

SPECIALIST STUDIES – SECTION C

C2.4 Meiofaunal Analysis of Namibian Offshore Sediments forming part of Verification Surveys for proposed marine dredging operations in ML 170

MEIOFAUNAL ANALYSIS OF NAMIBIAN OFFSHORE SEDIMENTS FORMING PART OF VERIFICATION SURVEYS FOR PROPOSED MARINE DREDGING OPERATIONS IN ML 170

BASELINE SURVEY

Prepared for:

Namibian Marine Phosphate (Pty) Ltd.

Prepared by:

Dr Simon Forster



July – August 2013

Physalia Ltd
Little Pook Hill, Heathfield Road, Burwash Weald, Etchingam, East Sussex, United Kingdom
Simon.forster@physalia.co.uk

Tel: + 44 (0) 1582 764 304

This report to be cited as:

Physalia (2013). Meiofaunal Analyses of Namibian Offshore Sediments forming part of Verification Surveys for proposed Benthic Mining Operations. Baseline Surveys, July - August 2013. A factual, baseline survey report prepared for Lwandle Technologies (Pty) Ltd, Cape Town, by Physalia Ltd, Consultant & Forensic Ecologists, Harpenden, UK.

CONDITIONS OF USE OF THIS REPORT

COPYRIGHT © NAMIBIAN MARINE PHOSPHATE (PTY) LTD 2014

ALL RIGHTS RESERVED

© NMP 2014. All rights to the intellectual property and/or contents of this document remain vested in Namibian Marine Phosphate (Pty) Ltd. (NMP). This document is issued for the sole purpose for which it is supplied. All rights reserved. No part of this publication may be reproduced, distributed, or transmitted in any form or by any means, including photocopying, recording, or other electronic or mechanical methods, without the prior written permission of Namibian Marine Phosphate (Pty) Ltd, except in the case of brief quotations embodied in critical reviews and certain other non-commercial uses permitted by copyright law. For permission requests, write to Namibian Marine Phosphate (Pty) Ltd, addressed "Attention: Administration Manager," at the address below.

Email: info@namphos.com
127 Theo-Ben Gurirab Street
Walvis Bay
Namibia

SUMMARY

- Physalia was approached by Lwandle Technologies (Pty) Ltd on behalf of their client Namibian Marine Phosphate to undertake analyses of the benthic meiofaunal communities present in sediment samples collected in the vicinity of a proposed offshore dredging operation. The survey site is situated on the Namibian continental shelf in approximately 200 metres of water.
- The survey area examined here, comprised 26 sampling stations. Sediments present in this area were predominantly fine to very fine silty sands with, in places, significant proportions of mollusc and/or brachiopod shell fragments.
- Of the meiofaunal groups present, the Nematoda (free-living roundworms) and harpacticoid Copepoda (microscopic, shrimp-like crustaceans) were processed and analysed quantitatively to species-level. The resulting data matrices were examined using a range of uni - and multivariate analytical techniques to describe and document patterns in the structures of their communities.
- Specialised, multivariate correlation analyses were then used to identify and describe quantitatively the relationships between the community structures and the measured sediment physico-chemical parameters.
- A total of 135 nematode and 36 harpacticoid copepod taxa was documented. With nematode species richness values of up to 42 species per sample and densities of up to 30,400 nematodes per litre sediment, these data are comparable to other offshore seabed sites recorded at similar water depths.
- Univariate distribution plots of nematode and harpacticoid copepod community data within the survey area were prepared and are presented and described.
- Multivariate analyses of the meiofaunal nematode communities revealed six robust, coherent clusters of communities comprising structurally-related species assemblages. Distributions of the multivariate clusters of these communities within the survey area were consistent with the presence of an environmental gradient oriented along an east - west axis. This is described.
- The correlation analyses identified the majority of elevated metal concentrations and finer sediment fractions as statistically significantly correlated with the largest nematode cluster located to the west of the survey area. The proportions of the coarsest sediment fraction (1,000 - 2,000 μm) correlated positively with a separate cluster of nematode communities. These were located along the eastern boundary of the sampling area. The authors conclude that sediment granulometry was the principal determinant of meiofaunal community structures in the 2013 verification survey.
- This verification survey has demonstrated that the meiofaunal assemblages will provide a robust means of assessing and tracking any changes in the seabed habitats that are associated with the proposed dredging operations and enable these to be placed into context with any changes in background conditions. Similarly, the same approach will enable recovery of mined areas to be documented.

contents

SUMMARY	ii
1 INTRODUCTION	1
2 MATERIALS AND METHODS	2
2.1 MEIOFAUNA ANALYSES	2
2.1.1 <i>Meiofaunal Sample Collection</i>	2
2.1.2 <i>Meiofaunal Sample Processing and Examination</i>	2
2.2 ANALYSES OF DATA MATRICES	3
2.2.1 <i>Standard Univariate Analyses</i>	3
2.2.2 <i>Multivariate Methods (Including ARESC Analyses)</i>	3
3 RESULTS AND DISCUSSION	9
3.1 NOTES ON TAXONOMY	9
3.2 COMMUNITY STRUCTURES AND SPATIAL DISTRIBUTIONS	9
3.2.1 <i>Univariate Community Data Distributions</i>	11
3.2.2 <i>Multivariate Analyses of Meiofaunal Community Data</i>	11
3.3 MULTIVARIATE CORRELATION (ARESC) ANALYSES	13
3.4 OTHER MEIOFAUNAL GROUPS	15
4 SUMMARY AND CONCLUDING COMMENTS	16
4.1 THE SURVEY AREA MEIOFAUNAL COMMUNITIES	16
4.2 SUITABILITY OF MEIOFAUNAL ANALYSES FOR MONITORING PURPOSES	17
5 REFERENCES	18
Annexure A: Taxonomic Lists of Invertebrate Species and their Identification Codes	20
Annexure B: Meiobenthic Community Data Tables	27
Annexure C: Univariate Distribution Plots for Key Meiobenthic Community Parameters	31
Annexure D: Multivariate Analyses of the Meiobenthic Nematode Community Structures	38
Annexure E: ARESC Analyses Identifying Relationships Between Community Structures and Measured Seabed Environmental Parameters	43

figures

Figure C1:	Numbers of meiofaunal nematode species recorded at each of the off shore Namibian verification survey sites during the survey.	32
Figure C2:	Total densities of meiofaunal nematode species recorded at the offshore Namibian verification survey sites.	32
Figure C3:	Diversity indices (Simpson's <i>D</i>) for the meiofaunal nematode communities recorded at the onshore Namibian verification survey sites, July/August 2013.	33
Figure C4:	Dominance (% abundance) recorded in the meiofaunal nematode communities present at the onshore Namibian verification survey sites, July/August 2013.	33
Figure C5:	Co-dominance (%) for the meiofaunal nematode communities recorded at the offshore Namibian verification survey sites, July/August 2013.	34
Figure C6:	Meiofaunal nematode community 1B/2A feeding type ratios recorded at the offshore Namibian verification sites, July/August 2013.	34
Figure C7:	Total number of meiofaunal harpacticoid copepod species recorded in the sediments at the offshore Namibian verification survey sites, July/August 2013.	35
Figure C8:	Densities of meiofaunal harpacticoid copepod communities represented in sediments at the offshore Namibian verification survey sites. July/August 2013.	35
Figure C9:	Diversity indices (Simpson's <i>D</i>) for meiofaunal harpacticoid copepod communities recorded at the offshore Namibian verification sites, July/August 2013.	36
Figure C10:	Dominance, the percentage abundance of the most abundant meiofaunal harpacticoid copepod species, recorded at the offshore Namibian verification survey sites, July/August 2013.	36
Figure C11:	K-dominance plots for the July / August 2013 Namibian meiofaunal nematode communities.	37
Figure D1:	Interpretation of Physalia Multivariate Analyses	38
Figure D2:	Two-way classification analyses	39
Figure D3:	Multivariate ordination analyses	40
Figure D4:	Distributions of structurally – related meiofaunal nematode communities	41
Figure E1:	ARESC plot showing mathematical relationship between meiofaunal communities and the sediment physico-chemical environment.	44

tables

Table 2.1:	Standard and more specialised community parameters for which spatial distribution plots have been prepared (see maps in Annexure C).	5
Table 3.1:	Comparison of meiofaunal nematode community data for the Namibian verification survey with those from an offshore North Sea survey undertaken in 2013 at 200 metres depth	10
Table A1:	Table presenting the full taxonomic list of meiofaunal Nematoda	20
Table A2:	Table presenting the full taxonomic list of meiofaunal harpacticoid Copepoda (Biramia)	25
Table B1:	Site-by-site results table for the communities of meiofaunal Nematoda	27
Table B2:	Site-by-site results table for the harpacticoid Copepoda communities	30
Table D1:	List of the indicator species	42
Table E1:	The formal ARESC analysis table	45

1 INTRODUCTION

Namibian Marine Phosphate (Pty) Ltd is currently undertaking a verification assessment following on from the environmental impact assessment (EIA 2102) for the proposed marine dredging operation that would centre on a site on the Namibian continental shelf, Mining Licence Area ML 170. The mineral material of interest is pelletal phosphorite and would involve dredging in approximately 200 metres of water. The consolidated verification survey will include assessments of the biological, chemical and physical conditions of the water column and benthic (seabed) habitats, as well as temporal studies of the prevailing water current regime designed to inform modelling of potential suspended sediment dispersion plumes.

Physalia Ltd. was contracted to undertake analyses of the communities of benthic meiofauna and to provide baseline and subsequent monitoring information relating to the effects of the proposed dredging activities on the benthic environment. Staff members at Physalia have been undertaking similar meiofaunal assessments and surveys at locations throughout the world since the mid-1980s and have produced numerous scientific papers/technical publications on the subject of meiofaunal ecology, eco-physiology and on the use of meiofauna as bio-indicators of environmental conditions.

The meiofauna comprises animals that live within sediments and are typically within the size range 50 µm and 1 mm in length (although some species with larger individuals exist). The benefits of meiofaunal analyses when assessing baseline conditions of a site and where monitoring, and documenting subsequent changes in conditions is required, can be summarised as follows:

- In a given habitat, meiofauna are usually an order of magnitude more abundant and more diverse than other invertebrate faunal groups. The high numerical abundance of individuals belonging to meiofaunal groups, such as the nematodes, means that the collection of scientifically robust, statistically valid samples is a simple, cost-effective process, minimising disturbance of existing habitats and maximising on information content.
- Meiofauna communities include species that occur throughout the entire spectrum of prevailing environmental conditions. These range from grossly polluted sites through to pristine habitats. They include tolerant, resistant species that are amongst the last to disappear as conditions deteriorate and are the first to reappear as conditions improve. Conversely, other more sensitive species are amongst the first to disappear as stresses increase and conditions begin to deteriorate. Analyses of meiofaunal communities can therefore be used to assess changes over the entire range of habitat conditions.
- Meiofaunal life-cycle times range less than 10 days (“egg-to-egg”) up to several years. This means that the structures of meiofaunal communities can respond rapidly to short-term changes in conditions as well as integrating the effects of, and providing information on, prevailing conditions over longer periods of time.
- Located nearer to the base of foodwebs than larger animal groups, populations of many meiofaunal species respond directly to any stresses that affect the nature and quality of their food supply (e.g. protozoans and ciliates (“microbes”) as well as algae, including diatoms). This means that changes in conditions are more rapidly expressed as changes in the relative abundances of these more sensitive meiofaunal species than in most other faunal groups.
- Living in the spaces between sediment particles and with relatively low mobility, meiofaunal animals are continuously subjected to the constraints of any materials that enter their

environment (e.g. contaminants or materials that lead to altered habitat conditions). The same is true of physical perturbations (e.g. sediment disturbance and smothering). As a result, analyses of meiofaunal communities provide greater spatial resolution of effects resulting from site-specific changes in habitat conditions than can be achieved by analysing more mobile and more patchily distributed animal groups.

Further details of the use of meiofaunal species as bio-indicators and their applications in industrial and other situations are given in Trett *et al.* (2009).

This report presents the results of the analyses of meiofaunal samples collected by Lwandle staff from 26 sites during the marine survey undertaken in July and August 2013. Site-by-site physico-chemical data provided by the Lwandle team enable a full suite of univariate and multivariate statistical analyses to be undertaken in parallel with the analyses of the meiofaunal assemblages. The data analyses employed in the present report also include ARESC analyses¹. These permit the key factors that shape the biological communities to be identified and ranked in order of statistical (and biological) significance.

2 MATERIALS AND METHODS

2.1 MEIOFAUNA ANALYSES

2.1.1 *Meiofaunal Sample Collection*

Sediment samples were collected from the Namibian continental shelf pelletal phosphorite dredging site Sandpiper-1 (SP-1) over the period 26th July to 3rd August 2013 using a box corer. Sub-samples for subsequent meiofaunal analyses were taken by inserting a hand corer into the sediment retained in the box corer. Samples were preserved in formalin (ca. 4% formaldehyde). A total of 27 samples from 26 sites was supplied to Physalia for analyses.

2.1.2 *Meiofaunal Sample Processing and Examination*

a Sample Separation

Standardised laboratory protocols developed and refined by staff at Physalia over the past 30 years were used for the extraction of the meiofauna from the preserved sediment samples. After re-coding, the volume of sediment in each sample was measured. The samples were homogenised in approximately 800 ml water. Initial separation was carried out using a modified, multiple Boisseau apparatus to elutriate the microscopic organisms from the bulk of the inorganic matrix. The first ("light") and subsequent ("heavy") meiofaunal fractions were collected on 50 µm mesh sieves immersed in flowing tap water (Flegg and Hooper, 1970).

¹ ARESC analyses - Technically the Assessment and Ranking of Ecologically Significant Contaminants. However, these analyses include assessments of correlations between the community structures *natural* physico-chemical parameters as well

Pooled meiofauna/silt fractions for each sample were further concentrated by a polymer density separation technique with centrifugation and the meiofauna re-collected onto 38 µm mesh sieves. The density separation technique was repeated and the separation efficiency estimated.

b Meiofaunal Harpacticoid Copepoda

Harpacticoid copepod shrimps were removed from the samples by hand using mounted 000-gauge needles (or single, mounted eyelashes; after David Hooper, Rothamsted Experimental Station, Harpenden). For microscopy, specimens were then mounted in Berlese's thin formulation medium prepared using the technique described by Humason (1979) or polyvinyl lactophenol (PVL; Physalia in-house formulation). The addition of a small quantity of lignin pink to the mountant aided location of the specimens on the slides. After clearing (approximately 3 days at room temperature), specimens were identified by means of 5th limb setotaxy (Lang, 1948) using Zeiss and Nikon differential interference microscopes, enumerated as numbers of individuals per species (or per "operational taxonomic unit" (= OTU)), and recorded as numbers per species per litre sediment per site.

c Meiofaunal Nematoda

Modified nematological techniques based on those of Bühner (1949), Baker (1953) and Cairns and Tarjan (1955) were used to process, handle and examine the remaining meiofauna, (primarily Nematoda - free-living roundworms). Specimens were processed to glycerol using a modified Seinhorst method (Seinhorst, 1959) in Syracuse watch glasses at 40 °C in a drying oven. Taxonomic microscope slides were then prepared for identification and enumeration. All microscopic examination was carried out using Zeiss Nomarski and Nikon differential interference contrast (DIC) microscopes. For the highly abundant nematodes, the first 100 specimens encountered were identified and counted. Remaining nematode specimens were then counted to allow the total densities of each species in each sample to be calculated and then recorded as numbers per species per litre sediment per sampling site.

2.2 ANALYSES OF DATA MATRICES

2.2.1 Standard Univariate Analyses

Table 2.1 below summarises and describes the standard univariate community parameters examined for the nematode and harpacticoid copepod communities. Maps and plots using scaled symbols for these parameters at each sampling site were prepared to enable spatial patterns and trends to be detected and examined (e.g. depression of species numbers or elevations of dominance values in the vicinity of the proposed dredging area).

To aid interpretation, the univariate data outlined in Table 2.1 below were superimposed onto survey maps and the values indicated by scaled symbols.

2.2.2 Multivariate Methods (Including ARESC Analyses)

With complex physico-chemical and faunal data matrices, multivariate analyses offer a practical means of screening and identifying/mapping spatial (and temporal) patterns of variation in the data. These analyses are used to recognise and describe the patterns of similarities in community

structures that develop wherever similar prevailing conditions act on diverse assemblages of species in marine systems. The techniques group structurally similar communities into “clusters” of assemblages. If these clusters are mapped back onto the survey area their distributions can then be examined. This enables the identification of sites at which different environmental conditions exist (e.g. effects of different sediment types, natural differences in sediment chemistry and any anthropogenic contaminants that may be present).

Two fundamentally different multivariate methods were used in the present study; a **classification technique** that “weighs” each community mathematically² and then groups them together to form clusters (hence its generic name cluster analysis) and an **ordination technique** that awards scores to each community and distributes them along different axes of variation³. The former produce results in the form of dendrograms whilst the latter produces graphs with communities grouped together in mathematical space. Distribution maps were prepared for the clusters identified in each set of multivariate analyses. These enable rapid assessment of the spatial patterns of groups of structurally-related species assemblages on the seabed in the 2013 verification survey area.

To aid ecological interpretation, an analysis based on **Monte Carlo permutation tests** (MCPTs; Sokal and Rohlf, 1995) was used to identify “indicator species” and to test the statistical significance of the relationships between these and the clusters of communities identified by the multivariate analyses (Dufrêne and Legendre, 1997).

² Based on Sørensen’s distance method and flexible β -linkage technique (Lance & Williams, 1967 & 1968) for both the inter- and subtidal survey data sets

³ Non-metric multidimensional scaling after Kruskal (1964a and 1964b) as modified by Mahler (1976)

Table 2.1: Standard and more specialised community parameters for which spatial distribution plots have been prepared (see maps in Annexure C).

Parameter	Description
Densities	Total numbers of animals per litre sediment
Species richness	Numbers of species per animal group per sampling site
Dominance	The percentage abundance of the most abundant species; a measure of stress-selection for tolerant, resistant species
Diversity indices	Simpson's index ⁴ integrating densities, dominance and numbers of species. This index is preferred as it gives more even weighting to abundant and rare species than other measures of diversity; see Peet, 1974 and Forster, 1998
Feeding type ratio	Nematodes only; the ratio of densities of non-selective deposit feeding species (e.g. detritus-feeders) to more sensitive specialist feeding types (e.g. diatom feeder and epigrowth feeders). Shows changes in the trophic (feeding) structure of nematode communities.
Selected features	Specific plots prepared to illustrate an ecological feature (e.g. changes in communities of species with particular physiological characteristics)

As its name suggests, Monte Carlo tests works by generating **large** numbers of random combinations, in this case of species (see Manly, 1997). From this it is possible to calculate the probability that the observed (real) patterns of relationships between each species and their clusters could have occurred by chance. By default, the robustness of the observed combinations can be determined. These results permit a more rigorous interpretation as the biologies, sensitivities, feeding requirements, physiologies and habitat requirements of indicator species can be used to corroborate the findings of correlation analyses described below.

To determine the statistical relationships between the community structures and the measured environmental parameters, **multivariate correlation analyses** (MCAs) have been employed. A modification of these analyses, termed ARESC analyses⁵, was developed by Dr. Beatriz Calvo Urbano (see Calvo Urbano in Trett *et al.*, 2000 and summarised in Trett *et al.*, 2009). These are based on ordination analyses of the community structures⁶. For present purposes, plots of the correlation lines

⁴ Simpson's diversity index D calculated as:

$$D = \frac{1}{\sum_{i=1}^S P_i^2}$$

Where: S is the number of species and P_i is the proportion of individuals of the i th species in the sample

⁵ ARESC = Assessment and Ranking of Ecologically Significant Contaminants. However, it also assesses and ranks natural factors such as the proportions of individual sediment particle size categories or the concentrations of total organic carbon.

⁶ Over 50 different random starting configurations of the real data were used in the multivariate correlation analyses. A 50-cycle Monte Carlo permutation test showed that the probability of achieving a similar low stress configuration of the data from a random combination of the variables was less than 0.05 and indicated that a three-dimensional analysis was the most appropriate for a given community data set. When completed, analyses showed that the minimum proportion of the variance present in the data sets explained by the axes (i.e. the coefficients of determination), was 0.814 (= 81.4% for the meiofaunal assemblages 3-dimensional solution). The environmental parameters were then correlated with the

for the environmental determinands have been prepared and are presented in Annexure E. The significance data were used to rank the environmental determinands (metal concentrations, proportions of sediment particle size fractions and total organic carbon contents) which can be used to indicate the importance of each parameter in the structuring of the faunal communities.

In the case of the ARESC analyses that were used to identify relationships between the meiofauna nematode community structures and the sediment physico-chemical parameters that were measured, the data were corrected to overcome the statistical issues that arise where multiple comparisons are made. These apply a mathematical correction to the results to prevent a false rejection of the null hypothesis that would lead to the identification of statistical significance where none exists. The differences can be seen in the ARESC summary table (Table E1; Annexure E) which presents the raw probability data (standard p -values) and the results from two different correction methods, the Benjamini and Hochberg (1995) technique ("BH") and the rather more draconian Benjamini and Yekutieli (2001) method ("BY"). In each case, the correlation coefficient values that are statistically significantly associated with the meiofaunal nematode community structures are identified in bold enabling the differences to be seen.

nematode community axes to provide information on the ecological (and statistical) significance of the physico-chemical parameters.

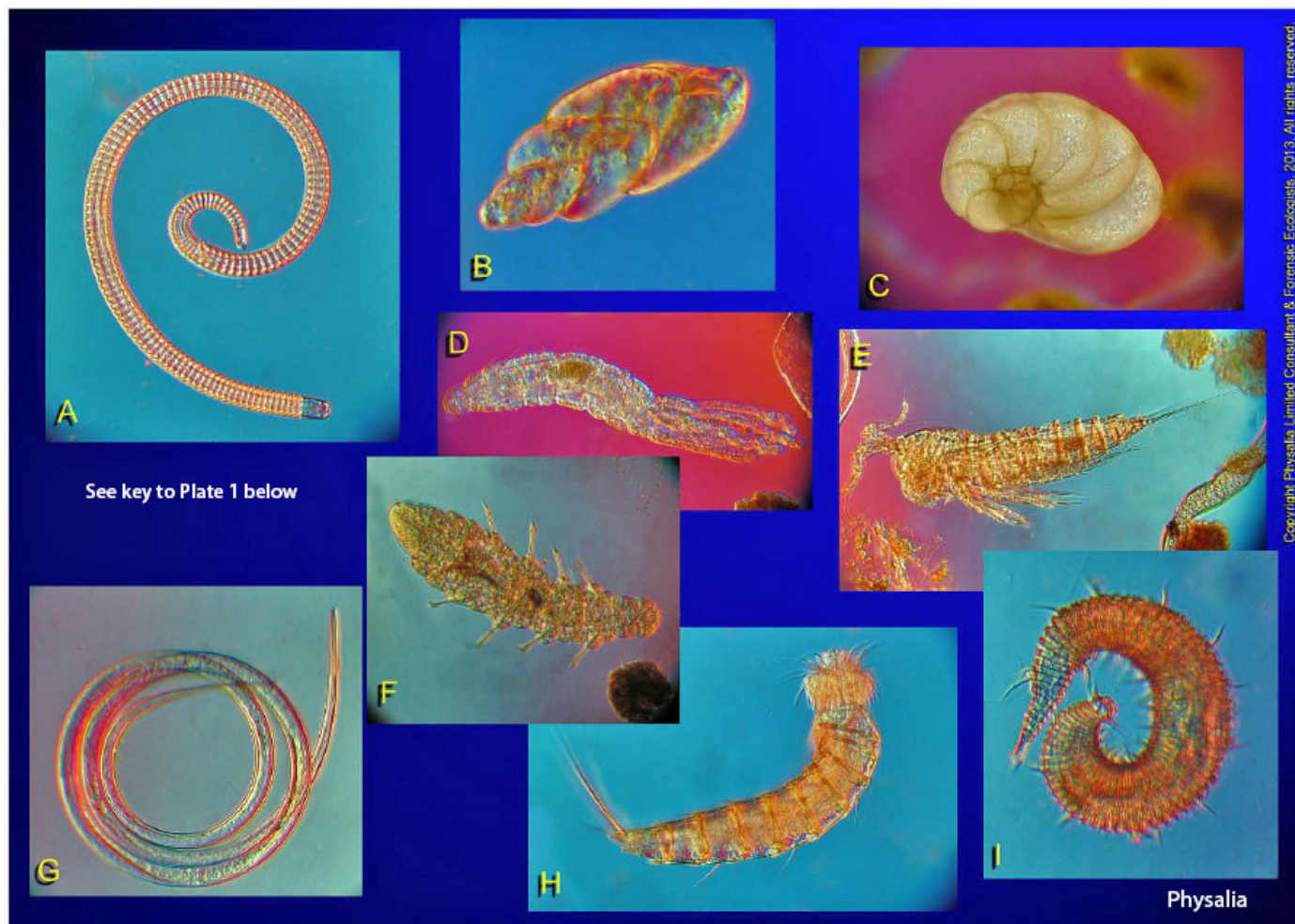


Plate 1: Meiofauna

Key to Plate 1:

- A** Members of the nematode family Ceramonematidae are easily recognized by their "chain mail" formed by overlapping circular plates. This species of *Ceremonema* was found at 12 of the 26 verification sites.
- B** Foraminifera were ubiquitous throughout the verification survey with specimens. A number of structural types were recorded including the Bolivinitid type shown here, as well as C, the planospiral "hyaline" species belonging to the family Elphidiidae
- D and F** Juvenile/neochaete polychaete annelid worms were recorded in all meiofaunal samples examined. These are temporary or transient meiofauna and, as such, do not provide suitably robust data for inclusion in meiofaunal biomonitoring. In the present survey, the mature polychaetes were studied as part of the larger macrofauna.
- E** A total of 36 harpacticoid copepod species was recorded in the verification survey with densities of up to 687 animals per litre. Due to the high fine silt content of the sediment, the majority of the copepods were large epibenthic species.
- G** Nematodes (free-living roundworms) were the most abundant meiofaunal group recorded. Densities >30,000 nematodes per litre sediment were recorded in the verification samples and included members of 135 species. This is a microbivorous *Halalaimus* species (probably *H. isaitshikovi*).
- H** The Phylum Kinorhyncha is considered exclusively meiobenthic, although kinorhynchs can occur in sediment associated with algal holdfasts. These animals were not abundant in the verification survey meiofauna samples and were observed at 3 sites only.
- I** This species of *Quadricoma* represented one of three genera within the family Desmoscolecidae. The family is characterized by the presence of cuticles annulated with desmen (thick rings of secretions and attached debris) that extend from the head to the tail.

3 RESULTS AND DISCUSSION

3.1 NOTES ON TAXONOMY

A total of 135 discernable nematode taxa was recorded during the present meiofaunal analyses, including representatives of 23 families. The taxonomic classification of these taxa is presented in Table A1, Annexure A.

Taxonomic descriptions of the nematode assemblages of West African offshore benthic habitats are poor and no comprehensive taxonomic lists and descriptions of nematode species are available for use as guides. Given the lack of meiobenthic studies in these habitats, it is highly likely that *a significant proportion of the nematode species in the verification survey area are “new to science”*. In order to determine which species has been described and which is currently un-described it would be necessary to review all of the current species description within the genera present. Such investigations were neither practical nor pragmatic. The purpose of the present study was to characterise the baseline, pre-dredging, conditions of the survey area and provide robust biological information against which post-dredging conditions can be compared. To this end, as with other sites that we have examined and monitored around the World, we have drawn and described each species encountered to create a set of site-specific observed taxonomic units (= OTU). In nearly all cases these have been ascribed to family and genus level based on the existing generic level definitions.

Beyond this, species level differences, based solely on the combination of standard taxonomic (= morphological) features present, have been used to define the individual OTUs and to ensure that valid comparisons are made between the sampling sites examined in the present survey. In each case full taxonomic drawings and descriptions have been prepared for the species encountered in the 2013 survey area to ensure consistency both within the current survey and with future surveys of this area. The same procedure was adopted for the meiofaunal harpacticoid copepod shrimp species. Tables A1 and A2 (Annexure A) list the taxonomic species for these meiofauna.

3.2 COMMUNITY STRUCTURES AND SPATIAL DISTRIBUTIONS

Table 3.1 below provides a summary of the status of the assemblages of meiofaunal nematode and harpacticoid copepod communities recorded in the 2013 verification survey area. In each case key, descriptive community parameters are presented. These include numbers of families and species per invertebrate group, ranges and means for total densities, species richness values and the diversity indices (descriptive indices that are based on a combination of the numbers of species present per sampling station, their densities and the distributions of these densities amongst the species present). In the case of the meiofaunal Nematoda, feeding type ratios have also been included. These descriptors allow changes in different nematode groups (“guilds”) to be examined and compared and serve here to provide a baseline against which changes in sediment physico-chemistry can be assessed⁷.

⁷ Prevailing conditions within sediments exert differential effects on nematode species with differing feeding preferences. For example, chemical stresses often militate against specialist feeders that exploit microbial epigrowth whilst populations of non-selective deposit feeders may be relatively unaffected. This is detected in the nematode feeding-type ratios (FTRs) as well as in the multivariate analyses feeding

In the present survey, total densities, species groups, species richness values and diversity indices for the nematode assemblages were comparable with those recorded in other offshore benthic sediments that we have examined in similar depths of water. Examples include parallels with species assemblages recorded in a recent *Physalia* survey in the North Sea offshore of Scotland in 200 metres water⁸. The data are compared in Table 3.2 below. Whilst nematode densities in the present survey were lower overall, the species richness and diversity values were remarkably similar.

Table 3.1: Comparison of meiofaunal nematode community data for the Namibian verification survey with those from an offshore North Sea survey undertaken in 2013 at 200 metres depth

Parameter	Offshore Namibia	Offshore North Sea
Mean Density (no.litre ⁻¹)	11,949	17,688
Max Density (no.litre ⁻¹)	30,400	36,800
Min Density(no.litre ⁻¹)	1,746	4,407
Mean Species Richness	29.77	30.25
Max Species Richness	42	44
Min Species Richness	14	18
Mean Diversity	11.30	12.18
Max Diversity	24.83	24.31
Min Diversity	4.47	2.06
Mean 1B/2A ratio	0.383	3.798
Max 1B/2A ratio	3.200	15.400
Min 1B/2A ratio	0.082	0.516

Interestingly, marked differences can be seen in the mean and ranges of the 1B/2A feeding type ratios between the two survey sites, with the present survey area yielding lower values than those for the North Sea study. As outlined in Table 2.1 above, this index is based on the density ratios of type 1B species (nematode species characterised by large, un-armed buccal cavities presumed to be associated with detritus feeders), and type 2A species (species with small buccal cavities armed with teeth, presumed to be selective epigrowth browsers and diatomivorous species). The assumptions relating to the feeding preferences of the nematode species are based on solely morphological features and several exceptions have been documented (see, for example, Moens and Vincx, 1997). One of the most common species recorded in the present (verification) survey was a *Paracomesoma* species (coded species 4). The elevated abundance of this species contributed strongly to the low 1B/2A ratios. This nematode has a deep but narrow, cuticularised buccal cavity with fine teeth at the anterior end. In nearly all studies this species has been classified as a type 2A nematode. Given its abundance in the Namibian sediments with elevated silt contents, it is quite likely that this *Paracomesoma* species does not feed selectively and should not be included in the type 2A category.

⁸ Survey of the meiofaunal communities of the Scanner and Braemar SAC sites, undertaken by *Physalia* for the UK governmental body Centre for Environment, Fisheries and Aquaculture Science (CEFAS)

Further consideration will be given to this, along with the feeding type classifications of other taxa, before the interpretation of the mining monitoring surveys is undertaken.

3.2.1 *Univariate Community Data Distributions*

A selection of descriptive, site-by-site univariate parameters for the meiofaunal nematode and harpacticoid copepod assemblages recorded in the 2013 survey are displayed as spatial plots with scaled symbols in Figures B1 to B10 (Annexure B). The distribution plots enable visual assessments of natural variation in each community parameter to be made and comparisons to be drawn between values in the proposed mining area and those at sites beyond. The degree of spatial variation (“patchiness”) that can be seen in the plots appears to relate primarily to differences in the sediment granulometry. Although individual plots have not been prepared for the individual granulometric fractions, the results of the ARES analyses, that assess correlations between community structures, provide strong supporting evidence for this (see Section 3.3 below).

From the *nematode* community parameter plots, several “textbook” examples of relationships can be seen between the different sets of ecological (community) descriptors. Note the inverse relationships between total nematode densities and species richness values (Figures C1 and C2) and again between nematode diversity and the community dominance/co-dominance values (Figure C3 and Figures C4/C5). In the case of the meiofaunal harpacticoid copepod communities, the parallel patterns were not observed (see Figures C7 to C10). This reflected the pre-dominance of active, mobile, epibenthic species. These larger harpacticoid species are typified by the Diosaccidae which migrate across the sediments in search of decomposing detrital materials and/or diatoms that have settled onto the seabed.

Figure C11 (Annexure C) presents cumulative species abundance curves for the July/August 2013 nematode assemblages. Also known as *k*-dominance curves, these provide a valuable means of visualising the dominance/diversity characteristics of the assemblages. Each assemblage (sample community) is represented by a plot of the species rank (*x*-axis; \log_{10} scale) against their cumulative percentage abundance (*y*-axis). Typically, the assemblage with highest species richness and highest evenness value, in this case the nematode community present in the sample from Site VS2, is represented by a sigmoid curve that is displaced down towards the bottom right of the plot. The community curves that lie in this area of the dominance-diversity “space” include the most diverse, species-rich assemblages present in the survey area.

In contrast, where selection pressures arising from prevailing environmental conditions within the sediments lead to a more restricted number of tolerant/resistant species, the low evenness/high dominance communities are displaced towards the top left of the dominance - diversity plot. In this instance, examples included the nematode assemblages present at sites VS5 and VS26.

During the subsequent mining monitoring program, the examination of *k*-dominance curves will provide a useful means to assess and visualise changes in the dominance/diversity characteristics of the meiofaunal communities associated with the mining activities.

3.2.2 *Multivariate Analyses of Meiofaunal Community Data*

Studies undertaken at sites around the world over the last 30 years have shown that, under similar prevailing conditions in contiguous habitats, meiofaunal nematode communities develop structurally

similar assemblages of species (see Trett *et al.*, 2000 and 2009). This observation is important and forms the basis for two powerful analytical/diagnostic tools. These are:

- a) The use of meiofaunal nematode assemblages to map sites that are subject to different prevailing environmental conditions and to identify and track changes in the physico-chemical parameters that shape the communities over time and
- b) Using advanced mathematical techniques, to rank and track the ecological importance of the measured environmental parameters that are responsible for shaping the meiofaunal nematode assemblages.

The former relies on the use of two fundamentally different sets multivariate techniques (outlined in Section 2.4.2 above) to identify patterns in the communities based on the species present at each site and their abundances (densities). The latter is achieved using multivariate correlation analyses to assess the strength of the relationships between the species assemblages and the measured environmental parameters. A brief guide to the interpretation of the classification and ordination analyses used to identify structurally-related related communities that are then mapped onto the survey area is presented in Figure D1 (Annexure D).

The results of the classification analyses are presented in Figure D2 in the form of a *two-way dendrogram*. The smaller, vertical dendrogram located on the left-hand side of the plot displays the relationships between the sample sites' nematode assemblages *and* identifies the *clusters* of the structurally-related communities (Clusters A to F; Figure D2). The larger, horizontal dendrogram represents the similarity linkages between the nematode species identified in the present survey based on *the samples in which they occurred and their densities in these samples*. This is summarised in the large density distribution matrix that depicts the densities of each species in each sample (colour coded from dark blue for the highest densities through to white for the lowest).

Note that Cluster E (light blue; Figure D2) comprises solely the meiofaunal assemblage present at Site 4 (S4). Although it exhibited similarities with the communities present in Clusters D and F, the sediments at Site 4 supported several species that were unique to this site (see species matrix in Figure D2 and indicator species analyses below).

The multivariate ordination studies (i.e. the non-metric multi-dimensional scaling (NMMDS) analyses), that were run in parallel to the classification analyses, confirmed the relationships between the meiofaunal nematode communities described above (see Figure D3; Annexure D). Note that the clusters were coherent and non-overlapping in the three-dimensional ordination space indicating structurally distinctive assemblages in each of the clusters. Taken together, the three community axes explained over 80% of the total statistical variation present in the nematode community data matrices. A minor difference between the two sets of multivariate analyses was the degree to which ordination analysis isolated Cluster F communities from their closest "relatives" i.e. the communities present in Clusters D and E.

Table C1 (Annexure D) presents the results of the mathematical indicator species analyses (ISAs). Based on Monte Carlo permutation tests, these identify species whose density distributions within the survey area show statistically significant correlations with the clusters of sample site communities identified in the multivariate analyses. Aside from characterising the ecology of the communities present in these clusters, the results provide a robust basis for the assessment of changes in the communities that occur as result of any natural/seasonal processes *and* those associated with the

effects arising from operational activities once these commence. Note that indicator species could not be identified for Cluster E as this comprised the single sampling site S4.

The clusters of structurally-related nematode assemblages identified by the classification analyses and corroborated by the ordination studies have been transposed onto the survey area chart (see Figure D4). This reveals a nematode community distribution pattern *that is consistent with the presence of an environmental gradient*. The closely-related Cluster D, E and F meiofaunal assemblages were present at sites located on the eastern side of the verification survey area. Amongst these, the Cluster F communities were confined to the five sampling stations that extended along the eastern side of the grid. The remaining nematode assemblages, i.e. those belonging to Clusters A, B and C, were identified at sites located in the western half of the survey area (Figure D4).

To determine the driver(s) for this pattern of community distributions we can turn to the results of the ARES analyses described in the following section.

3.3 MULTIVARIATE CORRELATION (ARESC) ANALYSES

The results of the ARES analyses that are used to assess the inter-relationships between structures of the seabed communities and the measured environmental parameters are presented in Figure D1 and Table D1 (Annexure E). From the ARES plot (Figure E1) it is clear that the majority of the elevated sediment metal concentrations recorded in the 2013 verification survey was associated with the finer sediment fractions. Note the orientation and lengths of the correlation lines for these sediment parameters. The direction of these lines indicates the communities with which the parameters are associated (in the present case, primarily with the group of communities belonging to Cluster A). The length of the individual correlation lines is a function of the strength of the associations and Table E1 identifies those correlations that are statistically significant once corrections have been made for potential “false positives” arising from multiple comparisons⁹.

Figure E1 shows a similar orientation of correlation lines for the majority of the metals and the finer sediment fractions. This is consistent with binding of metals by the surface charges present on fine, alumino-silicate (silt-clay) sediment particles. The concentrations of sediment vanadium and cadmium are interesting exceptions and elevated values were associated with sediments characterised by coarser particle size fractions (500 - 1,000+ μm ; Figure E1). The reason for this is unknown. However, whilst these metals may be of background, geological origin, there is a body of scientific information that details accumulation of vanadium and cadmium in some benthic invertebrates such as polychaete annelids and molluscs (see for example Fattorini *et al.*, 2010, Fattorini and Regoli 2012 and Erk *et al.*, 2005).

⁹ In statistical analyses, *multiple comparisons*, such as those that arise when considering which measured physico-chemical parameters are associated with the structures of the bio-indicator communities, can lead to serious problems. Put simply, the greater the numbers of measured parameters that are compared with the community axes, the greater the chance of rejecting the null hypothesis and, as a result, identifying a statistically significant association where none exists. To overcome this, compensatory techniques have been developed that require a stronger body of evidence for an individual comparison to be deemed “significant”, based on the numbers of inferences being made. Table E1 (Annexure E) provides uncorrected (raw) comparisons (standard *p*-values) for the relationships between sediment physico-chemical parameters and the community axes as well as the results of two different correction methods; the Benjamini and Hochberg technique - a standard, “moderate” approach and the Benjamini and Yekutieli technique - considered by some as rigorous and by others as over zealous.

In Section 3.2.2 above, it was noted that the spatial distribution of the clusters of the meiofaunal nematode assemblages within the 2013 survey grid was consistent with the presence of an environmental gradient. Cluster A, B and C communities were located at sites on the western side of the sampling area whilst the species assemblages in Cluster D, E and F were present at sites on eastern side. In the latter case, the Cluster F communities were confined to 5 sites located along the eastern edge of the survey area. Reference to Figure E1 indicates that the environmental gradient was primarily granulometric with the finest sediments located to the west and the coarsest sediments located to the east. It is noted that fine sediments correlated spatially with elevated concentrations of a range of sediment metals. This is consistent with alumino-silicate metal binding to the finest sediment fractions.

Table E1 (Annexure E) presents the statistical data for the relationships between the measured physico-chemical parameters and the three main axes of variance derived from the multivariate analyses of the meiofaunal nematode communities. Note that Axis 1 accounted for (“explained”) the highest proportion of the statistical variance in the meiofaunal nematode community structures (41.4%). Once corrections had been made for multiple comparisons 9 below), it was clear that sediment granulometry and the concentrations of several sediment metals were important determinants of the meiofaunal community structures.

Reference to Axis 1 shows that the proportions of finer particle size fractions (i.e. $\leq 63 \mu\text{m}$) were all statistically significantly correlated with the nematode communities in July/August 2013 (Benjamini and Hochberg correction; Table E1). Of these, both sets of correlation values corrected for multiple comparisons (“BH” and “BY” methods) identified the proportions of the 16 - 32 μm sediment fractions as the most important *granulometric* factor with respect to which species occurred where and at what densities. Note that the uncorrected data (headed *p*-value for Axis 1) also identified a relationship between coarser sediment fractions (125 - 500 μm and 1,000 - 2,000 μm) and ARESC Axes 1 and 2. With some meiofaunal species with life-cycles as short as 10-20 days, these might still be important factors in the shaping of the offshore meiobenthic communities over longer periods of time.

Although important, the strongest correlations between the meiofaunal nematode community structures and the quantified environmental parameters were not the sediment particle size fractions. Instead, these related to several of the chemical analytes. Structural variance in the nematode communities showed correlations with sediment concentrations of aluminium, arsenic, barium, cobalt, chromium, manganese, mercury, nickel and lead. The same elements were also identified in the uncorrected data with the single addition of zinc which was not found to be correlated with the nematode community structures when allowance was made for multiple comparisons (Table E1).

Note that neither the “BH” nor the “BY” sets of corrected data indicated statistically significant correlations between the sediment metal concentrations and the structures of the meiofaunal nematode communities for ARESC Axes 2 and 3. However, the uncorrected (“raw” *p*-value) correlation data indicated that cadmium and vanadium were associated with ARESC Axis 3 and that total organic carbon was associated with ARESC Axis 2 (Table E1). This implies that the nematode communities differed in their responses to these parameters when compared to the other (Axis 1) environmental parameters.

Examining these data, it appears likely that the distributions and concentrations of sediment TOC, cadmium and vanadium were not solely related to the sediments and that other localised

“processes” may have been involved. Sediment cadmium and vanadium may have been associated with patchy colonies of invertebrates (e.g. beds of polychaete worms, crustaceans and molluscs). As already noted in references cited above, the colonial species may have accumulated these elements in their tissues, shells and cuticles. In the case of organic carbon, elevated sediment values would have reflected the presence of biological tissues wherever beds of macroinvertebrate species had become established. Several published studies show that the stabilising effect of macrofaunal colonies results in accretion of organic/detrital materials that then become incorporated into the seabed. Similarly, the production of faeces and, in some cases, the secretion of mucus materials, that further serve to trap fine organic matter and bind it into sediments, would increase the net organic carbon contents, whilst fuelling microbial populations and increasing biomass still further (see, for example, Hughes and Gerdol, 1994).

Taken together, we believe that these could explain the differential nature of the relationships exhibited between the nematode communities and sediment cadmium, vanadium and total organic carbon contents when compared to other measured sediment physico-chemical determinands in the verification survey area.

3.4 OTHER MEIOFAUNAL GROUPS

The variety and abundance of meiofaunal groups other than nematodes and harpacticoid copepods observed during the taxonomic studies was surprisingly poor. However, polychaete annelid worms and foraminiferans (cased Protista) were found to be present in all samples examined. Most polychaete species present in the meiofaunal size fractions are temporary or transient meiofauna, and are usually represented by juveniles, rendering their taxonomy difficult. Assessment of the polychaete populations is, therefore, best undertaken on mature (adult) specimens present in the macrofaunal fractions (> 500 µm).

Amongst the Foraminifera a variety of test (“shell”) forms was observed during the meiofaunal sample analyses. It would therefore be possible in future surveys to undertake an assessment of the distribution of foraminiferan taxa based on structures of the tests. However, the persistence of tests in the sediment after the animals have died is an issue. Whilst viable tests are generally “glassy” in appearance and non-viable tests appear dull as a result of abrasion, the difference is not always clear and the success of such a study would rely on consistent interpretation of “live” and “dead” frustule appearance.

Occasional ostracods (“seed shrimps”), kinorhynchs (“spiney-crown worms”), ciliates and halacarid mites were also recorded during the taxonomic analyses of the nematode. However, these groups were present at low densities only and, based on the verification survey results, would not provide sufficiently robust data suitable for the assessment and monitoring of changes in benthic habitat conditions.

4 SUMMARY AND CONCLUDING COMMENTS

4.1 THE SURVEY AREA MEIOFAUNAL COMMUNITIES

The meiofauna analyses of the 26 verification sites demonstrated that abundant and diverse/species-rich nematode assemblages occurred in the benthic sediments at and within the vicinity of the proposed mining site. A total of 135 nematode taxa was documented with species richness values of up to 42 species per sample and densities of up to 30,400 nematodes per litre sediment. These data are comparable to other offshore seabed sites collected from similar depths of water.

The harpacticoid copepod assemblages were less diverse and abundant than the nematodes and a total of 36 taxa/species was recorded in the 26 verification samples, with up to 15 taxa documented in a single sample. Respectable densities of up to 687 harpacticoid copepods per litre sediment were recorded. Due to the high fine silt fraction contents of the sediments, the majority of the copepod species comprised larger, epibenthic species rather than the small, often vermiform, interstitial species that are usually more abundant in silt-free, “clean” sands.

The use of different multivariate analyses to examine the nematode communities revealed robust, coherent clusters of communities comprising structurally-related species assemblages. When these clusters were plotted onto the survey sampling site plan, distinctive zones of similar nematode communities were identified (see Section 3.2.2). In general, changes in the nematode community structures were orientated along an east-west, with Clusters A, B and C being located to the west of the survey area and Clusters D, E and F being located to the east.

The structural variation in the nematode communities correlated with the distribution of the measured physico-chemical parameters that were identified by the ARESC analytical techniques. The ARESC diagram (Figure E1) indicated a positive correlation between a suite of sediment metals, the finer silt fraction (< 63 µm) and the nematode communities within cluster A and a negative correlation with cluster F.

The statistical significance of the correlations between the nematode community structures and the physico-chemical parameters were calculated and are presented in Table E1. Of these correlations, the most statistically significant related to a suite of sediment metal concentrations. Statistically significant correlations between the fine silt (< 63 µm) fractions and the nematode community structures were also identified.

Given that the nematode communities present in Cluster A exhibited the highest diversity values recorded during the survey (and, hence, can be considered to the least “stressed”), it is likely that the main driver influencing the nematode community structures was sediment particle size characteristics rather than sediment metal concentrations. The majority of the measured sediment metals will adsorb readily to the charges present on the alumino-silicates of the fine silt fractions and will, therefore, be present at higher concentrations wherever fine silts occur. It is likely, in this case, that the significant correlation between the sediment metals and the nematode community structures is coincidental (due the metals’ associations with the fine sediments) rather than causal. This is supported by the highest correlation values identified by the analyses for sediment aluminium.

4.2 SUITABILITY OF MEIOFAUNAL ANALYSES FOR MONITORING PURPOSES

The verification survey reported here provides firm evidence that the benthic meiofaunal communities present in the vicinity of the proposed pelletal phosphorite mining site are appropriate bioindicators for monitoring and assessment of any operational impacts that might arise. The diversity and abundance of the principal meiofaunal groups, namely the Nematoda and harpacticoid Copepoda, will provide scientifically robust data enabling statistically valid ecological assessments. These will enable pre-mining baseline conditions to be established and described, the extent of the effects of the mining activities to be determined and, as operations move to new sites, the monitoring and documentation of the subsequent recovery of the formerly disturbed benthic habitats.

5 REFERENCES

This report to be cited as:

Physalia (2013). Meiofaunal Analyses of Namibian Offshore Sediments forming part of Verification Surveys for proposed Benthic Mining Operations. Baseline Surveys, July - August 2013. A factual, baseline survey report prepared for Lwandle Technologies (Pty) Ltd, Cape Town, by Physalia Ltd, Consultant & Forensic Ecologists, Harpenden, UK.

Baker, A.D. (1953). Rapid method for mounting nematodes in glycerine. *Canadian Entomology*, **85**: 77-78.

Benjamini, Y. and Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society*, **57**(1): 289-300

Benjamini, Y. and Yekutieli, D. (2001). The control of the false discovery rate in multiple testing under dependency. *Annals of Statistics*, **29**(4): 1165 – 1188.

Bouisseau, J.-P. (1957). Techniques pour l'étude quantitative de la faune interstitielle des sables. *Comptes Rendus du Congrès des Sociétés Savantes des Paris et des Départements*, 1957, 117-119.

Bührer, E.M. (1949). Technique for beheading and *en face* examination of nematodes and similar animal types. *Proceedings of the Helminthological Society of Washington*, **16**: 3-6.

Cairns, E.J. and Tarjan, A.C. (1955). Methylcellulose for the rapid preparation of temporary nematode head mounts. *Proceedings of the Helminthological Society of Washington*, **22**: 32.

Cobb, N.A. (1917). Notes on nemas. *Contr. Sci. Nematol.*, **5**: 117-128.

Dufrêne, M. and Legendre, P. (1997). Species assemblages and indicator species; the need for a flexible asymmetric approach. *Ecological Monographs*, **67**: 345-366.

Erk, M., Ruus, A., Ingerbrigtsen, K. and Hylland, K. (2005). Cadmium accumulation and Cd-binding proteins in marine invertebrates; A radiotracer study. *Chemosphere*, **61**: 1651-1664.

Fattorini, D., Notti, A., Nigro, M. and Regoli, F. (2010). Hyperaccumulation of vanadium in the Antarctic polychaete *Perkinsiana littoralis* as a natural chemical defence against predation. *Environmental Science and Pollution Research*, **17**(1): 220-228.

Fattorini, D. and Regoli, F. (2012). Hyperaccumulation of vanadium in Polychaetes. In *Vanadium - Biochemical and Molecular Biological Approaches*. Pages 73-92. Springer, Netherlands.

Forster, S.J. (1998a). *Aspects of the Ecology of Free-living Nematodes in a Temperate Intertidal Mudflat*. Ph.D. Thesis, University of London.

Flegg, J.J.M. and Hooper, D.J. (1970). Extraction of free-living stages from soil. In *Laboratory Methods for Work with Plant and Soil Nematodes*. Ed. Southey, J.F. Ministry of Agriculture, Fisheries and Food, Technical Bulletin No. 2, H.M.S.O.: London. 148 pp.

- Gerdol, V. and Hughes, R. G. (1994). Effects of *Corphium volutator* on the abundance of benthic diatoms, bacteria and sediment stability in two estuaries in south eastern England. *Marine Ecology Progress Series*, **114**: 109 - 115.
- Humason, G.L. (1979). *Animal Tissue Techniques*. 4th. Edition. Freeman and Co., San Francisco.
- Kruskal, J.B. (1964a). Multidimensional scaling by optimising goodness of fit to a non-metric hypothesis. *Psychometrika*, **29**: 1-27
- Kruskal, J.B. (1964b). Non-metric multidimensional scaling; a numerical method. *Psychometrika*, **29**: 115-129
- Lance, G.N. and Williams, W.T. (1967). A general theory of classification sorting strategies. I. Hierarchical systems. *Computer Journal*, **9**: 373-380
- Lance, G.N. and Williams, W.T. (1968). A general theory of classification sorting strategies. II. Clustering systems. *Computer Journal*, **10**: 271-277
- Lang, K. (1948). *Monographie der Harpacticoiden*. Naturhistorisches Reichsmuseum, Stockholm, pp. 1683.
- Manly, B.F.J. (1997). *Randomisation, Bootstrap and Monte Carlo Methods in Biology*. Second edition. Texts in Statistical Science. Chapman and Hall, London. 399 pp.
- Moens, T., and Vincx M. (1997). Observations on the feeding ecology of estuarine nematodes. *Journal of the Marine Biological Association of the United Kingdom*, **77**, 211-227.
- Peet, R.K. (1974). The measurement of species diversity. *Annual Review of Ecological Systems*, **5**: 285-307.
- Sokal, R.R. and Rohlf, F.J. (1995). *Biometry*. Third edition. Freeman and Co. Ltd. New York. 889 pp.
- Trett, M.W., Calvo Urbano, B., Forster, S.J., Hutchinson, J.D., Feil, R.L., Trett., S.P. and Best, J.G. (2000). Terrestrial Meiofauna and Contaminated Land Assessment. *Environmental Science and Technology*, **34**: 1594 -1602.
- Trett, M.W., Calvo Urbano, B., Forster, S.J. and Trett, S.P. (2009). Commercial Aspects of the Use of Nematodes as Bioindicators. Chapter 12 in *Nematodes as Environmental Indicators* eds. M.J. Wilson and T. Kakouli-Duarte, CAB International, Cambridge, pp. 275 - 326.
- Seinhorst, J.W. (1959). A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica*: **4**: 67-69.

Annexure A: Taxonomic Lists of Invertebrate Species and their Identification Codes

Table A1: Table presenting the full taxonomic list of meiofaunal Nematoda recorded by taxonomist team at Physalia in the July 2013 verification survey area sediments. The numbers shown beside each species are the unique identifier codes (UICs) ascribed by Physalia to Nematode taxa present in each survey region worldwide. These relate to specimens in the faunal reference collections and/or description maintained at Physalia. The UICs are essential for the multivariate (mathematical) analyses of the communities and appear in the site-by-site results tables (see Table B1; Annexure B).

Class Adenophorea; Sub-class Enoplia
Order Enoplida; Sub-order Enoplina

Family Thoracostomopsidae

- 130 *Epacanthion* species
- 118 *Paramesacanthion* species
(? *P. marei*)

Sub-order Trefusiina

Family Trefusiidae

- 102 *Halanonchus* species
- 95 Trefusiid species
(? *Rhabdocoma*)

Sub-order Oncholaimina

Family Enchelidiidae

- 1 *Bathyeurystomina* species

Family Oncholaimidae

- 105 *Oncholaimus* species
- 3 *Viscosia* species (? *V. elegans*)
- 31 *Viscosia* species
- 99 *Viscosia* species

Sub-order Ironina

Family Oxystominidae

- 12 *Halalaimus* species 1 (? *H. isaitshikovi*)
- 26 *Halalaimus* species 2 (? *H. capitulatus*)
- 43 *Halalaimus* species 3
- 45 *Halalaimus* species 4
- 94 *Halalaimus* species 5
- 25 *Nemanema* species 1
- 112 *Nemanema* species 2
- 6 *Oxystomina* species 1 (? *H. asetosa*)
- 70 *Oxystomina* species 2 (? *H. elongata*)
- 90 *Thalassoalaimus* species 1
- 131 *Oxystominidae* species (? *Nemanema*)

Class Chromadorea;

Sub-class Chromadoria

Order Chromadorida;

Sub-order Chromadorina

Family Chromadoridae

- 66 *Acantholaimus* species
- 14 *Actinonema* species 1
- 104 *Actinonema* species 2
- 29 *Innocuonema* species 1
- 98 *Innocuonema* species 2
- 16 *Prochromadorella* species (? *P. septempapillata*)
- 72 *Spiliphera* species

Family Cyatholaimidae

- 9 *Pomponema* species 1
- 20 *Pomponema* species 2
- 57 *Pomponema* species 3
- 71 *Pomponema* species 4
- 129 *Pomponema* species 5
- 36 Cyatholaimidae species 1

Family Ethmolaimidae

- 47 *Comesa* species (? *C. warwicki*)
- 63 *Filitonchus* species (? *F. ewensis*)

Family Selachinematidae

- 60 *Cheironchus* species
- 2 *Gammarus* species 1 (? *G. conicauda*)
- 38 *Gammarus* species 2
- 113 *Synonchiella* species
- 122 Selachinematidae species

Order Desmodorida;

Sub-order Desmodorina

Family Desmodoridae

- 121 *Catanema* species
- 32 *Chromaspirina* species (? *C. multipapillata*)
- 19 *Desmodora* species 1
- 64 *Desmodora* species 2
- 103 *Sigmophoranema* species
- 11 *Spirinia* species 1 (? *S. parasitifera*)
- 27 *Spirinia* species 2 (? *S. laevis*)
- 21 Desmodoridae species 1
- 23 Desmodoridae species 2
- 128 Desmodoridae species 3 (? *Chromaspirina*)

Family Microlaimidae

- 22 *Aponema* species 1

56	<i>Aponema</i> species 2
15	<i>Calomicrolaimus</i> species
35	<i>Microlaimus</i> species 1
73	<i>Microlaimus</i> species 2
80	<i>Microlaimus</i> species 3
92	<i>Microlaimus</i> species 4
132	<i>Microlaimus</i> species 5

Family Paramicrolaimidae

59	<i>Paramicrolaimus</i> species 1
84	<i>Paramicrolaimus</i> species 2

Order Desmoscolecida

Family Desmoscolecidae

34	<i>Quadricoma</i> species
96	<i>Tricoma</i> species

Order Monhysterida;

Sub-order Monhysterina

Family Monhysteridae

17	<i>Monhystera</i> species 1 (? <i>M. vulgaris</i>)
37	<i>Monhystera</i> species 2
52	<i>Monhystera</i> species 3
119	<i>Monhystera</i> species 4
74	Monhysterid species

Family Xyalidae

135	<i>Cobbia</i> species
7	<i>Daptonema</i> species 1
13	<i>Daptonema</i> species 2
30	<i>Daptonema</i> species 3 (? <i>D. psammoides</i>)
61	<i>Daptonema</i> species 4
85	<i>Daptonema</i> species 5
88	<i>Daptonema</i> species 6 (? <i>D. hirsutum</i>)
106	<i>Metadesmolaimus</i> species
33	<i>Theristus</i> species (? <i>T. ensifer</i>)
81	Xyalid species
115	Xyalid species

Sub-order Linhomoeina

Family Linhomoeidae

127	<i>Linhomoeus</i> species 2
44	<i>Metalinhomoeus</i> species 1
48	<i>Metalinhomoeus</i> species 2
49	<i>Metalinhomoeus</i> species 3
69	<i>Metalinhomoeus</i> species 4
93	<i>Metalinhomoeus</i> species 5
125	<i>Metalinhomoeus</i> species 6
10	<i>Paralinhomoeus</i> species 1
42	<i>Paralinhomoeus</i> species 2

54	<i>Paralinhomoeus</i> species 3
55	<i>Paralinhomoeus</i> species 4
101	<i>Terschellingia</i> species 1
28	Linhomoeidae species 1
51	Linhomoeidae species 2
39	Linhomoeidae species 3
77	Linhomoeidae species 4
107	Linhomoeidae species 5
117	Linhomoeidae species 6
120	Linhomoeidae species 7
123	Linhomoeidae species 8

Family Siphonolaimidae

89	<i>Siphonolaimus</i> species
----	------------------------------

Order Araeolaimida

Family Axonolaimidae

53	<i>Odontophora</i> species 1
78	<i>Odontophora</i> species 2
75	<i>Parodontophora</i> species

Family Comesomatidae

4	<i>Paracomesoma</i> species 1
50	<i>Paracomesoma</i> species 2
40	<i>Sabatieria</i> species (? <i>S. punctata</i>)

Family Diplopeltidae

100	<i>Campylaimus</i> species
82	Diplopeltidae species 1 (? <i>Morlaixia</i>)
83	Diplopeltidae species 2

Order Plectida

Family Aegialoalaimidae

97	<i>Aegialoalaimus</i> (? <i>A. elegans</i>)
5	<i>Cyartonema</i> species 1 (? <i>C. elegans</i>)
76	<i>Cyartonema</i> species 2
109	Diplopeltoides species
8	<i>Southernia</i> species
110	<i>Southernia</i> species (? <i>S. zosteriae</i>)
67	Aegialoalaimidae species (? <i>Cyartonema</i>)
116	Aegialoalaimidae species
124	Aegialoalaimidae species

Family Ceramonematidae

18	<i>Ceramonema</i> species 1
114	<i>Ceramonema</i> species 2
79	<i>Dasynemoides</i> species 1
91	<i>Dasynemoides</i> species 2
133	<i>Pselionema</i> species 1
68	<i>Pselionema</i> species 2

- 111 *Pselionema* species 3
- 126 *Pselionema* species 4

Family Leptolaimidae

- 58 *Deontolaimus* species
- 62 *Leptolaimus* species 1 (? *L. elegans*)
- 46 *Leptolaimus* species 2
- 24 *Leptolaimidae* species

Class Indet.; Sub-class Indet.

Order Indet.

Family Indet.

- 86 NAM.13.A (? *Cyatholaimidae*)
- 87 NAM.13.B
- 108 NAM.13.C

Table A2: Table presenting the full taxonomic list of meiofaunal harpacticoid Copepoda (Biramia) recorded by taxonomist team at Physalia in the July 2013 verification survey area sediments. The numbers shown beside each species are the unique identifier codes (UICs) ascribed by Physalia to harpacticoid copepod taxa present in each survey region worldwide. These relate to specimens in the faunal reference collections and/or description maintained at Physalia. The UICs are essential for the multivariate (mathematical) analyses of the communities and appear in the site-by-site results tables (see Table B2; Annexure B).

Phylum Biramia ("Crustacea")

Class Maxillopoda; Sub-class Copepoda

Order Harpacticoida; Sub-order Oligoarthra

Family Ameiridae

5	Ameirid sp. 1
14	Ameirid sp. 2 (? <i>Nitocra</i>)
20	Ameirid sp. 3
33	Ameirid sp. 4
34	Ameirid sp. 5

Family Cletodidae

1	<i>Enhydrosoma</i> species 1
2	Cletodid sp. 1
22	Cletodid sp. 2
23	Cletodid sp. 3
28	Cletodid sp. 4

Family Diosaccidae

31	Diosaccid sp. 2 (? <i>Stenhelia</i>)
35	<i>Stenhelia</i> sp. 3
3	<i>Typhlamphiascus</i> sp. 1
7	Diosaccid sp. (? <i>Bulbamphiascus</i>)
4	Diosaccid sp. 1 (? <i>Amphiascella</i>)
29	Diosaccid sp. 1 (? <i>Amphiascus</i>)
30	Diosaccid sp. 1 (? <i>Stenhelia</i>)
8	Diosaccid sp. 1
12	Diosaccid sp. 2 (? <i>Typhlamphiascus</i>)
10	Diosaccid sp. 2
15	Diosaccid sp. 3
24	Diosaccid sp. 4

Family Ectinosomatidae

13	<i>Ectinosoma</i> (<i>Ectin.</i>) sp. 1 (? <i>Ectinosoma</i>)
27	Ectinosomatidae sp.

Family Harpacticidae

36	Harpacticid sp. (? <i>Harpacticus</i>)
6	Harpacticid sp. 1

Family Laophontidae

9	Laophontid sp. 1
11	Laophontid sp. 2

17	Laophontid sp. 3
Family Thalestridae	
21	Thalestrid sp. 1 (? <i>Thalestris</i>)
26	Thalestrid sp. 3 (? <i>Idomene</i>)
Family Unknown	
19	NAM.7.13A
25	NAM.7.13B
32	NAM.7.13C (?Thalestridae)
16	NAM.7.13D (Miraciidae sp.)
37	NAM.7.13E (Miraciidae sp. 2)

Annexure B: Meiobenthic Community Data Tables

Table B1: Site-by-site results table for the communities of meiofaunal Nematoda present in the July 2013 verification survey area sediment samples. Species codes (in brackets) are followed by the numbers of animals belonging to that species recorded per litre sediment. The key to the species codes used is presented in Table A1 (Annexure A).

Site	Species Identification Code and Numbers per Litre Sediment	Species Richness	Densities	Feeding Type Ratios
VS 1	(1)135; (2)271; (3)406; (4)271; (5)813; (12)677; (14)542; (15)135; (16)135; (22)406; (27)1625; (28)135; (34)542; (35)542; (37)677; (40)813; (42)135; (43)135; (47)135; (52)135; (56)135; (57)406; (60)135; (62)1083; (66)135; (70)135; (72)406; (80)271; (95)135; (96)135; (97)406; (98)135; (99)271; (100)135; (101)135; (102)135; (103)271; (104)135; (105)135; (106)135; (107)135; (121)135	42	13804	0.37
VS 2	(1)123; (2)123; (3)123; (4)246; (5)676; (12)123; (13)123; (14)184; (18)123; (19)184; (22)492; (27)184; (32)61; (34)123; (35)246; (36)61; (37)184; (47)184; (50)123; (57)184; (60)61; (61)123; (62)369; (66)184; (69)61; (70)123; (79)123; (80)123; (91)61; (92)61; (97)61; (107)61; (108)246; (109)123; (110)61; (111)61; (112)61; (113)61; (114)123; (115)61; (133)61	41	6139	0.18
VS 3	(4)1583; (5)905; (11)226; (12)226; (18)226; (19)302; (20)226; (22)377; (25)151; (26)75; (27)151; (30)75; (32)75; (33)75; (35)151; (37)226; (46)75; (48)75; (50)528; (57)151; (62)302; (66)151; (70)151; (72)75; (75)75; (76)75; (83)151; (87)75; (108)226; (109)75; (116)151; (117)75; (118)75	33	7536	0.08
VS 4	(1)300; (2)600; (3)450; (4)2551; (5)1501; (6)300; (7)300; (8)150; (9)300; (10)600; (11)600; (12)600; (13)1201; (14)300; (15)600; (16)600; (17)150; (18)300; (19)450; (20)1201; (21)300; (22)1351; (23)150; (24)150; (25)750; (26)300; (27)150; (28)300; (29)150; (30)150; (31)150; (32)150; (33)150; (34)150	34	17405	0.34
VS 5	(4)3061; (5)106; (11)1161; (13)739; (14)106; (19)211; (20)1583; (25)633; (32)317; (33)1478; (45)106; (56)739; (62)106; (66)211	14	10557	0.36
VS 6	(2)239; (4)1317; (5)1556; (9)479; (11)120; (15)120; (19)359; (20)479; (24)120; (27)1077; (28)120; (32)120; (34)120; (35)239; (36)1077; (37)838; (38)120; (39)120; (40)1436; (42)479; (43)359; (44)120; (45)120; (46)120; (47)120; (48)120; (49)120; (50)120; (66)239	29	11973	0.54
VS 7	(2)47; (3)47; (4)608; (5)842; (9)234; (15)47; (16)47; (18)47; (20)187; (22)140; (25)47; (27)47; (29)47; (32)47; (35)47; (36)94; (37)187; (39)47; (40)94; (42)140; (47)94; (50)94; (51)47; (52)234; (53)94; (54)187; (55)94; (56)47; (57)94; (58)94; (59)47; (60)47; (61)94; (62)47; (63)47; (64)47; (66)47; (67)140; (68)47; (75)94	40	4779	0.35
VS 8	(4)4561; (5)1320; (11)240; (12)120; (19)120; (20)240; (22)1680; (25)120; (27)240; (30)120; (33)240; (34)120; (37)360; (42)120; (50)600; (56)240; (57)360; (61)120; (62)600; (69)240; (70)120; (71)120; (72)120	23	12121	0.10

SECTION C, SPECIALIST STUDIES
C2.4 Meiofaunal Analysis of Namibian Offshore Sediments

Site	Species Identification Code and Numbers per Litre Sediment	Species Richness	Densities	Feeding Type Ratios
VS 9	(2)161; (3)161; (4)5647; (5)1613; (10)484; (11)645; (13)161; (15)161; (18)161; (19)161; (20)807; (22)645; (23)161; (25)484; (27)323; (28)161; (30)161; (33)323; (35)161; (37)807; (42)161; (47)323; (50)323; (55)161; (57)645; (62)161; (66)161; (69)161; (71)161; (73)161; (74)161; (75)161; (76)161	33	16289	0.19
VS 10	(2)485; (4)9216; (5)1455; (10)485; (11)2183; (12)243; (15)243; (16)243; (18)243; (20)1455; (25)728; (30)728; (33)970; (40)243; (42)243; (43)243; (50)243; (56)1455; (57)243; (70)485; (72)1698; (75)243; (77)243; (78)243	24	24259	0.20
VS 11	(3)112; (4)1791; (5)896; (12)560; (19)224; (22)672; (25)336; (27)1232; (29)112; (30)112; (34)112; (35)448; (37)224; (40)1120; (43)112; (47)336; (60)112; (61)672; (62)672; (63)112; (76)224; (80)112; (85)112; (87)112; (96)112; (97)672; (99)112; (113)112; (116)112; (118)112; (121)112; (135)224	32	12095	0.57
VS 12	(3)78; (4)1947; (5)857; (6)78; (11)78; (12)78; (14)156; (18)78; (19)78; (20)78; (22)545; (25)234; (26)156; (27)234; (30)78; (34)78; (37)156; (47)467; (57)78; (61)234; (62)389; (63)234; (69)234; (70)156; (75)78; (80)78; (81)78; (82)156; (87)78; (97)78; (107)78; (119)78; (120)78; (121)78; (122)234	35	7871	0.21
VS 13	(2)86; (4)242; (5)225; (12)17; (16)17; (20)35; (22)52; (25)35; (27)17; (34)17; (35)17; (37)86; (50)35; (56)35; (61)52; (62)35; (70)35; (75)17; (81)104; (83)69; (87)69; (89)17; (98)35; (101)17; (120)17; (121)17; (123)52; (124)121; (125)156; (135)17	30	1746	0.85
VS 14	(1)91; (4)3282; (5)1003; (10)182; (11)274; (13)91; (18)91; (20)91; (22)638; (25)274; (27)182; (30)91; (32)91; (33)182; (35)91; (42)274; (50)274; (57)91; (62)547; (66)91; (70)274; (72)365; (76)91; (79)91; (80)182; (81)91; (82)182; (83)91	28	9298	0.18
VS 15	(1)120; (3)120; (4)3007; (5)962; (6)120; (10)120; (11)962; (12)601; (13)120; (19)120; (20)962; (22)361; (25)241; (27)120; (30)842; (32)241; (33)120; (42)241; (43)481; (46)120; (47)120; (50)361; (56)120; (57)481; (61)241; (62)241; (72)120; (80)120; (92)120; (93)120; (94)120	31	12145	0.29
VS 16	(2)894; (4)2235; (5)1564; (9)224; (11)3352; (12)1341; (13)1118; (16)224; (19)224; (20)3576; (25)447; (33)224; (40)224; (50)447; (53)224; (56)2011; (57)670; (62)447; (66)447; (72)894; (74)670; (79)224; (90)224; (98)447	24	22352	0.14
VS 17	(2)144; (4)144; (5)402; (6)29; (11)29; (12)29; (18)29; (20)57; (22)172; (27)287; (30)29; (32)29; (34)172; (35)287; (37)29; (40)172; (42)57; (47)115; (52)29; (61)29; (62)115; (66)29; (80)29; (81)29; (96)29; (101)29; (108)29; (110)29; (119)29; (121)29; (126)29; (127)29; (128)115; (129)29; (130)29	35	2877	3.20
VS 18	(2)644; (4)176; (5)526; (9)176; (14)58; (19)58; (22)58; (25)58; (27)58; (32)117; (34)58; (37)468; (40)58; (42)176; (45)58; (49)58; (50)58; (55)58; (56)292; (57)58; (61)58; (62)117; (66)117; (71)1638; (72)58; (81)58; (83)58; (121)176; (127)58; (131)234	30	5843	0.18

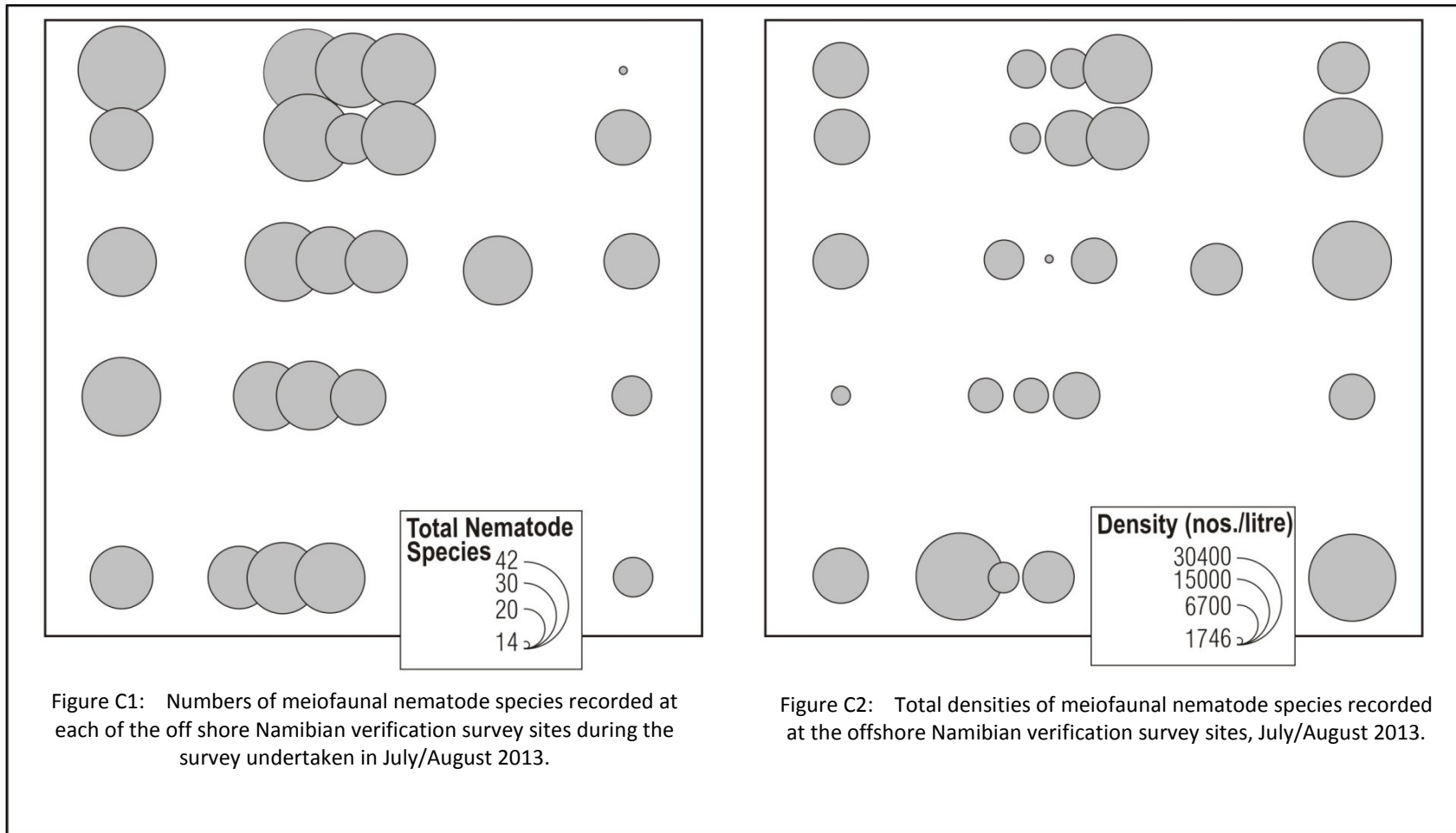
SECTION C, SPECIALIST STUDIES
C2.4 Meiofaunal Analysis of Namibian Offshore Sediments

Site	Species Identification Code and Numbers per Litre Sediment	Species Richness	Densities	Feeding Type Ratios
VS 19	(1)58; (2)115; (3)115; (4)2362; (5)518; (12)58; (19)58; (20)58; (22)58; (26)58; (27)173; (30)115; (33)115; (35)58; (37)461; (38)115; (40)115; (42)58; (47)58; (49)58; (50)173; (52)58; (53)58; (56)58; (76)58; (81)115; (83)230; (105)58; (119)58; (120)58; (121)58	31	5766	0.22
VS 20	(2)95; (3)95; (4)4474; (5)571; (12)190; (14)95; (16)95; (18)95; (19)190; (20)190; (22)666; (25)190; (27)571; (30)381; (33)190; (34)190; (35)190; (37)286; (38)95; (44)95; (50)381; (62)190; (70)190; (86)95; (87)95	25	9895	0.10
VS 21	(2)347; (4)2340; (11)1560; (12)347; (13)173; (16)173; (19)173; (20)520; (22)87; (25)87; (33)433; (40)173; (42)173; (50)87; (56)693; (57)260; (62)87; (66)173; (74)780; (87)87	20	8753	0.21
VS 22	(4)1028; (5)571; (11)114; (12)685; (14)343; (18)114; (19)114; (22)343; (25)114; (27)1028; (30)457; (34)914; (35)1942; (40)685; (43)228; (47)571; (50)114; (57)228; (61)114; (62)914; (66)114; (80)114; (90)114; (96)228; (97)228; (98)114; (109)114; (119)114; (132)114	29	11875	0.24
VS 23	(2)873; (4)7855; (5)2327; (12)1745; (13)1455; (14)291; (16)582; (18)291; (19)582; (20)1164; (22)2327; (25)291; (27)873; (33)291; (34)1164; (35)873; (37)291; (38)291; (39)291; (47)873; (61)291; (62)1164; (66)582; (70)291; (80)291; (83)873; (121)291; (124)291	28	28804	0.15
VS 24	(4)1181; (5)246; (10)49; (13)295; (16)49; (19)148; (22)295; (25)148; (27)295; (30)98; (37)98; (38)148; (40)98; (47)49; (50)148; (55)49; (56)98; (57)49; (61)49; (62)246; (68)49; (70)148; (83)98; (90)98; (91)49; (97)49; (107)148; (112)148; (119)98; (120)49; (121)49; (124)49; (128)49	33	4917	0.40
VS 25	(3)335; (4)2906; (5)1006; (7)112; (11)447; (12)335; (13)112; (16)112; (20)224; (22)1341; (25)335; (29)112; (30)112; (37)112; (38)112; (42)335; (46)447; (47)112; (50)559; (57)335; (62)559; (66)112; (69)112; (70)112; (72)112; (76)112; (79)112; (88)112; (89)112; (90)112; (91)112	31	11180	0.18
VS 26	(4)6992; (5)608; (6)304; (11)5776; (12)1216; (20)6384; (25)1520; (26)608; (27)608; (32)304; (33)2128; (50)304; (53)304; (56)912; (57)912; (62)608; (72)304; (75)304; (98)304	19	30400	0.13
July/August 2013 Survey				

Table B2: Site-by-site results table for the harpacticoid Copepoda communities present in the July 2013 verification survey area sediment samples. Species codes (in brackets) are followed by the numbers of animals belonging to that species recorded per litre sediment. The key to the species codes used is given in Table A2 in Annexure A.

Site	Identification Code and Number per Litre	No. Species	Density
VS 1	(6)14; (13)14; (27)14; (28)14; (29)14; (30)14; (31)43	7	127
VS 2	(4)18; (7)18; (10)18; (14)18; (16)18; (28)18; (29)18	7	126
VS 3	(32)4	1	4
VS 4	(1)9; (2)37; (3)37; (4)37; (5)19; (6)28; (7)102; (8)167; (9)93; (10)19; (11)19; (12)83; (13)19; (14)9; (15)9	15	687
VS 5	(3)18; (16)175; (33)18; (34)35	4	246
VS 6	(16)10	1	10
VS 7	(7)5; (13)5; (16)10	3	20
VS 8	(17)12	1	12
VS 9	(3)14; (4)14; (7)14; (8)70; (9)14; (10)28; (11)14; (17)42	8	210
VS 10	(7)19; (16)112; (19)112; (20)56	4	299
VS 11	(4)45; (17)45; (35)89	3	179
VS 12	(1)9; (36)17	2	26
VS 13	(1)38	1	38
VS 14	(7)5; (8)20; (10)10; (21)5; (22)5; (23)5	6	50
VS 15	(3)8; (6)8; (8)40; (17)16; (21)16; (24)8; (25)16	7	112
VS 16	(3)10; (5)10; (16)97	3	117
VS 17	(3)12	1	12
VS 18	(16)42; (37)8	2	50
VS 20	(5)21; (6)21; (8)42; (9)42; (17)21; (25)21; (26)104	7	272
VS 21	(16)147; (19)29	2	176
VS 22	(4)81; (9)40; (25)40; (30)81; (31)81	5	323
VS 23	(16)10	1	10
VS 24	(14)6	1	6
VS 25	(6)19; (8)134; (14)38; (16)19; (21)19; (24)19	6	248
VS 26	(7)4; (16)8	2	12

Annexure C: Univariate Distribution Plots for Key Meiobenthic Community Parameters



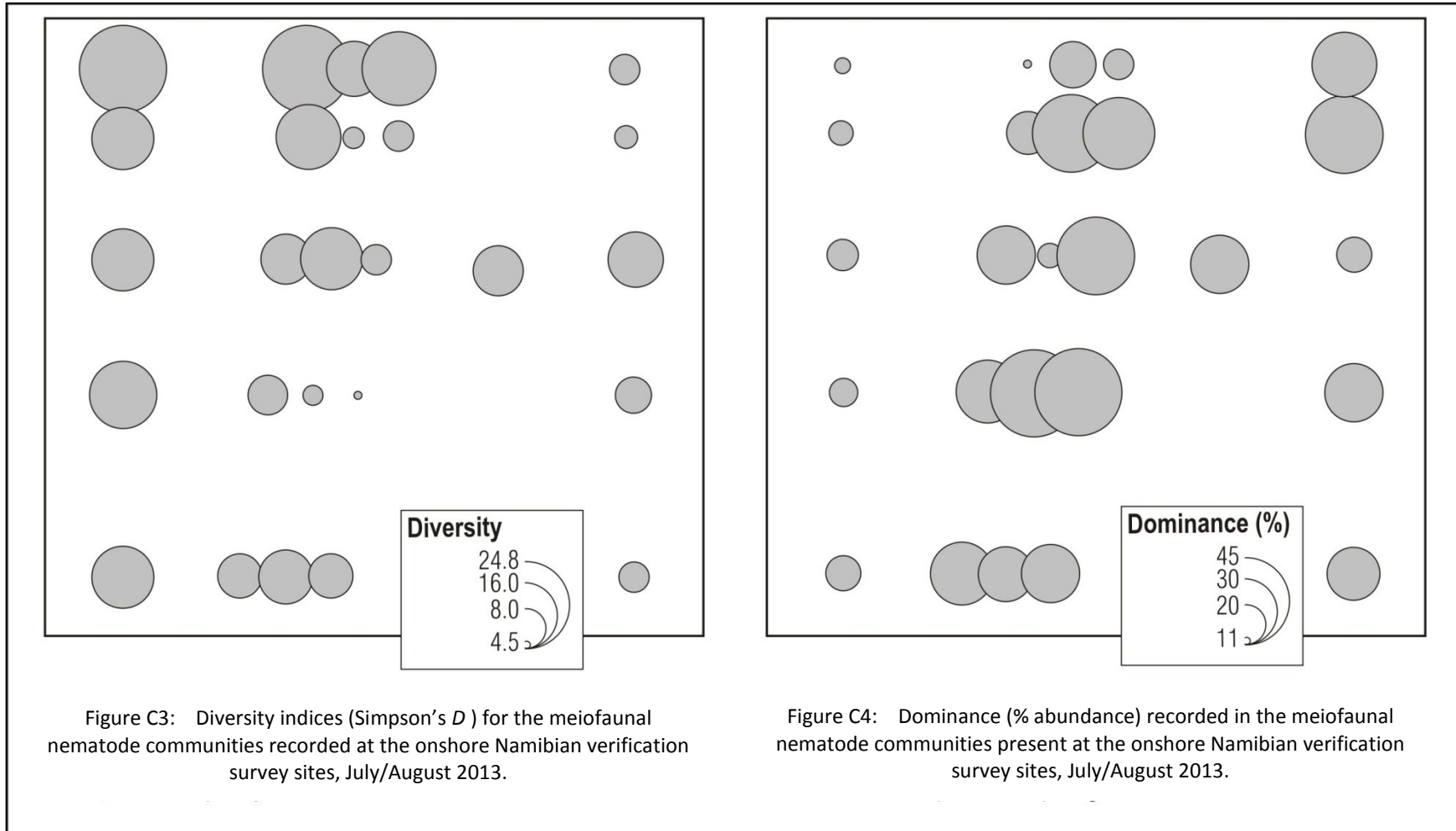
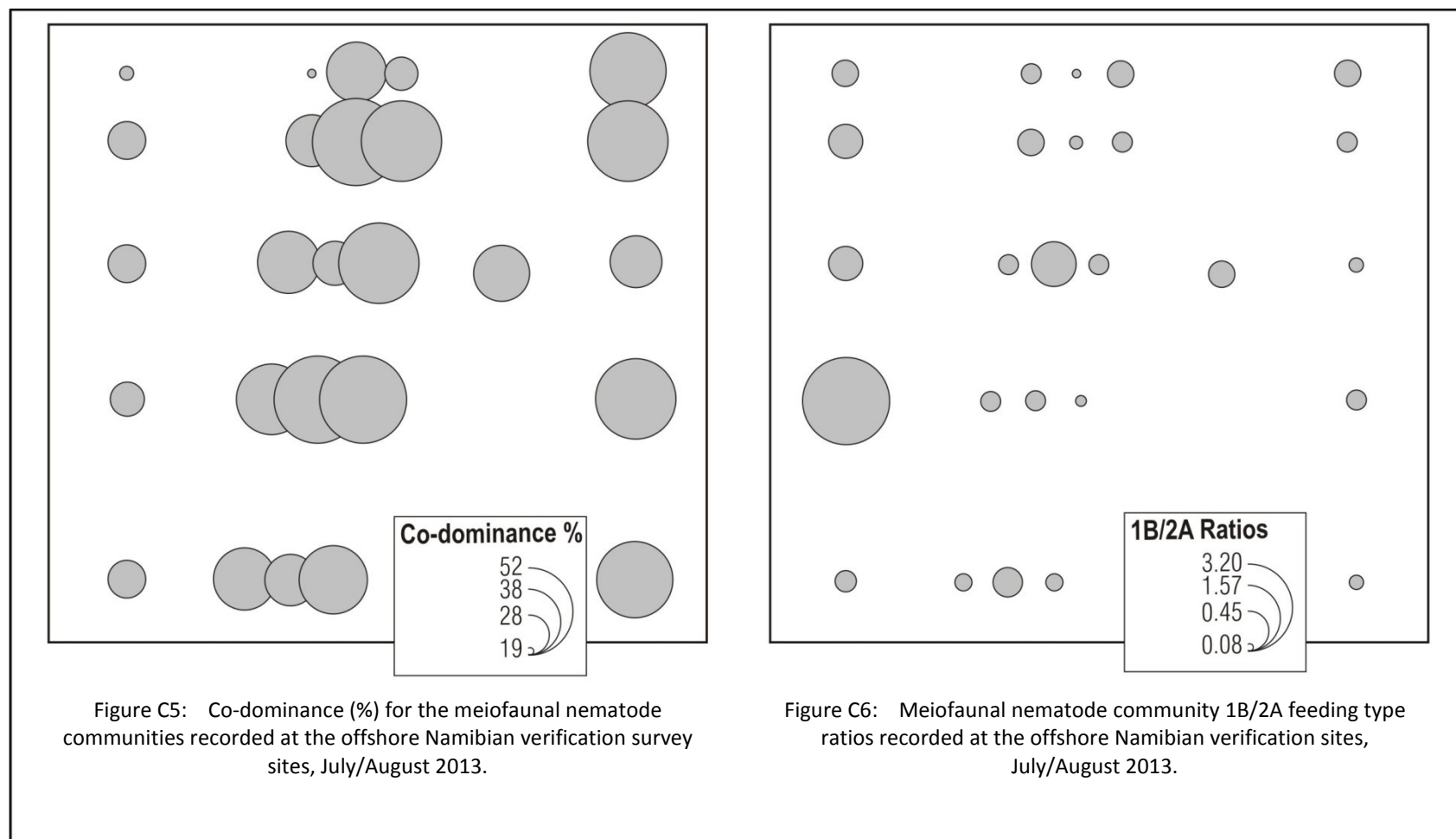


Figure C3: Diversity indices (Simpson's D) for the meiofaunal nematode communities recorded at the onshore Namibian verification survey sites, July/August 2013.

Figure C4: Dominance (% abundance) recorded in the meiofaunal nematode communities present at the onshore Namibian verification survey sites, July/August 2013.



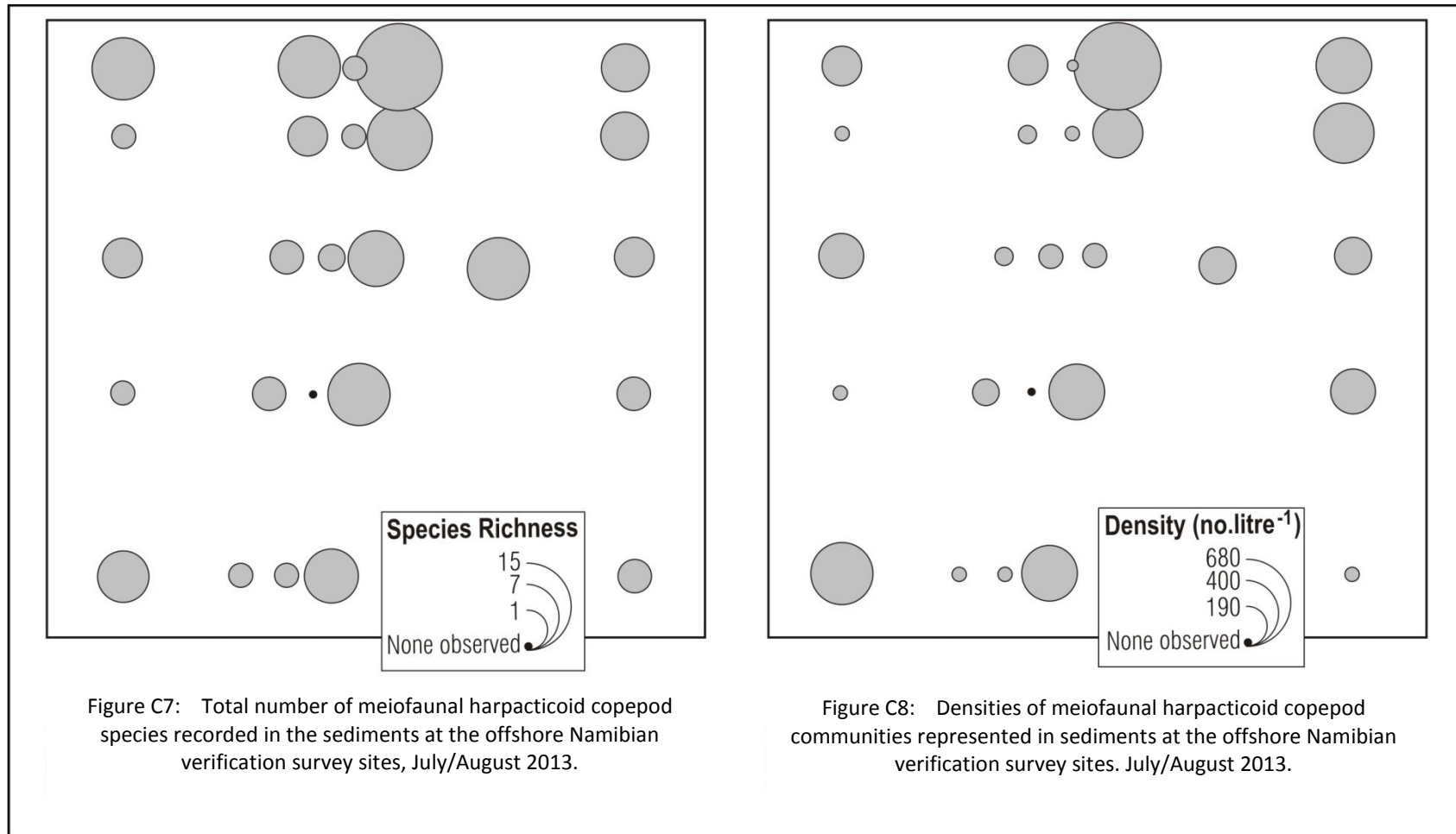


Figure C7: Total number of meiofaunal harpacticoid copepod species recorded in the sediments at the offshore Namibian verification survey sites, July/August 2013.

Figure C8: Densities of meiofaunal harpacticoid copepod communities represented in sediments at the offshore Namibian verification survey sites. July/August 2013.

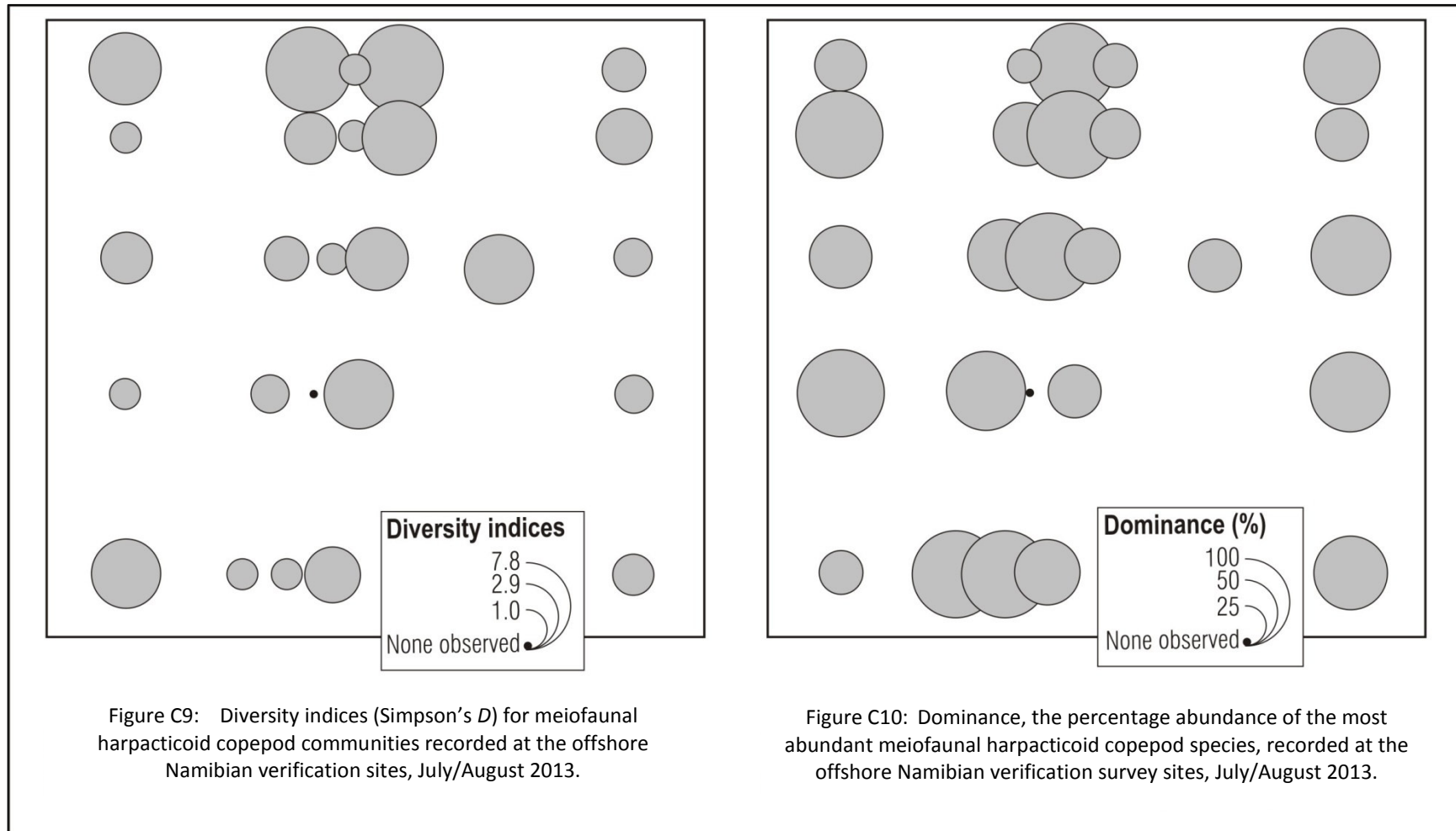


Figure C9: Diversity indices (Simpson's D) for meiofaunal harpacticoid copepod communities recorded at the offshore Namibian verification sites, July/August 2013.

Figure C10: Dominance, the percentage abundance of the most abundant meiofaunal harpacticoid copepod species, recorded at the offshore Namibian verification survey sites, July/August 2013.

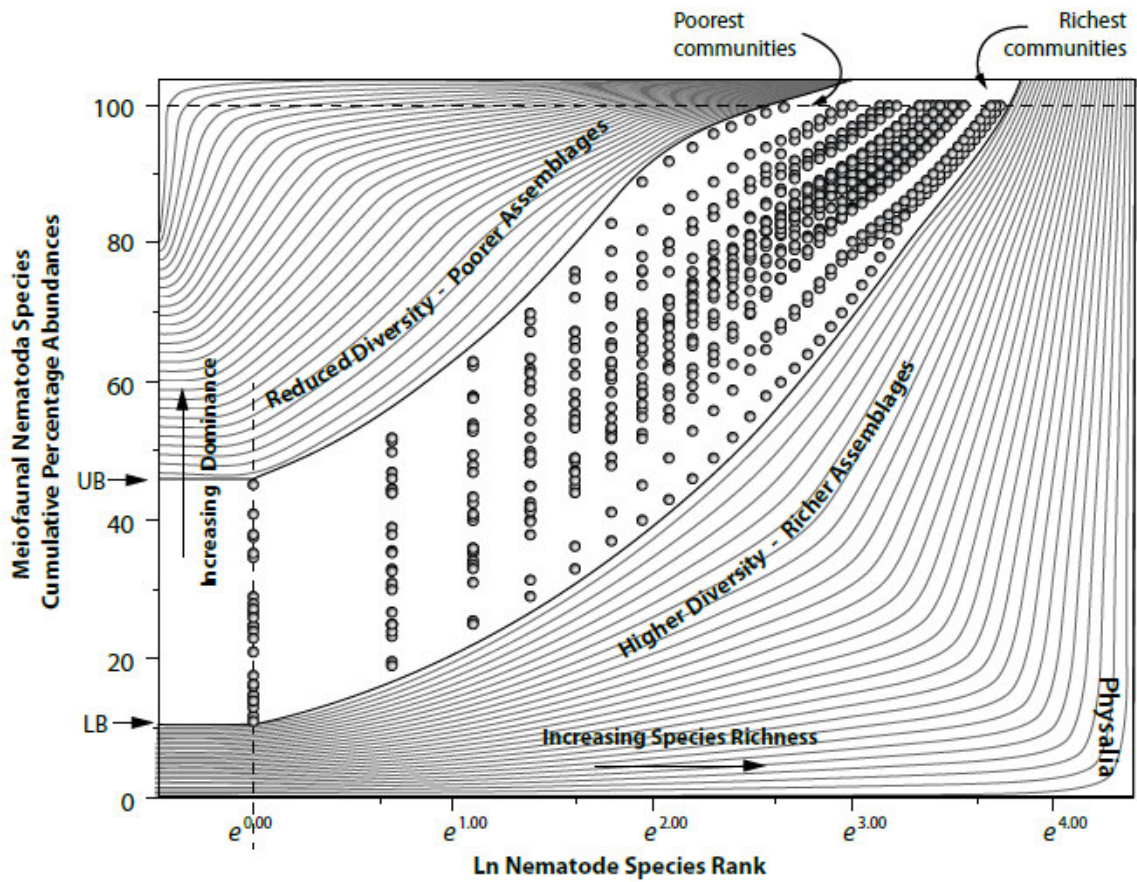


Figure C11: K-dominance plots for the July / August 2013 Namibian meiofaunal nematode communities. These comprise combined dominance: diversity curves for each of the nematode assemblages described by Physalia in the verification survey which forms part of the baseline assessment for proposed dredging operations. UB = upper dominance – diversity boundary (most ‘stressed’ of modified assemblage); LB = lower dominance – diversity boundary (least ‘stressed’ / most species rich assemblage).

Annexure D: Multivariate Analyses of the Meiobenthic Nematode Community Structures

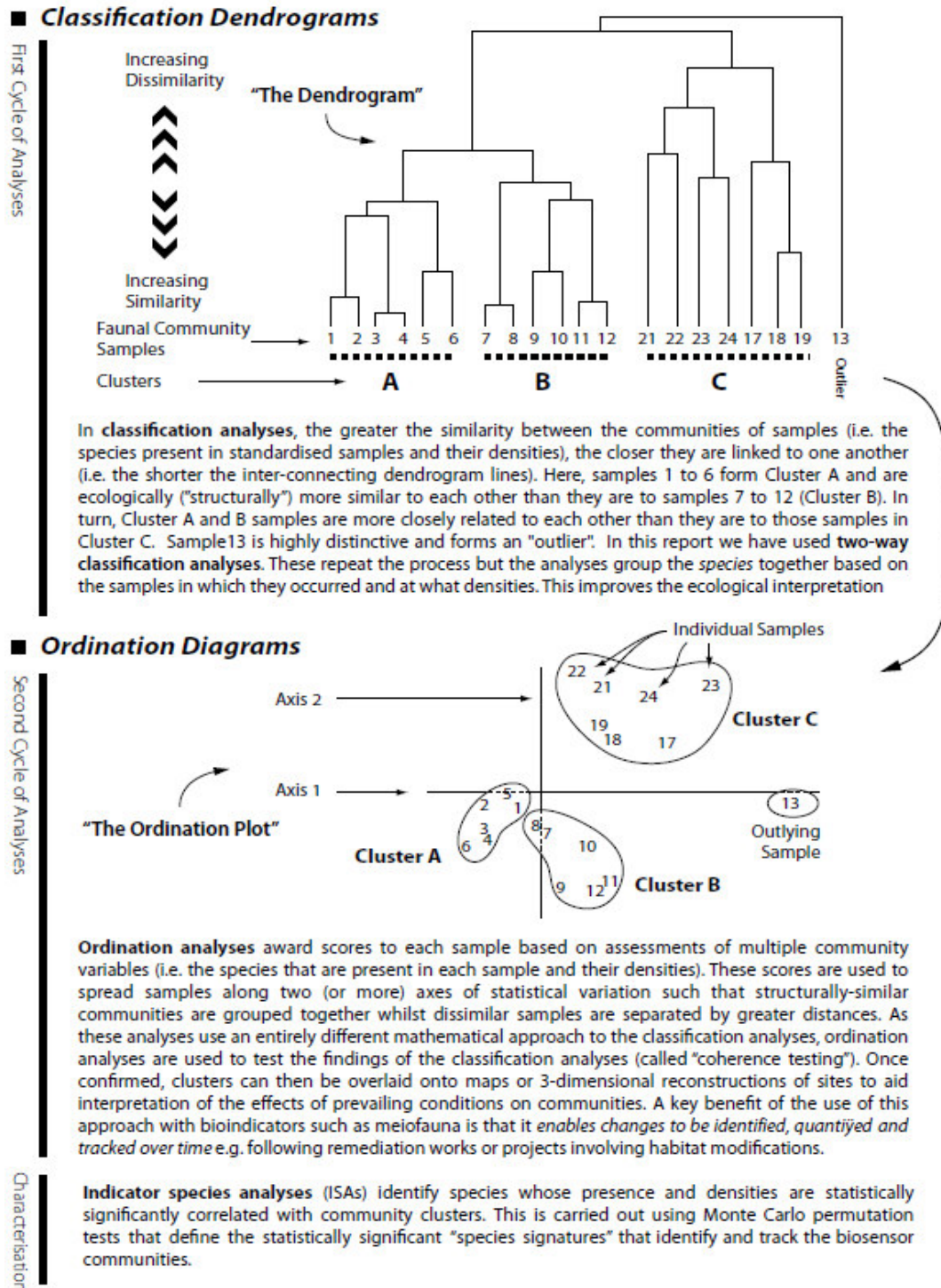


Figure D1: Interpretation of Physalia Multivariate Analyses

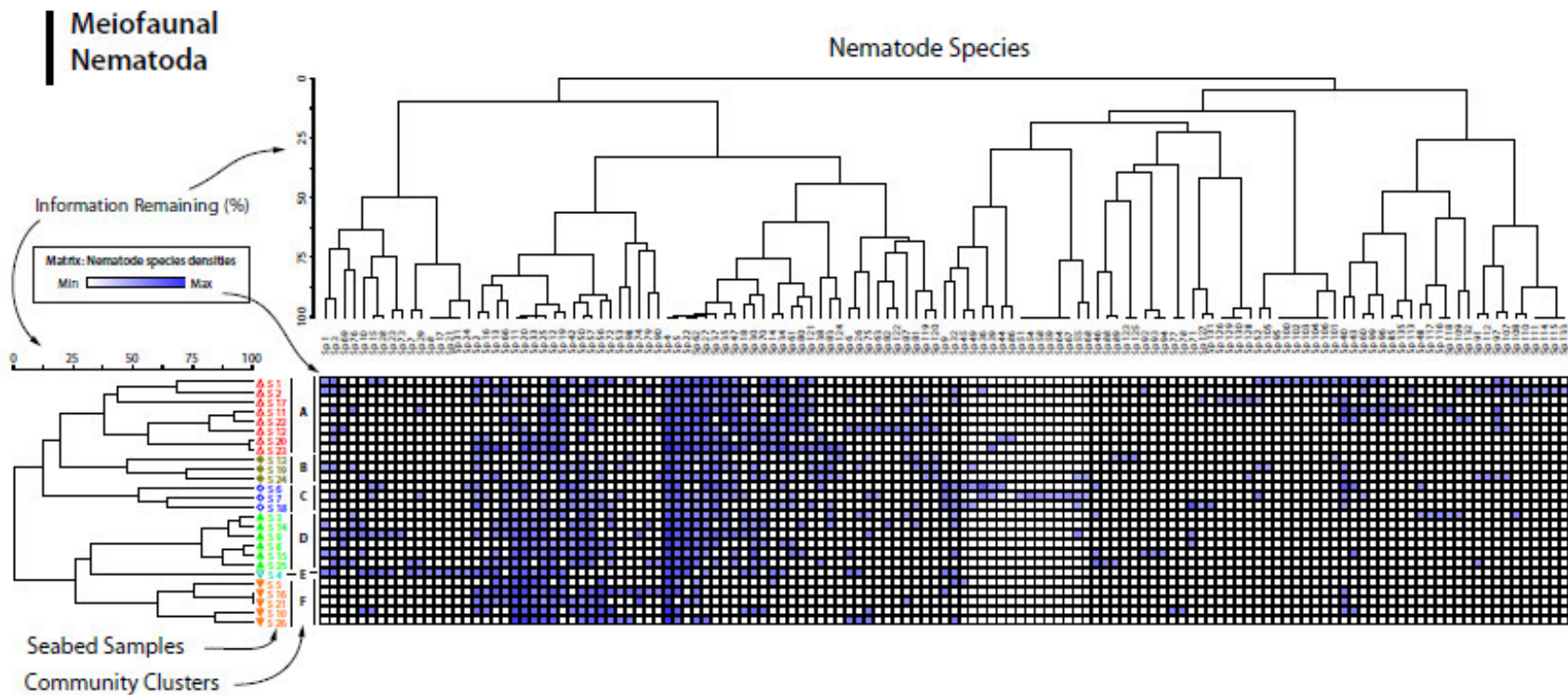


Figure D2: Two-way classification analyses (Sorensen's distance + flexible β – cluster analyses) identifying structural relationships between the communities of meiofaunal nematode worms (species / individuals > 38 μ m) that were present in the 2013 verification survey area). See Figure D3 for the parallel NMMDS analyses used to corroborate the relationships described here. See Annexure A for key to nematode species.

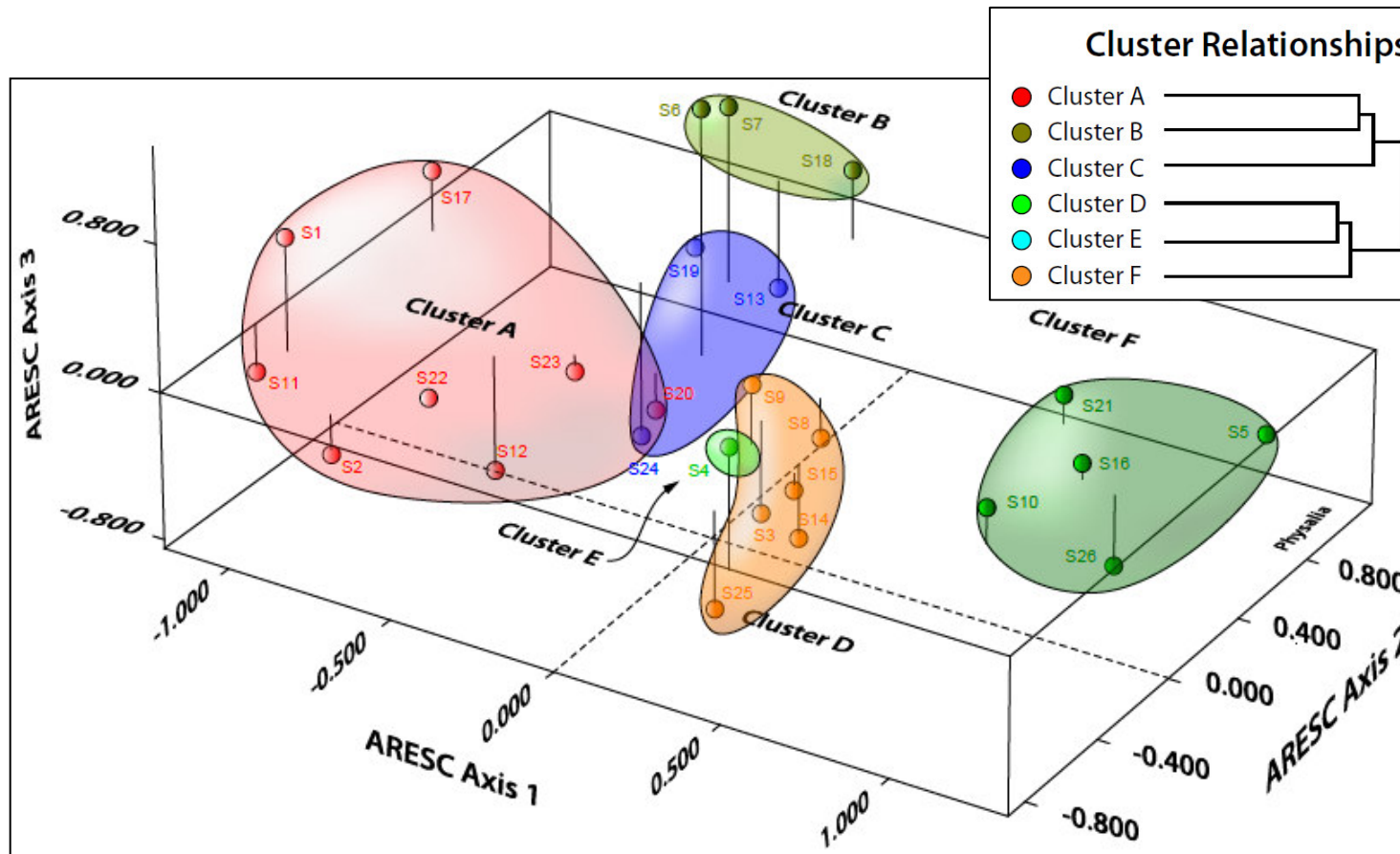


Figure D3: Multivariate ordination analyses (non-metric multi dimensional scaling) of the 2013 meiofaunal nematode communities (individuals > 38 38µm) present in the Namibian seabed survey area. Three axes shown here accounted for 81.4% of the total variation recorded in the communities

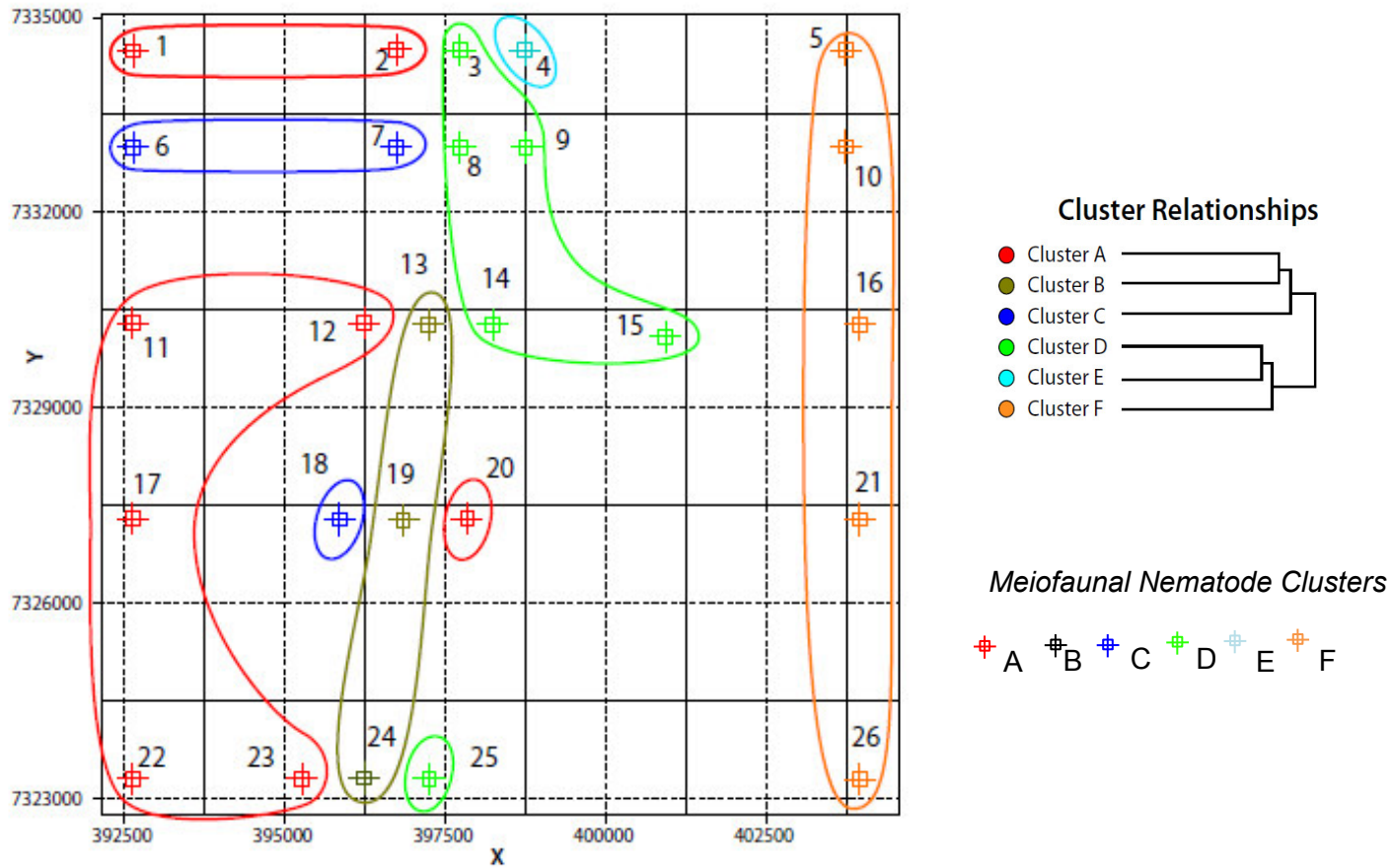


Figure D4: Distributions of structurally – related meiofaunal nematode communities recorded in the sediments offshore of Namibian in the July /August baseline survey

Table D1: List of the indicator species

MVA Cluster	Species Code	Indicator Value	Mean	St. Dev.	p-Value*	Indicator Species/Taxon/OTU
A	12	33.3	26.5	4.13	0.0270	<i>Halalaimus</i> species 1 cf. <i>H. isaitshikovi</i>
	14	48.0	23.4	10.07	0.0468	<i>Actinonema</i> species 1
	27	29.5	25.7	2.43	0.0408	<i>Spirinia</i> species 2
	34	54.1	25.3	8.38	0.0006	<i>Quadricoma</i> species
	62	27.6	24.2	1.31	0.0040	<i>Leptolaimus</i> species 1 cf. <i>L. elegans</i>
	80	62.5	24.1	8.96	0.0006	<i>Microaimus</i> species 3
	96	50.0	20.2	11.11	0.0138	<i>Tricoma</i> species
	97	44.8	22.0	11.51	0.0304	<i>Aegialolaimus</i> species cf. <i>A. elegans</i>
B	83	55.6	22.7	10.85	0.0218	Diplopeltidae species 2
	119	43.3	21.1	11.82	0.0236	<i>Monhystera</i> species 4
	120	86.9	20.6	11.64	0.0024	Linhomoeidae species 7
	124	53.6	20.4	11.25	0.0354	Aegialolaimidae species
C	9	83.8	20.4	11.61	0.0032	<i>Pomponema</i> species 1
	36	58.8	20.2	11.1	0.0118	Cyatholaimidae species 1
	39	53.5	20.2	11.16	0.0382	Linhomoeidae species 3
	45	50.7	20.0	10.76	0.0478	<i>Halalaimus</i> species 4
D	22	30.8	25.6	2.29	0.0068	<i>Aponema</i> species 1
	30	40.1	25.8	7.42	0.0156	<i>Daptonema</i> species 3 (cf. <i>D. psammoides</i>)
	76	40.3	21.7	11.82	0.0390	<i>Cyartonema</i> species 2
E	← Single community cluster; not possible to identify individual cluster-specific, statistically significant nematode indicator species could be identified by the analyses for this cluster					
F	11	45.9	26.2	6.78	0.0004	<i>Spirinia</i> species 1 (cf. <i>S. parasitifera</i>)
	20	34.0	26.2	3.41	0.0048	<i>Pomponema</i> species 2
	25	30.1	25.6	2.15	0.0112	<i>Nemanema</i> species 1
	33	48.0	25.1	7.96	0.0014	<i>Theristus</i> species (ascribed to <i>T. ensifer</i>)
	56	42.1	25.5	7.91	0.0220	<i>Aponema</i> species 2

Physalia

Table D1. List of the indicator species that exhibited statistically significant correlations with the clusters of the meiofaunal nematode communities described in the 2013 seabed survey area off the Namibian coast. Data derived from Monte Carlo permutation test-based indicator species analyses. *p-values relate to correlations between species and their respective clusters.

**Annexure E: ARESC Analyses Identifying Relationships Between
Community Structures and Measured Seabed Environmental
Parameters**

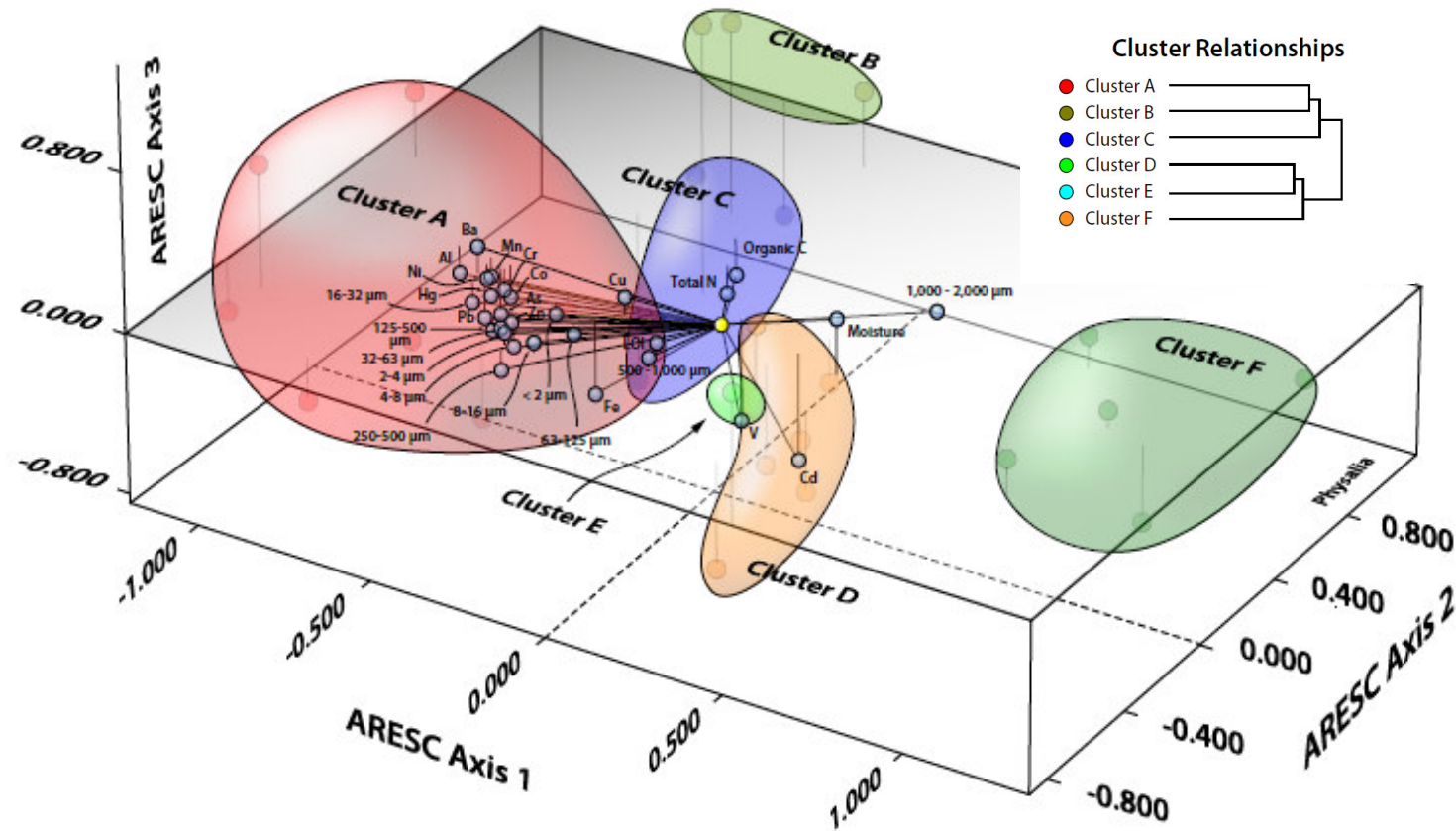


Figure E1: ARESC plot showing mathematical relationship between meiofaunal communities and the sediment physico-chemical environment.
 Note 1: higher metal concentrations with finer sediments (silt-binding): Cluster A communities (offshore) associated with non-selective deposit feeders, detritivores and microbivorous species. Note 2: High vanadium, cadmium, Total N and organic C found in coarser sediments

Table E1: The formal ARESC analysis table

Sediment Determinands	Axis1 (41.4% of total variance)			Axis2 (19.1% of total variance)			Axis3 (20.9% of total variance)		
	<i>p</i> -Value	BH	BY	<i>p</i> -Value	BH	BY	<i>p</i> -Value	BH	BY
Al	8.760E-06	2.540E-04	1.006E-03	9.771E-01	9.771E-01	1.000E+00	4.922E-01	9.693E-01	1.000E+00
As	1.408E-03	4.538E-03	1.798E-02	7.439E-01	9.318E-01	1.000E+00	2.681E-01	9.693E-01	1.000E+00
Ba	4.164E-04	3.019E-03	1.196E-02	5.986E-01	9.136E-01	1.000E+00	5.140E-01	9.693E-01	1.000E+00
Cd	2.504E-01	3.157E-01	1.000E+00	9.108E-01	9.665E-01	1.000E+00	5.121E-03	1.214E-01	4.808E-01
Co	1.156E-03	4.538E-03	1.798E-02	8.640E-01	9.637E-01	1.000E+00	4.401E-01	9.693E-01	1.000E+00
Cr	1.344E-03	4.538E-03	1.798E-02	7.851E-01	9.318E-01	1.000E+00	6.254E-01	9.693E-01	1.000E+00
Cu	2.657E-01	3.211E-01	1.000E+00	6.718E-01	9.318E-01	1.000E+00	6.892E-01	9.693E-01	1.000E+00
Fe	1.847E-01	2.551E-01	1.000E+00	4.248E-01	8.021E-01	1.000E+00	6.560E-02	6.342E-01	1.000E+00
Mn	2.826E-04	3.019E-03	1.196E-02	9.332E-01	9.665E-01	1.000E+00	6.342E-01	9.693E-01	1.000E+00
Hg	5.971E-04	3.463E-03	1.372E-02	4.410E-01	8.021E-01	1.000E+00	6.123E-01	9.693E-01	1.000E+00
Ni	3.823E-04	3.019E-03	1.196E-02	7.790E-01	9.318E-01	1.000E+00	7.356E-01	9.693E-01	1.000E+00
Pb	9.893E-04	4.538E-03	1.798E-02	5.230E-01	8.427E-01	1.000E+00	3.531E-01	9.693E-01	1.000E+00
V	8.912E-01	8.912E-01	1.000E+00	8.032E-01	9.318E-01	1.000E+00	8.370E-03	1.214E-01	4.808E-01
Zn	4.364E-02	7.176E-02	2.843E-01	5.065E-01	8.427E-01	1.000E+00	7.800E-01	9.693E-01	1.000E+00
Moisture	6.852E-02	1.046E-01	4.143E-01	7.602E-01	9.318E-01	1.000E+00	1.816E-01	9.693E-01	1.000E+00
TOC	3.294E-01	3.821E-01	1.000E+00	2.971E-02	4.413E-01	1.000E+00	3.636E-01	9.693E-01	1.000E+00
Total N	5.283E-01	5.892E-01	1.000E+00	1.674E-01	4.413E-01	1.000E+00	5.081E-01	9.693E-01	1.000E+00
LOI	7.760E-01	8.335E-01	1.000E+00	2.703E-01	6.030E-01	1.000E+00	8.037E-01	9.693E-01	1.000E+00
1,000-2,000 µm	3.633E-02	6.584E-02	2.608E-01	6.471E-02	4.413E-01	1.000E+00	9.943E-01	9.943E-01	1.000E+00
500-1,000 µm	8.475E-01	8.778E-01	1.000E+00	1.411E-01	4.413E-01	1.000E+00	8.314E-01	9.693E-01	1.000E+00
250-500 µm	1.139E-01	1.652E-01	6.544E-01	4.993E-02	4.413E-01	1.000E+00	9.411E-01	9.747E-01	1.000E+00
125-250 µm	4.454E-02	7.176E-02	2.843E-01	4.663E-02	4.413E-01	1.000E+00	8.046E-01	9.693E-01	1.000E+00
63-125 µm	2.045E-01	2.696E-01	1.000E+00	1.482E-01	4.413E-01	1.000E+00	9.006E-01	9.693E-01	1.000E+00
32-63 µm	1.824E-02	4.281E-02	1.696E-01	1.271E-01	4.413E-01	1.000E+00	9.025E-01	9.693E-01	1.000E+00
16-32 µm	2.162E-03	6.271E-03	2.484E-02	4.425E-01	8.021E-01	1.000E+00	8.385E-01	9.693E-01	1.000E+00
8-16 µm	2.409E-02	4.657E-02	1.845E-01	1.548E-01	4.413E-01	1.000E+00	6.323E-01	9.693E-01	1.000E+00
4-8 µm	2.067E-02	4.281E-02	1.696E-01	1.362E-01	4.413E-01	1.000E+00	6.712E-01	9.693E-01	1.000E+00
2-4 µm	1.279E-02	3.373E-02	1.336E-01	1.248E-01	4.413E-01	1.000E+00	8.141E-01	9.693E-01	1.000E+00
< 2 µm	1.934E-02	4.281E-02	1.696E-01	2.022E-01	4.886E-01	1.000E+00	8.697E-01	9.693E-01	1.000E+00

Table E1. The formal ARESC analysis table showing the relationships between measured physico-chemical parameters and the structures of the meiofaunal nematode communities in the 2013 marine survey area. Identification of statistically significant correlations is based on Pearson's correlation analyses for which the *p*-values have been corrected for multiple comparisons for each of the axes using the correction methods of Benjamini and Hochberg (1995) = "BH" and Benjamini and Yekutieli (2001) = "BY". Statistically significant raw and corrected *p*-values are highlighted as bold, underscored text for each of the nematode community axes to which they relate. *Total structural variance in the 2013 nematode communities explained by analyses = 81.4%*