensed regions of the Tasmanian fishery. (Note different scale on *y* axis).

The catch limit that occurred through quota management in 1998 had improved revenue from discounting for the summer months of post-quota years by about AUS$2 million annually, but little change for winter months which continues to incur loss of around AUS$1 million annually (Table 1). Overall, the quota management had improved revenue loss owing to colour by 24% to the current estimated value of AUS$6.67 millions/year.

Table 1 Trends in revenue loss owing to colour, collectively from all areas in the Tasmanian fishery. The average price differential (AUS/kg) between red and non-red lobsters (average of pale and white lobsters) and the average gross value of product (GVP, AUS$ millions/year) for the winter and summer periods over three time series are indicated. Total value indicated is the sum of summer and winter discount values averaged for each of the times series.

	Real Price Discount ($/kg, base year 2005)		Observed Real GVP Discount (AUS$ millions/annum)		
Year	Summer	Winter	Summer	Winter	**TOTAL**
1992-1995	6.18	5.05	7.70	1.10	**8.80**
1996-1999	4.83	2.09	5.83	0.97	**6.80**
2000-2005	5.63	2.84	5.24	1.43	**6.67**

2.4 Discussion

2.4.1 Spatial variation in colour

We observed a clear transition in shell colour of *J. edwardsii* with depth from a dark red colour in shallow depths to a pale cream colour at deeper depths across all three states. The rapidity in depth transition in shell colour was variable between regions and around Tasmania, but had a weak latitudinal trend, in a similar pattern to that which occurs with depth range of red lobsters' growth rates and size at onset of female maturity across this region (McGarvey et al. 1999; Gardner et al. 2006). The latter is thought to be driven by differences in temperature whereas the strong relationship we document between red shell coloration and depth appears to have a dietary basis, presumably because of changes in reef composition and in prey items with depth. Macro and encrusting algal assemblages which dominate inshore reef systems produce a wide variety of carotenoids which are then subsequently consumed by herbivores and larger reef dwelling animals such as lobsters who must acquire carotenoids dietarily (Goodwin 1960). Thus the concentration and intensity of red pigmentation in the exoskeleton is accumulated through the absorption of

dietary carotenoids and carotenoproteins and the subsequent synthesis into astaxanthin (Konosu and Yamaguchi 1994). Aquaculture studies have manipulated pigmentation by differing astaxanthin levels in artificial diets to produce different coloured juvenile *J. edwardsii* (Crear et al. 2002, 2003) and American lobster *Homarus americanus* (Tlusty 2005; Tlusty and Hyland 2005). Studies have also reported that both background colouration and photoperiod to have no effect on the colouration of juvenile southern rock lobsters (Stuart et al. 1996; Crear et al. 2003) and thus unlikely to be causative factors of colour variation in adult *J. edwardsii*. Therefore, the pale coloration deep waters rock lobsters around Tasmania, below the photic zone, most probably infers to a shift in dietary composition that is indicative of a shift in prey species or change in prey availability. Given that the diet of deep-water lobsters is unknown, shell colour could be used as a crude indicator of diet quality, at least in terms of carotenoid intake.

The variation we observed in the spatial and depth range of red lobsters may reflect the high variability in depth range and compositional differences in macroalgal communities across southern Australia. The penetration depth of light can vary in its quality and in its intensity, reaching the reef surface with an

overall trend for this depth range to extend into deeper water as wave exposure and water clarity increases (Edgar 2001). Depth range of algal communities may also be influenced by seasonal and temporal oceanographic processes such as localised up-welling events which are common in the southeast region of South Australia (Schahinger 1987). Furthermore, differences in geology and hydrology between these regions may also be a key factor as availability of homesites which provides adequate protection and food source are also highly variable.

In all states, red lobsters are still present at depths to 100 m, well below the photic zone, and in Victoria white lobsters are found in shallow-water depths of around 20–30 m. In these instances, movement of lobsters rather than diet may account for these observations. For example, in the spiny lobster *Panulirus cygnus*, colour change in the wild can occur during its intermoult period in association with an offshore migration event at the onset of maturity, termed “the whites run” (Melville-Smith et al. 2003). Large-scale migrations do not occur in Australian populations of *J. edwardsii* and thus do not contribute to the colour differences reported here (Gardner et al. 2003). However small-scale along-shore movement undertaken by immature females and males events are evident in some areas of the South

Australian (Linnane et al. 2005) and New Zealand fisheries (Annala and Bycroft 1993). Seasonal fluctuations in food resource and access to new spawning grounds are thought to be some of the reasons for these small-scale movement patterns and may explain some of the variation in the D50% red lobsters between adjacent areas of the Tasmanian fishery.

2.4.2 Impact of colour variation on the fishery

Fleet movements typically reflect attempts by fishers to find the optimal balance between costs (a function of catch rates) and revenue (a function of volume of product and beach price). The introduction of the quota systems in lobster fisheries across southern Australia has provided the incentive to shift fishing business strategy towards maximising catch value and minimising fishing costs in response to fixed volume of product (Bradshaw 2001). Fishers have the capacity to improve beach price by adjusting their effort in response to colour and seasonal differences in price. A general pattern is for fishers to target inshore areas for red lobsters in summer when supply is high and discounting for colour is greatest. In winter, fishers target locations with higher catch rates with less regard for colour because low supply leads to higher prices and less discounting

on the basis of colour. Ford's (2001) review of the Tasmanian Rock Lobster Industry 2 years after the introduction of the quota system found that there was already a trend of shifting catches to winter months. A socio-economic study on the impact of the Tasmanian quota system conducted 2 years after the introduction of quota, found only 25% of fishers had shifted their effort inshore to target red-coloured lobsters whereas 60% said they spent more time fishing in winter to seek higher prices when supply was more limited (Frusher et al. 2003). These trends in seasonal and spatial fishing patterns have altered little since then. For the 2003–05 period, fishing trends showed greater catch from shallow depth regions around Tasmania (with the exception of Area 7 and 8) whereas catch rates remained relatively low despite the intended stock rebuilding that was intended to occur though the quota introduction. Greater increase in catch rates in depths more than 50 m in all regions around Tasmania was seen during the post-quota years especially on the west coast. But despite high catch rates, catch levels from these depths were low and were mostly exploited during the winter months when the price differential between red and non-red lobsters was small. Similar trends in catch rates by depth were also evident in both South Australian and Victorian

fisheries (Department of Primary Industries 2003; Linnane et al. 2006).

Limited catches due to quota introduction resulted in a large reduction in the price differential of lobsters, which has increased the value of the industry by approximately AUS$2 millions for the post-quota years. While this is an economic improvement, the discounting practise continues to have a significant economic impact with the current estimated value of revenue loss at AUS$6.67 millions/year. Greater supply of pale lobsters during winter when demand for all coloured lobsters is high largely reduced the discounting value by around 50% (Table 1), despite the worsening weather conditions during winter months and lower catch rates at this time. Opportunities for further reductions to discounting and reducing the impact of fishing effort in the inshore areas are currently the major challenges facing all the state fisheries. Options include developing non-Asian markets such as those in the United States and Europe where colour of lobsters is less important, limiting harvest of pale lobsters to periods of high demand when colour preferences become less critical and the allocation of additional quota units for deep-water regions in order to shift effort away from inshore areas. One management option

currently under consideration in all three states is the sea-ranching of deep-water lobsters in shallow-water inshore areas (Gardner and van Putten 2008a). Translocation of pale and white coloured lobsters into areas of red coloured lobsters is aimed at achieving fast improvements to market traits such as growth, colour and condition which are assumed to be related to the diet of these animals. Thus, both the spatial and depth variability in lobster colour highlighted through this study and the current significant economic impact owing to colour grading estimated for the Tasmanian Fishery, provides valuable information for future management plans.

Chapter 3

Phenotypic variation among adult southern rock lobsters, and response to translocation

This Chapter is prepared for submission to ICES Journal of Marine Science as:

Chandrapavan A, Gardner C, Green B.S, Linnane A, Hobday D. Exploiting phenotypic plasticity to enhance the market traits of a spiny lobster.

Abstract

This study explored translocation as a method to increase the value of less marketable deep-water southern rock lobster *Jasus edwardsii*. Firstly, variation in the commercially important shell colouration and body shape between deep-water and shallow-water Tasmanian populations and among South Australian and Victorian populations was quantified. Deep-water *J. edwardsii* were pale coloured with longer walking legs but less meat content than shallow-water, red coloured *J. edwardsii*. Body shape traits were highly variable among deep-water populations across the three States and also between the sexes in each population. Deep-water lobsters were then translocated to a shallow-water inshore reef to determine if the observed variation in traits was plastic, and whether translocation could be used to improve the quality of deep-water lobsters. Translocated lobsters were monitored over a 14 month post-release period and during this time they changed from a pale/white colour to the more marketable red colour within one moult. Plasticity was observed in abdomen morphology but not leg morphology. The translocation experiment was successful in transforming pale/white deep-water lobsters into red lobsters with higher market value in a phenotypic response to altered environmental conditions. Translocation appears to have commercial

application for exploiting natural plasticity in market traits of lobsters to increase price.

3.1 Introduction

The price of live Australian southern rock lobster (*Jasus edwardsii*) in its main export markets in Asia can vary according to the colouration of the exoskeleton, overall body size and shape, degree of injury to appendages and its vitality after live transport. External appearances contribute to the price of lobsters sold live because consumer preferences are highly influenced by visual presentation. The most important market trait is shell colour, which varies systematically with capture depth and location throughout southern Australia (Chapter 2). Shallow-water *J. edwardsii* are typically red, while lobsters caught from depths greater than 30 m are paler and are commercially graded into categories of "pale" and "white" (Chapter 2). The fishing industry also differentiates lobsters on morphometric traits that reduce meat content. Characteristics associated with less meat yield such as long and spindly legs and/or abdomens that are narrow compared to their carapace

are believed to be more pronounced in deep-water pale lobsters than in shallow-water red lobsters.

The discounting of pale, deep-water lobsters based on colour and morphology is a standard practice for most of the year and greatest during summer months when supply is greater. Colour discounting reduced revenue by an estimated AUS$7 million or 12% of total revenue for the Tasmanian rock lobster industry in 2008 (Chapter 2). Discounting is also an issue for fisheries management as it influences fleet movements within the Tasmanian rock lobster fishery, and increases fishing pressure on the more depleted inshore stocks (Gardner et al. 2006). Management changes incorporating spatial elements are currently under review, with aims of increasing profitability and to reducing fishing pressure on inshore, more depleted reefs. One option under consideration is sea ranching through the translocation of pale-coloured, deep-water lobsters into inshore reefs and the economic and biological feasibility of this strategy is contingent upon translocated lobsters adopting the growth rates and phenotypic characteristics of the resident shallow-water population (Gardner and van Putten 2008a, b).

Differences in market traits may reflect differences in diet and/or adaptation to local habitat and through translocation. We investigate whether it is possible to harness the observed phenotypic differences between deep and shallow-water *J. edwardsii* to enhance its marketability. Our objectives for this study were twofold. Firstly, we examined the magnitude of phenotypic plasticity of the key market traits within the Australian southern rock lobster fishery by quantifying the variation in body-shape traits and shell colouration. Secondly, we translocated deep-water lobsters into a shallow-water reef inshore to test whether a change in habitat can induce a plastic response. We assessed this by comparing the market traits of the recaptured translocated lobsters with resident deep and shallow water populations.

3.2 Materials and methods

3.2.1 Lobster collection and translocation

Lobsters used for colour analysis

Natural variation in shell colour was determined from wild-caught, shallow-water (5 – 15 m) and deep-water (60 – 80 m) lobsters collected from Taroona Research Reserve (42.95°S, 147.34°E) and around Maatsuyker Island (43.38°S, 146.17°E) respectively in Tasmania (TAS).

Lobsters used for morphometric analyses

For analysis of spatial variation in body-shape traits, South Australian (SA) shallow-water lobsters (10 – 30 m) from Ringwood Reef (37.55°S, 140.05°E) and deep-water lobsters (40 - 100 m) from Robe (37.74°S, 139.84°E) and deep-water (70 – 110 m) lobsters from Victoria (VIC) (38.52°S, 141.53°E) were measured for comparisons with lobsters from the shallow and deep-water TAS sites (Fig 1). All lobsters analysed were mature adults and captured using baited pots and upon capture were measured, sexed, and clipped on one pleopod. To estimate leg length and meat yield, the fourth walking leg was removed by applying pressure to the base of the coxa to induce autotomy, and immediately placed in ice, then later stored in a -30˚C freezer. Lobsters with regenerated or damaged legs were not sampled. A sub-sample of the deep-water and shallow-water populations was photographed for measurements of shell colour and body-shape traits.

Translocation of lobsters

During November 2005, approximately 2000 adult lobsters (68 - 120 mm) were trapped from around Maatsuyker Island and translocated to Taroona Research Reserve. The reserve supports a large population of fast-growing, red coloured *Jasus edwardsii* protected from fishing. All translocated lobsters were individually coded with T-bar tags in the ventral surface of the first abdominal segment (coloured Hallprint T-bar anchor tag), clipped on one pleopod, measured, and sexed. Taroona Reserve

was resampled at 5, 8, 12 and 14 months after release, and at each time the same data was recorded for all recaptured translocated lobsters and fourth walking leg (non-injured, non-

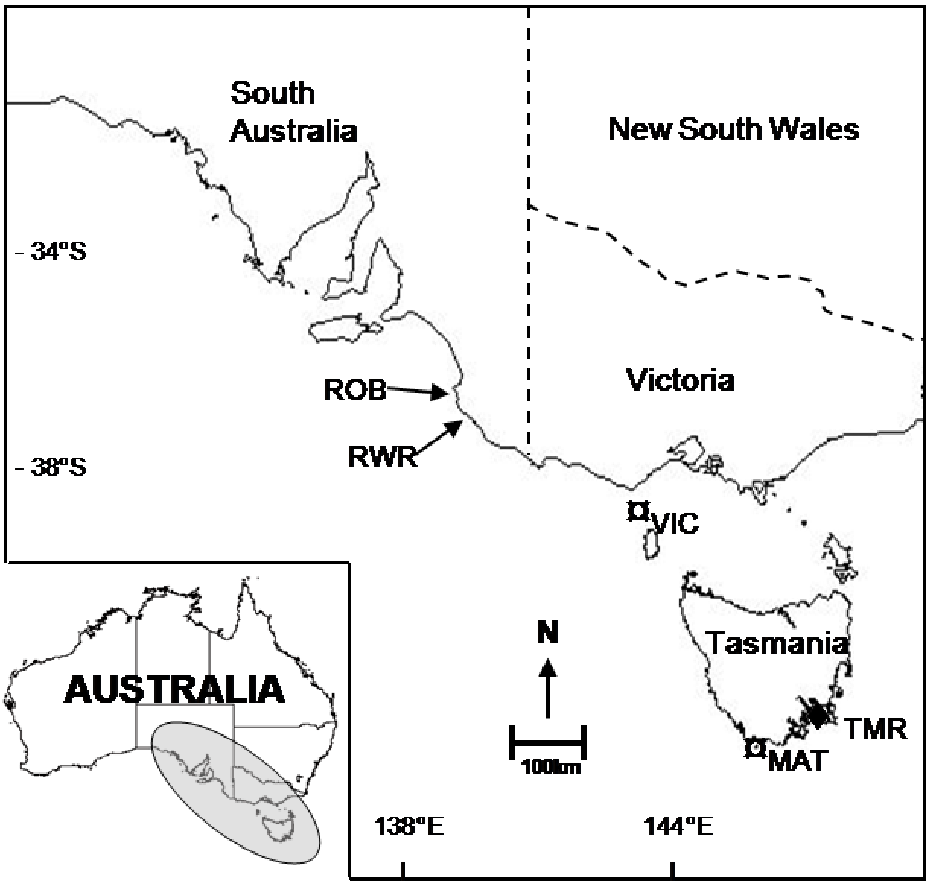


Fig 1. South-east region of Australia with deep and shallow-water sampling locations in Tasmania (MAT = deep-water site at Maatsuyker Island, TAR = shallow-water site at Taroona Marine Reserve), South Australia (ROB = deep-water site at Robe, RWR = shallow-water site at Ring-wood Reef) and Victoria (VIC = only deep-water site sampled). In Tasmania, lobsters for the translocation experiment were moved from MAT and released into TMR.

regenerated) was removed. If the same lobster was caught twice the remaining fourth walking leg in the pair was removed. A sub-sample of translocated lobsters (10 – 20 lobsters) was photographed for measurements of shell colour and body-shape traits. Moult growth increments of more than five millimetres and the partial or complete regeneration of the cut pleopod were used as moult confirmation (Ziegler et al. 2004).

3.2.2 Colour analysis

The range in natural colouration among red, pale and white lobsters was quantified by image analysis of digital photos taken of shallow-water and deep-water Tasmanian lobsters. Only hard-shelled lobsters with minimal carapace fouling were selected and colour was measured on five locations on the body, including the dorsal and lateral surface of the carapace, dorsal region of the abdomen closest to the carapace, the dorsal surface of the telson and the ventral surface of the sternum (Fig. 2). Colour changes in translocated lobsters were examined in all five body locations of recaptured moulted and non-moulted lobsters at each of the resampling surveys. The exoskeleton was blotted dry before being photographed with a Nikon Coolpix 5400 digital camera with a Nikon SL1 Macro Cool Light ring flash attached by a Nikon UR-E11step-down ring lens adapter (adapted from

Tlusty, (2005)). A black cloth hood was attached at the base of the ring flash to exclude external light. A 10 cm metal rod was also attached at the base of the camera to ensure constant distance from the exoskeleton. The camera was manually set under the macro operating function (shutter speed - 1/8 second, aperture - F4.4, picture quality - "normal", light metering - "spot", white balance - "speed light", sensitivity - "ISO100", image adjust and sharpness set to "auto"). Colour of the different body locations was quantified using the histogram function in Adobe Photoshop 7.0. Each image was opened in the RGB colour mode and a circular region (diameter of 1000 pixels) was measured for the mean values (darkest = 0 to lightest = 255) of each of the red, green and blue channels. These three individual channel values were added together to produce a single value for each image ranging from 0 – 765 (range of combined RGB value). This method was selected for analysis because we were only interested in the final colour produced by the three colour channels and not the changes or differences in the individual colour channels between the colour categories. In all five body locations the red channel dominated in intensity over the green and blue channels, therefore only variants of the colour red was present in our samples.

3.2.3 Morphometric analyses

Spatial and depth variation in leg length were compared among shallow and deep-water lobsters collected from TAS, SA and VIC sites. Prior to measurement, leg samples were thawed at room temperature and blotted dry. Periopods were measured along the dorsal length of the merus and the ventral length of the propodus (excluding the connective tissues). Total leg length (LL) was calculated from the combined lengths of the merus and propodus. To determine meat yield of the fourth walking leg, wet weight was recorded for the whole leg sample and the moisture content was determined by drying overnight at 100˚C and then reweighing the sample. Samples were then placed in a muffle furnace for three hours at 550˚C, and reweighed to determine the ashed weight. The difference between the wet and ashed weights provided an estimate of the combined moisture and organic weights of the meat content (g). Note that this method overestimates the meat yield as our calculations also include the water and organic content of the exoskeleton.

Spatial and depth variations in abdomen dimensions were assessed from digital image analysis using Image J v1.33 software (Wayne Rasband, National Institute of Health, USA,

http://rsb.info.nih.gov/ij/). Lobsters were restrained on a stable platform in a fully extended position underneath a camera stand fitted with an external light source. Photos were taken of the dorsal and ventral surface of the carapace and the abdomen region of each lobster. In order to minimise variation through different measurement techniques, carapace length was also calculated from the digital images. Tail measurements were taken from the ventral surface of the abdomen and included the anterior tail width (ATW) measured across the grooves between the pleurons of the first and second abdominal segments, posterior tail width (PTW) measured from the base of the sixth abdominal segment across the width of the sixth sternite, and the area of the tail inclusive of the ventral surface area of the abdomen covering the second to the sixth abdominal segment extending down to the anus (see Appendix for a diagram).

3.2.4 Data analyses

Colour data were normally distributed (Shapiro-Wilk W test) and variances were homogenous. Colouration of wild *J. edwardsii* between the three colour lobster categories was assessed among the five body locations using a MANOVA design, with Pillai's trace test statistic, followed by Tukey's post hoc analysis. Differences in shell colouration of the abdomen

between moulted and non-moulted translocated lobsters recaptured five months after release were analysed using a one-way ANOVA. There were insufficient lobsters in each moult category in subsequent surveys for statistical analyses. Analyses were performed using Statistica (V7.1 Statsoft Inc, Tulsa OK USA).

Allometric relationships among body-shape traits were examined using a combination of standardised major axis (SMA) and major axis (MA) regression analyses (types of Model II regression) using SMATR v2.0 software (Standardised Major Axis Tests and Toutines by D. Falster, D. Warton and I. Wright; http://www.bio.mq.edu.au/ecology/SMATR). These slope fitting techniques were considered most appropriate for describing bivariate growth relationships given all the measured variables (tail, leg and carapace measurements) had variation associated with them due to both measurement error and natural variability (Sokal and Rohlf 1981). The SMATR procedure is analogous in principal to ANCOVA, thus MA and SMA slopes were fitted between the desired two morphometric traits and tested for homogeneity of slopes and differences in slope elevation (if a common slope was present), followed by post-hoc pair-wise comparisons using the Wald statistic (for details of

statistical procedures see (Warton et al. 2006). All statistical analyses were tested for significance at $p = 0.05$.

All variables (tail, leg, carapace and meat yield measurements) were $\log_{10}$ transformed to achieve linearity and normality. Since tail morphometry is a secondary indicator of sexual maturity in female lobsters, only mature females were included in the analyses. An estimate of setation development of the pleopod was first determined to identify mature and immature females (Gardner et al. 2005). Further removals of immature females were based on observed data points that deflected and formed an angular delineation from the regression slope. Immature females were only detected in our samples from shallow-water TAS and deep-water SA lobsters. Allometric comparisons were made among Tasmanian populations (deep, shallow and translocated) and between deep and shallow-water sites (TAS, SA and VIC) separately for each sex and also between males and females (M and FM).

3.3 RESULTS

3.3.1 Shell colour variation

The natural shell colour of red lobsters had a RGB range of 100 – 250, while pale and white lobsters ranged from 350 to 800 (Fig. 2). Shell colouration varied significantly between all three market colour grades ($F_{10,92}$ = 49.5, p < 0.001) as well colouration between body locations ($F_{12,135}$ = 21.0, p < 0.001) with generally the dorsal surfaces more pigmented than the ventral surfaces (Fig 3). Five months after translocation, moulted lobsters were significantly darker than non-moulted lobsters, and both females and males had changed from pale or brindle coloration to the high market value red colouration (females, $F_{1,13}$ = 14.5, p = 0.01; males $F_{1,6}$ = 14.0, p = 0.01). By the end of the moulting period (10 months after translocation), all translocated lobsters had changed colour from pale or white to the red colour range. The timing of the colour change also suggests that translocated males had begun moulting earlier than resident males. Colour change was proportional in all body locations, therefore only the results for the tail region are presented (Fig. 4).

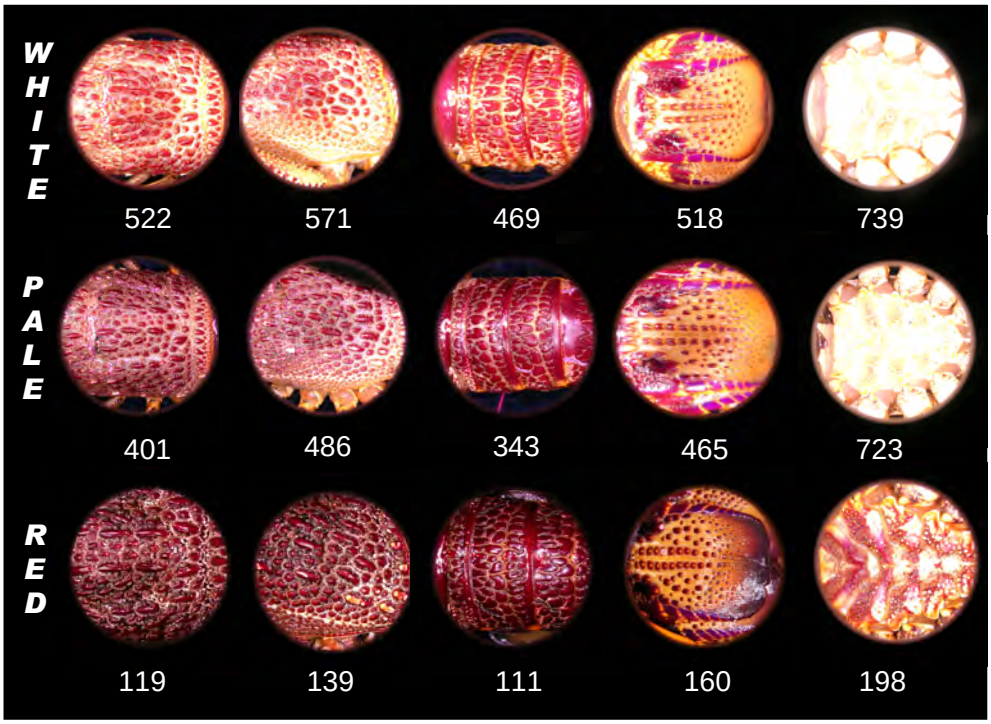


Fig 2. Examples of the five body locations in red (bottom row), pale (middle row) and white (top row) lobsters with their corresponding RGB value below each photo. From left to right: carapace-dorsal, carapace-lateral, tail, uropod, and sternum.

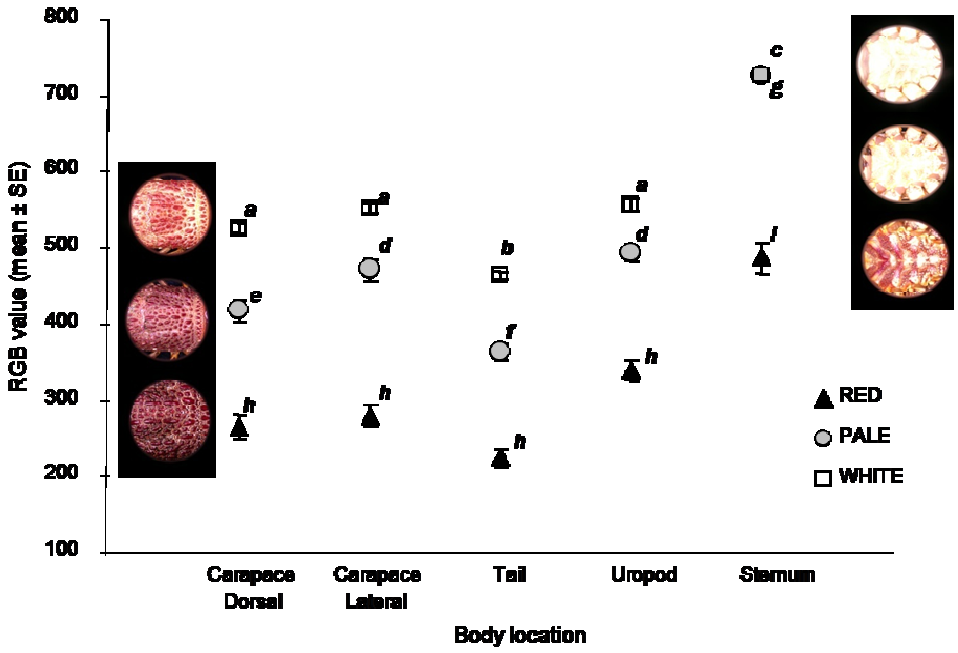


Fig 3. Natural colour variation (mean ± SE) of the five body locations of wild-caught red (n = 13), pale (n = 19) and white (n = 32) lobsters with image examples of the carapace-dorsal (left) and the sternum (right). Each colour category was significantly different from each other and body locations within each colour category that are significantly different in colour are indicated by different letters to the right of error bars (post-hoc Tukey's HSD test); white (*a,b,c*), pale (*d,e,f,g*), red (*h,i*). Error bars were often smaller than data symbols.

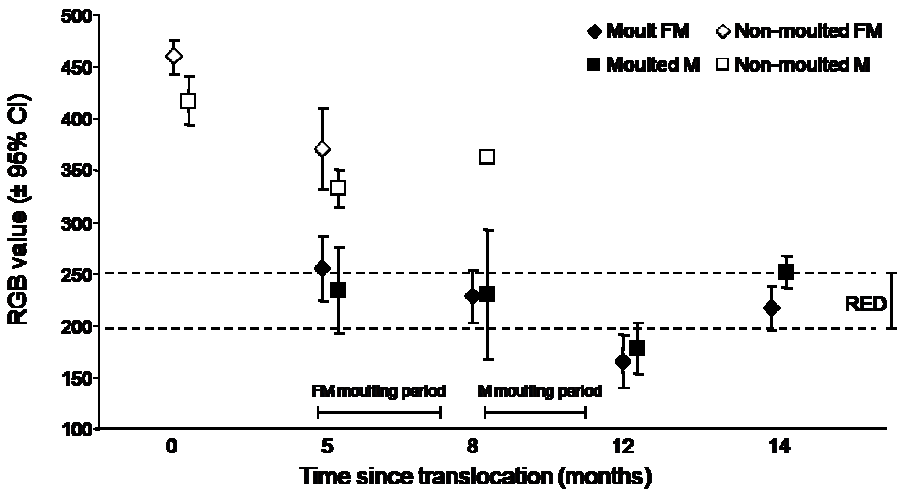


Fig 4. Mean colour differences (mean ± 95% confidence intervals) of the tail region between moulted (filled) and non-moulted (open) recaptured translocated female (diamond) and male (square) lobsters from four post-release sampling surveys. Moulting time periods for shallow-water male and female lobsters are indicated by horizontal bars above axis. RBG value range (mean ± 95% confidence intervals) for the tail region of red coloured lobsters (n = 13) are indicated by the dotted lines. RGB value for time zero was calculated from combined RGB values of the tail of pale and white lobsters (from Fig 2) (RGB range : 0 (darkest) – 765 (lightest)).

3.3.2 Body-shape variation

Deep-water male and female TAS lobsters had longer legs than shallow-water lobsters, but relative to their leg length, significantly less meat content than shallow-water lobsters (Fig. 5). Males had longer legs than females among TAS deep and shallow-water populations ($p < 0.05$, Table 1a). However, for any given leg length, meat yield was greater in shallow-water TAS females than males, but there was no differences in meat yield between the sexes of deep-water TAS lobsters ($p = 0.19$, Table 1b). After their first moult there was no difference in leg length of translocated lobsters (in either sex) compared to deep-water lobsters. The tail area (TA) of shallow-water TAS males was larger than the TA of deep-water males (Fig. 6a), but no difference detected among TAS females (Fig. 6b). There was an increase in the TA of translocated males to a similar size to that of shallow-water males (Table 2a). No change was detected in the TA of translocated females. In both sexes, the posterior tail width (PTW) relative to anterior tail width (ATW) was wider in deep-water TAS lobsters than in shallow-water TAS lobsters (Figs. 7a, 7b, Table 2b). In translocated males, the changes in the relative width of the PTW was not significantly different from shallow and deep-water males, and conversely in translocated females the

PTW was significantly less than deep-water lobsters and greater than shallow-water lobsters.

Deep-water TAS male lobsters had longer legs than VIC males while SA males were intermediate to TAS and VIC males (Fig. 5c). Among deep-water female populations, SA females had significantly shorter legs than TAS and VIC females (Fig. 5d). There were no differences in leg length between TAS and SA shallow-water males (Fig. 5e), but shallow-water SA females had longer legs than TAS females (Fig. 5f). Among deep-water sites, VIC lobsters had larger tail area than TAS and SA lobsters for both sexes. Tail area did not differ between SA and TAS deep and shallow-water populations (Figs. 6c, 6d). There was no difference in the width of the PTW between deep-water SA and VIC males but both populations had narrower PTW than TAS males (Fig. 7c). Conversely in deep-water females, the PTW was significantly different widths between all three sites. For any given ATW, the PTW was widest in TAS females followed by SA and narrowest in VIC females (Fig. 7d).

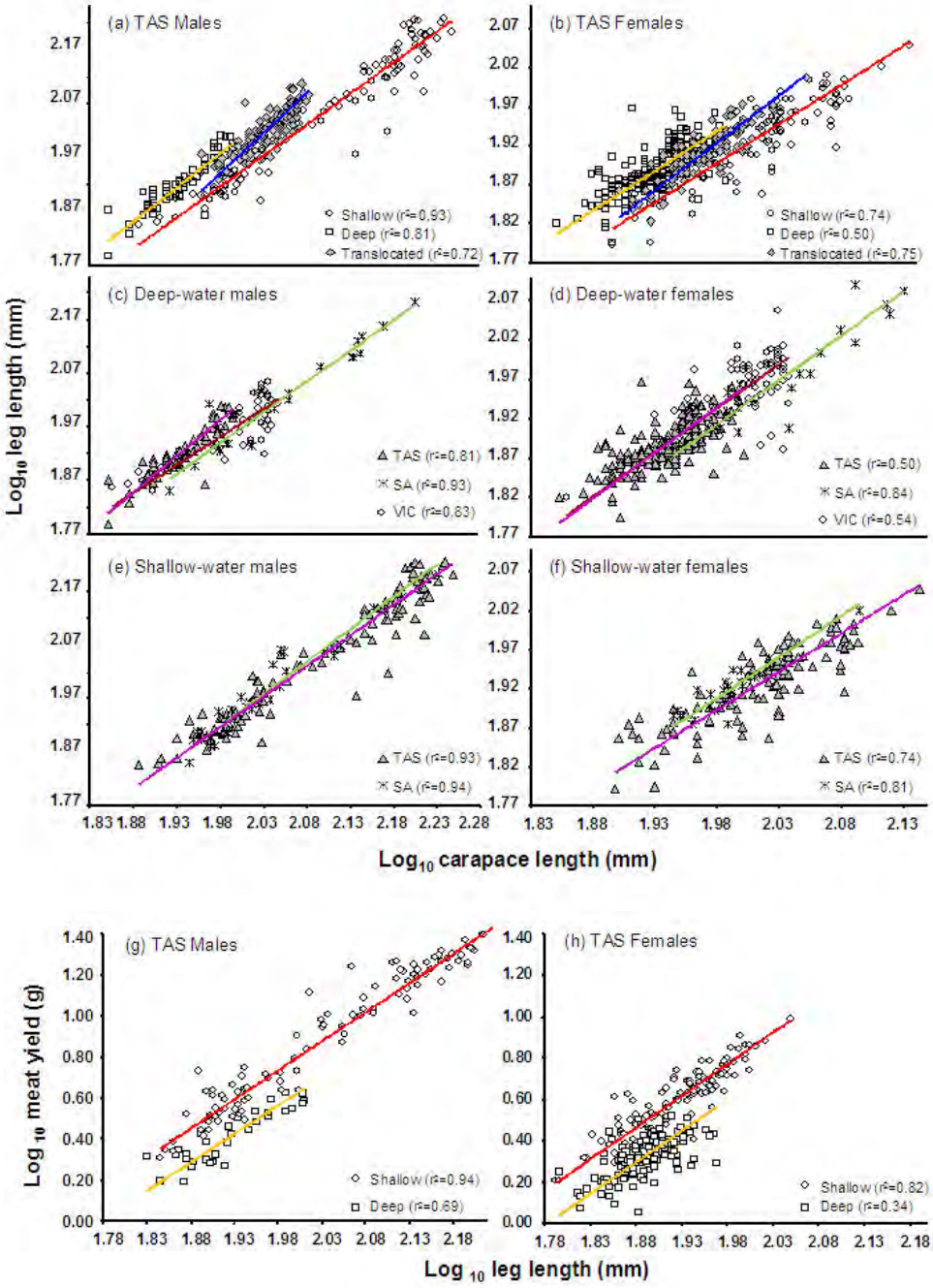


Fig 5. Leg length relative to carapace length of translocated (those which had moulted once when recaptured)(blue regression line) and resident shallow (red regression line) and deep-water (yellow regression line) lobsters from different sites (a) TAS males (b) TAS females (c) resident male deep-water lobsters from all states (d) resident female deep-water lobsters from all states (e) resident male shallow-water lobsters from all states (f) resident

female shallow-water lobsters from all states (TAS = purple, VIC = brown, SA = green regression lines) . Scatter plots of meat yield and leg length of TAS shallow-water and deep-water males (g) and (h) females are shown. Results of regression analyses based on these plots are presented in Table 1. Correlations values (r^2) are shown on the legend of each plot.

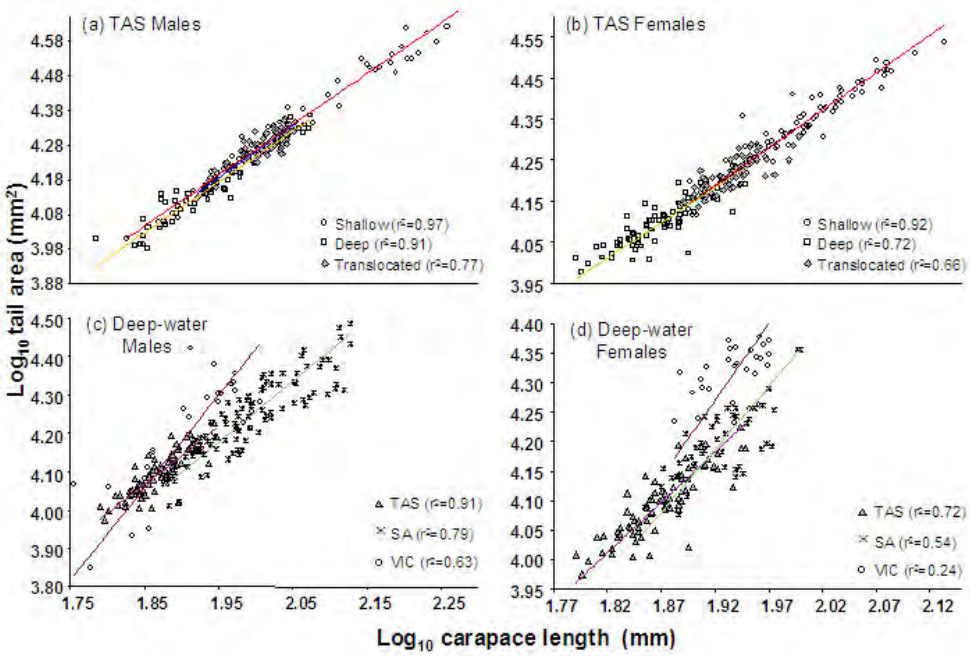


Fig 6. Tail area relative to carapace length of translocated (those which had moulted once when recaptured) and resident shallow and deep-water lobsters from different sites (a) TAS males (b) TAS females (c) resident male deep-water lobsters from all states (d) resident female deep-water lobsters from all states. No data were available from resident shallow-water lobsters in SA and VIC sites. Results of regression analyses shown in these plots are presented in Table 2a. Correlations values (r^2) are shown on the legend of each plot.

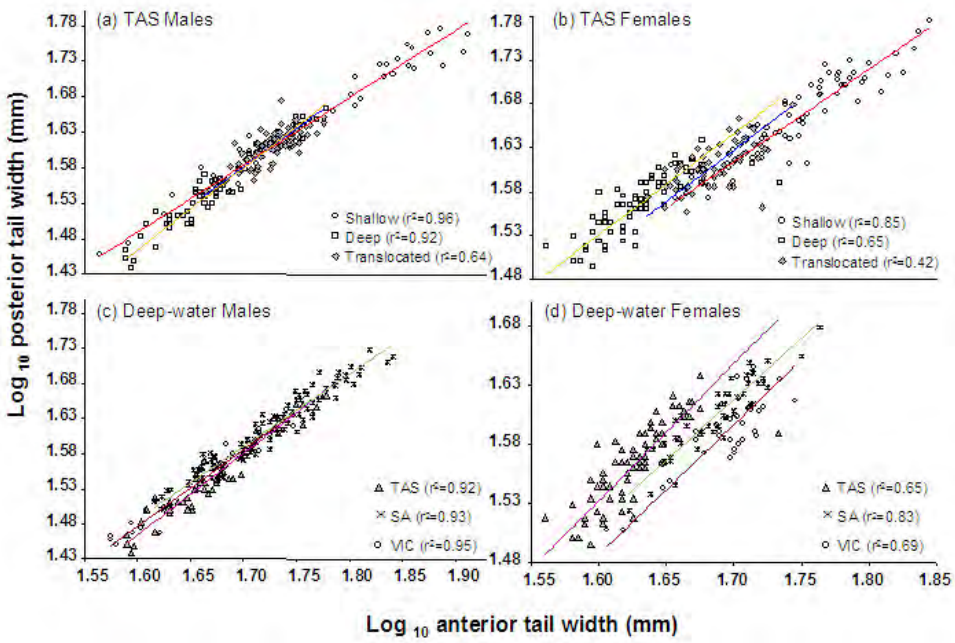


Fig 7. The posterior tail width relative to anterior tail width of translocated (those which had moulted once when recaptured) and resident shallow and deep-water lobsters from different sites (a) TAS males (b) TAS females (c) resident male deep-water lobsters from all states (d) resident female deep-water lobsters from all states. No data were available from resident shallow-water lobsters in SA and VIC sites.

Table 1. Regression statistics of the major axis (MA) regression analysis of (a) leg length and carapace length and standardised major axis (SMA) regression analysis of (b) meat yield and leg length. Results of comparisons between shallow, deep and translocated TAS populations and of deep and shallow-water lobsters are shown separately for each sex. Results of comparisons between males and females within each population are also given. Regression parameters of individual MA or SMA slope, common slope (CS) and intercept are indicated with (lower, upper 95% confidence intervals), differences in slope ($\Delta\beta$) and in elevation (ΔE) among populations were then tested for at the significance level of p = 0.05. Significant differences are indicated by different superscripts from post-hoc pair wise comparisons. Non-significance (p>0.05) indicated by the notation 'ns'.

a. LEG LENGTH	MA slope (± 95% conf.int)	Δ β, CS (± 95% conf.int)	intercept (± 95% conf.int)	Δ E
TAS Males				
Shallow	1.14 (1.08, 1.20) [a]		-0.34 (-0.47, -0.23)	
Deep	1.35 (1.17, 1.55) [b]	$p < 0.01$	-0.38 (-1.04, -0.32)	
Translocated	1.49 (1.33, 1.67) [b]		-1.00 (-1.35, -0.65)	
TAS Females				
Shallow	0.98 (0.87, 1.10) [a]		-0.40 (-0.27, -0.18)	
Deep	1.23 (1.07, 1.44) [b]	$p = 0.02$	-0.50 (-0.85, -0.14)	
Translocated	1.18 (1.06, 1.33) [b]		-0.43 (-0.69, -0.17)	
Males vs Females : $p_{shallow} = 0.02$; $p_{deep} = 0.42$, (CS = 1.30, Δ E $p < 0.01$) ; $p_{translocated} < 0.01$				
Deep-water Males				
TAS	1.35 (1.74, 1.55) [a]		-0.68 (-1.04, -0.32)	
SA	1.14 (1.01, 1.29) [ab]	$p = 0.03$	-0.32 (-0.60, -0.40)	
VIC	1.04 (0.92, 1.18) [b]		-0.11 (-0.37, 0.14)	
Deep-water Females				
TAS	1.24 (1.07, 1.44)		-0.50 (-0.85, -0.14) [a]	
SA	1.20 (0.98, 1.48)	ns. CS = 1.21	-0.45 (-0.94, -0.05) [b]	$p = 0.01$
VIC	1.19 (0.96, 1.49)	(1.01, 1.35)	-0.42 (-0.93, 0.09) [a]	
Males vs Females : $p_{SA} = 0.72$ (CS = 1.16, Δ E $p = 0.1$); $p_{VIC} = 0.02$				
Shallow-water Males				
TAS	1.14 (1.08, 1.20)	ns. CS = 1.16	-0.34 (-0.47, -0.23)	
SA	1.21 (1.01, 1.34)	(1.10, 1.21)	-0.48 (-0.72, -0.24)	ns.
Shallow-water Females				
TAS	0.98 (0.87, 1.10)	ns. CS = 1.16	-0.04 (-0.27, 0.18) [a]	
SA	1.05 (0.87, 1.28)	(1.10, 1.21)	-0.17 (-0.57, 0.23) [a]	$p < 0.00$
Males vs Females : $p_{SA} = 0.19$ (CS = 1.18, Δ E $p = 0.03$)				

b. MEAT YIELD	SMA slope (± 95% conf.int)	Δ β,CS (± 95% conf.int)	intercept (± 95% conf.int)	Δ E
TAS Males				
Shallow	2.83 (2.70, 2.96)	ns. CS = 2.82	-4.86 (-5.13, -4.58) [a]	
Deep	2.75 (2.26, 3.35)	(2.69, 2.95)	-4.90 (-5.94, -3.86) [a]	$p < 0.00$
TAS Females				
Shallow	3.07 (2.82, 3.33)	ns. CS = 3.06	-5.30 (-5.79, -4.81) [a]	
Deep	3.04 (2.68,3.43)	(2.85, 3.27)	-5.41 (-6.13, -4.71) [a]	$p < 0.00$
Males vs Females : $p_{shallow} = 0.09$ (CS = 2.88, ΔE $p = 0.02$); $p_{deep} = 0.19$ (CS = 2.70, ΔE $p > 0.05$)				

Table 2. Regression statistics of the major axis (MA) regression analysis of (a) tail area and carapace length and of (b) the posterior and anterior tail width. Results of comparisons between shallow, deep and translocated TAS populations and of deep and shallow-water lobsters are shown separately for each sex. Results of comparisons between males and females within each population are also given. Regression parameters as same as in Table 1.

a. TAIL AREA	MA slope (± 95% conf.int)	Δ β,CS CS (± 95% conf.int)	intercept (± 95% conf.int)	Δ E
TAS Males				
Shallow	1.47 (1.40, 1.54)	ns. CS = 1.48 (1.42, 1.54)	1.33 (1.18, 1.48) [a]	$p < 0.01$
Deep	1.48 (1.36, 1.61)		1.30 (1.06, 1.53) [b]	
Translocated	1.52 (1.37, 1.71)		1.22 (0.88, 1.56) [a]	
TAS Females				
Shallow	1.84 (1.72, 1.96)	ns. CS = 1.82 (1.72, 1.93)	0.66 (0.43, 0.90)	ns.
Deep	1.71 (1.49, 1.99)		0.89 (0.43, 1.35)	
Translocated	1.86 (1.56, 2.26)		0.62 (-0.04, 1.28)	
Males vs Females : $p_{shallow} < 0.01$; $p_{deep} = 0.07$ (CS = 1.53, ΔE $p < 0.01$); $p_{translocated} = 0.07$ (CS = 1.62, ΔE $p < 0.01$)				
Deep-water Males				
TAS	1.48 (1.36, 1.61) [a]	$p = 0.01$	1.30 (1.06, 1.53)	
SA	1.59 (1.43, 1.77) [a]		1.08 (0.74, 1.42)	
VIC	2.42 (1.80, 3.50) [b]		-0.41 (-1.89, 1.07)	
Deep-water Females				
TAS	1.71 (1.49, 1.99)	ns. CS = 1.84 (1.62, 2.10)	0.89 (0.43, 1.35) [a]	$p < 0.00$
SA	2.13 (1.63, 2.94)		0.09 (-0.08, 1.27) [a]	
VIC	2.63 (1.42, 8.71)		-0.77 (-4.43, 2.89) [b]	
Males vs Females : $p_{SA} = 0.05$; $p_{VIC} = 0.84$ (CS = 2.45, ΔE $p = 0.08$)				

b. TAIL WIDTH	MA slope (± 95% conf.int)	Δ β,CS (± 95% conf.int)	intercept (± 95% conf.int)	Δ E
TAS Males				
Shallow	0.95 (0.90, 1.01) [a]	$p < 0.00$	-0.03 (-0.13, 0.06)	
Deep	1.16 (1.08, 1.26) [b]		-0.40 (-0.55, -0.24)	
Translocated	1.09 (0.93, 1.27) [ab]		-0.26 (-0.56, 0.03)	
TAS Females				
Shallow	1.01 (0.96, 1.16)	ns. CS = 1.09 (1.00, 1.18)	-0.18 (-0.35, -0.00) [a]	$p < 0.00$
Deep	1.16 (0.97, 1.38)		-0.32, (-0.64, 0.01) [b]	
Translocated	1.18 (0.87, 1.62)		-0.37 (-1.00, 0.23) [c]	
Males vs Females : $p_{shallow} = 0.07$ (CS = 0.98, ΔE $p < 0.01$); $p_{deep} = 0.95$ (CS = 1.16, ΔE $p < 0.01$); $p_{translocated} = 0.67$ (CS = 1.11, ΔE $p < 0.01$)				
Deep-water Males				
TAS	1.16 (1.08, 1.26)	ns. CS = 1.08 (1.03, 1.12)	-0.40 (-0.55, -0.24) [a]	$p < 0.00$
SA	1.03 (0.98, 1.09)		-0.17 (-0.26, -0.07) [b]	
VIC	1.09 (0.99, 1.20)		-0.26 (-0.43, -0.09) [b]	
Deep-water Females				
TAS	1.16 (0.98, 1.38)	ns. CS = 1.08 (0.98, 1.19)	-0.32 (-0.64, 0.01) [a]	$p < 0.00$
SA	1.03 (0.90, 1.18)		-0.13 (-0.37, 0.10) [b]	
VIC	1.10 (0.86, 1.43)		-0.28 (-0.74, 0.19) [c]	
Males vs Females : $p_{SA} = 0.98$ (CS = 1.03, ΔE $p < 0.01$); $p_{VIC} = 0.91$ (CS = 1.09, ΔE $p = 0.01$)				

3.4 DISCUSSION

3.4.1 Natural variation in market traits

The observed variation in red colouration, and the relative growth differences between carapace length, leg length, tail area and tail shape between deep and shallow-water populations may reflect differences in diet and/or adaptation to the local environment driven by trade-offs between survival, growth and reproduction. Shell colouration is influenced by the availability of carotenoids derived from the diet (Rao 1985). The carotenoid astaxanthin is produced by macro-algae species and through its subsequent consumption by herbivores and larger predators it becomes the primary pigment in crustacean shell colour (Goodwin 1960; Meyers and Latscha 1997). In juvenile southern rock lobsters, manipulation of tissue carotenoid levels and shell colouration has been achieved through incorporating differing astaxanthin levels in artificial feeds (Crear et al. 2003). Tlusty and Hyland (2005) also produced similar results in juvenile American clawed lobsters *Homarus americanus* and further suggested that differences in the rates of carotenoid uptake and deposition in the cuticle could additionally control phenotypic colour variation. Aquaculture studies have also reported that

neither background colouration nor photoperiod affected colouration of juvenile southern rock lobsters (Stuart et al. 1996; Crear et al. 2003). Sea-cage experiments aimed at colour enhancement in pale, deep-water adult *J. edwardsii* had produced red lobsters with supplemented feeding, but colour improvement in starved, pale lobsters also occurred, with the authors concluding that the experimental lobsters consumed biofouling organisms from cage surfaces (Bryars and Geddes 2005). For adult *J. edwardsii* shell colour may depend on diet but it is unclear as to what extent colour expression reflects an adaptive plastic response to its physical environment or to physiological and behavioural stimuli.

There were morphological differences between deep and shallow-water Tasmanian lobsters when assessed by leg length, tail shape and tail area. These differences in body-shape imply differences in meat yield as evident from the analysis of one of the walking legs. The narrow tail shape reported for deep-water lobsters was however not observed and rather the reverse trend with shallow-water lobsters with narrower tails than and deep-water lobsters. There was substantial variation in the body-shape of *J. edwardsii* populations across the three states although not with any systematic pattern such as a latitudinal

trend. Allometric differences in morphology were also pronounced between the sexes. Sexual dimorphism may be responsible for the differences in tail characteristics, while differences in leg length and meat yield between the sexes could be due to differences in their metabolic rates. Similar differences have been observed in the clawed lobsters *Homarus americanus* and *Homarus gammarus,* where males and females from geographically different sites show variations in morphology despite their lack of movement and genetic differentiation (Debuse et al. 2001; MacCormack and DeMont 2003). The proposed causative factors of phenotypic plasticity were lobster density, water temperature, food availability and interactions among conspecifics. All of these factors potentially contributed to the observed differences between deep and shallow water lobsters in this study.

3.4.2 Market traits response to translocation and its commercial significance

Colour change in translocated *J. edwardsii* was a significant outcome of this study and consistent with colour change resulting from a change in diet. It contrasts with the colour transformation observed in the western rock lobster

Panulirus cygnus, which changes from a red to paler colouration prior to its migration to offshore breeding grounds (Melville-Smith et al. 2003). In this case, the distinct red to white colour transition in *P. cygnus* is assumed to have a genetically controlled mechanism (Wade et al. 2005). Changes in colour within a moult are also possible (Davis et al. 2005), but this is unclear from our results as increase in fouling during inter-moult masks any minor colour change. For deep-water *J. edwardsii* moulting was required following translocation in order for a more marketable red colouration and from a commercial point of view this increases its marketability and value.

Allometric changes in morphology after translocation varied among the body-shape traits and were also influenced by gender. The relative growth of the tail area of translocated males was similar to those of the resident shallow-water males after their first moult in the new habitat, demonstrating a high degree of plasticity. In another instance, the posterior tail width (PTW) of translocated females was statistically different but growth changes intermediate between deep and shallow-water females, while the PTW of translocated males were indistinguishable from both shallow and deep-water males. While there was no significant change in the leg length to carapace relationship in

translocated lobsters, graphical plots (Figs. 5a, 5b) indicate a transitional shift towards the resident population. Leg length may be less responsive to habitat change than the other traits examined and may require several moults for a complete transformation.

While there are many stock enhancement operations utilising hatchery reared juveniles (Bell et al. 2008; Zohar et al. 2008), the capture, translocation and release of adults in the marine environment is less common. However translocation has been adopted for similar value-adding strategies in other commercial species. For example, the roe size and quality of sea urchins are being enhanced in France by transplanting adults with poor quality gonads from polluted and barrens areas into areas dense in algal assemblages (Martin 2004). Abalone is another high value seafood product where transplant experiments had shown to improve the growth rates of stunted populations (Dixon and Day 2004). The success of all stock enhancement and sea ranching strategies depend on several associated factors including its economic feasibility (Bartley and Bell 2008), but for the Australian southern rock lobster industries, this study highlights translocations' potential in providing an opportunity to add value to its less marketable

product through improving its key market traits of colour and morphology.

Translocation appears to have commercial application for exploiting natural plasticity in market traits of lobsters to increase beach price. For deep-water *Jasus edwardsii* translocated to shallow-water, a single moult was sufficient to elicit significant changes in colour and a range of changes in body-shape traits as they grew in their new environment. Given colour has a higher priority over body-shape traits when setting the market price, there may still be a low risk of continued discounting by processors for translocated red lobsters with narrow tails or short legs. These issues needs to be addressed through further market research to assess industry and market response to market traits in translocated lobsters and determine if both the consumer and the processor are able to discriminate between legal size shallow and translocated lobsters. For the Australian southern rock lobster industry, this study highlights the potential of translocation to add value through transforming lobsters from undesirable phenotypes to desirable ones and offer a novel approach to fisheries management.

Chapter 4

Variation in the lipid and fatty acid content of adult southern rock lobsters, and response to translocation

This chapter previously published as:

Chandrapavan A, Guest M.A, Nichols P.D, Gardner C (2009). Translocating southern rock lobsters (*Jasus edwardsii*) from deep-water to shallow inshore water enhances nutritional condition through omega-3 long-chain polyunsaturated fatty acid content. Journal of Experimental Marine Biology and Ecology 375: 9-15.

Abstract

Deep-water southern rock lobsters (*Jasus edwardsii*) were translocated into shallow-water inshore reefs around Tasmania in an attempt to enhance their growth rates and market traits. We assessed changes in nutritional condition in adult, deep-water lobsters before and 12 months after relocation through variations in the lipid and fatty acid profiles in the hepatopancreas and muscle. Fatty acid compositions were similar between shallow and translocated lobsters and both were different from deep-water lobsters, suggesting a dietary difference between the deep and shallow-water lobsters, and a dietary change in deep-water lobsters after translocation. Nutritional condition indices, such as total lipid and triacylglycerol content, did not significantly vary between the lobster populations which may be due to within-population variability driven partly by differences in the moult stage of lobsters. Mean concentrations of fatty acids, lipid content and essential polyunsaturated fatty acids (PUFA) were higher in translocated lobsters than in both deep and shallow-water lobsters. Mean omega-3 long-chain PUFA content, in particular eicosapentaenoic acid (EPA, 20:5n-3) increased by 30% in the muscle of translocated lobsters, resulting in an enhanced nutritional value and a change in overall body condition. This

enhancement of key fatty acids, achieved through translocation, highlights the market potential of translocation for the commercial industry.

4.1 Introduction

The southern rock lobster (*Jasus edwardsii*) fishery in Tasmania currently operates below its optimal yield limit due to considerable regional and depth variation in biological traits of the lobsters (Gardner and van Putten 2008a, b). Within the fishery there are differences in key market traits such as shell colouration, size and body shape. Biological and market trait differences are greatest between the inshore shallow-water lobsters characterised by fast growth rates and bright red shell colour, and offshore deep-water lobsters characterised by slow growth rates and pale shell colour (Chapter 2). Considerable differences in size at sexual maturity (Gardner et al. 2006), seasonal catchability and possibly vitality during live transport occur between these populations and are further responsible for the greater fishing effort in inshore areas for the highly valued shallow-water lobster (Ford 2001). Biological variations between deep and shallow water *J. edwardsii* populations are assumed to

be related to differences in habitat and in particular the diet of these animals, but this has not previously been examined.

To increase the value, yield and sustainability of this resource, spatial management strategies aimed at optimising yield and marketability of the deep-water lobster stocks are currently being explored. One approach is stock enhancement through a form of sea-ranching. Large numbers of lower valued, sub-legal sized, adult, deep-water lobsters were translocated to shallow-water reefs inshore, where resident lobsters have a higher market value. Key variables in this mediated movement will be a change in diet and water temperature, although these were not directly measured. After their first moult event in the new habitat, translocated lobsters changed from a pale colouration to the marketable red colour of the resident lobsters, and growth increments showed a 2 to 3 fold increase (Chapter 6). What remains unclear is the role of diet in these transformations and to what extent nutritional condition of rock lobsters has been altered through translocation.

Indices of nutritional condition that are sensitive to dietary changes include total lipid content (energy reserves), the proportion of structural (phospholipid) versus storage lipid

(triacylglycerol), fatty acid composition and ratios of specific fatty acids (Kanazawa and Koshio 1994; Ju and Harvey 2004). These indices have been used to examine differences in body condition caused by dietary stress such as starvation in crustaceans (Jones and Obst 2000; Moore et al. 2000; Parslow-Williams et al. 2001), spatial variation within populations of the same and related species (Iverson et al. 2002, Murphy et al. 2002, Phillips et al 2003a, 2003b) and between wild and cultured populations (Navarro and Villanueva 2003; Nelson et al. 2005). For crustaceans, interpretation of nutritional information must also consider the physiological condition of animals such as reproductive and moult cycle (Chang and O'Connor 1983; D'Abramo 1997), and the functional role of the tissue analysed as these can alter the relative quantity and type of lipid reserves. For example, the digestive gland (hepatopancreas) of decapod crustaceans is primarily involved in the digestion and absorption of food material, lipid synthesis and storage, and in regulating energy metabolism (O'Connor and Gilbert 1968; Gibson and Barker 1979). Lipid reserves found in this gland are typically high and respond rapidly to changes in physiological and environmental parameters such as dietary stress caused by altered feeding rates, moulting and reproduction (Sargent 1974; McLeod et al. 2004). By contrast, muscular tissues such as

walking legs are low in lipid which is predominantly present in bio-membranes as phospholipids, and do not respond readily to short term changes in physiological and environmental parameters (Cockcroft 1997). This slow tissue turnover rate means they are useful in understanding the longer term dietary changes experienced by the animal (Corraze 1999).

In the present study, we quantified the lipid content, lipid class and fatty acid compositions of both the digestive gland and leg muscle of shallow-water, deep-water and recaptured translocated lobsters to examine differences in their nutritional condition through short-term and long-term changes, and to understand the dietary plasticity of *J. edwardsii* through the *in situ* change of lobster habitat and diet.

4.2 Materials and Methods

4.2.1 Translocation and lobster collection

One of the principal translocation release sites, Taroona Marine Reserve, is located in the Derwent Estuary in Tasmania and extends 800 m from the shoreline covering an area of 1.0 km^2 with a depth range of 1 to 15 m. The reserve supports high

densities of fast growing, red coloured *Jasus edwardsii.* Maatsuyker Island lies 10 km off the south coast of Tasmania in the southern ocean with a depth range down to 100 m and consists of rocky outcrops and patchy reefs (Fig. 1). These reefs are characterised by high densities of slow growing, pale coloured lobsters (Punt and Kennedy 1997).

In November 2005, approximately 2000 sub-legal sized lobsters (68-120 mm) of both sexes were captured using baited lobster traps at depths between 60-80 m around Maatsuyker Island. Lobsters were tagged ventrally in the abdomen (with a coloured Hallprint T-bar anchor tag), one pleopod clipped and bio-data recorded. Prior to release of translocated lobsters, digestive gland and leg muscle tissue samples of hard-shelled, male lobsters of varying sizes were collected from 10 shallow-water lobsters from the Taroona site and 9 deep-water lobsters from the Maatsuyker site. The Taroona site was surveyed twelve months later in November 2006, when translocated lobsters were recaptured and digestive gland and leg muscle samples were collected from 10 hard-shelled male lobsters. Selection of lobsters for analysis included those which had moulted at least once since release, and changed in shell colour from pale to

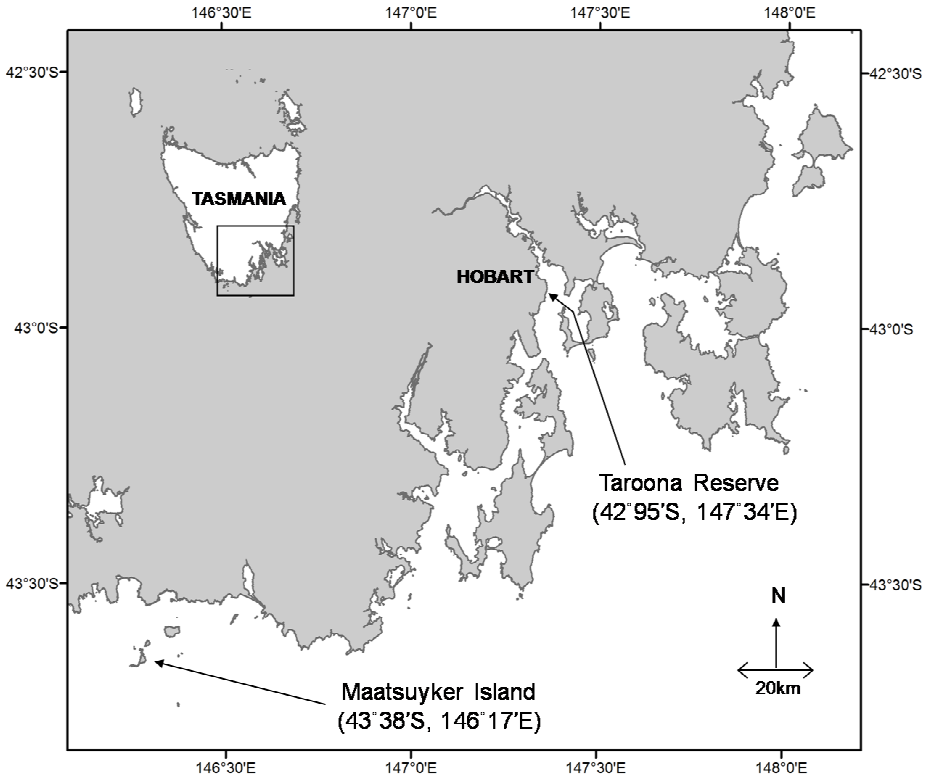


Fig 1. Map of south-east Tasmania showing the deep-water site (Maatsuyker Island) and the shallow-water translocation site (Taroona Reserve).

bright red colour. Moult growth increments of more than 5 mm and the partial or complete regeneration of the cut pleopod were used as moult confirmation (Ziegler et al. 2004). We compared triacylglycerol (TAG) concentrations in the digestive gland with blood refractive index (BRI) values of shallow-water and translocated lobsters to examine the influence of moult cycle. Haemolymph samples (1 ml) were taken from the pericardial sinus of only the shallow and translocated lobsters. Pigment

stage (PS) of the haemolymph was noted (Musgrove 2001) and then an aliquot of the sample was placed in a hand-held refractometer (Model UR-2, Industrial and Scientific Supply Co.) to measure blood refractive index. The blood protein/pigment stage index developed by Musgrove (2001) for *J. edwardsii* was used as an additional condition index. The BRI is directly proportional to the concentration of protein in blood of *J. edwardsii* (Oliver and MacDiarmid 2000; Musgrove 2001), which increases as body condition improves from the post-moult to pre-moult stage of the moult cycle. Haemolymph colour is indicative of the pigment astaxanthin, which increases in concentration during the late inter-moult to pre-moult stage and can be visually assessed for colour change. Both BRI and PS provide a crude indication on the moult stage, particularly in differentiating the beginning, middle and end phases of the cycle. Animals in post-moult to early-inter-moult stage will have low BRI values and PS of clear/grey colour. During the long inter-moult period as water is replaced by tissue growth in lobsters, BRI increases while the PS remains colourless. As lobsters approach the pre-moult stage, reabsorption of the old shell causes blood pigment to change progressively from clear to dark red colour and BRI levels are at their highest. Although we selected hard-shelled lobsters for analysis, these additional

indices provide more precise information on the moult condition of the lobsters. Animals were killed in freshwater before a single lobe of the digestive gland was dissected for lipid and fatty acid analyses. Muscle tissue from the fourth right or left walking leg was also removed and all samples stored in a –20˚C freezer prior to analysis.

4.2.2 Lipid and fatty acid analyses

All lobster digestive gland and muscle samples (as wet tissues) were quantitatively extracted overnight using a modified Bligh and Dyer (1959) one-phase methanol:chloroform:water extraction (2:1:0.65 v/v/v). Phases were separated the following day by addition of chloroform and water (final solvent ratio, 1:1:0.9 v/v/v methanol: chloroform: water). Lipids were recovered in the lower chloroform phase, solvents removed under vacuum, the concentrated lipid recovered through rotary evaporation at 40ºC, then weighed to obtain total lipid content. An aliquot of the total lipid extract (TLE) was analysed using an Iatroscan MKV TH10 thin layer chromatography flame ionisation detector analyser (Tokyo, Japan) to determine the proportions of individual lipid classes. A polar solvent system (60:17:0.1 v/v/v ratio of hexane: ether: acetic acid) resolved TAG, free fatty acids,

sterols and phospholipids. Peaks were quantified with DAPA Scientific Software (Kalamunda, Western Australia).

An aliquot of the TLE was transmethylated at 80ºC for 2 h in a 10:1:1 v/v/v mixture of methanol: hydrochloric acid: chloroform to produce fatty acid methyl esters (FAME). FAME were partitioned by the addition of water and extracted with 4:1 hexane: chloroform (v/v, 3 x 1.5 ml), the solvent removed under a stream of nitrogen, then silylated at 60ºC for 2h in N, O-bis-(trimethysilyl)-triflouracetamide (BSTFA). FAME were analysed by gas chromatography (GC) using a Agilent Technologies 6890N GC (Palo Alto, California, USA) equipped with an Equity™-1 fused silica capillary column (15 m × 0.1 mm i.d., 0.1µm film thickness), an FID, a split/splitless injector and an Agilent Technologies 7683 Series auto sampler and injector. Helium was the carrier gas, and pressure was maintained at 65 kPa. Samples were injected in splitless mode with an oven temperature of 120ºC, and temperature was increased to 250ºC at 10ºC / min, and finally to 270ºC at 3ºC / min. Peaks were quantified with Agilent Technologies ChemStation software (Palo Alto, California, USA). Individual components were identified by mass spectral data and by comparing retention time data with those obtained for authentic and laboratory standards. GC

results are typically subject to an error of up to ± 5% of individual component area. GC-mass spectrometric (GC-MS) analyses were performed on a Finnigan Thermoquest GCQ GC-mass spectrometer fitted with an on-column injector and using Thermoquest Xcalibur software (Austin, Texas, USA). The GC was equipped with an HP-5 cross-linked methyl silicone fused silica capillary column (50 m × 0.32 mm i.d.) of similar polarity to that used for GC analyses and helium was used as carrier gas.

4.2.3 Statistical analyses

A combination of analysis of covariance (ANCOVA) and multivariate analysis of covariance (MANCOVA) were used to compare total lipid content, TAG content and fatty acid compositions between shallow-water, deep-water and translocated lobster populations using lobster size (carapace length) as a covariate. All variables were normally distributed after transformations of $\log_{10}$ of digestive gland data and $\log_2$ transformation of leg muscle data (Shapiro-Wilk W test and normal probability plots) and there was no violation of the homogeneity of slopes assumption. Pillai's trace test was used as multivariate test of significance and significant effects were

further examined using Tukey's post-hoc analysis. Statistical analyses were performed using Statistica (V7.1 Statsoft Inc, Tulsa OK USA). All identified individual fatty acids (expressed as percentage of total fatty acids) were compared among deep-water, shallow-water and translocated lobsters using principal components analysis (PCA). PCA reduces the number of variables by producing components using linear correlations between variables to identify those fatty acids that contribute most to the separation between observed groups and was performed using PRIMER 6 software (PRIMER-E, Plymouth, UK).

4.3 Results

4.3.1 Total lipid content and lipid class composition

Mean total lipid content (mg g^{-1} wet wt.) of the digestive gland did not significantly vary between deep-water, shallow-water and translocated lobster populations ($F_{2,25}$ = 0.56, p = 0.58). The major lipid class in the digestive gland was triacylglycerol (TAG) followed by phospholipid (PL), free fatty-acid (FFA) and sterol (ST) (Table 1). TAG concentrations in the digestive gland did not vary between the lobster populations ($F_{2,24}$ = 0.41, p = 0.66). Total lipid content in the leg muscle

constituted less than 1% of the muscle (wet weight basis) and within populations was less variable than in the digestive gland. Phospholipids made up approximately 95% of the lipid content in the leg muscle of lobsters from each population, and the remaining lipid was ST, with minor proportions of FFA and TAG (Table 1).

Table 1. Total lipid content (mg g^{-1} sample wet weight) and lipid class composition (as % of total lipid) of the digestive gland and leg muscle expressed as mean ± SE from shallow-water, deep-water and translocated (recaptured after 12 months) lobsters. Size range indicative of the carapace length measurements, *n* = sample size; TAG = triacylglycerol; FFA = free fatty acid; ST = sterol; PL = phospholipid.

	Digestive Gland			Leg Muscle		
	Shallow	Deep	Translocated	Shallow	Deep	Translocated
n	10	9	10	10	9	9
Size range (mm)	110 – 160	85 - 132	96 – 118	118 - 147	78 - 97	96 – 118
(mg g^{-1} wet wt.)						
Total lipid	113.6 ± 19.4	107.1 ± 15.8	140.9 ± 26.4	8.8 ± 0.5	9.6 ± 0.5	9.5 ± 0.7
(percent comp.)						
TAG	68.8 ± 5.9	51.9 ± 9.6	59.7 ± 7.7	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.1
FFA	3.6 ± 0.7	10.5 ± 5.1	10.0 ± 2.8	0.2 ± 0.1	0.5 ± 0.1	0.5 ± 0.2
ST	0.8 ± 0.2	2.8 ± 0.8	1.6 ± 0.5	4.7 ± 0.4	3.9 ± 0.3	4.1 ± 0.4
PL	26.8 ± 5.3	34.8 ± 6.8	28.7 ± 5.5	94.9 ± 0.4	95.5 ± 0.3	95.2 ± 0.5

The median BRI values for shallow (1.339) and translocated lobsters (1.341) were similar (Fig 2b), indicating that most lobsters were in their late post-moult to early inter-moult stage of the moult cycle. Several translocated lobsters appear to be in their late inter-moult to pre-moult, indicated by their darker (reddish) blood pigment colour and higher BRI, and these same lobsters had higher TAG level (Fig 2a, b). Generally, lobsters in their late inter-moult and pre-moult stage are nearing the end of their maximum TAG accumulation phase and our results suggest that the low and high ranges in TAG concentrations in shallow-water and translocated lobsters (Fig 2a) may be associated with the early and late phases of the moult cycle respectively.

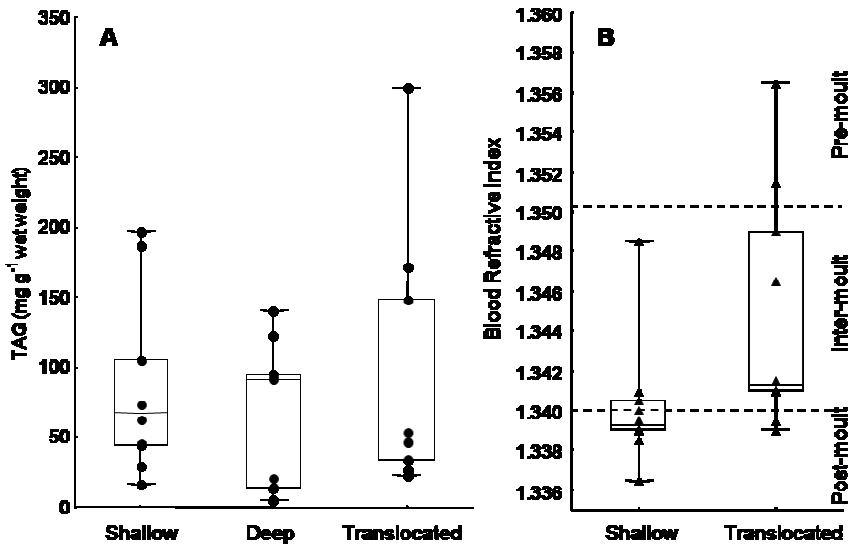


Fig 2. Body condition indices of shallow-water, deep-water and translocated (recaptured after 12 months) lobsters (A) TAG concentrations from the digestive gland and (B) Haemolymph refractive index values of only shallow-water and translocated lobsters (symbols indicate raw data). Also indicated are the broad BRI ranges associated with the moult cycle of *J.edwardsii* (see Musgrove 2001): Post-moult 1.335 – 1.3400; Inter-moult 1.3400-1.3450; Pre-moult 1.3460-1.3600. The box and whisker plot shows: box = 1st-3rd quantiles; centre line = median value; whiskers = minimum-maximum values. Note, no blood information collected for deep-water lobsters.

4.3.2 Fatty acid compositions

Digestive Gland

The most abundant fatty acids in the digestive gland (>5% of total fatty acids for all populations) were 16:1n-7c, 16:0, 18:1n-9, 18:1n-7, 18:0, 20:5n-3 (eicosapentaenoic acid: EPA), 20:1n-9+11 and 22:6n-3 (docosahexaenoic acid: DHA). Monounsaturated fatty acids (MUFA) were the dominant group of fatty acids (40.2 ± 4.9 % to 41.8 ± 1.9 %) with similar proportions of saturated fatty acids (SFA) (27.7 ± 1.7 % to 28.6 ± 1.9 %) and polyunsaturated fatty acids (PUFA) (29.6 ± 3.0 % to 31.8 ± 3.4%) (Table 2). Concentrations of total fatty acids (TFA) in the digestive gland did not vary significantly between deep water, shallow water and translocated populations ($F_{2,25}$ = 2.68, p = 0.88), nor did total concentrations of SFA, MUFA and PUFA ($F_{6,46}$ = 1.25, p = 0.30) (Fig. 3c). Analyses of selected (n-3) and (n-6) PUFA that typically indicate dietary differences also were not different between lobster populations ($F_{4,48}$ = 1.57, p = 0.197) (Fig. 3e). Mean concentrations of EFA was significantly higher in translocated lobsters than in shallow-water and deep-water lobsters ($F_{12,22}$ = 5.27, $p < 0.001$) (Fig 3a). Mean concentrations of EPA and AA were significantly higher in translocated lobsters than in shallow-water lobsters, but not different from deep-water

lobsters. Mean concentrations of DHA were higher in deep and translocated lobsters than in shallow-water lobsters (Fig 3g).

Leg Muscle

The most abundant fatty acids in the leg muscle were the same as those in the digestive gland with the addition of 20:4n-6 (AA). Both AA and EPA were found in higher proportions in leg muscle than in the digestive gland (> 5% and >10% of total fatty acids respectively) (Table 2). Concentrations of fatty acids in the leg muscle were less than 10% of the total fatty acids in the digestive gland. Although there was no significant difference in the concentrations of total fatty acids between shallow, deep and translocated lobsters ($F_{2,23}$ = 5.60, p = 0.10), PUFA were significantly higher in translocated than in shallow-water lobsters ($F_{6,42}$ = 3.99, p < 0.01, Fig. 3d). The sum of (n-6) PUFA was significantly higher in deep-water lobsters than in shallow and translocated lobsters, while the sum of (n-3) PUFA was higher in translocated lobsters than in shallow water and deep water lobsters ($F_{4,44}$ = 5.37, p < 0.01) (Fig. 3f). Concentrations of EFA were also higher in translocated lobsters than in shallow and deep-water lobsters ($F_{12,36}$ = 6.48, p < 0.01) (Fig. 3b).

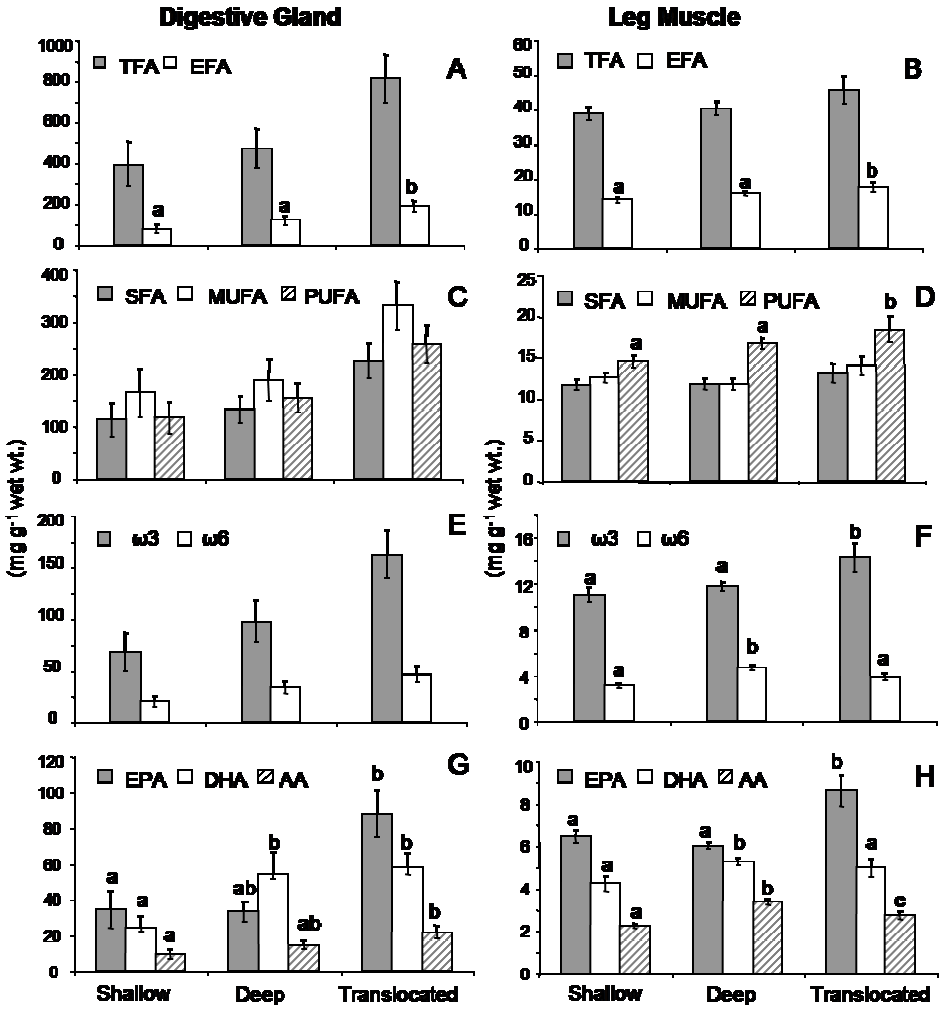


Fig 3. Comparisons of key fatty acid groups (A-F) and individual essential fatty acids (G-H) (mean ± SE, mg g^{-1} sample wet weight) from the digestive gland and leg muscle tissues of shallow-water, deep-water and translocated lobsters (recaptured after 12 months): TFA = total fatty acids; EFA = essential fatty acids; SFA = total saturated fatty acids; MUFA = total monounsaturated fatty acids; PUFA = total polyunsaturated fatty acids; ω3 = total omega-3 long-chain fatty acids; ω6 = total omega-6 long-chain fatty acids; EPA = Eicosapentaenoic acid; DHA = Docosahexaenoic acid; AA = Arachidonic acid. All groups were analysed using ANCOVA or MANCOVA and the results of the post-hoc Tukey's test are indicated by superscripts where significant difference among lobster populations are indicated by different (a, b, c) letters.

Eicosapentaenoic acid (EPA) was higher in translocated lobsters than in shallow and deep-water lobsters while DHA was slightly higher in deep-water lobsters, but not different between shallow and translocated lobsters. Arachidonic acid (AA) was significantly different between all lobster populations, and was highest in deep-water lobsters (Fig. 3h).

Leg muscle fatty acid profiles were grouped separately for each of the three populations on the PCA plot, although there was slight overlap between the FA profiles of translocated lobsters and shallow-water lobsters along the secondary axis (PC2, 23%, Fig 4). Deep-water lobsters were distinct from the other two populations along PC1, which accounted for 48% of the variance. Separation among groups along PC1 was driven strongly by 16:1(n-7)c and EPA while the main fatty acid causing separation between shallow and translocated lobsters along PC2 was 18:1(n-9), with additional influences from EPA and DHA.

Table 2. Fatty acid composition (as % of total fatty acids) of the digestive gland and leg muscle from shallow-water, deep-water and translocated *J.edwardsii*. Values are mean ± SE of those fatty acids (23 out of 63) with a mean value exceeding 1% in all groups ; LA, linoleic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Fald, fatty aldehyde derived from plasmalogen.

	Digestive Gland			Leg Muscle		
Fatty acid	Shallow (n = 10)	Deep (n = 9)	Translocated (n = 10)	Shallow (n = 10)	Deep (n = 9)	Translocated (n = 9)
14:0	4.1 ± 0.5	1.9 ± 0.4	3.0 ± 0.4	1.2 ± 0.1	0.4 ± 0.1	1.3 ± 0.1
16:1n-7c	5.3 ± 0.5	4.6 ± 0.4	6.8 ± 0.6	7.3 ± 0.2	4.5 ± 0.1	6.8 ± 0.4
16:0	10.2 ± 0.4	12.8 ± 0.3	11.0 ± 0.5	13.9 ± 0.3	14.1 ± 0.2	13.0 ± 0.2
br17:1	0.8 ± 0.1	1.3 ± 0.2	0.5 ± 0.1	0.9 ± 0.0	0.9 ± 0.2	0.9 ± 0.1
i17:0	0.5 ± 0.1	1.2 ± 0.2	0.6 ± 0.1	0.3 ± 0.0	0.7 ± 0.0	0.3 ± 0.0
17:1n-8/a17:0	1.0 ± 0.0	1.6 ± 0.2	1.0 ± 0.0	1.0 ± 0.1	2.1 ± 0.1	1.3 ± 0.0
16:0Fald	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	1.5 ± 0.1	1.3 ± 0.2	1.5 ± 0.2
17:0	0.6 ± 0.0	1.2 ± 0.1	0.7 ± 0.1	1.1 ± 0.1	2.0 ± 0.0	1.3 ± 0.0
18:2n-6 LA	1.0 ± 0.1	1.7 ± 0.1	1.0 ± 0.1	1.3 ± 0.1	1.4 ± 0.0	1.1 ± 0.1
18:1n-9	10.0 ± 0.7	12.9 ± 0.7	11.6 ± 1.2	14.9 ± 0.6	14.1 ± 0.2	12.9 ± 0.4
18:1n-7	7.0 ± 0.5	5.5 ± 0.3	7.0 ± 0.2	4.5 ± 0.1	3.9 ± 0.1	4.6 ± 0.1
18:0	6.4 ± 0.4	5.9 ± 0.4	6.2 ± 0.3	8.5 ± 0.2	7.5 ± 0.1	7.9 ± 0.2
18:0Fald	1.0 ± 0.1	0.8 ± 0.1	0.5 ± 0.1	1.3 ± 0.1	0.7 ± 0.2	1.4 ± 0.2
20:4n-6 AA	2.6 ± 0.2	3.6 ± 0.5	2.7 ± 0.2	5.7 ± 0.3	8.4 ± 0.2	6.1 ± 0.3
20:5n-3 EPA	8.6 ± 0.6	7.5 ± 0.4	10.7 ± 0.5	16.9 ± 0.3	15.1 ± 0.4	19.0 ± 0.3
20:2NMI	2.6 ± 0.5	1.2 ± 0.2	1.8 ± 0.1	0.5 ± 0.1	0.0 ± 0.0	0.1 ± 0.0
20:2n-6	1.1 ± 0.1	1.2 ± 0.2	1.2 ± 0.1	0.7 ± 0.0	1.1 ± 0.0	0.9 ± 0.0
20:1n-9/n-11	8.2 ± 0.7	7.4 ± 1.1	6.5 ± 1.2	1.7 ± 0.2	2.2 ± 0.1	2.4 ± 0.2
20:1n-7	2.8 ± 0.4	1.2 ± 0.1	2.8 ± 0.2	0.8 ± 0.1	0.4 ± 0.1	0.8 ± 0.1
22:6n-3 DHA	6.7 ± 0.6	10.9 ± 0.6	7.2 ± 0.6	10.9 ± 0.4	13.2 ± 0.2	11.0 ± 0.2
22:5n-3 DPA	1.7 ± 0.3	1.9 ± 0.1	1.6 ± 0.1	0.7 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
22:2NMI	1.3 ± 0.2	0.5 ± 0.2	1.2 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
22:1n-11/n-13	1.2 ± 0.6	2.4 ± 0.7	0.4 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Ratio (n-3)/(n-6)	3.3 ± 0.3	2.8 ± 0.3	3.6 ± 0.4	3.6 ± 0.2	2.5 ± 0.0	3.7 ± 0.1
Ratio EPA/AA	3.5 ± 0.4	2.4 ± 0.3	4.2 ± 0.5	2.9 ± 0.2	1.8 ± 0.0	3.2 ± 0.1
Ratio DHA/EPA	0.8 ± 0.0	1.5 ± 0.1	0.7 ± 0.1	0.6 ± 0.0	0.9 ± 0.0	0.6 ± 0.0

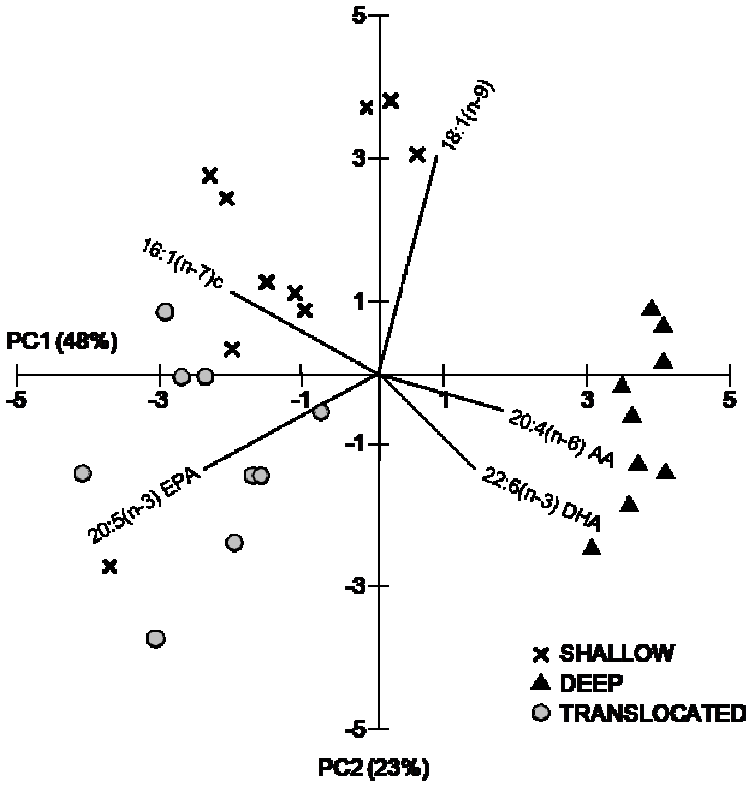


Fig 4. Two-dimensional PCA plot of the first two principal components derived from the leg muscle fatty acid profiles (as % of total fatty acids) of shallow-water, deep-water and translocated (recaptured after 12 months) lobsters. The percentage of variance explained by each component is indicated on the axis title. Key fatty acids responsible for the directional displacements are indicated by vectors.

4.4 Discussion

4.4.1 Fatty acid differences in *Jasus edwardsii*

Fatty acid profiles were different in lobsters from deep-water, shallow-water, and translocated populations. Overall FA compositions of translocated lobsters were more similar to those of shallow-water lobsters than to deep-water lobsters, while concentrations of some individual FA were more similar to deep-water lobsters (e.g. 20:1n-9+11). These differences in FA profiles are consistent with differences in diet between lobster populations at different depths and thereby confirm the change in diet in translocated lobsters. Identification of prey items from lipid signature analyses, as has occurred for a number of other marine species, may further resolve dietary differences between deep and shallow-water populations. Our results also showed that fatty acid profiles of deep, shallow and translocated lobsters were similar between muscle and hepatopancreas, demonstrating that a period of 12 months was sufficient (although not the maximum time period required) for the biochemical signature of the new prey items to be assimilated by translocated lobsters. This is supported by aquarium feeding trial studies where lipid profiles of adult *Jasus edwardsii* (tail and leg muscle) changed in response to different feed types after

four months (Nelson et al. 2005), and after three months in juveniles (whole body) (Johnston et al. 2003).

Both tissue types revealed concentrations of n-3 and n-6 FA were higher in deep than in shallow-water lobsters, and highest in translocated lobsters. The ratio of n-3/n-6 was also lowest in deep-water lobsters and markedly higher in shallow and translocated lobsters. Eicosapentaenoic acid (EPA) and DHA were the two main PUFA driving the separation of deep-water lobsters from shallow and translocated lobsters in our principal component analysis. These essential fatty acids play an important role in the growth and development of crustaceans, especially during the early larval and juvenile stages (Nelson et al. 2006). Essential fatty acid deficiency has been linked to slow growth rates and a decrease in feeding efficiency in several fish species and a reduction in egg production and deformities in larval morphology (Corraze 1999). The production of aquaculture feeds is a prime example of the importance of nutrient balance in achieving positive growth and condition outcomes. An imbalance can lead to competition between fatty acids and the inefficient synthesis of prostaglandins (D'Abramo 1997). Therefore it is possible that an imbalance in the proportions of EFA in the diet (e.g. AA/EPA, DHA/EPA ratios) in conjunction with insufficient

levels of essential nutrients may be contributing factors in the slower growth rates of deep-water lobsters.

4.4.2 Nutritional condition of *Jasus edwardsii*

Total lipid and TAG content were not different between the three lobster populations, despite differences in the fatty acid compositions. The most plausible reason for similarity in TAG and lipid is the influence of the moult cycle. For crustaceans, growth is achieved through the cyclic accumulation and depletion of organic reserves, a significant and important feature of decapod physiology (Passano 1960). During the long inter-moult period of the moult cycle, TAG reserves are accumulated for the next moult event. Lipid content is therefore constantly changing in addition to supplying the demands from reproduction, daily energetics, and environmental and seasonal fluctuations in food availability. In the present study, a high BRI in a number of translocated lobsters indicated they were in the inter-moult or pre-moult phase, while the majority were in the post-moult phase. These same lobsters had high levels of TAG. These differences in the moult phases within populations, especially among translocated lobsters, highlight the physiological heterogeneity among the lobsters used for analysis.

This highlights the importance of moult staging when quantifying lipid content, which many previous studies have overlooked.

Most interesting was the finding of higher concentrations of EFA in the hepatopancreas and muscle of translocated lobsters than in shallow or deep-water lobsters. In particular very high mean concentrations of total and selected PUFA groups in the muscle of translocated lobsters. Rates of metabolism, digestion and catabolism which are hormonally controlled (Santos et al. 1997) and driven by several factors such as temperature, light and diet (Childress et al. 1990), may change in response to a new habitat and the ensuing environmental changes. Translocated lobsters analysed in our study had experienced 12 months in the new environment and had also changed in appearance (Chapter 3) and growth (Chapter 6). Initial differences in growth rate may have been due to metabolic differences, assuming significant differences in metabolism were present between deep and shallow-water lobsters, although this is largely speculative at this point. Translocated lobsters may have experienced increased nutrient uptake or a greater absorption rate indicative of the higher concentrations of lipid and fatty acids in the digestive gland that

may reflect modifications to the digestive physiology due to greater quantity of food being digested. This may be a compensatory response whereby an increase in the size and volume of the digestive gland and enzyme activity occurred in response to the new habitat.

Leg muscle of translocated lobsters had higher concentrations of PUFA and in particular EFA than in shallow and deep-water lobsters. Given the accelerated growth in translocated lobsters over a short period of time, increased tissue synthesis including incorporation of associated lipid, may be a possible explanation for this observation which is similar to a compensatory response that is sometimes induced by a period of starvation or reduced nutrient intake (Ali et al. 2003). Compensatory responses can include hyperphagia, rapid weight increase, repletion of energy reserves and increased nutrient intake. For small and pale coloured translocated lobsters, risk from cannibalism is high. One way to avoid this situation is to eat and grow as fast as possible. This catch-up growth behaviour in habitats of high predation has been shown in several fish studies where there is a trade off between escape performance and growth acceleration (Alvarez and Metcalfe 2007). Since compensatory responses are reported to be short-lived, it is

unclear if this will be maintained over several moults by translocated lobsters or if it is regulated at optimal or maximal rates.

From a commercial and marketing point of view, increased concentrations of EFA in edible tissues are highly favourable. Recent studies have shown that consumption of oily fish has associated health benefits in humans, as they decrease cholesterol levels and the subsequent occurrence of cardiovascular disease (Gebauer et al. 2006; Jarvinen et al. 2006). In particular, the emphasis is on the richness of omega-3 long-chain PUFA, which in our study were 30% higher in concentration in the muscle of translocated lobsters than in shallow and deep-water lobsters. This is a significant and novel finding given the marketing issues surrounding the importance of omega-3 long-chain PUFA in cultured fish species comparable to their wild counterparts. Nelson et al. (2005) found the lipid and fatty acid content of muscle of cultured and wild *J. edwardsii* to be similar, but the animals differed in their quality attributes such as taste, texture and colour. Therefore enhanced nutrition in translocated lobsters may also implicate enhanced flavour and flesh quality thus providing the basis for further research.

4.5 Conclusion

Differences in fatty acid profiles between deep and shallow-water populations and the change in their FA profile after translocation highlights the dietary plasticity of adult *Jasus edwardsii.* Changes in key market traits, in particular the health benefiting omega-3 long-chain PUFA, were also observed, although it is presently unclear if these differences arise from nutritional condition and/or other factors. This study is the first to show nutritional enhancement through the *in situ* manipulation of lobster habitat. This finding suggests translocated deep-water lobsters have enhanced nutritional condition and quality, beyond that of legal-sized shallow-water lobsters, thus raising its market potential.

Chapter 5

Haemolymph condition variation among post-harvest adult southern rock lobsters, and response to translocation

This Chapter is *in press* with Marine and Freshwater Behaviour and Physiology:

Chandrapavan A, Gardner C, Green B.S. Haemolymph condition of deep-water southern rock lobsters translocated to shallow-water reefs.

Abstract

Deep-water southern rock lobsters were translocated to inshore reefs harbouring high-market value southern rock lobsters as part of an experimental spatial management strategy to enhance their market traits, growth and post-harvest condition. We assessed the haemolymph condition of deep-water lobsters before and after (over a period of 14 months) translocation for comparison to resident lobsters at the release site using a range of moult and haemolymph indices. Moult indices indicated deep-water lobsters were of similar moult stage to resident lobsters before translocation, they moulted earlier than resident lobsters in their new habitat but were of similar moult stage to resident lobsters when sampled 12 months after translocation. Comparisons of haemolymph parameters included total haemocyte count, serum protein and electrotypes such as magnesium and potassium, which were all significantly different between deep and shallow-water lobsters prior to translocation. These populations when sampled 12 months after translocation showed similar concentrations of all haemolymph parameters, suggesting an improved resilience to post-harvest stress in translocated lobsters. Benefits of translocation to inshore areas on the post-harvest condition of deep-water lobsters could be

due to improved body condition through the manipulation of habitat and diet but also from the reduced post-capture transport time and stress duration.

5.1 Introduction

The live trade of southern rock lobsters (*Jasus edwardsii*) from Australia to export markets in Asia began in the late 1980's and supplied by the three main State fisheries of Tasmania, South Australia and Victoria (Roberts 1994). The species is harvested from a range of depths, premium lobsters are caught from inshore shallow-water areas (< 30 m) where they are fast growing, red in colour and of high vitality for live transport. Lobsters harvested from offshore deep-water areas (> 30 m) are paler in colouration and thus sold at a discounted price (Chapter 2) but are also slow growing, and incur higher transport mortality. Maintaining low mortality rates is thus a priority as the condition of live lobsters affects its sale price and overall profitability of the fishing industry. While spatial differences in demographic traits such as growth (Punt et al. 1997), size at sexual maturity (Gardner et al. 2006) and shell colour (Chapter 3) have been documented for *J. edwardsii*, it is not clear if

distinctive physiological or biochemical differences exist between deep-water and shallow-water lobster populations or at least in their response to post-harvest stress.

Stress is a physiological response altering the physiological condition to beyond the normal range and can be either readily reversible if physiological disturbance are within the homeostatic capability of the lobsters, or it can be non-reversible, ultimately leading to death (Taylor et al. 1997). Stressors associated with live transport of crustaceans generally arise from the interchanges between immersions and emersions (air breathing) and exacerbated through capture and handling by fishing gear and crew, exposure to varying temperatures and oxygen availability, physical damage and interactions with other lobsters. Currently, processors rely on their visual assessment of lobster vigour (tail flipping, antenna response and general handling resistance) before lobsters are live transported, but quantitative measurements of condition can be assessed from oxygen uptake (Whiteley and Taylor 1992), stress hormones (Chang 2005), muscle metabolites (Speed et al. 2001) and haemolymph (blood) parameters (Moore et al. 2000; Fotedar et al. 2006; Lund et al. 2009). The crustacean haemolymph is the principal medium for transport and storage of a range of organic

and inorganic constituents and indices such as blood gases, blood pH, proteins, lipids, cells and ions (Paterson and Spanoghe 1997) are widely used measures of physiological condition. In addition to stress, haemolymph indices are also affected by the moult cycle, reproductive cycle and nutritional condition (Moore et al. 2000; Musgrove 2001), so it is important to take these factors into consideration when assessing post-harvest stress.

Translocation of deep-water southern rock lobsters into shallow-water reefs inshore is currently being explored as a fisheries enhancement strategy to increase their yield and improve marketability, including the live transport condition of deep-water lobsters. Large-scale experimental translocations have shown translocated lobsters to change from a pale to a red colouration, increase their growth rate and achieve enhanced nutritional value (Chapters 3, 4, 6) after their first moult in the new shallow-water habitat. High survival of translocated lobsters (Green and Gardner 2009) in synergy with enhanced biological traits imply improved physiological condition for deep-water lobsters after translocation, although how this translates to improved post-harvest condition is yet to be demonstrated. Thus the central aim of this study was to determine how translocation may have altered the post-harvest condition of deep-water

lobsters and if translocated lobsters share similar conditional profiles to resident lobsters. In this study we compare the post-harvest condition of deep-water and shallow-water *J. edwardsii* before and 12 months after translocation, as well as describe the temporal variability in the hemolymph condition of translocated and resident shallow-water lobsters over a 14 month post-translocation period.

5.2 Materials and methods

5.2.1 Translocation of *J. edwardsii*

During November 2005, 1998 sub-legal sized adult lobsters (68 - 120 mm) were captured from around Maatsuyker Island (60 – 80 m deep) (43.38°S, 146.17°E) using baited (barracouta and jack mackerel) lobster pots after a soak time of approximately 6 hours. Immediately upon capture, all lobsters to be translocated were tagged with an individually coded T-bar tag in the ventral surface of the first abdominal segment (coloured Hallprint T-bar anchor tag), had one pleopod clipped, and details of carapace length (CL), shell colour and sex recorded (bio-data). Captured lobsters were then kept on-board the research vessel

in a flow-though holding tank for approximately 48 hours while being transported to their release site (5 - 15m deep) at Taroona Marine Reserve (42.95°S, 147.34°E). Due to the logistical difficulties of haemolymph sampling on board the research vessel (weather, time and consistency with other surveys), a sub-sample of translocated lobsters (127 females (FM) and 72 males (M)) were land transported (out of water) back to the laboratory and placed into a re-circulating tank for 2-5 hours (time period between the first and last lobster sampled) before their haemolymph was extracted.

Also in November 2005, shallow-water lobsters (23 FM, 27 M) from the release site (Taroona Marine Reserve) were captured using baited pots after a soak time of approximately 12 hours. Captured lobsters had their size, shell colour and sex recorded and land transported back to the laboratory and placed in a re-circulating tank for 2-3 hours before their haemolymph was extracted. Taroona Reserve we re-surveyed using baited pots (as above) at 2, 3, 5, 8, 10, 12, 14 months after translocation. During each survey the bio-data was recorded for all recaptured translocated lobsters as well as a sub-sample of resident lobsters. Haemolymph samples were extracted after being transported back to the laboratory and allowed a period of 2-3

hours of acclimation in holding tanks. Haemolymph samples from resident lobsters were not collected from the 2 and 3-month post-translocation surveys. All haemolymph samples were placed immediately on ice and later stored in a -18ºC fridge. Moult growth increments of more than three millimetres and the partial or complete regeneration of the cut pleopod were used as moult confirmation (Ziegler et al. 2004).

5.2.2 Haemolymph collection and analyses

A haemolymph sample of 2 – 3 ml was extracted (using a 3 ml syringe, 22-gauge needle) by pericardial puncture and transferred to a sterile Eppendorf tube. Haemolymph pigment stage (1: colourless to 4.5: dark red) was assessed using the Southern Rock Lobster Blood Colour Reference Card developed by Musgrove (2001). The Pigment Stage Index (PS) was developed by Musgrove (2001) for the southern rock lobster *Jasus edwardsii*, and refers to the change in haemolymph colour during the moult cycle due to changes in astaxanthin concentrations in the haemolymph. From post-moult to inter-moult, colour changes from clear/pale blue through to pale yellow (PS 1 to PS 2.5) and progressively darken to a deep orange in pre-moult lobsters (from PS 3 to PS 4.5). This index can be

used to determine broadly the moult stage of a lobster where the darkening of the haemolymph colour corresponds with increasing pigment stage. All sampled animals were released alive back into Taroona Reserve.

A small aliquot of the haemolymph sample was then placed in a hand-held refractometer (Model UR-2, Industrial and Scientific Supply Co.) to measure the refractive index (RI). The blood RI values were converted to blood serum a protein concentration (as outlined by Musgrove 2001) which is a reliable index of physiological condition (Oliver and MacDiarmid 2001). For the analysis of total haemocyte counts (THC) which is another common measure of health condition, a 200 µl aliquot of haemolymph was mixed with 200 µl of Na-cacodylate based anti-coagulant in another eppendorf tube (4.28 g of Na-cacodylate added to 90 ml of distilled water, pH adjusted to 7.0 using 1.0 M HCl, 400 µl of stock 25% glutaraldehyde solution added and volume adjusted to 100 ml with distilled water). Tubes were well mixed prior to measurements of THC using a haemocytometer (Improved Neubauer, Bright-line, BLAUBRAND®).

Key haemolymph electrolytes critical for ion regulation in crustaceans was extracted from, the remaining whole

haemolymph that was allowed to clot overnight, and then macerated and centrifuged at 1600 *g* for 10 minutes. Using a sterile 1ml syringe, the serum portion was extracted and transferred to another sterile eppendorf tube. Analysis of serum extracts was measured for calcium (Ca), magnesium (Mg), and potassium (K) using a Cobas Mira Clinical Analyser. Extracts were diluted 1:4 for K and concentration determined by ion-selective electrode (ISE) and further diluted 1:8 for Ca and Mg, which were determined by colorimetric methods. Haemolymph electrolytes measurements were only analysed from a sub-sample of translocated and resident male lobsters captured in Nov 2005 and Jan 2007.

Multivariate analyses were used to compare haemolymph parameters (excluding PS index) between deep-water and resident lobsters before translocation (Nov 05), and between translocated and resident lobsters after translocation (Nov 06). Analyses were performed separately for the sexes due to differences in their moult stages during these sampling periods. Electrolytes were also analysed separately as these were only measured in males and were compared between Nov 05 and Jan 07. All variables were normally distributed and the Pillai's trace test was used as multivariate test of significance and significant

effects were further examined using Tukey's post-hoc analysis. Statistical analyses were performed using Statistica (V7.1 Statsoft Inc, Tulsa OK USA).

5.3 Results

The PS index provides a broad indicator of moult stage, and differences in mean PS values were not very large between populations in Nov 05 and in Nov 06, suggesting no difference in moult categories. That is, deep-water lobsters were of similar moult stage to shallow-water lobsters before translocation as well as 12 months after translocation. Concentrations of serum protein (SP) and total haemocyte counts (THC) varied significantly between deep-water and shallow-water populations before translocation (Females: $F_{2,47} = 28.97$, $p < 0.01$; Males: $F_{2,96} = 17.74$, $p < 0.01$) but no differences between translocated and resident lobster populations 12 months after translocation (Females: $F_{2,29} = 1.89$, $p = 1.68$; Males: $F_{2,32} = 1.86$, $p = 1.72$).

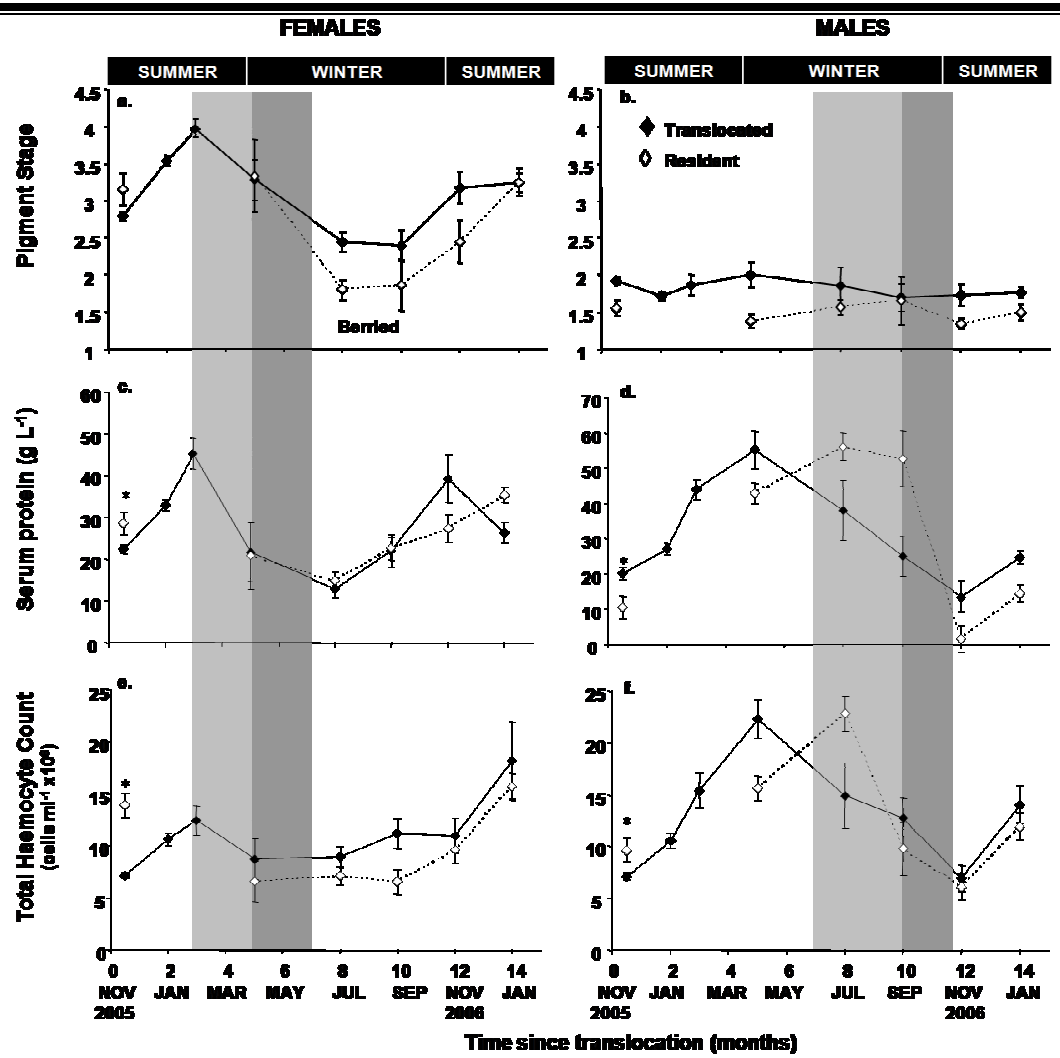


Fig 1. Temporal variability in the different haemolymph indices (mean ± SE)(pigment stage, serum protein and total haemocyte counts) sampled from resident (broken line) and translocated (solid line) female (left column) and male (right column) lobsters. Light grey area indicates the moulting period of translocated lobsters and dark grey area indicates the moulting period of resident lobsters. Significant difference between resident and translocated populations is indicated by * .

5.3.1 Pigment Stage

Growth moult increments, change in shell colouration and pleopod regeneration from recaptured tagged lobsters were used to estimate the moult period for translocated lobsters between sampling periods. The moult period for translocated females was between Feb 06 and April 06, and for translocated males we estimate moulting to occur between June 06 and Sep 06. These moult estimates were approximately 2-3 months in advance of the moulting by the resident population. In females, peak mean PS values of 4.0 (translocated) and 3.3 (resident) occurred prior to moulting and the lowest mean PS values 2.4 (translocated) and 1.8 (resident) occurred in the early inter-moult phase when the females were berried (carrying eggs) (Fig 1a). At the completion of the spawning period in Sept 06, PS values increased in the following months until Jan 07 when sampling ceased. In males however, no cyclic trends were observed for both translocated and resident populations (Fig 1b). Mean PS value did not exceed 2.0 on any of the sampling months over the 14-month period, indicating that sampled lobsters were in their inter-moult phase.

5.3.2 Serum Protein

Serum protein (SP) concentrations of translocated female lobsters followed a cyclic trend of rise and fall with highest peaks occurring 3 (Feb 06) and 12 months (Nov 06) after translocation (Fig 1c). For males, a cyclic trend was also observed with values peaking prior to moulting of resident lobsters (Sep 06), however the peak for translocated males occurred in Apr 06, well in advance of their estimated moulting period (Fig 1d). SP concentrations declined gradually after moulting in translocated males but more sharply in resident males. Before translocation, mean SP concentrations of deep-water females were significantly lower than resident females, but both similar 12 months after translocation. For males, mean SP concentrations were significantly higher then resident males before translocation but no differences detected 12 months after translocation.

5.3.3 Total Haemocyte Counts

Trends in THC reflected that of SP for males of resident and translocated populations however for females only a subtle cyclic pattern was detected (Fig 1e, 1f). For females, we observed

an increase in THC leading up to the moulting period of translocated females, then a decrease during post-moult, followed by a gradual increase in the subsequent months. This pattern was very similar to that of resident females. Mean THC was higher in deep-water lobsters than in resident lobsters before translocation (Nov 05) but were similar between translocated and resident populations 12 months after translocation.

5.3.4 Electrolytes

Concentrations of electrolytes were significantly different between deep-water and shallow-water males before translocation in Nov 05 ($F_{3,53} = 21.89$, $p < 0.01$) but were similar between translocated and resident males after translocation in Jan 07 ($F_{3,56} = 1.02$, $p = 0.39$). Concentrations of Ca were similar between populations before and after translocation, while Mg concentrations of deep-water lobsters were significantly higher than in resident lobsters and K concentrations were significantly lower than in resident lobsters in Nov 05 (Fig 2).

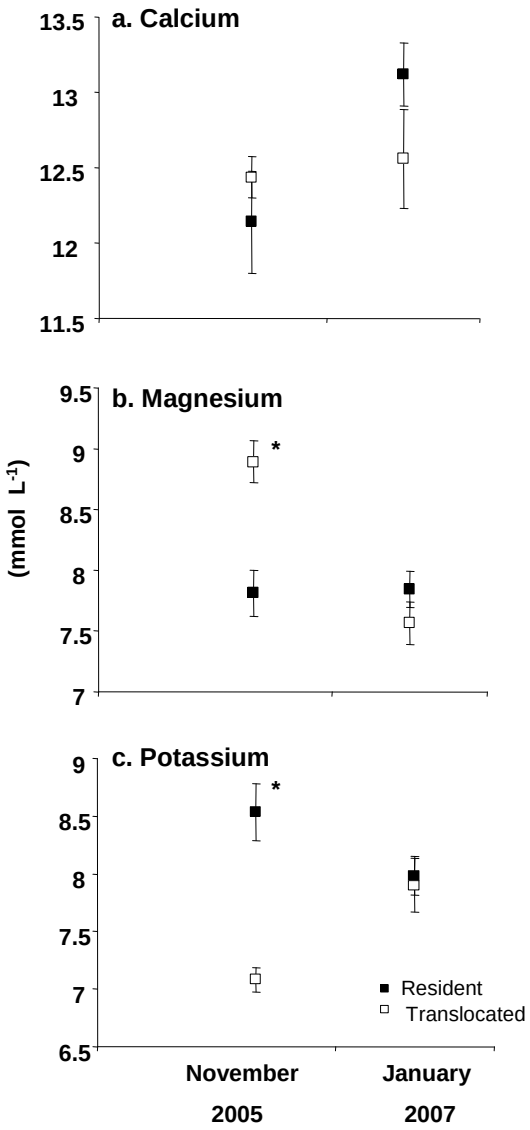


Fig 2. Comparisons of calcium, magnesium and potassium concentrations (mmol L^{-1}) between resident and translocated male lobsters sampled in November 2005 and January 2007. Significant differences between populations are indicated by *.

5.4 Discussion

The overall haemolymph conditional profile of deep-water lobsters was different from those of shallow-water lobsters prior to translocation, but the conditional profiles of translocated lobsters captured 12 months later were similar to resident lobsters. Haemolymph indices also showed clear temporal trends in physiological condition, largely influenced by the moult cycle.

Interpretation of the haemolymph indices relied on identifying the moult phases of all lobsters over the sampling period using the different morphological indicators (moult increments, pleopod regeneration, shell colour change) together with the PS index. This allowed us to account for the moult influence when assessing the overall haemolymph response to post-harvest stress. This step was important because crustacean physiology is dominated by the moult cycle and each stage is associated with changes in the composition of the blood (Maynard 1960; Mercaldo-Allen 1991). During pre-moult, a resorption of the exoskeleton occurs and the blood provides a temporary storage sites for these materials. Uptake of water at moult increases the haemolymph volume and rapidly dilutes

blood constituents. Water uptake occurs once the old exoskeleton begins to detach from the newly formed cuticle underneath. During post-moult, growth of muscle tissue reduces haemolymph volume and thus the concentrations of haemolymph components proportionally increase (Florkin 1960).

Overall trends in the haemolymph indices showed peaks and troughs associated with different moult stages, but these patterns were not always synchronised between translocated and resident lobsters since translocated lobsters moulted earlier than resident lobsters. For translocated females, the peaks and troughs in indices occurred at the beginning and end of the estimated moult period, thus showing a good corroboration between haemolymph condition and moult indices. For translocated males however, clear cyclic trends in the RI and THC that were indicative of the moult influence on the hemolymph condition were suppressed in the PS index. Morphological moult indicators estimated moulting to occur several months after the peaks in RI and BRI indices, which suggests either an extended pre-moult phase or a staggered moulting event among males. Despite the inconsistencies in moult estimates between indices, moult stages of translocated and resident lobsters were similar before translocation (Nov 05)

and 12 months after translocation (Nov 06). Thus haemolymph conditional differences between translocated and resident lobsters are most likely to be driven by factors other than its moult condition.

The total number of circulating haemocytes are generally elevated for a period of time during the post-harvest process in response to bacterial infections, physical injury and dehydration, but can decline gradually as an indication of worsening health condition (Jussila et al. 1997) resulting in very low THC at the moribund stage (Fotedar et al. 2006). For adult *J. edwardsii* the THC range that was subjective to moult stage was 5 – 25 x 10^6 cells/ml. Lavallée et al (2000) reported for *Homarus americanus*, an increase in THC from 20 to 30 x 10^6 cells/ml from capture to when they arrive for processing. Jussila et al. (1997) reported a range of 8.5 to 15.9 x 10^6 cells/ml for freshly arrived western rock lobsters to the processing factory, and a range of 5.3 to 5.6 x 10^6 cells/ml for healthy lobsters (after a 16 hour recovery). Lower THC of deep-water *J. edwardsii* males and females than resident lobsters from this study suggests a greater deterioration in the post-capture condition of deep-water lobsters.

Haemolymph serum protein (SP) concentrations responds to changes in the haemolymph volume through water uptake or fluid loss, thus lowest SP levels occur in post-moult lobsters when they ingest large volume of water to expand their newly formed exoskeleton (Oliver and MacDiarmid 2001). Blood pH and concentrations of organic and inorganic ions may also alter the total protein concentrations in response to stress (Paterson and Spanoghe 1997), while some proteins may be catabolised as an energy source after glycogen reserves are exhausted. Lavallée et al. (2000) reported a range of 18.6 to 99.8 g/L for *H. americanus* post-capture on boats, while Paterson et al. (2005) reported serum protein levels greater than 80 g/L for *P. cygnus* following simulated post-harvest handling. We found temporal trends in the serum protein concentrations of translocated and resident lobsters to reflect changes in the moult phases but never exceeding 60 mg/L. Since SP levels of deep-water lobsters was lower in females and higher in males than in resident lobsters, it is not clear from the SP index which population reflects a poor, healthy or declining health condition. Perhaps haemolymph indices sampled from dead or moribund lobsters may have provided a greater understanding on their deviations in health condition from lobsters unsuitable for live transport.

Emersion stress triggers a series of physiological disturbances interfering with the osmotic and ionic regulation as animals switch from aerobic to anaerobic respiration (Lignot et al. 2000). Thus a sign of good health is the ability to maintain tight regulation of ions during post-harvest. Calcium (Ca) ion concentrations change in the haemolymph during the buffering of acidosis, while magnesium (Mg) and potassium (K) concentrations respond to salinity changes and also increase in response to post-harvest stress caused by hypoxia which is indicative of body volume changes and how the antennal gland functions (Paterson and Spanoghe 1997). Paterson et al. (2005) reported significant deviations in Mg, K and Ca levels between western rock lobsters considered to be poor and good condition for live trade, but the same cannot be inferred for deep and shallow-water *J. edwardsii* without reference or baseline values. Both Mg and K were significantly different between deep and shallow-water males but its unclear if these deviations reflect poor ion regulation given the absence of reference values. Perhaps the sequence of the ion concentrations may provide an alternative approach to assess condition. For example, for resident males in Nov 05, the order of decreasing concentrations were Ca > K > Mg and this order was the same as in Jan 06, however the order for translocated males changed from Ca > Mg

> K in Nov 05 to Ca > K > Mg in Jan 06 which reflected those of resident males. This suggests ion regulation in deep-water males may have been compromised by post-harvest stress.

While this study did not monitor the haemolymph condition of lobsters throughout the entire post-harvest commercial process, significant deviations in health status between deep-water and shallow-water lobsters (before translocation) at an early stage of the process suggests that as physiological condition worsen during subsequent processing the likelihood of mortality for deep-water lobsters will be higher than for shallow-water lobsters. The similar condition profile between translocated and resident lobsters seems to suggest increased resilience to post-harvest stress in deep-water lobsters achieved through translocation. The impact of translocation on the physiological condition of deep-water lobsters may be due to change in diet and/or environmental influences, but from a commercial point of view other key benefits of translocation include the overall reduction in post-harvest transport time simply due to their closer proximity to land, thus reducing stress duration. Furthermore, current and related investigations do suggest that the body condition of deep-water lobsters have improved after translocation through increased growth rate

(Chapter 6), enhanced shell colour (Chapter 3) and nutritional condition (Chapter 4). By adopting the market traits of resident shallow-water lobsters, we assume this will translate to improved live transport condition in translocated deep-water lobsters.

6 Chapter

Growth rate variation among adult southern rock lobsters, and response to translocation

This chapter previously published as:

Chandrapavan A, Gardner C, Green B.S. (2010). Growth rate of adult rock lobsters *Jasus edwardsii* increased through translocation. Fisheries research 105: 244-247.

Abstract

Growth rates of southern rock lobster, *J. edwardsii,* decrease from north to south of Tasmania and also decrease with depth. Slow-growing lobsters from deep-water regions were translocated to shallow-water, inshore areas to examine the potential of translocation to increase yield for the Tasmanian Rock Lobster Industry. Within their first moult, translocated lobsters increased their growth rates, exceeding that of resident deep-water lobsters from the original site. Growth of translocated females increased to the extent that it exceeded resident shallow-water females in the first year post-release. The increased growth rate of deep-water southern rock lobsters after translocation and the magnitude of the growth changes achieved in a short period highlight the plasticity in growth of this species. Growth, and therefore size-at-age, is potentially determined by density effects, food availability or other unidentified environmental factors. Rapid increases in lobster growth achieved through translocation suggest that translocation could be applied to increase the productivity of the fishery.

6.1 Introduction

Variable growth of the southern rock lobster *Jasus edwardsii* is the basis of ongoing spatial management challenges across the Australian fishery (McGarvey et al. 1999). Around the coast of Tasmania, growth rates of *J. edwardsii* decrease from north to south of the State and also decrease with depth (Punt et al. 1997). For example, the mean annual growth increment for females of carapace length 75 mm is 1-2 mm in the southern regions while in the north of the State it is more than 20 mm per annum. Likewise for males of carapace length 75 mm, the mean annual growth increment is 10 mm in the southern regions and 22 mm in the north of the State (Punt et al. 1997). Differences in growth are also reflected in the spatial differences in L50% (length at which 50% of lobsters are mature) for female southern rock lobsters around Tasmania which is estimated to be 60-65 mm in the south and 100-105 mm in the north of the State (Gardner et al. 2006). Thus maturation at a smaller size in deep-water lobsters further inhibits their growth. Despite this spatial heterogeneity in growth and size at maturity, the Tasmanian Rock Lobster Fishery consists of a single fishing zone managed under a quota system with a State-wide size limit of 110 mm carapace length (CL) for males and 105 mm CL for females (Gardner et al. 2004). Under this management strategy, vast

differences in egg production, biomass levels and fishing effort are apparent between deep and shallow-water regions and from north to south of the State (Punt et al. 1997).

In addition to slower growth rates, deep-water lobsters are characterised by paler shell colouration and body-shape traits that contribute to a reduced market price when compared to red lobsters from inshore regions. Under a quota management system, commercial fishers maximise the value of their catch by heavily targeting inshore lobster stocks and only opportunistically utilise the deep-water stocks, such as in winter months when price of pale coloured lobster are more favourable (Chapter 2). Better tailoring of management to the biology of *J. edwardsii* is currently being explored through research, including harvest strategy evaluation of regional size limits and harvests. One management option under consideration to deal with spatial differences in the biology of *J. edwardsii* is the translocation of adult lobsters from low-growth areas to high-growth areas. If translocated deep-water lobsters survive and adopt the growth of resident shallow-water lobsters, translocation as a spatial management strategy has the potential to increase yield and exploitable biomass (Gardner and van Putten, 2008a). The first phase in exploring translocation as a

management tool was assessing its economic feasibility under assumed growth scenarios, and this predicted greatest increase in economic rent from the fishery when translocations occur between regions with greatest differences in growth (Gardner and van Putten, 2008b). The following phases of experimental translocation trials are underway in Tasmania to determine if translocated lobsters will adopt the biological characteristics of resident lobsters in the new habitat. In this paper we present results on the short-term growth response of translocated lobsters from a deep-water region in the south of the state to a shallow-water reef on the east coast of Tasmania.

6.2 Materials and methods

6.2.1 Translocation of lobsters

In November 2005, 1998 adult, male and female deep-water lobsters (68 – 120 mm CL) were trapped using baited metal pots in depths around 60 - 80 m from around Maatsuyker Island (43.38°S, 146.17°E) in the south of the State, and were transported in flow-through holding tanks onboard a research vessel and released into depths of 5 – 15 m at a marine protected area, Taroona Research Reserve (42.95°S, 147.34°E) on the east coast of Tasmania (Fig 1). All translocated lobsters were tagged

ventrally with T-bar tags (coloured Hallprint T-bar anchor tag), carapace length (CL) measurements and sex were recorded, and one pleopod was clipped as an indicator for moulting. Taroona Reserve was surveyed at 5 (April 2005), 8 (July 2005), 12 (November 2005) and 14 (January 2006) months after the translocation for the collection of growth data from resident and translocated lobsters. Ongoing tag-recapture research surveys at Taroona Reserve and around Maatsuyker Island provided growth information of the resident lobster populations for the years 2000 - 2005.

6.2.2 Data analyses

Data analyses of annual moult growth increments were restricted to setose (mature) females between 70 – 110 mm CL (at time of capture) and males between 70 – 130 mm CL. Only lobsters that were recaptured after the annual moulting season (April to May for females and September to October for males) were included, to ensure the lobster had moulted. Carapace length measurements were collected by numerous personnel over a number of years, and so we have assumed measurement errors of equal magnitude across all lobsters. Carapace length and growth increment data were transformed ($\log_{10}$) to achieve

normal distribution. Differences in growth moult increments were analysed using a combination of analysis of covariance (ANCOVA) using CL as covariate, and separate slope analyses (applied when the homogeneity of slopes assumption was violated). Pillai's trace test was used as multivariate test of significance and significant effects were further explored using Tukey's post-hoc analysis. All statistical analyses were performed using Statistica v7.1 (Statsoft Inc. Tulsa OK USA). Trends in growth differences among small (80-85 CL, well under the legal size) and large (Males; 100-105 CL, Females; 90-95 CL) those approaching the legal size) males and females are displayed using boxplots.

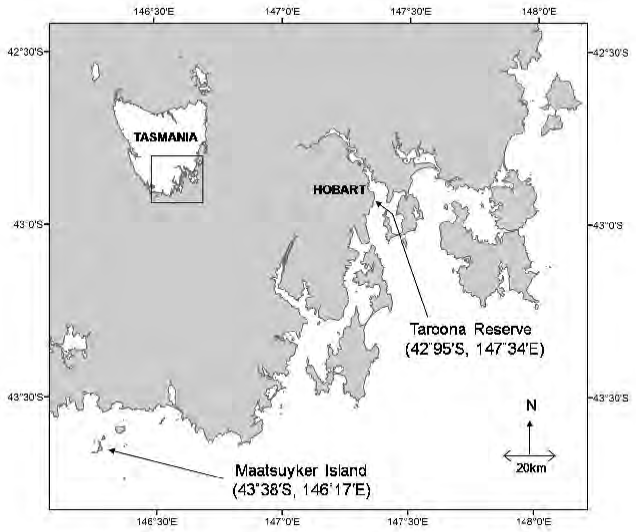


Fig 1. Map of south-east Tasmania showing the deep-water capture site (Maatsuyker Island) and the shallow-water translocation release site (Taroona Marine Reserve).

6.3 Results

Moult increments decreased with size and increments were highly variable across the size ranges for both males and females of the resident deep-water, resident shallow-water and translocated populations. Growth rates varied significantly among the three populations for both males ($F_{(2,559)} = 6.20$, $p < 0.001$) and females ($F_{(2,606)} = 4.48$, $p = 0.01$) (Fig 2). For male lobsters ranging between 70 and 130 mm CL, the mean (± SD) growth rate of translocated males (after their first moult) was significantly greater (8 ± 6.1 mm) than resident deep-water lobsters (4.9 ± 3.6 mm) but less than the growth rate of the resident shallow-water male lobsters (11 ± 4.9 mm). For female lobsters ranging between 70 and 110 mm CL, the mean (± SD) growth rate of translocated females were significantly greater (8 ± 4.2 mm) than resident shallow-water females (3 ± 2.5 mm) and resident deep-water population females (0.9 ± 0.8 mm).

Growth trends between small and large sized lobsters showed that for small males (80 – 85 mm CL), the median growth increment was higher in shallow-water males (13.0 mm) than in deep-water males (7.4 mm) but was highest in translocated males (15.4 mm; Fig 3a). For large males (100 – 105

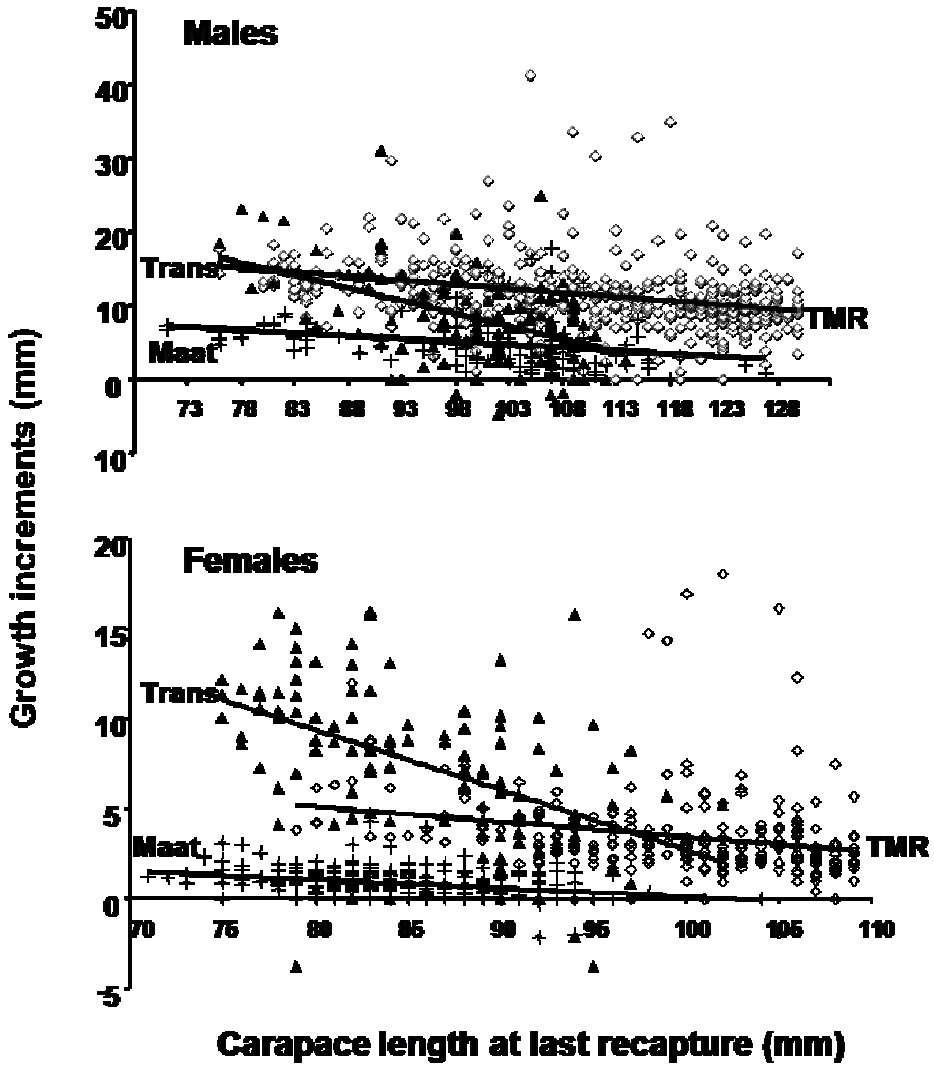


Fig 2. Growth regressions of resident male lobsters at Maatsuyker Is (Maat) (y = -0.082x + 13.17, r^2 = 0.06), resident males at Taroona Marine Reserve (TMR) (y = -0.118x + 27.49, r^2 = 0.06) and translocated male lobsters (Trans) (y = -0.341x + 42.34, r^2 = 0.24). Also shown are the growth regressions of resident females at Maatsuyker Is (y = -0.046x + 4.85, r^2 = 0.09), resident females at Taroona Marine Reserve (y = -0.172x + 21.76, r^2 = 0.16) and translocated female lobsters (y = -0.333x + 36.08, r^2 = 0.25).

mm CL), median growth increment was highest in shallow-water males (12.4 mm), followed by translocated males (7.1 mm) and lowest in deep-water males (3.1 mm; Fig 3b). Growth trends for small females (80 – 85 mm CL) showed that the median growth increment was highest in translocated females (8.8 mm), followed by shallow-water females (6.2 mm) and lowest in deep-water females with (1.0 mm; Fig 4a). For larger females (90 – 95 mm CL), median growth increment was also highest in translocated females (4.4 mm), followed by shallow-water females (3.0 mm) and lowest in deep-water males (0.6 mm; Fig 4b).

6.4 Discussion

This translocation experiment provided a unique opportunity to exploit the site-specific variation in growth rate in *Jasus edwardsii.* We were able to increase growth rates in the slow-growing, sub-legal portion of the population by moving them to areas of faster growth, and therefore increase the biomass of the stock. Growth in translocated females exceeded that of resident females of both the shallow and deep-water populations. This increases productivity and exploitable biomass

available to the fishery, given that commercial harvests are constrained by quota.

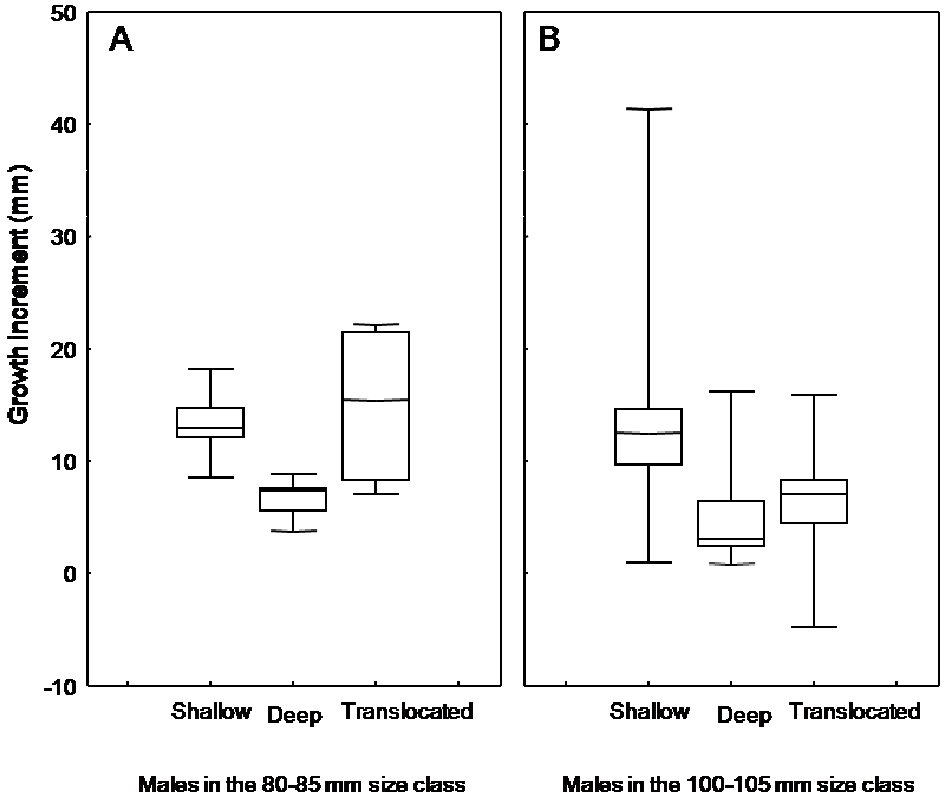


Fig 3. Comparisons of growth increments between deep, shallow and translocated male lobsters in the (A) 80 – 85 mm and (B) 100-105 mm size range. The box and whisker plot shows: box = 1st-3rd quantiles; centre line = median value; whiskers = minimum-maximum values.

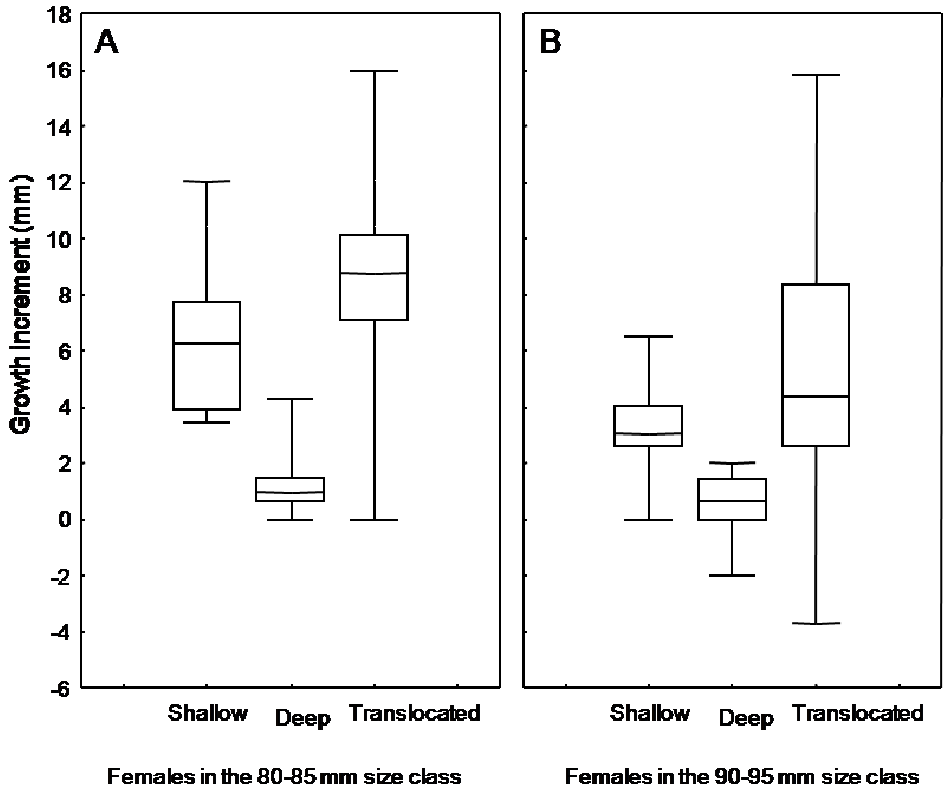


Fig 4. Comparisons of growth increments between deep, shallow and translocated female lobsters in the (A) 80 – 85 mm and (B) 95-95 mm size range. The box and whisker plot shows: box = 1st-3rd quantiles; centre line = median value; whiskers = minimum-maximum values.

Females are important to the fishery not only through contribution to exploitable biomass but also through egg production. Final implementation of translocation as a management tool needs to consider any implications for spawning stock biomass as translocated females receive less protection from size limits and enter the fishery sooner.

The overall economic feasibility evaluation of translocation identified both the operational costs of translocation and change in growth to increase with distance from Maatsuyker Island (Gardner and van Putten 2008b). Under the assumption that translocated lobsters would match the growth rate of resident lobsters, greatest yield gain resulted when growth differences between capture and release sites were large. We found growth increments of translocated lobsters to be greater than resident deep-water lobsters thus achieving the primary goal of translocation, but the magnitude of growth change varied among small and large males and females. This suggests that gains in economic yield through translocation may be a function of size at release and gender in addition to growth differences between regions. The key outcome from the experimental trials was that growth of small yet mature males and females exceeded the growth of resident males and females within the first 12 months

of translocation. Given this result, selectively translocating small sized lobsters to release locations at shorter distances may deliver the predicted yield as from translocating lobsters to high-growth areas in the north of the State, although this is yet to be substantiated from further experimental trials. Translocating small sized lobsters would however require additional effort for grading at sea thus increasing the cost of capture, but overall translocation costs will be reduced by the greater tonnages (from smaller sized lobsters) transported over smaller distances especially as catch rate of under-sized females is high.

Modelled translocation from Maatsuyker Island to Taroona predicted a 347% increase in total biomass, with assumptions of constant harvest rate, that lobsters adopt the growth rate of resident lobsters in the first year after translocation, and that release mortality is no greater than 10% (Gardner and van Putten 2008a). Increased growth from the first year after translocation presented here support the predicted increased biomass and mortality of translocated lobsters was similar to resident lobsters, and was less than 10% (Green and Gardner 2009). Given that none of the modelled scenarios considered growth of translocated lobsters to exceed that of the residents in the first 12 months, the results from this study would need to be

factored into future economic and biological evaluations for translocation operations. The magnitude of the growth changes in translocated lobsters in the current study is compelling in terms of its commercial implications, but longer term monitoring is needed to determine how growth rates change with subsequent moults, as this will influence the time period for translocated lobsters to enter the fishery.

Growth in crustaceans is moult related and discontinuous, and regional variations in moult growth increments and moult frequency have largely been attributed to differences in diet, environmental factors such as water temperature and density effects for major commercial lobster species (review by Wahle and Fogarty 2006 including *J. edwardsii* McGarvey et al. 1999). Therefore to understand how translocation will alter lobster stock dynamics, one must consider the mechanisms regulating growth. For instance, manipulation of lobster population densities through translocations has the potential to also change the growth of resident lobsters at the capture and release sites due to change in food availability and other density effects. For example, Dixon and Day (2004) demonstrated growth enhancement of a "stunted' abalone population by both density-reduction and

through translocation to habitats of faster growing abalone. Additional translocations of deep-water southern rock lobsters to sites of different densities are currently underway and the results of these experiments offer scope for quantifying density dependent growth in *J. edwardsii*. The role diet plays in the growth variation of *J. edwardsii* is also unclear but mean concentrations of lipid content and essential polyunsaturated fatty acids (PUFA) were higher in translocated lobsters than in deep and shallow-water lobsters suggesting enhanced overall body condition (Chapter 4). The change in diet through translocation is also apparent in the change in the shell colouration from pale/white to red due to the greater availability of dietary carotenoids in shallow-water habitats (Chapter 3). The combined results of colour and growth enhancement will add value and increase the productivity of deep-water recruits to deliver greater economic yield from the fishery.

Chapter 7

General Discussion

Translocation can improve yield and marketability of *Jasus edwardsii*

The specific aims of this thesis were to quantify and describe the variation in the key market traits and growth of the southern rock lobster *Jasus edwardsii* between deep and shallow-water populations, and assess how these traits respond to translocation. In addressing these aims, the current study 1) described the depth and latitudinal variation in the shell colouration of *J. edwardsii* and ascertained an economic impact value on the commercial industry 2) quantified differences in growth, shell colour, body-shape market traits, nutritional and post-capture condition between deep-water and shallow-water lobster populations and 3) demonstrated how translocation can improve marketability and increase growth of deep-water *J. edwardsii* over a short time period. In doing so, this study has highlighted the plasticity of these traits and how its exploitation through population manipulation presents a feasible option to value-add to the commercial industry and increase the exploitable biomass of the fishery.

Depth variation in the shell colour of *J. edwardsii* (Chapter 2) is an unusual occurrence among commercial lobster species and thus creates unique marketing issues. These marketing

issues are due to price discounting based on shell colouration in Asian country markets (where there is a high regard for the colour red), which dominate the live market trade. Several approaches have been considered in dealing with the marketing of different coloured lobsters. Alternate market destinations for deep-water *J. edwardsii* are under development through industry marketing initiatives in the USA while attempts at sea-cage grow out trials to enhance colour and improve growth delivered little success due to on-going feed, maintenance costs and poor lobster quality issues (Bryers and Geddes 1995). I have demonstrated that successful shell colour enhancement in *J. edwardsii* is however possible through translocation (Fig 1). Shell colour of all pale and white grades of *J. edwardsii* changed to the marketable red colour after its first moult in the new habitat (Chapter 3). For the commercial industry, colour enhancement through translocation can potentially eliminate the colour grading process and the estimated value of revenue loss at AUS$6.67 millions/year from price discounting can be recovered.

In addition to colour variation, this study is also the first to describe morphometric variation among Australian *J. edwardsii* populations. The study revealed significant differences

in body-shape traits such as leg length and abdomen shape across a depth gradient, however there were no complete transformations of these traits in the short-term of a single moult in translocated lobsters (Chapter 3). Deep-water southern rock lobsters are not graded based on body-shape differences alone since greater weighting is given to shell colouration, but shape is a contributing factor to price discounting nonetheless. Therefore it is presumed that any inferior body-shape traits that persist in translocated lobsters will be overridden by the improved marketability of the red shell colouration.

In addition to phenotypic variation in colour and morphology, differences in growth and body condition between deep and shallow-water lobster stocks also impact on the commercial potential of translocation. In addressing the commercial industry's observations that deep-water southern rock lobsters have poorer condition and lower survival during live transport than their shallow-water counterparts, the health and nutritional condition of deep-water, shallow-water and translocated lobsters were assessed through lipid and haemolymph indices (Chapters 4 and 5). An overarching factor which heavily influenced the outcome of all these analyses was the moult cycle which at times masked differences between

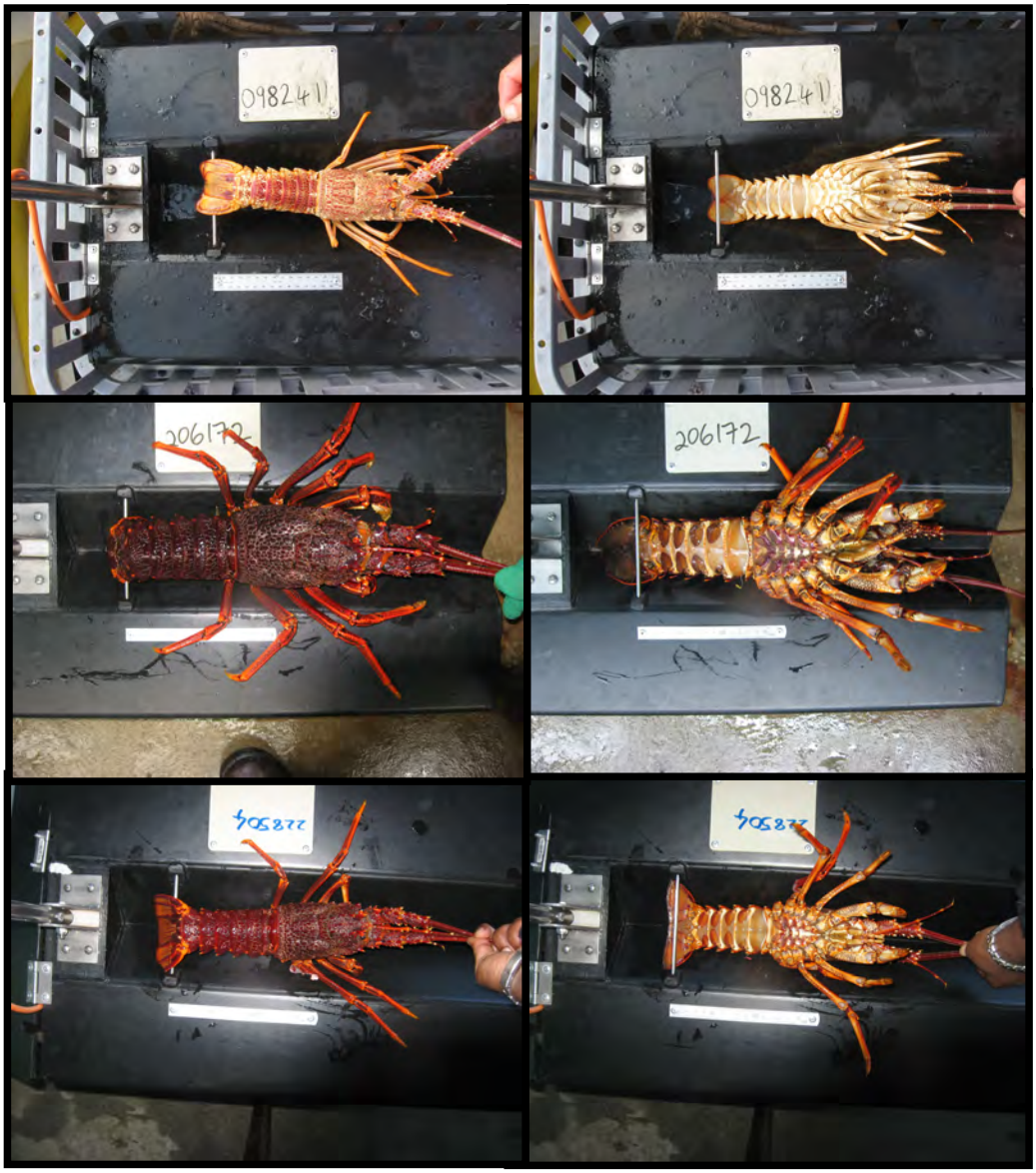


Fig 1. Dorsal (left) and ventral (right) view of a deep-water (top), shallow-water (middle) and translocated *Jasus edwardsii* caught after its first moult post-translocation (bottom).

populations and changes in translocated lobsters, although collectively all indices revealed translocated lobsters to be similar

in condition to resident lobsters after a period of 12 months. In particular the enhanced nutrition of translocated lobsters from the high amounts of omega3 fatty acid content provides an added marketing tool to further value-add to the product. Further research through simulated live-transport trials by Hawthorn (2009) found there was an improvement in live transport condition as translocated lobsters incurred lower mortality than deep-water lobsters. This further supports the results from my thesis that physiological and nutritional condition can be improved though translocation.

Potential benefits from translocation occur not only through changes in market traits but also through increased productivity with improvements in growth. Previous cost benefit analyses showed that benefits mainly accrue through increase in yield (Gardner and van Putten 2008b). My thesis demonstrated that the growth rate of deep-water males and females increased after translocation, with most lobsters matching and some achieving greater growth increments than resident lobsters in their first moult event (Chapter 6). Often the core challenge of many enhancement operations is for released individuals to survive and adopt the growth of wild individuals. Translocated individuals achieved both these goals in their new habitat and

thus overcoming some of the key challenges of enhancement strategies. Given the growth of *J. edwardsii* is highly plastic, manipulation of lobster stock densities through translocations is also likely to occur and thus the impact from density-dependent process would also need to be further evaluated. For Tasmania, yield gains are predicted to be economically feasible and beneficial from translocations between sites of extreme differences in growth rates and over short distances (Gardner and van Putten 2008a), but this prediction may change if translocated lobsters consistently exceed the growth of resident lobsters. Mechanisms regulating life-history traits and phenotypic expression in commercial lobster species are highly variable and are most probably a combination of environmental, behavioural, dietary and genetic factors acting upon each other. While identifying these specific drivers would have further clarified my results, unravelling the complex interaction between animal and habitat was beyond the capacity of this study and outside the scope of commercial interest.

Future of translocation as a fisheries enhancement strategy

"Translocations are powerful tools for the management of the natural and man-made environment which, properly used, can bring great benefits to natural biological systems and to man, but like other powerful tools they have the potential to cause enormous damage if misused"

(IUCN 1987)

The task of evaluating the potential risks against the benefits from translocations ultimately hinges on the economic, social, biological and environmental components that drive the fishery. The research presented in the thesis only assessed some of the biological outcomes from translocations to inform stakeholders of its application and provide quantitative data for its economical evaluation. Other aspects of translocation are equally imperative to its biological and economic feasibility:

- Related research underway has estimated that apparent survival of all translocated lobsters from Maatsuyker Island to Taroona Marine Reserve is similar to survival of resident lobsters (Green and Gardner 2009).
- Preliminary results from acoustic tracking data of post-release translocated lobsters indicate high site fidelity, overlap of home-ranges between resident and translocated lobsters and a slightly greater

foraging range than resident lobsters (B.S. Green unpublished data).

- There has not been a significant impact on reef ecology from increasing the density of lobsters (Hoare 2008) although density of lobsters was shifted closer to the natural state.
- Den sharing has been observed between translocated and resident individuals (Fig 2).
- Impact of translocation on statewide egg production and changes to egg quality are currently being investigated while any threat from a disease outbreak is also being assessed through examination of lobsters for pathogens.

Fig 2. A resident (left) and a translocated deep-water (right) *Jasus edwardsii* sharing a shelter at Taroona Marine Reserve. Photo by Tim Alexander

Although fisheries enhancement strategies using adults is less common than hatchery reared juveniles, they face many of the same challenges, concerns and constraints. Often the rationale for translocating adults sourced from the wild stocks serve to solve a specific issue rather than the broad goal of stock replenishment. For example, reproductively deficient individuals of queen conchs (*Strombus gigus*) from depleted inshore areas of the Florida Keys are being translocated to offshore regions where they once again develop normally and become reproductively active (Delgado et al. 2004). In Japan, the enhancement of (*Strongylocentrotus nudus*) roe quality and size was achieved by transplanting adults with poorly developed gonads from barrens habitats to kelp forests (Bell et al 2005). Stunted populations of greenlip abalone translocated to faster growth areas showed enhanced growth in response to greater food availability (Dixon and Day 2004). One of the drawbacks to translocation strategies is that proving its success requires long-term monitoring even if the short-term results seem promising. Thus adult translocations such as these are often documented as research attempts, while their commercial application is a drawn-out debated process.

Conclusion

" …the core challenge in understanding enhancement fisheries is to elucidate how the characteristics of the target population and its environment, fishing and enhancement technologies, and stakeholder behaviour interact and lead to particular outcomes "

Lorenzen (2008, pp 11)

The research presented in this thesis has addressed some of the key characteristics of the target population (translocated lobsters) in its new environment that will inform and aid stakeholders in planning future management decisions. The process towards implementation is currently underway with pilot commercial scale translocations being planned between deep and shallow-water sites within a single fishing area (refer to Fig 1 in Chapter 2). Stakeholders will review the outcome of these trials and all other research results before deciding on larger scale translocations from south to north of the State. The final adoption of translocation is envisaged to be a stepwise integration and used as a supplementary strategy with existing management arrangements, although the final implementation will hinge on the tradeoffs between the economic, social, biological and environmental components that drive the fishery.

In response to challenges presented by differences in the biological characteristics of *Jasus edwardsii* and aspects of its

resource management, the research presented in this thesis provides quantitative data on the spatial differences in market traits, body condition and growth and how these traits can be improved through translocation. While the success of translocation as a fisheries enhancement strategy is yet to be determined, in exploring its feasibility the current study has contributed and highlighted significant and interesting aspects of a lobsters' biology and its management.

References

Ali M, Nicieza A, Wootton RJ (2003). Compensatory growth in fishes: a response to growth depression. Fish and Fisheries 4: 147-190.

Alvarez D, Metcalfe NB (2007). The tradeoff between catch-up growth and escape speed: variation between habitats in the cost of compensation. Oikos 116: 1144-1151.

Annala JH, Bycroft B (1993). Movement of rock lobsters (*Jasus edwardsii*) tagged along the eastern coast of the North Island, New Zealand. NZ J Mar Freshw Res 27: 183-190.

Bartley DM, Bell JD (2008). Restocking, stock enhancement and sea ranching: Arenas of progress. Rev Fish Sci 16:357-365.

Bell JD, Rothlisberg PC, Munro JL, Loneragan NR, Masuda R (2005). Restocking and stock enhancement of marine invertebrate fisheries. Adv Mar Biol 49:1-370.

Bell JD, Leber KM, Blankenship HL, Loneragan NR, Masuda R (2008). A new era for restocking, stock enhancement and sea ranching of coastal fisheries resources. Rev Fish Sci 16:1-9.

Booth JD (2006). *Jasus* species. In: Lobsters–Biology, management, aquaculture and fisheries. Oxford, Blackwell publishing. 340-358.

Bligh EG, Dyer WJ (1959). A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911-917.

Bradshaw M, Wood L, Williamson S (2001). Applying qualitative and quantitative research: a social impact assessment of a fishery. Appl Geo 21: 69 85.

Bruce BD, Bradford R, Griffin D, Gardner C, Young J (2000). A synthesis of existing data on larval rock lobster distribution in southern Australia. FRDC project 96/107. 57pp.

Bryars SR, Geddes MC (2005). Effects of diet on the growth, survival, and condition of sea-caged adult southern rock lobster, *Jasus edwardsii*. NZ J Mar Freshw Res. 39: 251-262.

Caddy JF, Defeo O (2003). Enhancing or restoring the productivity of natural populations of shellfish and other marine invertebrates resources. FAO Fisheries Technical Paper 448. FAO, Rome.

Chang ES, O'Connor JD (1983). Metabolism and transport of carbohydrates and lipids. In: Bliss, D.E. (Ed.), The biology of crustacea. Academic Press, New York, pp. 263-287.

Chang ES (2005). Stressed-out lobsters: Crustacean hyperglycemic hormone and stress proteins. Integ Comp Biol 45: 43-50.

Childress JJ, Cowles DL, Favuzzi JA, Mickel TJ (1990). Metabolic rates of benthic deep-Sea decapod crustaceans decline with increasing depth primarily due to the decline in temperature. Deep-Sea Res Part A 37, 929-949.

Cockcroft AC (1997). Biochemical composition as a growth predictor in male west-coast rock lobster (*Jasus lalandii*). Mar Freshw Res 48: 845-856.

Colquhoun E, Archbold D (2009). Evaluating the performance of Australian marine capture fisheries. Final report to the FRDC – Resource Working Group.

Corraze G (1999). Lipid Nutrition. In: Guillaume, J., Metailler, R., Kaushik, S., Bergot, P. (Eds.), Nutrition and feeding of fish and crustaceans. Springer, London, pp. 111-130.

Crear B, Hart P, Thomas C, Barclay M (2002). Evaluation of commercial shrimp grow-out pellets as diets for juvenile southern rock lobster, *Jasus edwardsii*, influence on growth, survival, colour, and biochemical composition. J Appl Aqua 12: 43-57.

Crear BJ, Hart PR, Thomas CW (2003). The effect of photoperiod on growth, survival, colour and activity of juvenile southern rock lobster, *Jasus edwardsii*. Aquac Res 34: 439-444.

D'Abramo LR (1997). Triacyglycerols and fatty acids, Crustacean nutrition (Advances in World Aquaculture). World Aquaculture Society, Baton Rouge, pp. 71-107.

Davis JLD, Eckert-Mills MG, Young-Williams AC, Hines AH, Zohar Y (2005). Morphological conditioning of a hatchery-raised invertebrate, *Callinectes sapidus*, to improve field survivorship after release. Aquaculture 243:147-158

Debuse VJ, Addison JT, Reynolds AD (2001). Morphometric variability in UK populations of the European lobster. J Mar Biol Ass U K 81:469-474.

Delgado GA, Bartels CT, Glazer RA, Peterson NJB, McCarthy KJ (2004). Translocation as a strategy to rehabilitate the queen conch (*Strombus gigas*) population in the Florida Keys. Fish Bull 102:278-288.

Department of Primary Industries (2003). Rock lobster fishery management plan, 2003: Fisheries Victoria management report series, No 1. Victoria, Department of Primary Industries.

Dixon CD, Day RW (2004). Growth responses in emergent greenlip abalone to density reductions and translocations. J Shellfish Res 23:1223-1228.

Edgar GJ (2001). Australian marine habitats in temperate waters. Sydney, New Holland Publishers.

Fischer J, Lindenmayer DB (2000). An assessment of the published results of animal relocations. Biol Conserv 96:1-11.

Ford W (2001). Restructuring the Tasmanian rock-lobster fishery - the effect of two years of management under individual transferable quotas. Mar Freshw Res 52: 1641-1648.

Fotedar S, Evans L, Jones B (2006). Effect of holding duration on the immune system of western rock lobster, *Panulirus cygnus*. Comp Bio Physiol A - Mol Integ Physiol 143: 479-487.

Florkin M (1960). Blood chemistry. In: The physiology of crustacean I. Editor; Waterman T. Academic Press, New York. Vol 1, pp 141-159.

Frusher S, Eaton L, Bradshaw M (2003). Impact of management change to an ITQ system in the Tasmanian Rock Lobster Fishery. Hobart, Tasmanian Aquaculture and Fisheries Institute.

Garcia SM, Grainger RJR (2005). Gloom and doom? The future of marine capture fisheries. Phil Trans R Soc B 360:21-46.

Gardner C, Frusher S, Haddon M, Buxton C (2003). Movements of the southern rock lobster *Jasus edwardsii* in Tasmania, Australia. Bull Mar Sci 73: 653-671.

Gardner C, Mackinnon C, Haddon M, Frusher SD (2004). Tasmanian Rock Lobster Fishery 2002/03, Fishery Assessment Report. Tasmanian Aquaculture and Fisheries Institute.

Gardner C, Mills D, Frusher SD (2005). Does pleopod setation provide a measure of maturity in female southern rock lobsters *Jasus edwardsii*? Sci Mar 69:123-131.

Gardner C, Frusher S, Barrett N, Haddon M, Buxton C (2006). Spatial variation in size at onset of maturity of female southern rock lobster *Jasus edwardsii* around Tasmania, Australia. Sci Mar 70: 423-430.

Gardner C, van Putten I (2008a). The economic feasibility of translocating rock lobsters to increase yield. Rev Fish Sci 16:154-163.

Gardner C, van Putten I (2008b). Biological modelling of translocation as a management tool for a rock lobster fishery. Rev Fish Sci 16:81-90.

Gebauer SK, Psota TL, Harris WS, Kris-Etherton PM (2006). n-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. Am J Clin Nutr 83: 1526S-1535S.

Gibson R, Barker PL (1979). The decapod hepatopancreas. Oceanogr Mar Biol Annu Rev 17: 285-346.

Goodwin TW (1960). Biochemistry of pigments. In: Waterman TH (ed) The physiology of crustacea: metabolism and growth, Vol 1. Academic Press Inc., New York, p 101-140.

Green BS, Gardner C (2009). Surviving a sea-change: survival of southern rock lobster (*Jasus edwardsii*) translocated to a site of fast growth. ICES J. Mar. Sci 66: 656-664.

Griffith B, Scott JM, Carpenter JW, Reed C (1989). Translocation as a species conservation tool: status and strategy. Science 245:477-482.

Haddon M (2001). Modelling and quantitative methods in fisheries. Florida, Chapman & Hall.

Haddon M, Gardner C (2009). Rock lobster fishery assessment report 2007/08. Tasmanian Aquaculture and Fisheries Institute, Hobart.

Harrison AJ (2004). A report on the abalone and rock lobster post harvesting industry in Tasmania. Hobart, Tasmania Abalone Council. 121 p.

Hawthorne P (2009). Effects of deep-water hauling and translocation from deep-water to shallow-water inshore reefs on the physiological condition of southern rock lobster (*Jasus edwardsii*). Honours thesis, Flinders University, South Australia.

Hoare M (2008). Ecological effect of transplantation of deep-water rock lobsters. Honours thesis, Flinders University, South Australia.

Iverson SJ, Frost KJ, Lang SLC (2002). Fat content and fatty acid composition of forage fish and invertebrates in Prince William Sound, Alaska: factors contributing to among and within species variability. Mar Ecol Prog Ser 241: 161-181.

IUCN (1987). IUCN Position statement on translocation of living organisms: introductions, reintroductions and restocking. 22nd Meeting of the IUCN Council, Gland Switzerland,

September 1987 (http://iucn.org/themes/ssc/pubs/policy/transe.htm).

Jarvinen R, Knekt P, Rissanen H, Reunanen A (2006). Intake of fish and long-chain n-3 fatty acids and the risk of coronary heart mortality in men and women. Br J Nutr 95: 824-829.

Johnston DJ, Calvert KA, Crear BJ, Carter CG (2003). Dietary carbohydrate/lipid ratios and nutritional condition in juvenile southern rock lobster, *Jasus edwardsii*. Aquaculture 220: 667-682.

Jones PL, Obst JH (2000). Effects of starvation and subsequent refeeding on the size and nutrient content of the hepatopancreas of *Cherax destructor* (Decapoda : Parastacidae). J Crustac Biol 20: 431-441.

Ju SJ, Harvey HR (2004). Lipids as markers of nutritional condition and diet in the Antarctic krill *Euphausia superba* and *Euphausia crystallorophias* during austral winter. Deep-Sea Res Part II 51: 2199-2214.

Jussila J, Jago J, Tsvetnenko E, Dunstan B, Evans LH (1997). Total and differential haemocyte counts in western rock lobsters (*Panulirus Cygnus* George) under post-harvest stress. Mar Freshw Res 48: 863-867.

Kailola PJ, Williams MJ, Stewart PC, Reichelt RE, McNee A, Grieve C (1993). Australian Fisheries Resource. Canberra, Bureau of Resource Sciences. 155 p.

Kanazawa A, Koshio S (1994). Lipid nutrition of the spiny lobster *Panulirus japonicus* (Decapoda, Lalinuridae) - a review. Crustaceana 67: 226-232.

Konosu S, Yamaguchi K (1994). Colour and taste In: Spiny lobster management. Cambridge, Blackwell Scientific. pp. 495-501.

Lavallee J, Hammel KL, Spangler ES, Cawthorn RJ, Dohoo IR (2000). Descriptive statistics of fishing practices, postharvest health status, and transport conditions in the Prince Edward Island lobster (*Homarus americanus*) industry. J Shellfish Res 19: 265-274.

Lignot JH, Spanings-Pierrot C, Charmantier G (2000). Osmoregulatory capacity as a tool in monitoring the physiological condition and the effect of stress in crustaceans. Aquaculture 191: 209-245.

Linnane A, Dimmlich W, Ward T (2005). Movement patterns of the southern rock lobster, *Jasus edwardsii*, off South Australia. NZ J Mar Freshw Res 39: 335-346.

Linnane A, McGarvey R, Feenstra J (2006). Northern Zone rock lobster (*Jasus edwardsii*) fishery status report 2005/2006. West Beach, SARDI Aquatic Sciences.

Lorenzen K (2005). Population dynamics and potential of fisheries stock enahncment: practical theory for assessment and policy analysis. Phil Trans R Soc B 360:171-189.

Lorenzen K (2008). Understanding and managing enhancement fisheries systems. Rev Fish Sci 16:10-23.

Lund HS, Wang T, Chang ES, Pedersen LF, Taylor EW, Pedersen PB, McKenzie DJ (2009). Recovery by the Norway lobster

Nephrops norvegicus (L.) from the physiological stresses of trawling: Influence of season and live-storage position. J Exp Mar Biol Ecol. 373:124-132.

McGarvey R, Ferguson GJ, Prescott JH (1999). Spatial variation in mean growth rates at size of southern rock lobster, *Jasus edwardsii*, in South Australian waters. Mar Freshw Res 50: 333-342.

McLeod LE, Carter CG, Johnston DJ (2004). Changes in the body composition of adult male southern rock lobster, *Jasus edwardsii*, during starvation. J Shellfish Res 23: 257-264.

MacCormack TJ, DeMont ME (2003). Regional differences in allometric growth in Atlantic Canadian lobster (*Homarus americanus*). J Crust Biol 23:258-264.

Martin GAS (2004). Transplantation of large *Paracentrotus lividus*: A restocking strategy for an edible sea urchin. In: Lawrence JM, Guzman O (eds) Sea urchins: fisheries and ecology - Proceedings of the international conference on

sea-urchin fisheries and aquaculture. DEStech Publications, Pennsylvania, p 74-83.

Melville-Smith R, Cheng YW, Thomson AW (2003). Factors affecting colour change in 'white' western rock lobsters, *Panulirus cygnus*. J Exp Mar Biol Ecol 291: 111-129.

Mercaldo-Allen M (1991). Changes in the blood chemistry of the American lobster *Homarus americanus*, H. Milne Edwards, 1837, over the molt cycle. J Shellfish Res 10: 147-156.

Meyers SP, Latscha T (1997) Carotenoids. In: Crustacean nutrition (Advances in world aquaculture). World Aquaculture Society.

Moore LE, Smith DM, Loneragan NR (2000). Blood refractive index and whole-body lipid content as indicators of nutritional condition for penaeid prawns (Decapoda : Penaeidae). J Exp Mar Biol Ecol 244: 131-143.

Murphy KJ, Mooney BD, Mann NJ, Nichols PD, Sinclair AJ (2002). Lipid, FA, and sterol composition of New Zealand

green lipped mussel (*Perna canaliculus*) and Tasmanian blue mussel (*Mytilus edulis*). Lipids 37: 587-595.

Musgrove RJB (2001). Interactions between haemolymph chemistry and condition in the southern rock lobster, *Jasus edwardsii*. Mar Biol 139: 891-899.

Mynard DM (1960). Circulation and heart function. In: The physiology of crustacean I. Editor; Waterman TH. Academic Press, New York. Vol 1, pp 161-226.

Navarro JC, Villanueva R (2003). The fatty acid composition of Octopus vulgaris paralarvae reared with live and inert food: deviation from their natural fatty acid profile. Aquaculture 219: 613-631.

Nelson MM, Olley J, Crear BJ, Lewis T, Nichols PD (2005). Comparison of wild and cultured adult southern rock lobster, *Jasus edwardsii*: Growth, sensory analysis and oil composition. Food Australia 57: 499-508.

Nelson MM, Bruce MP, Nichols PD, Jeffs AG (2006). Nutrition of wild and cultured lobsters. In: Phillips, B.F. (Ed.),

Lobsters: Biology, Management, Aquaculture and Fisheries. Blackwell Publishing, Victoria, pp. 205-230.

Neter J, Kutner MH, Nachtsheim CJ, Wassermen W (1990). Applied linear statistical models. Chicago, Irwin.

O'Connor JD, Gilbert LI (1968). Aspects of lipid metabolism in crustaceans. Am Zool 8: 529-539.

Oliver MD, MacDiarmid AB (2001). Blood refractive index and ratio of weight to carapace length as indices of nutritional condition in juvenile rock lobsters (*Jasus edwardsii*). Mar Freshwa Res 52: 1395-1400.

Ovenden JR, Brasher DJ, White RWG (1992). Mitochondrial DNA analyses of the red rock lobster *Jasus edwardsii* supports an apparent absence of population subdivision throughout Australiasia. Mar Biol 112:319-326.

Parslow-Williams PJ, Atkinson RJA, Taylor AC (2001). Nucleic acids as indicators of nutritional condition in the Norway lobster *Nephrops norvegicus*. Mar Ecol Prog Ser 211: 235-243.

Passano LM (1960). Moulting and its control. In: Waterman, T.H. (Ed.), The physiology of crustacea. Academic Press Inc, London, pp. 473-536.

Paterson BD, Spanoghe PT (1997). Stress indicators in marine decapod crustaceans, with particular reference to the grading of western rock lobsters (*Panulirus cygnus*) during commercial handling. Mar Freshw Res 48: 829-834.

Paterson BD, Spanoghe PT, Davidson GW, Hosking W, Nottingham S, Jussila J, Evans LH (2005). Predicting survival of western rock lobsters *Panulirus cygnus* using discriminant analysis of haemolymph parameters taken immediately following simulated handling treatments. NZ J Mar Freshw Res 39: 1129-1143.

Pauly D, Watson R, Alder J (2005). Global trends in world fisheries: impacts on marine ecosystems and food security. Phil Trans R Soc B 360:5-12.

Phillips KL, Nichols PD, Jackson GD (2003a). Dietary variation of the squid *Moroteuthis ingens* at four sites in the Southern

Ocean: stomach contents, lipid and fatty acid profiles. J Mar Biol Ass U K 83: 523-534.

Phillips KL, Nichols PD, Jackson GD (2003b). Size-related dietary changes observed in the squid *Moroteuthis ingens* at the Falkland Islands: stomach contents and fatty-acid analyses. Polar Biol 26: 474-485.

Punt AE, Kennedy RB (1997). Population modelling of Tasmanian rock lobster, *Jasus edwardsii*, resources. Mar Freshw Res 48: 967-980.

Punt AE, Kennedy RB, Frusher SD (1997). Estimating the size-transition matrix for Tasmanian rock lobster, *Jasus edwardsii*. Mar Freshw Res 48: 981-992.

Rao KR (1985). Pigmentary effectors. In: Bliss DE, Mantel LH (eds) The Biology of crustacea: integument, pigments, and hormonal processes, Vol 9. Academic Press Inc., New York, p 395-462.

Roberts PR (1994). Export marketing of Australian and New Zealand Rock Lobster. In 'Spiny Lobster Management'.

(Eds BF Phillips, JS Cobb and J Kittaka) pp. 510-516. (Blackwell Scientific Publishing: Oxford).

Santos EA, Nery LEM, Keller R, Goncalves AA (1997). Evidence for the involvement of the crustacean hyperglycemic hormone in the regulation of lipid metabolism. Physiol Zool 70: 415-420.

Sargent JR (1974). The structure, metabolism and function of lipids in marine organisms. In: Malins, D.C., Sargent., J.R. (Eds.), Biochemical and Biophysical Perspectives in marine Biology. Academic press, London, pp. 149-212.

Sax DF, Smith KF, Thompson AR (2009). Managed relocation: a nuanced evaluation is needed. Trends Ecol Evol 24:472-473.

Schahinger RB (1987). Structure of coastal upwelling events observed off the south-east coast of South Australian during February 1983 – April 1984. Aust J Mar Freshw Res 38: 439-459.

Schlaepfer MA, Helenbrook WD, Searing KB, Shoemaker KT (2009). Assited colonization: evaluating contrasting management actions (and values) in the face of uncertainty. Trends Ecol Evol 24: 471-472.

Semmens J, Haddon M, Mackinnon C (2006). Tasmanian Rock Lobster Fishery assessment report 2004/2005. Hobart, Tasmanian Aquaculture and Fisheries Institute.

Sokal RR, Rohlf FJ (1981). Biometry. W.H Freeman and Company, New York.

Speed SR, Baldwin J, Wong RJ, Wells RMG (2001). Metabolic characteristics of muscles in the spiny lobster, *Jasus edwardsii*, and responses to emersion during simulated live transport. Comp Biochem Physiol B - Bioch and Mol Biol 128: 435-444.

Stuart T, Macmillan DL, Thomas M (1996). The effect of background colour on the colour of developing juvenile rock lobsters, *Jasus edwardsii* (Crustacea: Decapoda). Mar Freshw Behav Physiol 27: 269-273.

Taylor HH, Paterson BD, Wong RJ, Wells RMG (1997). Physiology and live transport of lobsters: report from a workshop. Mar Freshw Res 48: 817-822.

Tlusty M (2005). Use of digital colour analysis to assess variation within individual adult American lobsters (*Homarus americanus*) and the process of addition of colour in white lobsters. NZ J Mar Freshw Res 39: 571-580.

Tlusty M, Hyland C (2005). Astaxanthin deposition in the cuticle of juvenile American lobster (*Homarus americanus*): implications for phenotypic and genotypic coloration. Mar Biol 147: 113-119.

Turner K, Gardner C, Swain R (2002). Onset of maturity in male southern rock lobsters *Jasus edwardsii* in Tasmania, Australia. Invert Reprod Dev 42: 129-135.

Wade N, Goulter KC, Wilson KJ, Hall MR, Degnan BM (2005). Esterified astaxanthin levels in lobster epithelia correlate with shell colour intensity: Potential role in crustacean shell colour formation. Comp Biochem Physiol B 141:307-313.

Wahle RA, Fogarty MJ (2006). Growth and development: Understanding and modelling growth variability in lobsters. In: Lobsters: Biology, management, aquaculture and fisheries. Blackwell Publishing Ltd, Oxford. pp. 1-44.

Warton DI, Wright IJ, Falster DS, Westoby M (2006). Bivariate line-fitting methods for allometry. Biol Rev 81:259-291.

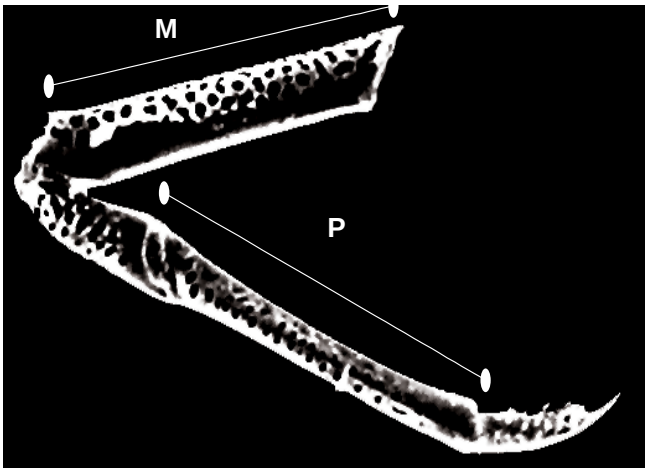
Whiteley NM, Taylor EW (1992). Oxygen and acid-base disturbances in the haemolymph of the lobster *Homarus-gammarus* during commercial transport and storage. J Crust Biol 12: 19-30.

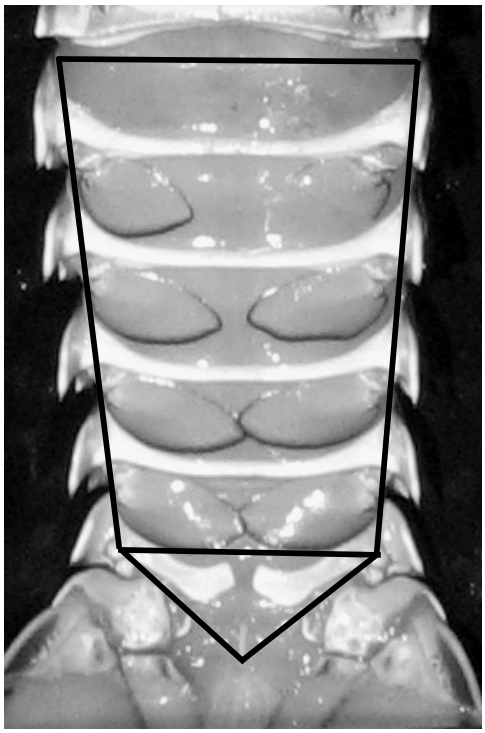
Wood R, Peat A, Galeano D (2007). Australian Fisheries Statistics 2006. Canberra, Australian Bureau of Agricultural and Resource Economics.

Ziegler PE, Haddon M, Frusher SD, Johnson CR (2004). Modelling seasonal catchability of the southern rock lobster *Jasus edwardsii* by water temperature, moulting, and mating. Mar Biol 145:179-190.

Zohar Y, Hines AH, Zmora O, Johnson EG, Lipcius RN, Seitz RD, Eggleston DB, Place AR, Schott EJ, Stubblefield JD, Chung JS (2008) The Chesapeake Bay blue crab (*Callinectes sapidus*): A multidisciplinary approach to responsible stock enhancement. Rev Fish Sci 16: 24-34

Appendix





Top: Drawing of the fourth walking leg of *J.edwardsii,* showing the measurement points taken for the merus (M) and propodus (P).
Bottom: Photograph depicting the points of measurements taken from the abdomen; A to B = tail width of the first abdominal segment, C to D = tail width of the last abdominal segment, ABDEC = abdominal area.