Measuring condition of lobsters to improve management of harvesting around periods of high transport mortality

C. Simon, T. Mendo, B. Green & C. Gardner

Project No. 2014/726

May 2015

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Measuring condition of lobsters to improve management of harvesting around periods of high transport mortality

Cedric Simon, Tania Mendo, Bridget Green, Caleb Gardner

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AUSTRALIAN SEAFOOD COOPERATIVE RESEARCH CENTRE

May 2015

IMAS INSTITUTE FOR MARINE AND ANTARCTIC STUDIES

UNIVERSITY of TASMANIA

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ISBN: 9781 8629 58128

The Australian Seafood CRC is established and supported under the Australian Government's Cooperative Research Centres Program. Other investors in the CRC are the Fisheries Research and Development Corporation, Seafood CRC company members, and supporting participants.

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Australian Government
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Non-Technical Summary

2014/726 - **Measuring condition of lobsters to improve management of harvesting around periods of high transport mortality**

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PROJECT OBJECTIVES:

1 Quantify differences in health, physiological and nutritional condition of pale and red male lobster stocks in four different fishing areas of Tasmania at the start of the fishing season.
2 Examine the differences in nutritional, physiological, health condition and survival of pale and red lobsters exposed to extended emersion exposure.

OUTCOMES ACHIEVED

This project provides the first comprehensive quantitative assessment of the health, physiological and nutritional condition of brindle and red lobsters from four different fishing areas of Tasmania at the start of the 2015 fishing season. Brindle lobsters sampled in the South East and South West of Tasmania were shown to have lower lipid reserves and higher magnesium than Orford lobsters but this difference did not affect their tail meat dry matter content and vulnerability to transport. The industry perception of brindle lobsters being in poorer condition could not be substantiated with the lobsters available to this study by differences in biological attributes relating to their survivorship.

Variation in nutritional condition based on the non-invasive Brix index assessment was found to be larger at the site level within area than between areas or within pots. This suggests the need to apply the assessment of condition to smaller scales than fishing areas in order to monitor accurately moult pattern and condition of lobsters. The Brix index in this study was not a good predictor of vulnerability to transport but correlated well to tail dry matter content, a likely proxy for meat yield.

The lobsters size (carapace length), baseline concentrations of haemolymph haemocyanin and bicarbonate were useful predictor of vulnerability to transport. This information may help improve the management of these lobsters especially if portable meters are used in the field or test kits are developed through future R&D. Lobsters that died after packing showed significantly higher lactate and lower pH values than those that survived, suggesting their haemolymph acid-base balance failed to compensate for the anaerobic metabolism. These lobsters also had significantly higher urea and potassium levels and significantly lower sodium and chloride ions indicating that dehydration, cell damage and possibly nitrogenous waste toxicity were other possible factors for their death. Thus, recommendations to minimise mortality risk would include ensuring lobsters have recovered from the ground transport stress in holding tanks for several days, minimising emersion duration and reducing temperature prior to packaging.

LIST OF OUTPUTS PRODUCED

- Peer-reviewed manuscript in preparation
- Two oral presentations at the Crustacean Society conference (TCS IAA2015):
    - An assessment of the nutritional, physiological and health condition of brindle and red adult *Jasus edwardsii* rock lobsters and their survival after extended emersion exposure
    - Measuring condition of lobsters (*Jasus edwardsii*) to improve management of harvesting around periods of high transport mortality

ACKNOWLEDGEMENTS

Field and laboratory technical support was provided by Neville Barrett, Justin Hulls, Colin Fry, Keerthi Elizabeth Chinappa and Michael Porteus. Special thanks to Ben Lawler for collection of lobsters, Michael Blake for letting us take lobster haemolymph samples at SALCO, Cambridge and to many volunteers who assisted with laboratory work and fieldwork throughout the project. We thank Dr Andrea Battison (CrustiPath) for her assistance with the shipment and analysis of haemolymph biochemistry. This research was supported under the Australian Research Council's Industrial Transformation Research Program (project number IH120100032).

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1. Introduction and Background

The live trade of southern rock lobsters (*Jasus edwardsii*) from Tasmania, Victoria and South Australia to export markets in Asia began in the late 1980s and has a gross revenue of AUD 67 million (2013-2014 fishing season) for Tasmania alone (www.Tasrocklobster.com). This species is harvested from a range of depths: premium lobsters tend to be caught in shallow-water areas and are bright red in colour ('reds') while lobsters harvested from deep-water areas are paler ('brindle', 'speckled' or 'pales') and are sold at a discounted price (Chandrapavan et al., 2009). The difference in price between red and brindles is driven by the Chinese market, and is a consequence of the cultural association of red with luck, happiness, and prosperity (Konosu & Yamaguchi, 2000).

Losses of 10% of lobsters during live transport are not uncommon and have a major financial impact. The fishing industry, including processors and operators are seeking ways to avoid this mortality. Mortality events occur mainly around the time of season openings in November and according to processors, are associated mainly with male brindle lobsters. Price often falls sharply at this time because of the effect of increase in supply, which combined with higher mortality rates impacting shipments that are received, can damage market reputation and be of financial risk to exporters. Managing the landing of poor quality lobsters is difficult because longer closed seasons would prevent landing of poor quality product but decreases income to business. Ideally the problem of lobster mortality in shipment would be managed by keeping the fishery open for extended periods but only landing lobsters with acceptable condition. The fishery tends to do this on a crude level by discounting the price of pale lobster. However this is not ideal because it impacts the price of some catches of pale lobsters that are in good condition, and also allows the shipment of some red lobsters of poor condition. Directly measuring condition is a step towards better management of this issue.

Processors rely on vitality (tail flipping, antenna response and general handling resistance) as a crude measure to assess condition prior to live-holding in anticipation for live transport to markets. Vitality of lobsters on arrival at the processing centre can be assessed quickly by trained processor staff and form the basis for the management of transport mortality issues. Lobsters of poor vitality are sold cooked, while lobsters of high vitality are graded, held for up to a week in tanks and packaged for live export (Michael Blake, Pers. Comm.). Nevertheless, assessment of vitality at processors does not necessarily prevent transport mortality in lobsters and moreover it does not explain which condition parameters might be more

important in explaining differential vulnerability in lobsters, and how these vary between locations in the wild.

Condition can be divided into several categories including physiological condition, nutritional condition, and health. All three categories can contribute to differences in vulnerability to transport stress and so measuring indicators of all three will increase the likelihood of identifying correlates or causes of vulnerability. Vitality is strongly affected by stressors associated with live transport including capture and handling by fishing gear and crew, exposure to varying temperatures and oxygen availability, physical damage and interactions with other lobsters. It is therefore biased towards a measure of physiological condition, which can be short-term. Stress reduces the physiological condition of lobsters to beyond the normal range but it can be readily reversible if the physiological disturbance is within the homeostatic capability of the lobsters, or it can be non-reversible, ultimately leading to mortality (Taylor et al., 1997). The study of crustacean haemolymph biochemistry, which includes a large range of organic and inorganic constituents, provides a measure of physiological condition (Paterson & Spanoghe, 1997). Indices such as haemolymph gases, pH, metabolites such as lactate and glucose, and electrolytes (Mg, K, Na) can provide useful information to the degree of stress in wild population, during live-holding and live transport (Chandrapavan et al., 2011). Additionally, haemocyanin is the oxygen-carrying protein in the haemolymph and hypoxia or lack of oxygen can result in an increase in haemocyanin in crustaceans (Defur et al., 1990).

Nutritional condition is related to the extent of energy reserves, which in rock lobsters is associated with lipid accumulation in the hepatopancreas as well as abdominal muscle dry matter and lipid content (Simon et al. 2015). Nutritional condition of lobsters however cannot be assessed visually (e.g., vitality) or morphometrically because the morphological changes in soft tissue that occur during reserve accumulation over the intermoult are masked by the carapace which remains fixed for the remaining duration of the moult cycle. The extensive use of hepatopancreas energy reserves before and after the moult, which correspond to a non-feeding period, results in poorest nutritional condition in the weeks following the moult (Simon et al., 2015). After the moult, the carapace takes time to harden (3-4 weeks) and shell hardness can be used as a tactile measure of condition. However carapace hardening occurs irrespectively of an improvement in nutritional condition which is associated with feeding (i.e., energy intake) and soft tissue growth (Simon et al., 2015). Therefore, lobsters with low energy reserves and a hard shell can be shipped inadvertently, especially at critical times such as after the male moult period.

Haemolymph protein concentration, and associated techniques to estimate it via refractometry (Chandrapavan et al. 2011), has been used as the main non-destructive index of nutritional condition in lobsters. Haemolymph protein concentration varies with a range of environmental stressors (see review in Fotedar & Evans, 2011), but also with the moult cycle, reproductive cycle and nutrition (Musgrove, 2001), so it important to take all these factors into account when considering its use as a predictor of vitality prior to shipment in live lobsters (Bolton et al., 2009).

One of the refractometry measurements is the Brix index. A project launched in 2004 in Canada (Atlantic Lobster Moult and Quality Project) has advanced the use of the Brix index as a tool to make management decisions with the American lobster *Homarus americanus* fisheries in Atlantic Canada. Approximately 141,311 lobsters have been sampled over the last decade as part of this project. The Brix index values are mapped each year for various locations and available publically via the following website: www.lobstermoult.ca. The website gives fishermen real-time information on nutritional condition of wild stocks and allows regulating fishing dynamically by catching lobsters in their optimum moult cycle position. Lobsters with low Brix index (<8) should generally not be stored for more than a few days for later sale, especially if they have also a soft-shell, as they are deemed of low quality and are best sold cooked locally. Lobsters with a hard-shell and a very high Brix index (>15) can also cause issues during holding or transport as they are close to moulting (i.e., freshly moulted lobsters are vulnerable to cannibalism and emersion) (Andrea Battison, Pers. Comm.). Cut-off values have been developed and are now used by the industry to make decision strategies around which area and time to fish to ensure the majority of lobsters have recovered from the moult and have the highest survival in transport. The precise cut-off values to improve survival in transport are industry-in-confidence but also likely species-specific so similar research on *Jasus edwardsii* is required.

Total haemocyte count (THC) is regarded as an important index of health, with healthy lobsters having higher THC counts than moribund lobsters (Jussila et al., 1997). Hypoxia and handling during transport has been associated with significant changes in haemocytes counts, which can accelerate disease conditions (see review in Fotedar & Evans, 2011). Additionally, total carotenoids in the haemolymph of crustaceans may be involved in removing cytotoxic radicals generated during an immune response (Cornet et al., 2007). Therefore, total carotenoids might provide an indication of the level of innate immune defences (Cornet et al., 2007).

Blood biochemistry profiles are routinely performed in free-living mammals and birds to assess condition but have only been recently applied successfully to free-living fish (Congleton & Wagner, 2006; Wagner & Congleton, 2004). The application of veterinary blood panels to rock lobsters can assist in analysing a wide range of haemolymph constituents within the same animal and may be the basis for predicting the vulnerability of particular groups of lobsters (Paterson et al., 2005). Comprehensive data on stress associated with capture, handling, live holding and transport, and on the interaction between stress and baseline condition of lobsters, may offer a better understanding of transport related mortality.

The current understanding and available data on differences in live transport mortality between pale and red lobsters is limited. The records kept by processors and feedback from overseas market do not allow a clear accounting of mortality issues, especially in regards to lobster colour. While significantly greater mortality rates during simulated live transport have been quantified for deep-water lobsters compared to shallow water lobsters, it is unclear to what extent distinctive physiological, nutritional or health condition differences in the wild would affect the response of pale and red lobsters to live-transport stress. In a 2005 study, significantly higher magnesium and lower potassium was recorded in pale lobsters compared to red lobsters in November but this disparity disappeared after translocation (Chandrapavan et al. 2011). While translocation provides several advantages such as increased growth rate, enhanced shell colour, and nutritional condition of lobsters, a better understanding of the potential issue with pale lobsters might help reduce transport mortality.

The research reported here aimed to measure the nutritional, physiological and health condition of pale and red lobsters in several areas around Tasmania at the typical time of high transport mortality. A comprehensive condition assessment was applied by measuring tissue proximal composition, Brix index, THC, pH, carotenoids, haemocyanin and another 25 haemolymph parameters of interest as part of advanced veterinarian blood panel assays.

1.1. Need

Losses of up to 10% of lobsters during live transport are not uncommon and have a major financial impact. The fishing industry, including processors and operators are seeking ways to avoid this mortality. As there is a clear industry perception deep-water pale lobsters are more susceptible to this mortality, this project will examine their physiological condition in great detail and compare it to shallow-water red lobsters.

1.2. Objectives

This project is a step towards the eventual goal of mapping condition of rock lobsters to enable harvesting operations to only land highest quality lobsters so that market reputation and price is maximised. We hope to be able to identify the causes of differences in condition then use this information to decide on the best management strategy. This data will increase our capacity to link the wild condition of lobsters to potential issues with lobsters at live holding facilities which could decrease their survival in transport. Specific objectives included:

1 Quantify differences in nutritional, physiological, and health condition of male lobsters in four different fishing areas of Tasmania at the start of the fishing season.
2 Examine the differences in nutritional, physiological, health condition and survival of pale and red lobsters exposed to extended emersion exposure.

2. Methods

2.1. Sampling locations

Male lobsters were collected from four different areas around Tasmania: South East, South West, Orford and Flinders Island (Fig. 1). Two to three sites were sampled in each area, lobsters sourced from an overnight fishing shot. Each shot involved the setting of 5-30 lobster traps (pots) by a commercial fishing vessel. Lobsters from the South West and South East were brindle in colouration, while in Flinders all lobsters collected were red (Table 1). In Orford, brindle and red lobsters were found at different depths allowing for colour comparisons in this area (Table 1). Colour was assigned following (Chandrapavan et al., 2009, Fig. 2), as either ‘Red’ or ‘Brindle’ (a combination of pale and white).

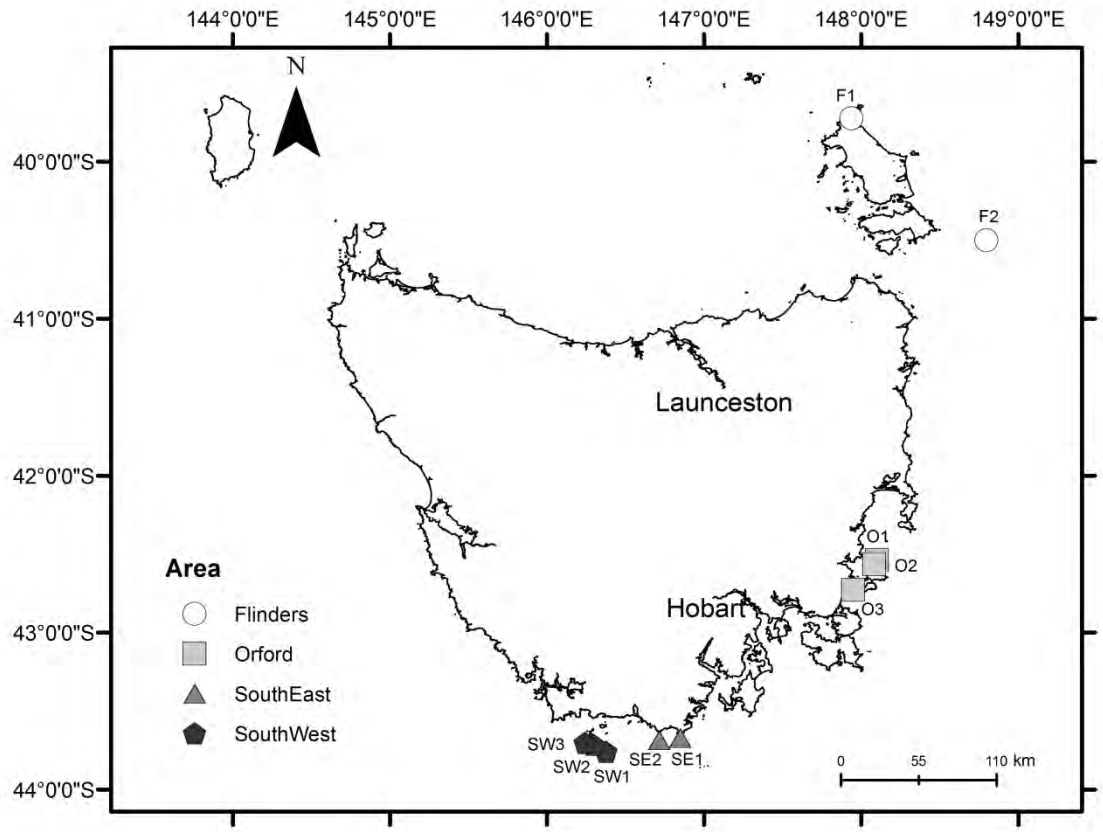

Fig. 1. Map of sampling sites at four different sampling areas (Flinders, Orford, South East and South West) and sites (F1, F2, O1, O2, O3, SE1, SE2, SW1, SW2, and SW3) around Tasmania.

Table 1. Lobster colour and average depth of sites located in different areas around Tasmania.

Area	Site	Average depth (m)	Colour
Flinders	F1	25	Red
	F2	25	Red
Orford	O1	40	Brindle
	O2	20	Red
	O3	20	Red
South East	SE1	89	Brindle
	SE2	88	Brindle
South West	SW1	96	Brindle
	SW2	99	Brindle
	SW3	84	Brindle

2.2. Tissue composition

Biochemical composition analyses of tissues were used to calculate various biochemical indices of nutritional condition and understand the relationship between these and haemolymph biochemistry parameters as well as Brix index for this species. A total of 46 male lobsters (South West n=16, South East n=8, Orford n=13, Flinders n=9) were destructively sampled immediately after taking the haemolymph sample by bringing them to a chill-coma on ice. Lobsters were kept frozen until dissection at −40 °C. Lobsters were weighed and the hepatopancreas (HP) and abdominal muscle (AM; entire muscle mass of the tail including extension into the cephalothorax) dissected out and weighed (± 0.01 g). The HP total wet weight was used to calculate hepatopancreas mass index (HP_I) as follows (McLeod et al., 2004):

HP_I (%) = HP tissue wet weight (HP_{WW}) / whole body wet weight (WB_{WW}) × 100

Dry matter content of the HP and AM tissue were analysed by weight change following freeze-drying to a constant weight as follows:

HP_{DM} (%) = HP tissue freeze-dried weight (HP_{DW}) / HP tissue wet weight (HP_{WW}) × 100
AM_{DM} (%) = AM tissue freeze-dried weight (AM_{DW}) / AM tissue wet weight (AM_{WW}) × 100

HP and AM samples were ground using a mortar and pestle and analysed for chemical composition. The two tissues were analysed for total nitrogen (%N) and total carbon (%C) (AM_C, AM_N, HP_C, and HP_N) by elemental analysis using an elemental analyser (PYRO cube, Central Science Laboratory, University of Tasmania). The carbon to nitrogen ratio (C:N) was calculated for each tissue (AM_{CN} and HP_{CN}, respectively for the abdominal tissue and the hepatopancreas). Total lipid (TL) was determined by the method of Bligh and Dyer (1959) using chloroform: methanol: water (1: 1: 0.9) and expressed in mg g^{-1} of HP_{WW} (HP_{TL}).

In order to determine if differences in nutritional condition of male lobsters differed amongst areas around Tasmania, lobsters (n=156) in different sites in each area were sampled non-destructively using a refractometer to measure Brix index.

2.3. Haemolymph sampling and analyses

Haemolymph was collected from the base of the 5th leg from all lobsters sampled in this study. A total of 2.5 ml was withdrawn using a 3 ml syringe and 26 G Terumo needle pre-chilled on crushed ice (Fig. 2). Whole haemolymph pH was measured using a Testo 205 temperature compensated pH probe. A total of 100 µl of whole haemolymph was added directly to an automatic temperature compensated digital refractometer to measure the Brix index (%) (Hanna HI96801, Hanna Instruments, Australia). The refractometer was zeroed using deionised water.

Fig. 2. Collection of haemolymph from the base of the 5th walking leg.

A total of 200 µl of whole haemolymph was added to 200 µl Baker's Formol Calcium anticoagulant (4% Formaldehyde, 2% NaCl, 1% calcium acetate) to estimate THC. Samples were placed on a slide haemocytometer with grids of 0.0625 mm^2 and three pictures of different grids were recorded using 100x magnification. Total haemocyte density was estimated using the software Image J (v1.48c) and calculated with the following equation:

THC = $\hat{y}_1$ x dilution factor x volume factor

Where $\hat{y}_1$ = average count of cells per grid, dilution factor = 1 and the volume factor was calculated as 80000.

Two aliquots of 1000 µl were centrifuged at 3,000 × *g* for 4 min, the haemocyte-free plasma removed into clean cryovials, and snap-frozen in liquid nitrogen. Samples were then stored

at −80 °C. One aliquot was used to determine haemocyanin and total carotenoids. Twenty µl of plasma were diluted 30 times in deionised water for analysis of oxygenated haemocyanin (OxyHc) at 334 nm (Hagerman, 1983) in a Synergy HT plate reader. An extinction coefficient of E=17.26 was used for OxyHc determination (Hagerman, 1983). Carotenoids were extracted from 60 µl of plasma by the addition of the same volume of ethanol, homogenization and the addition of 200 µl of methyl-tert-butyl ether (MTBE), following the methodology by (Cornet et al., 2007). The concentration of carotenoids was determined against a reference curve ranging from 0 – 50 ng μl^{-1} of a standard solution of astaxanthin in ethanol.

The second aliquot was shipped to Diagnostic Services at the Atlantic Veterinary College, University of Prince Edward Island, Canada, and analysed using a Cobas c501 automated biochemistry analyser (Roche Diagnostics Corporation, Indianapolis, IN, USA) for a full haemolymph profile consisting of bicarbonate (mmol l^{-1}), the following electrolytes and minerals (mmol l^{-1}): sodium (Na), chloride (Cl), potassium (K), magnesium (Mg), calcium (Ca), and phosphorus (P); and metabolites (mmol l^{-1}): glucose (Gluc), lactate (Lact), cholesterol (Chol), triglyceride (Trig), total protein (TP, in g l^{-1}), urea, creatinine (Creat, in µmol l^{-1}), and uric acid (Uric, in µmol l^{-1}).

2.4. Packaging experiments

Three packaging trials were carried out at the IMAS aquaculture facilities in Taroona (Table 1). In all trials, lobsters were allowed to recover from capture and transport-associated stress to the IMAS Taroona storage facilities for 4 to 6 days prior to experimental procedures in a 2000 l holding tank. After recovery, for each lobster, a sample of 500 µl of haemolymph was obtained and analysed for Brix index and pH. Carapace length, total weight and abdomen gap were recorded. The distal half of one pleopod was cut and kept for determination of moulting stage following (Musgrove, 2000). Groups of ten lobsters were packed into 20 kg Styrofoam shipping containers along with wood wool to restrict movement (Fig. 3). A foam mat was placed on the bottom, covered with wood wool, and then ten lobsters were placed, five on each side of the box, covered by more wood wool and two 1 l ice packs placed on top. A group of five or six lobsters were sampled as described above but instead of being packed into boxes they were released back into the holding tanks and used as controls for each trial. After a period of either 40 h (Trial A only) or 64 h (Trial A, B and C), boxes were opened, haemolymph samples taken as described above and live lobsters were put in crates into the holding tank at IMAS Taroona and survival monitored daily for four days. Controls

were sampled at the same time in a random manner. Temperature inside one Styrofoam box and in the holding tank was monitored every ten minutes with two submersible temperature loggers (Hobo U22-001 Water Temp Pro) during each trial. Dissolved oxygen ranged from 8.23 to 8.74 mg l^{-1} during the trials.

In Trial A, two different packaging durations were explored: three Styrofoam boxes were opened after 40 h and two after 64 h. Lobsters originating from the South East, the South West and Orford were packed to assess if there were any differences in survival between pale and brindle lobsters (Table 2). Only ten lobsters from Flinders were available for Trial B, and they were all red lobsters. In Trial C, in addition to the variables monitored for the previous two trials, THC was estimated and two extra aliquots of haemolymph were centrifuged, snap frozen and used to determine OxyHc, total carotenoids, various metabolites, ions and minerals (see *Section 2.3 Haemolymph sampling and analysis*).

Table 2. Lobster origin and number, dates when trial were conducted, temperature inside crates and inside holding tanks.

Trial	Origin	Date	Temperature range in boxes (ºC)	Sea water temperature (ºC)	Nº lobsters (+ controls)
A	SW, SE & Orford	9/12/2014	9.1 – 18.7	14.8 – 16.0	50 (+ 5)
B	Flinders	21/12/2014	7.8 – 19.0	15.5 – 17.0	10 (+6)
C	Orford	9/01/2015	7.0 − 17.6	16.3 – 18.1	30 (+6)

Fig. 3. Red and brindle lobsters in Styrofoam boxes used for packaging trials.

2.5. Statistical analyses

To assess whether the Brix index was a good indicator of nutritional condition in *J. edwardsii*, a t-test was used to test if the Pearson's correlation coefficient between Brix index and total protein in the haemolymph equalled zero (Quinn & Keough, 2002). To examine spatial differences in Brix index (as an indicator of nutritional condition in lobsters), a Nested ANOVA was used due to the hierarchical nature of the sampling design (sites inside areas) (Quinn & Keough, 2002). The main factor was the sampling Area (South West, South East, Flinders, and Orford) and the nested factor was the site inside each area. A nested ANOVA was also used in two sites (South West and South East) where there was enough replication to determine if there was any variation in lobster Brix index that could be explained at the level of the sampling pot.

Haemolymph biochemistry data were explored using factor analysis following a similar approach to a previous study on juvenile lobsters (Simon et al., 2015). Factor analysis is a method that determines whether variables in multivariate data sets can be partitioned into new sets of factors each consisting of two or more of the original variables that are more highly correlated with each other than with the variables in other factors (Tabachnick & Fidell, 2006). As exploratory factor analysis was used descriptively in the present study to summarise the relationships between variables, the assumption of multivariate normality was not in force (Tabachnick & Fidell, 2006). To assist with interpretation, factors were rotated by an orthogonal transformation (Varimax rotation). Factors with eigenvalues greater than 1 were retained. Variables were considered to contribute to a factor if the factor loading was greater than or equal to 0.5. Variables that did not load on any factor were removed from the analysis (Tabachnick & Fidell, 2006). Based on the exploratory analysis of the haemolymph, only ions and metabolites that were not in the same group as the Brix index (and therefore considered indicators of nutritional condition) were included the assessment of physiological condition

To identify if there were differences in nutritional condition among sites, $HP_{I,}$ HP, AM, total nitrogen and carbon in the hepatopancreas and in the tail, and lipids in the AM_{TL} and HP_{TL} and AM_{DW} of 46 lobsters collected from different sites were examined for differences using a MANOVA test. If differences between sites inside an area were insignificant, replicates were combined for analysis. There were strong correlations between several variables, therefore, only four variables ($HP_{I,}$ $AM_{TL,}$ $AM_{C:N}$ and $HP_{C)}$ were used. A sequential Bonferroni (Holm's method) was used to adjust the p-values from the pairwise contrasts among the reproductive

stages (Quinn & Keough, 2002). The Shapiro-Wilk Multivariate Normality Test was used to assess multivariate normality and the Box's M test was used to test homogeneity of covariance matrices using a p value <0.005 to reject the null hypothesis (Huberty & Petoskey, 2000). A canonical discriminant analysis followed the MANOVA to identify the variables that explained the differences in the centroid means for each site. Statistical analysis was conducted using the R software package (R development core team 2011, version 2.12.1).

To identify if there were differences in physiological condition of lobsters among sites, electrolytes, minerals and metabolites present in the haemolymph of 73 lobsters were examined. The full haemolymph profile showed correlations amongst different ions and descriptors of nutritional condition such as Brix index, dry matter and total lipid content of the abdominal muscle and hepatopancreas. Therefore, variables that were highly correlated were removed from the analysis. Calcium and phosphate showed a strong correlation with Brix index, therefore were assumed to be indicators of nutritional condition, rather than physiological condition. After careful removal of correlated variables, only four were left for the analysis: magnesium, potassium, urea and bicarbonates. Analysis was undertaken using a MANOVA followed by a canonical discriminant analysis as described above.

THC, oxygenated haemocyanin (OxyHc) and total carotenoids were compared using an ANOVA, followed by a Tukey's post-hoc test to determine if there were any differences amongst sites. Normality of residuals was assessed visually by plotting the residuals. Homogeneity of variances was assessed using the Bartlett's test (Bartlett, 1937). Tukey's post hoc tests were used to determine which sampling sites differed (Wright, 1992).

Differences in Brix index, pH, THC, total carotenoids, ions and minerals were compared before and after packaging, and against the control, in order to understand the effects of extended emersion exposure on lobster condition. A linear regression was used to examine how the treatment (pre-packaging and post packaging), the censored outcome (1 = dead, 0 = alive after packaging) and the packaging trial (A, B or C) affected each of these variables. The response variable was calculated as:

$$X\hat{\imath} = Xia - Xib$$

Where, $X\hat{\imath}$ = variable being measured (e.g. Brix index, pH), a=after packaging, b=before packaging.

If significant differences between dead and live lobsters were found, the model was re-run using only live lobsters to understand the effect of treatment on the response variable.

Due to logistical constraints, packaging trials were not run simultaneously, and therefore data was analysed separately for each trial. Mortalities of lobsters after packaging were treated as censored data (data for which the outcome is only partially known, where 1 = dead, 0= alive) and analyzed with the Cox proportional hazard model using Brix, pH, haemocyte density, carapace length and abdomen gap as covariates (Cox, 1972) in packaging trial A and B. In packaging trial C, in addition to the variables mentioned for the previous two trials, OxyHc, total carotenoids, metabolites, ions and minerals were used as covariates. Covariates were added and removed by stepwise selection using the Akaike's information criterion implemented in R (version 2.12.1). All statistical analyses used 0.05 as the critical probability level.

3. Results

3.1. Assessment of the nutritional, physiological and health condition of male lobsters at the start of the fishing season in four areas of Tasmania

3.1.1. Nutritional condition based on chemical composition of tissues

Nutritional condition in lobsters was significantly different amongst areas ($F=3.526$, df 3,36; $p=0.0007$). The first canonical discriminant dimension explained 91% of the variation in condition among areas and pairwise contrasts showed that Orford and the South East and Orford and the South West were significantly different ($F=15.82$, df 1,13; $p=0.001$, and $F=9.118$, df 1,21, $p=0.003$, respectively) (Fig. 4). These differences were mainly driven by HP_{TL} and $AM_{CN.}$ Lobsters from Orford were associated with greater values of HP_{TL} and AM_{CN} compared to lobsters from the South East and the South West (Table 3, Fig. 6).

Table 3. Mean (±S.E.) of nutritional condition indices for different areas around Tasmania.

Area	HP_{TL}	AM_{DM}	AM_{CN}
South West	128.00 (32.61)	23.55 (1.05)	3.06 (0.04)
South East	119.10 (23.02)	23.30 (0.60)	3.00 (0.01)
Orford	242.93 (83.78)	23.66 (1.40)	3.09 (0.05)
Flinders	158.47 (61.84)	23.56 (1.47)	3.07 (0.07)

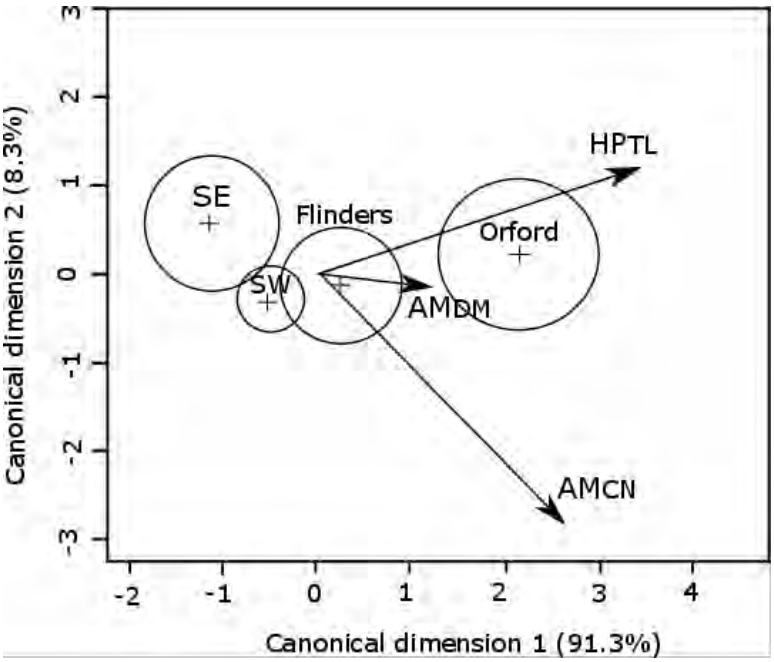

Fig. 4. The centroid means for each of the four Areas plotted in the first two canonical discriminant dimensions. The direction and length of the vectors show the strength and nature of the correlation with each variable and the canonical discriminant axes. The percent values for each axes is the percentage of variability among the four centroid means explained by each of the two axes.

3.1.2. Validation of the use of the Brix index to assess nutritional condition

Brix index was significantly related to HP_{TL} ($F=31.62$, $df = 1, 38$, $p<0.001$), AM_{DM}, ($F=81.03$, $df=1,44$, $p<0.001$) and total protein ($F=680.5$, $df=1, 48$, $p<0.001$) in the haemolymph (Fig. 5) and was therefore considered a good indicator for nutritional condition in lobsters. The Brix index correlated best with TP ($r^2=0.89$), followed by abdominal muscle dry matter content ($r^2=0.70$) and hepatopancreas lipid reserves ($r^2=0.40$) (Fig. 5).

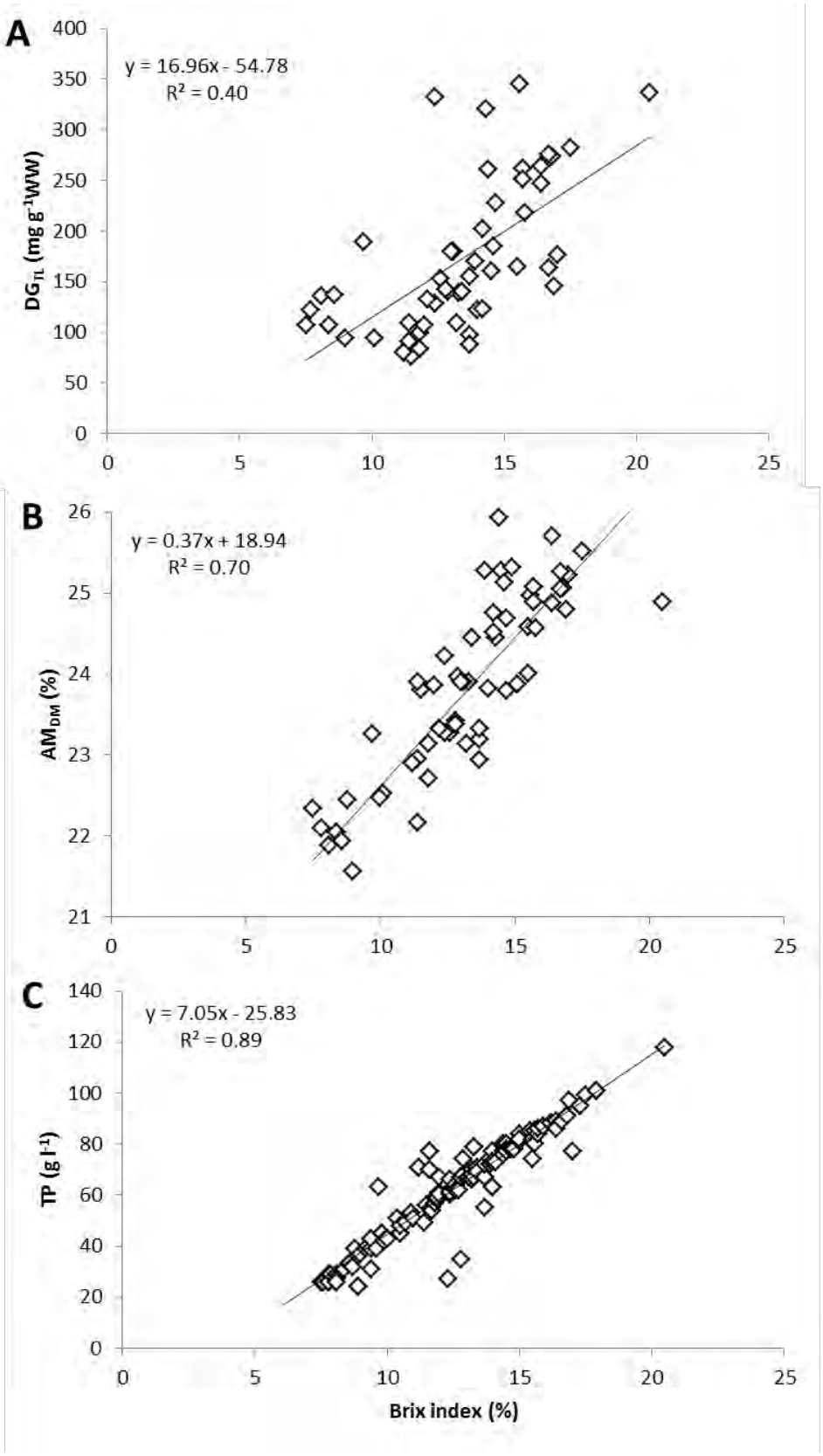

Fig. 5. Relationship between Brix index and A) HP_{TL} (hepatopancreas total lipid in wet weight) b) AM_{DM} (abdominal muscle dry matter content) and c) TP (total haemolymph protein). Regression lines and corresponding equations are shown.

3.1.3. Brix index as a non-invasive spatial indicator of nutritional condition

Assessment of Brix index allowed the incorporation of extra sites for the analysis of nutritional condition (site SW1, SE1, O1, and F1). Nutritional condition of male lobsters was not affected by lobster colouration ($F=0.006$, $df=1$, $p=0.94$). There was significant variation in Brix index between the replicate sites within each sampling area but there was no significant difference in Brix index among areas (Table 4). Significant differences between sites (Fig, 6; $F=2.59$, $df=9$, $p<0.009$) were driven mainly by one site in Orford (O1, north off Maria Island), which showed a lower mean Brix index compared to the South West (Fig. 6). This site was deeper (40 m) than the other two sites at Orford (20 m) and separated to its nearest site (O2) by 3.2 km (Table 5). Lobsters in this site were characterised by a brindle colouration. Also one site in the South East (SE2) differed in brix index compared to one site in the South West (SW1). At the level of sampling pot the nutritional condition of lobsters was very similar ($F=2.79$, $df=55$, $p=0.66$).

Table 4. Variation in Brix index within and between areas (Nested ANOVA).

Source of variation	df	MS	F	p
Area	3	5.944	1.106	0.348
Site (Area)	6	16.06	2.99	0.0081
Residual	143	5.37		

Table 5. Average depth, Brix index and number of replicate lobsters per site.

Area	Site	Average Depth (m)	Average Brix index	n	SD	Colour
Flinders	F1	25	12.28	6	2.21	Red
	F2	25	11.45	12	3.64	Red
Orford	O1	40	9.11	6	1.09	Brindle
	O2	20	13.5	14	3.84	Red
	O3	20	12.74	9	3.75	Red
South East	SE1	89	13.36	8	3.74	Brindle
	SE2	88	13.27	18	1.20	Brindle
South West	SW1	96	11.8	8	0.93	Brindle
	SW2	99	12.02	28	1.60	Brindle
	SW3	84	12.79	47	1.57	Brindle

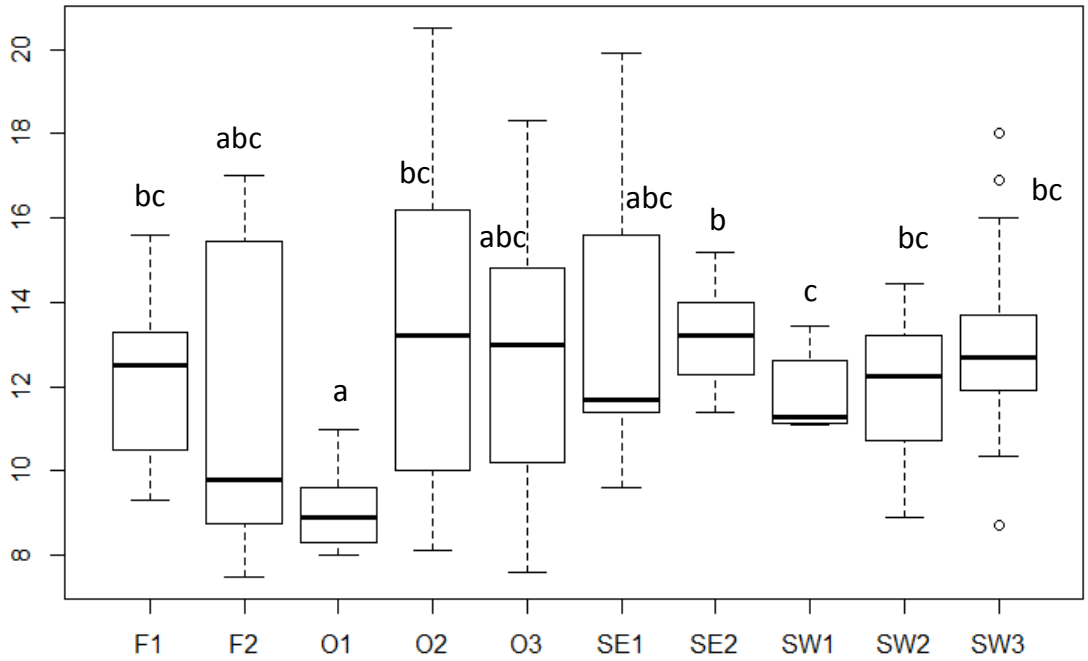

Fig. 6. Boxplot of Brix index amongst sites around Tasmania. The boxes enclose data falling between the 1st and 3rd quartile and the lines in bold represent the median in each location. The bars indicate the 95% confidence intervals of the median. Data points falling outside these ranges are plotted individually. Different letters above the bars indicate significant differences between sites.

3.1.4. Exploratory analysis of haemolymph biochemistry

Haemolymph biochemistry data were explored using factor analysis following a similar approach to a previous study on juvenile lobsters (Simon et al., 2015). Factor analysis is a method that groups variables that are highly correlated to each other and is used to simplify the interpretation of trends down to only a few factors (i.e., groups). Haemolymph biochemistry parameters of lobsters tend to strongly correlate because the concentrations measured are ultimately dependent on the individual lobster haemolymph volume (Simon et al., 2005). Factor analysis of the haemolymph biochemistry data resulted in five main factors explaining 75% of the cumulative variation (Fig. 7): Factor 1 accounted for 38% of the variation, and included highly correlated plasma parameters such as pH, phosphorus, creatinine, glucose, lactate, urea and uric acid. Factor 2 accounted for 14% of the variation and included Brix index, OxyHc, calcium, cholesterol, triglyceride and total protein; Factor 3

accounted for 10% of the variation and included sodium and chloride ions; Factor 4 accounted for 7% of the variation and included magnesium and carotenoid; and Factor 5 accounted for 6% of the variation and included bicarbonate and potassium. The last two factors could not be included in Fig.7.

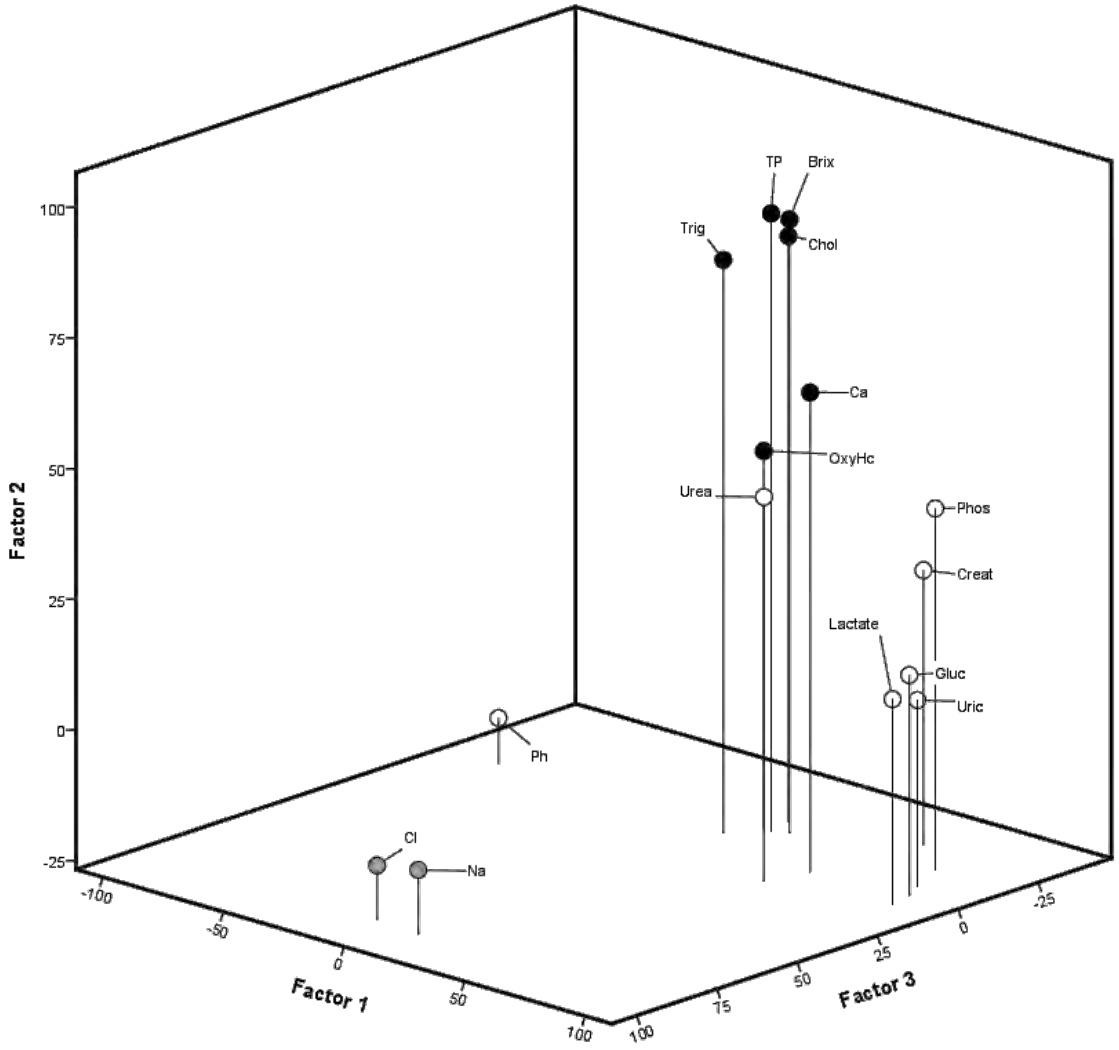

Fig. 7. Rotated factor loadings for haemolymph biochemistry data. Factor 1: white circles; factor 2: black circles; factor 3: grey circles.

3.1.5. Physiological condition

Physiological condition was different among sites (Fig. 8; $F=3.04$, $df=4,46$, $p<0.001$) but not between lobster colour ($F=0.270$, $df=1,46$, $p=0.846$). Differences among the sites were driven significantly by the amount of magnesium in the haemolymph. Lobsters from O3 and F2 were associated with lower levels of magnesium compared to lobsters from the South West. Pairwise contrasts showed significant differences between site F2 and SW ($F=5.822$,

df=1,21 p=0.026) and O3 and SW (F=7.68, df=1,20 p=0.016); F=9.037); and between O2 and F2 (F=9.572, df=4,37, <0.001).

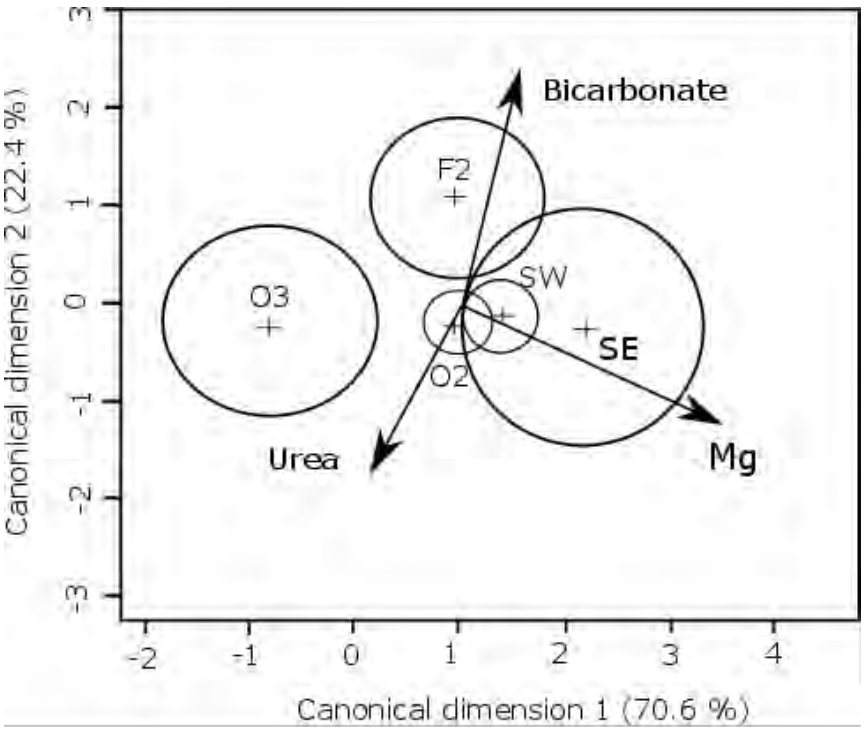

Fig. 8. The centroid means for each of the sites plotted in the first two canonical discriminant dimensions. The direction and length of the vectors show the strength and nature of the correlation with each variable and the canonical discriminant axes. The percent values for each axes is the percentage of variability among the centroid means explained by each of the two axes.

OxyHc content ranged from 0.01 to 1.17 (mmol l^{-1}). No significant difference in OxyHc content in lobster haemolymph was found among sites, areas, or lobster colour (F=1.658, df=5, p=0.171; F=1.527, df=3, p=0.224, and F=0.096, df=1, p=0.759, respectively).

3.1.6. Health

Total haemocyte count (THC) is regarded as an important index of health, with healthy lobsters having higher THC counts than moribund lobsters. The average THC was 6.8 million cells ml^{-1} and THC did not differ among sites, areas, or with lobster colour (F=0.83, df=5, p=0.53; F=1.10, df=3, p=0.35; and F=1.00, df=1, p=0.32, respectively). Total carotenoids content in the haemolymph was also quantified as part of the health assessment as it may indicate levels of innate immune defences. Total haemolymph carotenoids ranged from 0.33 to 5.63 ng μl^{-1} and did not differ among sites, areas or with lobster colour

(F=0.4524, df=5, p=0.808; F=1.208, df=3, p=0.553 and, F=0.994, df=1, p=0.324, respectively).

3.2. Compare vulnerability of pale and red lobsters associated with extended emersion exposure

3.2.1. Effect of emersion on nutritional, physiological and health condition

Emersion during packaging changed the haemolymph biochemistry of the lobsters. Emersion caused significant increases in levels of Brix index, glucose, lactate, uric acid, urea, bicarbonate, calcium, and magnesium (Fig. 9, Table 6). Lactate concentrations showed the greatest response, increasing about 3000 percent (30 fold) compared to levels before packaging. The next largest responses to packaging were in levels of urea and uric acid (around 1000 percent change). Haemolymph pH, and the concentration of potassium, chloride and sodium ions in lobsters that were packaged were not significantly different from control lobsters but did differ from lobsters that died (Table 6).

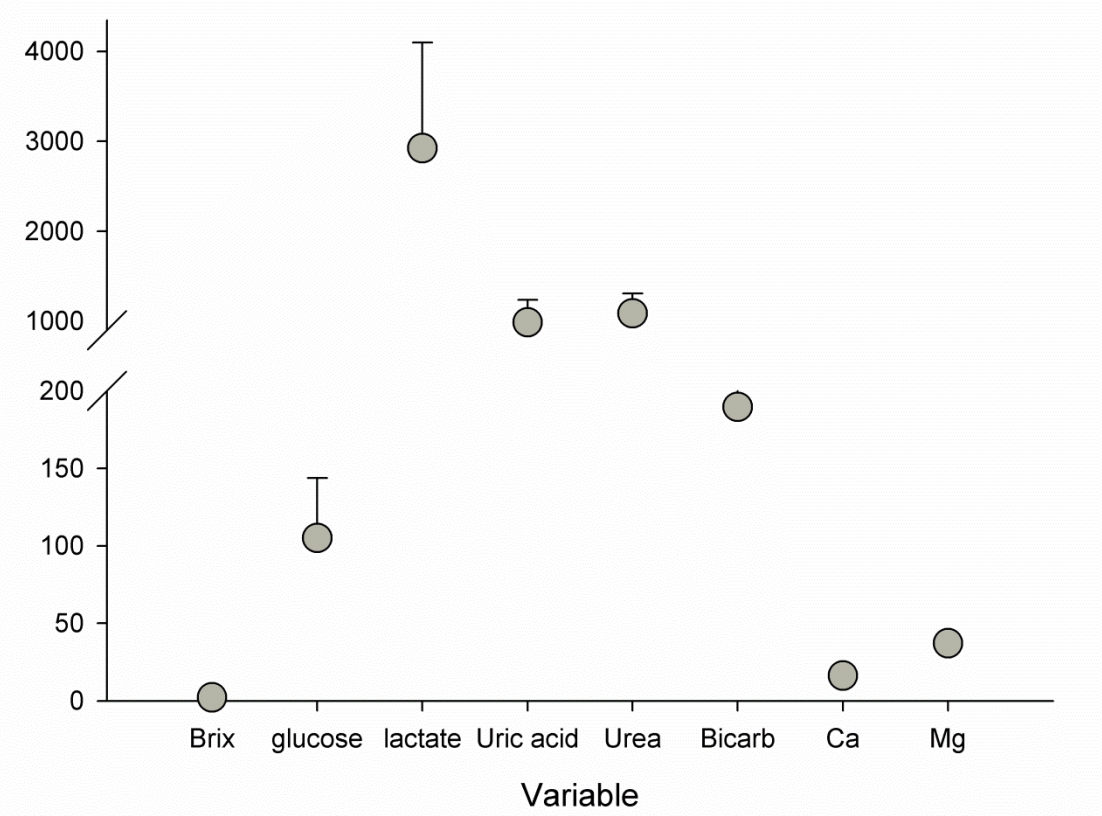

Fig. 9. Direction and mean change (Xia - Xib/ Xib*100) in response variable after packing. Only variables for which a significant effect of packaging was found are shown.

Table. 6. Average values +/- S.D. of different indicators of nutritional, physiological and health condition pre-packaging, post packaging in live lobsters, post-packaging in lobsters that died, and control lobsters that were not packed. Statistical test analysing the change in each variable after packaging between lobsters that died and survived ('status') and between control and packed treatments ('treatment') are shown. Statistically significant results are shown in bold.

Variable	Pre-packaging	Post-packaging alive	Post-packaging dead	Control	Status	Treatment
Brix index (%)	12.33+/-2.50	11.84+/-2.67	14.83+/-2.35	11.24+/-2.29	**t=4.465, df=2,75, p<0.001**	**t=3.75,df=1,44, p<0.001**
THC (10^5 cells ml^{-1})	76.75+/-36.99	66.79+/-36.14	69.76+/-44.58	72.46+/-30.07	t=-0.558,df=2,21, p=0.583	t=0.397, df=2,21, p=0.695
pH	7.70 +/- 0.10	7.66+/-0.16	7.33+/-0.30	7.71+/-0.17	**t=-4.55, df=3,68, p<0.001**	t=-0.94, df=6,65, p=0.348
Glucose (mmol l^{-1})	0.85+/-0.24	1.57+/-0.91	3.75+/-2.61	0.74+/-0.13	**t=2.54, df=2,23, p=0.018**	**t=3.04, df=1,12, p=0.010**
Lactate (mmol l^{-1})	0.21+/-0.23	3.8+/-4.34	15.87+/-7.43	0.38+/-0.23	**t=4.50, df=2,23, p<0.001**	**t=2.22, df=1,11, p=0.047**
Uric acid (µmol l^{-1})	25.17+/-17.24	136.44+/-89.23	209.75+/-120.69	30.5+/-19.47	t=1.17, df= 1,24, p=0.253	**t=3.03, df=1,24, p=0.005**
Urea (mmol l^{-1})	0.08+/-0.06	1.35+/-0.28	2.71+/-1.44	0.13+/-0.05	**t=2.81, df=2,23, p<0.01**	**t=2.25, df=2,23, p=0.033**
OxyHc (mmol l^{-1})	0.67+/-0.35	0.60+/-0.15	0.74+/-0.37	0.71+/-0.10	t=0.60, df=4,28,p=0.548	t=0.21, df=4,28,p=0.812
Carotenoids (ng μl^{-1})	2.20+/-1.81	2.26+/-1.53	2.93+/-2.06	2.75+/-2.05	t=1.03, df=4,27,p=0.312	t=0.03, df=4,27,p=0.976
Bicarbonate (mmol l^{-1})	7.57+/-3.44	19.38+/-6.49	13.48+/-8.58	8.36+/-5.72	t=-1.41, df=2,23, p=0.170	**t=2.50, df=2,23, p=0.017**
Calcium (mmol l^{-1})	15.18+/-0.95	17.24+/-3.31	17.50+/-3.68	14.99+/-0.50	t=-0.29, df=4,21,p=0.772	**t=2.29, df=1,24, p=0.030**
Magnesium (mmol l^{-1})	9.18+/-0.73	11.67+/-1.90	13.14+/-2.23	9.59+/-0.42	t=1.578, df= 1,24, p=0.129	**t=3.09, df=4,21, p=0.005**
Potassium (mmol l^{-1})	8.25+/-1.13	9.7+/-1.08	16.49+/-9.52	8.9+/-0.41	**t=2.17, df=1,20, p=0.041**	t=1.22, df=1,12, p=0.244
Sodium (mmol l^{-1})	523+/-43.17	514.33+/-16.30	463.25+/-54.05	500+/-31.45	**t=-3.36, df=4,17, p=0.002**	t=0.33, df=1,12, p=0.741
Chloride (mmol l^{-1})	527.82+/-66.47	502.67+-24.19	442.5+/-60.13	499+/-41.51	**t=-3.01, df=4,21, p=0.006**	t=0.14, df=4,21, p=0.88

3.2.2. Predicting the vulnerability of lobsters to extended periods of emersion

Survival was not different between red and brindle lobsters in Packaging Trial A for both 40 h and 64 h of emersion (χ^2=1.7, df=1, p=0.196). However, lobsters packed for 40 h showed significantly higher survival than those packed for 64 h (Fig. 10; χ^2=4.9, df=1, p=0.0275). The risk of death for lobster packed for 64 h was approximately 5 times the risk of death for lobsters packed for 40 h (Z=1.99, df=1, p=0.047). Parameters such as pH, Brix index, abdomen gap, THC or carapace length did not have a significant effect on the survival of lobsters (χ^2=1.16, df=4, p=0.884).

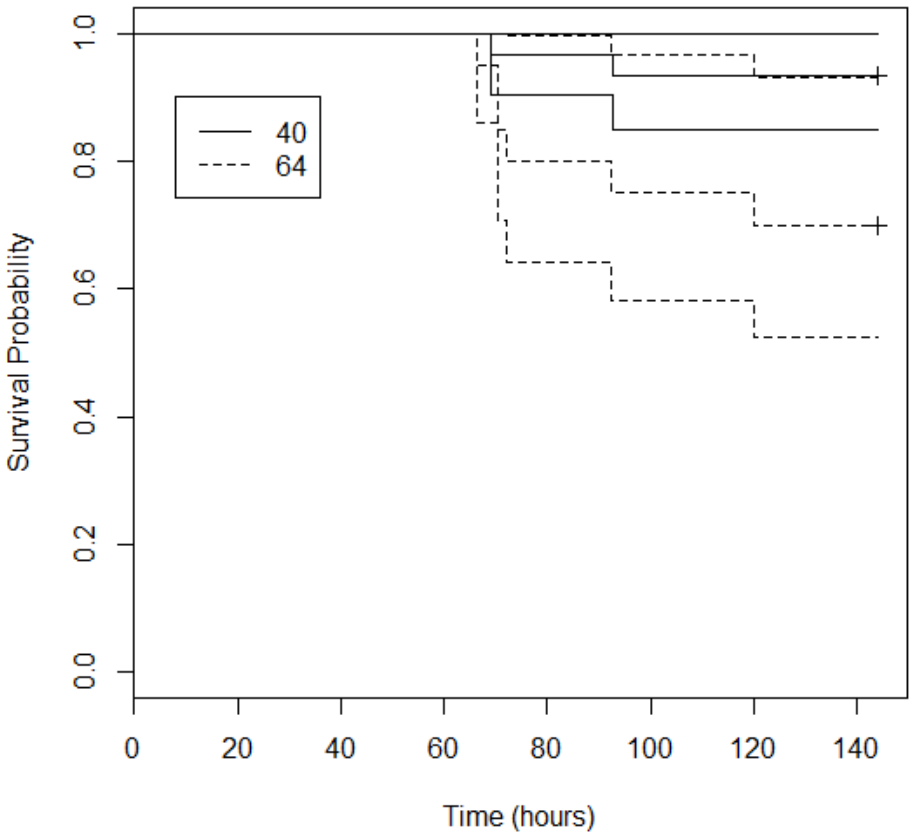

Fig. 10. Survival probability (and 95% confidence interval bands) of lobsters packed for 40 h (total number of lobsters = 30) and 64 h (total number of lobsters = 20).

In Packaging Trial B, which was for lobsters from Flinders Island, sex, pH, Brix index, abdomen, THC or carapace length did not affect survival of lobsters (χ^2=6.22, df=5, p=0.286).

In Packaging Trial C, lobster colour (Fig.11) or sex did not affect the survival of lobsters (χ^2=0, df=1, p=0.973 and χ^2=1.6, df=1, p=0.21). Smaller lobsters had an increased risk of dying compared to the sample average size (Z=-2.79, df=4, p=0.0053). Additionally, lobsters with a lower concentration of haemocyanin (OxyHc) had a greater relative risk of dying compared to the sample average, but this relationship was affected by the amount of bicarbonate in the haemolymph (Z=2.31, df=4, 0.021; Fig. 12). In lobsters with low concentrations of bicarbonate (i.e. lower quartile = 5.3 mmol l^{-1}) the risk of dying decreased more markedly with greater amounts of OxyHc than in lobsters with a higher concentration of bicarbonate (i.e. upper quartile = 9.0 mmol l^{-1}).

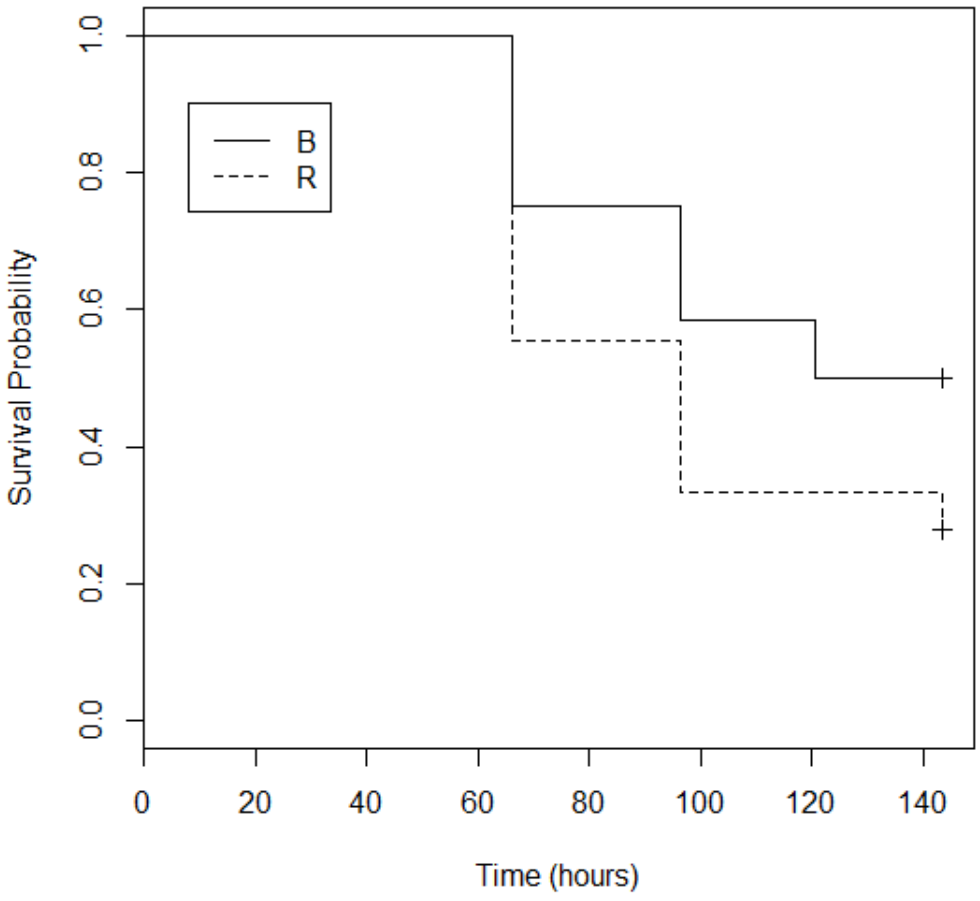

Fig. 11. Survival probability of red and brindle lobsters from Orford packed in Packaging Trial C. Packaging lasted 64h, after which mortality was assessed in lobsters recovering in flow-through tanks for up to 4 days

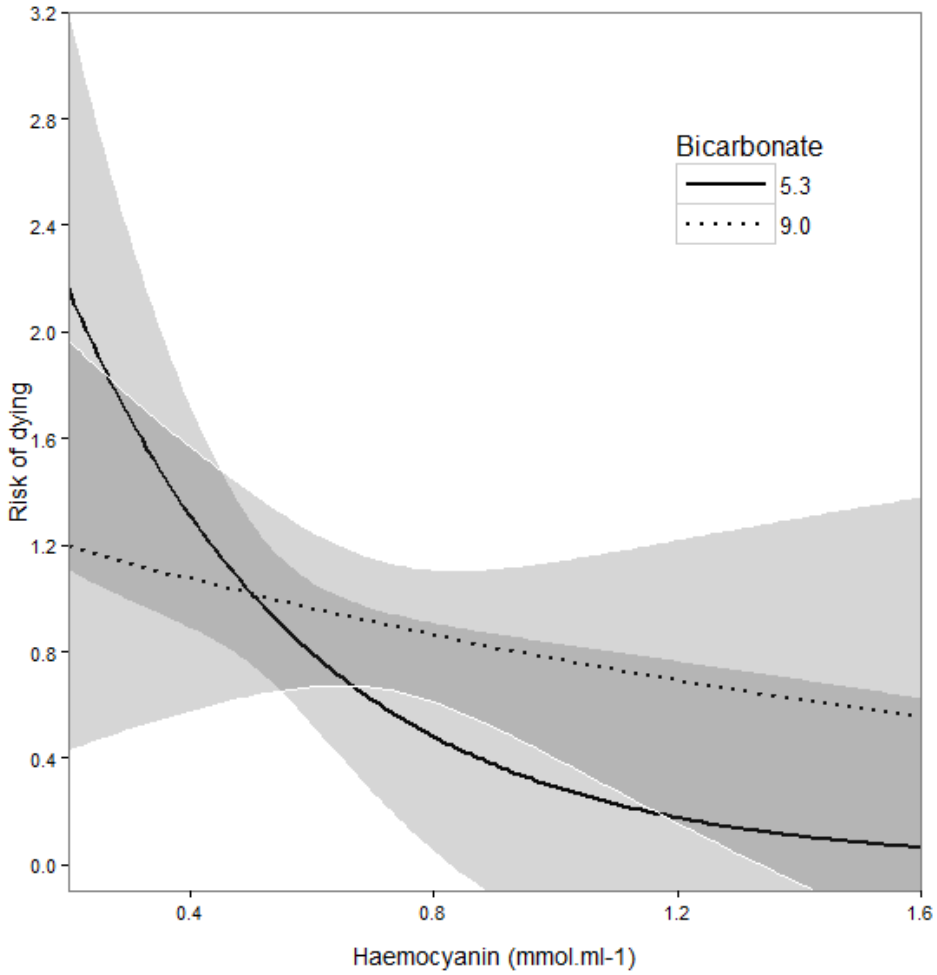

Fig. 12. Changes in the relative risk of dying of lobsters with increasing values of OxyHc (mmol l^{-1}) in lobsters with low (5.3 mmol l^{-1}) and high (9.0 mmol l^{-1}) levels of haemolymph bicarbonate, for an average individual measuring 102.5 mm in carapace length. Grey areas show 95% confidence intervals.

4. Discussion

Male lobsters in four areas of Tasmania (South West, South East, Orford and Flinders) differed in nutritional and physiological condition among areas, but not in health as indicated by total haemocyte counts (THC) and total carotenoids. The lobster hepatopancreas lipid reserves, as one of the main indicator of nutritional condition, differed between areas, while haemolymph magnesium concentration was the major difference in physiological condition between sites.

The Brix index correlated strongly to haemolymph total protein as well as to both hepatopancreas and abdominal muscle tissue composition in our study. Previous studies

have also demonstrated refractometry (e.g., Brix index, refractive index, density) to be an easy and affordable field method to estimate nutritional condition of crustaceans including lobsters (Ciaramella et al., 2014; Lorenzon et al., 2011; Oliver & Macdiarmid, 2001; Simon et al., 2015). In our study, the Brix index correlated better to abdominal muscle dry matter content than hepatopancreas lipid reserves suggesting it was a better indicator of the nutritional condition associated with the tail muscle (i.e., 'meat content') than lipid stored in the hepatopancreas for energy.

The hepatopancreas is typically high in lipid and responds rapidly to food availability, moulting and reproduction (McLeod et al., 2004). In contrast, changes in abdominal muscle tissue composition have received less attention as the current understanding is that this tissue is not as responsive to changes in physiological and environmental parameters (Cockcroft, 1997). However, the slow changes in muscle tissue can be useful in understanding the longer term dietary changes experienced by the animal and can be important from a commercial and market perspective as they affect an important aspect of edible meat yield and quality.

While lobsters from Orford had levels of hepatopancreas lipid that were twice as high as lobsters from the South East and South west, they were not different in terms of abdominal muscle dry matter (related to meat content) based on Brix index values across a larger sample size . Brix index did however vary within fishing areas at the level of specific sites. For example, lobsters collected from the deeper Orford site (O1), which were brindle-coloured, were in lower nutritional condition as measured by the Brix index than in other shallower sites of Orford and deeper sites in SE and SW. The Brix index did not differ between lobsters within pots, which supports its use as an indicator of site-specific abdominal muscle condition. The Brix index was found to correlate well to meat yield in American lobsters (Battison, 2014). Linking the Brix index to condition of lobsters is complex however as the relationship may vary from year to year depending on changes in factors such as water temperatures, productivity and timing of the moulting period. For example, in our sampling, male lobsters across all areas on the whole were in better condition (i.e., further recovered from the moult) in late November-December 2014 (average Brix index of 12.2) than previously noted from similar areas in November 2007 and 2008 (RI of 1.34 = Brix index of 6.0) (Chandrapavan et al., 2009, 2011).

The only physiological condition measure that was different among sites was magnesium concentration in the haemolymph. Lobsters collected from deeper areas (South West) were associated with greater values of magnesium, compared to lobsters from shallower areas

(Orford or Flinders). Similarly, Chandrapavan et al (2011) found greater where levels of magnesium in lobsters from deep areas in southern Tasmania than lobsters collected from a shallow reef at Taroona. Additionally, they found that when then deep water lobsters with higher magnesium were translocated to shallow reefs, within a year their magnesium levels were reduced to the same levels as resident shallow water lobsters (Chandrapavan et al., 2001). As levels of activity and the concentration of magnesium in the haemolymph are inversely related (Hagerman, 1973; Morritt & Spicer, 1993), these results suggest that lobsters in deeper areas have lower levels of activity than lobsters in shallower areas. Lower activity levels due to lower water temperature might explain the differences in hepatopancreas lipid content between areas, although this hypothesis needs further testing. While differences in magnesium were interpreted as arising from post-harvest stress in a previous study (Chandrapavan et al., 2011), the present results strongly indicate that while emersion can increase magnesium concentration by 50% (after 64h), differences in magnesium in wild lobsters are more likely related to water temperature and did not have an effect on survival when exposed to emersion in this study.

Total haemocyte count (THC) and total haemolymph carotenoid, which were used as indicators of health, did not differ among sites, areas or lobster colour. THC levels (mean = 7 million cells per ml) were similar to previously measured in male lobsters in November 2005 and 2006 (Chandrapavan et al., 2011). These levels are relatively low compared to the rest of the year were THC levels can be as much as three fold higher in late intermoult lobsters (Chandrapavan et al., 2011). This suggests that potential differences in health condition among areas are highly unlikely to explain the differences in survival after transport observed by processors. Accordingly, THC and carotenoids were not good predictors of lobster's vulnerability to transport. While changes in THC have been observed in crustaceans exposed to emersion (Jussila et al., 1997; Ridgway et al., 2006), in this study no significant change in THC was observed after packaging. Carotenoids have been recently associated to the level of innate immune defences in crustaceans (Cornet et al., 2007), however, under our study conditions, carotenoids in the haemolymph were not correlated with increased survival when exposed to emersion. While carapace colour in *Jasus edwardsii* is directly correlated to the amount of dietary carotenoids (Crear et al., 2002), our study demonstrates circulating levels of haemolymph carotenoids, which are likely of greater physiological role, were not different between brindle and red lobsters.

Our packaging trials showed no difference in survival between red and brindle lobsters. These findings were contrary to the general industry perception that brindles have a greater probability of mortality after shipment. Differences in survival rates have been reported

previously between lobsters from deep water and shallow waters in South Australia through storage, packing and shipping (Linnane & McGarvey, 2014) and through simulated packaging trials (Hawthorne, 2009). Unfortunately, nutritional, physiological or health condition was not assessed in previous research, making it difficult to determine which factors were most important in explaining the differences in mortality between studies. Hawthorne (2009) reported mortality rates ranging from 25 to 50% in lobsters originating from deep water after 30 hours of simulated packaging. In our study, 94% of lobsters survived after 40 hours of packaging, which strongly indicates that the lobsters in the present study had certain biological characteristics, or were better treated post-harvest, which improved their survival to extended periods of emersion. These observations are supported by the low economic losses to transport mortality experienced by processors this year (i.e. SALCO, Blake, Pers. Comm.).

Various haemolymph parameters increased following packaging for 64h (i.e., simulating a very long live transport duration to overseas market – more commonly around 40h). Our packaging trials did not incorporate a lobster pre-chilling phase employed by the industry so would be expected to produce a higher mortality rate, although pre-chilling did not increase survival of brindle lobsters in a study by Hawthorne (2009). Brix index and calcium, which are mainly associated with nutritional condition, increased by less than 10% after a 64 h emersion and this rise was most likely due to dehydration of lobsters. This suggests the Brix index can be applied to measure the nutritional condition of lobsters throughout the market chain with limited interference from emersion and stress. Glucose concentration doubled following emersion which is in agreement with crustaceans becoming hyperglycaemic under stressful condition (Paterson et al., 2005). Following emersion, anaerobic metabolism results in as much as a 30 fold increase in lactate. Lactic acid build-up results in a decrease in haemolymph pH which the lobster compensates for by increasing haemolymph bicarbonate content (possibly using minerals from the shell). While outside water, levels of nitrogenous wastes, which include uric acid and urea originating from protein digestion or nucleic acid catabolism, concurrently increased 10 fold as lobsters were unable to transform and excrete these as ammonia across the gills (Ciaramella et al., 2014). Lobsters that died after packaging showed significantly higher lactate and lower pH values than those that survived, suggesting their haemolymph acid-base balance failed to compensate for the acidification linked to anaerobic metabolism as found in a previous study (Paterson et al., 2005). These lobsters also had significantly higher urea and potassium levels and significantly lower sodium and chloride ions. This indicates that dehydration, cell damage and possibly nitrogenous waste toxicity were other possible factors for their death. While these trends are of interest, and provide important cues to understand lobster death, they do not allow

prediction of whether a particular lobster would die or not during packaging. At best, they indicate that ensuring lobsters have recovered from the ground transport stress in holding tanks for several days, minimising emersion duration, and reducing temperature prior to packaging, should theoretically decrease the build-up of anaerobic end-products (lactate) and nitrogenous waste (urea and uric acid). These steps are already well understood and implemented by lobster processors.

A pre-packaging indicator of vulnerability to transport would however be highly advantageous for the industry as it would allow decisions to be made regarding shipping lobster in particularly poor physiological condition. Paterson et al. (2005) found the haemolymph biochemistry of lobsters on arrival from a range of transport condition (e.g., emersed, submerged, humid air, sprayed) to be a good predictor of future mortality in a live holding facility but the relationships to differ between experiments/batches of lobsters. We attempted to use baseline haemolymph biochemistry from lobsters recovered for at least 4 days in flow-through tanks as a predictor of future mortality in transport, a more ambitious goal which would possibly allow the use of wild baseline data to reduce the landing of lobsters from sites with increased risk of death during live transport. We attempted to address this goal with a model including lobster size, haemocyanin and bicarbonate in the haemolymph and found this gave some guidance to estimate vulnerability of lobsters. When crustaceans are exposed to air (emersion), most species fail to maintain CO_2 excretion and they compensate for accumulating acid metabolites (e.g. lactate) by elevation of haemolymph bicarbonate (deFur, 1988; Taylor & Wheatly, 1980; Taylor & Whiteley, 1989). In *Jasus edwardsii*, this response is accompanied by mixed effects of accumulated uric acid and calcium that affects the oxygen affinity of haemocyanin (OxyHc) (Morris & Oliver, 1999), enabling lobsters to support extended periods of emersion. At lower haemocyanin content, higher bicarbonate is likely beneficial to compensate for acidification linked to a greater reliance on anaerobic metabolism as observed in this study. However, higher bicarbonate content was found to increase the risk of dying of lobsters having high haemocyanin content. The physiological mechanism behind the interaction between bicarbonate and haemocyanin concentration in the haemolymph evidenced in this study remains unclear.

5. Benefits and Adoption

This brief project was not able to complete the development of a screening test to prevent the landing and shipment of lobsters with higher risk of death during transport. An important factor here was that in the particular year and months of sampling, lobsters appeared to be

in above-average condition with no significant losses reported by processors. This was despite sampling during the period normally associated with problems in transport. As a result, no products or techniques could be developed and adopted from this research. Nonetheless, the project did have benefits in the form of insights into measurement of physiological processes and with some promising leads on how to screen lobsters. These methods require further development before they could be applied by industry. Promising results were found from:

1) Application of the Brix index to estimate non-invasively nutritional condition, particularly in regards to tail meat content. This may be of value for some markets where muscle content is important, noting that this is not the case in all markets. The close relationship between Brix index and nutritional condition, combined with the ease of sampling suggests that further sampling of Brix Index through the course of routine observer catch sampling for stock assessment may be worthwhile. This data could conceivably help in fisheries assessment (e.g., explaining years with unusually high or low growth, which would affect the number of undersize recruiting to the legal-sized stock). It could also be of value for screening of lobster unsuitable for transport although this requires ongoing data collection and future validation at a time when high transport mortality occurs.

2) There was evidence that baseline concentrations of haemolymph haemocyanin and bicarbonate could be used as a predictor of vulnerability to transport. This was a promising result for screening of lobsters but requires further testing especially during a year when lobsters were less fit and had higher levels of transport mortality. Use of these indicators would also only be possible if practical portable analysers could be used or test kits were developed. Portable blood gas analysers such as the AVL OPTI Critical Care Analyzer (5kg; 8h battery) are commercially available to measure electrolytes, pH, protein and bicarbonate in human blood (Schlebusch et al., 2001) and could be adapted to lobsters. A system of test strips, similar to portable diabetic or urine meters, may also be developed as part of future R&D to measure these variables and assist in improving their post-harvest handling.

6. Further Development

This project provided insights into measuring condition of lobsters for improving transport but further work is clearly required for adoption to be achieved.

Most importantly, further sampling of both Brix index and haemolymph biochemistry is required around periods of high mortality, ideally from moribund lobsters. This requires cooperation of processors, which is being sought through the lobster processors association. Low level but regular sampling of Brix index at sea would also be a valuable resource for identifying changes in nutritional condition of the stock in different areas of the fishery from year to year. Should high transport mortality be detected at some time in the future then this would provide an opportunity to test the application of this index.

Should this sampling show that measurement of haemocyanin and bicarbonate monitoring would be helpful for screening, a second step required is the adaption of portable blood gas analysers or the development of a sampling kit for use at sea. This could take the form of test strips as used for blood and urine testing in humans. Our results showed that haemolymph biochemistry was stable within sites, which means that sampling at sea by fishers would be practical – they'd only need to sample a few animals in each location to assess their physiological condition and ability to survive transport.

Our results also suggest possible application of the methods to two areas that would require further research. One was the measurement of meat yield using Brix index and development of a grading system based on quality. This may be of value for some niche markets. The other was the use of haemolymph biochemistry to fine-tune processing systems, such as recovery duration during re-tanking, pre-chilling temperature and transport air pressure on vulnerability to transport.

7. Planned Outcomes

The project had planned to collect baseline information about the haemolymph biochemistry of this species in the wild and its correlation to condition. It was a brief project with outcomes expected to be information rather than a fully implemented industry outcome.

Aside from the information summarised in this report, the project will also involve outputs of a peer-reviewed manuscript which is in preparation, two oral presentations at the Crustacean Society conference (TCS IAA2015) in Sydney (19-23 July), a popular article for wider circulation to industry, and a presentation to SRL Ltd.

8. Conclusion

Brindle lobsters sampled in the South East and South West of Tasmania had lower lipid reserves and higher magnesium than lobsters from Orford but this difference did not affect their tail meat dry matter content and vulnerability to transport which are more important market characteristics. The industry observation of poorer condition of brindle lobsters at the start of the season could not be detected by differences in biological attributes in this study. It is possible that this result is specific to this year and due to all lobsters recovering sufficiently from the moult prior the opening of the fishing season. It is also possible that environmental differences between years affect the relationship between site of harvesting and transport mortality. A longer study spanning several years may be required to understand temporal changes in the condition of lobster.

Variation in nutritional condition based on the non-invasive Brix index assessment was larger between sites within areas than between areas or within pots. This suggests the need to apply the assessment of condition to smaller scales than fishing areas in order to monitor accurately moult pattern and condition of lobsters. The Brix index in this study was not a good predictor of vulnerability to transport but correlated well to tail dry matter content, a likely proxy for meat yield. Measuring Brix index non-invasively could allow differentiation of product on the basis of meat yield which may be important in some markets. Fishermen could possibly estimate meat yield of catch from only a few pots given the low variation in condition observed at the pot level.

The lobsters size (carapace length), baseline concentrations of haemolymph haemocyanin and bicarbonate were useful predictor of vulnerability to transport. This information may help improve the management of these lobsters but would require practical portable meters or test kits to be developed, which could be the subject of future R&D. This information could allow processors or fishers to make decisions with batches, such as to release lobsters with very low haemocyanin and bicarbonate, or reduce transport by sending these lobsters to local markets only.

Lobsters that died after packaging showed significantly higher lactate and lower pH values than those that survived, suggesting their haemolymph acid-base balance failed to compensate for the anaerobic metabolism as found in previous research. These lobsters also had significantly higher urea and potassium levels and significantly lower sodium and chloride ions indicating that dehydration, cell damage and possibly nitrogenous waste

toxicity were other possible factors for their death. Thus, recommendations to minimise mortality risk would include ensuring lobsters have recovered from the ground transport stress in holding tanks for several days, minimising emersion duration and reducing temperature prior to packaging. These practices are already well understood and practiced by processors. Holding lobsters for periods between transport journeys, either from the boat to the processor or on separate legs of airfreight, could be expected to improve their physiological condition (i.e., lower lactate, urea, uric acid, glucose and increase pH). Further research around the minimum time required for the physiological condition to improve to back to wild levels is required.

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