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Satellite to Seafloor - The Global Biogeography of the Elasipodid Holothurians

by

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ABSTRACT

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Knowledge of the environmental factors driving species distributions in the deep-sea is essential for a better understanding of the biogeography of the abyssal benthos. Differences in the niches of elasipodid holothurians appear to exist at the level of species, genera and family. This study analysed the global distributions of elasipodid holothurians from records collected from online Natural History Collection databases, cruise reports and published literature.

A comparison of the distribution of four families of elasipodid holothurians, in relation to seven abiotic environmental factors found the families Psychropotidae and Elpidiidae to have the most different environmental distributions. POC flux, intra-annual seasonality and inter-annual variability in POC flux were the best explanatory factors for differences between the families. The Elpidiidae were generally found in areas with greater temporal variability. The potential for biogeographic schemes based upon the ecology of the upper ocean to inform management decisions for the deep sea was also investigated. Results suggest that Longhurst's 'case models' are more closely linked to the distribution of species and genera than Longhurst's biomes. Results also confirmed that provinces from the same ocean and latitude were more similar in terms of species composition, but that historical factors were equally capable of explaining the observed patterns and must be taken into account.

Population genetic studies of two species of Elasipodida; *Psychropotes longicauda* and *Oneirophanta mutabilis* were conducted using CO1 and 16S molecular markers. Multiple sympatric lineages were found in the Indian, Pacific and Atlantic Oceans in both species. Differences between sympatric lineages were greater than geographic differences within lineages. The potential for speciation in the Antarctic or Southern Ocean before multiple northwards dispersal events is considered the most plausible explanation for the observed patterns. Oceanic gyres therefore do not appear to be barriers to dispersal in these species.

The distributions of the elasipodid holothurians are therefore thought to be controlled by a combination of life-history, dispersal ability, temporal and quantitative variations in food supply and competitive interactions as well as historical processes relating to centres of origin for particular genera.

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Chapter 1. Introduction

1.1 An introduction to the field of biogeography

1.1.1 What is biogeography?

Biogeography is the study of the geographic distribution of organisms (Monge-Najera 2008). This includes the distribution of species, how groupings of species form distinct ecosystems and their geographical limits (Kaiser et al. 2005). Studies of biogeography may investigate large scale patterns in biodiversity or the distributions of individual species. At any one time, the geographic range size attained by any given species may potentially be limited by a number of factors, including the availability of suitable habitat, dispersal and establishment abilities, the presence or absence of competitors, predators and parasites, climatic and/or environmental tolerances and various historical considerations (Gaston 1996). Whilst geographical distributions and range sizes are one of the central subjects of ecology, recent studies have shown that it is difficult to apply universal rules to different groups of organisms (Macpherson 2003).

Biogeography is tied intimately to both ecology and phylogenetic biology. It seems logical therefore to consider biogeography as an integrative field that unites phylogeny and ecology to address important questions about the distribution of lineages and global patterns of diversity (Wiens and Donoghue 2004). However, in reality the field of biogeography remains a disjointed discipline, with major divisions between ecological and historical biogeography and between vicariance and dispersal biogeography (Fig.1.1). This often leads to polarisation within the field, which as discussed below, has generally been detrimental to the development of biogeography as a field (Cox and Moore 2010).

1.1.2 Historical vs. ecological biogeography

Ecological biogeography (or simply ecology) seeks causal explanations for the current distribution patterns of species over relatively small temporal and spatial scales, usually in terms of ecological processes. For example in terrestrial biogeography temperature, humidity, salinity, and the effects of other organisms are often viewed as key elements in the ecological biogeography of species (Monge-Najera 2008). Ecological biogeography attempts to answer questions such as: Why is a species confined to its present geographical range? What environmental and biological interactions restrict the current distribution of a species? (Cox and Moore 2010). Ecological biogeography started when early collectors recorded simple descriptions of the environment from which organisms were collected. However, it developed greatly in the 20th Century aided by advances in ecological theory and experimental approaches, such as ecophysiology. These improvements, along with ecological and behavioural studies and developments in statistical techniques helped biogeographers to understand the environmental requirements and niches of organisms (Cox and Moore 2010). Concepts from ecological biogeography are now being considered on much greater geographical scales than those associated traditionally with this field and form one branch of research of the relatively new field of 'macroecology'. Macroecology is considered in greater detail in *section 1.2.3*.

Historical biogeography usually concerns evolutionary processes on large, often global, scales over millions of years (Crisci 2001). It may ask questions such as: When did the taxon come to have its present distribution? How did geological and climatic events contribute to shaping that distribution? Why are some closely related species confined to the same regions, whereas others are widely separated? (Cox and Moore 2010). Historical biogeography is nowadays largely based on phylogeny (Wiens and Donoghue 2004) and is focused on vicariance events related to plate tectonics, and the timing and direction of dispersal events (discussed in the following section). However, there are at least nine different historical biogeographic approaches: centres of origin and dispersal, panbiogeography, phylogenetic biogeography, cladistic biogeography, phylogeography, parsimony analysis of endemicity, event based methods, ancestral areas, and experimental biogeography (Crisci 2001). These can be further subdivided into at least 30 techniques, so that at present historical biogeography lacks a coherent and comprehensive conceptual framework for handling the distribution of taxa in space (Crisci 2001).

Unfortunately, studies addressing ecological biogeography rarely incorporate historical factors into their research, even when it is crucial for addressing some of their central questions (Wiens and Donoghue 2004). Likewise, historical biogeography often ignores ecology, both in terms of the questions it asks and the answers it provides (Wiens and Donoghue 2004). However, ecology and history are 'indissolubly tied together' (Posadas et al. 2006) because large scale biogeographical patterns are also the product of ecological processes (Wiens and Donoghue 2004). Earth processes are dynamic and changes in environmental factors are continually occurring at ecological and evolutionary temporal scales (Crisci et al. 2006). A dichotomy between historical and ecological biogeography is therefore entirely artificial (Wiens and Donoghue 2004). Biogeography requires a new conceptual framework that integrates historical and ecological biogeography (Crisci et al. 2006).

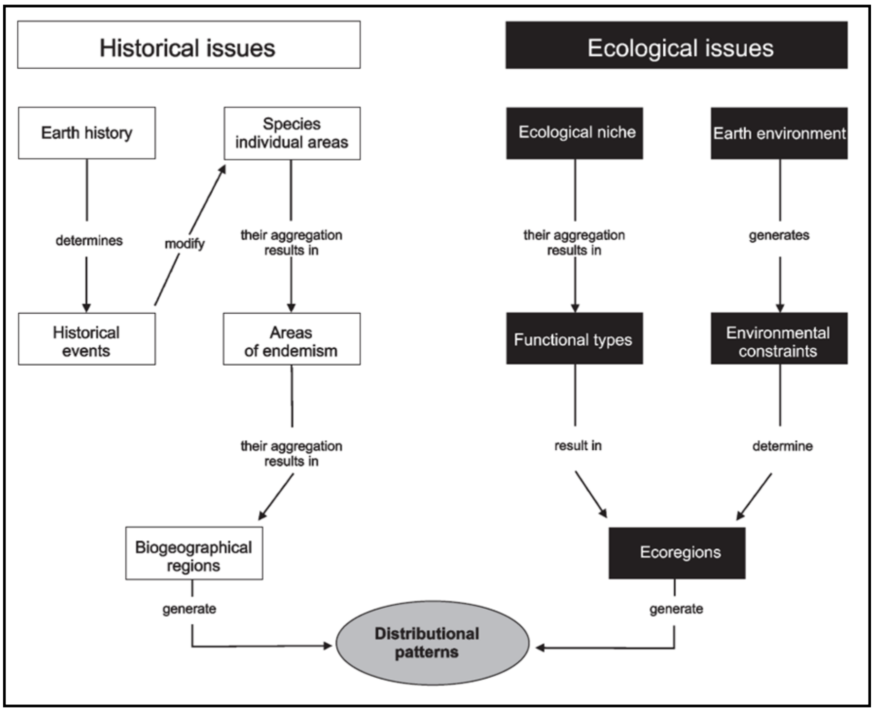


Figure 1.1 *Concept map showing how ecological and historical biogeographies separately contribute to our understanding of distributional patterns. From Crisci et al. (2006).*

1.1.3 Vicariance vs. dispersal biogeography

Historical biogeography is further subdivided into vicariance biogeography and dispersal biogeography. For centuries dispersal was the dominant explanation for the distribution of organisms (Crisci 2001). However, the validation of the plate-tectonics theory in the 1960s, combined with the spread of cladistic thinking, revolutionised biogeographic thinking from a dispersalist view to a predominately vicariant (paleogeographic) perspective (de Queiroz 2005, Cowie and Holland 2006). See *Figure 1.2* for a diagrammatic explanation of dispersal and vicariance.

Until recently, only vicariant mechanisms were considered to offer testable hypotheses because in essence all patterns could be explained by a dispersal hypothesis (de Queiroz 2005). Dispersal was viewed therefore only as adding 'noise' to the patterns generated by vicariance (Cowie and Holland 2006). The much quoted description of dispersalism as 'a science of the improbable, the rare, the mysterious and the miraculous' sums up attitudes to the study of dispersal in biogeography prior to more recent developments.

The proliferation of molecular based phylogenies in recent decades has reinforced dispersal as one of the major processes in creating concordant distribution patterns (Voelker 1999). In addition, (de Queiroz 2005) concluded that if plausible vicariance hypotheses were falsified then dispersal explanations are supported by default (de Queiroz 2005). Approximations of divergence times based on the molecular clock have been used to correlate crudely the timing of species cladogenesis with the timing of vicariant events (Sanmartin 2003); however also see (Emerson 2007) and (Bandelt 2008). In certain cases this has shown that the group studied is too young to have been affected by the presumed vicariant barrier, suggesting a more recent dispersal event in these taxa (Waters et al. 2000, Sanmartin 2003).

The impassioned debate between dispersal and vicariance biogeographers has also had an influence on the understanding of large scale biogeographic patterns; for example the theories of centres of biota origin and centres of biota accumulation.

Although new 'event-based parsimony methods' of biogeographical reconstruction are emerging which allow the consideration of both vicariance and dispersal (Ronquist 1997, Sanmartin 2003), the relative importance of vicariance and dispersal is still hotly debated.

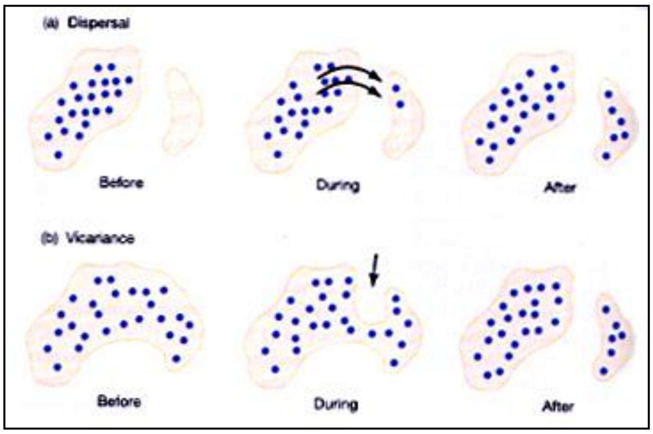


Figure 1.2 *Diagrammatic representation of a) Dispersal - individuals disperse from one or more ancestral populations resulting in the successful colonization of (a) new area(s) and b) Vicariance - an environmental or tectonic event separates taxa with a previously continuous range. (www.fiu.edu).*

1.1.4 A brief history of marine biogeography and our current state of knowledge

The first major contributions to marine biogeography were made in the 19th Century by James Dana who divided the surface waters of the World's oceans into simple biogeographic provinces based on their mean minimum temperature (Cox and Moore 2010). Shortly after, Edward Forbes proposed a biogeographical classification of the oceans with of 25 faunal provinces and five depth zones (Cox and Moore 2010). These provinces were arranged in nine latitudinal belts based on the characteristics of the seabed, local currents and depth. Forbes, therefore, was the first person to identify the now renowned Indo-Pacific hotspot of biodiversity (Cox and Moore 2010). In 1935 Ekman synthesised and reviewed marine biogeographical research at that time, and was one of the first researchers to discuss dispersal patterns, such as disjointed distributions caused by the formation of the Isthmus of Panama, and the disjointed distributions of species at mid-high latitudes in both hemispheres, on either side of, but not at the equator, see English translation of Ekman 1935 (Ekman 1953) and (Cox and Moore 2010). Ekman included work even on deep-sea zoogeography which is still referred to today (*see section 1.3.1*). The book '*Marine Zoogeography'* (Briggs 1974) is also seen by many as a seminal work, and built on the work of Ekman and others to propose a biogeographic scheme of the World's oceans, this time based on geographic areas of rapid species turnover. Clearly, biogeographic provinces do exist in marine environments. The spatial arrangement of the continents and oceans, combined with the influence of the latitudinal gradient of temperature, creates distinct areas characterized by geography, local circulation patterns and water properties (Levinton 2008). This much was obvious even to early biogeographers. Yet, as (Longhurst 2007) states in his book '*The Ecological Geography of the Sea*': "The results of 150 years of study of the distribution of the marine flora and fauna are so meagre that they permit us to predict comprehensively the characteristic assemblage of species likely to occur in *no* region of the ocean"(Longhurst 2007).

Even today the vast majority of broad-scale biogeographic studies deal with terrestrial organisms. Compared to terrestrial environments, the World's oceans are larger and more continuous, and more difficult to sample, so that marine biogeographical regions are less well pronounced (Rapoport 1994). Rapoport also argues that marine environments are more stable and provide less 'environmental stress' than terrestrial environments and that marine barriers are more porous that continental ones (Rapoport 1994). Furthermore, the life histories of marine organisms are often strikingly different from those of terrestrial organisms with a tendency towards a pelagic larval phase and therefore a greater capacity for dispersal (Macpherson 2003). These factors are thought to lead to greater individual ranges and greater cosmopolitanism in marine taxa (Rapoport 1994).

However, "that there are few recognised cosmopolitan species among echinoderms, molluscs, crustaceans and annelids must therefore mean either that the sea does indeed contain present and past barriers to gene flow or that sympatric speciation is common in marine environments" (Lessios et al. 2001). Indeed, some authors have highlighted the substantial similarities between the biogeography of terrestrial and marine taxa, especially when some of the more historical, vicariant processes affecting species biogeography are taken into account. (Heads 2005) argues that current dispersalist views of marine biogeography are often flawed and that most marine taxa show at least some degree of vicariant differentiation (Heads 2005). Specifically, he provides evidence to question the importance of dispersal ability in relation to the distribution and range size of marine organisms, a subject which is discussed in greater detail in later chapters of this thesis. Heads (2005) suggests that by using panbiogeographic analysis, biogeographically and geologically composite areas are evident in marine environments.

According to Longhurst the lack of progress stems from; 1) the lack of biogeographic method, 2) the tendency for marine biogeographers to ignore conflicting theories and ideas, 3) the cost of collecting samples from marine environments, and 4) the lack of isolation between natural regions. This has impeded the development of the science of marine biogeography relative to terrestrial biogeography (Longhurst 2007).

Longhurst also argues that previous proposals made for partitioning the ocean into geographic units based on the distribution of a single group of organisms or a single species, or even those based on a number of groups of organisms have been 'woefully inadequate' (Longhurst 2007). He suggests that in order to predict the distribution of characteristic pelagic biomes an alternative approach is needed.

In his seminal book 'The Ecological Biogeography of the Sea' Longhurst (2007) provides a new approach, based not on the distribution of individual groups, but on the ecological aspects of the marine environment which are known to be important for most marine animals. These data can be derived from remote sensing and the approach is described in greater detail in Chapter 1.

Alongside improvements in remote sensing parallel improvements in GIS technology, computerisation of natural history collections and statistical and analytical techniques are moving us towards a more synthesised approach to marine biogeography. These improvements are discussed in the following section and in later chapters of this thesis.

1.2 Recent developments in biogeographical methods

1.2.1 Computerisation of natural history collections and the development of distributed networks

Sparse knowledge of the number and distributions of species limits both our understanding of ecological and evolutionary processes and our ability to use this information for conservation planning (Graham et al. 2004). Natural History Collections (NHCs) are primarily public or university associated museums and herbaria which form a massive source of information, thought to be in the order of 2.5 billion specimens Worldwide, each with an associated "collecting event" describing the time and place where the specimen was sampled (Edwards et al. 2000, Graham et al. 2004). Specimen-based data held by NHCs provide the most comprehensive and reliable source of knowledge for most species (Ponder et al. 2001). The level of research utilising biological collections in museums/herbaria in an ecological or environmental context has risen substantially in the last 20 years (Pyke and Ehrlich 2010) for a variety of applied and theoretical applications (Graham et al. 2004). This is largely due to the increasing computerisation of NHC specimen data since the 1970s with up to 10% of all NHC specimens now recorded online (Graham et al. 2004). In addition, the development of "distributed networks" has allowed data from multiple institutions to be linked together (Graham et al. 2004). Each database continues to be owned and managed by the institution that holds the specimens, which serve as vouchers to verify the database records (Edwards et al. 2000). Therefore a number of national and international initiatives have evolved which aim to digitize all the distributional information gathered and held in NHCs (Hortal et al. 2008) and to make them available over a distributed network.

An example of this is the Global Biodiversity Information Facility (GBIF). GBIF is a multi-lateral initiative involving over 50 countries and 40 international organisations which has catalysed agreements on the standards and protocols required to make disparate datasets compatible and accessible (Edwards et al. 2000, Edwards 2004). Participating organisations have signed a Memorandum of Understanding stating that they will share biodiversity data and contribute to the development of increasingly effective mechanisms for making those data available via the internet (Matsuura 2009). However while the GBIF database as a whole is large, its coverage is patchy, with some areas and taxa well covered while others are absent (Yesson et al. 2007). In addition because biological collections were not designed originally for resolving ecological issues, they have inherent biases and limitations (Pyke and Ehrlich 2010). The relative positives and negatives of using NHC data are discussed further in Chapter 2. Despite these inherent issues NHC data have been successfully used to investigate a broad range of ecological questions including 1) determining potential species and community responses to past and future climate change (Guralnick 2007, Milanovich et al. 2010), 2) identifying priority areas for conservation (Minton and Perez 2010) and 3) predicting potential ranges of invasive species (Ba et al. 2010). Additionally NHC data have proved useful in two emerging fields of research; macroecology (Rodder et al. 2009, Hui et al. 2011) (see *section 1.2.5*) and species distribution modelling (e.g. Rodder and Lotters 2010, Vega et al. 2010).

1.2.2 Remotely sensed data

Remote sensing can be broadly described as “the measurement of reflected, emitted or back-scattered electromagnetic radiation from Earth’s surface using instruments stationed at a distance from the site of interest” (Roughgarden et al. 1991). Usually the instruments used for measurements are mounted on satellites or airborne platforms (Roughgarden et al. 1991). Traditional field ecological data do not transfer readily to regional or global extents (Kerr and Ostrovsky 2003) and such local data do not exist for many terrestrial and marine environments. The field of remote sensing has therefore provided the data sources and techniques necessary to study ecology on regional to global scales(Kerr and Ostrovsky 2003). Additionally, remote sensing provides longer term, more continuous data than has been achievable with ground-based measurements (Roughgarden et al. 1991).

Remote sensing has been broadly used in ecology for ~30 years now for a wide range of research including estimates of global net primary production, rates of evotranspiration, canopy structure, vegetation water content, frequency and extent of fire, land and ocean surface temperature and calculation of terrestrial vegetation indices (Roughgarden et al. 1991).

In the marine environment, satellites now routinely provide information representing conditions at the surface of the oceans at very short time intervals and specify these at relatively small spatial scales, allowing time sensitive mapping of significant relationships between physics and biology at a global scale (Longhurst et al. 1995, Longhurst 2007). A range of weather satellites now measures several features of the sea surface, such as surface roughness, elevation, topography, temperature and upwelling radiation and these global products are supported by modelled output representing a range of important ecological variables (Longhurst 2007). A thorough review of available satellite data for marine environments is provided by Longhurst (2007) and is discussed in greater detail in Chapter 2.

When combined with new GIS techniques and species distribution modelling, it is obvious that the new data derived from remote sensing, combined with these new tools of analysis, provides a new opportunity for studying marine ecological processes at much larger scales than was previously possible.

1.2.3 Macroecology

Macroecology stemmed from the search for broad patterns in nature, and a particular focus on global patterns of diversity (Clarke et al. 2007). It also has roots in geographical ecology. Macroecology is still viewed by some as a sub-discipline within biogeography (Fisher 2002) or ecology, whilst others insist that the fields are distinct (Brown 1999b, Blackburn and Gaston 2006).

Most ecological systems are extremely complex. They have many different kinds of organisms, each with its own unique attributes, such as anatomy, physiology, genetics, development and behaviour. All of them interact in complicated ways, and with their physical environment (Brown 1999b). Whilst research has allowed an understanding of the individual components of ecological systems, our knowledge of the organisational structure and dynamic behaviour of the systems is still rudimentary (Brown 1995, Brown 1999b, a). Macroecology seeks to address this.

Marquet (2002) summarised macroecology as the search for general and invariant principles underlying the diversity and variability of ecological systems. Brown (1995) described macroecology in a stricter sense as the *statistical* investigation of patterns of abundance, distribution, and diversity, and how these interact with environmental factors. Macroecology, therefore, includes aspects of ecology, biogeography, palaeontology and macroevolution (Brown 1995, Blackburn and Gaston 2006). Principal macroecological variables are local and regional abundances, regional distributions, body size, biomass and energy use by species (Blackburn and Gaston 2001). Some authors believe that the development of macroecology into a statistical, rather than a strictly geographical, approach is not desirable (Ruggiero and Hawkins 2006). They argue that in nature the evolutionary process is always entrenched within geographical space, and that evolution of form is not independent of time and location (Ruggiero and Hawkins 2006). However, Blackburn and Gaston (2006) argue that evolutionary and ecological studies progress without considering the geographical perspective and that many of the issues addressed by macroecology cannot be represented on a map (Blackburn and Gaston 2006). Indeed Brown (1999) argues that general statistical patterns provide clues to the operation of mechanistic processes which govern the structure and dynamics of complex ecological systems. It is this empirical approach of detecting general patterns that defines macroecology.

However, because of the scale on which it seeks to answer questions, macroecology has some inherent limitations. Large data sets, usually have

hundreds of individuals, species or “particles” of interest (Brown 1999b) and therefore focus on easily captured measurements, such as body size, abundance and parameters of the geographic range. It is usually limited to well studied groups. Perhaps the best studied of all macroecological patterns is the productivity species-richness relationship, which has now amassed a large body of literature (Waide et al. 1999, Willig et al. 2003, Storch et al. 2005).
Many of these patterns are mechanistically interlinked and do not occur in isolation. For example species richness generally declines with increasing latitude (the latitudinal diversity gradient) and is associated with a general increase in species range at higher latitudes (Rapoport’s rule) (Brown 1999b).

Clearly there has been considerable progress in showing that macroecological patterns hold across different taxonomic and functional groups, habitats, environments and geographic regions. However, there has been much less progress in identifying the mechanisms which underlie those patterns (Brown 1999b), which must be the true aim of macroecological research.

Recently macroecology has benefited from improvements in other fields such as remote sensing, increased sampling, powerful databases, and innovative software and analysis methods (discussed above) (Clarke et al. 2007). These are allowing the factors underlying macroecological patterns to be determined. For example, in a recent synthesis of marine biodiversity patterns Tittensor et al. (2010) detected a number of global patterns; coastal taxa showed a distinct peak in diversity in the Western Pacific and displayed latitudinal gradients along continental margins whilst oceanic species peaked in diversity at mid-latitudes. The only environmental factor to be a significant predictor of species diversity was found to be sea-surface temperature. Whilst general patterns of diversity exist, it was perhaps more difficult to identify underlying mechanisms (Tittensor et al. 2010).

It should be noted that while most animal phyla are marine and benthic, the majority of species that have been described are terrestrial (mainly insects). In particular the deep sea is extremely under represented by current sampling, relative to shallow water and shelf habitats. Pelagic areas of the World’s oceans have been extremely poorly studied relative to benthic environments (Webb et al. 2010). Consequentially, discussions of biogeography and biological diversity have tended to centre on terrestrial realm (Clarke et al. 2007). However some marine areas and taxa have been sufficiently sampled to investigate macroecological patterns. Examples include a Southern Ocean gastropod and bivalve molluscs (Clarke et al. 2007), marine mammals in the Pacific (Rosales-Nanduca et al. 2011), the seaweed *Halimeda* (Verbruggen et al. 2009) and the European soft sediment benthos (Webb et al. 2009).

1.2.4 Phylogeography and population genetics

Phylogeography is a field of study which investigates the historical, phylogenetic components of the spatial distribution of gene lineages (Avise 2004). It relates to the principles and processes which govern the geographical distributions of the lineages, particularly those within and between closely related species. As phylogeography tends to refer mostly to studies of conspecific organisms and closely related species, it could be considered a branch of population genetics (Avise 2009). However, phylogeography departs from classical population genetics by its particular focus on genealogy (not just allele frequencies) as revealed by historical genetic records in particular segments of DNA (Avise 2009). It therefore builds conceptual and empirical bridges between the formerly separate disciplines of population genetics and phylogenetic biology (Avise 2004). This young and fast growing field grew out of the new techniques of the 1980s that made it possible to determine DNA sequence variation from individuals across a species range, and hence to reconstruct gene genealogies (Emerson and Hewitt 2005). Phylogeography is essentially a subdivision of biogeography which serves to place traditional ecogeographic perspectives (Gaston et al. 2008) that stress the role of contemporary ecological pressures in shaping spatial distributions of organismal traits, into a broader temporal context (Avise 2009).

Population genetics investigates the changes in population allele frequencies resulting from mutation, genetic drift, gene flow, natural selection and sexual selection (Avise 2009) and can be used to study geographic differences between populations. Populations of virtually all species whether social or otherwise, exhibit at least some degree of genetic differentiation between populations (Ehrlich and Raven 1969). The scale and pattern of population structure can be seen as a balance between two opposing processes: genetic differences arising both within and between populations through random genetic drift and natural selection with gene flow acting to oppose such local differentiation through random mixing of alleles from differing local population (Slatkin 1987). The degree of population differentiation can be determined using F_{st} and related measures, for any type of polymorphic genetic marker (Frankham et al. 2002). Because genetic differences provide the raw material for natural selection, population genetic differentiation can also indicate the potential for local adaptation or speciation in the future (Bohonak 1999).

The genetics of marine populations is a subject that has made little progress compared with the genetics of terrestrial organisms (Feral 2002). However the application of increasingly sophisticated and accessible tools for generating and analysing molecular genetic information has resulted in a recent burst of empirical genetic data for marine organisms (Mathews 2006).

1.3 Biogeography of the deep sea soft sediment fauna

1.3.1 A brief overview of deep-sea soft sediment zoogeography and biogeography

Compared to shallow water, the distribution of higher taxa in the deep sea is relatively homogenous; whilst species may be restricted to one geographic area genera and higher taxonomic ranks tend to be cosmopolitan (Thistle 2003). However, geographical patterns in the distribution of species in the deep sea, especially those which inhabit the deep-sea floor, are poorly known (Thistle 2003). Our knowledge of large-scale biogeographic patterns in the deep sea is still based on only a few invertebrate taxa and limited sampling efforts relative to the vastness of the habitat (Rex et al. 2000, Thistle 2003). In addition, the lack of taxonomists to identify existing species and describe new ones further hinders our understanding (Thistle 2003). Research has shown that whilst some deep-sea species are widespread, others have much more limited distributions. This is one of the most recognisable patterns in nature and although the causes of these patterns are unknown, ecological differences among basins, dispersal limitations and history appear to be likely factors in the deep sea (Thistle 2003).

When the bottom fauna of the deep sea was first discovered its species were thought to have a World-wide distribution (Ortman, 1896 in Vinogradova 1997). This supposition seemed to be confirmed after analysis of specimens collected on the voyage of the *HMS Challenger* (Vinogradova 1997). Conclusions drawn from this cruise suggested that the deep sea was one single province inhabited by cosmopolitan species (Gage and Tyler 1991).

However, this was later refuted by Ekman (1953), who acknowledged that whilst genera are usually cosmopolitan, species usually belonged to their "special oceans" (Ekman 1953). However, Ekman (1953) also argued that range size increased with depth so that abyssal species were more widely distributed than bathyal or continental slope species. He went on to divide the abyss into four zoogeographic zones; the Atlantic, the Indo-Pacific, the Arctic and the Antarctic.

In his work on the deep-sea Elasipodida, an order of deep-sea holothurians discussed later in this chapter, Hansen (1975) discovered that relatively few species were cosmopolitan, but still concluded that wide geographic distributions were the norm for most deep-sea taxa (Hansen 1975). Despite this, by the 1970s, the theory of the cosmopolitan distribution of the deep-sea bottom fauna had been largely rejected (Vinogradova 1997). However, this does not mean that species with cosmopolitan distributions do not occur in the deep sea. Rather it indicates that cosmopolitanism is not the *rule* (Vinogradova 1997). In contrast to the findings of Ekman, Vinogradova found an increasing isolation of the deep-sea fauna with increasing depth of species occurrence. In the Antarctic region of the Atlantic at depths of <2000m about 70% of species have been found elsewhere. At depths of <3000m this number decreases to about 6% of species, while at 4000m there was no overlap (Vinogradova 1997). Corresponding figures for the Pacific are 60, 10 and 0%. Vinogradova (1997) also highlighted the important relationship between the vertical and geographical range of taxa. Vinogradova splits the deep-sea fauna into two distinct groups. Eurybathic deep-sea taxa (cnidarians, Echinoidea, Polychaeta, Decapoda, Ophiuroidea) mainly occur at bathyal depths, but some representatives extend down into abyssal depths. Species of eurybathic groups tend to have broad geographic distributions. Stenobathic deep-sea taxa (Isopoda, Echiura, Porifera, Asteroidea & Elasipodida) are not found at shallower depths, and tend to have more limited geographic distributions and display higher rates of endemism (Vinogradova 1997). Whilst no taxonomic group is likely to be composed of entirely eurybathic or stenobathic species, Vinogradova suggests a distinction between those comprised of less than 50% of truly abyssal species, and those comprised of more than 50% of truly abyssal species. She argues that only the latter group can truly be used to study patterns of abyssal zoogeography. These two categories reflect the two modes of colonization of the deep sea. The former group probably represent species that have recently colonized the deep sea and are closely related to shallow water taxon. The latter group are ancient deep-sea groups that have adapted to life at great depth and are clearly delimited from the slope fauna (Vinogradova 1997).

Dispersal has important consequences on both ecological and evolutionary time frames (Young et al. 1997a). In ecological time, dispersal potential can influence biological interactions such as competition and the ability of a species to persist in a variable environment (Pechenik 1999). Over evolutionary time, dispersal allows geographic range expansion, increases species longevity by reducing extinction rates in variable environments, reduces the effects of inbreeding depression and maintains genetic continuity between metapopulations, thereby reducing speciation rates (Pechenik 1999). Generally, it is assumed that species with planktotrophic development will have broader distributions than species with lecithotrophic development. This is because planktotrophic larvae can actively feed and therefore remain in the larval stage longer, allowing greater dispersal distances in currents. However, such assumptions may not be applicable to deep-sea organisms. (Young et al. 1997a) studied the effects of different kinds of larval dispersal on the biogeographies of echinoderms and ascidians in the deep Atlantic. They found that both the bathymetric and geographic ranges were greater for lecithotrophic than planktotrophic species in asteroids and echinoids. In comparing two groups with lecithotrophic larvae, they found that ascidians, which probably have a shorter larval period and therefore less dispersal potential, were present in fewer geographic regions than elasipodid holothurians, which are likely to have longer larval periods (Young et al. 1997a). The authors concluded that lecithotrophic development does not constrain dispersal in the deep sea, probably because species with planktotrophic development may be confined to regions of high detrital input (Young et al. 1997a). Additionally, consistently low temperatures in the deep sea may reduce species metabolism and slow their development, thereby increasing the dispersal abilities of lecithotrophic organisms. Consequently, lecithotrophy is the single overriding element in the life-history strategy of the deep-sea biota (Gage and Tyler 1991).

Species which enter the water column for part of their life history may be expected to be less constrained by topographic barriers surrounding ocean basins. This may be one of the reasons the biogeographical distributions of elasipodid species apparently show little correspondence to topography (Hansen 1975) (although also see Section 1.4 for swimming capability in Elasipodida). Dispersal mechanisms in deep-sea organisms are clearly important in shaping their biogeography.

Historical geological and oceanographic events are important in shaping the geographic distributions of abyssal species by controlling the nature and timing of dispersal (Smith et al. 2006). Throughout palaeoceanographic history the deep sea is known to have fluctuated widely in temperature, oxygen, nutrient input and circulation (Cronin and Raymo 1997, Rogers 2000, McClain and Hardy 2011). These fluctuations between warm, salinity structured, poorly ventilated oceans with high sea levels (S-state) and colder, more oxygenated oceans with lower sea-levels (P-state) have occurred throughout geological history and are thought to be linked to cycles of atmospheric temperature fluctuation (Rogers 2000). As a result it is thought that the deep-sea fauna is relatively young, owing to catastrophic global anoxic events as recently as 65.5mya during the Late Palaeocene/Early Eocene (Rogers 2000, Stuart et al. 2003). Consequently, it is thought that large-scale gradients of diversity developed during the Cenozoic (Thomas and Gooday 1996). Therefore much of the deep sea may be relatively young compared to terrestrial and shallow water habitats, and may have changed substantially during the last 50-million-year "ice-house" era (Stuart et al. 2003). Therefore, processes that occur over geological time frames are likely to have an effect on the present day geographic distributions of abyssal organisms.

Hansen (1975) found that the distributions of a small number of elasipodids could be related to ocean currents. Whilst this was more common in bathyal species, some abyssal species also appear to be affected. Hansen (1975) also noted the existence of some degree of similarity between the faunas of the North Atlantic and the South Western Pacific. A similar pattern can be seen in deep-sea copepods, suggesting that the faunal similarities might be due to transport of individuals by deep ocean currents (Hansen 1975). The currents implicated flow from the North Atlantic southward, giving off a water mass that flows eastward south of the Cape of Good Hope and across the southern part of the Indian Ocean to the south-western Pacific (Hansen 1975). Three of the elasipodid species common to the two regions (*Benthodytes typica*, *Psychropotes longicauda* and *Peniagone diaphana*) have been sampled as juveniles in pelagic nets (Hansen 1975). This highlights the importance of deep dispersal on the biogeography of abyssal organisms.

Hansen (1975) concluded that the known distribution of the Elasipodida can only to a very small extent be correlated with parameters of the physical environment. However, he did concede that one factor seemed to exert a great influence on the geographic distribution of deep-sea species: the variations in food supply to the bottom. Hansen suggested that in certain geographic regions the abundant supply of nutrients to the benthos could form the basis for a rich fauna of Elpidiidae, a family of holothurians thought to rely heavily on the flux of very fresh material to the deep-sea floor. Hansen also suggested that where little flux of organic matter occurs geographic isolation of high productivity areas surrounded by these 'barren' regions could occur (Hansen 1975). In the following section we review the environmental conditions at the deep-sea floor before considering which may play a role in shaping the distributions of deep-sea organisms.

1.3.2 The deep-sea environment

The deep sea is defined as beginning at the shelf break, usually at around 200m depth (Thistle 2003). Alternatively, in hydrographical terms, the deep sea maybe considered to be the domain below the permanent thermocline. This can be described as the transition layer in the water column in which temperature drops the most quickly with increasing depth until temperatures below 4°C are reached and the downward temperature gradients become small (Gage and Tyler 1991). The deep sea covers more than 65% of the Earth's surface (Sverdrup et al. 1942) with abyssal depths of 3000-6000m accounting for ~ 54% of the Earth's solid surface (Gage and Tyler 1991). In geographical extent the abyssal realm therefore represents the largest single biotic unit in the World (Menzies et al. 1973). Besides its enormous extent, the deep sea has some characteristic physical conditions which make it a unique environment which are briefly reviewed in the following paragraphs.

1.3.2.1 Light

Light intensity decreases exponentially with depth increasing owing to absorbance and scattering. The depth to which light penetrates depends upon the clarity of water. However, no solar light penetrates deeper than 1000m. Bioluminescence is the only form of light present, with the exception of vent and seep environments. The absence of solar light obviously results in a total absence of primary production in the deep sea and is perhaps one of the starkest differences between the abyss and more familiar environments (Thistle 2003).

1.3.2.2 Temperature and salinity

In the deep sea, unlike coastal waters, the physical characteristics of the environment lie generally within a very narrow range. The temperature of the waters below 2000m typically occur within the range of -1° to 4°C, with the exception of the Mediterranean Sea, the Red Sea, some other accessory seas and hydrothermal vent localities (Menzies et al. 1973, Gage and Tyler 1991). Salinity is also relatively constant with values falling between 34.8‰ and 34.65‰ (Sverdrup et al. 1942). Seasonal and other variations in temperature and salinity are negligible or absent (Menzies et al. 1973).

1.3.2.3 Pressure

Hydrostatic pressure is the most predictable physical variable in the deep sea (Gage and Tyler 1991). At any point in the ocean the pressure is a function of water density and depth. Generally, pressure increases one bar (or one atmosphere) for every 10m of depth, so that the pressure at 10,000m is equal to 1000 bars (Menzies et al. 1973).

1.3.2.4 Oxygen concentration

Oxygen enters the ocean at the surface by exchange with the atmosphere and additionally in the euphotic zone as a by-product of photosynthesis by marine plants (Thistle 2003). The descent of surface waters in Polar Regions carries the dissolved oxygen to the deep-sea floor. Oxygen concentrations are near saturation in abyssal waters (Gage and Tyler 1991). The variation in space and time of oxygen concentration on the scale of an individual organism is small in absolute terms and does not constitute an environmental challenge for organisms inhabiting the seafloor (Thistle 2003). There are two exceptions to the oxygen saturation of water overlying the deep-sea floor. The first occurs where the oxygen minimum layer (where decomposition and animal respiration reduce the oxygen concentration in mid-water at ~300-1000m depth) meets the upper continental slope. As a result, the bottom fauna can be reduced or eliminated (Thistle 2003), as observed in the Arabian Sea (Murty et al. 2009). The second can occur in enclosed basins (Gage and Tyler 1991), when oxygen deficient bottom water does not freely exchange with the surrounding oxygen rich water, because of a topographical barrier. Organic matter may settle in the basin and will be decomposed by microbes. If oxygen is consumed at a faster rate than it is supplied then bottom water oxygen concentrations may become oxygen-deficient relative to outside the basin. Low oxygen concentrations can also be found in areas far from the source of deep water masses, such as deep water in the North Pacific (Gage and Tyler 1991).

1.3.2.5 Topography

The general topography of the ocean floor is generated as a consequence of plate tectonics (Kaiser et al. 2005) and the sedimentation of organic and inorganic particles (Gage and Tyler 1991). The shelf break occurs at edge of the continental shelf, where the gradient of the sea floor suddenly increases, forming the continental slope. The gradient can vary greatly, and in certain areas, such as off the south west coast of the British Isles, it can be very steep (Kaiser et al. 2005) extending from 200m to 4000m depth. Typically however, the gradient is much less. The base of the continental shelf occurs between 2000 and 5000m and the relatively steep slope gives way to a gentler continental rise. Deeper than 4000m the seabed levels off resulting in the wide and relatively flat expanse of the abyssal plain. In some areas inactive ocean floor volcanoes (seamounts), which can rise several kilometres above the seabed, punctuate the abyssal plains. The ocean floor is also segmented by mid-ocean ridges, where new ocean crust is formed, typically around 2500m below sea level. At active margins, especially subduction zones, where one plate is subducted below another, trenches are formed reaching depths of between 6,000 and 11,000m, thereby representing the deepest points of the World’s oceans (Gage and Tyler 1991).

1.3.2.6 Sediment

Whilst the sediment across the vast majority of the ocean floor is soft, fine mud (Kaiser et al. 2005), hard substrates do occur, including exposed hard rock, phosphate deposits, manganese nodules and sulphide deposits around hydrothermal vents (Gage and Tyler 1991). Deep-sea sediments have two primary origins. Firstly terrigenous particles, derived from the weathering of rock on land, are transported to the sea by wind and rivers. Secondly, particles may be derived from planktonic organisms in the overlying water column (Thistle 2003). The vast areas covered by soft sediment in the deep sea usually fall into one of two categories, clay or biogenic oozes depending on the productivity of the overlying water column (Gage and Tyler 1991). Biological oozes are sediments which contain more than 30% by volume of plankton derived shells from organisms such as diatoms, radiolarians, foraminifers and coccolithophores (Gage and Tyler 1991). These can be further subdivided depending upon the organisms whose skeletons make up the ooze: siliceous oozes (primarily diatom skeletons), radiolarian oozes, foraminiferan oozes and pteropod oozes (Kaiser et al. 2005). These tend to vary geographically depending on the dominant organisms in the euphotic zone and the biogeochemical processes that break down the particles as they descend through the water column. Under extremely oligotrophic oceanic gyres red clay predominates, which contains volcanic ash and wind-transported volcanic dust (Kaiser et al. 2005).

The thickness of the sediment layer on the deep-sea floor varies greatly. It may exist only as a very thin layer on relatively new crust (near ocean ridges) or it may accumulate to thousands of metres of sediment where the crust is ancient, such as along passive margins (Gage and Tyler 1991). The continuing addition of organic matter to these sediments will be discussed in sections below.

1.3.2.7 Bottom currents

In the deep sea there are three main types of current. Firstly, so-called tidal currents can form regular ripple patterns on the deep-sea floor, even down to the base of the continental slope (Kaiser et al. 2005). The tides appear to have their major effect in a semi diurnal period, but they may also play a part in longer period cycles in bottom current energy in the deep sea (Gage and Tyler 1991). Secondly, much of the deep ocean floor is bathed in water masses which are formed in both the Antarctic and in the Greenland-Norwegian Sea of the Arctic Ocean. In these localities surface water cools and sinks forming Antarctic

Bottom Water (AABW) in the Southern Hemisphere and North Atlantic Deep Water (NADW) in the Northern Hemisphere (Gage and Tyler 1991). Areas along the route of transport of these northward and southward currents can experience persistent current activity (Gage and Tyler 1991). These currents also seem to be subject to periodic reversal ultimately resulting in 'benthic storms' in some localities (Gross et al. 1988). Thirdly, the Coriolis Current arises from the rotation of the earth and the relative movement of water in relation to the seabed (Kaiser et al. 2005). The friction between the seabed and the water moving over it is also responsible for the formation of the benthic boundary layer (BBL).

1.3.2.8 The benthic boundary layer (BBL)

The BBL constitutes those portions of the sediment and water column that are affected directly in the distribution of their properties and processes by the presence of the sediment- water interface (Boudreau and BB 2001). Friction between the seabed and the water column above it generates a vertically homogenous (but horizontally inhomogeneous) layer of varying thickness (usually tens of metres) capped by a region of strong density gradient (Gage and Tyler 1991). The upper limit of the BBL is defined by the height of frictional influence, which in the deep sea is normally equivalent to the Ekman scale (Gage and Tyler 1991). This layering is so stable that even quite large perturbations (e.g. caused by tides) may not disrupt it and it is critical to the ecology of benthic organisms that inhabit the sea-floor (Angel 2003). According to Smith and Hinga (1983), the BBL can be defined biologically as 'the sediment community and assemblage of organisms in the overlying water column associated with the bottom, within 100m of the seabed' (Smith and Hinga 1983). The benthic boundary layer community includes the bacterioplankton and zooplankton residing in the directly overlying water column, the near bottom or benthopelagic fauna and the community more closely associated with the sediment and hard substrata, collectively known as the benthos (Gage 2003).

1.3.2.9 Food Availability

As early as 1888 it was noted that “deep-sea organisms are nourished by a ‘rain’ of organic detritus from overlying surface water” (Agassiz 1888 in(Turner 2002). For many years it was assumed that fast-sinking particles such as the faecal pellets of zooplankton were the primary components of such a flux (Turner 2002). Sinking rates of fecal pellets can vary anywhere between less than 10m/day up to 2700m/day (Turner 2002). However, more recent evidence has shown that most fecal pellets are reprocessed in the water column by microbial decomposition or coprophagy (the consumption of faeces). Although they may constitute a major proportion of the flux in some regions it is now known that most of the vertical flux is due to marine snow or sedimenting phytoplankton blooms (Turner 2002). The term ‘marine snow’ is used to describe organic aggregates greater than 500µm in size (Alldredge and Silver 1988) which originate from a number of different sources including; abandoned larvacean houses, diatom and dinoflagellates flocs, fecal aggregates and aggregates of miscellaneous detritus. Other planktonic organisms and particles such as phytoplankton and bacteria can become attached to aggregates and enrich them. Until recently phytoplankton were not thought to comprise a major part of the flux of organic matter due to laboratory estimates yielding very low sinking rates. However, studies in the field have revealed that some ungrazed phytoplankton blooms can sink at surprisingly fast rates. In fact, such is the sinking efficiency of some phytoplankton blooms it is now known that they can descend directly into deep water.

1.3.3 POC flux to the deep-sea floor; Spatial and temporal variations in quantity and quality

Deuser and Ross (1980) were the first to report the flux of organic particles to the deep ocean, with data from sediment trap records showing that the flux at 3200m in the Sargasso Sea appeared closely related to the annual cycle of phytoplankton primary productivity. Using photo transects and time lapse photography Billett et al. (1983)discovered the presence of a patchy detrital layer on the sea floor of the Porcupine Sea bight in the North East Atlantic between 1370 and 4100m depth. This detrital layer was present between April and July and appeared to be absent at other times of year (Billett et al. 1983). The composition of the phytodetritus was investigated using a multiple corer to take sediment samples. Among the amorphous organic matrix intact cells were discovered that closely resembled the phytoplankton assemblages of early spring and summer of the overlying water column (Billett et al. 1983). These assemblages were dominated by diatoms in the spring and coccolithophorids in the summer months. Billett et al. (1983) estimated sinking rates of 100 to 150m/day, which was much faster than previous, laboratory deduced, sinking rates for phytoplankton blooms. Whilst the incorporation of phytoplankton cells into fecal pellets could have partially explained such rapid descent in the summer months, it would not explain these sinking rates for the spring months when zooplankton abundance was still low. Therefore, (Lampitt 1985) extended this work to show that such phytoplankton sedimentation was a regular occurrence during the summer months in the NE Atlantic. (Asper et al. 1992) investigated the effect of variations in upper ocean primary productivity on particle fluxes measured at several depths from the upper to the deep ocean in the Atlantic. They found that the productivity signal can be transferred rapidly to the deep sea via settling particles, yielding close temporal coupling between the surface and deep oceans.

Since the findings of 1980s in the NE Atlantic many areas of the World's oceans have been shown to have associations between surface productivity patterns and vertical flux of organic matter to the deep-sea floor. Areas where data has shown this phenomenon to occur, using either sediment trap data, seafloor photography or analysis of cores (or a combination of the three) include: the North Pacific (Scharek et al. 1999), the North East Pacific (Baldwin et al. 1998), the central equatorial Pacific (Smith 1996), the South West Pacific (Gupta and Kawahata 2003), the Panama Basin (Honjo 1982), the Arabian Sea (Haake et al. 1993), the Antarctic (DiTullio et al. 2000) and the NE Mediterranean (Danovaro et al. 2000).

Modern estimates suggest that typically, around 2% of the net primary production from the euphotic zone reaches the deep sea (Smith et al. 2008) with flux rates now confirmed at around 100-150m/day (Turner 2002). However, clear geographic and temporal patterns in the strength of seasonality of primary production influence the proportion of primary production that leaves the upper water column (Gooday 2002)(Gooday 2002). The transport of biogenic elements from surface waters to the deep-sea (pelagic and benthic) is known as the “biological pump”. A number of factors appear important in determining how great a proportion of the primary production in the euphotic zone is exported to depth, how quickly it is exported, and how labile that material will be when it reached the deep sea floor.

1.3.3.1 Magnitude of net primary production in the euphotic zone

The magnitude of NPP in surface waters was originally thought to be the main determinant of particle export to depth (Boyd and Trull 2007). Early algorithms for the estimation of the flux of POC to depth were simple functions of the amount of production of organic carbon at the surface or the amount exported from the base of the euphotic zone, scaled to depth. The coefficients of the equations were derived from sediment trap flux data from the eastern Pacific and north-western Atlantic (Suess 1980) and the north-east Pacific (Pace et al. 1987). However, the poor performance of these simple models in some situations suggests that the magnitude of primary production may not necessarily be the key determinant of downward POC flux (Boyd and Newton 1999).

1.3.3.2 Surface water temperature

In a modelling study by Laws et al. (2000) it was found that there was no correlation between the magnitude of primary production in surface waters and ratios of new production to total production (f ratio) or the ratio of export to production to total primary production (Laws et al. 2000). On the other hand temperature alone was accountable for 86% of the variance in e and f ratios (Laws et al. 2000).

The suggested mechanism is through differential respiration of assimilated carbon by bacteria at different temperatures (Rivkin and Legendre 2001). Lower water temperature limits the activity of microbial heterotrophic decomposers more than it limits the activity of autotrophic producers so that a greater fraction of production is not demineralised and is available for export. This process may also affect the quality of food arriving in the deep sea as the lack of surface recycling in colder surface waters may result in the phytodetritus being more labile (Lutz et al. 2007).

1.3.3.3 Community composition of the primary producers in the euphotic zone

Boyd and Newton (1999) hypothesised that the proportion of large cells in the phytoplankton assemblage might be a more influential factor regulating downward POC flux than the magnitude of primary production at the surface. They corroborate this with epipelagic observations and POC flux data from deep-moored sediment traps from six different ocean regions, used in conjunction with two standard versions of a food web/vertical flux model and published empirical depth/POC flux algorithms. The first model allows the direct sinking of large ungrazed algae from surface waters and the second model does not. The two sets of predictions were then compared to deep ocean flux measurements. Their findings indicate that deep ocean POC flux estimates based on primary production algorithms compared poorly with the observed POC fluxes. The version of the food web/ vertical-flux model permitting the direct sinking of ungrazed algae provided the most reliable predictor of POC flux for five of the six sites (Boyd and Newton 1999).

A number of models now incorporate some measure of phytoplankton community structure. Some divide production between functional size classes, which although not an entirely satisfactory proxy for community composition, ecological and biogeochemical processes are related to cell size (Brewin et al. 2011), especially the sinking rate and export of those communities to depth (Boyd and Newton 1999, Laws et al. 2000). Others identify the fundamental groups, such as the divisions seen between diatoms, small phytoplankton and diazotrophs in Moore et al.'s intermediate complexity model (Moore et al.

2002). Moore et al. found that generally, in areas with large diatom blooms the sinking carbon flux is high, and where small phytoplankton dominate the assemblage, sinking export is smaller (Moore et al. 2002). In addition efforts are being made to use remote sensing technology to identify dominant phytoplankton size classes, which will allow more complex models to be developed and applied globally (Brewin et al. 2011).

The community composition of the surface plankton has the potential to affect the quantity and quality of POC flux in a number of ways. For example sinking and aggregation rates, which may have consequences for the timing of the flux in relation to production, and the transformation of the phytodetritus through the water column, and subsequently how labile the detritus is when it reaches the deep-sea floor.

1.3.3.4 Variability in the efficiency of trophic transfer (seasonality)

The seasonality of production in the global oceans has long been a cornerstone of biological oceanography (Berger and Wefer 1990). In low latitudes, where nutrients are limiting to algal growth seasonal changes in wind fields are the primary factor forcing seasonality in primary production (Berger and Wefer 1990). However because the supply of solar irradiation and nutrients are (relative to high latitudes) fairly constant, environments are stable throughout the year and there is equilibrium between the activities of autotrophs and heterotrophs. Trophic transfer is therefore efficient and recycling is the dominant process in euphotic waters (Lutz et al. 2007). In mid-latitudes the azimuth of the sun and wind action control seasonality, and in high latitudes the forcing is primarily driven by the angle of the sun (Berger and Wefer 1990). Mid and high latitudes therefore generally experience more time varying physical conditions, solar radiation and nutrients resulting in temporal disequilibrium with new production dominating and autotrophic growth outpacing consumers (Lutz et al. 2007). In variable (seasonal) environments particulate matter is therefore more likely to escape consumption in the euphotic zone, resulting in a greater proportion of NPP being fluxed to depth in a more highly labile condition (Lutz et al. 2007). The seasonality of the arrival of phytodetritus in the deep sea was first noted in the Sargasso Sea (Deuser and Ross 1980, Deuser et al. 1981) and the north-east Atlantic and Porcupine Sea bight (Billett et al. 1983). These seasonal changes in flux are now known to be a feature of the continental margin and Open Ocean, eutrophic and oligotrophic settings (Gooday 2002).

Various indices have been developed to describe the seasonality of production and/or export. These include the Seasonality Index which is defined as the number of months required to accumulate half of the total annual primary production (Berger and Wefer 1990). The Flux Stability Index (FSI) was developed from a global synthesis of flux to depth measurements and is defined as the minimum time taken for 50% of the annual flux to be collected (Lampitt and Antia 1997a). Finally the Seasonal Variation Index (SVI) of Lutz et al. 1997 is a dimensionless descriptor of variability which is statistically similar to both the Seasonality Index and FSI (Lutz et al. 2007). The SVI is defined as the coefficient of variation, the standard deviation normalized to the average of either NPP or flux climatologies, usually calculated for a specific region (Lutz et al. 2007).

Lutz et al. (2007) were among the first to investigate the importance of seasonality in the efficiency of the biological pump. Using data from global sediment trap records and remotely sensed estimates of NPP and SST they developed mathematical techniques to compare the temporally discordant time series using NPP and POC flux climatologies. Results of seasonal production to flux analyses suggest that during bloom periods, the sinking fraction of NPP is typically half that of other seasons, possibly due to seasonally varying biodegradability or multi-season retention of POC. The relationship between variability in NPP and flux variability reverses with latitude, perhaps due to the highly seasonal, high magnitude NPP signal at higher latitudes (Lutz et al. 2007). More importantly Lutz et al. (2002, 2007) develop new algorithms for describing POC flux to depth describing labile and refractory flux components as a function of remotely sensed variables: NPP, NPP variability and SST, which result in more accurate predictions than previous flux to depth predictors (Lutz et al. 2007). Seasonal changes are thought to be particularly important when considering the link between POC flux and the benthos (Lampitt and Antia 1997a).

1.3.3.5 Algal aggregation

Billett et al. (1983) hypothesised that incorporation of phytoplankton cells in gelatinous matrices enhanced sedimentation rates. It has also been suggested that formation of mucous diatom flocs would accelerate sinking rates of diatoms away from surface strata with high populations of zooplankton during, or immediately prior to seasons of maximum grazing pressure (Smetacek 1985).

Diatoms in particular are capable of producing large quantities of exopolymers that have high efficiencies of coagulation (Alldredge and McGillivary 1991). These exopolymers generally enhance sinking velocities by promoting larger faster sinking aggregates, despite adding low density mass to aggregates (De La Rocha and Passow 2007).

Algal aggregation generally occurs when single cell concentrations reach critical concentration and are therefore more likely to form in regions with very high levels of productivity (Jackson 1990). Coagulation is less likely to occur or will occur less rapidly in regions where zooplankton and bacteria consume substantial parts of algal production (Jackson 1990).

1.3.3.6 Zooplankton grazing

We have already mentioned the destruction of surface primary producers by bacteria, the other important component that contributes to this destruction is zooplankton (Boyd and Trull 2007). Recently more effort has been placed on studying bacteria than the zooplankton in part because of the novelty of research into marine bacteria, but also because of the associated difficulties of conducting shipboard dietary experiments on vertically migrating zooplankton (Boyd and Trull 2007). Zooplankton grazing can affect aggregation, disaggregation, mineral scavenging and therefore the size, composition and character of sinking particles (De La Rocha and Passow 2007)

1.3.3.7 Mineral ballasting

The importance of ballasting minerals (silicate and carbonate biominerals and dust) has been recognised for determining the sinking rate of particles (Armstrong et al. 2002). It has been shown that the flux of organic matter at depths >1800m is in direct quantitative proportion to the fluxes of ballast minerals at these depths, but that there is no such proportionality at shallower depths (Armstrong et al. 2002). However this is a controversial topic and it has been suggested that this correlation is not linked in any way to causation. De la Rocha and Passow (2007) provide a more detailed investigation of the argument.

1.3.3.8 Degradation at depth

POC, calcium carbonate concentrations, number of aggregates per litre, average size of aggregates, total volume of aggregates per litre and organic content all decline rapidly below the euphotic zone(Gage 2003, De La Rocha and Passow 2007). However, whilst these degradation processes (driven by zooplankton and bacteria), are strongly active in the upper few hundred metres, most of the modification of flux to depth occurs in the upper 1km of the water column (Lutz et al. 2002). Fluxes to depths between 0.5km and 1km are highly correlated with fluxes to depths greater than 1km (Lutz et al. 2002). Also, it has been suggested that the composition of hydrolysable amino acids of sinking organic matter do not appear to change much between the euphotic zone and the seafloor, perhaps due to protection from biominerals (Ingalls et al. 2003).

1.3.3.9 Sources of error in measuring POC flux

These and other subsequent studies have also provided evidence for the existence of inter-annual variations in the magnitude of fluxes and in their timings. We now know that organic matter flux can vary seasonally (Billett et al. 1983), inter-annually (Baldwin et al. 1998) and over longer time scales (Smith et al. 2006). However, not all data find strong support for the existence of benthic-pelagic coupling. Lampitt and Antia (1997b)reviewed spatial and temporal data from sediment trap records from different oceans in order to deduce any regional characteristics in the seasonality of sediment flux. They investigated the variability of flux over annual cycles and expressed this as the Flux Stability Index (FSI). They then examined the relationship between the FSI and the vertical flux of material. Somewhat surprisingly, no significant relationship was found between FSI values and fluxes of dry mass, organic carbon, inorganic carbon or opaline silica (Lampitt and Antia 1997). Studies have found many sub-euphotic zone aggregates were derived from sources not tied to phytoplankton production cycles (Silver et al. 1998) and wide discrepancies between temporal trends in the primary production signal and those of the downward particulate organic carbon (POC) flux in the N central Pacific have been noted (Karl et al. 1996). Indeed transformation processes and advection have the potential to modify the transmission of surface signals to the deep sea so that benthic pelagic coupling may not always be tight (Asper et al. 1992). A study examining the macro and microscopic composition of phytodetritus on the abyssal sea floor at Station M in the NE Pacific found that sediment flux data generally did not reflect differences in composition between aggregates collected in August and September (Beaulieu and Smith 1998). They attributed this to the differing sampling resolution between sediment traps (10 days) and sea floor aggregates (probably containing much older material) and the inability of sediment traps to catch very large particles (Beaulieu and Smith 1998).

1.3.4 POC flux to the deep-sea floor; effects on the benthos

Despite the uncertainties in the processes which regulate the quantity, quality and timing of organic matter flux, geographical and temporal variations in the deposition of organic matter have been linked to a number of biological processes and patterns. These include the seasonal reproductive cycles in some deep-sea megabenthos (Tyler et al. 1993, Campos-Creasey et al. 1994, Ramirez-Llodra et al. 2002, Wigham et al. 2003b, Benitez-Villalobos et al. 2007, Galley et al. 2008), latitudinal distributions and abundances of megabenthos (Thurston et al. 1994, Thurston et al. 1998) and macrobenthos (Rex et al. 1993, Rex et al. 2000), geographic patterns in macrofaunal diversity (Levin and Gage 1998) and standing stock (Johnson et al. 2007) temporal patterns in macrofaunal community structure (Galeron et al. 2001), spatial and temporal patterns in benthic community structure (Lauerman and Kauffman 1998, Smith et al. 2001, Ruhl and Smith 2004, Hughes et al. 2007, Ruhl 2008, Wolff et al. 2011) and megabenthic activity (Bett et al. 2001).

However, other studies have failed to find a direct between the magnitude and/or seasonality of OMF and the biology of deep-sea organisms. Beaulieu and Smith (1998) found that a detrital layer on abyssal sea floor of the NE Pacific was composed of diatoms in summer and radiolarians during autumn, but found no significant correlation with echinoderm distributions and the abundance and composition of sea floor detritus. Sayles et al. (1994) studied a deep-sea site close to Bermuda. They found large seasonal variations in POC flux but no corresponding seasonality in sediment oxygen demand. A study at the Porcupine Abyssal Plain (PAP) in the NE Atlantic (~4200m depth) found a radical change in the abundance of invertebrate fauna over a period of 15 years (Billett et al. 2010). Actinarians, annelids, pycnogonids, tunicates, ophiuroids and holothurians increased significantly in abundance. However, there was no significant change in wet weight biomass. Two holothurian species, *Amperima rosea* and *Ellipinion molle* increased in abundance by more than two orders of magnitude (Billett et al. 2001). Increases in abundance were often accompanied by an associated decrease in mean body size. *A.rosea* was abundant over a large area of the PAP suggesting that the phenomenon was not a localized event. Billett et al. (2001) suggest that the observed changes in abundance, species dominance and size distributions were probably the result of environmental forcing through inter-annual variability and long-term trends in organic matter supply to the seabed. However, predicted and observed fluxes of organic matter to the PAP did not increase in the years prior to the "*Amperima* Event", or during it (Lampitt et al. 2001). Instead, Billett et al. (2001) suggest variation in the timing, quality or nutritional value of the organic matter as being responsible for the observed community changes. Therefore, not only is the quantity, variation and timing of organic matter flux important for shaping deep sea communities and biogeography, but also the quality of that food resource. A similar conclusion was drawn from a study investigating the differences between two areas of the abyssal Atlantic with very different bottom faunas; the PAP, and the Madeira Abyssal Plain (MAP)(Thurston et al. 1994). It was assumed, due to their very different community compositions, that the PAP received much greater, and more seasonal organic matter flux than the MAP and that this would affect the abundance and biomass of organisms living on the deep-sea floor at these sites. However data from sediment traps suggest that the sites actually received comparable amounts of OMF and seasonality was apparent at both the PAP and the MAP (Thurston et al. 1994). The authors concluded that the hypothesis that seasonality of organic matter supply can explain differences in benthic communities did not receive strong support from sediment trap data(Thurston et al. 1994). However, one clear difference between the sites was the nature of the particle flux to the deep-sea floor. There were aggregates on the deep sea floor at the PAP, but not at the MAP, so that macroscopic phytodetrital deposition was the difference between the two sites (Thurston et al. 1994). Repackaging of phytoplankton into larger aggregates occurs at PAP but not MAP. Thus, a combination of larger pellet size, less recycling and slower degradation rates at higher latitudes may explain the presence of phytodetritus at the PAP but not the MAP (Thurston et al. 1994). Therefore we need to understand more about the composition of organic matter, and how this will affect the deep sea benthos.

More recently, attention has focused upon which biochemical components of phytodetritus benthic deposit feeders are utilizing, as this may give clues as to the important qualitative aspects of OMF that have thus far been overlooked. Evidence suggests that sterols (Ginger et al. 2001)and chlorophyll and carotenoid pigments (Hudson et al. 2003, Wigham et al. 2003b) may be important for various biological processes for abyssal holothurians. As the organisms cannot biosynthesize these compounds themselves, the differential content of sterols and/ or pigments in phytodetritus reaching the seafloor could be an important factor explaining geographic differences in community structure and abyssal biogeography. Danavaro et al. (1999)compared benthic responses to particulate fluxes in different trophic environments; they found that dominance of protein fluxes over those of carbohydrate is specifically related to the productivity of the system being investigated. Therefore the availability of proteins may be an important factor regulating the abundance and distribution of deep-sea benthic consumers (Fabiano et al. 2001)and therefore, on a broader scale, biogeographic patterns in the abyss.

Whilst benthic pelagic-coupling is not yet well understood, it appears that there is, in many areas of the ocean, some degree of coupling between surface productivity and the distribution of deep-sea organisms. Whether this is due to the quantity, seasonality or quality, or more probably a combination of all three, we do not yet fully understand. Experimental work investigating the effects of quantity, quality and seasonality of OMF on the abundance, diversity, feeding behaviour and reproduction of the benthos, coupled with large scale studies investigating links between the biogeography of individual species and biogeochemical provinces will help us better understand the underlying processes linking OMF and abyssal biogeography.

1.3.5 Species diversity in the deep-sea: historical perspectives to modern day estimates

Early deep-sea discoveries into the existence of life in the deep sea were often accidental; John Ross discovered that the mud attached to a sounding line from 2500m in the Antarctic was "teeming with life", made relatively little impact on the supposition that the deep sea was a lifeless environment (Snelgrove and Smith 2002). Therefore, when Edward Forbes deemed the deep sea 'azoic' below 600m (Forbes 1844) (largely due to Forbes' unfortunate sampling location in the species-poor Aegean) despite previous evidence to the contrary, the concept of the deep sea being virtually absent of life was widely accepted (Anderson and Rice 2006).

Eventually, the great explorations of the HMS *Porcupine,* and HMS *Challenger* (1872-76) which encompassed multiple oceans, sampled at great depths and at thousands of locations extinguished earlier azoic paradigms and, rather, showed the deep sea to be species rich (Anderson and Rice 2006). R.R. Hessler and H.L. Sanders in the 1960s were among the first to quantify this richness in terms of the macrobenthos (Hessler and Sanders 1967, Clarke et al. 1969).

Today much debate surrounds total species numbers both in the deep sea and in terrestrial environments. It is now thought that the deep sea may be more diverse than shallow water communities, largely due to the highly diverse macrofauna in many areas. However, various methods of estimation and extrapolation result in estimates of diversity for the deep sea differing by orders of magnitude (Snelgrove and Smith 2002). Studies of diversity also focus on different scales, looking at both local and regional patterns, and the relationship between the two (Witman et al. 2004), so that different studies and environments can be difficult to compare. The deep sea is probably one of the most diverse ecosystems on Earth.

Initial expectations that the deep sea should be species-poor were based upon the assumption that the deep sea is a stable and environmentally homogenous habitat. In 1968, Sanders proposed the stability-time hypothesis to describe patterns of diversity in both shallow-water and deep-sea communities (Sanders 1968). The assumption that the deep sea is environmentally homogenous, on both a temporal and spatial axis, led Sanders to suggest that the unexpectedly high levels of diversity in the deep sea might be caused by a high level of coexistence through competitive niche partitioning. He argued that over large time scales, this would allow evolutionary diversification of deep-sea fauna. The stability-time hypothesis can be classed as an equilibrium theory. Equilibrium theories suggest that relatively unchanging conditions create a favourable habitat for the evolution of high diversity (Snelgrove and Smith 2002). The species-area relationship, which is often cited as a potential cause of high diversity in the deep sea, can also be classed as an equilibrium theory. According to this hypothesis, the large surface area of the deep sea reduces the likelihood of species with wide distributions becoming extinct over broad scales. This is because refugia are more likely to exist somewhere in their distribution, thus facilitating the diversification of that species (Abele and Walters 1979). It has also been suggested that low productivity, and therefore limited energy availability, could be one of the major causes of species diversity in the deep sea, by reducing rates of competitive interaction (Van Valen 1976).

In contrast to equilibrium theories, non-equilibrium theories are based upon the idea that dynamic processes allow competitively inferior species to coexist in a system with competitive dominants (Snelgrove and Smith 2002). Dayton and Hessler (1972) suggested that if disturbance or predation differentially remove competitive dominants from a community, species which are poor competitors will be allowed to coexist (Dayton and Hessler 1972). The intermediate disturbance hypothesis has also been cited as potentially increasing species diversity in the deep sea (Kukert and Smith 1992). It suggests that moderate environmental fluctuations prevent the attainment of equilibrium and therefore enhance diversity, but that extremely severe or frequent fluctuations may eliminate sensitive species and thus depress diversity (Connell 1978, Huston 1979). Non-equilibrium theories therefore require the existence of some variation in environmental factors in the deep sea. At the time of the Sander's development of the stability-time hypothesis such variations were not thought to be common, if they occurred at all. However, there is now a growing body of evidence pointing to local and global scale variations in the deep-sea environment (Stuart et al. 2003). On relatively small scales the deposition of terrestrial derived material, such as wood and other plant material create biogenic microhabitats on the deep-sea floor (Wolff 1979). Organisms themselves, such as hexactinellid sponges (Beaulieu 2001) and the skeletons of whales (Baco and Smith 2003) can also provide habitat for other species.

The burrows, mounds and tracks of megabenthos also create habitat structure in abyssal ecosystems (Smith et al. 2008). The majority of literature on deep-sea biodiversity is based upon the mechanisms underlying local species diversity and species coexistence at small scales (Levin et al. 2001). It has proven difficult to extend mechanisms of local community structure to regional patterns in any ecological system so far (Levin et al. 2001). Over larger spatial scales in the deep sea, heterogeneity is caused in some locations by powerful currents (Hollister and McCave 1984).

Spatial and temporal variation is also caused by changes in food input to the deep-sea floor in the form of sinking organic matter. Cycles of seasonal phytoplankton dynamics determine the quantity and composition of organic material deposited on the deep-sea floor (Kiriakoulakis et al. 2001). As early as 1880, the potential for this to cause geographic and temporal variation in food supply to the deep-sea floor was recognised: "Possibly there is at some places a periodical variation in the supply of food falling from above which may give rise to a little annual excitement among the inhabitants" (Moseley 1880). This insight was more or less ignored for a century until the 1980s when new technology provided evidence to suggest that there was indeed a seasonal flux of organic matter to the benthos in certain locations (Deuser and Ross 1980, Billett et al. 1983, Lampitt 1985) and that this flux was linked to cycles of primary productivity in overlying waters. This flux of organic matter is now thought to be one of the most important ecological factors controlling benthic communities in the deep sea (Smith et al. 2008) and it is discussed in much greater detail in the following chapters. Other regional scale processes affecting deep-sea diversity include boundary constraints, sediment heterogeneity, oxygen availability, hydrodynamic regimes and catastrophic physical disturbance (Levin et al. 2001). Regional scale diversity is also important because species diversity on a regional scale will affect local species diversity. In a study of sub tidal marine benthic communities, Witman et al. (2004) found that the richness of the regional species pool explained 73-76% of local species richness (Witman et al. 2004).

The relative importance of the above hypotheses and how they relate to empirical data on species diversity in the deep sea has been reviewed elsewhere (Levin et al. 2001, Snelgrove and Smith 2002). It is clear that no single hypothesis explains patterns of diversity in the deep sea. Small scale experiments will allow the investigation of specific questions regarding diversity, especially those relating to the importance of food availability and organic matter flux (Snelgrove and Smith 2002). However, the stochasticity and patchiness observed at small scales in deep-sea communities becomes resolved at larger scales (Levin et al. 2001). Therefore, more broad investigations into how geographic patterns of diversity relate to key environmental factors will also improve our understanding of the causes of diversity in the deep sea.

Additionally, our current knowledge of deep-sea species diversity remains largely based on traditional taxonomy, which examines the morphology of organisms. Very little is known about deep-sea biodiversity at the genetic level (see future chapters), so that with increasing research using molecular genetic data species diversity may be even higher than currently recognized (Etter et al. 1999).

1.4 The elasipodid holothurians

1.4.1 Historical significance

The Challenger Expedition 1872-1876 founded the knowledge of the deep-sea holothurians, as well as that of most other deep-sea groups, bringing back material from the Atlantic, Pacific and Indian oceans (Hansen 1975). The order Elasipodida was erected by Théel (Theel, 1882) comprising 52 species, 51 of which were new to science (Hansen 1975). The genera *Irpa* and *Kolga* were established by Danielssen and Koren (1882) after the *Vöringen* Norwegian North Atlantic Expedition 1876-1878 (Hansen 1975). Many more geographically specific voyages contributed to sampling of the World's oceans and to the knowledge of the Elasipodida (Hansen 1975). *Table 1.1* summarises these historical expeditions and the taxonomists who helped to describe and revise the elasipodid holothurians.

Whilst all these expeditions contributed to the knowledge of elasipodid holothurians, it was surely the circumnavigation of the World by the Galathea Expedition that furthered our understanding of the Elasipodida most significantly. The Galathea expedition sampled a total of 98 trawling or dredging stations, and focused particularly on the hadal fauna of the deep-sea trenches (Hansen 1975). Not only did Hansen improve our knowledge of the taxonomy of this group, but also their life history, biogeography and evolutionary origins. Recent major contributions to our knowledge of the systematics of the elasipodid holothurians have come from a number of research programs. The CROZEX project discovered a new genus and species of laetmogonid holothurian, *Gebrukothuria profundus* (Rogacheva et al. 2009) and a new species of elpidiid holothurian, *Peniagone crozeti* (Cross et al. 2009) from abyssal waters surrounding the Crozet Islands in the southern Indian Ocean. The MAR-ECO expedition to the northern Mid-Atlantic Ridge resulted in the description of four new species of elasipodid (Gebruk 2008). Detailed taxonomic work on the elpidiidae has also been carried out by Gebruk (Gebruk 1983b, a, Gebruk and Shirshov 1994, Gebruk et al. 2003a, Gebruk et al. 2003b).

Thanks to the work of the taxonomists the systematics of the Elasipodida are relatively well described. However due to their relatively simple morphologies and the damage that occurs to these relatively soft-bodied organisms upon their collection, it is likely that genetic techniques will improve the systematics of the Elasipodida. A recent study by O'Loughlin et al. (2011) found substantial intraspecific genetic geographic differentiation in Antarctic holothurians, including Elasipodid species. Multiple geographically differentiated "evolutionary significant units" (ESUs) in species from three Elasipodid families were found including the Elpidiidae (*Peniagone incerta*) Psychropotidae (*Benthodytes sanguinolenta*) and Laetmogonidae (*Pannychia moseleyi* & *Laetmogone wyvillethomsoni*) (O'Loughlin et al. 2011).

Contemporary work by deep-sea taxonomists and ecologists have given us a much clearer understanding of the life histories and ecological significance of elasipodid holothurians and their evolutionary history, which are reviewed briefly in the following sections.

1.4.2 Ecological relevance

Holothurians are the dominant epibenthic invertebrate taxon in many areas of the deep sea (Sibuet and Lawrence 1981, Billett 1991). At certain sites in the deep sea holothurians constitute up to 90% of the benthic megafauna (Iken et al. 2001). Holothurians are known to feed on the uppermost few millimetres of the sediment and it is thought that many species preferentially feed on relatively fresh phytodetritus (Billett 1991, Miller et al. 2000). Evidence suggests that not all holothurians utilise phytodetritus in the same manner, and they may feed in subtly different ways (Moore et al. 1995, Roberts and Moore 1997, Ginger et al. 2001, Hudson et al. 2003). This may allow elasipodid species to co-exist despite limited resources (Roberts and Moore 1997).

Although the mode of reproduction is not directly known for most holothurian species, and despite large variations in egg size within the Elasipodida (Hansen 1975) it is thought that most species probably have a lecithotrophic larval stage (Hansen 1975, Tyler et al. 1985b, Billett 1991). In shallow water organisms developmental mode is linked to dispersal ability, with planktotrophic development generally resulting in larger geographic and bathymetric ranges than lecithotrophic larvae (Scheltema 1986). However in the deep sea a lecithotrophic larval phase may increase the potential for survival and therefore dispersal in an extremely food-poor environment (Tyler et al. 1985b, Young et al. 1997a). Elasipodid holothurians are therefore theoretically capable of relatively wide scale dispersal. Additionally some species may also have a pelagic juvenile phase (Gebruk et al. 1997) which may further their potential for widespread dispersal. A wide range of life-histories are displayed amongst the elasipodid holothurians. For example, in a study of two Elpidiidae species collected from the north-east Atlantic; *Peniagone azorica* and *Peniagone diaphana*, it was found that despite both species producing gametes continuously, the population size structure of the cogeners was quite different, possibly relating to different depth ranges or differential predation pressures on juveniles (Tyler et al. 1985b). In a study of two Elpidiidae (*Protelpidia murrayi* and *Peniagone vignoni*) from the Antarctic continental shelf it was found that gametogenesis was synchronised and seasonal, probably caused by a summer food pulse in the Antarctic summer (Galley et al. 2008). Temporal and geographic differences in reproductive output (Billett and Hansen 1982, Wigham et al. 2003b, Ramirez-Llodra et al. 2005), biochemical composition

(Neto et al. 2006, FitzGeorge-Balfour et al. 2010) population structure (Billett et al. 2001, Wigham et al. 2003a, Wigham et al. 2003b) and community composition (Thurston et al. 1994, Billett et al. 2001, Bett et al. 2009, Wolff et al. 2011) have been noted for elasipodid holothurians.

However the magnitude and direction of changes vary greatly between families and even between species, with some species displaying a 'boom/bust' life history strategy (Uthicke et al. 2009) and others responding much more slowly (Bett et al. 2001). Whilst linking these to direct causal mechanisms has proven problematic, it is thought that temporal and spatial variations in the quantity and quality of food input to the deep sea is going to be the most important factor for the observed changes (Wigham et al. 2003a, Billett et al. 2010, Wolff et al. 2011). The reproduction and life histories of the families of the Elasipodida are considered in more detail in Chapter 3.

The existence of an entirely pelagic family within the Elasipodida has been known for some time; Family Pelagothuridae, reviewed in Gebruk (1990). However, it is now thought that swimming in adults of the elasipodid holothurians is a more common phenomenon than previously thought. The swimming of some species of Elasipodida has been known for some time (Hansen 1975) with members of the Elpidiidae being particularly well adapted to swimming (Tyler et al. 1985b). It appears swimming may be more common than previously thought; Rogacheva et al. (in preparation) have observed swimming for the first time in eleven more species of elasipodid holothurian, mostly from the family Elpidiidae, but also species of the family Psychropotidae. It is thought that many more genera and species are probably capable of swimming than have been observed so far (Rogacheva et al., in preparation). Swimming elasipodids can be roughly categorised into three groups: preferential swimmers, frequent swimmers and occasional swimmers. Swimming is probably an adaptation to reduce energy use in foraging for patchily distributed food in the deep sea (Rogacheva et al., in preparation). However, it is thought that all Elasipodid holothurians (except members of the Pelagothuridae) have to return to the benthos to feed. Holothurians therefore present themselves as an ideal candidate for studying the underlying mechanisms driving distribution patterns and biogeography of megafaunal organisms in the deep sea, particularly in relation to surface primary productivity and organic matter flux.

1.4.3 Evolutionary context

Hansen (1975) suggested that in groups which have been restricted to the deep sea for geological periods of time species have probably mainly arisen through speciation in the deep sea. In contrast in deep-sea species closely related to shallow water congeners, species may have also been added through relatively recent immigration from shallow water. The elasipodid holothurians probably belong to the former group and Hansen (Hansen) suggested that at least the suborder Psychropotina (including the Families Elpidiidae, Psychropotidae and Pelagothuridae) evolved entirely within the deep sea.

Gebruk (Gebruk 1994) proposed two main stages in the evolution of the deep-sea fauna of elasipodid holothurians. Based mainly on thorough taxonomic, systematic & paleontological review of various Elpidiidae genera, Gebruk suggests an early stage of shallow water origin and evolution of the Elasipodida linked with the Tethys Basin and a later stage linked with the Antarctic which involved the invasion from bathyal to abyssal depths (Gebruk 1994).

Although some debate still exists between centres or origin and centres of accumulation, it is probable that the richest marine fauna of the Jurassic, Cretaceous and Palaeogene was characteristic of the part of the Tethys Sea that included southern Europe and northern Africa (Mironov 2006). The richness of the fauna was at least equal to that of the recent fauna of the Indo-Malayan triangle (Mironov 2006). Figure 1.4 shows the position of the Tethys Sea and surrounding continents at the beginning of the Jurassic.

Table 1.1 *A Summary of historical expeditions whose findings contributed to our knowledge of the taxonomy and systematics of the Elasipodid holothurians, modified from Hansen (1975).*

Vessel	**Date**	**Geographic region**	**Author (s)**
Challenger	1872-1876	Global	Théel, 1882, 1886a
Vöringen	1876-1878	Norwegian Sea & adjacent North Atlantic	Danielssen & Koren, 1882
Ingolf	1895-1896	Northern N. Atlantic & SW Norwegian Sea	Heding, 1935, 1942
Godthaab	1928	West Greenland Seas	Mortensen, 1932
Travailleur & Talisman	1880-1883	North Atlantic	R.Perrier, 1902
Princesse Alice & *Hirondelle II*		North Atlantic	Von Marenzeller, 1893, Hérouard, 1902,1923
Caudan	1895	Bay of Biscay	Koehler, 1896
Albatross	1893	Western North Atlantic	Verrill, 1885
Blake	1877-1880	Western North Atlantic	Théel, 1886b
Atlantis	1938-1939	Western North Atlantic	Deichmann, 1940
Michael Sars		North Atlantic	Grieg, 1921
Albatross	1947-1948	Mid-Atlantic	Madsen, 1953
Valdivia	1898-1899	Eestern S. Atlantic, Antarctic, Indian	Ludwig & Heding, 1935, Heding, 1940
Investigator	1887-1901	Bay of Bengal, Arabian Sea	Koehler & Vaney, 1905
Siboga	1899-1900	Indonesian Seas	Sluiter, 1901b
Albatross	1891	West coast of Central America	Ludwig, 1894
Albatross	1902	Hawaiian Islands	Fisher, 1907
Albatross	1903	North Pacific coast of North America	Edwards, 1907
Albatross	1906	Japan	Oshima,1915,1916-1919
Albatross	1911	Baja California	Clark, 1913, 1923
Albatross	1899-1900, 1903-1905	Eastern Tropical Pacific	Clark, 1920
Francais	1903-1905	Antarctic	Vaney, 1906, 1914
Pourquoi-Pas?	1908-1910	Antarctic	Vaney, 1906, 1914
Belgica	1897-1899	Antarctic	Hérouard, 1906
Scotia	1902-1904	Antarctic	Vaney, 1908
S.Y.Antarctic	1901-1903	Antarctic	Ekman, 1927
Tanglin	1901-1903	Antarctic	Ekman, 1927
Vitiaz	1949	Various	Belyaev 1971
Galathea	1950-1952	Global	Hansen,1975
Elatanin	1962-1964	Antarctic	Agatep 1967 a,b

The most prominent trend of specialisation within the Order Elasipodida is the origin of swimming forms; namely the families of the Suborder Psychropotina (Gebruk 1994). The families of Suborder Deimatina (Deimatidae and Laetmogonidae) represent a less advanced stage of morphological specialisation (Gebruk and Shirshov 1994). However recent molecular phylogenetic work (Alt et al. in preparation, Gubili et al. in preparation) suggests that the family Deimatidae actually sits outside the order Elasipodida altogether.

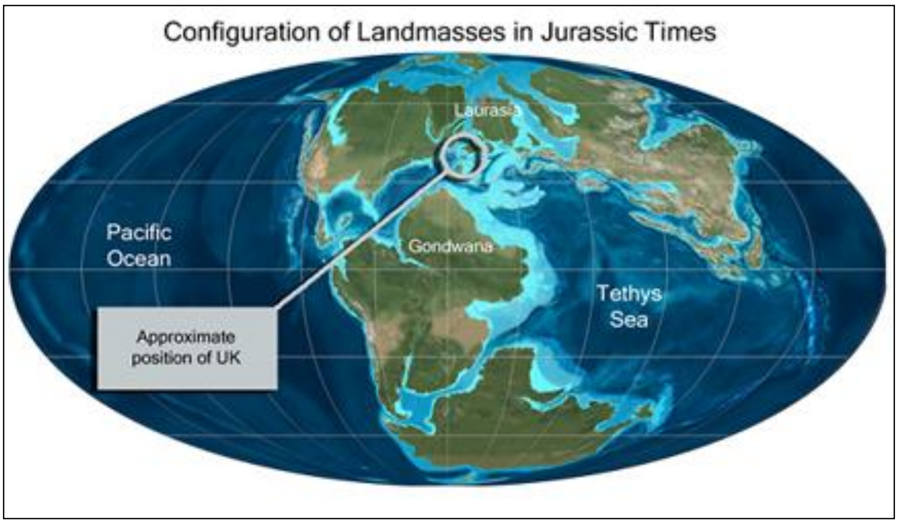


Figure 1.4 *The position of the continents 200mya showing the position of the Tethys sea and the surrounding continents.*
(http://www.thewhitbyseagull.co.uk/geology_of_whitby_history_pg01.html)

The geographic distribution of Elpidiid genera (which account for ~66% of total species in the Order Elasipodida) falls into four main groups and shows that the vast majority inhabit the Antarctic (Gebruk 1994). The more primitive genera are either distributed entirely within the Antarctic (*Rhipidothuria, Protelpidia*), or have circum-tropical or tropical distributions (*Psychrelpidia, Penilpidia* and *Psychroplanes*). The more morphologically advanced genera tend to have Antarctic and high & moderate latitudinal distributions (*Peniagone, Kolga, Elpidia, Ellipinion*) or Antarctic-Pacific (both coasts) distributions (*Scotoplanes* & *Amperima*) (Gebruk 1994).

It is therefore thought that the elasipodid fauna was widely distributed in the ancient Tethys Basin and was split into at least three parts following the breakup of the Tethys Sea into the Indo-Malayan, the Mediterranean and the West Indies sections, the latter remaining in the Panama Gulf region. By the end of the Tethys Sea era (Miocene), the elasipodids had very likely invaded bathyal waters. Bathyal Antarctic waters were invaded by elasipodid holothurians which moved along the South America continental slope from either the Caribbean or the Panama Gulf localities, where the primitive forms are still present today. The invasion into abyssal waters by the Antarctic bathyal fauna probably occurred just after the appearance of cold water in the Southern Ocean (just after the Middle Miocene). The distributional pathways from the Antarctic spread along the ocean floor in different directions, probably following the dominant deep-sea currents (Gebruk 1994). They invaded the North Pacific via both east and west Pacific coastlines and the North Atlantic. It is not thought any Arctic or North Atlantic elasipodids were derived directly from the post-Tethys Mediterranean. The close biotic affinity between the Antarctic and the deep sea is well documented (Mironov 1982, 2006, Brandt et al. 2007, O'Loughlin et al. 2011). This is thought to be due to the wide depth ranges of species related to the comparatively deep continental shelves, weak stratification of the upper ocean and substantial mixing of the water column (Brandt et al. 2007). For timings of connections between the Antarctic and other oceans see *Figure 1.5.*

The vertical distributions of the Elasipodida suggest a similar pattern. Primitive forms (*Rhipidothuria* & *Protelpidia*) show gradual colonization of abyssal Antarctic waters whereas more advanced and widely distributed elpidiid genera, linked with the Antarctic, have colonized abyssal and hadal depths (Gebruk 1994). The bathyal fauna of elpidiids consists exclusively of the most primitive genera, with one group being linked to Antarctic, and the other one tropical.

The Antarctic region is therefore seen as a hotspot of elasipodid and echinoderm diversity (Gebruk et al. 2003b, Rogacheva 2007). Around 30 morphospecies of elasipodid holothurian have been recorded in the deep

Southern Ocean and local species richness can be high, with up to seven species within one trawl (Brandt et al. 2007).

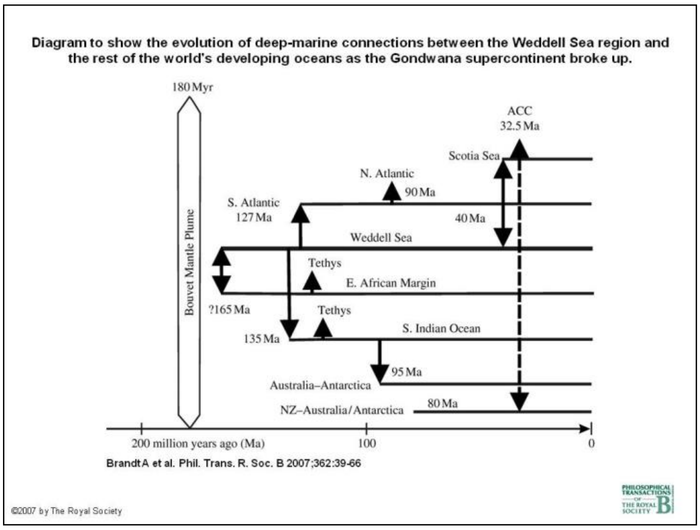


Diagram to show the evolution of deep-marine connections between the Weddell Sea region and the rest of the world's developing oceans as the Gondwana supercontinent broke up.

BrandtA et al. Phil. Trans. R. Soc. B 2007;362:39-66

Figure 1.5 *The timing of deep-sea connections between the Weddell Sea and other oceans, from (Brandt et al. 2007)*.

1.5 Aims and hypothesis

The aim of this study is to investigate elasipodid holothurian biogeography using two approaches. The first approach uses natural history collection data to investigate the distributions of elasipodid holothurians in relation to remotely sensed environmental variables, including estimates of food availability. The first approach tests two main hypotheses;

1) Elasipodid families, with different life-history traits have different environmental preferences at Ocean and global scales. The Elpidiidae are expected to be found in more variable environments than the other families.

2) Zonation of the marine environment based on pelagic ecology of the upper ocean which takes into account seasonality and quantity of phytoplankton blooms will be applicable to the biogeography of elasipodid holothurians through tight benthic-pelagic coupling.

The second approach is to use molecular markers to investigate the level of connectedness of abyssal cosmopolitan elasipodid holothurians. The two major hypotheses are tested

3) Cosmopolitan elasipodid holothurians show little genetic structure due to their potential for long distance dispersal, their large population sizes, and the connectedness of abyssal soft sediments on a global scale.

Any genetic structure present will be related to simple geographic distances between populations.

Chapter 2. Mapping Methods

2.1 The creation of GIS layers of biological and environmental data

Chapters 3 and 4 are both based upon a dataset of published and unpublished records of Elasipodid holothurians. Chapter 3 also relies on global maps of environmental variables thought to be potentially important to elasipodid holothurians. In this chapter the sources used to build both the distribution database and the maps of environmental variables are outlined. Additionally the statistical approaches for Chapters 2 and 3 are described, as they share many similarities.

2.1.1 Mapping the distribution of elasipodid holothurians

All available distribution records for elasipodid holothurians were downloaded from the Global Biodiversity Information Facility (GBIF) data portal http://data.gbif.org. Data was retrieved by searching for elasipodid records and selecting 'datasets with occurrences' and then downloading an Excel spreadsheet. The GBIF dataset had good global coverage, with records from all Oceans. Records from the Galathea expedition (Hansen 1975), up to very recent species discoveries, were present in the GBIF dataset, suggesting good temporal coverage. *Table 2.1* shows the main sources of data included in the GBIF dataset.

Additional important distribution records were added from the HMS *Challenger* (1872-76) expedition report on the Holothuroidea (Theel 1882). Data from a number of National Oceanography Centre, Southampton (NOCS) cruises were also added to the database from cruise reports. NOCS data covered the NE Atlantic and abyssal depths around the Crozet Islands in the southern Indian Ocean, collected as part of the CROZEX programme (Wolff et al. 2011). Long-term records for echinoderm species for Station M in the Northeast Pacific were added to the database from published literature (Lauerman and Kaufmann 1998, Ruhl and Smith 2004). A number of published and unpublished records from Russian research cruises as well as additional global records from museum records that were not present in the GBIF dataset were kindly provided by A. Rogacheva. Occurrence records were cross checked with other information in online databases and online museum records to ensure that it was as comprehensive as practically possible. These data form a strictly presence-only dataset, as in biogeographic analysis the recording of a species 'absence' from an area requires a detailed level of sampling not currently achievable in a global deep-sea dataset such as this.

All georeferenced records were converted to decimal degrees, and depth data to metres. The data quality of georeferenced collection points was screened in ArcGIS to detect any anomalies. Most records that were incorrectly georeferenced (such as those that were "terrestrial") were checked against original sources and corrected wherever possible. Where no reliable correction could be achieved, records were deleted. Duplicate records (those input from multiple sources) were removed from the database, as were any records missing latitude, longitude or depth of collection information.

Nomenclature was checked against the World Register of Marine Species (WoRMS: http://www.marinespecies.org/) and corrected as necessary. Ambiguous names were checked with taxonomic experts (D. Billett, A. Rogacheva, and A. Gebruk) and updated as necessary. Species maps in the form of shapefiles were created using ArcGIS v.10. *Figure 2.1* shows the distribution of all records in the final dataset.

A map of all sites where non-elasipodid holothurians were collected was compiled, in order to give a qualitative comparison of the distribution of elasipodid holothurians in relation to sampling effort. All non-elasipodid holothurian records were downloaded from GBIF, with only records from greater than 500m depth retained. Additional records of locations which sampled megafauna, which were collated as part of the Census of the Diversity of Abyssal MARine life (CeDAMar), were provided by C. Stuart (Stuart et al. 2008).

Table 2.1 *Sources of GBIF elasipodid holothurian records, where acknowledged within the GBIF data access agreement*

Secondary Source	**Primary Source**
Alaska Ocean Observing System	*As secondary*
Australian Antarctic Data Centre	*As secondary*
IFREMER - French Research Institute for Exploitation of the Sea	*As secondary*
GBIF-Sweden	*As secondary*
Museo Argentino de Ciencias Naturales	*As secondary*
National Chemical Laboratory, India	*As secondary*
US National Museum of Natural History	*As secondary*
Ocean Biogeographic Information System (OBIS)	Unspecified
	ChEss project
	NIWA
	University of California San Diego
OZCAM (Online Zoological Collections of Australian Museums) Provider	*As secondary*
Senckenberg	*As secondary*
The Danish Biodiversity Information Facility	*As secondary*
Yale University Peabody Museum	*As secondary*

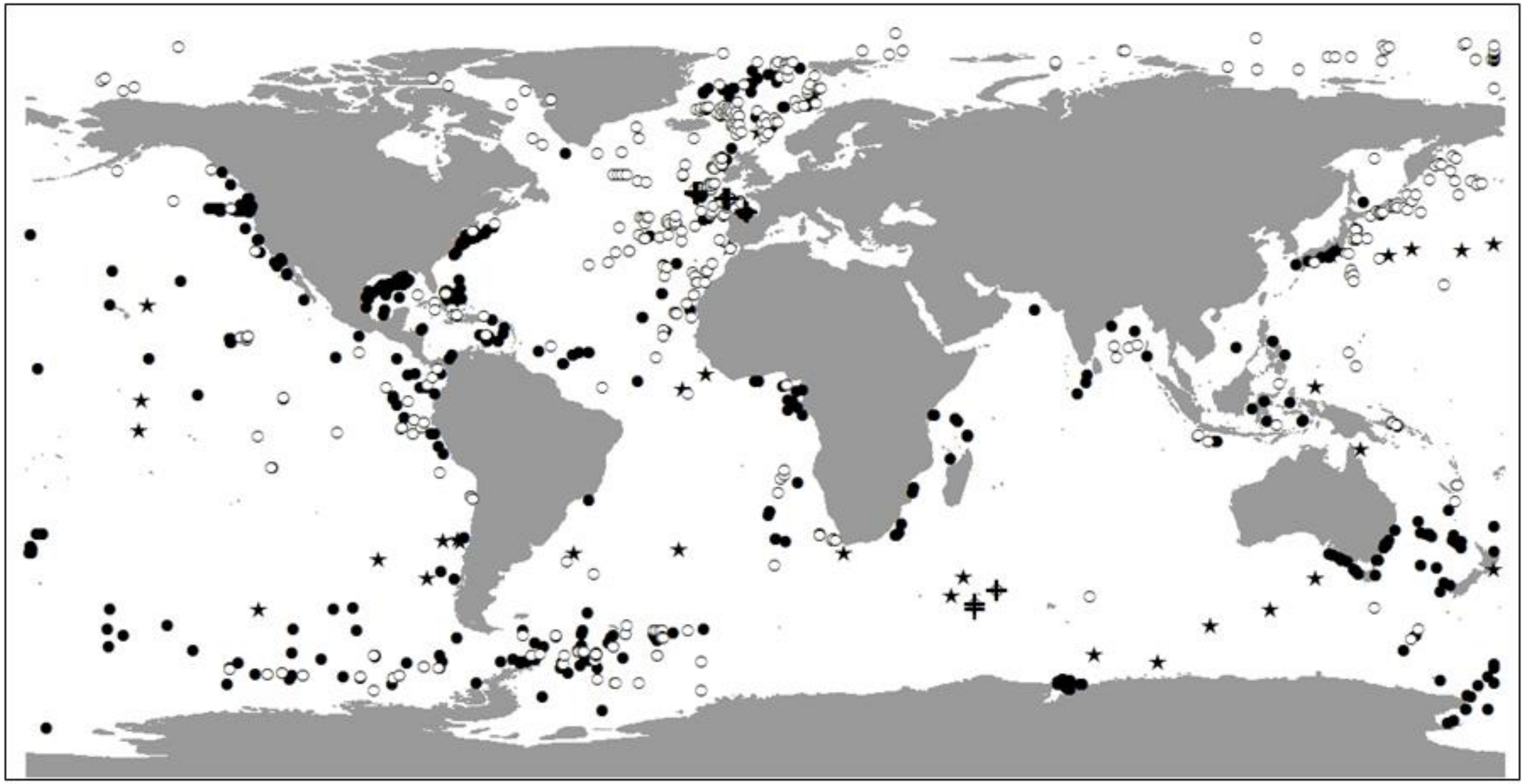


Figure 2.1 *Map of main data sources for the compilation of elasipodid holothurians. Closed circles represent GBIF records, open circles represent data from A. Rogacheva's database, crosses represent additional records from NOCS cruise reports, and stars represent additional records from the* HMS Challenger *expedition*

This dataset provided the method of collection, and only records which were obtained by trawl or epibenthic sled were retained, as megabenthic samples from cores would be biased towards only collecting smaller holothurians. Records were mapped in ArcGIS v.10. Anomalous records were corrected where possible, and removed where reliable data could not be sourced. A final data collection distribution map was constructed, also in ArcGIS v.10 (*Figure 3.34*) which is discussed in section *3.3.2*.

2.1.2 Mapping environmental data

Environmental 'layers' of environmental variables thought to be potentially important for holothurian distribution were created using remotely sensed data and mapped using ArcGIS v.10. Three environmental layers relating to quantity and variability of food availability and three layers related to the physical conditions experienced by organisms at the seafloor were created.

2.1.2.1 Particulate Organic Carbon flux to the seafloor

The quantity of POC flux to the seafloor was obtained from the Lutz (2007) dataset and the data layer, whose production is described below, was provided by D. Jones. As previously discussed, the Lutz algorithms describe the labile and refractory flux components as a function of remotely sensed NPP rates, NPP variability calculated from sediment traps, and SST, which predicts POC flux with accuracies greater than previous flux estimates (Lutz et al. 2007). The Lutz equations also take into account regional variations in the efficiency of the biological pump (Lutz et al. 2002). The method developed by Lutz for calculating POC flux to the seafloor is extremely complex, requiring multiple steps for different depths and regions, and for incorporating both satellite and sediment trap data. The main steps are briefly outlined below to give readers an understanding of the resulting data layer, but for a full understanding are referred to Lutz (2002, 2007).

1. First, average yearly POC flux regimes, or POC flux 'climatologies', are constructed using ~25 years of sediment trap POC flux observations from all over the world's Oceans. This involves multiple steps which must take into

account differences in the coverage (spatial and temporal) of sediment trap records. Sediment trap data had to be from below the local export depth (100-400m latitude dependent), be minimally affected by either benthic re-suspension or terrestrial detritus and had to describe at least one entire year of flux. This results in a minimum daily flux rate. Where more than one year of data is available, measurements were averaged into one climatological year.

2. NPP is estimated using Behrenfeld and Falkowski's (1997) approach: Seven years of 8-day satellite images at a 9km resolution between August 1997 and June 2004 and NASA SeaWiFs datasets. Satellite data for the same timeframe provide global SST estimates from the NOAA/NASA AVHRR Ocean pathfinder SST. Regional climatologies are developed:

3. The seasonal variation index (SVI) was then calculated for each region, using the following algorithm for *both* NPP and flux climatologies:

This expression defines the standard deviation of either NPP or POC flux

$$\mathrm{SVI} = \frac{\sigma(X)}{\overline{X}}.$$

This expression defines the average NPP or POC flux

Equation 4 – the SVI, which can be applied to NPP or POC flux (Lutz et al. 2007)

The SVI is a dimensionless description of variability which is defined as the standard deviation of POC flux or NPP (σ(X)), normalised to the average, POC flux or NPP ($\overline{X}$). The SVI is also known as the coefficient of variation and is statistically similar to other indicators of variability which, for NPP calculate the number of months required to accumulate one half the total annual primary production, or for POC flux, calculate the minimum time take for 50% of the annual flux to be collected.

4. The annual and seasonal transfer efficiency of a region is then calculated for different regions. In the Lutz model the annual transfer efficiency of a region is

calculated by the annual POC flux at depth *(z)* normalised to the annual NPP in overlying surface waters, resulting in an annual production *(p)* ratio.

This is shown in Lutz's equation 5:

This expression relates to average flux at depth, calculated from the flux climatology either from the sediment trap data or satellite data

$$p\ \mathrm{ratio}(z) = \frac{C_{\mathrm{flux}(z)}}{C_{\mathrm{npp}}}$$

This expression relates to average NPP from the climatologies described

Equation 5 – annual and seasonal transfer efficiency for a given region (Lutz et al. 2007)

6. Finally to quantify the relationship between satellite based data and sediment trap flux observations, annual production ratios are estimated using an exponential algorithm. Previously derived from the empirical fit between regional production and flux measurements (Lutz 2002), it is applied in this case to determine if flux can be approximated from satellite derived environmental parameters Lutz (2007). This function describes the labile and refractory components of the flux to depth below the depth of the export zone (z_e).

This coefficient describes the more refactive & therefore rapidly sinking portion of the flux.

$$p\ \mathrm{ratio}(z_e) = pr_d \exp\left(\frac{-z_e}{rl_d}\right) + pr_r$$

These coefficients measure flux that is available to decay and sinks more slowly.

Equation 6 – the labile and refractory components of flux to depth (Lutz et al. 2007)

The methods described by Longhurst (2007) and outlined above result in a global map of POC flux at the seafloor shown in Figure *2.2*.

2.1.2.2 Intra-annual seasonality of surface productivity

The Seasonal Variation Index is described above, in Equation 4. Whilst above it is used as a component to help accurately calculate the flux of POC to depth, it is also useful in its own right to directly assess the importance of variability of production on the biogeography of the benthos. Because intra-annual differences in POC flux have not been directly linked to changes in community structure of the megabenthos (Lauerman and Kauffman 1998) here we consider intra-annual seasonality in surface production to retain the seasonal signal. In this case Net Primary Production (NPP) was estimated using seven years (August 1997 - June 2004) of 8-day NASA SeaWiFs satellite ocean colour images at a 9km resolution and global sea surface temperature (SST) estimates from the NOAA/NASA AVHRR Ocean pathfinder SST using the methods described by Carr et al. (2006). Surface NPP was not included as an environmental variable in analyses, but provided a starting point for the estimation of inter and intra-annual seasonality, as described below. The global SVI layer was then created by normalizing the standard deviation in NPP to mean NPP on a 1° grid (Lutz et al. 2007) using the Lutz (2007) equation 4 as displayed above. Calculations were carried out in MATLAB. The resulting data is shown in *Figure. 2.3*.

2.1.2.3 Inter-annual variability of POC flux to the sea-floor

Inter-annual changes in megabenthic communities have been linked to inter-annual changes in POC flux. Therefore here we consider the inter-annual variability in POC flux to depth. Once again the SVI is applied, but in the instance it assesses inter-annual differences in POC flux to the seafloor. NPP was calculated from SeaWiFS data captured between 1998 and 2007, with NOAA/NASA AVHRR Ocean pathfinder SST from the same period and derived using the Carr et al. (2006) algorithm. The inter-annual seasonality in POC flux to the seafloor was created by calculating the year-to-year standard deviation of POC flux to the seafloor. The SVI, as described above, was then applied to this year- to- year deviation. Calculations were carried out in MATLAB. GIS compatible layers of POC flux, intra-annual seasonality of surface production and inter-annual variability of POC flux to the seafloor were then created and visualised in ArcGIS v.10. For visualisation purposes the symbology of *all* environmental layers (POC flux, intra and inter-annual seasonality, temperature, dissolved oxygen and salinity) was set to classified (20 classes) with natural breaks. The symbology in no way affects the underlying data or statistical analyses, but a standard method of data visualisation for environmental layers is used throughout this thesis for consistency of interpretation of maps provided. The results are shown in *Figure 2.4*.

2.1.2.4 World Ocean Atlas data - oxygen, salinity and temperature

Data on temperature, salinity and dissolved oxygen concentration were obtained from the World Ocean Atlas 2009 (WOA09), available through the National Oceanographic Data Centre (NODC) website: (http://www.nodc.noaa.gov/OC5/WOA09/pr_woa09.html). The World Ocean Atlas provides climatological maps of environmental variables at selected standard depth levels of the world ocean on a one-degree latitude-longitude grid (Locarnini et al. 2010). These climatologies are computed by objective analysis of all scientifically quality-controlled historical temperature data in the *World Ocean Database 2009*. For descriptions of data analysis procedures for i) temperature see Locarnini et al. (2010). ii) oxygen see Garcia et al.(2010) and iii) salinity see Antonov et al. (2010).

All files were downloaded in climatology_csv.tar format and the annual objectively analysed mean was extracted for each of the three WOA09 variables. Each variable has data 33 depth layers, with the first layer being the surface and the 33rd being 5500m. Point files for depth each layer were converted to raster files. For each environmental variable raster files of depth layers were combined using the ‘mosaic function’ in ArcGIS v.9.3 to form a map of the deepest data point for each layer. This effectively created a map of the environmental variable of interest on seafloor, or at the deepest available data point. These were then mapped on a 1 degree grid. WOA09 environmental layers were visualised in ArcGIS v.10 using a WGS 1984 projection. The resulting maps are shown in *Fig. 2.5*.

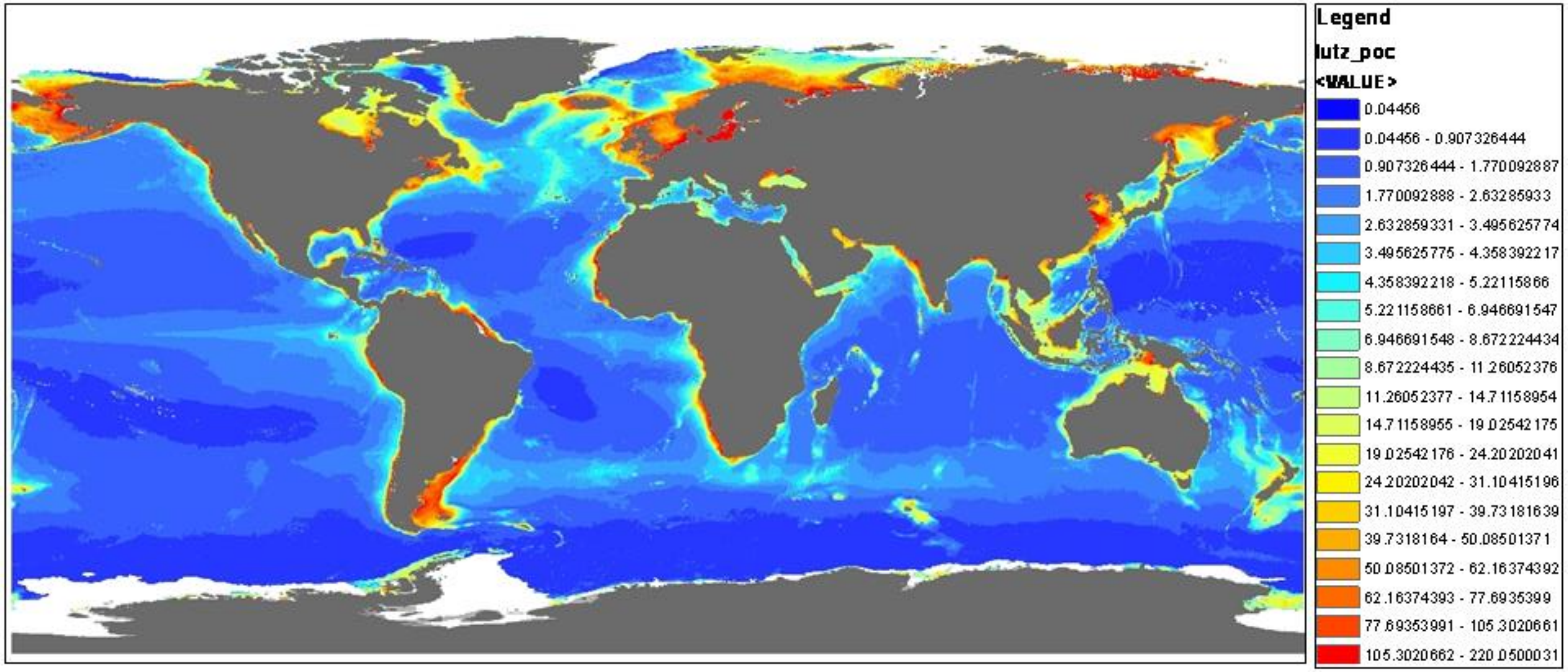


Figure 2.2 *Particulate Organic Carbon (POC) flux (mg carbon m2 day-1) to the seafloor as calculated by the Lutz (2007) algorithm.*

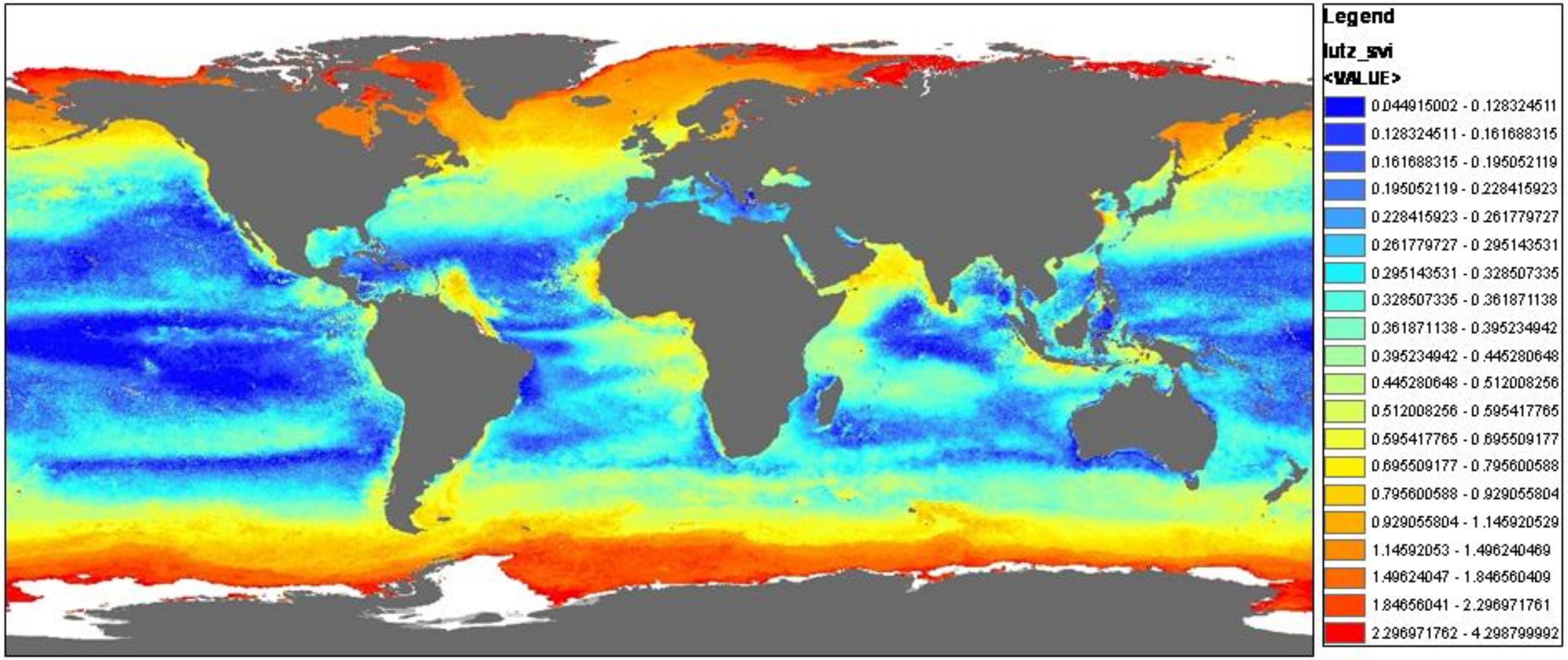


Figure 2.3 ***Intra***-*annual seasonality at the surface measured by the dimensionless Seasonal Variation Index as described by Lutz (2007).*

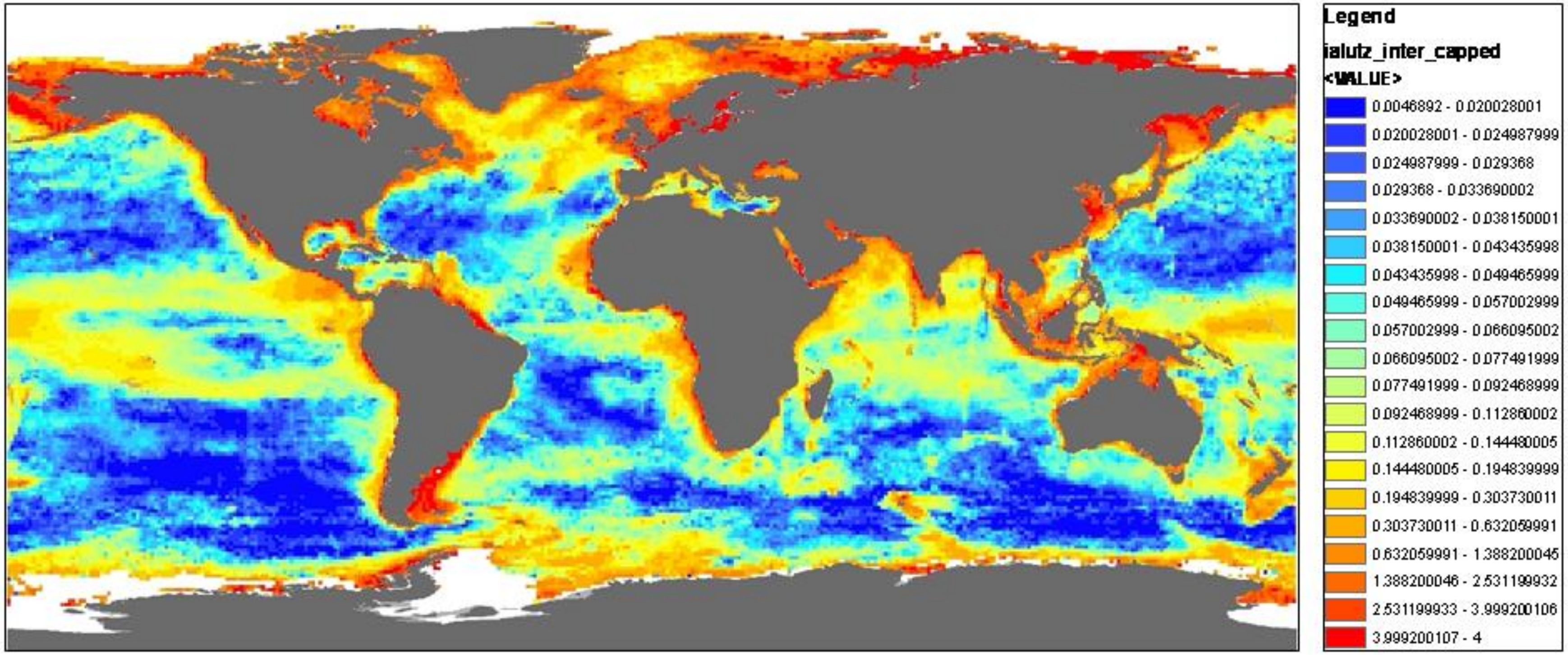


Figure 2.4 *Inter-annual variability of POC flux (Lutz 2007) to the seafloor calculated by the dimensionless Seasonal Variation Index (SVI)*

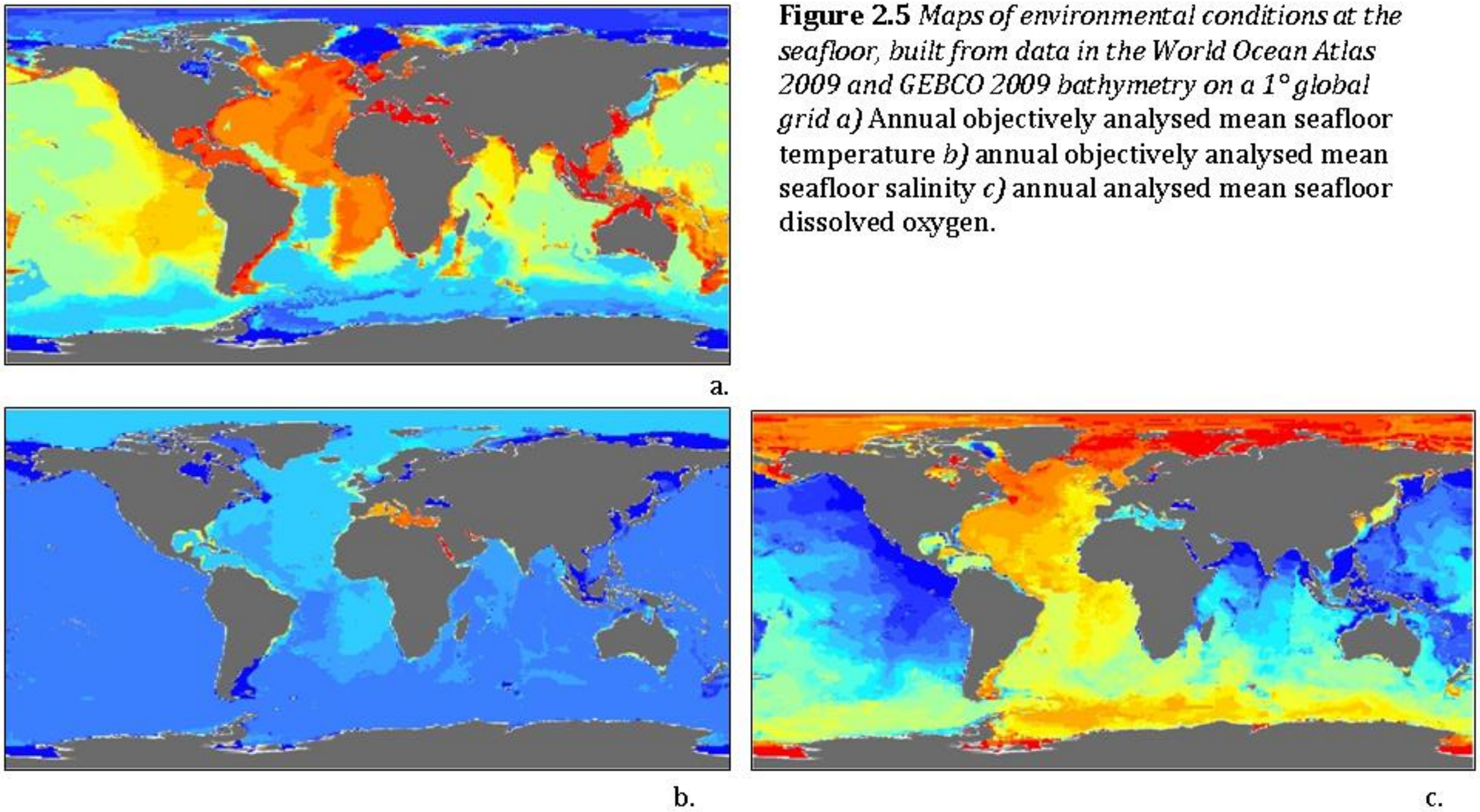


Figure 2.5 *Maps of environmental conditions at the seafloor, built from data in the World Ocean Atlas 2009 and GEBCO 2009 bathymetry on a 1° global grid a)* Annual objectively analysed mean seafloor temperature *b)* annual objectively analysed mean seafloor salinity *c)* annual analysed mean seafloor dissolved oxygen.

2.2 Differences in the environmental distribution of four families of Elasipodida

2.2.1 Environmental data extraction from holothurian collection sites

Biological records were mapped with the environmental layers created. Using the spatial analyst toolbox in ArcGIS v.10 data from each environmental layer was extracted for each holothurian data point and exported as an excel table. This resulted in a dataset of species occurrences, each associated with a set of environmental variables. This dataset formed the basis for the statistical analysis performed in Chapter 3.

Although records shallower than 500m had been removed, some areas deeper than 500m (usually continental shelf and Polar Regions) were missing WOA09 data as the estimation methods used by WOA09 are not suitable for estimating highly variable coastal and shallow shelf waters. Similar problems occurred for the data based on primary production. Therefore, after environmental data were extracted for each distribution point, a further round of data 'cleaning' took place, where any distribution records that were missing data for an environmental variable were removed from further analyses requiring point environmental data (Chapter 3). Such records were retained for those based on zoogeography (Chapter 4).

2.2.2 Statistical analyses

Extracted data was split according to depth band (500-2999m bathyal, 3000-5999m abyssal and 6000-10,000m hadal). These three independent data sets were then analysed separately. This resulted in three databases with the presence or absence of the four families of elasipodid holothurian at each collection site, associated with 7 environmental variables (mean POC, intra-annual seasonality, inter-annual seasonality, temperature, oxygen and salinity). Each site also had associated 'factors' which included which Ocean and which biome that site occurred in. The environmental matrices were input into PRIMER-E v.6.0 for multivariate analysis.

Draftsman's plots of the environmental variables across sites were created to look at the distribution of the data and subsequently environmental data (excluding depth) was log transformed. All environmental data were then normalised. The presence or absence of each holothurian family were entered as factors into Primer with present families scored as 1 and absent families scored as 0. Resemblance matrices based on Euclidean distance were created for the environmental data.

2.2.2.1 Principle Component Analysis

Principle Component Analysis (PCA) of the environmental data was carried out in PRIMER-E v.6 for bathyal, abyssal and hadal depth zones across all oceans and for the three depth zones within each ocean. Plots for each family were visualised by sorting the plot by factor (family). This could be used as a qualitative visual tool for assessing differences in the environmental distribution of each family.

2.2.2.2 ANOSIM analysis

In order to formerly test whether the environmental distribution of each family was statistically different from the environmental conditions experienced by the other families of the Elasipodida a one-way Analysis of Similarity (ANOSIM) was conducted in PRIMER-E v.6. ANOSIM is a non-parametric permutation procedure that is applied to the similarity matrix (in this case Euclidean distance computed from the environmental data). The ANOSIM computes an overall test statistic which reflects the overall differences between sites (or families, biomes, or oceans), contrasted with differences among replicates within sites (within families, biomes or oceans). The ANOSIM is based upon corresponding rank similarities, rather than on distances calculated in the resemblance matrices. This results in a test statistic 'R' which can lie in the range -1 to 1. R=1 if all replicates within sites are more similar to each other than any replicates from different sites. R= 0 if similarities between and within sites are the same and the null hypothesis is true. The test statistic is then recomputed under permutations of the sample labels (Clarke et al. 2006), in all cases here, where sample size allowed 999 permutations were carried out. Smaller permutation levels are reported. The significance of the R value is then calculated by comparing the actual value of R to the distribution of randomly-calculated R values in the permutation test.

2.2.2.3 Discriminant Function Analysis

Discriminate Function Analyses (DFA) were performed in SPSS v18 on log-transformed, normalised environmental variables. DFA is used to determine which variables discriminate between two or more naturally occurring groups. In this case it was used to determine which environmental variables discriminate between the four elasipodid families. DFA is computationally similar to ANOVA. The basic underlying idea is to determine whether the groups differ with regard to the mean of a variable, and then to use that variable to predict group membership (Statsoft 2012). The DFA then applies a multivariate significance test, which as in the case of a single variable compares the ratio of between-group variance in the data over the pooled within-group variance. If between-group variance is significantly larger then there must be significant differences between means. There are seven variables in this analysis (depth, POC flux, intra-annual seasonality, inter-annual variability, temperature, oxygen and salinity), resulting in a matrix of pooled within-group and covariance's (Statsoft 2012). These matrices are compared using multivariate F-tests (Wilks' lambda) to determine whether or not there are significant differences, i.e. it is a goodness of fit statistic. Where $p<0.05$ for Wilks lambda the multivariate test is a good fit for the data. In DFA for multiple variables, the number of discriminant functions calculated will be equal to the number of groups, minus one (three discriminant functions in this analysis of four families). DFA was performed for all oceans and for each ocean individually, for bathyal and abyssal depths, in the same manner as the ANOSIM analyses.

2.3 Biogeochemical zonation in the deep sea

2.3.1 Mapping and data layout

The shapefile containing the co-ordinates for Longhurst's biomes and provinces was downloaded from http://comlmaps.org. The shapefile was uploaded into ArcGIS v.10 and species distributions were added to the map. Using the 'Identify' tool in the ArcGIS toolbox, the name of the Longhurst biome and province that each holothurian record occurred in was extracted. Species occurrences were pooled by provinces and a similarity matrix of provinces based on Sorenson's similarity coefficient of presence and absence of elasipodid species was created. This was then repeated for genera.

2.3.2 Statistical analyses

2.3.2.1 Multiple Dimensional Scaling plots (MDS)

MDS plots based on the similarity matrices described above were constructed, and sorted by Ocean and by Longhurst's cases and biomes. This allowed a visualisation of any differences in species composition of the provinces which could then be tested using the ANOSIM methods described above.

2.3.2.2 Analysis of Similarity (ANOSIM)

In contrast to the analysis in Chapter 3, the analysis for Chapter 4 was based on matrices of species data, not environmental data. The 2-way ANOSIM was utilised to test for differences in species and generic differences in the composition of Longhurst's provinces between biomes, allowing for differences in Ocean and vice versa. A second ANOSIM was performed which tested for differences in species and generic differences in the composition of Longhurst's provinces between Longhurst's 'Cases', allowing for differences between Oceans and vice versa. As before, permutation tests were repeated 999 where possible. Smaller levels of permutation analysis are reported.

2.3.2.3 Hierarchical Clustering and Similarity Profile (SIMPROF) analysis

Simple agglomerative clustering based on group-average linking was performed on the same similarity matrices described above. This results in a dendrogram showing samples (or in this case provinces) grouped into successively smaller clusters of larger size with an associated similarity. The major difference between the clustering analysis and the ANOSIM analysis is that groups were identified *a priori* in the ANOSIM (Biome, Ocean, and Case) and specifically tested for. The cluster analysis is completely unstructured a priori and essentially lets the data tell its own story. A 'similarity profile' permutation was then applied to the cluster analysis looking for statistically significant evidence of genuine clusters. SIMPROF applies a permutation test at every node of the final dendrogram, testing to see if the group being subdivided has multivariate pattern.

2.3.2.4 Inverted cluster analysis

From the original dataset a second similarity matrix was built. Instead of calculating similarity in species or generic composition between provinces, similarities in distribution (in terms of the provinces they occupied) were calculated between species. CLUSTER analysis was performed, resulting in a dendrogram of species clusters. By referring to the maps of these species distributions, the geographic affinity of each group of species could be determined and compared to the multivariate pattern noted between provinces. Thus the species and genera responsible for relationships between provinces could be identified.

Chapter 3. Differences in the environmental distributions of four families of elasipodid holothurian; a global and ocean-scale analysis

3.1 Introduction

3.1.1 Niche theory and resource partitioning in deep-sea echinoderms

Environmental factors play important roles in controlling the distributions of individual species and even whole taxa. The ecophysiology of an organism determines its ability to cope with abiotic stresses such as cold temperatures or high pressure. Some of the most fundamental ideas about species distributional areas and how it related to their surrounding environment were proposed by Joseph Grinnell, nearly a century ago (Grinnell 1914, 1917). Hutchinson developed the concept of the niche into an abstract space, with axes corresponding to environmental factors that affect organismal performance; the niche is the mapping of population dynamics onto this space (Holt 2009). However Hutchinson also considered issues of species coexistence (Hutchinson 1957) and now numerous niche theories have expanded to include elements of competition, predation and parasitism. Most definitions, in their broadest sense, still identify the environments that allow a population to survive, differing on key points such as the types of variables used, the abstract objects constituting niches and the spatial and temporal scale of relevance (Soberon and Nakamura 2009).

According to the competitive exclusion principle, coexisting species must differ in their trophic niches when the environment is constant and homogenous, or when resources are limiting and competition occurs, otherwise patterns of resource use may overlap. Therefore co-existing species must; utilise the same resource, utilise the same resource in different ways, or utilise the same resource at a different time (Nagelkerken et al. 2006). Competition for food is thought to be a powerful evolutionary driver in the deep sea (Billett et al. 1988, Roberts and Moore 1997, Smith et al. 2008). Evidence suggests that differential utilisation of food resources in the food-limited deep sea occurs in elasipodid holothurians.

The Elasipodida are typically surface-living, highly mobile deposit feeders. This lifestyle is probably an advantage in the deep sea where food sources are likely to be patchily distributed (Massin 1982). Elasipodids typically feed on the upper 2mm of the sediment (Sibuet et al. 1982) leaving little or no trace (Massin 1982). This topmost layer of sediment is the most nutritive, possibly in organic carbon, but certainly in some proteins (Sibuet et al. 1982). Khripounoff and Sibuet (1980) identified 16 types of organic particle within the foregut of four species of elasipodid holothurian collected at bathyal and abyssal depths. The main nutritional sources identified were organo-mineral aggregates, faecal matter and organic incrustations on mineral compounds. Selection for the particles richest in bio-available compounds was found, with concentrations of organic carbon and nitrogen are four times and six times greater, respectively than that of the surrounding sediment (Khripounoff and Sibuet 1980).

3.1.2 Potential mechanisms for resource partitioning among the Elasipodida in a seemingly homogenous environment.

Subtle differences in tentacular structure between sympatric species may prevent competitive exclusion, and allow species co-existence through niche partitioning (Roberts and Moore 1997). Roberts and Moore (1997) therefore suggest tentacular diversity as a possible driver of diversity in deep-sea deposit feeders (*Fig. 3.1*).

Early studies involved interspecific comparisons of tentacle morphology to the type or size of particles present in the gut (Sokolova 1958, Hansen 1975, Khripounoff and Sibuet 1980). The size of particle ingested was thought to be linked to the nutritive value of that particle, as was gut fullness. Species thought to ingest food of low nutritive quality were generally expected to have fuller guts than more ‘selective’ species (Hansen 1975).

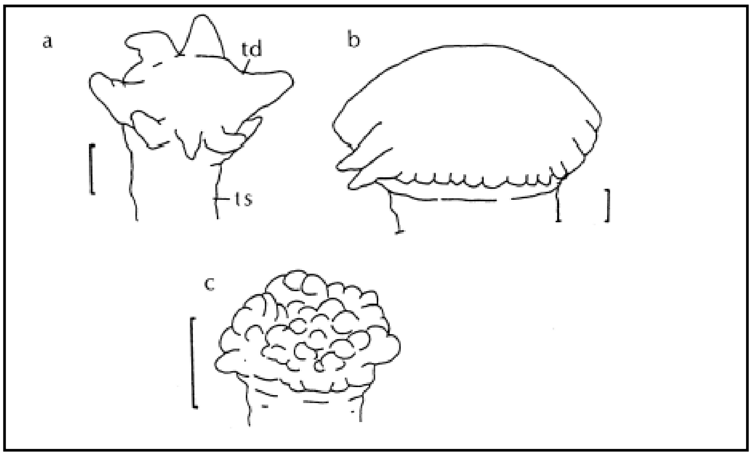


Figure 3.1 *Types of feeding tentacle in elasipodid holothurian a) digitate* Oneirophanta mutabilis*; b) peltate* Psychropotes longicauda*; c) peltate* Benthogone rosea*, from Roberts & Moore (1997).*

Another possibility for niche differentiation is that some species may target patches on the seafloor with rich bacterial floras Hansen (1975). This was corroborated by the findings of (Sibuet et al. 1982) who determined that bacterial abundance was higher in holothurian oesophageal contents than in the surrounding sediments. However distribution of bacterial activity profiles along the guts was not found to differ significantly between *P.longicauda* and *O.mutabilis* (Roberts et al. 2001). Evidence suggests that there are at least inter-familial differences in the bacterial composition of gut contents (Roberts and Moore 1997) and in the concentrations of certain enzymes, namely glycosidases, between individuals of different elasipodid families (Roberts et al. 2001).

Higher resolution information regarding resource partitioning in elasipodid holothurians has been provided by the use of pigment biomarkers. It has confirmed that selection for food quality, rather than quantity, is probably the main driver of niche partitioning in deep-sea echinoderms. Billett et al. (1988) conducted one of the first biomarker studies, and found that chlorophyll pigments have selection coefficients between 4 and 20. Wigham et al. (2003a) found differences in pigments from gut sediment profiles indicative of selective feeding in three elasipodid species from the PAP, and also found seasonal inter-species differences.

Wigham et al. (2003a) found no support for the link between selectivity and tentacle type. Instead they found species with very similar tentacle type to have very different pigment biomarker profiles, and species with very different tentacle morphology to have very similar pigment profiles. Instead Wigham suggests that the ability of a species to utilise a freshly deposited resource before it becomes mixed into the sediment as a potential mechanism for resource partitioning. FitzGeorge-Balfour et al. (2010) also used chlorophyll a to look at selectivity for fresh material in elasipodid holothurians at the PAP site in the north-east Atlantic. *Amperima rosea*, *Peniagone diaphana* (both Elpidiidae) and *Oneirophanta mutabilis* (Deimatidae) were all found to select for the freshest fraction of the sediment. When fresh material was scarce however, *O.mutabilis* was outcompeted and fed on more refractory material. The Psychropotid *Psychropotes longicauda* was found to be less selective, along with four non-elasipodid holothurians (FitzGeorge-Balfour et al. 2010). The concentrations of carotenoid pigments in the ovaries were also studied. The three species found to be selective feeders had high and consistent concentrations of carotenoids in the ovaries. *P.longicauda* and the non-elasipodid species had low and very variable carotenoid concentrations in the ovaries (FitzGeorge-Balfour et al. 2010). FitzGeorge-Balfour et al. (2010) suggested that the selectivity of a species is linked to its ‘feeding guild’ and this, in turn, is related to the organisms’ reproductive mode.

Indirect evidence of resource partitioning in deep-sea holothurians has also been provided by tracking elasipodid community changes at long-term time series. Unequal utilisation of food resources were highlighted by differential species responses to a changes in POC flux on an inter-annual time frame at Station M in the northeast Pacific (Ruhl 2008). Similar results have been found in the northeast Atlantic (Billett et al. 2001), suggesting the occurrence of niche separation between species. Furthermore Ruhl (2008) suggests that life history of the species involved has a 'partially deterministic' role in interspecific differences in response to changing food supply.

Iken et al. (2001) used stable isotope analysis to identify three 'trophic groups' of holothurian at the PAP which occupied different, but overlapping niches. The first of these groups (Group A) had low $\delta^{15}N$ values associated with feeding on fresh POM. It consisted of *Peniagone diaphana*, *Amperima rosea* and *Ellipinion molle*. This group is characterised by gelatinous body walls and a small body size, allowing them to be carried in the current, and for some (perhaps many) to actually swim. Iken et al. (Iken et al. 2001) hypothesised that this group was better adapted to foraging, finding fresh patches of POM more effectively even when flux to the seafloor was low. This was also supported by findings of the gut content analysis, previously discussed. Greater mobility would only be a benefit if phytodetritus was unevenly distributed, which is thought to be the case; it often gathers around mounds and depressions on the seafloor (Billett et al. 1983). Differences in species mobility have also been linked to resource partitioning in shallow water holothurian communities (Uthicke and Karez 1999). The second group identified by Iken et al. (2001) had higher $\delta^{15}N$ values, suggesting that more refractory material was being consumed. This group contained the larger species *Psychropotes longicauda* (Psychropotidae) *Deima validum validum* and *Oneirophanta mutabilis* (Deimatidae). It is thought that they have no swimming ability or reduced ability (*P.longicauda*) relative to the Elpidiidae, reducing their foraging ability. Also they may have increased enzymatic capabilities and/or bacterial flora which aids in the digestions of less fresh material. Finally Group C feed below the upper few millimetres, and some members are infaunal. Correspondingly they have the highest $\delta^{15}N$ values, confirming that these species are feeding on the most refractory of sedimentary material (Iken et al. 2001). Crucially, this discrimination was not linked simply to tentacular diversity but to a combination of factors including morphology (tentacle structure, body wall consistency, body extensions), mobility (buoyancy, swimming capability) and digestive properties (enzymatic activities, enteric bacteria) (Iken et al. 2001) (see *Fig.3.2*).

Clearly life history is an important aspect determining niche differentiation in the deep sea. The feeding guilds identified by Iken are well defined by the elasipodid families, with group A describing the morphology and behaviour of the Elpidiidae, and the Group B describing the morphology and behaviour of the Deimatidae and Psychropotidae.

3.1.3 Resource partitioning and reproduction in the elasipodid holothurians

There is now convincing evidence that a species ability to utilise food resources affects its reproductive capacity (Wigham et al. 2003a, Wigham et al. 2003b, FitzGeorge-Balfour et al. 2010). Reproduction and recruitment have been to be important in explaining temporal abundance dynamics which were in turn linked to changes in POC flux in the north east Pacific (Ruhl 2007).

As the feeding guild of a species is linked to its phylogeny, so is a species reproductive mode. The Elpidiidae tend to produce the smallest eggs ranging between 180μm and 400μm. The Psychropotidae tend to have the largest egg, between 1900 and 4mm. Some Psychropotid species are therefore thought to be direct developers. The Deimatidae and Laetmogonidae tend to produce eggs of intermediate size.

The size of the egg is also thought to link to the likelihood that a species reproduces seasonally. Seasonality is usually associated with eggs smaller than 200um and planktotrophic development. However, caution to general rules must again be expressed, as it has now been shown that some lecithotrophic deep-sea echinoderms do reproduce seasonally (Mercier and Hamel 2008).

There have been mixed results in the investigation of seasonality in reproduction in the elasipodids. The Elpidiidae have an opportunistic nature that may allow direct gametogenic response to pulses in food supply which allows them to thrive in physically unstable conditions (Galley et al. 2008). Early evidence for this came from the observation of large aggregations of the Elpidiid *Kolga hyalina* in the northeast Atlantic (Billett and Hansen 1982) (see also *Fig.3.3*).

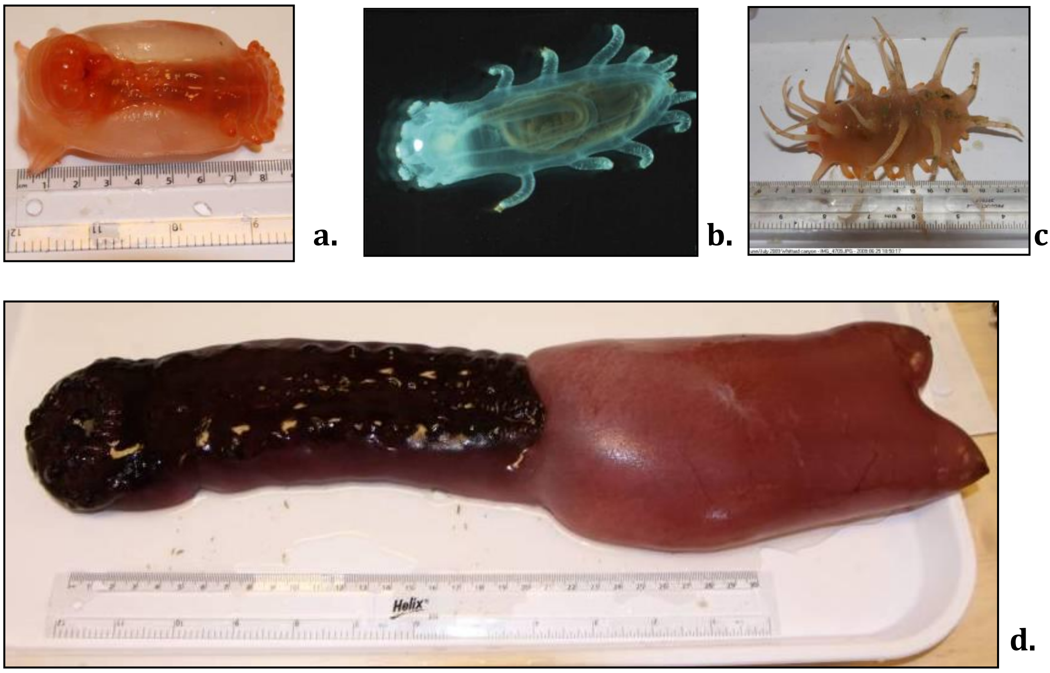


Figure 3.2 *Typical examples of members of Iken's group A; (a)* Peniagone *sp. from the Whittard Canyon, NE Atlantic and (b)* Kolga hyalina *from the mid-Atlantic ridge and Iken's group B; (c)* Deima validum *from the NE Atlantic and (d)* Psychropotes longicauda *from the NE Atlantic.*

Photo credits (a),(c),(d); Leighton Rolley; (b); *Encyclopædia Britannica Online*. Retrieved 16 March 2012, from http://www.britannica.com/EBchecked/media/139585

The elpidiid species *Protelpidia murrayi* and *Peniagone vignoni* displayed seasonal reproduction in response to large seasonal pulses of OMF on the Antarctic continental shelf. It is thought that the seasonal influx of food to *P.murrayi* results in synchronised gametogenesis followed by a future spawning period after some extended period of vitellogenesis. This would result in vitellogenesis in early winter, suggesting that sufficient food exists year-round, or that *P.murrayi* can store nutrients in its gonads or other body tissue.

Spawning for *P.murrayi* is thought to take place in June (Galley et al. 2008). *P.vignoni* on the other hand did not spawn all of the vitellogenic oocytes at once. Instead it displayed seasonality variability in the production of vitellogenic oocytes followed by a spawning period in October/November. Spawning is thought to continue until February/March (Galley et al. 2008). This may allow

P.vignoni juveniles to settle at the time of greatest OM flux, during January-March (Galley et al. 2008). Whilst such direct evidence is rare, the existence rapid changes of the abundance and size distribution of certain Elpidiid species such as *Amperima rosea* and *Ellipinion molle* in the north-east Atlantic suggest the ability to respond rapidly, if not strictly seasonally to large inputs of fresh organic matter (Billett et al. 2001, Wigham et al. 2003b, Billett et al. 2010). Similar dynamics have been shown in the north-east Pacific, with food-input related increases in abundance of certain elpidiid species being linked to a reduction in average body size. This is indirect evidence of a recruitment event linked to food input (Ruhl and Smith 2004, Ruhl 2007). It has been suggested that this 'boom-bust' reproductive strategy may be common in many other echinoderm species (Uthicke et al. 2009). However not all Elpidiidae display seasonality, the cogeners *Peniagone diaphana* and *Peniagone azorica* collected from the north-east Atlantic did not show any seasonality in their reproduction (Tyler et al. 1985b). Tyler and Billett (1988) recorded a maximum eggs size of 900µm in the Deimatidae. Accordingly with their relatively large egg size, the Deimatidae have low fecundity (Tyler and Billett 1988). There is no evidence of synchronous/seasonal breeding in the Deimatidae outside of the Gulf of Panama (Tyler and Billett 1988).

However significant differences in oocyte-size distribution and abundance in *Oneirophanta mutabilis* have been reported (Tyler and Billett 1988, Ramirez-Llodra et al. 2005) over longer time frames. These were thought to be related to the previously discussed '*Amperima* event' at the PAP and associated community changes (Ramirez-Llodra et al. 2005) which are thought to be driven by changes in the quality of food input (Billett et al. 2010).

In the Laetmogonidae Hansen (1975) noted an extremely wide range of egg sizes which were probably indicative of different reproductive modes within this species. In fact Hansen suggested that there may exist a correlation between egg size and depth of occurrence, but that this required further investigation (Hansen 1975).

Figure 3.3 *An aggregation of unidentified Elpidiid holothurians, www.bogleech.com.*

Indeed, in a study of two Laetmogonid species (*Laetmogone violacea* and *Benthogone rosea*) in the north-east Atlantic Tyler et al. (1985a) found interspecific variation in reproductive mode. *Laetmogone violacea* had a maximum egg size of 350µm whilst *Benthogone rosea*'s eggs reached 750µm. Tyler et al. suggested that *L.violocea* had an abbreviated larval stage, whereas *B.rosea* probably had direct development. Neither species displayed any evidence of seasonal reproduction (Tyler et al. 1985a).

The Psychropotidae generally have very large eggs, but some interspecific variation exists, which may be related to the depth of occurrence (Hansen 1975). Samples from the Galathea expedition suggest that lower bathyal-upper abyssal species such as *Psychropotes depressa and Psychropotes semperiana* may have smaller egg sizes (0.5-1.9mm) than truly abyssal species such as Psychropotes longicauda (4mm) (Billett and Hansen 1982).

The Psychropotids are therefore generally expected to have direct development. As predicted by their large egg size, the Psychropotidae have low fecundity (Tyler and Billett 1988). Direct development is generally thought to occur on the seafloor but it is thought that development of Psychropotid holothurians occurs in abysso and bathy-pelagic zones (Billett 1991). Although their eggs are smaller (although relatively still large) there is evidence that the bathyal Psychropotids also have direct development.

3.1.4. Niche conservatism and Hutchinson's duality; relating the niche to biogeographic space

The fact that species of the same family share more similar niches, occupy the same feeding guild and have similar reproductive strategies is unsurprising. Species often share ecological traits because of their shared evolutionary history so that niches of closely related species are often thought to be more ecologically similar than those of more distantly related species (Seligmann 1999, Hof et al. 2010). This is known as phylogenetic niche conservatism.

The relationship between a species niche and the geographic area it occupies is not always straightforward. Hutchinson severed all direct ties between the physical world and the abstract hyperspace in which he defined a species niche (Colwell and Rangel 2009). However, the concept of the niche is primarily useful when applicable to physical space so Hutchinson resolved the disjunction by defining a duality between niche space and 'biotope space'. Hutchinson described this duality by stating: "Any point in niche space can correspond to many points in the biotope, but not all points in niche space are represented in any given biotope" (Hutchinson 1978) (*Fig. 3.4*). Furthermore, the geographic range of a species reflects both its environmental tolerances and its geographical opportunities, now and in the past (Olalla-Tarraga et al. 2011). If, however, large-scale range limits are largely governed by slowly evolving environmental tolerances, then these limits will tend to be more similar among relates species (Olalla-Tarraga et al. 2011). Climatic niche conservatism has been defined as the tendency for species to retain aspects of their climatic niche over evolutionary time frames (Olalla-Tarraga et al. 2011).Whilst questions regarding the niche have been asked at a variety of taxonomic scales (Seligmann 1999) many studies have found that high values of among-species niche variance is explained at higher (family, order) taxonomic levels (Hof et al. 2010).

So far, studies of differences in the niches of elasipodid holothurians have been based on very small geographic scales, with much progress being made from time-series studies which focus on a single area. Here we aim to exploit Hutchinson's duality; by using existing data on species distribution in 'biotope space' (i.e. geographic occurrence records) and having environmental data to attribute to those occurrence records (Chapter 2) we can look at the distributions of the families in 'niche space' and ask whether there are differences between families in the environmental conditions occupied i.e. despite overlaps in distributions, do the elasipodid families have different realized niches at the global and ocean scale.

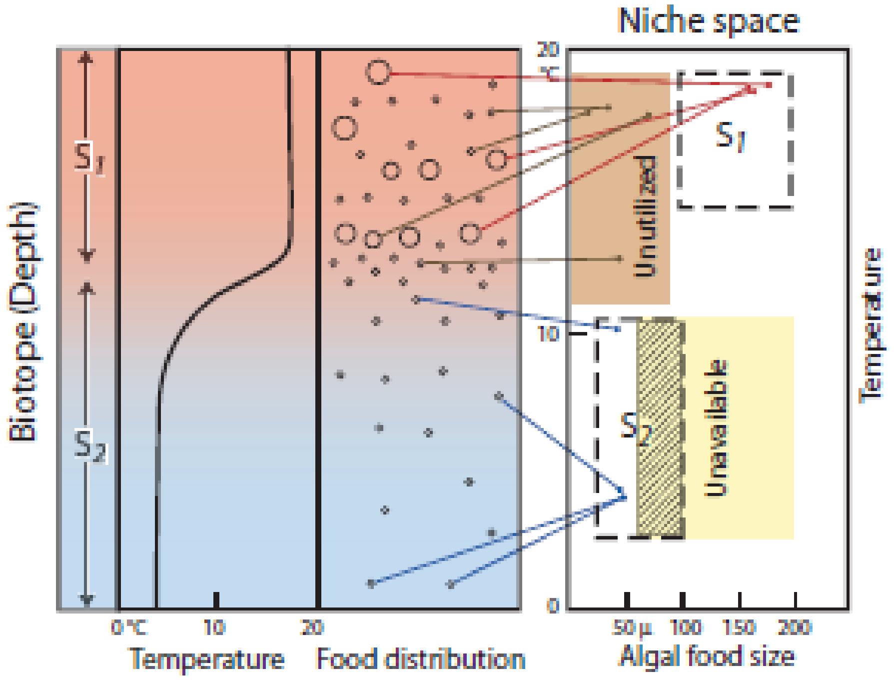


Figure 3.4 *The relationship between 'biotope space' and 'niche space' simplified for a temperate lake with algae and two different species of consumer (S_1 & S_2). The lake has a stratified water column and a strong summer thermocline. The two environmental factors which define the biotope are temperature and food size (both a function of depth) which correspond to the niche axes in niche space as shown on the right. It shows an unutilised niche in the biotope (small algae in warm water) and unavailable niche space that does not exist in the biotope (large algae in cold water. From (Colwell and Rangel 2009).*

3.2 Results

3.2.1 A brief overview of the geographical and vertical distributions of the four families of the elasipodid holothurians

The global geographical distributions (>500m) of the four families of elasipodid holothurian are shown in *Figures 3.5* (Deimatidae), *3.6* (Elpidiidae), *3.7* (Laetmogonidae) and *3.8* (Psychropotidae). The elasipodid family Pelagothuridae is excluded from this analysis. The distribution records used are from the database described in Chapter 2.

One of the most notable features of the distribution of the four families is that they overlap greatly. All families occur in all the major oceans. An exception is the Arctic Ocean where only the Elpidiidae are present. The Elpididiidae and the Psychropotidae are the best represented families in the database. The Laetmogonidae are important at bathyal depths on ocean margins but are generally absent from bathyal depths on ocean ridges in the central Atlantic and Pacific. Families are generally better represented on continental margins than in central oceanic regions. The reasons for this are considered in the discussion of this Chapter.

The Indian Ocean has fewer records than the other oceans. This is particularly noticeable for the Laetmogonidae and Deimatidae. Due to the small sample sizes available for the Indian Ocean it is therefore excluded from the individual Ocean analyses. Similarly hadal depths have fewer records than the bathyal and abyssal zones. Only two families have records at hadal depths; the Elpidiidae and the Psychropotidae. The Psychropotidae occur at hadal depths at fewer than five sites. Analysis of differences between depth zones within and between families were significant (results not shown). Therefore analyses were conducted for two seperate depth zones bathyal (500-3000m) and abyssal (3000-6000m). Due to low sample sizes hadal depths were excluded.

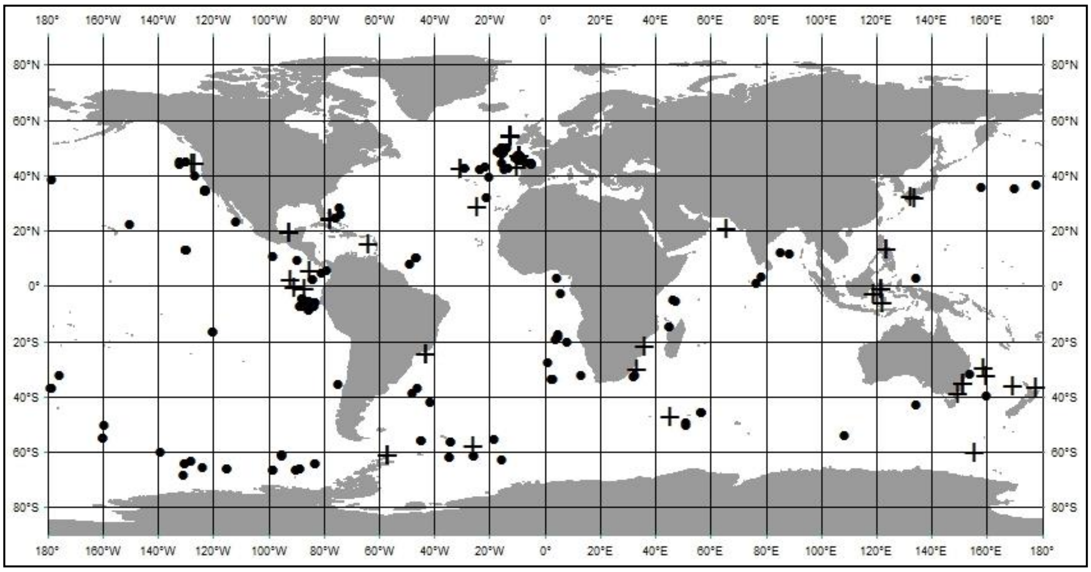


Figure 3.5 *The global distribution records of the family Deimatidae showing bathyal (500-3000m) records (crosses) and abyssal (3000-6000m) records (closed circles)*

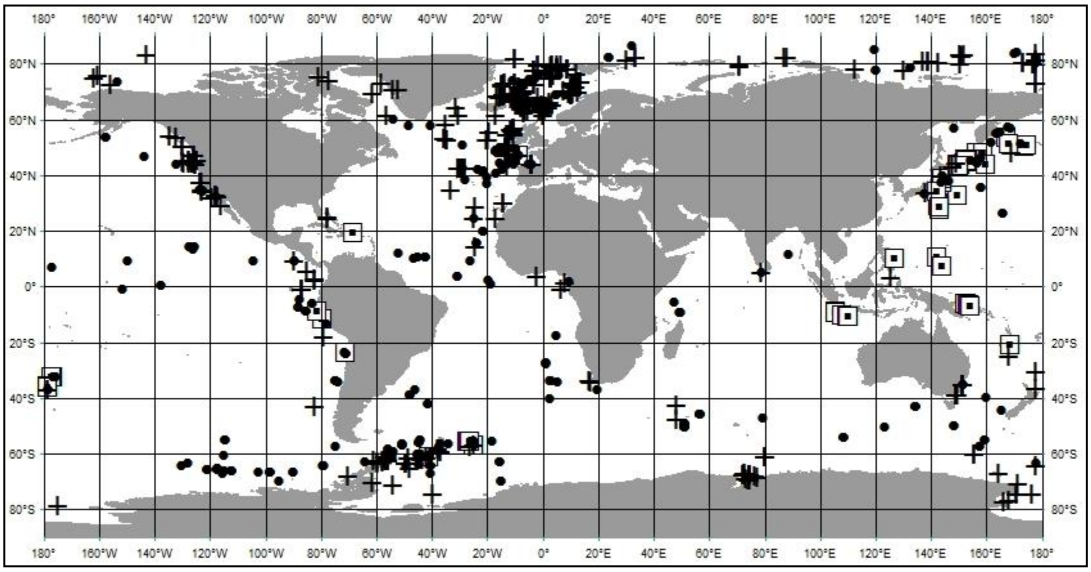


Figure 3.6 *The global distribution records of the family Elpidiidae showing bathyal (500-3000m) records (crosses), abyssal (3000-6000m) records (closed circles) and hadal records (6000-10,000m) (squares).*

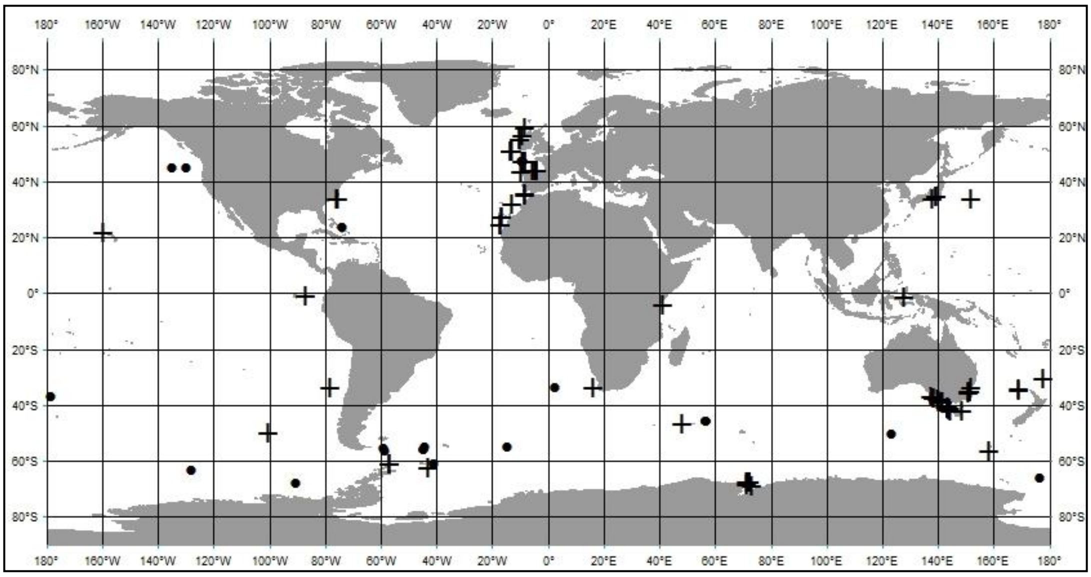


Figure 3.7 *Global distribution records of the family Laetmogonidae showing bathyal (500-3000m) records (crosses) and abyssal (3000-6000m) records (closed circles)*

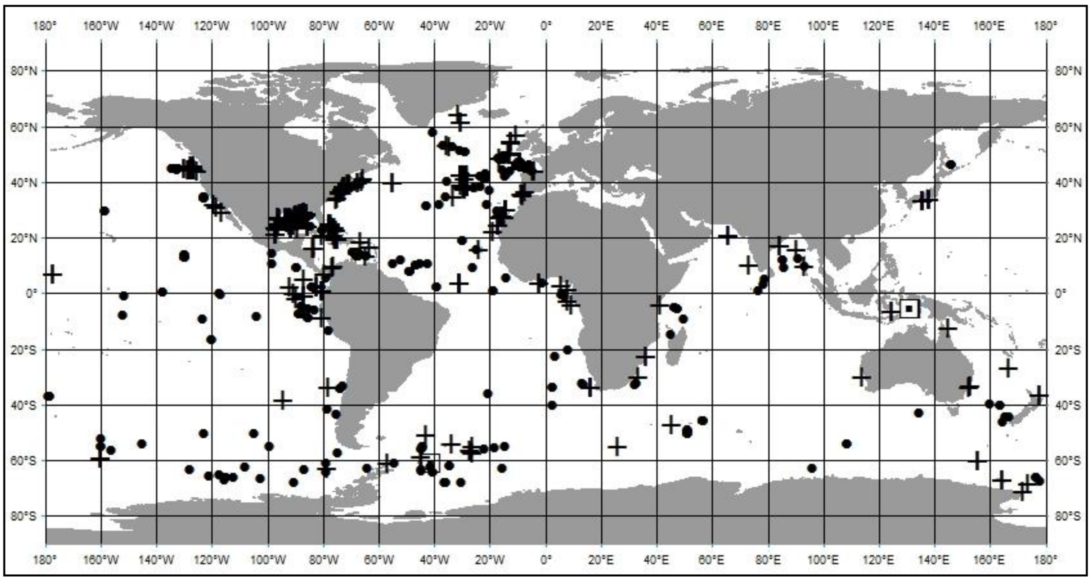


Figure 3.8 *The global distirbution records of the family Psychropotidae showing bathyal (500-3000m) records (crosses), abyssal (3000-6000m) records (closed circles) and hadal records (6000-10,0000m) (squares)*

3.2.2 The global environmental distributions of the four families of elasipdodid holothurians

3.2.2.1 Bathyal depths

The ANOSIM results found that the Laetmogonidae and Deimatidae had distributions that did not differ significantly from the distributions of the other families. In contrast ANOSIMs for the Elpidiidae ($p<0.005$) and Psychropotidae ($p<0.005$, *Table 3.1*) were significant. In the PCA plot the Elpidiidae dominated the left hand axis of the PCA plot, tending towards stronger intra-anuual seasonality, more oxygenated waters with lower temperatures. As well as these sites, records of the Elpidiidae were found throughout the range of conditions experienced by the elasipodids (*Fig. 3.9a*). The Psychropotidae were skewed towards the right hand side of the PCA plot, towards deeper sites with lower POC flux and weaker inter-annual variability (*Fig. 3.9b*). Their distribution was strikingly different to that of the Elpidiidae. PCA plots for the Deimatidae are found in *Fig. 3.9c* & *Fig. 3.9d*, Appendix A for a visual comparison.

The differences between the families was also confirmed by a statistically significant Discriminant Function Analysis (DFA) (Wilks' Lambda .483, $p<0.005$ (*Table 3.2.*). The first discriminant function (DF) accounted for 69.1% of the variance between families, the second and third DFs accounted for 27.9% and 3.1% of the variation respectively. Temperature (-.843) and intra-annual seasonality (.787) were the two variables with the greatest individual correlations with the first discriminant function (Table 3.2). The DFA plot for bathyal depths across all oceans (*Fig.3.10*) shows good separation between the Psychropotidae and Elpidiidae along the axis of the first DF, which supports the results of the PCA which showed the Elpidiidae tending towards colder highly intra-annual sites, and Psychropotidae towards less intra-annually variable sites.

The second DF correlated most closely with depth (.563) and POC flux (-.385) (*Table 3.2*) and could therefore be described as a measure of food quantity. The

DFA plot shows that the second DF separates the Laetmogonidae from the other families (*Fig. 3.11.a*), so that even within the bathyal zone, the Laetmogonidae have a significantly shallower mean depth.

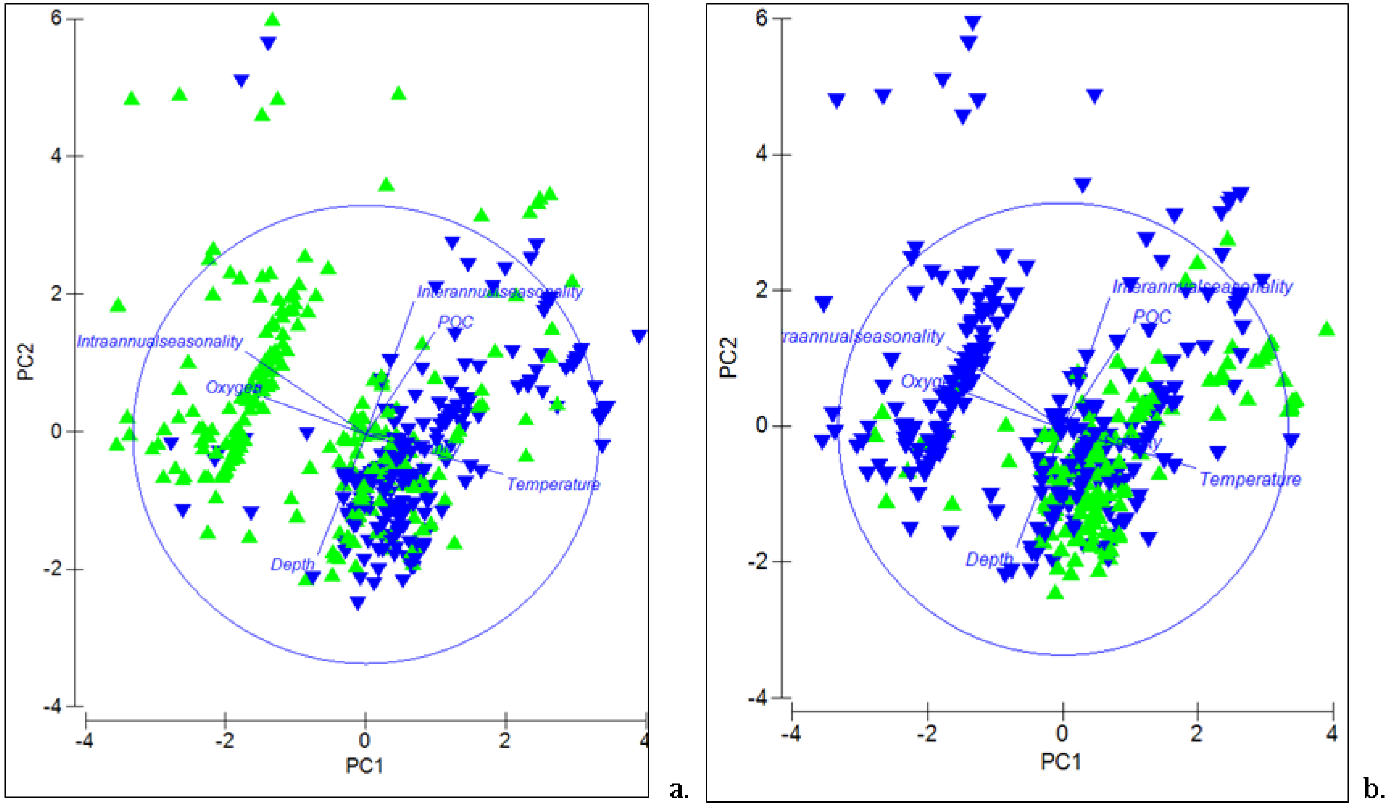


Figure 3.9 *Principle Component Analysis plots illustrating the environmental distribution of the four elasipodid holothurian families at* ***bathyal depths*** *(500-3000m) across* ***all oceans****. Distribution of all recorded elasipodid sites is shown in each figure with green triangles indicating the families presence at a site and a blue inverted triangle denoting the families' absence for; (a)* ***Elpidiidae*** *and (b)* ***Psychropotidae***

Table 3.1 *ANOSIM results comparing the distribution of each family against the environmental distirbution of the other families of the Elasipodida at bathyal abyssal depths across all Oceans*

Family	Depth zone	All Oceans	
		Global r	*p*
Deimatidae	**Bathyal**	-0.65	0. 82
	Abyssal	-0.033	0.99
Elpidiidae	**Bathyal**	**0.16**	**0.001***
	Abyssal	**0.027**	**0.001***
Laetmogonidae	**Bathyal**	-0.017	64.8
	Abyssal	0.024	36.5
Psychropotidae	**Bathyal**	**0.096**	**0.001***
	Abyssal	**0.033**	**0.001***

Table 3.2 *Discriminant Function Analysis (DFA) summary for bathyal and abyssal depths, across* ***all Oceans***

Depth zone	Wilks Lambda	Sig. (p)	DFA 1 % variation explained	DFA1 Largest absolute correlations	DFA 2 % variation explained	DFA 2 Largest absolute correlation
Bathyal	.483	.000*	69.1	**Temperature (-.843)**; intra-annual seasonality (.787);	19.1	**Depth (.563)**; POC (.-385)
Abyssal	.866	.000*	67.9	**Temperature (-.691);** intra-annual seasonality (.644)	24.5	**Depth (.601)**; POC (.585); salinity (.497); inter-annual variability (.301)

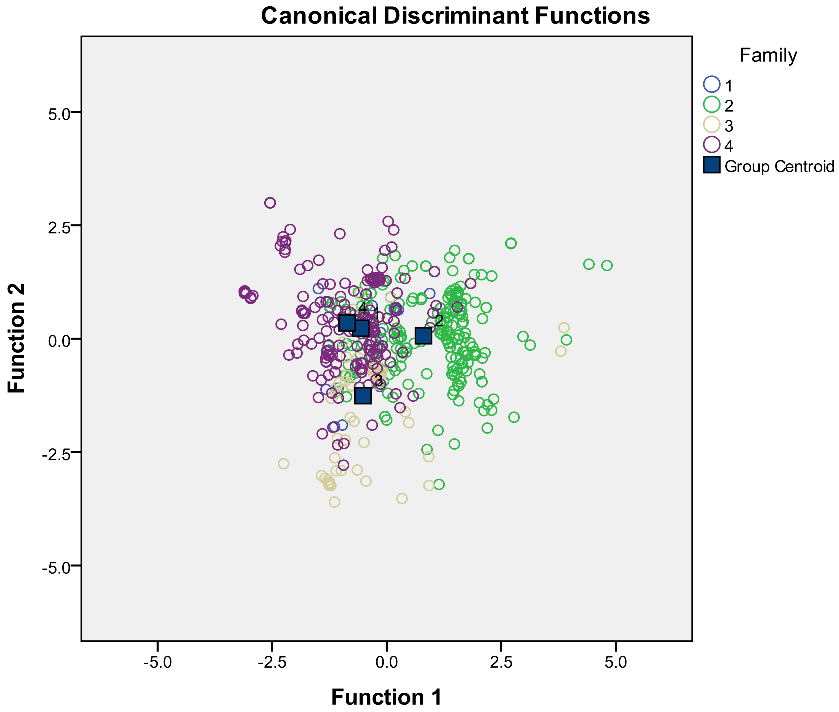


Figure 3.10 *Plot of the discriminant function scores (first and second DF) at each site for the four elasipodid families, where; (1)* Deimatidae*; (2)* Elpidiidae*; (3)* Laetmogonidae *and (4)* Psychropotidae *for **bathyal** depths for all oceans*

3.2.2.2 Abyssal depths

Similar results were found in the abyssal analysis. ANOSIMs for the Laetmogonidae Deimatidae were insignificant (*Table 3.1*). ANOSIMs for the Elpidiidae (p<0.005) and Psychropotidae were, once again, significant (p<0.005, *Table 3.1*). The PCA plot showed that the Elpidiidae were distributed throughout the PCA plot but also formed one group indicative of highly intra-annual, well oxygenated, sites that were not occupied by any of the other families (*Fig. 3.11a*). There was a tendency for the Elpidiidae to be positioned to the left of the PCA plot relating to deeper, lower POC flux sites with higher intra-annual seasonality (*Fig.3.11a*). The PCA plot for the Psychropotidae showed that the family displayed wide environmental tolerances (*Fig.3.11b*). However their distribution followed a similar trend to the one displayed at bathyal depths. Namely, having an almost opposite (though overlapping) environmental distribution to the Elpidiidae. They tended towards warmer sites with greater POC flux and stronger inter-annual variability. PCA plots for the Deimatidae and

Laetmogonidae are found in *Fig. 3.11c* and *Fig. 3.11d*, Appendix A. Results for the abyssal DFA were very similar to the results for the bathyal DFA (*Table 3.2*). Overall the environmental variables could discriminate between families (Wilks Lambda .866, $p<0.005$). 67.9 % of variation was described by the first discriminant function, with the second and third discriminant accounting for 24.5% and 7.6% of the variability respectively. Again temperature (-.691) and intra-annual seasonality (.644) were the factors which correlated most closely with the first discriminant function. The second discriminant function most closely correlated with depth (.601) and POC flux (.585) (*Table 3.2*). There were also smaller correlations with salinity and inter-annual variability. The third DF most closely correlated with oxygen. However, in contrast to the plot for bathyal depths (*Fig 3.10*) the families in the DFA plot for abyssal depths (*Fig. 3.12*) showed greater overlap.

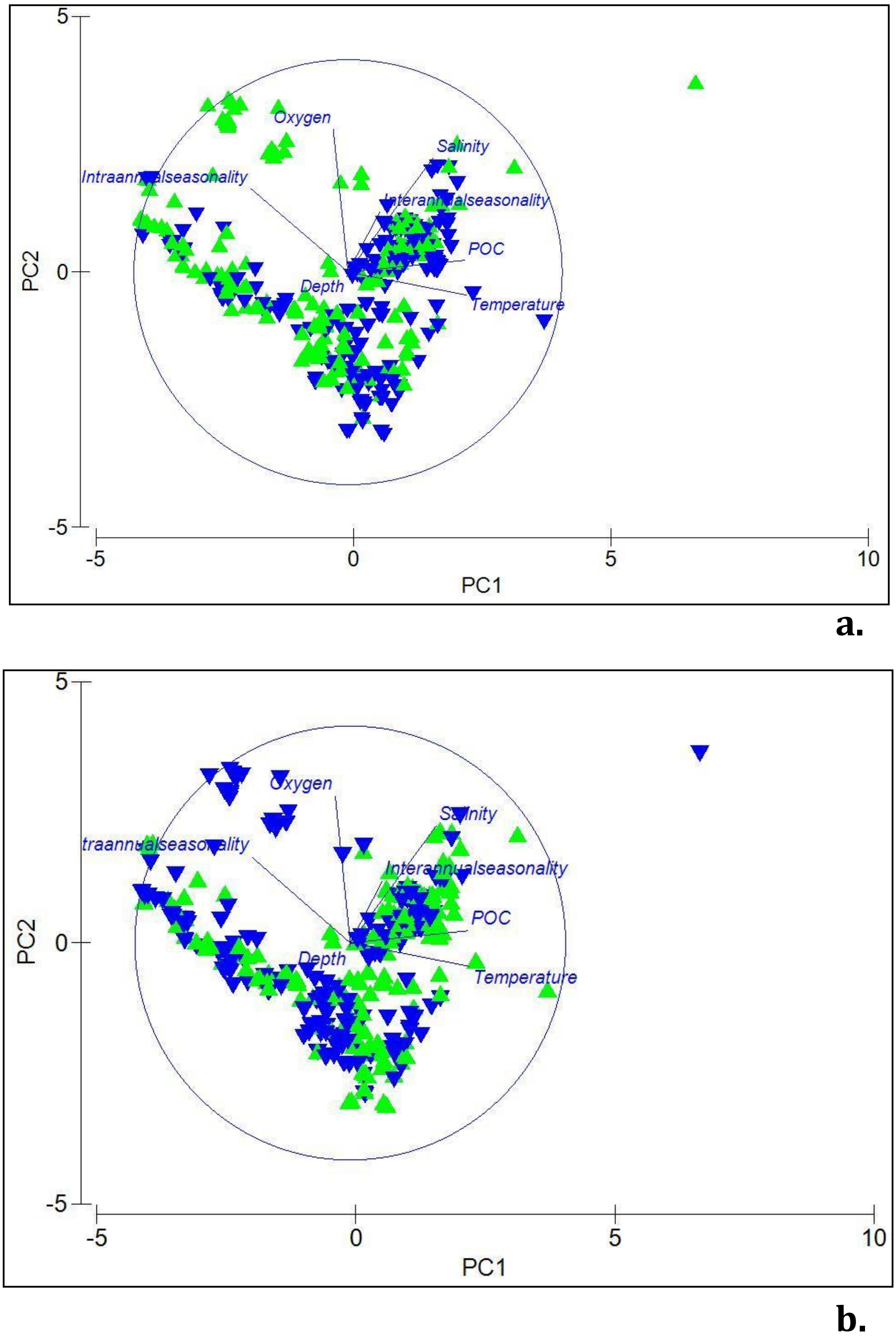


Figure 3.11 *Principle Component Analysis plots illustrating the environmental distribution of the four elasipodid holothurian families at* ***abyssal*** *depths (3000-6000m) across* ***all oceans****. Distribution of all recorded elasipodid sites is shown in each figure with green triangles indicating the families' presence at a site and a blue inverted triangle denoting the family's absence; (a)* **Elpidiidae** *(b)* **Psychropotidae**

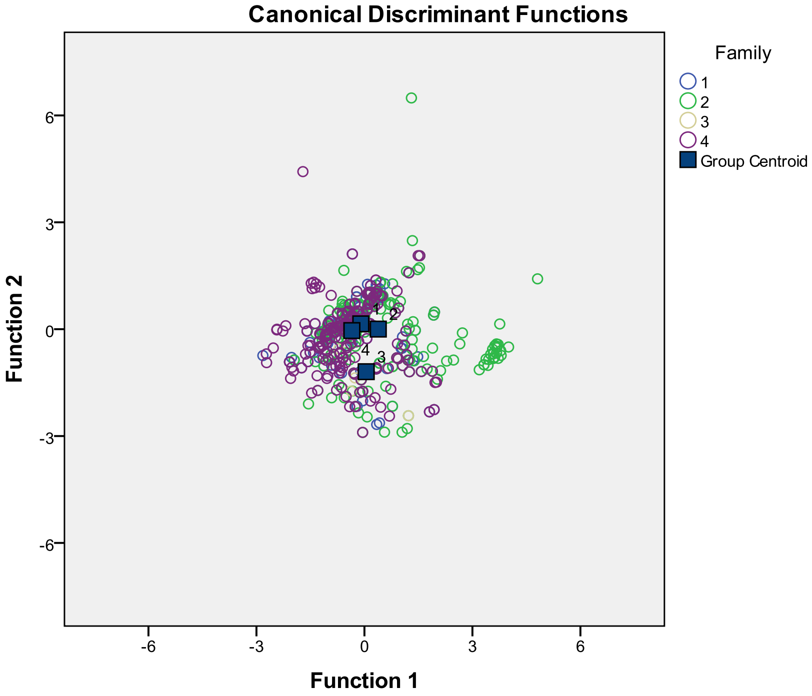
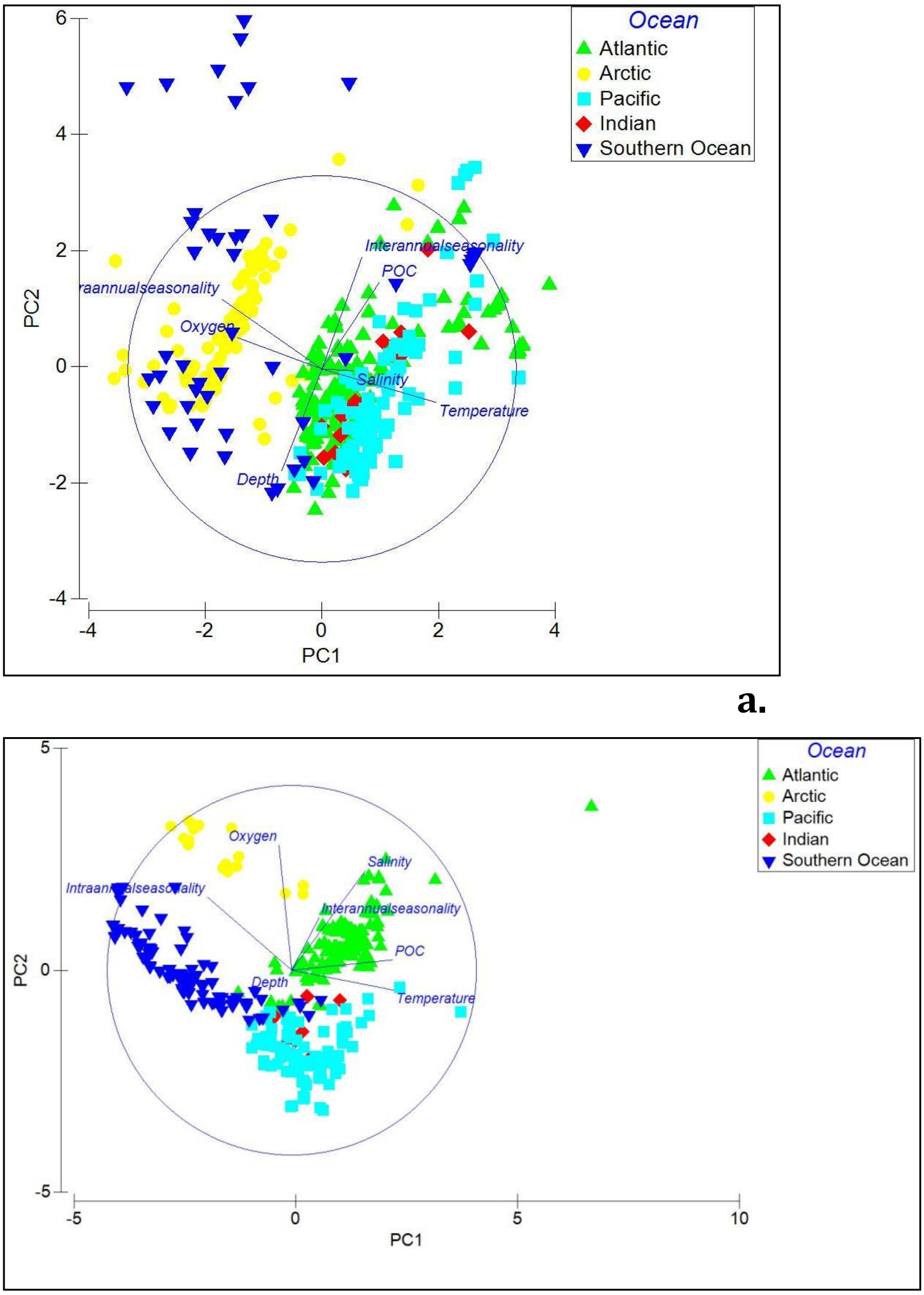


Figure 3.12 *Plot of the discriminant function scores (first and second DF) at each site for the four elasipodid families, where; (1)* Deimatidae*; (2)* Elpidiidae*; (3)* Laetmogonidae *and (4)* Psychropotidae *for* ***abyssal*** *depths for all oceans*

3.2.2.3 Geographic influence

When the PCAs of environmental variables experienced by the Elasipodida at bathyal (*Fig.3.13a*) and abyssal depths (*Fig.3.13b*) are sorted by Ocean there is a clear geographic signal. Separate oceans cluster together with very similar environmental conditions. The pattern is especially strong at abyssal depths (*Fig.3.13b*) where there is little overlap between the environmental conditions in each ocean.

Whilst the global analysis has considered the distribution of the Elasipodids out of geographical context, it is evident that geographical factors are influencing the results. In particular the high intra-annual seasonality and lower temperature which explain large proportions of the differences between families at bathyal and abyssal depths are driven by the existence of the Elpidiidae in the Arctic, and the absence of the other families in this region (see *Section 3.2.1*). Therefore analysis of data is next conducted for each ocean at bathyal and abyssal depths.



a.

b.

Figure 3.1 *Principle Component Analysis of the environmental variables experienced by the elasipodid holothurians across all oceans at (a) bathyal depths and (b) abyssal depths sorted by which ocean each site occurred in.*

Table 3.3 *ANOSIM results comparing the distribution of each family against the environmental distirbution of the other families of the Elasipodida at bathyal abyssal depths in the Atlantic, Pacific, Indian and Southern Oceans*

Family	**Depth zone**	**Atlantic**		**Pacific**		**Indian**		**Southern**	
		Global r	***p (%)***	***Global r***	***p (%)***	***Global r***	***p (%)***	***Global r***	***p (%)***
Deimatidae	**Bathyal**	0.027	33.2	-0.052	73.5	N/A	N/A	N/A	N/A
	Abyssal	**0.043**	**1.2***	0.004	39.4	-0.051	73	-0.057	96.9
Elpidiidae	**Bathyal**	-0.037	85.3	**0.078**	**1.4***	N/A	N/A	**0.098**	**2.0***
	Abyssal	**0.098**	**0.1***	**0.073**	**0.2***	0.156	11.4	0.02	24.4
Laetmogonidae	**Bathyal**	-0.0139	100	0.091	14.6	**0.253**	**1.6***	**0.356**	**0.2***
	Abyssal	N/A	N/A	N/A	N/A	N/A	N/A	-0.073	76.6
Psychropotidae	**Bathyal**	**-0.015**	**0.9***	-0.082	97.2	**0.158**	**4.5***	-0.169	99.5
	Abyssal	-0.015	86.9	**0.052**	**1.0***	0.079	21	**0.048**	**0.5***

Table 3.4 *Summary of the number of records included in the global analysis and analyses of individual oceans and depth bands. Ocean-depth combinations with fewer than five records are highlighted and are not included in detailed analyses*

Ocean	***All Oceans***		***Atlantic***		***Pacific***		***Indian***		***Southern***	
Family	*Bathyal*	*Abyssal*	*Bathyal*	*Abyssal*	*Bathyal*	*Abyssal*	*Bathyal*	*Abyssal*	*Bathyal*	*Abyssal*
Deimatidae	34	192	14	99	14	35	**2**	9	**3**	33
Elpidiidae	261	287	58	119	48	47	**2**	5	27	64
Laetmogonidae	69	16	38	**4**	11	**1**	7	**0**	10	10
Psychropotidae	179	263	123	140	33	46	7	12	11	49
Total	543	758	233	362	106	129	18	26	51	156

Table 3.5 *Discriminant Function Analysis summary for bathyal and abyssal depths in the Atlantic, Pacific, Indian and Southern Ocean*

Ocean	**Depth zone**	**Wilks Lambda**	**Sig.**	**DFA 1** % variation explained	**DFA1** Largest absolute correlations	**DFA 2** % variation explained	**DFA 2** Largest absolute correlation
Atlantic	Bathyal	**.474**	**.000***	71.3	**Intra-annual seasonality (.712);** temperature (.472); oxygen (.363)	24.6	**Depth (.347)**
	Abyssal	**.763**	**.000***	53.8	**Oxygen (.575);** Salinity (.564); temperature (.466); intra-annual seasonality (-.383)	40.0	**Depth (.705);** inter-annual variability (-.284)
Pacific	Bathyal	**.444**	**.000***	76.2	**Depth (.694)**; oxygen (.441)	20.9	**POC (.845)**; inter-annual variability (.663); intra-annual seasonality (.592); salinity (.522)
	Abyssal	**.761**	**.042***	86.9	**Depth (.762)**; temperature(.639); intra-annual seasonality(.543); oxygen (.489)	9.0	**Salinity (.596)**
Indian	Bathyal	.103	.200	-	-	-	-
	Abyssal	.682	.906	-	-	-	-
Southern	Bathyal	**.433**	**.016***	76.8	Salinity (.535); POC (.643)**	18.8	**Inter-annual variability (.888)**; oxygen (.770); POC (.732);temperature (.616)
	Abyssal	.892	.707	-	-	-	-

3.2.3 The Atlantic Ocean

3.2.3.1 Bathyal Atlantic (500-3000m)

When Atlantic records alone are considered (excluding any samples North of the Iceland Faeroe Ridge) the ANOSIM for the Deimatidae, Elpidiidae and Laetmogonidae are not significant ($p>0.05$, *Table 3.3*). Only the Psychropotidae have a markedly different distribution to the rest of the Elasipodida at bathyal depths in the non-Arctic Pacific ($p<0.01$, *Table 3.3*). On the left hand side of the PCA plot for the Psychropotidae (*Fig.3.14*) there is a cluster of sites associated with increased inter-annual variability and warmer bottom temperatures. These sites also have reduced intra-annual seasonality and POC flux relative to the other sites. Most of these sites are associated only with the Psychropotidae. However the Psychropotidae also inhabit a broad range of POC flux and intra-annual seasonality as they are also well represented in the right hand side of the plot. PCA plots for the Deimatidae, Elpidiidae and Laetmogonidae are found in *Fig. 4.14a,c* & *d,* Appendix A for visual comparison.

The DFA supports the results of the PCA and ANOSIM analysis. The first discriminant function is very similar to the first DF in the Atlantic analysis which included the Arctic data. DF1 explains 71.3% of the variation between families and is associated with intra-annual seasonality, temperature and oxygen (Table 3.5). The second DF explained 24.6 of the variation between families and was closely associated with depth (*Table 3.5*). *Figure 3.23* shows that the first DF discriminates between the Elpidiidae and Psychropotidae whilst the second DF seperates the Laetmogonidae from the other families. Intra-annual seasonality, which was the best correlate with the first DF, is very successful at discriminating between the Elpidiidae and the Psychropotidae (*Fig. 3.15*). However, it is much more difficult to see which families depth (the best correlate with the second DF) discriminates between (*Fig. 3.15*).

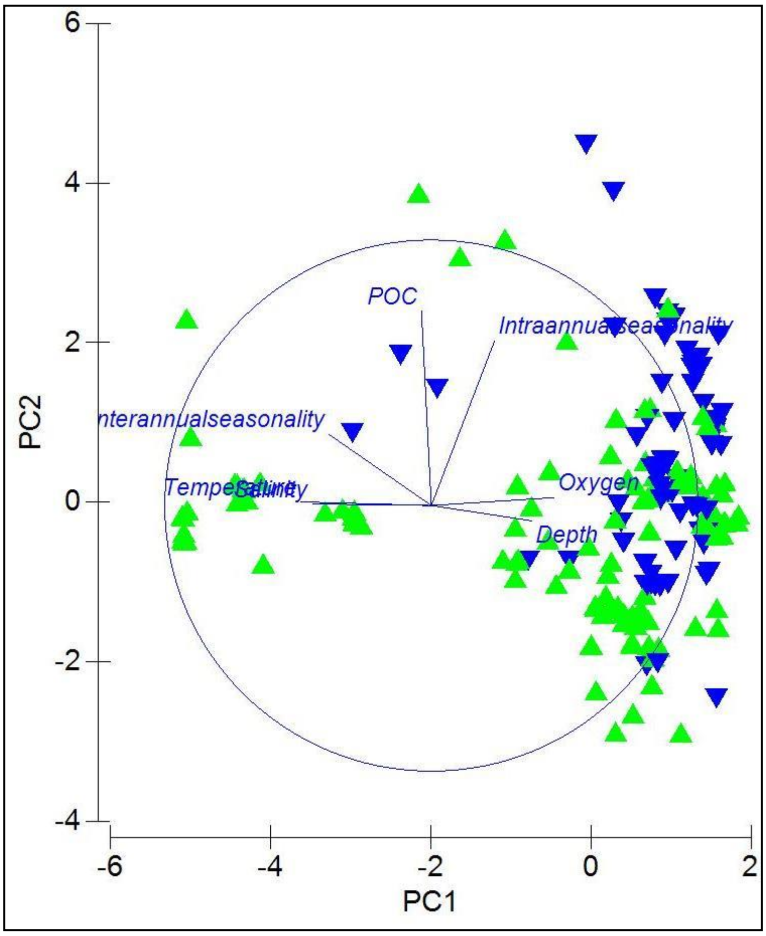


Figure 3.14 *Principle Component Analysis plots illustrating the environmental distribution of the four elasipodid holothurian families at* ***bathyal*** *depths (500-3000m) in the* ***Atlantic Ocean****. Distribution of all recorded elasipodid sites is shown in each figure with green triangles indicating the families' presence at a site and a blue inverted triangle denoting the family's absence for the* ***Psychropotidae***

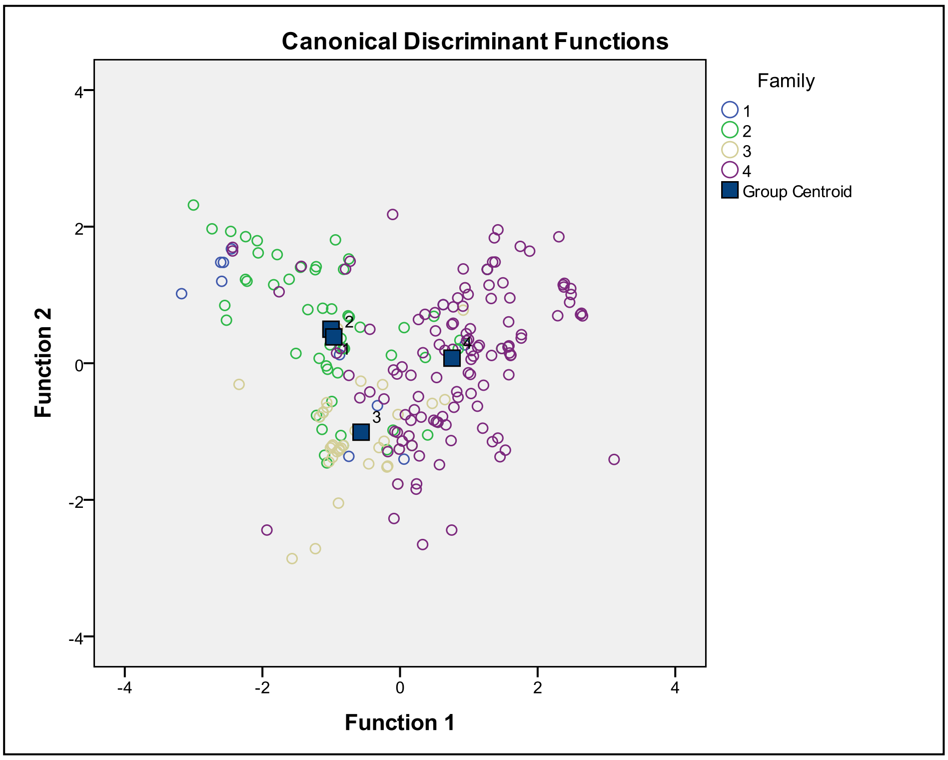


Figure 3.15 *Plot of the discriminant function scores (first and second DF) at each site for the four elasipodid families, where; (1)* Deimatidae*; (2)* Elpidiidae*; (3)* Laetmogonidae *and (4)* Psychropotidae *for* ***bathyal*** *depths in the* ***Atlantic Ocean***

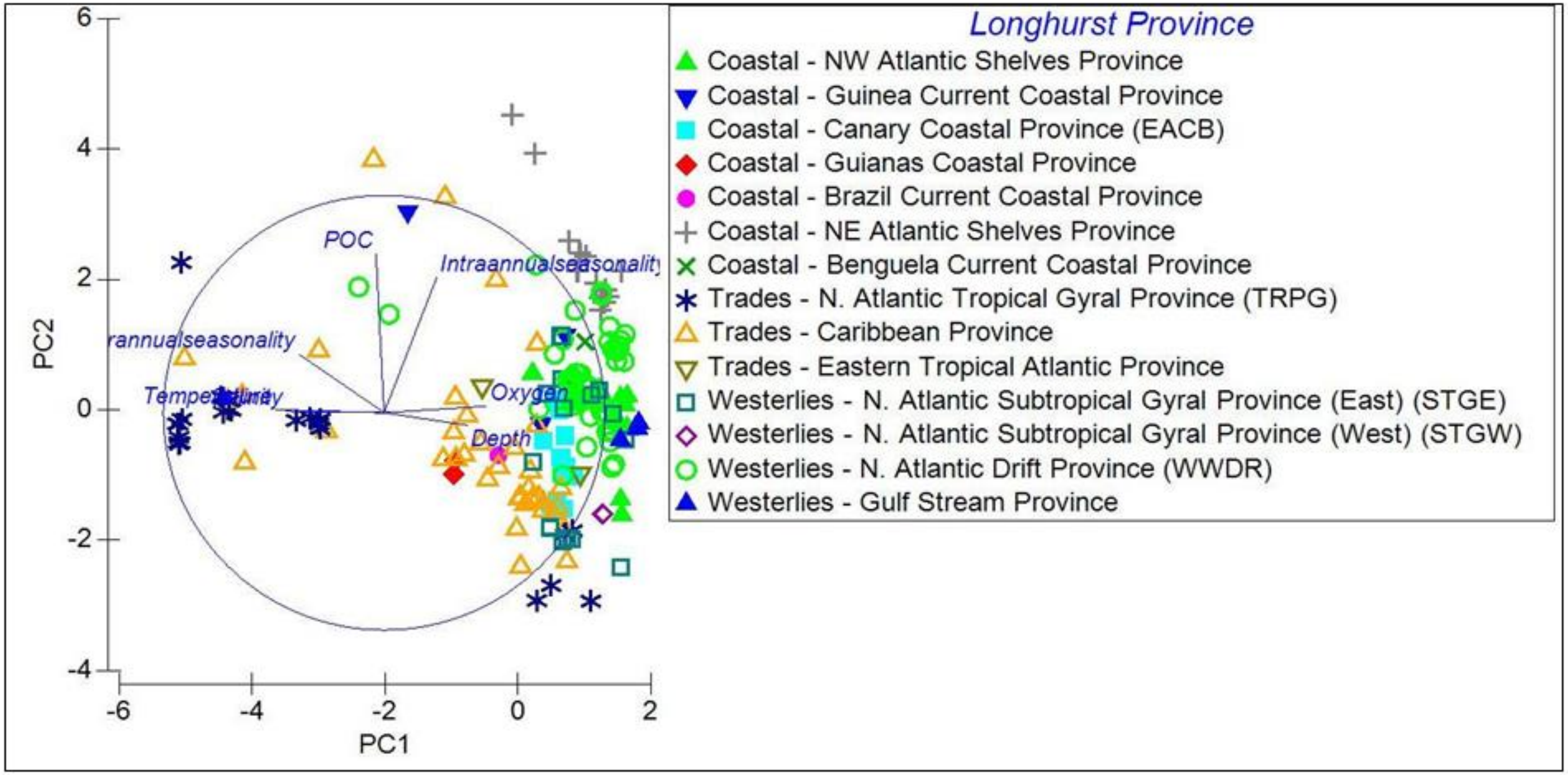
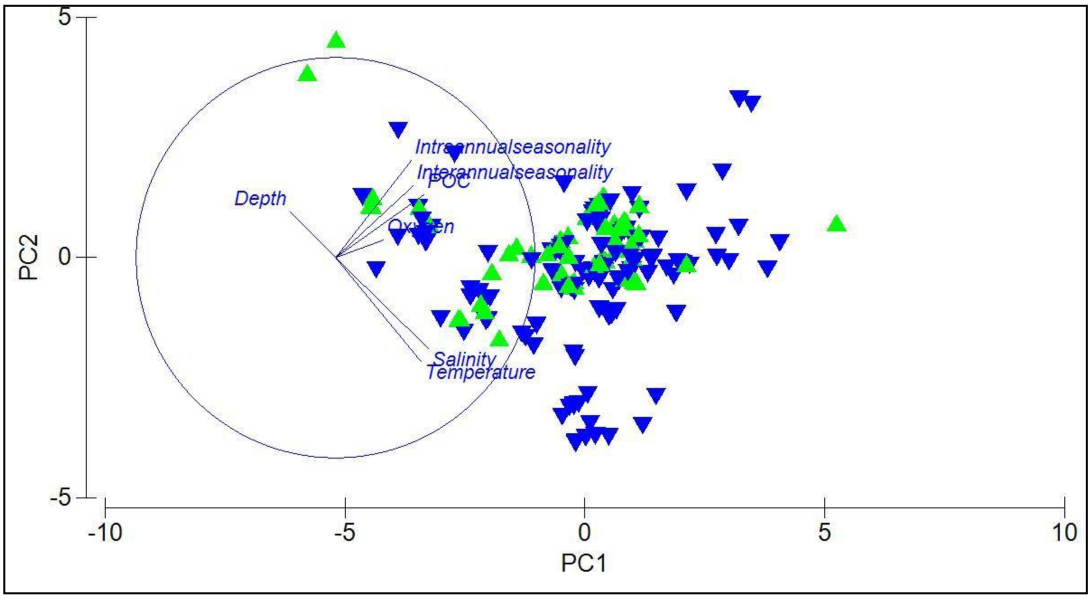


Figure 3.16 *Principle Component Analysis of the environmental variables experienced by the elasipodid holothurians at* ***bathyal*** *depths in the* ***Atlantic Ocean, minus Arctic records*** *sorted by Longhurst's Provinces*

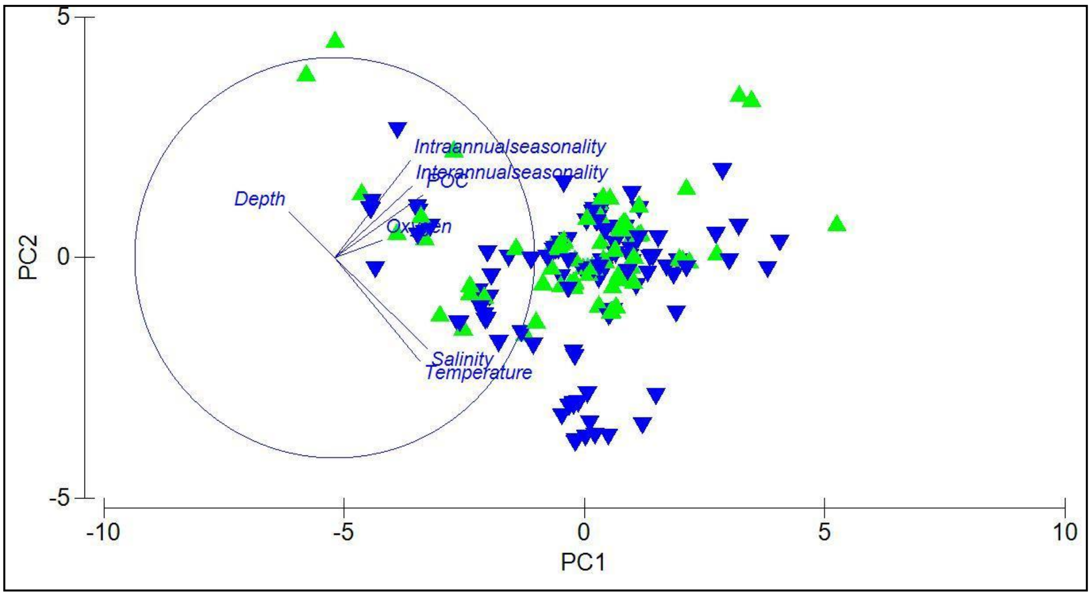
3.2.3.2 Abyssal Atlantic (3000-6000m)

In contrast to the results at bathyal depths, in the abyssal Atlantic the Deimatidae ($p<0.05$, *Table 3.3*) and Elpidiidae ($p<0.005$, *Table 3.3*) have significant ANOSIMs whilst the ANOSIM for the Psychropotidae was insignificant ($p>0.05$, *Table 3.3*) . The Laetmogonidae had very few records at abyssal depths (*Table 3.4*). The PCA plots for the Deimatidae shows that in relation to the distribution of the other families they were skewed towards the upper left of the PCA plot for abyssal non-Arctic Atlantic sites (*Fig. 3.17a*). Whilst the environmental axis are not easy to interperate, these sites appear to be related to deeper sites with higher POC flux and stronger intra-annual seasonality and inter-annual variability, lower temperature and salinity relative to the cluster of sites in the bottom right of the graph. The environmental distribution of the Elpidiidae was extremely similar to that of the Deimatidae at abyssal depths (*Fig. 3.17b*). They were skewed towards the right hand side of the plot, towards deeper, more oxygenated waters with stronger intra-annual seasonality. PCA plots for the Laetmogonidae and Psychropotidae are found in *Fig. 3.17c* and *3.17d*, Appendix A for a visual comparison.

The DFA was statistically significant (Wilks Lambda $p<0.001$), confirming that the environmental variables could be used to discriminate between the families (*Table 3.5*). The first DF explained only 53.8% of variation and correlations with environmental variables were weak; oxygen (.575), temperature (.466) and intra-annual seasonality (-.383). These are the same variables that were important at bathyal depths. Salinity was also correlated with the first DF at abyssal depths (.564). The second DF explained a further 40% of variation between the families. It is also closely associated with depth inter-annual variability at abyssal depths. The plot of the scores of the first two DFs for the four families (*Figure 3.18*) shows that the Elpidiidae and Psychropotidae are best seperated along DF1. Discerning which families are seperated by DF2 is difficult to visualise.



a.



b.

Figure 3.17 *Principle Component Analysis plots illustrating the environmental distribution of the four elasipodid holothurian families at* ***abyssal*** *depths (3000-6000m) in the* ***Atlantic Ocean****. Distribution of all recorded elasipodid sites is shown in each figure with green triangles indicating the families' presence at a site and a blue inverted triangle denoting the family's absence for; (a)* **Deimatidae***; (b)* **Elpidiidae**

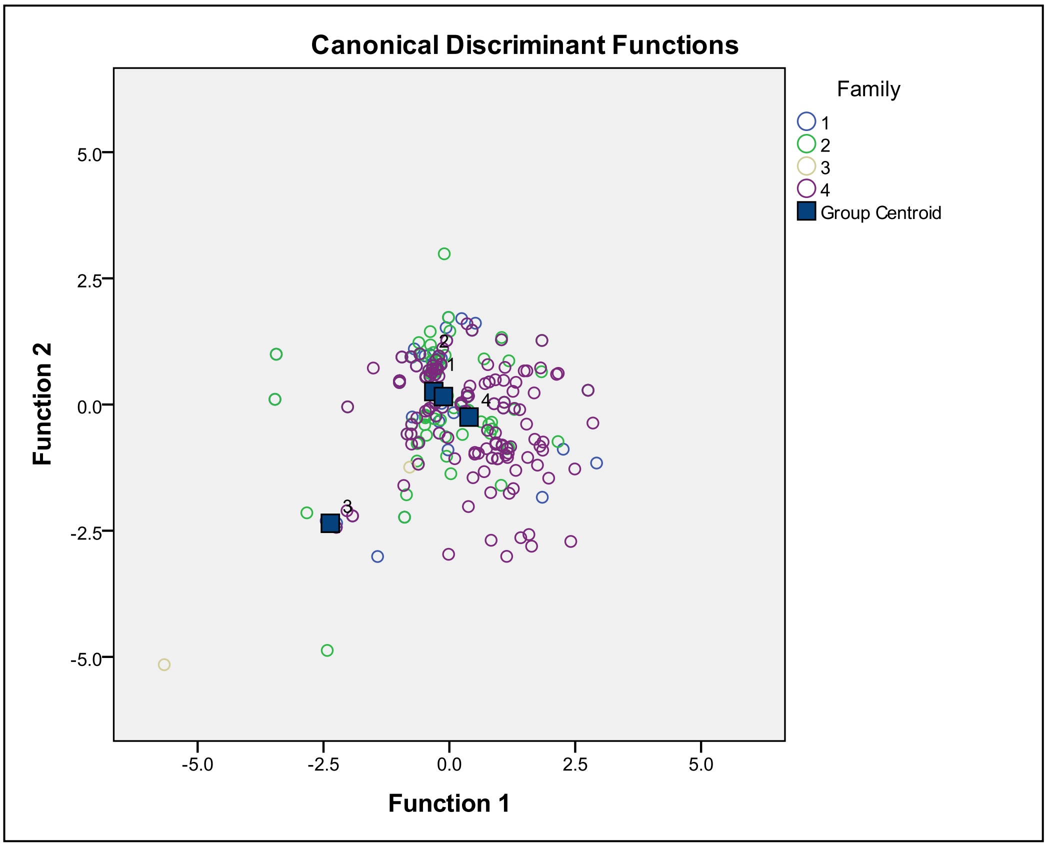


Figure 3.18 *Plot of the discriminant function scores (first and second DF) at each site for the four elasipodid families, where; (1)* Deimatidae*; (2)* Elpidiidae*; (3)* Laetmogonidae *and (4)* Psychropotidae *for **abyssal** depths in the **Atlantic Ocean***

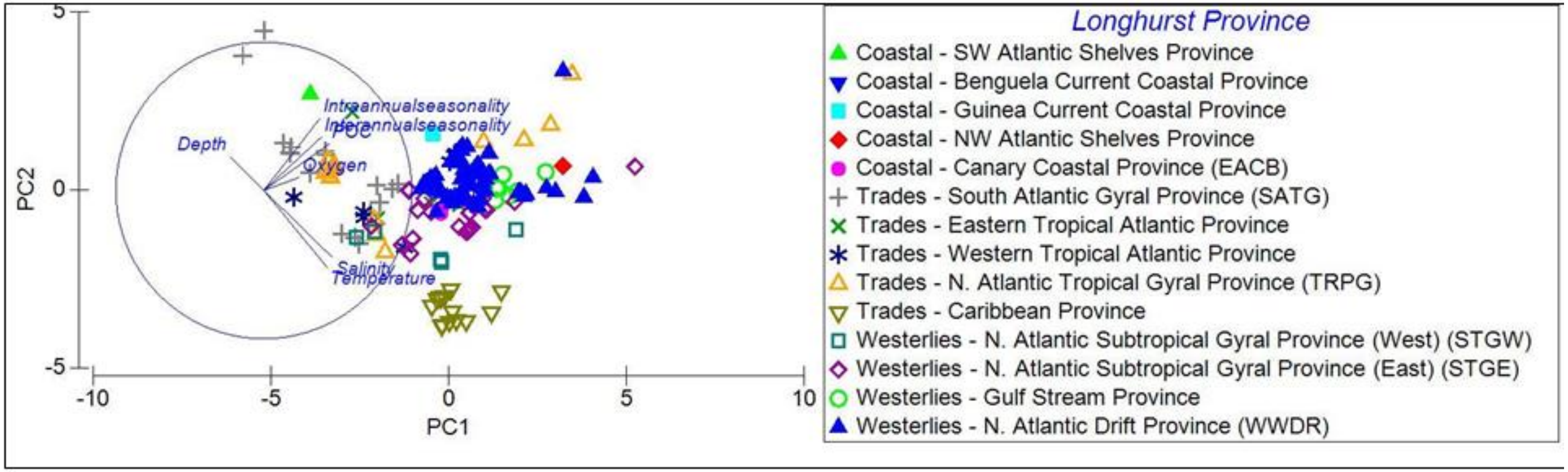


Figure 3.19 *Principle Component Analysis of the environmental variables experienced by the elasipodid holothurians at* ***abyssal*** *depths in the* ***Atlantic Ocean, minus Arctic records*** *sorted by Longhurst's Provinces*

3.2.3.3 Geographic context in the Atlantic

Figure 3.16 shows the bathyal PCA plot sorted by Longhurst The outlying cluster on the left hand side of the PCA plot that are, with the exception of a few sites, occupied by the Pschropotidae only represen two provinces 1) the North Atlantic Tropical Gyral Province and 2) the Caribbean province (*Fig3.29b*).

Figure 3.19 identifies the outlying cluster of shallower warmer, more saline sites are located in the Caribbean province are occupied only by the Psychropotidae. Sites from other provinces within the trade biome are occupied by the Elpidiidae and Laetmogonidae.

3.2.4 The Pacific Ocean

3.2.4.1 Bathyal Pacific (500-3000m)

At bathyal depths in the Pacific, the ANOSIM results showed that only the Elpidiidae had a significantly different distribution to the other families ($p<0.05$, *Table 3.3*). Relative to bathyal depths in the Atlantic the Elpidiidae are more evenly distributed throughout the PCA plot (*Fig. 3.20*). However the family did occupy a number of sites with greater POC flux and stronger intra-annual seasonality and inter-annual variability not occupied by the other families. PCA plots for the Deimatidae, Laetmogonidae and Psychropotidae are provied in *Figures 3.20b*, *c* and *d*, Appendix A, for a visual comparison.

Although the ANOSIM only detected differences among the Elpidiidae vs. All other families DFA was still significant (Wilks Llambda $p<0.001$) (*Table 3.5*). The first DF was responsible for 76.1% of between group variations. The DF correlated most closely with depth (.694) and oxygen (-.441). The second DF explained 20.9% of the variation and best correlated with POC flux (.845), inter-annual variability (.663) and intra-annual seasonality (.592). The DFA plot (*Fig. 3.21*) shows that the first DF (depth, oxygen) discriminates the Laetmogonidae from the other families. The lack of significance in the ANOSIM for the Laetmogonidae may therefore have been related to sample size (*Table 3.4*). The second DF separates the outlying Elpidiidae values from the other families.

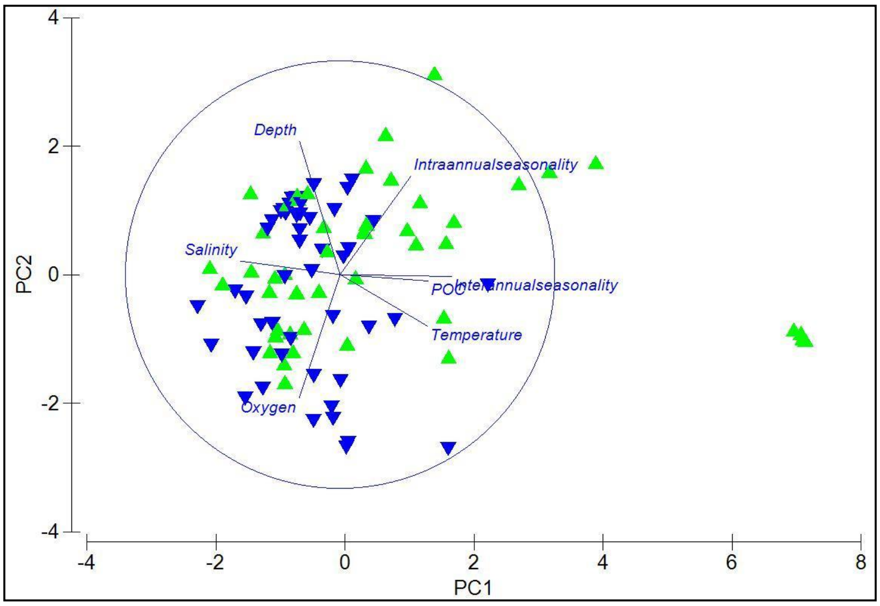


Figure 3.20 *Principle Component Analysis plots illustrating the environmental distribution of the four elasipodid holothurian families at* **bathyal** *depths (500-3000m) in the* ***Pacific Ocean****. Distribution of all recorded elasipodid sites is shown in each figure with green triangles indicating the families' presence at a site and a blue inverted triangle denoting the family's absence for the* **Elpidiidae**

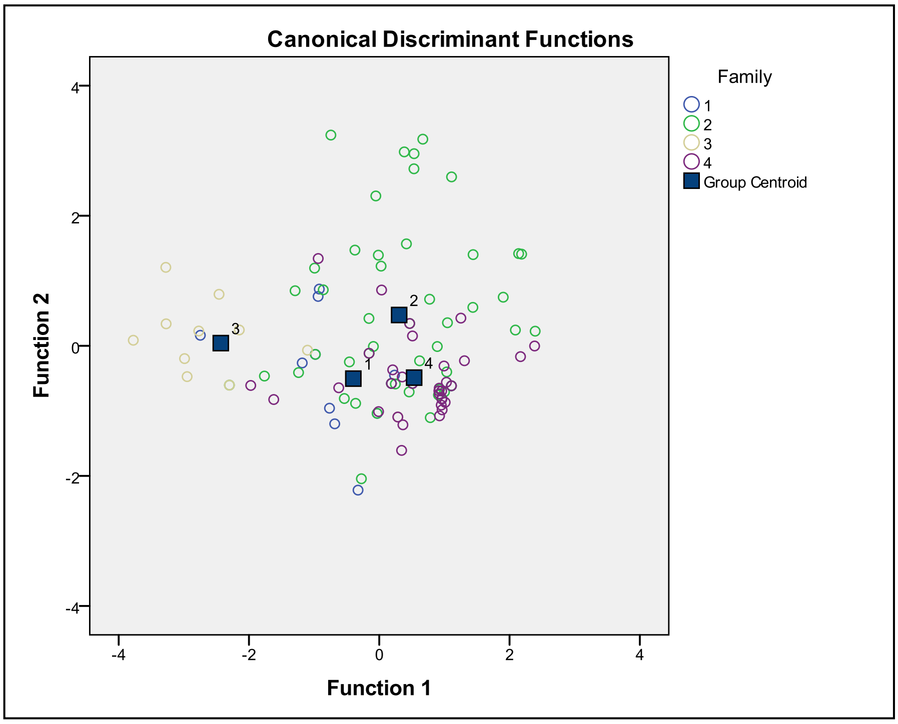


Figure 3.21 *Plot of the discriminant function scores (first and second DF) at each site for the four elasipodid families, where; (1)* Deimatidae; *(2)* Elpidiidae; *(3)* Laetmogonidae *and (4)* Psychropotidae *for* **bathyal** *depths in the* ***Pacific Ocean***

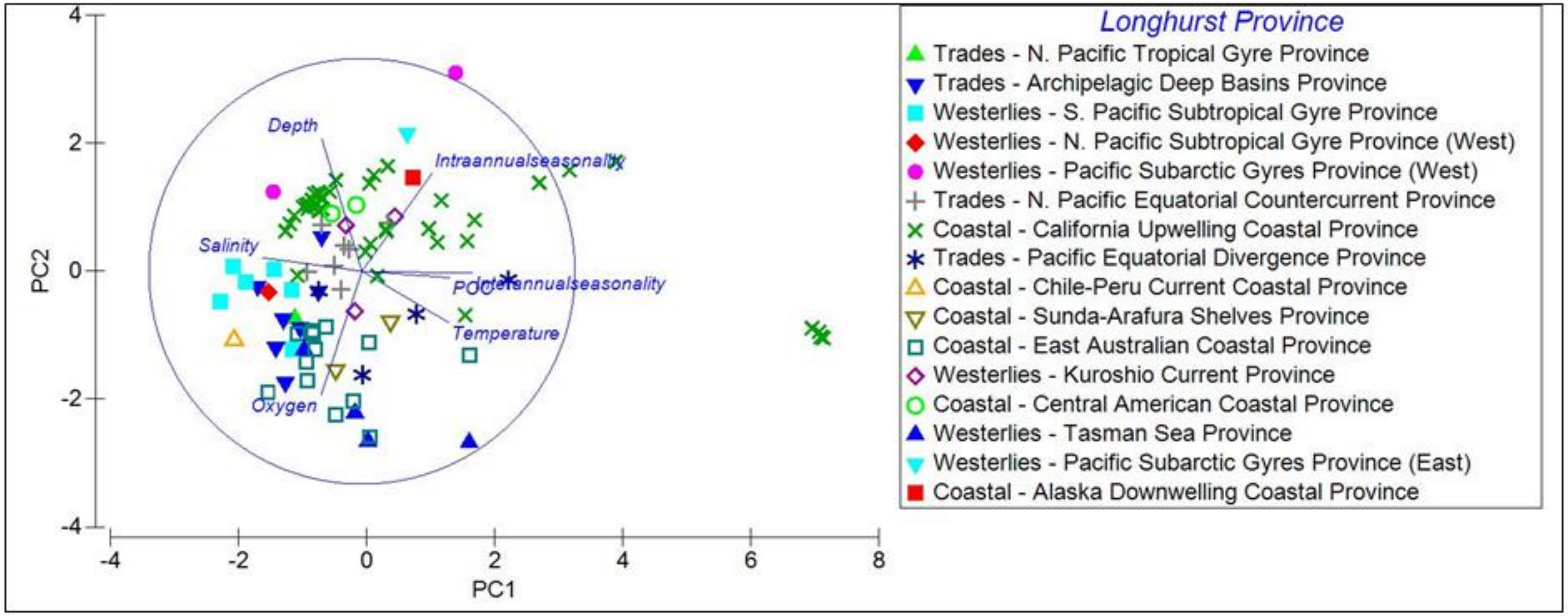
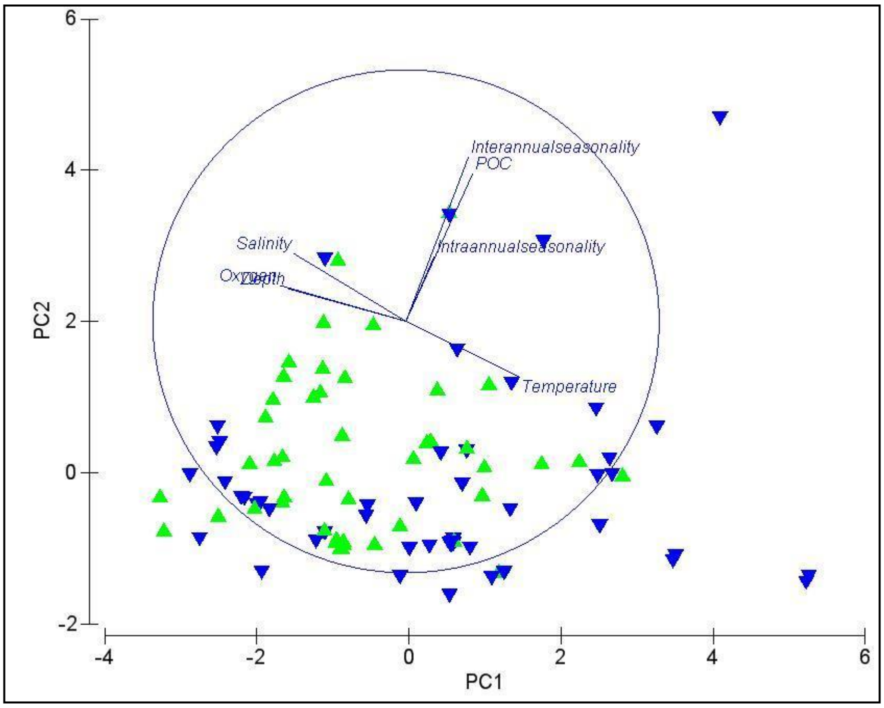


Figure 3.22 *Principle Component Analysis of the environmental variables experienced by the elasipodid holothurians at* ***bathyal*** *depths in the* ***Pacific Ocean*** *sorted by Longhurst's Provinces*

3.2.4.2 Abyssal Pacific (3000-6000m)

Results for the abyssal Pacific were similar to those for bathyal depths. The ANOSIM results found a significant difference in the distribution of the Elpidiidae ($p<0.05$, *Table 3.*3) but also the Psychropotidae ($P<0.05$, *Table 3.*3). The ANOSIM for the Deimatidae and was insignificant and the sample size for the Laetmogonidae was extremely small (*Table 3.4*).The PCA for the Elpidiidae shows that whilst the family was spread throughout the broad range of environmental conditions of the elasipodida they tend towards higher POC flux, intra-annual seasonality and inter-annual variability (*Fig.3.23a*). The PCA for the Psychroptidae shows that despite being present at some sites with high POC flux and intra-annual seasonality values, the Psychropotidae generally tended towards sites with low values of these variables (*Fig. 3.23b*). The PCA plots for the Deimatidae is provided in *Fig. 3.23c*, Appendix A for a visual comparison.

The DFA for the abyssal Pacific showed that there were significant differences between the sites occupied by the families (Wilks Llambda $p<0.05$, *Table 3.5*). The first DF explained most of the between group variance (86.9%). Individual correlations between the first DF and environmental variables were strongest for depth (.762), temperature (-.639), intra-annual seasonality (.543) and oxygen (.489). The second DF was responsible for 9% of the variation between groups and was most closely correlated with salinity (.596). The DFA plot (*Fig. 3.24*) shows that there is much overlap between the families in their DF scores. Whilst the Elpidiidae are somewhat seperated from the other groups along DF1, any further differentiation cannot be percieved. This suggests a complex combination of factors are necessary to describe the significant differences in the distributions of the families. The combination of the PCA plots and the DFA suggests that POC flux, inter-annual variability and intra-annual seasonality are important in differentiating between the families, but the presence of the Psychropotidae in the highest POC flux sites may have complicated results.



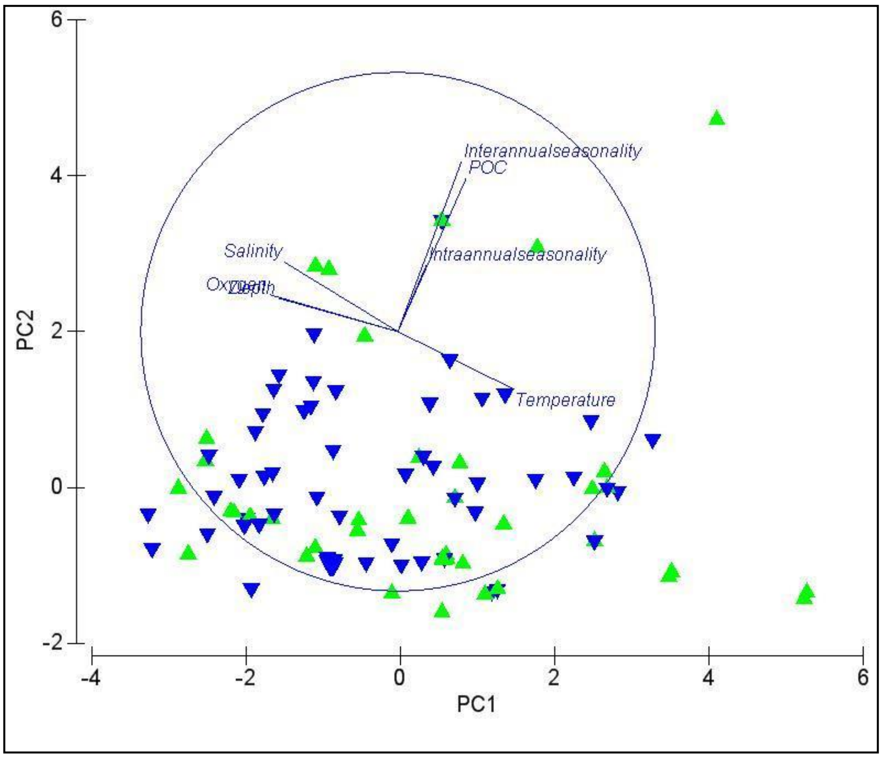


Figure 3.23 *Principle Component Analysis plots illustrating the environmental distribution of the four elasipodid holothurian families at* ***abyssal*** *depths (3000-6000m) in the* ***Pacific Ocean****. Distribution of all recorded elasipodid sites is shown in each figure with green triangles indicating the families' presence at a site and a blue inverted triangle denoting the family's absence for; (a)* **Elpidiidae**; *(b)* **Psychropotidae**

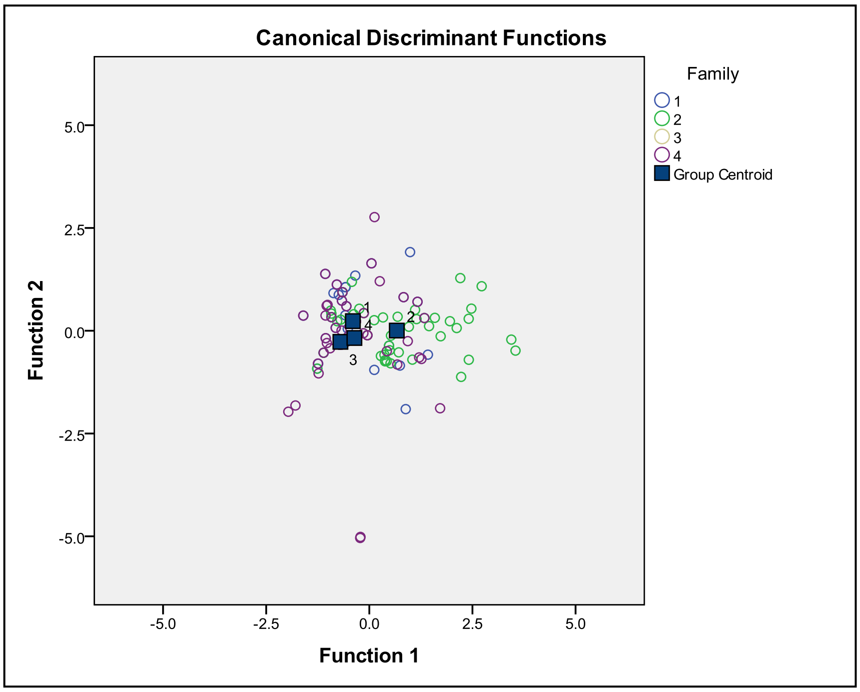


Figure 3.24 *Plot of the discriminant function scores (first and second DF) at each site for the four elasipodid families, where; (1)* Deimatidae; *(2)* Elpidiidae; *(3)* Laetmogonidae *and (4)* Psychropotidae *for* ***abyssal*** *depths in the* ***Pacific Ocean***

3.2.4.3 Geographic context in the Pacific Ocean

The bathyal PCA plot for Longhurst's provinces (*Fig. 3.22*) shows that the presence of only the Elpidiidae at sites with the strongest intra-annual seasonality is not related a single geographic factor. The sites are located in a number of seperate provinces including the N.Pacific Subtropical Gyre Province, the Pacific Subarctic Gyres Province and theCalifornia Upwelling Coastal Province. Similarly the presence of only the Elpidiidae at a small cluster of outlying sites on the right hand side of the bathyal PCA plot appears not to be related to geography. The sites belong to the California Upwelling Coastal Province (Coastal biome). Other families inhabit this Province, but were not found at sites with these high values of POC. Similarly differences in the distributions of the Psychropotidae and Elpidiidae do not appead to be related to any obvious geographic differences when their PCA plots are compared to the PCA plot of Longhurst's provinces (*Fig. 3.25*).

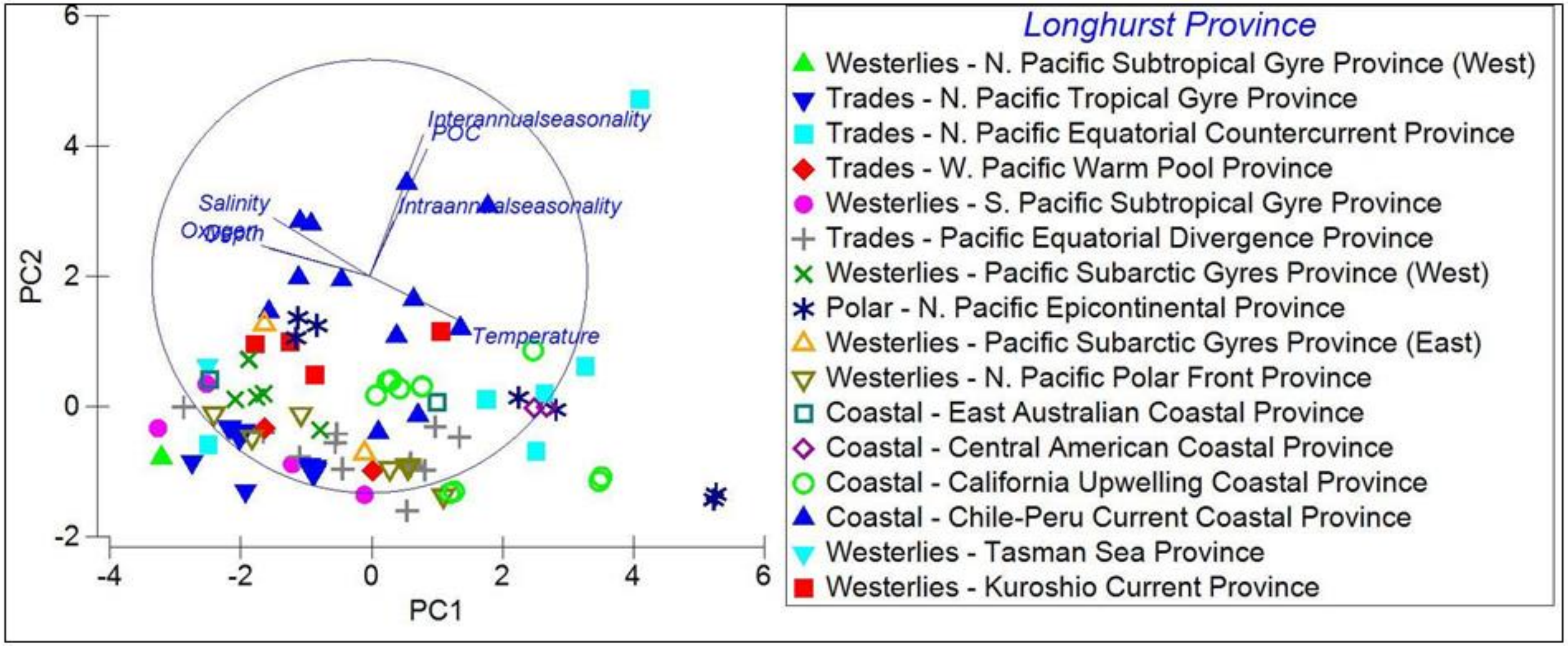


Figure 3.25 *Principle Component Analysis of the environmental variables experienced by the elasipodid holothurians at* ***abyssal*** *depths in the* ***Pacific Ocean*** *sorted by Longhurst's Provinces*

3.2.5 The Southern Ocean

3.2.5.1 Bathyal Southern (500-3000m)

At bathyal depths in the Southern Ocean the Elpidiidae ($p<0.05$, *Table 3.3*) and Laetmogonidae ($p<0.005$, *Table 3.3*) had statistically different distributions to the other families. Neither the Deimatidae nor Psychropotidae's distribution were found to be significantly different (*Table 3.3*). The PCA plot shows that the Elpidiidae were extremley widespread throughout the environmental conditions experienced by the Elasipodida in the bathyal Southern Ocean (*Fig. 3.26a*) However, the sites they were absent from tended to be shallower, warmer and with weaker intra-annual seasonality. The PCA plot for the Laetmogonidae shows that they were spread across a wide range of environmental conditions but that they did inhabit a number of outlying shallow sites with greater POC flux values (*Fig. 3.26b*). PCA plots for the Deimatidae and Psychropotidae are included in *Fig. 3.26c* and *d*, Appendix A for a visual comparison.

The DFA for the bathyal Southern Ocean was statistically significant (Wilks Llambda $p<0.005$, *Table 3.5*) confirming the existance of between-family differences. The first DF which explained 77.1% of the between-group variability was best correlated with total POC flux (.644), salinity (.535) and depth (.310). The second DF, which described 19.2% of the variability, correlated most strongly with oxygen (.745), inter-annual variability in POC flux (.745) and temperature (-.479) (*Table 3.5*). The plot of the DFA (*Fig 3.27*) shows that the first DF best discriminates between the Laetmogonidae and the other families. The second DF best discriminates between the Elpidiidae and Psychropotidae.

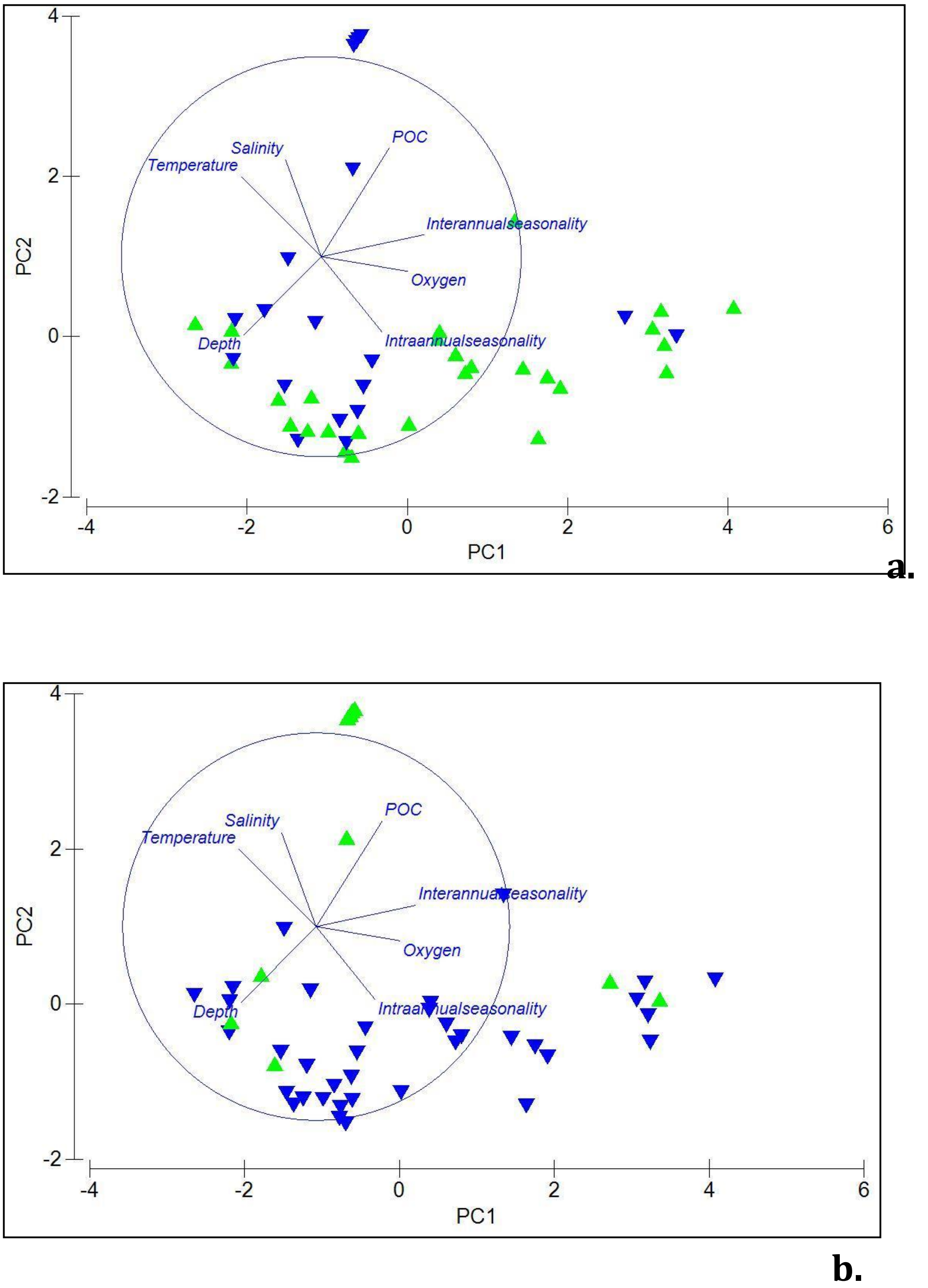


Figure 3.26 *Principle Component Analysis plots illustrating the environmental distribution of the four elasipodid holothurian families at* ***bathyal*** *depths (500-3000m) in the* ***Southern Ocean****. Distribution of all recorded elasipodid sites is shown in each figure with green triangles indicating the families' presence at a site and a blue inverted triangle denoting the family's absence for; (a)* **Elpidiidae** *(b)* **Laetmogonidae**

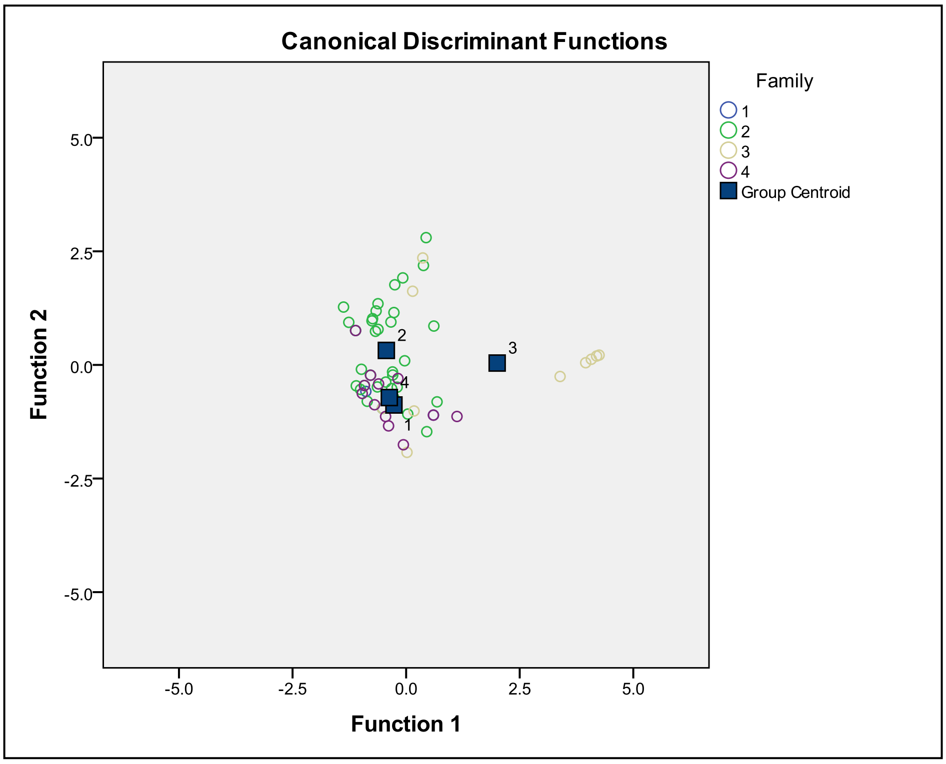


Figure 3.27 *Plot of the discriminant function scores (first and second DF) at each site for the four elasipodid families, where; (1)* Deimatidae*; (2)* Elpidiidae*; (3)* Laetmogonidae *and (4)* Psychropotidae *for **bathyal** depths in the **Southern Ocean***

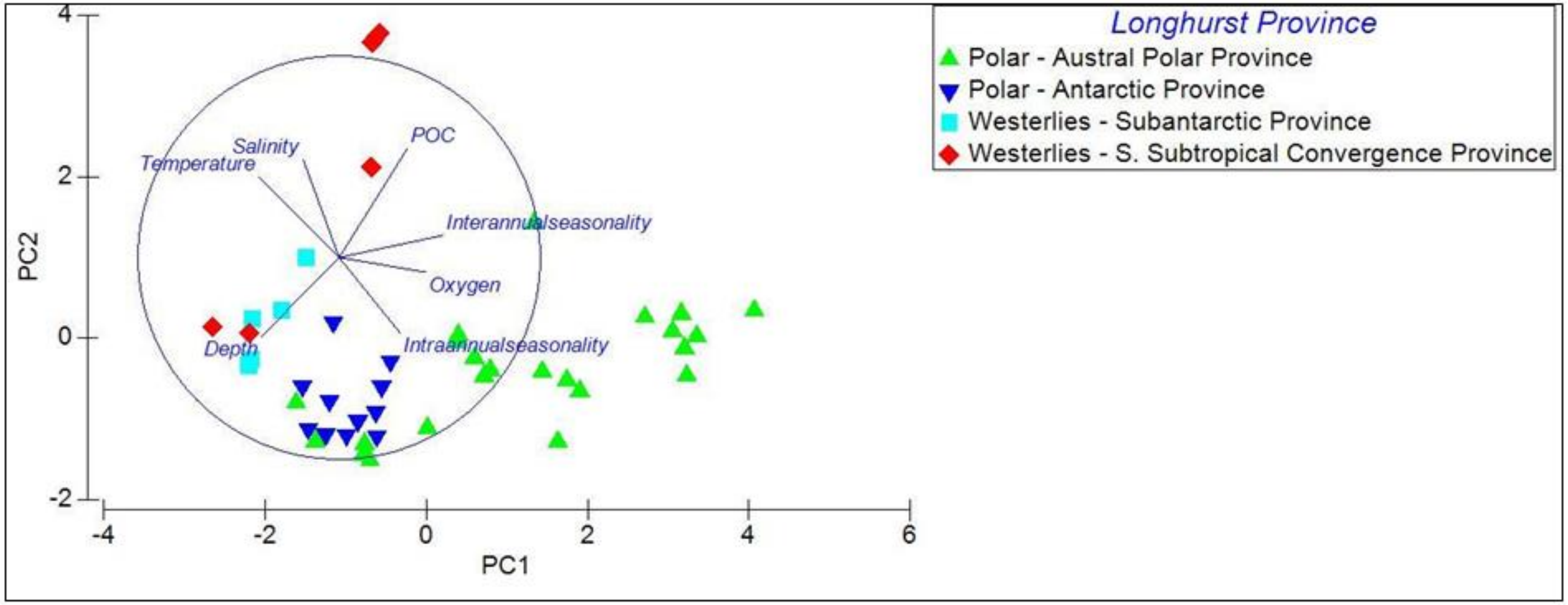
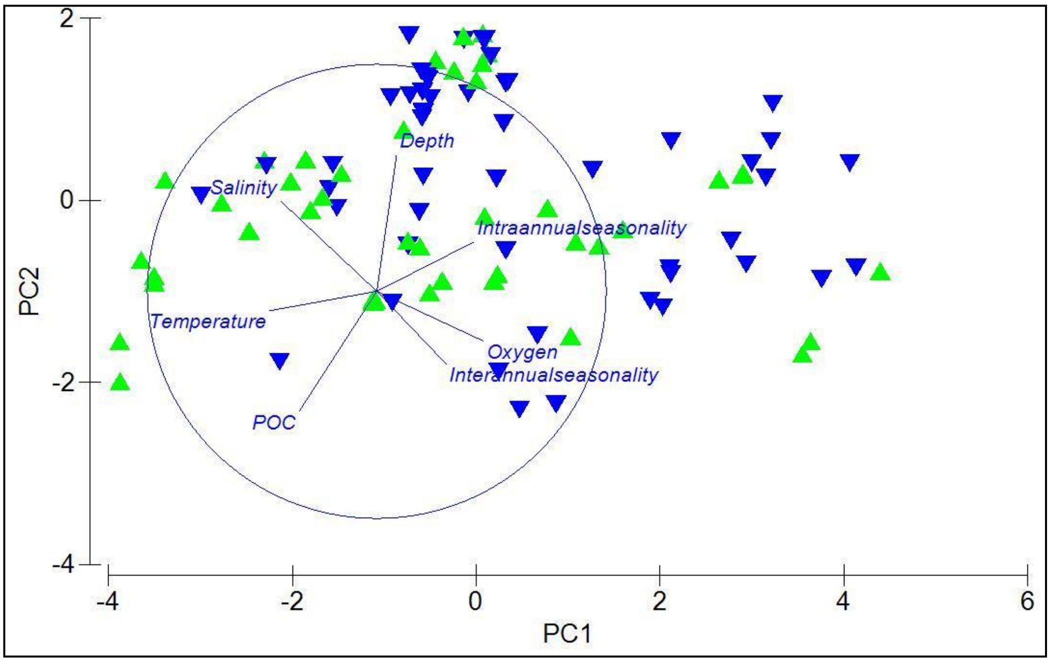


Figure 3.28 *Principle Component Analysis of the environmental variables experienced by the elasipodid holothurians at* ***bathyal*** *depths in the* ***Southern Ocean*** *sorted by Longhurst's Provinces*

3.2.5.2 Abyssal Southern (3000-6000m)

Families were generally much more widespread throughout the range of environmental variables encountered by the Elasipodida at abyssal depths in the Southern Ocean compared to bathyal depths. ANOSIMs for the Deimatidae, Elpidiidae and Laetmogonidae were insignificant (*Table 3.3*). The Psychropotidae were the only family with a significant ANOSIM in the abyssal Southern Ocean ($p<0.01$, *Table 3.3*). Whilst they inhabited sites across most environmental conditions, Psychropotids were skewed in the PCA plot towards greater depth and weaker intra-annual seasonality (*Fig.3.29*). PCA plots for the Deimatidae, Elpidiidae and Laetmogonidae are included in *Figs. 3.29b, c* and d, Appendix A for a visual comparison. However the DFA was not significant (Wilks Lambda $p>0.05$, *Table 3.5*). Despite this, differences can be seen in the DFA plot (*Fig. 3.30*) but due to the insignificance of the overall test, these differences cannot be attributed to any particular environmental variables.



d.

Figure 3.29 *Principle Component Analysis plots illustrating the environmental distribution of the four elasipodid holothurian families at* ***abyssal*** *depths (3000-6000m) in the* ***Southern Ocean****. Distribution of all recorded elasipodid sites is shown in each figure with green triangles indicating the families' presence at a site and a blue inverted triangle denoting the family's absence for the* ***Psychropotidae***

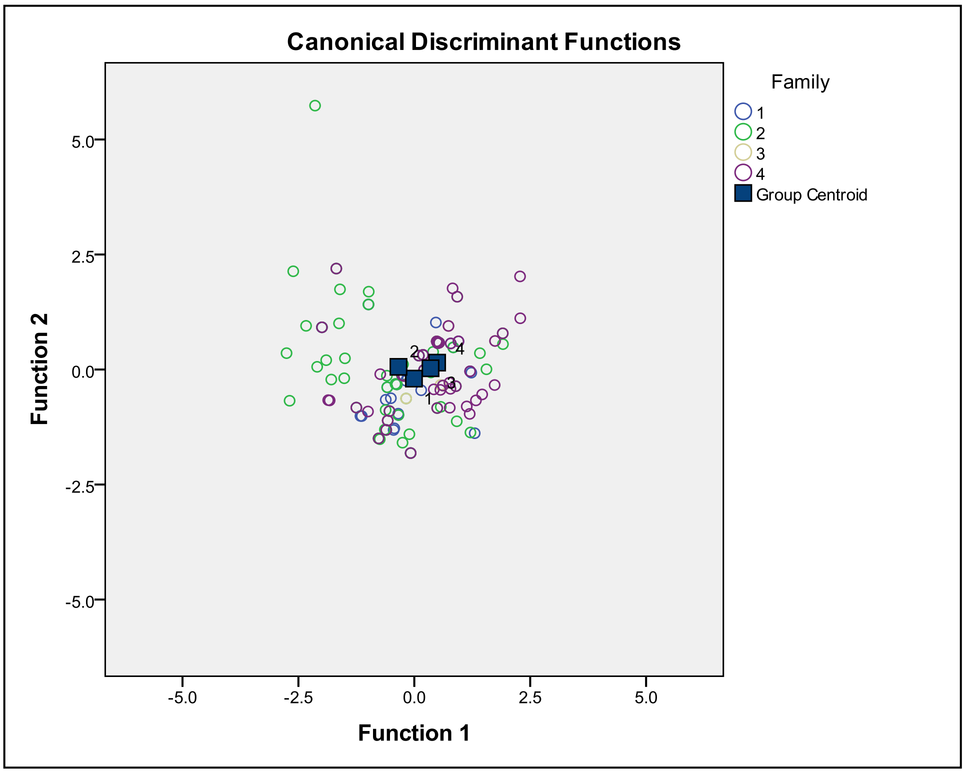


Figure 3.30 *Plot of the discriminant function scores (first and second DF) at each site for the four elasipodid families, where; (1)* Deimatidae*; (2)* Elpidiidae*; (3)* Laetmogonidae *and (4)* Psychropotidae *for **abyssal** depths in the **Southern Ocean***

3.2.5.3 Geographic context in the Southern Ocean

When the bathyal PCA plot is sorted by Longhurst's provinces (*Fig. 3.25*) the skew of the Elpidiidae to the bottom of the plot is related to its distribution in the two Polar provinces in the Southern Ocean, rather than the Southern Ocean provinces that fall within the Westerlies biome. The presence of the Elpidiidae at outlying sites associated with high intra-annual seasonality was related to their presence in the Polar Austral Province (*Fig. 3.25*). The Laetmogonidae were also found in a small number of sites in the province, as were the Psychropotidae. Therefore it appears that ecological rather than geographical reasons explain the presence of Elpidiidae at sites with strong intra-annual seasonality. Similarly, at abyssal depths the left-skew of Psychropotidae (*Fig. 3.29*) appears to be related to the separation between the Westerlies provinces which are represented on the left hand side of the plot (*Fig. 3.31).* However these are preferences, not absolute differences, suggesting they are ecologically, rather than biogeographically (e.g. topographically) based.

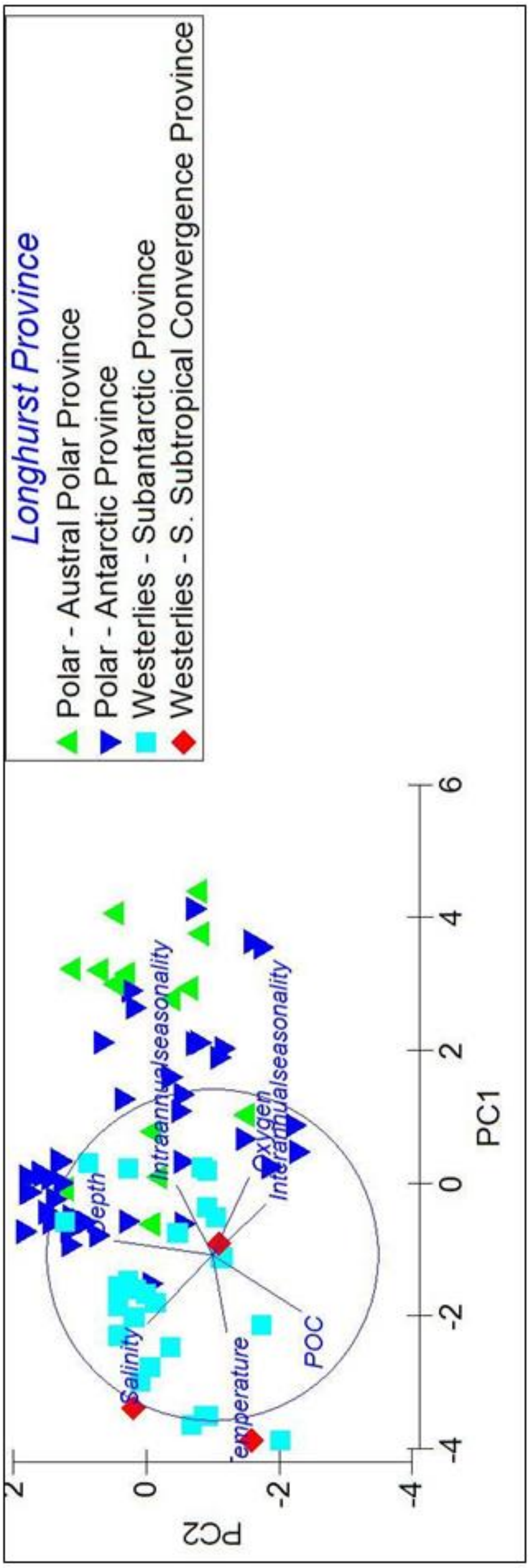


Figure 3.31 *Principle Component Analysis of the environmental variables experienced by the elasipodid holothurians at* ***abyssal*** *depths in the* ***Southern Ocean*** *sorted by Longhurst's Provinces*

3.3 Discussion

3.3.1 Environmental preferences of the four families; concurrence between ocean-scale and local-scale studies on the importance of food quantity, quality and variability

Whilst the degree and driver of differentiation between the elasipodid families varied between oceans and depth bands, one pattern was relatively universal at bathyal depths and often still evident at abyssal depths. This was the differentiation in the distributions of the Psychropotidae and Elpidiidae. The Deimatidae often had an intermediate environmental distribution, sometimes more similar to the Elpidiidae, sometimes the Psychropotidae. The Deimatidae were never as polarised as either the Elpidiidae or Psychropotidae however. Relatively few records of the Laetmogonidae were included in this analysis as their often shallow distribution led to a lack of coverage by the environmental datasets used, which often did not cover shallow bathyal depths in coastal waters.

The four families are known to co-exist at many well known sampling locations, such as the PAP in the NE Atlantic (Billett et al. 2001), Station M in the NE Pacific (Ruhl and Smith 2004) and the Crozet Islands in the southern Indian Ocean (Wolff et al. 2011). Certainly, their global distributions showed a great degree of overlap. However, significant levels of differentiation were notable in the bathyal and abyssal depths in the Atlantic and bathyal depths in the Pacific and Southern Ocean. Additional quantitative (ANOSIM) or qualitative (PCA plot) differences were evident for the abyssal Pacific and Southern Ocean. Where strong differentiation occurred, the Elpidiidae and Psychropotidae were generally differentiated along some axis of food availability or quality. The former were more likely to be found in areas of higher intra-annual seasonality, inter-annual variability or POC flux or a combination of these factors. Conversely, the Psychropotidae often tended towards weaker intra-annual seasonality, lower POC flux and reduced inter-annual variability. However it should be noted that both families had very broad environmental tolerances.

Sometimes these preferences were related to the presence of one family in a locality separated by a biogeographic barrier (e.g. the Arctic or Caribbean province) and the absence of the other families. More commonly it was related to a preference for different environmental conditions within a province, or provinces, also occupied by the other family. These large-scale environmental preferences agree with much finer resolution local-scale studies on feeding selectivity and temporal and spatial changes in community structure of elasipodid holothurians.

3.3.1.1 Intra-annual seasonality as a biogeographic driver in differences between the families

Intra-annual seasonality was an important factor in the differences between the families, particularly the Psychropotidae and Elpidiidae. Intra-annual seasonality was implicated as a key driver in differences at bathyal and abyssal depths in the global analysis. When individual oceans were examined it was found to be the primary driver of differentiation between the Elpidiidae and Psychropotidae in the Atlantic at bathyal depths, and was still an important variable at abyssal depths. The apparent importance of intra-annual seasonality as a driver of differences between families agrees with previous, anecdotal information; Hansen (1975) noted that the distribution of the genus *Elpidia* appeared to be restricted to highly seasonal areas. Its importance in the Pacific was less evident, but was implicated in contributing to differences at abyssal depths. At bathyal depths in the Pacific POC and inter-annual variability were more important. Intra-annual seasonality was a much less important factor in determining differences between the families in the Southern Ocean, but this may be due a lower range in intra-annual seasonality relative to the Pacific and Atlantic in the consistently high latitude Southern Ocean.

Intra-annual seasonality acts as a suitable proxy for food quality in the relatively course taxonomic grain of this analysis. As explained in the introduction, POC flux from seasonal environments is more likely to escape consumption in the euphotic zone, resulting in more labile material fluxing to depth (Lutz et al. 2007). However, differences in the distributions of individual species may rely on the spatial and temporal variability in the specific type of phytoplankton at the surface and therefore the biochemical composition of flux arriving at the seafloor. Mapping of food quality at the seafloor is not yet possible. Certain progress has been made in mapping patterns at the surface with research into the using remote sensing to identify of the main functional phytoplankton groups (Alvain et al. 2005, Nair et al. 2008). However, these techniques are still in their infancy, as is our understanding of the links between dominant phytoplankton functional group and the lability and composition of POC reaching the seafloor (Moore et al. 2002). A suitable proxy in the meantime is for freshness of the food arriving at the seafloor is the periodicity during which the flux occurred. Additionally intra-annual seasonality may be related to the spatial patchiness of food arriving on the sea floor (Billett et al. 1983).

There exists a great deal of evidence to support the importance of intra-annual seasonality in differences between families in the Atlantic. Two separate studies investigated gut-content chlorophyll a concentration and ovarian phytopigment (chlorophyll a and carotenoids) concentrations as biomarkers for feeding selectivity (Wigham et al. 2003a, FitzGeorge-Balfour et al. 2010). Wigham et al. (2003a) found that *Amperima rosea* (Elpidiidae) had a significantly different ovarian carotenoid profile to *Oneirophanta mutabilis* (Deimatidae) and *Psychropotes longicauda (*Psychropotidae). Chlorophyll *a* gut sediment analysis carried out by FitzGeorge-Balfour et al. (2010) showed that the Elpidiids *Amperima rosea* and *Peniagone diaphana* were found to feed selectively on the freshest material. These species also had relatively high carotenoid concentrations in their ovaries and consistent ovarian carotenoid profiles between individuals. Consistent ovarian pigment profiles, despite the temporally and spatially patchy nature of fresh phytodetritus at the PAP (Billett et al. 1983) suggests that *A. rosea* is actively searching for fresh food. In contrast *P. longicauda* was found to be much less selective than the Elpidiid species. *Psychropotes longicauda* had relatively low concentrations of carotenoids in the ovaries, with inconsistent carotenoid profiles between individuals. This suggested that *P. longicauda* was feeding on sediment indiscriminately, eating fresh food when it encountered it, and eating less fresh food when it was encountered. *Psychropotes longicauda* was found to be more similar in terms of pigment profiles to two non-elasipodid species studied than the Elpidiid species included in the analysis. *Oneirophanta mutabilis* was found to be intermediate between the Elpidiidae and the Psychropotidae. It displayed chlorophyll *a* concentrations more similar to the Elpidiidae, ingesting relatively fresh material. However, when fresh material was scarce *O. mutabilis* switched to more refractory material (FitzGeorge-Balfour et al. 2010). This agrees with current results which generally found the Deimatidae to have distributions spread throughout the range of environmental conditions experienced by elasipodid holothurians, but rarely inhabiting the sites at the 'extremes'. Only in the abyssal Atlantic did the Deimatidae have a statistically different distribution from the other families, where it had a very similar PCA profile to that of the Elpidiidae (*Fig. 3.17a*). Results from a study into the sterol chemistry of elasipodid holothurians from the same location have had similar findings. *Amperima rosea* was found to have a significantly different sterol distribution to *Oneirophanta mutabilis* and *Psychropotes longicauda*. Evidence of metabolites of the freshest components of the POC flux were present in the Elpidiidae species but not the Deimatidae or Psychropotidae (Ginger et al. 2000, Ginger et al. 2001).

Iken et al. (2001) used gut content analysis and stable isotope tracers to investigate food-web interactions, also at the Porcupine Abyssal Plain (PAP). Results provided a clear distinction in the freshness of POM ingested between species. Within the holothurians encountered at the PAP, the Elpidiid species *Amperima rosea, Ellipinion molle* and *Peniagone diaphana* clustered together with the lowest $\delta^{15}N$ values. Low $\delta^{15}N$ values are associated with feeding selectivity for the freshest components of the POM. *Psychropotes longicauda* on the other hand had much higher $\delta^{15}N$ values, suggesting that they are less selective feeders, ingesting more refractory material. Two members of the Deimatidae; *Oneirophanta mutabilis* and *Deima validum validum* also had higher

$\delta^{15}N$ values. Iken outlined three functional groups based on the level of selectivity for fresh phytodetritus as measure by stable isotope analysis only. Membership of these groups corresponds well with the systematics of the elasipodid families. Iken's Group A consisted of surface deposit feeders that preferentially fed on the freshest material. Members of Group A were primarily species of the Elpidiidae. Group B consisted of surface deposit feeders that fed on more refractory material and consisted primarily of species of the Psychropotidae and Deimatidae. Group C were sub-surface deposit feeders that fed on extremely degraded materials. No elasipodids fell into this category. Results from such studies suggest that a) food quality is an extremely important variable determining niche partitioning in elasipodid holothurians and b) some level of niche-conservatism between families may be occurring.

However, differences within families also occur which would help to explain the large spread of environmental tolerances observed within the families in the PCA plots. The trophic positions and seasonality of two Elpidiidae species (*Peniagone vignoni* and *Protelpidia murrayi*) on the West Antarctic Peninsula was examined using stable isotope analysis (Mincks et al. 2008). Whilst both species fell into the same trophic grouping, feeding on very fresh material, *P.murrayi* showed greater seasonal changes in the freshness of material selected for. This suggests that *P.vignoni* selects the freshest material year-round, whereas when the availability of fresh phytodetritus is low, *P.murrayi* switches to more refractory material (Mincks et al. 2008). In the study by Iken et al. (2001) at the PAP two Psychropotid species; *Psychropotes semperiana* and *Benthodytes sordida*, fell into the (primarily Elpidiidae) Group A. However the two Psychropotids exhibited the highest $\delta^{15}N$ values (most refractory material) within this group. Additionally, only the three Elpidiid species (*Amperima rosea, Ellipinion molle* and *Peniagone diaphana*) in Group A were found to have ingested considerable amounts of POM during periods of low POC-flux (Iken et al. 2001). Neither of the Group A Psychropotids or any members of Group B had high quantities of POM in their guts at these times. Therefore feeding selectivity studies at the PAP suggest that, despite apparent species-level differences at local scales, families are a suitable unit to be exploring functional differences in the utilisation of food resources i.e. niche partitioning. These species-level differences would also help to explain the large spread and overlap of the environmental tolerances within families found in this study.

Differences in distribution between the Elpidiidae and Psychropotidae could be explained by two different mechanisms; 1) some of the sites with low intra-annual seasonality were outside the range of tolerances that the Elpidiidae can survive and reproduce in and those with very high intra annual seasonality were outside of the range tolerances of the Psychropotidae and/or b) in areas of very high productivity the Elpidiidae outcompete the Psychropotidae for the freshest components of the flux and in areas with very low intra-annual seasonality of the flux the Psychropotidae outcompete the Elpidiidae.

Evidence for competitive interactions at the PAP has been found. Ginger et al. (2000) found that based on their sterol composition *A. rosea* and *Ellipinion molle* were capable of ingesting the total sterol flux to the PAP during the time period of the study, preventing its utilisation by other species. Ramirez-Llodra et al. (2005) found that the reproduction of *Oneirophanta mutabilis* may have been negatively affected by the dramatic increase in *Amperima rosea* on the PAP. Bett et al. (2001) found a significant negative correlation between the level of activity of megabenthic deposit feeders and the physical presence of phytodetritus in photographs of the seafloor. Through estimations of tracking rates Bett et al. (2001) suggest that megabenthic activity was enough to cause near total removal of all incoming POC flux in the NE Atlantic, suggesting, despite niche partitioning at the PAP, competition between deposit feeders is still strong.

Wigham et al. (2003b) suggest that the high fecundity *of Amperima rosea* along with the rapid growth and onset of sexual maturity, even in juvenile stages, allows *A. rosea* results in an opportunistic reproductive strategy, which may provide a competitive advantage at times of optimal quality of food flux. An opportunistic response to food supply has often been cited as the primary cause for dense aggregations of Elpidiid species in areas with highly variable food input. For example *Kolga Hyalina* in the NE Atlantic*, Achlyonice violaecuspidata* in the Weddell Sea (Gutt and Piepenburg 1991), *Elpidia glacialis* in the Arctic (Rogacheva 2007) and Weddell Sea (Gutt and Piepenburg 1991), *Peniagone willemoesi* from a canyon system in the Gulf of Mexico (Rowe and Menzel 1971), and *Scotoplanes globosa* from a canyon in Southern California (Smith and Hamilton 1983) and the Weddell Sea (Gutt and Piepenburg 1991).

Iken assigned physical characteristics that correlated with the ability of holothurians to select for the freshest components of the POC flux. Those that selected the freshest material (Group A as discussed earlier) had gelatinous body walls, a small body size and high mobility. Iken suggested that member of Group A invest more time in finding high quality food. Group B, which feed on more refractory material were larger, with lower mobility and eat whatever they encounter, rather than searching for higher quality food. The Psychropotidae appear to fall into this group. The Deimatidae are classed as Group B by Iken et al. (2001) but in reality probably fall somewhere between the two groups depending on prevailing competitive conditions (FitzGeorge-Balfour et al. 2010). Iken suggested that mobility and the ability of an organism to find 'fresh' or high quality food patches is therefore the main driver in niche differentiation in elasipodid holothurians. Clearly, competitive interactions are important in seasonally variable environments. Whether areas not occupied by some families constitute unsuitable, or just less suitable habitat is unclear. It is likely that competition plays an important role in areas which are suitable for all families. Whether in some areas competition is enough to exclude one family altogether is not yet clear. In summary, the ability to respond to local and regional changes in the *quality* of POC flux is probably related to mobility and reproductive/ life-history strategy. The strength of intra-annual seasonality may be a suitable proxy for spatial variability in freshness (or species/biochemical composition) of material arriving at the seafloor in the Atlantic. Alternatively it could be linked to patch dynamics and spatial variability in food. Both feeding selectivity driving niche partitioning and competitive interactions between (and within) families are likely to be important in determining the environmental distribution of the elasipodid families. The preference of the Elpidiidae for fresh material and the Psychropotidae for more refractory material is evident in their distributions in Atlantic Ocean. However, variability within families and potentially phenotypic plasticity within species and families, alongside temporally changing conditions, results in great overlap.

3.3.1.2 Inter-annual variability in food quantity and quality and changes to abundance and community composition

Inter-annual variability was an important factor in differentiating between the environmental conditions occupied by the Psychropotidae and Elpidiidae at bathyal depths in the Southern Ocean. In the bathyal Pacific, although not the primary correlate (which was POC), inter-annual variability was still an effective discriminatory variable. POC flux and inter-annual variability were extremely closely correlated at sites occupied by Elasipodida in the bathyal Pacific. POC flux and inter-annual variations were less closely correlated in the bathyal Southern Ocean. The mean POC and inter-annual variability was higher for sites occupied by the Elpidiidae than those occupied by the Psychropotidae in the bathyal Pacific. The same pattern was true in the Southern Ocean, but the differences between the families were much more driven by inter-annual seasonality, with only small differences in mean POC.

Results from long-term times series in the northeast Pacific confirm the increased importance of inter-annual variations over intra-annual seasonality (a reverse in the results found in the Atlantic). Station M in the NE Pacific experiences strong inter-annual and intra-annual seasonal fluctuations in the quantity (and probably the quality) of POC flux to the seafloor (Lauerman et al. 1996). Investigations into the correlation between the quantity of food supply and the abundance and distribution of epibenthic echinoderms did not detect any significant patterns over intra-annual time frames (Lauerman et al. 1996, Lauerman and Kauffman 1998). Subsequent longer- term studies have found significant correlations between inter-annual variations in POC flux and the community structure and population dynamics of the megabenthos (Ruhl and Smith 2004, Ruhl 2007, 2008). Inter-annual variations in the Pacific-basin Northern Oscillation Index (NOI) have been linked to abyssal food supply over a fifteen year period (Smith et al. 2009).

Results suggest differential reaction in oscillation indices between large and small holothurians. Positive oscillation indices result in greater upwelling and increased flux and vice versa for negative indices. On inter-annual time frames Ruhl and Smith (2004) found significant correlations between the NOI and species composition. A regime shift occurred between 1998 and 1999. The NOI was generally negative between 1989 and 1998 but switched to generally positive index values between 2000 and 2002. The abundances of four Elasipodida (the Elpidiids *Elpidia minutissima*, *Peniagone vitrea, Scotoplanes globosa* and the Psychropotid *Psychropotes longicauda*) were significantly different between the two time periods. Cross correlations revealed significant relationships between climate (lagged by ~14months) and POC flux (lagged by ~7months) and the abundances of *E. minutissima*, *P. vitrea* and *S. globosa*. Whilst *E. minutissima* and *P. vitrea* increased in abundance with decreased NOI and flux, *S. globosa* was rare during low NOI and flux conditions, but increased in abundance during higher NOI and flux conditions. *Psychropotes longicauda* and *Oneirophanta mutabilis* (Deimatidae) also showed smaller-scale increases in abundance with increased NOI and POC flux (Ruhl and Smith 2004, Smith et al. 2009).

Whilst reactions of epibenthic echinoderms were species specific, trends between families are apparent. Despite the variable direction of the change, a number of the Elpidiid species showed large changes in abundance in relation to inter-annual changes in POC flux (Smith et al. 2009). The larger holothurians of the Deimatidae and Psychropotidae showed smaller changes in abundance in relation to flux (Ruhl 2008). This highlights the facts that differences between families are driven by seasonality and inter-annual variations, not by total amount of POC flux. Quantity may be an important factor within families, especially in the Elpidiidae. Similar links between inter-annual variations in climate, known as the North Atlantic Oscillation Index and food supply to the abyssal community have been established in the North East Atlantic (Lampitt et al. 2010).

(Ruhl 2008) suggests that the smaller holothurians at Station M (Elpidiid species) increase during periods of low POC flux because they require less resource per unit area and body size is linked to energy use and community structure (Ruhl 2008). Therefore large-scale intra-familial similarities in reactions to changing food supply could be due in-part to similar energy requirements related to intra-familial conservatism in body size. However, this is unlikely to be a straight forward relationship, as body size is also related to mobility, reproductive mode and feeding selectivity in elasipodid holothurians. In a review of so called 'boom-bust' life history strategies in echinoderms Uthicke et al. (2009) identified 28 species which demonstrated large population density variations. The study defined large population density variation as a minimum fourfold increase (i.e. two doublings) or decrease to <0.25 (i.e. two halvings) of the initial population of a species. 10 out of the 28 species were holothurians. Five out of the 10 holothurians with boom-bust life histories were elasipodids, and all five of these elasipodids were Elpidiidae (*Amperima rosea, Ellipinion molle, Elpidia minutissima*, *Peniagone vitrea and Scotoplanes globosa*).

Whilst information on the larval form of these species is generally lacking, results from other species in the study suggested that the boom-bust life history strategy was related to statistically significant over representation of an ancestral type planktotrophic larvae (Uthicke et al. 2009). The authors argued that the presence of planktotrophic larvae is a high-risk high-gain strategy owing to a strong non-linear dependency of larval production on adult densities, a low potential for compensatory feedback mechanisms and the uncoupling of larval and adult ecology. A planktotrophic boom-bust reproductive strategy, coupled with strong species-specific preferences for specific carotenoid pigments (Wigham et al. 2003b, FitzGeorge-Balfour et al. 2010) help explain why species within a family may respond differently to local or regional changes in food quality. Spatially, and over longer time frames, the Elpidiidae as a whole dominate highly seasonal and inter-annually variable areas because although some species may decline during one 'type' of POC flux, another Elpidiid species may benefit during this time and vice versa. This may also help explain the co-existence of many Elpidiid species at individual sites, and perhaps the specious nature of the family as a whole.

3.3.1.3 Spatial changes in the quantity vs. seasonality of food supply

The suggestion from this study that in many areas the temporal variability of flux is as important as the quantity of flux in driving differences within the Elasipodida agree with smaller scale spatial studies. Two sites with similar abiotic settings, but different POC flux regimes situated 430km apart in the Southern Indian Ocean, close to the Crozet Islands were compared. One site (+Fe) was initially thought to have higher POC flux resulting from natural iron fertilization due to run-off from the Crozet Islands. The second site (HNLC) was thought to be relatively oligotrophic, occurring outside of the area affected by runoff from the Crozet Islands. Despite the proximity of the sites, they were found to have very different elasipodid community composition. Other groups, such as the ophiuroids differed little between sites. Whilst both sites were dominated by Elpidiidae, the +Fe site was dominated numerically by *Peniagone crozeti* and *Peniagone challenger.* The HNLC site was dominated by numerically by *Kolga nana, Peniagone willemoesi* and *Peniagone affinis*. In terms of biomass the +Fe site was dominated by *Psychropotes longicauda* whilst the HNLC sites biomass was dominated by *Peniagone affinis.* Abundance was six times greater at the +Fe site compared to the HNLC site and biomass was three times greater. The surprising dominance of the Elpidiidae at a site thought to be low in flux is explained by an unexpected finding in the sediment trap records at the HNLC site. As expected, the +Fe site experienced a prolonged flux of POC carried by currents from the Crozet bloom. This lasted at least 120 days originating from the spring/summer bloom in late 2004/early 2005. The HNLC site, which was thought to be oligotrophic with little intra-annual seasonality, actually experienced one very short pulse of downward flux.

The periodicity of events like this at the HNLC site is not known. Wolff et al. (2011) noted that it could be an annual or occasional occurrence. It is perhaps therefore unsurprising, given the short lived nature of the flux at the HNLC site, that it is dominated by a species that is known from other highly variable locations (Billett and Hansen 1982). Cumulative flux of organic carbon was still much greater at the +Fe site (41.1mmol m^2 (355days) and 14.1 mmol m^2 (352 days) at the HNLC site. The quality of the flux was also higher at the +Fe site. Therefore, despite the low total amount of POC flux arriving at the HNLC site, it still supported an elasipodid community because the flux was seasonal in nature.

The dominance of the Elpidiidae at both sites highlights their opportunistic nature and ability to respond quickly to different levels and temporal regimes of POC flux. The abundance of *Psychropotes* at the +Fe site, and its much lower abundance and biomass at the HNLC site agrees well with biochemical studies. If the flux event at the HNLC site is only occasional then the more refectory matter that *Psychropotes* feeds on may be too low in the sediment at the HNLC site. Because flux occurs over longer time periods at the +Fe site, although refractory, the sediments may be more nutritive and less refractory than those at the HNLC site. i.e. *Psychropotes* may feed on refractory material but it probably has a lower limit which is exceeded at the HNLC site. Additionally, *Psychropotes longicauda* cannot respond as quickly as the Elpidiid species to the flux, in terms of physically seeking out food patches (Iken et al. 2001) or being able to convert food pulses to increased fecundity (Wigham et al. 2003b) at the HNLC site and so is outcompeted.

3.3.1.4 Quantity of POC flux and differences between the families

Quantity of POC flux was the most important environmental factor for explaining differences between the Elpidiidae and Psychropotidae at bathyal depths in the Pacific. However, as previously noted, POC flux and inter-annual variability were extremely closely related in the bathyal Pacific. In the Pacific sites occupied by Elpidiidae with high values of POC flux were largely (but not exclusively) associated with the Californian Coastal Upwelling Province. The upwelling of nutrient rich waters along the east coast of the North America drives some of the strongest primary production in the world (Lutz et al. 2007) which is then translated to POC flux in coastal provinces. The Psychropotidae were also found in this province, but were not associated with sites with the highest POC or intra-annual seasonality. The ability of Psychropotid holothurians to exist in areas with low temporal variability in flux and therefore (in the Pacific) low POC flux has already been discussed. While increased POC flux is not a disadvantage per se, Psychropotes is less well equipped to compete in areas of high temporal variability and POC flux. It is possible that the Psychropotidae have some digestive mechanism that gives them an advantage in areas of lower POC flux. There is evidence that *Psychropotes longicauda* may have enhanced bacterial activity within its intestine that may allow microbial breakdown of a broader range of substrates than could be otherwise be achieved by the holothurian itself (Roberts et al. 2001). Similar mechanisms are now known for infaunal holothurians that feed on the most refractory material (Amaro et al. 2009). POC flux was also highly correlated with differences between the families at bathyal depths in the Southern Ocean. Once again differences between the families occurred along an axis related to both POC flux and inter-annual seasonality. Thus it is proposed that the Psychropotidae are better adapted than the other families at life in low-flux condition. A proposed mechanism for the adaptation to low-flux conditions could be this; 1) initial adaptation to higher flux (or seasonal) environments (Psychropotidae are believed to have originated in Antarctic – A. Gebruk pers. comm.) 2) maintenance of populations in low-flux areas from higher-flux (or more seasonal) areas in a source-sink dynamic 3) eventual adaptation of sink population to local conditions. This mechanism is described by (Hermsen and Hwa 2010).

Essentially, the environmental co-correlation between intra-annual seasonality, inter-annual variability and POC flux essentially means that the dynamics between the three, rather than any particular one, is important in the biogeography of elasipodid holothurians.

3.3.1.5. Temperature, Oxygen and Salinity

Temperature was found to be an important factor explaining differences between the families in the abyssal Atlantic when the Arctic was included. When the Arctic samples were removed, oxygen became the most important variable. When the Caribbean are also removed from analysis, it becomes clear that oxygen and depth (the two most important variables in the discriminant function analysis) are explaining a gradual transition from more oxygenated Westerlies provinces to lower oxygen, deeper trade provinces. There was no strong gradient in families in terms of food availability.

Salinity was found to be able to discriminate the families in the Southern Ocean. This is largely due to the absence of the Elpidiidae in a few sites within the South Subtropical Convergence and the presence of the Psychropotidae here. Therefore salinity is indicative of the differences in distribution of the two families across two water masses with different physical and biological conditions. It seems unlikely that it is salinity that is driving the patterns, but as it is the best differentiator between the water masses, it is the best at describing the differences between them. Salinity and POC are closely related and it is possible that the pattern witnessed is actually due to the high POC and low inter-annual seasonality of these sites. This demonstrates the problem of environmental co-correlation.

3.3.1.6 Depth

Despite the differentiation into depth zones in order to minimize the importance of depth, it was still found to be an important variable for differentiating between the families in the bathyal and abyssal Atlantic and Pacific. At bathyal and abyssal depths this relationship is driven by the Laetmogonidae who are a generally bathyal family, unlike the Deimatidae, Elpidiidae and Psychropotidae. However, even when the Laetmogonidae are excluded from the analysis (results not shown) depth is still and important discriminatory variable.

One of the most obvious results from these analyses is that environmental differentiation between the families is much stronger at bathyal depths compared to abyssal depths. *Figure 3.32* shows the range of environmental variables experiences by elasipodid holothurians at bathyal and abyssal depths across all oceans. The elasipodid holothurians experience a much wider range of environmental variables at bathyal compared to abyssal depths. Although analyses were conducted within depth bands so that the scale of analysis was relative, it means differentiation between the families would have to be occurring on a much smaller scale to be apparent in the abyssal zone compared to the bathyal zone. Additionally, all the environmental variables experienced at abyssal depths fall within those experienced at bathyal depths by the elasipodida. Therefore at abyssal depths it appears unlikely that families environmental tolerances would be reached, causing absences, which are essentially underpinning the differences between the families in this analysis. Additionally competitive exclusion is more likely to occur at the ‘extremes’ of the environments experienced by the Elasipodida (Hermsen and Hwa 2010).

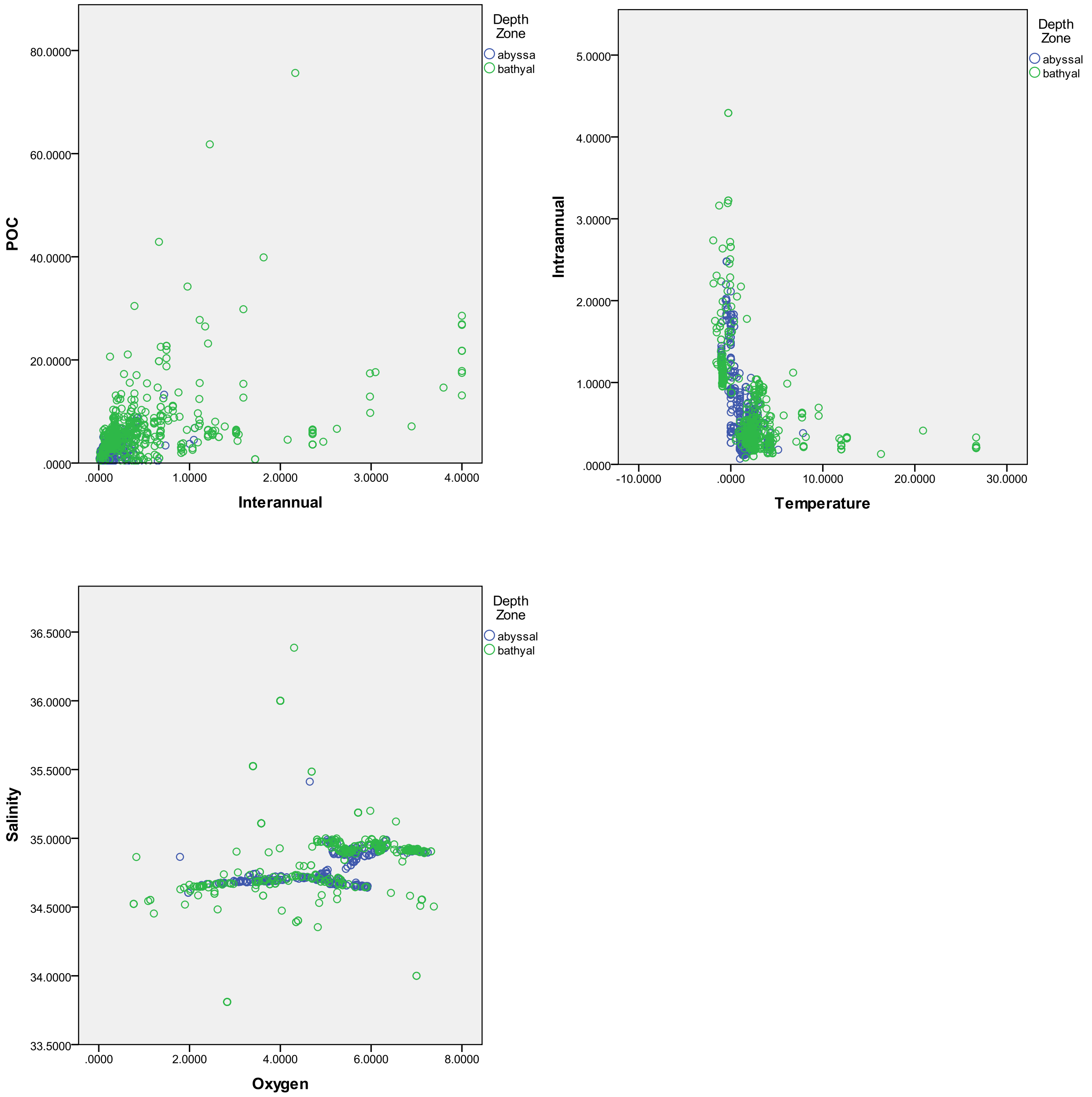


Figure. 3.32 *The environmental conditions experienced by the Elasipodida across all oceans at bathyal (green circle) and abyssal (blue circle) depths.*

3.3.2 Sampling issues

Much of our knowledge of temporal knowledge of the deep sea holothurians comes from long-term time series which have focused upon areas with high intra annual seasonality, inter-annual variability and relatively high POC flux. These areas tend to be relatively close to major research institutions and are not evenly distributed (*Fig. 3.33*). The benthic communities below gyral regions are relatively under-sampled. This is possibly because their communities are thought to be relatively depauperate and therefore 'less interesting'. Records from tropical regions and open ocean records tended to be the result of some of the early global expeditions, such as Challenger and Galathea. Additionally, because of lower population densities in food poor areas, a greater sampling effort would be required to characterise the fauna than would be necessary in areas with higher population densities. Therefore, gyral regions are under-sampled in the dataset presented here. Additionally there appear to be more records of elasipodid holothurians in gyral regions in the east Pacific relative to gyral regions in other oceans. In the Pacific the intense upwelling close to the coast, combined with the greater sampling in gyres may have increased the differences in POC flux between the families (*Fig. 3.33*).

This suggests that greater sampling in low POC areas may increase the importance of POC in the differences between the families of elasipodid holothurians. Sampling of central ocean gyres is particularly poor in the north and south Atlantic, the Indian Ocean and the western Pacific. *Figure 3.34* shows areas where non-elasipodid holothurians have been sampled (>500m) and also abyssal areas where megafauna have been sampled. Gyral areas in the Indian Ocean have been sampled, suggesting the absence of the elasipodid holothurians is ecologically driven (since there is no environmental barrier to their existence). However Atlantic gyres have been comparatively under-sampled by gears that could collect elasipodid holothurians. Future research must drive to improve spatial coverage of our knowledge of temporal and spatial drivers of faunal change in the deep sea. Greater sampling in gyres, combined with long-term time series such as those at the PAP and Station M in a variety if biogeographic settings would be optimal.

3.3.3 Barriers to dispersal or sharp boundaries in environmental variables? Lessons from the Arctic

The presence of the Elpidiidae in the Arctic and the apparent exclusion of the other families is one of the main drivers of the observed environmental differences between the families in the global-scale analysis in this study (*Fig.3.11* and *3.13*). However caution must be observed in interpretation of apparent absences. In biogeographic studies which rely on Natural History Collection data the definition of absence is strict. The absence of presence does not confirm the presence of absence. The fauna of the continental shelves of the Arctic are relatively well sampled through investigations led by Canadian, North American, Scandinavian and Russian Institutions (Clarke 2003, Piepenburg 2005). The abyssal fauna of the Arctic is less well known, largely due to the presence of permanent sea-ice over in surface waters overlying abyssal depths and the logistical difficulties this presents (Clarke 2003).

A recent revision of Arctic Elasipodids identified the presence of four species. These included three previously described species; *Elpidia glacialis*, *Elpidia heckeri* and *Kolga hyalina*. Additionally a new species was identified; *Elpidia belyaevi* (Rogacheva 2007). A fifth species, *Irpa abyssicola* has been recorded from the Nordic Seas but not the central Arctic (Gebruk et al. 2003a). The results of morphological parsimony analysis of North Atlantic and Arctic forms suggests that speciation in *Elpidia* occurred by depth isolation in the Arctic after invasion by a common ancestor invading via the Atlantic. The ancestral (bathyal) form evolved into *E.belyaevi* at bathyal depths, *E.heckeri* at abyssal depths and *E.glacialis* at sublittoral depths. Elpidiid holothurians are one of the dominant megafauna in Arctic communities at bathyal and abyssal depths (Rogacheva 2007). Numbers of Elpidiidae can reach tens of thousands per trawl (Rogacheva 2007). Thus, the absence of the Psychropotidae, Deimatidae and Laetmogonidae is thought to be true (D. Billett pers. comm.) but cannot yet be confirmed due to a lack of sampling in the abyss. Other elpidiid genera which have very broad geographic distributions in the deep sea such as *Peniagone*, *Amperima*, *Ellipinion* and *Scotoplanes* are absent in the Arctic (Hansen 1975).

Thus the Elasipodida appear to have a depressed diversity in the Arctic. Depressed Arctic diversity is also known for shallow water echinoderm species (Piepenburg 2000). There are several proposed explanations for depressed deep-sea diversity in the Arctic deep sea. Such explanations are not mutually exclusive.

The young age of the Arctic deep-sea fauna is often cited as a possible cause, having arisen relatively recently during the Pleistocene (Rogacheva 2007), so that the accumulation of species is still in the early stages. Unfortunately such theories are impossible to test as they occurred in the past and cannot be directly observed (Tyler et al. 2000). Results for the Elasipodida provide support for this hypothesis. Rogacheva (2007) notes that the high levels of morphological variability evident in Arctic *Elpidia* and *Kolga* suggest that the Arctic has been invaded relatively recently and speciation is an active process. This hypothesis does not entirely explain the absence of species with very broad distributions and predicted high dispersal ability in the Arctic. Dispersal into the Arctic by deep-sea obligates may have been slowed by the topography of the Arctic and the physical barriers between the Atlantic, Pacific and Arctic. The Iceland-Faeroe ridge (also known as the Greenland-Scotland or Greenland-Iceland-Faeroe Ridge) acts as a partial or complete barrier preventing the dispersal of organisms between the Atlantic and Arctic (*Fig.3.35*). This has been shown to affect the distribution of a number of benthopelagic organisms including amphipods (Weisshappel 2000), isopods (Svavarsson et al. 1993, Negoescu and Svavarsson 1997, Svavarsson 1997) and benthic foraminifera (Gudmundsson 1998). The shallow sills (~500m) of the ridge act as a physical barrier to deep-water species (Weisshappel 2000).

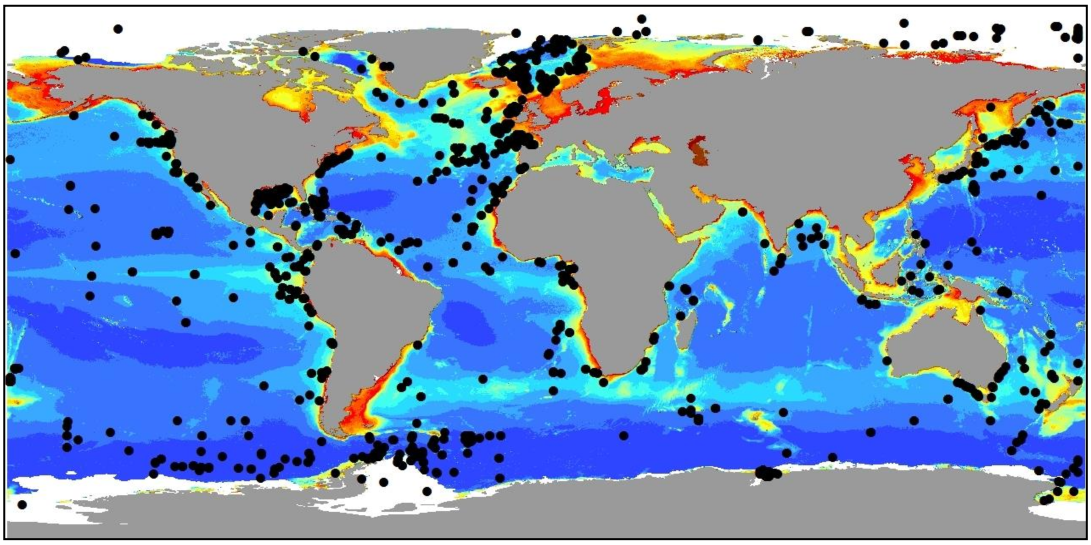


Figure 3.33 *Global distribution of elasipodid holothurian records >500m*

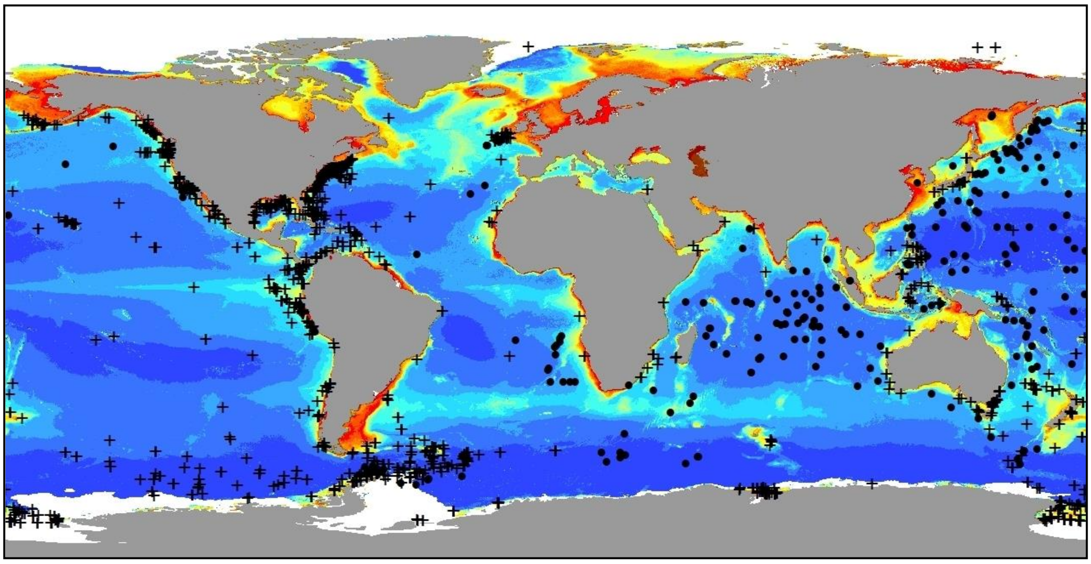


Figure 3.34 *Global distribution of non-elasipodid holothurians >500m in the GBIF database (crosses) and abyssal sampling sites that sampled megafauna (filled circles) (data courtesy of C. Stuart)*

Hansen was the first to suggest that the Iceland-Faeroe Ridge may form a barrier to the elasipodid holothurians (Hansen 1975). Some of the most frequent swimmers (*Peniagone diaphana*) and those best adapted to bathyal depths (the Laetmogonidae) are absent in the Arctic, suggesting mechanisms for overcoming the barrier are not linked to adult swimming ability or physiochemical adaptations to bathyal depths in adults. Research has shown that the range of a species pressure tolerance is higher in early life stages compared to that observed in adults (Young et al. 1997b, Tyler et al. 2000)

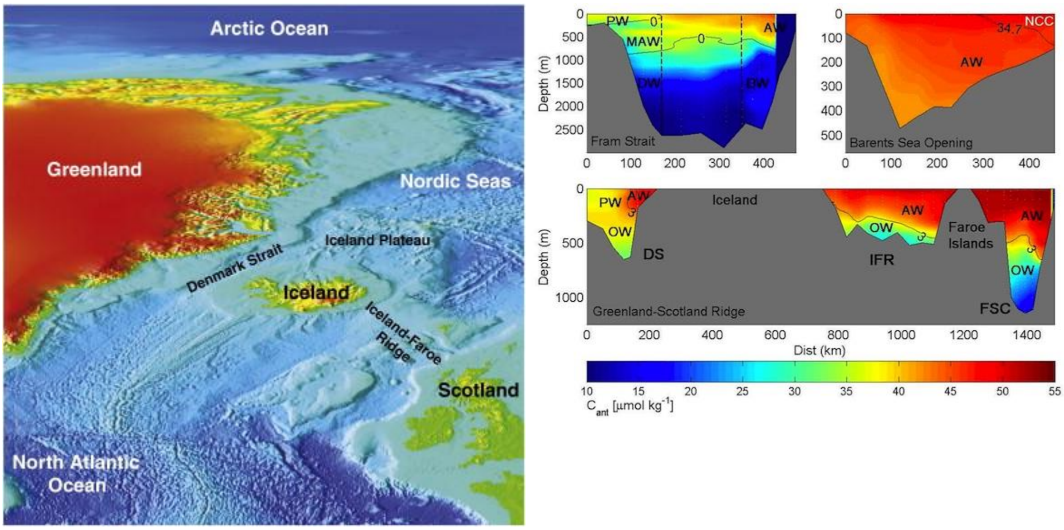


Figure 3.35 *Topography of the Iceland-Faeroe Ridge and deep water connections between the Atlantic and Arctic.*

This is corroborated by the discovery of juvenile Psychropotids (*Psychropotes longicauda*, *Psychropotes depressa*, *Psychropotes semperiana* and *Benthodytes sp*.) and Elpidiids (*Peniagone diaphana* and *Peniagone islandica*) in mid-water trawls 10 to 2750m off the seabed (Gebruk et al. 1997). However, despite the presence of all the above species in the North Atlantic, none are known from the Arctic.

Little is known about the dispersal ability or pressure tolerance of larvae in the Elasipodida. Most data relies upon the extrapolation between egg size, development mode and dispersal potential. The Elpidiidae generally have smaller eggs and tend to be more fecund than the other elasipodid families (Hansen 1975). Despite the small egg size

the Elpidiidae are still expected to have lecithotrophic larvae (Billett 1991) however the existence of planktotrophic larvae has also been proposed

(Uthicke et al. 2009) . Table 3.6 shows some published egg sizes of elpidiid holothurians. Both *Elpidia glacialis* and *Kolga hyalina* occur in the Arctic and have relatively small eggs at ~200µm. Two other elpidiid species from the NE Atlantic *Amperima rosea* and *Ellipinion molle* have egg sizes ~200µm but are absent in the Arctic. The Deimatidae and Laetmogonidae also have lecithotrophic larvae but are not found in the Arctic. The Psychropotidae should, in theory, have excellent dispersal potential. Their very large eggs may be buoyant and able to float to the surface, aiding dispersal (Gebruk et al. 1997). On the other hand, the direct development in this family may have hindered its ability to disperse into the Arctic. However, the relationship between eggs size and dispersal ability has been questioned in the Elasipodida (Young et al. 1997a). All of the families include species which have wide distributions, and those whose distribution appears to be more limited. Further research would be required to examine whether the small egg sizes of the Elpidiidae have given them an advantage in colonizing the Arctic, or whether their eggs have greater pressure tolerances than other families. In summary, with current evidence, there is no obvious mechanism by which the species of Elpidiidae found in the Arctic would have an advantage in overcoming the Iceland-Faeroe Ridge in terms of dispersal ability.

It is also possible that environmental factors may be affecting the distribution of the Elasipodida: It has been proposed that the deep Arctic will have low taxonomic richness related to latitudinal environmental gradients in temperature, productivity or seasonality (Rex et al. 1993). However, results were heavily skewed by depressed diversity in the Norwegian Sea.

The Norwegian Sea has been affected by historical biogeographic events (Lambshead et al. 2000), and is influenced by the Iceland-Faeroe Ridge. In studies of North Atlantic diversity which exclude Norwegian Sea data, a positive relationship between latitude and diversity has been discovered(Lambshead et al. 2000). This is thought to be related to gradients in productivity, and similar patterns have been noted in the Pacific (Lambshead et al. 2002). Therefore

Table 3.6 *Published records of Maximum egg size in the Elpidiidae*

Species	**Max. egg size (µm)**	**Ocean reproduction studied**	**Arctic?**	**Depth zone**	**Source**
Protelpidia murrayi	570	Southern	No	Slope/Bathyal	Galley et al. 2008
Peniagone vignoni	570	Southern	No	Slope/Bathyal	Galley et al. 2009
Peniagone azorica	370	Atlantic	No	Abyssal	Tyler et al. 1985
Peniagone diaphana	370	Atlantic		Abyssal	Tyler et al. 1985
Amperima rosea	200	Atlantic	No	Abyssal	Wigham et al. 2003
Ellipinion molle	200	Atlantic	No	Abyssal	Wigham et al. 2004
Kolga hyalina	240	Atlantic	Yes	Abyssal	Billett 1991
Elpidia glacialis	200	Atlantic	Yes	Abyssal	Hansen 1975

depressed Arctic diversity appears to be part of a steep cline, rather than a gradual gradient.

Temperature is the sharpest cline at abyssal depths (Fig. 3.36d). Echinoderm larvae are often associated with a certain water mass of specific temperature or salinity (Banse 1986). Furthermore, laboratory studies have shown that they are able to change their vertical distribution in the water column in relation to temperature by affecting changes in swimming ability or by inducing an inherent behavioural response (Daigle and Metaxas 2011)

Echinoderm larvae have been shown to display adaptive avoidance of low temperatures (Daigle and Metaxas 2011). If larvae are able to cross the ridge, the sharp cline in seafloor temperature may induce a behavioural response in some larvae, reducing the chance of their survival or settlement. However, it is unclear how the Elpidiidae would have an advantage in survival in cold conditions, unless they show greater phenotypic plasticity than the other families.

Intra-annual seasonality also increases northwards in a steady gradient (Fig. 3.36b). The only other area that reaches such strong intra-annual seasonality is the Southern Ocean. As this Chapter has shown, the Elpidiidae are particularly well adapted to areas with great temporal variability in POC flux. The Elpidiidae may therefore be the only family whose life-history allows for survival in the extremely variable environment of the Arctic. However, such strong variations exist in the Southern Ocean. Though differentiation between the families occurs here, overlap shows that members of the other families can exist in such seasonal environment. Alternatively the preference of the Elpidiidae for highly variable environments and evidence for adaptations for this environment may give them a competitive advantage in the Arctic over the other families. The high densities which they occur in (Rogacheva 2007) in the Arctic could be reducing food or biochemical components of food required by other families.

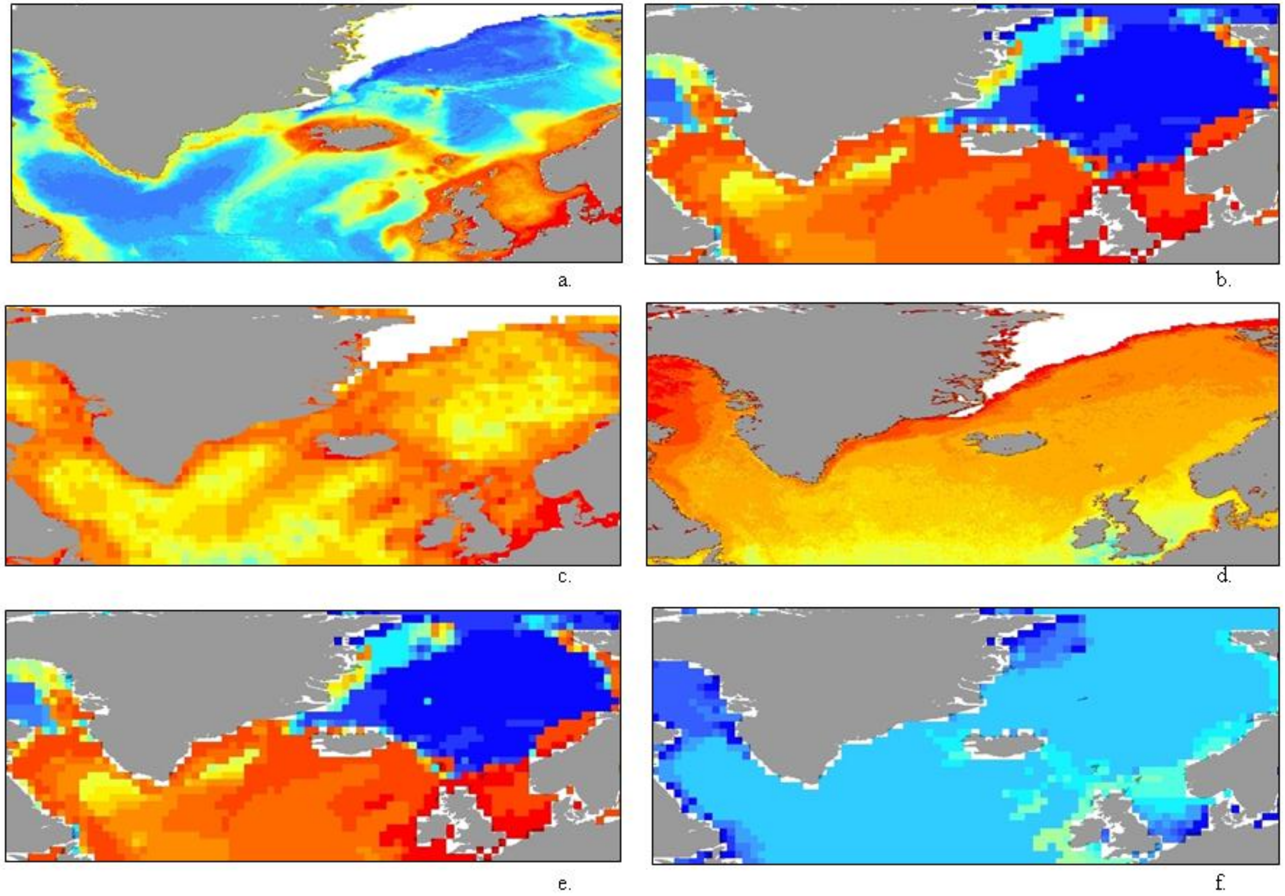


Figure 3.36 *Environmental conditions in the N Atlantic and Arctic in the vicinity of the Iceland-Faeroe Ridge a) POC flux; b) intra-annual seasonality; b) inter-annual variability; d) temperature; e) oxygen and f) salinity. All layers and scales as described in Chapter 2*

Therefore environmental preferences of the Elpidiidae could have given them an advantage in colonising the Arctic once the barrier of the ridge was overcome. Whilst environmental variables are therefore unlikely to be the driving force of the absence of the Deimatidae, Psychropotidae and Laetmogonidae, they could be acting as reinforcement to the partial physical barrier posed by the Iceland-Faeroe Ridge.

3.3.4 Conclusions

At the scale of individual oceans there is much overlap in the distribution of the families of elasipodid holothurians. The greatest differentiation exists between the Psychropotidae and the Elpidiidae, often along gradients of food variability. This agrees with local scale temporal biochemical and community composition studies. Differentiation is much greater at bathyal depths, where elasipodid holothurians experience a wider range of environmental conditions. Differences at abyssal depths do occur, but are harder to relate to single environmental factors. Physical barriers to dispersal exist in the deep-sea. Such physical barriers are semi-permeable, but may be reinforced by the existence of different environmental conditions on either side of the barrier. Greater sampling effort is needed underneath central ocean gyres and open ocean areas generally. This may increase the observed level of environmental differentiation between the Psychropotidae and Elpidiidae at abyssal depths, or it may detect the "bottom limit" of food quantity and/or seasonality/quality for elasipodid holothurians. Temporal variability is a more important control on elasipodid community composition. A better understanding between the effect of intra-annual and inter-annual variability of phytoplankton production on the species composition, physical form the biochemical composition of flux is critical for understanding both temporal community dynamics and biogeography of the abyssal deposit feeding fauna. Additionally improved knowledge of the reproductive mode of elasipodid families, especially the prevalence of lecithotrophy and planktotrophy is essential for understanding competitive dynamics and resilience to highly dynamic temporal environments. Inter and

Table 3.7 *Summary of statistical analyses of environmental differentiation in Chapter 3*

Ocean	**Depth Zone**	**ANOSIM statistically significantly different elasipodid families, from the overall elasipodid distribution**	**Discriminant Function Analysis (DFA) significance**	**DFA best correlates**
Atlantic	Bathyal	Psychropotidae	Significant	Depth
	Abyssal	Deimatidae, Elpidiidae	Significant	Depth, Inter-annual variability
Pacific	Bathyal	Elpidiidae	Significant	POC, inter-annual variability, intra-annual seasonality, salinity
	Abyssal	Elpidiidae, Psychropotidae	Significant	Salinity
Indian	Bathyal	Laetmogonidae, Psychropotidae	Non-Significant	-
	Abyssal	-	Non-Significant	-
Southern	Bathyal	Elpidiidae, Laetmogonidae	Significant	Inter-annual variability, Oxygen, POC, temperature
	Abyssal	Psychropotidae	Non-Significant	-

intra-familial resolution is required. Niche conservatism is not thought to be an important factor driving differentiation between families. Conservatism in life-history strategy is likely to be more important, although it is thought that variation in reproductive mode exists within families. In fact differentiation within the Elpidiidae to different biochemical components of the flux may allow species co-existence. Studies into biochemical feeding preference of other con-familial species would allow us to understand whether niche differentiation is an important driver of diversity in elasipodid holothurians.

Chapter 4. Are biogeographical provinces evident in surface waters applicable to deep sea organisms?

4.1 Introduction

The theoretical division of the ocean into distinct biogeographic regions has been debated since the 19th Century (Forbes 1844, Wallace 1876). Biogeographic provinces are categories for comparing and contrasting biogeochemical processes and biodiversity between ocean regions (Oliver and Irwin 2008). There is now a considerable body of work supporting the existence of marine biogeographic provinces. Many suggested zonation patterns have focused upon the benthos of coastal waters and continental shelves (Briggs 1974, Briggs and Bowen 2012, Spalding et al. 2012), whilst some have focused on open-ocean pelagic (Longhurst 2007) or deep-sea benthic zonation (Vinogradova 1959, 1997, Sokolova 2000). Three broad approaches have been applied to identifying biogeographic provinces; 1) those that base divisions on taxonomic or zoogeographic factors i.e. the distributions of the organisms dictate the limits of the provinces 2) those that are identified largely by abiotic or ecological features and 3) those that contain political boundaries where zonation are designed with a specific management objective.

Delineation of biogeographic provinces based on zoogeography often rely on the percentage of endemic species in selected groups of fish or invertebrates (Ekman 1953, Briggs 1974, Briggs and Bowen 2012) or by groups of overlapping species boundaries (Van Den Hoek et al. 1995). Early biogeographic delineations for deep-sea organisms were largely of this first type. Such zonations were often based upon single taxa that were of particular interest to the authors, with limited geographic coverage (Ramirez-Llodra et al. 2010). Criticisms of such approaches include the lack of generality of such patterns (Longhurst 2007), the problems of determining provinces based on species rarity (Adey and Steneck 2001) and problems of removing the influence of spatial variability in sampling effort (Adey and Steneck 2001). Whilst early studies included the entire abyssal zone as one large province with largely cosmopolitan species (Bruun 1957). Further investigation led (Ekman 1953) to suggest the existence of four deep-sea provinces; the Atlantic, the Indo-Pacific, the Arctic and the Antarctic. Based on the extensive findings of Russian research, Vinogradova proposed a zoogeographic regionalisation of the abyss including three regions; the Pacific-North-Indian, the Atlantic and the Antarctic. These were then subdivided into six sub-regions and finally into eight provinces based on observed faunal distributions (Vinogradova 1959, 1997) (Fig. 1.3). Vinogradovas' provinces were based on % endemism and similarity between areas. Additionally Vinogradova believed the topography of the deep sea played a large part in creating observed faunal patterns. Accordingly, Vinogradova's zoogeographic regions were separated to a great extent by submarine ridges, which were thought to constitute barriers to dispersal for abyssal species (Vinogradova 1959). According to (Thistle 2003) the topography of the deep sea 'sets the stage' for the biogeography of abyssal organisms. Within major ocean basins the habitat is more or less continuous at slope depths, along isobaths, with the exception of relatively small features such as submarine canyons. Below 2500m the mid-ocean ridges and submarine mountain ranges divide the major oceans into separate regions and below 3500m the deep-sea floor is composed of isolated basins (Thistle 2003).

However (Hansen 1975) found that the topography of the ocean floor at abyssal depths had very little influence on the distribution of the Elasipodida and their distribution contradicted the scheme proposed by Vinogradova. A major exception is the Wyville Thomson Ridge which separates the Atlantic and Arctic. It appears to form an impenetrable barrier to practically all of the elasipodid holothurians of the North Atlantic (Hansen 1975), excluding them from the Arctic Basin.

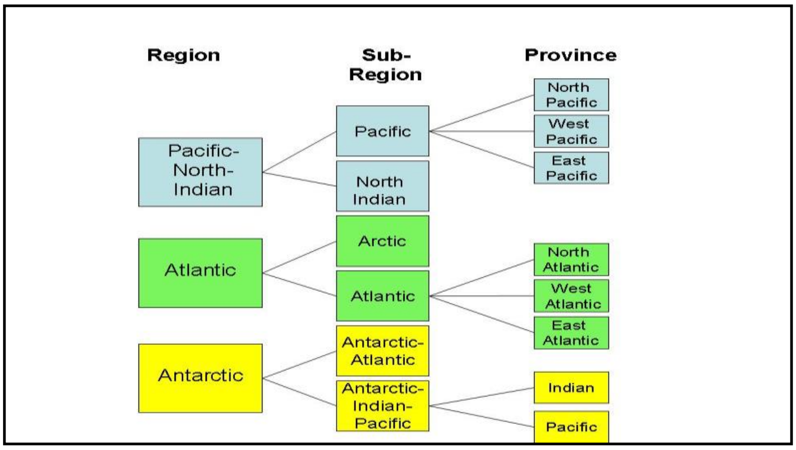


Figure 4.1 *Vinogradova's division of abyssal zoogeographic regions, sub-regions and provinces based on faunal distributions.*

Hansen also adds that topographic barriers such as ridges often exert a pronounced influence on the hydrographic conditions in the basin, making it difficult to separate topographic effects from hydrographical ones (Hansen 1975). Menzies et al. (1973) proposed zoogeography which included five large zones that largely agreed with previous work, dividing the World Ocean into Pacific, Arctic, Atlantic, Indian and Antarctic zones. However, the 13 provinces and 17 regions based on topography and temperature of the ocean floor that Menzies et al. (1973) suggested had little biological support, according to Vinogradova (1997). This led Vinogradova to suggest that zoogeographic constructions based on changes in environmental factors, but not on studying species ranges, was an incorrect approach. However, Vinogradova does concede that environmental factors are important in determining abyssal species distributions. In particular Vinogradova recognised the potential for 'trophic conditions', linked to surface water productivity, to affect species ranges and zoogeographic patterns. Extensive work on the effect of 'trophic conditions' on the megabenthos by Russian researchers has shown clear differences between species occupying eutrophic and oligotrophic regions (Sokolova 2000). Latitudinal turnover in species composition, which is not related to topography or hydrography, is another example of environmental factors affecting abyssal zoogeography (Vinogradova 1997).

It is now acknowledged that physical and biological forces interact in the marine environment to create a complex emergent seascape commonly characterized by conservative (temperature and salinity) and non-conservative (ocean colour and nutrient concentration properties (Oliver and Irwin 2008). Despite this complexity, such factors are being increasingly used to delineate biogeographic provinces. For example (Adey and Steneck 2001)used a temperature-space-time integrated model to develop biogeographic regions for benthic marine algae. Biogeographic provinces based upon zoogeographic data are desirable in areas wherever data is sufficient (Spalding et al. 2012). However where data is severely lacking, such as in the deep sea, zonation based on readily available environmental data may be more suitable (Spalding et al. 2012). At the very least, such zonation acts as a testable hypothesis for existing and future species distribution data.

Perhaps the most thorough application of ecological and abiotic factors to the subdivision of the Oceans was by Alan Longhurst (Longhurst et al. 1995, Longhurst 2007). The main catalyst for this development was the development of sensors carried on earth-orbiting satellites allowed a new and much more in depth partitioning of the oceans into biogeochemical biomes and provinces (Longhurst 2007). The CZCS obtained data representing the surface colour of the oceans and thus was able to show phytoplankton chlorophyll distribution (Longhurst 2007). When combined with information on the parameters of the photosynthesis-light relationship, the sun angle and cloudiness, ocean colour information can provide an estimate of global net primary production in the ocean (Longhurst et al. 1995). Additionally, there exists a general relationship between phytoplankton biomass (given as chlorophyll concentration) and the characteristic dimension of phytoplankton cells, which tend to be smaller in less productive water and larger in more productive water. This means that a simple global chlorophyll field has the potential to inform us about a wide range of ecological characteristics of the organism, whose pigment is observed (Longhurst 2007). In a study by (Longhurst et al. 1995), an examination of all available regional and seasonal images of the surface CZCS chlorophyll field for characteristic, observable, and repetitive regional patterns, both spatial and temporal, of surface chlorophyll enhancement at the surface of the ocean (Longhurst 2007) was undertaken. Longhurst et al. (1995) then considered this alongside data on the regional oceanography of all parts of the ocean and critical studies of plankton ecology, particularly those describing the growth, consumption and/or sinking of phytoplankton blooms. The result was the description of four types of primary biome which describe

broad ecological characteristics of latitudinal areas common across the world's oceans (Longhurst et al. 1995, Longhurst 2007). Two of these biomes relate primarily to open ocean areas; the Westerlies biome and the Trades biome. The names of these biomes relate to the prevailing winds under which they are situated. The Polar biome relates to areas north of the Polar Front in the northern Hemisphere and those areas south of the Polar Front in the Southern Hemisphere. The Coastal biome includes coastal regions and shallow seas in low-mid latitude areas. Whilst the provinces form an interesting biogeographical hypothesis at the species level, the distribution of functional groups at a taxonomic level greater than that of species, may be expected to differ between biomes. A description of the main ecological features of each biome follows, as this is the primary determinant of the ecological characteristics of the provinces within.

The Westerly biome is found at higher latitudes (*Fig. 4.2*) and can be further subdivided into ecological conditions under Longhurst's Case 2 or Case 3 scenarios of production. Case 2 areas are generally higher latitude areas under the influence of stronger Westerly winds with a deep mixed layer. A temperature specificity in the Atlantic and a salinity specificity in the Pacific delineate between Case 2 and Case 3 regions (Longhurst 2007). In Case 2 there is a nutrient limited spring peak, with a second peak associated with reduced herbivory in the later summer. Production and consumption are somewhat uncoupled in these areas. Case 3 Westerly areas are open-ocean mid-latitude areas where winter westerly winds are lighter than in case 2. Primary production rates are low and seasonality is weak. There is a winter-spring peak in production rate, but chlorophyll only shows a broad peak in winter but does not track the spring surge. This is because of greater coupling between production and consumption rates (Longhurst 2007).

The Trade biome can also be subdivided into areas which fall under two separate production scenarios; Cases 4 and 5. Case 4 is defined by a relatively small amplitude response to trade wind seasonality. These are generally low latitude areas where geostrophic adjustment of zonal equatorial current systems. This results in low values of primary production rate and phytoplankton biomass and weak seasonality. There is a minor increase in PP rate forced by the geostrophic response of the pycnocline to seasonality in trade wind stress, and also to Ekman divergence and suction, especially at the equator. Strong coupling exists between primary production and consumption. Case 5 prevails where much larger amplitude responses occur as a result of Monsoon or Monsoon-like reversal of trade winds. In its most extreme form, in the northwest Indian Ocean, the seasonal reversal in prevailing winds results in a reversal of the monsoon currents, down to as much as 1000m depth. This results in an ecosystem-scale shift between oligotrophic and eutrophic conditions. Whilst wind stresses comparable to those in Westerlies during winter, the result is a much shallower mixed layer depth. Despite this, four seasons are detectable, due to small changes in the pycnocline and its relative position to the photic zone. Productivity is not light limited and responds rapidly to coastal upwelling and other processes associated with the reversal in trade winds. Production and Consumption are once again, closely coupled (Longhurst 2007).

The Polar biome and the Coastal biome are each defined by only one model; Cases 1 and 6 respectively. The Polar biome is the only biome whose production rate is largely limited by irradiance. Beyond the Polar Fronts of each hemisphere, a halocline defines the surface mixed layer within a relatively isothermal water mass. The pycnocline is mixed deepest during the winter months in open ocean areas which are clear of ice. A single light-limited maximum in PP rate is reached around the summer solstice following which summer oligotrophic conditions develop. A decoupling exists between consumption and production during later summer in the Polar biome. Whilst the production rate continues to decrease a second peak in chlorophyll occurs during late summer as migratory consumers descend into deeper waters for the winter (Longhurst 2007).

Finally, Longhurst defines the Coastal Biome as being characterised by intermittent production, but limits its application mostly to the four eastern boundary upwelling areas at low-moderate latitudes. Here coastal winds during summer result in deep nitrate rich waters being entrained into the photic zone, resulting seasonally high PP rate values. This results in a seasonal 'march' of upwelling from low to mid-latitudes during the some and from mid to low latitudes in the winter. A central region of more or less continual upwelling also occurs, where chlorophyll accumulation is balanced by advection and consumption. Coastal provinces are also locally affected by coastal asymmetry, geomorphology and tidal forcing.

In order to capture the fine detail observed in the satellite images and of the surface chlorophyll field, each biome was then further subdivided, forming 57 biogeochemical provinces, in order to reflect the regional structure of the pelagic ecosystem (*Fig. 4.4*). Longhurst (2007) determined these smaller provinces on the basis of characteristics of regional circulation and stratification, bathymetry, river discharges, characteristic coastal wind systems, location of islands and the distribution of land masses.

Longhurst's Provinces have now been shown to successfully describe biogeographic patterns in a number of taxa from a number of marine habitats with a range of life histories. These include high dispersal pelagic organisms such as billfish and tuna (Reygondeau et al. 2012) and leatherback turtles (Fossette et al. 2010), revealing an unexpectedly strong link between these communities and physical and chemical characteristics of the oceans. Communities of benthic polychaetes in European Seas were also found to closely correlate with Longhurst's biogeographic provinces in a comparison of different classification systems (Arvanitidis et al. 2009). A weak but significant relationship was found between Longhurst's provinces and global and pelagic and benthic bacterial community composition (Zinger et al. 2011). Additionally Longhurst's provinces have been found to be useful to predict geographic patterns of properties of pelagic consumer communities (Jennings et al. 2008) and have applications for fisheries management (Chassot et al. 2011).

"To what extent can the logic of an open ocean partition be relevant to the biogeography of shelf organisms?" is a question posed by Longhurst himself (Longhurst 2007). The process of benthic pelagic-coupling, described extensively in Chapters 1&2 forms the link between the biogeographical subdivisions of surface waters proposed by Longhurst and biogeographic processes in the deep sea. Indeed, province designations by Longhurst have been found to be linked to the quantity and temporal variability of flux of organic matter to the deep-sea. (Lampitt and Antia 1997a) explored the utility of using the eight planktonic climatological categories proposed by Longhurst (1995) as a means to examine flux and its variability. They discovered that each category had a characteristic level of variability associated with it, with highest levels (most stable, least seasonal variation) in the tropics and highest levels in the Antarctic (least stable, most seasonal variation. Waniek et al. (2005) found that province specific limitation of primary production in the upper ocean successfully predicted the particle flux to depth in three provinces in the northeast Atlantic. Similarly global datasets of biogeochemical fluxes have been found to be concordant with Longhurst's scheme (Boyd and Doney 2003, Ducklow 2003). Clearly biogeographical provinces are mirrored to some extent by the environmental conditions in the deep-sea.

Research is beginning to show that Longhurst's provinces are also mirrored by the distributions and biodiversity of some deep-sea organisms and communities. In an indirect assessment investigated the relationship between surface production in the western North Atlantic using SeaWiFs satellite colour imagery, and the standing stock of deep-sea macrobenthos (Johnson et al. 2007). They found that estimated POC flux at depth accounted for 62-67% of the variance in benthic standing stock. Longhurst's proposed biogeographic scheme has also been more directly detected in the distribution of deep-sea fauna. Longhurst's provinces were a better explanatory variable than either depth or simple surface primary productivity in ostracods collected from the surface to 2000m depth in the Atlantic (Angel et al. 2007). Similarly (Macpherson 2002) found large-scale patterns of diversity of pelagic and benthic organisms at 0-2000m depth to relate closely to the limits suggested by Longhurst's (1995) provinces along gradients on either side of the Atlantic. Evidence of Longhurst's provinces have also been noted in the abundance of abyssal polychaetes and cumaceans (Paterson et al. 1998, Watling 2009) and densities of abyssal benthic foraminifera in the Atlantic (Gooday 1996) along transects spanning two or more of Longhurst's' provinces.

Previous studies have therefore focused on macrofauna in the Atlantic, with few tests of Longhurst's provinces in the Pacific, Indian and Southern Oceans. Even fewer have addressed more than two sites for the megafauna (Thurston et al. 1998). Additionally, the affect of Longhurst's biomes and cases on the distributions of pelagic and benthic organisms has been almost entirely ignored.

Because of known ecological (Billett et al. 2001, Wigham et al. 2003a, Hughes et al. 2007, FitzGeorge-Balfour et al. 2010) and related distributional differences (*Chapter 3*) between the species and genera of the elasipodid holothurians in relation to various aspects of the availability and quality of organic matter flux they provide an excellent model taxa with which to test the utility of Longhurst's zonation. Therefore the following analysis uses the species distribution database described in Chapter 2 (*Sections 2.1 and 2.4*) to compare similarities in the taxonomic composition of the provinces, cases and biomes.

This study tests four hypotheses. The first is that the species composition of Longhurst's provinces will be significantly different between Longhurst's biomes and cases whilst statistically allowing for differences between Oceans and that vice versa; differences between species composition of Longhurst's provinces will be significantly different between Oceans, whilst statistically allowing for differences between Longhurst's biomes and cases. The second is that the generic composition of Longhurst's provinces is significantly different between Longhurst biomes and cases whilst allowing for differences between Oceans but that the opposite is not true; generic composition of Longhurst's provinces does not differ significantly differ between Oceans, whilst statistically allowing for differences between Longhurst's biomes and cases. The third hypothesis tests the utility of Longhurst's provinces. Because of the lack of discrete quantitative samples with which to formally test between provinces, it is simply expected that the species composition of Longhurst's provinces should be more similar to other provinces which are geographically proximate and ecologically similar than they are either to geographically distant or ecologically different provinces. Finally the fourth hypothesis, which also tests the utility of Longhurst's provinces, is that species with similar distributions should form groups that are largely coherent with oceans, and within oceans, with Longhurst biomes or cases.

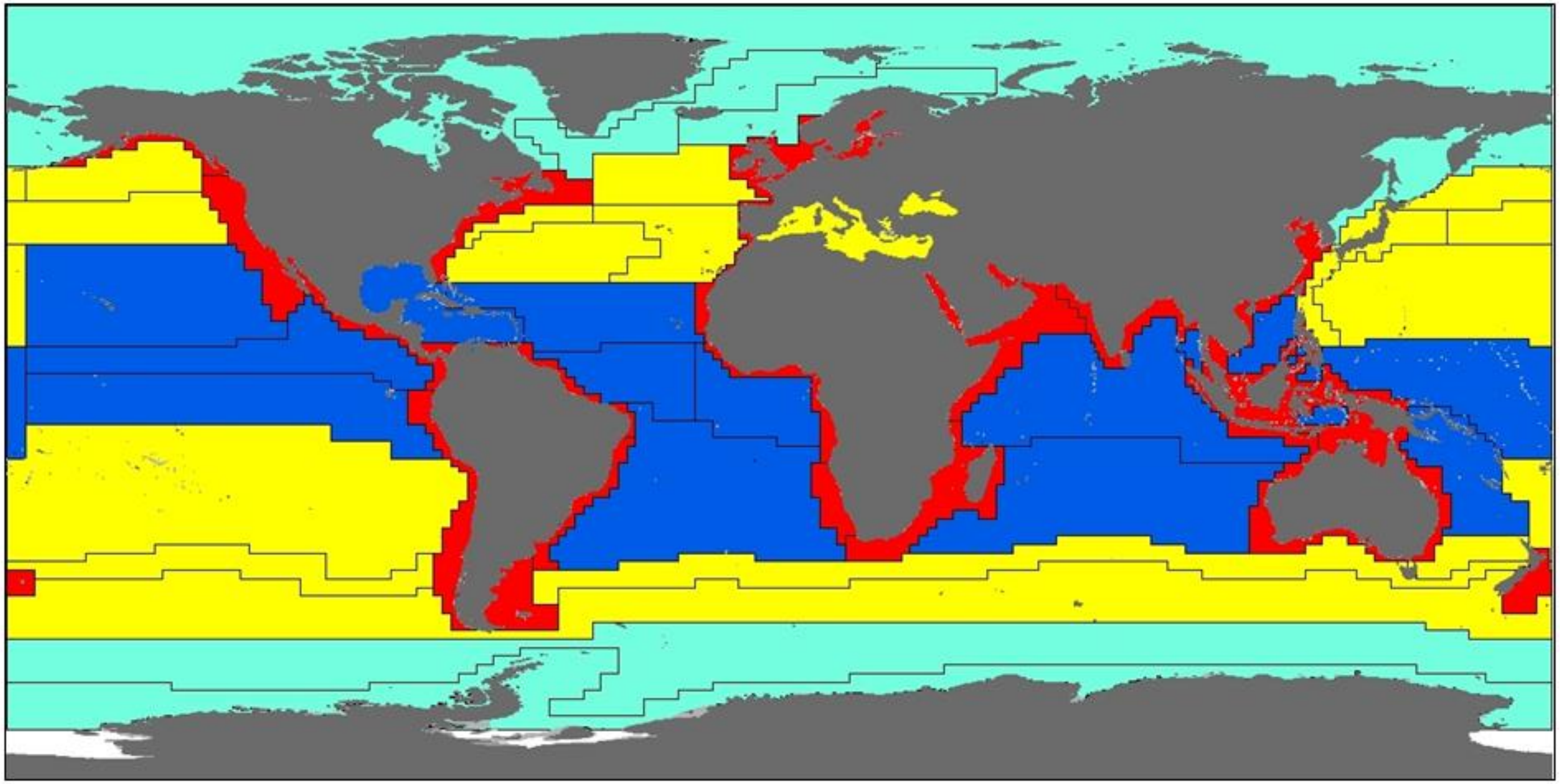


Figure 4.2 *The distribution of Longhurst's (1997, 2007) provinces (outline in black) and biomes. Dark blue provinces are within the Trades biome, yellow provinces are within the Westerlies biome, light blue provinces fall within the Polar biome and red provinces are within Longhurst's Coastal biome*

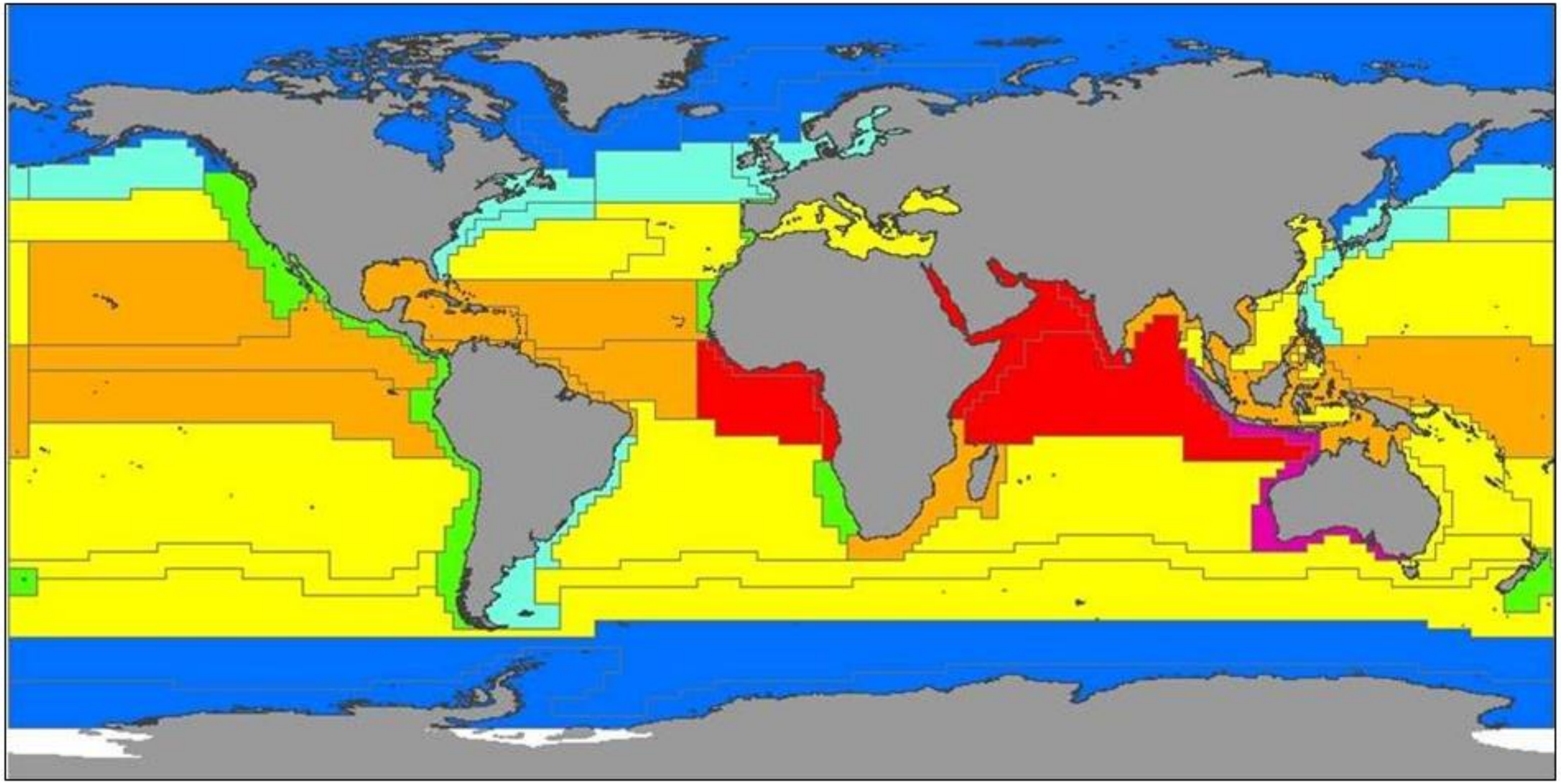


Figure 4.3 *The distribution of Longhurst's (1997, 2007) provinces (outline in black) and cases. Dark blue provinces fall under Case 1, light blue provinces fall under Case 2, yellow provinces fall under Case 3, orange provinces fall under case 4, red provinces fall under Case 5 and green areas fall under Longhurst's Case 6 scenario. Purple provinces were not assigned to a Case by Longhurst due to a lack of long-term data.*

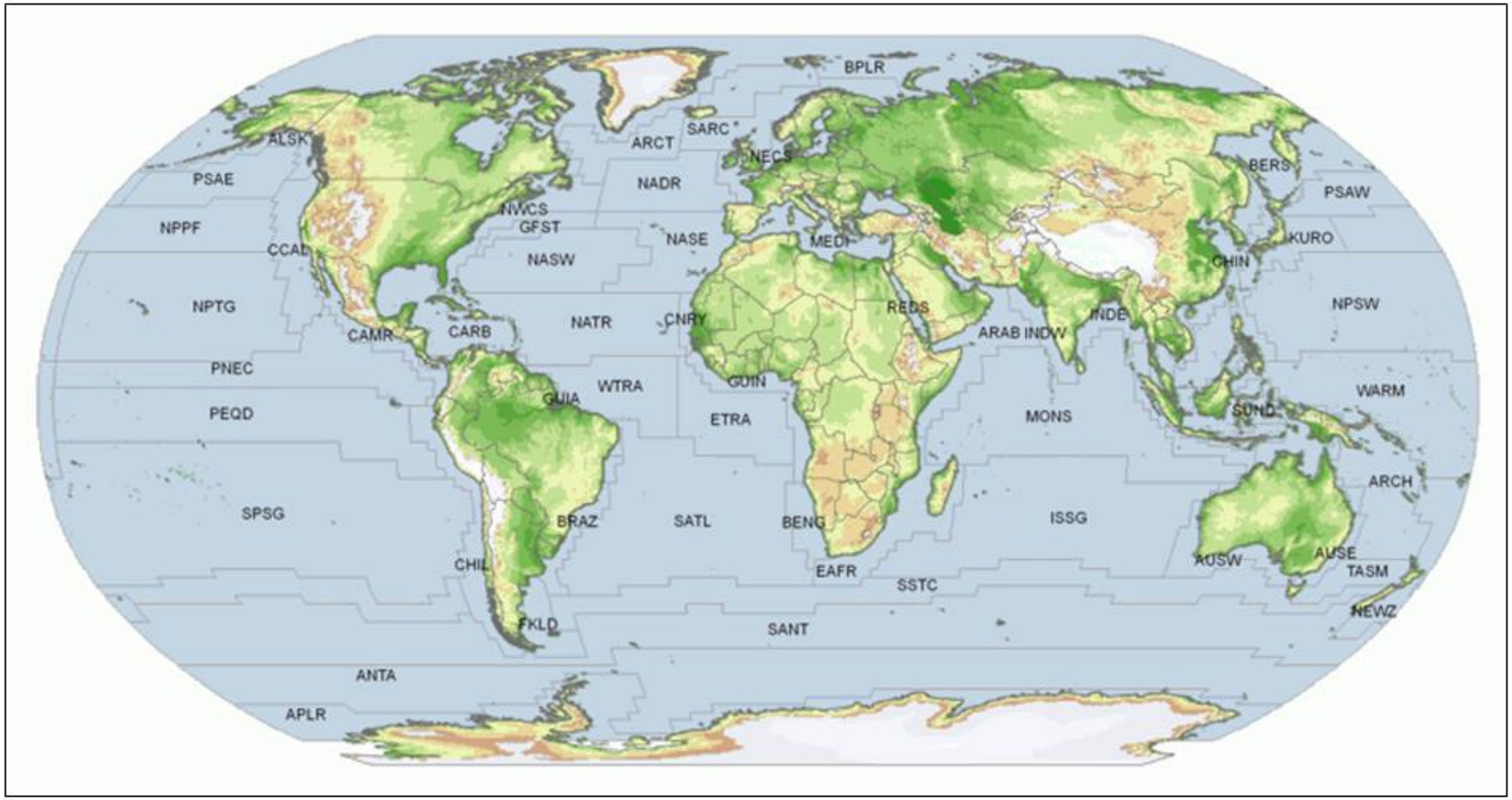


Figure 4.4 *The distribution of Longhurst's biogeochemical provinces from* http://www.vliz.be/vmdcdata. *Province codes refer to provinces descriptions given in* ***Table 4.1***

Table 4.1 *Province codes and description of Longhurst's biogeochemical provinces*

Province code	Province description	Province code	Province description
ALSK	Coastal - Alaska Downwelling Coastal Province	BERS	Polar - N. Pacific Epicontinental Province
AUSW	Coastal - Australia-Indonesia Coastal Province	ARCH	Trades - Archipelagic Deep Basins Province
BENG	Coastal - Benguela Current Coastal Province	CARB	Trades - Caribbean Province
BRAZ	Coastal - Brazil Current Coastal Province	ETRA	Trades - Eastern Tropical Atlantic Province
CCAL	Coastal - California Upwelling Coastal Province	MONS	Trades - Indian Monsoon Gyres Province
CNRY	Coastal - Canary Coastal Province (EACB)	ISSG	Trades - Indian S. Subtropical Gyre Province
CAMR	Coastal - Central American Coastal Province	NATR	Trades - N. Atlantic Tropical Gyral Province (TRPG)
CHIL	Coastal - Chile-Peru Current Coastal Province	PNEC	Trades - N. Pacific Equatorial Countercurrent Province
CHIN	Coastal - China Sea Coastal Province	NPSW	Trades - N. Pacific Subtropical Gyre Province (West)
EAFR	Coastal - E. Africa Coastal Province	NPTG	Trades - N. Pacific Tropical Gyre Province
INDE	Coastal - E. India Coastal Province	PEQD	Trades - Pacific Equatorial Divergence Province
AUSE	Coastal - East Australian Coastal Province	SPSG	Trades - S. Pacific Subtropical Gyre Province
GUIA	Coastal - Guianas Coastal Province	SATL	Trades - South Atlantic Gyral Province (SATG)
GUIN	Coastal - Guinea Current Coastal Province	WARM	Trades - W. Pacific Warm Pool Province
NECS	Coastal - NE Atlantic Shelves Province	WTRA	Trades - Western Tropical Atlantic Province
NEWZ	Coastal - New Zealand Coastal Province	GFST	Westerlies - Gulf Stream Province
ARAB	Coastal - NW Arabian Upwelling Province	KURO	Westerlies - Kuroshio Current Province
NWCS	Coastal - NW Atlantic Shelves Province	MEDI	Westerlies - Mediterranean Sea, Black Sea Province
REDS	Coastal - Red Sea, Persian Gulf Province	NADR	Westerlies - N. Atlantic Drift Province (WWDR)
SUND	Coastal - Sunda-Arafura Shelves Province	NASE	Westerlies - N. Atlantic Subtropical Gyral Province (East) (STGE)
FKLD	Coastal - SW Atlantic Shelves Province	NASW	Westerlies - N. Atlantic Subtropical Gyral Province (West) (STGW)
INDW	Coastal - W. India Coastal Province	NPPF	Westerlies - N. Pacific Polar Front Province
ANTA	Polar - Antarctic Province	PSAE	Westerlies - Pacific Subarctic Gyres Province (East)
ARCT	Polar - Atlantic Arctic Province	PSAW	Westerlies - Pacific Subarctic Gyres Province (West)
SARC	Polar - Atlantic Subarctic Province	SSTC	Westerlies - S. Subtropical Convergence Province
APLR	Polar - Austral Polar Province	SANT	Westerlies - Subantarctic Province
BPLR	Polar - Boreal Polar Province (POLR)	TASM	Westerlies - Tasman Sea Province

4.2 Results

4.2.1 Statistical differences in species and generic composition of provinces from different Oceans, Longhurst biomes and Longhurst cases

4.2.1.1 Species

Visual projections of differences between provinces large overlap between the species composition of provinces in different biomes (*Fig. 4.4a*). When the same plot is sorted by Ocean (*Fig.4.4b*) it seems apparent that there is greater discrimination in species composition of provinces between oceans. Clustering also exists between Longhurst's cases (*Fig.4.4c*) although overlap also occurs here.

Two-way crossed ANOSIMs results confirm these observations. The species composition of provinces were significantly different between Biomes, when Oceans were accounted for (R= 0.207, $p<0.005$), and significantly different between Oceans, when Biomes had been accounted for (R=0.363, $p<0.005$). However, the higher R–value between Oceans, suggests that differences in species composition is greater between Oceans than between biomes. In order to investigate the influence of the Arctic fauna on the overall results, the ANOSIM was repeated, without Arctic provinces included, this is because of the existence of substantial topographic barriers to dispersal, which may skew the results (see Chapter 3). When the three Arctic provinces were removed, the 2-way ANOSIM found Ocean differences to be significant, with differences between the Biomes no longer significant (R=0.095, $p>0.05$). Pairwise comparisons of biomes in the 2-way ANOSIM analysis are shown in *Table 4.2a* but are not discussed as the overall test statistic was not significant. The pairwise comparisons for oceans show that the only significant differences are between the Pacific and Atlantic (*Table 4.2b*), although the Atlantic and Southern have a high R-value but a low number of possible permutations, suggesting lack of statistical significance due to small sample size (*Table 4.2b*).

When Longhurst's Cases (rather than Biomes) were investigated with a two-way crossed ANOSIM (Ocean, Longhurst Case) it was found to be a more powerful factor at discriminating between the species composition of provinces than 'Biome' (R= 0.315, p<0.001). However in the Ocean- Case 2-way ANOSIM it should be noted that the significance of the Ocean as an explanatory factor in the similarity between provinces also increased (R=0.383, p<0.002). Whilst pairwise analysis found statistically significant differences in the species composition between Case 1 and all other Cases (results not shown), when the Arctic was removed, differences remained; Case 1 provinces (polar irradiance mediated production peak- strong seasonality) were significantly different from Case 3 provinces (winter-spring production with nutrient limitation-weak seasonality) (*Table 4.3*). Also, high R-values between Case 1 (polar) provinces and provinces in Case 3, 4, 5 and 6 suggest strong differentiation exists despite the lack of statistical significance. After removal of the Arctic, only 3 Polar provinces remain resulting in a low number of possible permutations (*Table 4.4*). This results in low-power in significance tests between the polar and other provinces, so that high R-values are probably better indicators of differentiation than statistical significance (Clarke et al. 2008).

The pairwise analysis of the Ocean, biome 2-way crossed analysis found no significant difference in species composition between Trade and Westerlies provinces, once differences between oceans had been accounted for (*Table 4.2a*). However, when the relationship is broken down further into Longhurst 'Cases' significant differences emerge between Case 2 (nutrient limited spring production peak – strong seasonality) provinces which are generally found in the Westerly biome and Case 3 (winter-spring production with nutrient limitation – weak seasonality) and Case 4 (small amplitude response to trade wind shift – weak seasonality) provinces which are generally found in Trade Provinces (*Table 4.3*).

Significant differences also exist between cases found in the same Longhurst biome. Cases 2 and 3, which both generally occur in the Westerlies biome (Clarke et al. 2008) were found to be statistically significant (*Table 4.3*).

4.2.1.2 Genera

Visual projections of differences between provinces appear to show an opposite pattern to those observed in the species level analysis. Whilst there is relatively little similarity in generic composition between provinces in the same Ocean (*Fig. 4.5b*), provinces of the same biome seem more coherently clustered, suggesting generic composition between provinces of the same biome are more similar than between provinces of different biomes (*Fig. 4.5a*), albeit with much overlap. Longhurst's cases appear to offer a further level of clustering, again with overlap (*Fig.4.5c*).

At the generic level the once the Arctic provinces were removed, neither biome (ANOSIM, R=0.047, p>0.2) or ocean (ANOSIM, R=0.091, p>0.1) were found to have significantly different generic composition in provinces. Pairwise comparisons are shown between biomes (*Table 4.5a*) and Oceans (*Table 4.5b*) but are not discussed as the overall tests found them not to be significant.

Two-way crossed ANOSIMS of Ocean and Case however, found Ocean to be an insignificant factor (R=0.165, p>0.05), but Case to be significant (R=0.161, p<0.005).

Pairwise comparisons (*Table 4.6*) find that Case 1 (polar irradiance mediated production peak-strong seasonality) to significantly different in generic composition of its provinces to Case 4 (small amplitude response to trade wind shift – weak seasonality). Differences between Case 1 and Case 3 provinces are also indicated by the high- R value. The lack of statistical significance is probably due to the low number of possible permutations (*Table 4.7*). Case 2 (nutrient limited spring production peak – strong seasonality), which is generally found in the Westerly biome was statistically different from the generally Trade biome Case 4 (small amplitude response to trade wind shift – weak seasonality).

4.2.1.3 Summary

Overall the greatest differences in species composition of Longhurst's provinces was found between Oceans and Cases. The strongest differences occurred between high latitude Case 1 and Case 2 provinces and low latitude Case 3 and 4 provinces. The difference between case 2 and Case 3 is particularly noteworthy as both occur in Longhurst's Westerlies biome. Provinces found in Cases at lower latitudes (3, 4, 5, 6) had very similar species composition. Provinces in different Oceans and Longhurst biomes did not have significantly different generic compositions. However provinces in different Longhurst case models did have statistically different generic compositions. Pairwise comparisons revealed differences between the high latitude cases 1 and 2 compared to the low latitude case 4 provinces. Differences also occurred between case 1 and case 3. The within-biome differences between case 2 and 3 that occurred at the species level, did not occur at the level of genera.

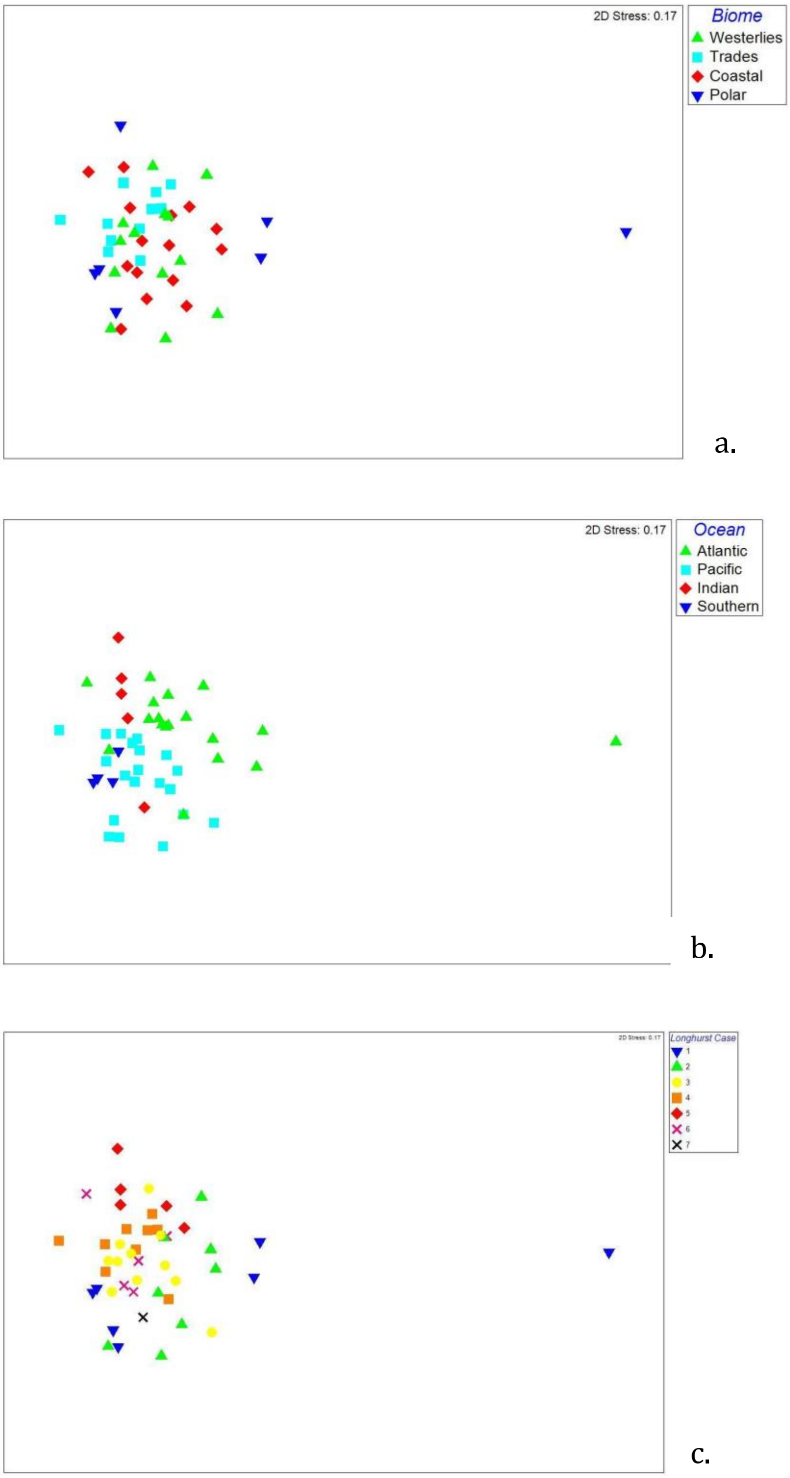


Figure 4.4 *MDS plots depicting similarity between the species composition of Longhurst's provinces. Each marker represents an individual province. Provinces are colour coded by a)* Longhurst biome, *b)* Ocean *and c)* Longhurst case

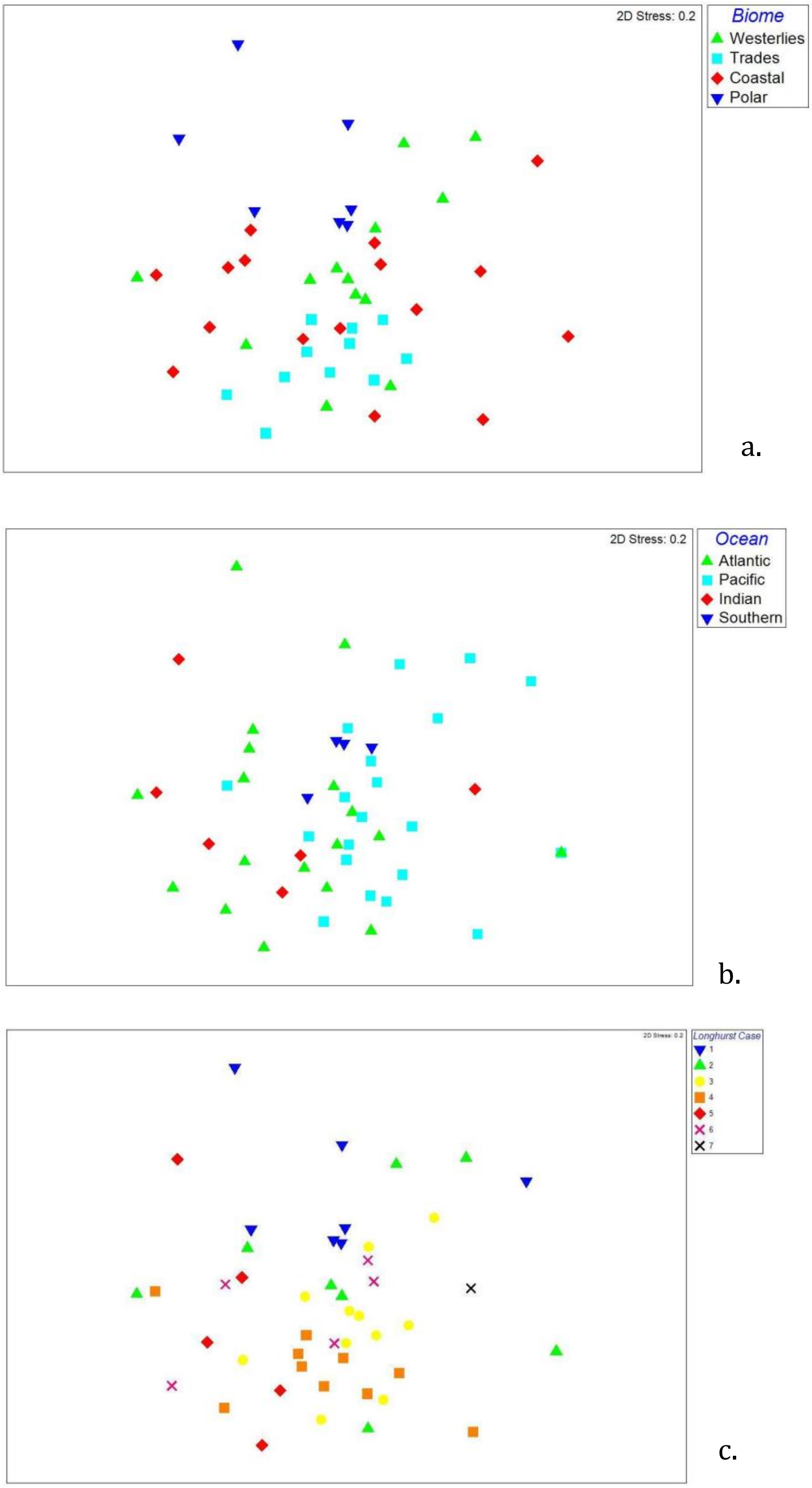


Figure 4.5 *MDS plots depicting similarity between the generic composition of Longhurst's provinces. Each marker represents an individual province. Provinces are colour coded by a)* Longhurst biome, *b)* Ocean *and c)* Longhurst case

Table 4.2 *Pairwise comparisons for 2-way crossed ANOSIM analysis investigating differences in species composition of Longhurst's provinces between biomes and oceans from presence-absence data using Sorenson's coefficient where Arctic data have been excluded and;* a) *shows comparisons between biomes once ocean differences have been accounted for and* b) *shows comparisons between oceans once biomes have been accounted for. Stars (*) denote statistical significance where p<0.05*

Biome comparison	**R Statistic**	**Significance (p)**	**Possible Permutations**	**Actual Permutations**	**Number >= Observed**
Coastal, Polar	0.25	0.156	32	32	5
Coastal, Trades	0.089	0.154	1463616	999	153
Coastal, Westerlies	0.056	0.265	360360	999	264
Polar, Trades	0.46	0.333	6	6	2
Polar, Westerlies	-0.015	0.625	24	24	15
Trades, Westerlies	0.103	0.157	99792	999	156

a.

Ocean comparison	**R Statistic**	**Significance (p)**	**Possible Permutations**	**Actual Permutations**	**Number >= Observed**
Pacific, Indian	0.175	0.153	720	720	110
Pacific, Atlantic	0.405	0.001*	71351280	999	0
Pacific, Southern	-0.041	0.648	108	108	70
Indian, Atlantic	0.318	0.056	504	504	28
Indian, Southern	1	0.333	3	3	1
Atlantic, Southern	0.643	0.133	15	15	2

b.

Table 4.3 *Bottom diagonal shows* **p-values** *of pairwise comparisons for 2-way crossed ANOSIM analysis investigating differences in species composition of Longhurst's provinces between Longhurst's 'cases' once the effects of oceans were accounted for, excluding Arctic provinces. From presence-absence data using Sorenson's coefficient. Top diagonal shows* **R-values** *from the same analysis. Higher R-values denote greater differentiation between Longhurst's cases. Statistically significant p-values highlighted with *. See Table 4.4 for associated sample size which may affect significance*

	Case 1	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**Case 6**
Case 1 - single production peak, light limited, strong seasonality		-0.268	0.667	0.545	-	0.583
Case 2 - nutrient limited spring peak, later summer peak, strong seasonality	0.867		0.296	0.432	0.036	0.284
Case 3 - winter-spring weak production peak, weak seasonality	0.012*	0.013*		0.014	-0.125	0.052
Case 4 - small amplitude response to trade wind seasonality, weak seasonality	0.143	0.007*	0.425		0.57	0.166
Case 5 - large amplitude response to monsoon trade wind reversal, strong seasonality	-	0.381	0.700	0.075		0
Case 6 – intermittent production related to boundary upwelling at low-mid latitudes	0.100	0.091	0.365	0.134	0.667	

Table 4.4 *Possible and Actual permutations for 2-way crossed ANOSIM results above. A low number of permutations suggests a small sample size. Pairwise comparisons with a high R-value but non-significant p –value in Table 4.3 are likely due to small sample size as denoted by low possible and actual permutations*

Cases	Possible Permutations	Actual Permutations	Number >= Observed
1, 6	10	10	1
1, 4	21	21	3
1, 3	84	84	1
1, 2	15	15	13
6, 4	560	560	75
6, 3	840	840	307
6, 5	3	3	2
6, 2	735	735	67
4, 3	4620	999	424
4, 5	40	40	3
4, 2	7056	999	6
3, 5	10	10	7
3, 2	11760	999	12
5, 2	21	21	8

Table 4.5 *Pairwise comparisons for 2-way crossed ANOSIM analysis investigating differences in generic composition of Longhurst's provinces between biomes and oceans from presence-absence data using Sorenson's coefficient where Arctic data have been excluded and;* a) *shows comparisons between biomes once ocean differences have been accounted for and* b) *shows comparisons between oceans once biomes have been accounted for. Stars (*) denote statistical significance where p<0.05*

Biome comparison	**R Statistic**	**Significance (p)**	**Possible Permutations**	**Actual Permutations**	**Number >= Observed**
Coastal, Polar	-0.045	0.6	40	40	24
Coastal, Trades	0.042	0.306	1829520	999	305
Coastal, Westerlies	0.011	0.425	360360	999	424
Polar, Trades	0.6	0.333	6	6	2
Polar, Westerlies	-0.308	0.875	24	24	21
Trades, Westerlies	0.093	0.15	99792	999	149

a.

Ocean comparison	**R Statistic**	**Significance (p)**	**Possible Permutations**	**Actual Permutations**	**Number >= Observed**
Pacific, Indian	0.195	0.146	1980	999	145
Pacific, Atlantic	0.154	0.033*	71351280	999	32
Pacific, Southern	-0.104	0.639	108	108	69
Indian, Atlantic	-0.147	0.816	1260	999	815
Indian, Southern	1	0.333	3	3	1
Atlantic, Southern	-0.036	0.6	15	15	9

b.

Table 4.6 *Pairwise comparisons for 2-way crossed ANOSIM analysis investigating differences in generic composition of Longhurst's provinces between Longhurst's 'cases' and once the effects of oceans were accounted for, excluding Arctic provinces. From presence-absence data using Sorenson's coefficient. Top diagonal shows* **R-values** *from the same analysis. Higher R-values denote greater differentiation between Longhurst's cases. Statistically significant p-values highlighted with *. See Table 4.7 for associated sample size which may affect significance*

	Case 1	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**Case 6**
Case 1 – single production peak, light limited, strong seasonality		-0.268	0.515	0.773	-	0.5
Case 2 – nutrient limited spring peak, later summer peak, strong seasonality	0.867		0.195	0.314	-0.036	0.103
Case 3 – winter-spring weak production peak, weak seasonality	0.071	0.073		-0.022	0.083	0.147
Case 4 – small amplitude response to trade wind seasonality, weak seasonality	0.048*	0.024*	0.563		0.229	0.257
Case 5 – large amplitude response to monsoon trade wind reversal, strong seasonality	-	0.619	0.400	0.210		0
Case 6 – intermittent production related to boundary upwelling at low-mid latitudes	0.2	0.301	0.219	0.080	0.667	

Table 4.7 *Possible and Actual permutations for 2-way crossed ANOSIM results above. A low number of permutations suggests a small sample size. Pairwise comparisons with a high R-value but non-significant p –value in Table 4.6 are likely due to small sample size as denoted by low possible and actual permutations*

Cases	Possible Permutations	Actual Permutations	Number >= Observed
1, 6	10	10	2
1, 4	21	21	1
1, 3	84	84	6
1, 2	15	15	13
6, 4	560	560	45
6, 3	840	840	184
6, 5	3	3	2
6, 2	735	735	221
4, 3	4620	999	562
4, 5	100	100	21
4, 2	7056	999	23
3, 5	10	10	4
3, 2	11760	999	72
5, 2	21	21	13

4.2.2 Cluster and Similarity profile test (SIMPROF) for species-level analysis

4.2.2.1 Between-province cluster-analysis

The results of the species-level cluster analysis confirm those of the MDS – that provinces closer to each other geographically tend to be more similar in terms of species composition. However at a broader level four main groupings of provinces can be identified. Group 1 consists of North Atlantic provinces, Group 2 of South West and North Pacific samples, Group 3 of mid to low latitude provinces from all three Oceans and Group 4 Southern Ocean and East Pacific provinces.

In the cluster analysis Group 1 is more different to the other groups than the other groups are to each other. Group one consists of entirely Atlantic provinces, which can be split further into (1a) which is comprised of the Gulf Stream, NW Atlantic Shelves and NE Atlantic Shelves provinces and (1b) which is comprised of the three Atlantic- Arctic provinces; the Boreal Polar, the Atlantic Sub-Arctic and the Atlantic-Arctic provinces. It is noteworthy that all provinces in Group 1a fall under Longhurst's Case 2 whilst those in group 1b fall within Longhurst's Case 1. Whilst Group 1 is statistically supported by the SIMPROF ($p<0.05$), the further division into the Arctic and North Atlantic is not.

Group 2 is an entirely Pacific group, which can be further subdivided into 2a and 2b. The former is comprised entirely of North Pacific provinces; the Pacific Sub-Arctic Gyres West, the North Pacific Epicontinental, the Pacific Sub-Arctic Gyres East and the Alaska Downwelling provinces. The latter is almost entirely comprised of southwest and west Pacific provinces; the Tasman Sea, the East Australian, the Archipelagic Deep Basins, the Sunda-Arufa Shelves, the Kurushio Current, the New Zealand Coastal and the Australian-Indonesian Provinces. Somewhat surprisingly, the only exception to the geographical proximity of provinces in group 2b is the inclusion of the SW Atlantic Shelves Province in this group. As well as the obvious geographic differentiation between 2a and 2b, and ecological differentiation also occurs. Group 2a is comprised of provinces described by Longhurst's Case 1 and Case 2 scenarios. Group 2b can be further subdivided again into two further groups – one is comprised primarily with Case 2 provinces (SW Atlantic Shelves province, NZ coastal province), the other Case 3 and 4 provinces (Tasman Sea, East Australian Coastal Province, Archipelagic deep basins province, Sunda-Arufara shelves province). This group is also comprised of primarily bathyal species, so differences between group 2 and other groups could also be depth related. The differences within the group appear to be more driven by ecology however.

Group 3 is comprised of provinces from all three oceans. Unlike other groups, differences within the major clusters are often statistically significant. Three major sub-groups exist. Subgroup 3a is comprised of three provinces from three different oceans. All lie at low-latitudes, falling into Longhurst's Case 4 (N. Pacific Tropical Gyre province), Case 5 (Indian Monsoon Gyre province) or Case 6 (Benguela Current province). Subgroup 3b is a low to mid latitude Atlantic grouping. It extends as far north as the North Atlantic Drift Province and as far south as the Eastern and Western Tropical Atlantic provinces. The SIMPROF finds statistically significant substructure within sub-group 3b with significant differences between pairs of provinces. Provinces within pairs are usually adjacent. Group C is an Indian Ocean group comprised of the NW Arabian upwelling and E Africa Coastal province. The majority of the provinces in Group 3 belong to Longhurst cases 4 or 5. Only one Case 2 province is present in this group; the North Atlantic Drift Province. Therefore the group 3 can be thought of as an ecologically defined grouping, with geographic substructure. Species compositions of provinces at low latitudes in separate oceans are more similar to each other than they are to provinces at high latitudes from the same ocean. However, provinces from low latitudes in the same ocean are more similar to each other than to low latitude provinces from other oceans (SIMPROF $p<0.05$).

Group 4 is generally a Southern Hemisphere high-latitude plus East Pacific cluster. It can be split into three major groupings. 4a is comprised of provinces from the Southern Ocean and South Atlantic. 4b is comprised of low–mid latitude East Pacific provinces. 4c is comprised of two geographically disjunct provinces with similar ecologies. Group 4 suggests strong biogeographical ties between the Southern Ocean and the South Atlantic, and the Southern Ocean and East Pacific. These groups of provinces have many species in common, despite being geographically quite separate, especially in the case of the East Pacific. However, within this group, the relationships between smaller groupings of provinces appear to be related to ecology. For example, sub-group 4a includes a further statistically significant split into three smaller groupings (SIMPROF, $p<0.05$); the first contains South Subtropical Convergence province and the South Atlantic Gyral province. These are both described by Longhurst's Case 3 model. The second contains the Antarctic Polar province and the Austral Polar province, both of which fall under the Case 1 model. The third contains only the Subantarctic province, which is described by Longhurst's case 3. Sub-group 4b is comprised of a group of provinces which all fall under Longhurst's Case 6 or Case 4 model. Group 4c contains the South Pacific Subtropical Gyre province, and the North Pacific Polar Front province. Both are Longhurst Case 3.

4.2.2.2 Between-species inverted cluster analysis

An inverted cluster (similarity of distribution over Longhurst's provinces between species) analysis was performed on the same dataset to identify clusters of species with similar distributions (*Fig. 4.7.).* Working backwards, by mapping these groups, we can then identify which species contribute to the geographic groupings of provinces observed in the original cluster analysis. Because many species have broad ranges spanning multiple oceans and provinces it is not expected that the two dendrograms be mirror images of one another.

There are seven major groupings within the species inverted cluster (*Fig. 4.7.*). Group 1 species occur in Sub-Antarctic and Antarctic provinces. Whilst some species have distributions which extend into lower latitudes in other Oceans, many are limited to the Southern Ocean. Splits within the group are related to which other oceans species are found in, or in the Case of 1b, the group is found in the Sub-Antarctic province but not the Austral Polar or Antarctic provinces. Group 1 is composed of Psychropotid and Elpidiid species only. Species in

Group 1 in the inverted cluster (Fig.4.7.) are responsible for cluster 4a in the original cluster analysis (Fig.4.6.).

Group 2 in the inverted cluster is comprised of species that are found in the Southern Ocean and East Pacific. Group 2ai is comprised of cosmopolitan species such as *Psychropotes longicauda, Oneirophanta mutabilis* and *Laetmogone violacea* which are found in many oceans, and at high and low latitudes as well as in the Southern Ocean and East Pacific. Within the Southern Ocean this group of species is found in all three Antarctic polar provinces. In contrast Group 2aii is restricted to the Sub-Antarctic and East and West Pacific. Group 2b is found in mid-latitude provinces in the Pacific and Indian Oceans, whilst 2c is found in the Antarctic and East Pacific only. Therefore species in group 2 of the inverted cluster (*Fig.4.7.*) appear to responsible for forming Groups 4b and 4c in the original cluster dendrogram (*Fig.4.6*).

Species in Group 3a and 3b are primarily found in the NW Pacific, with a few species showing broader distributions. The species in this cluster are all Elpidiidae. A cluster of NW Pacific provinces is not evident in the province cluster. Instead provinces occupied by these species are spread out amongst groups 1, 2 and 3 in the original cluster analysis (*Fig. 4.6.*).

Group 4 is comprised of species with Atlantic or Atlantic-cosmopolitan distributions. Group 4ai are restricted to the NE and E Atlantic. These species are primarily from the family Elpidiidae. In contrast, group 4aii are found in the East and West Atlantic, with some species having a global distribution. A key difference between the broadly distributed species in Group 4ai and those described above in group 2ai is that they are not found in the Southern Ocean or high latitudes in the Pacific. The Psychropotidae dominate this group with 5 out of the 8 species coming from this family. Other families are represented by single species; *Deima validum* (Deimatidae), *Benthogone rosea* (Laetmogonidae) and *Peniagone diaphana* (Elpidiidae). Group 4b is generally restricted to lower latitudes in the Atlantic compared to group 4ai but does not inhabit the Caribbean or show a cosmopolitan distributions such as those in Group 4aii.

The Atlantic mid-latitude distribution of these species is reflected in Group 3b of the original cluster analysis. Additionally, the cosmopolitan nature of group 4ai could explain the existence of three Oceans within group 3 of Fig. 4.6.)

Group 5 (*Fig.4.7.*) in the invert-cluster is comprised of only two species; *Psycheotrephes exigua* and *Peniagone wyvilli.* They are restricted to mid-latitudes in the Pacific and Atlantic Oceans and they may also contribute to the grouping of low-latitude provinces in Group 3 of the original cluster analysis (Fig.4.6.).

Group 6 of the inverted cluster is found in the SW and Indo-Pacific. This group is comprised of three species of Orphnurgus (Deimatidae); *O. vitreus*, *O.glaber* and *O.protectus* four species of Laetmogonidae (*L. maculata, L.ijimai and L. fimbriata B. Fragilis*) and a single Psychropotid (*Benthodytes sibogae*). These species are therefore likely to be important in defining Group 2b in the original cluster analysis (Fig. 4.7.) They do not explain the affinity between the SW Pacific and SW Atlantic noted in the original cluster however.

Finally, group 7 in the inverted-cluster (Fig.4.7) is comprised of 5 species of Elpidiidae; *Elpidia heckeri, Elpidia belyaevi, Kolga hyalina, Peniagone azorica* and *Irpa abyssicola*. All are restricted to high latitudes in the Atlantic and Arctic. This group thus corresponds to Group 1 in the original cluster analysis (*Fig. 4.6*).

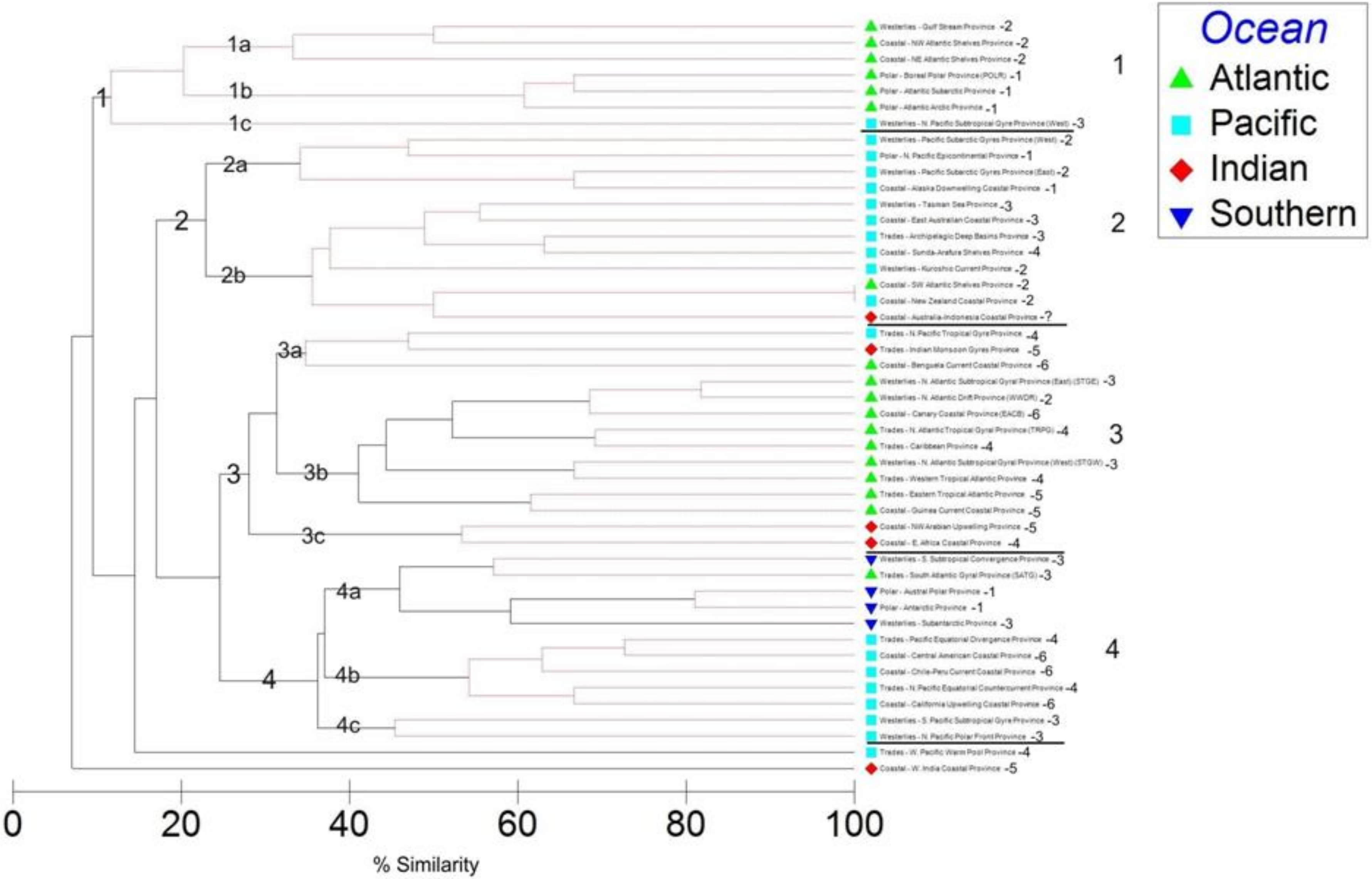


Figure 4.6 *Cluster analysis showing similarity between species composition of Longhurst's provinces using presence-absence of elasipodid holothurians. Dendrogram branches marked with a dark black line represent significant clusters, as determined using the SIMPROF routine in PRIMER-E. Symbols represent which Ocean the province is in, numbers after the province refer to which Longhurst Case model best describes them*

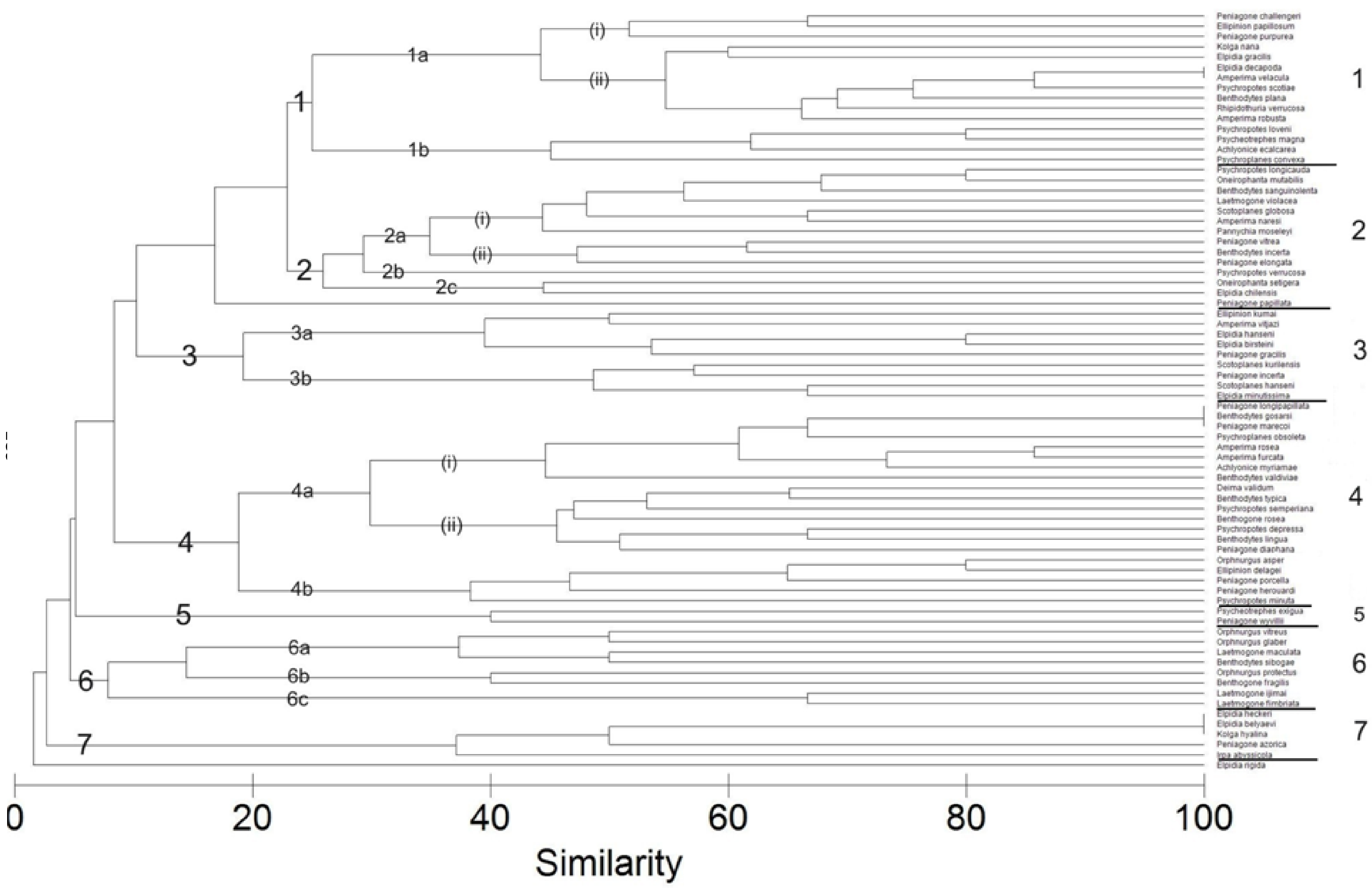


Figure 4.7 *Dendrogram of inter-specific similarity in species distribution between elasipodid species which were identified from two or more biogeographic provinces. The species distributions fall into seven primarily geographic groupings; 1) Antarctic/Southern Ocean; 2) East Pacific 3) NW Pacific; 4) Atlantic; 5) Atlantic-Pacific low latitudes; 6) SW Pacific 7) Sub-Arctic/Arctic*

4.3 Discussion

4.3.1 Are Longhurst's biomes a suitable classification system for the distribution of elasipodid holothurians?

Longhurst's biomes are supposed to be representative of fundamentally different and characteristic latitudinal zones of the Oceans (Longhurst 2007). Longhurst's principle argument was that processes that force stratification of the surface layer determine different phytoplankton routines, resulting in large scale differences in pelagic ecology between the biomes (Longhurst 2007). Despite this, no relationship between the compositions of Longhurst's biomes could be found in terms of species or genera. A lack of such a relationship is surprising, based upon our knowledge of the coupling of flux to the deep sea in relation to Longhurst's provinces (Lampitt and Antia 1997a, Boyd and Doney 2003, Ducklow 2003, Waniek et al. 2005), and the tie between seasonality of food input and the spatial (See Chapter 3)and temporal (Billett et al. 2001, Ruhl and Smith 2004, Ruhl 2007, 2008) distribution and abundance of elasipodid holothurians. However, the inclusion of areas with very different regimes in terms of seasonality within single biomes is a potential cause for the lack of significance of Longhurst's biomes. The significance of differences between Cases from different biomes and from the same biome agrees with this theory. Longhurst's cases further subdivide the biomes into areas of more similar seasonality therefore they may be more representative of the aspects of pelagic ecology that affect benthic deep-sea organisms. Intra-annual seasonality is one of the key drivers in differences in the distribution of elasipodid holothurians (Chapter 3) so that it is likely the strong differences within the biomes could be masking a true differentiation between species and generic composition of provinces in different broad-scale environments. Whilst a small body of literature has directly tested the suitability of Longhurst's provinces for describing the distribution of pelagic and benthic organisms (Paterson et al. 1998, Angel et al. 2007, Watling 2009, Reygondeau et al. 2012) to date no similar analysis has been applied to Longhurst's biomes or cases.

A gradient in differences between non-Arctic Case 1 provinces and those of the low-seasonality Westerlies Case 3 provinces exists. Case 1 and case 2 were not found to differ significantly. Whilst Case 1 was found to be different to Case 3 at the level of species and genera, Case 2 was found to have significant but weaker differentiation to Case 3 at the level of species but not genera. Species and generic composition differed strongly between cases 1 and 2 vs. Case 4. This suggests a latitudinal gradient in differences between Longhurst's cases. Latitudinal patterns have been noted in deep-sea benthic organisms previously (Lambshead et al. 2000, Rex et al. 2000) but such studies were based upon abundance or diversity data rather than presence-absence data.

4.3.2 Which taxa are contributing to differences between Longhurst's cases and what can this tell us?

The inverted species cluster showed that clusters of species at higher latitudes were often associated with Elpidiidae species which were restricted to these regions, such as cluster 1 and cluster 7 in *Fig. 4.7*. Unlike in the Northern Hemisphere where strong physical barriers prevent interchange between Case 1 provinces and Case 3 provinces, deep-sea organisms in the Case 1 provinces of the Antarctic and North Pacific face no major physical barriers. Although the combined effects of the Antarctic Polar Front and the Circumpolar Current are potential isolating mechanisms (Thornhill et al. 2008), many elasipodid holothurians have extensive global distributions that include the Antarctic provinces. In contrast to Cluster 1 which was generally restricted to the Antarctic and was dominated by Elpidiidae, the species in cluster 2 in *Fig. 4.7* are often cosmopolitan, as well as being found in the Antarctic. The proportion of Elpidiidae in Group 2 is much lower, although some species are still present. This suggests that mechanisms causing differences between Case 1 and 2 provinces and cases at lower latitudes may be disproportionally influenced by the distribution of Elpidiidae species. As discussed in Chapter 3, the distribution of some species of Elpidiidae may be restricted to areas of highly variable food-supply. Areas of lower variability may result in stronger barriers to dispersal to the Elpidiidae compared to the other families (Hansen 1975).

Differences between Case 2 vs. Case 3 and 4 provinces were much more difficult to associate with particular groups of species, in part because the differentiation was much weaker.

4.3.3 The influence of historical biogeographic processes on observed patterns

Whilst it is tempting to infer that differences in community composition are caused by the factors that differentiate Longhurst's cases (phytoplankton ecology and seasonality in particular), other factors which co-correlate with these patterns must also be considered. For example there may be differences in the low-latitude composition of elasipodid holothurians that are related to historical, rather than ecological factors. Historical events have been found to confuse latitudinal gradients in species diversity in the deep sea (Lambshead et al. 2000, Lambshead et al. 2002, Rex et al. 2005). Historical biogeographic processes have also been implied as a potential cause of unexplained variation in global patterns of seafloor abundance and biomass (Thurston et al. 1998, Wei et al. 2010). Whilst species composition would be expected to respond differently to either diversity, abundance or biomass, the potential for historical biogeographic processes to affect observed patterns must be considered. The CLUSTER analysis and associated inverted cluster analysis, where the data is allowed to 'tell its own story' (Clarke et al. 2008) show evidence of historical processes affecting observed patterns.

As previously mentioned, the species-level clustering of provinces (*Fig. 4.6*) shows that the Atlantic and Pacific each have two distinct clusters, one found comprised generally of high latitude provinces (cluster 1, *Fig. 4.6*) and one which consists of low latitude provinces (cluster 3b, *Fig. 4.6*). The Atlantic clusters 1 and 3b are composed of primarily Case 1 and 2 provinces and primarily Cases 3, 4, 5 and 6 provinces respectively. In the Pacific clusters 2 and 4b are comprised of provinces from cases 1, 2 & 3 and cases 4 and 6 respectively (*Fig. 4.6*). These differences account for the statistical significance of differences between Oceans and Longhurst Cases. However, if we look at the distribution of the provinces which make up these clusters, it is apparent that there is another mechanism at work. The high latitude (Cases 1-3) group of Pacific samples are located in the northwest Pacific and the southwest Pacific, whereas the low latitude group is found primarily in the eastern Pacific. These eastern provinces happen to all fall into cases 4 and 6 and are generally found at mid-low latitudes. Therefore differentiation between Longhurst's cases in the Pacific appears actually to be caused by differentiation between the east and west Pacific. Differentiation between the east and west pacific in shallow water organisms is well reported (Ekman 1953, Briggs 1974). It is generally thought to be caused by the large expanse of abyssal ocean, known as the East Pacific Barrier. However (Madsen 1961) suggested that the East Pacific Barrier is also an important zoogeographical boundary for the abyssal fauna. He describes the region (stretching from the western North Pacific to the east of Polynesia) as a 'poor feeding ground for benthic animals...comprised of desert-like areas'. Additional differences between the east and west Pacific could be depth related; the main cluster of species that delimit the southwest Pacific (cluster 6, *Fig.4.7*) are comprised primarily of bathyal species from the families Laetmogonidae and Deimatidae, although those generally found in the NW Pacific (cluster 3 Fig. 4.7.) are abyssal. As well as separation by the East Pacific barrier the east & west Pacific may have separate evolutionary histories. (Gebruk 1994) postulates that the first stage of the evolution of the Elasipodid fauna involved the Tethys basin and which would have split into the Indo-Malayan, the Mediterranean and the West Indian (now Panama Gulf) regions. Furthermore the Tethys fauna would have most likely been bathyal. Therefore (Madsen 1961) suggests that the bathyal elements of primarily abyssal faunas may be the most ancient, and furthermore in the Porcellanasteridae, such bathyal elements are restricted to the Indo-Pacific. In the Elasipodida, the species which differentiate the southwest Pacific are indeed bathyal, and generally restricted to low latitude areas and often to the Indo-Pacific. This suggests that closer affinities between low-latitude provinces could be due to a shared evolutionary history, rather than similar ecologies.

Historical events could also explain the close affinity between the East Pacific and Southern Ocean found in the cluster analysis. (Gebruk 1994) proposed that after the spread from various Tethys centres of origin, some Elasipodida dispersed down the East coast of America, into the Antarctic. After this they descended to abyssal depths before dispersing northwards, one pathway being along the East Pacific. Additionally (Madsen 1961) suggests that spreading of the of the abyssal fauna occurs along the food-rich areas close to continents.

Within the Atlantic, the difference between cases could equally have historical biogeographic, rather than strictly ecological causes. The separation of the Arctic from the rest of the Atlantic is expected, based on the existence of only 3 species of elasipodid from this region (Rogacheva 2007), for reasons discussed in Chapter 3. Differences between the Atlantic, the Sub-Arctic and the Arctic have skewed results of latitudinal gradient analyses in species richness and confused the diversity-productivity relationship in previous deep-sea studies (Lambshead et al. 2000, Lambshead et al. 2002, Rex et al. 2005). Additionally the division of the North Atlantic fauna in the North East Atlantic Shelves, North West Atlantic Shelves and Gulf Stream provinces from the lower latitude is almost certainly caused by depth. The three species that these areas have in common; *Laetmogone violacea*, *Benthodytes lingua* and *Psychropotes depressa* are all known from bathyal depths. Finally, a group of low-latitude species in the Atlantic that have extensive distributions throughout the Caribbean and Western Atlantic (4aii, *Fig.4.7*) as well as low latitudes in other oceans, could be a further example of the Tethys-derived fauna described by (Gebruk and Shirshov 1994). This is also suggested by this group of species absence from the Southern Ocean (*Fig. 4.18*, Appendix B). However the existence of several genera from all four families suggests a shared evolutionary history is not necessarily causal for the observed distribution of this group. Therefore the division between Case 1 and 2 provinces and Cases of lower latitudes (especially 3 and 4) may be due to historical and depth related factors in addition to ecological factors.

Despite the evidence of historical processes separating low and high latitude cases and provinces, species from these sometimes disjunct environments may have evolved to suit the conditions in which they occur, in which case although historical reasons were the primary cause of their distributions, ecological factors maintain their current distribution (Hermsen and Hwa 2010). Additionally patterns could simply relate to clusters of sampling effort, with differing levels of sampling effort at different latitudes on the West and East coasts of each continent.

4.3.4 The effects of presence-only data and sampling coverage

Perhaps one of the main problems associated with confirming ecologically driven reasons for differentiation in the species composition of fauna in this study is the fact that it is based upon presence-absence data. Those species which tend to exist in the low-latitude provinces and areas of lower seasonality also tend to be the most broadly distributed species. Cluster 2ai of *Figure 4.7* is comprised of six cosmopolitan species; *Psychropotes longicauda*, *Oneirophanta mutabilis*, *Benthodytes sanguinolenta*, *Laetmogone violacea*, *Scotoplanes globosa* and *Amperima naresi* (*Fig.4.13*, Appendix B). These species are found in the Southern Ocean, as well as at low-mid latitudes in other oceans. Some are generally restricted to shelf environments (e.g. *L. violacea*), others to abyssal depths along productive coastal margins (e.g. *S. globosa*) and some are found in low productivity regions in the middle of ocean gyres (*P. longicauda*, *O. mutabilis*). Therefore despite their presence, they are not unique to low productivity regions and so will not induce such provinces to cluster more closely. A second group containing widely distributed species exists in cluster 4aii of *Fig. 4.7,* and the distributions of these species are mapped in *Fig. 4.18* of Appendix B. This cluster contains species such as *Deima validum*, *Benthodytes typica*, *Benthodytes typica* and *Peniagone diaphana*. In contrast to cluster 2ai, those in 4aii of *Fig. 4.7* are not found in the Southern Ocean. Many of these species are found in low latitudes of the Atlantic and Pacific, so will contribute to differences between Case 1 and lower latitude cases, but they are found in provinces of very varying ecology throughout the Atlantic, so differences between case 2 and case 3 and 4 models will be weak.

Therefore the presence-absence nature of the data-set presented here is probably not ideal for answering the questions at hand. The presence of a species at a site does not guarantee that the ecological conditions are ideal for that species. The source-sink hypothesis suggests that an organism may inhabit a habitat that is unsuitable, providing there is continuous immigration from a source area (of more suitable habitat) which exceeds the rate of mortality in the sink habitat (Hermsen and Hwa 2010). Therefore presence-absence of species or genera may be a poor way of assessing differences between areas of different habitat. Species richness, evenness, abundance and biomass are potentially more sensitive means of detecting differences in the faunal characteristics of Longhurst's provinces, cases and biomes. Other studies have failed to find firm evidence of Longhurst's provinces. (Gibbons 1997) identified six biogeographic provinces in the South Atlantic based on presence-absence of euphasids. Whilst Antarctic, Sub Antarctic, cold temperate and Argulhas current. However the data failed to identify coastal and tropical biogeochemical provinces described by Longhurst. The author attributed this to adjoining areas having many species in common, and only differing in their quantities (Gibbons 1997). However, biogeographic studies rely on the geographic 'gaps' filled by historical records which are essential for improving geographic coverage in large-scale analyses (Prendergast et al. 1993). Additionally, if sustained immigration to a sink occurs, it is possible for species to adapt to sink conditions and in time, to develop viable populations (Hermsen and Hwa 2010).

Unlike previous studies, we did not specifically test for differences between Longhurst's provinces for a number of theoretical and logistical reasons. Firstly, previous studies have tested specifically between Longhurst's provinces by testing to see if individual samples are more similar within or between provinces. However, such studies have been based on quantitative studies of macrofauna (Angel et al. 2007, Watling 2009) where individual samples contain a number of species at varying abundances. Although such studies contain a certain level of pseudo replication (Lambshead et al. 2000), due to the relative paucity of data available, this is usually overlooked, or at least considered in post-hoc discussions. Because the data here are based on presence-absence data for megafauna, many sites (akin to samples in the macrofauna studies) contain only one species. If many sites within a province contain that same one species (e.g. *Amperima rosea* in the north Atlantic Drift province) then similarities between provinces will be inflated. Other studies have attempted to bypass this problem by pooling records into 5 degree grid cells, for example (Gibbons 1997). This will reduce the pseudo replication to more acceptable levels, but in most of these analyses the grid cell boundaries do not match those of the provinces under investigation. This has led to problems attributing differences in faunal composition to the provinces under test (Gibbons 1997). Additionally a large number of elasipodid holothurians have distributions spanning more than one ocean. Several species are cosmopolitan, and many have the potential for long distance dispersal (Tyler and Billett 1988). Therefore whilst it may be expected that provinces close to each other would generally be more similar to each other than those further away, it would not necessarily be expected that sharp differences exist between provinces, especially considering the presence-absence nature of the dataset. Despite this, statistical differences between pairs of provinces, particularly at low latitudes were found. Some individual provinces were found to be statistically different from their nearest neighbour; the Subantarctic province was in a significant cluster with the Antarctic and Austral Polar provinces, and yet formed a statistically significant grouping from them. Statistical differences between provinces became less common, so that similarity between geographic provinces increased in the higher latitude clusters for both the Pacific and Atlantic. This could be related to sampling effort in relation to latitude, or latitude related differences in species range sizes (McClain and Hardy 2011).

4.3.5 Conclusions

In summary, this study has found that the species composition of Longhurst's provinces does differ between Oceans and ecologically different areas even when presence-absence data are compared. At higher taxonomic levels, generic composition of provinces between Oceans is not statistically significant, but differences between ecologically different areas are still tractable. However cluster analysis reveals that historical biogeographic processes and uneven sampling effort make it difficult to accept hypotheses based on differences in species composition being controlled by ecology. Whilst presence-absence data is sub-optimal for looking at fine-scale differences between provinces, it is necessary when geographic coverage of quantitative data is poor, such as in the deep sea. Presence-absence data are capable of defining large-scale biogeographic areas, and in some cases can distinguish between individual provinces.

Chapter 5. Global phylogeography of *Psychropotes longicauda* and *Oneirophanta mutabilis*

5.1 Introduction

Some major differences exist in the environment and level of genetic structure observed in species from shallow water environments compared to those in the abyssal deep sea. Zoogeographic hypotheses about the level of connectedness of the abyssal zone have varied from assumptions of widespread cosmopolitanism (Bruun 1957, Hansen 1967) to those of fine scale subdivision (Vinogradova 1959, Menzies et al. 1973). Some authors predicted very high levels of genetic structure and cryptic speciation to be discovered in the deep sea (Knowlton 1993).

For most of the deep ocean we lack basic information about the spatial and temporal scales of population differentiation and the forces that might lead to population divergence and speciation (Zardus et al. 2006). Population structure reflects the interplay of physical, ecological and physiological isolating mechanisms (Zardus et al. 2006). Therefore, a lack of large physical isolating mechanisms does not preclude the existence of population structure in the deep sea. Barriers to gene flow in the abyss may not have been recognised previously owing to insufficient coverage of geographic scales over which they might be expected to occur (Benzie 1998). Additionally, ecological or physiological isolating mechanisms could be important in shaping population structure in the deep sea. Studies in the deep sea have so far been limited to a very narrow range of taxa, so that there is little way of knowing whether the patterns observed are generally applicable. Research has focused on two main themes in recent decades; structuring within and between species living in chemosynthetic environments and depth related patterns in the deep-sea soft sediment macrofauna.

The genetic population structure of vent species has been reviewed in detail (Vrijenhoek 1997, Tyler and Young 1999, Ramirez-Llodra et al. 2007).

Cosmopolitanism is thought to be unlikely in vent species, which show high levels of endemism, due to the spatially and temporally patchy nature of their environment (Van Dover 2012). Studies of population structure have therefore generally focused on intra-specific patterns within ridge systems. Both isolation by distance (Vrijenhoek 1997) and island models (Craddock et al. 1995, Creasey et al. 1996) are applicable to vent organisms, suggesting that mechanisms affecting population structure may be species specific. There is also evidence for vicariant events affecting the genetic structure of vent organisms (Tunnicliffe and Fowler 1996, Matabos et al. 2011). Mode of dispersal may also influence the genetic structure of vent species, as in other environments (Tyler and Young 1999, Shank and Halanych 2007). Other factors affecting the population genetics of vent communities include 1) the geological characteristics of a vent, 2) the successional position of a species in space and time on a vent (which in turn may affect population abundance and local meta-population dynamics), 3) proximity of other chemosynthetic environments, 4) geographic and hydrographic isolation, and 5) local topography and currents (Vrijenhoek 1997).

Soft sediment communities have been much more poorly studied, perhaps because previous ocean paradigms suggested that deep-sea sediments were environmentally homogenous, temporally invariable and well connected. Early genetic studies of deposit-feeding megabenthos using allozymes did little to challenge such ideas. Population structure of two deep-sea holothurians *Benthogone rosea* and *Benthodytes typical* from the eastern-Atlantic and western-Atlantic were investigated for geographic population structure (Bisol et al. 1984). The study found very few polymorphisms suggesting populations of each species must be very large with gene flow occurring over very large distances (Bisol et al. 1984). However, allozyme electrophoresis often lacks the resolution to distinguish intraspecific differences (Parker et al. 1998) and has been replaced by more powerful methods (see below).

More recently genetic studies have found that deep sea species which previously were considered to be cosmopolitan are, in fact, cryptic species complexes. In the deep-sea mollusc *Frigidoalvania brychia* genetic analysis revealed that the mean haplotype divergence within this one species alone was greater than the mean haplotype divergence for complete genera of shallow water gastropods (Etter et al. 1999). The results were particularly surprising because specimens were collected over a narrow depth range (500-1100m) and were separated by only tens of kilometres geographically. This led to more detailed studies of bathymetric and geographic patterns of population structure in the macrofauna (Etter et al. 2005, Zardus et al. 2006). Four species of protobranch bivalve that are numerically dominant at different depth ranges (upper bathyal, mid bathyal, lower bathyal and lower bathyal to abyssal) were investigated for depth related patterns of divergence (Etter et al. 2005). Upper to mid bathyal species showed more pronounced geographical variation than the lower bathyal and abyssal species. Stronger differentiation in bathyal populations relative to abyssal populations was attributed to stronger environmental gradients and greater biotic and abiotic heterogeneity at upper bathyal depths (Quattro et al. 2001, Etter et al. 2005, Zardus et al. 2006). In particular, strong gradients in decreasing nutrient input with increasing depth, and the related changes in benthic standing stock, may influence patterns of genetic structuring (Etter et al. 2005). There is also biogeographic evidence to suggest that some abyssal populations might be sinks from nearby source bathyal populations and thus have little potential for evolutionary divergence in situ (Rex et al. 2005).

A ‘mid bathyal’ bivalve species, *Deminicula atacallena*, studied initially by Etter et al. (2005), was investigated subsequently throughout the Atlantic Ocean at a variety of depths (Zardus et al. (2006). Whilst isolation by distance was thought to explain differences between basins, depth related divergence was much stronger. Populations at different depths within the same basin showed greater differences than populations at the same depth in different ocean basins. Similar depth-related patterns have been found in a morpho-species of the sea star *Zoraster fulgens* in the Porcupine Seabight (Howell et al. 2004). Greater genetic structuring was evident between populations separated by only 1000m along a bathymetric depth gradient compared with individuals separated by 900km along the same isobath. They suggest that reproductive isolation along depth gradients occurred in relation to factors such as temperature, pressure and food availability (Howell et al. 2004).

The few studies of genetic structure in abyssal environments have shown conflicting results. A species of deep-sea isopod with a broad bathymetric and geographic range was found to have strong geographic structuring, showing multiple clades at bathyal *and* abyssal depths (Raupach et al. 2007). In contrast, genetic structuring within basins was almost absent in an abyssal protobranch bivalves in the North and South Atlantic Ocean (Chase et al. 1998, Etter et al. 2011). Only modest genetic structuring was present between the eight basins studied (the American, West European, Guyana, Canaries, Sierra Leone, Angola, Cape and Argentine) (Etter et al. 2011). Major topographic features such as the Mid-Atlantic Ridge were found to disrupt gene flow. Whilst the geographic scale on which structuring occurs may be different in bathyal and abyssal environments it is too early to draw conclusions about levels of population structure in abyssal organisms. In order to gain a better understanding of population genetic structure and factors leading to speciation at abyssal depths, future research must focus on species which are strictly abyssal.

5.1.1 Genetic population structure in shallow water echinoderms

Traditionally, marine populations were expected to demonstrate little genetic population structure due to a lack of robust physical barriers and the existence in many species of a larval stage aiding dispersal (Vermeij 1978) . However, the existence of cryptic speciation is now known for a wide range of taxa in the marine environment, including species with poor and excellent potential dispersal ability (Knowlton 1993, Palumbi 1994, Neigel et al. 2007). Therefore the potential for speciation in marine environments is much higher than previously thought (Palumbi et al. 1997).

A large body of work now exists which investigates population structure in, predominantly, shallow-water echinoderms. Genetic structure has been detected within species on a number of scales including those separated only by a few kilometres (Clouse et al. 2005), at within-ocean scale (Williams and Benzie 1997, Arndt and Smith 1998, Benzie 1998, Landry et al. 2003, Addison and Hart 2004, Maltagliati et al. 2010, So et al. 2011) and at the between-ocean scale (Benzie 1999, Lessios et al. 1999, Uthicke et al. 2004, Yasuda et al. 2009). Few have found genetic continuity on the scale of hundreds to thousands of kilometres along continental shelves, but sharp genetic breaks in the same species over much smaller distances related to barriers to dispersal (Banks et al. 2007, Hunter and Halanych 2010). Others have shown high dispersal with weak genetic structure associated with isolation-by distance rather than due to distinct biogeographical breaks (Uthicke and Benzie 2003). Finally, some studies have failed to detect population structure, even at ocean-basin scales (Bisol et al. 1984, Uthicke et al. 2004, Muths et al. 2006). Causes of such divergences have been equally diverse. Allopatric causes of genetic population structure have included altered current patterns during the Pleistocene (Uthicke et al. 2004), the location of modern day land barriers and ocean currents (Lessios et al. 1999, Banks et al. 2007, Yasuda et al. 2009, Hunter and Halanych 2010), vicariance events (Lessios et al. 2003), upwelling areas (Lessios et al. 2003), topography (O'Loughlin et al. 2011), and separation in separate glacial refugia (Boissin et al. 2008).

5.1.2 Genetic population structure in holothurians

The vast majority of molecular studies on holothurians have focused on shallow water, commercially valuable species. A comparative study of genetic diversity and population structure of *Cucumaria miniata* and *Cucumaria pseudocurata* found that dispersal ability, currents and possible vicariant events were important (Arndt and Smith 1998). Both species occur over similar ranges of the north-eastern Pacific. *Cucumaria miniata,* which has a pelagic non-feeding larval stage lasting 2 weeks, was found to be panmictic throughout its range. In contrast, *C. pseudocurata*, a brooding species with no pelagic phase, displayed significant genetic disjunction between northern and southern populations, thought to correspond either to the separation of the Californian and Alaska currents, or to the formation of separate northern and southern refugia during Pleistocene glaciation events (Arndt and Smith 1998).

In another study, two species of holothurian *Stichopus chloronatus* and *Holothuria atra* from the western Pacific and western Indian Ocean, were found to have little genetic population structure, despite the existence of asexual reproduction in both species (Uthicke et al. 2001). However, other species show greater than expected levels of population structure, and even cryptic speciation. *Holothuria scabra*. is a commercially important species in many developing countries throughout the Indo-Pacific region (Uthicke and Benzie 2001). It reproduces sexually, producing planktotrophic larvae that spend 10 to 14 days in the water column. Despite the potential for high gene flow populations along the north-east coast of Australia can be grouped into three genetically distinct stocks. Whilst the divergences were not sufficient to delineate separate species, the results have important implications for the management of the fishery (Uthicke and Benzie 2001). An investigation using molecular techniques was used to overcome confusion on the taxonomic status of Indo-Pacific shallow water holothurians, or teatfish (Uthicke et al. 2004). The study identified a complex of at least 3 species(Uthicke et al. 2004). One colour morph, the white teatfish, was found to have a wide distribution over the tropical Indo-Pacific region. It may even be a species complex in itself, as indicated by large sequence divergences. The authors also identified two allopatric species of black teatfish that had not been separated by taxonomists. *Holothuria whitmaei* has a Pacific distribution, whilst *H. nobilis* occurs only in the Indian Ocean. This allopatric speciation was thought to have occurred during the Pliocene when the oceans were separated and current patterns were altered (Uthicke et al. 2004).

Sympatric speciation has also been identified in shallow-water holothurians. Kan-No and Kijima (2003) used gel electrophoretic techniques to investigate genetic differentiation among three colour variants, red green and black, of the Japanese sea cucumber *Stichopus japonicus*. Results showed that the red morphotype was genetically different from the green and black varieties, which were more closely related. Red-type individuals inhabit slightly different sedimentary habitats to the green and black type. Therefore, reproductive isolation may have occurred through microhabitat differentiation (Kan-No and Kijima 2003).

Studies of deep-sea holothurians from bathyal depths in the Antarctic have revealed that cryptic speciation (allopatric, sympatric, bathymetric) is surprisingly common (O'Loughlin et al. 2011). A number of elasipodid holothurians were included in the study. There were 29 species complexes, which included 39 nominal species and 71 Evolutionary Significant Units (ESUs). ESUs were designated for groups in which population structure is not at a significant enough level to allow complete species divergence. Most ESUs were allopatric (i.e. geographically disparate). These included the elasipodid holothurians *Peniagone incerta* (Elpidiidae) and *Benthodytes sanguinolenta* (Psychropotidae). However two other elasipodid holothurians of the Family Laetmogonidae; *Laetmogone wyvillethomsoni* and *Pannychia moseleyi* had ESUs that were sympatric. Where ESUs are sympatric, species can be considered separate species under the biological species concept (O'Loughlin et al. 2011). In the case of *L. Wyvillethomsoni* this pattern was related to differences in depth of collection (O'Loughlin et al. 2011). This indicates agreement with previous studies suggesting depth-related differentiation in bathyal species (Etter et al. 2005, Zardus et al. 2006)

Such a range in levels, scales and causes of population structure in echinoderms highlights the need to understand the ecology, evolutionary biology, and historical and current distribution of the particular species under investigation

5.1.3 Target species

This study focuses on two primarily abyssal species of elasipodid holothurian; *Psychropotes longicauda* (Psychropotidae) and *Oneirophanta mutabilis* (Deimatidae). Both species are thought to be cosmopolitan, inhabiting every major ocean, with the exception of the Arctic. *Oneirophanta mutabilis* is split into two sub species; *O.mutabilis mutabilis* which is globally distributed and

O.mutabilis affinis which is though to be limited to a very small area of the equatorial east Pacific near Panama.

Both *P. longicauda* and *O. mutabilis* have quite variable morphology. Body colour, number of dorsal papillae and length of the unpaired appendage (in *P. longicauda*) and the number of ventro-lateral tubefeet (in *O. mutabilis*) show great variability not associated with geography. Other features, or combinations of features, do appear to vary geographically. For example calcium carbonate deposits in Atlantic specimens of *P. longicauda* are different to those of specimens from other locations, most strikingly from those in the east Pacific. Specimens from the Kermadec region have intermediate deposits between those seen in the Atlantic and Pacific. Additionally one particular form of spicule found in specimens of *P. longicauda* from the Weddell Sea and south Atlantic were not seen from any other location (Hansen 1975). However, Hansen believed that because variation was so great within regions, as well as between regions, that geographic subdivision of either species was inadvisable, with the exception of the Panama Basin sub species of *O. mutabilis*. Moreover, Hansen (1975) synonymised a number of previously described species under *P. longicauda* and *O. mutabilis*, reaffirming their supposedly cosmopolitan distributions. O*neirophanta mutabilis mutabilis* has a maximum egg diameter of 950µm and is thus thought to produce lecithotrophic larvae. Lecithotrophic larvae are thought to have the potential for wide dispersal in the deep sea (Young et al. 1997a). *Oneirophanta mutabilis affinis* is known to display intra-ovarian brooding, with possible reproductive periodicity. It should be noted that this behaviour was only noted for one locality (Panama Basin) and no indication of brooding behaviour, has been reported for *O. mutabilis mutabilis* (Ramirez-Llodra et al. 2005).

Psychropotes longicauda possesses one of the largest eggs of all echinoderms with a maximum diameter of 4mm. It is thought that *P. longicauda* has direct development in the abyssopelagic environment (Gebruk et al. 1997), leading to a very wide dispersal potential (Billett 1991). Large lipid-rich eggs provide buoyancy and an energy store for prolonged development (Billett 1991).

Hansen (1975) suggests that this is an adaptation which ensures a long pelagic development independent of food uptake from the plankton. It is thought juveniles settle on the seafloor when they attain a body length of about 35mm (Billett 1991). The congeneric species *P. semperiana* has been recorded swimming above the seafloor (Hansen 1975).

5.1.4 Target markers

Animal phylogeography is dominated by mitochondrial DNA (mtDNA) (Emerson and Hewitt 2005) and literally thousands of studies have reached conclusions about patterns of gene flow, genetic structure, historical demography and species delineation on the basis of mtDNA alone (Zink and Barrowclough 2008). Animal mtDNA accumulates nucleotide substitutions at a much greater rate than single copy nuclear DNA (Brown et al. 1979). The reasons for this are complex and are discussed by (Avise 2004) but in most taxa there is great nucleotide sequence variation, a prerequisite for phylogeographic analyses (Avise 2009). Mitochondrial DNA therefore provides information primarily on historical processes for intraspecific matrilineal relationships, but also allows insights into the importance of contemporary gene flow (Avise 2004). Because mtDNA does not undergo the ‘shuffling effects’ of meiosis and syngamy, which affect nuclear DNA during sexual reproduction, the genetic variation of mtDNA in populations is usually accountable to mutation alone (Avise 2004). Emerson and Hewitt (2005) suggest that an ideal phylogeographical study would combine nuclear, cytoplasmic (e.g. mtDNA), sex-linked, autosomal, conserved and neutral sequences, including examples with high and low mutation rates. However, the progress of the development of nuclear markers has been hampered by their slow rate of molecular evolution, difficulties associated with isolating nuclear haplotypes from diploid organisms, and the process of intragenic recombination (Avise 2009). Therefore this study focuses on two mitochondrial markers; Cytochrome Oxidase subunit 1, and 16S rRNA.

The most frequently used tool for investigating population structure in animals is a 650-700 base fragment of the 5' end of mitochondrial gene cytochrome oxidase subunit I (CO1) (Hajibabaei et al. 2007). The use of CO1 bar-coding techniques on zoological specimens was initiated by Hebert et al., (2003) who showed that more than 98% of species pairs (from a total of 13,000 congeneric pairs from 11 phyla) showed greater than 2% sequence divergence. A study which investigated the utility of CO1 bar-coding in echinoderms found a mean intraspecific divergence of 0.62% and a mean congeneric divergence of 15.33% (Ward et al. 2008). It was found that 97.9% of the 191 species studied could be distinguished by their DNA barcodes (Ward et al. 2008). CO1 has been used successfully to study the population structure and phylogeography a number of echinoderms. These include the phylogeography of the pantropical sea urchin *Eucidaris* in relation to land barriers and ocean currents (Lessios et al. 1999), global population structure, speciation and phylogeography in *Diadema* sea urchins (Lessios et al. 2001), cryptic speciation in the subtidal brittle star *Acrocnida brachiata* (Muths et al. 2006) and the molecular phylogeny of eastern Pacific sea cucumbers (Arndt et al. 1996).

16S is a region of mitochondrial rRNA which, unlike other RNA markers, has an extremely fast rate of sequence evolution (Shouche and Patole 2000). This makes it an excellent target marker for high resolution intra-specific studies of population structure (Hillis and Dixon 1991). 16S markers have been successful in detecting intra-specific population structure in deep-sea organisms (Howell et al. 2004) and investigating interspecific and intraspecific population structure in shallow-water holothurians, including detecting cryptic species (Uthicke et al. 2010).

5.1.5 Aims and hypotheses

This study aims to investigate whether intra-specific geographic structuring occurs in abyssal organisms with 'cosmopolitan' distributions and the potential for large scale dispersal. Levels of population structure were compared with bathyal species to understand whether abyssal species have reduced levels of structuring. Finally, the level of connectedness between three disjunct geographic locations (Porcupine Abyssal Plain in the NE Atlantic, Station M in the NE Pacific, and Crozet in the Southern Indian Ocean) was determined to understand potential paths of dispersal.

5.2 Materials and Methods

5.2.1 Sampling and sample collation

Overall, 43 samples of *Psychropotes longicauda* and 42 samples of Oneirophanta mutabilis spanning three oceans (Atlantic, Pacific and Indian) were obtained (*Table 5.1*). Samples were collected between 1955 and 2005. Samples of both species from the Porcupine Abyssal Plain and *P. longicauda* from the Whittard Canyon were collected during National Oceanography Centre cruises using semi-balloon otter trawl or Remotely Operated Vehicle (ROV). On recovery of the trawl all samples were taken to a cold room (~4°C) and dissected. Muscle or gonad tissue was removed, avoiding contact with gut contents. Samples were cleaned using 100% ethanol, then frozen using liquid nitrogen and transferred to -20°C a -80°C for long terms storage. Samples from the NE Pacific were provided by Scripps Benthic Invertebrate Collection and Monterey Bay Aquarium Research Institute. They were collected during trawls conducted as part of the Station-M long term pulse-chase time series. Whole specimens were preserved in 100% ethanol until dissection for muscle tissue. Samples of both species from two sites (+FE, HNLC) in the Crozet region of the Southern Indian Ocean were collected by semi-balloon otter trawl as part of the CROZEX study on the effect of natural iron fertilisation on the benthic fauna. Samples were processed as for the NE Atlantic. Additional samples from the Pacific, Atlantic and Indian Oceans were provided by the Shirshov Institute of Oceanology. These samples included some of the oldest tissue samples used in the analysis.

5.2.2 Genomic DNA Extraction

Between 10 and 25mg of tissue (depending upon the amount of tissue available) was placed in 1.5ml micro centrifuge tube for total DNA extractions with a DNeasy Bloody and Tissue Extraction Kit (Qiagen) following the manufacturers guidelines with the following adjustments: Tissue samples were cut into small pieces and once ATL buffer and proteinase K had been added a small disposable pestle was used to further macerate the tissue before leaving to lyse in a thermal heat block overnight. The final concentration of DNA in each sample was quite variable, largely depending on the starting amount of tissue, the age of the tissue and the method of storage. Where only a small amount of tissue was available DNA concentrations tended to be quite low, however in general where more than 20mg of tissue was available the concentration of the elutants was between 50 and 400ng/µl. In samples where only tiny amounts of tissue were available, the Macherey Nagel Tissue XS DNA extraction kits were used. This resulted in a smaller volume but more concentrated DNA elutant.

5.2.3 DNA amplification and sequencing

A 481 and 515 base-pair fragment of cytochrome oxidase 1 (COI) was amplified for *P. longicauda* and *O. mutabilis* respectively. Echinoderm specific primers were used for *P.longicauda* (Hoareau and Boissin 2010)(Table 5.2). Polymerase Chain Reactions (PCR) were carried out using the Multiplex PCR kit (Qiagen) and reaction conditions were optimised as follows; 94°C for 12 min, 35 cycles of 94°C for 35 sec, 40-50°C for 70 sec, 72°C for 55 sec, followed by 72°C for 10 min on a Bio-Rad (Herts, UK) *MyCycler™* thermocycler. Additional species specific COI primers were designed for use in more degraded samples of *P.longicauda* (*Table 5.2*). Optimised reaction conditions using the Multiplex PCR kit (Qiagen) were; 94°C for 12 min, 35 cycles of 94°C for 35 sec, 48°C for 70 sec, 72°C for 55 sec, followed by 72°C for 10 min on a Bio-Rad *MyCycler™* thermocycler. Species specific primers were also designed for amplification of *O. mutabilis* COI (*Table 5.2*) with PCR conditions identical to those used for the species specific *P. longicauda* COI primers. All the above reactions were carried out with ~40ng genomic DNA per reaction.

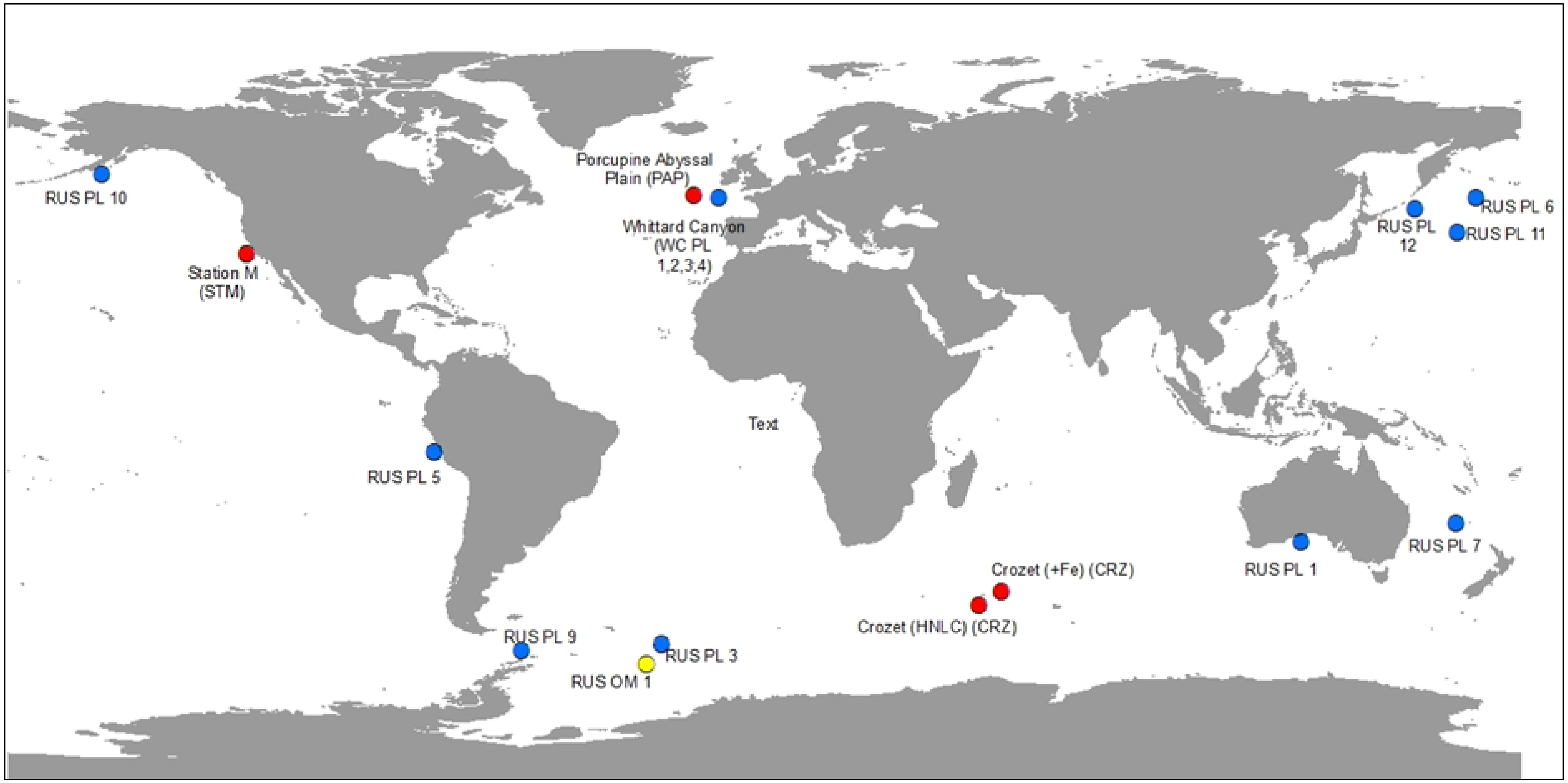


Figure 5.1 *Sampling locations of molecular samples where both species (red circles)* P. longicauda *only (blue circles) or* O. mutabilis *only (yellow circle) were collected.*

A 418 and 400 base-pair fragment of the large subunit 16S ribosomal DNA (16S rDNA) was amplified for *P. longicauda* and *O. mutabilis* respectively. The 16S primers of Palumbi et al. (1991) were used for amplification. A separate reverse primer was designed for use with more degraded samples of DNA. Reaction conditions used for 16S reactions were the same as those used for the echinoderm specific primers described above using ~20ng total genomic DNA per reaction. All PCRs for both genes were carried out with the addition of a negative control in order to detect the presence of contamination. Amplified products were run out on a gel. PCR products were purified using QIAquick™ PCR purification kit (Qiagen) following the manufacturer's instructions. PCR products with multiple products were gel extracted using a QIAQuick Gel Extraction Kit (Qiagen).

5.2.4 Data Analysis

Sequences were manipulated and translated using MEGA 4.0 (Tamura et al. 2007). Sequences were then aligned using ClustalX 2.1 (Larkin et al. 2007) and were amended by eye. ARLEQUIN 3.1.1 (Excoffier 2005) was used to compute summary mtDNA statistics, such as the number of haplotypes, the number of polymorphic sites, haplotype diversity (h), nucleotide diversity (π) and their associated standard deviations (SD) (Nei 1987). Haplotype networks displaying genealogical relationships between mtDNA haplotypes were examined using a median joining network (Cassens et al. 2003) with NETWORK 4.6 (Bandelt 2008).

Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992) was performed to infer genetic subdivision and clustering of populations within oceans (within and among sampling localities) in *P. longicauda*. As *O. mutabilis* samples only came from the three primary sampling sites (PAP, Crozet, Station M) AMOVAs exploring variation partitioned within and between sampling sites within oceans was not possible. AMOVAs pooling individuals from different combinations of Oceans together (Atlantic-Pacific vs. Indian, Atlantic-Indian vs. Pacific, Indian-Pacific vs Atlantic) were therefore performed in order to determine levels of variation within and between combinations of oceans for *O.mutabilis* with 'Ocean' as each population. For comparative purposes, these were also completed for *P.longicauda*, but including the individual populations, rather than individuals pooled among Oceans.

Population divergence among different localities were investigated using pairwise *F*-statistics based on Tamura-Nei corrected sequence divergences among haplotypes, with a gamma correction (Tamura and Nei 1993). A non-parametric permutation procedure (10,000 permutations) was used to test whether *F*-statistics are significantly different from zero (ARLEQUIN 3.11). A Bonferroni correction was applied to correct for multiple pairwise comparisons, and significance of p-values adjusted accordingly.

Phylogenetic analyses for each species were conducted for both genes separately and in combination MrModeltest 2.3 (Nylander 2004) was used to select the most appropriate model of evolution. Bayesian analyses was conducted using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). Analyses were run for one million generations, sampling every 100 generations. Additionally, maximum-likelihood (ML) analyses were performed on PhyML 3.0 (Guindon and Gascuel 2003) under 100 replications.

For tree building *Benthodytes gosarsi* (Psychropotidae) was used as the outgroup for *P. longicauda*, whilst *Deima validum validum* (Deimatidae) was used as the outgroup for *O. mutabilis*. Maximum-likelihood (ML) analyses were performed using PhyML 3.0 (Guindon & Gascuel, 2003). Analyses were run for 500 replications.

Finally, levels of intra-specific variation were examined following calculations of pairwise genetic distances between groups identified by the combined Bayesian trees using MEGA 4.0 (Tamura et al. 2007).

Table 5.1 *Ocean of occurrence, general location and year of collection for all individuals used in this study. Table includes both species and provides metadata on amplification success for both molecular markers used for phylogenetic analysis.*

Species	Sample	Ocean	Region	Location	Sampling Year	Sampling depth	CO1	16S
Psychropotes longicauda	PAP PL 1	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2004	4841	Yes	Yes
Psychropotes longicauda	PAP PL 2	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2004	4841	Yes	Yes
Psychropotes longicauda	PAP PL 3	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2004	4841	Yes	Yes
Psychropotes longicauda	PAP PL 4	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2004	4841	Yes	Yes
Psychropotes longicauda	PAP PL 5	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2004	4841	Yes	No
Psychropotes longicauda	PAP PL 6	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2005	4841	Yes	No
Psychropotes longicauda	PAP PL 7	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2005	4841	Yes	No
Psychropotes longicauda	PAP PL 8	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2005	4841	Yes	No
Psychropotes longicauda	WC 1 (8)	Atlantic	NE Atlantic	Whittard Canyon	2009	3592	Yes	Yes
Psychropotes longicauda	WC 2	Atlantic	NE Atlantic	Whittard Canyon	2009	3592	Yes	Yes
Psychropotes longicauda	WC 3	Atlantic	NE Atlantic	Whittard Canyon	2009	3592	Yes	Yes
Psychropotes longicauda	WC 4	Atlantic	NE Atlantic	Whittard Canyon	2009	3592	Yes	Yes
Psychropotes longicauda	RUS PL 3	Atlantic	S Atlantic	S. Sandwich Islands	2002	3948	Yes	No
Psychropotes longicauda	RUS PL 9	Atlantic	SW Atlantic	Drake Passage	2002	3720	Yes	No
Psychropotes longicauda	CRZ PL 1	Indian	S Indian	Crozet (+Fe)	2005	4270	Yes	Yes
Psychropotes longicauda	CRZ PL 2	Indian	S Indian	Crozet (+Fe)	2005	4270	Yes	Yes
Psychropotes longicauda	CRZ PL 3	Indian	S Indian	Crozet (+Fe)	2005	4270	Yes	No
Psychropotes longicauda	CRZ PL 4	Indian	S Indian	Crozet (+Fe)	2005	4270	Yes	No
Psychropotes longicauda	CRZ PL 5	Indian	S Indian	Crozet (+Fe)	2005	4270	Yes	Yes
Psychropotes longicauda	CRZ PL 6	Indian	S Indian	Crozet (HNLC)	2005	4191	Yes	No
Psychropotes longicauda	CRZ PL 7	Indian	S Indian	Crozet (HNLC)	2005	4191	Yes	Yes
Psychropotes longicauda	CRZ PL 8	Indian	S Indian	Crozet (HNLC)	2005	4191	No	Yes
Psychropotes longicauda	CRZ PL 9	Indian	S Indian	Crozet (HNLC)	2005	4191	Yes	Yes
Psychropotes longicauda	RUS PL 1	Indian	SE Indian	Great Australian Bight	1976	2755	Yes	No

Psychropotes longicauda	STM PL 1	Pacific	NE Pacific	Station M	2004	4110	Yes	No
Psychropotes longicauda	STM PL 2	Pacific	NE Pacific	Station M	2004	4065	Yes	Yes
Psychropotes longicauda	STM PL 4	Pacific	NE Pacific	Station M			No	Yes
Psychropotes longicauda	STM PL 5	Pacific	NE Pacific	Station M			Yes	No
Psychropotes longicauda	STM PL 6	Pacific	NE Pacific	Station M			Yes	Yes
Psychropotes longicauda	STM PL 8	Pacific	NE Pacific	Station M			Yes	No
Psychropotes longicauda	STM PL 9	Pacific	NE Pacific	Station M			Yes	No
Psychropotes longicauda	STM PL 10	Pacific	NE Pacific	Station M			Yes	Yes
Psychropotes longicauda	STM PL 11	Pacific	NE Pacific	Station M			Yes	No
Psychropotes longicauda	STM PL 12	Pacific	NE Pacific	Station M			Yes	No
Psychropotes longicauda	STM PL 13	Pacific	NE Pacific	Station M			Yes	No
Psychropotes longicauda	STM PL 14	Pacific	NE Pacific	Station M			Yes	No
Psychropotes longicauda	STM PL 15	Pacific	NE Pacific	Station M			Yes	No
Psychropotes longicauda	RUS PL 5	Pacific	E Pacific	Peru-Chile Trench	1968	4200	Yes	No
Psychropotes longicauda	RUS PL 6	Pacific	NW Pacific	NW Pacific	1955	6282	Yes	No
Psychropotes longicauda	RUS PL 7	Pacific	SW Pacific	Norfolk Island	1975	6135	Yes	No
Psychropotes longicauda	RUS PL 10	Pacific	N Pacific	Aleutian Islands	1969	5740	Yes	No
Psychropotes longicauda	RUS PL 11	Pacific	NW Pacific	NW Pacific	1966	5525	No	Yes
Psychropotes longicauda	RUS PL 12	Pacific	NW Pacific	NW Pacific	1966	5240	Yes	No
Oneirophanta mutabilis	PAP OM 1	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2004	4841	Yes	Yes
Oneirophanta mutabilis	PAP OM 2	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2004	4841	Yes	Yes
Oneirophanta mutabilis	PAP OM 3	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2004	4841	Yes	Yes
Oneirophanta mutabilis	PAP OM 5	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2004	4841	Yes	Yes
Oneirophanta mutabilis	PAP OM 6	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2004	4841	Yes	No
Oneirophanta mutabilis	PAP OM 12	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2004	4841	Yes	No
Oneirophanta mutabilis	PAP OM 13	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2004	4841	Yes	No
Oneirophanta mutabilis	PAP OM 14	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2004	4841	Yes	No
Oneirophanta mutabilis	PAP OM 15	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2004	4841	Yes	No

Oneirophanta mutabilis	PAP OM 16	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2004	4841	Yes	No
Oneirophanta mutabilis	PAP OM 17	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2004	4841	Yes	No
Oneirophanta mutabilis	PAP OM 18	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2004	4841	Yes	No
Oneirophanta mutabilis	PAP OM 19	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2004	4841	Yes	No
Oneirophanta mutabilis	PAP OM 20	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2004	4841	Yes	No
Oneirophanta mutabilis	PAP OM 21	Atlantic	NE Atlantic	Porcupine Abyssal Plain (PAP)	2004	4841	Yes	No
Oneirophanta mutabilis	STM OM 1	Pacific	NE Pacific	Station M	2004	4110	Yes	Yes
Oneirophanta mutabilis	STM OM 2	Pacific	NE Pacific	Station M	2004	4065	Yes	No
Oneirophanta mutabilis	STM OM 3	Pacific	NE Pacific	Station M			Yes	No
Oneirophanta mutabilis	STM OM 4	Pacific	NE Pacific	Station M			Yes	No
Oneirophanta mutabilis	STM OM 5	Pacific	NE Pacific	Station M			Yes	No
Oneirophanta mutabilis	STM OM 7	Pacific	NE Pacific	Station M			Yes	No
Oneirophanta mutabilis	STM OM 9	Pacific	NE Pacific	Station M			Yes	No
Oneirophanta mutabilis	STM OM 11	Pacific	NE Pacific	Station M			Yes	No
Oneirophanta mutabilis	STM OM 12	Pacific	NE Pacific	Station M			Yes	No
Oneirophanta mutabilis	STM OM 13	Pacific	NE Pacific	Station M			Yes	No
Oneirophanta mutabilis	STM OM 14	Pacific	NE Pacific	Station M			Yes	No
Oneirophanta mutabilis	STM OM 15	Pacific	NE Pacific	Station M			Yes	Yes
Oneirophanta mutabilis	STM OM 26	Pacific	NE Pacific	Station M			Yes	No
Oneirophanta mutabilis	STM OM 29	Pacific	NE Pacific	Station M			Yes	No
Oneirophanta mutabilis	STM OM 32	Pacific	NE Pacific	Station M			Yes	No
Oneirophanta mutabilis	STM OM 33	Pacific	NE Pacific	Station M			Yes	No
Oneirophanta mutabilis	STM OM 34	Pacific	NE Pacific	Station M			Yes	No
Oneirophanta mutabilis	STM OM 35	Pacific	NE Pacific	Station M			Yes	No
Oneirophanta mutabilis	STM OM 36	Pacific	NE Pacific	Station M			Yes	No
Oneirophanta mutabilis	CRZ OM 1	Indian	S Indian	Crozet (+Fe)	2005	4275	Yes	No
Oneirophanta mutabilis	CRZ OM 3	Indian	S Indian	Crozet (+Fe)	2005	4275	Yes	No
Oneirophanta mutabilis	CRZ OM 4	Indian	S Indian	Crozet (+Fe)	2005	4275	Yes	No
Oneirophanta mutabilis	CRZ OM 5	Indian	S Indian	Crozet (+Fe)	2005	4270	Yes	Yes

Oneirophanta mutabilis	CRZ OM 6	Indian	S Indian	Crozet (+Fe)	2005	4270	Yes	No
Oneirophanta mutabilis	CRZ OM 7	Indian	S Indian	Crozet (+Fe)	2005	4270	Yes	No
Oneirophanta mutabilis	CRZ OM 8	Indian	S Indian	Crozet (+Fe)	2005	4270	Yes	No
Oneirophanta mutabilis	RUS OM 1	Atlantic	S Atlantic	S. Sandwich Islands	2002	4544	Yes	No

Table 5.2 *Primer sequences used for the amplification of 16S and COI in Psychropotes longicauda and Oneirophanta mutabilis; is for the forward and R for the reverse primer. The same primers were used to amplify 16S in both species.*

Species	**Primer**	**Targeted Gene**	**Sequence (5'-3')**	**Source**
Psychropotes longicauda	CO1hb10F	CO1	ACTGCCCACGCCCTAGCAATGATATTTTTTATGGTNATGCC	Hoareau & Boissin, 2010
Psychropotes longicauda	CO1hb10R	CO1	TCGTGTGTCTACGTCCATTCCTACTCTRAACATRTG	Hoareau & Boissin, 2010
Psychropotes longicauda	PLCO1F	CO1	TATGGCTTTYCCACGKATG	Gubili *et al.* 2011
Psychropotes longicauda	PLCO1R	CO1	AAGTTTCTTGCTTTCCTCTRT	Gubili *et al.* 2011
Oneirophanta mutabilis	OMCO1F	CO1	ATTGRCTTRTMCCCCTTATG	This Study
Oneirophanta mutabilis	OMCO1R	CO1	TCTTTCTTSCCSCTATAGTG	This Study
Oneirophanta mutabilis	OMCO1F2	CO1	RTMCCCCTTATGATAGGRGCYC	This Study
Oneirophanta mutabilis	OMCO1R2	CO1	ATTGCTACCATTGCATATACCAT	This Study
Both species	16Sa (F)	16S	CGCCTGTTTATCCAGATCACGT	Palumbi *et al.* 1991
Both species	16Sc (R)	16S	AGGGCTTTAATGGACGAACAG	Gubili *et al.* 2011

5.3 Results

5.3.1 Psychropotes longicauda

5.3.1.1 Descriptive statistics for *P. longicauda*

Forty individuals of Psychropotes longicauda were successfully sequenced for a 481 bp section of COI (*Table 5.1*). Forty eight polymorphic (variable) sites and 22 haplotypes were identified (*Table 5.7,* Appendix C). The absence of indels and stop codons suggests that no Nuclear Copies of Mitochondrial DNA (NUMT) were present. Overall haplotype diversity was high (0.9192 ± 0.0285) with a relative nucleotide diversity of 0.0325 ± 0.016 (*Table 5.3*). The Indian Ocean (Crozet and Great Australian Bight) had the highest haplotype diversity, followed by the Pacific and the Atlantic (Table 5.3). Patterns of nucleotide diversity followed a similar pattern.

Nineteen *P. longicauda* individuals were successfully sequenced for a 418 bp section of 16S mtDNA (*Table 5.1*). Sequences contained eleven polymorphic sites and seven haplotypes (*Table 5.8*, Appendix C). Overall haplotype diversity was still high (0.8262 ± 0.0635) although nucleotide diversity was somewhat lower (0.0059 ± 0.0037) (*Table 5.3*). For 16S the haplotype diversity was highest in the Pacific, followed by the Indian Ocean, which was considerably lower (*Table 5.3*). The Atlantic once again had the lowest haplotype diversity (*Table 5.3*). Nucleotide diversity was highest in the Pacific Ocean, followed by the Atlantic, and the Indian.

5.3.1.2 Genetic structuring in *Psychropotes longicauda*

Geographic population structure statistics

Statistically significant differences between all pairwise ocean combinations were found for COI Φ_{ST} values (*Table 5.4*). However using 16S, only Φ_{ST} values between the Atlantic- Indian and Pacific- Indian showed statistically significant population differentiation (*Table 5.4*). The Φ_{ST} values themselves were highest between the Atlantic and Indian Ocean and lowest between the Atlantic and

Pacific in both genes. However, AMOVA analysis of COI data showed that when finer substructure is taken into account, the proportion of variation explained by the differences between the Oceans is actually insignificant. Instead, differences between populations with oceans and differences within populations both explained significant proportions of observed variation. No comparable analysis of 16S was possible due to small sample sizes of the populations within regions. AMOVAs using pooled combinations of Oceans, but maintaining internal population structure were investigated (*Table 5.5*). Tests of differences between regions (Atlantic-Pacific vs. Indian, Indian-Pacific vs. Atlantic, Indian-Atlantic vs. Pacific) were all insignificant. Again, both differences between populations within regions and differences within populations explained significant proportions of the variations in COI and 16S. The highest percentage of between-group variation was explained by the Indian-Pacific vs. Atlantic grouping in COI. This grouping also resulted in the lowest percentage of variation being explained by within population differences; however it was not statistically significant (Table 5.5). The structure used for AMOVAs is clarified in *Table 5.15*, Appendix C.

Haplotype networks

The COI haplotype network revealed the presence of five main lineages within the 22 mtDNA COI haplotypes (*Fig. 5.2*). Major clades were separated by over ten mutational steps. Three clades are composed of haplotypes from the same ocean, whereas two are composed of haplotypes from more than one ocean. Additionally, well separated clades exist sympatrically in the same location. The first clade contains samples from three different locations in the Pacific (NE, E and NW Pacific). This group is separate by over ten mutational steps to a single individual from the Drake Passage and by eight mutational steps to the next clade, composed of Crozet (Indian Ocean) and S. Atlantic individuals. This clade is then separated by fifteen mutational steps to an individual from the North Pacific. This individual is separated by four mutational steps to a clade composed of NE Atlantic samples and two singletons from the SW Pacific and the Great Australian Bight in the Indian Ocean. The remaining clade is composed entirely of Indian Ocean samples from the Crozet Islands in the Indian Ocean.

Bayesian and Maximum Likelihood phylogenetic analysis

Here we shall only consider the combined Bayesian phylogram (*Fig. 5.3*) because the individual COI and 16S phylograms are congruent (*Figures 5.9 and 5.11,* Appendix C). The clades in the tree agree with those identified by COI haplotype groups and have been labelled 1-6. Clade 1 is the pan-Pacific clade (north east, north west, north and east) identified in the haplotype network. It is basal in the Bayesian combined phylogram. Clade 2 is comprised of individuals from Crozet in the Indian Ocean and a single sample from the South Atlantic and is also basal Together with clade 1, clade 2 forms Group A. Clades 3-6 are monophyletic but with much internal structuring. Clade 3 is a single sample from the Drake Passage and is separated from the next three clades it therefore forms a group in itself, Group B. The following three clades; 4,5 and 6 also share a common ancestor and form Group C. Clade 4 is composed of samples from Crozet in the Indian Ocean only. Clade 5 is restricted to the northeast Pacific. The final clade, clade 6, contains all samples from the north-east Atlantic including samples from the PAP and the Whittard Canyon. Clade 6 also contains samples from the Great Australian Bight in the Indian Ocean, the south-west, the north-west and the north Pacific.

The COI and 16S Maximum likelihood trees (*Fig 5.10 and 5.12,* Appendix C) are congruent, so here we discuss only the combined gene maximum likelihood tree (*Fig. 5.8,* Appendix C). The Maximum Likelihood (ML) phylogram generally agreed with the results of the Bayesian analysis. There are differences in the resolution of clades 1 and 2 which are poorly resolved in the ML tree. Both trees support the existence of multiple clades at Crozet in the Indian Ocean and Station M in the NE Pacific. They also both agree with the close relationship between geographically disjunct samples from the Pacific, Indian and Atlantic Oceans in clade 6.

Distance measures

Intra-specific variation between clades identified by the combined Bayesian tree (*Fig. 5.3*) ranged between 1.8 and 5.2% (*Table 5.6*). Pairwise distances between clades 1, 2 and 3 (Pan-Pacific, S. Indian, S. Atlantic) were small, ranging between 0.6-2.2%. The largest between group distances (4.7-5.2%) occurred between clades 1 and 2 (Pan-Pacific and S. Indian respectively) vs. Clades 4 and 5 (S. Indian and Station M in the NE Pacific). Therefore some of the most divergent clades within *P. longicauda* occur sympatrically. Clades 5 and 6 (NE Pacific and Atlantic/Pacific respectively) only have small percentage between group divergence at 1.7%. Group 6 is actually slightly less divergent from groups 1, 2 and 3 than are groups 4 and 5.

5.3.2 Oneirophanta mutabilis

5.3.2.1 Descriptive statistics for *O. mutabilis*

Forty two individuals of *Oneirophanta mutabilis* were successfully sequenced for a 515 bp section of COI (*Table 5.1*). Overall, 103 polymorphic sites were indentified, forming 33 individual haplotypes (*Table 5.9*, Appendix C). Overall COI haplotype diversity was high (0.971 ± 0.018) with a relative nucleotide diversity of 0.0650 ± 0.032 (*Table 5.3*). The Indian Ocean had the highest haplotype diversity, followed by the Atlantic then the Pacific. Nucleotide diversity was highest in the Pacific, followed by the Atlantic, then Indian Ocean. No indels or stop codons were present, suggesting an absence of Nuclear Copies of Mitochondrial DNA (NUMTs).

Only seven *O. mutabilis* individuals were successfully sequenced for 16S, so results must be interpreted with extreme caution (*Table 5.1*). 26 polymorphic sites were identified, resulting in six haplotypes (*Table 5.10*, Appendix C). Despite low sample sizes the 16S haplotype diversity was relatively high (0.952 ± 0.096). Nucleotide diversity was relatively low (0.022 ± 0.014). The 16S summary statistics for individual Oceans are presented in *Table 5.3* for completeness, but results are not discussed due to the low sample sizes.

5.3.2.2 Genetic structuring in *Oneirophanta mutabilis*

Geographic population structure statistics

Φ_{ST} values for COI between pairwise comparisons of Oceans showed a significant differentiation between the Atlantic and Pacific (*Table 5.4*). No other pairwise comparisons were significant, for either gene, although the extremely small sample sizes for 16S must be born in mind. AMOVAs investigating population structure between and within Oceans were not possible due to a lack of secondary sub structure (only one ocean had been sampled for more than one geographic locale). However comparisons between pooled Ocean groups using 'Ocean' as the 'population' parameter (i.e. where the regional comparison is Pacific-Atlantic vs. Indian, between population would be Pacific vs. Atlantic vs. Indian and the within population would all samples within each ocean) were possible (Table 5.5). The results confirmed that little variation is explained between 'regions'. For the Indian-Pacific vs. Atlantic analysis and the Atlantic-Pacific vs. Indian analysis significant amounts of variation were explained between and within populations. However, for the Atlantic-Indian vs. Pacific only within-population variation was statistically significant. See *Table 5.15*, Appendix C for AMOVA layout.

Haplotype network

Five putative clades are identifiable in the COI haplotype network for *O. mutabilis* (*Fig. 5.4*). The first clade is comprised entirely of individuals from the NE Atlantic. The second clade is comprised of individuals from Station M in the NE Pacific. The NE Atlantic and Station M clades are separated by over 25 mutational steps. A group of more closely related clades follows. One is comprised of species from the Atlantic, Pacific and the Indian Oceans. A second clade is formed by samples from the Indian Ocean and NE Pacific. A singleton from the South Atlantic then forms a clade on its own. The final clade is comprised only of Indian Ocean samples.

Bayesian analysis and Maximum Likelihood phylogenetic analyses

The combined gene phylogram (*Fig. 5.5*) is discussed here as the 16S and COI phylograms were concordant (*Fig. 5.15 and Fig 5.17,* Appendix C*).* The results of the Bayesian analysis support the clades identified by the haplotype network and suggest the existence of some deep divisions within *O. mutabilis*. The combined gene phylogram shows the cluster of Crozet (Indian samples) to be basal and separate from all other samples. It therefore forms a separate group, group A. A lone sample from the Atlantic sector of the Southern Ocean (RUS-OM-1-clade 2) is separated from all other clades and is intermediate between Group A and a monophyletic grouping of all other samples. It is therefore assigned its own group; Group B. Finally all other samples fall into Group C. Group C is internally structured, with two sub-groups, C (i) and C (ii). Sub-group C (i) is further split into two sub-clades. All together Group C contains four clades. The Bayesian phylogram supports the existence of three separate clades in the NE Pacific, three in the Indian Ocean and two in the NE Atlantic. The clustering of an Indian Ocean individual (CRZ OM 7) amongst individuals from the NE Atlantic has a high posterior probability (0.98). All other clades are restricted to a single Ocean, although separate clades from the same Ocean are usually more closely related to clades of other oceans than the sympatric clades from their own Ocean. The combined Maximum likelihood tree (*Fig. 5.14*, Appendix C) largely agrees with the major clades identified by the Haplotype network and the Bayesian combined gene phylogram. Some slight differences occur in internal substructure, but as bootstrap support was generally low throughout the tree, we will not discuss Maximum Likelihood trees further.

Distance measures

Intra-specific variation between clades identified by the combined Bayesian tree for *O.mutabilis* (*Fig. 5.5*) ranged between 3.2 and 8.4% (*Table 5.6*). Clade 1 was the most divergent, separated from other clades by 6.4-8.4%. Clade 7 (NE Atlantic) is the next most divergent clade, diverging from clades 2-6 between 3.9 and 6.2%. Clades 2,3,4,5 and 6 are less divergent, between 3.2 and 4.2%. Sympatric clades in the NE Pacific (4, 5, and 6) diverge between 3.6 and 3.9%.

Sympatric clades within the NE Atlantic are 3.9% divergent and sympatric clades at Crozet in the Indian Ocean (1, 3, and 5) diverge by 1.8-5.2%.

Table 5.3 *Measures of genetic diversity (CO1 & 16S) for* Psychropotes longicauda *and* Oneirophanta mutabilis. *K, number of individuals; n, number of haplotypes; h, haplotype diversity; π, nucleotide diversity; SD, standard deviations are in brackets; p, number of polymorphic sites*

Population	**All**	**Atlantic**	**Pacific**	**Indian**
Parameters *P.longicauda* CO1				
K	40	14	17	9
n	22	6	9	7
h (±SD)	0.9192 (±0.0285)	0.6044 +/- 0.1499	0.8235 +/- 0.0840	0.9167 +/- 0.0920
π (±SD)	0.032494 (±0.016456)	0.011944 +/- 0.006830	0.027887 +/- 0.014773	0.032034 +/- 0.017957
p	48	27	32	32
Parameters *P.longicauda* 16S				
K	19	8	5	6
N	7	3	3	2
h (±SD)	0.8246 (±0.0635)	0.4643 +/- 0.2000	0.8000 +/- 0.1640	0.5333 +/- 0.1721
π (±SD)	0.005863 (±0.003710)	0.002432 +/- 0.002068	0.008821 +/-0.006260	0.001289 +/- 0.001412
p	11	4	7	4
Parameters *O.mutabilis* CO1				
K	42	16	19	7
n	33	14	12	7
h (±SD)	0.971 (±0.018)	0.975 +/- 0.035	0.871+/- 0.073	1.000 +/- 0.076
π (±SD)	0.0650 (±0.032)	0.064 +/- 0.033	0.072 +/-0.037	0.062 +/-0.036
p	103	90	79	72
Parameters *O.mutabilis* 16S				
K	7	4	2	1
n	6	3	2	1
h (±SD)	0.952 (±0.096)	0.833 (+/- 0.222)	1.000 (+/- 0.500)	1.000 (+/-0.000)
π (±SD)	0.022 (±0.014)	0.010 (+/- 0.008)	0.033 (+/- 0.034)	0.000 (+/- 0.000)
P	26	7	13	0

Table 5.4 *Pairwise comparisons from CO1 and 16S for Psychropotes longicauda and Oneirophanta mutabilis from separate oceans. ΦST values using Tamura & Nei (1993) are given below the diagonal. Significance values (p) were generated after 10,000 permutations. Significant pairwise tests after Bonferroni correction for multiple pairwise tests are depicted with a star symbol (*)*

Psychropotes longicauda								
Gene	**CO1**				**16S**			
Region	Atlantic	Pacific	Indian		**Region**	Atlantic	Pacific	Indian
Atlantic	-	**0.0004***	**0.0001***		Atlantic	-	0.102	**0.000***
Pacific	0.300	-	**0.003***		Pacific	0.134	-	**0.013***
Indian	0.482	0.287	-		Indian	0.727	0.436	-

Oneirophanta mutabilis								
Gene	**CO1**				**16S**			
Region	Atlantic	Pacific	Indian		**Region**	Atlantic	Pacific	Indian
Atlantic	-	**0.013***	0.191		Atlantic	-	0.205	0.999
Pacific	0.135	-	0.067		Pacific	0.234	-	0.999
Indian	0.046	0.120	-		Indian	0.698	0.196	-

Table 5.5 *AMOVA results (Weir et al. 1984) for differences within populations, between populations in groups and between different groupings of oceans. Significant results after 10,100 permutations. Details of populations given in Haplotype tables; Fig 5.15, Appendix C. Populations analogous with 'regions' in Table 5.1*

Gene		**CO1**			**16S**		
Species	**AMOVA Grouping**	**Among groups**	**Between populations within groups**	**Within populations**	**Among groups**	**Between populations within groups**	**Within populations**
P.longicauda	Indian vs. Pacific vs. Atlantic	3.61	**30.64*****	**65.75*****	-	-	-
	Indian-Pacific vs. Atlantic	9.4	**27.01*****	**63.58*****	2.260	**37.41****	**60.33*****
	Indian-Atlantic vs. Pacific	3.400	**31.92*****	**65.32*****	-24.120	**57.49*****	**66.64*****
	Pacific-Atlantic vs. Indian	-8.53	**39.29*****	**69.24*****	20.240	**23.24***	**56.52*****
O.mutabilis	Indian-Pacific vs. Atlantic	-0.67	**6.82***	**93.84*****	2.62	9.42	87.96
	Indian-Atlantic vs. Pacific	5.480	1.870	**92.65*****	-3.500	14.330	89.170
	Pacific-Atlantic vs. Indian	-3.21	**7.85*****	**95.36*****	1.56	10.94	87.5

Table 5.6 *Percent nucleotide divergences (Tamura e al. 2004) for a) Psychropotes longicauda and b) Oneirophanta mutabilis between clades identified by the Bayesian combined gene phylogram. Divergences estimated by Maximum Composite Likelihood with variance estimate after 500 bootstrap replications. Mean percent pairwise nucleotide divergences are shown on the bottom diagonal with associated standards errors on the top diagonal. Mean within group percent pairwise nucleotide divergence are shown on the diagonal and are shaded grey*

	1	**2**	**3**	**4**	**5**	**6**
1	0.1	0.6	0.6	1.1	1.2	1.1
2	1.8	0.3	0.7	1.1	1.1	1.0
3	1.8	2.2	-	1.0	0.9	0.9
4	5.1	5.0	4.2	0.1	1.0	1.0
5	5.2	4.7	3.8	3.7	0.2	0.6
6	4.4	4.0	3.1	4.0	1.7	0.2

a.

	1	**2**	**3**	**4**	**5**	**6**	**7**
1	1.3	1.8	2.0	2.2	2.1	2.2	2.2
2	6.4	-	1.0	1.1	1.0	1.0	1.4
3	7.5	3.5	2.3	0.9	1.1	1.2	1.1
4	8.4	3.8	3.5	2.0	1.1	1.0	1.4
5	7.8	3.4	3.9	3.8	0.8	1.1	1.7
6	8.4	3.2	4.2	3.6	3.9	0.6	1.3
7	8.1	5.0	3.9	5.3	6.2	4.6	0.7

b.

Figure 5.2 *Media-joining network of mtDNA (COI) haplotypes of* Psychropotes longicauda. *The size of the circles corresponds to the relative haplotype frequencies. Small black circles represent hypothetical intermediate haplotypes. Mutational steps are indicated by dashed lines*

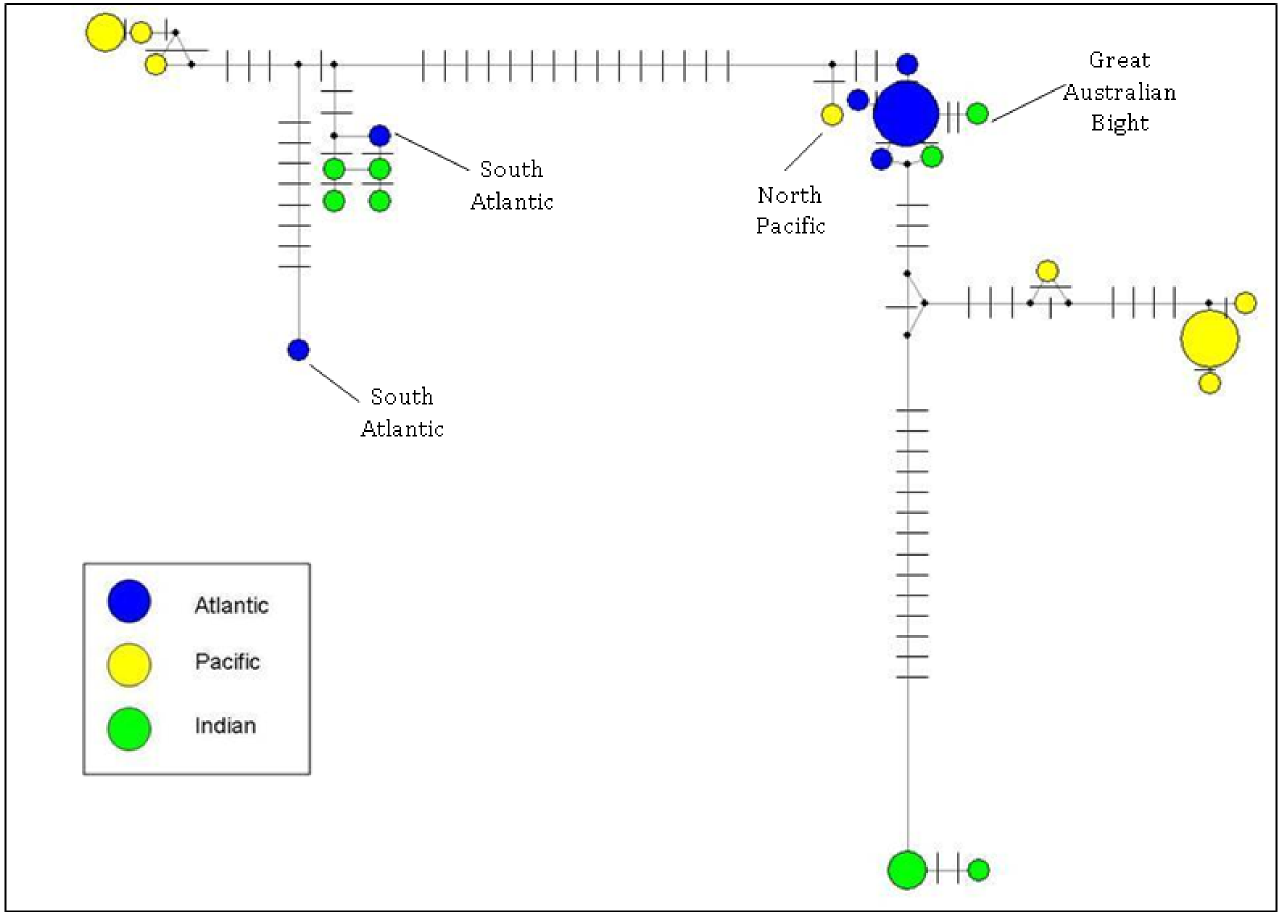


Figure 5.5 *Bayesian combined gene tree depicting genetic relationships among* Oneirophanta mutabilis, *inferred from mtDNA COI sequences (alignment length of 896bp). The tree is rooted with* Deima validum validum *and values at nodes indicate their prior probability. Values under 0.6 are not shown. Clades are numbered 1-7 for ease of discussion and relate to clades 1-7 in pairwise divergence analyses.*

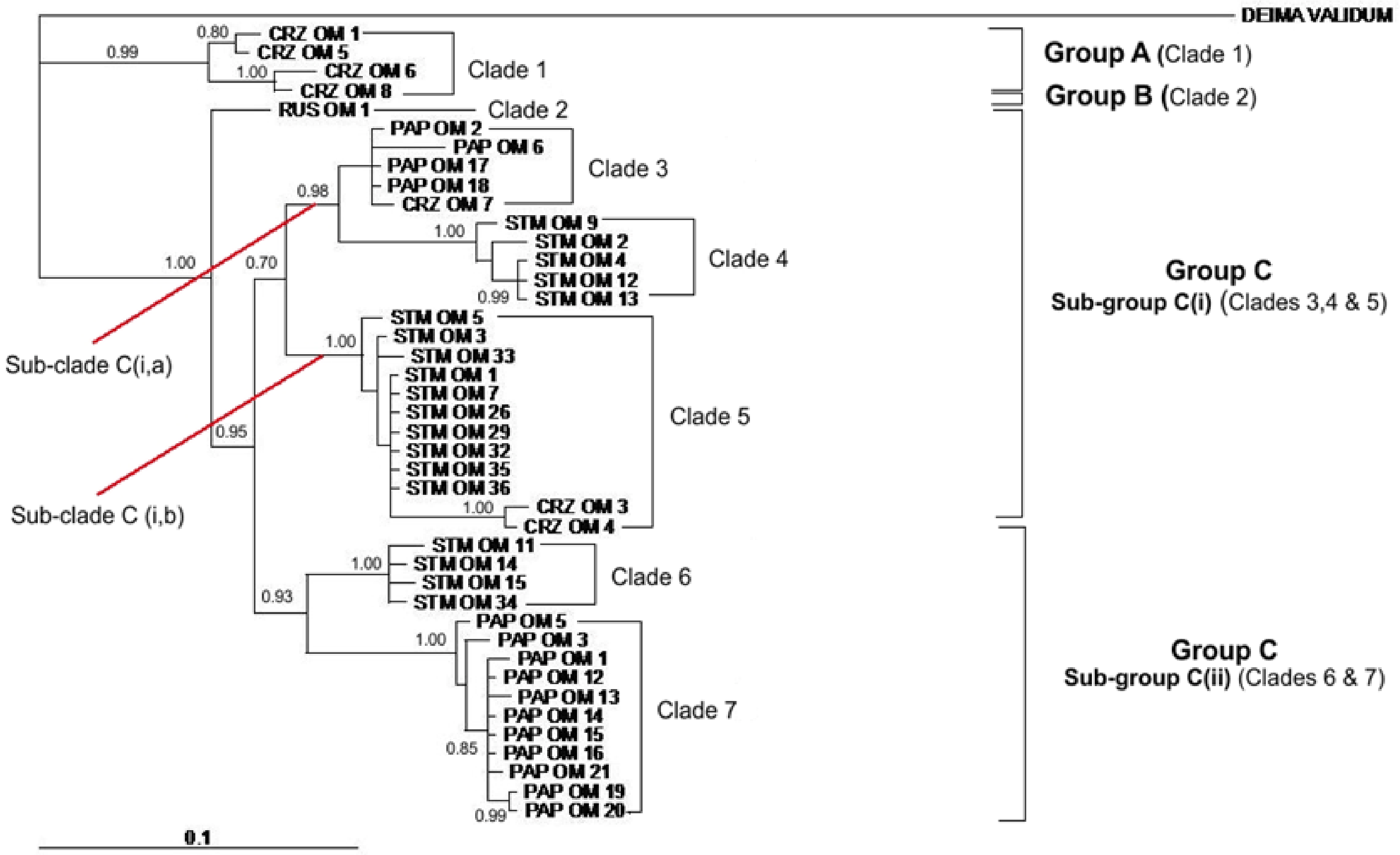


Figure 5.4 *Media-joining network of mtDNA (COI) haplotypes of* Oneirophanta mutabilis. *The size of the circles corresponds to the relative haplotype frequencies. Small black circles represent hypothetical intermediate haplotypes. Mutational steps are indicated by dashed lines*

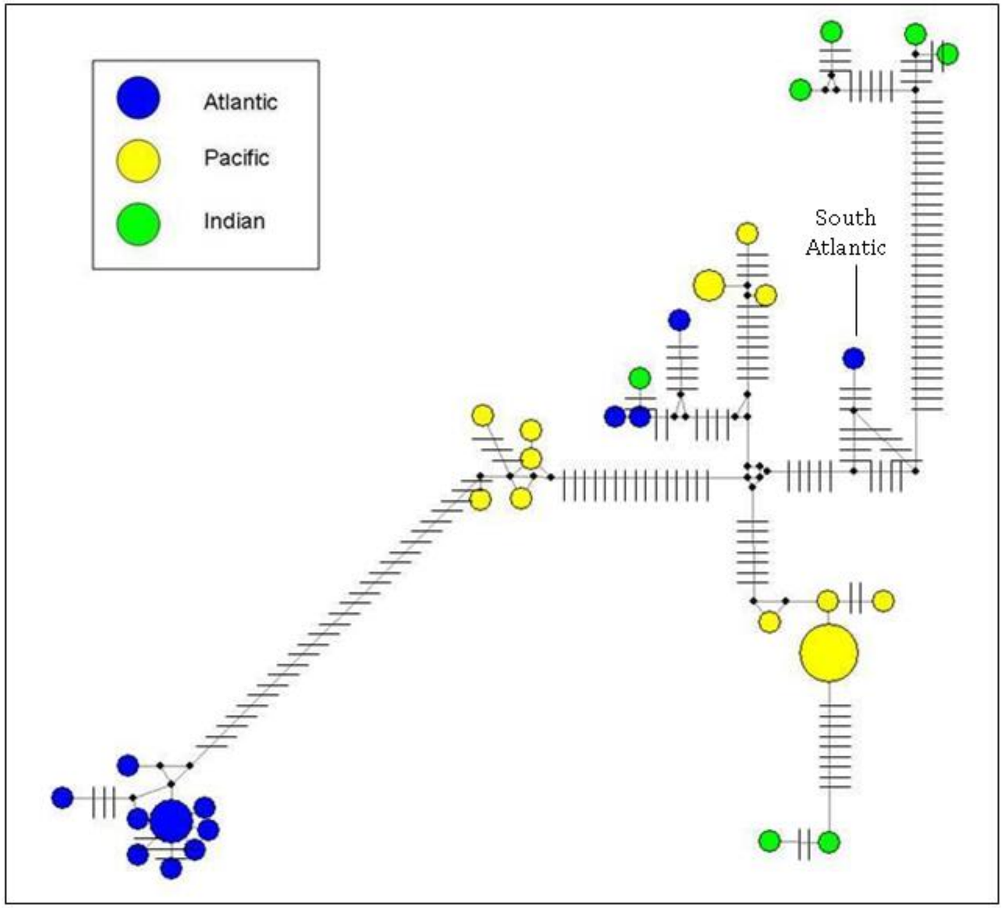
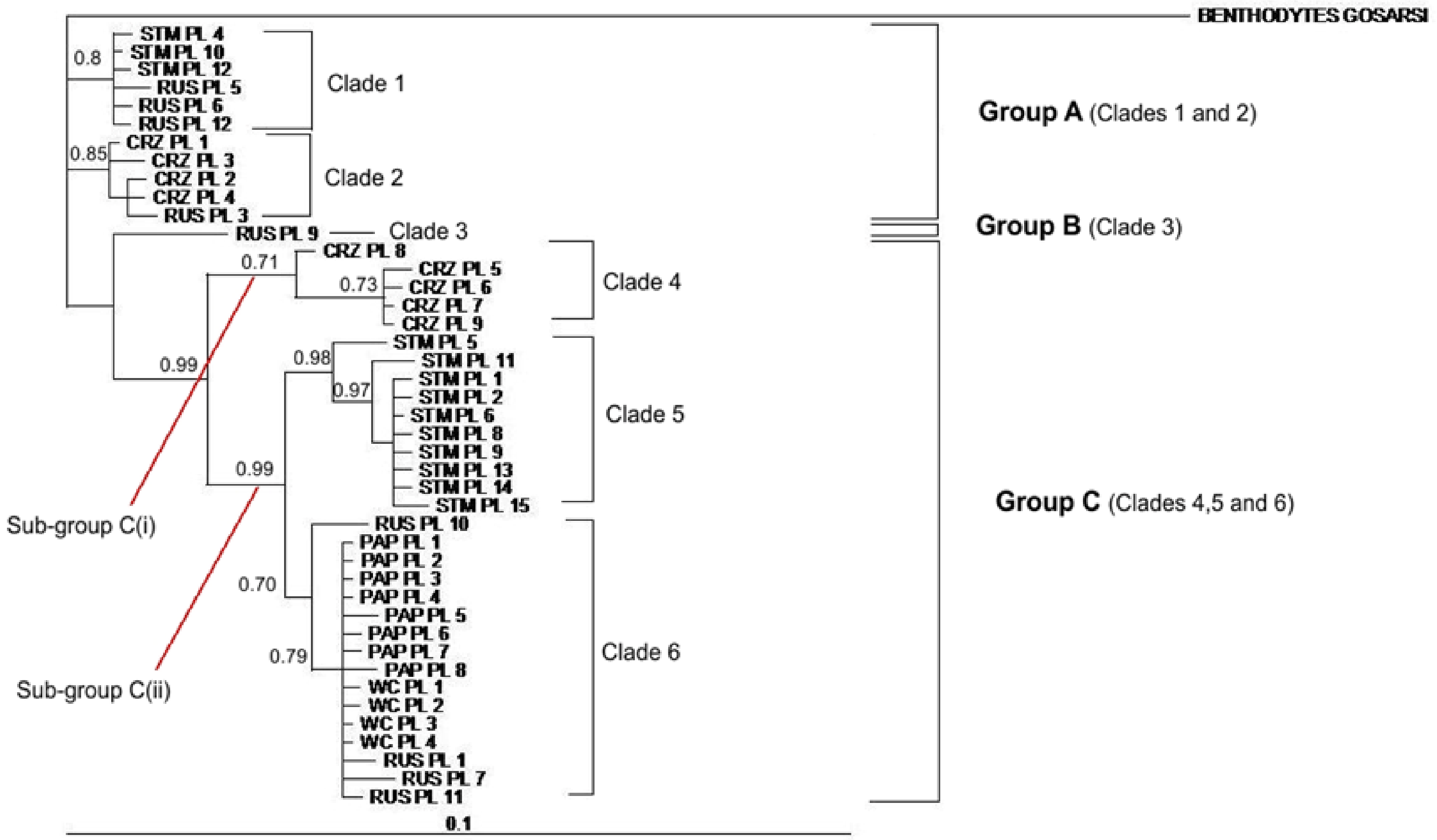


Figure 5.3 *Bayesian combined gene tree depicting genetic relationships among* Psychropotes longicauda, *inferred from mtDNA COI sequences (alignment length of 899bp). The tree is rooted with* Benthodytes gosarsi *and values at nodes indicate their prior probability. Values under 0.6 are not shown. Clades are numbered 1-6 for ease of discussion and relate to clades 1-6 in pairwise divergence analyses*



5.4 Discussion

5.4.1 Geographic population structure

The significant Φ_{ST} values support the existence of geographic population structure for *Psychropotes longicauda* between oceans. The strongest differentiation occurred between the Atlantic and Indian Oceans while the weakest differences occurred between the Pacific and Indian Oceans (*Table 5.4*). For *Oneirophanta mutabilis* only pairwise comparisons between the Atlantic and Pacific Oceans were significantly different and Φ_{ST} values were much lower in all comparisons. For *O. mutabilis* the Indian-Pacific Ocean comparison had a similar absolute Φ_{ST} value as the Atlantic-Pacific analysis. The lack of statistical significance may be related to the relatively small sample size in the Indian Ocean (*Fig. 5.1*). Strikingly, the Atlantic and Indian Oceans, which were the most differentiated for *P. longicauda*, were the least differentiated for *O. mutabilis.* These results are in agreement with other studies which have found genetic structure on within and between ocean scales at abyssal depths (Zardus et al. 2006, Etter et al. 2011).

The AMOVA and Bayesian analyses showed that the genetic structures in *P. longicauda* and *O. mutabilis* are more complicated than simple between-ocean geographic structure. The AMOVA analyses were not significantly different between oceans for both species; most of the differentiation was accounted for by within-population differences. This was more evident in the Bayesian analysis for each species, which revealed the existence of multiple sympatric lineages within oceans, and even within sampling sites. This within-ocean genetic divergence disrupted the simple pattern of between-ocean vs. within-Ocean structure measured by the Φ_{ST} statistic because of the variance within oceans. Despite this, the combined gene phylograms of the Bayesian analyses (*Figs. 5.3* and *5.5*) show that geographic population structure does exist in both species. For example, in *P. longicauda* (*Fig. 5.3*) the phylogram revealed at least two well- separated lineages, labelled as Groups A and C in *Figure 5.3*. A singleton from the southern ocean (RUS-PL-9) forms an intermediate group between Groups A and C so is labelled as a separate group (Group B). Group A is comprised of two clades; Clade 1 is distributed at Station M and throughout the Pacific and Clade 2 is found at Crozet and in the South Atlantic. As mentioned above Group B is comprised of only one sample, which forms clade 3 in the phylogram (*Fig. 5.3*). Group C is comprised of two sub-groups; sub-group (Ci) of Group C is comprised of a single clade; clade 4 which is found at Crozet. Subgroup (Cii) of Group C splits into two separate clades; clade 5 which is found only at Station M and clade 6 which contain all the PAP samples, but also a number of samples from the northern Indian Ocean, the southwest, north and northwest Pacific. Such a structure reveals the sympatric distribution of separate lineages of *P.longicauda* at Crozet (Clade2, Group A and clade 4, Group C), Station M (Clade 1, Group A and Clade 5, Group C) and in the north Pacific (Clade 1, Group A and Clade 6, Group C). Such a complex pattern of sympatry therefore explains the lack of significant differences found between, as opposed to within Oceans in the AMOVA (Table 5.5). However within single lineages geographic structure does exist. In group A there is a split into a Pacific clade (clade 1) and an Indian-Atlantic clade (clade 2). In group C individual clades represent the southern Indian Ocean (clade 4) the NE Pacific (clade 5) and the Atlantic-Pacific (clade 6). Therefore whilst within *P.longicauda* lineages are sympatric and not geographically structured, within lineages, geographic structuring does exist.

Structuring was even more complicated in *O.mutabilis*. Again the population structure reveals two well separated lineages (Groups A and C) with an intermediate singleton which forms its own group (Group B). Group A is formed of one clade (clade 1) and is found at Crozet. Group B, as mentioned above, is a singleton from the Atlantic sector of the Sub-Antarctic Southern Ocean. Group C is highly structured itself. It splits into two sub-groups; C (i) and C (ii). Sub-group C(i) splits into two sub-clades, C(i,a) and C(i,b).The first of these sub-clades is split into two clades, one which is found at PAP and in one sample at Crozet (clade 3) and a second clade at Station M (clade 4). Sub-clade C (i,b) is comprised of one clade (clade 5) found at Station M and Crozet. Sub-group C (ii) is comprised of two clades. One is found at Station M (clade 6) and the other at the PAP (clade 7). Sympatry exists between separate lineages and within finer structure within major lineages. For example, whilst groups A and C both occur at Crozet, within group C there are two clades (clade 5 and clade 3) from the same sub-group C(i) but different sub-clades C(i,a) and C(i,b). Sympatry also occurs at Station M with 3 clades (4, 5, and 6) all occurring there, despite belonging to separate sub-clades (4 vs.5) or sub-groups (4&5 vs. 6). Sympatry of 2 clades (clades 3 & 7) of *O.mutabilis* from separate sub-groups (C(i) and C(ii) respectively) also occurs at the PAP. However within sub-group C(ii) and within sub-clades in sub-group C(i) geographic structuring does occur, with clades generally relating to a geographic area.

Complex population structure involving the existence of multiple lineages within sites has been found in echinoderms previously. In some cases this has been associated with asexual reproduction limiting dispersal capabilities and allopatric speciation followed by secondary contact (Boissin et al. 2008). In others it has been associated with a recent population expansion followed by divergent selection for habitat preferences, combined with a short pelagic phase and associated poor dispersal capability (Muths et al. 2006). However both species under consideration have the (apparently realised) potential for long distance dispersal so that sympatry cannot be simply due to restricted gene flow over small spatial scales.

The following analysis, therefore, considers the individual phylogeographies of the two species based on the results of the Bayesian combined gene analyses and pairwise distance comparisons, in order to propose possible mechanisms which could result in the observed patterns.

5.4.2 Southern Origins

P. longicauda has two unresolved, closely related basal clades which together form Group A (clades 1 and 2, *Fig. 5.3*). Clade 1 is distributed throughout the

Pacific (NW, N, NE and E). Clade 2 is distributed at low latitudes in the Southern Hemisphere, being found both in the Crozet region of the Southern Indian Ocean and one specimen from the South Atlantic. As in *P. longicauda*, a basal southern Indian Ocean (Crozet) Group (Group A) is present in *O. mutabilis* (comprised of one clade; clade 1 *Fig. 5.5*). Both species also have an intermediate group (Group B) which occupies the position between basal Group A and more derived Group C. Group B in *P. longicauda* is comprised of a single sample from the Drake Passage (Clade 3, *Fig. 5.3*). Group B in *O. mutabilis* is comprised of a single sample from the South Atlantic (Clade 2, *Fig. 5.5*).

This suggests that both species have origins in the Southern Hemisphere and agrees with patterns of evolution and dispersal that have been proposed for the Order Elasipodida. Based on the morphological taxonomy of the Family Elpidiidae, (Gebruk 1994) suggested that there were two main stages in the evolution of the Elasipodida; The Tethys and Antarctic stages. The first, the Tethys stage, involves a vicariant event in the Miocene which split the ancestral shallow water elasipodids in the Tethys basin into at least three groups, the Indo-Malayan, the Mediterranean and Tropical Pacific in the Panama Gulf, following the various geological and oceanographic events of the Tethys Sea era. . Divergence at bathyal depths then occurred from the Panama Gulf (or possibly the Caribbean) to the Antarctic. The second, Antarctic stage (Gebruk 1994), involved the invasion of abyssal depths, driven by expanding ice sheets forcing bathyal fauna to retreat to deeper refugia (Rogers 2007). The Antarctic is now acknowledged as a centre of origin for many deep-sea species (Briggs 2003, Rogers 2007). Shallow water and slope species are thought to have colonized abyssal depths during the late Mesozoic and Cenozoic epochs (Strugnell et al. 2008), coinciding with a change in deep-water circulation, caused by a reduction of warm saline deep water flow to the Southern Ocean (Flower and Kennett 1994). This resulted in an increased stability of the East Antarctic Ice Sheet (EAIS) (Flower and Kennett 1994) and a switch from greenhouse to icehouse conditions (Rogers 2000, Rogers 2007). The resulting sinking of cold, saline water adjacent to the Antarctic continent and its subsequent movement northwards at abyssal depths has resulted in the dispersal from the Antarctic to the rest of world's ocean for many invertebrate families and genera. As well as the Elasipodida, an Antarctic origin has been suggested for deep-sea isopods (Kussakin 1973, Brandt 1992, Held 2000, Brandt et al. 2007), octopods (Strugnell et al. 2008) and macrofauna (Gage 2004). However, whilst the basal position of the Crozet and South Atlantic specimens in both *P. longicauda* and *O. mutabilis* suggest a Southern origin, as the current analysis lacks true Antarctic fauna for either species this cannot be confirmed, being found in the Sub-Antarctic rather than the Antarctic itself. The presence of an additional basal pan-Pacific clade (clade 1) in *P. longicauda* suggests that the 'basal' groups may actually represent the species well after the first northwards dispersals. Future work including samples from the Antarctic are required in order to verify the hypotheses of Gebruk (1994).

5.4.3 Multiple sympatric clades at Crozet

As well as basal lineages present at Crozet (Group A in both species) *P.longicauda* and *O.mutabilis* also show additional, more derived clades at this site. In *Psychropotes longicauda* this second Crozet lineage (clade 4) is basal within Group C (*Fig. 5.3*), forming sub-group C(i) ,with subsequent divergence into Atlantic and Pacific clades (Clades 5 and 6, *Fig.5.3*) forming sub-group C(ii). This suggests that genetic differentiation within the Group C lineage had occurred within the Southern Hemisphere prior to the species dispersal northwards. In *Oneirophanta mutabilis*, although the additional lineages at Crozet are spread throughout the tree, the existence of a split within Group C clade resulting in multiple lineages existing in the northeast Pacific and northeast Atlantic may also suggest speciation in the Southern Hemisphere (probably Antarctica) before multiple northwards dispersal events. In-situ intra-specific genetic differentiation and cryptic speciation in the Antarctic has been revealed recently by molecular studies of a number of species in shallow-water, shelf and deep-sea environments. For example, genetic analysis of a direct-developing Antarctic nudibranch *Doris kerguelensis* revealed explosive radiation and cryptic speciation with 29 sympatric mitochondrial lineages in the Drake Passage area at intertidal to bathyal depths (Wilson et al. 2009).

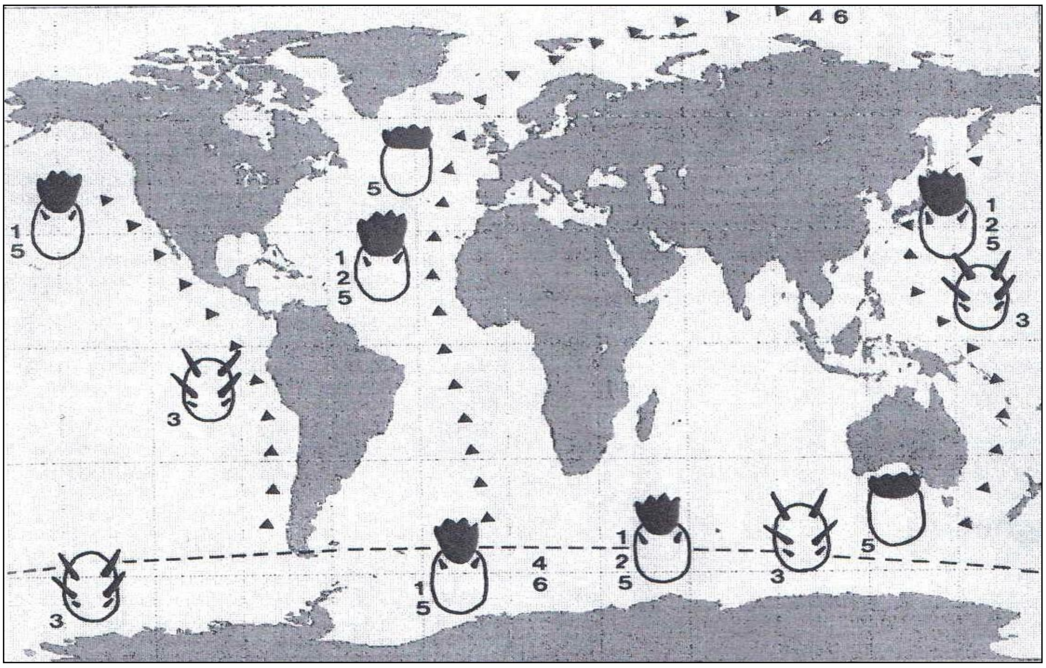


Figure 5.6 *Geographical dispersal pathways from Antarctica, based on morphological analyses of the distribution of the Elpidiidae, from (Gebruk 1994). Similar dispersal lineages are postulated for causing patterns of geographic genetic structuring in* Psychropotes longicauda and Oneirophanta mutabilis

The origin of these lineages was not related to depth, but was correlated with allopatric events caused by repeated glacial cycles. The glacial events were caused by periodic Milankovitch cycles (Clarke et al. 2004) and the repeated expansion and contraction of the Antarctic ice sheets (Wilson et al. 2009). The expansions and contractions resulted in pulses of species range contraction into refugia and subsequent expansion and secondary contact, causing the observed modern-day sympatry of many genetically divergent populations or sister species which formed in separate refugia (Rogers 2007). Repeated glacial cycles in the Antarctic and associated allopatric events have been termed the 'Antarctic diversity pump' (Clarke et al. 2004). These fluctuations have been affecting Antarctica for approximately 35 million years, and in the last 2.4 million years, there may have been as many as 50 to 60 glacial-interglacial cycles, creating huge potential for complex genetic patterns in Antarctic marine organisms (Wilson et al. 2009). The Antarctic diversity pump has been associated with genetic structuring in a number of benthic and deep-sea Antarctic fauna such as bivalves (Linse et al. 2007) isopods (Held 2000), amphipods (Baird et al. 2011), pycnogonids (Leon 2001) and octopods (Allcock et al. 2011, Strugnell et al. 2012).

Echinoderm species have previously been found to show complex genetic structuring and sympatric lineages in the Antarctic. For example, initial studies of specimens of an Antarctic crinoid with a purportedly circumpolar distribution, *Promachocrinus kerguelensis*, from the Atlantic sector of the Southern Ocean was found to be composed of multiple highly divergent lineages existing sympatrically (Wilson et al. 2007). Further sampling from other locations around Antarctica confirmed that the sympatric lineages were indeed highly divergent and all had circumpolar distributions (Hemery et al. 2012). Significant genetic population structure was also found in the brittle star *Ophionotus victoriae* despite the potential for long-distance dispersal via a planktotrophic larval stage (Hunter and Halanych 2010). Additionally many deep-sea holothurians once thought to have circum-Antarctic distributions are now known to demonstrate significant allopatric subdivision, including deep-sea Elasipodids (O'Loughlin et al. 2011).

Therefore, the existence of multiple lineages at Crozet in *P.longicauda* may be due to repeated colonisations from divergent Antarctic populations. The Antarctic and Southern Indian Ocean are thought to be faunistically well connected (Vinogradova 1997). Additionally, the Crozet region is thought to be located on the border between two deep-sea sub-regions, the Antarctic-Atlantic and the Antarctic-Indian-Pacific (Vinogradova 1997). This border passes along the South Atlantic Ridge from the Cape of Good Hope, through the Crozet Region, to Enderby Island (Vinogradova, 1997). Therefore, it may be an area where allopatrically differentiated lineages from the Antarctic meet. Despite occurring in the same location, the two lineages at Crozet have an average nucleotide divergence of 5% suggesting substantial separation (see *Section 5.4.6* on cryptic species). Similar processes could explain the existence of divergent lineages occurring in the northeast Pacific and northeast Atlantic. . This is reviewed in the following section.

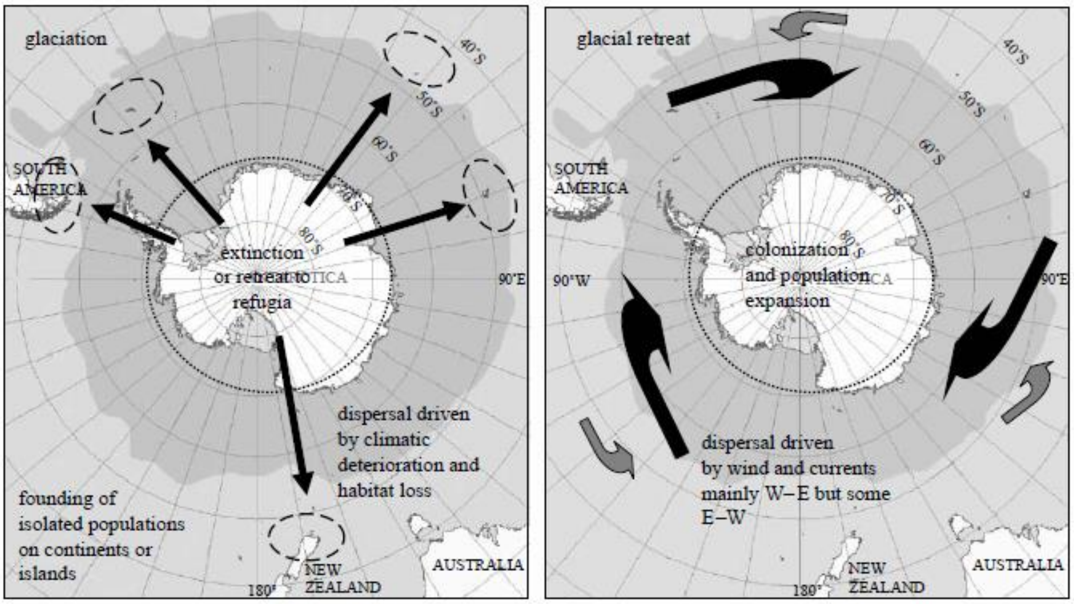


Figure 5.7 *A schematic for the Antarctic Diversity Pump depicting the mechanisms causing genetic population structure in Antarctic and Southern Hemisphere species over glacial cycles illustrating major processes during; a) glacials and b) inter-glacials. From (Rogers 2007)*

5.4.4 Atlantic-Pacific differentiation

Starting from the Southern Indian Ocean (and Pan-Pacific in *P.longicauda*) both species split broadly into northeast Pacific and Atlantic populations. In *P.longicauda* this occurs only once, in group C, where sub-group C *(ii)*, splits into clades 5 (Station M) and 6 (PAP-Pacific) from a common ancestor who is present at Crozet (subgroup C(i)). In *O.mutabilis*, as explained above, this differentiation is more complicated. In *O.mutabilis* Group C sub-group C *(i)* splits into two sub-clades; sub-clade C (*i,a*) with clade 4 in the Pacific and clade 3 in the Atlantic and Indian Oceans. Sub-group C (ii) also splits into a northeast Pacific clade (clade 6) and a northeast Atlantic clade (clade 7). In both species there are three possible scenarios for the differentiation of the northeast Pacific and 'Atlantic' clades; 1) Dispersal from an Antarctic (or at least Southern Hemisphere) common ancestor (or ancestors) up the (probably East) Pacific coast and the (probably East) coast of the Atlantic respectively. 2) An Atlantic-Pacific common ancestor being divided by the rise of the Isthmus of Panama and 3) Dispersal from the north Pacific via the Bering Seaway to the north Atlantic or vice versa.

The first scenario of dispersal to the Atlantic and Pacific separately from an Antarctic common ancestor has been considered in detail above. It is the mechanism considered most likely for the Elpidiidae by (Gebruk 1994). This scenario could also explain divergences between the Atlantic and the Pacific clades within major lineages, including the complicated pattern observed in *O. mutabilis*: If multiple ancestral species existed in the Southern Hemisphere and multiple northwards dispersal events occurred, then existence of 3 clades from 2 groups at Station M could be at least partially explained (*Fig. 5.5*).

The second possible scenario involves widely distributed groups dispersing from the Southern Hemisphere into the Pacific and Atlantic. However these populations would remain genetically connected until the rise of the Isthmus. The Isthmus rose ~3.5 MYA, but in addition there had not been a deep-water connection for millions of years prior to this (Vinogradova 1997). Genetic population structure of abyssal soft sediments caused by the rise of the isthmus has not been recorded previously, apart from in relation to the evolutionary history of vent fauna (Connelly et al. 2012). Neither P. Longicauda nor O. mutabilis are located in the Caribbean, despite the occurrence of 2 congeners *Psychropotes* (*P. depressa* and *P. semperiana*) there. The genus *Oneirophanta* is entirely absent from the mid and northwest Atlantic. Additionally, the splits between Pacific and Atlantic clades in the various sub-clades of *O.mutabilis*, although not calibrated by a molecular clock, appear to have occurred at different times. If the differentiation was caused by the Isthmus acting on multiple cosmopolitan lineages originally derived from the Southern Hemisphere, then these splits would presumably would have occurred more or less simultaneously. Whilst not conclusive, current evidence suggests that a split caused by the Isthmus is unlikely as the general rule for Pacific-Atlantic splits.

The third scenario, of dispersal through the Arctic is known in shallow-water echinoderms (Palumbi and Kessing 1991). The Bering land bridge created a barrier to the dispersal of marine animals between the north Pacific and north Atlantic until it was flooded approximately 3.5MYA (Palumbi and Kessing 1991). Since then the direction of dispersal has generally been from the North Pacific to the North Atlantic. Although dispersal has been noted in the sub tidal urchin *Stronglyocentrotus pallidus* (Palumbi and Kessing 1991) the Bering Strait is currently only 91m deep at its deepest point (Hu et al. 2008), and is a major barrier for deep sea species. Similarly, the effects of the Iceland-Faeroe Ridge have already been discussed in Chapter 3. Additionally, the absence of the Psychropotidae and Deimatidae from the Arctic (see Chapter 3) suggests this route is unlikely and morphological analyses of the Elpidiidae confirm separate Pacific and Atlantic pathways are likely in the Elasipodida (Gebruk, 1994).

The most likely scenario for the genetic differentiation between Atlantic and Pacific populations of *P. longicauda* and *O. mutabilis* within major lineages is thought to be the existence of a southern hemisphere ancestor in each species (or multiple ancestors in *O. mutabilis*), which then dispersed into the north Atlantic and north Pacific separately.

In Group C in *O. mutabilis* the split between within sub-group C(i) into sub-clades C(i,a) and C(i,b) resulting in two separate clades found at northeast Pacific could potentially be explained by speciation in the Southern Hemisphere before separate lineages disperse northwards, or alternatively by a West Pacific and East Pacific dispersal route which then met at Station M. These hypotheses are not mutually exclusive and could also occur in tandem. Whilst an East Pacific route was thought more likely in the Elpidiidae, (Gebruk 1994) acknowledged the potential for dispersal along both margins.

5.4.5 Recent global dispersal events

In *Psychropotes longicauda* the existence of samples from the southwest, north and northwest Pacific, plus a northern Indian Ocean sample within the primarily northeast Atlantic clade (Clade 6, *Fig. 5.3*) suggests a recent dispersal event or ongoing genetic connectivity. The haplotype network (*Fig. 5.2*) suggests that within this clade the northeast Atlantic is basal, and the samples from other oceans are separated from the basal haplotype by only one or two mutations. In *O. mutabilis* the inclusion of individuals from Crozet within a primarily northeast Atlantic clade (clade 3, *Fig. 5.5*) and within a primarily northeast Pacific clade (clade 5, *Fig. 5.5*) also points towards ongoing long distance dispersal. However the genetic differences between major (sometimes sympatric) lineages alongside the general trend of geographic structuring within-lineages suggest such events are relatively sporadic, rather than constant contact between a panmictic single global population.

According to our results, despite different dispersal capabilities; lecithotrophic larvae in *O. mutabilis* with an egg size of ~950 μm (Billett 1991) and direct development with an extremely large egg - c. 4mm - with potential for long distance pelagic dispersal in *P. longicauda* (Billett 1991) the potential for global dispersal may be realised in both species. Such dispersal, as mentioned above, appears to be sporadic rather than continual or in a stepping-stone manner. However greater sampling resolution is required both in terms of geography and the types of molecular marker used to confirm these initial findings.

The existence of multiple sympatric lineages and substantial geographic structuring, relatively (but not entirely) unrelated to simple allopatric or distance related events, was not expected based on other deep-sea organisms (Chase et al. 1998, Etter et al. 2005). However, similar patterns have been noted in other cosmopolitan marine species, including species thought to have good dispersal abilities and those with more limited dispersal. Multiple cryptic lineages existing sympatrically have been observed in the brooding brittle star *Amphipholis squamata* (Sponer and Roy 2002). Additionally, within lineages, very close relationships, indicated by low sequence divergence, were found between New Zealand and South African populations, and between New Zealand and North Atlantic populations, suggesting high population connectivity over long distances (Sponer and Roy 2002). This is extremely similar to the unexpected grouping of NE Atlantic *P. longicauda* with individuals from the Northern Indian, SW, NW and N Pacific. The authors concluded that different lineages have sporadically colonized New Zealand by inter-oceanic dispersal (Sponer and Roy 2002). Similarly multiple colonization events of the Hawaiian Islands by the upside down jellyfish *Cassiopea*, one from the Indo-Pacific and another from the Western Atlantic have resulted in the existence of two highly divergent cryptic lineages (Holland et al. 2004). The existence of multiple lineages of the bivalve *Macoma balthica* in the Atlantic Ocean was thought to be caused by multiple dispersal events from the Pacific via the Arctic (Luttikhuizen et al. 2003). In an estuarine copepod thought to be globally distributed, major sympatric differences were found with multiple cryptic lineages (Lee 2000). As for *P. longicauda* and *O. mutabilis*, populations within lineages separated by >5000km were found to be highly connected genetically (Lee 2000).

Similar results have been found recently in deep-sea free-living nematodes, where closely related lineages were found to exist in sympatry, whilst the lineages themselves were found to be distributed widely, showing some population structure on ocean-basin scales in some lineages and cosmopolitan distributions in others (Bik et al. 2010).

5.4.6 Cryptic species

The level of divergence that warrants a species level difference, known as the species-specific threshold (Lefebure et al. 2006), is topic of great discussion (Wu 2001, Shaw 2002, DeSalle et al. 2005, Hebert and Gregory 2005, Gomez et al. 2007, Ward et al. 2008). A careful morphological and genetic analysis of Australian land snails found a threshold of 5-7% genetic divergence agreed with traditional taxonomic species delineation (Koehler and Johnson 2012). In a study of the cryptic speciation in Antarctic holothurians (including Elasipodida) (O'Loughlin et al. 2011) defined a species complex as clades whose members either 1) share the same morpho-specific identification, or 2) have some morphological differences but are within 5% pairwise sequence divergence in COI. Other studies of echinoderm population genetics support this value. In a global study of the genetic divergence within and among species in the asteroid genus *Coscinasterias* distances of 0-3% were reported within morpho-species, and distances of 4.1-16.6% between morpho species (Waters and Roy 2003). They suggested a sub-specific status for Atlantic and Mediterranean populations of *Coscinasterias tenuispina* based on a sequence divergence of 1.5%. Additionally within species distances of up to 8% occurred between Australian populations of *C. muricata* indicating a species complex.

In a study of deep-sea and shallow water species of the sea urchin genus *Sterichinus* distributed North and South of the Antarctic Circumpolar Current (ACC) found that four morpho-species were only 0.2-0.6% differentiated. In contrast a fifth morpho species was ~5% differentiated from the other four species. Therefore the 'clumping' of the four poorly delineated morpho-species into one was suggested, whilst the fifth species, *S. neumayeri*, was upheld (Diaz et al. 2011). In a study of two holothurian species from the East coast of North America and Canada, genetic differences of ~1.2% within *Cucumaria miniata* were considered to be indicative of population structure, whereas genetic distances of ~6.1% between populations of *C. pseudocurata* were thought to indicate the presence of a species complex. (Muths et al. 2009) reported genetic differentiation of less than 1% within morphological 'varieties' of *Ophiothrix fragilis* in the northeast Atlantic. The authors concluded that the varieties were redundant and not linked to genetic population differentiation. However, a cryptic species level differentiation of 18.6% was found between Atlantic and Mediterranean populations that were entirely unreported from a morphological viewpoint.

Differences within *P. longicauda* ranged from 1.7-5.2% between clades. Whilst geographically disparate populations with intermediate divergence values are difficult to place along the continuum from interbreeding population to totally separate species, those lineages which are >4% divergent but sympatrically distributed can be thought of as approaching a species level difference. Therefore the Groups A and C can be thought of as a species-level difference, although concurrence from a larger number of samples and molecular markers would be needed to confirm this. Detailed morphological reanalysis of *P. longicauda* is also needed in order to determine whether post-hoc morphological differences can be assigned to these species, or determine if they are 'cryptic' in the truest sense of the word. Divergences of up to 8% between the lineages described by the Groups of *O. mutabilis* suggest cryptic speciation in this species is even more advanced. The clade that forms the basal Crozet group (Group A) is 6.4% diverged from its most closely related group (Group B) and between 7.5-8.4% divergent from the clades which form Group C. Therefore a species level difference is suggested between Group A and Group C. However, as mentioned above, greater sampling resolution and investigation with a larger range of molecular markers, preferably including nuclear genes, is needed to confirm these results. Whilst differences between some other pairs of clades within lineages are greater than 5%, the existence of genetic intermediaries suggests that, whilst speciation is probably occurring, it would be premature to assign a species level difference. Excluding Group A, sympatric clades generally reach a differentiation of 3.8%. Therefore functionally sympatric clades even within the same lineage appear to genetically disjunct.

5.4.7 Conclusions

Complex patterns of genetic structuring have been found in both *Psychropotes longicauda* and *Oneirophanta mutabilis* that do not fit previous expectations of simple ocean-basin isolation-by-distance differences. Whilst geographic genetic structuring does exist, this is complicated by the existence of multiple sympatric lineages at many sampling sites. Often, the differences between sympatric lineages at one site are greater than differences between populations within lineages separated by tens of thousands of kilometres. It is suggested that both species may have spread northwards from (perhaps) multiple lineages the Antarctic. Greater sampling effort in the Southern Hemisphere is needed to confirm this. Results from COI and 16S genes suggest there may be species-level differences between some lineages in *Oneirophanta mutabilis*, and close to species-level differences in *Psychropotes longicauda*. However, larger sample sizes, greater geographic resolution and the inclusion of nuclear genes will benefit future analyses in order to confirm the preliminary results presented here.

Chapter 6. Synthesis and conclusions

Traditional views of deep-sea biogeography predicted very large range sizes with few barriers to dispersal for the majority of taxa (McClain and Hardy 2011). Whilst patterns from seamounts and hydrothermal vents have identified higher than expected levels of endemicity, the potential dispersal ability of many species, combined with reduced metabolic requirements in the cold waters of the deep-sea suggests that many species may indeed have large distributions spanning multiple oceans (McClain and Hardy 2011). Additionally mechanisms that are thought to be potential barriers to the dispersal of marine organisms, such as topography, currents and strong environmental gradients, are often found to be semi-permeable (McClain and Hardy 2011). For the deep-sea soft-sediment benthos, primary productivity or flux to depth is thought to be one of the most important environmental gradients in the food-poor deep sea (Smith et al. 2008).

A number of studies have investigated the effects of gradients of primary productivity on diversity, abundance and community composition of macrofauna on a within-ocean scale (Rex et al. 1993, Lambshead et al. 2000, Rex et al. 2000, Lambshead et al. 2002, Rex et al. 2005, Angel et al. 2007). However relatively few have investigated such patterns in the megafauna and those that have tend to test for differences between only two sites, thought to be in different productivity regimes (Thurston et al. 1994, Thurston et al. 1998, Wolff et al. 2011). Recently (McClain and Hardy 2011) suggested a number of areas of deep-sea biogeography that needed further research. These included; 1) the assessment of the effects of productivity patterns on biogeography; 2) improved biogeographic schemes and 3) population genetics and phylogeography for all deep-sea habitats and especially for the abyssal plains (McClain and Hardy 2011). Here we will review the evidence from this study in the light of these suggested avenues of research.

6.1 Assessment of the effects of productivity patterns on biogeography

Evidence from long-term time series and geographic studies suggested that the distribution of elasipodid holothurians was particularly reliant on various aspects of primary productivity, and its transfer to the deep sea. The abundance, size distribution and community structure of elasipodid holothurians is known to react temporally and spatially in changes to food input, linked to regimes of primary production in surface waters (Billett et al. 2001, Ruhl and Smith 2004, Ruhl 2007, Wolff et al. 2011). Studies suggested that differences existed within the Elasipodida existed with regard to their selectivity and life history (Iken et al. 2001) which could potentially affect their global distributions. Therefore the elasipodida presented themselves as ideal candidates for studying abyssal soft-sediment biogeography.

Environmental differentiation in the ocean and global-scale geographic distributions of the Elpidiidae and Psychropotidae were detected for the first time in this study. Environmental factors related to food input (intra-annual seasonality, inter-annual seasonality and quantity of POC flux) were found to be the best descriptors of differences between the Psychropotidae and Elpidiidae, confirming existing anecdotal and observational information about the distribution of the Elpidiidae in relation to areas of high seasonality (Hansen 1975, Billett and Hansen 1982).The differences in the distributions of the Elpidiidae and Psychropotidae also agrees with knowledge of their reproduction, mobility, body size, behaviour and temporal and local spatial changes in abundance in relation to food-supply shift between oceans (Tyler and Billett 1988, Billett et al. 2001, Iken et al. 2001, Wigham et al. 2003a, Wigham et al. 2003b, Ruhl and Smith 2004, Ruhl 2007, 2008, Billett et al. 2010, FitzGeorge-Balfour et al. 2010, Wolff et al. 2011). Different aspects of food input were more influential in discriminating between the two families in different oceans, with intra-annual seasonality being the most strongly correlated with differences between the two families in the Atlantic Ocean and POC flux and inter-annual seasonality as the most important factors in the Pacific and Southern Oceans.

Finally, it is apparent that strong barriers do exist for the Elasipodida, but that these require a number of combining factors such as topography and strong clines of environmental variables, combined with differences in suitability of habitat on either side of the barrier. Such barriers have thus far only been noted in the Arctic, where habitat on north of the Iceland-Faeroe Ridge is at the extreme of conditions experienced by the Elasipodida. Similar gradients may be evident in other regional seas such as the Mediterranean, but this requires further study. Even then, such barriers will prove semi-permeable over evolutionary time, but may continue to reduce diversity.

6.2 Improved biogeographic schemes

Analysis of Longhurst's zonation also provides evidence for differences between high latitude regions and lower latitude regions with associated differences in seasonality, inter-annual variations and total POC flux. Additionally, differences between areas of high seasonality appear to be related to increased presence of geographically limited Elpidiidae species. Whilst the high latitude fauna of different oceans were quite different to each other, the species compositions of low-latitude areas were more similar to each other than to high latitudes of their respective Oceans. This suggests a fauna typical to low-latitude regions, which may be under represented in the Elpidiidae. However, analysis of similarities in species distributions suggests historical factors may also be influencing the observed patterns of differentiation along seemingly environmental gradients. Biogeographic groupings of species formed major clusters for the a) the Arctic, b) the Southern Ocean, c) the southwest and northwest Pacific d) the east Pacific and Southern Ocean, including many cosmopolitan species e) the northeast Atlantic and d) the low-latitude Atlantic, including the Caribbean and low latitudes in other oceans. This suggests observed differences in Longhurst cases could be related to historical factors, combined with uneven sampling effort.

The finding that differences in distributions of species in high latitude and low-latitude areas may be influenced by historical influence has been noted before

(Lambshead et al. 2000, Lambshead et al. 2002, Rex et al. 2005). However, to the author's knowledge, no previous studies have observed the interaction between ecological and historical patterns on a global scale in deep-sea soft sediment organisms. In the debate about whether biogeographic zonation in the deep sea should be based upon the zoogeographic distribution of taxa (Vinogradova 1997) or on ecologically-coherent environmentally delimited areas (Longhurst 2007) such combined approaches are extremely informative. Results suggest that both bottom-up zoogeographic and top-down ecological approaches should be undertaken simultaneously, as the competing hypotheses that underpin the two paradigms may be working simultaneously to produce the observed patterns. However, it should be noted that presence-data is more suited to the zoogeographical analyses methods, and Longhurst-case scale analysis whilst differences between provinces may be more related to shifts in abundance and diversity. The evidence from this study suggests that there are differences in between some aspects of zonation proposed by Longhurst, but that the cosmopolitan distribution of many species combined with historical effects on species distribution, including information on abundance of organisms may help to further delineate the provinces. The wealth of information contained in Natural History Collections, most of which are now available online, should be utilised in order to help answer questions regarding deep-sea biogeography. Such data has limitations and must be applied cautiously and with regard to the sampling effort and taxonomic skill that accompanied it. Such Natural History Collections are now benefiting a great range of ecological and biogeographical research (Graham et al. 2004, Cotterill and Foissner 2010).

6.3 Population genetics and phylogeography for all deep-sea habitats and especially for the abyssal plains

The pattern of genetic population structure observed in two cosmopolitan elasipodid holothurians *Psychropotes longicauda* and *Oneirophanta mutabilis* questions previous paradigms that predict genetic structuring that relates to simple ocean-basin separation of populations in abyssal organisms (Etter et al.

1999, 2005, Etter et al. 2011). Much cryptic differentiation is observed that suggests mechanisms exist, even in highly motile abyssal organisms that cause substantial genetic structure. However, evidence is also provided that suggests that these species are capable of dispersal over very wide geographic (inter-ocean) scales. The existence of several related but distinct lineages at several sampling sites is not known from any previous studies of deep-sea soft-sediment fauna. It provides evidence for supporting two deep-sea paradigms that were previously thought to be mutually exclusive. The first is that geographic population structure does occur on ocean scales. The second is that potential for long-distance dispersal is realised in some cosmopolitan abyssal species. Previous theories would have expected long distance dispersal to prevent geographic structuring through the maintenance of gene flow over evolutionary times frames. Instead, mechanisms for creating gene flow appear to be limited to discrete geographic areas, which have acted as centres of genetic diversity followed by repeated outward dispersal. The importance of such mechanisms has been noted in other marine species (Strugnell et al. 2008, Strugnell et al. 2012). There is evidence that such a mechanism may not be restricted, within the Elasipodida, to the two species examined (O'Loughlin et al. 2011).

6.4 Summary of main findings

- There are ecological differences in the environmental conditions occupied by the Psychropotidae and Elpidiidae, especially with regard to the ‘extremes’ of their ranges.
- Elpidiidae tend towards more variable environments in terms of intra-annual seasonality and inter-annual variability.
- Psychropotidae can survive in areas with lower variability which are often (but not always) associated with lower overall food supply.
- These differences agree with known differences between the families in terms of mobility, reproduction and feeding selectivity.
- There is evidence that intra-annual seasonality is more important in causing faunal gradients in the Atlantic, whereas POC flux and inter-annual variability is more important in the Pacific and Southern Oceans.

- Longhurst's 'cases' are a more suitable scheme for zonation than biome at the level of species and genera, but ocean differences are also important at the species level.
- Evidence suggests provinces are a useful biogeographic zonation, although species distributions usually span a number of provinces.
- Within oceans smaller differences occur between provinces at higher latitudes than at lower latitudes, although this may be related to sampling.
- The largest differences occur between polar fauna and the fauna of low-latitude provinces.
- Sampling bias and historical factors heavily influence the findings of global and ocean-scale studies and must be incorporated into interpretations.Genetic population structure does exist in cosmopolitan deep-sea holothurians.
- Simple geographic structure exists within lineages, but multiple lineages may exist within the same morpho-species and these may be sympatric.

6.5 Limitations of the study and future work

One of the major limitations of this, and other ocean-global scale analyses, is that findings are largely influenced by the spatial distribution of sampling effort (Lambshead et al. 2000, Watling 2009). This problem is not specific to presence-absence data, but also affects analyses based upon abundance and diversity.

As well as being geographically biased, sampling effort has been environmentally biased. Far more sampling effort of the megabenthos has occurred in areas with highly variable seasonal and inter-annual production and POC flux regimes e.g. PAP, Station M and Crozet. Studies which have attempted to compare high POC flux environments with low POC flux environments (Thurston et al. 1998, Wolff et al. 2011) have generally found unexpected 'pulses' of food to the supposedly oligotrophic comparison site. The few, rather sporadic samples from Ocean gyres tend to be the result of historical research cruises. Future research requires better sampling in the centre of ocean gyres, in places of low flux, but primarily low intra and inter-annual seasonality. Sampling effort in these areas will need to be comparably higher than that in more productive regions due to the lower abundance and biomass in gyres. Therefore greater spatial coverage will be needed in order to sample a comparable portion of the species pool to that in coast and higher latitude areas.

Additionally, in order to test the boundaries of provinces, continuous sampling across province boundaries must be carried out (Watling 2009).

Because species distributions usually spanned a number of Longhurst's provinces, data which also includes abundance information will help elucidate finer scale patterns where gradient in environmental conditions cause shifts in abundance of biomass, rather than causing presence or absence. Few Natural History Collection datasets include standardised abundance datasets. Although a major challenge, such a move would greatly enhance future global biogeographic investigations.

The existence of long-term time series in multiple oceans has vastly improved our ability to understand common mechanisms affecting the benthos, especially those related to temporal trends in changes to POC flux. However, currently the two major sites (PAP in the northeast Atlantic and Station M in the Pacific) are under broadly similar production regimes. Long-term time series under very different productivity regimes (polar, tropical, ocean gyre, monsoon) would greatly enhance our understanding of the controls on deep-sea organisms.

Whilst current information points to intra-annual seasonality being a key environmental factor determining the distribution of elasipodid holothurians, and other fauna, it is thought that it acts as a proxy for food quality and the physical form and distribution of food. Two approaches are needed to further test these assumptions. The first is to experimentally test the effects of different food 'qualities' on the reproduction of different species. Results suggest that differences between the Psychropotidae and Elpidiidae should be most extreme. Also such feeding experiments which focus on Elpidiidae with different egg sizes would also help shed light on the boom-bust life history thought only to effect species with ancestral planktotrophic larvae as suggested by (Uthicke et al. 2009). However such experimental work in abyssal environments is extremely costly, so that initial studies on shallow-water proxies may be desirable initially. The second approach is to compare temporal and geographic shifts in Elasipodid presence-absence (or abundance wherever possible) in relation to emerging data on global maps of the distributions of phytoplankton functional groups. Such a variable could be included in the analysis carried out in Chapter 3 and may help shed light on the mechanism by which intra-annual seasonality affects abyssal holothurians.

Finally, a detailed phylogeny of the Elasipodid holothurians alongside population genetic studies will help us to better understand the evolutionary origins of species and genera and to understand whether the patterns observed in two species observed thus far are common to most cosmopolitan species. Genetic studies will also help identify centres of origin for different elasipodid taxa, which will greatly enhance our ability to understand whether observed patterns are due to historical or ecological causes.

Appendix A

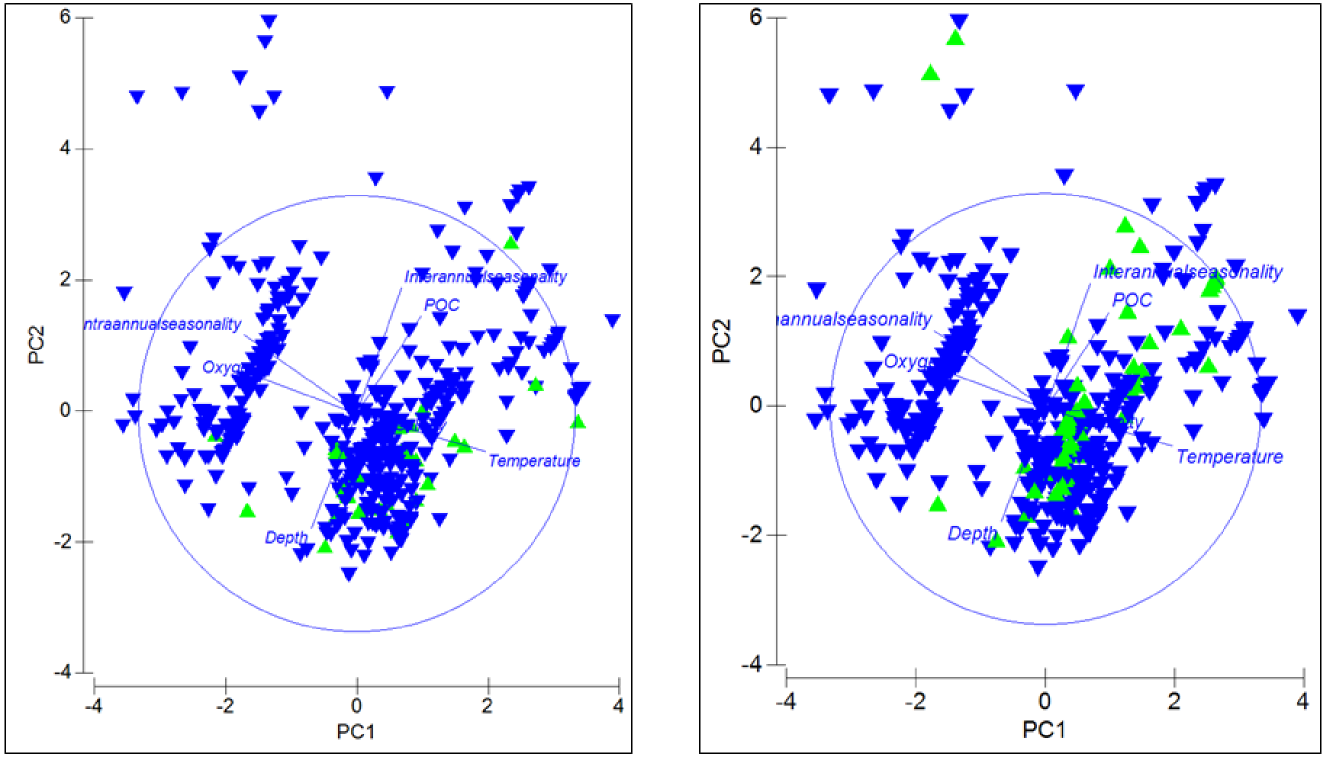


Fig. 3.9 *Principle Component Analysis plots illustrating the environmental distribution of the four elasipodid holothurian families at* ***bathyal*** *depths (500-3000m) across* ***all oceans****. Distribution of all recorded elasipodid sites is shown in each figure with green triangles indicating the families presence at a site and a blue inverted triangle denoting the families' absence for; (c)* ***Deimatidae*** *and (c)Laetmogonidae*

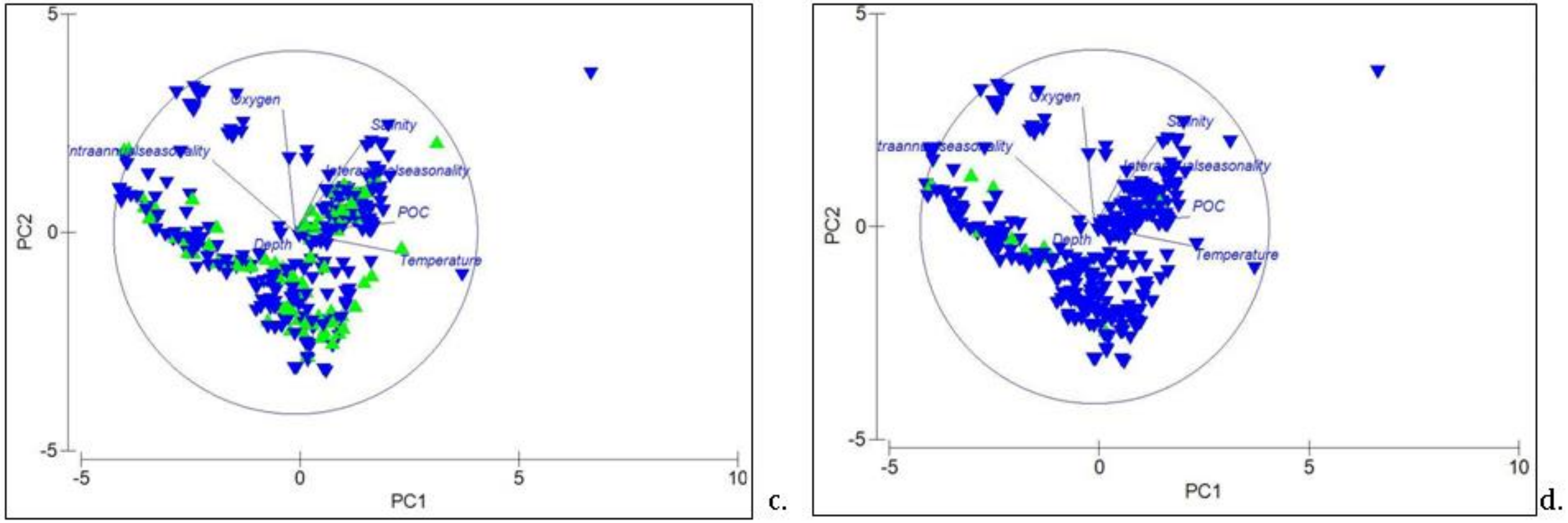


Fig. 3.11 *Principle Component Analysis plots illustrating the environmental distribution of the four elasipodid holothurian families at* ***abyssal*** *depths (3000-6000m) across* ***all oceans****. Distribution of all recorded elasipodid sites is shown in each figure with green triangles indicating the families' presence at a site and a blue inverted triangle denoting the family's absence; (c)* ***Deimatidae*** *and (d) Laetmogonidae*

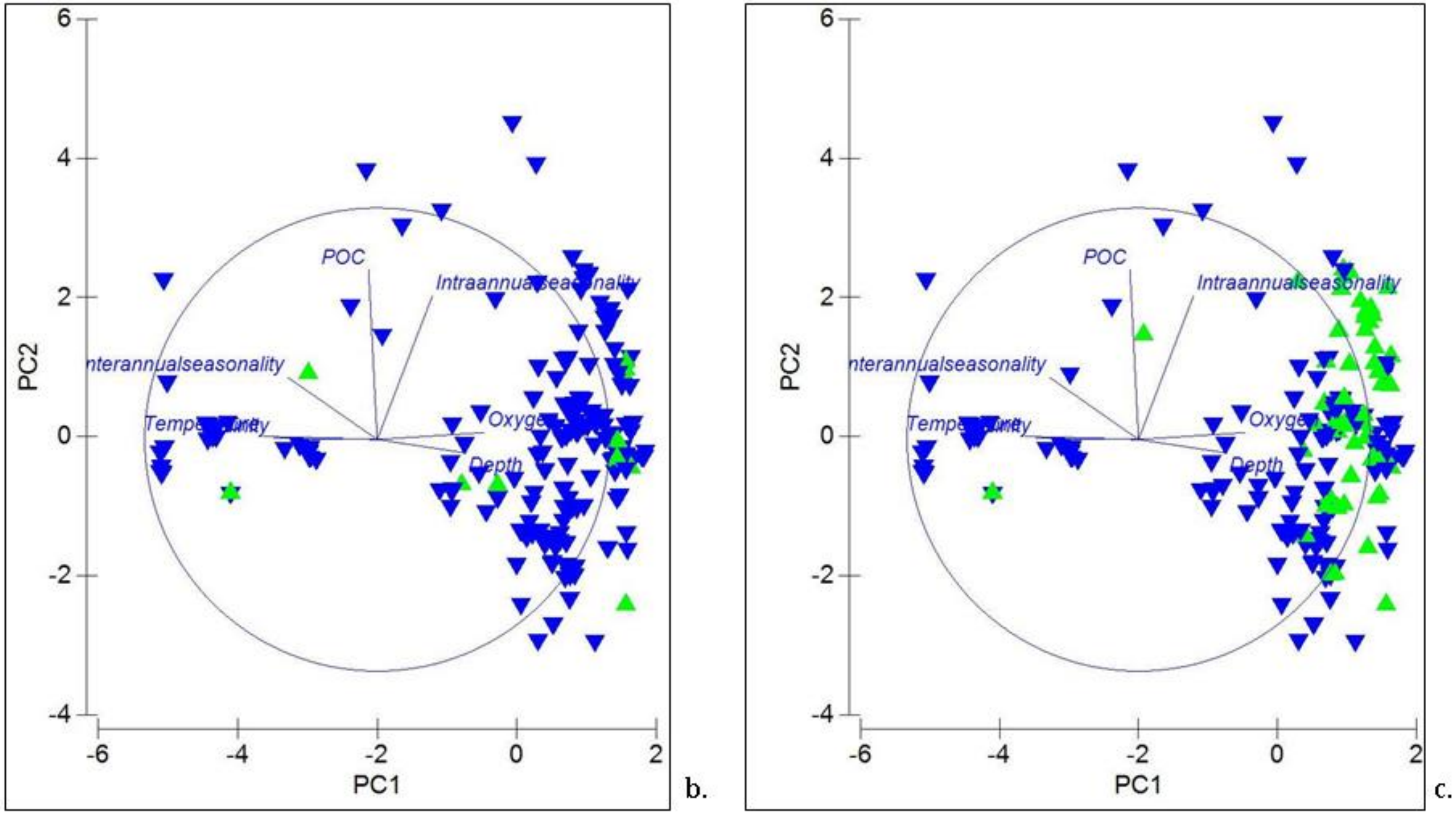


Fig. 3.14 Principle Component Analysis plots illustrating the environmental distribution of the four elasipodid holothurian families at ***bathyal*** *depths (500-3000m) in the* ***Atlantic Ocean****. Distribution of all recorded elasipodid sites is shown in each figure with green triangles indicating the families' presence at a site and a blue inverted triangle denoting the family's absence for; (b)* **Deimatidae***; (c)* **Elpidiidae**.

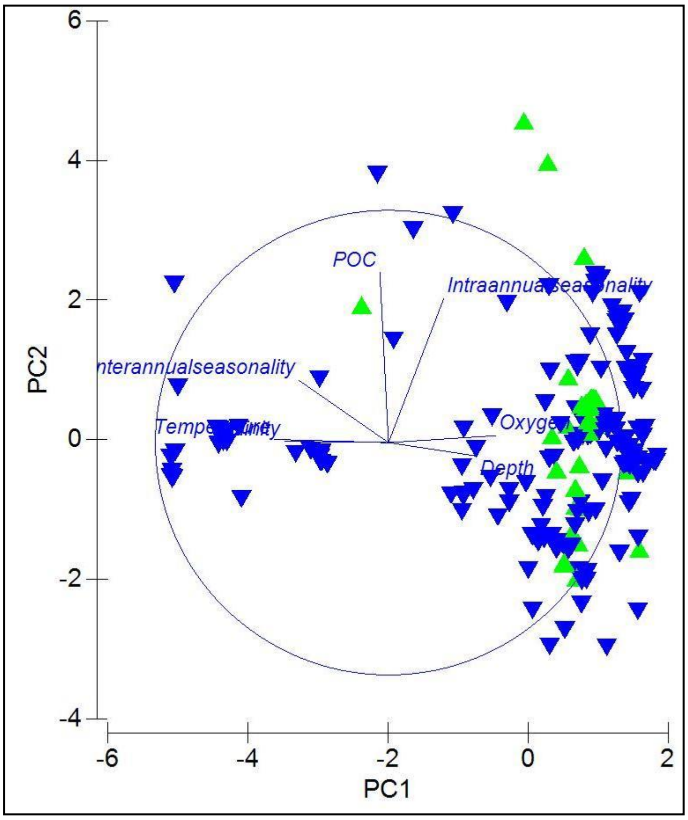


Fig. 3.14 Cont. *Principle Component Analysis plots illustrating the environmental distribution of the four elasipodid holothurian families at* ***bathyal*** *depths (500-3000m) in the* ***Atlantic Ocean, minus Arctic records****. Distribution of all recorded elasipodid sites is shown in each figure with green triangles indicating the families' presence at a site and a blue inverted triangle denoting the family's absence for; (d)* ***Laetmogonidae.***

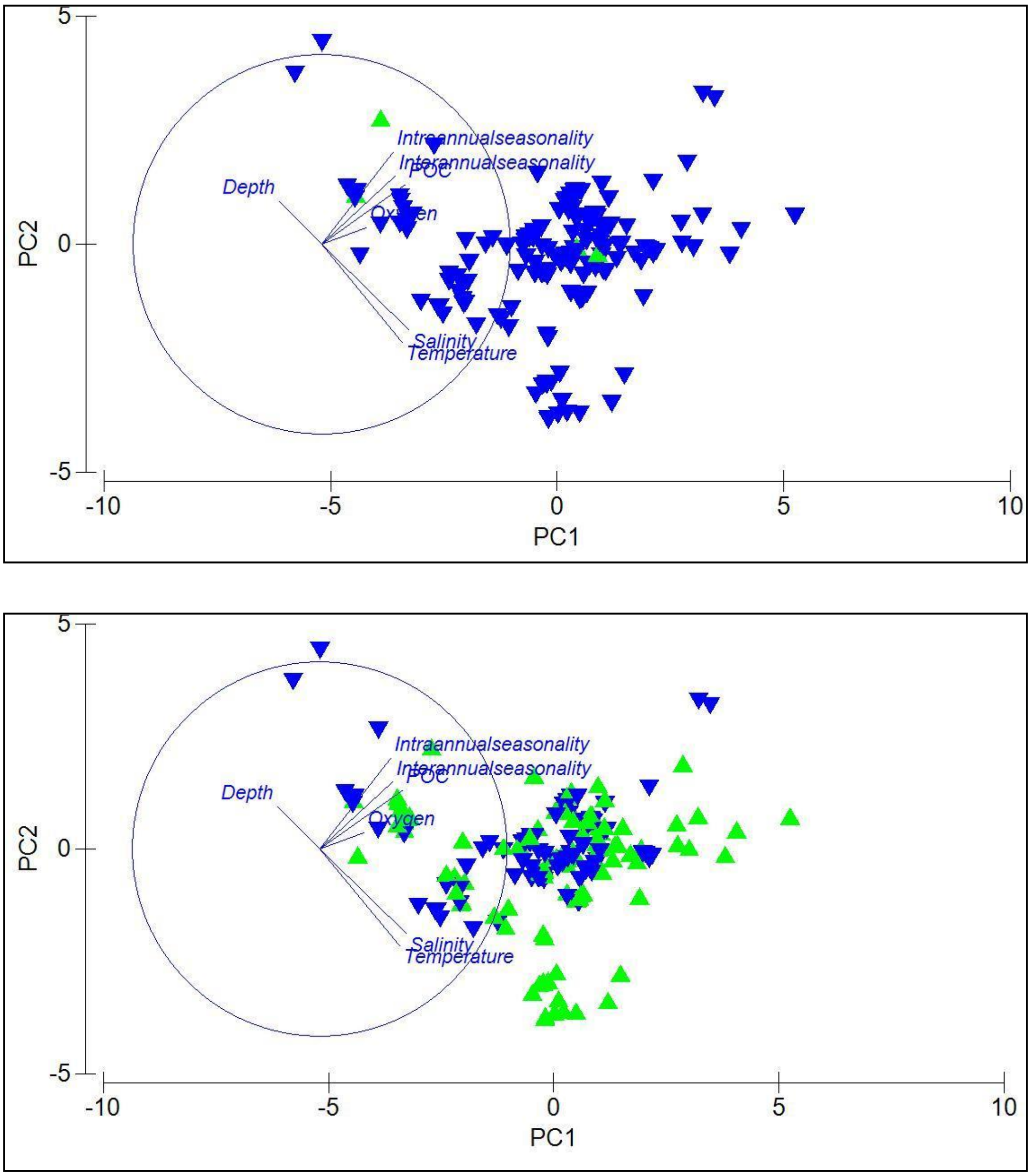
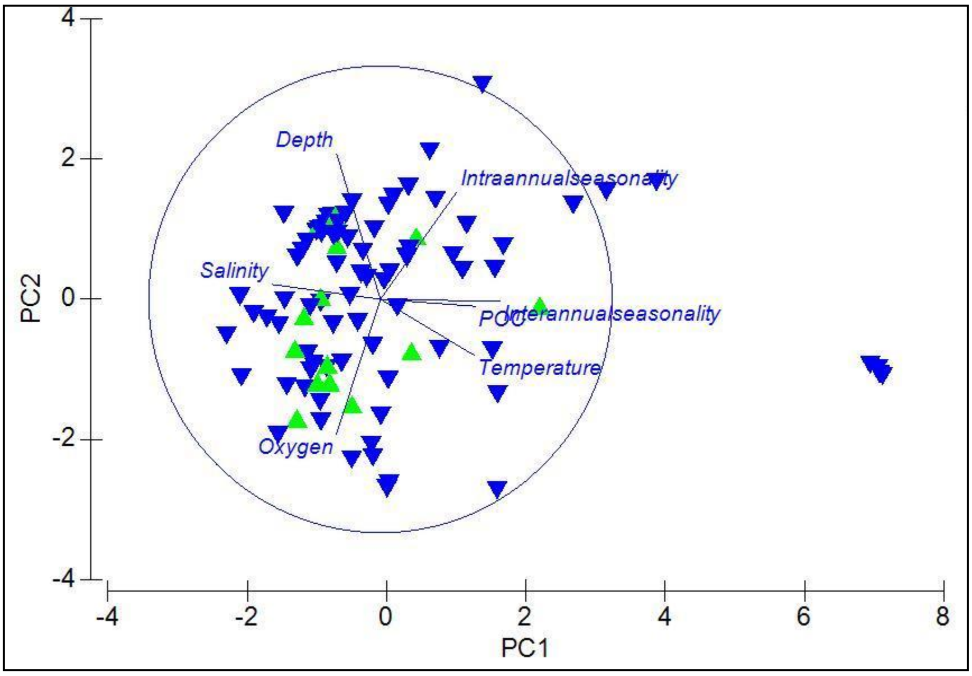


Fig. 3.17. *Principle Component Analysis plots illustrating the environmental distribution of the four elasipodid holothurian families at* ***abyssal*** *depths (3000-6000m) in the* ***Atlantic Ocean****. Distribution of all recorded elasipodid sites is shown in each figure with green triangles indicating the families' presence at a site and a blue inverted triangle denoting the family's absence for; (c)* ***Laetmogonidae*** *and (d)* ***Psychropotidae****.*



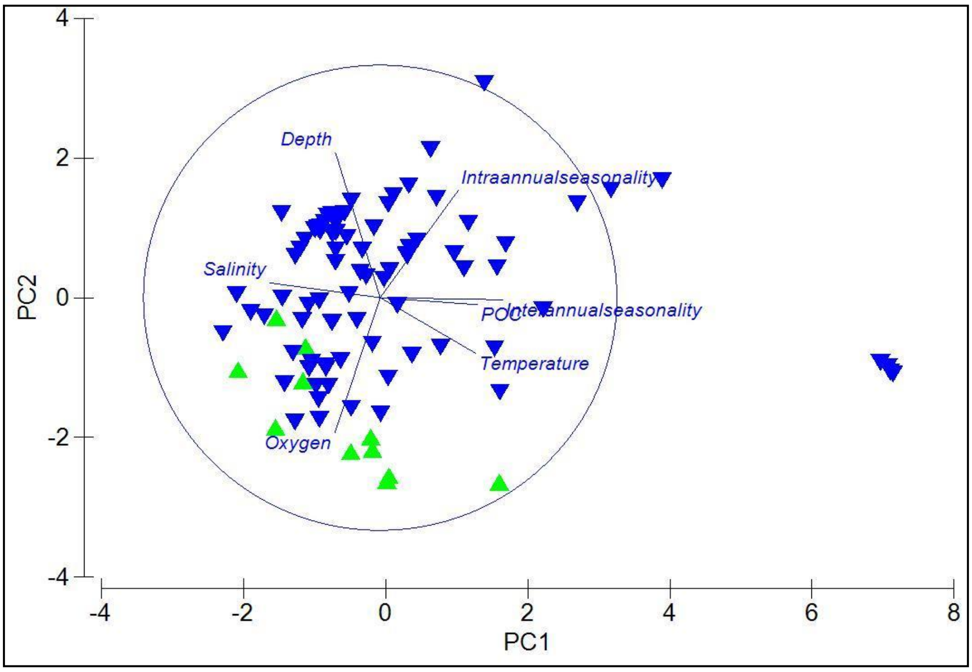


Fig. 3.20 *Principle Component Analysis plots illustrating the environmental distribution of the four elasipodid holothurian families at* ***bathyal*** *depths (500-3000m) in the* ***Pacific Ocean****. Distribution of all recorded elasipodid sites is shown in each figure with green triangles indicating the families' presence at a site and a blue inverted triangle denoting the family's absence for; (b)* **Deimatidae** *and (c)* ***Laetmogonidae.***

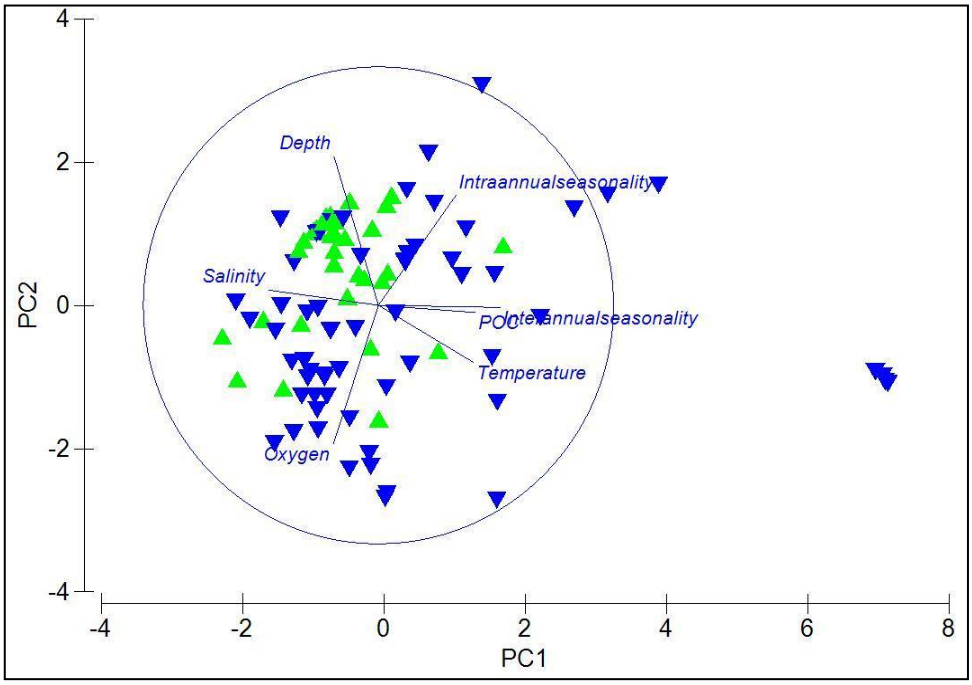


Fig. 3.20*. *Cont. *Principle Component Analysis plots illustrating the environmental distribution of the four elasipodid holothurian families at* ***bathyal****depths (500-3000m) in the* ***Pacific Ocean****. Distribution of all recorded elasipodid sites is shown in each figure with green triangles indicating the families' presence at a site and a blue inverted triangle denoting the family's absence for;(d)* ***Psychropotidae****.*

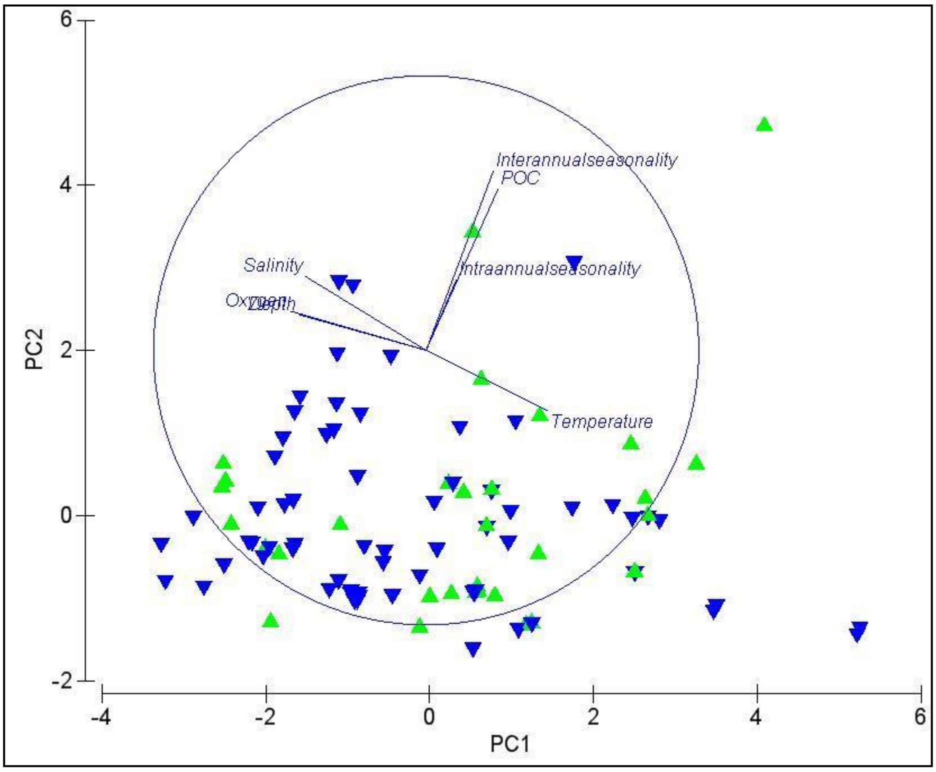
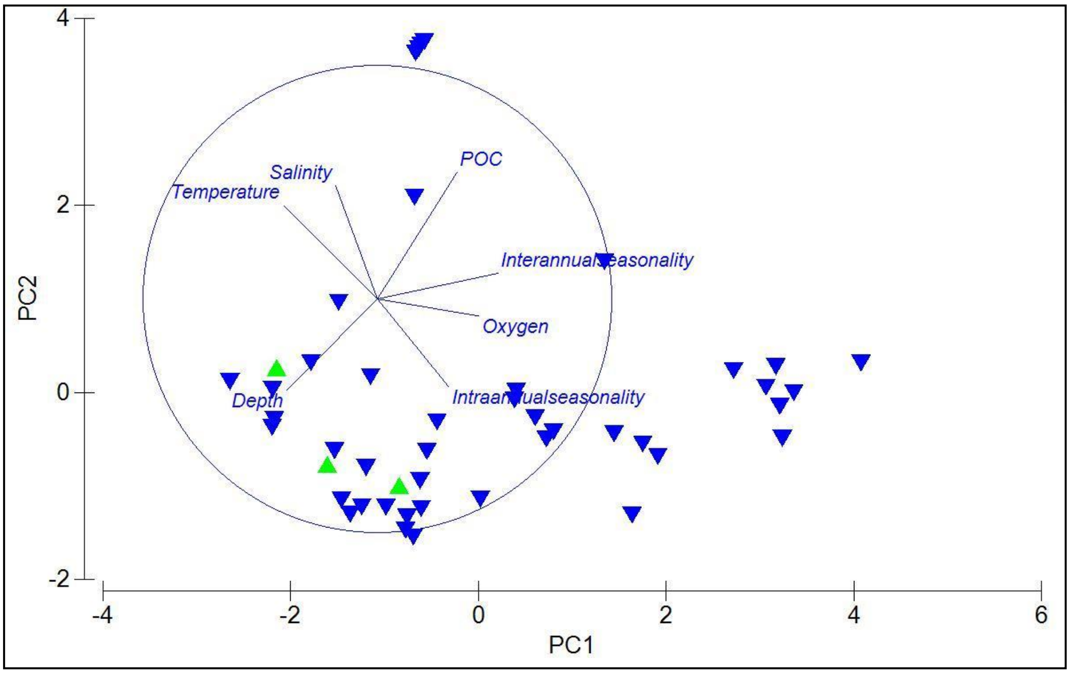


Fig. 3.23 *Principle Component Analysis plots illustrating the environmental distribution of the four elasipodid holothurian families at* ***abyssal*** *depths (3000-6000m) in the* ***Pacific Ocean****. Distribution of all recorded elasipodid sites is shown in each figure with green triangles indicating the families' presence at a site and a blue inverted triangle denoting the family's absence for; (c)* **Deimatidae***.*



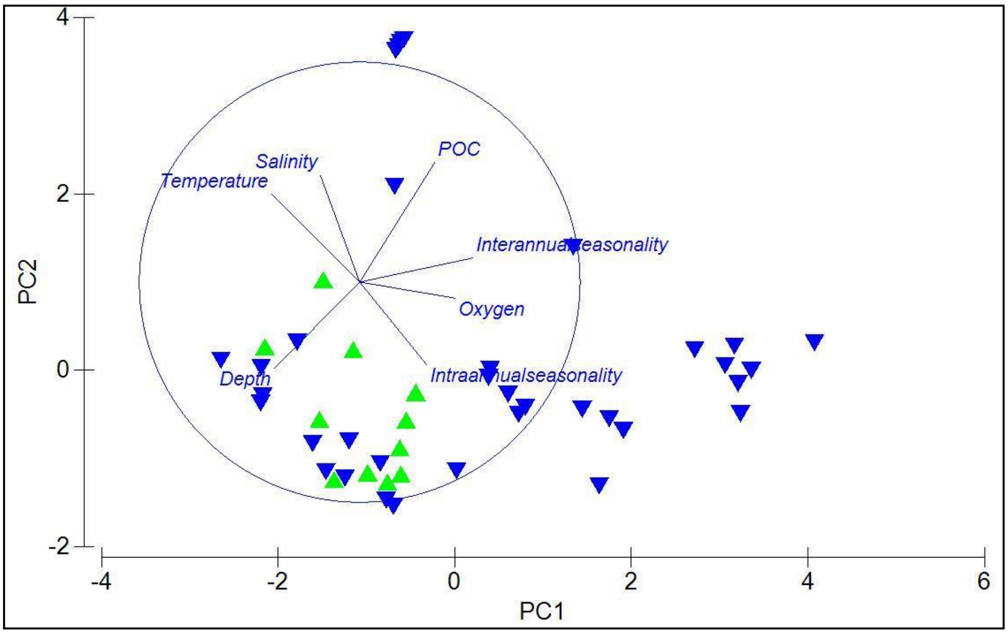


Fig. 3.26. *Principle Component Analysis plots illustrating the environmental distribution of the four elasipodid holothurian families at* ***bathyal*** *depths (500-3000m) in the* ***Southern Ocean****. Distribution of all recorded elasipodid sites is shown in each figure with green triangles indicating the families' presence at a site and a blue inverted triangle denoting the family's absence for; (c)* ***Deimatidae*** *(d)* ***Psychropotidae.***

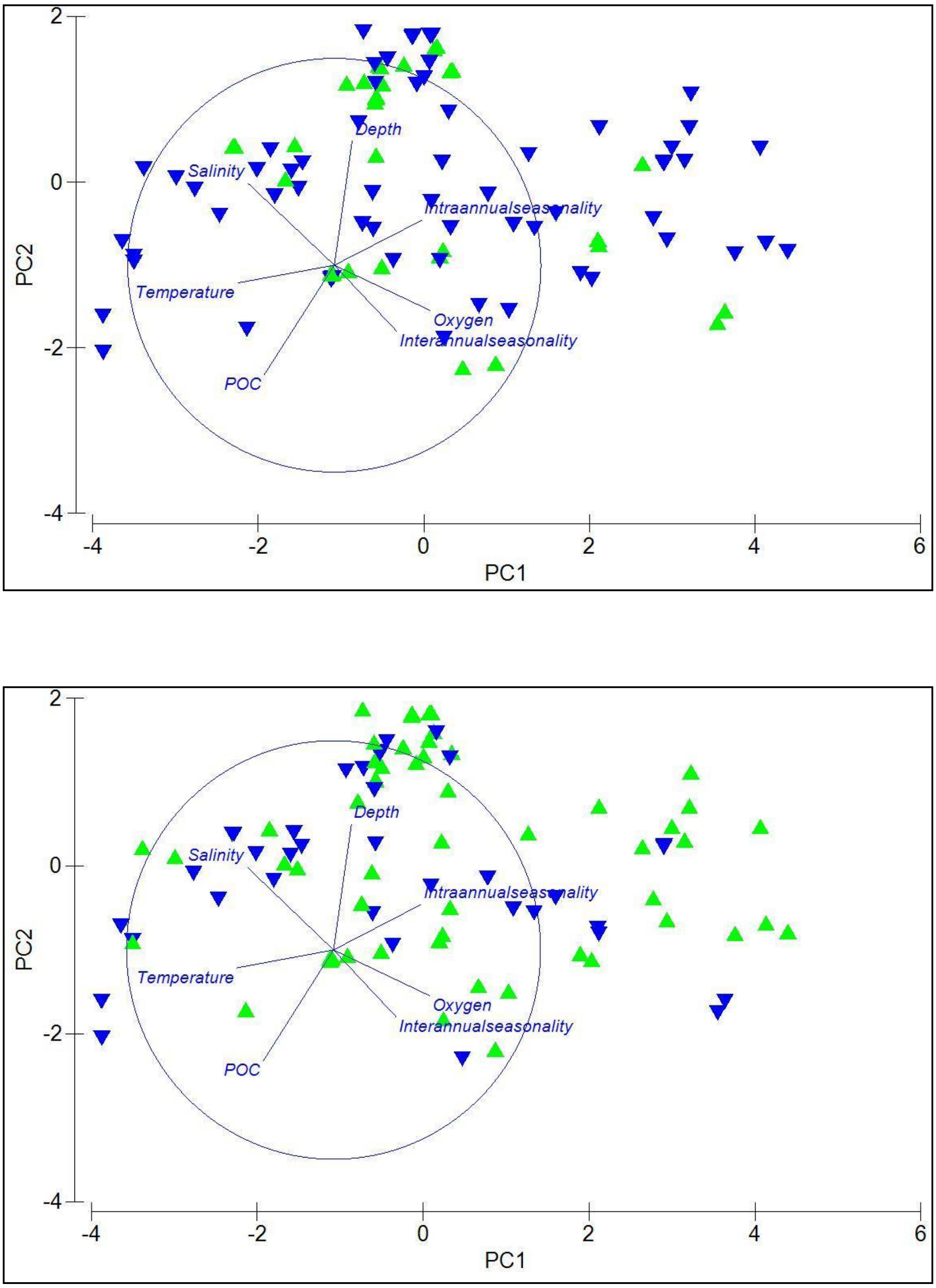


Fig. 3.29 *Principle Component Analysis plots illustrating the environmental distribution of the four elasipodid holothurian families at* ***abyssal*** *depths (3000-6000m) in the* ***Southern Ocean****. Distribution of all recorded elasipodid sites is shown in each figure with green triangles indicating the families' presence at a site and a blue inverted triangle denoting the family's absence for; (b)* **Deimatidae***; (c)* **Elpidiidae.**

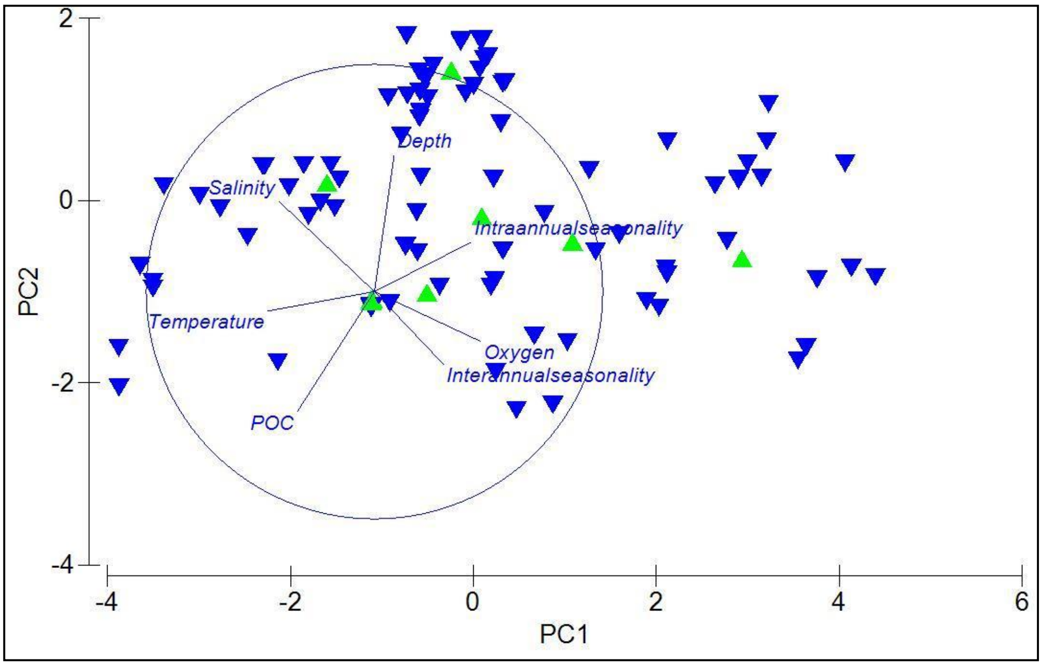


Fig. 3.29**. **Cont. *Principle Component Analysis plots illustrating the environmental distribution of the four elasipodid holothurian families at **abyssal** depths (3000-6000m) in the **Southern Ocean**. Distribution of all recorded elasipodid sites is shown in each figure with green triangles indicating the families' presence at a site and a blue inverted triangle denoting the family's absence for; (d) **Laetmogonidae**.*

Appendix B

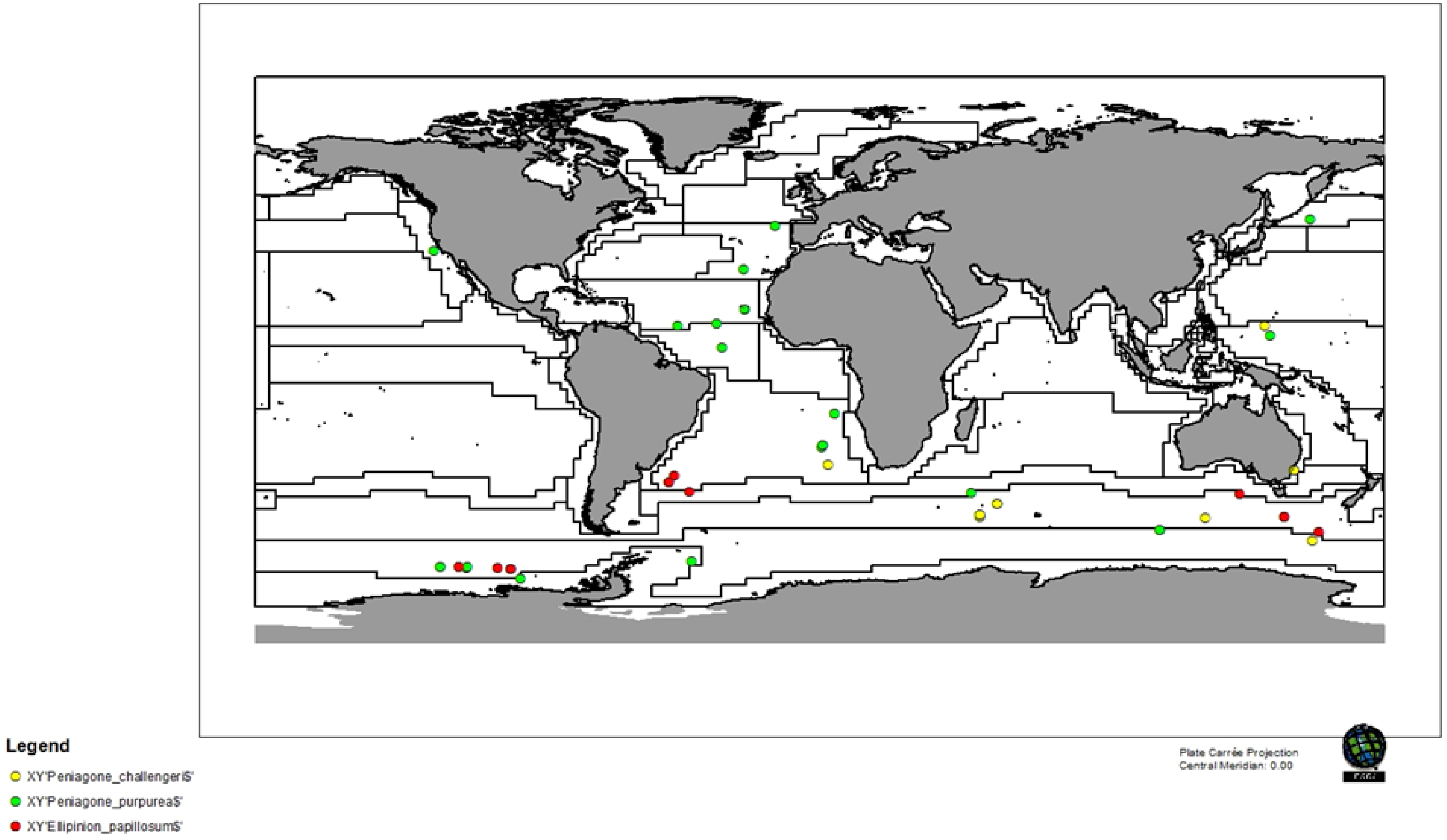


Figure 4.10 *The distribution of the species that form cluster* ***1ai*** *in the species-level inverted cluster (Chapter 4, Fig.4.7.)*

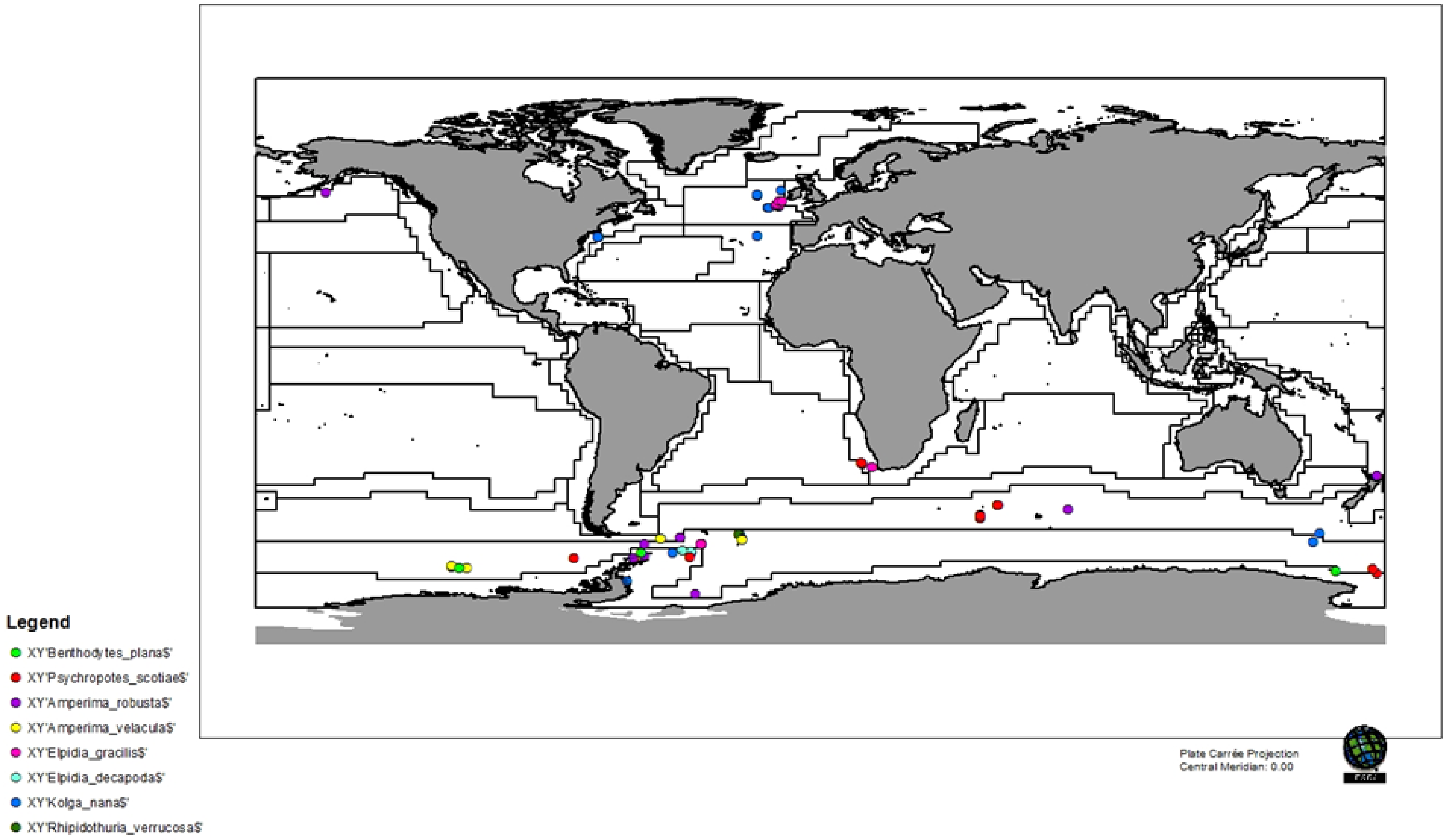


Figure 4.11 *The distribution of the species that form cluster* ***1aii*** *in the species-level inverted cluster* (*Chapter 4*, Fig 4.7)

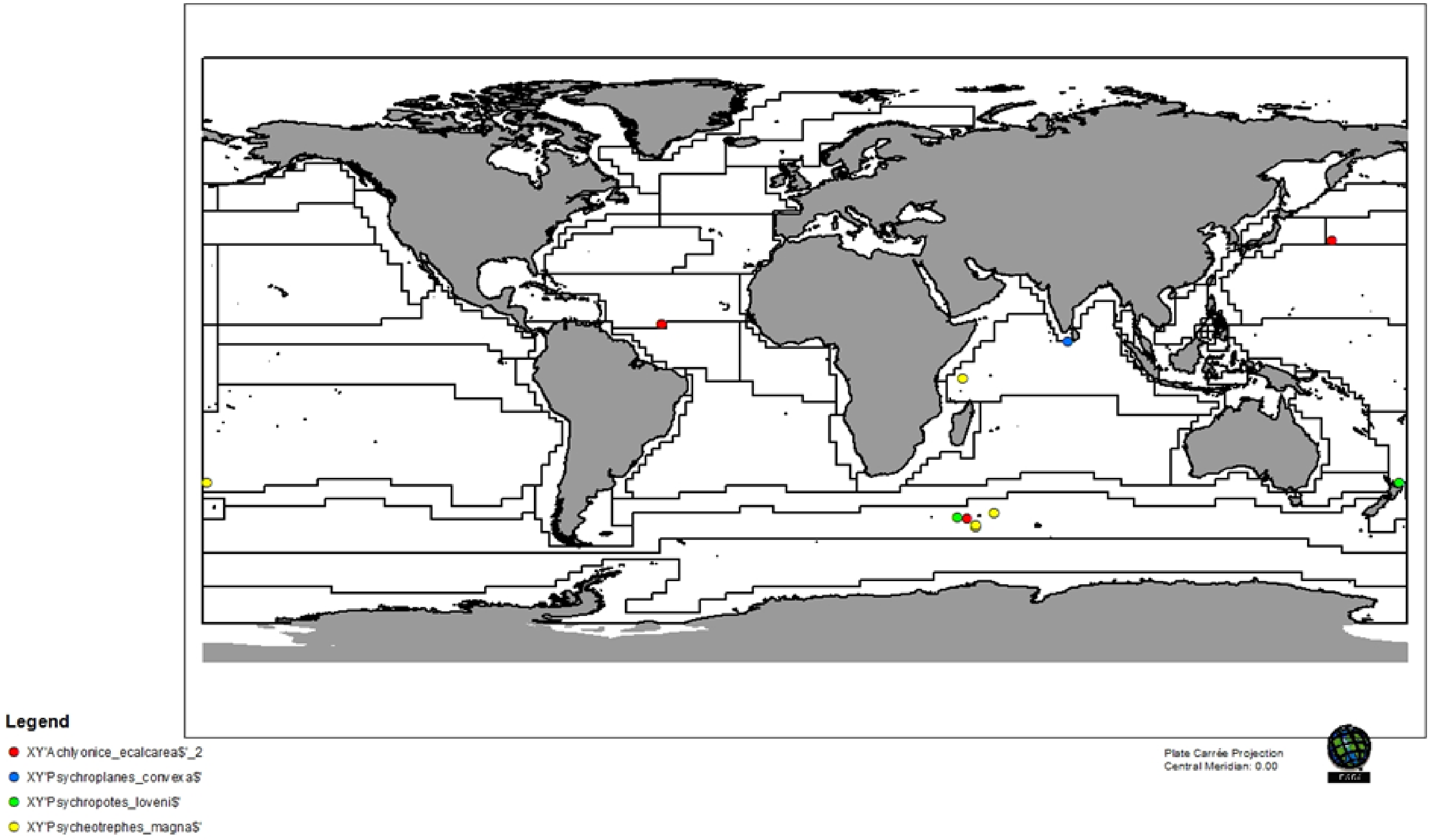


Figure 4.12 *The distribution of the species that form cluster* ***1b*** *in the species-level inverted cluster* (*Chapter 4*, Fig.4.7.)

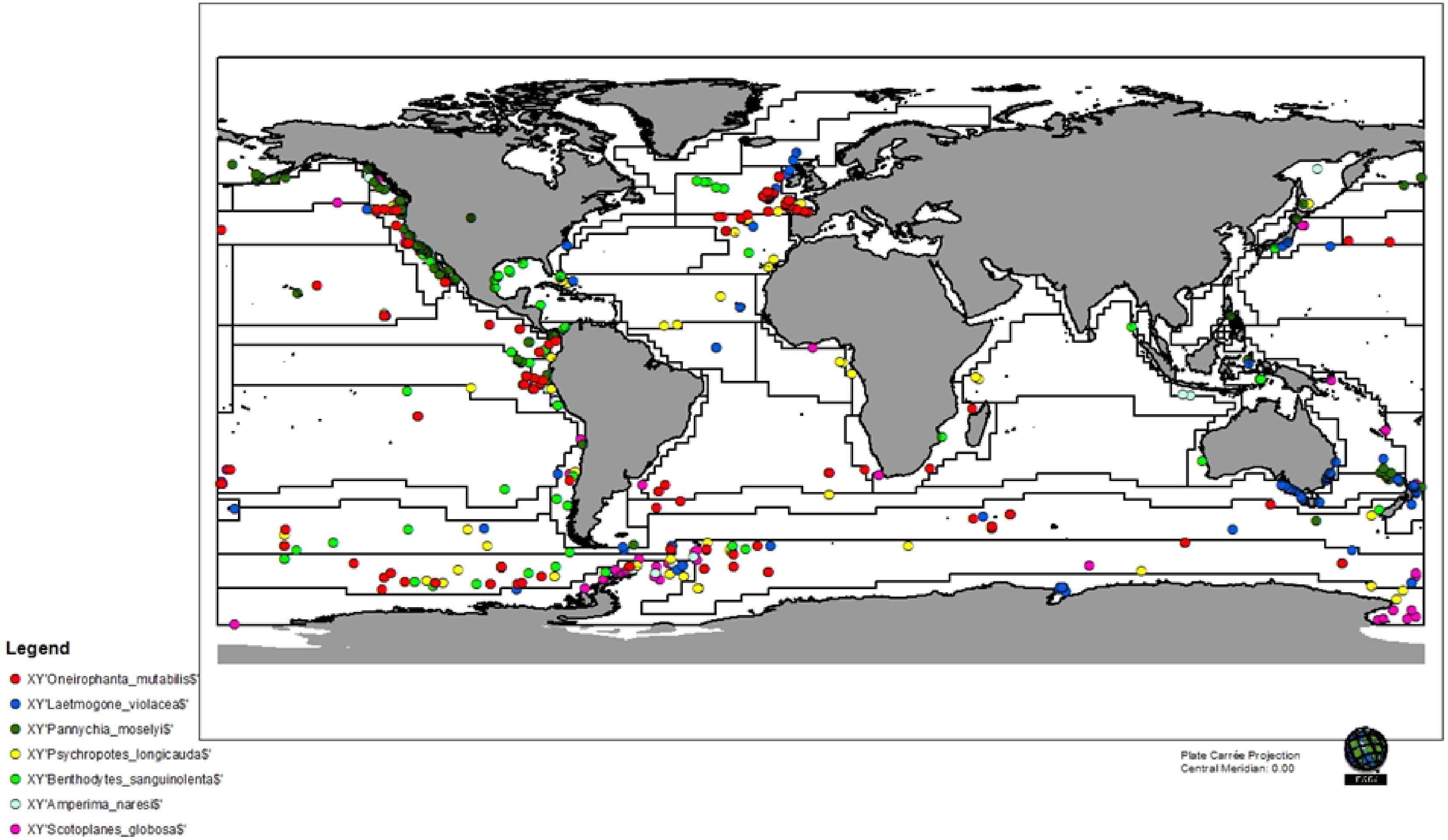


Figure 4.13. *The distribution of the species that form cluster* ***2ai*** *in the species-level inverted cluster* (*Chapter 4*, Fig. 4.7)

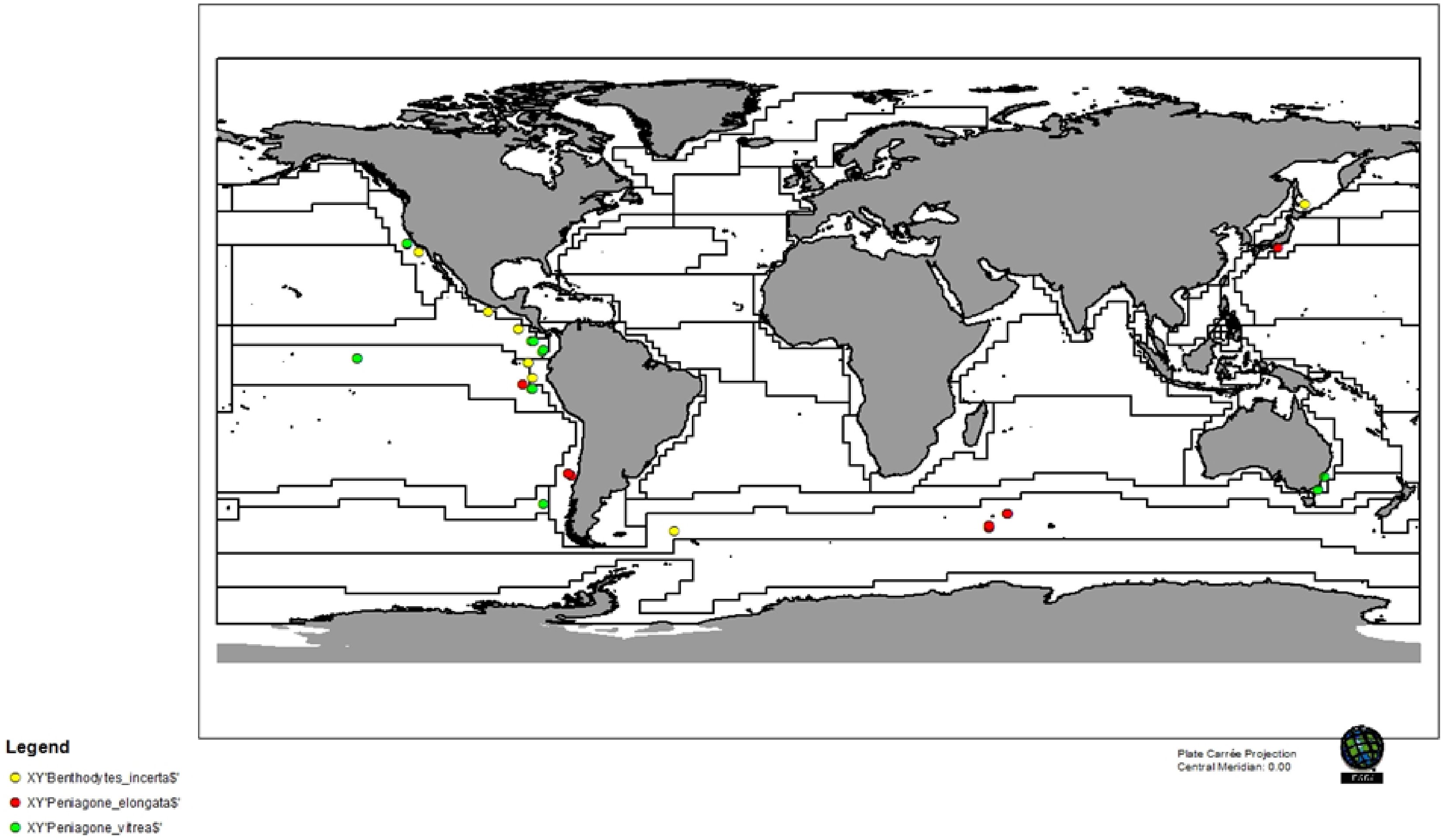


Figure 4.14 *The distribution of the species that form cluster* **2aii** *in the species-level inverted cluster* (*Chapter 4,* Fig.4.7.)

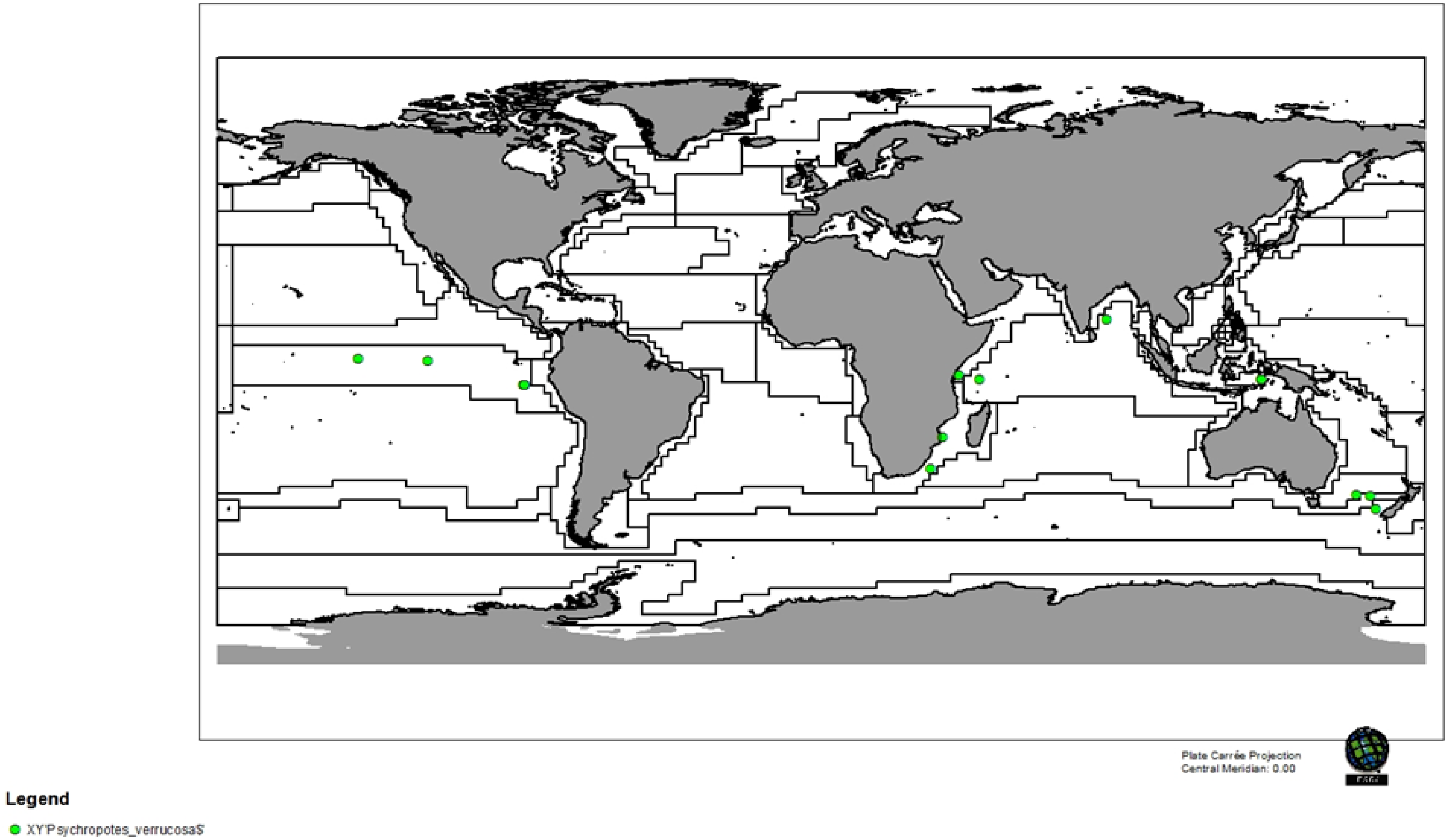


Figure 4.15 *The distribution of the species that form cluster* **2b** *in the species-level inverted cluster* (*Chapter 4*, Fig.4.7.)

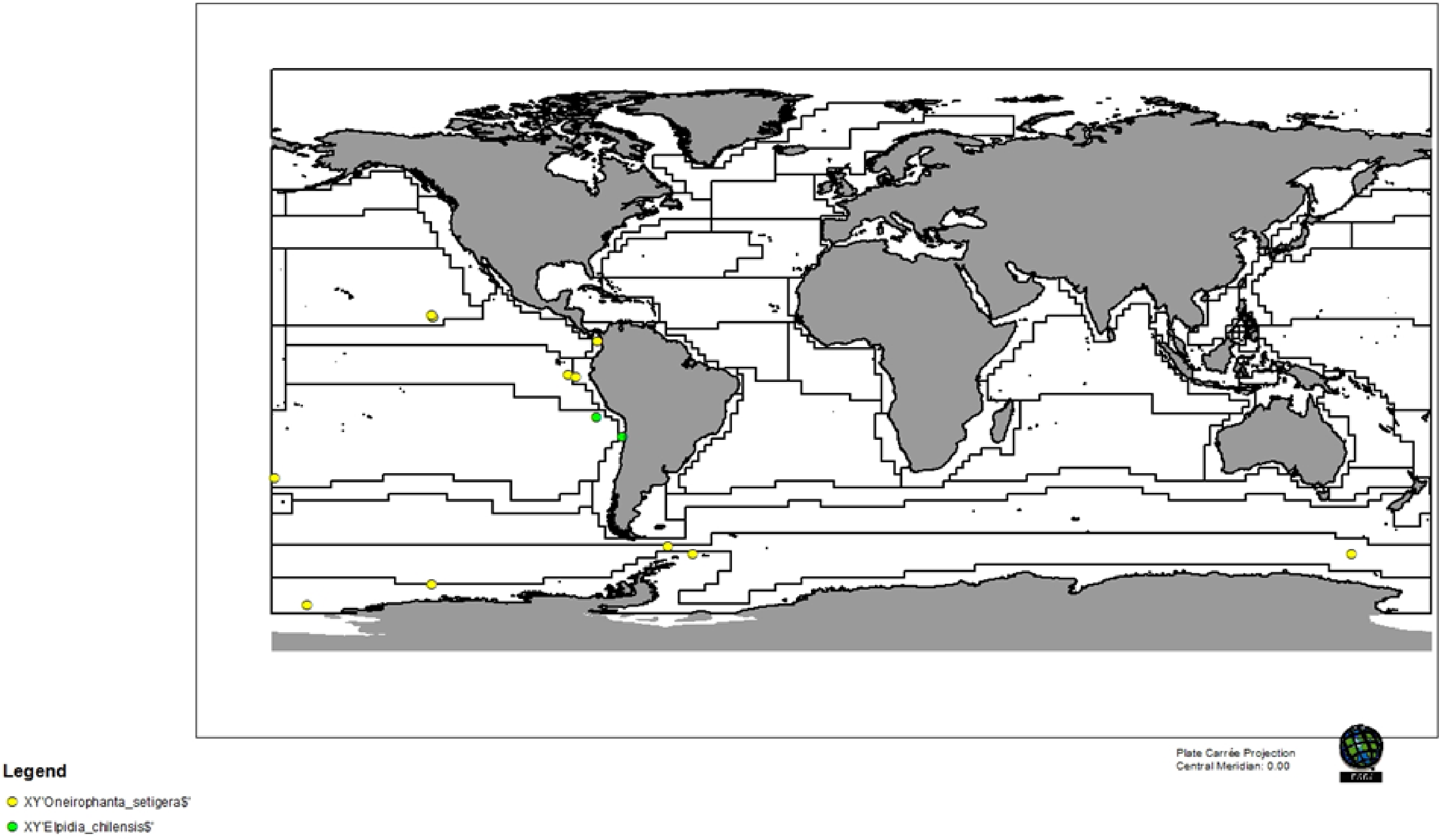


Figure 4.16 *The distribution of the species that form cluster* **2c** *in the species-level inverted cluster* (*Chapter 4*, Fig.4.7.)

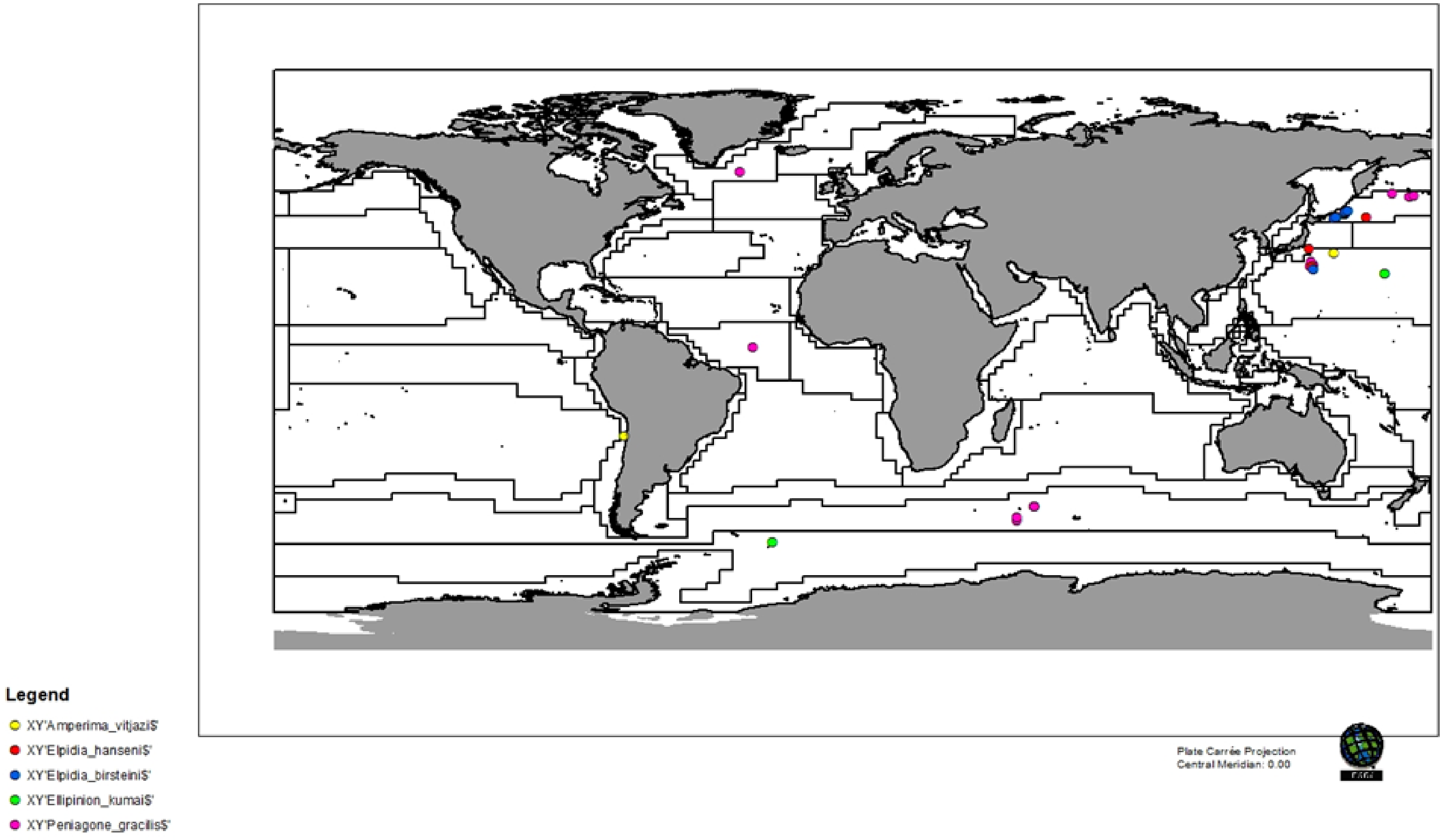


Figure 4.17 *The distribution of the species that form cluster* ***3a*** *in the species-level inverted cluster* (*Chapter 4*, Fig. 4.7)

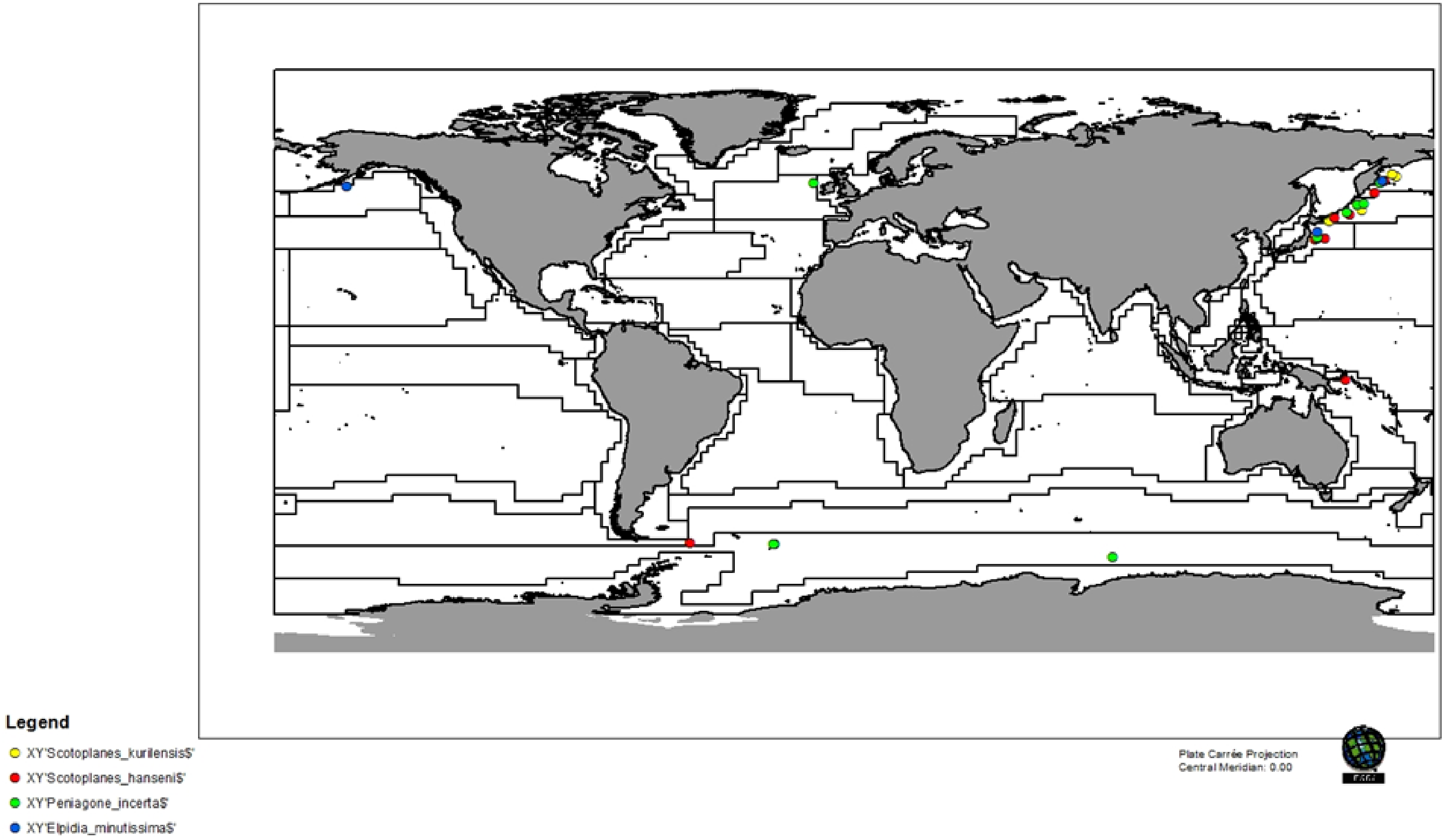


Figure 4.18 *The distribution of the species that form cluster* ***3b*** *in the species-level inverted cluster* (*Chapter 4*, Fig. 4.7)

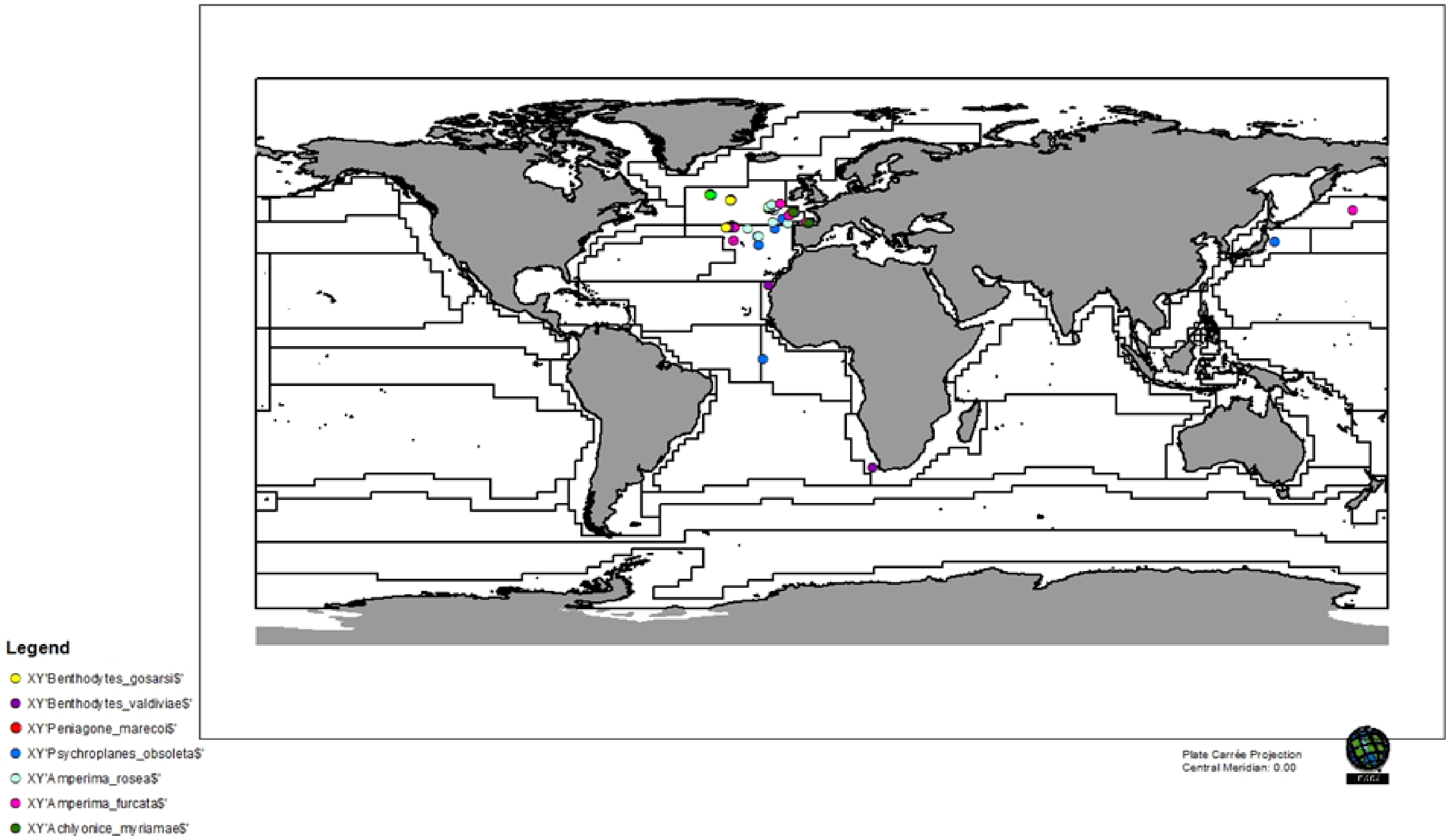


Figure 4.19 *The distribution of the species that form cluster* ***4ai*** *in the species-level inverted cluster* (*Chapter 4*, Fig. 4.7)

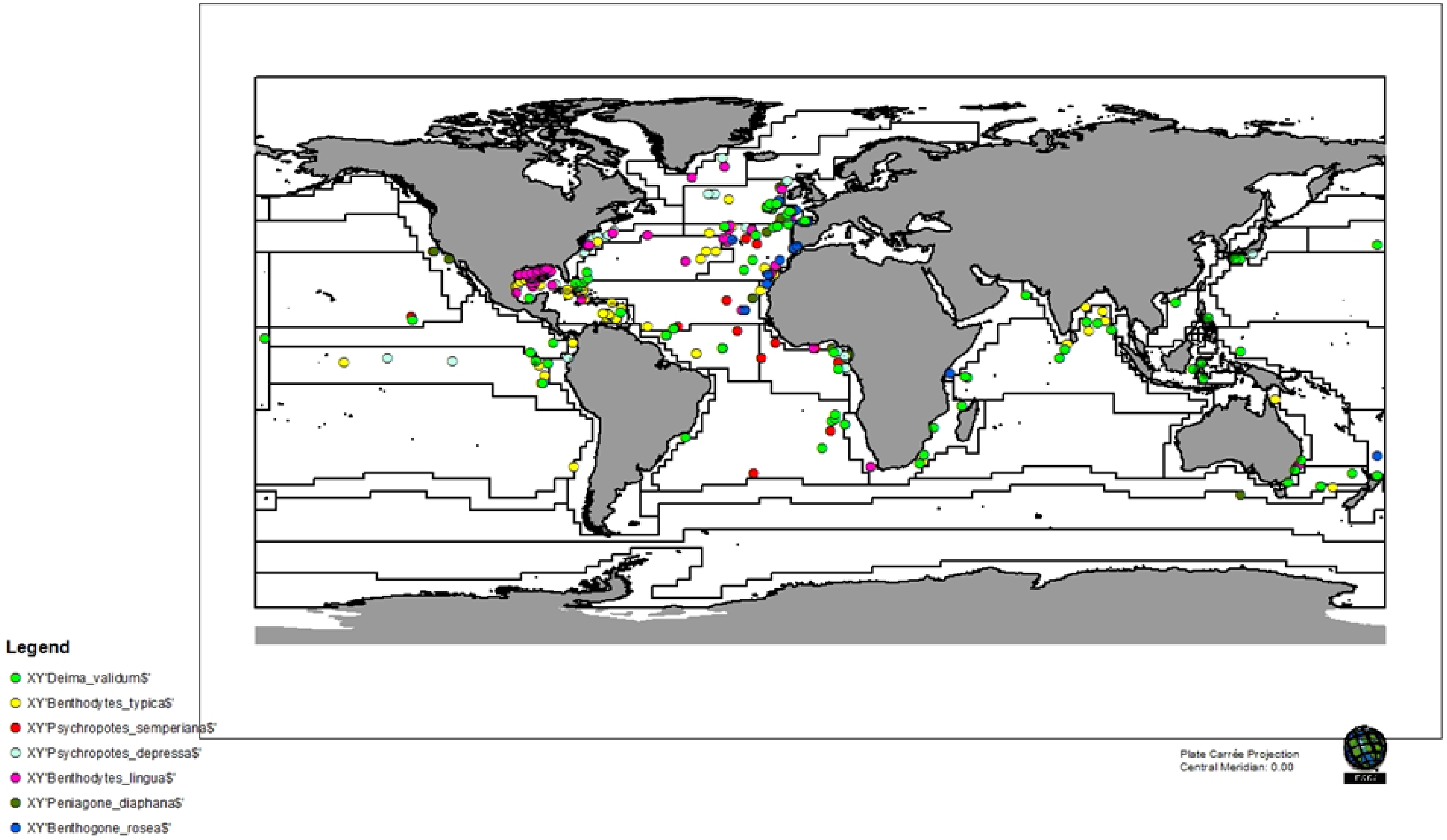


Figure 4.20 *The distribution of the species that form cluster* ***4aii*** *in the species-level inverted cluster* (*Chapter 4*, Fig. 4.7)

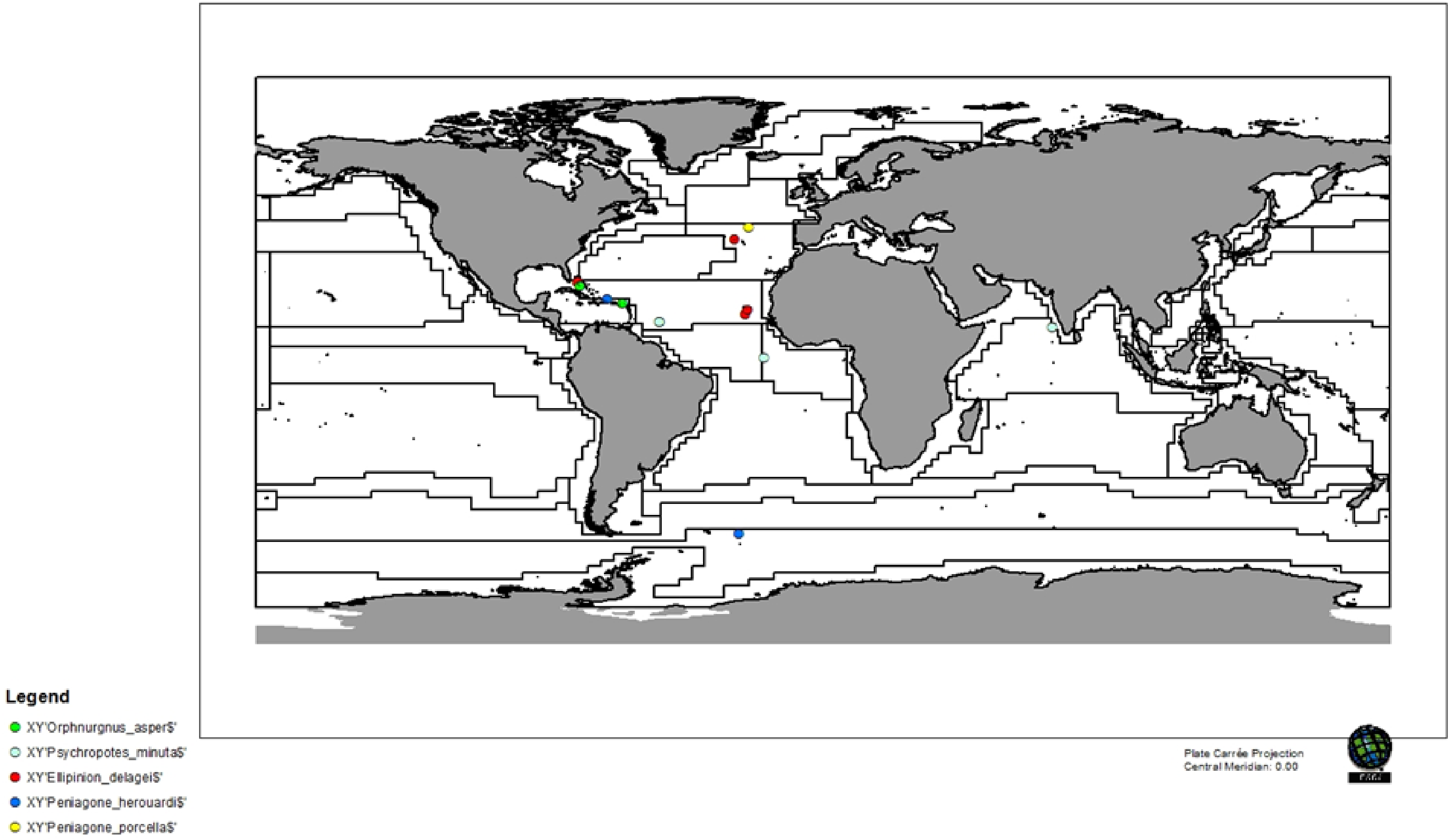


Figure 4.21 *The distribution of the species that form cluster **4b** in the species-level inverted cluster* (*Chapter 4*, Fig. 4.7)

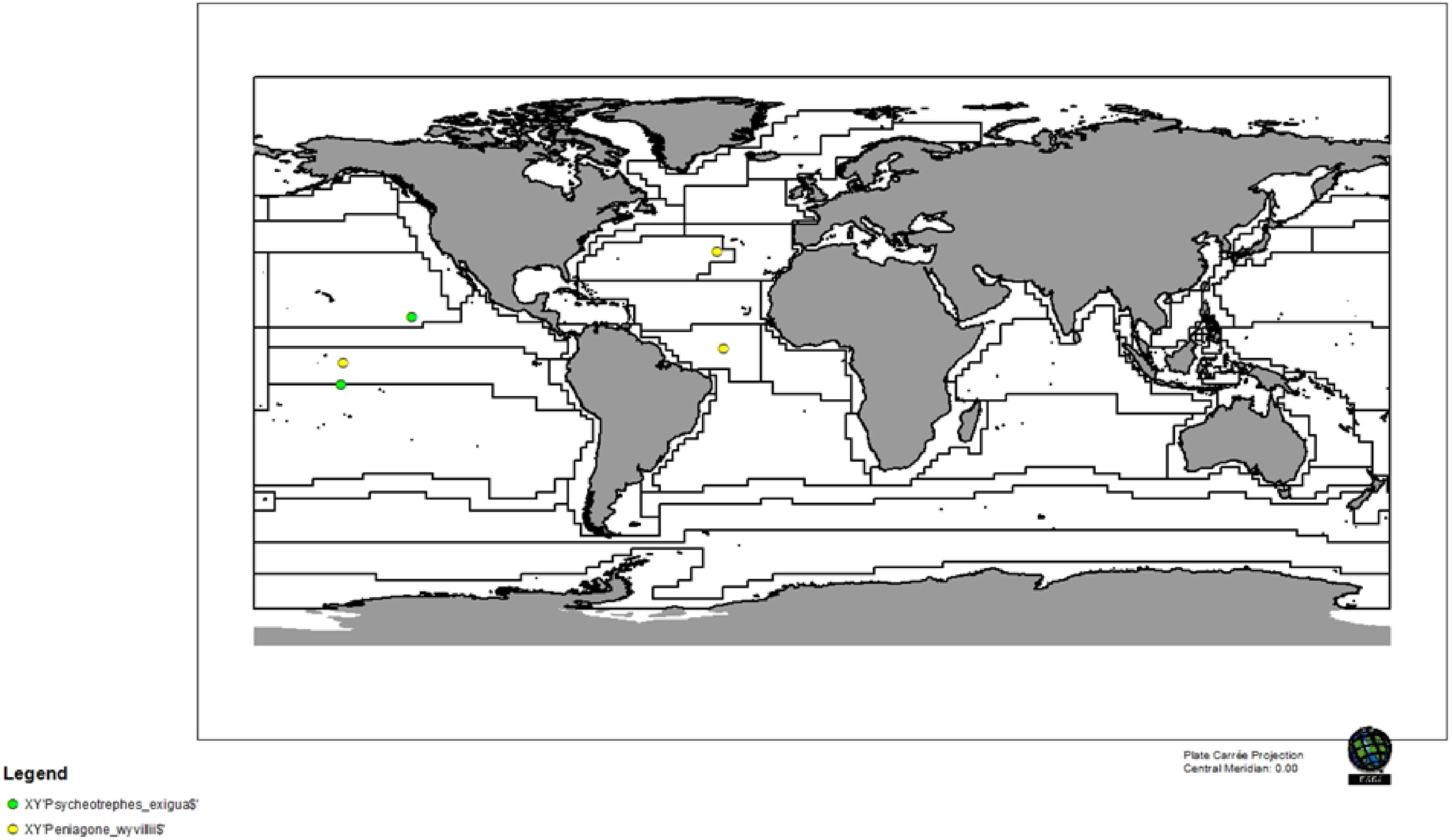


Figure 4.22 *The distribution of the species that form cluster 5 in the species-level inverted cluster* (*Chapter 4,* Fig. 4.7)

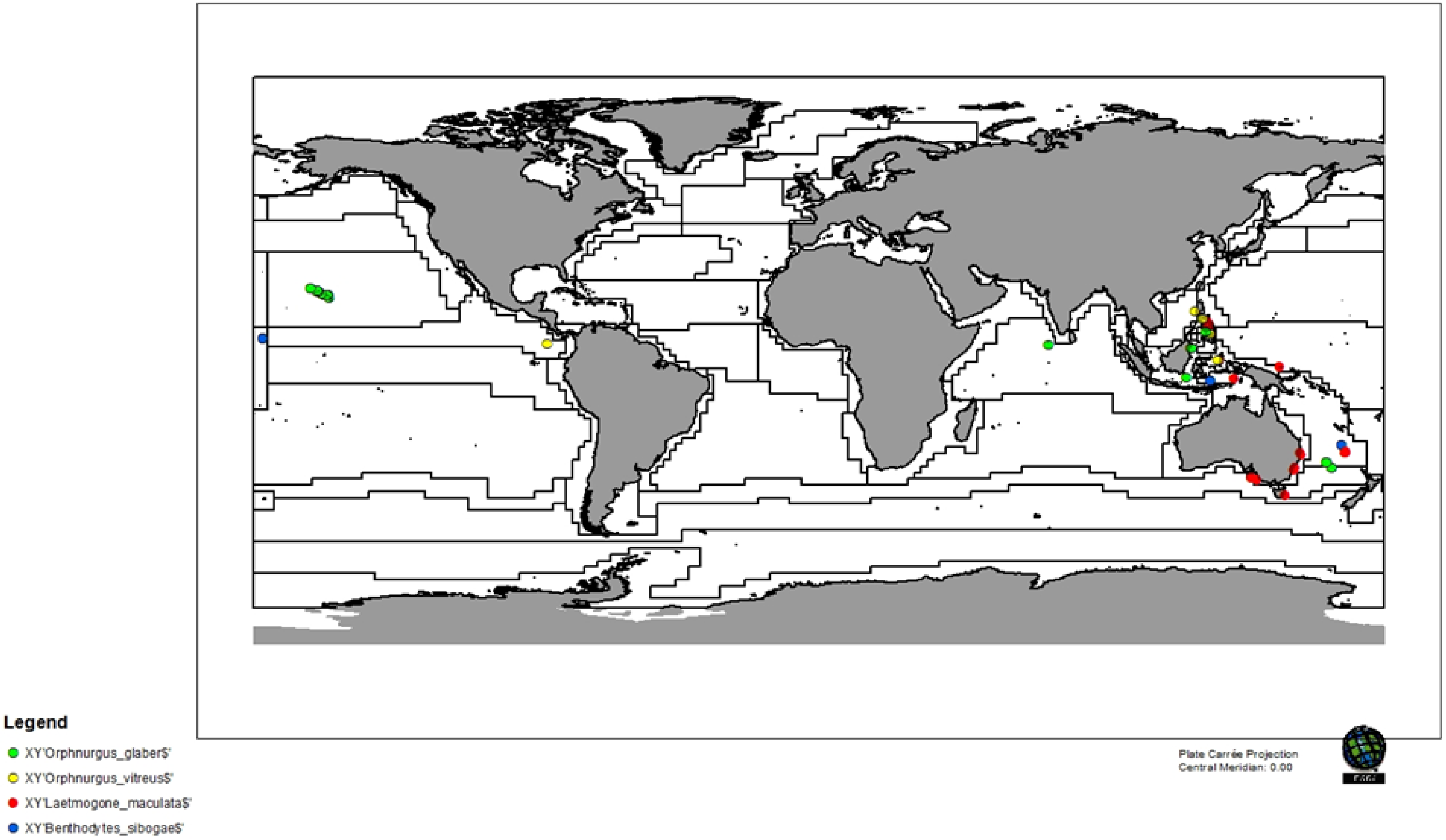


Figure 4.23 *The distribution of the species that form cluster 6a in the species-level inverted cluster* (*Chapter 4*, Fig. 4.7)

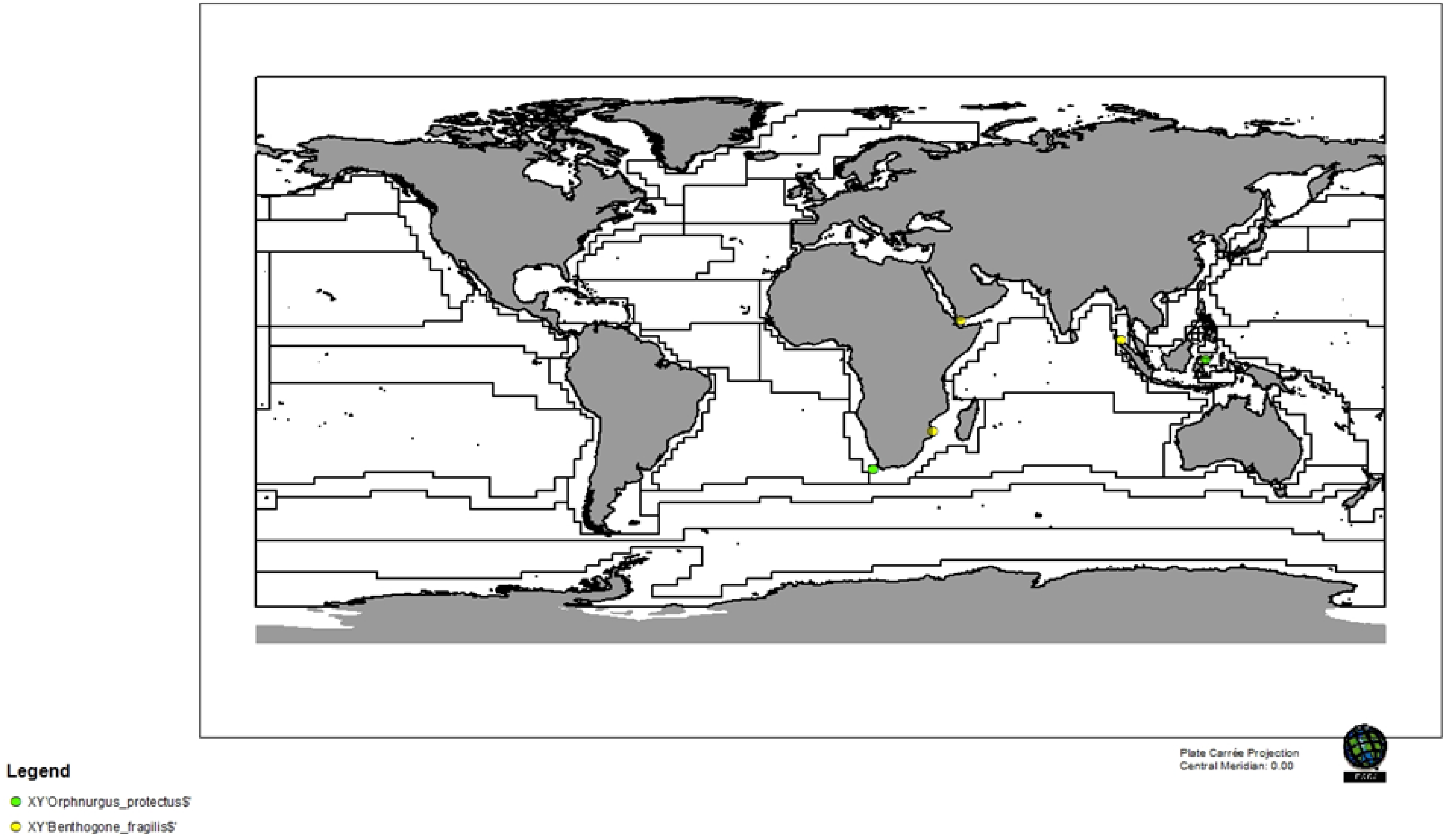


Figure 4.24. *The distribution of the species that form cluster **6b** in the species-level inverted cluster* (*Chapter 4*, Fig. 4.7)

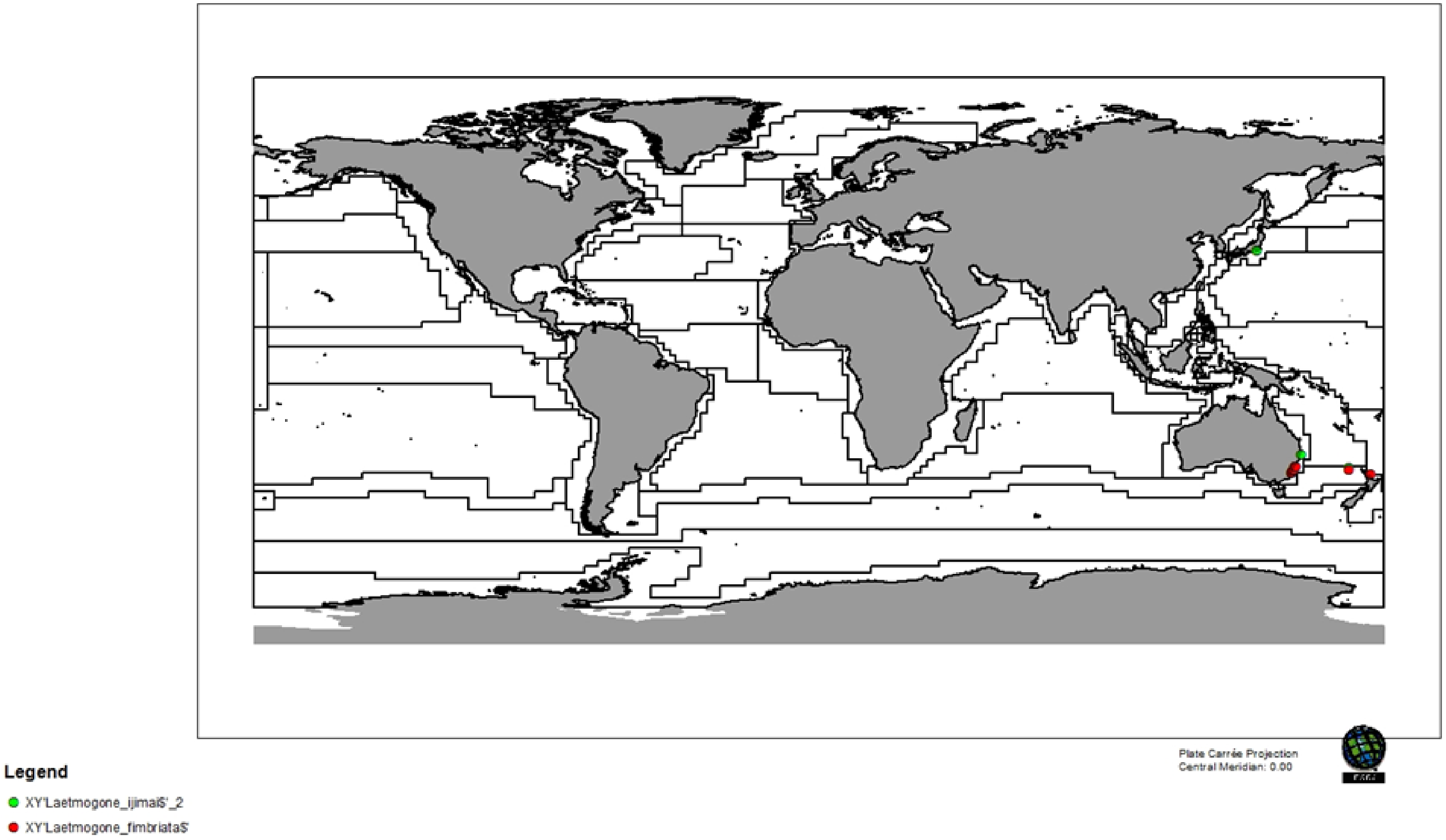


Figure 4.25 *The distribution of the species that form cluster* **6c** *in the species-level inverted cluster* (*Chapter 4*, Fig. 4.7)

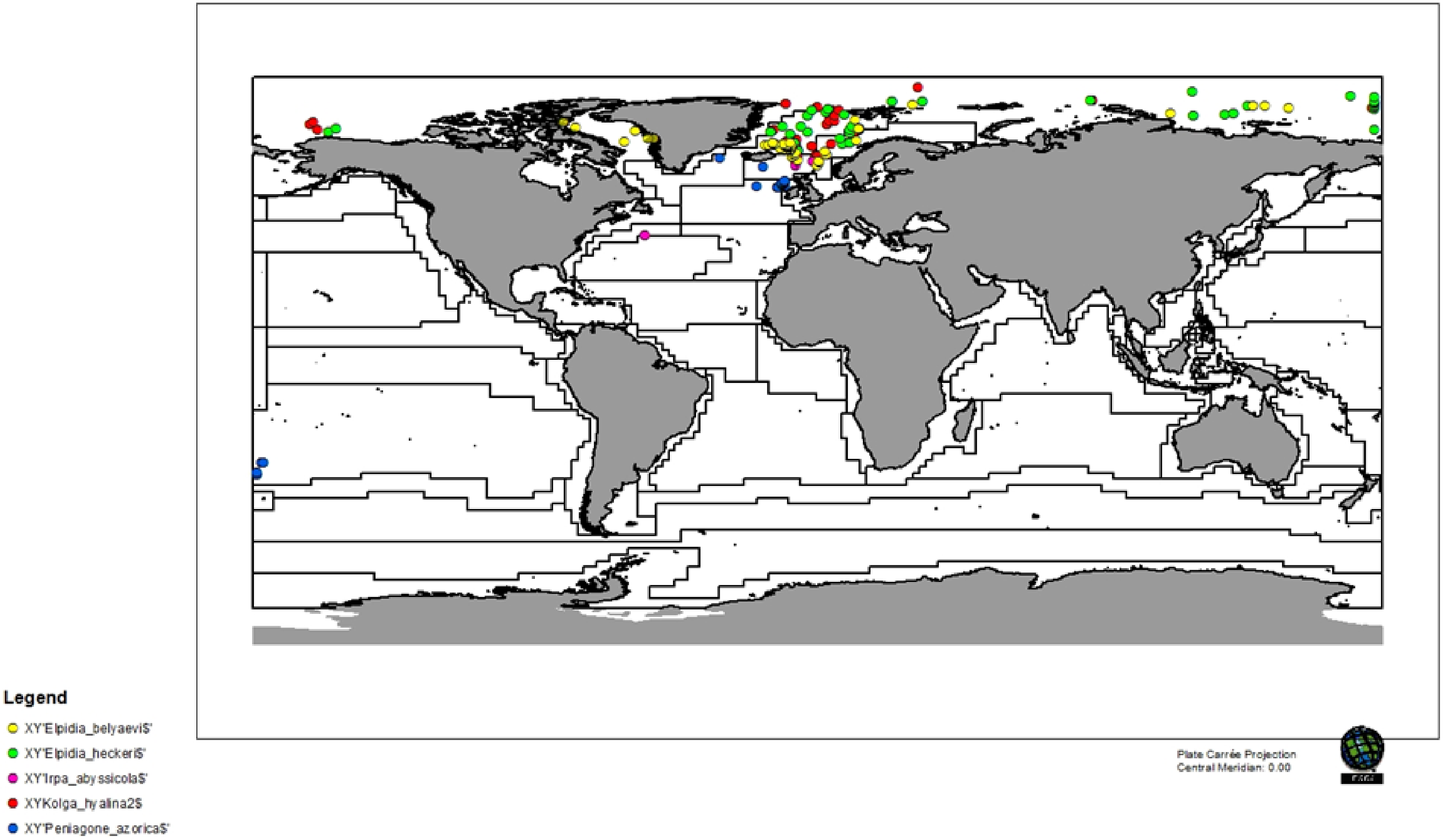


Figure 4.26 *The distribution of the species that form cluster 7 in the species-level inverted cluster (Chapter 4, Figure 4.7)*

Appendix C

Table 5.7 *Polymorphic nucleotide positions of cytochrome oxidase subunit I (29 haplotypes) in* Psychropotes longicauda*. (.) indicates nucleotide identity and N unknown bases*.

	1333344677899911111111112222223333333333333444444	
	5067928128401901222444579024783344778889992444578	
	5706912761278902045826147036614940	
H_1	CCCCTTCAAGACACAATTATCTCATACTTAATTTCATATCCCATTTAA	9
H_2	CCCCTTCAAGACACAACTATCTCATACTTAATTTCATATCCCATTTAA	1
H_3	CCCCTTCAAGACACAATTATCTCATACTTAATTTCATATCCCGTTTAA	1
H_4	CCCCTTCAAGACACAATTATCTCATACTCAATTTCATATCCCATTTAA	1
H_5	CCCCCTCAAAATAAAATTATCATATACTTAACTTCATATTCCGCTTCA	7
H_6	CCCCTTAAAGATAAAATTATCACATACTTAATTTCATATTCCGTTTCA	1
H_7	TTCCCTTGAAGCACCATAGCTACGTATCCTATCTCGTATTTCATTAAT	3
H_8	CCCCNTCAAAANAAAATTATCACATACTTAACTTCGTATTCCGCTTCA	1
H_9	CCCCCTCAAAATAAAATTATCATATACTTAACTTCATGTTCCGCTTCA	1
H_10	TTCCTTTGGAACACCCTTATTACGTATCCATTCTCGTATTTCATTAAA	1
H_11	TTCCTTTGGAGCACCCTTATTACGTATCCATTCCCGTATTTCATTAAA	1
H_12	TTCCTTTGGAACACCCTTATTATGTATCCATTCTCGTATTTCATTAAA	1
H_13	TTCCTTTGGAGCACCCTTATTACGTATCCATTCTCGTATTTCATTAAA	1
H_14	CTTATTTAAAGCCAAACTATTACGTATTTAATTTTAATATTTACTTTC	1
H_15	CTTCTTTAAAGCCAAACTATTACGTATTTAATTTTAATTTTTACTTTC	3
H_16	CCCNTTNAANACACAATTATCTCATACTTAATTTCATATTCCATTTAA	1
H_17	TTCCTTTGAAGCACCCTTATTACGTATCCATTCTCGTATTTCATTAAA	1
H_18	TTCCTTTGAAACACCATAGCTACGTATCCTATCTCGTATTTCATTAAC	1
H_19	TTCCCTTGAAACACCATAGCTACGTATCCTATCTCGTATTTCATTAAT	1
H_20	CCCCTCCAAGACACAATTATCTCACACTTAATTTCATATCCCATTTAA	1
H_21	CTCATTTGAAATACCATTGTTACATGTTTAATCTCGTATTTCATTAAT	1
H_22	CCCCTTCAAANCACAATTATCACATACTCAATTTCATATCCCATCTAA	1

Table 5.8 *Polymorphic nucleotide positions of 16S mRNA (7 haplotypes) in* Psychropotes longicauda*. (.) indicates nucleotide identity and N unknown bases.*

	11111222222 66688226679 35745697834	
H_1	TA---TTAT-T	2
H_2	TT---TTTA-T	4
H_3	TT---CTTA-T	2
H_4	ATTTTCCTA-T	1
H_5	AT-T-CTTA-T	7
H_6	AT-T-CTTATT	1
H_7	TA---TTAT-C	2

Table 5.9 *Polymorphic nucleotide positions of* cytochrome oxidase subunit I *(33 haplotypes) in* Oneirophanta mutabilis. *(.) indicates nucleotide identity and N unknown bases.*

	411 12333334556 7778 8899 9 1111111111 1111 1222222 222 2222 222222222233 333 33333333 3333 3333 344 44444444 444 444444444555 0696124576284069258145002344555678882223344456666778888899000112345556677888890112233344556678899 99011 03432814532147369284786356847016795845739976258473902589432814079231736817049812	
H_1	CGAATTCACAAAGGAAACTTTTTTATTTGTTTGCTTCTTACCATCTGATTATTTTTAATCTAATTTTTCTTTGAGTTATTCCGTACTATCAACATCAAAGGCT	7
H_2	CGAATTCACAAAGGAAACTTTTTTATTTGTTTGCTTCTTACCATCTGATTATTTTTAATCTAATTTTTCTTTGAGTTATTCCTTACTATCAACATCAAAGGCT	1
H_3	CGAATTCACAAAGGAAACTTTTTTATTTGTTTGCTTCTTACCATCTGATTATTTTTAATCTAATTTTTCTTTGAGCTATTCCTTACTATCAAAATCAAAGGCT	1
H_4	CGAATTCACAAAGGAAACTTTTTTATTTTTTTGCTTCTTACCATCTGATTATTTTTAATCTAATTTTTCTTTGAGTTATTCCTTATTATCAACATCAAAGGCT	1
H_5	CACATTTACAAAGATGACTTTTTTATTTGTTTGCTTCTTACCATCTGATTATTCTTACTCTGACTTTTCCTTGAGCCATTCTGTAATATTAACATCAAAGGCT	1
H_6	CACATTTACAAAGATGACTTTTTTATTTGTTTGCTTCTTACCATCTGATTATTCTTACTCTGACTTTTCTTTGAGTCATTCTGTAATATTAACATCAAAGGCT	1
H_7	CAAATCTGCAAAGAAAACTTTTTTATTTTTTTAATTTTTCCCACCTGCTTATTCTTAATCTAACTTTTCTCTAAGTTATTCTTTAACATCAAAACCAAAGCTT	1
H_8	CAAATCTACAAAGAAAACTTTTTTATTTTTTTAATTTTTCCCACCTGCTTATTCTTAATCTAACTTTTCTCTAAGTTATTCTTTAACATCAAAACCAAAGCTT	1
H_9	CAAATCTACAAAGAAAACTTTTTTATTTTTTTAATTTTTCCCACCTGCTTATTCTTAATCTAACTTTTCTCTAAGTTATTCTTTAACATCAACCCCAAAGCTT	1
H_10	CGCAACTACAAAGAAAACTTTTTTATTTTTTTAATTTTTCCCACCTGCTTATTCTTAATCTAAATTTTCTCTAAGCTATCCTTTAACATCAAAACCAAAATTT	1
H_11	CGAATTTACAAAGAGAACTTTTTCATTTTTTTAATTTTTTCCACTTGATCATTCTTACTCTAATCTTTCTTTAGGTTATTCTTTAACACCAGAACCAGAATCT	2
H_12	CGACTTTACAAAGAGAACTTTTTCATTTTTTTAATTTTTTCCACTTGATCATTCTTAATCTAATTTTTCTTTAGGTTATTCTTTAACACCAGAACCAGAGTCT	1
H_13	CGAATTTACAAAAGGAGCTTTTTCATTTTTTTAATTTTTTCCACTTGATCATTCTTAATCTAATCTTTCTTTAGGTTATTCTTTAACACCAGAACCAGAGTCT	1
H_14	CGTATTCATAAAAGAGACCTTCTTATTATTTTACTTTTTATCATCTGATTATCCTCATTCTAATCTTTCTTTAAGCTATTCTGTAACATCAGTATCAAAAATT	1
H_15	CGTATTCATAAAAGAGACTTTCTTATTATTTTACTTTTTATCATCTGATTATCCTCATTCTAATCTTTCTTTAAGCTATTCTGTAACATCAGTATCAAAAATT	1
H_16	CATATTCATAAAAGAGACTTTCCTATTATTTTACTTTTTATTATCTAATTATCCTCATTCTAATCTTTCTTTAAGCTATTCTGTAACATCAGTATCAAAAATT	1
H_17	CATATTCATAAAAGAGACTTTCTTATTATTTTACTTTTTATCATCTGATTATCCTCATTCTAATCTTTCTTTAAGCTATTCTATAACATCAGTATCAAAAATT	1
H_18	CATAATCATAAAAGAGACTTTCTTATTATTTTACTTTTTATCATCTGATTATCCTCATTCCAATCTTTCTTTAAGTTATTCTGTAACATCAGTATCAAAAATT	1
H_19	CACTTTTACCAAAGAAACTTTTTTATTATTTTATTTTTTACCATCTGATTATTCTCAATATAACTTTTATTTAAGTTATTATTTAACATCAACATCAAAAACT	1
H_20	CATCTTTACCATAGAAATTTTTTTATTCTCTTAATCTTCATTACCTACCTATCCCTAATCTAATTTTTCTTTAAGCTCTTCTGCAACATCATTTCCGGAATTC	3
H_21	CATCTTTACCATAGAAATTTTTTTATTCTCTTAATCTTCATTACCTACCTATCCCTAATCTAATTTTTCTTTAAACTCTTCTGCAACATCATTTCCGGAATTC	1
H_22	CATCTTTACCATAGAAATTTTTTTATTCTCTTAATCTTCATTACCTACCTATCCCTAATCTAGTTTTTCTTTAAGCTCTTCTGCAACATCATTTCCGGAATTC	1
H_23	CATCTTTACCATAGAAATTTTTTTATTCTCTTAATCTTCATTACCTACCTATCCCTGACCTAATTTTTCTTTAAGCTCTTCTGCAACATCATTTCCGGAATTC	1
H_24	CATCTTTACCATAGGAATTTTTTTATTCTCTTAATCTTCATTACCTACCTATCCCTAATCTAATTTTTCTTTAAGCTCTTCTGCAACATCATTTCCGGAATTC	1
H_25	CACCTTTACCATAGAAATTTTTTTATTCTCTTAATCTTCATTGCCTACCTATCCCTAATCTAATTTTTCTTTAAGCTCTTCTGCAACATCATTTCCGGAATTC	1
H_26	CGTCTTTACCATAGAAATTTTTTTATTCTCTTAATCTTCATTACCTACCTATCCCTAATCTAATTTTTCTTTAAGCTCTTCTGCAACATCATTTCCGGAATTC	1
H_27	CATCTTTACCANAGAAATTTTTTTATTCTCTTAATCTTCATTACCTACCTATCCCTAATCTAATTTTTCTTTAAGCTCTTCTGCAACATCATTTCCGGAANTC	1
H_28	CATCTTTACCACAGAAATTTTTTTATTCTCTTAATCTTCATTACCTACCTATCCTTAATCTAATTTTTCTTTAAGCTCTTCTGCAACATTATTTCCGGAATTC	1
H_29	CACCTTTACCATAGGAATTTTTTTGTTCTCTTAATCTTCATTACCTACCTATCCTTAATCTAATTTTTCTTTAAGCTCTTCTGCAACGTCATTTCCGGAATTC	1
H_30	TATTTTTACTTAAAAAACTCCTTTTACCTTCCAAGCAAAATCATCAGTTTCCTTTTAAAATAAATTTAATTCAAGTCCCTCTTTATCATCGAACATAACGCCT	1
H_31	TATTTTTACTTAAGAAACTCCTTTTACCTTCCGATCAAAATCATCAGTTTCCTTTTAAAATAAATTTAATTCAAGTCCCTCTTTATCATCGAACATAACGCCT	1
H_32	TATCTTTACTTAAAAAACTCCTTTTACCTTCCAATCAAAATTATCAAACTCCTCTTAAAATAAATTCAATTTAAGTCACTCTTTGTCATCGAAAATAACGCCT	1
H_33	TATTTTTACTTAAGAAACTCCTTTTACCTTCCAATCAAAATTATCAACCTCCTCTTAAAATAAATCCAATTCAAGTCACTCTTTGTCATCGAAAATAACGCCT	

Table 5.10 *Polymorphic nucleotide positions of 16S mRNA (6 haplotypes) in* Oneirophanta mutabilis*. (.) indicates nucleotide identity and N unknown bases.*

	51111111112222222222223333 54566778991224445556788999 7806384481472361566855367	
H_1	CTTGCCCTTATCAATGTCA-AGGAAG	1
H_2	CTCACCCTAATCAATGTCC-TGGAAG	1
H_3	CTTATTCTTATCAATGTCC-AGGAAG	2
H_4	CTTATTCTTATTAATCCCC-AAACCC	1
H_5	CTTATTATTATCAACGTTA-AGGAAG	1
H_6	TCTATCCCGGCTGCTGTTATTGGAAG	1

Table 5.11 *Haplotype distribution by geographic sampling location of* Psychropotes longicauda *(n=40) COI haplotypes, with sample size and haplotype numbers. The NE Atlantic is a mixture of Porcupine Abyssal Plain and Whittard Canyon samples.*

Ocean	**Atlantic**			**Pacific**					**Indian**		
	Population										
Haplotype	NE Atlantic	S Atlantic	Drake Passage	NE Pacific	NW Pacific	N Pacific	E Pacific (Peru-Chile Trench)	SW Pacific (Norfolk Island)	Crozet	Great Australian Bight	**Total**
Hap 1	9										9
Hap 2	1										1
Hap 3	1										1
Hap 4	1										1
Hap 5				7							7
Hap 6				1							1
Hap 7				2	1						3
Hap 8				1							1
Hap 9				1							1
Hap 10									1		1
Hap 11									1		1
Hap 12									1		1
Hap 13									1		1
Hap 14									1		1
Hap 15									3		3
Hap 16										1	1
Hap 17		1									1
Hap 18							1				1
Hap 19					1						1
Hap 20								1			1
Hap 21			1								1
Hap 22						1					1
Total individuals	**12**	**1**	**1**	**12**	**2**	**1**	**1**	**1**	**8**	**1**	**40**

Table 5.12 *Haplotype distribution by geographic sampling location of* Psychropotes longicauda *(n=19) 16S mRNA haplotypes, with sample size and haplotype numbers*.

Haplotype	**Location**				**Total**
	NE Atlantic	**Crozet**	**NE Pacific**	**NW Pacific**	
Hap 1		2			2
Hap 2		4			4
Hap 3			2		2
Hap 4	1				1
Hap 5	6			1	7
Hap 6	1				1
Hap 7			2		2
Total	**8**	**6**	**4**	**1**	**19**

Table 5.13 *Haplotype distribution by geographic sampling location of* Oneirophanta mutabilis *(n=42) COI mRNA haplotypes, with sample size and haplotype numbers*.

Haplotype	Location				Total
	NE Atlantic	Crozet	NE Pacific	South Atlantic	
Hap 1			7		7
Hap 2			1		1
Hap 3			1		1
Hap 4			1		1
Hap 5		1			1
Hap 6		1			1
Hap 7	1				1
Hap 8	1				1
Hap 9		1			1
Hap 10	1				1
Hap 11			2		2
Hap 12			1		1
Hap 13			1		1
Hap 14			1		1
Hap 15			1		1
Hap 16			1		1
Hap 17			1		1
Hap 18			1		1
Hap 19				1	1
Hap 20	3				3
Hap 21	1				1
Hap 22	1				1
Hap 23	1				1
Hap 24	1				1
Hap 25	1				1
Hap 26	1				1
Hap 27	1				1
Hap 28	1				1
Hap 29	1				1
Hap 30		1			1
Hap 31		1			1
Hap 32		1			1
Hap 33		1			1
Total	**15**	**7**	**19**	**1**	**42**

Table 5.14 *Haplotype distribution by geographic sampling location of* Oneirophanta mutabilis *(n=7) 16S mRNA haplotypes, with sample size and haplotype numbers.*

Haplotype	**Location**			**Total**
	NE Atlantic	**Crozet**	**NE Pacific**	
Hap 1	1			1
Hap 2			1	1
Hap 3	2			2
Hap 4	1			1
Hap 5			1	1
Hap 6		1		1
Total	**4**	**1**	**2**	**7**

Table 5.15 *AMOVA design for* Psychropotes longicauda *and* Oneirophanta mutabilis. *Analysis of* P.longicauda *included testing for differences within and between individual populations within Oceans. This was not possible for* O.mutabilis *as only one population per ocean was sampled.*

Species	**Groups**	**Populations**
Psychropotes longicauda	Atlantic vs. Indian-Pacific	NE ATLANTIC/ S ATLANTIC/ DRAKE PASSAGE
		NW PACIFIC / N PACIFIC /PCTRENCH /NORFOLKISL / CROZET / GAB
	Indian-Atlantic vs. Pacific	CROZET / GAB/NEATLANTIC/ SATLANTIC/DRAKEPASS
		NWPACIFIC / NPACIFIC /PCTRENCH /NORFOLKISL
	Pacific vs. Atlantic/Indian	NWPACIFIC / NPACIFIC /PCTRENCH /NORFOLKISL/ NEATLANTIC/ SATLANTIC/DRAKEPASS
		CROZET / GAB
Oneirophanta mutabilis	Indian-Pacific vs. Atlantic	INDIAN/PACIFIC
		ATLANTIC
	Indian-Atlantic vs. Pacific	INDIAN/ATLANTIC
		PACIFIC
	Pacific-Atlantic vs. Indian	PACIFIC/ATLANTIC
		INDIAN

Fig. 5.8 *Maximum Likelihood phylogeny of* Psychropotes longicauda *inferred from combined COI and 16S region sequences. The tree is rooted with* Benthodytes gosarsi*. PAP = Porcupine Abyssal Plain, northeast Atlantic, WC= Whittard Canyon, northeast Atlantic STM= Station M, northeast Pacific, CRZ= Crozet, southern Indian, RUS= samples collected by Russian cruises, see* Fig. 5.1*.*

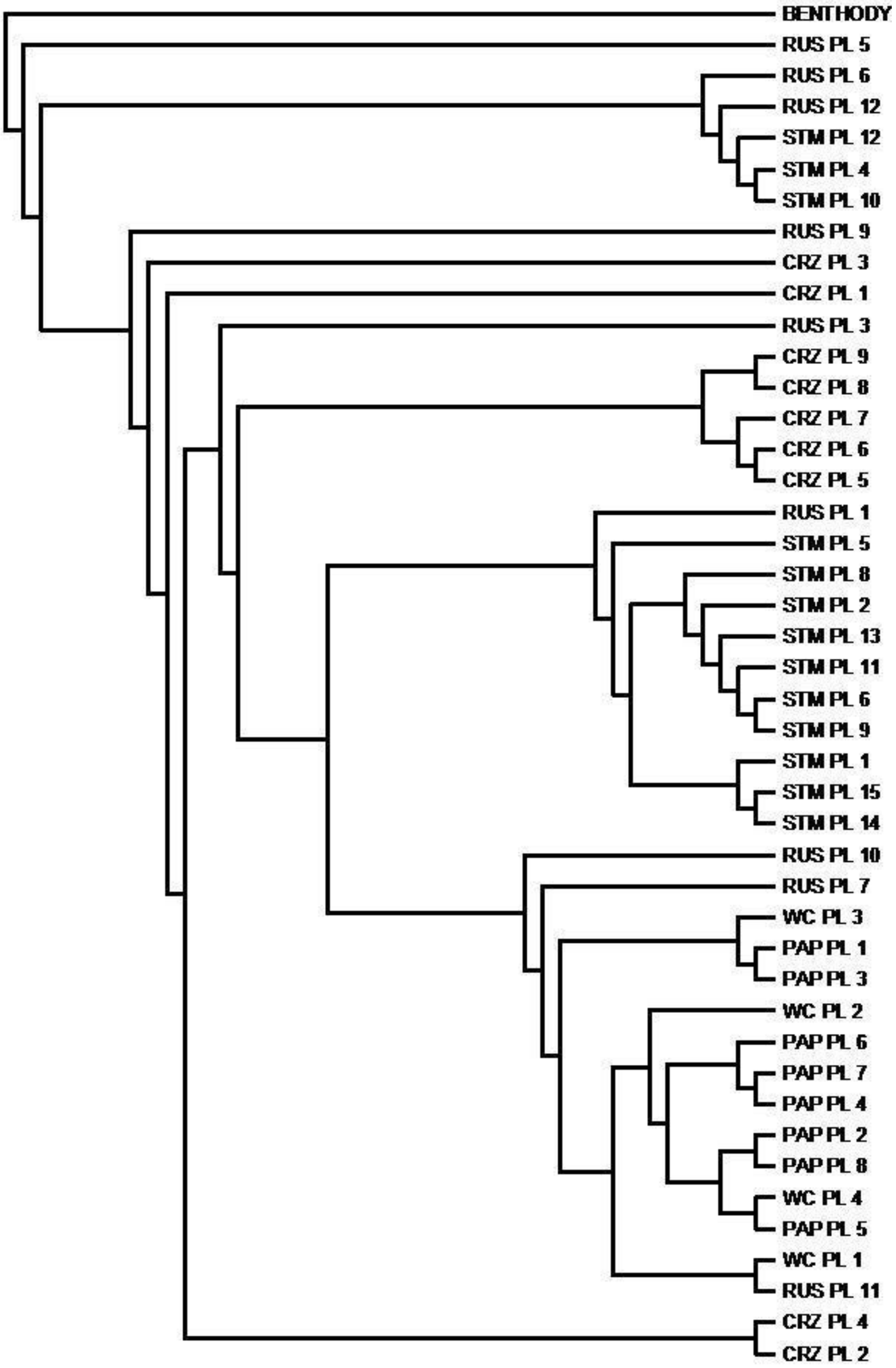


Fig. 5.9 *Bayesian phylogeny of* Psychropotes longicauda *inferred from COI sequences. The tree is rooted with* Benthodytes gosarsi*. PAP = Porcupine Abyssal Plain, northeast Atlantic, WC= Whittard Canyon, northeast Atlantic STM= Station M, northeast Pacific, CRZ= Crozet, southern Indian, RUS= samples collected by Russian cruises, see Fig. 5.1.*

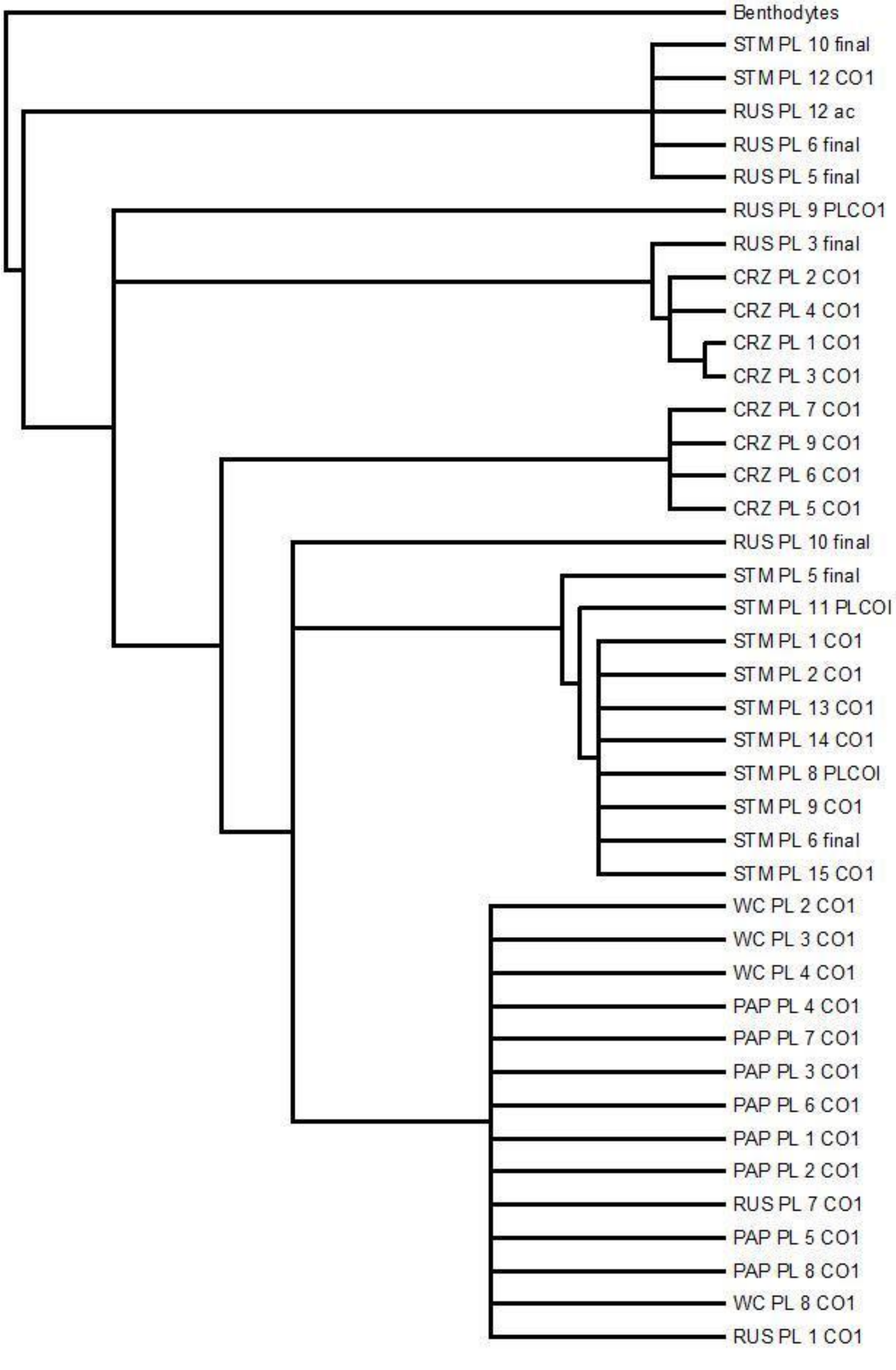


Fig. 5.10 *Maximum Likelihood phylogeny of* Psychropotes longicauda *inferred from COI sequences. The tree is rooted with* Benthodytes gosarsi*. PAP = Porcupine Abyssal Plain, northeast Atlantic, WC= Whittard Canyon, northeast Atlantic STM= Station M, northeast Pacific, CRZ= Crozet, southern Indian, RUS= samples collected by Russian cruises, see* Fig. 5.1

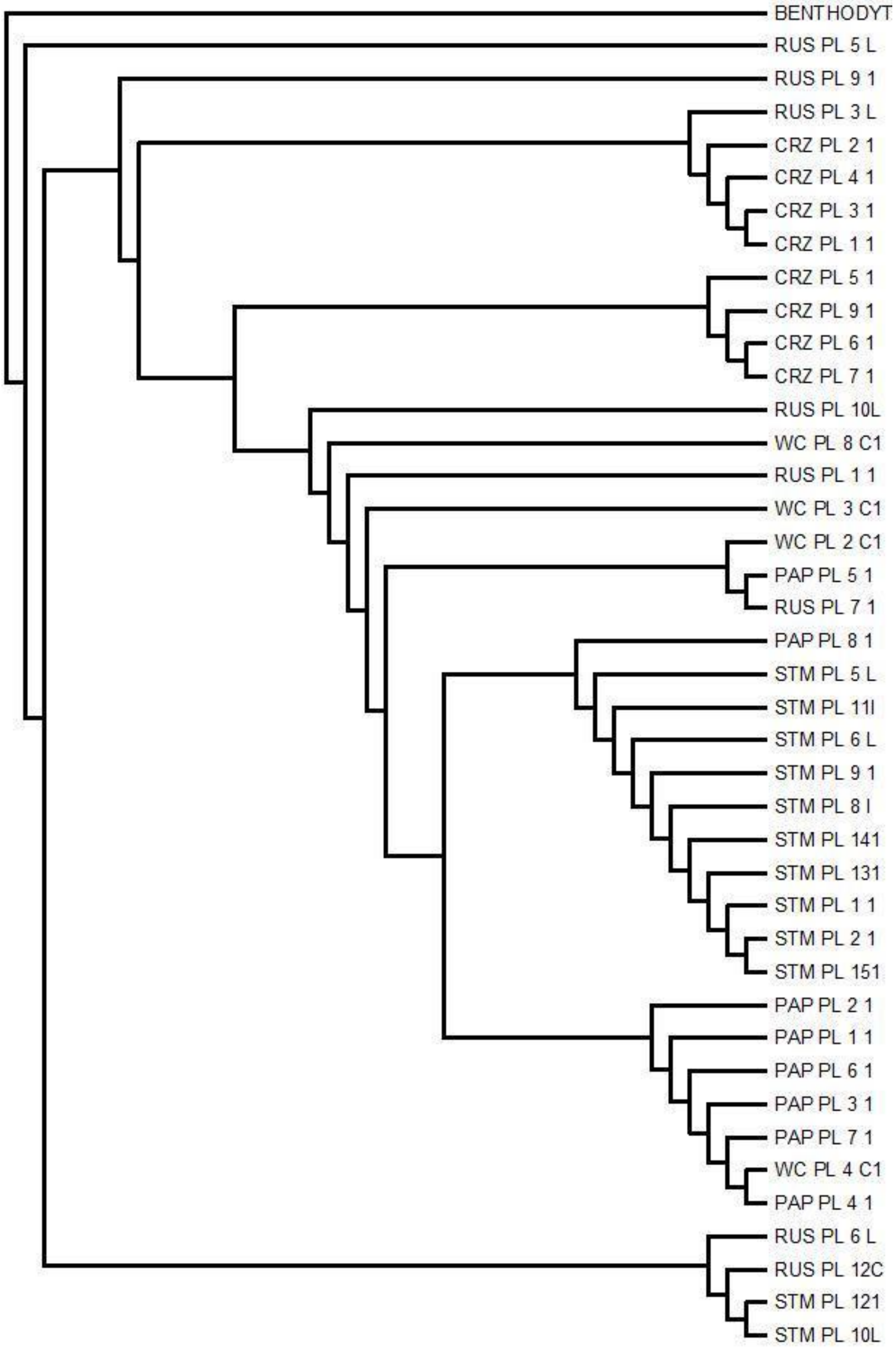


Fig. 5.11 *Bayesian phylogeny of* Psychropotes longicauda *inferred from 16S sequences. The tree is rooted with* Benthodytes gosarsi*. PAP = Porcupine Abyssal Plain, northeast Atlantic, WC= Whittard Canyon, northeast Atlantic STM= Station M, northeast Pacific, CRZ= Crozet, southern Indian, RUS= samples collected by Russian cruises, see Fig. 5.1*

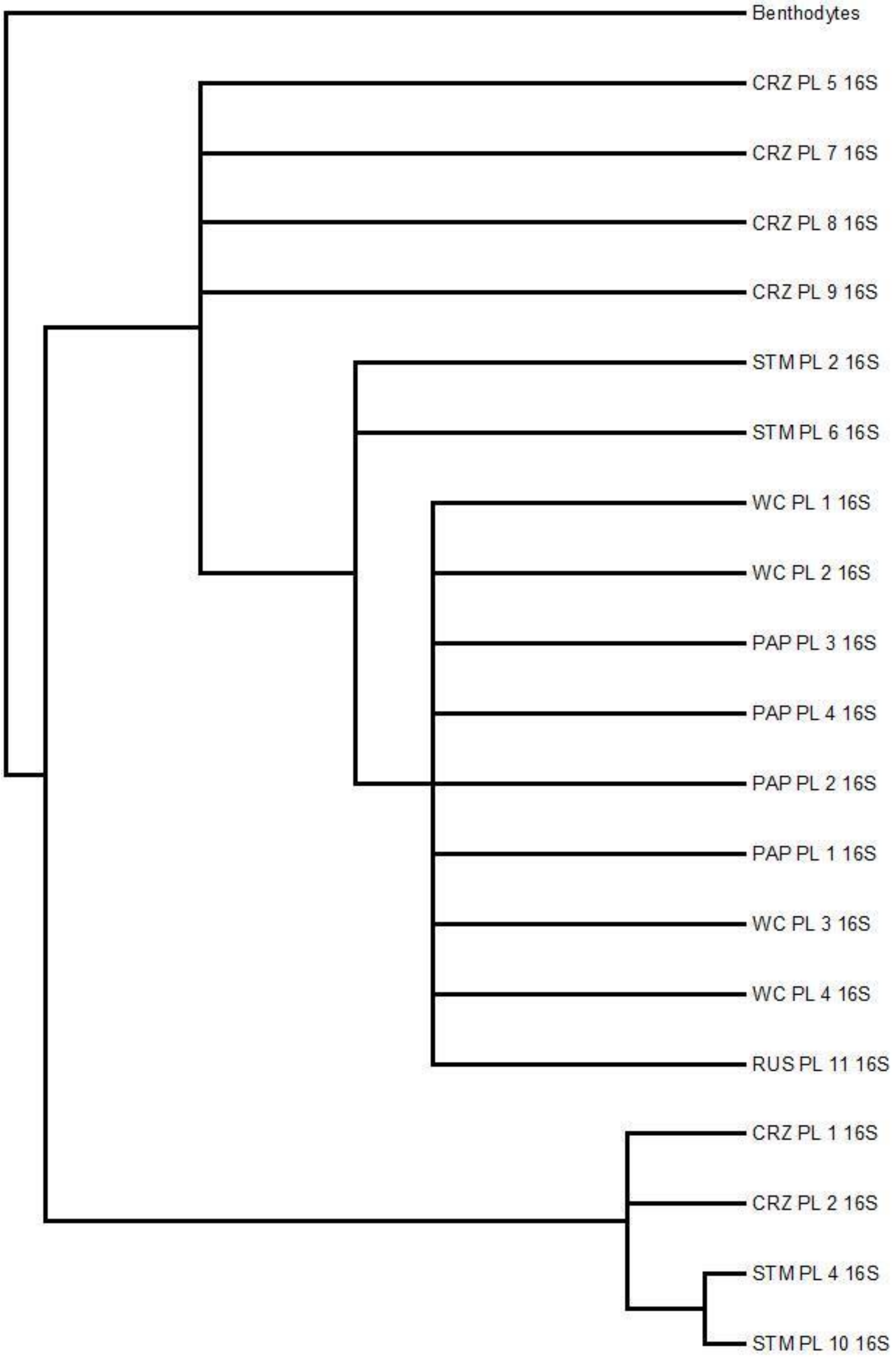
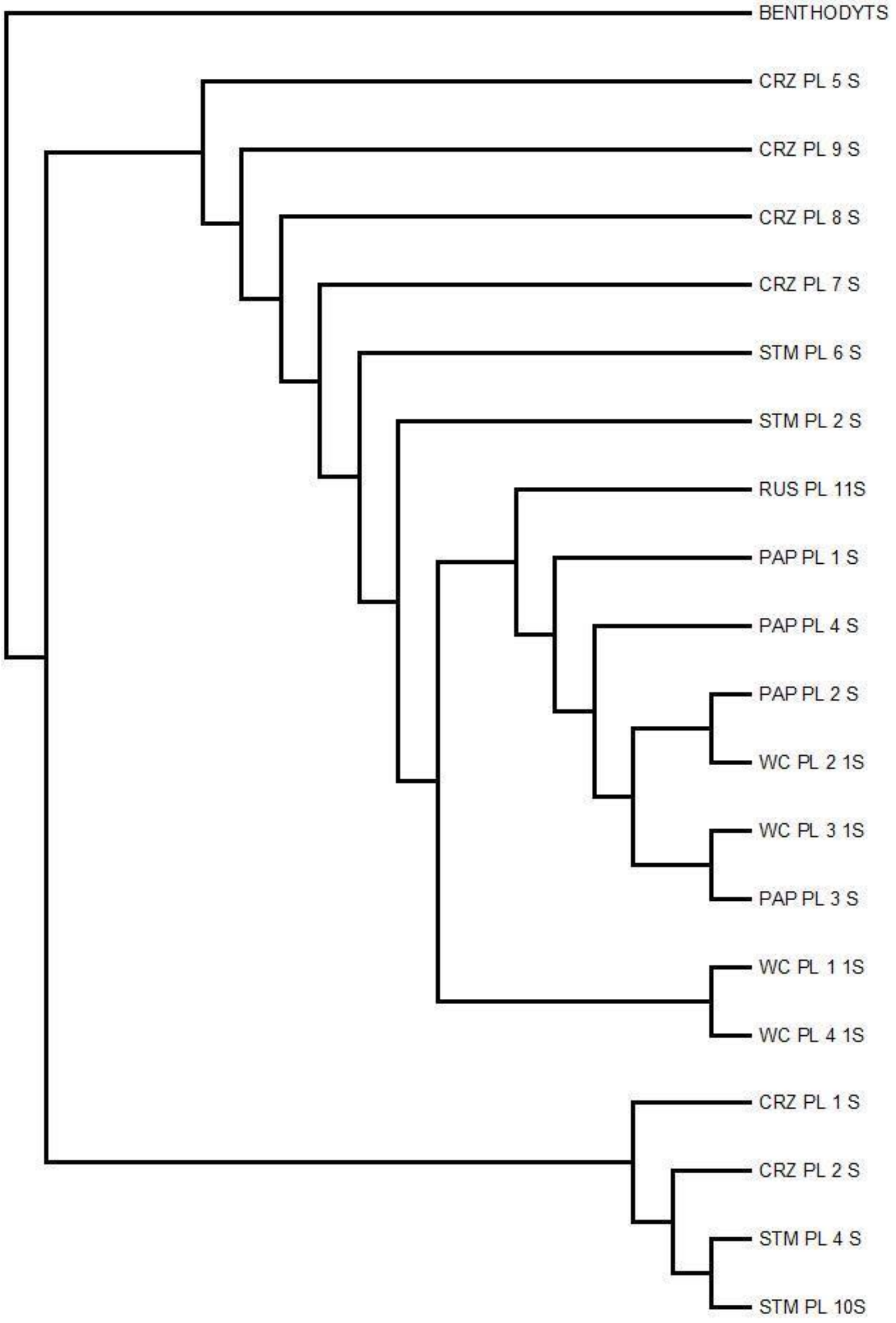


Fig. 5.12 *Maximum Likelihood phylogeny of* Psychropotes longicauda *inferred from 16S sequences. The tree is rooted with* Benthodytes gosarsi*. PAP = Porcupine Abyssal Plain, northeast Atlantic, WC= Whittard Canyon, northeast Atlantic STM= Station M, CRZ= Crozet, southern Indian, northeast Pacific, RUS= samples collected by Russian cruises, see* Fig. 5.1.



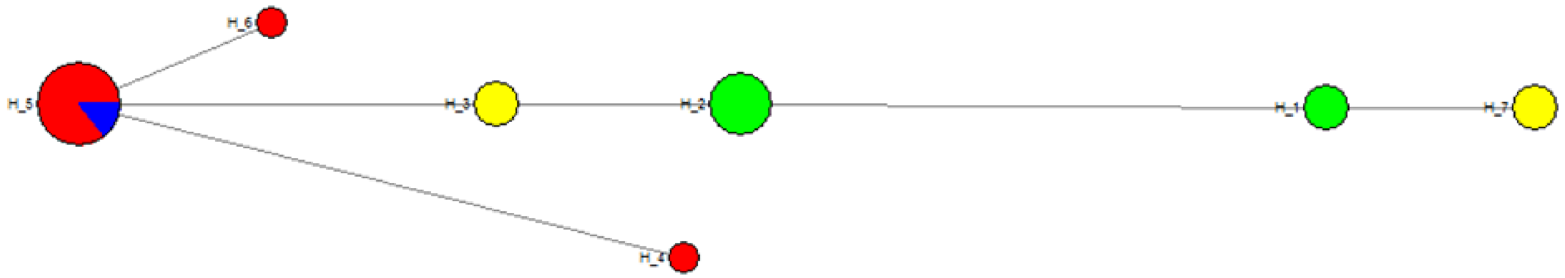


Fig. 5.13 *Median-joining network of 16S rDNA haplotypes of* Psychropotes longicauda *Circle size corresponds to the relative sample size of haplotype frequencies.*

Fig. 5.14 *Maximum Likelihood phylogeny of* Oneirophanta mutabilis *inferred from combined COI and 16S region sequences. The tree is rooted with* Deima validum validum*. PAP = Porcupine Abyssal Plain, northeast Atlantic, STM= Station M, northeast Pacific, CRZ= Crozet, southern Indian , RUS= samples collected by Russian cruises, see Fig. 5.1.*

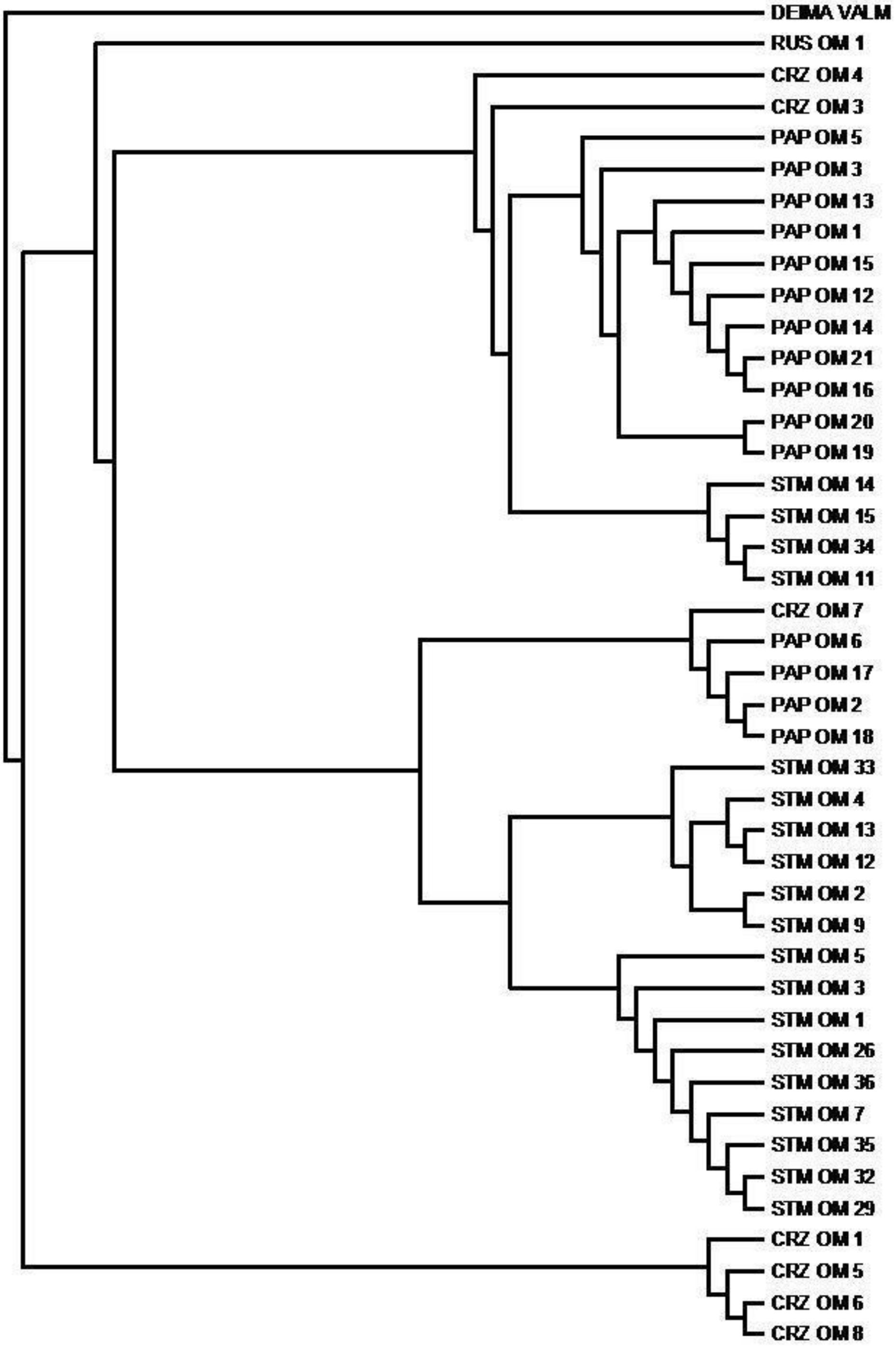


Fig. 5.15 *Bayesian phylogeny of* Oneirophanta mutabilis *inferred from COI sequences. The tree is rooted with* Deima validum validum*. PAP = Porcupine Abyssal Plain, northeast Atlantic, STM= Station M, northeast Pacific, CRZ= Crozet, southern Indian , RUS= samples collected by Russian cruises, see Fig. 5.1.*

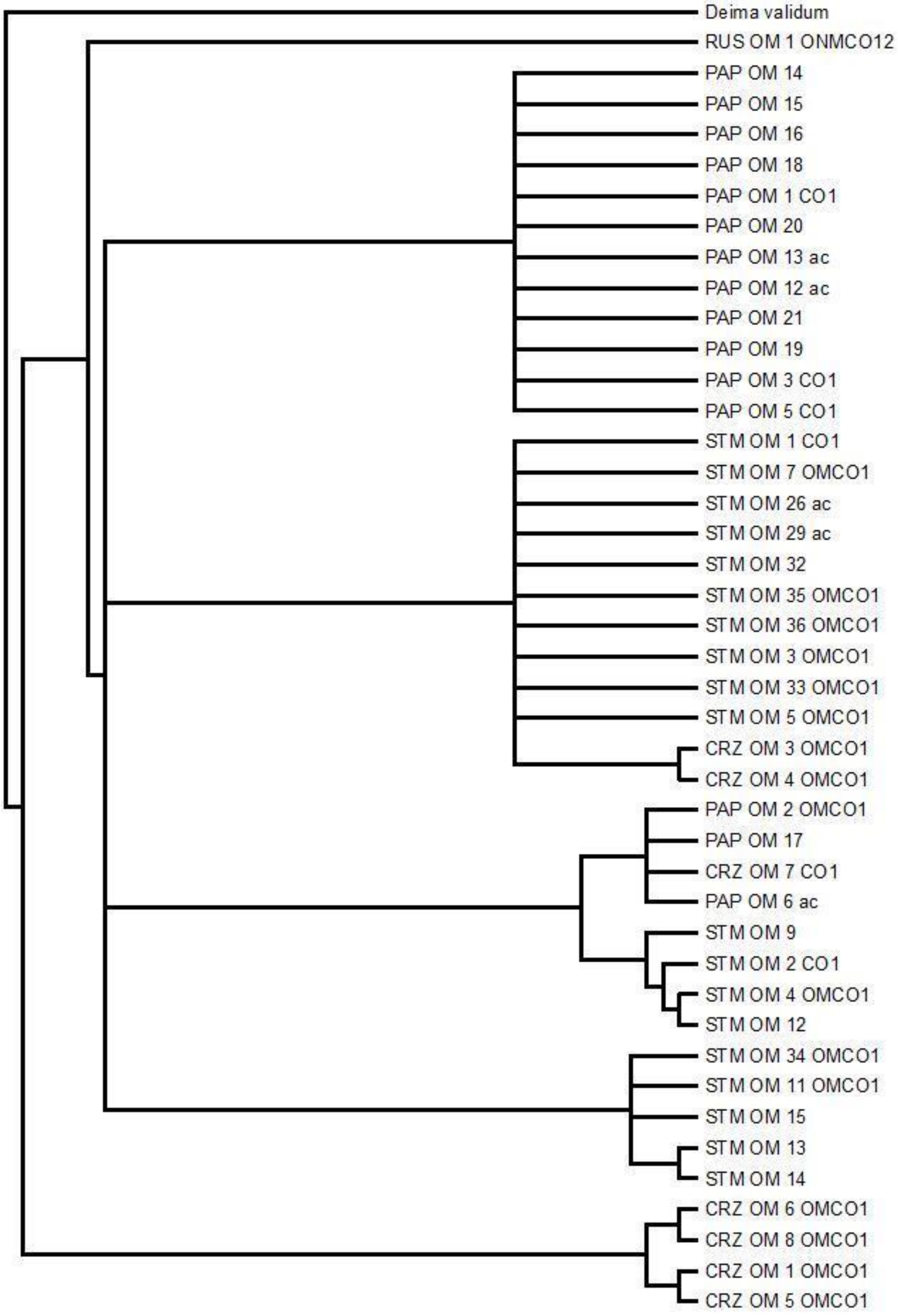


Fig. 5.16 *Maximum likelihood phylogeny of* Oneirophanta mutabilis *inferred from COI sequences. The tree is rooted with* Deima validum validum*. PAP = Porcupine Abyssal Plain, northeast Atlantic, STM= Station M, northeast Pacific, CRZ= Crozet, southern Indian, RUS= samples collected by Russian cruises, see* Fig. 5.1.

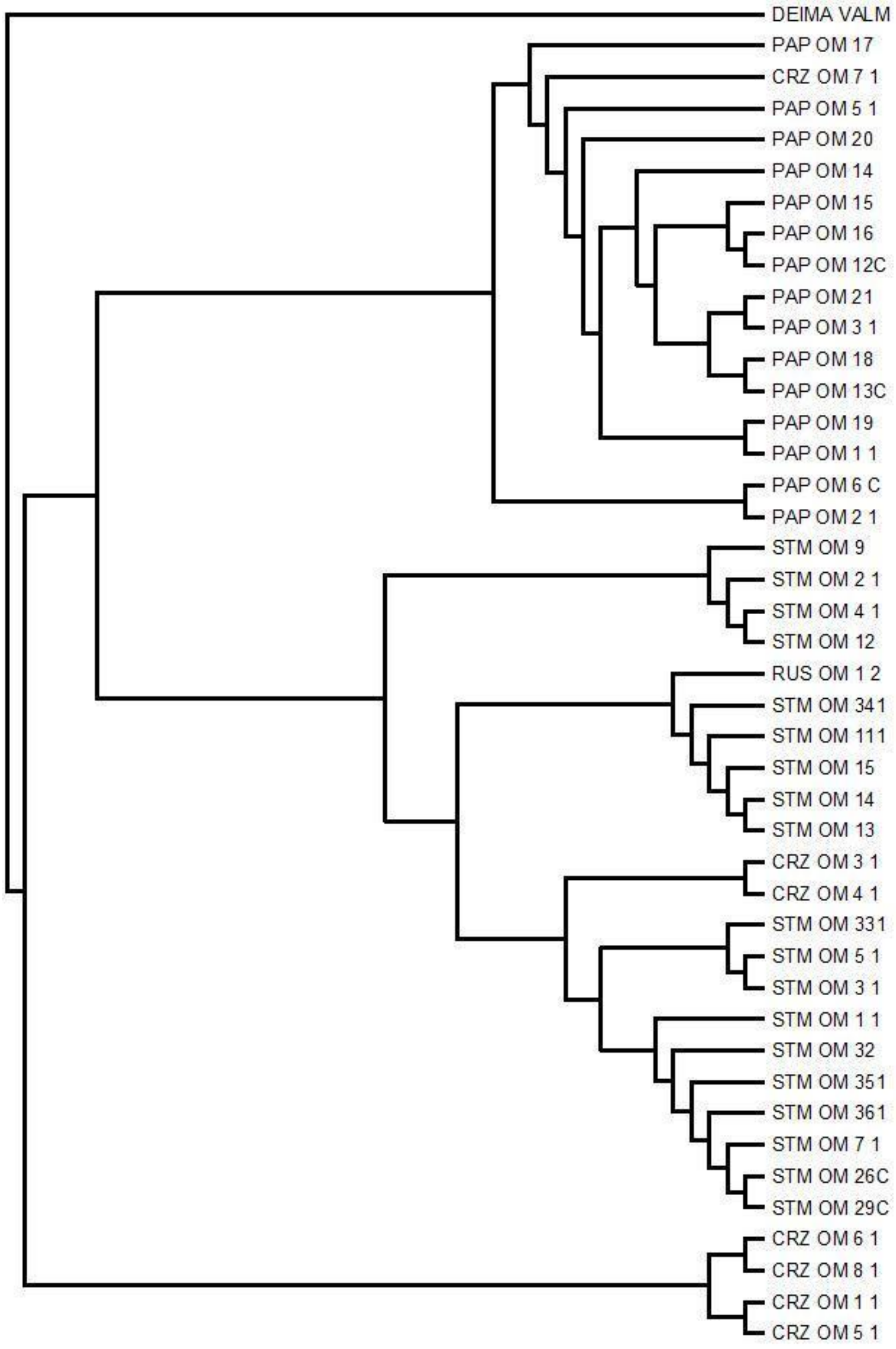


Fig. 5.17 *Bayesian phylogeny of* Oneirophanta mutabilis *inferred from 16S sequences. The tree is rooted with Deima validum validum. PAP = Porcupine Abyssal Plain, northeast Atlantic, STM= Station M, northeast Pacific, CRZ= Crozet, southern Indian Ocean.*

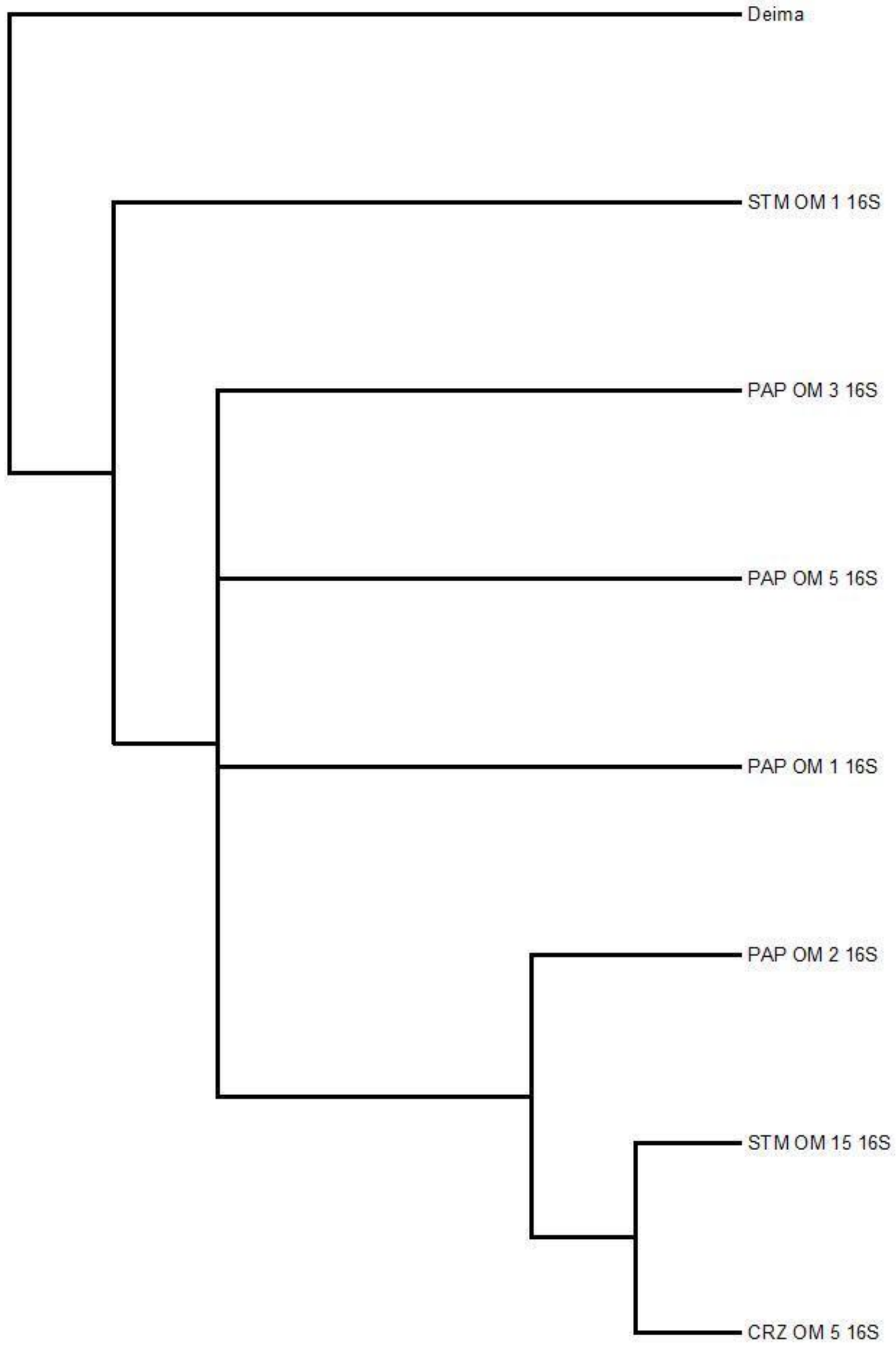
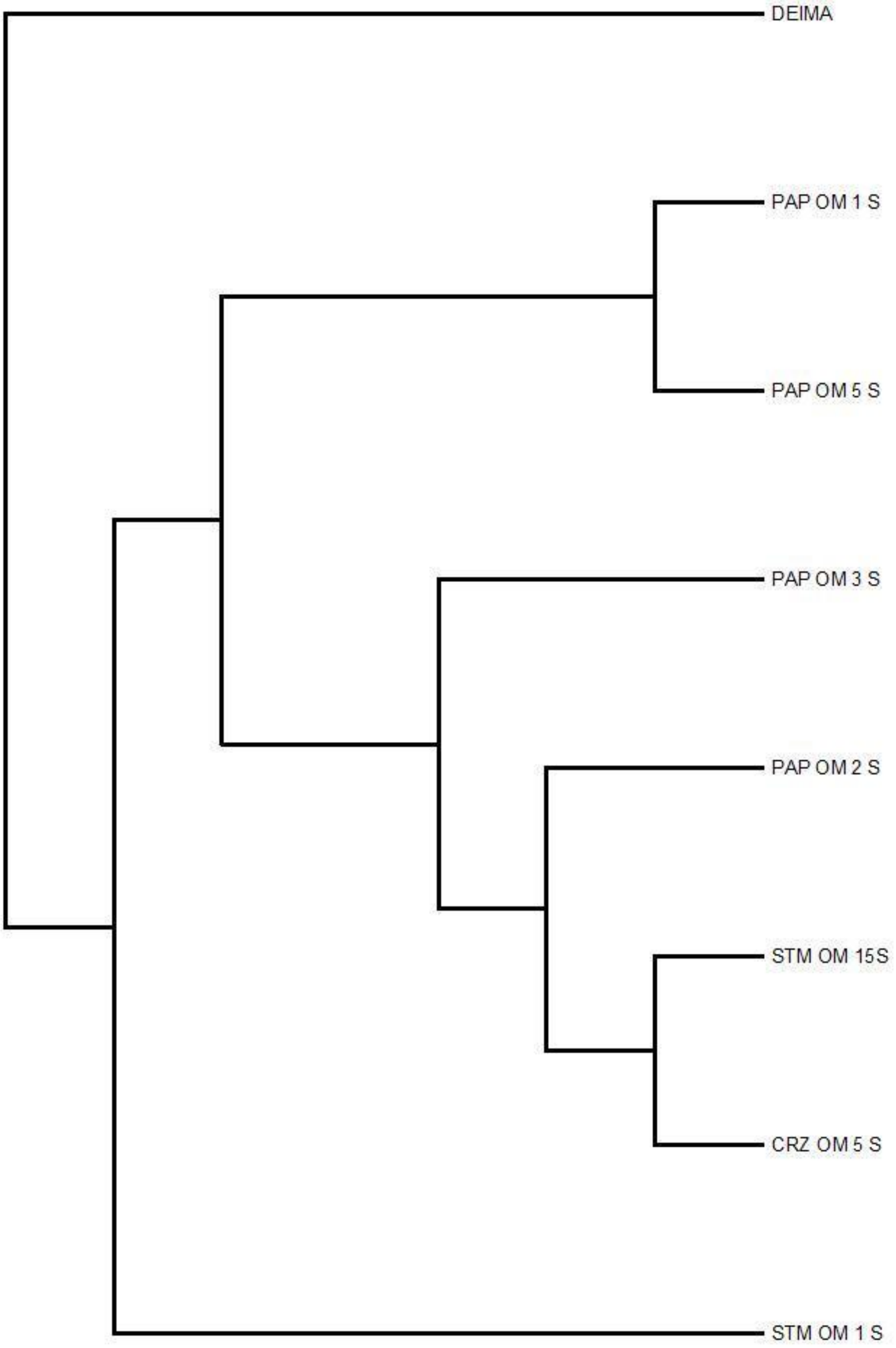


Fig. 5.18 *Maximum likelihood phylogeny of* Oneirophanta mutabilis *inferred from 16S sequences. The tree is rooted with* Deima validum validum*. PAP = Porcupine Abyssal Plain, northeast Atlantic, STM= Station M, northeast Pacific, CRZ= Crozet, southern Indian.*



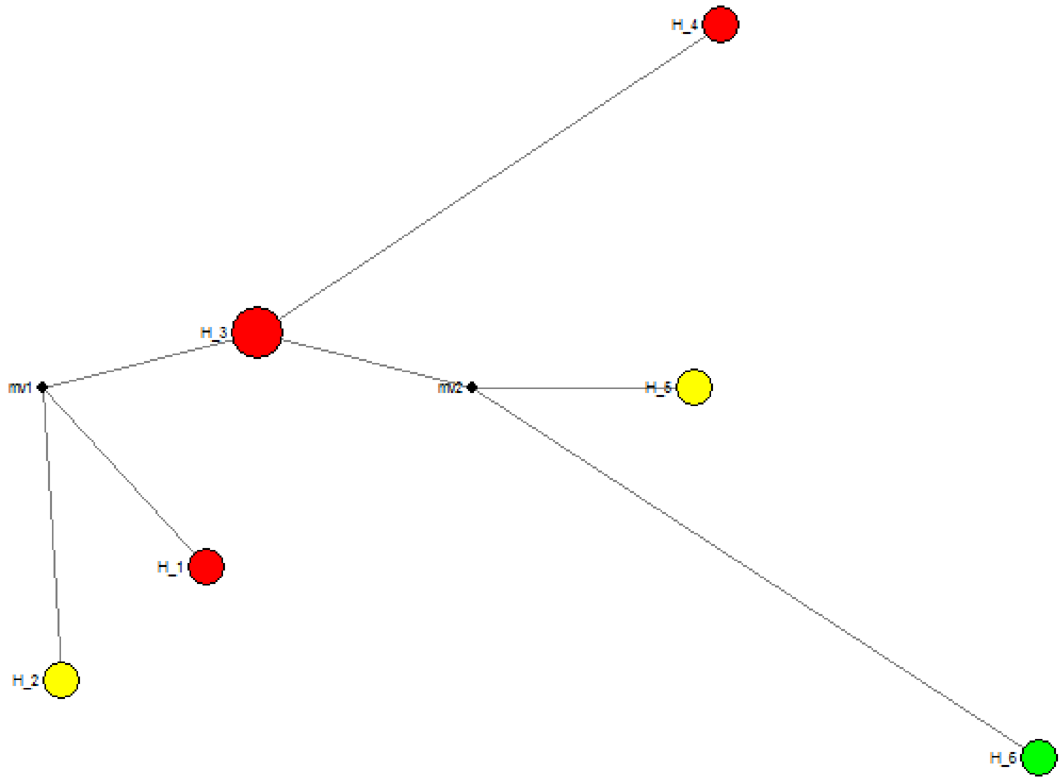


Fig. 5.19 *Median-joining network of 16S rDNA haplotypes of* Oneirophanta mutabilis*. Small black circles represent hypothetical intermediate haplotypes. Circle size corresponds to the relative sample size of haplotype frequencies*.

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