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What lies within; Annelid polychaetes found in micro-habitats of coral/carbonate material from SW Indian Ocean seamounts

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Abstract

Individual corals, coral framework and rubble are generally quite abundant on seamounts, with carbonate materials derived from an unknown source also being found. These micro-habitats are known to often harbour higher abundance of smaller sized fauna and thus potentially increases the biodiversity of that region. However, very few studies have examined what may reside within the coral and carbonate structures themselves, if anything at all. Samples that were collected on five seamounts during a research cruise to the South West Indian Ocean Ridge in 2011, were examined opportunistically in order to determine if there were any animals to be found inside the hard coral framework/rubble material. The hard material was dissolved away using 10% acetic acid and was examined on a twice daily basis to remove any animals that had been released through the dissolution process. There were a surprising number of phyla found inside the micro-habitats created by the coral rubble/framework material including sponges, brachiopods and a number of different polychaete families. As the polychaetes were more numerous, they were investigated more closely and a total of 34 different families were identified, with the Syllidae being found in every sample examined. The methodology presented here highlights that a “controlled” weak acid dissolution can be used to release the fauna from deep inside the coral rubble/framework material. Frequent removal (twice daily or more often) of liberated material meant that the fauna released were exposed to the acid for a minimum amount of time resulting in specimens in better condition and with more identifiable
features. The preliminary results also illustrate the importance of sampling the dead coral framework/rubble/carbonate material, not only to identify them for what they are, but to look more closely at the fauna residing inside the structure themselves.

Keywords: endocryptolithic fauna, South West Indian Ocean Ridge, seamounts, coral, macrofauna, polychaetes

1. Introduction

Yesson et al. (2011) predicted that there were >100,000 seamounts and knolls spread throughout the world’s oceans and it has been estimated that less than 0.5% of them have been studied biologically and not in any detail (Ramirez-Llodra et al. 2010). The studies that have been undertaken have tended to focus on the larger, more fragile corals and sponges, with some, more recent studies, looking at the macrofauna inhabiting the soft sediments found on seamounts (Chivers et al 2013, and refs therein) or living freely on the larger sessile fauna (Serpetti et al. in press this vol.). Even fewer studies have examined the internal structures of individual corals and the often dead framework/rubble to investigate if any small fauna reside within the cavity structures of the polyps themselves.

Work in the late 1970s by Hutchings and Weate (1978) found few individuals after the material was crushed and this may potentially be one reason why little work has been undertaken to investigate the coral and carbonate material further in this way. In studies undertaken by Jensen and Frederiksen (1992) and Mortensen and Fossa (2006), they too cracked individual coral branches into smaller pieces to extract fauna residing there. However, one can only assume that they did not find many individuals as they do not present results specifically for the
endocryptolithic fauna. The authors of this paper have experience of crushing shallow carbonate material from the summit of an Atlantic seamount (130 m) and subjecting it to careful, time-consuming examination to extract all observed fauna. Despite this, previously undetected smaller fauna were subsequently found to be present after removing the carbonate by dissolution. Brock and Brock (1977) dissolved shallow water coral reef carbonate in an agitated solution of 4% nitric acid. More recent work has focussed on carbonates formed in association with seep sites. Levin et al (2015) studied the macrofauna inhabiting the authigenic carbonates at methane seeps off Costa Rica. They found that species diversity, particularly of polychaetes and gastropods, was higher on rocks that were exposed to active seepage and that the composition between carbonate material and seep sediments was quite different (Levin et al. 2015). Other studies looking at fauna in association with carbonate structures have all focussed on material obtained from seeps in the Marmara Sea (Ritt et al. 2010), the del Mar seep off southern California (Grupe et al. 2015) and the Nile Delta (Ritt et al. 2011). These studies have found conflicting results with some finding high faunal densities (Ritt et al. 2010) whereas others report low faunal densities (Grupe et al. 2015).

The limited studies that have been undertaken have also removed the fauna from coral/carbonate material in differing ways. Faunal extraction has mainly been undertaken by placing the carbonate material either in seawater (Leviten 1976) or freshwater (Clausade 1970) to encourage the fauna to crawl out to then be preserved, sorted and identified. Other methods to remove the fauna have included more crude manual extraction by physically breaking up and chipping at the smaller pieces of material (e.g. Grassle 1973; Hutchings and Weate 1978), as well as the use of nitric acid to dissolve coral and extract the boring polychaetes (Tribollet et al. 2002). Hutchings and Weate (1978) only recommended using an acid dissolution
technique for rapid extraction of the fauna as they felt that the fauna could be partially or fully dissolved in acid.

1.1 Objective

Previous work undertaken by Narayanaswamy and Lamont on Ampere seamount in the NE Atlantic found a high density and diversity of macro-infauna concealed within the large fragments of carbonate collected. This preliminary study led us to further investigate the material collected from the seamounts on the South West Indian Ocean Ridge (SWIOR). The samples that were used in this study were primarily collected for other analyses and were only latterly examined opportunistically in order to determine if there were any animals remaining inside the hard coral/carbonate material after onboard removal of all visible fauna.

The overarching aim of this study was to reveal the fauna undetected on, and especially concealed within, the coral/carbonate material that had been collected. Hutchings and Weate (1978) in a similar study did not recommend the use of glacial acetic (99%) to extract the organisms because of concerns of damaging the fauna but did not report any trials with dilute acid. We employed a dilute (10%) acetic acid for dissolution in order to extract the endocryptolithic fauna with a view to determining (i) the existence of fauna living within individual corals, framework/rubble and carbonate material itself, and (ii) the quantity and diversity of any macrofauna, in this instance specifically annelid polychaetes. This method had been trialled earlier on carbonate material collected from the summit (~130 m) of Ampere seamount (located in the Cape Verde Archipelago (Chivers et al. 2013)) and the preliminary results proved to be promising. With the SWIOR material, the overall polychaete composition and quantity per unit weight of carbonate framework/rubble material were investigated in
relation to the type of micro-habitat they were collected from as well as across the five seamounts.

In the SWIOR programme, specific definitions were given to the terms framework, rubble and carbonate. Framework was defined as coral material that still retained its 3-dimensional structure (i.e. branches) (Figure 1a), but a single sample may contain several types of coral. Rubble was specified as material where the original structure of the coral is lost, i.e. the branching system is no longer apparent (Figure 1b). Stylasterid stem as the name suggests was given to the hydrocoral structure where possible (Figure 1c). Carbonate was classified as a hard mass of material (Figure 1d) where the carbonate itself came from an undescribed source. The carbonate mass sometimes contained obvious shell debris.

2. Materials and methods

2.1. Sample collection

Five seamounts, Coral, Melville, Middle of What, Sapmer and Atlantis were sampled along the South West Indian Ocean Ridge (Figure 2), during RRS James Cook cruise JC066 in 2011. The seamounts lie along the ridge in a northeast – southwest orientation, with Atlantis Bank found at the end of the ridge in the northeast, and Coral seamount found at the opposite end. The summits of the seamounts are found at different depths, with Middle of What having a summit depth at ~1000 m, whilst Coral and Melville have summits at ~200 m.

Many samples as well as HD video footage were obtained with the German ROV, Kiel 6000. The scoop used by the manipulator arm on board the ROV Kiel 6000 collected coral rubble, framework, individual pieces of dead coral and carbonates as well as other sediment material to look in general at the macrofauna living on the seafloor. In addition the HyBIS grab
(Narayanaswamy et al. 2016) was used to collect larger quantities of material from the different seamounts. This grab was designed primarily for geological samples and recovers well washed material because the grab, when closed, is not fully sealed and hence the sediment sample acquired is strongly agitated during recovery.

Onboard ship all visible fauna were picked off the recovered material and preserved in 70% ethanol. These removed individuals were allocated numbers in accordance with the NHM guidelines set out at the beginning of the cruise (Rogers and Taylor 2012). The solid framework/rubble/carbonate material and stylasterid stems (the subject of this paper) was separated into two fractions of >4 mm and >0.25 mm<4 mm using stacked sieves. The >4 mm residue samples analysed in this paper were preserved either in 100% ethanol only or initially fixed in 4% formaldehyde, before being transferred onboard ship to 80% IDA alcohol.

2.2 Acid dissolution

The individual stylasterids, coral framework/rubble and carbonate material were visually screened for any remaining visible fauna attached to the surface of the samples and removed including bryozoans, bivalves or other molluscs. The acetic acid for the dissolution technique was 100% GPR RECTAPUR supplied by McQuilkin & Company in the UK. Prior to acid dissolution being undertaken, each sample was photographed and the wet weights of the >4 mm fraction (+/- 1 g) were obtained.

The material was left in 10% acetic acid solution until the solid material content was dissolved to a size below ~1 mm. The container was left partially open in a fume cupboard while the carbonate was dissolving to avoid build up of CO₂ that would stop the dissolution process of the carbonate. The sample was decanted at least twice every 24 hours and a copious volume of
freshwater used to flush out liberated material. A 4 mm sieve was used to retain the solid
material with a 250 µm mesh sieve retaining the macrofauna included in the liberated material.
The undissolved sample was then returned to fresh acid solution. The change to a fresh
aqueous acid solution each time reduced dissolution time to less than three days. On occasion
sieving was undertaken more frequently if the reaction was releasing a lot of material in order
to reduce damage especially to any calcareous fauna from coming into contact with the acid.
This process was continued until no more solid carbonate component remained. The material
retained on the sieve was washed in freshwater before being placed in 70% ethanol. The fauna
were then sorted, counted and identified using a Wild Heerbrugg M5 stereo microscope.

3. Results

3.1. General observations

Coral seamount was the southernmost of the group of seamounts (Figure 2) sampled from
which four processed ROV scoop samples were collected (Table 1). The samples comprised
mainly coral framework and rubble, with the external structure being coated in manganese
deposit. The largest of the Coral seamount samples was framework material weighing 3.6 kg
wet weight collected from 739 m water depth. The internal space of the coral framework was
dominated by colonising sponges. This sample in addition also yielded many other filter
feeding fauna notably brachiopods, cirripeds and Sabellidae and Serpulidae annelids.

The samples from Melville seamount were obtained by using the HyBIS grab. These samples
were composed of what has been defined as coral rubble (Table 1) with some shell particles
present. All were less intensely coated in manganese deposit. One large sample of rubble (>5.3
kg wet weight, 980 m water depth) yielded almost 50 undamaged Pholoidae and Polynoidae
polychaetes.
Three samples were collected by the ROV scoop from Middle of What seamount. These samples were separated into framework and rubble (Table 1) and much of the material was well coated in manganese deposit. Brachiopods and polychaetes were most abundant at the two stations sampled, with bryozoans also occurring at the shallower station.

Sapmer received less attention on the expedition as a result of time constraints (Rogers and Taylor 2012) and only one sample is reported here collected with the ROV scoop. This material contained no coral framework or rubble and was mainly composed of carbonate pieces with some shell material and with little if any manganese deposit. Only polychaetes, brachiopods, isopods and ophiuroids were collected from this seamount, with brachiopods and then the polychaetes being the most abundant fauna.

Atlantis Bank, the most northern of the seamounts, was sampled using the ROV scoop and the samples collected ranged from coral rubble, through to stylasterid stems and carbonate pieces (Table 1). This was similar to Sapmer, but the pieces of carbonate were larger by comparison, and again had little manganese deposit. A number of different fauna were collected from the dissolution process with polychaetes, sponges and ophiuroids being the most commonly found.

3.2. Polychaete standing stock

As polychaetes were the most frequently observed fauna, the rest of paper will focus solely on this group.

The numbers of polychaetes found varied across the seamounts, but also varied depending on the micro-habitat in which they were found (Figure 3a). The numbers of individual polychaetes
found per kg of material ranged from 25 ind./kg at Middle of What - Rubble to, >800 ind./kg at Atlantis – Stylasterid. However, most of the micro-habitats sampled contained <150 ind./kg of material (Figure 3b). The biomass recorded did somewhat reflect the pattern observed with the abundance, whereby the greater number of individuals collected, the higher the biomass (Figure 3b). The highest biomass was recorded from coral rubble material collected from Atlantis seamount, with almost 1.3g/kg being found for 274 polychaete individuals per kg of rubble. Biomass from three samples was not able to be measured.

3.3. Polychaete composition

A total of 34 different polychaete families were extracted from the framework/rubble/carbonate material and stylasterid stems, with the Syllidae and Sabellidae being the most commonly occurring families found in the material dissolved at each seamount (Table 2; Figure 4 a-d). There were five families (Accrocirridae, Apistobranchidae, Chaetopteridae, Lacydoniidae and Terebellidae) which were only represented by one individual and there were some individuals that could not be identified to family level (termed Family indet).

The number of polychaete families found within each microhabitat varied considerably; 5 - 11 families found in coral framework, 8 – 22 families found in rubble material, 3 – 6 in stylasterid stems and 3 – 11 in carbonate material (Table 2). The samples with only three families present were both collected from Atlantis seamount, one from a stylasterid stem and the other from the carbonate material, whilst the most diverse samples came from the two rubble samples (980 m and 985 m) from Melville seamount which yielded 16 and 22 polychaete families respectively.

Further analysis was undertaken to determine the numbers of individual for each polychaete family found per kg of material with Figure 4 illustrating all the polychaete families found in
each micro-habitat. As is clear from Figure (4 a-d) the Syllidae, Paraonidae and Sabellidae were the most abundant families in all the microhabitats, with the exception of the stylasterid stems from Atlantis seamount, whereby the Paraonidae were ~5 times more abundant, and the Sabellidae ~10 times more numerous than the Syllidae.

4. Discussion

Endocryptolithic fauna (defined as fauna living within a concretion) are a generally unnoticed, disregarded group of fauna both in the deep sea as well as in shallow water. In some places these fauna may increase estimates of the numbers of individuals and the diversity of that region. To date deep-sea research has tended to focus on the more accessible soft sediments for all faunal size classes, whilst hard substrata because of their very nature, have been sampled mainly for larger megafauna.

The preliminary results highlighted above indicate that there can be a great number of individuals residing within the coral framework/rubble/carbonate structures and stylasterid stems and that future studies should, where possible, also aim to investigate this “hidden” fauna. As in many deep sea habitats, polychaetes were quite abundant in the different microhabitats although there was quite a range between and within the framework/rubble/carbonate material as well as within the stylasterid stems. With this relatively small dataset it is very difficult to establish any clear patterns as to whether more polychaetes were extracted from one type of micro-habitat over another. For example the three different stylasterid stems from Atlantis seamount harboured very differing numbers of individuals ranging from 36 ind./kg to >880 ind./kg of material. The same is also true for coral rubble material, where the numbers of individuals ranged from 46 ind./kg to >270 ind./kg of material.
The composition of polychaete families that were found in the coral framework/rubble/carbonate material and stylasterid stems was very similar to those found living on and around the same material on the SW Indian Ocean Ridge. Serpetti et al. (in prep.) found a total of 40 polychaete families, however, like the results presented here the two most numerically abundant families living on and around the coral-carbonate material were the Syllidae and Sabellidae. By contrast this current study found an additional two families, Magelonidae and Pilargiidae, that were not found by Serpetti et al. (in prep.). In this study we found that the Syllidae were collected in every micro-habitat and at each seamount, and accounted for between 6 and 40% of the total number of polychaetes collected. In this study we found that the Syllidae were collected in every micro-habitat and at each seamount, and accounted for between 6 and 40% of the total number of polychaetes collected. Chivers et al. (2013) in their study of macrofauna of Senghor seamount, NE Atlantic, also found that the most abundant polychaete family was the Syllidae accounting for 34% of the total number of individuals collected. Sabellidae were the second most abundant family in this study and accounted for between 2 and 75% of the total number of polychaete individuals, whilst in Chivers et al’s (2013) study, the Sabellidae accounted for just 5% of the total number of annelid individuals. Surprisingly, the Spionidae which are often highly abundant in deep sea habitats, were only found in four of the samples, and only from the rubble and once in a stylasterid stem. It is difficult to determine with any degree of confidence whether any of the polychaetes extracted from the material exhibit an obligate or facultative association with their host. From other studies, some Polynoidae species have been described as having a facultative commensal association e.g. Harmothoe imbricata in association with e.g. Asteroidea, (Martin & Britayev, 1998) whilst other species have an obligate association e.g. Gorgoniapolynoe caeciliae in association with Octacorallia (Martin & Britayev, 1998). Serpetti et al. (in press this vol.) also found G. caeciliae in their samples collected from the SWIOR, living on the branches of the
octocorals. However, Jensen and Frederiksen (1992) did not find any species either living exclusively on *Lophelia pertusa* or having an obligate association with it.

The acid dissolution technique employed here was advised against by Hutchings and Weate (1978) over concerns that the fauna would be damaged, making them impossible to identify. However, we found that with careful monitoring of the dissolution process, and removal from the acid at least twice every 24 hours, that the fauna, could be extracted relatively intact and thus were still identifiable (Figure 5).

In other work, where macrofauna from authigenic carbonates located at cold seeps have been extracted, the fragments have initially been placed in water to encourage the fauna to leave their carbonate habitat (e.g. Grupe et al. 2015; Levin et al. 2015). In previous work undertaken on shallow water carbonate material (~130 m) collected from the summit of Ampere seamount Narayanaswamy and Lamont first trialled this extraction method onboard ship and although some fauna were removed in this manner, not all were removed. Subsequent manual crushing of the carbonate material into smaller pieces, followed by the acid dissolution process outlined above, revealed the presence of many more individuals and taxa from those same concretions. Through these combined extraction techniques, more than 750 polychaete individuals were extracted per kilogramme of carbonate material found at the shallow station on Ampere seamount in the NE Atlantic (Narayanaswamy & Lamont (unpubl)). The material collected from the SWIOR seamounts was much deeper by comparison, with the shallowest station occurring at a depth of 668 m on Sapmer. In addition the age of the coral framework/rubble/carbonate material and stylasterid stems may also influence the phyla and number of individuals found. Although difficult to quantify here, as it is difficult to ascertain how long the material has been dead for. Carbon dating analysis taken of large barnacle shells
collected from some of the seamounts, found that the shells had a radiocarbon age BP of
>54,000 years as dated by the Scottish Universities Environmental Research Centre AMS
Facility (SUERC 2012). The barnacle plates were covered in a similar layer of manganese to
the framework and rubble, so we may be able to estimate that they are of similar ages
considering the time frame for deposition of manganese. Like Hutchings and Weate (1978),
we would expect that the older the material the greater the faunal colonisation. The
endocyrtolithic community may however, eventually destroy the framework/rubble material
completely through its endless burrowing.

It is difficult to compare the results presented here with other studies undertaken as samples in
this study have been standardised by wet weight, whilst studies undertaken on carbonate
material from seep sites have been standardised by area (Levin et al. 2015 and references
therein). In addition the methodology of the studies have also been quite different, with the
present study employing a weak acid dissolution technique, and other studies placing the
coral/carbonate material in fresh water (e.g. Grupe et al. 2015; Levin et al. 2015, Ritt et al.
2015).

Two studies in the early 1990s and mid-2000s investigated the fauna found in association with
the cold water coral *Lophelia pertusa* in Faroese and Norwegian waters (Jensen & Frereiksen
1992; Mortensen & Fossa 2006). One aspect of their studies looked at the fauna inside the
individual coral branches, however, neither study indicates if the individual coral branches that
they crushed were dead or alive and further more did not make a direct comparison between
them and therefore makes any similarities or differences with our study on the polychaetes
presented here very difficult. Jensen and Frederiksen (1992) did find some species of
Sabellidae and Paraonidae that are known to be boring polychaetes. Our study also found that these two families were present in every micro-habitat investigated.

The few studies that have looked at macrofauna associated (living on) with coral rubble and coral framework have found extremely high biodiversity (Buhl-Mortensen et al. 2010). It is possible that extraction of the endocryptolithic fauna would increase the estimation of overall diversity of these complex habitats yet further. However, none of the studies discussed throughout this paper have looked at the fauna that has been extracted through dissolution of the framework/rubble/carbonate material or even individual stylasterid stems.

Future work looking at endocryptolithic fauna would benefit from employing the dissolution technique described here to ensure, as far as possible, extraction of all the fauna from the framework/rubble/carbonate material. The numbers individuals and families found within the framework/rubble/carbonate may be related to the age of the material from which they were extracted, with the older the material the more fauna and taxa predicted to be found. Where possible, ageing of this material may give an indication as to whether greater numbers of individuals and taxa are found in older material or not. Finally, studies such as those undertaken by Levin et al. (2015), investigating the trophic structure of the fauna may provide information as to whether the fauna are feeding within the framework/rubble/carbonate itself or on other material.

Acknowledgements
The hard substrata of seamounts is difficult to sample and we are indebted first to the Master and crew of the R.R.S. James Cook for providing the platform and facilities to enable samples to be obtained from this remote location. We are also indebted to the GeoMar ROV Kiel 6000
team who operated their gear with skill to achieve excellent bottom images as well as scoops and grabs of bottom material that could not have been obtained in any other way. Software processing of some of the photographs used the software programme ‘Combine ZP’ made freely available by Alan Hadley, to whom we are indebted. We are very grateful to the SWIOR team who helped wherever possible onboard the ship and in particular to Adam Chivers who undertook much of the sample processing, as well as to Alex Rogers and Gordon Paterson the other two co-investigators of this project without whom this work would not have been possible. This work was funded through a NERC grant NE/F005563/1 to the lead author. We would also like to thank two anonymous referees for their extremely helpful comments.

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Table 1: The habitat type sampled at each station and seamount along with the total numbers of polychaete individuals and biomass per kilogramme of coral related material from across the ridge.

<table>
<thead>
<tr>
<th>Seamount</th>
<th>Depth (m)</th>
<th>Latitude (S)</th>
<th>Longitude (E)</th>
<th>Gear type</th>
<th>Habitat type</th>
<th>Abundance (N/kg)</th>
<th>Biomass (g/kg)</th>
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<tbody>
<tr>
<td>Coral</td>
<td>739</td>
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<td>46° 43.85</td>
<td>ROV Scoop</td>
<td>Coral framework</td>
<td>46</td>
<td>&lt;0.01</td>
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<td>Coral framework</td>
<td>80</td>
<td>N/A</td>
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<td>Coral rubble</td>
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<td>Carbonate fragments</td>
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<td>ROV Scoop</td>
<td>Stylasterid stem</td>
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Table 2: The presence/absence of the 34 polychaete families collected from the different micro-habitats and seamounts sampled including the mean number of families (± S.E.) found per habitat type. (MoW = Middle of What Seamount)
Figure 1. Pictures illustrating the four main different micro-habitats found and sampled from the seamounts along the SWIOR; a) Coral Framework, b) Coral Rubble, c) Stylasterid stem, and d) Carbonate material.
Figure 2. Cruise map highlighting the locations of the five seamounts visited on the RRS James Cook JC066 cruise where most sampling was undertaken. The five seamount sites were, from north to south, Atlantis Bank, Sapmer Bank, Middle of What, Melville Bank and Coral (modified figure from Letessier et al., 2015).
Figure 3. The polychaete standing stock, a) abundance and b) biomass per kg of material from coral framework/rubble, stylasterid stems and carbonate material from across the five seamounts sampled across the SWIOR. C = Coral seamount, MoW = Middle of What Seamount, M = Melville Bank, S = Sapmer Bank and A = Atlantis Seamount.
Figure 4 (a-d). Each figure illustrates the number of polychaetes collected per Kg of material for all the polychaete families extracted from each micro-habitat and each seamount; a) coral rubble material, b) coral framework material, c) Stylasterid stems and d) carbonate material.
Figure 5. Composite photographs illustrating a) the range of the polychaete family Syllidae that have been extracted through the dissolution process, and the level of intactness of these b) Syllidae, and c) Sabellidae specimens.