Assessing Short-term Movements of Western Rock Lobsters by Analysis of Carbon and Oxygen Isotope Ratios in their Exoskeleton

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OBJECTIVES:

? To investigate the factors affecting the carbon/oxygen isotope composition of the exoskeleton of western rock lobsters as a location specific chemical signature for this species.
? To apply these results as a method for determining the locality of a lobsters origin, at the time of its most recent moult.

NON-TECHNICAL SUMMARY:

This project was aimed at using a chemical technique to understand the movements of western rock lobsters during their 'whites' phase. The technique was considered to have useful possibilities, for monitoring the migration of 'whites' lobsters. The 'whites' phase, is a stage in the life cycle when this species migrates offshore to the deeper water breeding grounds. These migrating animals, which are pale pink in colour – hence the reference to 'white', are around legal minimum size and sometimes cross management zones in the course of their offshore movement. The tagging technique investigated in this study, involved the chemical analysis of calcium carbonate, a compound that is the main inorganic structural material of the rock lobsters exoskeleton. Calcium carbonate contains atoms of oxygen and carbon which each occur in different stable isotopic proportions. These isotopic proportions can be attributed to different environmental influences, such as the temperature in which the rock lobster lives, the food that it eats, among other factors. If these environmental influences change from geographic location to geographic location, (for example, along the coastline, or with depth) then potentially a chemical technique that employs these stable isotopes as a location-diagnostic tool can be used to distinguish rock lobsters from different areas based on the isotope ratio of the oxygen and carbon found in their exoskeleton.

This chemical tagging technique would be superior to the currently adopted practice of physical tagging, as the tags would be natural, indestructible and not suffer from loss or interference. Further negative effects of physical tagging, for example reduced growth rate which may impact on migration, would also be alleviated.

Initially, the project set out to understand the fundamentals of the magnitude and range of values and the variations of the carbon and oxygen isotope ratios found in the calcium carbonate of the exoskeleton of western rock lobster. The success of the project relies on these fundamentals being understood, predictable and of significant variability such that discrimination of different rock lobsters from different management areas can be evaluated.

The experiments showed that the isotope ratios varied to a significant degree within the exoskeleton of an animal and between animals of similar ontogeny. Isotope ratios were found to be similar between the sexes of rock lobsters of similar ontogeny.

Finally, the distinguishing of rock lobsters from different areas using this technique was the focus of this project. Subsequent to the fundamentals of the chemical tagging method being understood and proven to be a readily reliable method, the application of this chemical technique as a research tool was investigated by application of the method to wild stocks.

Large overlapping ranges of both oxygen and carbon isotope values were found for individual rock lobsters within and among different management zones along the West Australian coast. These overlapping values suggest that on an animal-to-animal and zone basis, the use of stable isotopes to distinguish animals from different management zones would not be possible.

This chemically based research tool was not forthcoming in experimentation, due to a number of fundamental scientific principles and processes that must more fully be understood and developed further prior to application in a management sense. These include, a deeper understanding of the mechanism by which the rock lobster sequesters the oxygen and carbon from the natural environment, the mode of formation of the calcium carbonate and possible perturbing influences during calcification events and the role of the organic components to the structural make up of the exoskeleton and their influence on the isotope ratios. Finally due to the large degree of isotope ratio variability throughout a rock lobsters exoskeleton and also between rock lobsters with similar characteristics, statistically several rock lobsters rather than the animal-to-animal method used here could prove pivotal to the successful application of isotope ratio results as a research tool.

KEYWORDS: isotope, *Panulirus cygnus* carbon, oxygen, movement, migration, rock lobster

ACKNOWLEDGEMENTS

Jonathon Blaxell (Laboratory Technician) and Melinda Ranaldi (Technical Officer) performed all the experimental work described in this report. In addition, Melinda Ranaldi performed various statistical appreciations of the data, was the overseeing officer of the project and collated the data presented in this report.

Dr John Edmonds (National Institute of Environmental Studies, Japan), together with Drs Trevor Bastow and Roy Melville-Smith conceived and wrote the successful research proposal for this project, based on preliminary pilot studies.

Vitolds Gailitis (CSIRO Land and Water, Perth, Australia) performed all the carbon and oxygen stable isotope analyses in this report.

Finally, we thank the Fisheries Research and Development Corporation for the financial resources provided, without which this project would not have been possible.

BACKGROUND

Studies involving the physical tagging (internal anchor tags) of western rock lobsters, *Panulirus cygnus* (Melville-Smith *et al.*, 1998, FRDC Project 96/108) have previously been undertaken to investigate longshore and offshore migration by these animals. These studies have revealed in general that western rock lobsters are relatively sedentary. However, during their whites phase (a phase that is reached just prior to the animals attaining maturity) the animals move from the inshore nursery grounds to the offshore breeding grounds. A small number of these animals taking part in the offshore migration also undertake extensive movements in a northwesterly direction. These longshore movements have been a focus of interest to the management of the western lobster fishery as well as fishers, because they result in the movement of animals between management zones.

Some of the tagging funded by the FRDC Project 96/108 was aimed at establishing the extent of movement between these management zones. Results from this work have been criticised by fishers because there was a widespread belief that some fishers in the more northerly zones avoided reporting recaptured tagged lobsters so as to 'play down' the extent of the movements of white lobsters between zones.

Previous studies of stable isotope ratios in the carbonate of teleost otoliths have shown that oxygen isotopes are deposited at or close to equilibrium with ambient seawater and have been used to distinguish mixing and non-mixing fish stocks (Edmonds and Fletcher, 1997, Edmonds *et al.*, 1999). If this were to be the case in crustacean exoskeletons, then known differences in the temperatures of inshore areas (the areas where juvenile lobsters moult into whites) over different areas of the coast, would be expected to provide the basis for differences in oxygen isotopic compositions of the exoskeletons of lobsters in those areas. Additionally, stable carbon isotopes in the exoskeleton carbonate are likely to be dependent on factors such as dissolved inorganic carbon, temperature, and metabolic and kinetic effects. Likewise, stable carbon isotopes can potentially be used to contribute to a location specific signature.

The purpose of this project was to examine the possible factors that may influence the carbon and oxygen isotopic composition in lobster exoskeleton carbonate. Should these factors be determined and understood the next step was to apply these principles to determine the origin of white western rock lobsters, which migrate within a moult cycle. The aim of the work was to produce a natural tag from the isotopic signature, thus the technique would have many

advantages over the visual tagging methods because it would be independent of the non-reporting problems that have been mentioned above, as well as tag loss, damage and mortality caused by the tagging process.

Furthermore, should the project prove successful for lobsters, this method has the potential for application to the study of the movements of other species of crustaceans which have long intermoult periods and even terminal moults, for example, in the case of some crab species.

NEED

The reporting of visible anchor tags by commercial fishers is believed to be biased in some circumstances. Consequently, there is a need to develop a fishery-independent technique of identifying the origin of migrating white lobsters so as to address the extent of movements of these animals between management zones, without having to rely on visible tags.

OBJECTIVES

- To investigate the factors affecting the carbon/oxygen isotope composition of the exoskeleton of western rock lobsters as a location specific chemical signature for this species.
- To apply these results as a method for determining the locality of a lobsters origin, at the time of its most recent moult.

METHODS

Reagents and Animals

Sodium hypochlorite (12.5%, Sigma Chemicals) was used as the stock reagent and diluted as needed. Rain water was purified by passage through an ion exchange resin prior to glass distillation at constant temperature. Seawater was sourced off the coast at Watermans, W.A. on a constant flow basis and filtered through sand filters, prior to introduction to the animal aquariums at ambient sea temperatures, 18-22°C or at a constant temperature of 25°C. Western rock lobsters (*Panulirus cygnus*) (carapace length, 70-80mm) were sourced from locations along the Western Australian coastline. Rock lobster pueruli were collected from puerulus collectors and grown through several moults in aquariums. The mussel (*Mytilus edulis*) was used as the staple diet of all the experimental animals and sourced from commercial contractors in Cockburn Sound, Western Australia. Stable isotope ratios, $^{13}C:^{12}C$ and $^{18}O:^{16}O$ were acquired using standard mass spectrometric techniques (CSIRO Land and Water, Perth) and the values are reported in standard ? notation (per mil, ‰) relative to PDB-1 standard (Epstein *et al.*, 1953).

Chemical Preparation of the Exoskeleton for Isotope Ratio Analysis

In a typical sample preparation procedure; the rock lobster was sacrificed by cooling in a freezer, the exoskeleton was removed from the animal, scrubbed, washed with distilled water and placed in a desiccator under reduced pressure for at least 24 hours to dehydrate the material prior to physical and chemical degradation. The exoskeleton was then reduced to a powder in a blender and approximately 100 mg transferred to labeled 5 ml plastic vials. Each portion was treated carefully with ~10% sodium hypochlorite (NaOCl) solution to remove residual organic material from the mixture. After approximately 5 minutes, the exhausted sodium hypochlorite was removed and a further portion of fresh sodium hypochlorite added. This process was

continued with progressively longer reaction times 10, 60 and to ~240 minutes. During the initial applications of sodium hypochlorite the vials were immersed in a cold water bath to minimise the rapid exothermic reaction which ensues.

After this reaction sequence the resultant calcium carbonate ($CaCO_3$) was rinsed with distilled water four times, with thorough mixing, leaving the sample to stand for a least 5 minutes between rinses. The samples were then dried under reduced pressure in a desiccator for at least 24 hours. To check that no organic components remained in the sample, a small portion of the dried powder was dissolved in hydrochloric acid (2M). Should the presence of any remaining organic material be found in the solution, further treatment of the powdered sample with NaOCl for a further ~240 minutes was performed.

The samples were submitted for stable carbon and oxygen (*?* ^{13}C and *?* ^{18}O) isotope analyses.

Experiments designed to examine the efficiency of the chemical technique for the removal of organic components of the exoskeleton

A series of experiments were undertaken to gauge the efficiency and completeness of the removal of organic components using sodium hypochlorite and the sample preparation protocol described above. These experiments were devised in order to evaluate the efficiency of the organic compound removal process and also to obviate confounding isotope ratio results from extraneous organic material which could potentially bias the results.

An artificial calcium carbonate / organic material sample was prepared by mixing either chitin or chitosan as the organic component with calcium carbonate of known isotope ratio, in this case calcium carbonate obtained from red emperor (*Lutijanus sebae*) otolith was used. The ratio of the organic component to the calcium carbonate was 3:7. Four samples each of chitin / calcium carbonate and chitosan / calcium carbonate were used. The starting mass of the samples was 100 mgs. The sample preparation protocol above was used and the carbon and oxygen stable isotope ratios acquired.

Experiments designed to examine the uniformity or variation of the isotope ratio within specific body parts, 'Intra-body part' variation

The uniformity of the isotope ratio for a specific body area of a rock lobsters exoskeleton was examined. Experiments consisted of sampling particular body areas, for example the dorsal carapace or the abdominal plates, at a number of different points for one particular lobster. The aim was to see if the isotope ratio varied for a particular body part for this specific animal, that is, to measure the 'intra-body part' variability.

The dorsal carapace and abdominal plates were taken from one randomly selected lobster collected from the Abrolhos Islands. For each body part, five randomly chosen samples of approximately 100 mgs were sectioned and the samples were treated using the above sample preparation protocol. The resultant white calcium carbonate powder was analysed for the carbon and oxygen isotopic ratios.

Experiments designed to examine the uniformity or variation of the isotope ratio between particular body parts, 'Intra-animal' variation

The variation of the isotope ratio from six different regions of a rock lobsters exoskeleton was examined. Experiments consisted of sampling particular body regions, antenna, dorsal abdomen, dorsal carapace, front right leg, rear left leg and the telson for one particular lobster.

The aim was to see if the isotope ratio varied for particular body parts for this specific animal, that is, to measure the 'intra-animal' variation.

The antenna, dorsal abdomen, dorsal carapace, front right leg, rear left leg and the telson were taken from one randomly selected lobster collected from Garden Island. For each of the six body parts, samples of approximately 100 mgs were collected and the samples were treated using the above sample preparation protocol. The resultant white calcium carbonate powder was analysed for the carbon and oxygen isotopic ratios.

Experiments designed to examine the uniformity or variation of the isotope ratio between the sexes of the western rock lobster

Similar to the 'intra-animal' variation experiment, the isotope ratio of both a male and female rock lobster, collected from the same geographic region and of similar size, was examined. The experiments consisted of sampling particular body regions, namely antenna, dorsal abdomen, dorsal carapace, front right leg, rear left leg and the telson for both the male and female lobsters collected from Jurien. The aim was to see if the isotope ratio varied for a particular body part between the sexes, and to measure this variation.

The antenna, dorsal abdomen, dorsal carapace, front right leg, rear left leg and the telson were taken from both the male and female lobsters collected from Jurien. For each of the twelve body parts, samples of approximately 100 mgs were collected and the samples were treated using the above sample preparation protocol. The resultant white calcium carbonate powder was analysed for the carbon and oxygen isotopic ratios.

Experiments designed to examine the effect of temperature on the isotope ratio

Two experiments were performed concurrently and designed to examine the effect of temperature at the time of moulting on the isotope ratio. Twelve juvenile laboratory-reared lobsters (carapace length 25-30 mm) were kept in a continuous flow through aquarium system, six of which were held at a constant temperature of 25°C and the remaining six at ambient seawater temperature (variable 18°C to 22°C). All of the lobsters were fed a similar diet of fresh mussels daily. The lobsters were monitored for one full moult cycle, at which stage three of the lobsters at ambient temperature and three of the lobsters held at constant temperature were sacrificed five days post-moult. The dorsal carapace and abdominal plates were removed from each of the animals, and subjected to the sample preparation protocol and analysed. The remaining three lobsters at ambient temperature and three lobsters held at constant temperature were monitored for a further full moult cycle and sacrificed five days post-moult. Again, the dorsal carapace and abdominal plates were removed from each of the animals, subjected to the sample preparation protocol and analysed.

RESULTS / DISCUSSION

The ultimate aim of this project was to produce a research tool, which employed chemical techniques to examine the coastal origin of lobsters suspected of undertaking the northwesterly migration of using the stable isotopes of carbon and oxygen. The intrinsic value of developing such a research tool relies on the fact that the marking of the animal in this natural, non-intrusive way, does not allow the tag to be interfered with. In order to develop such a research tool, it was first necessary to achieve an understanding of possible limitations of this technique by examining some of the fundamental traits of the carbon and oxygen isotopes which form the basis of this method.

The scientific basis for this project can be found in the inherit nature of the stable isotopes to be examined. This in principle, is governed by the environment in which the stable isotopes are incorporated into the carbonate matrix of the exoskeleton (Aharon, 1991). As an example, at it's most simplistic, it has been shown that changes in the oxygen isotope ratio can be related to changes in temperature, thus carbonate formed at a certain temperature has an oxygen isotope ratio which in principle is governed by this temperature at which it is incorporated into the carbonate (Wefer and Berger, 1991). If this is the main underlying biogenetic trait which governs the changes in the oxygen isotope ratio for carbonates occurring in poikilothermic organisms, and other perturbing effects are considered small and uniform during deposition, then conceivably the sourcing of carbonate from different temperature regimes will have different oxygen isotope ratios. Thus, a fundamental tenet of this project is, that if a western rock lobster which is caught at Garden Island in say, 10-15 metres of water (Figure 1), should have a different oxygen isotope ratio to a similar size lobster caught in Jurien (at a similar depth, Figure 1) or further north, by virtue of the fact that the seawater temperature would be different. The *proviso* to this rule is that the rock lobsters have lived in their respective areas for at least one moult and indeed the water temperatures are significantly different. Further expansion of this paradigm from oxygen stable isotopes to carbon stable isotopes in the carbonate, which can be shown to have a relationship to the carbon isotope ratios of the carbon found in the food consumed (Gauldie, 1996), allows a multi-dimensional approach to the development of this research tool. Thus, taking the example above, if the Garden Island rock lobster feed principally on molluscs sourced from seagrasses, while the rock lobster from

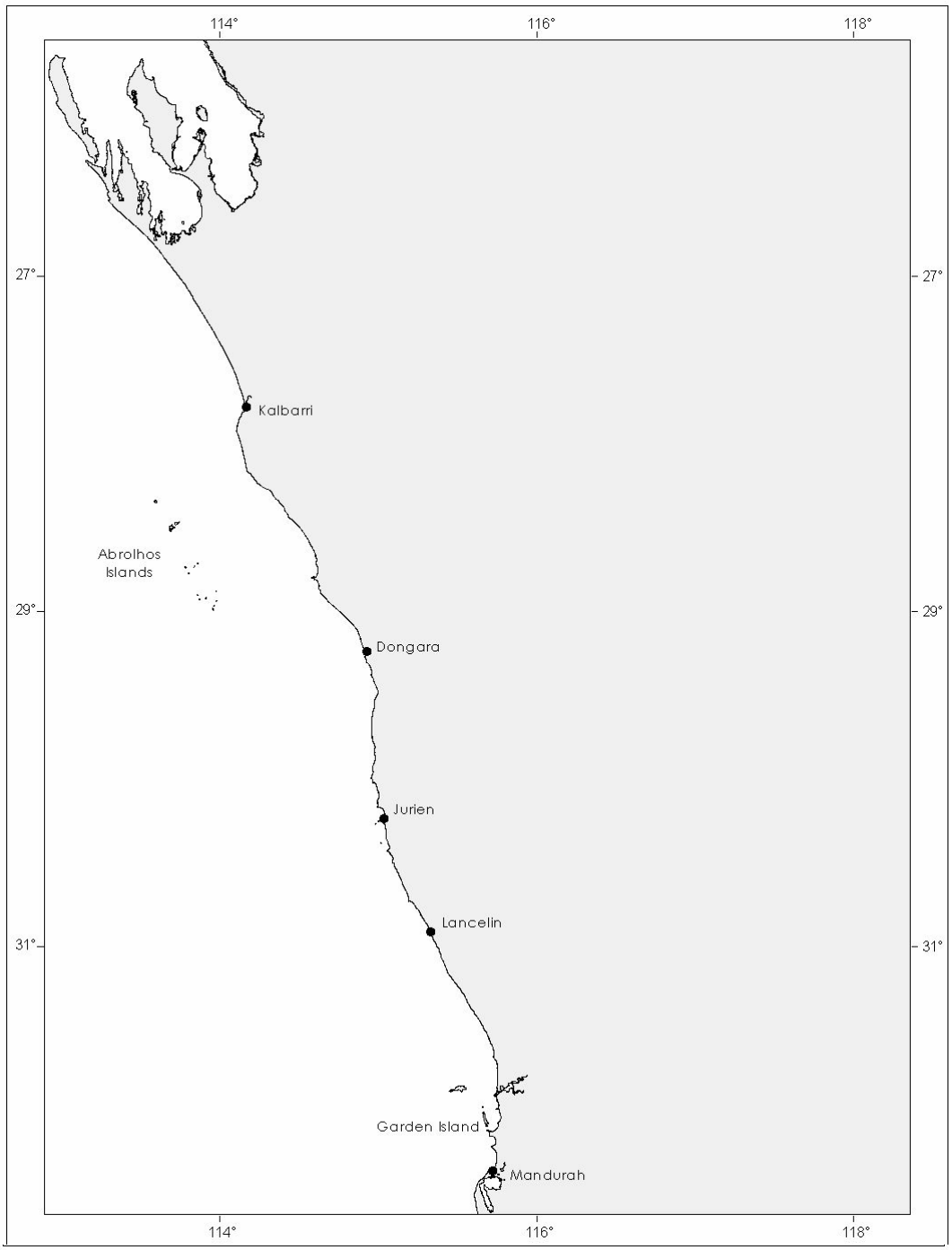

Figure 1. Portion of the West Australian coastline showing the location of where rock lobsters were sourced for this project.

Jurien feed principally on the remains of coralline algae and say the remains of dead fish, then again the carbon isotope signatures found in the carbonate for the respective exoskeletons of the two animals would be different. Thus, the oxygen and carbon ratios together would provide a means of distinguishing the two animals, (i.e. provide a natural tag).

A fisheries research tool using these stable isotope biomarkers can be proposed based on the above principle. For example, if the Garden Island rock lobster above, moves from this region to a more northly region offshore of Lancelin, where it is caught, then intuitively the isotopes in the carbonate of it's exoskeleton should be different to a rock lobster which has been a resident

of this offshore region of Lancelin. The *proviso* to this generalisation is that there are sufficient and significant isotope discriminating effects between these two regions and that the incorporation of the isotopes in the rock lobsters results in unique values to the extent that the underlying signature values do not over lap between the regions.

Table 1. Stable isotope results for the examination of the removal of organic components from calcium carbonate (‰ PDB).

Sample Type	? ?^{13}C ‰ PDB	? ^{18}O ‰ PDB
Otolith $CaCO_3$ Standard	-3.33	-0.26
$CaCO_3$ / Chitosan	-3.30	-0.28
$CaCO_3$ / Chitosan	-3.36	-0.30
$CaCO_3$ / Chitosan	-3.33	-0.29
$CaCO_3$ / Chitosan	-3.39	-0.34
$CaCO_3$ / Chitin	-3.32	-0.22
$CaCO_3$ / Chitin	-3.36	-0.28
$CaCO_3$ / Chitin	-3.35	-0.24
$CaCO_3$ / Chitin	-3.35	-0.34

Evaluation of the chemical method for removal of the organic component of the exoskeleton.

Treatment of the freshly excised exoskeleton from a rock lobster with 10% sodium hypochlorite (bleach) was required to remove the organic materials (proteins, carotenoids, chitin, etc.) in the exoskeleton, which could potentially bias or confound the carbon and oxygen isotope results. In order to measure the efficiency of this method, a standard bleaching protocol (*vide sopra*) (Pingitore *et al.*, 1993) was adopted and applied to mixtures of calcium carbonate of known isotope ratio with chitosan (deacylated chitin) and chitin (a biopolymer consisting of unbranched chains of ?-(1->4)-2-acetamido-2-deoxy-D-glucose, which commonly occurs in the integument of marine arthropods) i.e. an artificial exoskeleton matrix (Stevenson, 1985). Clearly from the data shown in Table 1 and Figure 2, the removal of the organic material from the calcium carbonate was an efficient and readily repeatable process, the mean for chitin/$CaCO_3$ & chitosan/$CaCO_3$ samples were ?^{13}C = -3.34‰ and ?^{18}O = -0.28‰ while the $CaCO_3$ reference was ?^{13}C = -3.33‰ and ?^{18}O = -0.26‰. It is noted however, that in most carbonate skeletons, organic matrix materials are finely disseminated throughout the carbonate in minute, intercrystalline and intracrystalline voids and complete removal of the organics is impossible without dissolution of the carbonate (Gaffey and Bronnimann, 1993). Gaffey and Bronnimann also showed that the use of 5% sodium hypochlorite on their samples reduced the TOC content from 10.78% in the untreated sample to, on average 0.55% post treatment. They also note that the sodium hypochlorite method is clearly the most effective method for removal of the organic material (Gaffey and Bronnimann, 1993).

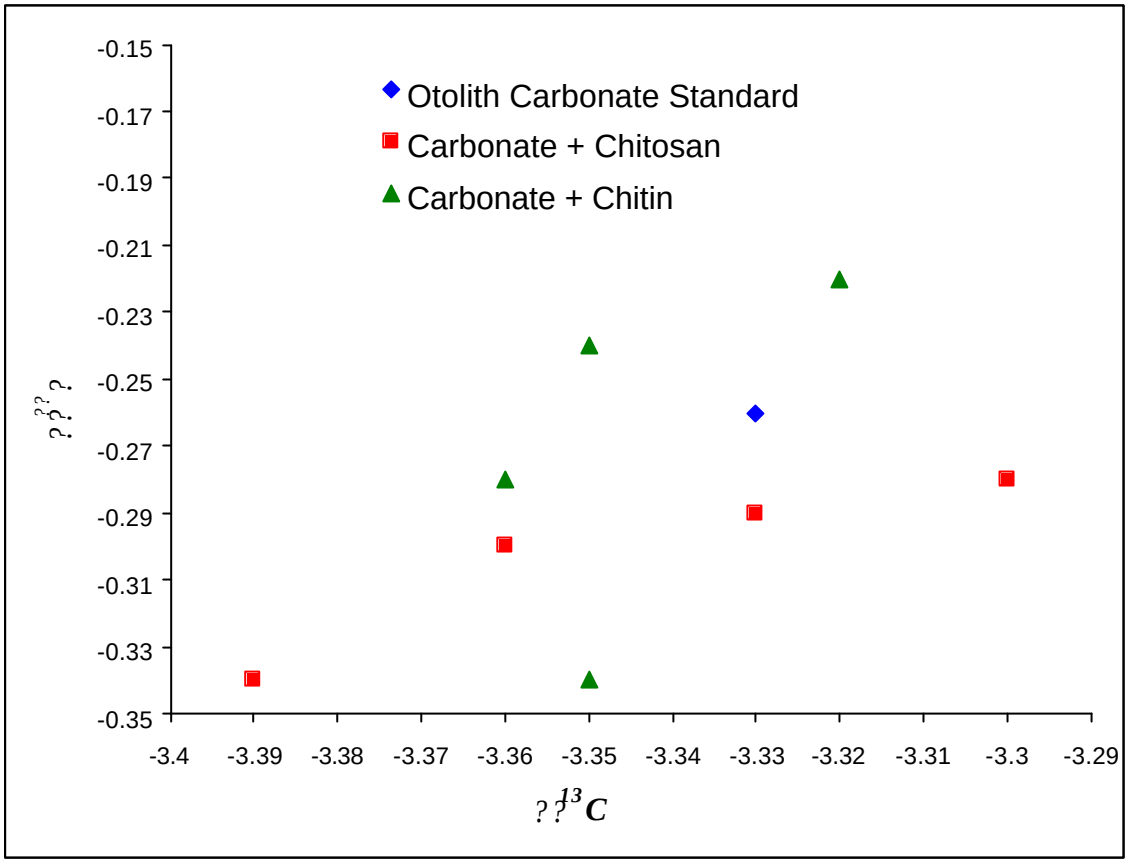

Figure 2. Graph of stable isotope results for the examination of the removal of organic components from calcium carbonate (‰ PDB).

Intra-body part variation

Analysis of the carbonate carbon and oxygen isotope ratios at different points for a specific body part was needed to establish the degree of variation of the isotope ratios within a particular body part. The data from the analyses at five points on each of the dorsal carapace and the abdominal plates for one animal from the Abrolhos Islands are shown in Table 2 and Figure 3. Clearly, the degrees of variation in the dorsal carapace ? ?^{13}C = 0.64‰ and ? ?^{18}O = 0.87‰ and the abdominal plates ? ?^{13}C = 0.52‰ and ? ?^{18}O = 0.71‰ were unexpectedly large for both body parts. A thorough explanation as to the underlying reason for this unusual result was not investigated, but suffice it to say that a combination of physiological factors and more thorough understanding of the mechanism by which the rock lobster generates it's exoskeleton, i.e. the mechanism of biosynthetic production of the calcium carbonate / organic material matrix, could potentially elude to the variation in the isotope ratios found here. Furthermore, a larger number of samples for each body part with averaging and standard deviation treatments, could potentially furnish carbon and oxygen isotope values indicative of each body part. Also sampling of more than one animal, from different geographic locations in addition to sampling from more areas of the rock lobsters body would enable a more descriptive account of the initial results reported here. As this type of experimentation was not only timely, but also costly, and went beyond the initial premise of the research proposal, it was not investigated further.

Table 2. Stable isotope results for examination of 'Intra-body part' variation (‰ PDB).

Sample Type	? ?^{13}C ‰ PDB	? ^{18}O ‰ PDB
Dorsal Carapace-1	-3.16	-2.26
Dorsal Carapace-2	-2.98	-2.01
Dorsal Carapace-3	-3.62	-2.86
Dorsal Carapace-4	-3.61	-2.88
Dorsal Carapace-5	-3.60	-2.68
Abdominal Plate-1	-3.33	-2.25
Abdominal Plate-2	-3.39	-2.35
Abdominal Plate-3	-3.01	-1.71
Abdominal Plate-4	-3.52	-2.42
Abdominal Plate-5	-3.00	-1.99

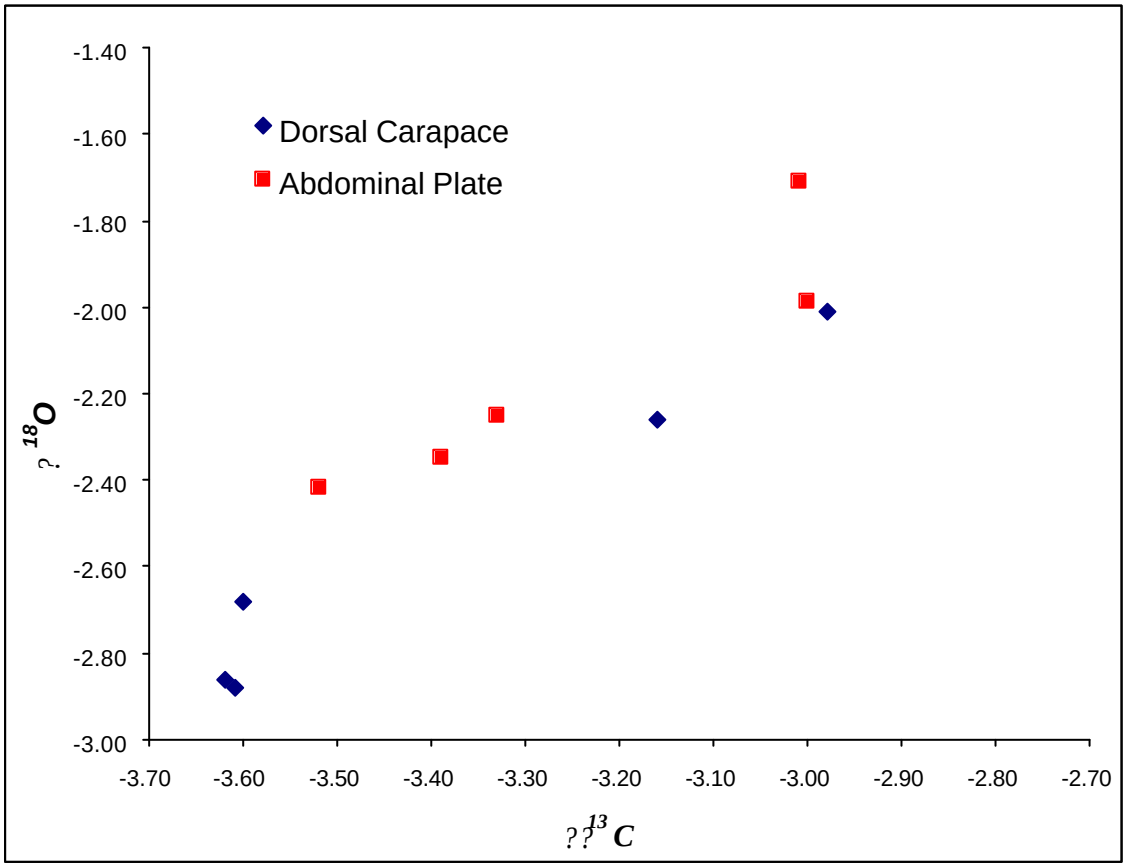

Figure 3. Graph of stable isotope results for the examination of 'Intra-body part' variation (‰ PDB).

Intra-animal variation

Further to the large variation in isotope ratio within a body part, an understanding of the intra-animal variation or the variation between body parts was undertaken. Sampling and analysing six anatomically independent exoskeleton parts of a rock lobster collected from Garden Island was conducted (Table 3 and Figure 4). Variation in the oxygen isotope ratio was significantly large, ??^{18}O = 2.16‰, while that for the carbon isotope ratios was of a similar size found for intra-body part variations, ??^{13}C = 0.80‰. These unusual large ranges of isotope values defied expectation and require further experimentation as to the reason why they occur. As this further experimentation went beyond the scope of the research project in it's present form, no further studies were instigated in order to further understanding the reason behind the values attained here.

Table 3. Stable isotope results for examination of 'Intra-animal' variation (Garden Island rock lobster) (‰ PDB).

Sample Type	??^{13}C ‰ PDB	?^{18}O ‰ PDB
Antenna	-2.60	0.29
Dorsal Abdomen	-2.27	0.63
Dorsal Carapace	-3.07	0.60
Front Right Leg	-2.73	0.40
Rear Left Leg	-3.02	-1.53
Telson	-2.71	-0.35

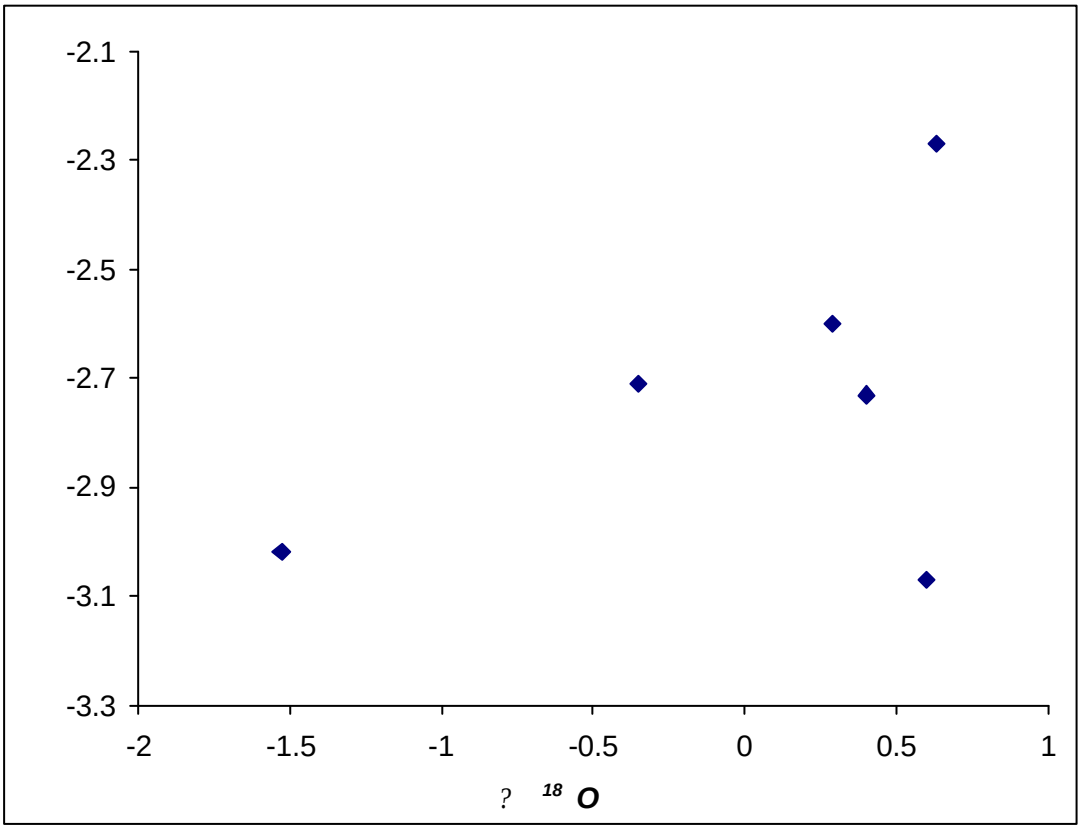

Figure 4. Graph of the stable isotope results for examination of 'Intra-animal' variation (Garden Island rock lobster) (‰ PDB).

Variation of isotope ratio between the sexes

Variation of the isotope ratio with the sex of the rock lobster was also investigated. A male and female rock lobster of similar size from Jurien was used. Exoskeleton from six independent anatomical regions of the rock lobsters were excised and analysed, (Table 4 and Figure 5). Not surprisingly, for both the male and female rock lobsters examined, there were large variations in the carbon and oxygen isotope ratios for the six body parts sampled (*vide sopra*, "Intra-animal variations") male : ??^{18}O = 1.70‰, ??^{13}C = 0.90‰, female : ??^{18}O = 1.36‰, ??^{13}C = 0.88‰. However, the mean of these ratios for each sex were comparable, male ?$^{18}O_{mean}$ = 0.24‰, and ?$^{13}C_{mean}$ = -3.13‰, female : ?$^{18}O_{mean}$ = 0.11‰, and ?$^{13}C_{mean}$ = -2.98‰. Clearly, averaging of a number of exoskeleton samples for both male and female rock lobsters suggests that there is no significant difference with sex in either the carbon or oxygen isotope ratios.

Table 4. Sexual variation of stable isotope (Jurien rock lobster) (‰ PDB).

Sample Type	Sex	??^{13}C ‰ PDB	?^{18}O ‰ PDB
Antenna	Male	-3.51	-0.12
Dorsal Abdomen	Male	-3.49	-0.51
Dorsal Carapace	Male	-3.17	0.71
Front Right Leg	Male	-2.61	-0.2
Rear Left Leg	Male	-2.75	0.35
Telson	Male	-3.26	1.19
Antenna	Female	-2.92	-0.25
Dorsal Abdomen	Female	-3.16	-0.19
Dorsal Carapace	Female	-3.54	-0.23
Front Right Leg	Female	-2.66	-0.16
Rear Left Leg	Female	-2.83	0.37
Telson	Female	-2.75	1.11

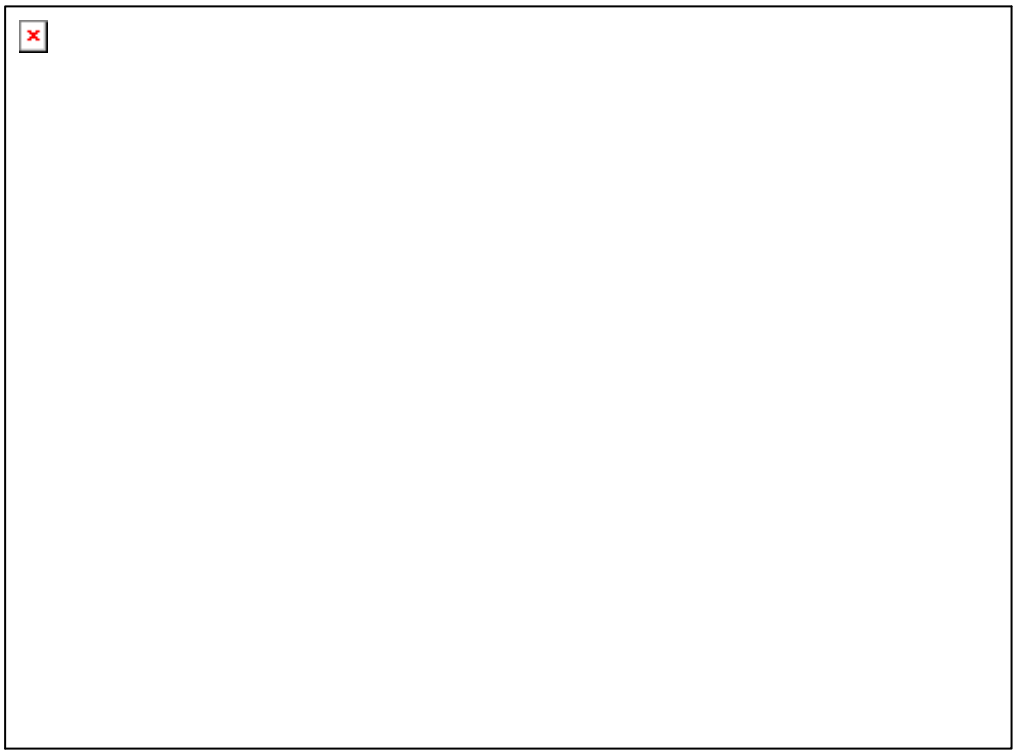

Figure 5. Graph of the stable isotope result variations with sex of the rock lobster (‰ PDB).

Table 5. Stable isotope results from the experiments designed to examine the effect of temperature on the isotope ratio (‰ PDB).

Sample Type	Temperature	Moult	$\delta^{13}C$ ‰ PDB	$\delta^{18}O$ ‰ PDB
Dorsal Carapace	Ambient	*First*	-1.97	-1.27
Dorsal Carapace	Ambient	*First*	-2.18	-1.55
Dorsal Carapace	Ambient	*First*	-2.36	-1.68
Abdominal Plates	Ambient	*First*	-1.99	-1.61
Abdominal Plates	Ambient	*First*	-1.98	-1.34
Abdominal Plates	Ambient	*First*	-2.83	-1.85
Dorsal Carapace	25ºC	*First*	-2.68	-2.09
Dorsal Carapace	25ºC	*First*	-2.69	-2.13
Dorsal Carapace	25ºC	*First*	-2.16	-1.53
Abdominal Plates	25ºC	*First*	-2.46	-1.69
Abdominal Plates	25ºC	*First*	-2.42	-1.97
Abdominal Plates	25ºC	*First*	-2.28	-1.54
Dorsal Carapace	Ambient	*Second*	-1.52	-1.95
Dorsal Carapace	Ambient	*Second*	-1.63	-1.92
Dorsal Carapace	Ambient	*Second*	-1.45	-1.61
Abdominal Plates	Ambient	*Second*	-1.77	-2.20
Abdominal Plates	Ambient	*Second*	-1.52	-1.33
Abdominal Plates	Ambient	*Second*	-1.77	-2.01
Dorsal Carapace	25ºC	*Second*	-1.96	-2.49
Dorsal Carapace	25ºC	*Second*	-1.24	-0.58
Dorsal Carapace	25ºC	*Second*	-2.04	-2.74
Abdominal Plates	25ºC	*Second*	-2.35	-2.83
Abdominal Plates	25ºC	*Second*	-1.09	-0.38
Abdominal Plates	25ºC	*Second*	-2.12	-2.85

The effect of temperature on the isotope ratio

In order to measure the effect of temperature on the isotope ratio of the exoskeleton, juvenile rock lobsters (carapace length from 25-30mm) were grown through several moults and fed the same diet of mussels during this period. Subsequent to this period, six rock lobsters were kept under isothermal conditions at 25?C. The rock lobsters kept at ambient seawater temperatures experienced a temperature range from 18-22?C during the course of the experiment. All lobsters continued to be fed mussels. Lobsters were sacrificed five days post-moult after the first and second moults under these temperature regimes. The data is presented in Table 5 and in Figure 6. Interestingly, the means of the oxygen isotope ratios showed a small but significant and expected trend of greater depletion with increasing temperature, first moult $?^{18}O_{ambient}$ = *-1.55‰* compared with $?^{18}O_{25C}$ = *-1.83‰* and second moult $?^{18}O_{ambient}$ = *-1.84‰* compared with $?^{18}O_{25C}$ = *-1.98‰*. Puzzlingly, comparison of the first moult with the second moult oxygen isotope means for the rock lobsters held at isothermal temperatures showed a small but significant change which is similar in magnitude to that seen for the second moult variation between the temperature regimes, i.e. first moult $?^{18}O_{25C}$ = *-1.83‰* compare with second moult $?^{18}O_{25C}$ = *-1.98‰*.

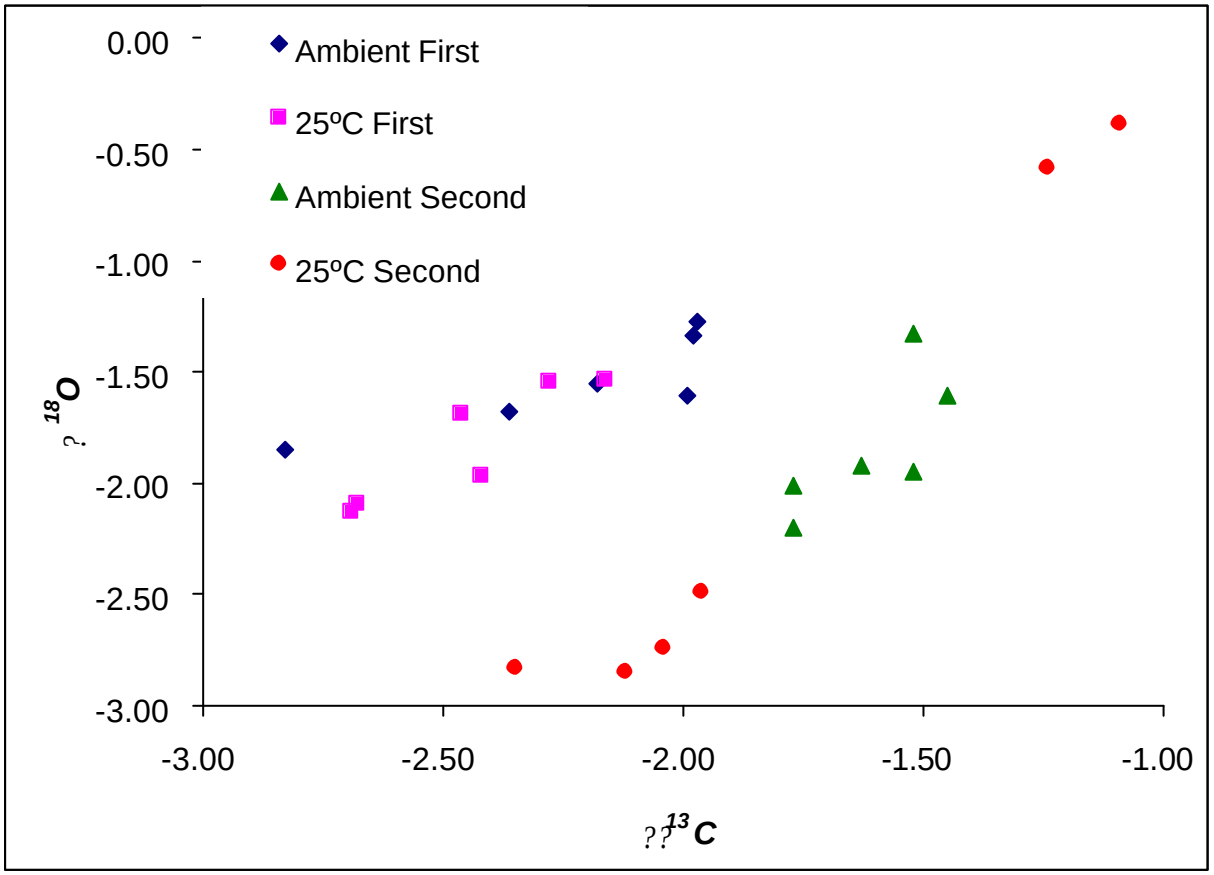

Figure 6. Graph of the stable isotope results from the experiments designed to examine the effect of temperature on the isotope ratio (‰ PDB).

In the context of this project, can the stable isotopes of carbon and oxygen in the calcium carbonate of the exoskeleton of the rock lobsters be applied as a fisheries research tool?

The results presented thus far clearly show that the stable isotopes of the carbonate in the exoskeleton for individual animals have a large range of values within a body part, for different body parts, and with temperature, while the isotope ratio between sexes of the animals of similar size and geographic location are comparable. The results of these preliminary experiments constitute *prima facie* evidence to the effect that the use of exoskeleton carbonates isotope ratios, as a research tool would prove impossible on an animal-to-animal basis.

To illustrate this point, collection of wild rock lobsters from Garden Island (two females), Lancelin (two females), Jurien (one male and one female), and Dongara (two females and two males) was conducted. Exoskeleton was excised from six anatomically different regions of each of the rock lobsters; *vide sopra* intra-animal experiments, and the stable isotope ratios of oxygen and carbon determined for each of these samples. The results were averaged for each animal and the data presented in Figure 7. Clearly, from the results found, the large deviation of stable isotopes for individual animals results in an overlapping of the values achieved for many of the regions where the rock lobsters were sourced. Thus, expectantly, the method fails to adequately discriminate between regions based on the isotope ratios found for the individual animals.

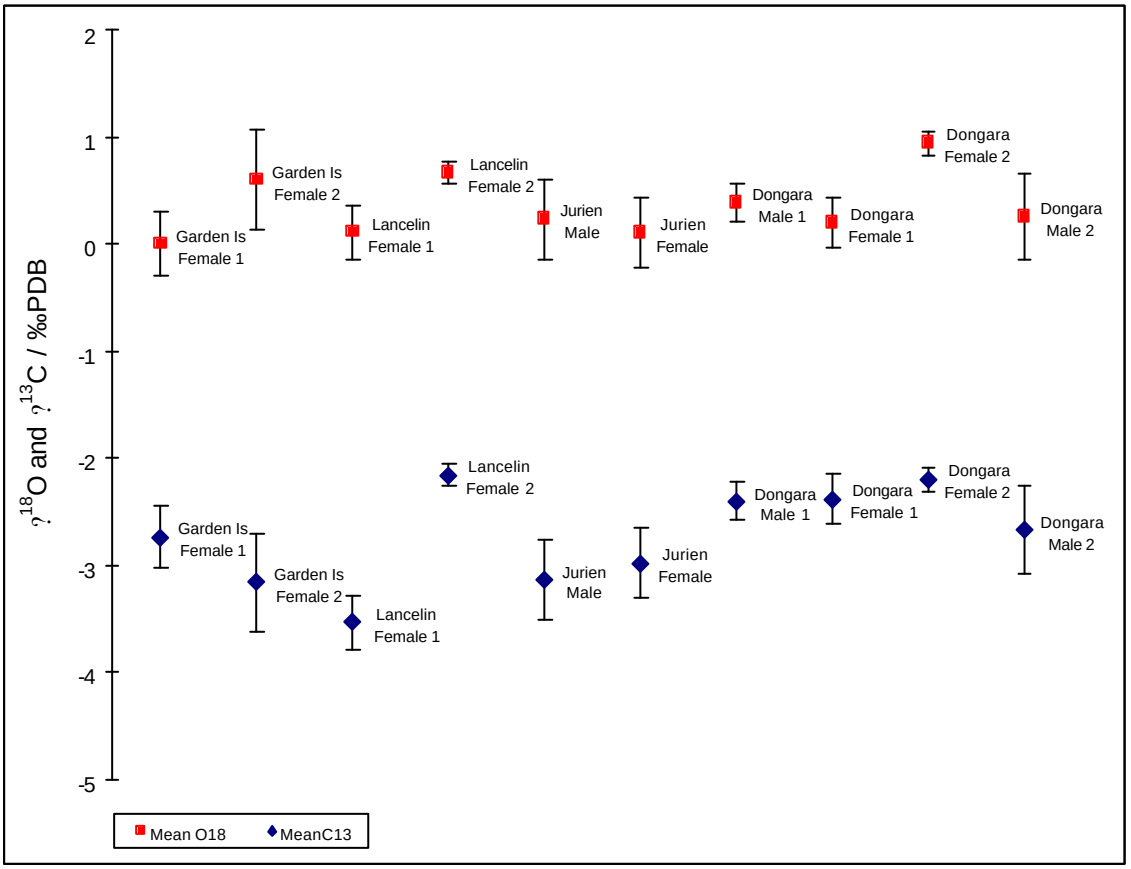

Figure 7. Graph of averaged stable isotope results (with standard deviations) for rock lobster from different locations on the West Australian coastline.

Comparison of intra-animal variation, variation of isotope ratio between the sexes and pilot study

Comparison of the isotopic carbonate results from the intra-animal variation and the variation of isotope ratio between the sexes is inconsistent. Both sets of results have data relating to the isotopic variation of the animals body parts (Tables 3 and 4). However, the data in Table 3 was used to select the body part to be used for this study. Table 3 shows that the lightest oxygen isotope ratio values were observed in the legs and telson and the heaviest in the carapace. Therefore, from these results the carapace was chosen as the body part to be used for the study. The premise for this selection was that higher oxygen isotope ratio values would represent body areas were the carbonate was deposited more slowly and in so would represent the surrounding water temperature (location). However, when the data in Table 4 is examined in the same context it is evident the telson and front legs now have the highest oxygen isotope ratio values and the carapace has some of lightest values.

The results in Table 4 suggest that the telson (and legs) have the highest oxygen values and in hindsight may have been a better body part to use as a proxy for water temperature (location). This also agrees with the location of these body parts on the animals. The legs and telson are at the extremities of the animal and would therefore be expected to have slower carbonate growth and in so a better proxy for water temperature (and location). The pilot study to this work examined the carbon and oxygen isotope ratio values of the rear legs (because they were easily obtained from fisherman) to identify any locational trends. The pilot study showed some trends with the oxygen isotope ratio values and water temperature, which suggests that the rear legs may be better proxy (than the carapace) for water temperature.

CONCLUSION

It is clear from the results of the experiments conducted here that the initial hypothesis being tested was not true for the body part used. Consequently, it was not possible to develop a technique to distinguish the origins of rock lobsters, which utilised carbon and oxygen isotope ratios of the carbonate found in the exoskeleton of the western rock lobster. This chemically based research tool, while simplistic and desirable in concept was not forthcoming in experimentation due to a number of fundamental scientific principles and processes that must more fully be understood and developed further prior to application in a management sense. These include, a deeper understanding of the mechanism by which the rock lobster sequesters the oxygen and carbon from the natural environment, the mode of formation of the calcium carbonate and possible perturbing influences during calcification events and the role of the organic components to the structural make up of the exoskeleton and their influence on the isotope ratios. Finally due to the large degree of isotope ratio variability throughout a rock lobsters exoskeleton and also between rock lobsters with similar characteristics, statistically several rock lobsters rather than the animal-to-animal method used here could prove pivotal to the successful application of isotope ratio results as a research tool.

FURTHER DEVELOPMENT

Studies directed towards the development of a research tool to assess migration in western rock lobsters, or indeed other crustaceans that have long inter-moult periods would need to address critical areas of the animals biology in the first instance and subsequently on the experimental design. For example, an understanding of the physiological factors and a more thorough appreciation of the mechanism by which the rock lobster generates it's exoskeleton, i.e. the mechanism of biosynthetic production of the calcium carbonate / organic material matrix, could

potentially elude to the variation in the isotope ratios found here. Furthermore, a larger number of samples for each body part with averaging and standard deviation treatments, could potential furnish carbon and oxygen isotope values indicative of each body part. This may allow a better understanding of the mechanisms by which the carbonate is deposited and a body part (e.g. legs, telsons) that depicts water temperature (location) more accurately. Also sampling of more than one animal, from different geographic locations and sampling from more areas of the rock lobsters body would allow a more descriptive discussion of the results reported here.

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