FRDC 2021-020: Stable isotopes: a rapid method to determine lobster diet and trace lobster origin?

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PREAMBLE

As part of a wider research project on the diet of Southern Rock Lobster, we became aware of industry interest in the potential of using stable isotope analysis to identify origin of lobsters post-harvest. This report details one of two parallel projects, with the aim to understand the value of stable isotopes as a method to trace lobster origin and to develop suitable sampling method to use in an industry setting. The second project (the lead author's PhD) uses stable isotopes and other diet analysis methods to investigate lobster diet in the wild to determine the predatory role in controlling the range-extending Longspined Sea Urchin. The PhD thesis will be published early 2024.

EXECUTIVE SUMMARY

Scientists at IMAS conducted a stable isotope study on Southern Rock Lobster (*Jasus edwardsii*) collected from Southern Australian sites to determine whether or not capture site could be determined post-harvest.

Background

The project was undertaken because of interest to industry - will this technology allow rapid post-harvest determination of capture site? Such a method would be of great value to the Southern Rock Lobster industry as it could allow isolation of stock abnormalities and validation of catch data.

Aims

There were two major aims for this project:

1. To assess if stable isotope signatures can be used to trace lobster origin

2. To detail a suitable method which could be used to collect this data in an industry setting, should the results prove valuable

These two aims were directly related to the industry's interest in the method, these took priority for this project and report and we provide conclusions on these.

Parallel research aiming to use stable isotopes to evaluate lobster diet will be publish as part of the lead author's PhD program in 2024.

Methodology

The project was split into two parts:

1. A sample set of lobsters taken from 8 sites around Southern Australia were used to assess the viability of using stable isotopes to trace lobster origin post-harvest. Each lobster's isotope signature was measured and then assigned a "most-likely" site of origin based on the signatures of others in that site. We then looked at how accurate our assignment process was.

2. Lobsters from the wild and tank experiments were sampled to determine whether there was any difference in isotopic signature between different tissues within the same individual (i.e.

does the leg muscle vary from the dorsal muscle or from the shell?). Wild lobsters were also sampled across different size classes and depths to assess whether these factors influence mean isotopic signature within a capture site.

Key Findings
Our key findings are:

- δ^{13}C and δ^{15}N isotopes from lobsters can be used to gain information on the origin of catch but not on a fine geographic scale. Accuracy of origin assignment is heavily dependent on site specific reference data and prior known information. For example, δ^{13}C and δ^{15}N isotopes can be helpful in validating (yes/no) the possibility of a lobster originating from a labelled origin but would be limited in determining a location without any prior reference.

- We show the use of leg tissue for isotope analysis in lobsters is highly correlated with dorsal tissue, allowing for non-lethal/non-destructive sampling in future

- We show that depth does not necessarily influence isotope signature within a fishing site (this requires more site-specific sampling)

- We show that in some sites, size class of lobster can influence isotopic signature - this is not the case at all sites, but we recommend sampling within size classes for reference datasets

- We found that isotope assimilation time is within months, therefore recommend similar sampling time (i.e. same season) for reference and test samples

Implications for relevant stakeholders
This research is relevant to multiple stakeholders:

INDUSTRY & MANAGERS
We found that although there is some differentiation between lobster isotope signatures of different capture sites, stable isotope analysis is unlikely to be able to trace exact origin of lobsters post-harvest. Due to the wide variation in the isotopes and lots of overlap between sites, isotope signature alone will not categorically assign lobsters to capture site with a high level of accuracy. Depending on the geographical scale required, isotope signatures can be accurate in assigning to larger regions (such as state, rather than sites within a state), but this still depends on the regions to be distinguished.

SCIENTISTS & PRACTITIONERS
In future, we recommend the use of non-lethal sampling for stable isotope analysis of Southern Rock Lobsters. Isotope variation can occur between tissues within individuals, between sites, seasons and depths so we recommend designing isotope studies accordingly.

Recommendations

We recommend non-lethal samples, such as a lobster leg rather than a dorsal muscle, can be used for isotope analysis. For tracing purposes and from the results we show here we recommend using size- and season-specific reference datasets but not necessarily depth-specific.

Larger sample sizes for reference isotope datasets would improve our understanding of the variance in signature associated with each site. Similarly, sampling lobsters at much deeper depths (i.e. 100m) will help to inform sampling protocols.

KEY WORDS

Stable isotope analysis; origin tracing; seafood; Southern Rock Lobster (*Jasus edwardsii*);

INTRODUCTION

Background – why stable isotopes?

The isotopic signature of marine organisms varies depending on differences in the isotopic composition of their diet and/or physical conditions of their habitat, e.g. nutrient availability in surrounding water. Naturally, these factors such as diet and habitat conditions vary geographically due to prey/diet availability, proximity to cities, etc. and therefore typical isotopic signature of organisms also varies geographically. By measuring the isotopic signature from an individual (e.g. the signature of a muscle tissue sample), it is possible to infer the geographic origin of the individual by matching the isotope signature with other individuals of the same species originating in the same area (Trueman, 2019).

Previous work on stable isotope signatures across spatial gradients in Southern Rock Lobsters (*Jasus edwardsii*) around Tasmania suggested variation in signatures was greatest between samples from a meso-scale spatial distance (i.e. 100-1000m difference, rather than 10 km difference) (Guest et al., 2010). Here we investigate lobster isotope signatures on a broader geographical scale (>100km) by comparing interstate and regional samples.

Stable isotopes have been used successfully to discriminate origin of wild caught seafood products at local and broad geographic scales (see Cusa et al., 2021). Many studies on seafood focus on finfish, but invertebrate studies do exist (e.g. for the Norwegian Lobster, *Nephrops norvegicus* (Slesser and Trueman, 2021)). Using isotopic signatures to trace geographic origins requires reference datasets which provide the known/expected isotope compositions and variance for all potential areas the catch may have originated from. If the average isotope signatures are distinct between different sites, we can work out the probability a sample is from a particular site. This can be done both distinctively, by employing data from specified sites (as we do in this report), or continuously, over wide geographic scales using spatial mapping and modelling (for more on this, see section 2.6 in Trueman (2019)).

In marine organisms, carbon and nitrogen are the most commonly used elements to describe isotopic signatures. Trueman (2019) found the average accuracy of using these elements to infer geographical origin of *Gadus morhua* (Atlantic Cod) was 95.1%. Sulphur can also be used as a third element, but for the extra expense it was shown to add limited extra outcomes in that study (94.5% average accuracy). Due to expense and limited added benefit of extra isotopes, we only used carbon and nitrogen isotope in this project.

Aside from potentially high accuracy of assignment, another benefit of using stable isotope analysis as a method for seafood origin testing is the small individual sample size required for stable isotope analysis - only 0.5mg of lobster tissue is required for processing. In the Rock Lobster industry, aesthetic appeal is an important feature of the market product, so it was important to show here that a non-destructive sample (e.g. a piece of leg muscle) will be viable to conduct this analysis. However, current professional opinion recommends using muscle from under the dorsal carapace due to its long-term nature as opposed to a leg which are readily lost and regrown as a defence mechanism in lobsters (pers comm. Mazumder). We investigate variation of signatures between tissues within the same individual lobster, particularly dorsal muscle and leg muscle, as this has been shown to vary in other spiny lobster species (Díaz et al. 2012). We expect our Southern Rock Lobster samples to reflect work in closely related Eastern Rock Lobster (*Sagmariasus verreauxi)* where isotopic signatures in leg muscle and antennae tissue were found to be closely correlated with those in abdominal muscle (Day et al., 2022).

If stable isotope analysis proves useful to industry, a common sampling protocol would be required for the creation of isotope reference datasets and for testing samples. Generally, at about $30 a sample for carbon and nitrogen analysis, this method is relatively cheap but the time from sampling to results may be important, here we aim to determine and estimate of time required for processing.

As isotope signatures are a reflection of predator diet and local habitat conditions, we expect that tissue compositions may vary temporally as well as spatially. This could be important for food traceability in terms of the isotope reference datasets – if seasonal changes occur this would need to be included in sampling guidelines and reference datasets to be separated accordingly. Similarly, depth is also expected to influence diet, so we also assess samples from the same location but different depths to evaluate depth influence on isotope signatures.

Using isotopes to answer questions about animal origin

There is huge importance on the type of question asked when using isotopes to identify animal origin (Trueman, 2019; Trueman and St John Glew, 2019). For example, notice the difference in 1) "is this lobster from site X?" and 2) "where is this lobster from?". With more demanding questions we increase assumptions and decrease accuracy and precision: For question 1 we must have an idea of the characteristics of lobsters from a reference isotope data set from site X, and the answer could be yes or no. But for question 2 we need to know the isotopic signatures of lobsters from all possible locations. To know what the typical characteristics in site X are, we need a reference dataset: reference samples that accurately represent the isotopic variance within a spatial area.

When using isotopes to answer questions about animal origin we are making multiple assumptions of the data, and these need to be reported and made clear to the user. We are assuming that spatial factors are the only possible source of isotopic difference between reference dataset and unknown samples. If you can address the question of "is this lobster from site X?" rather than "where is this lobster from?", it will be the least demanding and make the least assumptions on the reference dataset. For this reason, we anticipate the best use of stable isotopes for lobster origin tracing will be validating whether or not the individual in question is likely to have come from a specific location - this could potentially be fishing zone/state/territory depending on the need for the tracing. For further reading see Trueman and St John Glew (2019); Hobson (2008).

NEED

Tracing lobster origin

The need for determining whether stable isotopes are a useful tool for diet analysis and identification of catch location is a priority for the Southern Rock Lobster (SRL) fishery. If proved useful, this technology could provide rapid identification of origin and therefore allow for isolation of stock abnormalities – such as areas with known toxic algal blooms, which has potential to save a lot of time and money for fisheries management. Stable isotope analysis could also be used as a tool for traceability to strengthen food security.

OBJECTIVES

1. **Tracing lobster origin:** To ascertain if stable isotopes can be used to identify catch location of individual lobsters post-harvest across SE Australia.

2. **Method optimisation and sampling protocol:** To determine the level of stable isotope variation within individual lobster tissues (e.g. muscle vs shell) and identify a non-lethal and minimally destructive method of sampling for stable isotopes.

METHODS

Sample Collection

Lobsters for origin tracing

All 116 lobsters used for origin tracing were collected by local fishers in South Australia, Victoria and Tasmania for FRDC project 2020-092. With permission, we took a 1cm^3 piece of tissue from the frozen sample of homogenised white tissue for isotope analysis.

Mazumder et al. (2008) indicated that minimum of five samples are required to describe characterise signatures of local populations of marine invertebrates, following this we took ten samples from each site and where possible, five of these were deep and five were shallow (relative to depths available at each site). The potential for variation in samples due to temporal change was minimised by confirming the samples used here were all collected between January-March 2021.

The map shown in Figure 1 shows the locations where lobsters were trapped for the study. Colours represent lobster fishing blocks. We purposely chose sites that are geographically separated by distance and also those that fall into distinct lobster fishery blocks.
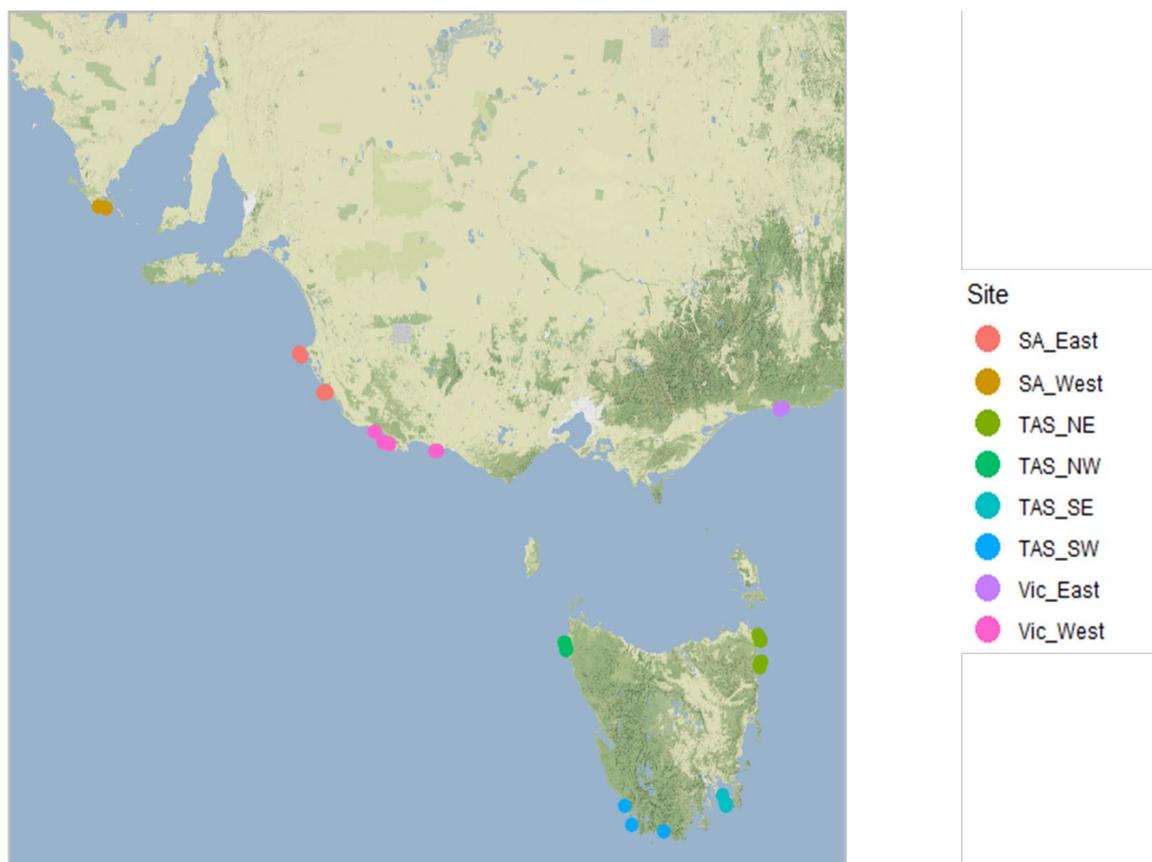
Figure 1: Map of southern Australian coast with points marking sample fishing location in eight different fishing sites

Lobsters for method optimisation

Juvenile lobsters (carapace length 47-101mm) held in tanks at IMAS labs since puerulus stage were used in a controlled diet feeding experiment. This study was to determine the effectiveness of non-lethal sampling and assimilation time of changing diet. At the beginning of the experiment, we destructively sampled five individuals for dorsal and leg samples. For the first 12 months, legs were removed from live lobsters at four-month intervals and then a final destructive sampling took place at 18 months. All lobsters that had legs removed survived.

Prior to the beginning of the experiment, all lobsters had been fed on defrosted Blue Mussels (*Mytilus galloprovincialis)*. After the initial sampling, lobsters were split into three tanks - one continued to have Blue Mussels (*M. galloprovincialis*), the second tank were then fed defrosted Longspined Sea Urchin (*Centrostephanus rodgersii,* harvested from the Tasman peninsula) and the third tank fed defrosted Australian Sardines (*Sardinops neopilchardus*).

Dorsal samples were collected by removing a section of dorsal muscle from beneath the carapace, a section of dorsal carapace was also collected. Leg samples were processed by removing muscle from carapace.

Sample preparation for isotope analysis

Isotope analysis in this study was the same for both groups of lobsters.

Each tissue or carapace sample was stored in a glass vial in a 60°C drying oven for at least 24 hours. After 24 hours samples were weighed until they reached a continuous weight implying all moisture had been removed from the sample. Once samples were dry, they were ground into fine homogeneous powder using mortar and pestle. Samples were then weighed (0.45-0.55mg) into tin capsules and sent to the UTAS Central Science Laboratory, Hobart for analysis. Equipment was cleaned with ethanol between samples to avoid contamination (note: this is actually not best practise, for future reference - ethanol can significantly influence the δ^{13}C isotope reading - in this case, we assessed results for anomalies and confirmed this has not affected our results (pers. comm. Dietz)).

Carbon and nitrogen stable isotopes were analysed using flash combustion isotope ratio mass spectrometry (varioPYRO cube coupled to lsoprime100 mass spectrometer) at the Central Science Laboratory, University of Tasmania (Australia). The nitrogen produced during combustion is directly fed into the mass spectrometer and measured against a reference gas. Carbon dioxide is trapped in the meantime and released into the mass spectrometer after the nitrogen isotope measurement is done and the system retuned accordingly.

Stable isotope abundances are reported in delta (δ) values as the deviations from conventional standards in parts per mil (‰) from the following equation:

$$[\; \delta \text{ X (‰)} = (R_{sample}/R_{standard} - 1) \times 1000)\;]$$

where X = ^{13}C or ^{15}N and R = the ratio ^{13}C/^{12}C or ^{15}N/^{14}N. δ^{13}C and δ^{15}N values are reported respective to PDB (Pee Dee Belemnite) and atmospheric air, respectively. International reference standards with known isotopic composition (USGS 25, USGS 40, USGS 41, IAEA-N1 and IAEA-N2, NBS 21, USGS 24 and ANU-NAT-76H) were used to correct for instrumental drift and Quality Assurance purposes. The analytical performance of the instrumentation, drift correction and linearity performance were calculated from the repetitive analysis of these standards. Precision was 0.1% for both isotopes.

Timescale of sampling

The time taken for the process from collection of the sample is approximately 10 minutes for dissections and sample preparation followed by 24 hours in the oven to dry and then approximately 30 minutes per sample to grind and weigh. Priority analysis at a lab would reduce time to results, but on average we waited about 2 weeks. This does not include transport of samples before or after processing.

Data analysis

All data analysis was conducted in R through R Studio. Stable isotope data is available at https://doi.org/10.25959/MCAM-JZ40.

Tracing lobster origin

To assign lobsters back to their point of origin we followed two steps:

1. Using standard area ellipses (SEAs) and Bayesian modelling to assess variation between sites.

   "SIBER" and "MIXSIAR" packages were used to find and assess the accuracy of the standard areas ellipses (Jackson et al., 2011). Archived "bivariate" package and discrete assignment script adapted from Trueman and Slesser (2021: still awaiting preferred reference) used probability density function (PDF) analysis to define the most likely source of origin for the lobster isotope signatures.

2. Precision and accuracy testing of assignment to lobster origin. This report relies on the methods outlined in Trueman (2019). These data were used to calculate the assignment accuracy associated with assigning lobsters back to their known origin sites based on a Monte Carlo re-sampling method. 25% of known samples were taken at random to use as test samples and the remaining used as reference samples for the fishing sites. Probability density functions (PDFs) were used to best predict which site each test sample had originated from. The re-sampling and assignment of lobsters was repeated 1000 times.

Method optimisation and sampling protocol

We compared isotopic signature between lobster size, depth and different tissue types. Difference between isotope signatures among lobster size classes and capture depth at each site was investigated using two-way ANOVAs. The similarity of isotope signatures in different lobster tissues (within the individuals) was assessed using linear models.

RESULTS

Tracing lobster origin

To illustrate the potential use of stable isotope analysis to discriminate between Rock Lobster fishing sites in South-eastern Australia, carbon and nitrogen stable isotope compositions of lobster tissue are displayed in Figures 2 & 4.
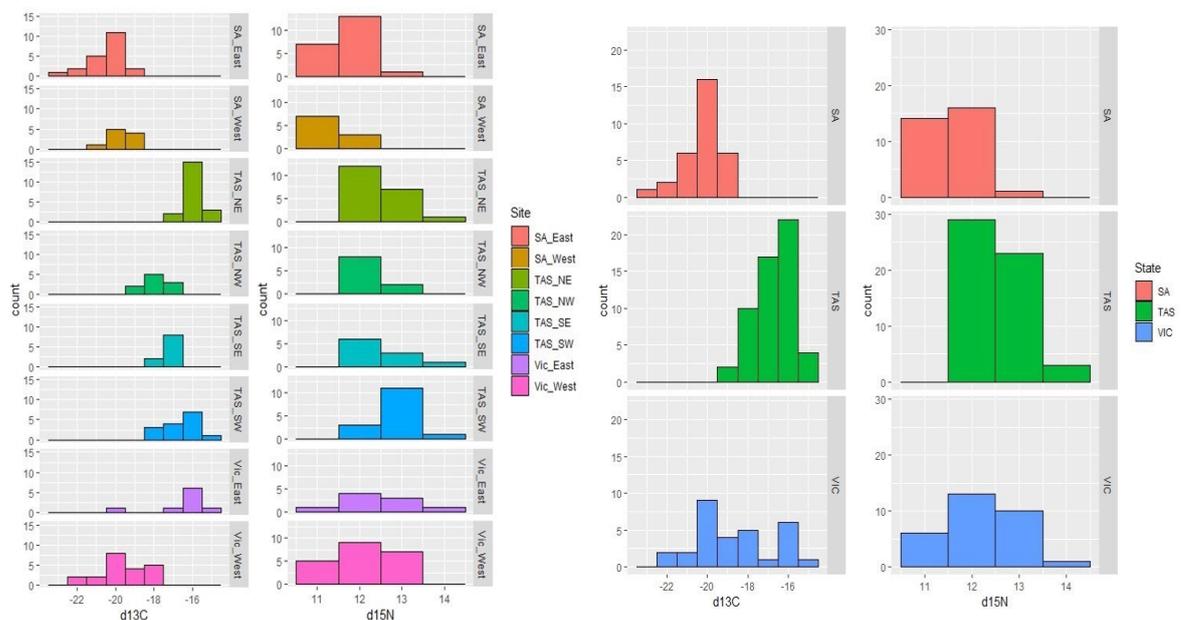
Figure 2: Histograms of the range of δ^{13}C and δ^{15}N in lobster dorsal tissue at each site

Figure 3: Histograms of the range of δ^{13}C and δ^{15}N in lobster dorsal tissue in each state

Figure 2 shows the δ^{13}C isotope is more variable between fishery regions than the δ^{15}N isotope. The range in δ^{13}C is large -24 to -14, compared to a relatively small range in δ^{15}N. Figure 3 shows the same data but grouped by state rather than individual site: South Australia has lower average δ^{13}C levels than Tasmania, but Victoria has a much wider range. There is much less distinction between sites and states in the δ^{15}N isotope (Figures 2 & 3).

The Standard Ellipse Areas (SEAs) in Figure 4 show the 'core area' of the isotopic niche width of Southern Rock Lobster in each of the eight samples fishing areas - these represent the 95% confidence limits in which we would expect lobster isotopes from this site to fall. Note the separation between Tasmanian sites (blue-green) and the South Australia sites (red-orange), this distinction is made clearer in Figure 5 in which we have removed sites and just grouped samples by state. Interestingly, although the Victorian SEA overlaps both Tasmania and South Australia in Figure 5, in Figure 4 it is clear than eastern Victoria (purple) overlaps with Tasmanian sites and western Victoria overlaps with South Australian sites. The map shown in Figure 1 shows that this overlap makes sense geographically - the closest sites have the most similar isotope signatures.

To determine the confidence we have in the size of the SEAs, the SIBER package in R allowed the use of a Bayesian approach to estimate variance and calculate confidence values. Using this approach we can robustly compare between populations stating for example that we are 95% certain that Vic-East have a larger isotopic niche than Tas NW (Figure 6). This is largely driven by the large isotopic niche width seen in the Vic-East site (Figure 4).
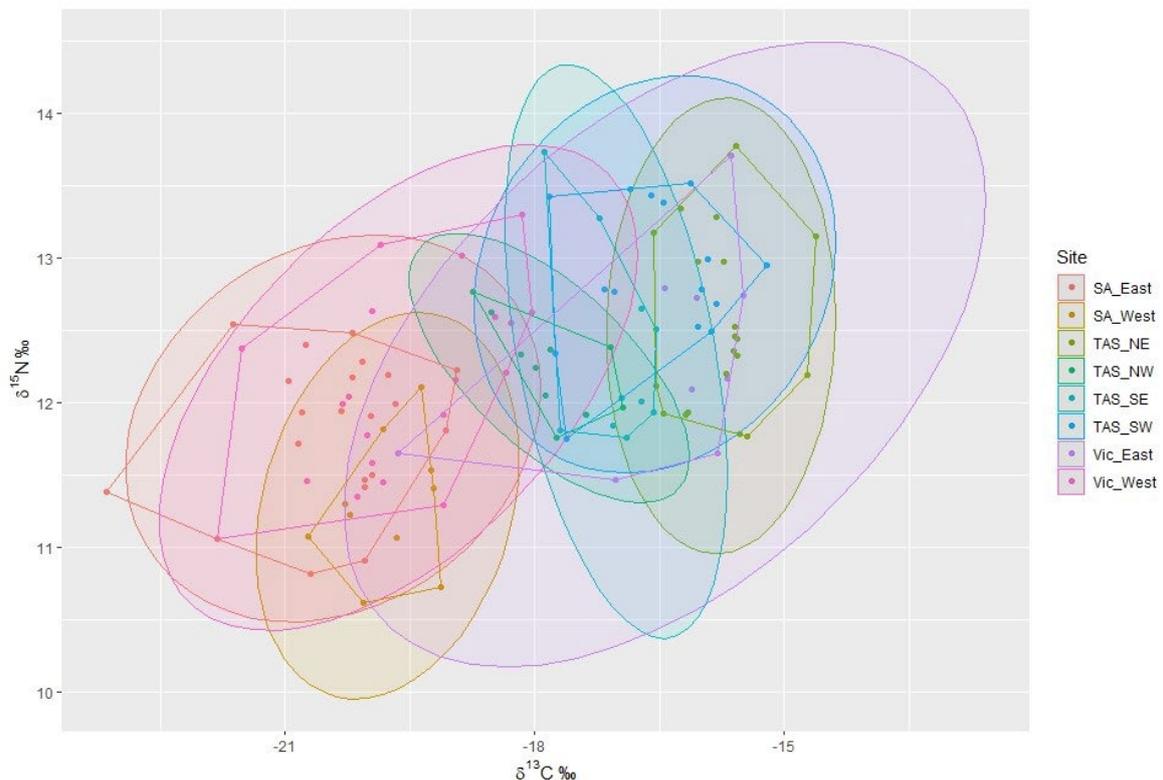
Figure 4: Isoplot showing the individual samples, standard area ellipse (SEA) calculated as 95% confidence level and convex hull for each site in the study.
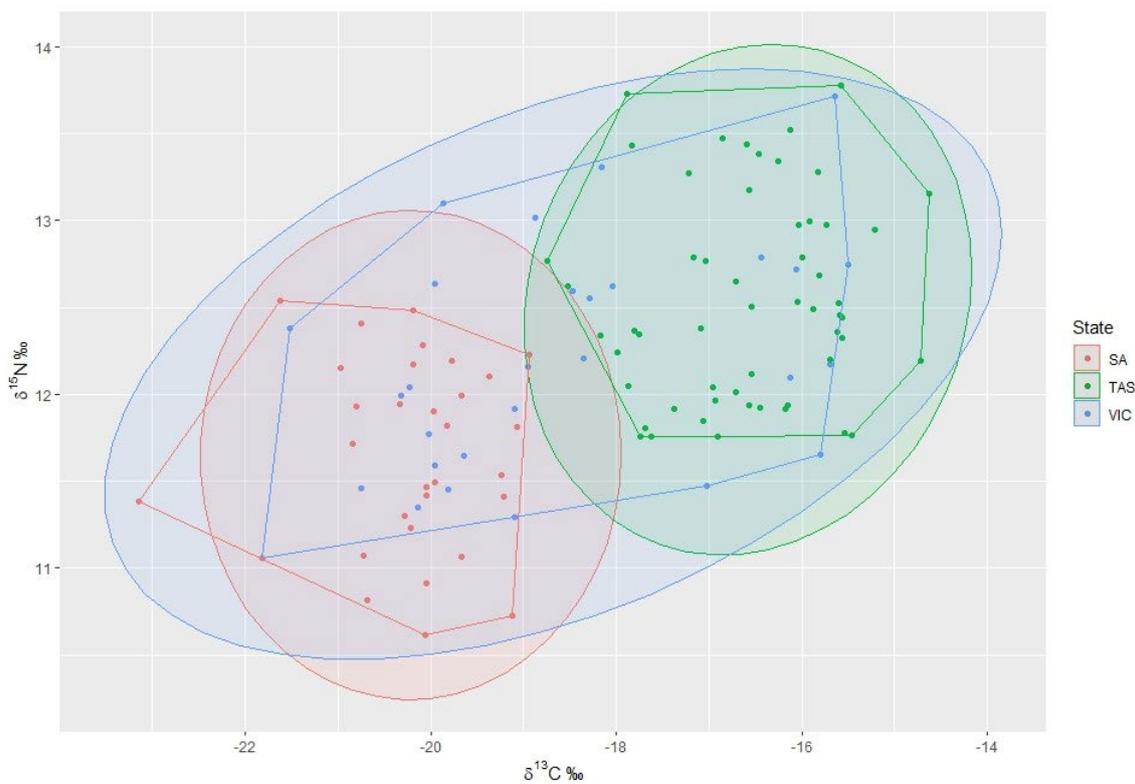
Figure 5: Isoplot showing the individual samples, standard area ellipse (SEA) calculated as 95% confidence level and convex hull grouped by state.
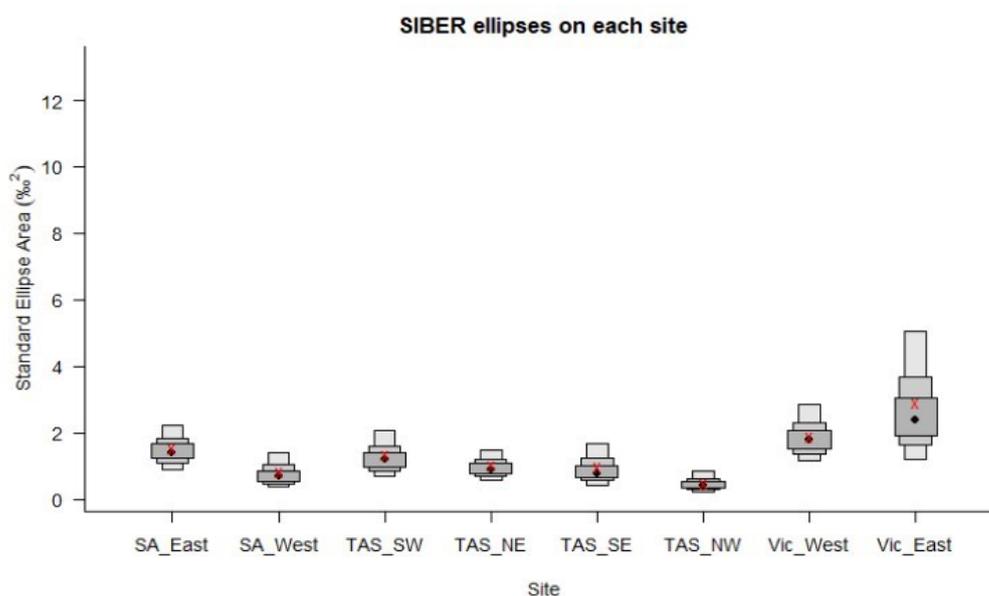
Figure 6: SIBER Density Plot showing the distribution of ellipses for each site as a density plot. The gradations denote 95, 75 and 50% credibility intervals. Black points are the mode of the Bayesian Ellipse (SEAb) values (of 1000 samples). Red crosses represent the maximum likelihood SEA-c.

Precision and accuracy testing

Over 1000 re-sampling events, the average accuracy of assignment of lobsters to known locations for all eight fishing sites was low at 36%. As Figure 5 suggests, the removal of Victorian lobsters from the dataset allowed for much higher average assignment accuracy with 54% accuracy of assignment to site, and 99% accuracy of assignment to state between South Australia and Tasmania.

Method optimization and sampling protocol

Isotope composition in different sized lobsters

Lobster size class did significantly influence δ^{13}C and δ^{15}N values in some sites (Table 1 & Figure 7). In the Vic-West site, lobster size classes were distinct in both δ^{13}C and δ^{15}N, in SA-East, δ^{15}N was different between size classes.
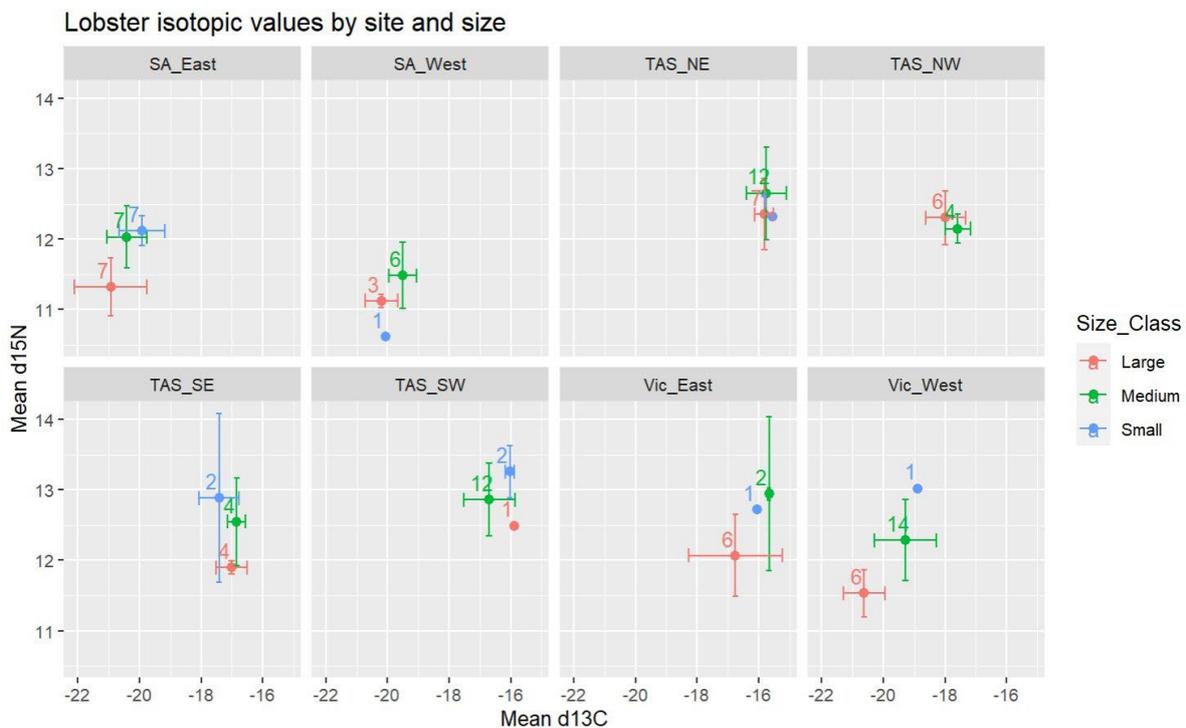
Figure 7: Isoplot showing the mean δ^{13}C and δ^{15}N values coloured by size class of lobster and faceted by site. Error bars show standard deviation. Numbers represent number of samples in that group.

Table 1: Two-way ANOVA table for size class by each site and isotope

| Isotope	Site	Effect	DFn	DFd	F	p	p<.05	ges
d13C	SA_East	Size_Class	2	93	2.8	0.066		0.057
d13C	SA_West	Size_Class	2	93	0.834	0.437		0.018
d13C	TAS_NE	Size_Class	2	93	0.05	0.951		0.001
d13C	TAS_NW	Size_Class	1	93	0.61	0.437		0.007
d13C	TAS_SE	Size_Class	2	93	0.35	0.705		0.007
d13C	TAS_SW	Size_Class	2	93	0.992	0.375		0.021
d13C	Vic_East	Size_Class	2	93	1.523	0.224		0.032
d13C	Vic_West	Size_Class	2	93	6.405	0.002	*	0.121
d15N	SA_East	Size_Class	2	93	5.135	0.008	*	0.099
d15N	SA_West	Size_Class	2	93	1.463	0.237		0.03
d15N	TAS_NE	Size_Class	2	93	0.848	0.432		0.018
d15N	TAS_NW	Size_Class	1	93	0.224	0.637		0.002
d15N	TAS_SE	Size_Class	2	93	2.958	0.057		0.06
d15N	TAS_SW	Size_Class	2	93	0.85	0.431		0.018
d15N	Vic_East	Size_Class	2	93	2.534	0.085		0.052
d15N	Vic_West	Size_Class	2	93	6.211	0.003	*	0.118

Isotope composition in lobsters from different depths

There appeared to be little variation in isotopes by depth at most sites (Figure 8). We were limited by the depths of the samples already collected, but had lobsters caught from depths ranging from 7.5m to 58m. The greatest depth difference within a single site was 40m in Vic-West, the least was 8m in Tas-SE, hence the need to separate by site to investigate depth relative to site. We found a significant difference in δ^{13}C between deep and shallow sites at SA-East and a difference in δ^{15}N at TAS-SW (Table 2). However, even when there was a large difference between deep and shallow sites (e.g. 35m at Vic-West), there was no meaningful difference in the isotope composition.
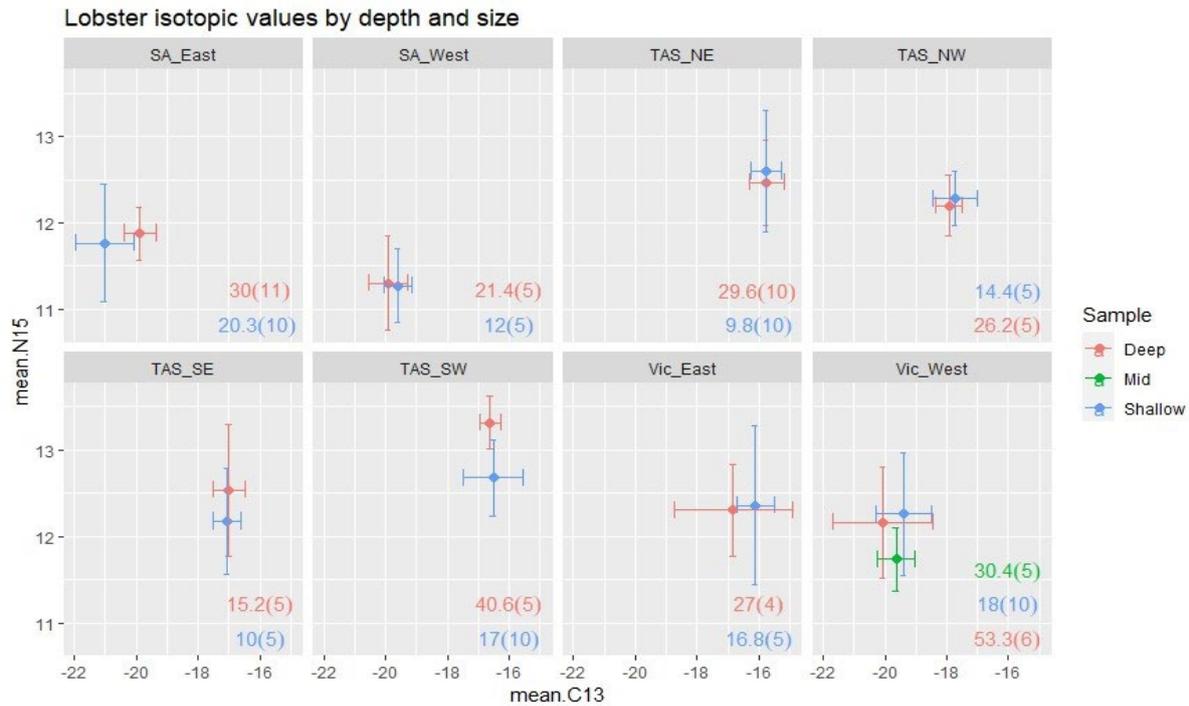
Figure 8: Isoplot showing the mean δ^{13}C and δ^{15}N values coloured by fishing depth category of lobster and faceted by site. Error bars show standard deviation. Coloured numbers represent average depth in metres of the group and numbers in parentheses show number of samples in that group.

Table 2: Two-way ANOVA table for depth class by each site and isotope. Average depths for each depth class at each site included.

Isotope	Site	Effect	DFn	DFd	F	p	p<.05	ges	Deep	Shallow	Mid
d13C	SA_East	Sample	1	99	10.358	0.002	*	0.095	30	20.3	NA
d13C	SA_West	Sample	1	99	0.372	0.544		0.004	21.4	12	NA
d13C	TAS_NE	Sample	1	99	0.000138	0.991		1.39E-06	29.6	9.8	NA
d13C	TAS_NW	Sample	1	99	0.148	0.702		0.001	26.2	14.4	NA
d13C	TAS_SE	Sample	1	99	0.01	0.921		9.93E-05	15.2	10	NA
d13C	TAS_SW	Sample	1	99	0.043	0.836		0.000435	40.6	17	NA
d13C	Vic_East	Sample	1	99	1.676	0.199		0.017	27	16.8	NA
d13C	Vic_West	Sample	2	99	1.328	0.27		0.026	53.3	18	30.4
d15N	SA_East	Sample	1	99	0.209	0.648		0.002	30	20.3	NA
d15N	SA_West	Sample	1	99	0.006	0.941		5.60E-05	21.4	12	NA
d15N	TAS_NE	Sample	1	99	0.252	0.617		0.003	29.6	9.8	NA
d15N	TAS_NW	Sample	1	99	0.05	0.824		0.000503	26.2	14.4	NA
d15N	TAS_SE	Sample	1	99	1.022	0.315		0.01	15.2	10	NA
d15N	TAS_SW	Sample	1	99	4.26	0.042	*	0.041	40.6	17	NA
d15N	Vic_East	Sample	1	99	0.022	0.883		0.000221	27	16.8	NA
d15N	Vic_West	Sample	2	99	1.466	0.236		0.029	53.3	18	30.4

Isotope composition in leg vs. dorsal tissue and carapace

Isotope signatures from lobster legs and lobster dorsal tissue were strongly correlate in both δ^{13}C (Figure 9) and δ^{15}N (Figure 10). We also compared the isotope signatures of lobster dorsal tissue and dorsal carapace and found a positive correlation but much less uniform in both isotopes (Figures 11 & 12, Table 3). Lobsters in this data set were primarily tank based (30 individuals) and some wild (10 individuals).
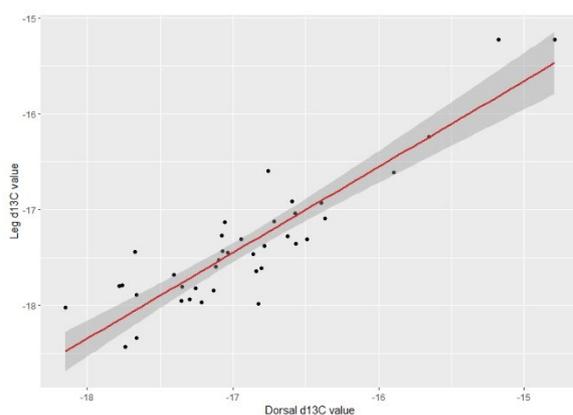

Figure 9: Scatterplot showing the relationship between δ^{13}C isotope in lobster leg and dorsal tissue in 39 samples (9 wild and 30 tank). One data point was removed due to being a significant outlier. Line shows the linear regression. R-squared value of 81%.
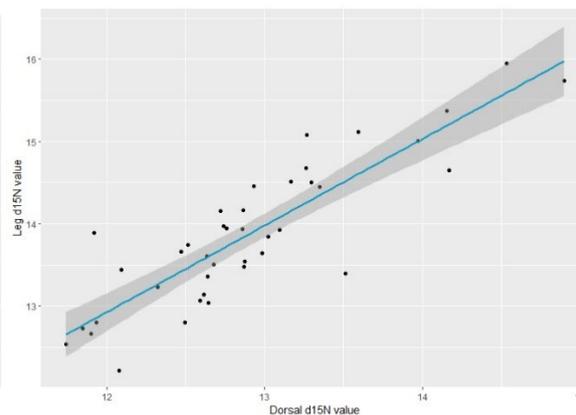
Figure 10: Scatterplot showing the relationship between δ^{15}N isotope in lobster leg and dorsal tissue in 40 samples (10 wild and 30 tank). Line shows the linear regression. R-squared value of 75%

Table 3: Linear model results and r-squared values for tissue comparisons

Model	Estimate	Std. Error	t-value	p-value	Multiple r-squared
lm(δ^{13}C dorsal ~ δ^{13}C leg)	0.90403	0.07229	12.505	7.44E-15	0.8087
lm(δ^{15}N dorsal ~ δ^{15}N leg)	0.7141	0.06666	10.71	4.87E-13	0.7512
lm(δ^{13}C dorsal ~ δ^{13}C dorsal_cara)	0.30587	0.07993	3.827	0.000471	0.2781
lm(δ^{15}N dorsal ~ δ^{15}N dorsal_cara)	0.41318	0.09158	4.512	6.02E-05	0.3488

Importance of timely sampling: change in isotope signature over time

At five sampling intervals, from June 2020 - December 2021, 78 leg samples were taken from captive lobsters in different diet treatments. These were used to investigate change in isotopic signature over time after a known dietary alteration. From Figure 13 it is clear that four months post diet change, there is separation between treatments in δ^{13}C isotope but not so much in the δ^{15}N isotope. By eight months there is clear distinction between the urchin diet treatment and the other two, and more separation between δ^{13}C and δ^{15}N values. At 12 months, there is clear difference between lobsters fed mussels (original food) and the new diets, but lobsters on the sardine diet had caught up to lobsters fed urchins in δ^{15}N values, the two are still distinct in δ^{13}C values. The lobsters which

remained on the mussel diet the whole year have not fluctuated much, as we would expect. Through to 18 months, after four months, fish and urchin-fed lobsters remain at higher δ^{15}N value than mussels.

At 18 months there seems to be a steep increase in δ^{15}N values, seen in all three treatments, currently we are uncertain as to what may have caused this.
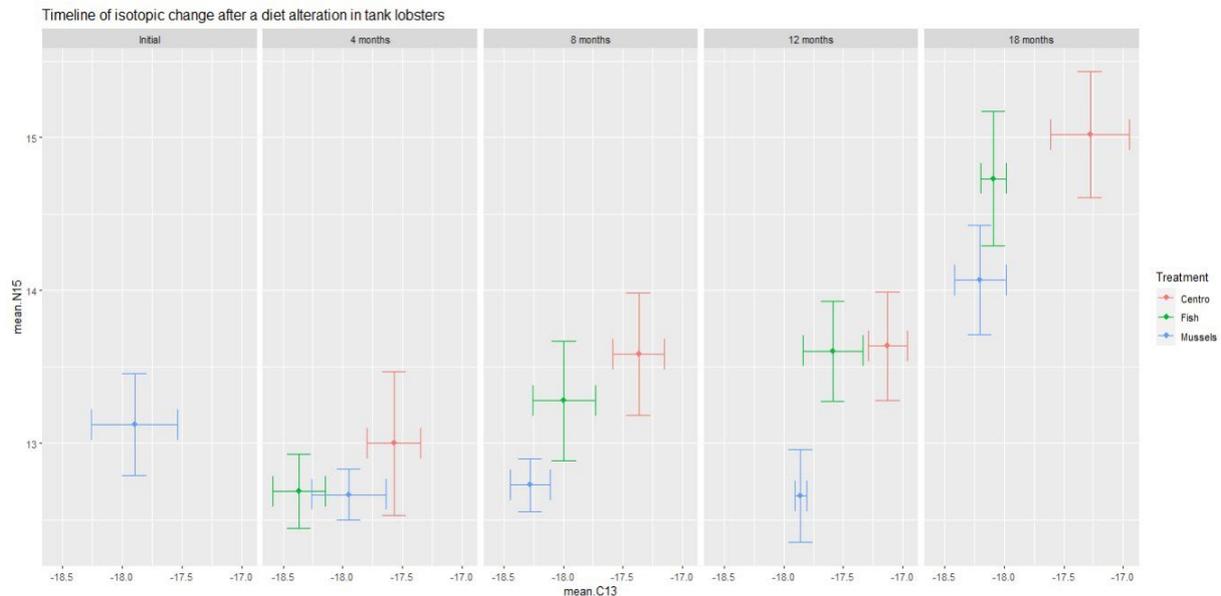

Figure 13: Isoplot timeline showing the change in isotopic signature of captive lobsters after a diet alteration in four-monthly intervals. Colour represents diet.

DISCUSSION

Isotopes are a powerful tool in the field of tracing, diet determination and the future of the fishing industry. This study represents analysis of three separate data sets which used stable isotopes in lobsters to investigate multiple questions.

Firstly, it is promising from our origin tracing study, that in such a relatively small geographic area, compared to the Southern Rock Lobster's entire range, we can still define discrete isoscapes depending on where lobsters originate. Although the isotopic signature of lobsters do not necessarily fall within the anthropogenic boundaries of state and territory lines, they do cluster in a spatially explicit arrangement (Figure 4). Rather than state boundaries, isotopes relate more to available diet for lobsters and the nutrients which make up their diet and habitat and this is likely to be dependent on nutrients provided by ocean currents (pers comm. Dietz 2022). The map in Figure 17 shows that the ocean currents around Southern Australia are largely aligned with the isotope clusters we described - the East Australian Current hits east Victoria and Tasmania, whereas the West Leeuwin current hits South Australia and West Victoria. We see mixing zones and overlapping isoscapes along coastlines and in the path of ocean currents. Geographically, this data makes sense. These results have parallels with genetic work identifying population differences between groups of the same species on either side of the Bassian Isthmus (e.g. York *et al.* 2008), which we historically isolated east-west due to the land bridge. In the case of stable isotopes however, the signature of an individual is related to its lifetime and so the historical isolation is unlikely to be the cause of the

population differences. However, it would be warrant more research into the stable isotope structure within the Bass Straight to see how the isotopes are influenced by the mixing of currents around this area.

Secondly, our experiment to determine the difference in isotopic signatures between tissues in the same individual allow us to make recommendations on sampling in the fishery - lobster leg tissue gives much the same result as lobster dorsal tissue, without lethal sampling. The implication of this is that if this technique was adopted by the commercial fishery, for example for market tracing purposes, the aesthetic nature of the product would not need to be harmed. Instead of breaking open the lobster carapace to collect a dorsal sample, which would decrease its market value, a single leg could be taken from the animal to reduce aesthetic damage.

Source tracing

Here we show that the accuracy of assignment to origin using eight potential sites in SE Australia was low, but increased when the number of potential sites the test subjects could originate from was reduced. By using the isoplots (Figures 4 & 5) we can see which areas we have the potential to distinguish between, and which areas have more variation/overlap - it is possible to distinguish South Australia from Tasmania with confidence, but Victoria has a lot of overlap over the other states.

Very rarely does source tracing rely on only one tracing system (e.g. stable isotopes), here we can see that isotopes could be useful as a first point of call to check if the individual sample in question is likely or not to have come from the site/area claimed by the labelling. Next, the process would need other tracing methods such a DNA profiling in order to reliably assign the sample to a site with high enough accuracy (Gopi et al., 2019). Similarly though, other tracers cannot be used solo, so stable isotopes as a rapid method to assign lobsters in likely or unlikely to have come from a site could still be useful on certain scales.
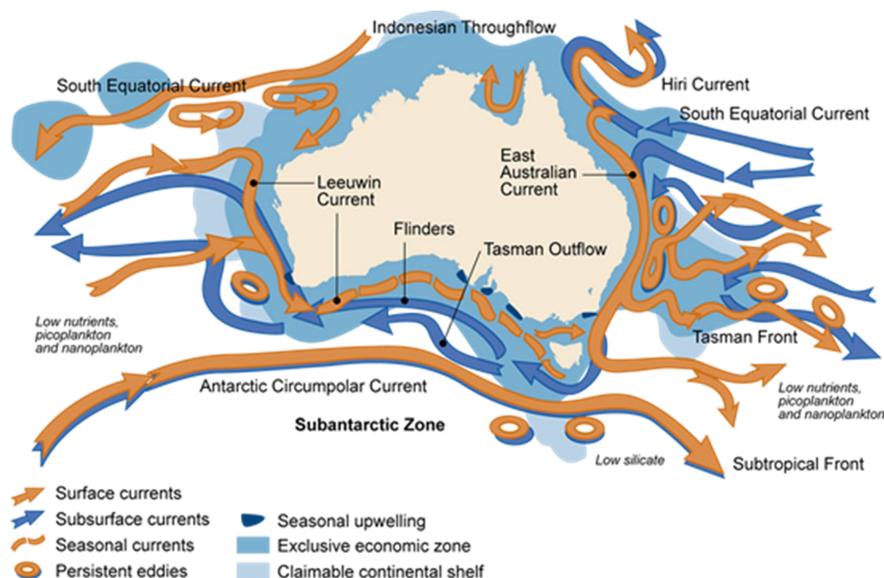

Figure 17: Ocean currents around Australia. By © Commonwealth of Australia 2013, CC BY 3.0 au, https://commons.wikimedia.org/w/index.php?curid=36006341

For the sake of marking lobsters as safe or unsafe based on a localised toxic algal bloom (TAB), the validity of using isotope analysis would be case specific. For clarity, in this analogy stable isotope analysis is not being used to detect the presence of toxic algae. As described, we can only confidently use isotopes as a negative confirmation, such as clearance of safe lobsters which are not from a TAB hotspot. At this stage we cannot confidently determine a positive origin identification due to variation and overlap in sites and a lack of reference datasets. For example, if there is a known TAB outbreak in North-east Tasmania we can analyse isotopes from a legs of all lobsters coming into a central processor and confidently say if the signatures fall within the NE-Tas range or not - but if they do this would also include overlapping regions such as Vic-east and Tas-SE (see Figure 4). In this case we could remove all potential affected lobsters from the market, but lobsters from safe sites with similar isotopes would also be condemned. Due to the localised and time dependent nature of toxic algal blooms this may or may not be useful, depending on the reference datasets at hand at the time. It would be worth comparing the processing and analysis time and costs to other TAB detection methods to justify the use of stable isotopes commercially.

Rather than specifying where a lobster has originated from, reference datasets such as this could be used to reliably say where the lobster is *not* from. This could prove useful in market tracing such as for quality control and food labelling.

It is clear that the variation within sites is causing lots of overlap, larger sampling size could help to verify this variation.

Sampling methods

We have shown high correlation in isotope signatures between lobster dorsal tissue and leg tissue and positive but lower correlation in tissue and carapace. The implication of this is that rather than lethal sampling to acquire stable isotope samples, we can collect sufficient isotope signatures from leg samples. Similar work on different spiny lobster species also concluded leg tissue was a suitable non-lethal alternative for isotope analysis (Díaz et al. 2012; Day et al., 2022). **Therefore we suggest if stable isotope work is continued, the use of leg tissue for reference datasets and test samples.**

We anticipated depth to have an influence on isotope signature in lobsters as different diet components are available at different depths. However, we see that depth rarely has an impact on δ^{13}C or δ^{15}N values within each site. In the two sites that did show a difference in depth, only one isotope caused the difference - so could still be suggestive of origin from the second isotope. It would be advantageous to have larger sample sizes across wider range of depths before concluding whether depth influences isotope signature. **For this reason we would not necessarily recommend the need to sample at the same depth based on our results, but continuing with caution and keeping depth in mind when interpreting results.**

In some locations we found size to sometime be determinant of δ^{13}C and δ^{15}N values within a site. This could be expected as different size lobsters may feed on different diets. Interestingly, when difference was significant, we found smaller sized lobsters at a higher δ^{15}N value than large lobsters. In the locations where we found a significant difference in size (Vic-West and SA-East), it would be worthwhile to see whether unusual diet items were available (for example, in a highly fished zone there could be high quantities of leftover bait). Regardless of the reason for differing isotopic signatures between lobster sizes, this is reason **to recommend sampling to same-sized lobsters for reference datasets and test samples** until more research is conducted.

We used captive feeding trials to investigate the assimilation time of changing diet and the annual fluctuation of isotope signatures in lobsters (Figure 13). For 12 months, lobsters which were maintained on the initial mussel diet had no change in isotope signature, whereas the lobsters fed urchins or fish displayed a changed isotope signature after the diet swap. This change was seen as soon as four months into the experiment for δ^{13}C in urchin-fed lobsters and eight months for δ^{13}C and δ^{15}N in fish-fed and urchin-fed lobsters. It is interesting after four months, fish and urchin-fed lobsters remain at higher δ^{15}N value than mussels, this could be related to the low trophic level of mussels as filter feeders compared to urchins (grazers) and fish (predators). If the mussel-fed lobsters had remained the same throughout the whole experiment (including 18 months) we may have recommended that year-round sampling could be considered, however due to the dramatic change in δ^{15}N values at 18 months we need longer term, multi-year experiments to investigate this further. Reasons for the dramatic change in δ^{15}N values could be related to nutrients in the water or perhaps rapid growth in juvenile lobsters, these hypotheses require further study.

Further, this timeline experiment simulates the potential of changing diet in the wild due to seasonal prey availability/preference, as we see in Figure 13 the change in isotope signature after a change in diet might impact the ability to identify origin if not sampled at the same time of year (dependent on diet change in the wild). Seasonal sampling in wild fishing sites would be needed to confirm if this is the case. In this study of isotopes in lobsters across southeast Australia we only sampled between January-March, similar sampling in a different season is warranted to infer any influence of seasonal change. Further, diet is far more varied in the wild than in our controlled captive feeding trials and so there is potential for wider isotopic variation in wild samples. For this reason, currently **we would recommend wild sampling during similar seasons (within 2-3 months) for reference datasets and test samples when aiming to identify origin.**

CONCLUSIONS

- Given the potential influence of region, season, depth, ontogeny and diet – these results suggest that stable isotopes will be challenging to use as a reliable tracer of lobster origin.

- Isotopes could be useful in part-validation of lobster origin on a wide geographical scale if reference datasets were collated and kept up to date.

- To use stable isotopes in the SRL fishery, leg tissue rather than destructive dorsal tissue samples will suffice.

RECOMMENDATIONS

As it stands, to directly infer an origin from just an isotope signature with no prior information would be difficult, if not impossible - due to overlap and variation within and between sites. However, isotopes could be a useful tool in a series of origin identification techniques to create a clearer picture of where an animal may have come from. Larger sample sizes for reference datasets would be useful for describing variance of signatures at each site.

Sampling methods

As discussed above, we make four recommendations in relation to sampling methods:

1. The use of leg tissue for reference datasets and test samples

2. Not necessarily the need to sample at the same depth based on our results, but continuing with caution and keeping depth in mind when interpreting results

3. Sampling the same-sized lobsters for reference datasets and test samples until more research is conducted

4. Seasonal sampling for reference and test samples when aiming to identify origin

Use another element as a third isotope?

Although other studies have used a third tracer, often sulphur - we would not recommend the addition of another tracing isotope to the process without having a valid ecological reason why. For example, in the terrestrial realm we expect sulphur to vary due to anthropogenic influence such as fertilised farmland, however in the ocean this is not necessarily the case. Oxygen isotopes however could be useful across latitudinal gradients as they are known to vary with ocean temperature - but this would not be seen in the lobster tissue, it would be in the calcium carbonate of the carapace. However, Glendenning et al. (2000) found that oxygen and carbon stable isotopes were not useful to distinguish lobsters from different management zones. Hence, adding more isotopes will not necessarily clarify the view we have, unless there is clear ecological and spatially defined reason to use them.

Recent work by Doubleday et al. (2022) suggests that oxygen stable isotopes from marine animals' hard tissue (i.e. lobster carapace in this scenario) are variable in marine systems and correspond to the animal's latitude. They suggest δ^{18}O would be most valuable across large spatial gradients (100s to 1000s km), which may provide differentiation between southern sites such as Tasmania and south coast mainland sites in South Australia or Victoria. This would be worth further investigation; however, it should be noted that while carbon, nitrogen and sulphur isotopes can be measured in one analytical run, analysis of oxygen requires a different analytical set up. This would add significant cost for analysis.

Further development

If time and funding allowed, we could create a continuous isospace map for the whole of the *Jasus edwardsii* range, such as those seen in St. John Glew et al. (2019), however this would be costly and not necessarily helpful in answering industry specific questions. Instead we could highlight sample areas of interest and maintain detailed reference datasets for validating labelled samples, Trueman (2019) suggests updates would be required every 5 years.

It would be interesting to further investigate the concept of isotopes matching ocean currents. For example, whether lobsters from the east coast New South Wales would show similar isotopic signatures to Tasmania and east Victoria, or whether further west in South Australia would match eastern sites in the state. In this project, we coincidentally sampled an area where different continental currents combined, making this a particularly interesting study site. Further work could include samples from other rock lobster species around Australia's coastline, or Southern Rock Lobster samples from New Zealand.

In other commercial fisheries, as marine farming and aquaculture continues to expand, a major source of validation questioning will be between wild versus farmed animals. This is where stable isotopes could play a huge role the difference between farm-fed animals and wild animals will likely be evident in the isotope signature. Due to the unlikely commercialisation of aquaculture in this species, this may not be valid here.

EXTENSION AND ADOPTION

Presented results to SRL board 24th Oct 2022.

Presented at ASFB Conference 2022.

Possibility to present at The International Conference & Workshop on Lobster Biology and Management 2023 should funding allow.

PROJECT MATERIALS DEVELOPED

Poster and slideshow of results.

Stable Isotopes
A rapid method to trace lobster origin?

Jennifer Smith*
John Keane, Caleb Gardner, Craig Mundy and Michael Oellermann

Stable isotope signatures of animals reflect their local environment and diet. In cases where isotope signatures vary geographically, isotopes can be used to match animals to their origins. Determining whether stable isotopes are a useful tool for identifying the catch location of Southern Rock Lobster is a priority for the fishery. If proven useful, this technology could allow for rapid identification and isolation of stock abnormalities (e.g., toxic algal blooms), which has potential to save significant time and money for fisheries management.

This project aimed to:

1) Assess if stable isotopes can be used to identify catch location of individual lobsters post-harvest

2) Determine the level of stable isotope variation within individual lobster tissues and identify a non-lethal and minimally destructive method of sampling for stable isotopes

1) Tracing lobster origin

δ13C and δ15N isotopes from lobsters can be used to gain information on the origin of catch but may not be useful to identify origin on a fine geographic scale. The isoplot and histograms show that lobsters in SA sites cluster around the lower δ13C and δ15N and lobsters in TAS sites cluster at a higher δ13C and δ15N. Interestingly, although the Victorian ellipse overlaps both Tasmania and South Australia, it is clear that eastern Victoria overlaps with Tasmanian sites and western Victoria overlaps with South Australian sites. This makes sense geographically; the closest sites have most similar isotopic signatures.

Accuracy of assignment to origin is heavily dependent on site specific reference data and prior known information. For example, accuracy of assignment to site was 36% when all eight locations were used for reference. However, the removal of Victorian lobsters from the dataset allowed for much higher average assignment accuracy with 54% accuracy of assignment to site, and 99% accuracy of assignment to state between South Australia and Tasmania.

Isoplot showing all samplings from eight locations

Standard area ellipses (SEAs) were calculated to the 95% confidence level for each site and allow us to see the variation in the signature of samples at each location.

More overlap of sites means less likelihood of differentiating between them.

Note the difference between SA and TAS sites, versus the overlap of VIC East with TAS and the overlap of VIC West with SA.

2) Future data collection

Non-lethal/non-destructive sampling

The use of leg tissue for isotope analysis in lobsters is highly correlated with dorsal tissue

✓ For future stable isotope work, use leg tissue for reference datasets and test samples

Lobster depth

Depth does not necessarily influence isotopic signature (requires more site-specific sampling)

! Sampling at the same depth may not be necessary but continue with caution and keep depth in mind for results

Lobster size

At some sites, size class of lobster can influence isotopic signatures

✓ Sample same-sized lobsters for reference datasets and test samples

Sampling time

Isotope assimilation time is within months

✓ For reference and test samples, sample at similar time (i.e., same season)

*Any questions? Send them to je.smith@utas.edu.au

Graphic by Visual Knowledge
www.visualknowledge.design

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