Project Description

I. Rationale: The hadal zone, which is comprised primarily of ocean trenches, represents the deepest marine habitat on Earth (6000-11,000m), accounting for the deepest 45% of the global ocean. Much of our knowledge of hadal biology is derived from two sampling campaigns in the 1950s (the Danish G*alathea* and the Soviet *Vitjaz* expeditions). These exploratory campaigns culminated in an initial catalogue of hadal species but they did not strategically sample at comparable depths or with sufficient replication to permit intra- or inter-trench comparisons upon which to draw ecological conclusions regarding demography or spatial population dynamics. Far from being devoid of life as originally perceived (Forbes & Austen 1859), additional opportunistic observations have since stimulated the hypotheses that the hadal zone hosts a substantial diversity and abundance of fauna with a high degree of endemism (Wolff 1960; 1970). However, as a result of historical factors and severe technical challenges associated with the extremes of hydrostatic pressure and distance from the sea surface, hadal systems remain among the most poorly investigated habitats on Earth.

The hadal zone cannot be considered as simply a continuation of the deep-sea environment because when depths exceed 6000 m the habitat splits into clusters of disjunct and isolated trenches. Furthermore, there is compelling evidence that food supply (in the form of particulate organic carbon; POC) accumulates along trench axes as a result of the characteristic V-shaped topography funnelling resources downwards (Itou 2000; Otosaka & Noriki 2000; Danovaro *et al*. 2003; Itoh *et al*. 2011). The combination of extremely high hydrostatic pressure, accumulation of food along trench axes and geographical isolation is believed to have resulted in these habitats having a high degree of endemism, and an extraordinarily high abundance of certain taxa and yet exclusion of other faunal groups.

Areas of specific and often extreme topography such as seamounts, canyons and trench systems foster a diverse range of habitats for deep-sea life (Vetter & Dayton 1998; Shank 2004; Tyler *et al*. 2009; Shank 2010; Clark *et al*. 2010; DeLeo *et al*. 2010). Geological, oceanographic, and biological interactions that can create and maintain "biological hotspots" in the ocean, which can ultimately control regional biodiversity, biogeography and the evolution of the deep-sea fauna (Weaver *et al*. 2004; Shank 2004). These same processes may however also serve to limit productivity, endemism and the diversification of populations among habitats. A number of hypotheses, encapsulated below, have been put forward to advance our ability to explain these observations in different regions of the oceans, including the influences of hydrographic boundaries, depth and species-specific life history differences, co-evolution and climate variation (Ruhl & Smith 2004; Jamieson *et al*. 2010).

A recent international meeting of leading trench scientists (*Trench Connection*, Tokyo, 2010) and a review of hadal environments (Jamieson *et al*. 2010) have highlighted the need to differentiate between the variables of hydrostatic pressure (and associated variables), food supply, and trench topography as drivers of community structure (including species diversity and endemism). Discerning between these key factors is currently impossible given the lack of data for any of them individually, but results and insights from a systematic co-located investigation of each will provide the currently needed foundation for understanding the formative processes that structure hadal ecosystems. It is therefore a high priority to pursue scientific inquiry in the deepest yet grossly under sampled environments on Earth (Webb *et al*. 2010). Given the fundamental scientific discovery and impact expected from basic systematic observations of trench environments, why haven't these data already been collected?

The majority of faunal samples retrieved from hadal depths have been opportunistic, if not haphazardly collected with non-standardised, semi-quantitative gear such as grabs, trawls and traps, unable to document robustly the structure of benthic communities and provide major advances in hadal ecological studies. These few samples have provided a wealth of information about the basic biology of some trench organisms (e.g., Wolff 1960, 1970). This notwithstanding, the lack of deep-submergence technologies to conduct systematic seafloor imaging and sampling surveys has prevented the scientific community access to quantitative faunal distributions, compositions, and an absence of environmental and physiological data

in space and time, which have hindered the application and development of essential ecological theory in this environment (Belyaev 1989).

The successful development of the fully functional Hybrid Remotely-Operated Vehicle *Nereus* (Fig. 1; Bowen *et al*. 2009) has now prompted scientists from around the world to come together in this proposal to identify the foremost standing questions in hadal science. In short, the ecological and evolutionary significance of depth/pressure adaptations, food-supply/trophic relationships, and topographically and depth-driven isolation of trench fauna pose fundamental questions form the basis of our overarching hypotheses below. Through our proposed Hadal Ecosystems Study (HADES) program, we will determine the composition and distribution of hadal species, the role of hadal pressures (piezolyte concentrations, enzyme function under pressure), food supply (distribution of POC with the abundance and biomass of trench organisms, metabolic rates/energetic demand), and depth/topography (spatial connectivity of populations) on impacting community structure.

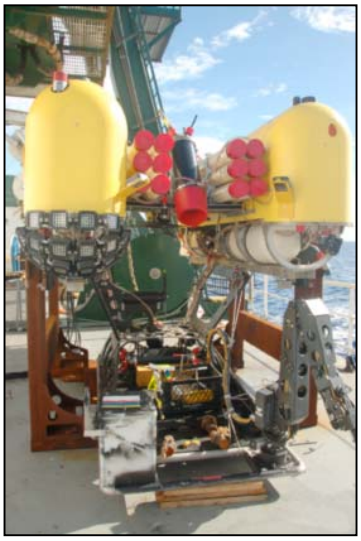


Figure 1. The newly-developed *Nereus* in ROV mode in May 2009 showing ceramic pressure housings, lateral and vertical thrusters, ROV-mode flotation spheres, robotic arm, digital imaging cameras, and LED lighting arrays.

II. Hypotheses

1. Differences in hadal megafaunal community structure occur as a function of depth, and in particular between topographically distinct hadal trench and abyssal plain habitats.
2. Seabed concentrations of organic carbon and bacterial biomass within the trench increase with depth, reaching maximums along the trench axis.
3. Megafaunal community structure within the trench changes as a function of seabed concentrations of organic carbon and bacterial biomass.
4. Depth and topographic differences between trench and abyssal environments have promoted the formation of genetically distinct species, and population genetic divergences vary with depth and habitat heterogeneity such that hadal populations exhibit greater genetic structure than those at abyssal depths.
5. Metabolism of hadal organisms can be predicted by depth and the nature of their predator-prey interactions, such that species with image-forming eyes (e.g., fishes) will have much lower metabolism than shallow water relatives.
6. Protein-stabilizing osmolytes, such as trimethylamine oxide (TMAO), vary as a function of depth, indicative of depth limitations in abyssal and hadal species.

III. Objectives: To address these hypotheses, we will investigate the major environmental drivers of trench ecology. Megafaunal composition and distribution will be examined as a function of depth and location by systematic high-definition imaging and sediment/faunal sampling transects from abyssal to full trench depths both along and perpendicular to the trench axis. Data from this sampling will enable us to explore the relationship between POC and benthic bacterial biomass, and faunal community structure. Population genetic approaches and the identification of evolutionarily independent lineages will assess the role of depth and topography in a trench and adjacent abyssal plain in promoting the formation of species and their populations. Physiological constraints will be investigated by examining in-situ respiration of selected fauna and tissue concentrations of such protein stabilizers as trimethylamine oxide (TMAO), and the structural adaptations of macromolecules. We feel these objectives represent an achievable and powerful combination of current technological capability, scientific understanding and theory, and the expertise of an international consortium of scientists. Our objectives are to:

1. Determine the composition, abundance, and diversity (i.e., community structure) of megafauna across the deepest 6000m in the Kermadec trench and surrounding abyssal plain.
2. Quantify the distribution of particulate organic carbon and bacterial biomass in trench and abyssal

plain sediments and

3. Relate carbon/biomass distribution to megafaunal distribution, density, depth and topography.
4. Determine whether topographic features (trenches and plains) and/or depth variation (and their predicted environmental heterogeneities) promote speciation/endemism and/or genetic divergence through genetic isolation of multiple species' populations.
5. Determine if the metabolic/respiration rates of trench fauna relate to depth and/or resource availability.
6. Determine whether or not the depth-range over which a species can live depends in part on pressure-counteracting osmolytes ("piezolytes").

IV. Anticipated Results

Through pursuing these objectives, we anticipate, *a priori*, the following results:

1. Community structure will significantly differ with depth, particularly between on axis and off axis surveys; suggesting that depth and trench topography play a significant role in controlling hadal species distribution and connectivity.
2. Concentrations of organic carbon within the trench will increase with depth, reaching a maximum along the trench axis owing to the accumulation of resources therein; sites of comparable depth in the axis will have greater sediment total organic carbon than sites perpendicular to the axis.
3. Benthic biomass and the abundance of epibenthic megafauna will be greater in the trench axis than at comparable depths perpendicular to the axis and that as depth increases from the abyssal plain to the deepest point this difference will become greater.
4. Genetic divergence across multiple species (hosting different inferred modes of larval dispersal) will be smaller in the abyssal than the hadal region, and genetic connectivity among populations will be significantly greater along the axis, than between the trench and abyssal populations.
5. The metabolic rates and enzyme activities of trench animals will be similar to those at abyssal depths but in some cases much lower than phylogenetically related shallower living animals. Within the trench (on and off axis stations) metabolic rates will remain similar intra-specifically so that changes in population density will be a direct function of food availability rather than fluctuating individual energetic demand.
6. Concentrations of pressure-counteracting osmolytes will increase with depth (to a maximum in bony fish around 8500m), and types will vary among species in accordance with their depth distributions.

V. Framework: Technical challenges associated with surveying and sampling extreme depths have prohibited this inquiry. As testament to these challenges (and lack of capable assets) was the 2003 at-sea loss of the only (at that time) full-ocean depth remotely-operated vehicle (ROV), *Kaiko*, after 20 dives to ~11000 m in the Mariana Trench (Momma *et al*. 2004). Now, the only scientifically proven vehicle in existence is the Hybrid ROV (HROV) *Nereus,* which is fully operational and capable of long-distance transits (>10 km at full-ocean depth), sampling sessile and mobile organisms, and sediments at full-ocean depth (Bowen *et al*. 2009; see WHOI Facilities). Additionally, advanced hadal-landers and mini-baited traps are now available and proven operational at full ocean depth (Jamieson *et al*. 2009bc, 2011; Fujii *et al*. 2010; see Aberdeen Facilities). To address the current lack of ecological understanding of trench environments, we propose to utilize these new state-of-the-art technologies, an international consortium of researchers and educators from 7 Institutions (University of Aberdeen, NIWA, NOC, UH, Whitman College, and WHOI) and our *a-priori* hypotheses under this framework to undertake the most comprehensive investigation to date of the biology and ecology of hadal megafauna in a single trench.

Given the historical knowledge of trenches around the world and ongoing research within our consortium, our objectives are best met when focussed on conducting them in the Kermadec Trench. The Kermadec Trench (SW Pacific) is a hadal environment that provided data for the earliest appreciations of trench ecosystems (Wolff 1960) and where some of the most recent trench research has been (and is being) undertaken (Blankenship *et al*. 2006, Blankenship & Levin 2007; Jamieson *et al*. 2009abc, 2011). Also key to enabling HADES-Kermadec is the immediate scientific leverage that comes from the "wealth" of historical data, and both active and anticipated research on the taxonomy, ecology, and evolution of fauna

at seamounts, slopes, vents, and canyons in the vicinity of the Kermadec Trench. The regional biogeography is reasonably well known (CANZ 2008) and a 6-year program involving our NIWA co-PIs (AR, MC with TS) is underway to understand the faunal differences between deep habitats in the region. The Kermadec Trench has a gradient in depth both across its axis and along its length, and lies in close proximity to a variety of other habitat types in the Kermadec region (seamounts, ridges, slope, and abyssal plain). As such, extensive comparisons between these habitats and the trench and abyssal environments can be made, placing the hadal fauna in a direct ecological context for the first time. Our co-PIs (AJJ *et al*.) currently have two funded field programs that will take place in the Kermadec Trench in the next two years to investigate scavenging fauna between 7 and 10 km deep (see Jamieson Letter of Collaboration). Our proposed research is designed to integrate into these ongoing studies for additional comparative value on the roles that pressure, topography, food supply, and isolation play in shaping faunal abundance, diversity, and endemism in the deep sea. As such, the HADES PIs will participate in these expeditions and coordinate research to accomplish our stated objectives.

The Kermadec Trench is the fifth deepest trench with a maximum depth of 10,047 meters (Angel 1982). It is 1500 km long with a mean width of 60 km and exhibits the characteristic V-shape cross section common to hadal trenches. The southernmost tip of the trench lies approximately 120 km off the coast of New Zealand. The trench is located under the South Pacific Subtropical Gyre (SPSG) province, which has an average primary production rate of 87 $g.C.m^{-2}.yr^{-1}$ (Longhurst *et al*. 1995). It is one of the coldest trenches in the world (~1.5°C) due to the incursion of deep-water originating from Antarctica. Recent years have seen the first stages of a new interest in benthic sampling within the trench, with the focus to date being mainly on the recovery of amphipoda and the imaging of fish and decapods (Blankenship *et al*. 2006, Jamieson *et al*. 2009bc, 2011). Prior to these studies, only 8 trawls have been carried out at hadal depths in the Kermadec Trench (Belyaev 1989) with only limited success. Nevertheless, these 8 trawls managed to recover >90 species from as deep as 10,015m. Figure 2 indicates that despite a low number of samples, most major faunal groups are present and at least four diverse groups (amphipoda, holothurioidea, polychaeta and bivalvia) extend to the deepest parts.

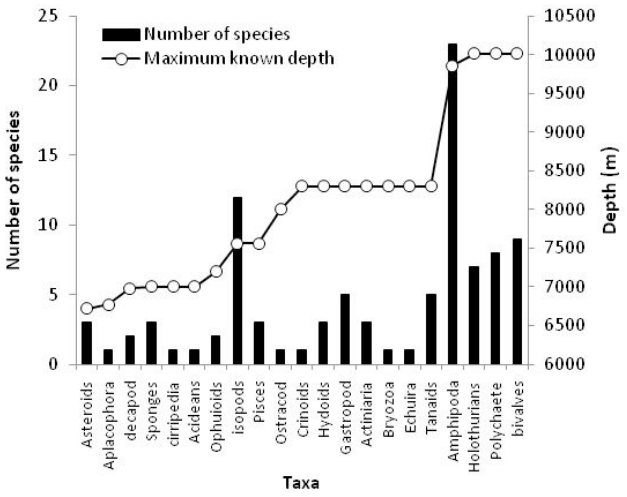


Figure 2. The number of species (95) and maximum depths of all known megafauna in the Kermadec trench.

VI. Proposed Research

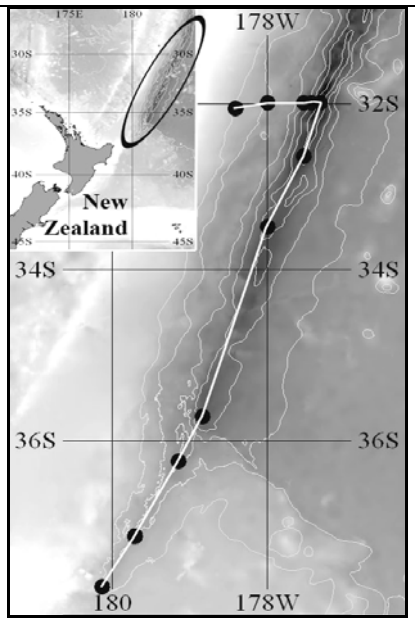
1. Hadal and Abyssal Community Structure

Objective: Determine the composition, abundance, and diversity (i.e., community structure) of megafauna across the deepest 6000m in the Kermadec trench and surrounding abyssal plain.

Testing our hypotheses and completing our objectives relies on careful site selection to disentangle the potential influence of depth and topography-induced food distribution. To determine whether the distribution of hadal megafauna is intrinsically linked to organic matter accumulation at the trench axis or merely a function of depth, we will perform our sampling along two transects: one along the trench axis and one perpendicular to the trench axis but both covering an equal depth gradient (Fig. 3). Thirteen stations have been identified based on these criteria and GIS analysis of trench topography to limit any adverse effects of extreme internal topography (i.e. internal escarpments). The on-axis imaging transect will focus on a 4, 5, 6, 7, 8, 9 and 10 km site in the southern sector of the trench. The off-axis transect will begin at the previous 10 km site and run west at the same depth intervals to 4 km. The off-axis transect was chosen via a combination of equal gradient across the depth range and included an area of equal

depths to the trench axis (the min depth to the east is 6 km). To characterize megafaunal community structure, *Nereus* will perform high-definition imaging and sampling transects. Methodological details can be found in Section VII.

SITE	Axis	Depth	Latitude	Longitude
St. 01	ON	4000	179°49'30"E	37°44'34"S
St. 02	ON	5000	179°42'48"W	37°06'12"S
St. 03	ON	6000	179°05'54"W	36°10'47"S
St. 04	ON	7000	178°52'25"W	35°49'28"S
St. 05	ON	8000	178°10'33"W	34°07'09"S
St. 06	ON	9000	177°32'13"W	32°36'55"S
St. 07	ON	10000	177°18'02"W	31°57'07"S
St. 08	OFF	9000	177°21'00"W	31°55'49"S
St. 09	OFF	8000	177°23'39"W	31°54'42"S
St. 10	OFF	7000	177°29'47"W	31°53'15"S
St. 11	OFF	6000	177°34'23"W	31°52'45"S
St. 12	OFF	5000	177°53'58"W	31°51'23"S
St. 13	OFF	4000	178°18'30"W	31°47'58"S



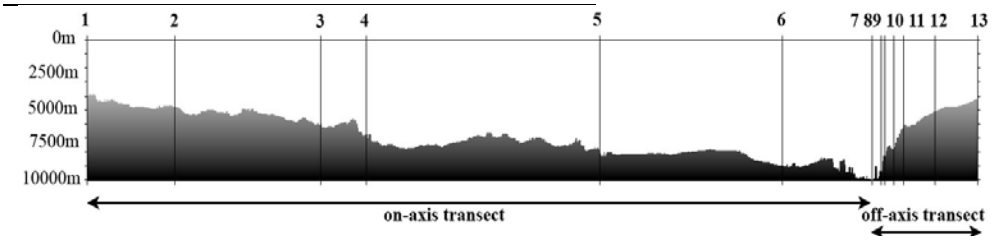


Figure 3. The survey and sampling design of the proposed HADES-Kermadec program. The on and off-axis locations of the 13 survey and sampling stations and their geographic setting in the Kermadec Trench and adjacent abyssal plain are shown. Also shown below is a bathymetric profile indicating the locations and depth coverage of the on-axis and off-axis transects.

2. Distribution of Food Supply and Megafauna

Objective: Quantify the distribution of particulate organic carbon and bacterial biomass in trench and abyssal plain sediments and relate this to megafaunal distribution, density, depth and topography.

Most heterotrophic organisms within trenches receive energy from surface-derived POC. Chemosynthetic bacterial communities also exist at hadal depths (Fujikura *et al*. 1999), but these are not expected to play a major role in general hadal ecology owing to their spatially-restricted distribution (Jamieson *et al*. 2010). The vertical flux of POC decreases exponentially with depth (De La Rocha & Passow 2007; Buesseler & Boyd 2009). This is thought to explain the observed reduction in faunal biomass from the surface to 6000m (Sibuet *et al*. 1989; Rex *et al*. 2006; Wei *et al*. 2010). The relationship between benthic bacterial biomass and depth across this range is less clear; these variables show no evidence of correlation at the global-scale (Rex *et al*. 2006; Wei *et al*. 2010), but highly significant, positive correlations do exist between benthic bacterial biomass and POC flux across a range of contrasting deep-sea environments (Deming & Carpenter 2008). Co-linearity between depth and resource availability from the surface to abyssal depths confounds our ability to differentiate between the effects of these variables on the vertical distribution of marine organisms.

The notion that energetic and nutritional resources accumulate along trench axes (*e.g*. Danovaro *et al*. 2003) suggests that the negative relationship between depth and POC concentrations breaks down at hadal depths. This provides a unique opportunity to distinguish between the effects of depth and resource availability on benthic biomass. We hypothesize that, in the absence of hydrostatic pressure limitations,

the biomass maxima of benthic bacteria and deposit-feeding organisms within a hadal trench should both occur at the trench axis owing to the accumulation of resources therein. Anecdotal evidence supports this hypothesis: Holothurians are present at the trench axes in abundances much greater than observed relationships to 6000 m (Wolff 1970; Belyaev 1989; Fig. 4). Other taxa, such as amphipods, are extremely prevalent in the trenches (Hessler *et al*. 1978; Blankenship *et al*. 2006); their population density increases exponentially with depth (Jamieson *et al*. 2009c; Fig. 4). Hadal amphipods are opportunistic scavengers (Jamieson *et al*. 2009c; 2010), however, sediment and bacteria are regularly found in their guts suggesting that detritivory, bactivory and possibly also predation are their principal feeding modes (Blankenship and Levin 2007). It follows that the elevated amphipod abundances at the greatest depths likely reflects their reliance upon the benthic food web and hence the accumulation of POC at the trench axis. Nevertheless, the lack of quantitative information on the abundance and distribution of hadal organisms and their food makes it currently impossible to robustly prove or refute this idea. The area of the trench's seafloor decreases logarithmically with depth (Fig. 4) with synoptic increases in megafauna abundance, which reverses the declining abundance with depth trend observed to 6000 m. We therefore further hypothesize that the holothurians and amphipod populations in the trench are intrinsically linked to accumulated resources. We expect that sampling equivalent depths on and off the axis will allow us to confirm this hypothesis by isolating depth and topographic position as factors.

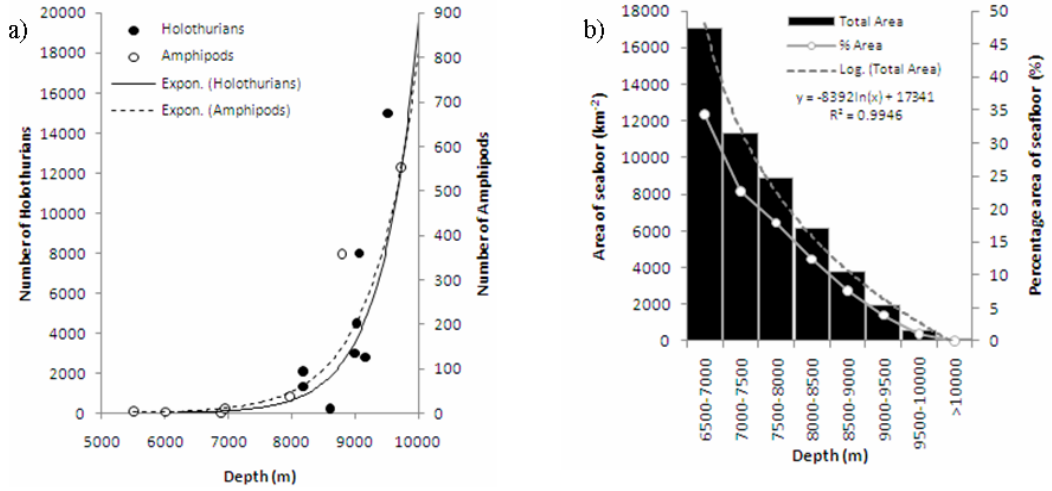


Figure 4. (a) Exponential increase with depth of holothurians numbers recovered by trawl (Wolff, 1970) a visible numbers of amphipods using baited cameras (Jamieson *et al*. 2009c). (b) The total area of seafloor (km^{-2} and %), in 500m depth bins, decreases logarithmically with depth in the Kermadec Trench.

3. Genetic Divergence and Connectivity with Depth

***Objective*:** Determine whether topographic features (trenches and plains) and/or depth variation (and their predicted environmental heterogeneities) promote speciation/endemism and/or genetic divergence through genetic isolation of multiple species' populations.

A characteristic feature of hadal environments is the perceived large degree of local endemism. Such endemism is considered a product of both the isolation of trenches and also the extreme selection pressures associated with hadal environments and concomitant potential for rapid divergence and speciation. The mechanisms or factors that drive high degrees of local endemism in some taxa while others are ubiquitous are often difficult to discern. Evolutionary theory would predict that endemism reflects population isolation and localised selection pressures while ubiquity is a consequence of dispersal. The almost complete absence of information on hadal population genetic and phylogeographic structure across such scales means such an assertion is entirely speculative. Studies of several abyssal fauna have found genetic differentiation across similar depths for example, molluscs (Etter *et al*. 1999, 2005; Peek *et al*. 2000; Quattro *et al*. 2001), corals (France *et al*. 1996; Le Goff-Vitry 2004a, b), the

munnopsoid isopod *Betamorpha fusiformis* (Raupach *et al*. 2007), and the abyssal protobranch bivalve *Ledella ultima* (Etter *et al*. 2011).

In contrast, several studies have shown that vertical depth separation more than horizontal spatial separation is promoting genetic differentiation (Etter *et al*. 2011). This genetic differentiation "by depth" has been documented for several abyssal fauna (reviewed in Creasey & Rogers 1999) including gastropods (Siebenaller 1978), amphipods (Bucklin *et al*. 1987, France 1994, France &Kocher 1996), and bivalves (Chase *et al*. 1998; Zardus *et al*. 2006). In many cases, high genetic differentiation with depth may result in species complexes that are morphologically similar but genetically distinct (Creasey and Rogers 1999; Raupach *et al*. 2007). In addition, levels of population divergence vary with depth such that bathyal organisms appear to exhibit much greater population structure than do those from abyssal depths (France & Kocher 1996; Zardus *et al*. 2006). This Depth-Differentiation Hypothesis (DDH; Etter *et al*. 2005) suggests that, based largely increased environmental heterogeneity, the continental margin/bathyal regions may be the preferred locations of adaptive radiation. The obvious contrast between bathyal and abyssal patterns suggests that the ecological and evolutionary forces that promote population differentiation decrease with depth. The reasons why divergence is lower at abyssal than bathyal depths has been attributed to the abyssal environment being less variable, with weaker environmental gradients, reduced environmental heterogeneity and lower diversity at abyssal depths may limit population differentiation (Etter *et al*. 2011). This may also be the case in the comparison of abyssal to hadal environments and levels of divergence. However, the increased heterogeneity of the trench geomorphology, with hard and soft substrates, and increased/channeled food supply, may provide explanations for higher levels of genetic divergence in hadal systems. Our proposed work will document environmental variables in concert with our genetic studies to provide the first comparative test of the "DDH hypothesis" between hadal and abyssal depths.

To assess the level of genetic differentiation (nuclear and mitochondrial genomes) and connectivity in hadal and abyssal environments, we will examine the connectivity of trench and adjacent abyssal populations through phylogeographic and population genetic analysis of within-species genetic structure from *Nereus* sampling transect collections, an array of baited traps, sediment sample collections, and opportunistic samples obtained from previous expeditions in neighbouring habitats. For the cosmopolitan amphipod species, we already have data on genetic variation from several sources: 1) mitochondrial DNA sequences such as ND5/6 that mutate at a sufficient rate to provide intra-specific differences and; 2) a suite of 12 microsatellite markers that we have recently developed. Data will be analysed using both standard landscape genetic and coalescent frameworks allowing historical and contemporary ecological dispersal to be teased apart, and ultimately providing an understanding of connectivity in relation to topography and distance. Moreover, on-going characterization of the *Eurythenes* transcriptome (Piertney & Jamieson, unpublished) will provide a large number of nuclear single nucleotide polymorphisms (SNPs) that can be used to augment existing markers. In addition, such markers will facilitate so-called outlier analysis within a population genetics framework, which will identify those gene regions showing greater divergence than expected under neutral theory, and hence under the influence of selection. We will use DNA barcoding techniques (16S and COI) to classify hadal megafauna from which phylogenies will be produced (e.g., Shank *et al*. 1999). This genetic data will then be correlated to morphology and ecology (local density, density-size distributions and environmental conditions) data. Given our previous experience, it is expected that multiple cryptic and non-cryptic species new to science will be sampled and subsequently described.

4. Metabolic rates of trench fauna

***Objective*:** Determine if the metabolic/respiration rates of trench fauna relate to depth and/or resource availability.

In order to understand what drives the distribution and abundance of the dominant trench fauna, we must also know the energetic demands of the individuals and populations. Metabolic rates can be used to construct models of the flow of energy and materials in an ecosystem (Childress & Thuesen 1992; Smith,

1992; Christiansen *et al*. 2001; Smith *et al*. 2001). In the deep sea, studies have assumed that energetic demands can be extrapolated from data on shallow living animals by using models of the mass and temperature dependence of metabolic rate (Mahaut *et al*. 1995), or they have been based on a handful of measurements of representative taxa (Smith, 1992; Smith *et al*. 2001). It remains unclear whether the energetic demands/metabolism of the trench fauna can be extrapolated from work on shallower living animals.

Studies of some animals such as pelagic and benthopelagic fishes, crustaceans and cephalopods show order of magnitude lower metabolic rates in deep-sea species (to ~2000m) compared to shallow water animals (Drazen & Seibel, 2007; Seibel & Drazen 2007). However, for some benthic fishes, a few amphipods, and crabs, there are no apparent differences in metabolism after the effects of habitat temperature and body size are taken into consideration (Seibel and Drazen 2007; Drazen *et al*. in revision). An exhaustive analysis of echinoderm respiration rates also showed no apparent trend with depth (deepest known echinoderm respiration rate from 4,100 m), when the effect of temperature was eliminated (Hughes *et al*. submitted). It is unlikely that pressure explains these data. Enzymes adapted to high pressures can be inefficient (Somero and Siebenaller 1979), which can lead to lower metabolic rates. However, capacity adaptations allow an organism to maintain a level of performance regardless (Hochachka and Somero 2002; see also section below). This is confirmed by constant levels of enzymatic activity in the brains and hearts of fishes regardless of depth (Childress &Somero 1979; Sullivan & Somero 1980; Siebenaller *et al*. 1982). Studies of metabolic rates under varying pressure show no effect in fishes or crustaceans (Meek & Childress 1973; Childress 1977; Belman & Gordon 1979). One hypothesis to explain lower metabolic rates in some deep-sea animals is that the animals have evolved lower routine rates in order to match a low food supply (Childress 1971; Smith & Hessler 1974; Collins *et al*. 1999; Treude *et al*. 2002). The low rate of metabolism in an abyssal amphipod was hypothesized to be an adaptation to low food supply (Treude *et al*. 2002) and a variety of animals respond to low rations by depressing routine metabolism (Sullivan and Smith, 1982; Christiansen & Diel-Christiansen, 1993; Yang and Somero, 1993). Interestingly, photographic observations of the behavior of liparid fishes living in the Kermadec trench with potentially greater food supply than the abyssal plain, suggest relatively high activity (Jamieson *et al*. 2009). If food supply is a determinant of individual metabolism, then we expect higher metabolic rates in animals found along the trench axis, irrespective of depth.

An alternative is the Visual Interactions Hypothesis (VIH; Childress 1995; Seibel & Drazen 2007). In brightly lit surface waters animals are able to detect both predators and prey at a great distance (Lythgoe 1988) and must have higher metabolic rates and greater locomotory capabilities for long chases or escapes. In the dimly lit and sparsely populated deep-sea, predators and prey do not interact as frequently or over as large a distance, relaxing the need for locomotory capacity which reduces metabolism. This argument is supported by the fact that sighted taxa exhibit depth related metabolic declines but non-visual groups such as holothurians do not (Seibel & Drazen 2007). In contrast, recent data on non-visual copepods found declines from the epipelagic to the abyssopelagic (Ikeda *et al*. 2006). The VIH would predict that nonvisual hadal animals would have rates of metabolism similar to their shallow living relatives (at the same temperature) and visual hadal animals would have rates much lower than shallow living animals but similar to those in bathyal and abyssal habitats because there is effectively no visible light below ~1000m to warrant further metabolic reductions (Warrant & Locket 2004). However, in previous studies it has been difficult to separate the covarying effects of food supply, temperature, light, pressure, and oxygen on the observed trends. The trench ecosystem provides a unique opportunity in which food supply may actually show an inverse relationship with depth (increases towards the axis) while light levels, temperature and oxygen will vary little. This allows for a robust test of the two hypotheses to explain variation in metabolic rates.

We will use an in-situ respirometer to measure the metabolism of megafauna at each of the proposed stations along and across the trench axis. Muscle samples will also be examined for the rates of key enzymes of intermediary metabolism. These enzyme activities have correlated well with metabolic rates

(Childress & Somero 1979; Hochachka & Somero 2002; Dalhoff 2004). Regardless of which hypothesis is correct the metabolism data will help us understand how food supply regulates the distribution and density of the hadal megafauna. If respiration remains consistent for a species regardless of food availability then we could expect that changes in population density would be a direct function of food supply mediated by competitive interactions (see objective 2). The derived data on metabolism and associated energetic demands will provide important information for future food web and carbon budget modelling of trench ecosystems.

5. Physiological pressure adaptations

Objective: Determine whether or not the depth range over which a species can live depends in part on pressure-counteracting osmolytes ("piezolytes").

One of the defining characteristics of deep-sea habitats is hydrostatic pressure, which can have large perturbing effects on biological molecules. The ability to adapt to high pressure as well as to different pressures may be an important limit on species distributions. Membranes and proteins from deep-sea organisms have been found to have structural adaptations which confer pressure resistance (Hochachka & Somero 1984). However, many proteins such as homologs of actin from deep species, while having evolved some pressure resistance, still retain some sensitivity. Others such as the enzyme pyruvate kinase (PK) are highly pressure sensitive even in homologs from deep-sea animals. In recent years a different adaptation to pressure has been hypothesized involving "piezolytes." These are small organic solutes first discovered as organic osmolytes, solutes accumulated by most marine organisms to prevent osmotic shrinkage of their cells by osmoconforming to the environment (osmotic pressure of about 1000 mOsm). The main osmolytes in animals are: (1) neutral amino acids (taurine, glycine, etc.) and the methylamines glycine betaine and trimethylamine oxide (TMAO; the source of the "fishy smelling" trimethylamine) in most marine invertebrates; and, (2) urea and TMAO in Chondrichthyes (e.g., sharks, skates, etc.). In contrast to these osmoconformers, most marine Osteichthyes (bony fish) are osmoregulators, maintaining an internal osmotic pressure of about 300-400 mOsm. Shallow bony fish do contain TMAO but only at about 40-50 mOsm.

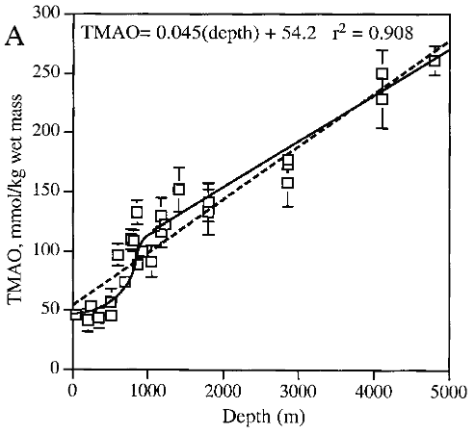


Figure 5. TMAO in bony fishes as a function of depth. Dashed line shows a linear fit for all the data. Solid curve shows a sigmoidal (third-order polynomial) fit for 0–1,400 m.

Such organic solutes are thought to be selected as osmolytes over inorganic solutes because the latter can perturb macromolecules while the former usually do not; i.e., they are "compatible" with cellular functions (Brown and Simpson 1972). More importantly, many osmolytes actually stabilize macromolecules and can counteract perturbants (Yancey 2005). This is of fundamental importance to deep-sea adaptation, because analyses of deep-sea bony fishes, elasmobranchs, shrimp and crabs have revealed that TMAO contents increase with depth (Kelly & Yancey 1999, Samerotte *et al*. 2007) (Fig. 5). In bony fish, internal osmotic pressure increases with depth as of result of increasing TMAO content. In osmoconformers, as TMAO increases with depth, other osmolytes decrease in osmotic compensation—urea in elasmobranchs and glycine in decapod crustaceans (Kelly & Yancey 1999). In deep-sea holothurians, as free amino acids decline with depth, the rare polyol scyllo-inositol and an unidentified solute increase (Yancey *et al*. 2002). Most importantly, in the laboratory, TMAO (but not glycine) counteracts the perturbing effects of hydrostatic pressure on enzyme kinetics (including PK) and protein stability and assembly (including actin) (Yancey and Siebenaller, 1999; Yancey *et al*. 2001, 2004; Yancey, 2005). Scyllo-inositol is a stabilizer that, among other effects, can block amyloid-beta formation (McLaurin *et al*. 2000). Its effects on proteins under pressure are not known. Other osmolytes were found to be accumulated by some deep-sea bacteria when pressurized, and were

dubbed "piezolytes" (Martin *et al*. 2002). Thus, we hypothesize that TMAO (and other stabilizing osmolytes), acting as piezolytes, help organisms adapt to the deep-sea, and the ability to accumulate such solutes may limit depth distributions.

However, osmolyte analyses have only been done on fishes down to 4900 m and holothurians and crustaceans down to 2900 m. What happens at greater depths, particularly in the trenches at nearly 11,000 m? Do concentrations of stabilizing osmolytes continue to increase in proportion to depth down to the trench bottom? At such depths the concentrations required for protein function may be in excess of what can be supported osmotically. In bony fishes if the trend of increasing TMAO with depth shown in Figure 5 were to be extrapolated to greater depths, the point at which the fishes would become isosmotic with seawater is about 8000- 8500 m, roughly the depth of the deepest fishes observed or captured (Nielsen 1977, Jamieson *et al*. 2009). It may be physiologically difficult to elevate TMAO above this level. If there is a limit to the accumulation of TMAO, are there other solutes in the deepest animals (e.g., holothurians, amphipods) that are more effective at counteracting pressure? Or have their proteins evolved greater structural resistance to high pressure? Through testing our hypotheses, we will address these questions.

VII. Operational and Methodological Approaches

Operations Overview and Investigator Responsibilities: HADES-Kermadec will conduct the activities and operations described below and as represented in Figure 6.

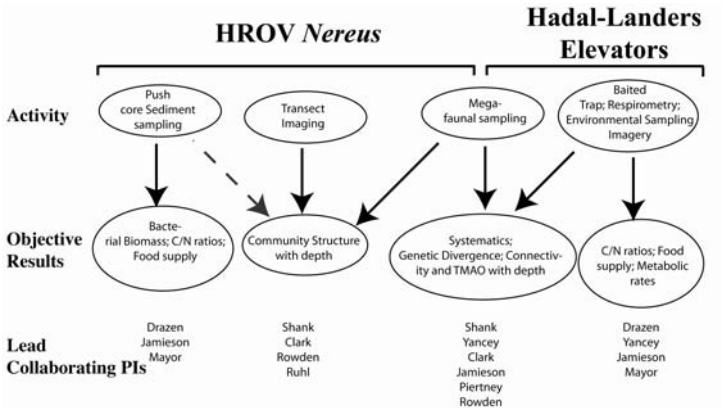


Figure 6. HADES-Kermadec overview indicating HROV *Nereus* and Hadal-Lander/ Elevator operations, objective comparative datasets with depth and topographic position on and off axis, and responsible lead US investigators and their collaborators in this program.

HROV *Nereus* and Hadal-Lander Operations Plan: The HADES-Kermadec expedition is proposed for the optimal weather window, the austral spring of 2012. At each of the sites in Fig. 1, the hadal landers and elevator will be deployed then the *HROV Nereus* will dive to the elevator location with aim of achieving 12 hours of bottom time at each station (Table 1). Upon reaching the seafloor, the HROV will locate the elevator and conduct systematic grid searches for target species in which to deposit into the respirometer (4 hours allocated to these tasks). Following respirometer activation, *Nereus* will conduct replicate imaging transects (3 hours). On completion of each, *Nereus* will return along the approximate navigated three transect lines to collect the observed constituent fauna, and obtaining push cores (5 hours allocated). Once back at the transect start position (the Hadal-Lander/elevator), the HROV will start the ascent to the surface. Then the elevator, traps and Hadal-Lander (see Aberdeen Facilities) will one by one be released from the seafloor by acoustic command from the ship. The charging period of *Nereus* (10.5 hours) can take place simultaneously with the recovery of the free-fall systems to increase ship-time efficiency. Also, while the free-fall systems are being recovered and *Nereus* is charging, the samples from the dive will be prepared, sorted and fixed accordingly. The landers can be quickly turned around - servicing batteries, retrieval of samples, and reballasting.

Table 1. Deployment activities with time allocation (hours) at each site over our 21 day HADES expedition. The total working station time is ~21 days. Note the HROV charge time occurs simultaneously with elevator, lander and trap recovery (10.5 hours).

Station	1	2	3	4	5	6	7	8	9	10	11	12	13
Depth (km)	4	5	6	7	8	9	10	9	8	7	6	5	4
Axis	ON	ON	ON	ON	ON	ON	ON	OFF	OFF	OFF	OFF	OFF	OFF
Deploy Lander	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Deploy Traps	1	1	1	1	1	1	1	1	1	1	1	1	1
Deploy Elevator	1	1	1	1	1	1	1	1	1	1	1	1	1
HROV descent	4	5	6	7	8	9	10	9	8	7	6	5	4
Locate elevator	1	1	1	1	1	1	1	1	1	1	1	1	1
Elevator set-up	3	3	3	3	3	3	3	3	3	3	3	3	3
Video transect	3	3	3	3	3	3	3	3	3	3	3	3	3
Coring/Slurping	5	5	5	5	5	5	5	5	5	5	5	5	5
HROV ascent	4	5	6	7	8	9	10	9	8	7	6	5	4
Elevator ascent	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Lander/Trap recovery	8	8	8	8	8	8	8	8	8	8	8	8	8
Total Hours	33	35	37	39	41	43	45	43	41	39	37	35	33

More specifically, at each site, *Nereus* will conduct 1.5 km of high-definition video transects running parallel to the trench axis along a given contour at 0.15 $m.s^{-1}$. The 1.5 km will be divided into three replicate transects each of which will start at randomly selected sites within 1 km radius of the elevator with direction determined by local conditions. *Nereus* will perform random sampling transects between the image transect stations whereby epibenthic organisms are collected using either scoop or slurp gun and deposited into a container. The specimens will be used as outlined in our proposed research, including for taxonomy, physiology, groundtruthing the image analysis, as well as phylogenetic and population genetic analysis. At the start and end of each transect replicate, *Nereus* will obtain 6 push cores for organic matter content and bacterial biomass analysis (total 24 cores per dive). One set (4 tubes) of replicate sediment cores per site will be sliced at 1cm horizons and frozen at -80°C, while other replicate tube cores (2) will be processed with nested sieves such that the 300 and 45 μm fractions are retained and processed for macrofauna and meiofauna using standard methods (Neira *et al*. 2001). This set of organisms will be chilled, photographed and split preserved in molecular grade ethanol and -80°C frozen for potential future investigation outside the scope of our immediate program. Small tissue samples of constituent of all faunal species will be preserved and frozen in liquid nitrogen and transferred to -80°C pending organic osmolyte content and enzymatic analysis after the cruise.

1. Community assessment from transect image analysis: Video imaging transects will be conducted at less than 0.5 m altitude from the seafloor using broadcast high-definition video imaging and LED lighting. The seafloor area of the video frame will be calculated from the field-of-view width multiplied by distance travelled. Epifaunal megafauna observed on the video will be identified to the lowest practicable taxonomic level, counted and sized. Voucher specimens from the collecting transects will be indentified, measured and weighed prior to preservation (and genetic analysis), and will be used in aiding identification and calculating biomass from the video transects. Resulting megafaunal species composition, abundance and biomass data, and derived diversity measures, from each individual video transect will be appropriately partitioned and calculated to allow statistical comparisons within and between the factors pressure (depth strata) and topographic setting (location). The statistical routines ANOSIM and SIMPER of the multivariate software package PRIMER will be used to test and describe the effects of the main factors on community structure. The routine RELATE will be used to examine the extent to which community structure is affected by pressure, depth, slope, substratum type, sediment organic carbon content and bacterial biomass. Data for these variables will be forthcoming from instrumentation aboard *Nereus*, analysis of the video images, multibeam data, and the sediment cores collected. Comparable univariate analyses will be conducted using appropriate measures of species diversity (the type of diversity metrics used will ultimately depend upon the degree of variability in the seafloor area imaged by each individual transect) and standard ANOVA and regression analysis.

2. Environmental Data and samples from Hadal-Landers: One limitation of using a ROV for specimen collection is their inability to image and capture faster moving demersal fauna such as decapods, fish and amphipods. Therefore, prior to the *Nereus* dive, the autonomous Hadal-Lander and trap array will be deployed. These systems descend to the seafloor, unattached to the ship. The camera lander will take high-resolution time-lapse images obtaining very accurate response times of scavenging and bait-attending fauna for theoretical population estimates following Sainte-Marie & Hargrave (1985). It also provides data to map the distribution of bait-attending species, most of which are gregarious and not observed using ROVs. This lander will also record salinity, temperature, pressure, and current speed/ direction during the deployments. A lander array of six small free-fall closable traps will be baited with locally-sourced tuna flesh and deployed in a line running parallel to the trench axis along a contour with traps spaced 500 m apart. Samples from the trap array will be indentified, counted, measured, weighed, sexed and staged to produce a bathymetric gradient of the these population parameters. The trap samples will be preserved in molecular grade ethanol with the exception of liquid nitrogen fixed tissue samples for TMAO and enzyme analysis. The fixed trap specimens will then be made available for taxonomy (plus new description where applicable) and made available for the phylogenetic and phylogeographic analysis.

3. Genetic divergence and connectivity: In order to identify species populations and define the geographic distributions of sampled (and obtained*) taxa, first-order molecular systematic and phylogenetic analyses will be performed using proven approaches and software packages (e.g., PAUP* 4.0b10 [Swofford 2002]), MrBayes [Huelsenbeck & Ronquist 2001]) to generate phylogenetic trees from nucleotide alignments. Our phylogenies will involve comparisons at the intrageneric level, and thus we do not anticipate problems with assessing homology for nucleotide alignments of protein-coding genes. Standard parsimony, likelihood, and Bayesian analyses will be conducted on taxa in order to assess their evolutionary relationships (e.g., Shank *et al*. 1998, 1999). Where multiple genes have been sequenced, we will run analyses on single gene and concatenated datasets in a combined analysis. Topological robustness (i.e., support of nodes) will be assessed using nonparametric bootstrap methods (Felsenstein 1985) and likelihood ratio tests (Huelsenbeck & Rannala 1997). Once species and their populations with depth have been identified through phylogenetic assessment, we will assess the historical connectivity over topographic features and depth strata using a suite of analyses as in Cho and Shank 2010. Analyses of molecular variance (AMOVA) will utilize pairwise Φ_{ST} values to test for the geographic/genetic isolation of populations within the trench, between the trench and abyssal plain, and grouped based on depth strata. To detect and examine genetic structure on the scale of 'adjacent depth strata,' a series of AMOVA will be performed with a sliding window (Plouviez *et al*. 2009; Cho and Shank 2010), grouping three 'adjacently'-sampled depth strata populations together per run, moving downslope. In addition statistical parsimony and nested clade analysis will be used to infer the demographic history of the species and historical rates of gene flow will be assessed using coalescent methods in the software package MIGRATE using the Bayesian framework (Beerli & Felsenstein 2001).

* We will make full use of reference museum and private collections of taxonomically similar fauna from other trenches around the world. We fully recognize the invaluable information that can be gathered by including these samples for understanding the potential isolation occurring between trenches (see our introduction), and will make every opportunity toward this end.

4. Quantifying food resources and benthic bacterial biomass: Upon retrieval of sediment cores, the 0-1, 1-2, 2-3, 3-5, 5-10 cm depth horizons will be sectioned and stored frozen (-80 °C) until analysis. Food availability, described as the bulk quantities of organic carbon and nitrogen and bacterial biomass, will be determined from freeze dried and decalcified (Hedges & Stern 1984) sediment samples with a Fisons NA 1500 elemental analyser using sulphanillic acid as a standard. Benthic bacterial biomass will be calculated from sediment concentrations of the bacterial biomarker phospholipid fatty acids (PLFAs) i14:0, i15:0, ai15:0, i16:0 (Gontikaki *et al*. 2011; Mayor *et al*. submitted). In brief, PLFAs will be extracted and purified from known quantities of freeze-dried sediment samples (Bligh & Dyer 1959; White *et al*. 1979) and subsequently derivitized to yield fatty acid methyl esters (FAMEs). FAMEs will be quantified using

GC-FID (Agilent Technologies 6890N). Bacterial biomass will be calculated using the well-established PLFA:C conversion factor of 0.056 gC PLFA/gC biomass (Brinch-Iversen & King 1990), assuming that the aforementioned biomarker PLFAs represent 10% of the total (Gontikaki *et al*. 2011; Mayor *et al*. submitted). Data exploration will be undertaken to determine the most appropriate form of statistical analysis (Zuur *et al*. 2010). We anticipate that the relationship between bacterial biomass, depth, location and bulk POC will be examined using linear mixed-effects statistical models that account for inherent correlations that exist between replicate observations made at each sampling location (Mayor *et al*. 2010). We will also use GIS software (ArcGIS v10; ERSI, USA) to generate maps of trench resource accumulation zone based on these data.

5. Direct measurements of energetic demands: We will construct a *Nereus*-operated six-chamber respirometer to measure the energetic demand of hadal (deep-sea) megafauna. The system will be deployed on a depth-adapted elevator to the seafloor. We anticipate using, at each site, one chamber as a control, three chambers for holothurians, and two chambers for amphipods, these animals being the dominant megafauna (Jamieson *et al*. 2010). *Nereus* will collect holothurians from the seafloor using its suction sampler and scoop, place them in the chambers and seal them. *Nereus* will place bait (occluded in fine mesh to prevent feeding) in the chambers to attract them, then remove the bait and seal the chamber.

The respirometry system will be adapted from systems used to 3000 m (Drazen, 2005; Drazen & Yeh, submitted). Each chamber will be equipped with 11km rated modified Aanderaa oxygen optode/temperature sensors, and a small water pump with electronics in a titanium pressure housing. Chambers will be 250-500ml and will be easy to service at sea between deployments based on initial results. Based on volume and anticipated metabolic rates of ~0.1-1.0 μmol O_2 g^{-1} hr^{-1} (Seibel and Drazen, 2007) incubations should be from 10-14 hours so that an ample oxygen depletion can be recorded.

Metabolic enzyme assays will be conducted on all species used in the respirometery experiments. Trapping will also allow data collection from more mobile animals such as liparid fishes, larger amphipods, and decapods shrimps. Metabolic enzyme assays, as proxies for metabolism (reviewed in Hochachka & Somero 2002; Dalhoff 2004), will follow those described in Drazen (2002) and Drazen *et al*. (in revision) in a temperature-controlled spectrophotometer at the ambient temperature of collection. Lactate dehydrogenase (LDH) and pyruvate kinase (PK) will be used as indicators of anaerobic metabolism and LDH as indicative of burst locomotor capacity. Citrate synthase (CS) is a key regulatory enzyme in the Krebs cycle and its activity generally scales with whole animal metabolic rate, thus its activity will be a good indicator of aerobic metabolism. Malate dehydrogenase (MDH) is also an important component of the Krebs cycle and it may also be involved with redox balance in the cytoplasm.

6. Physiological pressure adaptations: Two types of analyses will be done using frozen muscle samples from freshly collected animals on dry ice, and stored at -80˚C. First, in the first summer, osmolyte/piezolyte analysis will be done. The samples will be homogenized and deproteinated with perchloric acid or ethanol, then analyzed for TMAO by a standard colorimetric method and by high-performance liquid chromatography, which detects numerous potential organic osmolytes including other methylamines, polyols, and amino acids. This process will continue in the second summer, in which solutes not identifiable by this method will be isolated by HPLC and analysed by NMR and mass spectrometry. All methods have been used successfully for many years in the Yancey laboratory (e.g., Kelly & Yancey 1999; Yancey *et al*. 2002). The second type of analysis will occur in the third summer: key proteins including PK and actin will be tested for stability and kinetics under pressure with and without relevant osmolytes. This testing involves tissue extracts placed in pressure chambers and spectrophotometric measurements, as done previously (e.g., Siebenaller & Yancey 1999; Yancey *et al*. 2001). Tissues of shallow and moderate-depth holothurians, crustaceans, and fish (frozen at Whitman College) will be tested for comparison. The combined results of osmolyte/piezolyte composition and protein pressure sensitivities should allow us to determine whether pressure has a role in faunal depth distributions.

IX. Broader Impacts

Results will be disseminated broadly to the scientific community and the public: Deep-sea trenches and the potential communities they support have fascinated the public's imagination for more than a half of a century. The PIs associated with this proposal have been (and will be) involved in a myriad of educational and public outreach activities over the years that have reached literally millions of individuals. Shank co-developed a high school and undergraduate educational CD on hydrothermal vents, and the web-based learning portals, Oceanexplorer.gov and DiveandDiscover.com. He operates a high school and undergraduate training program in his laboratory, and is currently developing 6 museum exhibits on ocean life. Results of his research efforts have been featured in Science, Discover Magazine, National Public Radio, National Geographic, as well as on several nationally broadcast documentaries (*e.g.*,the Discovery Channel, BBC, National Geographic Television, PBS). For HADES-Kermadec, he is working with National Geographic who is committed to develop a major National Geographic television event special (*See Letter of Support*) featuring our proposed expedition and its science- with a global reach of 410 million households globally. Given the focus of the proposed studies, we plan to involve, all in conjunction with NSF's web portal, museums and aquaria (e.g., in public education institutions throughout the US, Britain, and Germany) together with science educators and the network of NSF COSEE Centers (Centers for Ocean Sciences Education Excellence) directly through "real time" satellite networking tied directly to the websites below, providing daily data logs, images, streaming video, etc. In addition, the results of this research will be posted online on Yancey's deep-sea website (people.whitman.edu/~yancey/deepsea.html) at Whitman College. Yancey is the developer and maintainer of this educational website on deep-sea biology since 1997; it is the top hit on a Google search of "deep-sea biology" and is used widely by teachers and students, who send many email queries every week. The site was selected in 2002 by the National Science Teachers Association under NSF guidelines as a K-12 science resource. Drazen is actively engaged in collaborations with K-12 educators (e.g., NSF RET program) serving schools from economically disadvantaged areas of Honolulu, Hawaii. Many of the students are from minorities and groups underrepresented in the sciences. He also works intimately with several museums and non-profit educational institutions to develop educational materials on the deep sea.

Education and public outreach activities will continue to be an integral part of all of our ongoing studies, and we believe that our past records attest to our deep commitment to continue these activities. In addition, a trench ecology and biogeographic web-based education module will be constructed prior to the expedition(s) for incorporation into these curricula and web portals, including DiveandDiscover.com. These web portals bring oceanographic research to classrooms and the general public and already contain considerable material that is pertinent to conveying research of the deep ocean. In addition, at no cost to the present proposal, we will develop a new seagoing educational web site targeted at middle school, college students and the general public, in collaboration with INDEEP, a follow up to the Census of Marine Life, CenSeam and ChEss projects, (MC and AR were the Secretariat of CenSeam, and TS and AR served on steering committees of both). As founding INDEEP members, we will bring abyssal and hadal science to the broader scientific community. We will present our results at national and international meetings, and publish our results in respected peer-reviewed journals. With the anticipated collection of unique and numerous specimens (added to over 550,000 individuals from > 550 deep-sea species from more than 93 vent, seep, seamount, and slope sites from Atlantic, Pacific, and Indian Oceans presently in our cryo-repository), we will actively pursue developing a Biotic Survey & Inventories program, in partnership with the ALL Species Foundation, the Alfred P. Sloan Foundation, and other parts of NSF to support Planetary Biodiversity Inventories worldwide, and providing full access to these data by the at large scientific community. Lastly, in addition to the collection of some of the deepest-living species in the world, we will include the proper preservation for international exhibition, including the National Museum of Natural History, the National Maritime Museums in the United Kingdom, and an international travelling exhibit.

International Consortium For Hadal Science: training the next generation of marine scientists: Putting this proposal together, we have combined the ideas and efforts of senior, junior, and postdoctoral

scientists, students, engineers, and educators to focus on the fundamental questions of the geology, ecology, and evolution of trench systems. Through this, we have formed an international consortium of researchers and educators from 6 Institutions (Aberdeen University, NIWA, NOC, UH, Whitman College, and WHOI) and received intense partnering interest from others. In response, we would like to extend this to the broader community of scientists and educators with the development of a dedicated web portal/list serve, and via a 2-day open Hadal Symposium and Data Results Workshop in Woods Hole, MA. Scientists will post and present their interests and results. Students and postdocs will, as part of their training and mentorship by our PIs, present and discuss data collection and analyses. In addition to this workshop, the principle investigators and associated students and postdocs will meet by web conference every 6 months and in person in year 2 and 3 with one of those coinciding with the 13th Deep-Sea Biology Symposium at NIWA. We will present recent findings and current thinking with the aim of synergizing findings from each partner. The progress meetings will prepare for publication, coordination of our Student/Postdoc exchange program, and dissemination of information. See supplementary documentation for Mentoring Programs for Postdoctoral scientists. Lastly, our use of the *HROV Nereus* will advance this vehicle's use for deep-sea biologists, drive advances in further technologies for conducting rigorous hadal and abyssal science, and further the capabilities of this vehicle system as well as off-shoot technologies for use by the broader scientific community.

X. Results from Relevant Prior Support:

Tim Shank: Collaborative Research: Investigation of a New Class of Hydrothermal Systems; The Lost City Hydrothermal Field: A Peridotite-Hosted Off-Axis System at 30°N on the Mid-Atlantic Ridge:OCE0136871 $171,000 1/1/03- 12/31/03, with D. Yoerger. We characterized the macrobiology of the Lost City Hydrothermal Vent Field, an off-axis peridotite-hosted hydrothermal system on the Atlantic Massif (800m) as part of an intense interdisciplinary study (June 2003) to understand the linkages among hydrothermal alteration of the mantle, geochemistry, and biological activity within this novel environment. Genetic studies elucidated relationships between Lost City and MAR fauna to shed light on the role of depth, fluid chemistry, and the presence of non-endemic species in controlling community composition as well as the role of Lost City-type vent fields in serving as stepping stones or evolutionary refugia for chemosynthetic fauna along the Mid-Atlantic Ridge. Immediate results communicated via NPR, Science News, Science Online, as well as Kelley *et al*. (*Science*, 2005) and Kelley & Shank (2010).

Jeff Drazen: An investigation of patterns of deep-sea demersal fish metabolism and feeding rates. OCE0727135. 9/07-9/11 $594,089. We developed a novel in situ respirometer and measured fish metabolism to a depth of 3000m to comprehensively evaluate competing hypotheses used to predict animal metabolic rates in benthic and benthopelagic fishes. Results were augmented by enzymatic measurements of trap and trawl caught fishes from 100-3000m. Our results found great depth related differences in benthopelagic species but not in benthic species. They create a predictive framework for understanding the energetic demands of demersal fishes along the continental slope. Certain fishes were also found to be adapted specifically to the oxygen minimum zone and our studies help identify the potential metabolic impacts of predicted OMZ expansion. One paper has been published from this project, two are in revision and four more are in preparation. This project trained 2 graduate students, gave shipboard opportunities to 4 more and trained 4 undergraduate students.

Paul Yancey: An absence of sharks in the abyss: ecological or physiological limitations? (ROA subcontract on JD's grant OCE0727135, 6/09-6/10, $27,197). We obtained 14 species of elasmobranchs from about 50 to 2200 m and analysed them for their main osmolytes, particularly TMAO (a protein stabilizer) and urea (a protein destabilizer). Using also previous data from a skate from 2900m, we found that urea contents decrease linearly with depth while TMAO contents conversely increase, but with a possible plateau near 3000m. A possible limit to TMAO accumulation may explain why these animals are very rare below 3000m and non-existent below about 4000m. One undergraduate was supported (laboratory and shipboard experience and a talk at the International Deep-sea Biology Symposium in Iceland, 2010) and she is first author of a paper on this work, currently in review.

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