Diet quality and muscle tissue location influence consumer-diet discrimination in captive-reared rock lobsters (*Panulirus cygnus*).

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Abstract

Fundamental to the accuracy of stable isotope analysis in trophodynamic studies is the ability to predict discrimination between a consumer and its diet. Despite the widespread use of stable isotope analysis in trophic ecology, uncertainty still surrounds the factors affecting consumer-diet discrimination. Here we present evidence that diet quality and location of muscle tissue analysed affects the consumer-diet discrimination for the western rock lobster, *Panulirus cygnus*. Consumer-diet $\delta^{15}N$ and $\delta^{13}C$ discrimination for western rock lobster tail tissue were 1.67-2.97‰ and 2.92-3.60‰ respectively, with $\delta^{13}C$ discrimination differing to values reported in the literature. Differences in nitrogen and carbon discrimination were observed between tail and leg tissue of lobsters of 1.22‰ and 1.13‰ respectively. Diet quality was also found to affect consumer-diet discrimination, with high protein pilchard diet leading to lower $\delta^{15}N$ and higher $\delta^{13}C$ discrimination. Diet quality should be considered as a factor that has the potential to affect consumer-diet discrimination when interpreting results from stable isotope studies.

Introduction

The ability to predict consumer-diet discrimination in stable isotopes of carbon and nitrogen (differences in $\delta^{15}N$ and $\delta^{13}C$ between a consumers' tissue relative to its diet (Minagawa and Wada 1984; Robinson 2001)) has allowed ecologists to unravel complex trophic interactions (e.g. Rounick and Winterbourne, 1986, Davenport and Bax, 2002). Predictable patterns in consumer-diet δ13C discrimination helps identify the ultimate sources of primary production that support consumers (DeNiro and Epstein 1978), while patterns in consumer-diet δ15N discrimination allow us to determine the trophic position occupied by consumers (DeNiro and Epstein 1981; Post 2002).

Early studies indicated $\delta^{13}C$ consumer-diet discrimination exhibited little variability (0-1‰ between trophic levels) (DeNiro and Epstein 1978; McConnaughey and McRoy 1979), however more recently, measures of consumer-diet $\delta^{13}C$ discrimination have been suggested to be more variable than first thought (between -10‰ and 1.3‰) (Gannes et al. 1997; McCutchan Jr et al. 2003; Crawley et al. 2007). Similarly, consumer-diet $\delta^{15}N$ discrimination has been observed to be highly variable. Vanderklift and Ponsard (2003) observed $\delta^{15}N$ discrimination to range from -3.22 to 5.89‰, while Post (2002) determined $\delta^{15}N$ discrimination to range from 0.5 to 5.5‰.

A number of factors have been identified as contributing to variability in $\delta^{15}N$ and $\delta^{13}C$ discrimination. Variation in consumer-diet $\delta^{13}C$ discrimination has been suggested to depend on organism diet, respiration rate and tissue type (Hobson and Clark 1992; Pinnegar and Polunin 1999; Hobson and Bairlein 2003; McCutchan Jr et al. 2003). Similarly, variation in $\delta^{15}N$ discrimination have been shown to vary depending on organism diet, mode of excretion, taxon, nutritional condition and tissue type (Fantle et al. 1999; Pinnegar and Polunin 1999; Ponsard and Averbuch 1999; Vanderklift and Ponsard 2003).

The effect of muscle tissue type and diet quality on consumer-diet discrimination is known to be large, but unpredictable (eg. Fantle et al. 1999; Pinnegar and Polunin 1999). While tissue type has been determined to affect both $\delta^{13}C$ and $\delta^{15}N$ discrimination (DeNiro and Epstein 1978; DeNiro and Epstein 1981; Tieszen et al. 1983; Pinnegar and

Polunin 1999; Schmidt et al. 2004; Seminoff et al. 2006), considerably less research effort has focused on the influence of the location of muscle tissue on discrimination (Pinnegar and Polunin 1999). Muscle tissue is a common tissue used for ecological studies conducted on many taxa including fish, birds, mammals, and crustaceans (Fry and Parker 1979; Bunn et al. 1995; Davenport and Bax 2002). If the $\delta^{13}C$ or $\delta^{15}N$ discrimination is variable for the same tissue type taken from different parts of the body, this will affect results gathered from trophodynamic studies.

The effect of diet quality on consumer-diet discrimination is also largely unknown, despite the fact that diet quality may vary spatially or temporally in the wild. Knowledge of the effect of diet quality on consumer-diet discrimination will be useful in refining discrimination values for consumers where diet quality is known to vary on spatial or temporal scales.

Due to known variability in discrimination with tissue type, measures of discrimination must be used corresponding to the tissue type sampled to ensure accurate interpretation of results from ecological studies. While variation in discrimination between tissue types of an organism are well documented (Meyer-Rochow et al. 1992; Hobson 1995; Pinnegar and Polunin 1999; Seminoff et al. 2006), few studies have sought to determine the variability within muscle tissue taken from different parts of an organism. Pinnegar and Polunin (1999) determined that variability between muscle tissues of an organism can be considerable, prompting us to compare discrimination between white muscle tissues in our study.

Diet quality has been shown to affect consumer-diet $\delta^{13}C$ and $\delta^{15}N$ discrimination (Dittel et al. 2000). Quality of diet items available to consumers in the wild is known to vary both spatially and temporally (eg. Joll and Phillips 1984). Thus, the effect of diet quality on the consumer-diet discrimination should be investigated to account for observed variability in discrimination with diet quality that may otherwise introduce error into results from trophic studies. Carbon to nitrogen ratios (C:N) can be used to indicate diet quality (Fantle et al. 1999). As nitrogen is mostly present as protein, diets with a low C:N ratio contain a greater proportion of protein and are of higher quality to a consumer relative to diets with a high C:N ratio (Fantle et al. 1999).

We use the western rock lobster (*Panulirus cygnus* George) to determine the effect of location of muscle tissue and diet quality on consumer-diet discrimination. The western rock lobster is a spiny lobster species distributed along the west coast of Western Australia between Cape Leeuwin (34° 22' S, 115° 8' E) and North West Cape (21° 48' S, 114° 9' E) (Chittleborough 1970). This species is highly abundant along its distributional range, forming the basis of Australia's largest single species fishery with over 14 000 tonnes caught during the 2004/2005 fishing season (Fletcher and Head 2006). Western rock lobsters are known to consume a wide variety of plant and animal material, including coralline algae, seagrass, and a wide variety of macroinvertebrate fauna (Joll and Phillips 1984; Edgar 1990; Jernakoff et al. 1993).

The current study aims to determine the effect of muscle tissue location and diet quality on the consumer-diet discrimination of western rock lobsters. Specifically we will test the following null hypotheses. (i) Muscle tissue location does not affect consumer-diet discrimination, and (ii) Diet quality does not affect consumer-diet discrimination.

Materials and Methods

Pre-Experiment

Lobsters were collected as post-puerulus and raised on a diet of pellets and the mussel, *Mytilus edulis* (Johnston et al. 2007). Prior to the experiment commencing, lobsters were kept in 4 circular tanks, 1 m diameter, 0.8 m deep for 30 days to acclimate. At the commencement of experiments, lobsters were juveniles, approximately 2 years post-puerulus and between 54.6 and 61.1 mm carapace length (CL).

Experimental design

Following acclimation, individual lobsters were randomly allocated to one of 8 tanks (0.6 m × 0.4 m × 0.44 m). Each tank was split into two compartments (0.3 m × 0.4 m × 0.4 m) using a shade-cloth and PVC screen, with one lobster per compartment. The PVC screen prevented exchange of food particles but not water between compartments. Exchange of water between compartments was important as spiny lobsters are gregarious animals (Atema and Cobb 1980; Cobb 1981), with conspecific detection occurring via chemical cues (Zimmer-Faust and Spanier 1987).

Plastic mesh was attached to partitions in the center of tanks to form a roof under which lobsters could shelter. Flow rates of the tanks were maintained at 72 L. hr^{-1}. Lighting was ambient (approx 14 hours light/ 10 hours dark). Seawater input was directly from the ocean and unheated, meaning water temperatures were consistent with temperatures experiences in coastal lagoons in this region during this time of year (between19 °C to 21 °C over the course of the experiment). The experiment was run for 119 days (17 weeks), from 16th January 2006 until 15th May 2006.

Diet Manipulation

Prior to commencement of experiment, 4 lobsters were sacrificed and tail and leg tissue samples taken for stable isotope analysis. The remaining 16 lobsters had one of four diets randomly allocated to them. Diets of differing qualities (C:N ratio) were used. We used C:N ratios as a proxy for diet quality, as a diet with low C:N ratio will presumably have a higher proportion of protein (Dittel et al. 2000).

Diets fed to lobsters were (i) mussels (*Mytilus edulis*) (supplier – Blue lagoon mussels, Rockingham, Western Australia), (ii) Australian pilchards (*Sardinops sagax*) (WA bait supply, O'Connor, Western Australia), (iii) coralline algae (*Amphiroa gracilis*) (collected from Marmion lagoon (31° 44′ S, 115° 40′ E)) and, (iv) a mussel/ coralline algae mix (mussels and coralline algae fed to lobsters on alternate weeks). These diets are representative of diets consumed in the wild. All diets were collected at the same time and frozen to minimise variation in isotopic signature over time. Frozen diet samples did not differ in isotopic signature between t0, t65, and t119 (one-way ANOVAs; $p > 0.20$).

The pilchard diet was significantly higher in $\delta^{15}N$ than both mussels and coralline algae (one-way ANOVA; $F_{2,9} = 57.87$, $p < 0.001$) and lower in $\delta^{13}C$ relative to mussels and algae (one-way ANOVA; $F_{2,9} = 2.14$, $p = 0.029$), whilst coralline algae had a higher C:N ratio than mussels and pilchards (one-way ANOVA; $F_{2,12}$, $p = 0.03$) (Table 1). Mussels and coralline algae did not significantly differ in $\delta^{15}N$ or $\delta^{13}C$ (Tukey test, $p > 0.05$). Lobsters were fed daily to excess, with uneaten food removed prior to the addition of fresh food. At the commencement of the experiment (t0) and 32, 64, 96, and 119 days later a randomly chosen leg was removed to identify changes in $\delta^{15}N$ or $\delta^{13}C$ over time. Limb loss amongst crustaceans is a natural process termed autotomy (Robinson et al. 1970). At the conclusion of the experiment, samples of tail tissue were taken from all lobsters for stable isotope analysis.

Assessment of lobster condition

At the conclusion of the experiment, blood protein concentration of lobsters was determined using a protein refractometer. Blood protein concentration provides a measure of lobster nutritional condition (Dall 1974), allowing the condition of lobsters fed the different diets to be compared. This comparison was made using one-way ANOVA.

Tissue Analysis

Leg and tail white muscle tissues were washed using de-ionised water before being dried in an oven at 60°C for 72 hours. Tissue was then ground in a ball mill grinder before being stored in centrifuge tubes in a dessicator. $\delta^{15}N$ and $\delta^{13}C$ were measured by continuous-flow isotope ratio mass spectrometry using ANCA-NT (Europa Scientific, Crewe, UK) interfaced with a 20-20 isotope ratio mass spectrometer (Europa Scientific, Crewe, UK). Lobster, mussel and pilchard samples were analysed in dual isotope mode, allowing $\delta^{15}N$ and $\delta^{13}C$ to be determined simultaneously. We used fish flesh standardised against IAEE reference materials (IAEA-CH-6, IAEA-N-1, IAEA-N-2, USGS40, USGS41, USGS24) as our internal standard for SI analysis. Coralline algae samples were analysed for $\delta^{15}N$ prior to treatment with 1M HCl to remove inorganic carbonates, then re-analysed for $\delta^{13}C$. Analytical precision of the instruments was 0.04 (s.e) and 0.07 (s.e) for $\delta^{15}N$ and $\delta^{13}C$ respectively.

Data Analysis

As leg and tail tissue was taken from the same experimental lobster at the completion of the experiment, a split-plot ANOVA was used to compare $\delta^{15}N$ and $\delta^{13}C$ discrimination (at 119 days) with the factors diet (fixed factor, four levels), and tissue location (fixed factor, two levels). Where differences between factors were detected, post hoc Tukey tests were used to determine which levels of factors were significantly different.

A two-way repeated measures ANOVA was performed with diet type and time as factors to determine if $\delta^{15}N$ or $\delta^{13}C$ of leg tissue changed between sampling times for lobsters fed different diets. Bonferonni pairwise comparisons and separate one-way repeated measures ANOVAs were used to further investigate significant differences highlighted in the two-way design. All data were first checked for homogeneity of variance using Levene's test before analysis and were found to be homogenous.

Comparison of results to wild captured lobsters

To ensure any differences in $\delta^{15}N$ and $\delta^{13}C$ discrimination between tail and leg tissue observed in the laboratory were applicable to wild populations, 40 lobsters were captured from wild populations and had leg and tail tissue analysed. Differences in isotopic values between leg and tail tissue ($\delta^{15}N_{tail} - \delta^{15}N_{leg}$ and $\delta^{13}C_{tail} - \delta^{13}C_{leg}$) were compared between laboratory and wild caught lobster using a one-way ANOVA.

Results

Assessment of lobster condition

The blood protein concentration of lobsters from different treatments did not differ (Table 2), indicating the health of lobsters fed different diets was comparable at the end of the experiment.

Variation in $\delta^{15}N$ and $\delta^{13}C$ discrimination between muscle tissues

Consumer-diet $\delta^{15}N$ discrimination for lobster tail tissue was significantly lower than discrimination for lobster leg tissue (Table 3; Figure 1a). Consumer-diet $\delta^{13}C$ discrimination for lobster tail tissue was significantly higher than observed discrimination for lobster leg tissue (Table 4; Figure 1b). Lobster leg tissue was found to have higher C:N ratio than lobster tail tissue ($p<0.001$) (Table 5).

Differences in between tail and leg $\delta^{15}N$ and tail and leg $\delta^{13}C$ of 40 wild caught lobsters were of the same magnitude as differences observed amongst laboratory reared animals (Table 6). As the ANOVA was not significant, the observed differences in $\delta^{15}N$ and $\delta^{13}C$

of tail and leg tissue were identical between lobsters collected from the field and those raised in the laboratory.

Effect of diet on $\delta^{15}N$ and $\delta^{13}C$ discrimination

Significant differences in $\delta^{15}N$ and $\delta^{13}C$ discrimination were observed between lobsters fed different diets (Tables 3 and 4). Lobsters fed the pilchard diet showed significantly less $\delta^{15}N$ discrimination for both leg and tail tissue than lobsters fed on other diets (Post hoc Tukey test, $p<0.001$, df = 3; Figure 1a) but exhibited significantly higher $\delta^{13}C$ discrimination than lobsters fed coralline algae or the mixed coralline algae/mussel diet (Tukey test, $p < 0.05$, df = 3; Figure 1b). Lobster fed the mussel diet exhibited higher $\delta^{13}C$ discrimination than those fed coralline algae (Tukey test, $p<0.05$, df = 3; Figure 1b).

Changes in $\delta^{15}N$ and $\delta^{13}C$ of leg tissue over time

Results from repeated measures ANOVA indicated significant changes occurred in leg muscle $\delta^{15}N$ values over time as well as differences between lobsters fed different diets (Table 7; Figure 2a). Observed changes over time were consistent between diets, indicated by non significant interaction between factors time and diet. Bonferonni pairwise comparisons between times averaged over diets indicated that $\delta^{15}N$ concentration in leg muscle rose significantly between t0 and t32 ($p<0.01$) and between t32 and t96 ($p<0.05$) but then did not rise further (ie. dropped between t96 and t119 ($p<0.01$)). The final recorded concentration was significantly higher than at the beginning of the experiment ($p<0.05$). In contrast to the results for $\delta^{15}N$, a significant interaction existed between time and diet for leg muscle $\delta^{13}C$ values, indicating change in $\delta^{13}C$ over time depended upon diet (Table 8; Figure 2b). As a consequence, separate one-way repeated measures ANOVAs were performed for each diet type to investigate the effect of time. None of these tests were significant at $p<0.05$ using Bonferonni corrected p-values ($p<0.05/4$), indicating no change in $\delta^{13}C$ over the 119 day period for lobsters fed individual diets.

Discussion

Both location of muscle tissue and diet quality affect consumer-diet discrimination in western rock lobsters. Both null hypotheses are therefore rejected. Location of muscle tissue affects consumer-diet discrimination, with leg tissue consistently higher in $\delta^{15}N$ and lower in $\delta^{13}C$ relative to tail tissue regardless of diet or whether lobsters were laboratory reared or field caught. Diet quality also affects consumer-diet discrimination. $\delta^{15}N$ discrimination was determined to be lower for lobsters fed pilchards (high quality diet) relative to lobsters fed coralline algae. $\delta^{13}C$ discrimination was determined to be higher for lobsters fed pilchards relative to lobsters fed other diets. $\delta^{15}N$ of leg muscle tissue showed a pattern consistent with asymptotic change while $\delta^{13}C$ of leg muscle tissue showed no change over the 119 day period. As lobsters were observed to be actively feeding during this time, a conclusion was reached that 119 days is sufficient for $\delta^{15}N$ and $\delta^{13}C$ to reach a new stable level.

Variation in $\delta^{15}N$ and $\delta^{13}C$ discrimination between muscle tissues

This study has revealed that for the western rock lobster, *Panulirus cygnus*, tail and leg muscle differ in their discrimination of $\delta^{15}N$ and $\delta^{13}C$. Diet-tissue $\delta^{15}N$ discrimination of lobster tail tissue ranged between 1.67 ‰ and 2.97 ‰, dependant on diet type, whilst $\delta^{15}N$ discrimination of lobster leg tissue ranged between 2.87 ‰ and 4.22 ‰, dependant on diet type. Observed differences in $\delta^{15}N$ discrimination between tail and leg muscle tissue (mean difference of 1.22 ‰) may account for up to half a trophic level in ecological studies and thus this factor has the potential to influence conclusions on trophic structure if it is not controlled for when investigating food webs.

$\delta^{13}C$ discrimination between diet and tail tissue in this study ranged between 2.92 ‰ and 3.60 ‰, whilst discrimination between diet and leg tissue ranged between 1.95 ‰ and 2.21 ‰. The mean observed difference in discrimination was 1.13‰ across all diets. Thus, controlling the location of muscle tissue used in ecological studies is important as observed differences in $\delta^{13}C$ discrimination between muscle tissues exceeds the $\delta^{13}C$ discrimination observed for one trophic level in ecological studies. Differences in isotope values observed between muscle tissues from the laboratory study paralleled differences observed amongst wild populations, indicating findings from the laboratory are applicable to field populations and have applications for food web studies involving western rock lobster.

Differences in $\delta^{15}N$ and $\delta^{13}C$ discrimination between tissue types have been previously found for many species (DeNiro and Epstein 1978; DeNiro and Epstein 1981; Hobson and Clarke 1992; Bearhop et al. 2002; Cherel et al. 2005; Seminoff et al. 2006). Further, variable discrimination between different muscle tissue types has been determined among fish whereby $\delta^{15}N$ and $\delta^{13}C$ discrimination differed between white and red muscle tissue

(Pinnegar and Polunin 1999). The current study has demonstrated that $\delta^{15}N$ and $\delta^{13}C$ discrimination is also variable amongst white muscle tissue from different body parts on the same individual. As white muscle tissue is commonly used by ecologists in trophic studies (e.g. Bunn et al. 1995; Davenport and Bax 2002), researchers should recognise the potential for differences in white muscle location to affect consumer-diet discrimination. To account for demonstrated differences in discrimination between muscle tissue location, discrimination values specific to the muscle tissue chosen for analysis should be used when calculating results and using models in analysis of stable isotope data.

Differences in discrimination of $\delta^{15}N$ and $\delta^{13}C$ in different muscle tissues might be related to differences in tissue composition since different compounds vary in their $\delta^{15}N$ and $\delta^{13}C$ signatures. Lipid rich tissues have a lower $\delta^{13}C$ than a protein rich tissue since lipids are depleted in ^{13}C relative to proteins (Tieszen et al. 1983). It is possible that following digestion, proteins are preferentially assimilated into tail tissue of the western rock lobster thereby increasing the concentration of ^{13}C in the tail. A comparison of C:N ratios of lobster leg and tail tissues reveals leg tissue has a higher C:N ratio relative to tail tissue (Waddington and MacArthur, unpublished data), perhaps indicating that tail tissue has a higher concentration of proteins, thereby lending some support to this idea.

Effect of diet on $\delta^{15}N$ and $\delta^{13}C$ discrimination

$\delta^{15}N$ and $\delta^{13}C$ discrimination was also found to differ with diet. Lobsters fed pilchards displayed less $\delta^{15}N$ discrimination but higher $\delta^{13}C$ discrimination between diet and tissue than those fed other diets. Differences in $\delta^{15}N$ and $\delta^{13}C$ discrimination of individuals fed diets of differing quality have been demonstrated for the blue crab, *Callinectes sapidus* (Fantle et al. 1999; Dittel et al. 2000) and the anomopod crustacean, *Daphnia magna* (Adams and Sterner 2000). In these examples, higher discrimination of δ^{15}N was observed for animals fed low quality diets with high C:N ratios; the proposed explanation for this being that the high C:N diet provides insufficient N for metabolic needs and thus tissue N reserves are utilized, raising concentration of δ^{15}N as the lighter δ^{14}N is preferentially excreted (Gannes et al. 1997). Whilst lobsters fed the lowest quality diet, coralline algae, exhibited higher discrimination of δ^{15}N than those fed pilchards, lobsters fed mussels also exhibited higher discrimination whilst not differing significantly to pilchards in C:N. Results suggest that even different diets controlled for C:N may be discriminated differently and that the composition of individual compounds (e.g. amino acids) within diets may be important in determining the degree of fractionation (Schmidt et al. 2004).

Values for consumer-diet δ^{15}N discrimination of lobster tail tissue (range 1.67‰ to 2.97‰; mean all diets 2.57‰) are lower than the value of 3.4‰ (range of 3-5‰) for δ^{15}N discrimination reported in the literature (DeNiro and Epstein 1981; Minagawa and Wada 1984; Post 2002) and commonly utilized in ecological studies (Kling et al. 1992; Hecky and Hesslein 1995). Our range of values for δ^{15}N discrimination encompassed the estimate of 2‰ for δ^{15}N discrimination reported by Vanderklift and Ponsard (2003) for 21 crustacean taxa. Similarly, values for consumer-diet δ^{13}C discrimination of lobster tail tissue (range 2.92‰ to 3.60‰; mean all diets 3.20‰) differed to those reported in the literature. These values for δ^{13}C discrimination exceed the range 0-1‰ suggested by

DeNiro and Epstein (1978) for discrimination between trophic levels. Similarly large $\delta^{13}C$ discrimination values have been determined for other ectothermic organisms. Values of 2.0‰ to 3.4‰ for $\delta^{13}C$ have been determined for fish tissue (Hesslein et al. 1993; Pinnegar and Polunin 1999; McCutchan Jr et al. 2003), while variation in $\delta^{13}C$ of between -10 ‰ and -2 ‰ have been reported for the amphipod, *Allorchestes compressa* (Crawley et al. 2007).

The range of $\delta^{15}N$ leg tissue-diet discrimination determined from our study is 2.87‰-4.22‰; mean all diets 3.79‰. These values encompass the average of 3.4‰ reported in the literature for $\delta^{15}N$ discrimination (DeNiro and Epstein 1981; Minagawa and Wada 1984; Post 2002). However, the value reported in our study exceeds the average of 2‰ reported by Vanderklift and Ponsard, (2003) for crustacean taxa. As with tail tissue $\delta^{13}C$ discrimination, the values for lobster leg tissue $\delta^{13}C$ discrimination (1.95‰-2.21‰; mean all diets 2.07‰) exceed the values 0-1‰ reported in the literature for $\delta^{13}C$ discrimination (DeNiro and Epstein 1978; Post 2002; McCutchan Jr et al. 2003). Models incorporating measures of consumer-diet discrimination for the analysis of lobster trophodynamic relationships should validate discrimination values used to accurately represent ecological relationships.

Conclusions

This study further highlights the complexity surrounding selection of appropriate consumer-diet discrimination factors for trophodynamic studies. In addition to factors such as mode of excretion, taxon, nutritional condition, respiration rate and tissue type, our research suggests location of muscle tissue must also be considered when selecting tissue for analysis. In situations where significant differences in the quality of the food available to (eaten by) the organism exist, diet quality must also be considered as a factor affecting $\delta^{13}C$ and $\delta^{15}N$ discrimination. Our values for $\delta^{13}C$ consumer-diet discrimination and $\delta^{15}N$ discrimination of leg tissue were found to differ to those reported in the literature. To increase the confidence associated with the application of naturally occurring stable isotopes in ecological studies, species-specific values for discrimination are preferable to using values derived from a number of other species.

Acknowledgements

We wish to thank Danielle Johnston for providing experimental lobsters and Kylie Cook for help feeding the lobsters. We thank Diana Walker and Mat Vanderklift for helpful comments on the manuscript. This project was funded by the School of Plant Biology at the University of Western Australia, the School of Natural Sciences at Edith Cowan University, and The Strategic Research Fund for the Marine Environment (SRFME). All procedures were approved by the animal ethics committee at The University of Western Australia (Approval number RA/3/100/478), and authorized under state government permits.

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Figures

Figure 1: $\delta^{15}N$ discrimination (a) and $\delta^{13}C$ discrimination (b) between diet and muscle tissue for lobster fed four different diets.

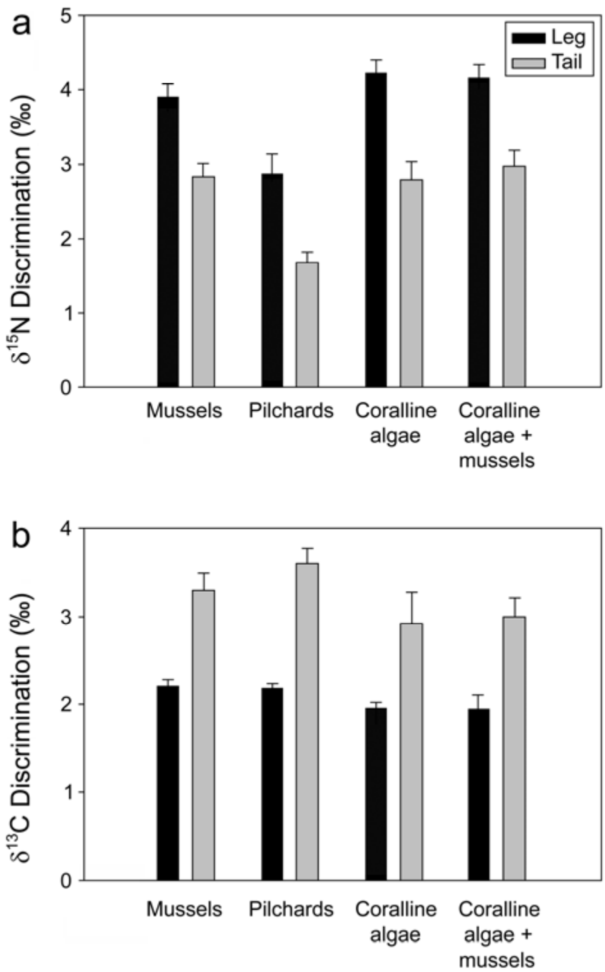
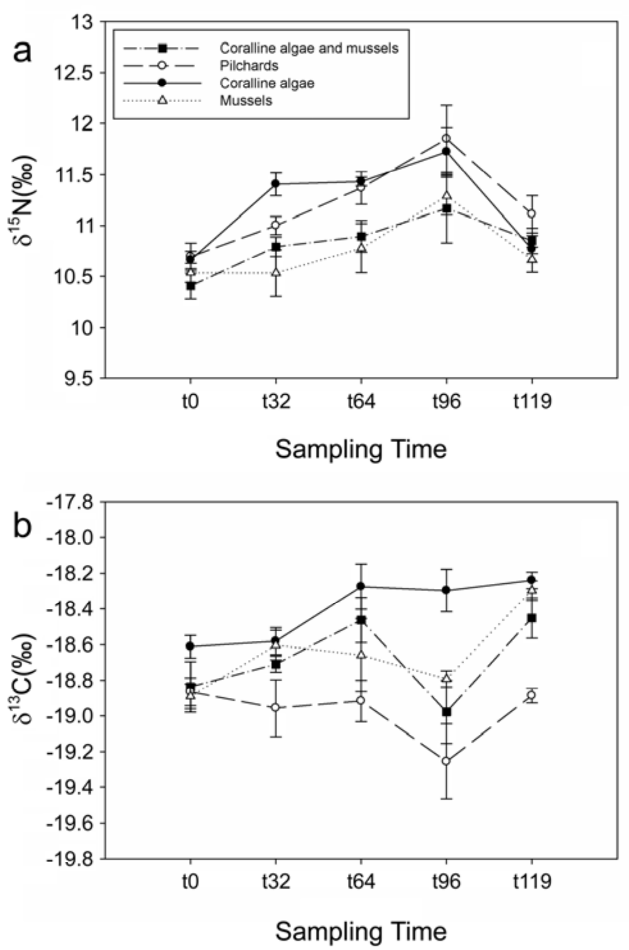


Figure 2: Change in $\delta^{15}N$ (a) and $\delta^{13}C$ (b) concentration of leg muscle tissue from lobsters fed four different diets. t = time since diet switch.



Tables

Table 1: Diets fed to experimental lobsters.

Diet item	$\delta^{15}N$ (± s.e.)	$\delta^{13}C$ (± s.e.)	Diet Quality (C:N Ratio) (± s.e.)
Pilchards (*Sardinops sagax*)	8.25 ± 0.04‰	-21.14 ± 0.01‰	3.80 ± 0.06
Mussels (*Mytilus edulis*)	6.76 ± 0.1‰	-20.51 ± 0.19‰	4.54 ± 0.16
Coralline algae (*Amphiroa gracilis*)	6.54 ± 0.12‰	-20.19 ± 0.56‰	7.03 ± 0.52

Table 2: ANOVA of blood protein concentration of lobsters at t119.

Factor	df	SS	MS	F-value	*p-value*
Blood protein concentration	3	727	242	0.74	*0.55*
Error	12	3946	329		
Total	*15*	*4673*			

Table 3: Split-plot ANOVA for $\delta^{15}N$ discrimination (tissue × diet).

Factor	df	SS	MS	F-value	p-value
Diet	3	8.98	2.99	25.06	0.000
Residual (Diet)	12	1.43	0.12		
Tissue	1	11.96	11.96	299.17	0.000
Tissue × Diet	3	0.14	0.04	1.13	0.375
Residual (Tissue)	12	0.48	0.04		

Table 4: Split-plot ANOVA for $\delta^{13}C$ discrimination (tissue × diet).

Factor	df	SS	MS	F-value	p-value
Diet	3	1.14	0.38	5.12	0.016
Residual (Diet)	12	0.89	0.07		
Tissue	1	10.23	10.23	157.91	0.000
Tissue × Diet	3	0.24	0.08	1.26	0.332
Residual (Tissue)	12	0.78	0.06		

Table 5: Split-plot ANOVA comparing C:N ratio (tissue × diet).

Factor	df	SS	MS	F-value	*p-value*
Diet	3	0.06	0.02	2.736	*0.090*
Residual (Diet)	12	0.08	0.01		
Tissue	1	0.53	0.53	204.928	*0.000*
Tissue × Diet	3	0.00	0.00	0.444	*0.726*
Residual (Tissue)	*12*	*0.03*	*0.00*		

Table 6: Difference in $\delta^{15}N$ and $\delta^{13}C$ of leg and tail tissues compared between laboratory reared animals and lobsters collected from the field.

Isotope	Factor	df	SS	MS	F-value	p-value
$\delta^{15}N$	Lobster origin	1	0.37	0.37	1.30	0.258
	Residual	86	24.41	0.28		
	Total	87	24.78			
$\delta^{13}C$	Lobster origin	1	1.18	1.18	2.650	0.107
	Residual	86	38.22	0.44		
	Total	87	39.39			

Table 7: Two-way repeated measures ANOVA showing differences in $\delta^{15}N$ of lobster leg muscle tissue over time and for lobsters fed different diets. Since Mauchly's test indicated a violation of sphericity, degrees of freedom marked '*' have been adjusted using the Huynh-Feldt correction.

Factor	df	SS	MS	F-value	p-value
Diet	3	3.42	1.14	4.13	0.032
Residual (Diet)	12	3.31	0.27		
Time	3.537*	7.71	2.18	21.60	0.000
Time × Diet	10.612*	1.48	0.14	1.39	0.217
Residual (Time)	42.449*	4.28	0.10		

Table 8: Two-way ANOVA showing differences in $\delta^{13}C$ of lobster leg muscle tissue over time and for lobsters fed different diets.

Factor	df	SS	MS	F-value	p-value
Diet	3	3.32	1.11	11.754	0.001
Residual (Diet)	12	1.13	0.09		
Time	4	1.49	0.37	8.291	0.000
Time × Diet	12	1.10	0.09	2.045	0.040
Residual (Time)	48	2.16	0.04		