

# **DIET OF THE TRISTAN ROCK LOBSTER *JASUS TRISTANI* FOLLOWING THE 2011 SOYA SPILL AT NIGHTINGALE ISLAND**

by

**LUKE DOUGLAS SCOTT JONES**

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**University of Cape Town**

**Supervisor: Dr Laura Blamey**

**Co-Supervisors: Emeritus Prof George Branch and Dr Ander M. de Lecea (University of KwaZulu-Natal)**

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## DECLARATION

I, Luke Jones, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the University of Cape Town.

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Signed

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Date

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## Abstract

The spiny lobster *Jasus tristani* inhabits the Tristan da Cunha Island archipelago in the South Atlantic Ocean 2,400km from the West coast of South Africa. It is commercially exploited across the archipelago and is the main economic source of income for the local government as it accounts for nearly 80% of the local gross domestic product. The commercial rock lobster industry was established in 1949, and the fishery is currently recognised as sustainable, and as a result was awarded a Marine Stewardship Council Certification in 2011. That same year however, the sinking of the OLIVA at Nightingale Island spilt 60,000 tonnes of soya beans (*Glycine max*), greatly affecting the local benthic environment, and with probable consequences for the local food web, including the diet of *J. tristani*. It is still unclear whether the soya beans are still on the seafloor at Nightingale Island as there have been no scientific surveys conducted since the spill. Using samples from 2015, I assessed whether the diet of the lobsters from Nightingale Island differs from that of lobsters from the unaffected Tristan and Inaccessible Islands, and whether there is any evidence of soya in the diet of the lobsters from Nightingale Island. In addition, I examined whether diet differed between lobsters of small or large size, and between shallow and deep depths. In total, 540 lobsters were sampled across the three islands, and a combination of gut content and stable isotope (SI) analysis was conducted to assess the dietary components of the lobsters. Gut fullness was significantly less at Nightingale Island suggesting there may be less food available on the reef. Statistical analyses showed that diet differed between all islands, depths and sizes, although this was difficult to ascertain from visual multidimensional scaling plots as diet showed considerable overlap and variability both within and between islands, depths and sizes. The stable isotope analysis showed

significant differences in nitrogen levels among the three islands, and significant differences in the carbon signatures between the size and depth categories. There was no evidence of soya content in the gut, nor did SI signatures show evidence of a C3 carbon source (i.e. soya beans) supporting the diet of the lobsters at Nightingale Island. This is possibly due to the time that had elapsed between the sinking of the OLIVA in 2011 and the sampling the lobsters in 2015. The rock lobsters from Nightingale Island may have been more attracted to the baited traps if food was limited there, which makes this study important in interpreting the effects of the soya bean spill on catch rates as well as the possible consequences for the local ecosystem.

## 1. Introduction

Spiny lobsters (or rock lobsters) occur in a wide range of habitats and depths, and constitute some of the most important marine fishing resources in many parts of the world (Phillips 2013). Over 90 countries worldwide currently fish or market spiny lobsters as a natural resource and over 70% of the global catch is sourced from countries in the Caribbean Sea, south-eastern Atlantic Ocean and East Indian Ocean (Glass 2014).

In addition to their commercial importance, spiny lobsters are also important ecologically and can play a vital role in their ecosystems, which can range from the deep ocean to shallow littoral habitats (Phillips et al. 1994). They are important prey items of larger predators such as sharks, fish, otter and seabirds (Joll and Phillips 1984, Cox et al. 1997, Crawford et al. 2008), but can also play a predatory role themselves. In shallow coastal zones, spiny lobsters are major predators of various benthic species, including urchins, bivalve, crabs, polychaetes, sponges and seaweeds (Mayfield et al. 2000, Haley et al. 2011). However, studies have shown that spiny lobsters are also opportunistic feeders (Andrew 1999, Leber et al. 2008, O'Rourke et al. 2012). Despite broad diets, some species, e.g. the South African rock lobster *Jasus lalandii*, show preference for certain prey types (Mayfield et al. 2001, Haley et al. 2011), yet still have the ability to survive and maintain dense populations on unusual prey items, such as sponge and barnacle recruits (Barkai and Branch 1988b). The Tristan rock lobster *Jasus tristani* has been reported to consume a greater percentage of kelp and sessile invertebrates such as sponges, hydroids, and bryozoans, compared to other *Jasus* species, which is likely to be a result of the

general paucity of benthic fauna in the Tristan da Cunha Archipelago (Pollock 1991, Phillips 2013). Spiny lobsters that show a preference for certain prey may have profound effects on their ecosystem, particularly in terms of benthic species composition and size frequency distributions of the preferred prey items (Heydorn 1969, Pollock 1979, Cobb and Phillips 2012), while others that are generalist predators, foraging over large areas (e.g. *Panulirus argus*), may have diffuse effects on benthic communities (Cox et al. 1997).

The Tristan da Cunha islands form a small chain of very remote islands located in the South Atlantic Ocean, ca. 2,400 km from South Africa and are regarded as the most remote inhabited archipelago in the world. The volcanic islands of Tristan da Cunha, Nightingale, Inaccessible and Gough support the endemic Tristan rock lobster *J. tristani*, which occurs from 0-200m and has been commercially and recreationally fished by the local population since 1949, except during 1961 to 1963 when the entire population of the archipelago was evacuated due to a volcanic explosion (Pollock 1991). The islands rise from the abyssal depths to the surface and the considerable depths between the islands create a natural barrier that separates the Tristan lobster populations from each other (Glass 2014). The subtidal benthic environment at the Tristan da Cunha island chain is described as impoverished with low faunal species diversity (Roscoe 1979, Pollock 1991, Andrew and Ryan 2007). Seaweeds dominate the shallow subtidal (0-3m) and the kelp *Laminaria pallida* is dominant in the deeper waters (10-40 m) (Pollock 1991). The sea urchin *Arbacia dufresnii* is considered to be the most abundant benthic herbivore, ranging from the shallows down to about 50m (Roscoe 1979). The fauna living in these kelp forests include a variety of fish, the rock lobster *J. tristani*, the urchin *A. dufresnii*, whelks



*Argobuccinum* sp, anemones, sponges, barnacles, hard tubeworms and encrusting bryozoans among others (Pollock 1991, Scott & Tyler 2008). The commercial exploitation of *J. tristani* is economically the most significant source of income for the Tristian da Cunha archipelago, accounting for over 80% of government revenue per annum (Glass 2014). There are currently two distinct fishing sectors present at the island chain, which share the same markets and resources: a vessel-based fishery and an island-based fishery (Pollock et al. 2000). The vessel-based fishery is conducted by large commercial vessels from Cape Town that catch and process the lobsters on board. The island-based fishery is solely restricted to local fishermen, who employ two main fishing methods, namely hoop nets from dinghies and traps from boats, and process the catch in factories on land (Ryan 1991).

On the 16<sup>th</sup> of March 2011, the bulk carrier *OLIVA* ran aground at Nightingale Island, where 1,500 tonnes of oil and diesel spilled into the surrounding environment. Two days later on the 18<sup>th</sup> March the carrier broke up and sank along with 65,000 tonnes of raw soya bean (*Glycine max*) on board. The lobster fishery was subsequently closed for one season, along with a reduced Total Allowable Catch for the 2011/12 season (Glass 2014). This was a precautionary measure until surveys could be carried out to assess the toxicity of the oil spill and impact of the soya beans on the substrate. Following the sinking of the *OLIVA* and subsequent spread of the soya beans along the seafloor, a thick black sludge formed on the benthic environment and rock lobster catch per unit effort (CPUE) initially declined (Glass 2014). The effects on the rest of the ecosystem are unknown, but rock lobster CPUE has since increased and for

the past three seasons, it has been 2-3 times greater than it was before the soya spill (Glass 2014).

Reasons for the increase in CPUE are not known but two hypotheses have been put forward: (1) the rotting soya destroyed most of the benthos and available food sources, resulting in hungry lobsters that were overly attracted to the baited lobster traps or (2) the soya beans acted as a food source and contributed to increased productivity of the system and subsequently the lobster population. This study serves as a first attempt to address what effect the soya spill might have had on rock lobster populations at Nightingale Island, by analysing rock lobster diet after the soya spill, and comparing this diet to rock lobster from the two nearby unaffected islands (Tristan and Inaccessible). Rock lobsters from Gough Island were not available at the time of this study.

To test the above hypotheses, a combination of gut content and stable isotope analyses were used to assess the composition and diversity of the diet for the rock lobsters at each of the three islands. The two approaches have different benefits and limitations, so it was attractive to use a combination of the two techniques to generate complementary results. Gut content analysis gives a snapshot of recent rock lobster feeding activity over the past 48-72hrs, but has various limitations (Olsson et al. 2008). One of the major limitations is that gut content analysis is biased by the different digestion rates of various food items, as they are preserved to different degrees inside the rock lobster gut passage. Although soft prey items are digested easily, various prey items that possess hard shells or skeletons, e.g. urchins, can remain in the stomach for longer periods of time and have a higher chance of being recorded (Crehuet et al.

2007). Common prey items for rock lobster, such as invertebrate flesh, are digested at a fast rate and their identification can be difficult, and thus might not be recorded in the stomach content analysis (Correia 2003). Stable isotope analysis obtains data on assimilated food sources over a longer period of time (Olsson et al. 2008). The use of stable isotopes analysis allows for the differentiation between the resources ingested (i.e. those found in the gut) and those assimilated by the rock lobster, a very important difference from an energetic point of view (Crehuet et al. 2007). Lancaster and Waldron (2001) argue that rock lobsters, as omnivores, will encounter signatures from all food sources consumed and therefore it can be difficult to distinguish the contribution of individual food sources from the isotopic signals in lobster. However, it is possible to distinguish between the contributions of different food sources to the isotopic signals in rock lobster by combining stable isotope analysis and Bayesian mixing models (Olsson et al. 2008).

In addition, organic matter entering the system from different sources generally should have distinctive isotope carbon signature (de Lecea et al. 2013). This makes stable isotopes an ideal tool to try and understand whether the soya beans have contributed to the diet of the rock lobster or not. This is especially true in distinguishing between terrestrial and marine primary producers (Goñi et al. 1998). The carbon isotope signature of the soya beans should be the same as C<sub>3</sub> terrestrial plants, and as a result the rock lobsters from the soya-affected Nightingale Island should have a specific stable isotope signature if they consumed the soya beans. Additionally, as the islands are small, the chances are very low of other terrigenous organic matter entering the system and skewing the results.

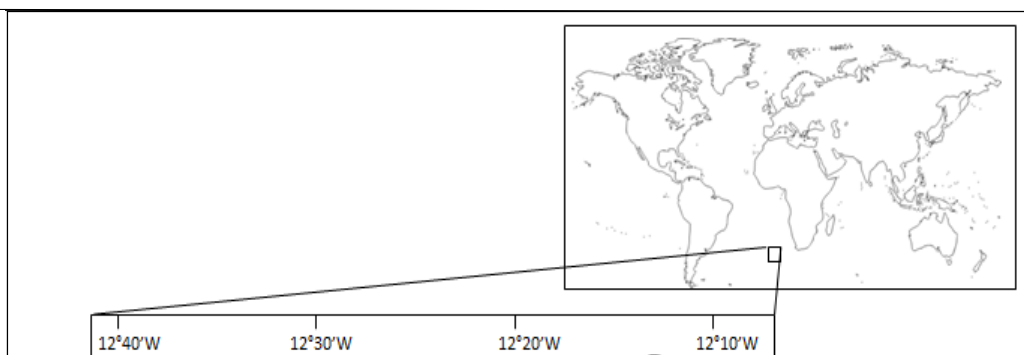
Using a combination of gut content and stable isotope analysis, this thesis addressed the following questions:

- (1) Does rock lobster diet differ between soya-impacted (Nightingale) and non-impacted (Tristan and Inaccessible) islands?
- (2) Is there evidence for soya in rock lobster diet at Nightingale Island?
- (3) Following the soya spill, are the rock lobster diets at Nightingale, Tristan and Inaccessible Islands different from those reported in earlier studies?
- (4) Does diet differ between the two depth zones sampled or between small and large lobsters?

## 2. Materials and Methods

### 2.1 Study Site

Tristan da Cunha Island group lies in the middle of the South Atlantic ocean, approximately midway between southern Africa and South America, at 37°05'S, 12°17'W, about 1,660 nautical miles (nm) west from Cape Town, South Africa (Fig. 1). The island group comprises four volcanic islands located on the outer slope of the Mid-Atlantic Ridge: Tristan da Cunha, Nightingale, Inaccessible and Gough Islands. Three of these islands are grouped closely together to form the Tristan Group – these are Tristan da Cunha Island, with the smaller, uninhabited Nightingale and Inaccessible Islands located roughly 20nm to the west and south west respectively. Gough Island is located approximately 200nm from Tristan da Cunha Island.



*Figure 1: Location of Tristan da Cunha Island Group in the South Atlantic Ocean*

## *2.2 Data Collection*

Tristan rock lobsters were collected from Tristan, Inaccessible and Nightingale Islands by the commercial fishing vessel *Edinburgh*, a South African registered vessel, between February-May 2015. At each Island, two size classes of lobster were collected (count 20 and count 40) from two different depth zones (0-30m and 60-80m). 'Count 20' and 'Count 40' lobsters are categorised by the fishing industry as the total number of lobsters that the fishermen can fit into a box. For simplicity, I refer to these two sizes classes as 'large' (Count 20) and 'small' (Count 40). Sixty lobsters per size class per island were collected at 60-80m and 30 lobsters per size class per island were collected at 0-30m. In total, 540 lobsters were collected. Rock lobster bait-protected traps were deployed from the fishing vessel at 60-80m, and similar, but smaller traps were deployed from

power-boat vessels at 0-30m. The rock lobsters were then processed, with the tails being removed for export while the remainder of the bodies (head and thorax containing the stomach) were immediately packed, frozen and transported to the University of Cape Town and stored in a -20° C freezer.

### 2.3 Rock Lobster Gut Analysis

#### a. Gut analysis

Frozen rock lobsters were placed in cold water to thaw and the sex of each lobster and its carapace length (CL) were recorded. The gastric-mill stomachs were then removed and the total mass of each stomach was recorded using a 0.05g electronic balance. Stomach contents were then flushed into a petri dish, and the stomach membrane was weighed and the gut fullness index (GFI) was calculated as follows:

$$GFI = \frac{\text{Total stomach mass (g)} - \text{stomach membrane mass (g)}}{\text{Total stomach mass (g)}} \times 100$$

The contents of each stomach were viewed under a binocular dissecting microscope (10.5 x magnification) and prey items were identified as far as possible from diagnostic fragments in the stomach content. The frequency of occurrence and volume of each prey item were then recorded. Percentage volume contribution of each prey item was calculated using the following equation (Hyslop 1980, Williams 1981):

$$\text{Percentage Volume} = \frac{\text{Volume of prey item}}{\text{Total Volume of gut contents}} \times 100$$

Data from male and female rock lobsters were pooled given that (1) very few

females were caught in this study and (2) a study on a sister species (*Jasus lalandii*) revealed no difference in diet between male and female rock lobsters (Mayfield et al. 2000).

#### b. Statistical Analysis

A multifactorial analysis of variance (ANOVA) and *post hoc* Tukey test were used to assess mean lobster GFI between islands, depth and lobster size class. Given that rock lobsters were caught using baited traps, I expected to find bait (fish) inside the guts and so initial analyses checked for the proportion of lobster stomachs that (i) were empty, (ii) contained fish, (iii) contained fish and prey and (iv) contained no fish. Two sets of percentage volume data were then calculated – one in which fish were included and the other in which fish were excluded. Dietary analyses were done on the percentage volume data without fish using the software PRIMER with PERMANOVA+, given that fish are unlikely a natural prey item for rock lobsters. The data were square-root transformed and a Bray-Curtis similarity measure was used to create a resemblance matrix. Multidimensional scaling (MDS) plots and permutation-based analysis of variance (PERMANOVA) were performed to assess (1) diet between the three islands (island treated as a fixed factor) and (2) diet across depth (depth treated as a fixed factor) and (3) diet across size (size treated as a fixed factor). PERMDISP was used to test for the homogeneity of dispersion in the data to assist interpretation of PERMANOVA results. Similarity Percentage (SIMPER) analyses were used to identify prey items responsible for any differences in the MDS or PERMANOVA analyses. Dietary diversity was calculated using the Shannon Weiner index and then assessed using a one-way ANOVA across the three islands and t-tests across depth and size categories.

A two way ANOVA and t-test were used to compare the location, size and depth for the carbon and nitrogen isotope results. Additionally, a Tukey's *post-hoc* multi comparison of means test results was also used to compare the carbon and nitrogen isotope signatures across the three islands.

## 2.4 Isotope Analysis

### a. Sample preparation and statistical analysis

Differences in rock lobster diet between the three islands were also assessed using stable isotope ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) signatures. Ten replicate rock lobsters per island for the Count 20 (large) size class, 60-80m depth were used for the analysis of the isotope signatures. For each rock lobster, a section of tissue was removed from the front right antennae, along with a random sample of the unprocessed (whole) stomach contents and a sample of the most frequent prey items in the gut contents.

The tissue and prey items were oven dried at 50°C for 48 hours. Prey items collected from the stomachs were homogenised and 2N HCl was added to the samples containing  $\text{CaCO}_3$  and left for 24 hours to soak. The samples were then rinsed with filtered water through a filtration tower to remove any excess HCl, and oven dried again at 50°C for 48 hours. All samples were then homogenised and weighed into tin capsules with approximately 0.5 mg required for analysis. Samples were then sent to iThemba Laboratories, Pretoria for analysis. The carbon and nitrogen analyses were conducted on a Flash HT Plus elemental analyser coupled to a Delta V Advantage isotope ratio mass spectrometer by a ConFloIV interface, and the isotope values were calculated against an in-house



standard (Merck Gel), provided by the CSIR. The reported precision for the standards run for the analyses was 0.50 permille for N and 0.23 permille for C.

Bayesian mixing model MixSIAR version 1.0 (Stock and Semmens, 2013) with uninformative priors was used to determine the proportional contribution of each diet item found in the stomachs. The individual diet items analysed from the stomach, were set as “*sources*” in the mixing model, while the lobsters from each of the islands were set as “*consumers*”, with the islands set as “*community*” to differentiate each group of lobsters. Although it was suspected that the fish sampled from the guts was not a natural part of the lobster’ diet, I nonetheless used them in the Bayesian mixing model. I did this as a means to check the mixing models validity, i.e. if the models indicated that the fish were a large portion of the natural diet, that the results could not be trusted. One-step fractionation factors of 0.4-1‰ for  $^{13}\text{C}$  (Post, 2002), were used to estimate appropriate enrichment factors. Although the mass spectrometer provides  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from the same sample, I did not use the prey nitrogen isotope values due to some of the samples being too small to obtain reliable  $\delta^{15}\text{N}$  values. Therefore the mixing model is based on one isotope ( $\delta^{13}\text{C}$ ), although the  $\delta^{15}\text{N}$  values, for the lobster only, are presented graphically and independently analysed statistically.

Lipid correction models for chemical extraction were not used in this study. De Lecea and de Charmoy (2015) concluded that the best possible method to obtain accurate isotopic signatures was to analyse two sets of samples: a treated  $\delta^{13}\text{C}$  sample and an untreated  $\delta^{15}\text{N}$  one. However, because of the small

size of most of the items extracted from the lobsters guts replication was not possible.

### 3. Results

#### 3.1 Lobster Size and Gut Fullness Index (GFI)

At all three islands, male rock lobsters made up ca. 95% of the catch and thus sexes were pooled in all the analyses. Gut fullness (GFI) of rock lobsters differed by only 3.7% among the islands (Fig. 2), but the mean GFI of 24.4% at Nightingale Island was significantly less than the values of 27.1% and 28.1% at Tristan and Inaccessible islands respectively (Fig. 2a, Table 1). GFI was not significantly different between depths, except at Inaccessible Island, hence a significant island:depth interaction (Fig. 2b, Table 1), while small lobsters had significantly fuller guts compared to large lobsters across all islands (Fig. 2c, Table 1). Males were predominately encountered in the samples, with 95% at Nightingale, 94% at Inaccessible and 93.9% at Tristan Island. Furthermore 96.4% of the samples were males in the deep depth category, and 92% in the shallow category. Additionally 89% were males in the small size group and 99% in the large size group.

*Table 1:* ANOVA results comparing mean GFI across islands (Inaccessible, Nightingale and Tristan), depth (shallow and deep) and size class (count 40 and count 20). Significant values are highlighted in bold.

<i>Source of Variation</i>	<i>Df</i>	<i>Sum Sq</i>	<i>Mean Sq</i>	<i>F value</i>	<i>P-value</i>
Island	2	1029	515	21.784	< <b>0.0001</b> ***

Depth	1	74	74	3.116	0.0783#
Size	1	14116	14116	597.443	< <b>0.0001</b> ***
Island:Depth	2	299	149	6.318	<b>0.0020</b> **
Island:Size	2	1990	995	42.121	< <b>0.0001</b> ***
Depth: Size	1	6	6	0.236	0.6275
Island:Depth:Size	2	21	10	.437	0.6465
Residuals	41	9711	24		

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Significant Codes    #<0.1    \**P*<0.05    \*\**P*<0.01    \*\*\**P*<0.001

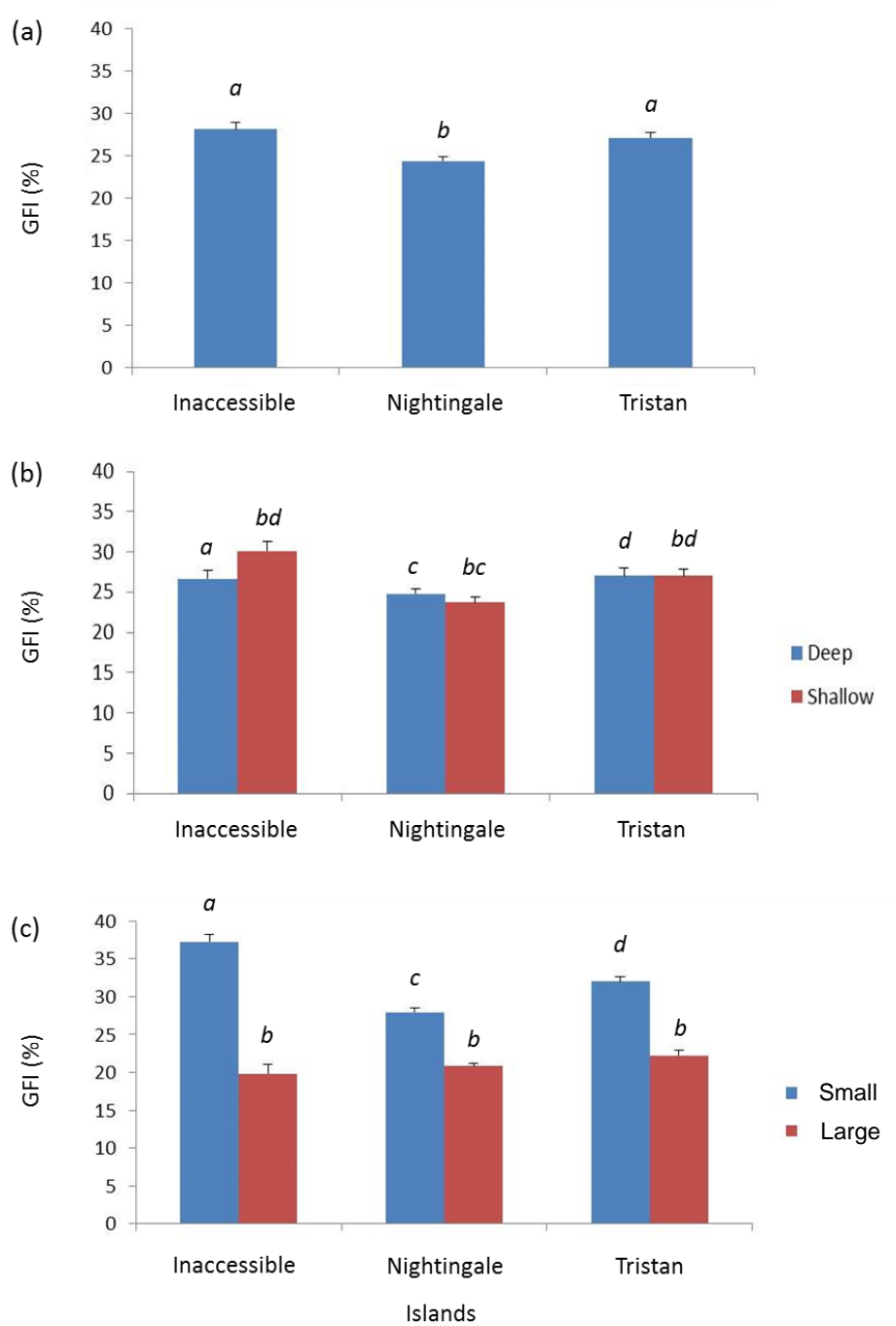


Figure 2: Mean Gut Fullness Index (GFI) expressed as a percentage for (a) the three islands across all depths and sizes (b) deep and shallow depth categories and (c) ‘small’ (count 40) and ‘large’ (count 20) categories. The standard error is shown in the length of the error bars, and italicized letters indicate significant differences reported by ANOVA and *post-hoc* Tukey tests at the  $p < 0.05$  level.

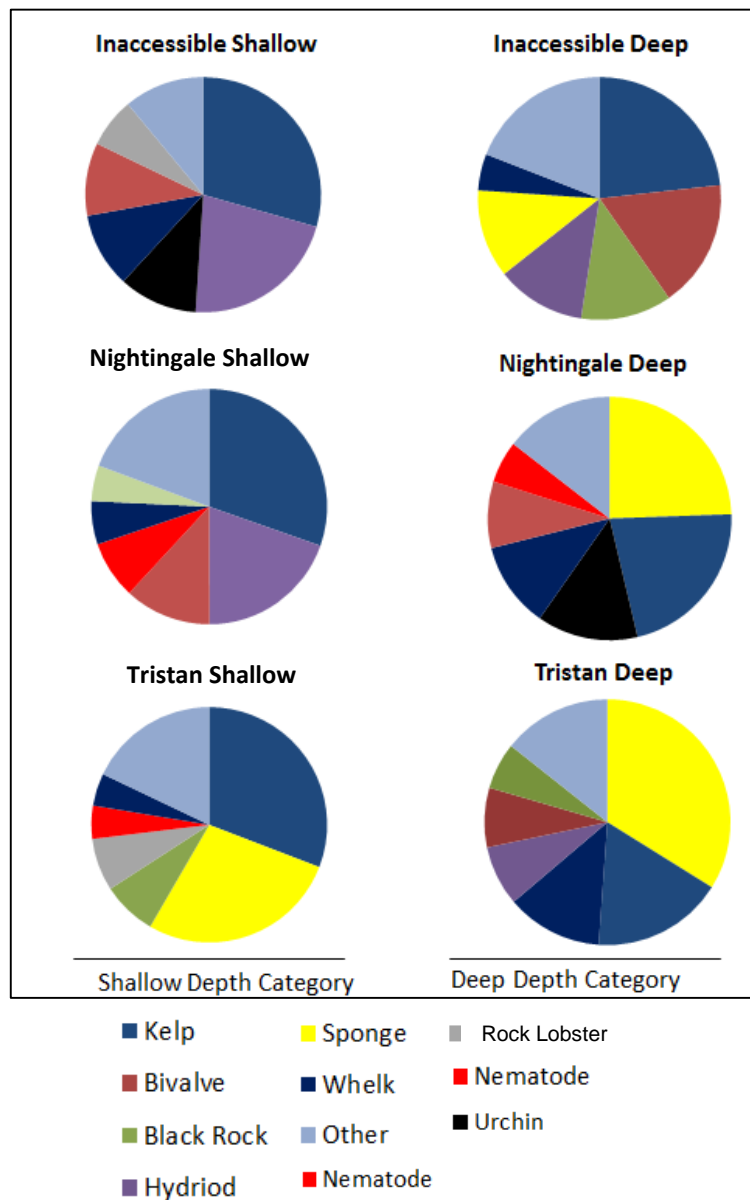
### 3.2 Dietary Analysis

Very few lobster stomachs were completely empty (3.5 % overall, Appendix A4). Fish bones and remains of fish flesh/skin were found in over 75% of the stomach samples, although the majority of these contained other prey items as well, particularly in the small lobsters (Appendix A4). Only 23.9% of stomach samples did not contain any fish in the gut (Appendix A4). The prevalence of fish in the gut was generally greater at Tristan and Nightingale islands, but no obvious indication that reliance on fish was greater at Nightingale specifically (Appendix A4).

As it is almost certain that the fish in the lobster guts originated from the bait in the lobster traps, initial analyses were run with and without fish in the dietary data and, as expected, rock lobster diets between islands were more similar when fish was included in the analyses compared to when fish was excluded from the analyses (Table 2). To obtain a better understanding of the differences in diet across islands depth and size, results presented here excluded fish from the data.

There was a wide range of prey items found in the gut contents across the three islands and, as there was no obvious difference between size classes, data for size classes were pooled in what follows. The most frequent gut contents included kelp, bivalve, hydroid, sponge, urchin, nematode, lobster, whelk/unknown flesh and incidentally ingested small pieces of black sand (Fig. 3). Kelp, hydroid and sponge were the most common prey items across the three islands (Fig. 3). Kelp was found in lobster stomachs from both the shallow and deep depths, whereas sponges were mostly found in lobsters from the

deep, except at Tristan Island, where kelp and sponge dominated in both depths (Fig. 3). Urchins were more prevalent in the diet of rock lobsters from Inaccessible Island shallow and Nightingale Island deep depth categories and bivalves and hydroids were more common in the lobsters from Inaccessible and Nightingale Islands compared to Tristan Island.



*Figure 3:* Proportion of the most common prey items found in rock lobster stomachs at the Inaccessible, Nightingale and Tristan Islands, for both shallow and deep depth categories. Fish were excluded from the analyses.

To provide an overview of lobster diets across islands, depths and size classes, the data were pooled to show the data of the lobster diets across the islands, depth and size categories as averages. Tristan Island clustered towards the centre of the MDS plot while the other two islands were more spread out (Fig.4a), indicating that lobsters from Inaccessible and Nightingale Islands had differing diets from those at Tristan Island. Diets of lobsters from the deep depth, particularly those of the large size class, appeared more similar than those from the shallow depth (Fig. 4b). The diet of the lobsters across the size classes was diverse, although larger lobsters appeared to have a more similar diet (Fig.4c).

When analysing the raw data, MDS plots showed little difference in diet among islands, or between depths and sizes (Fig. 5). There was a large amount of overlap in lobster diet across the three islands, suggesting that diets weren't that different, but were individually variable, because for all islands the percentage similarity within each island was less than 20%. Mean dietary diversity was greatest at Inaccessible Island (0.43) followed by Nightingale Island (0.40), both of which were significantly greater than mean dietary diversity at Tristan Island (0.28, Table 3). MDS plots of raw data also showed a high degree of overlap and no clear difference in diet between depths (Fig. 5b) or between sizes (Fig.5c). Mean percentage similarity was also low (14-17%) between size and depth classes (Figs 5b,c). Dietary diversity was significantly greater in the deep depth (Table 3), with no difference in dietary diversity between lobster sizes (Table 3). Even when the data for islands were analysed in separate groups (i.e. deep only, shallow only), MDS plots still showed little difference in diet, and a high degree of overlap between the islands (Fig 5d,e).

Despite MDS plots showing no clear differences in diet between islands, size or depth (Fig. 5), PERMANOVA results indicated that there were significant differences in diet between islands ( $p = 0.001$ ), size ( $p = 0.001$ ) and depth ( $p = 0.001$ ) and the interactions thereof (Table 4). As there were contrasting results between MDS plots and PERMANOVA, a PERMDISP analysis was carried out to check for homogeneity in the dispersion of data from the centroid, because if the dispersion is unequal between factors (e.g. island), this can bias the PERMANOVA results. The PERMDISP results indicated equal spread in the data from the centroid between the three islands, and between depth and size ( $p > 0.05$  in all cases, Table 5). As there was no significant difference in spread/dispersion found in the PERMDISP results, the PERMANOVA results, showing significant difference of diet between islands, diet and size can be considered robust.

*Table 2: Average similarity (expressed as a percentage) of prey items between the islands for all data (with fish) compared to data excluding fish.*

<i>Type of Data</i>	<i>Average Similarity (%)</i>
<b><i>All Data</i></b>	
Inaccessible	20.57
Nightingale	34.69
Tristan	41.60
<b><i>Data Excluding Fish</i></b>	
Inaccessible	16.23
Nightingale	13.70
Tristan	18.67



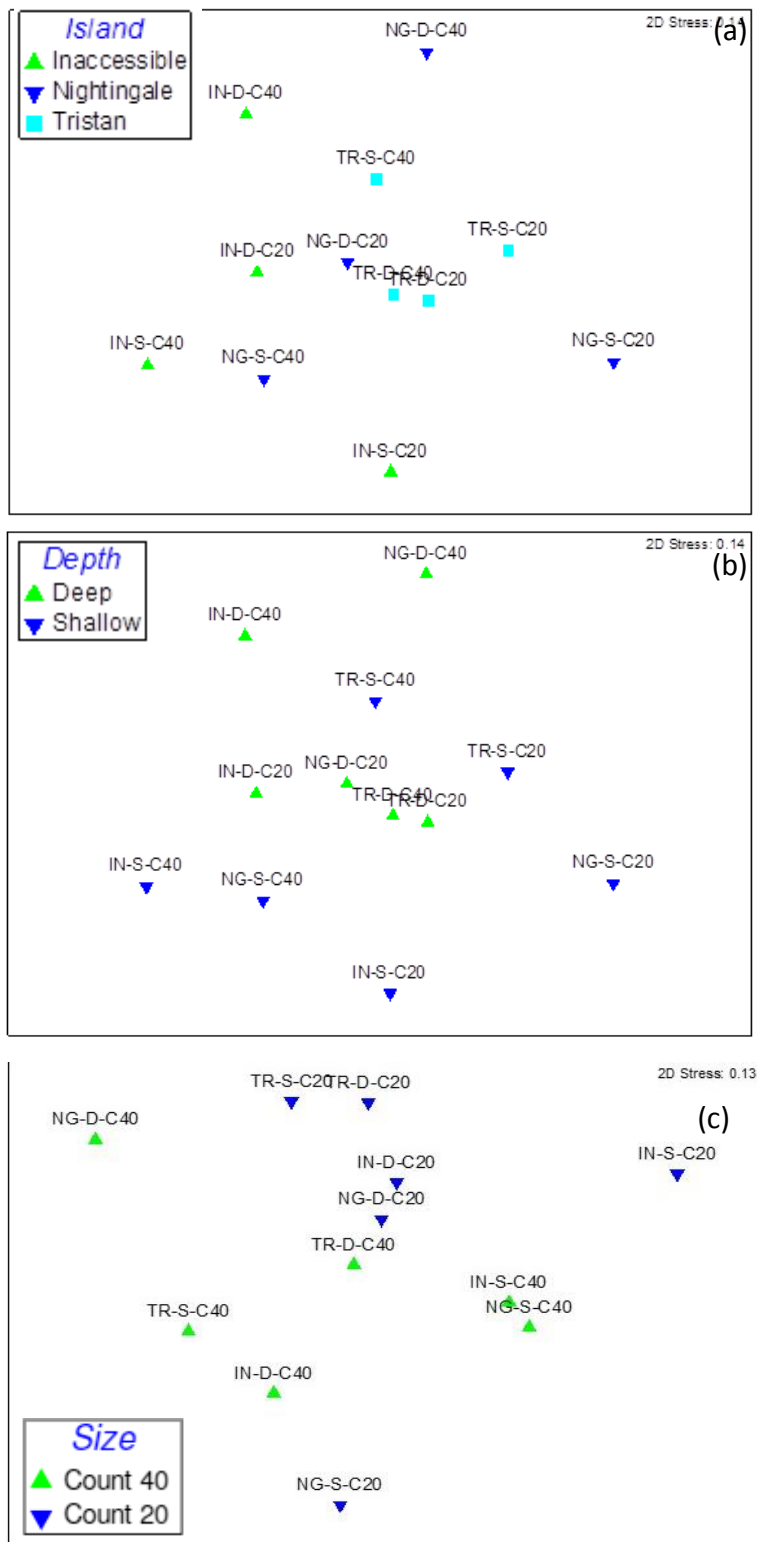


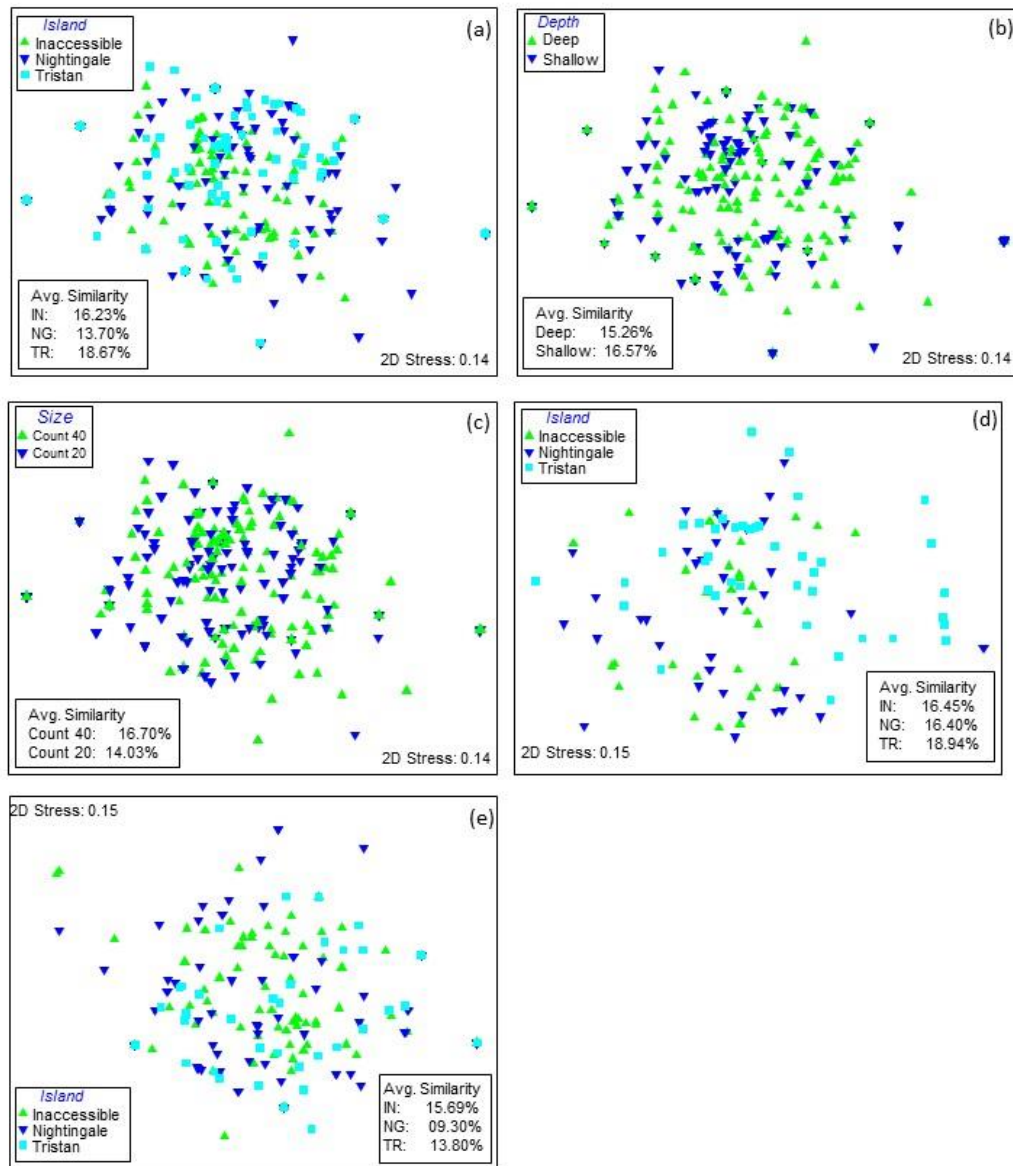
Figure 4: MDS plot of dietary data comparing (a) the three islands (b) depth and (c) size. Data were averaged for each island, depth and size class. IN = Inaccessible, NG = Nightingale, TR = Tristan, D = Deep, S = Shallow, C20 = large and C40 = small lobsters.

Table 3: Mean dietary diversity ( $H'$ ) calculated using the Shannon Weiner index and results of a one-way ANOVA (t-test) to compare mean diversity across each island (size and depth category). Significant results are highlighted in bold.

Diversity ( $H'$ )			ANOVA and t-test results			
	Mean	SE		F	df	P
<b>Island</b>			<b>Island</b>	4.928	2	<b>0.008</b>
Inaccessible	0.431	0.035	IN and NG			0.842
Nightingale	0.404	0.037	IN and TR			<b>0.008</b>
Tristan	0.282	0.032	NG and TR			0.049
	Mean	SE		t	df	P
<b>Depth</b>			<b>Depth</b>	2.271	341	<b>0.024</b>
Deep	0.415	0.027				
Shallow	0.32	0.03				
	Mean	SE		t	df	P
<b>Size</b>			<b>Size</b>	0.428	341	0.669
Large	0.386	0.031				
Small	0.369	0.027				

Table 4: PERMANOVA results comparing diet across island, depth, size and the interactions thereof. Significant results are highlighted in bold.

Source of Variation	Df	Sum Sq	Mean Sq	Pseudo-F	P (perm)
Island	2	40169	20085	6.114	<b>0.001</b>
Depth	1	20271	20271	6.1706	<b>0.001</b>
Size	1	14240	14240	4.3347	<b>0.001</b>
Island: Depth	2	21854	10927	3.3263	<b>0.002</b>
Island: Size	2	36742	18371	5.5923	<b>0.001</b>
Depth:Size	1	26133	26133	7.9551	<b>0.001</b>
Island:Depth:Count	2	19320	9659.9	2.9406	<b>0.003</b>
Residuals	323	1.0611E6	3285		

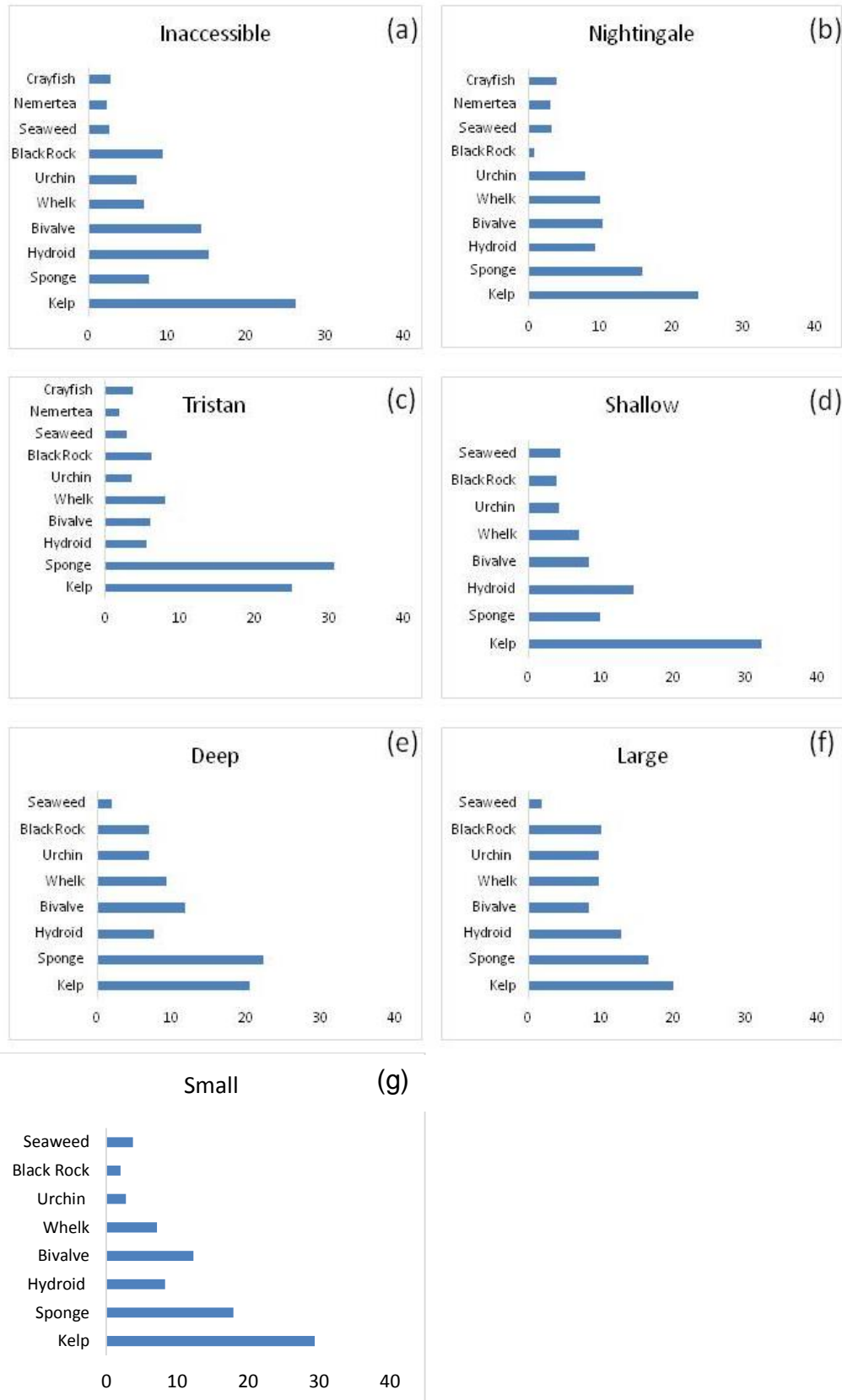


*Figure 5:* MDS plots comparing dietary data from individual rock lobsters across (a) the three islands regardless of depth and size (b) depth, regardless of island and size (c) the two size classes, regardless of island and depth, (d) the three islands for shallow depth only and (e) the three islands for deep depth only. Average similarities for each island, depth or size are expressed as a percentage. IN = Inaccessible, NG = Nightingale, TR = Tristan, D = Deep, S = Shallow, Count 20 = large lobsters and Count 40 = small lobsters.

SIMPER analyses revealed that the average dissimilarity between islands, depth and size was great (85-86% in all cases), suggesting that diets were in fact quite different. Prey items that contributed most to the average dissimilarity between islands, depth and size are shown in Fig. 6. Kelp was the most common food item found across the three islands, as well as across depth and size. This was followed by sponges, hydroids, whelks and bivalves. Lobsters from Inaccessible Island contained a large proportion of kelp, bivalve, hydroid and black rock (Fig 6a), while Nightingale also had kelp, but more sponge and less bivalve, hydroid and black rock (Fig. 6b). Rock lobsters from Tristan Island contained lots of kelp and sponge, and less urchin, bivalve, whelk and hydroids, and black rock (Fig. 6c). Lobster stomachs from shallow depths contained lots of kelp and hydroids (Fig. 6d), while those in the deeper depths had less kelp and hydroid, but more sponge (Fig. 6e). Large lobsters had less sponge, kelp, seaweed and bivalve compared to small lobsters, and instead more urchin, hydroid, whelk and black rock (Figs. 6f,g). Cannibalism was very low across the islands, as rock lobster accounted for a very low mean percentage volume of the samples.

*Table 5:* Results from the PERMDISP analysis comparing dispersion in the data from the centroid between islands depth and size. Significant results are highlighted in bold.

<i>Pairwise Comparisons</i>	<i>F Value</i>	<i>P (perm)</i>
<b><i>Islands</i></b>	<b>2.6722</b>	<b>0.132</b>
Inaccessible - Nightingale		4.9E-2
Inaccessible - Tristan		0.661
Nightingale - Tristan		0.131
<b><i>Depth</i></b>	<b>0.77343</b>	<b>0.416</b>
Deep-Shallow		0.446
<b><i>Size</i></b>	<b>0.81004</b>	<b>0.425</b>
Small-Large		0.442

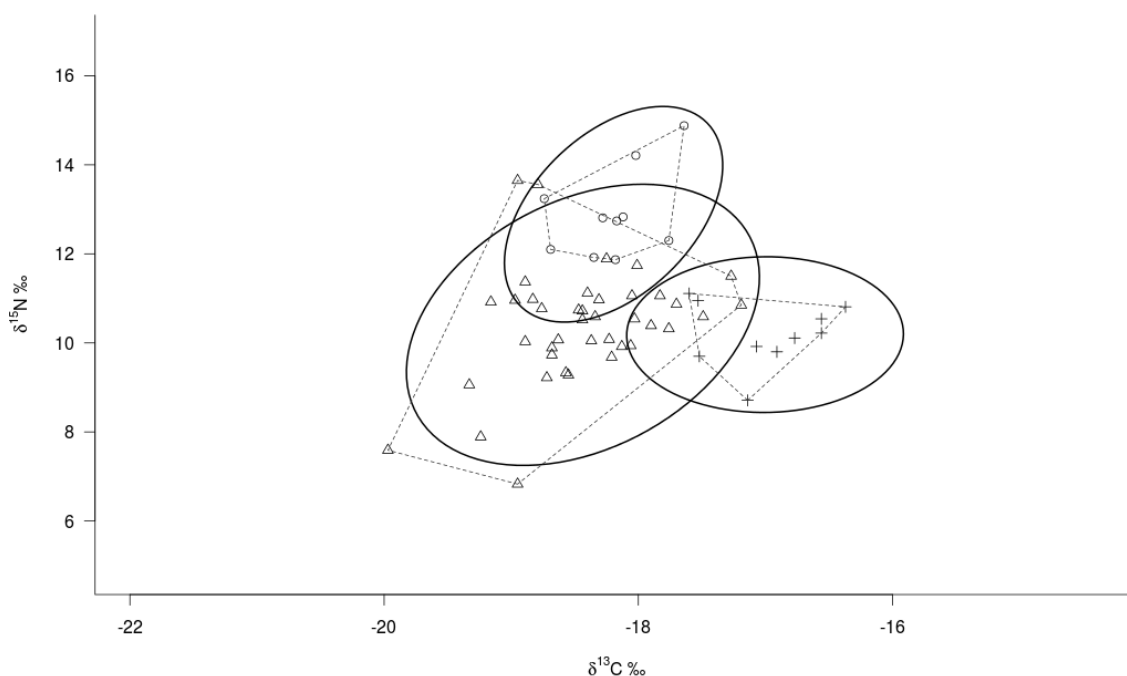


Percentage Volume (%)

Figure 6: Mean percentage volume of prey items contributing most to the dissimilarity in lobster diet across (a) Inaccessible (b) Nightingale (c) Tristan Islands (d) shallow and (e) deep depth categories and (f) large and (g) small size categories

### 3.3 Stable Isotope Analysis

The carbon and nitrogen isotopic signatures for the sampled rock lobster are shown in Fig. 7, where it is evident that isotopic signatures for Tristan and Inaccessible Island overlap with Nightingale Island, but Tristan and Inaccessible Island cluster away from each other. The depleted carbon values at Inaccessible Island averaged  $-19\text{‰}$ , compared to  $-18\text{‰}$  at Nightingale Island and  $-17\text{‰}$  at Tristan Island, where the carbon signatures were more enriched in carbon. It is also evident that the lobsters from Inaccessible Island were more enriched in nitrogen compared to Tristan and Nightingale Island, which were more similar to one another. Nightingale Island had the largest range of nitrogen values, from  $7.1\text{‰}$ - $13.8\text{‰}$ , while Tristan Island had the lowest range of  $8.5\text{‰}$  –  $10.8\text{‰}$ .



*Figure 7:* The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the isotope signatures of the sampled lobsters at the three islands. The small circles represent Inaccessible Island, triangles represent Nightingale Island and the crosses represent Tristan Island.

Table 6: ANOVA results comparing island, size and depth for the carbon and nitrogen results for the stable isotope. Significant results are highlighted in bold

ANOVA and t-test results					
	<i>Df</i>	<i>Sum Sq</i>	<i>Mean Sq</i>	<i>F Value</i>	<i>P-value</i>
<b>Island</b>					
$\delta^{15}N$					
Island	2	42.25	21.123	15.08	<b>&lt; 0.0001 ***</b>
Residuals	57	79.86	1.401		
$\delta^{13}C$					
Island	2	10.02	5.02	10.23	<b>0.0001***</b>
Residuals	57	27.91	0.49		
<b>Size</b>					
$\delta^{13}C$					
Size	1	0.045	0.0449	0.136	0.714
Residuals	38	12.508	0.3292		
$\delta^{15}N$					
Size	1	1.03	1.029	0.612	0.439
Residuals	38	63.88	1.681		
<b>Depth</b>					
$\delta^{13}C$					
Depth	1	4.579	4.579	21.82	<b>0.0001***</b>
Residuals	38	0.21			
$\delta^{15}N$					
Depth	1	2.87	2.868	1.756	0.193
Residuals	38	62.04	1.633		
Significant Codes	#<0.1	* <i>P</i> < 0.05	** <i>P</i> < 0.01	*** <i>P</i> < 0.001	

The ANOVA results in Table 6 showed that there was a significant difference in carbon signatures between depths and islands ( $p=0.0001$ ). However, there was no significant difference between sizes ( $p=0.714$ ). No significant difference in nitrogen signatures was measured between size and depth ( $p>0.05$ ), but there was a significant difference among islands ( $p < 0.0001$ ). The *posthoc* TUKEY test (Appendix A7) found that there was a significant difference in the nitrogen signatures between Inaccessible and Tristan Islands ( $p=0.0001$ ) and between

Inaccessible and Nightingale Islands ( $p=0.0001$ ), but no significant difference between Nightingale and Tristan Islands ( $p=0.3655$ ). There was also a significant difference in the carbon signatures between Tristan and Inaccessible Islands ( $p=0.0001$ ) and between Tristan and Nightingale Islands, but not between Nightingale and Inaccessible Islands ( $p=0.264$ ). Among the possible prey items, lobster (i.e. cannibalised) had the most enriched carbon values, followed by nematode and whelk. Seaweed, with  $-27.1\text{‰}$  and sponge  $-26.4\text{‰}$  had the most depleted carbon values.

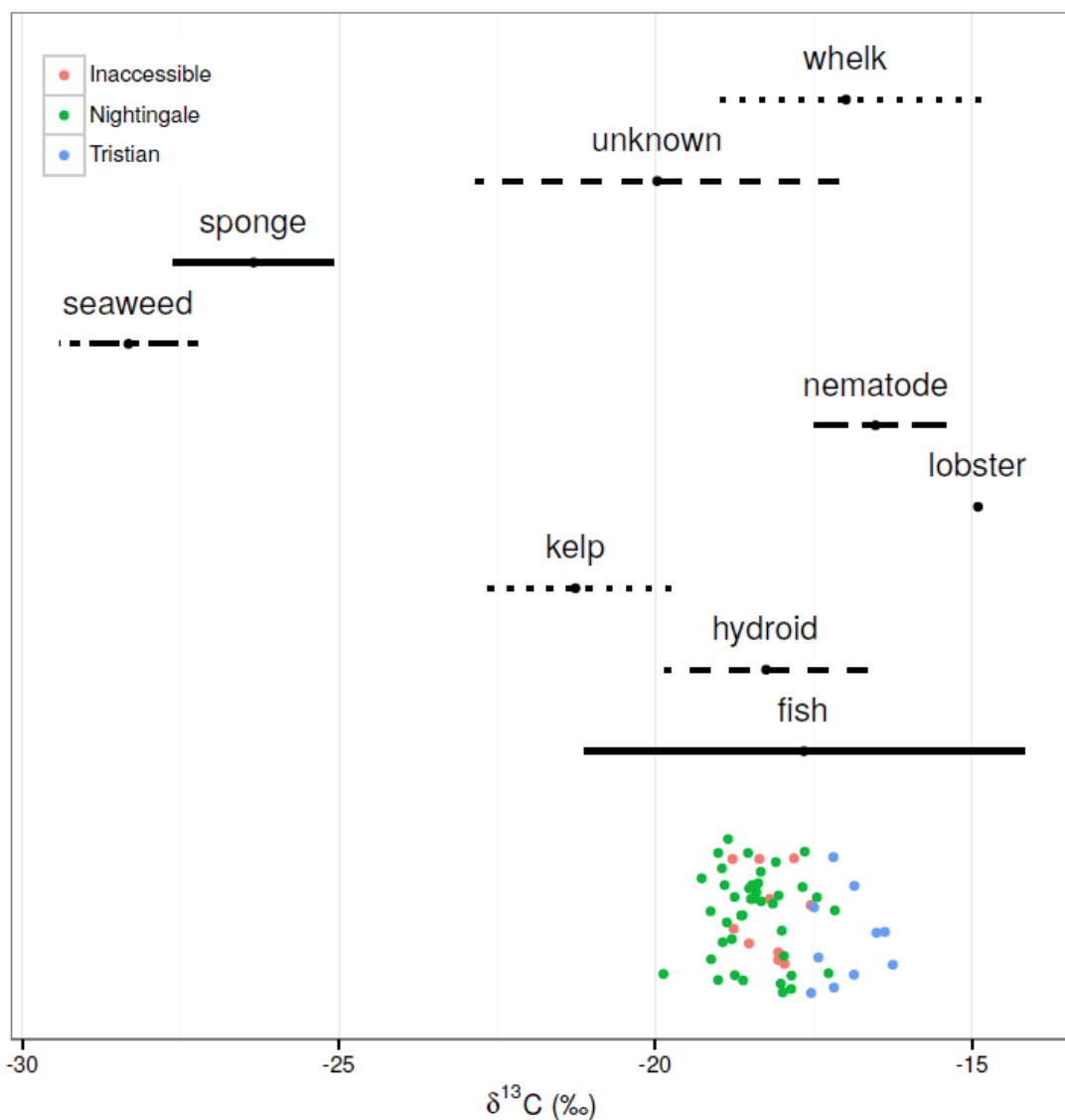
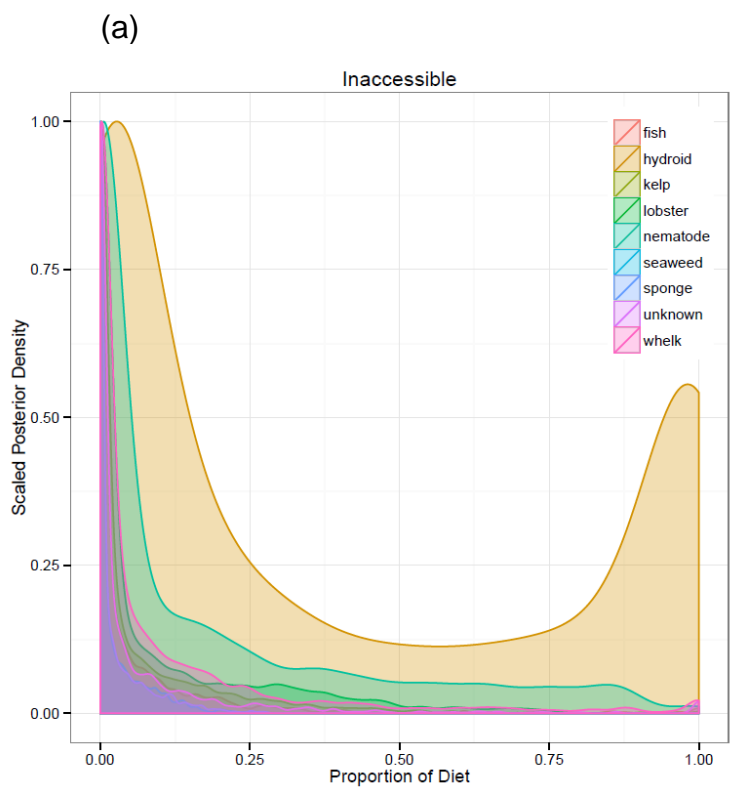


Figure 8: Stable isotope input for carbon across Inaccessible, Tristan and Nightingale Island. Consumer data are shown in colour and possible prey items are labelled above.



Mixing models were used to calculate the importance of each prey item to the lobster diet (Fig 9; for full results see Appendix Table A5). We accept that the model yielded valid results, on the grounds that fish, the most common item in the guts of lobsters, was not part of the natural diet and would not, therefore have been reflected in the isotope values. As such the results indicated different diet preferences depending on where the lobster came from, explaining the grouping seen in Fig. 7. At Inaccessible Island, hydroid constituted the highest proportion of diet ( $0.386 \pm 0.407$ ), followed by nematode ( $0.169 \pm 0.251$ ) and whelk ( $0.105 \pm 0.2$ ). At Nightingale Island, hydroid contributed the highest proportion of the diet ( $0.411 \pm 0.426$ ), along with whelk ( $0.126 \pm 0.238$ ) and unknown prey item ( $0.104 \pm 0.22$ ). At Tristan Island, nematode made up the highest proportion in the diet ( $0.406 \pm 0.408$ ), followed by whelk ( $0.215 \pm 0.338$ ) and lobster ( $0.139 \pm 0.219$ ).



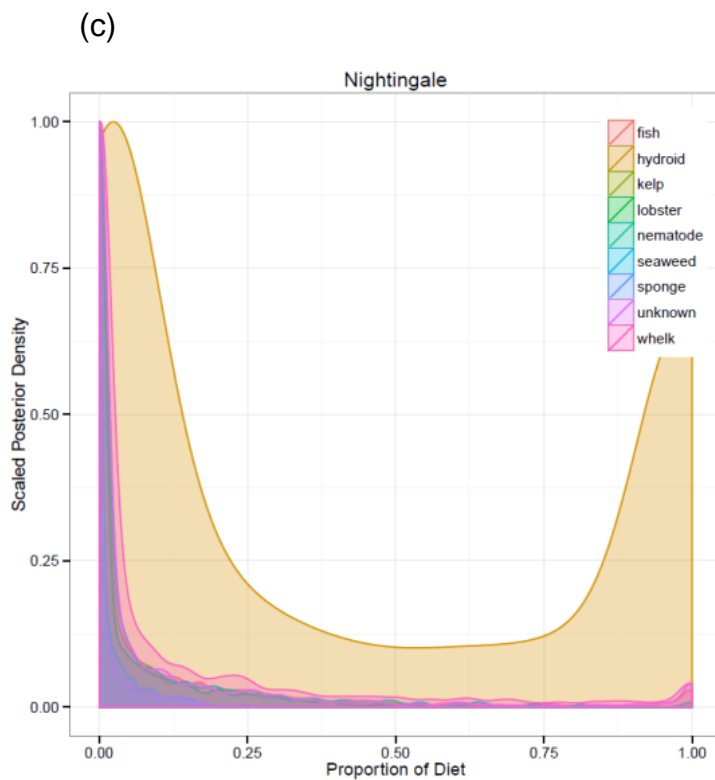
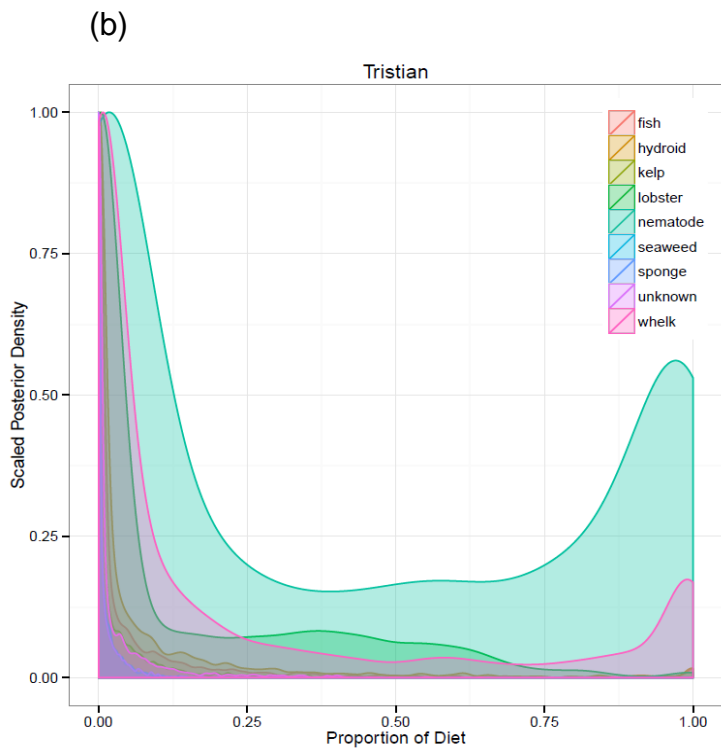


Figure 9: Posterior plots of lobster diet based on the isotope signatures for (a) Inaccessible (b) Nightingale and (c) Tristan Islands. Refer to appendix Table A5 for full result ranges

#### 4. Discussion

This study is the first to analyse the diet of the Tristan rock lobster *Jasus tristani* at Nightingale Island following the sinking of the *OLIVA* in 2011, and compare this to the diet of rock lobsters at unaffected, neighbouring islands.

There are concerns about analysing gut contents from rock lobsters that have been caught using baited traps (e.g. Griffiths et al. 2000), because the lobsters may feed on the bait (even although it is protected), or on invertebrates that have been attracted into the trap. While I am aware of these problems, the use of traps to secure the lobsters was imperative for three reasons: 1) the number of rock lobsters required for this study, 2) the remoteness of the study area and 3) the depth from which the lobsters needed to be collected, which collectively made it unfeasible to sample the animals by diving. To counter these concerns, firstly, the contribution to the diet of animals that might have been attracted to the traps was very small (<1%) and, secondly, I ran analyses of diet both with fish included and fish excluded because of the likelihood that fish in the gut contents would have been derived from the bait.

A high proportion of fish was found in the rock lobster stomachs, across all three islands, yet fish is generally not part of the natural diet of rock lobster (Pollock 1991, Góes and Lins-Oliveira 2009, Griffiths et al. 2000, Mayfield et al. 2000). Fish remains found in the lobster stomachs were not unexpected because of the likelihood that the lobsters would access the fish bait in the traps. I recognise that because of this, there are problems associated with using baited traps in diet studies, but this method was the only feasible option given the remoteness and depth of the study area. Fish remains included small teeth, much like those of hake *Merluccius* sp. teeth, and given that hake heads are used as bait in the

lobster traps, I attributed the presence of fish in lobster stomachs to bait from the lobster traps, despite the traps being bait-protected. For this reason, the gut data were analysed excluding fish. Furthermore, stable isotope analyses demonstrated that fish was a very low contributor to the background natural diet of *J. tristani* thereby providing further validation for excluding fish from the gut content analyses.

The two techniques of dietary analysis employed in the current study gave differing outcomes for the proportional contribution of prey to lobster diet. Gut content analysis indicated that kelp, sponge and hydroid were the main components of the diet across the three islands, whereas the stable isotope analysis showed that hydroid and nematode were the most important contributors. Observed differences likely reflect the different dietary time scales of the two techniques (i.e. SI = long term while gut contents = a point in time, (Overman and Parrish 2001, Waddington et al. 2008) and the variability in evacuation rates of prey from lobster guts. Additionally, the results may have been affected by the high percentage of lobsters feeding on the fish bait, which may have pushed through some prey items from the gut so that they were not detected. To overcome this problem, one could employ molecular methods on lobster faecal matter as was successfully done in a study by Redd et al. (2008). In addition, it is important to consider that the mixing models were run on only the carbon isotope results, making them weaker than if carbon and nitrogen isotopes had been used.

Differences in sex ratio of the catches likely reflect the catchability of male and female lobsters, which may be linked to moulting (e.g. McKoy and Esterman

1981, Goñi et al. 2003). Given that female *J. tristanii* moult between January-March and subsequently lower their food intake (Glass 2014), it was not surprising that their male counterparts dominated the catches. Gut fullness was significantly lower at Nightingale Island, which might be expected if more females (moulting and not feeding) were caught there. However, the female:male sex ratio was similar across all islands (ca. 1:20). The PERMANOVA and SIMPER analysis suggest that diets differed between island, size and depth. This was perhaps somewhat evident in MDS plots of the averaged data (Fig. 4), but not in the MDS plots from individual rock lobster data (Fig. 5), where there was a large overlap in diet between islands, depth and size, and high variability (low percentage similarity) within. The differences in diet were difficult to assess visually in the individual rock lobster data (Fig. 5), possibly due to the low percentage similarity of diet within each island, i.e. individual variation in the amount and type of prey each lobster consumed, and because of the large sample size, the MDS can compress the information from the similarity matrix and give a distorted view of the data (Clarke and Warwick 2001). Therefore an MDS of the averaged data (Fig. 4) helps provides a clearer understanding of lobster diet among islands and between depths and sizes.

Carbon and Nitrogen signatures were significantly different among islands, and between depths (carbon signature only), thus supporting the PERMANOVA and SIMPER results. However, only the diet of the lobsters at Tristan Island was different to that of Nightingale and Inaccessible. Reasons for this difference in diet at Tristan Island are unclear, but I hypothesise it is due to the high within-site variability in the diet. An alternative hypothesis is that, as Tristan da Cunha is the only inhabited island, perhaps human interference in this island (such as

building a small harbour, sewage water, etc.) might have caused a change in the marine ecosystem, or that the ecosystems are naturally different among islands. Nematodes represented the main contribution of the natural diet at Tristan Island, but were not identified as a highly abundant prey item in rock lobster from Nightingale or Inaccessible Islands. The high abundance of nematodes in the diet at Tristan Island could reflect low species diversity and therefore limited availability of prey for the lobsters to feed on. Rock lobster diet at Nightingale and Inaccessible Islands exhibited a high representation of hydroids in the stable isotope signature analysis, which correlates with the stomach content analysis, representing 14.9% of diet at Inaccessible and 11.1% and Nightingale.

At each island and at each depth, lobster diet was highly variable, with significant overlap between location, size and depth. Rock lobsters from Tristan Island showed a higher degree of similarity in their diet than those from the other two islands, and stable isotope analyses found that lobsters from Tristan Island had a more enriched carbon isotope signature compared to lobsters from Nightingale and Inaccessible Islands.

Enriched values may reflect a higher inshore and benthic foraging diet (17 ‰) and are not restricted to the input of carbon from pelagic phytoplanktonic origin (Couturier et al. 2013). Carbon can be used to trace the origins of organic matter within an organism in a food web (Kohn 2010). For instance, C<sub>3</sub> plants (including soybeans)  $\delta^{13}\text{C}$  values range between -22.00 and -33.00 ‰ (O'Leary 1988), while C<sub>3</sub>  $\delta^{15}\text{N}$  values range from -7 to 7 ‰ (Kelly 2000). These values are very different from marine phytoplankton signatures, which range from -19.1

to -22 ‰ for  $\delta^{13}\text{C}$  and 3 to 12 ‰ for  $\delta^{15}\text{N}$  (Gearing et al. 1984, Owens 1987). If the range of carbon isotopic composition was taken into consideration, and with an enrichment of about 0.4 ‰ per trophic level (Post 2002), then the results indicate that at most, C3 plant material contributed trivially to the food web. The significant differences in carbon isotopic signatures among the islands could be attributed to the different carbon isotopic structures of benthic organisms in the different environments. However, more importantly, there is a clear indication that soya beans are not currently being consumed by rock lobsters at Nightingale Island and either rotted away, dispersed or had a very short-lived influence.

It was predicted that the level of impact on the benthic environment at Nightingale Island from the grounding of the *OLIVA* and the subsequent spill of the soya bean in 2011 would currently be very difficult to assess, due to the time delay of 4 years since the incident. At the time, it was reported that the sinking of 60,000 tonnes of soya beans formed a thick sludge on the benthic environment (A. James, Ovenstone Fisheries, pers. comm.), and the CPUE of the lobster industry initially declined as a result, so as a precaution the fishery was closed. However, after the reopening of the fishery approximately a year later, the CPUE increased and by 2014 was 2-3 times greater than before the spill (Glass 2014). Reasons for the increase in CPUE are still unclear but there was no significant difference in the carbon values from the stable isotope analysis between Inaccessible and Nightingale Islands, indicating that soya beans do not play a part in the current food web at Nightingale Island. As the spill occurred four years ago, any remnants of the soya beans would have been either previously consumed as a dietary item by the benthic species, including

lobster, or eradicated from the environment by natural wave movement. An earlier study immediately following the soya spill would have been more useful in identifying the impact of soya beans on the diet of the lobsters at Nightingale Island. Collectively, consideration needs to be given to the facts that (1) no soya beans were observed in the gut content analysis, (2) the isotope analysis also indicated that soya had not contributed to the diet, (3) there was a long time frame of 4 years since the soya spill, and (4) the diet of lobsters sampled at Nightingale did not stand out significantly from Inaccessible and Tristan Islands (with all three islands differing significantly from each other, and Nightingale and Inaccessible showing more similarity to one another than to Tristan). Therefore it can be concluded that if the soya bean spill did have an impact on the benthic environment, it is no longer apparent and exerted no influence on the diet of the lobsters at Nightingale Island. Although there was a significant difference in diet of lobsters sampled at Inaccessible and Tristan Island in the stable isotope signatures, this is likely due to differences in benthic composition. Two possible explanations advanced above for the increase in CPUE at Nightingale Island following the spill are (1) that the soya beans may have acted as an additional food source for a short period of time and increased productivity, or (2) that the soya spill negatively affected benthic prey and lobsters were therefore more likely to be attracted to traps. The latter seems more likely, as any alteration of productivity would have been unlikely to be manifested so rapidly in CPUE due to the slow growth rates. However, there was nothing in the diet that suggested a difference in diet at Nightingale Island only and not at the other two islands. Gut fullness was, however, significantly lower at Nightingale island, so perhaps food is less abundant there than at the other two non-impacted islands.



The diet of lobsters at Nightingale Island, four years after the soya spill, was similar to that described in a previous study conducted in the early 1990s. Pollock (1991) examined a small sample size of 32 lobster stomachs, 27 from Nightingale Island and 5 from Inaccessible Island. A quantitative analysis of Pollock's data of food consumption could not be conducted because of the small number of samples. However, a qualitative analysis allowed major food items to be identified, and no difference in these items was found between the two islands. Pollock (1991) showed that the sea urchin *Arbacia dufresnii* and kelp were the main benthic organisms present across the three islands on rocky surfaces between 4-50m where lobsters also occur. Furthermore, filter-feeding organisms were not present on rock surfaces, but whelks and barnacles occurred in rock crevices and caves where a large proportion of the lobster population was found (Pollock 1991). This description of the benthic composition of the islands would explain the dietary composition of the lobsters sampled by Pollock (1991), in which main prey items included kelp, urchins, barnacles, whelks and other macroalgae.

The results from this study found comparable results to those of Pollock (1991): Similar prey items were found across all three islands, and kelp was one of the most common items. I also found urchins and barnacles in the stomachs I analysed, but they were not as common as in the Pollock (1991) study. I did record other seaweeds, but only in trace amounts. While similar prey items were recorded in both my study and that conducted by Pollock in 1991, it is difficult to make a direct comparison or evaluate any differences in diet between the time periods due to the very small and uneven sample size, and the fact that lobsters from Pollock (1991) may have come from shallower depths.

Across the globe, lobster diet is known to differ depending on lobster size, depth and habitat. Ontogenetic changes in diet have been observed in the clawed lobster *Homarus americanus* (Lawton and Lavalli 1995), the spiny lobster *Panulirus homarus* (Berry 1971) and the flat back lobster *Stereomastis sculpta* (Cartes and Abello 1992). Depth-related dietary changes have also been observed in *Polycheles typhlops* with diet changing as the abundance of prey species alters (Cartes and Abello 1992). However, Mayfield et al. (2000) found that *Jasus lalandii* diets did not differ with depth, and species richness and diversity were very similar across depths. I found that dietary composition and diversity did differ between the shallow and deep depth, and diet, but not dietary diversity, did differ between large and small lobsters.

Spiny lobster diet is often quite broad and generally includes polychaetes, molluscs, crustaceans, bivalves, echinoderms and sponges (Ennis 1973, Elnor and Campbell 1987, Lawton and Lavalli 1995, Mayfield et al. 2000). The diet of *J. tristani* is similar, except that macroalgae (especially kelps) appear to be an important food source to them and they are more of an omnivorous opportunistic feeder. The prevalence of sponge as a major prey item seems unusual in that it does not appear to be very nutritious and has been regarded as an unorthodox prey item for *Jasus lalandii* (Barkai et al. 1996), and something that is common in its diet when benthic reefs are depauperate (Haley et al. 2011), as is the case with the reefs of the Tristan Da Cunha island cluster (Scott and Tyler 2008, Scott 2010a,b), where there are very few prey items of high nutritional value. Barkai et al. (1996) have shown that *J. lalandii* has all the necessary enzymes to digest

sponges, but lack the laminarinase and alginase necessary for breakdown of kelps.

Commercial fishing for rock lobster, the only commercial fishing industry in the island archipelago, may have an impact on the rest of the ecosystem by removing lobsters, a main predator in the system. In previous studies (Waddington et al. 2008), differences in spiny lobster abundance, often related to exploitation intensity, have been shown to influence prey abundance in New Zealand (Shears and Babcock 2002), Tasmania (Pederson and Johnson 2006), South Africa (Barkai and Branch 1988a,b, Blamey and Branch 2012, Blamey et al. 2013) and California (Tegner and Levin 1983). At present though, the Tristan commercial and recreational lobster fishing industry is considered to be sustainable and managed conservatively with a CPUE at Tristan Island of 180 tonnes/per year, compared to 52 tonnes/per year at Nightingale Island and 53 tonnes per/year at Inaccessible Island (Glass 2014). This indicates a larger population of lobsters at Tristan Island compared to the outer islands, which may explain why Scott and Tyler (2008 p.22) reported the Tristan nearshore benthos as “*extremely impoverished in terms of species numbers*”. Scott (2010b) found that the habitats and marine communities of the shallow subtidal and deeper areas at Inaccessible Island were different to those of Tristan and Nightingale Islands. Inaccessible Island is exposed to stronger currents and wave activity, and is dominated by seaweeds that thrive in extremely exposed conditions (Scott 2010b).

The rock lobsters sampled across the three islands appear to be opportunistic omnivorous feeders, as was found in previous studies of other species of

lobster, which concluded that they are mobile nocturnal crustaceans that feed on most available food sources (Andrew 1999, Leber et al. 2008, O'Rourke et al. 2012). This was confirmed in the range of prey types found in the gut content analysis and the stable isotope results. The feeding behaviour is also similar to that of *Palinurus elephas* in the north-west Mediterranean, which studies have found to be a generalist, opportunistic feeder that is omnivorous and changes its prey as a function of the composition of the benthic community (Goñi et al. 1998). Although kelp and sponge were the most common prey items identified in my study, the diversity of species found in the gut shows opportunistic feeding in the lobsters, taking advantage of any prey that may be accessible in the benthic environment. Carbon and Nitrogen isotope values from lobsters at Tristan and Inaccessible Island did not overlap with each other, but both overlapped with Nightingale Island. This indicates the lobsters at Nightingale Island might have a more varied diet. This variety in diet was also shown in the stable isotope results, the large carbon and nitrogen ranges could possibly indicate that within the species organisms were feeding at different trophic levels.

## Conclusion

From visual analyses of gut content, this study has shown there were slight but significant differences in the diet of *J. tristani* among the three Islands, and between size and depth categories. The rock lobsters at Tristan Island showed a higher degree in similarity in the diet compared to Nightingale and Inaccessible, as shown in the MDS plots and the stable isotope analysis where Tristan had a more enriched carbon isotope signature. The study also found

similar prey items in the lobsters to those detected in previous studies. Even though there was a difference in lobster diet between the three islands, the absence of soya beans in the gut contents, and no obvious C3 plant carbon signature in the stable isotope analysis, such as would be expected from soya bean, indicate that currently there is no evidence of soya in the rock lobster diet at Nightingale Island. I was unable to determine if soya beans could have played a role in the natural diets of the rock lobsters prior to the sampling done in 2015. Gut fullness was significantly less at Nightingale Island and it may be that lobsters have less food available there since the sinking of the soya beans. To conduct future studies on whether the soya spill of the *OLIVA* at Nightingale Island did have an impact on the rock lobster fishery, I would undertake an underwater photographic study of the wreck and the surrounding area, to provide evidence of whether the black sludge is still present, and whether differences in benthic composition exist at various distances from the wreck.

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## Appendices

A1: ANOVA results comparing rock lobster carapace length across islands (Inaccessible, Nightingale and Tristan), depth (shallow and deep) and size class (count 40 and count 20). Significant figures are highlighted in bold

<i>Source of Variation</i>	<i>Df</i>	<i>Sum Sq</i>	<i>Mean Sq</i>	<i>F value</i>	<i>P-value</i>
Island	2	2276	1138	78.119	<b>&lt; 0.001</b>
Depth	1	4	4	0.296	0.586
Island:Depth	2	507	253	17.402	<b>&lt;0.001</b>
Island:Count	2	753	376	25.846	<b>&lt;0.001</b>
Depth:Count	1	6	6	0.384	0.536
Island:Depth:Count	2	15	8	.529	0.589
Residuals	411	5987	15		

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Significant Codes . <0.1 \**P* <0.05 \*\**P* <0.01 \*\*\**P* <0.001

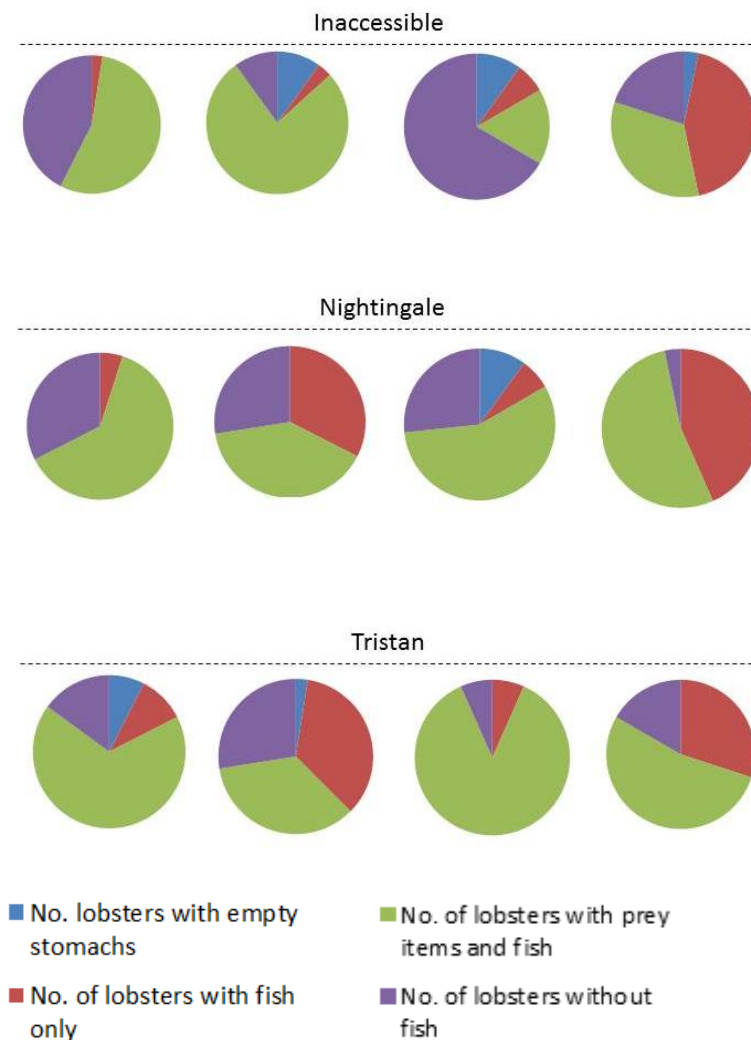
A2: TUKEY multi comparison of means test results comparing means of rock lobster carapace length across islands (Inaccessible, Nightingale and Tristan). Significant figures are highlighted in bold

<i>Island</i>	<i>diff</i>	<i>lower</i>	<i>upper</i>	<i>P value</i>
Tristan- Inaccessible	1.922347	0.858012	2.986681	<b>&lt;0.0001</b>
Nightingale - Inaccessible	5.576821	4.514491	6.639152	<b>&lt;0.001</b>
Nightingale-Tristan	3.654475	2.571762	4.737188	<b>&lt;0.001</b>

A3: TUKEY multi comparison of means test results comparing means of GFI across islands (Inaccessible, Nightingale and Tristan). Significant figures are highlighted in bold

<i>Island</i>	<i>diff</i>	<i>lower</i>	<i>upper</i>	<i>P-value</i>
Tristan-Nightingale	2.683377	1.304415	4.062338	<b>&lt;0.001</b>
Inaccessible-Nightingale	3.693405	2.340404	5.046406	<b>&lt;0.001</b>
Inaccessbile-Tristan	1.010028	-.345526	2.365582	0.187

**DEEP COUNT 40   DEEP COUNT 20   SHALLOW COUNT 40   SHALLOW COUNT 20**



A4: Proportion of rock lobster stomachs that were empty, contained only fish, contained fish and prey items and contained no fish, for each island, depth and size class.

A5: Mixing model Bayesian statistics for the sampled lobsters across the three islands (Nightingale, Inaccessible and Tristan)

	Mean	SD	2.50%	5%	25%	50%	75%	95%
<b>Overall Population Diet</b>								
Fish	0.106	0.094	0.004	0.008	0.035	0.081	0.151	0.293
Hydroid	0.138	0.110	0.005	0.01	0.051	0.111	0.199	0.352
Kelp	0.105	0.092	0.003	0.006	0.033	0.078	0.151	0.293
Lobster	0.125	0.106	0.004	0.007	0.039	0.097	0.185	0.340
Nematode	0.139	0.114	0.006	0.01	0.05	0.11	0.196	0.366
Seaweed	0.082	0.083	0.002	0.005	0.024	0.055	0.113	0.252
Sponge	0.084	0.086	0.002	0.003	0.021	0.057	0.118	0.256
Unknown	0.101	0.095	0.002	0.004	0.028	0.075	0.142	0.297
Whelk	0.121	0.101	0.005	0.009	0.044	0.094	0.174	0.316
<b>Lobster Diet per Island</b>								
<i>Inaccessible</i>								
Fish	0.062	0.142	0	0	0	0.004	0.06	0.283
Hydroid	0.386	0.407	0	0	0.014	0.175	0.879	1.000
Kelp	0.066	0.122	0	0	0	0.006	0.079	0.318
Lobster	0.092	0.156	0	0	0	0.004	0.121	0.442
Nematode	0.169	0.251	0	0	0	0.026	0.245	0.767
Seaweed	0.026	0.044	0	0	0	0.002	0.034	0.123
Sponge	0.027	0.049	0	0	0	0.002	0.034	0.136
Unknown	0.069	0.159	0	0	0	0.004	0.061	0.345
Whelk flesh?	0.105	0.200	0	0	0	0.010	0.115	0.591

*Nightingale*

Fish	0.098	0.208	0	0	0	0.004	0.089	0.587
Hydroid	0.411	0.426	0	0	0.008	0.182	0.959	1.000
Kelp	0.07	0.144	0	0	0	0.003	0.074	0.354
Lobster	0.061	0.119	0	0	0	0.002	0.069	0.306
Nematode	0.082	0.166	0	0	0	0.003	0.082	0.451
Seaweed	0.025	0.047	0	0	0	0.002	0.029	0.129
Sponge	0.023	0.043	0	0	0	0.001	0.024	0.127
Unknown	0.104	0.220	0	0	0	0.004	0.091	0.644
Whelk Flesh?	0.126	0.238	0	0	0	0.007	0.133	0.741

*Tristan*

Fish	0.068	0.163	0	0	0	0.003	0.053	0.365
Hydroid	0.082	0.179	0	0	0	0.005	0.073	0.459
Kelp	0.029	0.061	0	0	0	0.002	0.031	0.142
Lobster	0.139	0.219	0	0	0	0.005	0.239	0.612
Nematode	0.406	0.408	0	0	0.006	0.233	0.872	1.000
Seaweed	0.012	0.023	0	0	0	0.001	0.013	0.055
Sponge	0.013	0.026	0	0	0	0.001	0.014	0.065
Unknown	0.036	0.088	0	0	0	0.001	0.032	0.193
Whelk Flesh ?	0.215	0.338	0	0	0	0.024	0.262	0.999

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A6: TUKEY multi comparison of means test results comparing the carbon and nitrogen signatures across the three islands. Significant figures are highlighted in bold

	<i>diff</i>	<i>lower</i>	<i>upper</i>	<i>P-value</i>
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**D15N**

NG and TR	1	-0.43553	1.57853	0.3655
IN and TR	3	1.364197	3.911803	<b>0.0001***</b>
IN and NG	2	1.05947	3.07353	<b>0.0001***</b>

**d13C**

NG and IN	0	-0.2053	0.9853	0.264
TR and IN	1	0.5869	2.093	<b>0.0001***</b>
TR and NG	1	0.3546	1.5453	<b>0.0001***</b>

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Significant Codes	#<0.1	* <i>P</i> <0.05	** <i>P</i> <0.01	*** <i>P</i> <0.001
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