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# The London Workshop on the Biogeography and Connectivity of the Clarion-Clipperton Zone

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## Workshop Report

# The London Workshop on the Biogeography and Connectivity of the Clarion-Clipperton Zone

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## **Abstract**

### **Background**

Recent years have seen a rapid increase in survey and sampling expeditions to the Clarion-Clipperton Zone (CCZ) abyssal plain, a vast area of the central Pacific that is currently being actively explored for deep-sea minerals (ISA, 2016). Critical to the development of evidence-based environmental policy in the CCZ are data on the biogeography and connectivity of species at a CCZ-regional level.

### **New information**

The London Workshop on the Biogeography and Connectivity of the CCZ was convened to support the integration and synthesis of data from European Union (EU) CCZ projects, supported by the EU Managing Impacts of Deep-Sea Resource Exploitation (MIDAS) and EU Joint Programming Initiative Healthy and Productive Seas and Oceans (JPI Oceans) projects. The London Workshop had three clear goals: (1) To explore, review and synthesise the latest molecular biogeography and connectivity data from across recent CCZ cruises from both contractor and academia-funded projects; (2) To develop complementary and collaborative institutional and program-based academic publication plans to avoid duplication of effort and ensure maximum collaborative impact; (3) To plan a joint synthetic data publication highlighting key results from a range of planned molecular biogeography/connectivity publications. 32 participants attended the workshop at the Natural History Museum in London from 10-12 May 2016. Presentations and discussions are summarised in this report covering (1) overviews of current CCZ environmental projects, (2) policy and industry perspectives, (3) synthesis of DNA taxonomy and biogeography studies, (4) summaries of the latest population genetic studies, (5) summaries of the latest broader morphological context, (6) an overview of publication and proposal plans to maximise collaborative opportunities and finally a series of workshop recommendations.

### **Keywords**

Deep-sea biology, deep-sea mining, biodiversity, phylogeography, population genetics, European Union Framework 7, discussion meeting

### **Date and place**

10-12 May 2016, Natural History Museum, London

## List of participants

Same as author list.

## Introduction

Recent years have seen a rapid increase in survey and sampling expeditions to the Clarion-Clipperton Zone (CCZ) abyssal plain, a vast area of the central Pacific that is currently being actively explored for deep-sea minerals (ISA 2016). For signatory nations to the United Nations Convention on the Law of the Sea (UNCLOS), the commercial exploration or exploitation of areas of the seafloor beyond national jurisdiction is regulated in combination by the International Seabed Authority (ISA), established under UNCLOS, and national governments that act as the Sponsoring State to commercial or other organisations that enter into contract with the ISA. There are now 15 contracts signed with the ISA for polymetallic nodule exploration, 8 of these signed in the last 10 years and the most recent by UK Seabed Resources Ltd (UKSRL) for its second contract in March 2016 (ISA 2016). The 6 million km<sup>2</sup> CCZ is the most active area worldwide for deep-sea mining exploration, and the ISA is currently developing a new environmental regulatory framework for mineral exploitation to be published in 'zero-draft' form in 2016 (S Brager, ISA, pers. comm.).

Critical to the development of evidence-based environmental policy in the CCZ are data on the biogeography and connectivity of species at a CCZ-regional level. With this in mind, the London Workshop on the Biogeography and Connectivity of the CCZ was convened to support the integration and synthesis of data from European Union (EU) CCZ projects, supported by the EU Managing Impacts of Deep-Sea Resource Exploitation (MIDAS) and EU Joint Programming Initiative Healthy and Productive Seas and Oceans (JPI Oceans) projects, individual EU-based contractors and the Natural History Museum in London, a leading centre for marine biodiversity research.

## The challenge of biogeography and connectivity in the CCZ

The Clarion-Clipperton Zone is so called as it lies between the Clarion and Clipperton Fracture Zones, topographical highs that extend longitudinally across almost the entire Pacific (Fig. 1). There is no strict definition of the region, but it has come to be regarded as the area between these fracture zones that lies within international waters and encompasses the main areas of commercial interest for polymetallic-nodule mining. Exploration licenses issued by the ISA extend from 115° W (the easternmost extent of the UK-1 claim) to approximately 158° W (the westernmost extent of the Chinese COMRA claim). We therefore adopt here a working definition of the CCZ as the box: 13° N 158° W; 18° N 118° W; 10° N 112° W; 2° N 155° W.

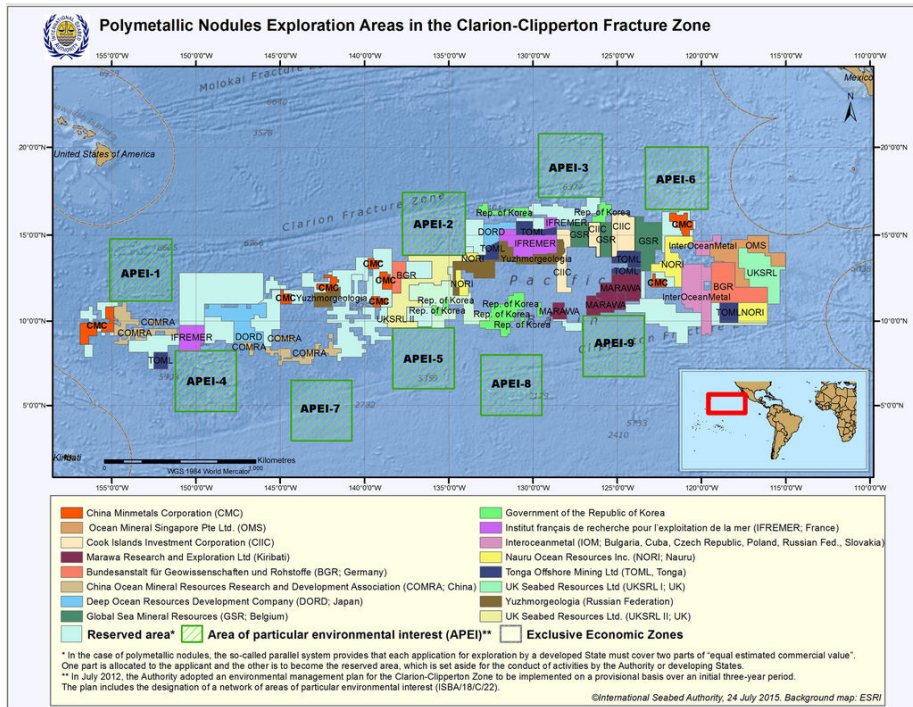


Figure 1.

Exploration contract areas for polymetallic nodules in the Clarion-Clipperton Zone, central Pacific Ocean. Areas of Particular Environmental Interest (APEIs) numbered according to the latest International Seabed Authority data (Source: Stefan Brager, ISA), see section in this report: 'Key Outcomes and Discussion'. Image credit: International Seabed Authority, 2015.

Conducting regional-level studies of the biogeography and connectivity of the CCZ is an immense challenge for several reasons: e.g., (1) the great depth and long distances to home ports, (2) the great physical heterogeneity of the region (the CCZ is not a homogenous abyssal plain of mud, it is a region of 6 million km<sup>2</sup> with a bathymetric variation of at least 1200 m and punctuated by many thousands of seamounts) and (3) there are almost no data on habitat characteristics such as food availability or prevailing oceanographic currents. However, these are only minor issues compared with the greatest problem: the almost complete lack of taxonomic synthesis or standardisation across the region based either on traditional morphological data or modern DNA (Glover et al. 2015). An example is given through a simple search of the Ocean Biogeographic Information System (OBIS, 2016) centred on a 300,000 km<sup>2</sup> (5°) area of ocean between the Netherlands and the United Kingdom providing 182,939 records of polychaetes, a common benthic animal in all oceans. In comparison, the same size box centred on the UK-1 exploration claim area provides only four polychaete records, none of which are benthic (Fig. 2). The lack of taxonomic synthesis has resulted in a situation where biologists are unable to identify animals collected and successive research cruises, both contractor and academic-led, are unable to provide identified species occurrence data to global database

systems such as OBIS or the Global Biodiversity Information Facility (GBIF). In turn, this has prevented a synthesis of data to produce for example a biogeographic map of the CCZ.

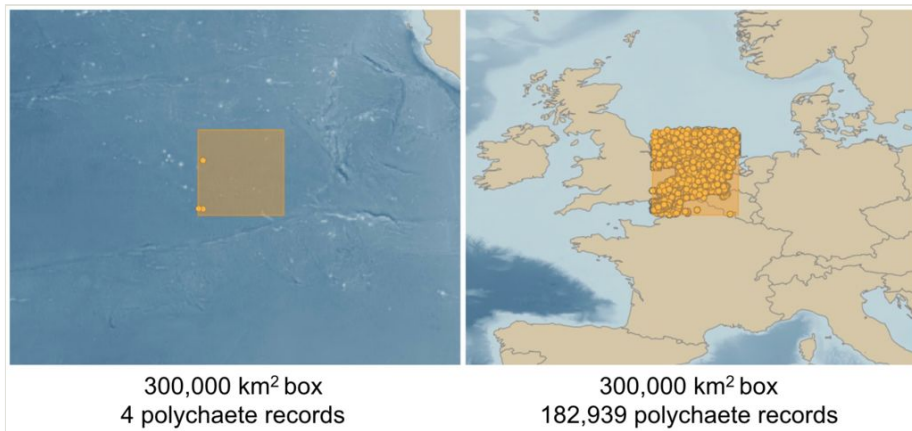


Figure 2.

There is an almost complete absence of published, databased benthic species records from the Clarion-Clipperton Zone (CCZ), despite over 30 years of intensive oceanographic and geological research in the area. Here illustrated as an example are the current species records for a 300,000 km<sup>2</sup> (5°) box centred on the North Sea and Eastern Channel in Europe, where the Ocean Biogeographic Information System (OBIS 2016) provides 182,939 records of polychaetes, a common benthic animal in all oceans. By contrast, the same size box centered on the eastern CCZ provides just 4 polychaete records, none of which are benthic.

Despite these problems, new data are now emerging from both academia and contractor-led CCZ programs that utilise genetic data in the form of short DNA sequences from typical invertebrate markers such as cytochrome oxidase I mitochondrial gene (COI), 16S mitochondrial ribosomal RNA coding genes and 18S or 28S nuclear genes. Recent publications from the BGR-led and UKSRL-led projects have made these data available on the open data repository NCBI GenBank (Janssen et al. 2015, Glover et al. 2016b, Dahlgren et al. 2016) (Fig. 3). Although full taxonomic descriptions of these fauna are still lacking in most instances, the availability of genetic data does permit a regional-level analysis of putative species as these data become available. In addition, a large number of DNA sequences and morphological data have been obtained from a range of cruises that are currently unpublished.

The London Workshop was expressly designed to try and overcome these challenges by bringing together researchers from a range of EU projects working on molecular data from the CCZ to share findings and plan future publications and synthetic activities.

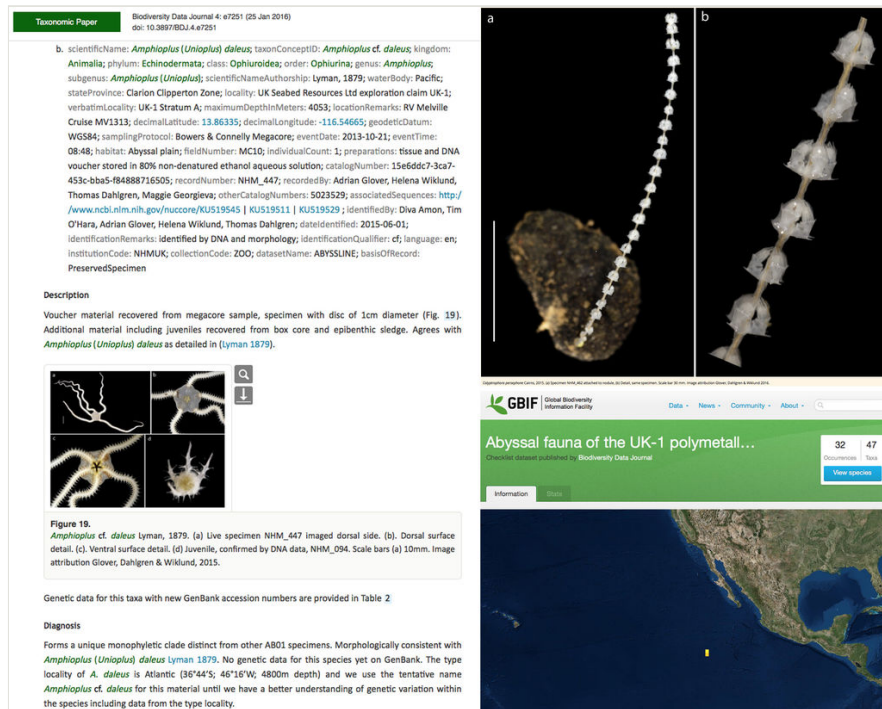


Figure 3.

Recent publications on the Clarion-Clipperton Zone are making imagery and genetic data available in public databases that will allow future workers access to these data and the voucher materials, for regional-level syntheses and further DNA sequencing if needed. Examples are recent taxonomic data papers on the Echinodermata (Glover et al. 2016b) (image left) and Cnidaria (Dahlgren et al. 2016) (image top right). Data associated with these papers is automatically uploaded to the Global Biodiversity Information Facility (GBIF, bottom right). See Workshop Recommendations.

## Aims of the workshop

### Goals

The London Workshop had three clear goals:

1. To explore, review and synthesise the latest molecular biogeography and connectivity data from across recent CCZ cruises from both contractor and academia-funded projects
2. To develop complementary and collaborative institutional and program-based academic publication plans to avoid duplication of effort and ensure maximum collaborative impact
3. To plan a joint synthetic data publication highlighting key results from a range of planned molecular biogeography/connectivity publications



During discussions regarding the workshop it also became apparent that it was important to review historical knowledge of CCZ biogeography based on morphological data, new data from visual survey tools and taxonomic descriptions alongside the new molecular data – this is discussed in more detail in section “Workshop Recommendations” below.

## London Workshop: The Agenda

The agenda is presented here as planned in order to provide transparency of discussion and a useful guide to those planning future similar workshops. Summaries of talks and discussion are provided in the following section “Key outcomes and discussions”.

Tuesday 10 May 2016

1200 Lunch in the Natural History Museum North Hall

1300 **Adrian Glover:** Welcome, meeting logistics and participant introductions

1330 **Adrian Glover:** Overview of meeting goals and agenda, discussion

1400 Session 1: Project Overviews

*The purpose of Session 1 was to introduce each relevant EU CCZ project from the point of view of molecular biogeography and connectivity data.*

1400 **Pedro Martinez:** Overview of the German exploration claim (BGR) recent cruises and data collected

1420 **Thomas Dahlgren & Pedro Martinez:** Overview of the JPI-Oceans cruise program and data collected

1440 **Ann Vanreusel:** Overview of biological and environmental sampling in the GSR (Belgian) exploration area

1500 **Lenaïck Menot:** Overview of the IFREMER (France) environmental studies

1520 Tea/coffee break

1540 **Daniel Jones & Sergi Taboada:** Overview of the NERC-MIDAS RRS James Cook JC120 cruise to APEI-6, including overview of molecular collecting

1600 **Adrian Glover:** Overview of the ABYSSLINE (UK Seabed Resources Ltd (UKSRL)) data on molecular biogeography and connectivity

1620 Session 2: Policy and Industry Perspectives

1620 **Stefan Brager:** The policy perspective: The International Seabed Authority and environmental management of the CCZ



1640 **Ralph Spickermann:** *The industry perspective: Manganese nodules in the Pacific ocean, path from exploration to exploitation*

1730 End of Day 1: Icebreaker social at the Hereford Arms pub, South Kensington.

Wednesday 11 May 2016

0900 Arrival and summary of Day 1, overview of Day 2 agenda.

0910 **Thomas Dahlgren:** Discussion Starter: Recent molecular connectivity papers in the deep sea, lessons learned

0930 Session 3: DNA taxonomy and biogeography within CCZ exploration claim areas

*The purpose of Session 3 was to review new data on DNA taxonomy from the CCZ, that is the formal or informal description of species using DNA barcodes, and the examination of the distribution of those species within exploration areas.*

0930 **Pedro Martinez:** *Molecular taxonomy and biogeography within the German (BGR) exploration area*

0950 **Helena Wiklund:** *Molecular taxonomy and biogeography within the UKSRL, Ocean Mineral Singapore (OMS) and Area of Particular Environmental Interest (APEI) #6.*

1010 **Paulo Bonifacio:** *Diversity and distribution patterns of Polynoidae (Annelida) across the CCZ*

1030 Tea/coffee break

1050 **Lara Macheriotou:** *Deep-sea Nematoda of the CCZ – preliminary insights*

1110 **Andrew Gooday:** *Foraminifera species diversity within the the UKSRL and OMS exploration areas*

1130 **Magdalena Błazewicz:** *Abyssal Tanaidacea – (not) stunning cryptic diversity*

1150 **Andrea Waeschenbach:** *Abyssal Bryozoa – first results from UKSRL area and comments on CCZ projects*

1210 Discussion session

1300 Lunch break

1400 Session 4: Intra-specific population connectivity in target taxa across the CCZ

*The purpose of Session 4 was to review new data on population genetics and connectivity within populations, for which new data are starting to emerge from recent CCZ projects. These data are extremely limited but have the potential to offer*

*statistically-robust estimates of connectivity for the first time. Typically, 'target' taxa are chosen that have large enough sample sizes.*

**1400 Heiko Stuckas:** *Population structure and demography in deep-sea species using molecular markers*

**1420 Thomas Dahlgren:** *Population structure in target taxa (Annelida, Mollusca and Echinodermata) from the UKSRL, OMS and APEI-6 areas*

**1440 Pedro Martinez:** *Population structure in Ophiuroidea from the CCZ*

**1500 Sarah Schnurr:** *Phylogeographic patterns in Isopoda from the JPI Oceans project*

**1520 Uwe Raschka:** *Phylogeographic patterns in Harpacticoida from the JPI Oceans project*

**1540** *Tea/coffee break*

**1600 Sergi Taboada:** *Molecular connectivity of the sponge Tethyda sp. nov. within the CCZ using microsatellites*

**1620 Pedro Ribeiro:** *Using RAD sequencing (RADseq) data to investigate population genetic structure in the deep sea*

**1640** *Session 5: The broader biogeographic context based on morphology and molecules*

*The purpose of the final data session was to review data on the broader biogeographic context of the CCZ, particularly based on historical morphological data coupled to new molecular studies. In particular, what new identified species records of actual described species can tell us about ranges at what scales.*

**1640 Adrian Glover:** *Annelida, Mollusca, Echinodermata: Actual species identifications from the CCZ using historical morphological data: some examples*

**1700 Daniel Kersken:** *Porifera of the CCZ*

**1720 Andrew Gooday:** *Foraminifera, the broader biogeographic context*

**1740 Gordon Paterson:** *The problem and challenge of rarity in the abyss*

**1800** *Discussion*

**1900** *Workshop Dinner, Ognisko Restaurant, South Kensington.*

Thursday 12 May 2016

**0800** *Principal Investigator break-out session to agree on conceptual framework for a synthesis paper*

0845 Workshop Photograph

0900 Summary of Day 2, discussion and overview of Day 3 agenda

0930 Session 6: Summary tables and data synthesis session

*The purpose of the final summary session was to review what data are currently available (published or made available on open databases), what data are in the process of being published, and what data could be shared in collaborative, synthetic publications or reports.*

*0930 Session 6 discussion and production of summary table of planned publications*

*1030 Tea/coffee break*

*1100 Continuation of summary table production*

*1200 Round-table discussions on future cruises and grant proposals for report*

*1230 General workshop recommendations – summary table for report*

1300 Workshop close

## Key outcomes and discussions

### Participant composition and overview of workshop goals

The workshop opened with participant introductions and a discussion of the proposed agenda. 32 participants attended the workshop, based on invitation sent to the MIDAS, JPI Oceans and EU-led contractor programs. The meeting was also advertised via the MIDAS news feed and the INDEEP email alert. The workshop was over-subscribed, with an initial capacity of 25, but the venue was expanded to accommodate the 32 accepted invitees. The meeting was maintained within the EU-led projects in order to achieve a manageable size to facilitate discussion, although it was noted that the potential number of attendees could have been much higher if the workshop had been expanded to include non-EU contractors and Sponsoring State research programs. The workshop participants agreed that this could be the subject of a future workshop or specialist session at an international meeting (see Workshop Recommendations).

23 of the 32 attendees had specialist taxonomic knowledge in particular phyla (Fig. 1), with Annelida and Crustacea contributing 26% of the expertise each, with the remaining groups (Cnidaria, Echinodermata, Foraminifera, Mollusca, Nematoda, Porifera and Bryozoa) forming the remainder relatively evenly. It was noted that there was no experts on fish, microbial communities, or pelagic taxa present at the meeting.

In terms of professional status, the meeting was dominated by Principal Investigators (56%), then post-graduate students (25%) and Post-docs (19%) (Fig. 4). Nations represented in order of the number of attendees were the UK, Germany, Poland, Portugal, Belgium, France and Sweden, with 2 attendees representing the United Nations. The gender balance was 50:50.

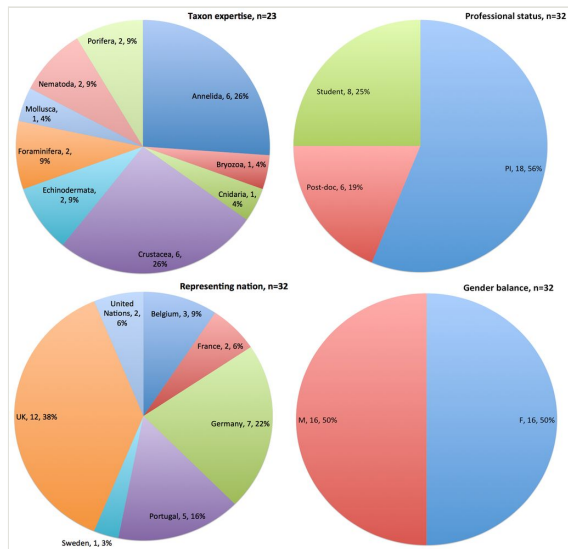


Figure 4.

Composition of participants in the London Workshop on the Biogeography and Connectivity of the Clarion-Clipperton Zone, based on taxon expertise (only estimated in 23 participants), professional status, representing nation and gender (from 32 participants).

Following participant introductions, the workshop commenced with an overview from Adrian Glover on the workshop goals. Glover pointed out the rapid growth in exploration activity in the CCZ (e.g. the number of exploration contracts has more than doubled in the last 10 years (ISA 2016), and the need to start to bring together contractor and academia-led programs to help provide regional synthesis in our biodiversity knowledge of the region. The need for regional-level understanding has been highlighted in a review of the CCZ Environmental Management Plan (CCZ-EMP) (Seascope Consultants 2014) and in a recent methodological overview of DNA taxonomy methods for the CCZ (Glover et al. 2015). It has also been highlighted in three ISA workshops on taxonomic standardization in meiofauna, macrofauna and megafauna from the CCZ (ISA 2013, ISA 2014, ISA 2015). Despite all these past reviews and recommendations, it was noted that the funding available for regional-level taxonomic work is still lacking as it falls outside the remit of individual contractor or Sponsoring State environmental programs, and indeed the London Workshop itself had no funding, with individual attendees finding travel funding from their own institutional program budgets to attend (see Workshop Recommendations).

There was agreement on the overarching goals of the workshop:

1. To explore, review and synthesise the latest molecular biogeography and connectivity data from across recent CCZ cruises from both contractor and academia-funded projects
2. To develop complementary and collaborative institutional and program-based academic publication plans to avoid duplication of effort and ensure maximum collaborative impact
3. To plan a joint synthetic data publication highlighting key results from a range of planned molecular biogeography/connectivity publications

There was also general agreement on the need for a synthesis paper, although the delivery mechanism for this third goal remained unclear at the start of the workshop, and is discussed further below.

### **Session 1: An overview of recent CCZ cruises and projects of relevance to molecular biogeography and connectivity**

Extensive collection for DNA taxonomy and biogeographic analysis first started in the CCZ in 2003-2004 as part of the Kaplan project (Smith et al. 2008) and has continued with the BGR, IFREMER, UKSRL and Belgian (GSR) exploration activities. DNA success rates (successful extraction, amplification and sequencing) with the Kaplan project were very low owing to technological and funding restrictions at the time, and coupled with low sample numbers resulted in few useful genetic sequences. Nevertheless, these data were important in advising the ISA during the development of the first marine protected area (MPA) regional management plan for the CCZ, the APEI network (Smith and al. 2008, Smith et al. 2008, Fig. 1). The Kaplan DNA success rates were in the region of 11-29% for polychaetes (Smith and al. 2008), in comparison with the UKSRL ABYSSLINE project, which is now 96% (Glover et al. 2015). The majority of CCZ projects are now achieving good DNA success rates, particularly following careful preservation protocols (Glover et al. 2015) and the use of new sampling devices such as the epibenthic sledge (Brenke 2005).

At the London Workshop, Pedro Martinez provided an overview of the recent German (BGR) environmental work in their exploration claim area. Martinez explained that BGR have two claim areas, one in the eastern CCZ and one in the western CCZ, and the site in the eastern CCZ has been much more intensively explored (Fig. 5). The first cruise was in 2010 and they have continued since then on a more-or-less annual basis using the R/V Kilo Moana or R/V Sonne. The initial focus of activities was to try and survey the whole area, but the emphasis has now switched to focussing on two sites – one likely important for mining (Impact Reference Area) and another that may form the basis of a Preservation Reference Area. They now have three years of collecting samples and data from these sites, which are likely to be useful to inform future environmental management of the region and the development of guidelines for environmental management.

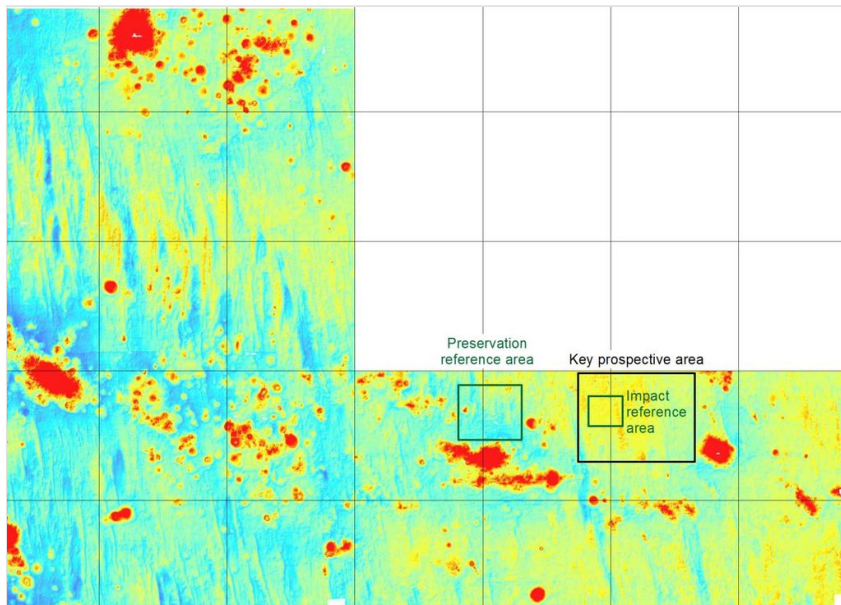


Figure 5.

The BGR-Germany polymetallic nodule exploration claim area in the eastern CCZ, illustrating the two areas in which survey work has concentrated in recent years - the proposed preservation reference area (PRA) and impact reference area (IRA). Image source: Federal Institute for Geosciences and Natural Resources (BGR, Germany) and Pedro Martinez, Senckenberg Institute (Rühlemann et al. 2011).

Pedro Martinez also outlined the JPI Oceans program aboard the R/V Sonne (Martinez Arbizu 2015). This is not a BGR project, but in fact a collaborative EU-wide project utilising funding from a range of national instruments, with the sea-time funded by the German government. A major goal of the JPI Oceans program was to re-visit a site in the East Peru Basin (not the CCZ) to investigate the impacts of the Disturbance and Recolonisation (DISCOL) experiment conducted in 1989 (Borowski 2001), with two of the JPI Oceans cruises dedicated to this (SO-242-1 and SO-242-2). A third cruise (SO-239) was dedicated to sampling the BGR, Interoceanmetal (IOM), French (IFREMER), Belgian (GSR) and APEI-3 areas in the CCZ (Fig. 6), with a portion of the molecular connectivity work being undertaken and funded by the Swedish Research Council FORMAS through Thomas Dahlgren at the University of Gothenburg. As well as sampling the exploration areas for fauna through collection and imagery, additional goals were to re-visit tracks (assumed to be made by a dredge) in the former USA Ocean Minerals Corporation (OMCO) area that is now part of the French area and to investigate the seamount fauna in the CCZ. Martinez outlined some of the preliminary results from the sampling of the tracks and the seamount fauna, and highlighted the importance of determining baseline conditions in the APEIs, in particular ensuring that the APEIs were similar to potentially mined regions (see Workshop Recommendations).

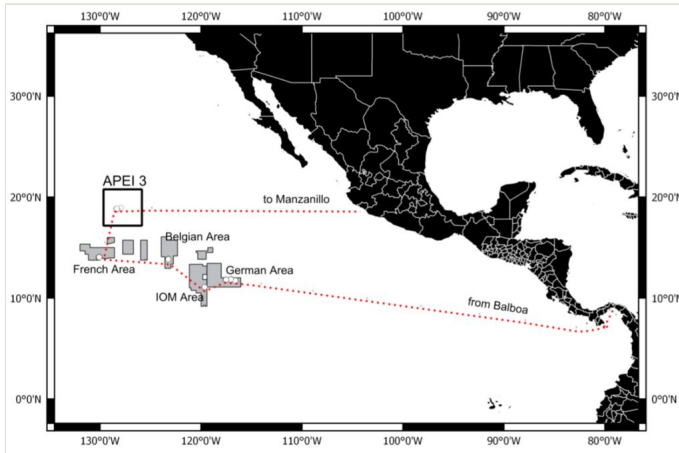


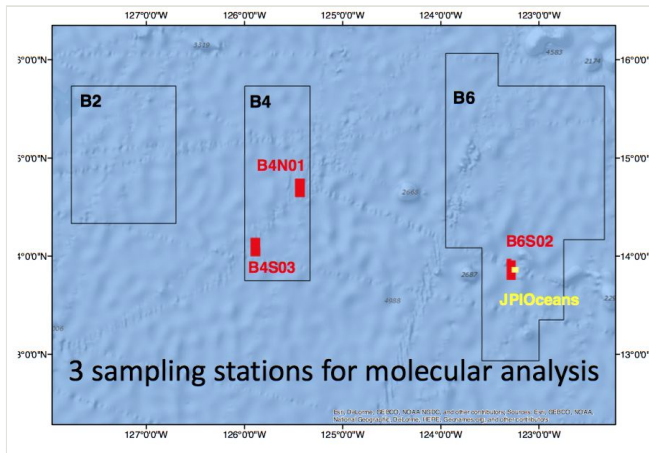
Figure 6.

Outline of the JPI Oceans 'SO-239' research cruise aboard the R/V Sonne 10 March – 30 April 2015 to the eastern CCZ (Image from Short Cruise Report: Martinez, 2015).

Discussion at the Workshop about the JPI Oceans project was mainly focussed on the data from the tracks and the impacts on connectivity. For example, David Billett raised the question of the distance at which visible (sedimentation on nodules) impacts of the old OMCO tracks were still visible. Daniel Jones pointed out that a significant portion of their work with Autonomous Underwater Vehicles (AUVs) is focussed on developing high-resolution mapping of the impacts from disturbances. Discussion moved on to the importance of understanding the hydrodynamics of these sites and the potential role of larval dispersal and larval biology, raised by Ana Hilario. Marina Cunha pointed out that a more precise working definition of 'connectivity' would be useful, with recent reviews published in the literature (see Workshop Recommendations).

Environmental sampling from the Belgian (GSR) contracted area was reviewed by Ann Vanreusel. Sampling has taken place in the Belgian area in the eastern CCZ (between the German and French areas) during GSR cruises in 2014, 2015 and as part of SO-239 in 2015. Molecular samples were obtained from 'multiple core' and 'box core' samples for meiofauna (DESS preserved) and macrofauna (ethanol preserved) in samples from three areas within the Belgian claim (Fig. 7). These samples came only from the 2015 cruise, and the SO-239 cruise (the earlier 2014 cruise did not collect molecular samples). Research has focussed on the nematodes. As some other researchers have found, success rates with COI were quite low (34%), but some sequences were obtained, with success rate for 18S at 61%. There have been very few attempts to sequence nematodes so these data are of potentially great significance. Vanreusel highlighted some interesting trends which were the subject of discussion. For example, the prevalence of rare species (see Workshop Recommendations) and the prevalence of high numbers of congeneric species in nematodes. The concept that mining activities and subsequent plumes might actually enhance connectivity by releasing animals into the water column was reviewed briefly.





the south-east corner of APEI-6, but also made a brief visit to the UK-1 area (Fig. 8). The cruise program utilised a wide variety of sampling methodologies, most relevant to the London Workshop were the macrofaunal and megafaunal specimen collection from box core and trawl, the AUV photography collection (see Workshop Recommendations), the collections from the scavenging amphipod traps and eDNA analysis based on sediments. There was some discussion of the issue of standardisation of megafaunal imagery, which is becoming a significant issue across a range of CCZ projects (see Megafaunal Imagery section and Workshop Recommendations). Jones pointed out that NOC were already actively collaborating with the University of Hawaii and JPI Oceans team on this, and in addition developing automated systems for the counting of nodules (Schoening et al. 2016). Some discussion ensued on the importance of nodule density and cover, and the need for regulators to understand that a range of habitat types must be protected, not just single-block areas. For example, many of the CCZ sites are characterised by gentle ridges, troughs and relatively flat areas, in which nodule density, cover and faunal composition may vary.

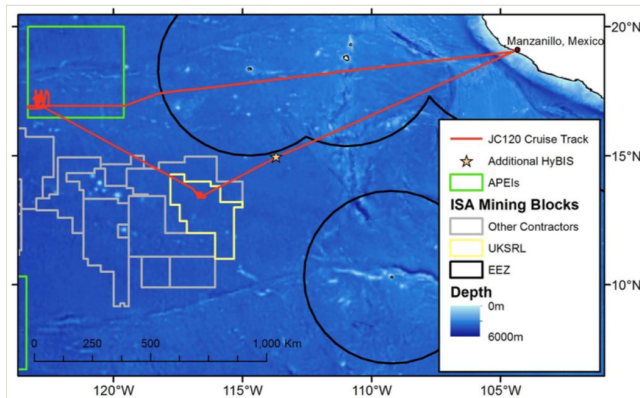


Figure 8.

The eastern CCZ, illustrating the cruise track of the RRS James Cook MIDAS JC-120 cruise to sample APEI-6 (green box) and a short sampling station in the northern sector of UK-1 (yellow box). Image source: Daniel Jones, National Oceanography Centre, UK.

The macrofaunal and megafaunal DNA component of the JC120 samples is being funded directly by the MIDAS through Work Package 4 (Task 4.1) and being led by Sergi Taboada, Gordon Paterson and Adrian Glover in collaboration with Daniel Jones. Taboada provided an overview of the relevant data that is being provided. Samples were obtained from box core, mega core, Hybis ROV, Agassiz trawl and amphipod traps. 462 samples were taken for DNA analysis, 90% of them from the APEI-6 and 10% from UK-1. 81 species were determined based on barcoding analysis, 15 of them annelids (Fig. 9). The intention is that the sequences will be pooled with samples being analysed in Glover's lab from the UKSRL ABYSSLINE project with connectivity studies published collaboratively. In addition Taboada outlined a major component of the MIDAS-funded project which is to undertake a detailed microsatellite DNA-based project on one of the more common nodule-dwelling sponges, a

species currently being described (*Timea* sp.). Further details are provided in the population genetics section below. A discussion point raised by David Billett that arose from Taboada's talk concerned the relative need for imagery versus collecting, in particular given the high cost associated with ROV cruises. A general consensus was the need for both sampling and imagery, highlighted further in the sections of this report – Megafaunal Imagery and Workshop Recommendations.

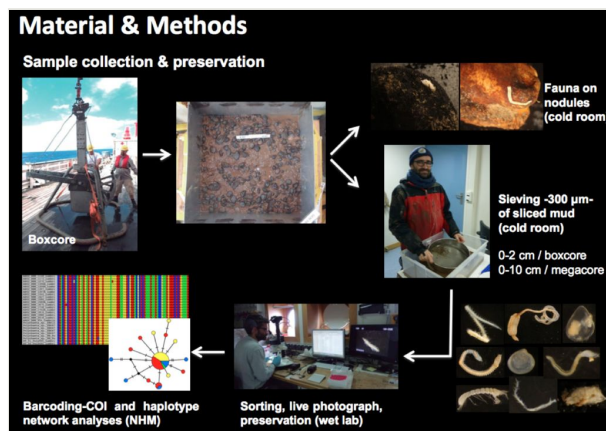


Figure 9.

The DNA taxonomy field pipeline for the Managing Impacts of Deep Sea Resource Exploitation (MIDAS) cruise aboard RRS James Cook (JC120). Image source: Sergi Taboada, Natural History Museum, UK.

Adrian Glover provided the final project overview of the day with a discussion on the molecular connectivity and biogeography parts of the ABYSSLINE project, funded by UKSRL. This is a contractor baseline survey that is being undertaken through a collaborative partnership with a globally-distributed academic partner network, coordinated and led by Craig Smith at the University of Hawaii. Partners in the UK include the National Oceanography Centre and the Natural History Museum. The project has been running since mid-2013, with cruises taking place in October 2013 (R/V Melville) and February-March 2015 (R/V Thomas G Thompson). The sampling design consists of a series of 30x30km boxes within which randomised sampling takes place using a wide range of equipment, the most relevant to the DNA work being the epibenthic sledge, 'box core', 'multi core' and ROV (Smith et al. 2013). To date, 3 boxes have been sampled, 2 within the UK-1 exploration area and 1 within the UK-1 reserved area, which is contracted to Ocean Minerals Singapore (OMS) under a joint venture between OMS and UKSRL (Fig. 10). The three boxes, UK-1 Stratum A, Stratum B and OMS Stratum A differ to some extent in their seabed habitat, with UK-1 Stratum B having a large number of seamounts (Fig. 11). Molecular (DNA) parts of the project cut across several different PI programs within ABYSSLINE (Table 1). Glover, together with Helena Wiklund and Thomas Dahlgren are leading the work on the macrofauna and megafauna, excluding Crustacea. Pedro Martinez is leading the work on the Crustacea and meiofauna. Andrew Gooday is leading the work

on the Foraminifera and Matthew Church (University of Hawaii, not present at the workshop) is leading the microbial work. All of these projects involve DNA work. More detailed outlines of the macrofauna, megafauna and foraminifera work from ABYSSLINE are provided later in this report from Glover and Gooday, and a summary of the DNA sampling techniques for the macrofauna provided in Glover et al. (2015).

Table 1.

Summary of Clarion-Clipperton Zone (CCZ) EU-contractor/academia led projects with a significant molecular biology (DNA taxonomy or population genetics) component

Project Name	Time Period	Cruises & Year	Principal DNA Work	Key DNA Publications
KAPLAN	2003-2004	R/V New Horizon 2003; R/V Umitaka-Maru 2004; R/V L'Atalante 2004	Macro- and meiofauna DNA taxonomy	Smith et al 2008
BGR (Germany)	2010-ongoing	R/V Sonne and R/V Kilo Moana; ~Annual since 2010	Mega-, macro- and meiofauna DNA taxonomy & connectivity	Janssen et al 2015
JPI-Oceans	2015-ongoing	R/V Sonne 2015	Mega-, macro- and meiofauna DNA taxonomy	In progress
GSR (Belgium)	2014-ongoing	2014, 2015 (JPI-Oceans)	Meiofauna	In progress
Ifremer (France)	2004-ongoing	R/V L'Atalante 2004; R/V L'Atalante 2012	Mega-, macro- and meiofauna DNA taxonomy	Janssen et al 2015
MIDAS (NOC- NERC)	2015	RRS James Cook JC120 2015	Mega-, macrofauna DNA taxonomy & connectivity	In progress
ABYSSLINE (UKSRL)	2013-ongoing	R/V Melville 2013; R/V Thomas G Thompson 2015	Mega-, macro- meio- and microbial fauna DNA taxonomy & connectivity	Glover et al 2015; Glover et al 2016; Dahlgren et al 2016

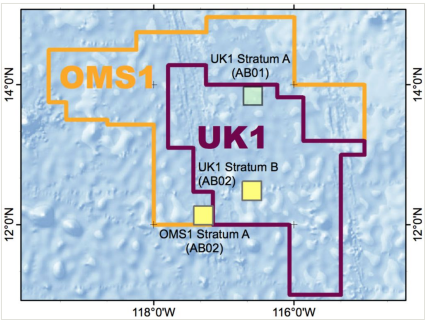


Figure 10.

The UK Seabed Resources Ltd (UKSRL) Abyssal Baseline (ABYSSLINE) survey area in the UK-1 exploration area in the eastern CCZ, with the UK-1 and Ocean Mineral Singapore (OMS) reserved areas highlighted. The survey design consists of replicated 30x30km boxes within the exploration area within which randomised sampling using a range of equipment is undertaken (Smith et al 2013). Image source: Craig R Smith, ABYSSLINE Project.

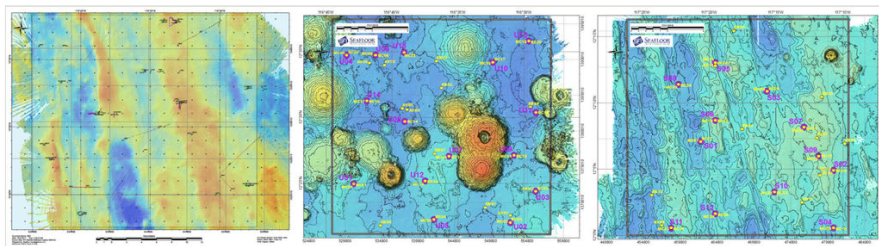


Figure 11.

Contrasting seafloor topography in the three Abyssal Baseline (ABYSSLINE) 30x30km survey boxes sampled to date, left to right, UK-1 Stratum A, UK-1 Stratum B and Ocean Mineral Singapore (OMS) Stratum A. Image source: Craig R Smith, ABYSSLINE Project.

## Session 2: The policy and industry perspective

Although primarily a data-discussion workshop, it was thought important to include reasonable time on policy and industry perspectives in order to direct discussion and to provide direct two-way dialogue with the development of the regulatory framework at the ISA. With this in mind, Stefan Brager (ISA) and Ralph Spickermann (UKSRL) were invited to the workshop to contribute from these perspectives.

Stefan Brager presented the policy background for contractor investments into the study of biogeography and connectivity in the CCZ. To protect the Common Heritage of Mankind, the Law of the Sea has mandated the ISA to create rules and regulations to ensure protection of the marine environment during deep-sea mining. The 'Environmental Management Plan for the Clarion-Clipperton Zone' (ISBA/17/LTC/7, ISA 2011) is such a binding document. It was adopted in 2012 and is currently up for review. Besides creating the nine existing Areas of Particular Environmental Interest (APEI) (Fig. 1), it calls for a suite of additional measures, several of which require biogeographical input such as the designation of the Impact and Preservation Reference Zones within each claim area. A review of the functionality of the APEI will need to look at the size of the areas as well as at the operational and management objectives with sufficient biological connectivity being one of the key issues.

Dr Brager's presentation resulted in several discussion points being raised at the London Workshop. There was a short discussion on how aware (or not) the academic scientific community is with regard to the various regulatory developments, in particular important dates for reviews, milestones, adoptions of regulations and changes etc. Examples are the need for review of the APEIs, the deadline for that, and the need for draft exploitation guidelines, due in July 2016. A policy brief for the academic community may be of use (see Workshop Recommendations). Brager challenged the scientists in the room to answer if they felt the goals of the CCZ EMP (ISA 2011) are being met, in particular whether the APEI should be changed or not. Adrian Glover noted, based on anecdotal evidence only, that most scientists he had spoken to felt uncomfortable that there are no APEIs in the middle of the CCZ, and that they are only distributed around the edges of the main

contracted areas. He questioned whether there could be justification for putting smaller APEIs in the middle of the CCZ, avoiding contracted areas, but covering different habitat types. Glover pointed out that the APEIs are based on a range of assumptions and models following Wedding et al. (2013). It may be possible that many of these assumptions and models can be updated, but this needs funded work undertaken by the scientific community (see Workshop Recommendations). Daniel Jones pointed out that preservation areas and set-aside regions further afield may be required, and Kirsty McQuaid pointed out the work they are doing with Kerry Howell at the University of Plymouth to use a habitat-modelling approach to determine if APEIs are representative of mining areas. Gordon Paterson noted that access to data from each claim is required by the scientific community to enable this kind of finer-scale habitat modelling, and Steffi Kaiser pointed out that the sediment characteristics between claim areas was often quite different, and these types of environmental parameters need to be recorded.

Following this discussion, Ralph Spickermann (UKSRL) addressed the workshop from the industry perspective. His presentation outlined the path from exploration to exploitation from the contractor point of view. Spickermann pointed out that seabed minerals are essentially a capital-intensive new industry, with two key business enablers being the regulatory framework and the environmental responsibility. From an industry perspective, the future exploitation regulations (currently under discussion at the ISA) must be commercially viable and environmentally sustainable, stable and predictable for an infant industry, reflecting of the technical risk relative to terrestrial ventures and with a simple royalty structure. In terms of resource certification (i.e how much of a resource there actually is), this can follow already well-established protocols such as the Canadian NI 43-101 or equivalent. Spickermann finished with a brief overview of the ABYSSLINE consortium environmental baseline work, highlighting the cruises (mentioned by Adrian Glover earlier in his overview) and the broad range of studies being carried out, and emphasising that the value of sample richness is only realised upon analysis and publication, not just collection. This latter point is broadly supported by the science community and particularly relevant to the CCZ where there has been a long history of biological sample taking without appropriate funding models to work up the samples (see Workshop Recommendations).

### **Session 3: Synthesis of DNA taxonomy and biogeography within CCZ exploration claim areas**

The third session of the London Workshop addressed what has been the main focus of attention in recent DNA work in the CCZ: what we term in this report 'DNA taxonomy'. There is often a mixture of use of the terms DNA taxonomy and DNA barcoding in the literature. In its strictest sense, DNA barcoding refers to the identification of a species by sequencing a known marker gene, often the COI marker, and comparing this against known databases or libraries (Hebert et al 2003). In contrast, DNA taxonomy (at least as we define it) is the creation of that database or library. This is particularly important in the deep sea, where we have seen in recent CCZ examples (Janssen et al. 2015; Glover et al. 2016a; Dahlgren et al. 2016) there are no identified, vouchered reference sequences



whatsoever, so no 'barcoded' specimens can actually be identified using the currently available libraries. New taxa that are recovered from the CCZ and sequenced, with those sequences made available to the community are effectively being 'described' taxonomically, albeit in an informal or formal sense depending on if a name is provided. This problem has led to the use of the concept of 'Molecular Operational Taxonomic Units' or MOTUs, used in Janssen et al. (2015). In this case, MOTUs can be distinguished from one and another using barcoding gap or phylogenetic analysis, but not necessarily identified to name. The use of the term MOTU is not universal – some authors (Glover et al. 2016a; Dahlgren et al. 2016) refer to phylogenetically-distinct clades based on genetic evidence as species, citing the phylogenetic species concept (see Workshop Recommendations).

The session started with Pedro Martinez highlighting results from the joint BGR-Ilfremer efforts to the German and French exploration areas. Data from the BGR cruise SO205 in 2010 and the BIONOD Ifremer cruise in 2012 have been published in Janssen et al. (2015). The paper reports COI data from 556 polychaete and 150 isopod samples. A typical finding from the study is the large number of singleton species, for example out of 233 polychaete 'MOTUs' determined by gap analysis, 138 were represented by only a single specimen. A similar pattern was observed for the isopods. Also typical was the confirmed presence of a small number of broadly-distributed species - 28 polychaete species were found in both the BGR and Ifremer areas, separated by a distance of 1300km and a depth difference of 697m (Janssen et al. 2015). For isopods, only two species were found to be present in both areas, which remarkably were thought to be brooding species with limited dispersal abilities (Fig. 12). Given that the vast majority of the species were present at only one site, the question arises as to how many of these species are truly restricted in their distributions and how many have just not yet been found elsewhere owing to undersampling. This is likely to only be answered through collaborative work across the entire region.

Helena Wiklund provided an overview of the DNA taxonomy work on the UKSRL ABYSSLINE project. The project team (Wiklund, Adrian Glover and Thomas Dahlgren) are working on a collection of 3312 individually databased and photographed megafaunal and macrofaunal specimens (excluding Crustacea) from the two ABYSSLINE cruises conducted in 2013 and 2015 (Fig. 13). To date all the sequencing has been completed from the first cruise, it is ongoing for the second cruise, and papers have been published on the overview methodologies (Glover et al. 2015), the echinoderms (Glover et al. 2016b), the cnidarians (Dahlgren et al. 2016) and megafauna (Amon et al. 2016). Further publications are in progress (see Publication Plans, below). With the collaboration of the Molecular Collections Facility of the NHM and Bioinformatics team, a sample archiving and open-data pipeline has been developed to allow colleagues, scientists, contractors, regulators and future generations access to NHM-housed material collected on project ABYSSLINE. This will also permit molecular-based real taxonomy to be undertaken with adequate curation and protection of DNA extracts and voucher specimens providing type material to new species names.



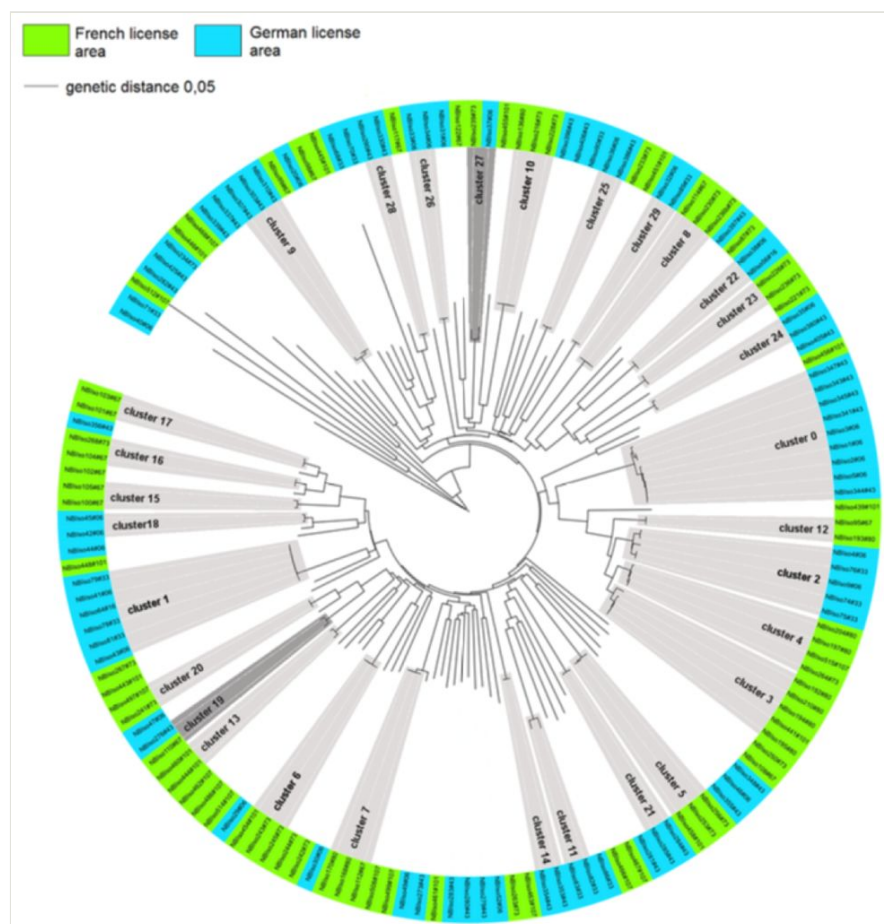


Figure 12.

Analysis reproduced from Janssen et al. (2015) highlighting the large number of 'MOTUs' unique to individual claim areas in a study of isopods from the BGR and Ifremer regions, only two taxa were found to be shared across the claim regions.

At the London Workshop, Wiklund outlined some of the new data from the second ABYSSLINE cruise (AB02) and in addition analysed these data alongside new unpublished data from the MIDAS-JC120 cruise (see above). In general, some evidence for broad species ranges was found in a range of taxa when regional-level data was included from the UK-1, OMS, Ifremer and BGR exploration contract areas. However, it was pointed out that it is only possible to demonstrate presence, not absence, given the likelihood of under-sampling. This is a common problem thread that runs through much of the regional-level analyses being undertaken at present (see Workshop Recommendations). Discussion focussed on this, and in addition Lenaick Menot pointed out that it would be very interesting to focus some efforts on these broadly-distributed species to examine their functional traits, for example. Wiklund pointed out that as new data accumulate, new 'target taxa' emerge

such as the pycnogonids, which seem to be quite broadly-distributed. Adrian Glover pointed out that we need to at some point stop thinking about the CCZ in terms of the contract boxes, and start to think more broadly about the region and the drivers of heterogeneity in general.



Figure 13.

Selected live images of specimens recovered from the UK Seabed Resources Ltd (UKSRL) Abyssal Baseline (ABYSSLINE) research cruise to the eastern Clarion-Clipperton Zone, February-March 2015. Image credit: Adrian Glover, Thomas Dahlgren, Helena Wiklund.



Figure 14.

Nematoda of the Clarion-Clipperton Zone, recovered from abyssal sediments during the JPI Oceans 'SO-239' cruise aboard the R/V Sonne in February-March 2015. Image credit: Lara Macheriotou, University of Ghent.

Paulo Bonifácio, together with Lenaick Menot and Lenka Neal, presented new data on the diversity, distribution and connectivity of polynoid worms across the CCFZ. The study aims to describe new species of deep-sea polynoids using morphology complemented with molecular data, evaluating monophyly of the subfamily Macellicephalinae and examining the genetic connectivity for widely distributed species found among different sampled areas (BGR, IOM, GSR, Ifremer and APEI#3). Samples were collected using an epibenthic

sledge, box core (0.25m<sup>2</sup>) and ROV from the French and German cruise programs. Preliminary results suggested the presence of 40 morphotypes of Polynoidae, with the subfamily Macellicephalinae being the most abundant and species rich. Further, *Bathyaufvelia* sp. A, was found in 4 of the 5 studied areas indicating no evidence for a biogeographic barrier and the connectivity appears to be high between the areas at least 100 km apart from each other. Bonifácio concluded that their study exemplifies the need for a dual approach in taxonomy, phylogeny and connectivity studies, i.e combining morphological and genetic data.

Lara Macheriotou presented molecular barcoding work completed thus far relevant to the biodiversity and population connectivity of free-living Nematodes of the CCZ. These samples were collected during the SO-239 (JPI Ocean EcoResponse, March-April 2015) cruise which visited four contractor licences (BGR, IFREMER, IOM, GSR) and APEI#3, as well as the GSR-led campaign (GSRNOD15A, September-October 2015) to their respective claim. Despite being very preliminary results, these data are providing the first initial insights to the nematofauna of the CCZ based on DNA. Most prominent was the large discrepancy in generic diversity of Nematodes collected using methodologies pertaining to meiofaunal versus macrofaunal taxa, the latter being significantly lower than the former. Data specific to the most abundant and widespread genera (*Halalaimus*, *Phanodermopsis*) confirm these are cosmopolitan and tentatively point to the possibility of an endemic deep-sea species from genus *Halalaimus*. Discussion of the Macheriotou presentation focussed on a couple of issues: firstly the reduced success rate with COI relative to 18S (noted by many other researchers across a range of taxa) and secondly the interesting observations of the large numbers of macrofaunal-sized nematodes found, particularly in the box core samples.

Andrew Gooday presented an overview of the UKSRL ABYSSLINE studies on benthic foraminiferal diversity with a focus on the new data based on DNA. Samples were obtained from all the ABYSSLINE survey boxes (see above). Morphological analyses of only eight preserved coretop samples revealed very high levels of species richness, with over 500 morphospecies recognised and many others recorded from shipboard sorting (Fig. 15). Molecular analyses conducted by Maria Holzmann (U. Geneva) have had mixed success. Sequences were obtained from 85 of the 99 xenophyophores analysed, leading to a 14-fold increase in the total number of known xenophyophore sequences and confirming that these giant protists are monothalamous foraminifera. The success rate for other foraminiferal groups ranged from 2 to 53%. Results for the calcareous rotaliids are consistent with global distributions for some species. On another front, High-Throughput Sequencing of DNA and RNA from 2-gram sediment samples by Franck Lejzerowicz (U. Geneva) yielded high-quality sequence data for 374 samples that may provide an alternative solution to the time-consuming sorting and morphological analysis of foraminifera. Discussion focussed on the excitement of these new DNA data which have for so long been missing from groups such as xenophyophores – Adrian Glover pointed out the importance of the study given that only 68 xenophyophore species are described worldwide, and 35 of these are known from the ABYSSLINE study. They are also unusual and good for public engagement in understanding the marine biology of the region.



Figure 15.

Xenophyophores of the Clarion-Clipperton Zone, recovered from abyssal sediments during the two UKSRL Abyssal Baseline (ABYSSLINE) cruises aboard R/V Melville and R/V Thomas G Thompson in 2013 and 2015. Image credit: Andrew Gooday and Aurélie Goineau, National Oceanography Centre, UK.

Magda Błażewicz outlined the latest data on the tanaidaceans, a quite common crustacean found in abyssal sediments that lives in self-constructed tubes. Błażewicz is working on samples from the eastern CCZ (JPI Oceans project) together with material from another west Pacific project, the KuramBIO program. Generally, tanaidaceans are thought to have quite restricted distributions owing to their virtually sessile lifestyle (building the tubes) and reproductive strategy that lack planktonic stages throughout their lifetimes. Out of 67 specimens of the genus *Pseudotanaids* (Fig. 15), apparently the most abundant tanaidacean genus from the CCZ, DNA (COI) was obtained from 41. She has emphasised that success ratio in extracting and amplifying DNA was substantially higher from fresh material (extraction done onboard). Applying automatic procedures to delimit species (e.g. ABGD, GYMS) it has been demonstrated that *Pseudotanaids* is represented by 12 species in the CCZ and that the genus itself is not monophyletic. The molecular results are confirmed by morphological analyses. It was observed that only two species were broadly-distributed in the CCZ (between IOM and BGR) while there was no taxon in common across both the CCZ and KuramBio. Data are in preparation for a publication.

Figure 15 *Pseudotanaids* of the Clarion-Clipperton Zone, recovered from an EBS sample during the JPI Oceans 'SO-239' cruise aboard the R/V Sonne in February-March 2015. Image credit: M. Błażewicz, University of Łódź, Poland

Andrea Waeschenbach provided an overview of deep-sea and abyssal Bryozoa, including some preliminary data from some of the first DNA studies to be conducted in the CCZ as part of the UKSRL ABYSSLINE project. A general feature of deep-sea bryozoans is they are usually attached to hard substrates, with colonies raised above the substrate (Fig. 16). Examples of this have been observed in a remarkable new species of cyclostome bryozoan observed in the AB01 samples from the ABYSSLINE project. Waeschenbach provided an overview of the various deep-sea bryozoan projects that are ongoing. With regard to the CCZ, data are so far preliminary, but the majority of samples are returning good sequences and many appear to be new species. A discussion ensued on the importance of getting funding for taxonomic work on the less numerous CCZ taxonomic groups such as the bryozoans, pycnogonids and others (see Workshop Recommendations).

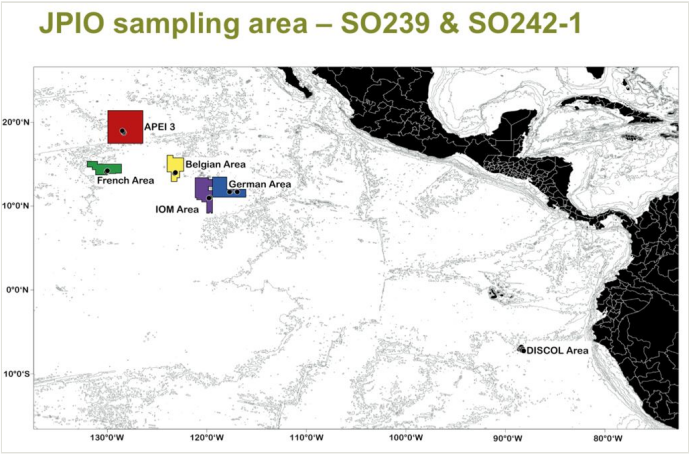


Figure 16.

A new species of cyclostome bryozoan from the eastern CCZ UKSRL ABYSSLINE project. Image credit: Adrian Glover, Thomas Dahlgren and Helena Wiklund. Identification by Dennis Gordon via Andrea Waeschenbach.

Sarah Schnurr presented an overview of the DNA taxonomy and biogeography in the isopods from the CCZ, which are dominated by the Asellota. These are generally understood to be brooding species with limited powers of dispersal, with distribution and gene flow dependent on passive and active migration of adults. Schnurr presented data from the JPI Oceans SO-239 and SO-242-1 cruises that took place in 2015 to the eastern CCZ (SO-239, see above) and the east Peru Basin DISCOL area (SO-242-1, see above, Fig. 17). Several hundred COI, 16S and 18S sequences have been obtained already from these samples, and preliminary data show some shared species between different exploration areas. These data will be the subject of a future publication (see Publication Plans, below).





**Figure 17.**  
Sampling stations in the eastern CCZ and east Peru Basin for the joint JPI Oceans cruises SO-239 and SO-242 utilised for phylogeographic studies of the asellote isopods being conducted by Sarah Schnurr, Senckenberg Institute. Image source: Sarah Schnurr, Senckenberg Institute.

Tasnim Patel spoke about her work on Amphipoda (Table 2) based on samples from the JPIO research cruise SO242-1 covering both the CCZ and DISCOL regions as part of her PhD studies. She is investigating whether habitat type, regional processes, different types and intensities of disturbance affect species diversity, dispersal and connectivity. Approximately 60,000 specimens were collected from the CCZ and DEA. These have been morphologically sorted at RBINS and 27 species have been identified thus far. Tasnim is now analysing the COI gene in two model species; *Paralicella caperesca* and *Abyssorhomene gerulicorbis* to test for cryptic diversity with results expected in June 2016. After this, she will attempt restriction-site associated (RAD) Next-Generation-Sequencing to test for population connectivity at various spatial scales.

**Table 2.**  
Summary of Clarion-Clipperton Zone (CCZ) projects studying DNA taxonomy and biogeography with key parameters and publications.

Taxon Group	Current lead scientist(s)	Cruise/area	Markers used	Key Publications
Isopoda, Polychaeta	P Martinez, A Janssen	BGR, Ifremer	COI	Janssen et al 2015
Polychaeta, Mollusca, Cnidaria, Echinodermata & misc.	H Wiklund, A Glover, T Dahlgren, J Taylor, S Taboada	UK-1, OMS, APEI-6	COI, 16S, 18S, 28S	Glover et al 2016; Dahlgren et al 2016
Polynoidae	P Bonifácio, L Menot, L Neal	BGR, IOM, GSR, Ifremer, APEI-3	COI, 16S	In preparation

Nematoda	L Macheriotou	BGR, Ifremer, GSR, IOM, APEI-3	COI, 18S	In preparation
Foraminifera	A Gooday	UK-1, OMS	18S	In preparation
Tanaidacea	M Blazewicz	eastern CCZ	COI	In preparation
Bryozoa	A Waeschenbach, P Taylor, A Glover, H Wiklund, T Dahlgren	UK-1, OMS, APEI-6	COI, 16S, 18S, 28S	In preparation
Amphipoda	T Patel	BGR, Ifremer, GSR, IOM, APEI-3, DISCOL	COI, and RADseq	In preparation
Isopoda (JPI Oceans)	S Schnurr	BGR, Ifremer, GSR, IOM, APEI-3, DISCOL	COI, 16S, 18S	In preparation

Table 3.

Summary of Clarion-Clipperton Zone (CCZ) projects studying population genetics with key parameters and publications.

Target Taxa for Pop Gen	Current lead scientist(s)	Cruise/area	Markers used	Key Publications
<i>Paralacydonia</i> sp, Sigalionidae sp, <i>Bathyglycinde</i> sp, misc. Polychaeta, <i>Macrostylis</i> sp, Desmosomatidae sp, <i>Eugerdella</i> sp	H Stuckas, P Martinez, A Janssen	BGR	COI	In preparation
<i>Bathyglycinde profunda</i> , <i>Paralacydonia</i> sp, <i>Lumbrinerides</i> sp, Maldanidae, Nereididae, Ophiuroidea, Mollusca ( <i>Nucula</i> sp)	T Dahlgren, A Glover, H Wiklund	UK-1, OMS, APEI-6	COI	In preparation
Ophiuroidea	P Martinez	BGR, IOM, GSR, Ifremer, APEI-3, DISCOL	COI	In preparation
<i>Pseudotachidius bipartitus</i>	U Raschka	BGR, Ifremer, GSR, IOM, APEI-3	COI	In preparation



<i>Timea</i> or <i>Hemiassterella</i> sp	S Taboada, A Glover, G Paterson, H Wiklund, T Dahlgren	UK-1, APEI-6	microsatellites and NGS	In preparation
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#### Session 4: Synthesis of intra-specific population connectivity in target taxa across the CCZ

The second major data session of the London Workshop was focussed on individual target taxa, in which detailed population genetic analyses have been carried out. These data are extremely new and nothing has yet been published from the CCZ. In most instances, researchers are investigating a small number of taxa in which sufficient sample numbers are available from individual locations, and analysis can be undertaken using rapidly-evolving markers such as COI on the genetic heterogeneity of the populations, measured by analysis of haplotype diversity and analysis of demographic patterns. These analyses can also sometimes be used to infer patterns of speciation (Glover et al. 2005) or provide more robust evidence of conspecificity over broad spatial scales (Georgieva et al. 2015).

Thomas Dahlgren opened the session by providing a historical overview of population connectivity studies in the deep sea. In the early period of deep-sea exploration, the deep sea was considered a broadly stable and homogenous environment that would favour low genetic diversity (e.g. Bretsky and Lorenz 1969). However, early allozyme data soon suggested there were relatively high levels of genetic diversity in some taxa (e.g Ayala et al. 1975). Since the discovery of hydrothermal vents, the efforts of researchers have focussed mainly on hydrothermal vents, ephemeral habitats and hard substrates such as seamounts. Remarkably, Dahlgren pointed out that although there have been dozens of papers on genetic connectivity at these 'island-like' deep-sea habitats, there are only three published studies from soft-sediment abyssal habitats (Etter et al. 2011, Janssen et al. 2015, Gubili et al. 2016). Even more remarkably, only (Etter et al. 2011) and (Gubili et al. 2016) includes detailed population data, and the latter publication is still in press. (Etter et al. 2011) showed that for an abyssal bivalve mollusc, there was remarkably little genetic variation within ocean basins, but some differentiation between basins. (Gubili et al. 2016) showed that the although the supposedly-cosmopolitan species *Psychropotes longicauda* was in fact a species complex, the separate lineages within the clade could have very broad distributions.

The Dahlgren presentation at the London Workshop opened up discussion, chaired by Adrian Glover and some key unanswered questions relevant to the CCZ were proposed (see Workshop Recommendations):

1. How do we go from **indirect** evidence of connectivity based on genetics to **direct** evidence based on functional traits and larval biology?
2. What **oceanographic data** (currents, models) are available to support indirect evidence of connectivity?

3. What **genetic markers** should we use to infer connectivity, and how does this vary between taxonomic groups?

Gordon Paterson raised the point that at what point do cryptic species matter if they perform the same ecological function? Ann Vanreusel pointed out that there are studies that show cryptic species are important at a functional level, and Adrian Glover pointed out that his lab are currently working on looking at functional differences measured by stable isotope analysis in cryptic polychaete species (PhD Student Madeleine Brasier). David Billett raised an interesting question as to whether we should be looking at also north-south patterns across the CCZ as well as east-west, and the effects of depth. Andrew Gooday noted that another variable that may structure populations is the calcium-carbonate compensation depth (CCD).

Heiko Stuckas provided an overview of haplotype diversity methods applied to deep-sea taxa (Fig. 18). New data were presented from Stuckas on the population connectivity of polychaetes and isopods from the BGR claim area at scales of 10-100 km. The COI data have been collected from five polychaete species and five isopod species that have been intensively sampled from the BGR Preservation Reference Area and Impact Reference Areas as well as some sites further afield (100-150km from these areas, but within the BGR exploration contract). The data show interesting diversity and demographic patterns that are the subject of a publication in preparation. Discussion focussed on whether these patterns could be seen to be representative of the CCZ as a whole. Lenaick Menot pointed out that isopods would be expected to have lower dispersal capabilities being brooders, and that we don't yet have representation from a range of functional groups.

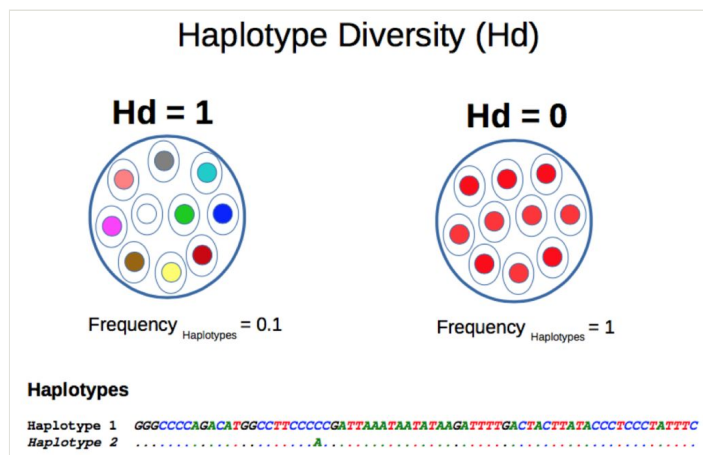


Figure 18.

An explanation of the study of haplotype diversity (Hd). Hd is measured by calculating the frequency of different haplotypes (genetically distinct sets of genes defined here by single nucleotide polymorphisms). The measurement of Hd and the frequency distribution of haplotypes can provide information on the genetic diversity of the population, the presence of speciation and demographic patterns. Image: Heiko Stuckas, Senckenberg Institute.

Thomas Dahlgren continued the population session with an overview of the study of 'target taxa' from the UKSRL ABYSSLINE project (Fig. 19). The new data come from the UK-1, OMS and APEI-6 sectors of the CCZ, and Dahlgren and the ABYSSLINE team have added in published data from Janssen et al 2015 into their analysis where possible. Currently, five polychaetes, two ophiuroids and one mollusc are being studied in detail, while a separate microsatellite study is ongoing for one of the nodule-dwelling sponges (see Taboada, below). The ABYSSLINE, MIDAS and JPI Oceans data are being studied together by Dahlgren to enhance spatial coverage and bring a more regional-view to CCZ connectivity, and publications are currently in preparation (see Publication Plans). Discussion of the Dahlgren presentation followed a similar pattern to that of Stuckas, with the main issue raised of what we are going to be able to say about non-target taxa – i.e those that are under-represented in samples. Gordon Paterson raised concern about the lack of meiofaunal target taxa, and Adrian Glover raised concern about the lack of megafaunal target taxa (see Workshop Recommendations).



Figure 19.

An example of a 'target taxon' being studied in the Clarion-Clipperton Zone for detailed population genetic study, *Paralacydonia* sp. This is a likely new species to science, but is abundant in a range of CCZ exploration areas. Image credit: Adrian Glover, Helena Wiklund and Thomas Dahlgren.

Pedro Martinez outlined population studies on ophiuroids being led by the Senckenberg team. Ophiuroids are extremely common in the CCZ, and are often the most recognised metazoan in video transects (Fig. 20). One of the outcomes from the ISA workshop on megafaunal taxonomy is that species ranges cannot be estimated from imagery alone, so Martinez focussed efforts on SO-239 to collect ophiuroids to confirm identity and establish population studies. One of the common ophiuroids, normally referred to as *Ophiomusium* cf. *glabrum* (discussed in Glover et al. 2016a) is in fact two species, and further analysis since the Glover et al paper by Tim O'Hara (Museum Victoria) has confirmed this. Adrian Glover pointed out that they left this out of the 2016 paper as O'Hara is planning a taxonomic publication on these taxa. Martinez discussed the new data also in the context

of data from the DISCOL area and the KuramBIO area in the west Pacific. Publications are in preparation. Discussion focussed on one of the main questions that has arisen before, as to why some taxa show evidence of both cryptic speciation and also broad species ranges.

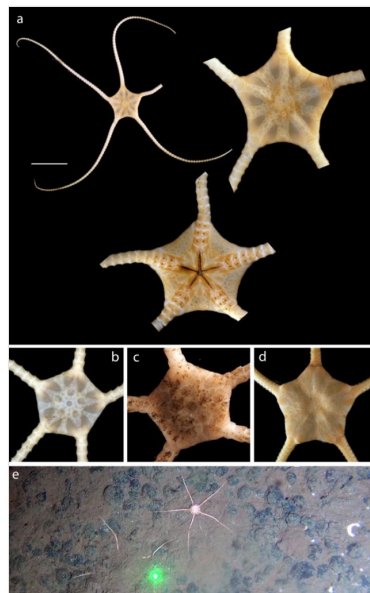


Figure 20.

The species often referred to as *Ophiomusium* cf. *glabrum* which is a likely cryptic species complex in CCZ samples, but is present in large numbers and is recognisable (at least to genus) in image surveys. This is a current target taxon for population studies. Image source: (Glover et al. 2016b).

Uwe Raschka presented an overview of detailed study of a species of harpacticoid copepod, *Pseudotachidius bipartitus* first described from the Beaufort Sea, Alaska, but recorded from the CCZ in recent surveys. 90 individuals have been sampled, with COI sequences obtained from 57 individuals from across the BGR, IOM, GSR and Ifremer contract areas. Publications on the genetic diversity and cryptic speciation are in preparation.

Pedro Ribeiro presented a preliminary population genetics analysis of a vent mussel species on the Mid-Atlantic Ridge using next-generation sequencing of RAD tag libraries (RADseq). The main purpose of this presentation was to provide an overview of the advantages, as well as methodological and analytical challenges, of using this technique to investigate population genetic structure in the deep sea. RADseq can be more time-efficient compared to other methods, and yield large numbers of single nucleotide polymorphism (SNP) markers, thus holding the potential to uncover levels of genetic structure usually not attainable by traditional methods. However, this technique requires high DNA integrity of samples to construct good quality RAD tag libraries, which in turn are

instrumental for sequencing success. Furthermore, discovery and validation of SNP markers can be particularly challenging and may require investing some time for developing the bioinformatics skills necessary to properly explore the data and the array of bioinformatics tools available.

Sergi Taboada presented preliminary data from a new MIDAS-funded study of the detailed population genetics of a relatively newly-discovered and overlooked species of nodule-dwelling sponge (Fig. 21). These small but abundant sponges were first noted by the ABYSSLINE team on the R/V Melville cruise in October 2013, and remained for some time in databases as 'Porifera sp. A'. Since that time, the team has been collaborating with colleagues at the National University of Singapore (Swee Cheng Lim) who are working on a subset of samples from the OMS exploration area part of the reserved area of the UK-1 contract also sampled as part of ABYSSLINE. Lim has identified the species as likely belonging to a new species in the genus *Timea* or *Hemiasasterella* and a species description is in preparation. Meanwhile Taboada and colleagues at the Natural History Museum are conducting a next-generation sequencing (NGS) approach to study genetic connectivity in the animal, as preliminary data show that COI is not a variable region in Porifera. The sponge is an ideal candidate for connectivity studies as it is common, easily identifiable (once a description is published), sessile, filter-feeding, with lecithotrophic larvae and is easily counted to establish population densities (using box cores). Taboada outlined the preliminary data currently being worked on, and publications in preparation.

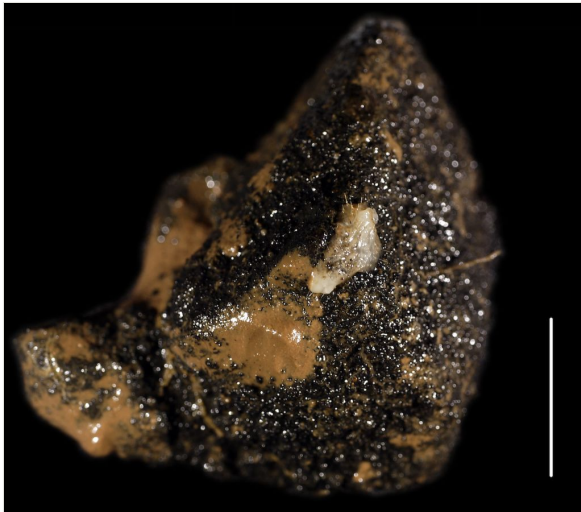


Figure 21.

A new species of sponge (white object on nodule), first noted on the R/V Melville ABYSSLINE cruise in October 2013, currently in description (Lim et al, National University of Singapore) possibly a new genus related to *Hemiasasterella*, that is now the subject of detailed investigation by the MIDAS and ABYSSLINE CCZ teams. The scale bar is 1 cm. Image credit: Adrian Glover, Thomas Dahlgren, Helena Wiklund.

## Session 5: Synthesis of the broader biogeographic context based on morphology and molecules

The purpose of the final data session at the London Workshop was to integrate the new studies of molecular biogeography and connectivity with the broader biogeographic context, in particular data based on morphology. Historically this has been from collected samples in formalin that are unsuited to DNA work; more recently there has been a surge of interest in trying to understand broad ranges of species based on imagery (ISA 2013).

Adrian Glover presented an overview of the 'express Taxonomy' approach used in the ABYSSLINE project to make raw taxonomic data (species morphology, genetic data and natural history observations) available from recent cruises even before species description is possible. These data are being published by the team in open-access data journals throughout the project. Glover highlighted the importance of morphological data in allowing comparisons with historic collections and species records (see Workshop Recommendations). These comparisons have in many instances supported potentially broad 'cosmopolitan' species distributions, but genetic data are in most cases lacking from species type localities (the location of original description). Glover explained how the team had been dealing with this by careful examination of type locality and/or material, and use of open nomenclature abbreviation 'cf.' to highlight cosmopolitanism as an untested hypotheses in many instances. An example was presented that links the new DNA data to historical collections and knowledge – that of *Nucula profundorum*, a protobranch mollusc that is abundant in abyssal samples. Samples of *N. profundorum* obtained from the ABYSSLINE program were assigned to *N. profundorum* based on direct morphological comparison with type material held in the NHM collections. Interestingly, on sequencing the specimens, they did not match the published sequences of *N. profundorum* on GenBank, and it is likely that the sequences on GenBank (recorded at much shallower depths and a long distance from the *N. profundorum* type locality) are erroneously identified. These matters will be clarified in future publications, but it highlights the importance of improving the quality of data on GenBank and checking the type locality of species that are identified (see Workshop Recommendations).

Daniel Kersken presented an overview of the Senckenberg work on Porifera from the JPI Oceans cruise SO239. The project was focussed not just on DNA work but also on providing an image-based catalogue of the Porifera in the CCZ. Samples were obtained from 15 ROV stations in depths of 1700-5000m in the eastern CCZ from the main SO239 sample areas. A large number of species were recovered, which are the subject of detailed morphological taxonomy coupled with DNA barcoding. Future projects include an attempt at next-generation sequencing and radiocarbon dating of *Saccocalyx pedunculatus* to determine the age of the sponges, as well as video-based annotation along ROV transects.

Andrew Gooday presented an overview of the broader biogeographic context for the Foraminifera (Fig. 22). Foraminifera have a superb fossil record and are thus widely used to reconstruct knowledge of ancient oceans. In this sense, understanding modern foraminiferal biogeography can also provide a 'deep-time' perspective on deep-sea

biodiversity. Knowledge from shallow-water studies is suggestive that endemism is high in forams (e.g. Culver and Buzas 1998), and that endemism decreases with increasing depth. In some cases, broad ranges are now backed up by genetic evidence, but there are now so many new deep-sea foraminiferal taxa, in many cases very rare, that making general statements on distributions within the groups is not yet possible (Gooday and Jorissen 2012)



Figure 22.

Some well-known deep-water foraminifera that are considered cosmopolitan based on morphology. Images by Andrew Gooday, Aurélie Goineau, ABYSSLINE project.

Gordon Paterson contributed to the session with a talk on the issue of rarity in the deep sea. Understanding species ranges is critical to the assessment of extinction risk, and part of this issue is related to rare species: are they particularly at risk? Paterson showed how almost all deep-sea biodiversity datasets demonstrate a pattern of rarity – that is large numbers of singleton species. Even when modelled against sampling effort, the number of rare species does not seem to decline. Paterson noted that much better definitions of rarity are needed, and is currently working on a meta-analysis of this problem using CCZ data. These show that rare species are the most represented in CCZ samples (in terms of percentages of species) but locally rare (but widely distributed) species also make up a similarly large percentage. So species may be locally rare, but actually widespread and hence common, irrespective of the abundance.

### Analysis of megafauna based on imagery

Although not part of the original London Workshop brief, discussions at the workshop also focussed to some extent on information that can be gleaned from imagery surveys. These are increasingly being used by contractors and academic cruises to gain broad-scale information on species abundance and diversity. Recent ISA workshops have recommended that imagery surveys are backed up by sampling (ISA 2013). However, the increasing prevalence of these datasets, the continued lack of megafaunal sampling and



the urgent need for better understanding of megafaunal distribution patterns suggests that imagery data must be considered in broad-scale biogeographic study, where possible. At the Workshop, Pedro Ribeiro highlighted some preliminary data from the CCZ with regard to imagery survey for corals obtained during the JPI Oceans cruise SO239, both from nodule areas and seamounts in their vicinity. Ribeiro highlighted the low degree of species overlaps between coral faunas from different survey locations and the significant number of singleton observations as clear indications that a higher sampling effort is required to obtain a sharper picture of biogeographic/connectivity patterns in the area. Daniel Jones also highlighted the extensive AUV photographic dataset collected on JC120 for megafaunal analysis. Not present at the Workshop, but invited, were Craig Smith and Diva Amon who are leading a survey of the megafauna based on ROV and AUV sampling associated with the ABYSSLINE project. A summary of their views follows.

The megafauna (typically organisms >2 cm in smallest dimension) constitute an important component of the biodiversity in the abyssal CCZ and play a significant role in deep-sea ecosystem function. Knowledge of biogeography and population connectivity are essential for effective environmental management of nodule mining, including the design of marine protected areas.

The study of megafaunal biogeography and connectivity requires collection of information on megafaunal biodiversity via imagery as well as physical samples of organisms, over a range of spatial scales within and beyond the CCZ, followed by morphological and molecular comparisons. The first studies now being published based on imagery data suggest there are many species new to science in the CCZ (Amon et al. 2016), likely requiring a major effort for connectivity and biogeographic studies. The extremely limited collection of megafaunal specimens in the CCZ thus far has severely hampered reliable species identifications, and in turn estimation of species richness and species ranges via detailed morphological and molecular analyses. Although a number of imaging surveys have been conducted to characterize megafaunal diversity and biogeography within the CCZ, varying image quality, and lack of voucher specimens, makes comparisons between studies and accurate assessment of species ranges very problematic. Differences in image quality have resulted from variations in equipment used (e.g., camera resolution, energy of light sources) and survey procedures (e.g., altitude and speed), which may be inevitable given the difference in budgets and oceanographic resources across projects. This influences the resolution to which megafauna can be identified, as well as reliable assignment of individuals to morphotypes. Critical needs for advancing our understanding of biogeography and connectivity in the CCZ and the broader Pacific include: (1) the collection of megafauna specimens to provide species vouchers, as well as material for population genetic studies, and (2) development of standardized megafaunal image atlases to facilitate reliable identification of morphospecies across projects and regions (see Workshop Recommendations).

## Summary tables and discussion

The final day of the London Workshop (Figs 23, 24) was focussed on (1) the scope and outline of a potential synthesis paper, (2) the collation of summary tables outlining future publication, cruise and proposal plans from the participants and (3) workshop recommendations. The Principal Investigators met and agreed in general a proposed outline of a synthesis paper, to be taken forward by the lead authors and discussed elsewhere. The summary of proposed publications (Table 4) was an extremely useful exercise, outlining several opportunities for collaboration through sample and/or data sharing and in general helping to align work programs to reduce redundancy. In general, it was felt that there was a highly level of complementarity amongst the proposed work plans and the Workshop participants were in general optimistic about the future knowledge that will be made available from the CCZ in the next 2-3 years.

Table 4.

Summary table produced during the London Workshop on the Biogeography and Connectivity of the Clarion-Clipperton Zone outlining proposed publications during the next three years, designed to facilitate collaboration and complementarity of activities in the region.

<b>Taxon or Concept Focus</b>	<b>Regional Focus</b>	<b>Lead Institution</b>	<b>Probable Lead Author</b>	<b>Study type</b>	<b>Notes/ Collaborations</b>
<b><i>Taxonomy &amp; biogeography</i></b>					
Annelida	DISCOL	U Aveiro	Ravara	Biodiversity and taxonomy	Potential to collaborate with UK-1 samples (Contact Adrian Glover)
Annelida (Data Paper)	UK-1A	NHM London	Wiklund	DNA Taxonomy Catalogue	in prep
Bryozoa (Taxonomy)	UK-1A, UK-1 B, OMS, APEI-6	NHM London	Waeschenbach	Taxonomy	analysis ongoing
Cnidaria (Black corals, pennatulids, alcyonaceans)	BGR, GSR, IOM, APEI-3, IFREMER	IMAR - Azores	Ribeiro	Taxonomy and Biogeography	Several papers
Cnidaria (Data Paper)	UK-1A	NHM London	Dahlgren	DNA Taxonomy Catalogue	in press
Crustacea (Amphipoda)	BGR, IFREMER, APEI-3, IOM, GSR, DISCOL, UK-1	Belgian Institute	Patel	Biogeography and evolution	Potential to collaborate with UK-1 samples (Contact Tammy Horton)

Crustacea (Amphipoda)	UK-1 A, UK-1 B, OMS, APEI-6	NOC Southampton	Horton	Taxonomy	Potential to collaborate with T Patel (Belgian Institute)
Crustacea (Amphipoda)	UK-1A, UK-1 B, OMS, APEI-6	Senckenberg	Mohrbeck	Taxonomy and population genetics	
Crustacea (Cirripedia)	BGR, IFREMER, APEI-3, IOM, GSR, DISCOL, UK-1	Belgian Institute	Kerkoff	Taxonomy	Potential to collaborate with UK-1, BGR samples (contact P Martinez)
Crustacea (Copepoda - benthic)	BGR, IOM, GSR, IFREMER, UK-1 A, UK-1 B, APEI-6	Senckenberg	Raschka	Biogeography	Potential to collaboate with JC120 (contact S Taboada)
Crustacea (Copepoda - benthic)	BGR, IOM, GSR, IFREMER, APEI-3	Senckenberg	Khodami	Phylogeny	
Crustacea (Copepoda)	BGR, IOM, GSR, APEI- 3	Senckenberg	Khodami	Barcoding, diversity and population genetics	
Crustacea (Isopoda)	BGR, IFREMER	Senckenberg	Janessen	DNA Taxonomy Catalogue	
Crustacea (Isopoda)	BGR, IOM, GSR, IFREMER, APEI-3, DISCOL, APEI-3, UK-1 A, UK-1 B	Senckenberg	Schnurr	Biodiversity and biogeography	Potential to collaboate with JC120 (contact S Taboada)
Crustacea (Isopoda)	BGR, Atlantic	Senckenberg	Kaiser	Taxonony, phylogeny, new genus	

Crustacea (Isopoda)	BGR, IOM, GSR, IFREMER, APEI-3, Atlantic	Senckenberg	Schnurr	Phylogeny	
Crustacea (Ostracoda)	BGR, IFREMER, APEI-3, IOM, GSR, DISCOL, UK-1 A, UK 1-B, APEI6	Belgian Institute	Patel	Biogeography and evolution	Potential to collaborate with UK-1, BGR samples (contact P Martinez)
Crustacea (Tanaidacea)	BGR, GSR, IOM, APEI-3, IFREMER, Atlantic, APEI-6, UK-1 A, UK-1 B, OMS	U Łódź	Błażewicz	Biodiversity, taxonomy, phylogeny	Potential to collaborate with UK-1, BGR samples (contact P Martinez); GSR (contact A Vanreusel)
Echinodermata (Data Paper)	UK-1A, UK-1 B, OMS, APEI-6	NHM London	Glover	DNA Taxonomy Catalogue	Update to Glover et al 2016 with new taxa
Echinodermata (Holothuroidea)	DISCOL, CCZ	Senckenberg	Martinez	DNA Taxonomy	
Echinodermata (Ophiuroidea)	UK-1, APEI	Mus Victoria	O'Hara	Taxonomy	Description of <i>Ophiosphalma</i> sp
Ecology - Megafauna (CCZ Seamounts)	BGR, GSR, APEI-3	IMAR - Azores	Ribeiro	Biogeography	Based on DNA
Foraminifera - Xenophyophores	UK-1 A, UK-1 B, OMS, APEI-6, Russian (YMG)	NOC Southampton	Gooday	Biodiversity and biogeography	
Foraminifera - Xenophyophores	UK-1 A, UK-1 B, OMS, APEI-6	NOC Southampton	Gooday	Taxonomy	maybe split into two papers

Misc. Taxa (Data Paper, Pycnogonida, Brachiopoda, Sipuncula...)	UK-1A	Uni Research	Dahlgren	DNA Taxonomy Catalogue	in prep
Mollusca	DISCOL, UK-1 A, UK-1 B, OMS, APEI-6	U Aveiro	Rodrigues	Taxonomy	Potential to collaborate with UK-1 samples (Contact Adrian Glover)
Mollusca (Data Paper)	UK-1A	NHM London	Wiklund	DNA Taxonomy Catalogue	in prep
Nematoda	BGR, IFREMER	Senckenberg	Miljutin	Taxonomy Catalogue	
Nematoda	BGR, IOM, GSR, IFREMER, APEI-3, UK-1 A, UK-1 B, OMS, APEI-6	U Ghent	Macheriotou	Biodiversity and biogeography	Potential to collaborate with UK-1, BGR samples (contact P Martinez)
Porifera	BGR, IOM, GSR, IFREMER, APEI-3	Senckenberg	Kersken	Taxonomy	
Porifera	BGR, IOM, GSR, IFREMER, APEI-3	Senckenberg	Kersken	DNA phylogeography	after first paper above
Porifera ( <i>Timea</i> / <i>Hemiassterella</i> taxonomy)	OMS	NUS Singapore	Lim	Taxonomy	in prep
Tanaidacea	DISCOL	U Aveiro	Esquete	Biodiversity and taxonomy	
<b>Methodology</b>					
Foraminifera	OMS, UK-1 A, UK-1 B	U Geneva	Pawlowski	HTS Methodology	
<b>Ecology</b>					

Echinodermata (Ophiuroidea)	BGR, IOM, GSR, IFREMER, APEI-3	U Aveiro	Hilário	Life History	
Ecology - Macrofauna	GSR	U Ghent	Desmet	Community structure	
Ecology - APEI#6 overview	APEI-6	NOC Southampton	Jones	Ecological overview of the APEI	JC120 and ABYSSLINE
Ecology - eDNA	APEI-6	NOC Southampton	NOC Southampton	eDNA water column and sediment	
Ecology - Macrofauna	DISCOL	U Aveiro	Cunha	Community structure	
<b>Connectivity</b>					
Annelida & Crustacea	BGR, IFREMER	Senckenberg	Janssen	Population Genetics	
Connectivity - Population Genetics	UK-1A, UK-1 B, OMS, APEI-6	Uni Research	Dahlgren	Population Genetics	analysis ongoing
Connectivity - Workshop Synthesis	CCZ	NHM London	Glover	Synthesis	
Echinodermata (Ophiuroidea)	UK-1A, UK-1 B, OMS, APEI-6, APEI-3, GSR, BGR	Senckenberg	Martinez	Population Genetics and Taxonomy	NHM to pass ophiuroid sequences to Senckenberg
Foraminifera - Xenophyophores	OMS, UK-1 B, IFREMER	U Geneva	Lejzerowicz	eDNA global synthesis	
Porifera ( <i>Timea</i> / <i>Hemasterella</i> populations)	UK-1A, OMS, APEI-6	NHM London	Taboada	Population Genetics	analysis ongoing



Figure 23.

The London Workshop on the Biogeography and Connectivity of the Clarion-Clipperton Zone discussing data in the Board Room of the Natural History Museum. Image: Adrian Glover.



Figure 24.

The London Workshop on the Biogeography and Connectivity of the Clarion-Clipperton Zone on the main steps of the Natural History Museum below the bust of Charles Darwin. Workshop participants: Back row, left to right: Ralph Spickermann, Stefan Brager. Second from back row, left to right: Pedro Ribeiro, Sergi Taboada, Lara Macheriotou, Ann Vanreusel, Magda Blazewicz, Sarah Schnurr, Helena Wiklund. Third from back row, left to right: Andrew Gooday, Daniel Jones, Lenaick Menot, Pedro Martinez, Steffi Kaiser, Ana Colaço, Sahar Khodami, Marina Cunha, Ana Hilario. Third from back row, left to right: Adrian Glover, Paulo Bonifacio, Kirsty McQuaid, Aurélie Goineau, Heiko Stuckas, Thomas Dahlgren. Front row, left to right: Daniel Kersken, Uwe Raschka, Tasnim Patel, Clara Rodrigues, Gordon Paterson, Amber Cobley. Not present for photo: Andrea Waeschenbach and David Billett. Image credit: T Cruise.



The only confirmed cruise plans indicated by the group were for BGR cruises in May 2016 and May 2017. In terms of grant proposals, Adrian Glover highlighted the ongoing work to develop a Strategic Programme Area (SPA) for the Natural Environment Research Council that would focus on deep-sea resource extraction in general, potentially the CCZ could be part of this. At the time of writing, no decisions have yet been made on this proposal. Pedro Martinez indicated that there is interest in developing a second iteration of the JPI Oceans project, but the timeline is not yet known. This may involve technology and mining tests. Some discussion ensued on the idea to develop a broad cross program proposal on taxonomy (see Workshop Recommendations).

## Conclusions

### Workshop Recommendations

The London Workshop on the Biogeography and Connectivity of the CCZ concluded with a short discussion on Workshop Recommendations, summarised here:

#### Funding and Planning

- The most critical funding priority for CCZ research on biogeography and connectivity is high-quality taxonomic work that includes species descriptions based on phylogenetic inference, specimen and data archiving to permit future molecular work and equal weight given to all taxonomic groups and size classes
- Taxonomic funding proposals should consider international programs that could deliver taxonomic funding across a range of contractor projects, not just within single ones
- Future international projects on the CCZ must include funding to ensure integrating workshops are fully costed within those projects as they play a vital role in developing collaborations and minimising redundancy of activities
- Future Workshops on CCZ connectivity should be expanded to include non-EU contractors and additional international programs
- Areas of Particular Environmental Interest (APEIs), the majority of which are completely unstudied, should be a priority area for research programs on biogeography and connectivity, helping to deliver an improved regional-level understanding of the CCZ
- Assumptions in the models used for the development of APEIs should be updated as soon as possible with new data with a funded program of research
- The CCZ academic community should be better informed of policy developments and regulatory milestones
- Any biological sampling programs that are taking place in the CCZ must include a funded program of research to work up the samples through to publication and data/specimen archiving

## Research

- Connectivity (e.g ecological, population, genetic) should be more precisely defined in CCZ projects and proposals
- Efforts should be undertaken to examine connectivity in rare species, either through NGS approaches or more broad sampling to determine if rare species are common in under-sampled habitats or refugia
- ROV or AUV imagery of megafauna should be improved through (1) collection of voucher materials to confirm conspecificity through DNA sampling, (2) improved resolution, in particular the use of high-quality scientific ROV able to hover and acquire quality imagery and (3) development of regional atlases of megafauna that are available to all contractors and academic programs
- Ongoing and future efforts for the development of automated software tools for video analysis are also fundamental and must be encouraged. Given the huge amount of video data gathered by ROV and AUV surveys, automated methods are essential to be able to produce comprehensive community analyses on a timely manner.
- Working species concepts for new CCZ fauna should be based where possible on phylogenetic species concepts using molecular data in combination with morphological characterization
- New approaches to connectivity study should be developed using new data on larval biology, functional traits (e.g to establish connectivity amongst a range of trait parameters) and physical oceanography (e.g currents and modeled currents)
- New genetic markers and methods to study connectivity should be developed that are appropriate to the fundamental questions posed

## Data and Archiving

- Publications on the DNA taxonomy or phylogeography of the Clarion-Clipperton Zone should ensure that raw data in the form of genetic sequences, images, locality information and voucher material access are archived in global online repositories (GenBank, OBIS, GBIF, WoRMS)
- Morphological data (e.g type material in museum collections, taxonomic descriptions and new morphological information) must be used, where possible, alongside molecular data to (1) improve knowledge of putative species ranges, (2) improve taxonomic descriptions that rely on DNA data and (3) check the type material of published species and to use a precautionary approach in estimating species ranges

## Funding program

European Union Framework 7 'Managing Impacts of Deep Sea Resource Exploitation (MIDAS)' and Joint Programming Initiative Healthy and Productive Seas and Oceans (JPI Oceans).

## Hosting institution

Natural History Museum, London

## Author contributions

All authors with the exception of D Amon and C Smith participated in the Workshop. Amon and Smith sent a contribution to the report for inclusion in text form. Adrian Glover led the workshop organisation, chaired the sessions and drafted the manuscript. Amber Cobley took notes at the Workshop. All authors commented and edited the manuscript. The views expressed in this paper are those of the authors and do not necessarily reflect the position of the International Seabed Authority.

## Conflicts of interest

None.

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