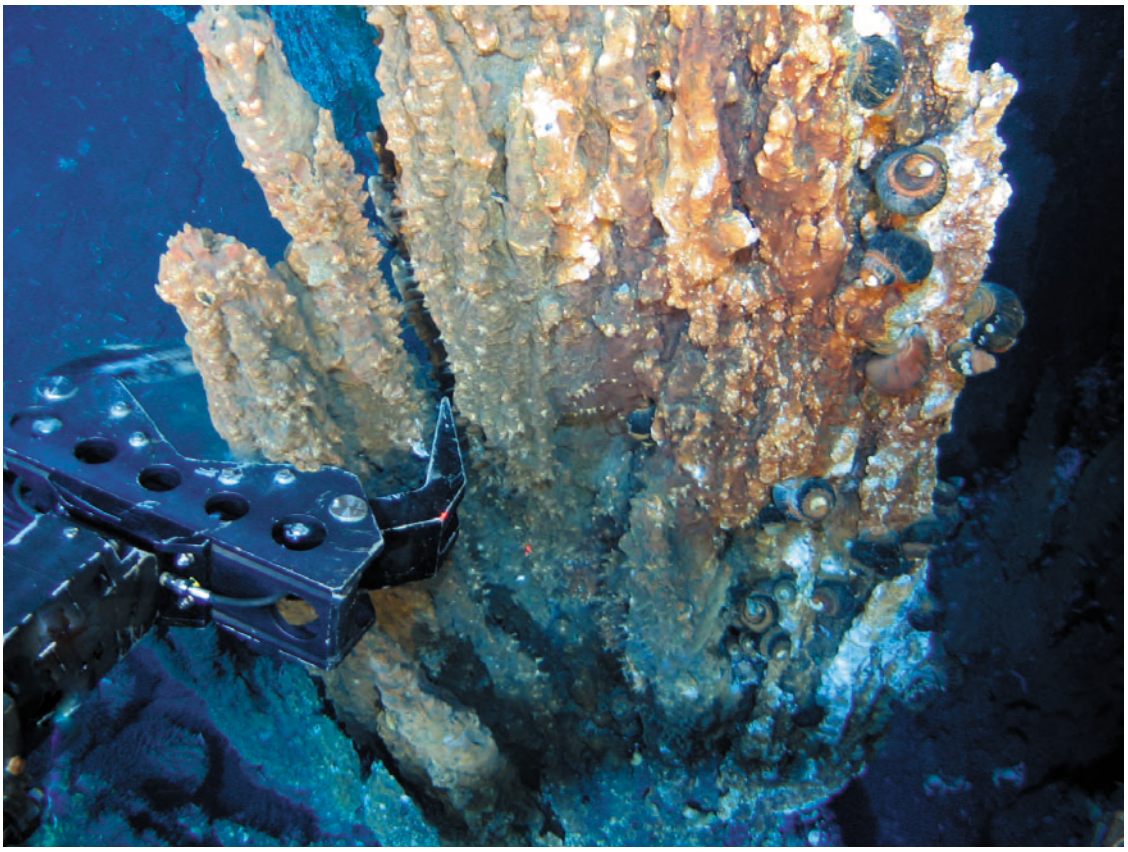

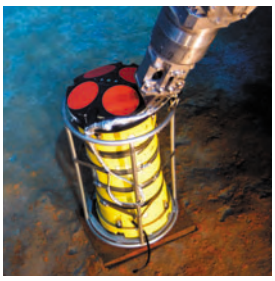

ENVIRONMENTAL IMPACT STATEMENT

Nautilus Minerals Niugini Limited

Solwara 1 Project

Volume B
Appendices 4 - 7

September 2008
CR 7008_9_v4

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APPENDICES 4 - 7

CR 7008_09_v4
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Version:	**Details:**	**Approved:**
CR 7008_09_v1	Initial draft to client	July 2008
CR 7008_09_v2	Second draft to client	August 2008
CR 7008_09_v3	Third draft to client	September 2008
CR 7008_09_v4	Final for exhibition	September 2008

Appendices

Appendices 1 to 3:	1	Baseline Environmental Study Eastern Manus Basin, Papua New Guinea – Module 1 Preliminary Scoping Study
	2	Baseline Environmental Study Eastern Manus Basin, Papua New Guinea – Module 2 Detailed Scoping Study
	3	Oceanography at Solwara 1
Appendices 4 to 7:	4	Characterization and comparison of macrofauna at inactive and active sulphide mounds at Solwara 1 and South Su (Manus Basin)
	5	Macroinfauna of Active and Inactive Hydrothermal Sediments From Solwara 1 and South Su, Manus Basin, Papua New Guinea
	6	Quality Including Trace Elements of Sediments from the SuSu Knolls, Manus Basin, Bismarck Sea, Papua New Guinea
	7	Water and Sediment Characterisation and Toxicity Assessment for the Solwara 1 Project
Appendices 8 to 15:	8	Juvenile Amphipod Whole Sediment Test Report
	9	Elutriate Testing Report Solwara 1 Project, Incorporating Phase 1: Effect of Holding Time; Phase 2: Effect of Temperature
	10	Biomass, Biodiversity and Bioaccumulation Desktop Study
	11	Modelling the Dispersion and Settlement of Sediment Removal Operation Prior to Mining at the Solwara 1 Mining Lease, Papua New Guinea
	12	Modelling the Dispersion of the Returned Water Discharge Plume from the Solwara 1 Seabed Mining Project Manus Basin, Papua New Guinea
	13	Prediction of underwater noise associated with a proposed deep-sea mining operation in the Bismarck Sea
	14	The Potential for Natural Disasters being Triggered by Mineral Extraction at the Solwara 1 Seafloor Hydrothermal Vent Site
	15	Stakeholder Consultation

Appendix 4

Characterisation and Comparison of Macrofauna at Inactive and Active Sulphide Mounds at Solwara 1 and South Su (Manus Basin)

Characterization and comparison of macrofauna at inactive and active sulphide mounds at Solwara 1 and South Su (Manus Basin)

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Table of Contents

Chapter 1

Executive Summary and Recommendations

Biological sampling during the Nautilus Minerals Luk Luk 07 Research Campaign in March and April 2007 focused on studies of species composition and community structure at inactive and active sulphide mounds, with emphasis on comparison of these attributes between a proposed mining zone, Solwara 1, and a proposed reserve, South Su. The parallel sampling design and successful sampling effort represents the most comprehensive sampling of vent sites in Manus Basin and of inactive sulphide mounds to date. Quantitative sampling with explicit attention to similar sampling efforts between Solwara 1 and South Su allows for robust analysis of diversity and community structure.

Regions of the mitochondrial COI gene of selected taxa were sequenced to determine phylogenetic alliances of Manus Basin specimens to specimens from nearby basins (e.g., Fiji, Lau) and to initiate a reference library of DNA 'barcodes' to facilitate species identifications. Some biomass dominant species have ranges that extend across multiple back-arc basins, others occur in another basin but not all, and still others are so far known only from Manus Basin. Until the genetic database is extended geographically and taxonomically, this level of assessment of faunal alliances is provisional.

Species richness and the composition of the biomass-dominant taxa in samples from inactive sulphide mounds were the same at Solwara 1 and South Su. Species-effort curves indicate that the fauna of inactive sulphide mounds is incompletely characterized – each additional sample recovers additional species new to the list. Differences in the character of the fauna of inactive sulphide mounds between sites were due to different assemblages of rarely sampled species (i.e., fewer than 5 specimens recovered in sampling efforts at each site). We expect that species colonizing inactive sulfide mounds will be found elsewhere in the deep sea, but the taxa of deep-sea hard substrata are poorly known. Studies of invertebrates colonizing hard substrata of seamounts in the southwest Pacific are dominated by suspension feeders, but may be endemic to local seamount clusters (Richer de Forges et al. 2000). Representatives of the bamboo coral genus *Keratoisis* are reported from a number of Pacific seamounts (data from SeamountsOnline; http://seamounts.sdsc.edu; Stocks 2005), but their taxonomic relationship to the Manus Basin species remains to be determined. Other taxa dominant at Manus Basin inactive sulfide mounds, including barnacles in the genus *Vulcanolepas* and cladorhizid sponges in the genus *Abyssocladia*, do not appear in the

SeamountsOnline database (*Vulcanolepas*) or are only reported from a very few locales (e.g., *Abyssocladia* from a single locale). Stocks (2004) provides a summary of seamount biodiversity.

Active vent sites at Solwara 1 and South Su were dominated by 3 habitat zones defined by their biomass-dominant species: *Alviniconcha* sp., *Ifremeria nautilei,* and *Eochionelasmus ohtai.* A number of other invertebrates live in association with these biomass dominants, including numerically dominant limpets, polychaetes, and shrimp. The relative abundance of the limpet *Lepetodrilus schrolli* was much greater at South Su than at Solwara 1, accounting for significant differences in univariate and multivariate measures of diversity and community structure. With the exception of *L. schrolli*, there was no significant difference in species richness, diversity, or community structure in quantitative samples from active sulphide deposits of Solwara 1 and South Su. Invertebrate assemblages were distinctive among the habitat zones, but differed primarily in the relative abundances of the numerically dominant species rather than in the composition of the numerical dominants. As in samples from inactive sulphide deposits, rare species (fewer than 5 individuals collected) make up most of the species list for any habitat. The lack of differentiation of community structure within similar habitats between two sites separated by less than 5 km is consistent with observations of community structure within mussel beds between vent sites separated by similar or greater distances on the East Pacific Rise.

A major difference between Solwara 1 and South Su was the presence at South Su of mussel beds and tubeworm clusters. These are important elements of the symbiont-hosting invertebrate assemblage of vent ecosystems and are of interest to the scientific community. The survey approach used in this study does not allow an assessment of why mussels and tubeworms should be at South Su and not at Solwara 1. Other taxa of interest to the scientific community observed during the Luk Luk cruise include the bone-eating worm *Osedax* sp., endosymbiont-hosting thyasirid bivalves, vent-dwelling echiurans, and a number of undescribed species of barnacles, limpets, shrimp, polychaetes, among others. Taxonomic experts consulted during this study are engaged in preparing formal descriptions of new species.

Recommendations

1) Particularly given the cost of field sampling programs, material in the collections should be used to effect to understand more about the biology and autecology of representative taxa. Priorities include broad dissemination of

survey results in the peer-reviewed literature, characterization of symbioses in selected taxa (thyasirids, nuculanids), publication of taxonomic descriptions, preparation of illustrated keys to species identification of the region for parataxonomic studies by Papua New Guinea and other scientists, and publication of gene sequences in GenBank. Illustrated keys, photos, video, and databases should be made available on an international web site (e.g., the Nautilus web site), to which links can be made from Papua New Guinea web sites and the international web sites such as the Census of Marine Life Biogeography of Chemosynthetic Ecosystems (ChEss) web site: (http://www.soc.soton.ac.uk/chess). An archival collection of specimens from Manus Basin vents should be deposited at an appropriate venue in Papua New Guinea where Papua New Guinea scholars can have access to it.

2) Subsequent sampling efforts (in Manus Basin or elsewhere) should strive to increase the scope of the 'barcode' library and should include analysis of multiple mitochondrial and nuclear gene markers.

3) Especially for species where individuals are abundant and where samples come from geographically discrete areas where the degree of genetic exchange is of interest, representative samples (tissues from 30 or more individuals of the same species at each site) should be preserved for DNA analyses at the population level, with relevant metadata (sample location, depth, images, description, etc). These samples and their analysis will allow for development of an understanding of the duration, extent, and direction of genetic exchange between regions.

4) Major gaps in our understanding of invertebrate assemblages on inactive and active sulphides include growth rates, recruitment rates, life history strategies, and other measures and indicators of population dynamics. The potential impact of mining operations differs depending on a myriad of factors, among which the ability of populations to recover through recruitment (both larval recruitment and recruitment through migration) and rapid growth rates are key. For active sulphides, we expect that recovery of invertebrate populations will be rapid, provided the supply of recruit to the system is not disrupted and that the geophysical setting quickly returns to pre-mining conditions. Because invertebrate species have different modes of dispersal and recruitment potential, we expect there to be a transition period of several years (1 to 3?) post-mining, during which populations characteristic of active sulphide mounds return and reorganize to a condition (in terms of biomass and biodiversity) that resembles the pre-mining state. This prediction is made in ignorance of the succession pattern that may take place when a large area cleared of biota becomes available for colonization. For inactive sulphides, we expect return to pre-mining conditions to take many years, if not decades. In part, this expectation is due to presumed slow growth rates

of corals and other non-vent invertebrates, as well as to a presumed lag time before inactive sulfide mounds of sufficient stability are regenerated in the mined area and become available to colonists.

5) The degree of endemicity of taxa occurring at inactive sites is impossible to assess without survey of hard, non-hydrothermal substrata. For some species where the taxonomy is poorly resolved, traditional morphometric methods and molecular methods should be employed in tandem.

6) Extended training (Professional Masters Degree or PhD) of one or more Papua New Guinea scholars in the ecology of deep-sea hydrothermal systems would contribute intellectual capital in this field of study in a region (SW Pacific) where deep-water resources are rich, varied, and exploitable and where wise management practices will be invaluable to the national heritage.

7) Enhanced interdisciplinary studies (physical, chemical, and biological) would facilitate measurements that might provide insight into why certain key species (e.g., tubeworms, mussels) occur in some places and not in others.

8) Monitoring of the impact of test mining and mining activities on the biota will require a strategic and efficient plan that might best be ratified and undertaken by a team of acknowledged experts in studies of deep-sea ecosystems that captures all of the major elements of the system (sediment, corals and other suspension-feeding inhabitants of inactive sulphide mounds, sessile and mobile vent faunas). Nautilus-sponsored workshops are effective in this regard, as will be participation ininternational meetings such as the WHOI Morss Colloquium Series on "Deep-Sea Mining of Seafloor Massive Sulfides: A Reality for Science and Society in the 21st Century" (late April, early May 2009.

9) The 17-18 Apr 2008 San Diego workshop sponsored by Nautilus Minerals brought forward new thinking about mitigation strategies, including exploration of the feasibility of transplants of biomass dominants and their associated invertebrates to retain genetic diversity, development of effective recruitment ’traps’ as a means of reseeding barren habitat, and construction of stable inactive sulfide mounds using mine tailings.

Chapter 2

Acknowledgements

We acknowledge the support for fieldwork and shore-based analyses from Nautilus Minerals, including an internship at the Duke University Marine Laboratory for Martha Mungkaje. The at-sea component of the effort was made possible through the professional efforts of the Captain and crew of Global Marine's CS Wave Mercury, the UTEC survey staff, the Helix Offshore staff, the operators of the ROV, and Nautilus personnel, especially Dr. Sam Smith. Additional support for this project was provided through Duke University, including Duke stipends for Summer Independent Study Students: Rebecca Jones and Katie Yang, part-time technical salary for Carol Logan, partial support for Van Dover, and contributions from the Marine Conservation Molecular Facility (Tom Schultz and Jens Carlsson) of the Duke University Marine Laboratory. Molecular work was supported in part by a TAWNI (Training Awards for New Investigators) grant for a molecular genetic internship to Patrick Collins from the Census of Marine Life's ChEss Program. This TAWNI internship was completed in the laboratory of Dr. Robert Vrijenhoek, Monterey Bay Aquarium Research Institute. We thank Dr. Vrijenhoek for his generosity in making his laboratory available to us and to Dr. Joe Jones and Ms Shannon Johnson in the Vrijenhoek lab for their technical support. We are very grateful for freely given assistance of taxonomic specialists around the world for species identifications. They, their specialties, and their home institutions are listed here.

Name	Specialty	Home Institution
Dr. Louise Allcock	Octopus DNA	Queens University, Northern Ireland
Dr. Ramlall Bisewar	Echiurans	University of Kwa Zulu Natal, Republic of South Africa
Dr. Daniel Desbruyeres	Polychaetes	Ifremer, France
Dr. Darryl Felder	Mud Shrimp	University of Louisiana, USA
Dr. Gonzalo Giribet	Thyasirids	Harvard University, USA
Ms. Shannon Johnson	Gastropod DNA	Monterey Bay Aquarium Research Institute, USA
Dr. Diana Jones	Barnacles	Museum of Western Australia
Dr. Tomo Komai	Shrimps	Natural History and Museum Institute, Japan
Dr. Rafael Lemaitre	Hermit Crab	Smithsonian Institution, USA
Ms. Janina Lerhke	Echiuran DNA	Berlin Free University, Germany
Dr. Enrique MacPherson	*Paralomis*	Centro d'Estudis Avancats de Blanes, Spain
Dr. Francoise Monniot	Ascidians	Museum of Natural History, France
Dr. Richard Nemeth	Fish	University of the Virgin Islands, USA
Dr. William Newman	Barnacles	Scripps Institution of Oceanography, USA
Dr. Bruce Robison	Fish	Monterey Bay Aquarium Research Institute,, USA
Dr. Greg Rouse	*Alaysia*	Scripps Institution of Oceanography, USA
Dr. Michel Segonzac	Shrimps	Ifremer, France
Dr. Janet Voight	Octopus	The Field Museum, USA
Dr. Anders Waren	Gastropods	Swedish Museum of Natural History, Sweden

Chapter 2a

General Field Methods

Samples were taken between the 23rd of March and the 27th of April using a Perry Slingsby ST200 Series ROV aboard Global Marine System's *CS Wave Mercury*. Survey operations were carried out by UTEC Survey Ltd. The ROV was modified for the macrofaunal sampling to hold a bio-box in the forward storage compartment. This wooden crate was four chambered; each chamber held a custom built scoop and could be closed by a hydraulically operated sliding lid.

Scoops were constructed from 20-cm diameter PVC pipe with a PVC pipe-base glued on. Steel handles were screwed on the sides to facilitate use with the ROV manipulators. The scoops were painted initially black and then yellow and numbered to aid recovery. Samples were placed into the biobox's chambers with the 7-function ROV arm. The ROV had two sets of two suction chambers; each set had its own slurp gun. Each chamber had a mesh size of 250μm that could be increased to 2 mm depending on the targeted fauna.

Tickler nets and luckybags were also used to collect specimens. Tickler nets consisted of a 0.25-m^2 quadrant constructed out of PVC pipe with a steel handle and a loose coarse meshed net behind. The ticklers were lengths of cable tie attached so as to 'tickle' shrimp and other mobile fauna from inaccessible areas and into the net. The luckybag consisted of a scoop in which the PVC base was replaced by sacking held on by hose clips.

Large segments of rock or chimney were transported to and dropped into the chimney bin with the 5-function ROV arm. The chimney bin consisted of six wheelie bins in a steel cage that could be lowered or raised independently of the ROV.

Chapter 3

Genetic Characterization of Macrofaunal Taxa at Solwara 1 and South Su

Aim: Genetic characterization of key invertebrate taxa of inactive and active sulphide mounds at Solwara 1 and South Su.

Objectives: To build a database for genetic identification invertebrates associated with active and inactive sulphide mounds in Manus Basin and to compare gene sequences of selected taxa between Manus Basin and other back-arc basins in the southwest Pacific.

Methods: The genetic effort focused primarily on taxa from active hydrothermal vents, and especially (but not exclusively) on those genera for which genetic sequences have already been reported in the literature for specimens elsewhere to determine the degree of endemicity of the Manus representatives. At least five specimens from selected taxa were identified to the lowest possible taxonomic rank and digitally photographed. Small tissue samples from each specimen were dissected, preserved in labeled centrifuge tubes containing 95% ethanol (ETOH), catalogued, and stored. Specimens were then fixed in 10% borax-buffered formalin for 24 h and stored in 70% ETOH. A total of 271 individuals from 34 morphologically identified species were sampled in this manner for the genetic sequencing effort. Tissue samples for DNA work are archived at the Monterey Bay Aquarium Research Institute (MBARI). Specimens from which the tissues were removed are archived at the Duke University Marine Laboratory.

DNA extraction and sequencing were undertaken at MBARI under the supervision of Dr. Robert Vrijenhoek. Small tissue samples (2 to 5 mm^3) were used for DNA extraction using DNeasy kits (QIAGEN Inc., Valencia, CA). QIAGEN's spin-column protocol was used for purification of total DNA. Polymerase Chain Reaction (PCR) methods were used to amplify mitochondrial cytochrome oxidase subunit 1 genes (COI) using CO1, LCO primers (Folmer et al. 1994) and Gala primers (Jones & Macpherson 2007). Primer sequences:

COI (*COIF 5' TCM ACT AAT CAY AAR GAY ATT GGN AC 3' & COIR 5' CCD CTT AGW CCT ARR AAR TGT TGN GG 3'*)

LCO (*LCO1708 5' GCY CCA GAT ATA GCW TTC CC 3' & LCO1490 5' TAA ACT TCA GGG TGA CCA AAA AAT CA 3'*)

Gala (*Gala COIF 5' CAT CAC TWA GWT TRA TYA TTC GAG CAG AA 3' & Gala COIR 5' GAA YAG GRT CTC CTC CTC CTA C 3'*).

A total reaction volume of 25 µl was prepared using 12.5 µl of GoTAQ® green master mix (green buffer, $MgCl_2$, Enzyme dNTPs), 7 µl H_2O, 2.5 µl Bovine Albumin Serum (BSA), 1µl DNA and 1 µl of each primer. The PCR amplification profile used was 94°C for 5 minutes, 40 cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 2 minutes, and a final extension at 72°C for 5-10 minutes for the COI primers. LCO primers were used mostly for crustaceans, with annealing temperature of 50°C. The Gala primer was used only for squat lobsters, with an annealing temperature of 52°C. Successfully amplified DNA extractions as determined by gel electrophoresis were chosen for sequencing.

Sequencing reactions were carried out using BDTv3.1. A total reaction volume of 10 µl was prepared using 0.5 µl Big Dye v3.1, 2 µl 5x sequencing buffer, 0.33 µl 3.2 pmol/µl primer, 1-2 µl DNA, 6.17-5.17µl H_2O. The PCR amplification profile used was 35 cycles (range 25-40 cycles) at 96°C for 10 seconds, 50°C for 5 seconds, 60°C for 4 minutes.

PCR products were pelleted by adding 5 µl H_2O, 2 µl 125 mM EDTA, and 2 µl 3M sodium acetate to each sample. Sample well plates were subsequently covered with foil, inverted, and left at room temperature for 15 minutes, after which they were spun for 30 minute at 3000 x g. After spinning, plates were inverted, drained onto a paper towel, and re-spun at 185 g. Ethanol (70%) was then added to each samples, plates were centrifuged for 15 minutes at 1650 g, and plates were again inverted, drained, and re-spun at 185 g. Samples not loaded into the sequencer within 24 h were covered with foil and stored at -20°C. Samples for sequencing were re-suspended in 10 µl formamide, followed by a 1-minute spin at 2000 g.

Sequencing was carried out using an ABI 3100 capillary sequencer (Applied Biosystems Inc., Foster City, CA). Sequences were coarsely aligned with *Sequencher v.4.2* (Gene Codes Corporation, Ann Arbor, MI, USA) and finely aligned by eye. Sequence data was analyzed for the nearest genetic neighbor using the nucleotide BLAST program

and GenBank (US National Centre for Biological Information) database. This analysis was optimized for 'somewhat similar sequences' (Blastn), using the database 'Others (nr etc.)'. Maximum identity (MI)- the "percentage coverage" or "maximum score" - for a set of aligned segments to the same subject sequence is reported. MI matches of ≥ 99% are assumed to represent a genetic match at the species level.

Results: A total of 126 individuals from 26 morphologically identified species were successfully sequenced (Table 3.1). Maximum identities ≥ 99% (matches to GenBank sequences at the species level) were documented for 6 species: *Alviniconcha* sp. 1 (8 individuals), *Alviniconcha* sp. 2 (1 individual), *Ifremeria nautilei, Bathymodiolus manusensis, Arcovestia ivanovi, Paraescarpia* sp., and *Munidopsis lauensis*. The differentiation of the morphological *Alviniconcha* species into two genetic species suggests these are cryptic species, i.e., species that are difficult to distinguish morphologically but that are readily distinguished based on gene sequences. CO1 gene sequences of an additional 6 morphological species had closest matches to GenBank sequences of species in the same genus (*Lepetodrilus, Alaysia, Lamellibrachia, Prionspio, Alvinocaris, Lebbeus*); sequences of an additional 2 genera (*Nereis* sp., *Opaepele* sp.) matched GenBank sequences of species in the same families.

Discussion: GenBank is surprisingly unpopulated with sequences of hydrothermal-vent species, making it difficult to assess phylogenetic alliances for more than a few species. Sequence data obtained in this project will be invaluable for consistent identifications of taxa between sampling efforts by non-taxonomic experts and for identification of cryptic species. An important contribution of this study will be the addition of sequence data, together with sample metadata (geographical location, depth date of collection), to the GenBank database.

Where there are genetic sequence matches between the GenBank database and material analyzed from the Luk Luk cruise, we can comment on biogeographic relationships of some hydrothermal-vent species. Of particular interest is the phylogenetic match of certain species collected from Manus Basin with species collected from nearby back-arc basins, especially Fiji and Lau Basins. CO1 gene sequences of *Alviniconcha* sp. 1 and *Alviniconcha* sp. 2 from Solwara 1 and South Su match those reported for North Fiji by Suzuki et al. (2006b) and are distinct from those of *Alviniconcha* sp. reported from Lau Basin (Suzuki et al. 2006b). COI gene sequences of *Ifremeria nautilei* from Solwara 1 and South Su match those reported for specimens collected from Manus, North Fiji, and Lau Basins (Suzuki et al 2006a). Gene sequences of *Arcovestia ivanovi* match those of *A. ivanovi* collected from the PACMANUS and DESMOS sites in Manu Basin (Kojima et al. 2003) and from North Fiji vents (Southward et al.

2002). Gene sequences of *Munidopsis lauensis* from Solwara 1 and South Su match those of specimens collected from Fiji, Lau, and Manus Basins and from Brothers Seamount (Kermadec-Tonga Arc; Cubelio et al. 2007; Cubelio et al. in press). *Bathymodiolus manusensis* is so far only known from Manus Basin, and is phylogenetically allied more closely to *Bathymodiolus thermophilus* of the East Pacific Rise than it is to *B. brevior* of Lau and Fiji Basins based on similarities in a 586 base-pair sequence of the COI gene (McKiness et al. 2005). The conclusion is that while some dominant biomass species have ranges that extend across multiple back-arc basins (*Ifremeria nautilei*, *Munidopsis lauensis*), others are so far known only from Manus and North Fiji Basins and not from Lau Basin (*Alviniconcha* species 1 and 2, *Arcovestia ivanovi*), and still others are even more endemic, so far known only from Manus Basin (*Bathymodiolus manusensis*). One can expect differing degrees of endemicity in other Manus Basin vent species as well. Within Manus Basin, we estimate that 10 of the 17 biomass-dominant invertebrate species reported by Desbruyeres et al. (2006a) at active sulfide mounds occur at Solwara 1 and at least 13 of the 17 occur at South Su. This estimate remains to be confirmed by more detailed analyses.

COI gene sequences of a few individuals are useful for confirmation of species identifications and species ranges, but they reveal nothing about population structure and degree of interaction among populations. For Solwara 1 and South Su, it is evident from morphologic and genetic analyses that they share the same dominant species and that these species have ranges that extend to adjacent back-arc basins. Population genetic approaches provide powerful tools for assessing the size of the gene pool and the extent of genetic isolation of one population from another and would allow one to document whether populations at Solwara 1 and South Su represent well-mixed genetic populations or whether there is an isolating mechanism that might preclude repopulation of one site by another.

A number of undescribed morphological species (and even some previously unidentified genera) were recognized in samples from the Luk Luk campaign [including 1 new species of tubeworm and at least 4 new species of gastropod mollusks (depending on further resolution of morphological and genetic data), 3 new species of bivalves, 7 new species of shrimp, plus new species in other phyla], and additional taxonomic characterizations are pending. The taxonomic nomenclature of a number of Manus Basin species is confusing, with parataxonomists sometimes suggesting a variety of names for what are very likely the same species (Appendix II). Taxonomic ‘errors’ also likely occur where morphological features are similar but where genetic differentiation is sufficient to indicate the cryptic species (as, for example, seems likely for *Amphisamytha galapagensis*).

Table 3.1 Species identified using traditional taxonomic (morphological) methods and GenBank species match. n/a: not available; f/r: forward and reverse primers used in the PCR reaction; MI: maximum sequence identity, a measure of similarity of nucleotide sequences**;** *: species match; **: genus match ***: family match.

Morphological ID	Number Sampled	Number Sequenced	Primer	Closest GenBank Match	MI (%)	
Keratoisis sp.	10	0	n/a	n/a	n/a	
Abyssocladia sp.	10	0	n/a	n/a	n/a	
Lepetodrilus schrolli	10	6	CO1 f/r	*L. fucensis*	76-83	**
		1	CO1 f/r	*L. gordensis*	77	**
Olgasolaris tollmanni	10	6	CO1 f/r	*Neritilia cavernicola*	79-82	
		2	CO1 f/r	*Neretina violacea*	78	
Shinkailepas tufari	10	2	CO1 f/r	*Nerita atramentosa*	81-83	
		1	CO1 f/r	*N. cavernicola*	82	
		1	CO1 f/r	*Echinolittorina feejeensis*	79	
		1	CO1 f/r	*E. cineria*	78	
		1	CO1 f/r	*E. natalensis*	77	
		1	CO1 f/r	*Cyathermia naticoides*	77	
Eosipho sp.	10	7	CO1 f/r	*Buscyon sinistrum*	86	
Alviniconcha sp.	10	8	CO1 f/r	*Alviniconcha* sp. 1	98-99	*
		1	CO1 f/r	*Alviniconcha* sp. 2	99	*
Ifremeria nautilei	10	8	CO1 f/r	*Ifremeria nautilei*	97-99	*
Provanna sp.	5	0	CO1 f/r	n/a	n/a	
Nuculanoidea n. gen., n. sp.	10	1	CO1 f/r	*Nuculana pella*	75%	
Bathymodiolus manusensis	5	5	CO1 f/r	*B. manusensis*	99%	*
Bonelliidae sp.	5	3	CO1 f/r	*Urechis caupo*	79%	
Nereis sp.	10	5	CO1 f/r	*Neanthes japonica*	79	***
mussel commensal	3	3	CO1 f/r	*N. japonica*	80-81	
Branchinotogluma segonzaci	10	5	CO1 f/r	*N. japonica*	80-81	
Branchinotogluma trifurcus	10	6	CO1 f/r	*N. japonica*	80-81	
		2	CO1 f/r	*Branchipolynoe* sp.	78-89	
Thermopolynoe branchiata	10	3	CO1 f/r	*Arabella semimaculata*	80-81	
Branchipolynoe sp.	5	1	CO1 f/r	*Branchypolynoe* sp.	90	
Alaysia sp.	5	2	CO1 f/r	*Alaysia* sp.1	90, 93	**
		2	CO1 f/r	*Alaysia* sp.4	93, 94	**
Arcovestia ivanovi	5	5	CO1 f/r	*Arcovestia ivanovi*	98-99	*
Lamellibrachia sp.	3	3	CO1 f/r	*Lamellibrachia* sp.	98	**
Prionspio sp.	5	2	CO1 f/r	*Prionospio* sp.	87	**
Paralvinella sp.	5	0	CO1 f/r	n/a	n/a	
Paralvinella unidentata	10	0	CO1 f/r	n/a	n/a	
Paralvinella fijiensis	10	0	CO1 f/r	n/a	n/a	
Terrebellidae sp.	5	0	CO1 f/r	n/a	n/a	
Eochionelasmus ohtai	10	2	LCO/LCO	*Calantica sp.inosa*	81	
		3	LCO/LCO	*Balanus glandula*	78	
Poecilasma cf. *kaempferi*	10	0	LCO/LCO	n/a	n/a	
Vulcanolepas parensis	10	5	LCO/LCO	*Neoverruca* sp. OK15	83	
Alvinocaris sp.	5	3	LCO/LCO	*Alvinocaris* sp. TVG29-*2*	87-92	**
Opaepele sp.	10	1	LCO/LCO	*Chorocaris vandoverae*	94	***
Lebbeus sp.	5	4	LCO/LCO	*Lebbeus carinatus*	86-93	**
Munidopsis lauensis	10	9	Gala	*M. lauensis*	98-100	*
Austinograea alayseae	10	4	LCO/LCO	*Chorila longipes*	78-82	

Chapter 4

Macrofaunal Communities at Inactive Sites, Manus Basin

Aim: Characterization and comparison of invertebrate macrofauna at inactive sites, Solwara 1 and South Su, Manus Basin (Figs. 4.1, 4.2).

Objective: To build an inventory of invertebrates associated with inactive sites, including species-abundance data, specimen archives, photo archives, and genetic archives and to compare two sites within Manus Basin: Solwara 1 SW1) and South Su (SS).

Methods: 'Inactive' habitats (sites) were defined by the absence of vent endemic species that host endosymbionts (i.e., *Ifremeria nautilei*, *Alviniconcha* sp., or *Bathymodiolus manusensis*) and by their lack of visually evident warm-water or black-smoker effluents. Sites selected for study were colonized by macroscopic organisms, including bamboo coral, carnivorous sponges, stalked barnacles, or hydrozoan mats, that allowed for representative sampling without destruction of the entire assemblage. Suitable sampling sites (discrete inactive habitats at least 10 m from an active vent site; Table 4.1) were scarce at South Su (n = 12 sites sampled), the smaller of the two study areas, compared to Solwara 1 (n = 15 sites sampled).

Video transects were undertaken at each site where possible to provide an overview of the faunal assemblages. For the smallest sites (sometimes a single sulphide spire projecting from the sedimented seabed), a series of photographs characterized the setting. A 0.25-m^2 photo-quadrat was used to document inactive sulphide faunas, but the images proved to be of little value for analysis of community structure because the majority of fauna were either infaunal or minute and were only discovered after sorting through sample residues. Video and still photos are archived at Nautilus Minerals.

At inactive sites, emphasis was placed on retrieval of at least 5 representative specimens of the biomass-dominant taxa for identification and gene sequencing and on collection of qualitative samples from surfaces within the 0.25 m^2 quadrats using a slurp /scrape/slurp method or by recovering pieces of the substratum.

On deck, samples were immediately chilled, sieved through a 0.025-mm aperture sieve and either processed or stored at 4ºC for < 2 hours. Samples for DNA sequencing were treated as described in Chapter 3. Remaining samples were fixed in 10% borax-buffered formalin for 24 h and stored in 70% ethanol. In the laboratory at the Duke University Marine Laboratory, preserved samples were rinsed with fresh water and sorted under light in white plastic trays. Species were identified to the lowest possible taxon and enumerated. A voucher collection was created and used for the provisional labeling of unidentified species, e.g., amphipod species 1, 2 and 3. Individual species were stored in labeled vials in 70% ETOH. This collection is maintained at the Duke University Marine Laboratory in trust for Nautilus Minerals, who in turn hold the samples in trust for the people of Papua New Guinea, with specimens dispensed on loan to individuals with taxonomic expertise in diverse groups. To date, 19 experts at 16 institutions have been consulted (Table 2.1) A number of new species descriptions and range extensions are expected to result from this work.

Data from qualitative samples were used to calculate species densities using cumulative species-effort curves. PRIMER v6 (Clarke & Warwick 2001) was used to randomize the sample data and to eliminate the effect of sample order (randomization operations = 999, without replacement). Effort is expressed as the cumulative number of samples. Where comparisons of species density among samples are made, effort is standardized to the level of the smallest number samples in any one collection. When the species-effort curve for a given local reaches an asymptote, a complete inventory of species for the local has been obtained.

Results: Inactive sites at Solwara 1 and South Su host the same biomass- and numerically dominant taxa. The most conspicuous species were the suspension-feeding corals (primarily *Keratoisis* sp.), stalked barnacles, (especially *Vulcanolepas parensis*), hydroids (various species), and carnivorous sponges (*Abyssocladia* sp.) (Fig. 4.3; Table 4.2; Appendix IA). Areas surrounding Solwara 1 and South Su inactive sites were heavily sedimented and hosted benthic invertebrates (notably brittle stars); worm castings were common.

Inactive sites were generally of two kinds: bare, vertically oriented deposits dominated by conspicuous stands or patches of sessile organisms, or toppled, horizontally oriented deposits covered with sediment and colonized by infaunal taxa, including annelids, amphipods, and bivalves.

A total of 91 species were recovered from inactive sites from Solwara 1 and South Su (Table 4.2). The final number of taxa recovered is pending identifications by specialists. The number of species recovered from Solwara 1 (65 species) is greater than that recovered from South Su (58 species). For a given sampling effort (n = 12 samples), however, there is no difference in species richness between Solwara 1 and South Su (Figure 4.4). From the species-effort curves (Figure 4.4), each stepwise increase in sampling effort at inactive sites resulted in recovery of an additional 2 to 3 species not previously recorded.

Inactive sites at Solwara 1 and South Su shared 31 species, including 11 species of cnidarians, 23 species of mollusks (18 gastropods and 5 bivalves), 16 species of annelids, and 32 species of arthropods (7 barnacles, 8 amphipods). Most species (> 80%) were represented by fewer than 5 specimens. Numerical dominants in qualitative samples (in decreasing abundance) were *Abyssocladia* sp. sponges, an unidentified species of amphipod, two species of stalked barnacles (*Vulcanolepas parensis* and *Poecilasma* cf. *kaempferi*), squat lobsters (*Munidopsis lauensis*), lepetodrilid limpets, and thyasirid clams. A surprising number of taxa (n = 11) from inactive sulphide deposits were previously generally considered to be endemic to active hydrothermal sites (Table 4.2).

Nearly identical distribution of taxa within major taxonomic groups at both sites (arthropods: SW1 = 40%; SS = 41%; mollusks: SW1 = 26%; SS = 31%; annelids: SW1 = 21%; SS = 22%; Fig 4.5) further underscores the similarity of their macrofaunal assemblages.

Three species of large, mobile animals were photodocumented from inactive sulphide deposits at both Solwara 1 and South Su: the giant sea spider *Collossendeis* sp., the benthic ctenophore *Tjalfiella tristoma*, and the hermit crab *Parapagurus richer*. Fish such as Congridae sp., Halosauridae sp., Ophidiidae sp., *Rhinochimaera pacifica(?)* and *Hydrolagus trolli(?)* were observed in the vicinity of inactive sites at both Solwara 1 and South Su (Appendix III).

Discussion: Overall, there were no obvious differences in the invertebrate fauna associated with inactive sites of Solwara 1 and South Su. Species richness was the same at each site and species abundant in samples from Solwara 1 (i.e., > 10 individuals) were also present in samples from South Su, and vice versa. Species lists from both sites were dominated by uncommon (occurring in only a few samples) or rare species (<5 individuals per sample), and species-effort curves demonstrate that the sampling effort did not capture the complete species list at either Solwara 1 or South Su.

Stands of carnivorous sponges (*Abyssocladia* sp.), bamboo corals (*Keratoisis* sp.), barnacles (*Vulcanolepas parensis*, *Altiverruca* sp., *Poecilasma* cf. *kaempferi*) and hydroids were the most conspicuous of the invertebrate taxa colonizing inactive sites at both Solwara 1 and South Su. Large 'Venus Fly-Trap' anemones (Actinoscyphiidae sp.) and red coral (*Alcyoncea* sp.) were observed and photodocumented at both mounds (Appendix III) and, although not abundant, were also conspicuous members of the sessile assemblage at inactive sites. These taxa are interpreted to be opportunists, taking advantage of an enriched food source in an otherwise nutritionally poor environment. In a general sense, this Manus inactive site assemblage is similar to that described for seamounts, where dense coral and sponge meadows are prominent biogenic features that rely on enhanced delivery of particulate material in flow regimes associated with topographic relief. Debate currently focuses on whether these biogenic features of seamounts are oases and biodiversity hotspots (McClain 2007).

The lack of comparable sampling efforts on hard substrata removed from the influence of hydrothermal activity in Manus Basin makes it difficult to establish the detailed biogeographic and biodiversity context of the invertebrate assemblage of the Solwara 1 and South Su inactive sites. Caution should be used in applying the term 'endemic' to members of this assemblage, given this under-sampling.

There is a paucity of knowledge regarding invertebrate assemblages of inactive sites; biologists have favored study of the active vent faunas instead. One report, based on preliminary survey of inactive sulphide deposits of Gorda Ridge (northeast Pacific Ocean), noted the surprising abundance of suspension-feeding invertebrates, comprised of solitary tunicates, brisingid sea stars, crinoids, sponges, anemones, and brachiopods (Van Dover et al. 1990). While the suspension-feeding nature of the invertebrates associated with inactive sulphides at Gorda Ridge is shared with that of Manus Basin inactive site invertebrates, the visual impact of the two sites is different due to the paucity of large corals at Gorda Ridge relative to Manus Basin sites.

Recent submersible studies near, but not at, active hydrothermal vents in North Fiji and Lau Basins (Vacelet 2006) report two species (*Asbestopluma formosa*, *Abyssocladia dominalba*) of putatively carnivorous sponges in the recently recognized family Cladorhizidae. The Manus Basin species of *Abyssocladia* appears to be morphologically distinct from *A. dominalba* of North Fiji and Lau Basins, based on the shape of the body and the terminations of the styles radiating from the body. Members of the cladorhizid family of sponges typically lack or have modified choanocytes (flagellated cells that generate feeding currents in sponges) and instead feed on small crustaceans

(copepods) captured by specialized spicules. Sustenance of dense populations of *Abyssocladia* sp. at inactive sites in close proximity to active hydrothermal vents may thus depend on enriched populations of small crustaceans at the vents.

The Keratoisidinae bamboo corals are a poorly known, phenotypically diverse group of deep-water corals. A recent study of DNA haplotypes revealed 14 species in 88 specimens collected from seamounts and flat bottom areas in deep water of the southwest Pacific Ocean (Smith et al. 2004). Contrary to reports of a high degree of endemism in bamboo corals, the haplotypes had large ranges, suggesting either that bamboo corals were more widespread than previously believed or that the regions of mitochondrial DNA sequenced do not record recent speciation events. Bamboo corals represent biogenic habitat that support associated invertebrates (e.g., the '*Keratoisis* anemone' reported here). Life histories (age, growth rates, longevity) of deep-water bamboo corals are poorly known, although there is good evidence that they can attain ages on the order of hundreds of years (Andrews et al. 2005). The age of a *Keratoisis* sp. specimen collected from nearly 1500 m on Davidson Seamount (120 km southwest of Monterey, California) was estimated radiometrically to be greater than 100 years, with a growth rate of 0.1 mm/yr (Andrews et al. 2005); a growth rate of 0.05 mm/yr was reported for a *Keratoisis* species from 1000 m off southern Australia (Ron Thresher, cited in Andrews et al. 2005). Given stable isotope evidence that bamboo corals on inactive sulphide mounds derive a significant portion of their organic sulfur from chemoautotrophic production (presumably by advection, although the hypothesis autocthonous sources of chemoautotrophic production cannot be rejected at this time; Van Dover et al. 1990; K Erickson and CL Van Dover, unpublished data), it is possible that their growth rates are more rapid than those of bamboo corals relying solely on the drift of photosynthetically derived organic material. Characterization of the degree of endemicity, growth rates, age, reproductive attributes (including colonization rates), longevity, and susceptibility to increased particulate loads of Manus Basin *Keratoisis* sp. would supply key metrics for understanding the impact of mining operations on benthic invertebrates.

Among the most numerous species in samples from inactive sites at Solwara 1 and South Su were amphipods (amphipod sp. 2) in the family Stenothoidae. Representatives of this family are also reported from vents on the Mid-Atlantic Ridge, where they live in association with sponges, hydrozoans, gorgonians, and colonial tunicates and outside the direct influence of venting fluids (Bellan-Santini 2005). In Manus Basin, amphipod sp. 2 was associated with hydrozoan mats at inactive sites.

Eleven barnacle species were provisionally identified from active sulphide mounds and inactive sites in Manus Basin, with the majority of them (7 species) found at inactive sites. This large diversity of barnacles is exceptional and is consistent with a western Pacific center of origin for a variety of taxa, including barnacles (Briggs 1996, 1999; W Newman *pers. comm.*). Most of the Manus Basin barnacles, particularly those found in areas peripheral to active venting (e.g., on inactive sites) are not necessarily vent obligates; as with other peripheral taxa, they may occur on hard substrata elsewhere in the surrounding deep sea.

Bivalves were surprisingly well represented in samples from inactive sites. The most numerous bivalve species in the sample collections from Solwara 1 and South Su was the unidentified thyasirid species, which belongs to a group known to host thiotrophic endosymbionts (Dando & Southward 1986, Fisher 1990) and reported from sedimented habitats at the Logatchev hydrothermal vent field on the Mid-Atlantic Ridge (Southward et al. 2001). The Manus Basin species is also assumed to host thiotrophic endosymbionts, which would imply that there is available dissolved hydrogen sulphide even at 'inactive' sites. Where a sediment layer caps a hydrothermally active region, it may cause warm vent fluid to travel horizontally until it reaches a joint where it can rise buoyantly, e.g., at the interface between sediment and a hard substratum like a sulfidic or basaltic outcrop. The bivalve populations warrant further study to confirm the presence of endosymbionts and to characterize the chemical environment. Shell beds of a large (10 cm maximum length) thyasirid belonging to the endosymbiont-bearing genus *Conchocele* (DuFour 2005) were observed in sediments at the base of active sulphide mounds. Sediment-hosted hydrothermal systems are atypical, with Middle Valley and Guaymas Basin being the most well known examples (Van Dover 2000). Indications of the presence of *Conchocele ?novaeguiniensis* sp. in sediments near inactive mounds, as well as populations of live thyasirids and of worms in the vent-endemic genus *Paralvinella* in sediments of Solwara 1 and South Su point to diffuse flux of hydrothermal fluids through the sediments and the existence of a habitat type not known at most mid-ocean ridge vent systems. Thyasirid clams are generally associated with cold seeps (Imhoff et al. 2003; Sahling et al. 2003), but this may reflect their need for sedimentary habitats, which are more typical of seeps on continental margins than of vents on oceanic spreading centers, rather than a requirement for a seep environment *per se*.

A number of species reported in samples from inactive sites at Solwara 1 and South Su are generally recognized as vent-endemic taxa. These include limpets (*Olgasolaris tollmani*, *Lepetodrilus* sp., and *Shinkailepas* sp.), coiled

gastropods (*Eosipho* sp., *Alviniconcha* sp., and *Desbruyeresia* sp.), polychaetes (*Archinome* sp. and *Amphisamytha* sp.), shrimp (*Opaepele* sp. and an unidentified Alvinocarid shrimp), and squat lobsters (*Munidopsis lauensis*). Two of the mollusk species (*Eosipho* sp. and *Alviniconcha* sp.) were juveniles and one of these, *Alviniconcha* sp., hosts bacterial endosymbionts that depend on vent fluids for autotrophic primary production. Recruitment of 'vent taxa' to adjacent but non-vent sites has been reported in colonization studies (Van Dover et al. 1988; Mullineaux et al. 1998), presumably followed by selective survival in optimal habitats. *Eosipho* sp. (a scavenging and/or predatory gastropod) and squat lobsters are often observed peripheral to active vents (Van Dover, personal observation); their presence on nearby inactive sites is thus not surprising. Given the low numbers of these species in samples collected at inactive sitess compared to their abundances at active vents, their distribution is interesting, but not important in the maintenance of high-density populations at vents. It is possible that the smaller, uncommon specimens were contaminants left behind in the sampling gear after work at active vent sites.

Table 4.1. Sample number, date, time, depth, and geographical coordinates of inactive sites at Solwara 1 and South Su, Manus Basin.

Sample Number	Date (2007)	Time	Depth (m)	Latitude	Longitude	Easting	Northing
				SOLWARA 1			
1	26/03	22:53	1503	-3.789997	152.095867	399607	9581033
2	26/03	01:09	1593	-3.790017	152.089788	398932	9581030
3	26/03	15:11	1606	-3.790415	152.089527	398903	9580986
4	28/03	14:46	1596	-3.790651	152.090256	398984	9580960
5	28/03	02:30	1597	-3.790651	152.090229	398981	9580960
6	06/04	23:05	1510	-3.788629	152.094112	399412	9581184
7	05/04	14:27	1518	-3.788873	152.094049	399405	9581157
8	06/04	15:23	1509	-3.790015	152.095894	399610	9581031
9	22/04	18:33	1513	-3.790141	152.095569	399574	9581017
10	23/04	00:32	1575	-3.784783	152.092486	399231	9581609
11	24/04	09:48	1575	-3.784719	152.092288	399209	9581616
12	26/04	11:24	1537	-3.789256	152.096930	399725	9581115
13	26/04	20:21	1540	-3.789093	152.097110	399745	9581133
14	27/04	20:55	1576	-3.784602	152.092252	399205	9581629
15	27/04	22:00	1577	-3.784728	152.092261	399206	9581615
16	27/04	23:00	1575	-3.784728	152.092432	399225	9581615
				SOUTH SU			
1	11/03	04:15	1437	-3.812611	152.103993	400512	9578534
2	11/03	05:58	1440	-3.812339	152.104065	400520	9578564
3	11/03	10:08	1403	-3.811291	152.104787	400600	9578680
4	14/04	02:49	1443	-3.812592	152.104020	400515	9578536
5	14/04	01:35	1435	-3.812394	152.104074	400521	9578558
6	14/04	03:02	1445	-3.812611	152.104056	400519	9578534
7	15/4	12:50	1342	-3.808747	152.102637	400361	9578961
8	14/04	21:44	1315	-3.808786	152.105483	400677	9578957
9	27/04	03:18	1452	-3.812556	152.103957	400508	9578540
10	27/04	04:37	1448	-3.812674	152.103975	400510	9578527
11	27/04	05:08	1437	-3.812384	152.104038	400517	9578559
12	26/04	23:31	1451	-3.812629	152.103975	400510	9578532

Table 4.2. Taxa and abundances in qualitative samples from inactive sulphide deposits at Solwara 1 (SWI) and South Su (SS), Manus Basin. Asterisk (*): taxa generally considered to be endemic to active hydrothermal vents.

	SWI	SS
PORIFERA		
Abyssocladia sp	172	120
CNIDARIA		
Hydrozoa sp. 1	1	1
Hydrozoa sp. 2	1	0
Hydrozoa sp. 3	1	0
Hydrozoa sp. 4	0	1
Hydrozoa sp. 5	1	0
Hydrozoa sp. 6	1	0
Keratoisis sp.	1	1
Actinaria sp. 1	1	1
Actinaria sp. 2	1	1
'*Keratoisis* anemone'	1	1
Edwardsia sp.	0	1
BRACHIOPODA		
Brachiopoda sp.	1	0
MOLLUSCA	0	0
Solengastres sp.	8	1
Gastropoda sp. 1	0	2
Gastropoda sp. 2	0	1
Gastropoda sp. 3	0	1
Gastropoda sp. 4	0	1
Gastropoda sp. 5	1	0
Gastropoda sp. 6	1	0
Philine sp.	1	0
Diaphana sp	0	2
Neomphalid n.gen.	0	1
**Lepetodrilus schrolli*	2	44
Anatoma sp.	3	1
**Olgasolaris tollmani*	3	1
**Shinkailepas* sp.	2	1
**Eosipho* juv.	2	0
**Alviniconcha* juv.	1	0
**Desbruyeresia* sp.	5	0
Provanna sp.	0	1
Vitrinellidae sp.	0	9
Nuculanoidea n. gen., n. sp.	3	6
Bivalvia sp. 1	0	1
Thyasiridae sp. 1	4	16
NEMERTEA		
Nemeretea sp.	1	0
PLATYHELMINTHES		
Platyhelminthes sp.	1	0
SIPUNCULA	0	0
Sipuncula sp.	1	0
ECHINODERMATA		
Ophiura sp. 1	0	1
Ophiura sp. 2	4	1
Crinoidea sp.	1	0
Holothurian sp.	1	0
ANNELIDA		
Echiura sp.	1	0
**Archinome* sp.	1	0
Ophryotrocha sp.	0	1
Lumbrinereis sp.	1	0

Glyceridae sp.	1	0
Hesionidae spp.	3	1
Nereis sp.	1	0
Polynoidea (damaged)	3	2
Polynoidea sp. 1	1	0
Polynoidea sp. 3	0	1
Sabellidae sp.	0	1
Maldanidae sp.	1	1
Capitellidae sp.	0	1
Orbiniidae sp.	4	1
Spionidae sp.	5	3
Terebellidae sp.	0	5
**Amphisamytha* sp.	3	1
ARTHROPODA		
Lohmannellinae sp.	1	0
Pycnogonida sp.	1	0
Copepoda spp.	2	5
Harpactacoid copepod sp.	0	1
Scalpellomorph sp. 1	0	1
Scalpellomorph sp. 2	1	0
Trianguloscalpellum michelottanum	1	0
Vulcanolepas parensis	53	9
Glyptelasma sp.	0	1
Poecilasma kaempferi	8	6
Altiverruca sp.	26	12
Eurodella sp.	0	1
Tanaidacea spp.	1	2
Isopod sp. 1	1	3
Isopod sp. 2	2	2
Isopod sp. 3	3	0
Isopod sp. 4	0	1
Amphipod indet (damaged)	0	1
Amphipod sp. 1	3	7
Amphipod sp. 2	23	42
Amphipod sp. 3	0	1
Amphipod sp. 4	0	1
Amphipod sp. 5	1	0
Amphipod sp. 6	6	0
Lysianassidae sp. 1	2	0
Lysianassidae sp. 2	1	0
Mysidacea spp.	1	0
Nematocarcinus sp.	2	0
*Alvinocarididae sp.	0	1
**Opaepele* sp.	3	0
Anomura sp.	0	1
Munida magniantennulata	4	2
**Munidopsis lauensis*	4	18

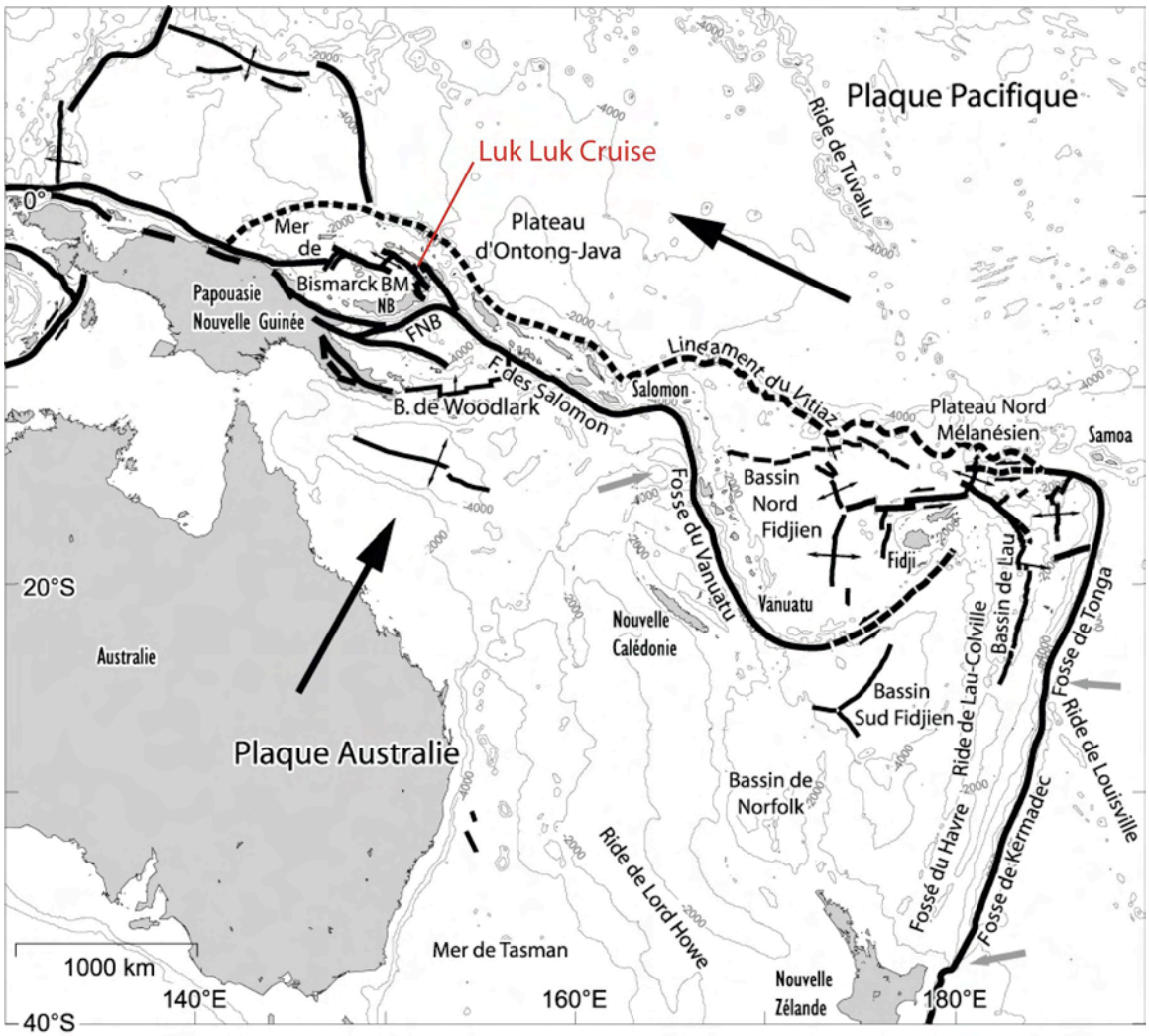

Figure 4.1. Geomorphological context of the Bismarck Sea (Manus Basin), North Fiji Basin (Bassin Nord Fidjien), and Lau Basin (Bassin de Lau). 'Luk Luk Cruise' marks the general location of Solwara 1 and South Su. Arrows indicate plate motion. From Ruellan & Lagabrielle (2005).

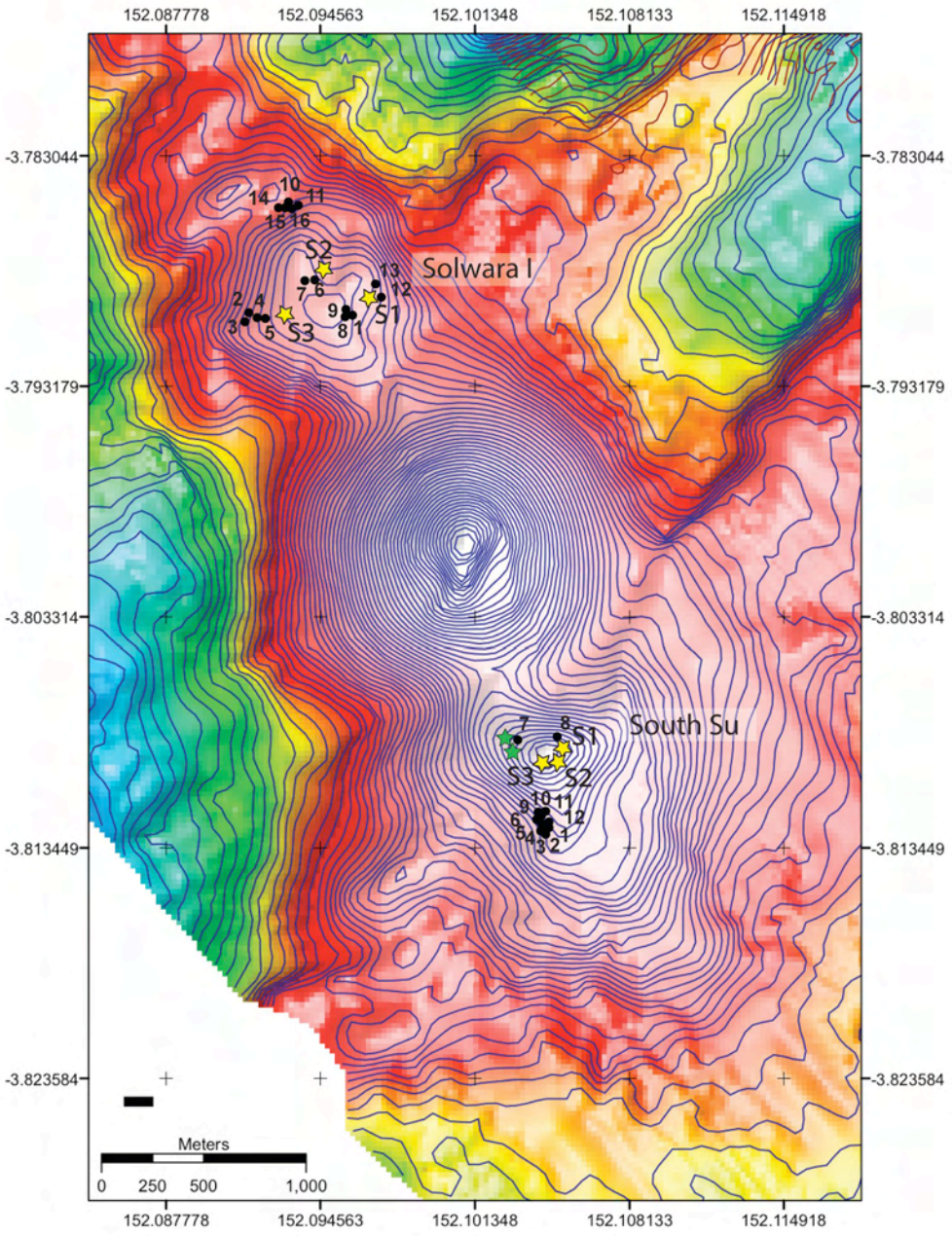

Figure 4.2. Sample locations at Solwara 1 and South Su study areas, Luk Luk Cruise (March-April 2006). Basemap from Nautilus Minerals. Yellow stars: active sites; green stars: location of tubeworm assemblages; numbered, filled circles: location of inactive samples.

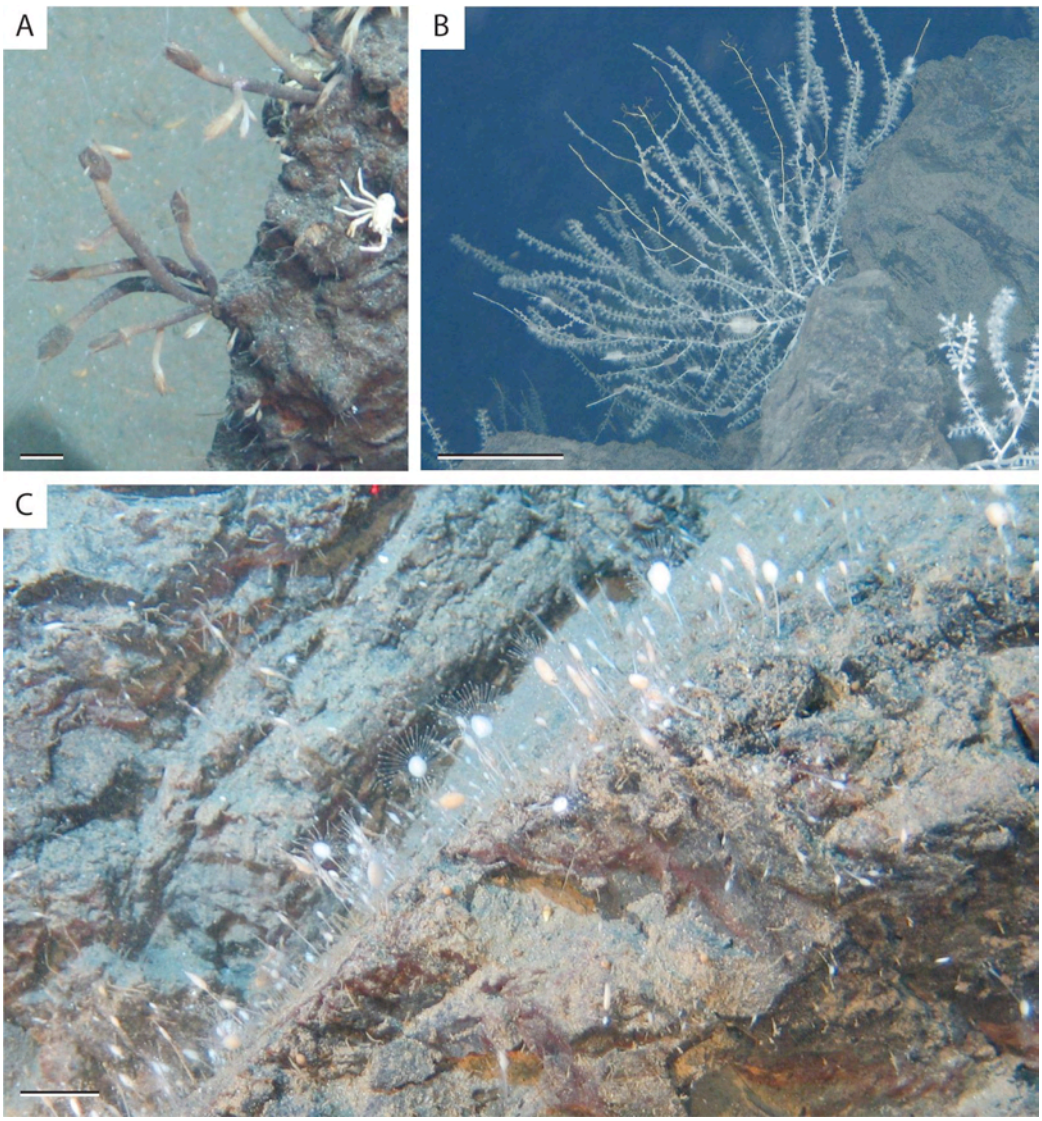

Figure 4.3 Characteristic taxa at inactive sites of Solwara 1 and South Su (Manus Basin). A: *Vuclanolepas parensis* (scale bar = 1 cm); B: *Keratoisis* sp. (scale bar = 10 cm); C: *Abyssocladia* sp. (scale bar = 5 cm).

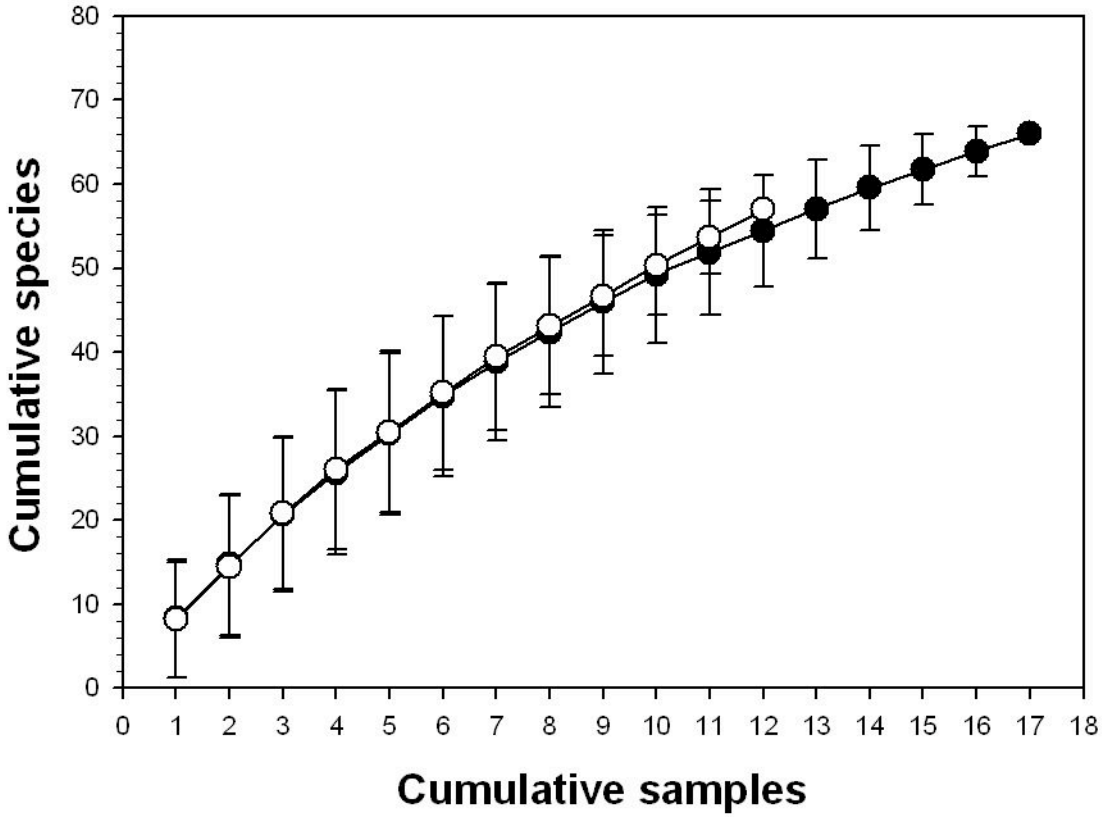

Fig 4.4. Species-effort curve for samples from inactive sites at Solwara 1 (filled circles) and South Su (open circles). Error bars are standard deviations derived during randomization of sample order. Each sample area was approximately 0.25 m^2.

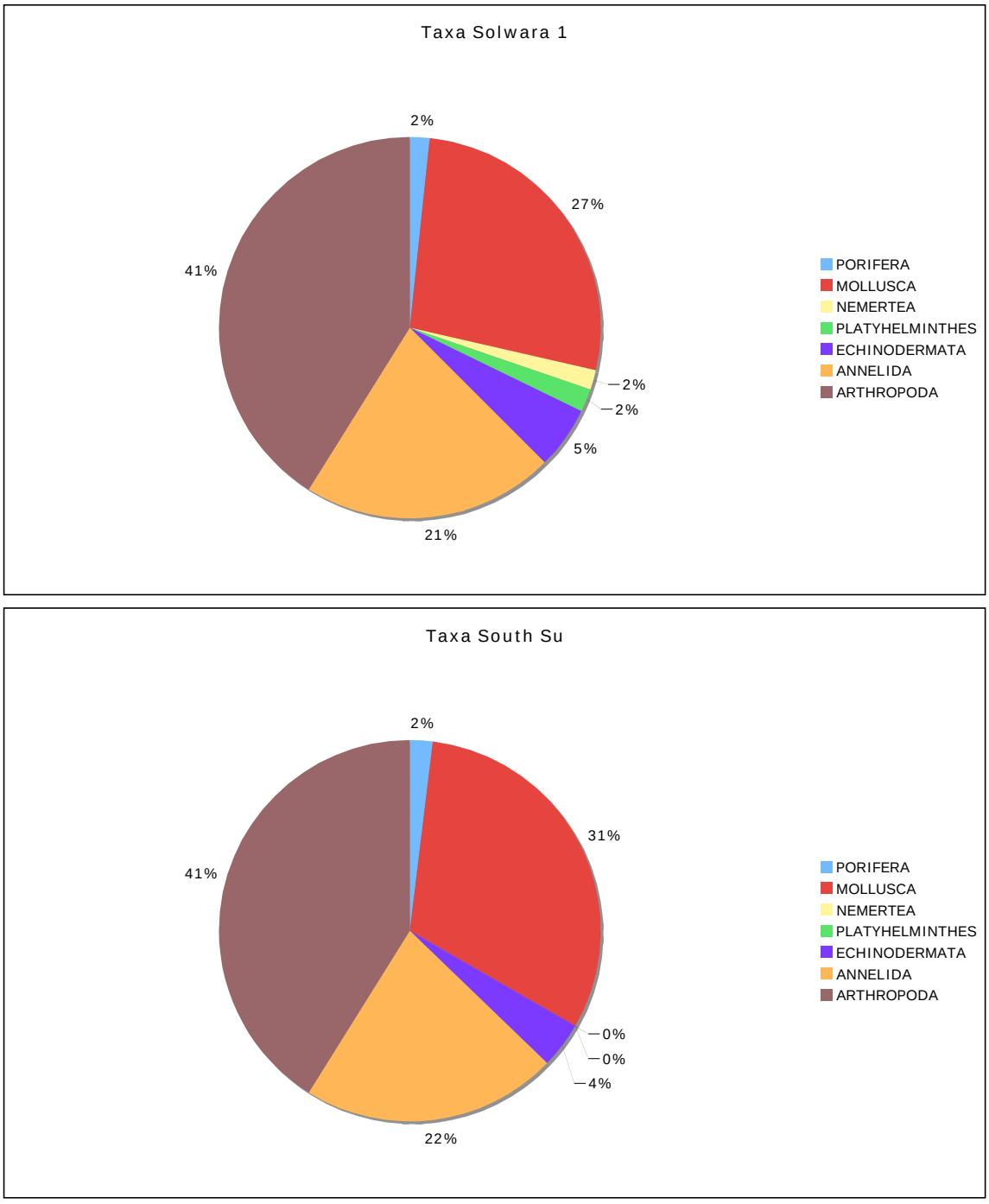

Figure 4.5. Distribution of major taxonomic groups at inactive sites of Solwara 1 and South Su.

Chapter 5

Macrofaunal Communities at Active Sulphide Mounds, Manus Basin

Aim: Quantitative characterization and comparison of the macrofaunal invertebrate fauna of active sulphide mounds at Solwara 1 and South Su.

Objectives:

- To build a quantitative inventory of invertebrates associated active sulphide mounds, including a species-abundance database, specimen archives, and photo archives.

- To compare community structure at Solwara 1 and South Su using species effort curves, univariate statistics, and multivariate statistics (multi-dimensional scaling and cluster analyses).

Methods: 'Active' sulphide habitats (sites) were defined by the presence of vent endemic species that host endosymbionts (i.e., *Ifremeria nautilei, Alviniconcha sp., Bathymodiolus manusensis*) or by barnacles in the genus *Eochionelasmus* and by the presence of visually evident warm water or black-smoker effluents. Three sites (20-m diameter) were chosen for quantitative sampling at Solwara 1 and at South Su (Fig. 4.2; Table 5.1). These sites were selected by consensus of the scientists, based on the criterion of having sufficient biomass to allow three 'replicate samples' from each of the three, pre-designated, dominant species patches, herein referred to as *Alviniconcha* (hairy snails), *Ifremeria* (black snails), and *Eochionelasmus* (barnacles) (Figs. 5.1). Patches of these three species were almost non-overlapping, representing distinct microhabitats within which a variety of other macro-invertebrates lived.

Sites were photodocumented before and after sampling. Three 'replicate' samples were collected within each species patch, resulting in a nested array of 3 replicate samples at each of 3 species patches at each of 3 sites, i.e., 27 samples at Solwara 1 and 27 samples at South Su (Fig. 5.2). Sampling impact in all instances was estimated to be <10% of the total habitat.

Standard sampling routines were followed: For *Alviniconcha* and *Ifremeria* patches, areas of roughly 0.25 m^2 were initially lightly suctioned with the onboard slurp gun (also known as the suction sampler) of the remotely operated vehicle (ROV). A PVC scoop was used to collect larger specimens, which were placed within a closeable 'bio-box'. To complete the sampling effort, the sample zone was more vigorously suctioned into the same chamber used for the initial suctioning. This entire procedure was referred to as as the "slurp-scoop-slurp" method. On deck, samples were immediately chilled, sieved through a 0.025-mm aperture sieve and either processed or stored at 4ºC for < 2 hours. For *Eochionelasmus* patches, sections of sulphide chimneys with ≥ 0.25 m^2 barnacle coverage (outlined using a quadrat) were broken off and placed in the chimney bin for transfer to the surface. Sections of the chimney were processed at 4ºC. Records of the labeled, but unprocessed, chimneys were taken by digital still camera. All epibiota within a 0.25-m^2 area, delineated by a quadrat, were picked off the chimney segment and processed. Additional, qualitative samples were collected using a scoop sample ("lucky bag"); these were samples of opportunity that focused on capturing the maximum diversity, including mussels, tubeworms and their associated fauna.

Sample material was fixed in 10% borax-buffered formalin for 24 h and stored in 70% ethanol. At the Duke University Marine Laboratory, preserved samples were rinsed with fresh water and sorted under light in white plastic trays. Species were identified to the lowest taxon possible and enumerated. Initial identifications were made using Desbruyères et al. (2006b) followed by review of original descriptions and consultation with taxonomic experts. A voucher collection was created and used for the provisional labeling of unidentified species, e.g., amphipod species 1, 2, and 3. This voucher collection is maintained at the Duke University Marine Laboratory, with specimens dispensed on loan to individuals with taxonomic expertise in diverse groups.

Data Analyses. Data from quantitative samples only were used to calculate species densities using cumulative species-effort curves. PRIMER v6 (Clarke & Warwick 2001) was used to randomize the sample data and to eliminate the effect of sample order (randomization operations = 999, without replacement). Effort is expressed as the cumulative number of samples.

Standard univariate indices [species richness, abundance, Shannon's diversity index (H_{loge}'), and Pielou's evenness index (J_{loge}'); Hayek and Buzas 1997] were calculated using using the DIVERSE subroutine in PRIMER v6 (Clarke & Warwick 2001) summarize community structure at each site. H' is a measure of uncertainty, where the maximum

uncertainty occurs when each of the species is equally represented (Hayek & Buzas 1997); the index increases by adding species or by increasing species' evenness. J' provides a measure of evenness, i.e., the extent to which individuals are equally partitioned between species (Hayek & Buzas 1997) and varies from 0 (all individuals in a single species) to 1 (individuals evenly distributed among multiple species). 'Habitat' species – i.e., *Alviniconcha* sp., *Ifremeria* sp., and *Eochionelasmus* sp. – were included in these calculations. Differences in mean indices for samples from each habitat were compared between Solwara 1 and South Su using paired t-tests ($\alpha = 0.05$).

Multivariate statistical analyses, using the species-abundance matrices based on 'quantitative' samples from active sulphides at Solwara 1 and South Su, were also undertaken using Primer v6. As in the univariate statistical analyses, 'habitat' species were included in these multivariate characterizations, unless otherwise indicated. The Bray-Curtis similarity measure was used for both cluster analysis and multi-dimensional scaling (MDS). The Bray Curtis computation is not affected by joint absences, i.e., this coefficient of similarity depends only on species that are present in one or the other (or both) samples and not on species that are absent from both samples. Stress values, which describe the quality of the representation of the multidimensional relationships of data on a 2-dimensional plane, are reported for each MDS plot. Stress factors < 0.2 are considered to give a good representation (Clarke 1993).

Transformations of abundance data were explored to determine the effects of the transformations on different aspects of community structure, including (1) no transformation: sensitive to changes in the abundance of the numerically dominant species; (2) square root transformation: detects effects of species with mid-abundance ranges without being overly influenced by either dominant or rare species; and (3) $\log_{10}(x+1)$: allows the less-abundant species to exert some influence on the calculation of similarities (Clarke and Warwick 2001). These transformations are useful where a single species is overwhelmingly numerically dominant. There is no right or wrong transformation – transformations are merely tools to assist in interpretation of similarities and differences in community structure among samples, habitats, and sites, and between Solwara 1 and South Su. Only log (x + 1) abundance data transformation for samples from *Eochionelasmus* habitats are reported, since this was the only transformation that provided additional insight in comparison of community structure between Solwara 1 and South Su. In some multivariate analyses, habitat species and the extremely abundant limpet species, *Lepetodrilus schrolli*, were excluded to test for differences in community structure based on the residual taxa.

SIMPER analysis (PRIMER v.6) was used to determine species responsible for generating differences in community structure within and between sites. Analysis of similarity (ANOSIM subroutine of Primer v.6) was used to test for statistically significant differences in faunal assemblages between two sites. The ANOSIM procedure provides a formal test of the null hypothesis of "no significant difference in overall community structure among the two sites" and is analogous to the standard parametric statistical analysis of variance (ANOVA; Clarke and Warwick 2001). ANOSIM is based on a non-parametric permutation procedure applied to the Bray-Curtis similarity matrix underlying the ordination of samples (Clarke & Green 1988; Clarke 1993). This test involves calculation of a test statistic, R, which reflects the magnitude of difference in Bray-Curtis similarities among/between sites, and ranges between 0 and 1. Clarke & Gorley's (2006) interpretation of the R statistic for pairwise groups: R > 0.75 = groups well separated (i.e., a big difference in community structure); R > 0.5 = groups overlapping but clearly different; R > 0.25 = groups barely separable.

Results: Active sulphide deposits at Solwara 1 and South Su shared three visually similar sub-habitats associated with biomass-dominant organisms, namely *Alviniconcha* sp., *Ifremeria nautilei*, and *Eochionelasmus ohtai* (Fig. 5.1). These sub-habitats are zoned in concentric bands, with *Alviniconcha* in the center, surrounded by a middle band of *Ifremeria*, and an outer band of *Eochionelasmus*. This concentric zonation is correlated with fluid flux, fluid chemistry, and temperature along a gradient from the center of the ring to the periphery. Qualitative samples and observations added habitats and species to the quantitative sampling effort: Shrimp (*Opaepele* spp.), brachyuran crabs (*Austinograea* sp.), and three species of polynoid scale worms (*Branchinotogluma trifurcus*, *Thermopolynoe branchiata*, *Branchinotogluma segonzaci*) were associated with *Alviniconcha* and *Ifremeria* habitats. Squat lobsters (*Munidopsis lauensis*), shrimp (*Lebbeus* spp., *Alvinocaris* sp., *Nematocarcinus sp*), brachyuran crabs (*Austinograea* sp.) and small clumps of stalked barnacles (*Vulcanolepas* spp.) were associated with *Eochionelasmus* habitats. At South Su, mussels (*Bathymodiolus manusensis*) and tubeworms (*Alaysia* n. sp. and *Arcovestia ivanovi*) shared the outer ring with *Eochionelasmus*. Predatory buccinid gastropods (*Eosipho* sp.) were observed in small numbers around the base of sulphide structures. In sedimented areas at the bases of chimneys, there were large numbers of the echiuran *Alomasoma belyaevi* and of an unidentified bivalve in the Nuclanoidea grouping. Various fish such as *Lepidion ?schmidti*, *Hexatrygon ?bickelli* and *Psychrolutes marcidus* were observed among sulphide deposits chimneys (*H. ?bickelli*), feeding on vent animals (*L. schmidti*) or in small numbers at the base of chimneys (*P.*

marcidus). *Pyrolycus manusanus*, a vent-edemic zoarcid eelpout, was common at Solwara 1 and South Su, though it was more frequently observed at South Su.

A total of 49 species (24,805 individuals) were collected in quantitative samples from Solwara 1 and South Su, with 23 of these species shared by both sites (Table 5.2; Appendix IB, IC, ID). Solwara 1 quantitative samples returned 33 species (7,278 individuals), with 10 species in these samples not present at South Su. South Su quantitative samples returned 39 species (17,527 individuals), with 16 species in these samples not present at Solwara 1. The number of species is provisional and is expected to increase as external experts validate identifications. The difference of more than 10,000 individuals in total macroinvertebrate abundance between Solwara 1 and South Su samples is due to the enormous numbers of *Lepetodrilus schrolli* in *Alviniconcha* and *Ifremeria* habitats at South Su. Solwara 1 samples were numerically dominated by *Lepetodrilus schrolli* (n = 1,971 individuals), *Eochionelasmus ohta*i (n = 1,938), and *Olgasolaris tollmanni* (n = 1,657 individuals). Most species at Solwara 1 (18, or 53%) were uncommon, with fewer than 5 individuals total in all samples (Table 5.2). South Su samples shared the same numerical dominants as Solwara 1 [*L. schrolli* (n = 13,189 individuals), *E. ohtai* (n = 1,571 individuals), and *O. tollmanni* (n = 910 individuals)]. Sixteen species (44%) in South Su samples were uncommon, with fewer than five individuals in all samples (Table 5.2). At both sites, the hierarchy of abundance within major taxa was mollusks (60 to 80% of individuals) > crustaceans (13 to 30% of individuals) > annelids (4 to 13% of individuals) (Figure 5.3).

For any given sampling effort, species richness was higher at South Su than at Solwara 1 (Figure 5.4). Within *Ifremeria* and *Eochionelasmus* habitats, species richness was also higher at South Su than at Solwara 1; within *Alviniconcha* habitats, species richness was higher in samples from Solwara 1 than in samples from South Su (Figure 5.5). At the maximum sampling effort (9 samples), species richness increased the most (2 new species for each additional sample) in samples from *Ifremeria* habitats at South Su and in samples from *Alviniconcha* habitats at Solwara 1.

Species density was low [mean: 6 to 10 species (±1) per sample] in all 3 habitats, with no significant differences observed between Solwara 1 and South Su (Tables 5.3, 5.4, 5.5). There were also no significant differences in univariate measures of diversity (H' and J') between Solwara 1 and South Su within *Alviniconcha* or *Eochionelasmus* habitats. Mean H' and J' values (Tables 5.3, 5.4, 5.5) were significantly greater in samples from *Ifremeria* habitats at Solwara 1 (H': 1.391 ± 0.053 std. err.; J': 0.699 ± 0.031 std. err.) compared to values for

samples from South Su (H' :0.638 ± 0.084 std. err., J': 0280 ± 0.036 std. err.). These diversity differences were driven by the greater numbers of *Lepetodrilus schrolli* in samples from South Su, which resulted in an uneven distribution of a large proportion of the individuals in a single species.

Similarity analysis of species-abundance data for quantitative samples from Solwara 1 and South Su (all habitats combined within sites) show no pattern of differentiation that can be inferred to correspond to site-specific differences (Figs. 5.6, 5.7). This is supported by the Global R value of 0.10 in an analysis of similarity between the two sites, i.e., community structure as measured by species composition and relative abundances in quantitative samples from all habitats cannot be distinguished between the two sites. When *Lepetodrilus schrolli* and habitat species (*Alviniconcha, Ifremeria, Eochionelasmus*) are excluded from multivariate analyses, Solwara 1 and South Su samples are not separable from one another (Global R = 0.03), although samples are separable by habitat [*Alviniconcha:Ifremeria* R = 0.549; *Alviniconcha:Eochionelasmus* R = 0.834; *Ifremeria:Eochionelasmus* R = 0.861 (Figure 5.8B)]. *Ifremeria* habitats supported more *Olgasolaris tollmannii*, the limpet that commonly occurs on the whorls of *Ifremeria nautilei*, and more *Amphisamytha galapagensis*, compared to *Alviniconcha* and *Eochionelasmus* habitats, while *Alviniconcha* habitats support greater densities of shrimp (*Chorocaris vandoverae*) than *Ifremeria* or *Eochionelasmus* habitats (Table 5.6).

There was a greater variation in species-abundance attributes of samples from within sites than between sites. Species-abundance matrices for samples from *Alviniconcha* habitats were the most dissimilar, with typically < 50% overall similarity (Fig. 5.8).

Solwara 1 and South Su samples from *Alviniconcha* habitats were barely separable (Global R = 0.32) using multivariate methods (Fig. 5.8, 5.9). Bubble plots (Fig. 5.8) showing the corresponding abundances of *Lepetodrilus schrolli* within samples indicates that the slight separation of samples among sites was largely driven by the greater abundance of *L. schrolli* at South Su (45% contribution to dissimilarity; Table 5.8). Greater average abundances of the shrimp *Opaepele* sp. at South Su contributed another 20% to the dissimilarity, as did the lesser abundances of *Alviniconcha* spp. (9%) and the limpet *Olgasolaris tollmanni* (8%; Table 5.8).

Samples from *Ifremeria* habitats were readily distinguished between Solwara 1 and South Su (Global R = 0.80; Figure 5.8, 5.10), again driven primarily by the greater abundance of *Lepetodrilus schrolli* at South Su (contributing

to 81% of the dissimilarity) and, to a much lesser extent, by the lower abundance of *Olgasolaris tollmanni* at South Su (9% of the dissimilarity; Table 5.8).

Samples from *Eochionelasmus* habitats could not be distinguished between sites (Global R = 0.11; Figure 5.8, 5.11), although with a [log (x+1)] transformation of abundance data to decrease the influence of the dominant species, Solwara 1 samples could be distinguished from South Su samples (Global R = 0.45). In the transformed data analysis, dissimilarities were the consequence of the greater average abundance of *Lepetodrilus schrolli* (23% contribution to dissimilarity) and lower average abundances of *Opaepele* sp. (13%), and of two polychaete species, *Amphisamytha galapagensis* (13%) and *Nereis* sp. (10%) at South Su (Table 5.8).

Discussion: This study represents the most comprehensive and systematic survey of active hydrothermal systems in Manus Basin to date, building on faunal studies of Galkin (1997), Hashimoto et al. (1999) and Erickson (2006). At least 20 new species have been added to the species list for active vent sites, excluding records of larger transients such as fish and octopus.

Diversity in *Alviniconcha, Ifremeria,* and *Eochionelasmus* habitats at Solwara 1 and South Su sites appears to be low relative to that observed in habitats (mussel beds, tubeworm thickets) in other active systems around the world (Table 5.9). Mussel beds (*Bathymodiolus thermophilus*) and tubeworm thickets (*Riftia pachyptila*) on the East Pacifc Rise support more than 50 invertebrate species (Van Dover 2003), compared to 25 or fewer invertebrates in Manus Basin snail and barnacle habitats. Species richness is greater in mussel beds of Fiji and Lau Basins (34-35 species) than in Manus Basin snail and barnacle habitats (Blake & Van Dover, unpublished). Sampling methods and effort were variable among these sampling programs, but given the intensity of sampling effort during the Luk Luk cruise, lower species richness at Manus Basin seems real, particularly with respect to East Pacific Rise vents. This low alpha diversity is likely a consequence of multiple factors, including lower habitat complexity and productivity. A key point is that while alpha diversity may be low in Manus Basin, the contribution of alpha diversity in Manus to beta and gamma diversity is significant.

Community structure at Manus Basin active vents is visually similar to that of Fiji and Lau Basins, with clear alliances between Manus, Lau, and Fiji Basin faunal composition at the taxonomic level of genus (Desbruyères et al. 2006a). Important distinctions are evident at the species level (e.g., *Bathymodiolus manusensis* in Manus Basin, but

B. brevior in Lau and Fiji basins). Population genetic analyses of selected vent taxa (e.g., *Ifremeria nautilei, Lepetodrilus schrolli, Olgasolaris tollmani, Amphisamytha galapagensis*) shared between back-arc basins would permit assessment of the degree and direction of regional genetic exchange.

Both Solwara 1 and South Su active sites are similar in faunal zonation and makeup. They differ primarily in the superabundance of the limpet *Lepetodrilus schrolli* in samples from the South Su site, especially within *Ifremeria* habitats. Orders of magnitude differences in abundances of epifaunal gastropods are typical of other vent systems. For example, at East Pacific Rise hydrothermal vents, the epifaunal gastropod *Cyathermia naticoides* (Govenar et al. 2005) varied between 5 and nearly 2000 individuals per m^2 of tubeworm (*Riftia pachyptila*) surface within a given tubeworm field. Correlations between invertebrate abundance and fluid flux or fluid chemistry within and between vent habitats have been documented for mussel and tubeworm communities (e.g., Van Dover 2003, Govenar et al. 2005). It seems likely that a difference in fluid chemistry between Solwara 1 and South Su is a factor in the difference in abundance of *L. schrolli* observed between the two sites, although alternative explanations, including differential delivery of larval recruits, cannot be eliminated. Population genetic analyses of selected taxa shared between Solwara 1 and South Su (as for basin-to-basin exchange) would permit assessment of the degree and direction of local genetic exchange.

Multivariate analyses of species-abundance data from Solwara 1 and South Su samples highlight overlap in community structure, especially within *Alviniconcha*, and *Ifremeria* habitats. This is similar to what is observed in tubeworm clusters and mussel beds at active vents on the East Pacific Rise (Govenar et al. 2005): Though major biogenic habitats at active vents are typically discrete or interdigitating and are correlated with differences in fluid flux and chemistry, numerically dominant invertebrates associated with these biogenic habitats are not restricted to a single habitat. When the biomass (*Alviniconcha* sp., *Ifremeria nautilei, Eochionelasmus ohtai*) and numerical dominants (*Lepetodrilus schrolli*) are removed from the analysis, differences among habitats become apparent, with *Chorocaris vandoverae* characteristic of *Alviniconcha* habitats, *Olgasolaris tollmani* and *Amphisamytha galapagensis* characteristic of *Ifremeria* habitats, and *Amphismytha galapagensis* and *Vulcanolepas* sp. characteristic of *Eochionelasmus* habitats. This fine-scale partitioning of habitat use by the non-habitat-forming species reflects physiological and/or ecological processes that control species' distributions.

Most species at Solwara 1 and South Su active vents, regardless of habitat, are rare or uncommon (see definitions, p. 16), with fewer than 5 individuals per m^2 of habitat. Predominance of rare species in species lists is typical of vent communities in general (e.g., Van Dover 2003, Govenar et al. 2005). Rare species may be more abundant either during other phases of the life cycle of a vent system or in other vent settings yet to be discovered; they may even be taxa that are not restricted to vent systems but that are not yet known from elsewhere on hard substrata in the deep sea due to the immense sampling bias toward hydrothermal systems. The paucity of formal species descriptions, the lack of regional taxonomic keys, and the relatively few species-diagnostic genetic sequences deposited in internet databases for material from back-arc basin hydrothermal systems in particular and from the deep sea in general makes cross-comparisons among habitats and between laboratories challenging, especially for rare (low abundance) species belonging to taxonomically diverse and difficult groups (e.g., hesionid polychaetes, lysianassid amphipods).

The presence of abundant mussels (*Bathymodiolus manusensis*) and small populations of vestimentiferan tubeworms (*Alaysia* n. sp. and *Arcovestia ivanovi*) at South Su but not at Solwara 1 is a key finding that underscores our lack of understanding of environmental features and processes that control the distribution of biomass dominants with the system. There may be a lesson from studies of vent communities in the Eastern Pacific, where studies of succession following a volcanic eruption in 1991 led to a new understanding of pioneer species (especially the tubeworm *Tevnia jerichonana*), secondary colonists (*Riftia pachyptila*), and overgrowth competitors (mussels, *Bathymodiolus thermophilus*) (Shank et al. 1998). At any given moment, a vent field on the East Pacific Rise is in one or another of these stages of succession, with change driven by both physico-chemical and biological progressions. We do not yet understand succession in southwestern Pacific back-arc basin vent settings. Mussels and tubeworms at South Su may be a stage in a successional sequence, but they may also be indicators of a microhabitat not present at Solwara 1, they may reflect mesoscale processes relating to larval dispersal, or they may be a consequence of some other set of circumstances.

Table 5.1. Site data for quantitative sampling at active sulphide deposits of Solwara 1 and South Su and other sites of interest.

Site	Date (2007)	Depth (m)	Latitude	Longitude	Easting	Northing
Solwara 1						
1	23/03-04/04	1525	-3.789866	152.096743	399702.56	9581086.11
2	25/03-23/04	1516	-3.788099	152.094605	399464.89	9581281.11
3	03/04-24/04	1560	-3.790879	152.091273	399095.22	9580973.44
South Su						
1	08/04-14/04	1308	-3.809219	152.105063	400628.67	9578947.78
2	08/04-14/04	1323	-3.809768	152.105303	400655.33	9578887.11
3	09/04-14/04	1308	-3.809494	152.104967	400618.00	9578917.44
Other sites of interest						
Lamellibrachia			-3.808961	152.102698	400366.00	9578976.00
Alaysia and *Arcovestia*			-3.809314	152.102833	400381.00	9578937.00
Paralvinella in sediment			-3.909255	152.105619	400702.00	9567895.00
Conchocele shells			-3.807489	152.104690	400587.00	9579139.00

Table 5.2. Summary of species-abundance distributions by habitat (*Alviniconcha, Ifremeria, Eochionelasmus*) at Solwara 1 and South Su. Juv = juvenile.

	SOLWARA 1			Total N	SOUTH SU			Total N
	Alvin	***Ifrem***	***Eochio***		***Alvin***	***Ifrem***	***Eochio***	
PORIFERA	0	0	0	**0**	0	0	0	**0**
Abyssocladia sp.	0	1	0	**1**	0	6	0	**6**
CNIDARIA	0	0	0	**0**	0	0	0	**0**
Hydrozoa sp.	2	1	0	**3**	0	0	0	**0**
Keratoisis sp.	0	0	0	**0**	0	1	0	**1**
MOLLUSCA	0	0	0	**0**	0	0	0	**0**
Gastropoda sp.	0	0	0	**0**	2	0	0	**2**
Neomphalid n. gen., n sp.	0	0	0	**0**	58	166	0	**224**
Bathyacmaea jonassoni	0	0	0	**0**	0	1	0	**1**
Lepetodrilus schrolli	212	1586	173	**1971**	1043	11360	786	**13189**
Olgasolaris tollmanni	105	1552	0	**1657**	52	858	0	**910**
Shinkailepas spp.	4	1	4	**9**	16	1	0	**17**
Puncturella sp.	0	0	0	**0**	0	1	0	**1**
Alviniconcha spp.	184	30	0	**214**	96	1	0	**97**
Ifremeria sp.	26	230	0	**256**	14	131	0	**145**
Provanna n. sp.	1	0	0	**1**	6	0	1	**7**
Nuculanoidea n. gen., n. sp.	0	2	0	**2**	1	0	0	**1**
Bathymodiolus manusensis	0	0	0	**0**	2	0	16	**18**
Nemertea	0	0	0	**0**	0	0	0	**0**
Nemertean sp.	1	0	0	**1**	0	0	0	**0**
Annelida	0	0	0	**0**	0	0	0	**0**
Polychaete sp. 1	9	0	0	**9**	0	0	2	**2**
Polychaete sp. 2	0	0	0	**0**	0	0	2	**2**
Polychaete sp. 3	0	0	0	**0**	0	0	2	**2**
Archinome rosacea	0	0	1	**1**	0	0	0	**0**
Hesionidae sp.	0	0	0	**0**	0	1	0	**1**
Nereis sp.	0	0	35	**35**	0	0	6	**6**
Branchipolynoe sp.	0	0	0	**0**	0	0	5	**5**
Branchinotogluma sp.	0	0	0	**0**	0	3	1	**4**
Branchinotogluma segonzaci	1	0	0	**1**	0	1	0	**1**
Branchinotogluma trifurcus	6	252	0	**258**	11	109	2	**122**
Thermopolynoe branchiata	0	0	0	**0**	0	7	0	**7**
Spionidae spp.	1	0	0	**1**	0	0	0	**0**
Prionspio sp.	5	0	0	**5**	1	2	1	**4**
Spiochaetopterus sp.	3	0	0	**3**	0	0	0	**0**
Paralvinella juv	2	0	0	**2**	0	0	0	**0**
Paralvinella sp.	2	0	0	**2**	0	0	0	**0**
Paralvinella unidentata	2	0	0	**2**	8	0	0	**8**
Paralvinella fijiensis	17	3	1	**21**	1	0	0	**1**
Ampharetidae sp.	0	0	0	**0**	0	0	4	**4**
Amphisamytha galapagensis	8	490	97	**595**	17	327	132	**476**
Terebellidae sp.	0	0	14	**14**	0	0	1	**1**
ARTHROPODA	0	0	0	**0**	0	0	0	**0**
Ostracod	0	0	2	**2**	0	0	0	**0**
Harpactacoid spp.	30	0	1	**31**	35	0	2	**37**
Eochionelasmus ohtai	0	2	1836	**1838**	1	25	1545	**1571**

Ashinkailepas sp.	0	0	0	**0**	0	0	1	**1**
Vulcanolepas parensis	0	0	116	**116**	0	0	21	**21**
Opaepele sp.	50	169	0	**219**	426	173	0	**599**
Tanaidacea sp.	1	0	0	**1**	0	0	0	**0**
Isopoda sp.	2	0	0	**2**	0	0	0	**0**
Amphipoda spp.	1	0	0	**1**	0	2	1	**3**
Alvinocaris sp.	0	0	0	**0**	0	4	0	**4**
Munidopsis lauensis	0	0	0	**0**	1	0	0	**1**
Austinograea alayseae	2	2	0	**4**	6	19	0	**25**
TOTAL SPECIES	25	14	11	**33**	20	22	19	**39**
TOTAL INDIVIDUALS	677	4321	2280	**7278**	1797	13199	2531	**17527**

Table 5.3. Univariate statistics for quantitative samples from *Alviniconcha* habitats. S: number of species; N: number of individuals; H': Shannon diversity index; J': Pielou's evenness index. *: significant difference in mean values, pairwise t-test, $p < 0.05$.

Site	Sample	S	N	H_{loge}'	J_{loge}'
Solwara 1					
1	1	10	83	1.559	0.677
1	2	3	15	0.628	0.571
1	3	4	68	0.869	0.627
2	1	4	55	1.108	0.800
2	2	5	32	1.256	0.780
2	3	13	68	1.925	0.751
3	1	8	32	1.619	0.779
3	2	7	172	1.176	0.604
3	3	12	161	1.337	0.538
mean		7	*76	1.275	0.681
std error		1.2	18.5	0.132	0.033
South Su					
1	1	3	13	0.790	0.719
1	2	4	33	1.381	0.996
1	3	11	176	1.207	0.504
2	1	9	222	1.088	0.495
2	2	8	93	1.302	0.626
2	3	7	102	1.035	0.532
3	1	9	175	1.485	0.676
3	2	8	327	1.013	0.487
3	3	14	661	1.270	0.481
mean		8	*200	1.175	0.613
std error		1	66	0.071	0.056

Table 5.4. Univariate diversity statistics for quantitative *Ifremeria* habitat samples. S: number of species; N: number of individuals; d: Simpson's diversity index; H': Shannon diversity index; J': Pielou's evenness index. *: significant difference in mean values between Solwara 1 and South Su, pairwise t-test, $p < 0.05$.

Site	Sample	S	N	H_{loge}'	J_{loge}'
		Solwara 1			
1	1	10	922	1.425	0.619
1	2	8	279	1.404	0.675
1	3	6	443	1.271	0.710
2	1	5	137	1.453	0.903
2	2	7	382	1.272	0.654
2	3	8	812	1.497	0.720
3	1	11	395	1.710	0.713
3	2	10	596	1.326	0.576
3	3	5	361	1.162	0.722
mean		8	*481	*1.391	*0.699
std error		1	84	0.053	0.031
		South Su			
1	1	10	688	1.004	0.436
1	2	15	2724	0.648	0.239
1	3	13	1863	0.912	0.356
2	1	9	1226	0.351	0.160
2	2	8	1890	0.389	0.187
2	3	8	751	0.831	0.400
3	1	10	2032	0.296	0.128
3	2	9	1328	0.657	0.299
3	3	8	706	0.656	0.315
mean		10	*1468	*0.638	*0.280
std error		1	236	0.084	0.036

Table 5.5. Univariate diversity statistics for quantitative *Eochionelasmus* habitat samples. S: number of species; N: number of individuals; H': Shannon diversity index; J': Pielou's evenness index. *: significant difference in mean values between Solwara 1 and South Su, pairwise t-test, $p < 0.05$.

Site	Sample	S	N	H_{loge}'	J_{loge}'
Solwara 1					
1	1	6	253	0.757	0.423
1	2	6	267	0.375	0.209
1	3	6	228	1.195	0.667
2	1	7	358	0.405	0.208
2	2	3	139	0.295	0.268
2	3	5	123	0.932	0.579
3	1	6	181	0.683	0.381
3	2	4	178	0.724	0.522
3	3	8	556	0.730	0.351
mean		6	254	0.677	0.401
std error		1	45	0.095	0.054
South Su					
1	1	4	356	0.801	0.578
1	2	9	536	0.982	0.447
1	3	3	319	0.364	0.331
2	1	8	339	0.794	0.382
2	2	10	80	1.283	0.557
2	3	4	113	1.066	0.769
3	1	8	211	1.028	0.494
3	2	11	292	0.915	0.382
3	3	7	288	0.817	0.420
mean		7	282	0.894	0.484
std error		1	46	0.084	0.045

Table 5.6. Species contributing most to dissimilarities (contribution > 5%) between habitats at active vents (Solwara 1 and South Su data combined); SIMPER analysis.

	Average Abundance	**Average Abundance**	**% Contribution to Dissimilarity**	**Cumulative % Contribution**
	Alviniconcha	***Ifremeria***		
Olgasolaris tollmanni	9	134	47	47
Amphisamytha galapagensis	1	45	21	68
Chorocaris vandoverae	26	19	12	80
Branchinotogluma trifurcus	1	20	8	88
Neomphalid n. sp.	3	9	4	92
	Alviniconcha	***Eochionelasmus***		
Chorocaris vandoverae	26	0	26	26
Amphisamytha galapagensis	1	13	19	45
Vulcanolepas sp.	0	8	13	58
Olgasolaris tollmani	9	0	12	70
Copepoda spp.	4	0	6	76
	Ifremeria	***Eochionelasmus***		
Olgasolaris tollmani	134	0	53	53
Amphisamytha galapagensis	45	13	17	70
Branchinotogluma trifurcus	20	0	9	79
Chorocaris vandoverae	19	0	7	86

Table 5.7. Species contributing most to similarity (to cumulative > 90%) within samples from Solwara 1 and South Su active sulphide deposits, by habitat; SIMPER analysis.

Site	Species	Average Abundance	% Contribution to Similarity	Cumulative % Contribution
	***Alviniconcha* habitats, untransformed abundance data**			
Solwara 1	*Alviniconcha* sp.	20	67	67
	Olgasolaris tollmanni	12	15	82
	Lepetodrilus schrolli	24	8	90
South Su	*Lepetodrilus schrolli*	116	53	53
	Opaepele	47	24	77
	Alviniconcha sp.	11	17	94
	***Ifremeria* habitats, untransformed abundance data**			
Solwara 1	*Lepetodrilus schrolli*	176	39	39
	Olgasolaris tollmanni	172	35	73
	Amphisamytha galapagensis	54	14	87
	Branchinotogluma trifurcus	28	6	93
South Su	*Lepetodrilus schrolli*	1262	87	87
	Olgasolaris tollmanni	95	6	94
	***Eochionelasmus* habitats, untransformed abundance data**			
Solwara 1	*Eochionelasmus ohtai*	204	89	89
	Opaepele sp.	13	4	93
South Su	*Eochionelasmus ohtai*	172	73	73
	Lepetodrilus schrolli	87	20	94
	***Eochionelasmus* habitats, log(x + 1) transformed abundance data**			
Solwara 1	*Eochionelasmus ohtai*	5	51	51
	Opaepele sp.	2	20	70
	Amphisamytha galapagensis	2	13	84
	Lepetodrilus schrolli	2	10	94
South Su	*Eochionelasmus ohtai*	5	44	44
	Lepetodrilus schrolli	4	30	74
	Amphisamytha galapagensis	2	13	88
	Opaepele sp.	1	6	94

Table 5.8. Species contributing most to dissimilarities (to cumulative > 90%) between samples from Solwara 1 and South Su active sulphide deposits, by habitat; SIMPER analysis.

Species	Solwara 1 Average Abundance	South Su Average Abundance	% Contribution to Dissimilarity	Cumulative % Contribution
***Alviniconcha* habitats, untransformed abundance data**				
Lepetodrilus schrolli	24	116	45	45
Opaepele	6	47	20	65
Alviniconcha sp.	20	11	9	74
Olgasolaris tollmanni	12	6	8	81
copepod sp.	3	4	4	85
limpet A	0	6	3	88
Ifremeria nautilei	3	2	2	90
Paralvinella fijiensis	2	0	2	92
***Ifremeria* habitats, untransformed abundance data**				
Lepetodrilus schrolli	176	1262	81	81
Olgasolaris tollmanni	172	95	9	90
***Eochionelasmus* habitats, untransformed abundance data**				
Eochionelasmus ohtai	204	172	50	50
Lepetodrilus schrolli	19	87	32	82
Amphisamytha galapagensis	11	15	7	89
Opaepele sp.	13	2	5	94
***Eochionelasmus* habitats, log(x + 1) transformed abundance data**				
Lepetodrilus schrolli	2	4	23	23
Opaepele sp.	2	1	13	36
Amphisamytha galapagensis	2	2	13	48
Nereis sp.	1	0	10	58
Bathymodiolus sp.	0	1	8	66
Eochionelasmus ohtai	5	5	6	72
Terebellidae sp.	0	0	4	76
Branchipolynoe sp.	0	0	3	79
polychaete sp.	0	0	3	82
Amphisamytha sp. 2	0	0	2	84
Shinkailepas tufari	0	0	2	86
copepod sp.	0	0	2	88
Branchinotogluma trifurcus	0	0	2	89
polychaete sp.	0	0	1	91

Table 5.9. Comparison of univariate diversity measures for selected active vent habitats.

Site	Habitat	S	H_{loge}'	J_{loge}'	Citation
Northern East Pacific Rise (9N)	mussel bed	61	1.61	0.55	Van Dover 2003
Southern East Pacific Rise (17-18S)	mussel bed	57	2.25	0.65	Van Dover 2003
Northern East Pacific Rise (9N)	tubeworms	46	1.66	0.51	Govenar et al. 2005
Mid-Atlantic Ridge (Snake Pit)	mussel bed	23	1.80	0.70	Turnipseed et al. 2004
Lau Basin	mussel bed	34	0.52	0.22	Blake 2006
Fiji Basin	mussel bed	35	0.53	0.21	Blake 2006
Manus Basin (Solwara 1)	hairy snails	25	1.27	0.68	this study
Manus Basin (Solwara 1)	black snails	24	1.39	0.70	this study
Manus Basin (Solwara 1)	barnacles	22	0.68	0.40	this study
Manus Basin (South Su)	hairy snails	20	1.17	0.61	this study
Manus Basin (South Su)	black snails	23	0.64	0.28	this study
Manus Basin (South Su)	barnacles	19	0.89	0.48	this study

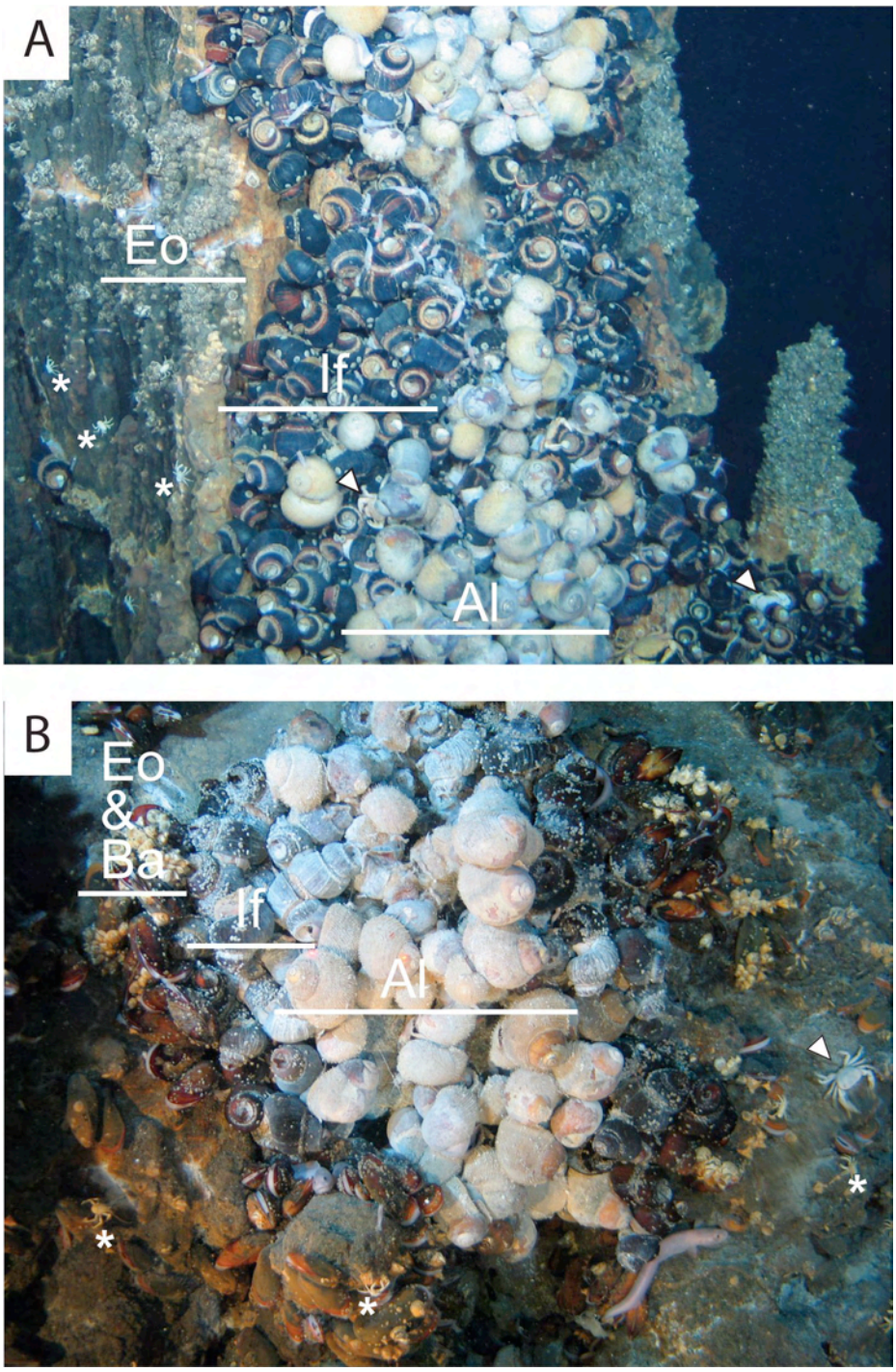

Figure 5.1. Sampling habitats. A. Solwara 1. B. South. Al: *Alviniconcha* (hairy snails)*;* If: *Ifremeria* (black snails)*;* Eo: *Eochionelasmus* (barnacles); Ba: *Bathymodiolus* (mussels). Arrowheads: brachyuran crabs (*Austinograea alayseae*); asterisks: *Munidopsis lauensis*. Note that the image from South Su is somewhat anomalous in that it highlights an assemblage in which mussels and barnacles make up the outer zone of dense biota. The more typical situation at South Su was as in (A), with barnacles alone comprising the outermost zone.

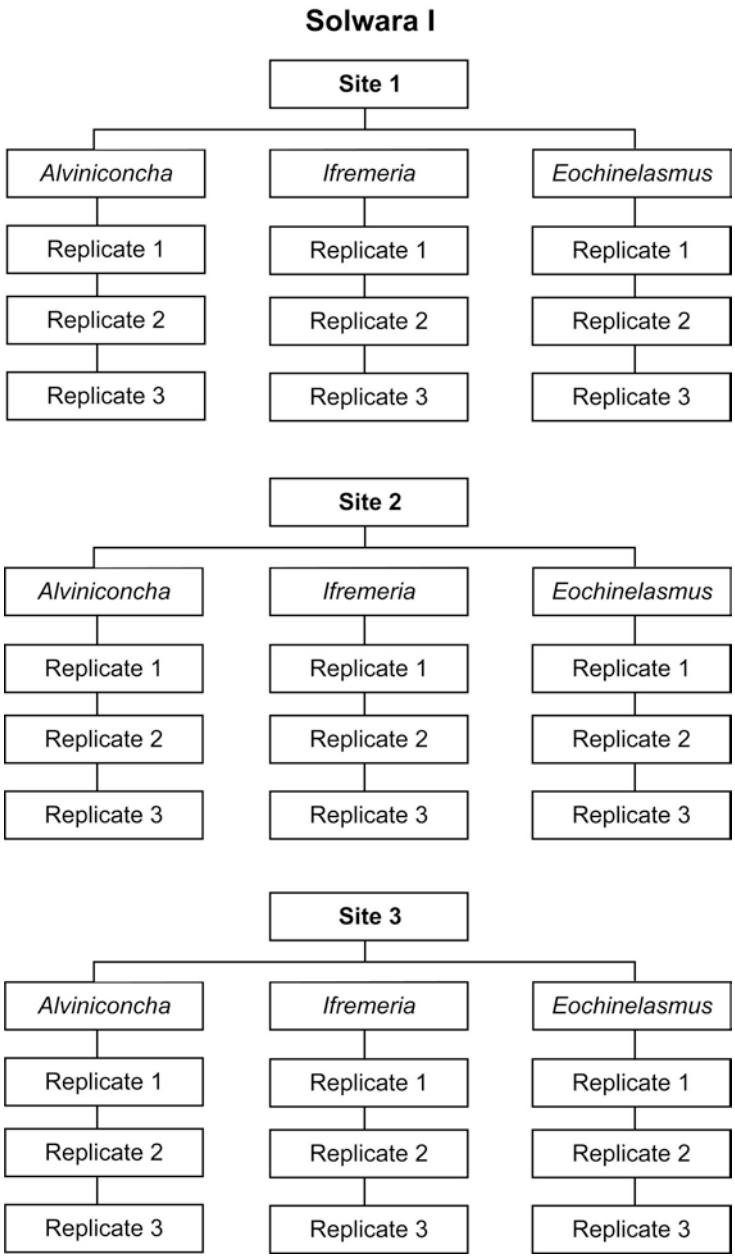

Figure 5.2. Sampling design for 'quantitative' sampling from active sulphide deposits at Solwara 1. An identical sampling scheme was conducted at South Su.

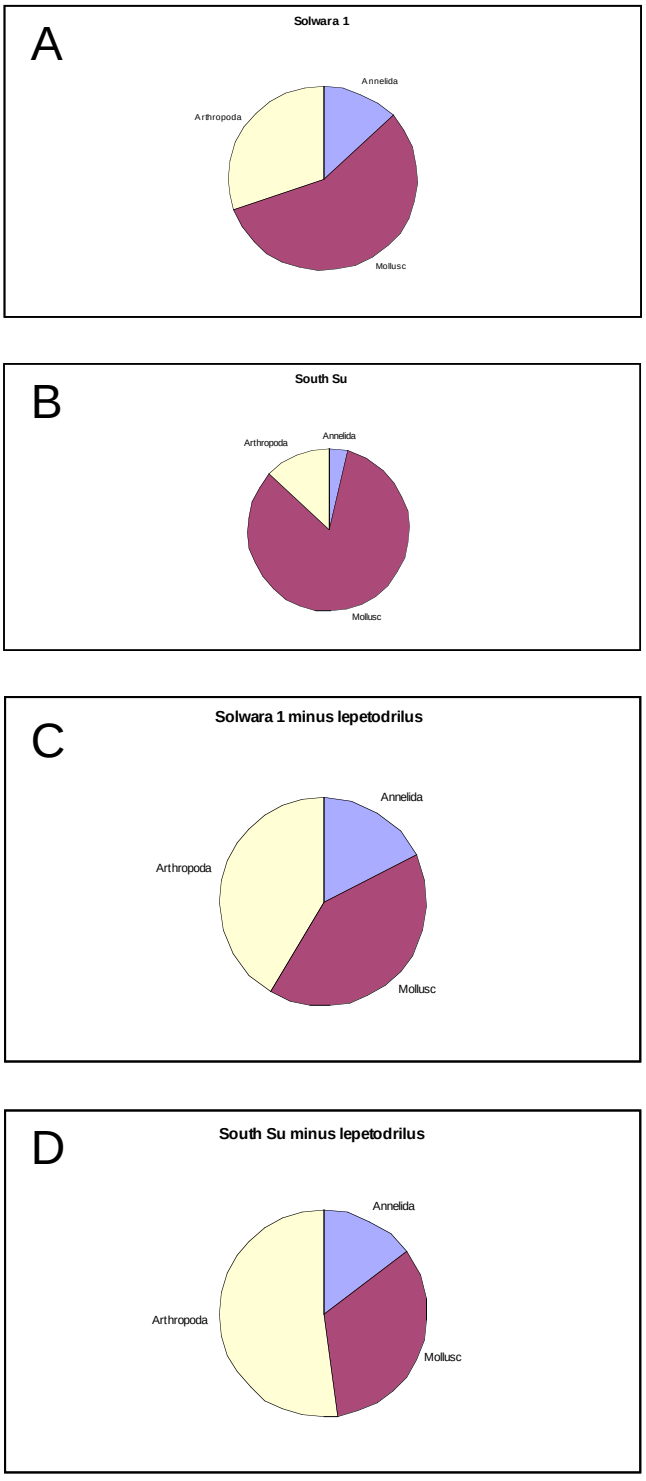

Figure 5.3. A. Relative abundance of major taxa at Solwara 1 active sulphide deposits. B. Relative faunal abundances of major taxa at South Su active sulphide deposits. (C, D) as in (A, B), but with limpets (*Lepetodrilus schrolli)* removed.

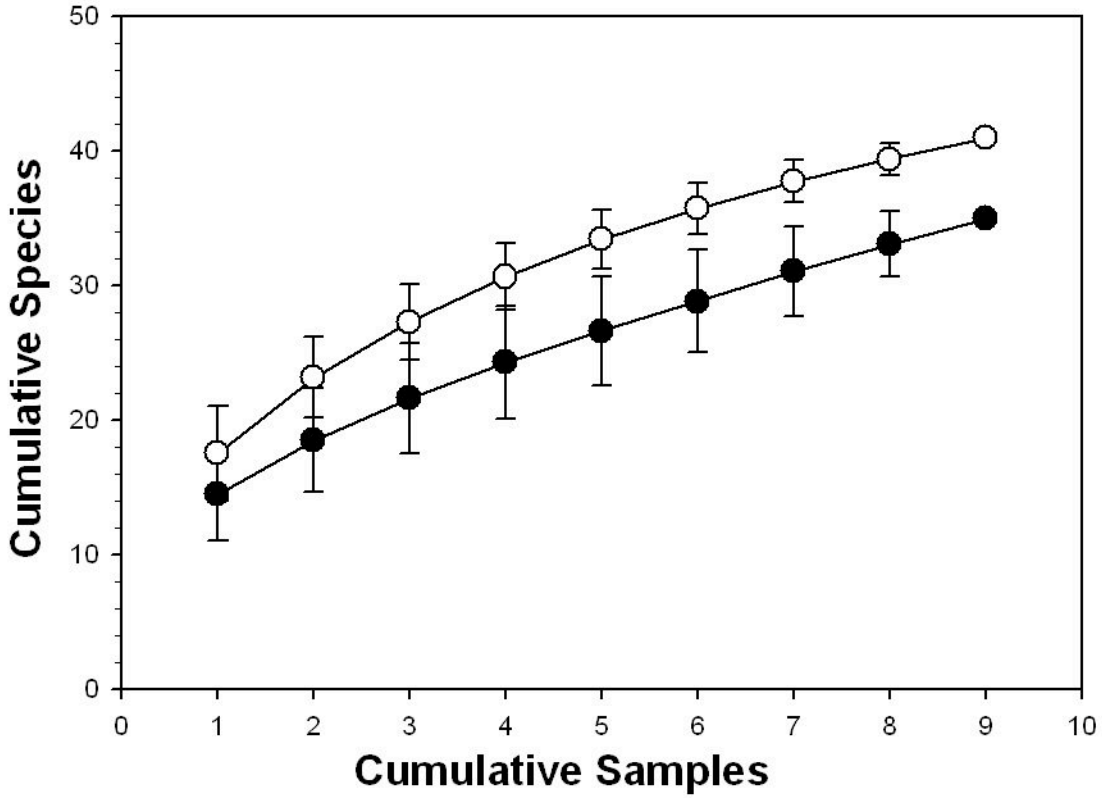

Figure 5.4. Species-effort curve for samples from active sites at Solwara 1 (filled circles) and South Su (empty circles). Error bars are standard deviations derived during randomization of sample order. Each sample area was approximately 0.25 m^2.

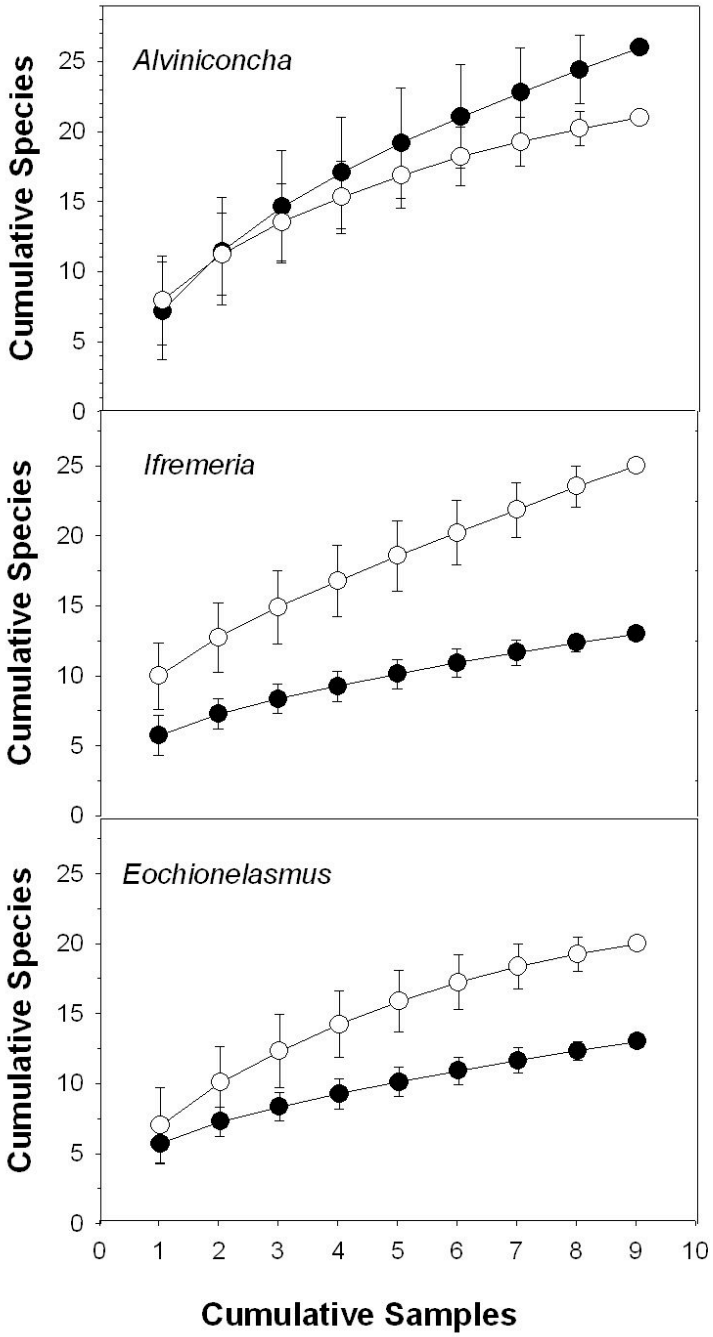

Figure 5.5. Species-effort curves for *Alviniconcha*, *Eochionelasmus*, *and Ifremeria* habitats at active sulphide deposits of Solwara 1 and South Su. Error bars are standard deviations derived during randomization of sample order. Each sample area was approximately 0.25 m^2.

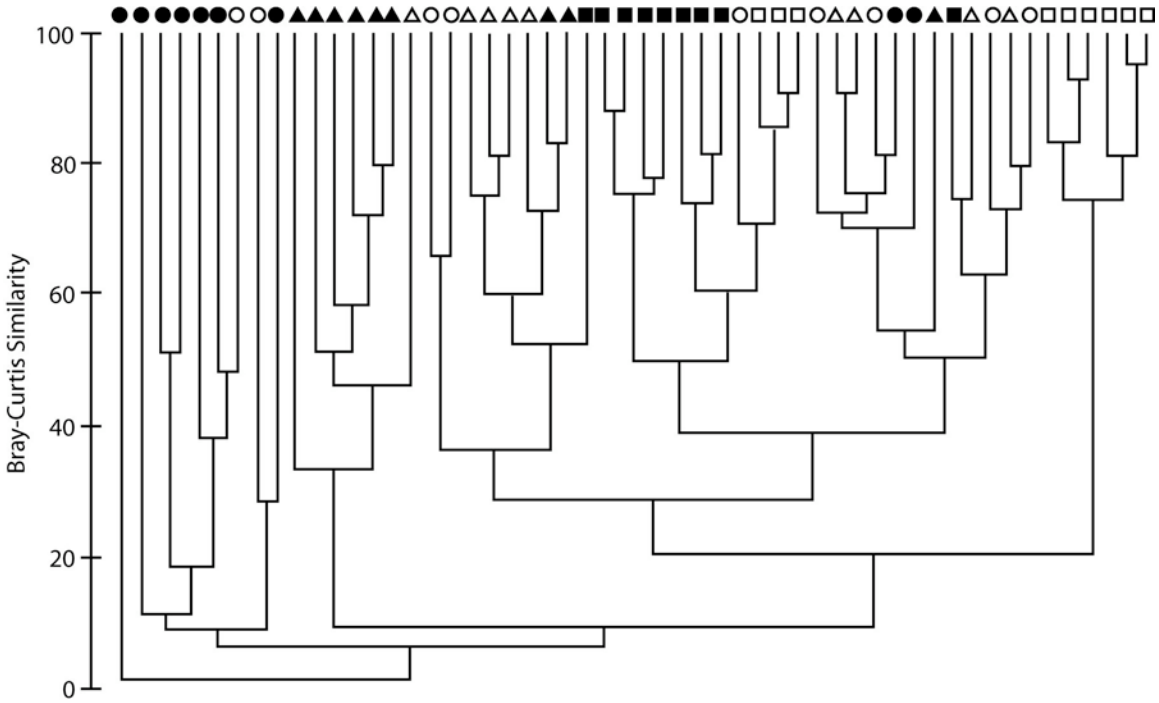

Figure 5.6. Cluster analysis of Bray-Curtis similarities of species-abundance matrices excluding habitat species (*Alviniconcha, Ifremeria, Eochionelasmus*; untransformed abundance data). Filled symbols: Solwara 1; open symbols: South Su; circles: *Alviniconcha* habitat samples; squares: *Ifremeria* habitat samples; triangles: *Eochionelasmus* habitat samples.

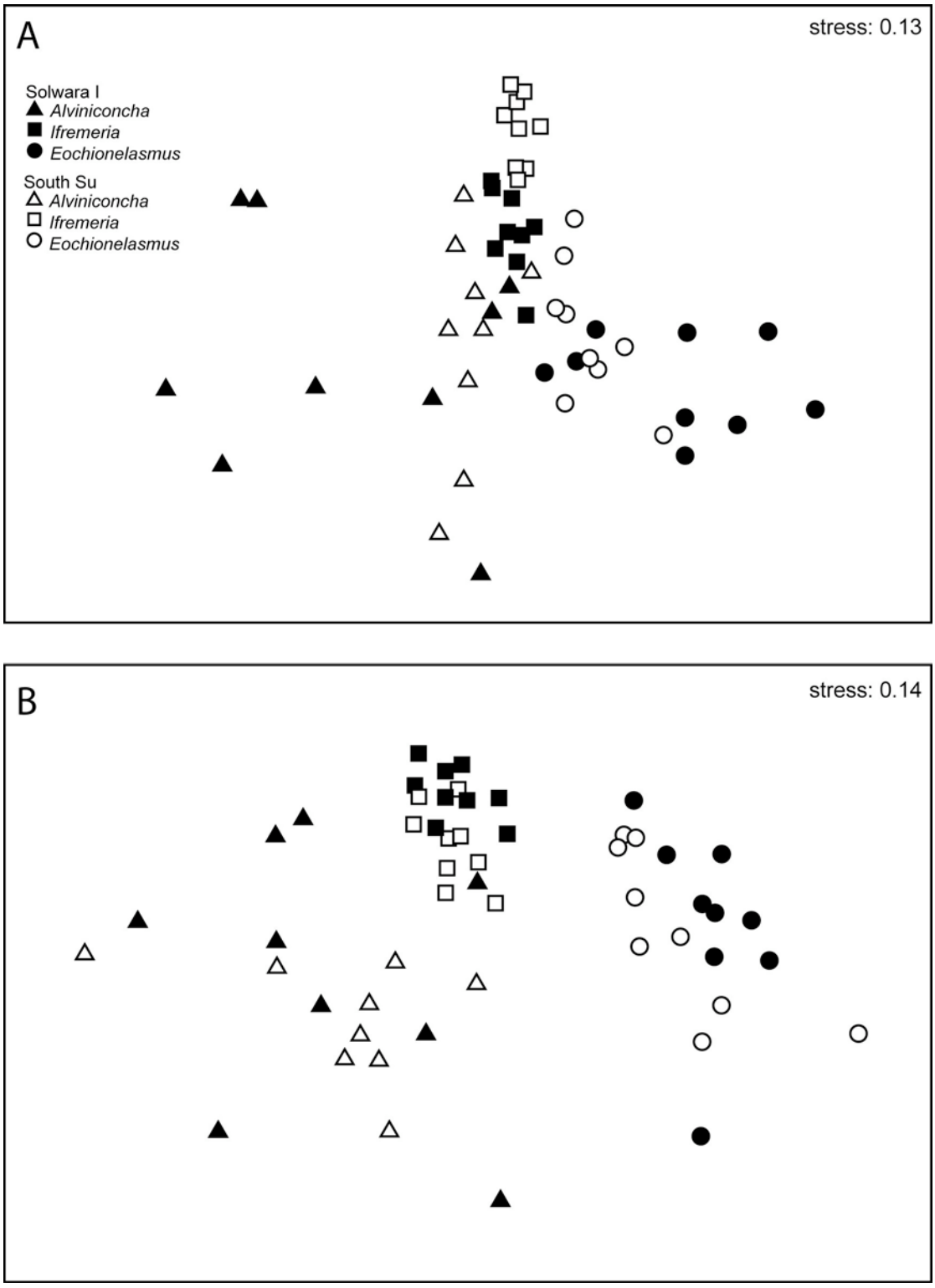

Figure 5.7. MDS analysis of species-abundance data. A. 'Habitat species' (*Alviniconcha, Ifremeria, Eochionelasmus*) excluded. B. Habitat species and *Lepetodrilus schrolli* excluded. Legend is the same for A and B.

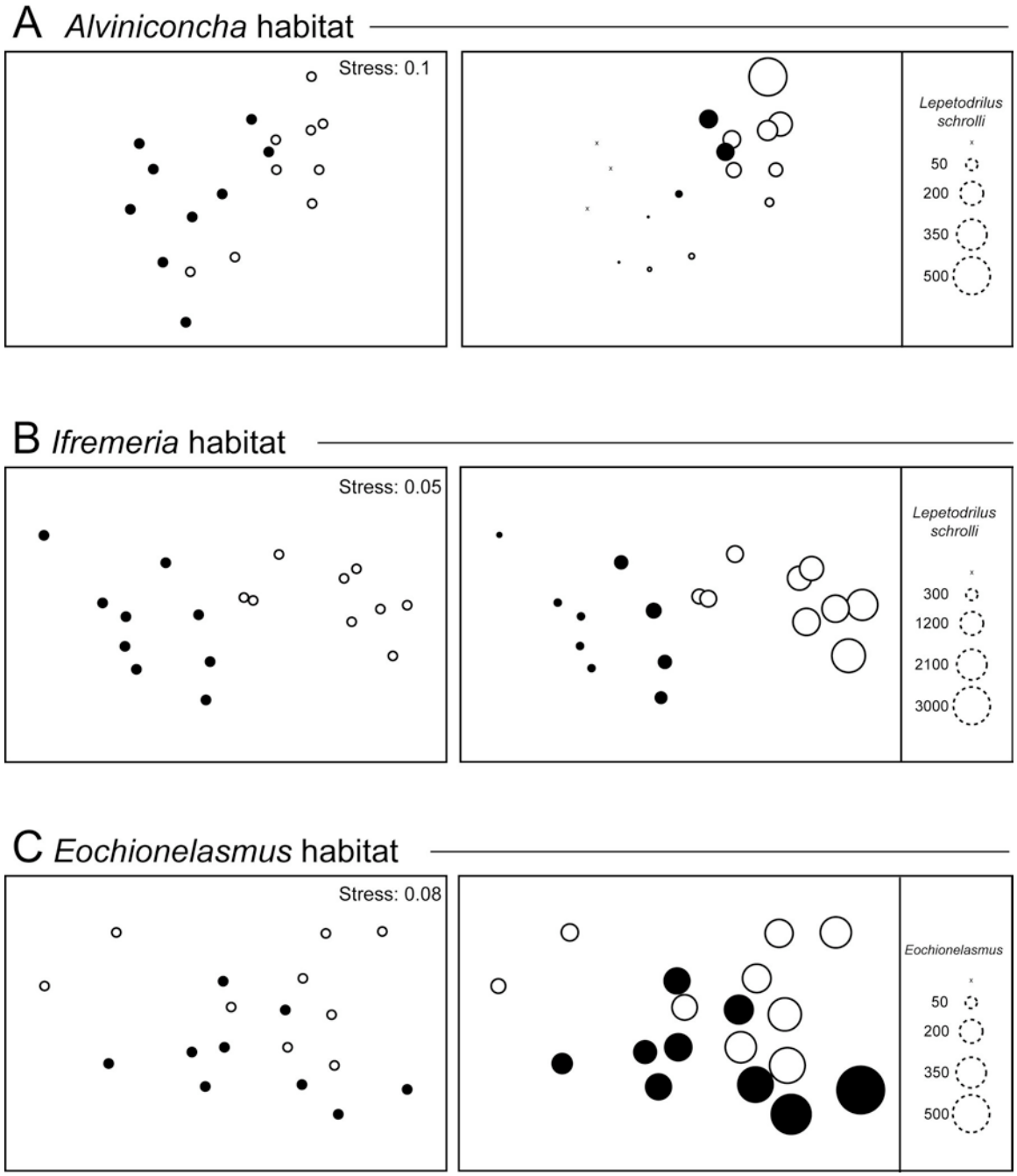

Figure 5.8. MDS analysis of species-abundance matrices by habitat (left-hand side) and bubble plots illustrating densities of the numerically dominant species for each habitat. Filled circles: Solwara 1; open circles: South Su.

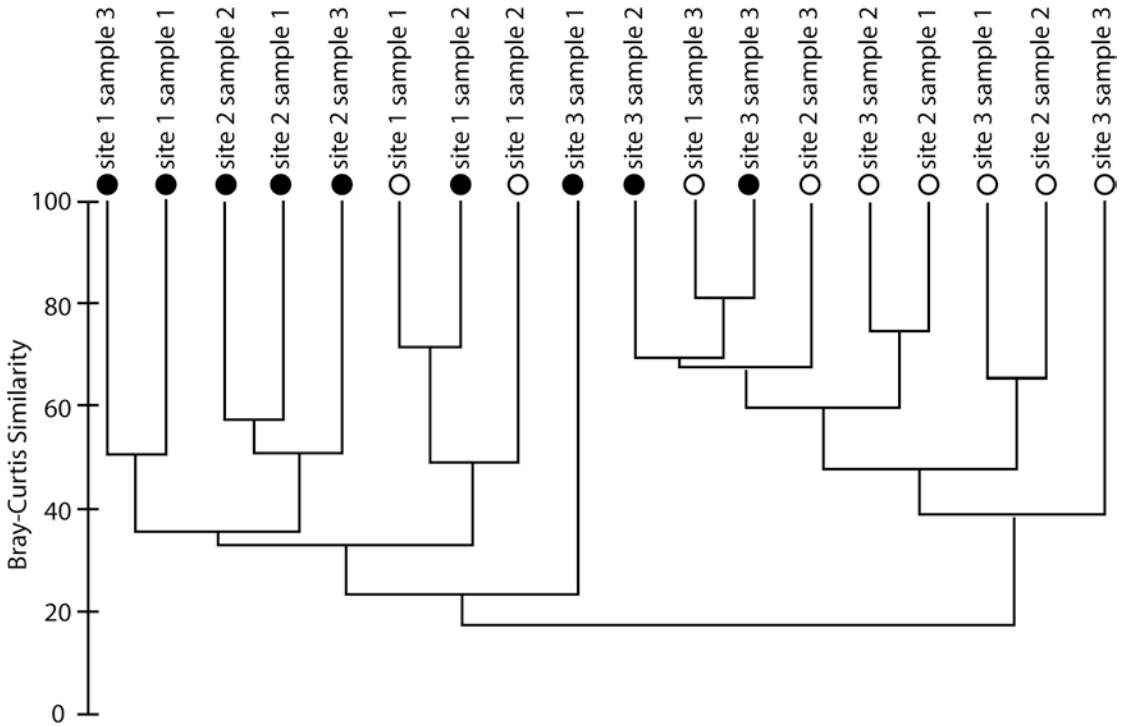

Figure 5.9. *Alviniconcha* habitats. Cluster analysis of Bray-Curtis similarities of species-abundance matrices (untransformed abundance data). Filled circles: Solwara 1; open circles: South Su.

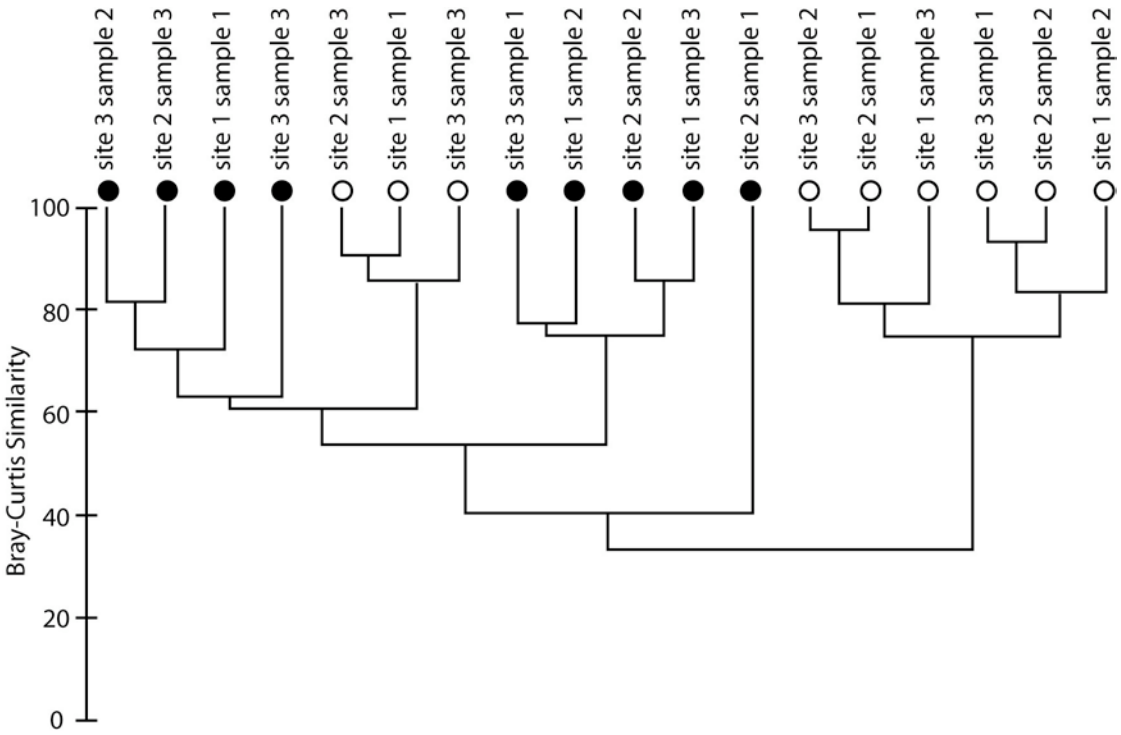

Figure 5.10. *Ifremeria* habitats. Cluster analysis of Bray-Curtis similarities of species-abundance matrices (untransformed abundance data). Filled circles: Solwara 1; open circles: South Su.

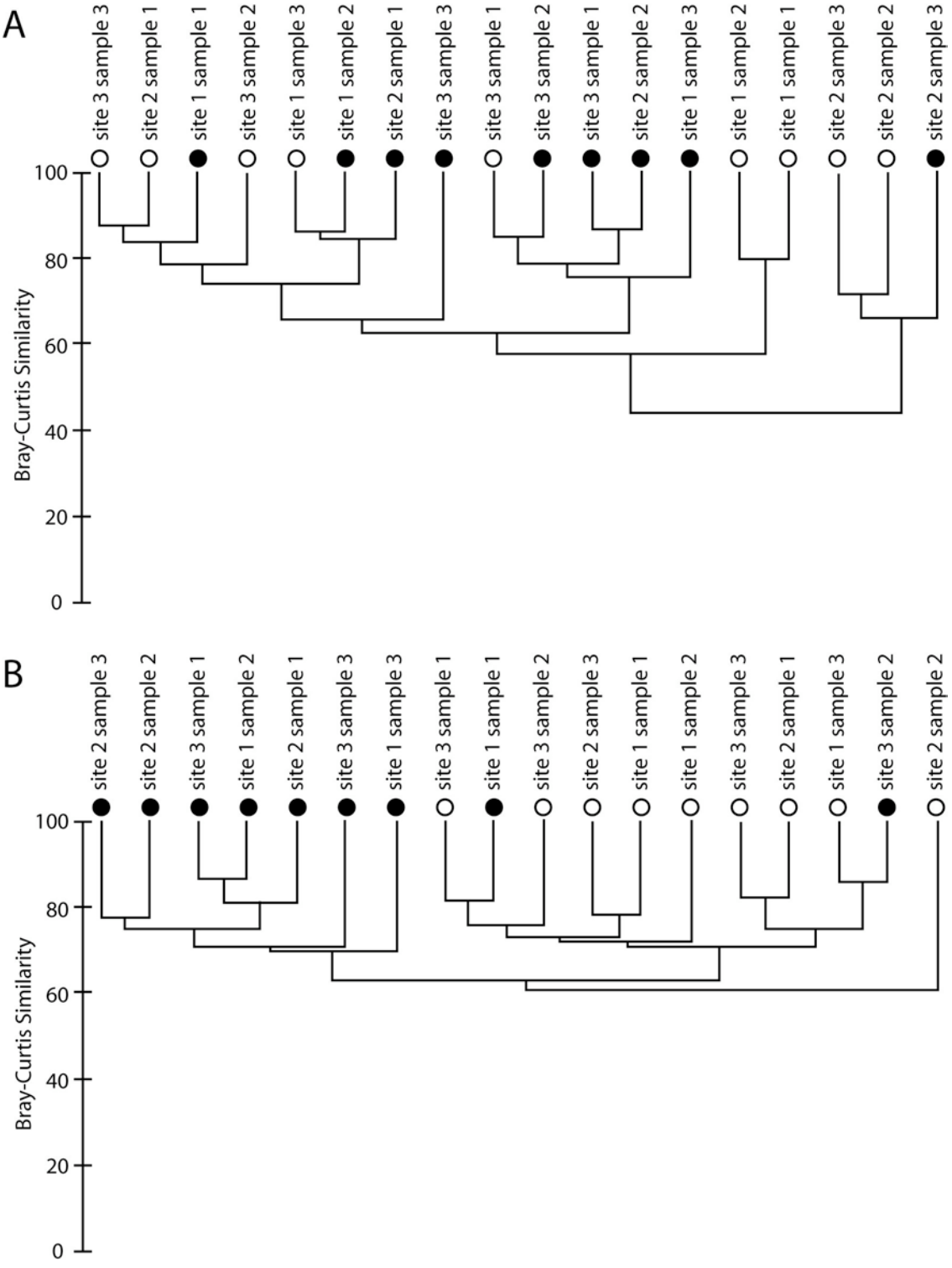

Figure 5.11. *Eochionelasmus* habitats. **A.** Cluster analysis of Bray-Curtis similarities of species-abundance matrices (untransformed abundance data). **B.** *Log (x + 1)* transformed abundance data. Filled circles: Solwara 1; open circles: South Su.

Chapter 7

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Appendix IA. Species-abundance (number of individuals sampled per 0.25 m^2) matrix for inactive sulfide habitats at Solwara I and South Su, Manus Basin. P: Present but not countable.

	Solwara 1																South Su											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
PORIFERA																												
Abyssocladia sp.	0	9	13	0	30	74	0	31	0	0	15	0	0	0	0	0	0	0	94	2	10	0	6	0	1	0	0	7
CNIDARIA																												
Hydrozoa sp. 1	P	P	P	0	0	0	0	P	0	P	0	0	P	0	0	P	0	0	0	P	P	0	P	0	0	0	P	P
Hydrozoa sp. 2	0	0	0	0	0	0	0	P	0	P	0	0	P	P	0	P	0	0	0	0	0	0	0	0	0	0	0	0
Hydrozoa sp. 3	0	0	0	0	P	0	0	0	0	0	P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrozoa sp. 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	P	0	0	0
Hydrozoa sp. 5	0	0	0	0	P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrozoa sp. 6	0	0	0	0	0	0	0	0	0	P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Keratoisis sp.	0	P	P	P	0	0	0	0	P	P	0	0	P	0	0	0	P	P	P	P	P	P	0	0	0	0	0	0
Actinaria sp. 1	0	0	0	0	0	0	P	0	0	0	0	0	0	0	P	0	0	0	0	0	0	0	P	0	0	0	0	0
Actinaria sp. 2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
"Keratoisis anemone"	0	P	0	P	0	0	0	0	P	P	0	0	0	P	0	0	P	P	0	0	0	P	P	0	0	0	0	0
Edwardsia sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
BRACHIOPODA																												
Brachiopoda sp.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
MOLLUSCA																												
Solengastres sp	0	0	0	0	0	0	0	0	0	6	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Gastropoda sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Gastropoda sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Gastropoda sp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Gastropoda sp. 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Gastropoda sp. 5	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gastropoda sp. 6	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Philine sp.	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diaphna sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Neomphalid n.gen.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Lepetodrilus sp.	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	1	12	21	0	8	0	0	0	2	0
Anatoma sp.	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Olgasolaris tollmani	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Shinkailepas spp.	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Eosipho juv.	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Alvinoconcha juv.	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Desbruyersia sp.	0	0	1	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Provanna sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Vitrinellidae sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	1	0
Nuculanoidea n.gen	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	5	0

Bivalvia sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Thyasiridae sp. 1	0	0	0	0	0	0	1	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	0
NEMERTEA																												
Nemeretean sp.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PLATYHELMINTHES																												
Plathyhelminthes sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
SIPUNCULA																												
Sipuncula sp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ANNELIDA																												
Bonelliidae sp	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Archinome sp.	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ophryotrocha sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Lumbrinereis sp.	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glyceridae sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Hesionidae spp.	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Nereis sp.	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Polynoidea (indet)	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Polynoidea sp.2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Branchipolynoe sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Sabellidae sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Maldanidae sp.	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Capitellidae sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Orbiniidae sp.	0	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Spionidae sp.	1	0	0	0	2	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0
Terebellidae sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	3	0
Amphisamytha sp.	0	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
ARTHROPODA																												
Lohmannellinae sp.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pycnogonida sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Copepoda	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	1
Harpactacoid copepod	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Scalpellomorph sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Scalpellomorph sp. 2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trianguloscalpellum michelottanum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Vulcanolepas parensis	0	0	0	0	29	23	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	5	0	0	0	0
Glyptelasma sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Poecilasma kaempferi	0	2	0	0	0	0	0	0	0	4	0	0	0	2	0	0	3	2	0	0	1	0	0	0	0	0	0	0
Altiverruca sp.	0	1	15	0	3	0	0	0	2	1	0	0	3	0	0	1	0	0	0	0	3	0	0	0	00	0	0	9
Eurodella sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
Tanaidacea sp.	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1

Isopod sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1
Isopod sp. 2	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Isopod sp. 3	0	0	0	0	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Isopod sp. 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Amphipod indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Amphipod sp. 1	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	5
Amphipod sp. 2	0	0	2	0	1	0	0	8	0	1	1	0	1	9	0	0	0	0	2	10	0	0	0	0	0	0	25	5
Amphipod sp. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Amphipod sp. 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Amphipod sp. 5	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amphipod sp. 6	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lysiannasidae sp. 1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lysiannasidae sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mysidacea	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nematocarcinus sp.	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Alvinocarididae sp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Opaepele n. sp.	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anomura sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Munida magniantennulata	0	0	1	0	0	0	0	0	0	1	0	0	0	2	0		0	0	0	0	0	0	0	0	1	0	0	1
Munidopsis lauensis	0	0	0	0	0	0	1	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	18	0	0	0	0	0
ECHINODERMATA																												
Ophiura sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Ophiura sp. 2	0	0	0	0	0	0	0	0	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Crinoidea sp.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Holothurian sp.	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix IB. Species-abundance (number of individuals sampled per 0.25 m^2) matrix for *Alviniconcha* habitats.

Mound	**Solwara 1**									**South Su**								
Site	**Site 1**			**Site 2**			**Site 3**			**Site 1**			**Site 2**			**Site 3**		
Replicate	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
CNIDARIA																		
Hydrozoa sp.	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
MOLLUSCA																		
Gastropod sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Lepetodrilus schrolli	15	0	2	0	1	0	0	102	92	3	7	116	154	29	75	53	183	423
Neoamphalid n.gen	0	0	0	0	0	0	0	0	0	0	0	0	8	4	4	17	5	20
Olgasolaris tollmanni	5	0	1	19	10	27	0	40	3	0	0	8	3	1	4	3	7	26
Shinkailepas tufari	1	0	0	0	0	0	1	0	2	0	0	0	0	0	0	15	1	0
Alviniconcha spp.	40	12	35	25	14	12	7	13	26	9	9	18	8	6	8	10	12	16
Ifremeria nautilei	2	0	0	10	1	3	0	9	1	0	0	1	1	1	3	1	0	7
Provanna n. sp.	0	0	0	0	0	0	1	0	0	0	0	5	0	1	0	0	0	0
Nuculoidea sp.	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Bathymodolis manusensis	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
NEMERTEA																		
Nemertean sp.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
ANNELIDA																		
Polychaete sp. 1	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0
Nereis sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Branchinotogluma segonzaci	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Branchinotogluma trifurcus	1	2	0	0	0	2	0	1	0	0	0	0	0	0	3	0	0	8
Spionidae sp.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Prionspio sp.	0	0	0	0	0	5	0	0	0	0	0	0	1	0	0	0	0	0
Spiochaetopteris sp.	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Paralvinella sp.	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
Paralvinella juv	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Paralvinella unidentata	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
Paralvinella fijiensis	2	0	0	0	0	0	13	0	2	0	0	0	0	0	0	0	0	1
Amphisamytha galapagensis	0	0	0	0	0	0	0	6	2	0	0	0	13	0	0	0	0	4
ARTHROPODA																		
Copepod sp.	0	0	30	0	0	0	0	0	0	0	8	1	0	0	0	0	0	26
Eochionelasmus ohtai	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Tanaidacea sp.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Isopod (indet)	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Amphipod sp.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Opaepele n. sp.	14	1	0	0	6	0	1	0	28	1	9	22	32	47	5	74	117	119
Munidopsis lauensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Austinograea alayseae	0	0	0	1	0	0	0	0	1	0	0	1	2	0	0	1	1	1

Appendix IC. Species-abundance (number of individuals sampled per 0.25 m^2) matrix for *Ifremeria* habitats.

Mound	**Solwara 1**																	
Site	**Site 1**			**Site 2**			**Site 3**			**Site 1**			**Site 2**			**Site 3**		
Replicate	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
PORIFERA																		
Abyssocladia sp.	0	0	0	0	0	0	1	0	0	0	6	0	0	0	0	0	0	0
CNIDARIA																		
Hydrozoa sp.	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Keratoisis sp.	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
MOLLUSCA																		
Bathyacmaea jonassoni	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Lepetodrilus schrolli	276	97	124	45	102	308	103	312	219	490	2316	1462	1143	1733	555	1924	1139	598
Neomphalid n. gen.	0	0	0	0	0	0	0	0	0	0	78	35	0	0	1	4	33	15
Olgasolaris tollmanni	429	114	226	43	191	254	118	136	41	109	218	152	12	86	138	38	51	54
Shinkailepas tufari	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Puncturella sp.	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Alviniconcha spp.	14	7	0	0	2	6	0	1	0	0	0	0	0	1	0	0	0	0
Ifremeria nautilei	74	28	41	15	11	21	16	14	10	19	18	20	8	18	9	18	13	8
Nuculoidea sp.	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ANNELIDA																		
Hesionidae sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Branchinotogluma sp.	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0
Branchinotogluma segonzaci	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Branchinotogluma trifurcus	57	8	19	8	18	27	39	43	33	18	22	18	6	2	23	11	5	4
Thermopolynoe branchiata	0	0	0	0	0	0	0	0	0	2	1	3	0	0	1	0	0	0
Prionspio sp.	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
Paralvinella fijiensis	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
Amphisamytha galapagensis	41	23	30	26	57	105	72	78	58	26	32	71	42	39	22	29	53	13
ARTHROPODA																		
Eochionelasmus ohtai	0	1	0	0	0	0	1	0	0	0	0	25	0	0	0	0	0	0
Amphipod sp.	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
Alvinocaris sp.	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0
Opaepele sp.	27	1	3	0	0	89	40	9	0	21	25	65	10	8	2	4	25	13
Munidopsis lauensis	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	1	3	0
Austinograea alayseae	0	0	0	0	0	0	1	1	0	1	1	6	0	3	0	2	6	0

Appendix ID. Species-abundance (number of individuals sampled per 0.25 m^2) matrix for *Eochionelasmus* habitats.

Mound	**Solwara 1**																	
Site	**Site 1**			**Site 2**			**Site 3**			**Site 1**			**Site 2**			**Site 3**		
Replicate	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
MOLLUSCA																		
Lepetodrilus schrolli	48	4	9	1	0	0	5	45	61	183	287	30	90	5	29	37	26	99
Shinkailepas tufari	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
Provanna sp.	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Bathymodiolus manusensis	0	0	0	0	0	0	0	0	0	0	0	0	3	2	2	5	3	1
ANNELIDA																		
Polychaete sp. 1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
Polychaete sp. 2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Polychaete sp. 3	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Archinome rosacea	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nereis sp.	5	1	18	5	0	1	4	0	1	0	1	0	0	0	0	4	1	0
Branchinotogluma sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Branchinotogluma trifurcus	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0
Branchipolynoe sp.	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	1	0
Prionspio n. sp.	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Paralvinella fijiensis	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Ampharetidae sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2	0
Amphisamytha galapagensis	5	4	43	7	2	19	9	0	8	8	37	0	3	9	21	17	35	2
Terebellidae sp.	0	1	11	0	0	0	0	0	2	0	0	0	0	1	0	0	0	0
ARTHROPODA																		
Ostracod sp.	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Copepod sp.	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Eochionelasmus ohtai	191	246	140	328	129	82	151	127	442	164	203	286	236	53	61	144	218	180
Ashinkailepas sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Vulcanolepas sp.	3	11	7	14	8	20	11	2	40	0	4	3	3	4	0	2	2	3
Amphipod sp.	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

Appendix II. Comparison of species lists for Manus Basin deep-water sites.

	† Observed/ sampled *** qualitative "Inactive"** **□ quantitative "Active"** **• qualitative "Active"**		**† observed only** *** inactive sample sites** **• active and inactive sites**	*** DESMOS only** ***† Both Sites**	***Active sites** **† Inactive sites**
Citation	**This Study**		**Desbruyeres et al. (2006b)**	**Erickson (2007)**	**Hashimoto et al. (1999)**	**Galkin (1997)**
Location	**SW 1**	**SS**	**Manus Basin**	**Solwara 1**	**PACMANUS/DESMOS**	**Manus Basin**
PORIFERA						
Abyssocladia sp.	* □	* □		*Abyssocladia dominalba**		
				*Cladorhiza abyssicola**		
						Hyalonema sp.
CNIDARIA						
Hydrozoa sp. 1	* □	*		*Acryptolaria* sp.*		
Hydrozoa sp. 2	*			Halecium n. sp. 1 *		
Hydrozoa sp. 3	*			*Stegolaria geniculata**		
Hydrozoa sp. 4		*		Sertularella n. sp.*		
Hydrozoa sp. 5	*					
Hydrozoa sp. 6	*					
						Rhodaliidae†
Keratoisis sp.	*	* □		Keratoisis sp.*		
						Candidella sp.†
			Pacmanactis hashimotoi			Actinaria n. sp.*
Actinaria sp. 1	*	*		Anthozoa sp. 2	Anthozoa? Type-1	Actinaria sp. 1†
Actinaria sp. 2	*	*			Anthozoa? Type-2	Actinaria sp. 2†
Actinaria sp. 3	†	†			Anthozoa? Type-3	
Actinaria sp. 4	†				Anthozoa? Type-4*	
Actinaria sp. 5		†				
?Cerianthus sp. 1	†	†				
?Cerianthis sp. 2	†	†				
?Cerianthis sp. 3						
Alcyoncea sp.	†	†				
Actinoscyphiidae sp	†			*Actinosctyphia* cf. *saginata*† *		
Keratoisis anemone	*	*		*Keratoisis* Anthozoa sp. 1*		
Edwardsia sp.		*				
Tjalfiella tristoma	†					
BRACHIOPODA						
Brachiopoda sp. 1	*				Brachiopoda	

MOLLUSCA

Solengastres sp	*	*				
					Gastropoda type-1	
					Gastropoda type-2*	
					Gastropoda type-3	
					Gastropoda type-4	
					Gastropoda type-5	
					Gastropoda type-6	
					Gastropoda type-7	
					Gastropoda type-8	
					Gastropoda type-9	
					Gastropoda type-10	
					Gastropoda type-11*	
Gastropoda sp. 1		*				
Gastropoda sp. 2		*				
Gastropoda sp. 3		*				
Gastropoda sp. 4		*				
Gastropoda sp. 5	*					
Gastropoda sp. 6	*					
Adeuomphalus sp.		•				
Philine sp.	*					
Diaphna sp.		*				
Bathyacmaea jonassoni		□			*Bathyacmaea* sp. *†	
Lepetodrilus sp.	*	*				
Lepetodrilus schrolli	□	□	*Lepetodrilus schrolli*	*Lepetodrilus schrolli*	Lepetodrilidae type-1*†	
Lepetodrilus n. sp.		•			Lepetodrilidae type-2*†	
			Symmetromphalus hageni			
						Pyropeltidae ? n. sp.*
Neomphalid n. gen., n. sp.		* □			Unidentified limpet	
Anatoma sp.	* •	* •				
Olgasolaris tollmani	* □	* □	*Olgasolaris tollmani*	*Olgasolaris tollmanni*		
Shinkailepas sp	*	*			*Shinkailepas* sp	
Shinkaliepas sp. 1	*					
Shinkailepas sp. 2	*					
Shinkailepas tufari	□	□		*Shinkailepas tufari*		
Puncturella sp.		□				
Margarites sp.		•			*Margarites* sp.	
"Margarites sp.*" juv.*	•					

Eosipho juv.	*					
Eosipho sp.	•	•		*Eosipho desbruyeresi*	Buccinidae?	Buccinacea *
Alviniconcha juv.	*					
Alviniconcha sp. 1	□	□		*Alviniconcha* sp.	*Alviniconcha* sp.	*Alviniconcha hessleri**
Alviniconcha sp. 2	□	□				
Ifremeria nautilei	□	□	*Ifremeria nautilei*	*Ifremeria nautilei*	*Ifremeria* sp.	*Olgaconcha tufari**
			Desbruyersia marianaensis			
			Desbruyersia melanioides			
			Desbruyersia spinosa			
Desbruyeresia n. sp.	*•			*Desbruyeresia* n. sp.1		
			Provanna nassariaeformis			
Provanna n. sp.	□	* □		*Provanna* n. gen. sp. 1	*Provanna* sp.*†	
				Provanna buccinoides		
			Phymorhynchus starmeri			
Turridae sp.	•				*Phymorhynchus* sp.*†	Turridae sp.*
Vitrinellidae sp.		*				
Muricidae: Trophoninae		•				
Bivalvia sp. 2		*				
Nuculanoidea n. gen., n. sp.	* □	* □				
Thyasiridae sp. 1	*	* •			Thyasiridae gen. sp.	
Thyasiridae sp. 2	†	†				
					Calyptogena sp.*	
Conchocele (shells)		†			*Conchocele* sp.*	
			Sinepecten segonzaci			
					Unidentified bivalve*†	
Bathymodiolus? tangaroa		†				
Bathymodiolus manusensis		□			*Bathymodiolus* sp	
Benthoctopus	† •	†				
NEMERTEA						
Nemeretean sp.	* □					
PLATYHELMINTHES						
Platyhelminthes sp.	*					
SIPUNCULA						
Sipuncula sp.	*					

ANNELIDA						
Bonelliidae sp	* •	†				
Polychaete sp. 1	□	□				
Polychaete sp. 2		□				
Archinome sp.	* □			*Archinome roacea*		
Dorvillea sp		•				
Ophryotrocha sp.		*				
Lumbrinereis sp.	*					
Glyceridae sp	*					
Hesionidae spp	*	* •		*Hesiospina vestimentifera*	Hesionidae gen. sp.	
					Shinkai sp.*	
Mussel commensal					*Iheyomytilidicola* sp.	
Nereis sp.	* □	□		Nereidiae gen. sp.	Nereidiae gen. sp.*†	
					Protomystides sp.	
					Polynoidae type-1	
					Polynoidae type-2	
Polynoidea (indet)	*	* •			Harmothoinae gen. sp.	
Polynoidea sp.2	*					
				Lepidonotopodus sp.	*Lepidonotopodus* sp.	
Branchinotogluma sp.		□			*Branchinotogluma* sp.	
			Branchintogluma marianus			
Branchinotogluma segonzaci	□	□	*Branchinotogluma segonzaci*	*Branchinotogluminae* cf. *segonzaci*	*Opisthotrochopodus* sp.*†	
				Opisthotrochopodus cf. *segonzaci*		
Branchinotogluma trifurcus	□	□		*Branchinotogluminae* cf. *trifurcus*		
Thermopolynoe branchiata		□	*Thermopolynoe branchiata*	*Thermopolynoe* cf. *branchiata*		
Branchipolynoe sp		* □			*Branchipolynoe* sp	*Branchipolynoe* n. sp.*
						*Iphionella n. sp.**
Sabellidae sp.		*				
					Serpulidae gen. sp.*	
Osedax sp.	†					
Alaysia sp.		†				
Arcovestia ivanovi		†	*Arcovestia ivanovi*		*Arcovestia ivanovi*†	
					Escarpia sp.†	
Lamellibranchia sp.		†			*Lamellibranchia* sp.	Lamellibrachiidae?*
					Ridgeia sp.*†	
Maldanidae sp.	*	*		Maldanidae		

Nicomache sp		•		*Nichoache arwindissoni?*		
				Axiothella sp.		
Capatellidae sp.		*			Capatellidae sp.	
Orbiniidae sp.	*	*				
					Trochochaetidae gen. sp.	
Spionidae sp.	* □	*			Spionidae gen. sp.	
Prionspio sp.	□	□				
Spiochaetopteris sp.	□			Chaetopteridae		
Terebellida sp.		*				
Paravlinella sp.	†					
Paralvinella juv	□					
Paralvinella (indet)	□				*Paralvinella* sp.	
			Paralvinella hessleri	*Paralvinella hessleri*		*Paralvinella hessleri**
Paralvinella unidentata	□	□		*Paralvinella* cf. *unidentata*		
Paralvinella fijiensis	□	□		*Paralvinella fijiensis*	Ampharetidae gen. sp.	
Ampharetidae sp.	*	* □				
Amphisamytha galapagensis	□	□	*Amphisamytha galapagensis*	*Amphisamytha galapagensis*		*Amphisamytha galapagensis**
Terebellidae sp.	□	□				
Ploycirrus sp.		•				
ARTHROPODA						
*Lohmannellina*e sp.	*					
Pycnogonida sp.	*					
Collossendeis sp.	†	†				
Ostracod	□					
Copepoda	*	*				
Harpactacoid copepod	□	* □				
Parasitic copepod	•					
Eochionelasmus ohtai cf. *manusensis*	□	□	*Eochionelasmus ohtai*	*Eochionelasmus ohtai* cf. *manusensis*	*Eochionelasmus ohtai* cf. *manusensis*†*	*Eochionelasmus ohtai**
?Ashinkailepas sp.		□				
Scalpellomorph sp. 1		*		Scalpellomorph n. sp. *		
Scalpellomorph sp. 2	*					
Trianguloscalpellum michelottanum	*					
Vulcanolepas parensis	* □	* □		*Vulcanolepas* cf. *parensis*•	*Neolepas* sp. type-1	
				*Neolepas cf. zevinae**		
					Neolepas sp. type-2*	
Glyptelasma sp.		*		*Glyptelasma* sp.*		

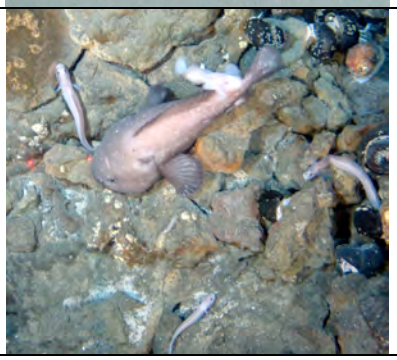
Poecilasma kaempferi	*	*		*Poecilasma* cf. *kaempferi**		
Altiverruca sp.	*	*		*Altiverruca* sp.*		
				*Neoverruca sp.**		
Eurodella sp.		*				
Tanaidacea sp	* □	*				
Isopod (indet)	□					
Isopod sp. 1	*	*				
Isopod sp. 2	*	*				
Isopod sp. 3	*					
Isopod sp. 4		*				
Munnopsidae	†	†				
Amphipod sp. (indet)	□	□				Amphipoda †
Amphipod indet (damaged)		*				
Amphipod sp. 1	*	*				
Amphipod sp. 2	*	*				
Amphipod sp. 3		*				
Amphipod sp. 4		*				
Amphipod sp. 5	*					
Amphipod sp. 6	*					
Lysiannasidae sp. 1	*					
Lysiannasidae sp.2	*					
Mysidacea	*					
Nematocarcinus n. sp.	*				*Nematocarcinus* sp.*	
Alvinocarididae sp.		*				
Shinkaicairis n. sp.						
Alvinocaris sp.	†	† □			*Alvinocaris* sp.*	
Opaepele n. sp.	* □	□				
Chorocaris n. sp.	* □	□	*Chorocaris vandoverae*	Chorocaris vandoverae•	*Rimicaris*	*Chorocaris vandoverae**
Lebbeus sp.1	†	†		*Lebbeus* n. sp.*		
Lebbeus sp.2	†	†				
Astacidea sp.		†				
Parapagurus richeri	†					
Anomura sp.		*				
Paralomis sp.	†	†		*Paralomis* cf. *spinosissima*† *		
Munida magniantennulata	* •	*				
Munidopsis lauensis	* •	* □	*Munidopsis marianica*	*Munidopsis lauensis* •	*Munidopsis lauensis**†	*Munidopsis* sp.*†

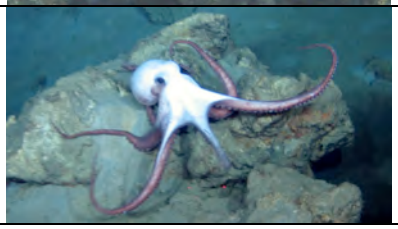
Munidopsis starmer	•	•		*Munidopsis starmer* •	*Munidopsis* sp.	
Austinograea alayseae	□	□	*Austinograea alayseae*	*Austinograea alaysae*	*Bythograeidae* gen. sp.	*Austinograea alayseae**
ECHINODERMATA						
						Henricia sp.†
					Echinoidea type-1	
					Echinoidea type-2	
Ophiura sp. 1		*		Ophiura sp.*	Ophiuroidea type-1	Ophiuroidea†
Ophiura sp. 2	*	*		Ophiacantha sp.*	Ophiuroidea type-2	
Brisingidae	*					*Freyella* sp.†
						Comatulida†
Holothurian sp.	*					
Enypniastes sp.	†			*Enypniastes* cf. *exemia*† *	Holothroidea type-1	Pelagothuridae†
			Chiridota hydrothermica	*Chiridota hydrothermica**	Holothroidea type-2	*Chiridota* n. sp.*
HEMICHORDATA						
Fimbrora ?calsubia		†				
Pyrosoma sp		†				
CHORDATA						
Rhinochimaera ?pacifica	†	†				
Hydrolagus ?trolli	†	†				
Hexatrygon ?bickelli	†	†				
Lepidion schmidti	†					
Pyrolycus manusanus	†	†	*Pyrolycus manusanus*	*Pyrolycus* cf. *manusanus* • †	Zoarcidae gen. sp.*†	
Psychrolutes marcidus	†	†				
Aphyonidae sp.	†	†				
Congridae sp.	†	†				
Ophidiidae sp.	†	†				
Halosauridae sp.	†	†				
Ogcocephalidae sp.		†				
TOTAL TAXA	**178**		**23**	**54**	**70**	**28**

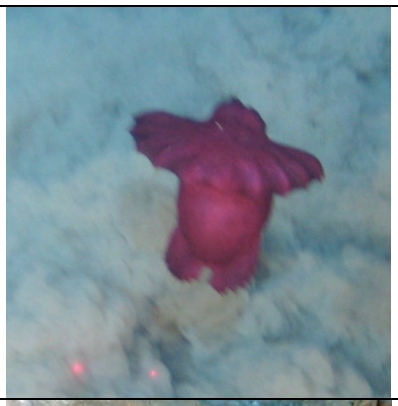
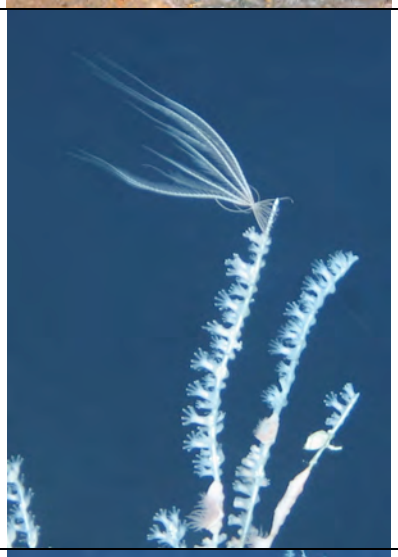
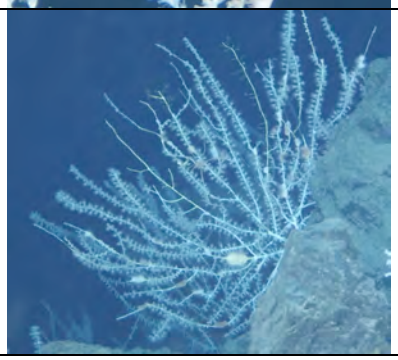
Appendix III

Photolog, Luk Luk Cruise

	*Hydrolagus ?trolli* (Pointy Nosed Blue Chimaera)
	*Hydrolagus ?trolli* from the front. Laser dots: 10cm.
	*Rhinochimaera ?pacifica* (Pacific Spookfish)
	*Lepidion ?schmidti* (Gray Hakling) Circumglobal deepwater continental shelf codfish. This fish was over 1 m in length.

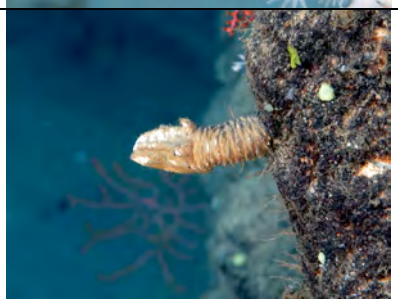
	*Hexatrygon ?bickelli* (Six-Gilled Stingray
	Congridae sp. (Conger Eel)
	Ophidiidae sp.
	*Psychrolutes marcidus* (center) (Fathead Fish or Blob Sculpin) surrounded by *Pyrolycus manusanus* (Eelpout) Laser dots: 10 cm

	Ogocephalidae sp. Laser dots: 10 cm
	Halosauridae sp. Rarely observed alive. Laser dots: 10 cm
	Aphyonidae sp. unidentified brittle star
	Actinopterygian sp.
	*Benthoctopus* sp.
	*Benthoctopus* sp. showing differing coloration in a larger individual

	*Enypniastes* sp. (Swimming Sea Cucumber) Laser dots: 10 cm.
	Crinoid on sediment, immediately prior to swimming off.
	Crinoid feather star positioned atop a stand of *Keratoisis* sp. coral.
	*Keratoisis* sp. Coral with epibiotic anemones and an unidentified anomuran crab visible.

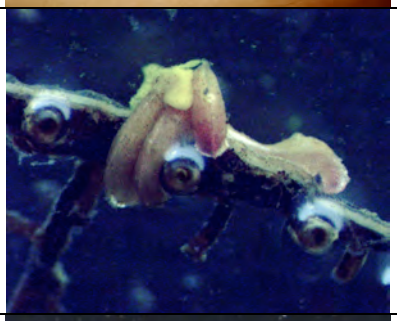
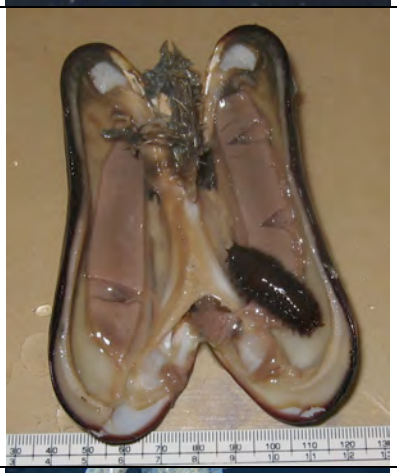
	*Actinoscyphiidae* sp. (Venus Flytrap Anemone)
	Actinaria sp. 1 (centre) with *Bathymodiolus manusensis* *Ifremeria nautilei* *Eochionelasmus ohtai* *Alvinocaris* sp. *Pyrolycus manusanus*
	Cerianthus sp. 1
	Cerianthus sp. 2 with a xenophyophore, a giant unicellular protozoan, to the right.

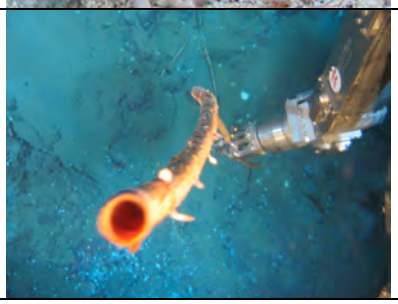
	*Alcyoncea* sp.
	*Alcyoncea* sp.
	Actinaria sp. 1
	*Parapagurus richeri* hermit crab with an unidentified anemone.

	Two *Poecilasma kaempferi* with unidentified hydroids on dead a *Keratoisis* sp. branch.
	*Triangulosalpellum ?michelottanum* (Solitary Massive Barnacle) The barnacle is about 10 cm long, with a diameter of 2.5 cm.
	*Vulcanolepas parenis*
	*Nematocarcinus* sp. shrimp (large individual) with assorted *Lebbeus* sp. (small red) and a single *Alvinocaris* sp. (white) shrimp. Mingled among them are *Munidopsis lauensis.*
	*Austinograea alaysea*

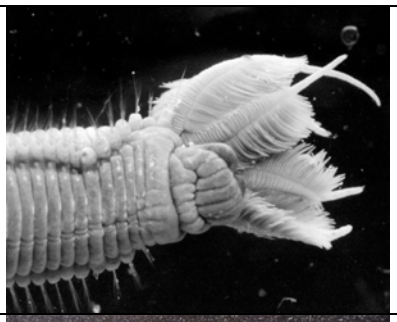
		*Tjlfiella* sp. (Benthic Ctenophore) Fishing tentacles retracted.
		Scattered *Conchocele* sp. shells. ~75 mm shell length.
		Nuculanoidea sp. (Nut Shell) Ubiquitous in sediment near vents (5 mm in length)
		*Neoamphalid* n. gen., n. sp. Lives on rocks adjacent to tubeworms *Alaysia* sp. and *Arcovestia ivanovi.*
		*Neoamphalid* n. gen., n. sp.

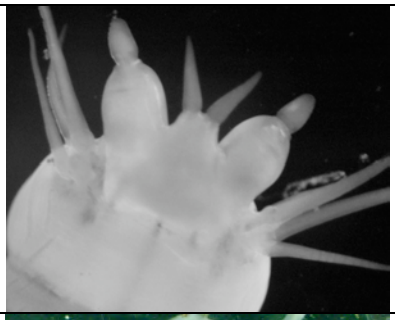
	*Neoamphalid* n. gen., n. sp.
	*Shinkailepas* sp. 1. Found on active vent chimneys.
	*Shinkailepas tufari*. Found on active vent chimneys.
	*Trophoninae* sp. Found away from the hot fluids on the vent periphery.
	*Ifremeria nautilei* (Black Snail)
	*Alviniconcha* sp. On a crust of *Neoamphalid* n. gen., n. sp. Laser dots: 10 cm.

	*Eosipho sp.* (Scavenging Gastropod) Occurs in areas of hot venting and in drifts of dead *Alviniconcha* and *Ifremeria* snails. Up to 70 mm in length.
	Solengasters sp. Entwined around a hydroid stem.
	*Anatoma* sp. A common gastropod found living among the hydrozoan mats in the vent periphery.
	*Bathymodiolus manusensis* *Branchipolynoe* sp. commensal polychaete
	*B. tangoroa*? Unlike *B. manusensis* these mussels were observed higher up on pillars.

	Scattered *B. manusensis* mussels in the sediment with a mixed stand of *Alaysia* sp. and *Arcovestia ivanovi*. Some *Munidopsis launensis* are also visible.
	*Paralomis* sp. Feeding on *Vulcanoleps parensis*. Some *Alaysia* sp. is also visible. Fiji/Lau *Paralomis* sp. are white. Laser dots: 10 cm.
	*Alaysia* sp. extracted from its tube.
	A single *Arcovestia ivanovi* in a warm current surrounded by neoamphalid limpets, *Opaepele* sp. shrimp, and *B. manusensis* mussels.
	*Lamellibranchia* sp. tube (2.7-m length; no animal) extracted from the sediment. *Vulcanolepas parensis* barnacles can be seen growing on the column.

		Tip of *Lamellibranchia* sp. tube from which a live worm was removed.
		*Osedax* sp. (Bone-Eating Worm) On quadruped (pig?) remains. First record of *Osedax* sp. in the southern hemisphere and on substratum other than whale bones.
		Gray hakling with numerous Bonnellidae echiuran proboscises visible across the substrate.
		Bonnellidae echiuran proboscis retracting, highlighting green coloration. The proboscis can extend 1 m over the sediment.

	*Paralvinella unidentata* (Palm Worm) Living in warm fluids, sometimes under *Alviniconcha* sp. aggregations. Up to 50 mm in length.
	*Paralvinella fijiensis* (Palm Worm) Distribution overlaps with that of *P. unidentata.*
	*Paralvinella* sp. (Palm Worm) In burrows where warm fluids diffused upward through the sediment.
	*Prionspio* sp., 15-mm long, living among *Paralvinella* spp.

	*Branchintogluma trifurcus* (Scale Worm) Free-living among *Ifremeria nautilei* and *Alviniconcha* sp. *R*arely exceednig 15 mm in length.
	*Branchinotogluma segonzaci* (Predatory Scale Worm) Up to 5 cm length, found especially around areas of hot fluid flow and bacterial matting.
	Close up of the head of *Nereis* sp. showing palps and antennae. A close relative of the coastal rag worm. Common away from the hot vent fluids.
	Terebellidae sp. A substantial polychaete (>10 cm length) living in tough, parchment-like tubes among *Eochionelasmus ohtai* barnacles.
	*Amphisamytha galapagensis* (15mm) with a large copepod parasite attached to its rear. This worm was found everywhere, attached to *Ifremeria nautilei*, among *E. ohtai,* and among hydrozoan matting.

		A small *Pyrosoma* sp., a colonial pelagic tunicate, photographed near the vents. This individual was about 10 cm in width.
		*Collessendeis* sea spider with a leg span in excess of 30 cm.
		Hydrozoan matting with a few *Abyssocladia* sp. sponges.

Appendix 5

Macroinfauna of Active and Inactive Hydrothermal Sediments from Solwara 1 and South Su, Manus Basin, Papua New Guinea

Macroinfauna of Active and Inactive Hydrothermal Sediments From Solwara and South Su, Manus Basin, Papua New Guinea

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Contents

List of Tables

List of Figures

List of Appendices

INTRODUCTION

Deep sea floor sediments are known to host a wide diversity of infaunal invertebrates (Rex 1983). Any new region in the deep sea, not sampled previously is certain to yield novel species that are undescribed. Because the Papua New Guinea region has not been subject to broad benthic surveys of sediment communities, it is not possible to determine whether the fauna at South Su and Solwara sites are limited to those local sites, are characteristic only of hydrothermal sediments, or are more broadly distributed over a larger area or range of habitats.

Most hydrothermal systems have substrates that are exposed rock (volcanic or precipitated mineral deposits) with little or no sediment cover. Yet hydrothermal areas where venting occurs through thick sediment layers represent distinctive habitats (hot muds) hosting unique infaunal organisms (Petrecca and Grassle 1990; Grassle & Petrecca 1994, Junniper and Tunnicliffe 1997). Hydrothermal activity in ridge-crest environments is characterized by low sedimentation rates involving the chemical interaction of seawater with basalts, whereas in regions of high sedimentation, hydrothermal fluids also react with sediments that blanket the seafloor, yielding distinct hydrothermal fluid composition and environmental conditions. These conditions likely promote microbial activity and generate an enhanced food supply for fauna relative to background (ambient) sediments. Thus, they potentially may support a specialized fauna.

The task. Sampling was conducted to examine macrofaunal invertebrate assemblages (>0.3 mm) in active and inactive hydrothermal sediments near sulfide precipitates at 2 locations in Papua New Guinea: Solwara I (3^{o} 47’ 18.7” S 152^{o} 5’ 38.2” E; 1504 m -1634 m) and South Su (3^{o} 48’ 33.2” S 152^{o} 6’ 16.4’E; 1312 m – 1430 m) (Fig. 1A). Solwara I is in the Bismark Sea about 50 km N of Rabaul. South Su is about 20 km SE of Solwara I. Active/inactive status was determined by proximity to actively venting sulfide chimeys. The goal was to characterize the infaunal macrobenthic community with respect to abundance (density), biomass, species composition, diversity and similarity among locations. Information about distributions, lifestyles or taxon features that could influence resilience or potential to recover from mining disturbance was also sought.

Fig. 1 A. Location of Solwara 1 and South Su sites in the Bismarck Sea, eastern Manus Basin, Papua New Guinea.

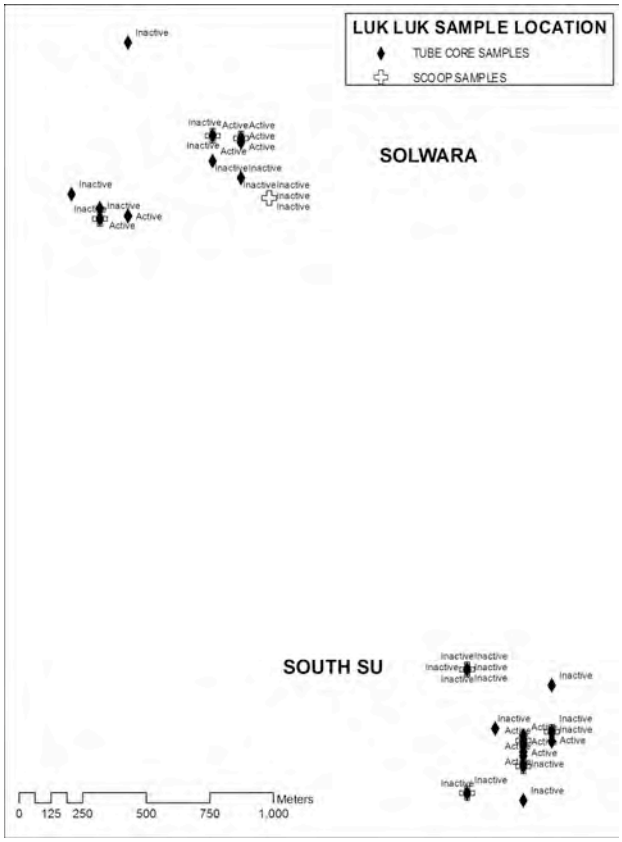


Fig. 1B. Relative position of active and inactive core (diamond) and scoop (cross) samples taken from Solwara and South Su sites.

METHODS

Sampling. Samples were collected on board the Wave Mercury during March and April 2007. Hydrothermal sediments were sampled using a Triton series ROV in tube cores 7 cm in diameter (38.5 cm^2 surface area). Thirty-five tube cores were collected (Table 1). When possible, cores were sectioned vertically at intervals 0-1, 1-2, 2-3, 3-5 and 5-10 cm to examine vertical distribution of fauna within the sediment column. However, core depth in the sediment varied from 3 to 10 cm, so not all fractions were available for all cores. Since no animals were recovered below 5 cm, and 94% of all fauna were present in the top 3 cm, core depth was not considered a significant factor in this analysis and all cores were used for determination of density, biomass and composition. However, only tube cores that recovered sediments to at least 5 cm were included in the quantitative analysis of macrofaunal vertical distribution. In addition to tube cores, 16 scoop samples were collected to provide additional material for faunal characterization, 2 from each of the active sites and 6 from each of the inactive sites (Table 2). These collected near-surface sediments from a larger area of sediment than tube cores. Relative locations of cores and scoops from active and inactive sediments at the Solwara and South Su areas are shown in Figure 1b.

Sample processing. On board ship tube core samples were preserved unsieved (0-10 cm) and the fraction > 10 cm was sieved on a 0.3 mm mesh with filtered seawater. All scoop samples were sieved on a 0.3 mm mesh prior to preservation. All samples were preserved in 8% buffered formalin and seawater. In the laboratory at Scripps samples were resieved on a 0.3 mm mesh and invertebrates were sorted from retained material under a dissecting scope at 12x magnification. Animals were counted, identified, then weighed wet (having been in dilute formalin) on an analytical balance to obtain biomass. Each taxon was weighed separately. No attempt was made to remove shells or hard parts. Photographs were taken of dominant species. In the case of the tanaids, bivalves and isopods, these were forwarded to systematists along with specimens.

A non-parametric Kruskall-Wallis test followed by an *a posteriori* Student's t-test was performed to evaluate differences in macrofaunal density and biomass among sampling sites, as data did not follow a normal distribution. Multivariate analyses of community similarity and dissimilarity between the two sites and between active and inactive sites were conducted using PRIMER Software (v.6.2). Specifically the statistical routines MDS, ANOSIM and SIMPER were employed. Macrofauna diversity analysis involved determining species richness (S) and Shannon's diversity index (*H'*) for logs at the base 10.

RESULTS

Tube core analysis

Densities. Faunal densities were extremely low for the bathyal depths studied (1300-1600 m). Only 220 individuals belonging to 15 species were collected in 35 tube cores taken in this study. Densities (individuals/m^2) were 935, 445, 3,740 and 909 at Solwara active,

Solwara inactive, South Su active and South Su inactive sites respectively (Table 3). South Su active sediment had higher macrofaunal densities than the other 3 sites, which were not significantly different from one another (Kruskall-Wallis chi sq. (3 df) = 11.94; P=0.01). Within each study area, the active site densities were higher than the inactive site densities, by a factor of 2 at Solwara and by a factor of 4 at South Su; however, this difference between active and inactive site densities was significant only at Solwara,

Biomass. Wet weight in the 4 study areas ranged from 0.25 to 50.53 g/m^2 (Table 3). In contrast to density, the Solwara active biomass (0.25 g/m^2) was lower than at the inactive site (1.07 g/m^2), though this difference was not statistically significant. The South Su active site (50.53 g/m^2) had biomass nearly 100 times higher than the inactive site (0.52g/m^2). South Su active macrofaunal biomass was significantly higher than that observed at the 3 other sites (Kruskall-Wallis Chi sq. (df 3) = 15.2; P=0.002). Biomass dominants were the tanaids *Paraleptognathia* sp. and *Pseudotanais sp.* (41% of total) in active Solwara sediments, a sigalionid polychaete (81%) in Solwara inactive sediments, *Prionospio* (*Minuspio*) sp. in active South Su sediments, and a nuculanoid bivalve (52%) inSouth Su inactive sediments. At most of the sites only a few species comprised most of the biomass in tubecores.

Composition. A full listing of tube core fauna is given in Appendix I, and summarized in Table 3. Tanaids and nuculanoid bivalves were the dominant taxa at both Solwara sites and at the South Su inactive site. The tanaids belong to 2 species: nr *Paraleptognathia* sp. (Family Anarthruridae, Subfamily Akanthophoreinae) and *Pseudotanais* sp. (Family Pseudotanaidae). The spionid polychaete *Prionospio (Minuspio)* sp. was the dominant taxon at the active South Su site, comprising 77% of the total number of individuals. Other taxa present included polychaetes from 7 families, peracarid crustaceans (gammarid amphipods, cumaceans, isopods), and limpets (*Lepetodrilus*) (Table 3). Although they were not counted as macrofauna, a single species of allogromid (protozoan – soft-bodied foraminiferan) was also very abundant in active South Su hydrothermal sediments. Despite overlap in major taxa, the South Su and Solwara communities were different. Of the 9 species collected at Solwara and the 10 species at South Su, only 4 species were collected in common (the tanaid *Paraleptognathia* sp., the nuculanoid bivalve, *Cossura* sp. and an isopod). Even within a site there were only 2-3 species in common between the active and inactive sites.

Assemblage composition differed between active and inactive sites (ANOSIM: Solwara P=0.03, SIMPER 81% dissimilarity; South Su P=0.004; SIMPER 71% dissimilarity), and between the two active sites (ANOSIM: Solwara vs South Su P=0.001; SIMPER 85% dissimilarity) but not between the two inactive sites (ANOSIM: Solwara vs South Su P=0.30). Difference in densities of tanaids, nuculanoid bivalves and *Prionospio* sp. accounted for most of the among-site differences. Within a site, among-core homogeneity was greatest at the South Su active sediments (49% similarity) and least in Solwara inactive sediments (6% similarity) (Fig. 2) (see Appendix II for ANOSIM and SIMPER results).

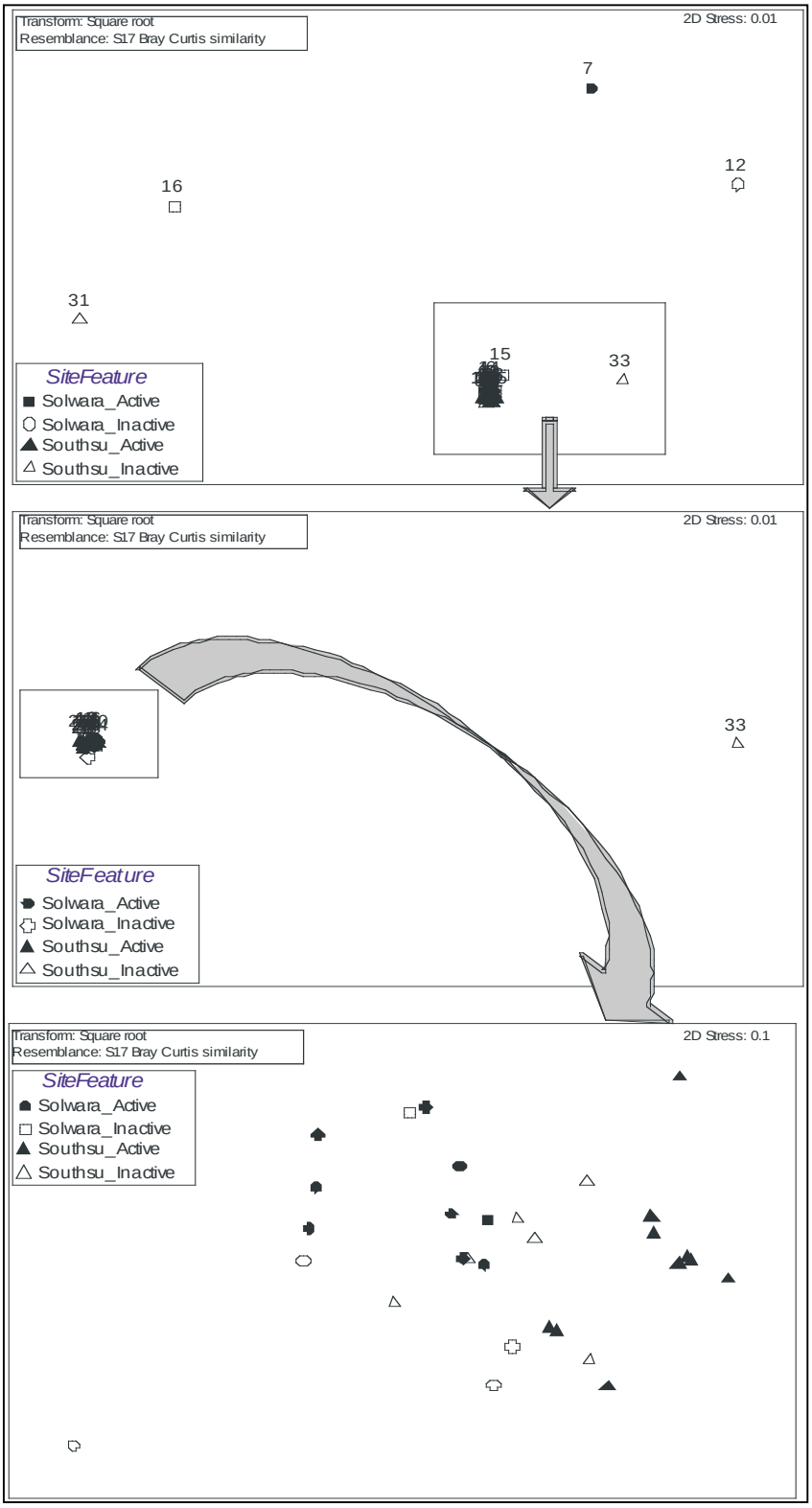


Figure 2. MDS Plot of macrofaunal composition in Solwara and South Su active (solid symbols) and inactive sediments (open symbols).

Vertical Distribution. Most of the fauna was collected in the top 2 cm of the sediment column; this proportion was 100%, 82%, 83% and 89% at the Solwara active, Solwara Inactive, South Su active and South Su inactive sites, respectively. In general macrofauna had deeper dwelling depths in the inactive than active Solwara sediments, and in the active than inactive South Su sediments (Fig. 3).

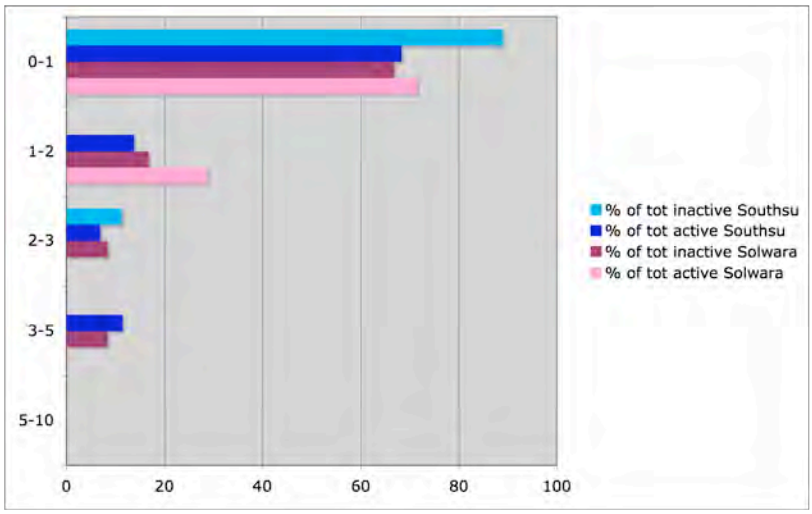


Fig.3. Vertical distribution of macrofauna in hydrothermal sediments at Solwara and South Su active and inactive sites.

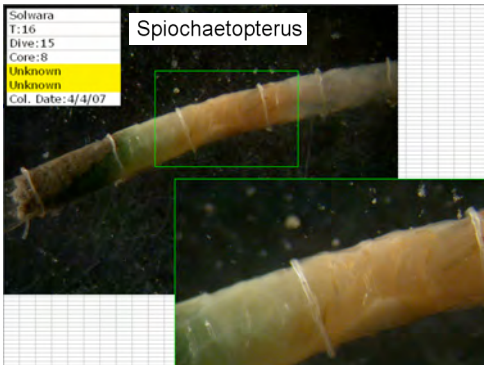
Diversity. Species richness was greatest at the Solwara active and South Su inactive sites. Species richness per core was 2.50 and 2.13, and H' was 0.35 and 0.31 respectively at these sites. In contrast species richness per core was only 1.0 and H' was 0.09 at Solwara inactive, with intermediate values (S = 1.90, H' = 0.17) at the South Su active site. Rank 1 dominance, a parameter inversely correlated with diversity, was highest at South Su active sediments (*Prionospio (Minuspio)* sp. 77%), and at Solwara inactive sediments (*Paraleptognathia* sp. 58%), and slightly lower (but still high) in South Su inactive sediments (nuculanoid bivalves 43%) and active Solwara sediments (*Paraleptognathia* sp. 36%).

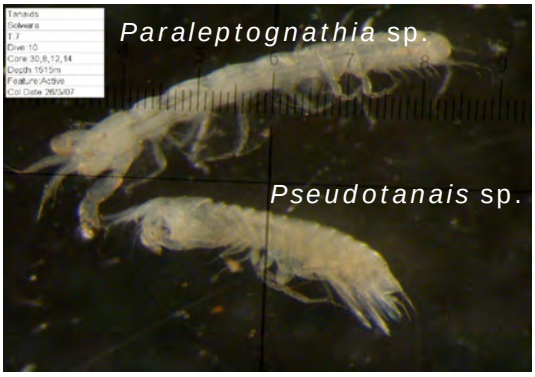
Feeding modes. Despite the small number of species present, a diversity of feeding modes are represented in the hydrothermal sediments examined. The polychaetes include subsurface-deposit feeders (*Heteromastus* sp. and Lumbrineridae), surface-deposit feeders (*Prionospio* and *Cossura*), and carnivores (Sigalionidae, Nereididae and Glyceridae). Most of the crustaceans are probably omnivorous (detritivores and scavengers), the limpet is a grazer, and the nuculanoid is a deposit feeder. Images of dominant and unique taxa are presented in Fig.4.

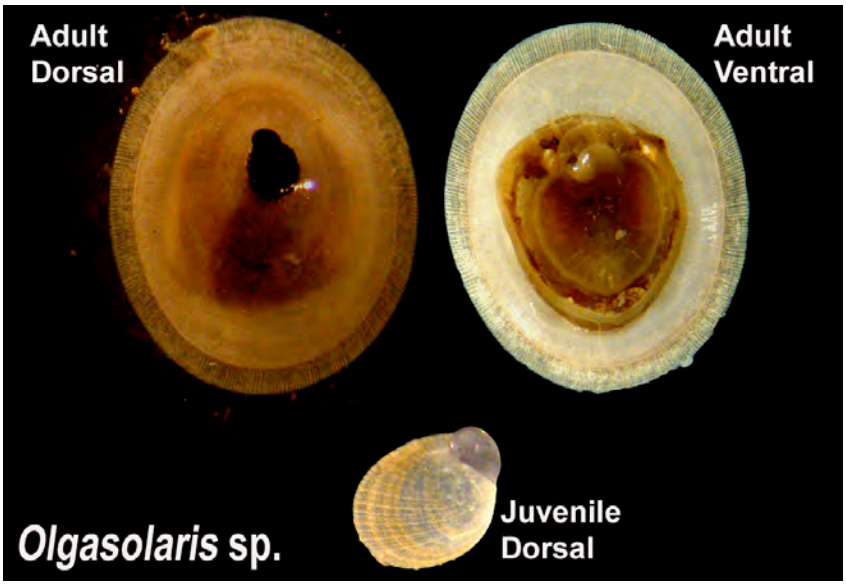
Scoop Samples.

Scoop samples, which are non quantitative, contained a total of 664 individuals belonging to more than 31 taxa (some groups have not yet been identified to species) (Table 4). A full listing of fauna in each scoop sample is given in Appendix 2. Thirty-

Figure 4. Dominant macrofaunal taxa collected at Solwara and SouthSu sites.





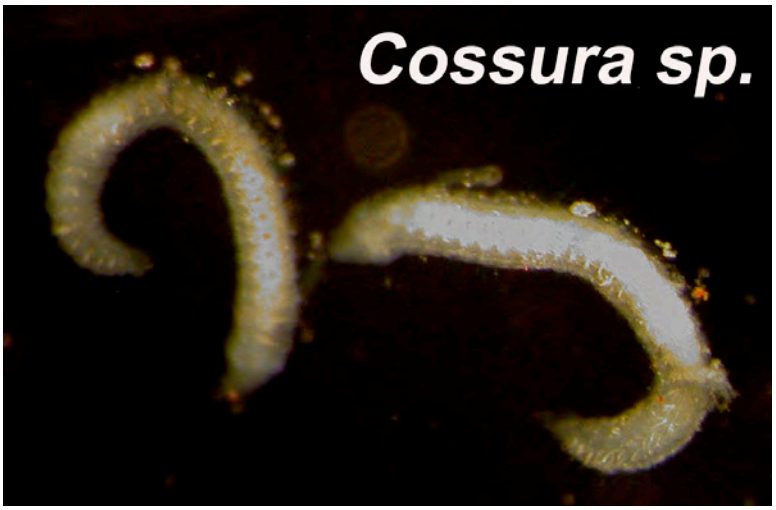

Adult
Dorsal
Adult
Ventral
Juvenile
Dorsal
Olgasolaris sp.

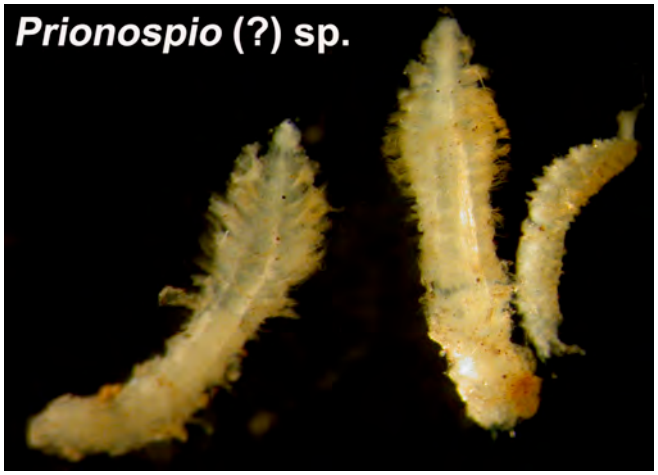

Lepetodrilus sp.


Alvinocarid


Nicomache sp.


Heteromastus sp.

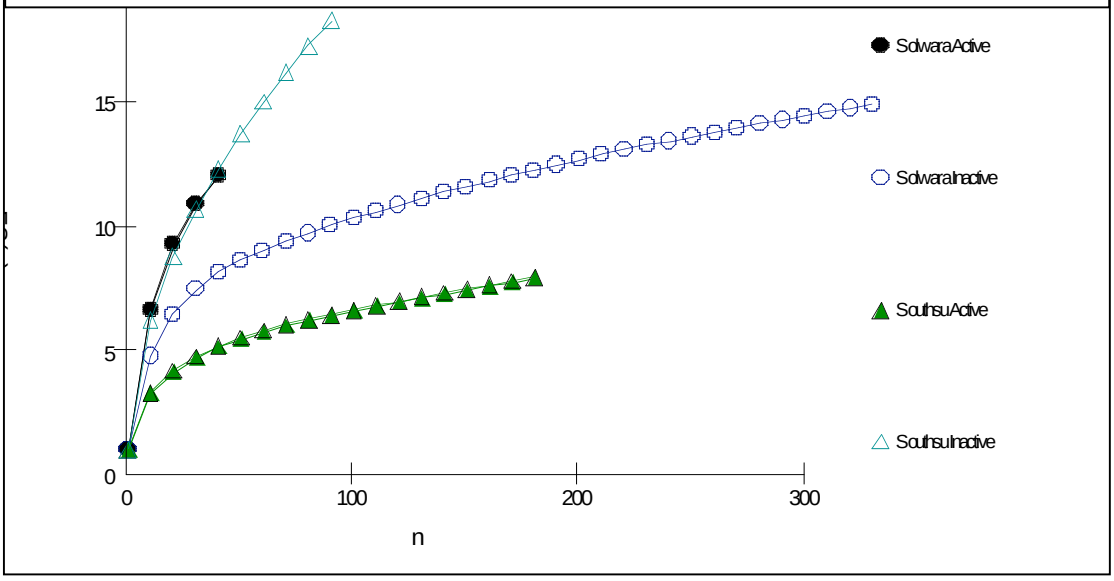

Cossura sp.


Prionospio (?) sp.

seven percent of the individuals collected were tanaids (*Paraleptognathia* sp. and *Pseudotanais* sp.) and 24% were nuculanoid bivalves (nr *Pseudomalletia dunkeri* Smith, *1885*) (Table 4). In general, dominant taxa in scoops (Table 4) resembled those in tube cores (Table 3). Tanaids were the most abundant group at the Solwara sites (2 species) and nuculanoids (1 sp.) were most abundant at South Su. Isopods belonged to 3 species in 3 families: *Notoxenoides* sp. (Paramunnidae), *Janirella* sp. (Janirellidae), and *Ilyarachna* sp. (Munopsidae). All 3 are new to science.

Rarefaction analyses conducted on pooled scoop samples revealed greatest (and similar) diversity (Es_{41}=12) at Solwara active and South Su inactive sediments, intermediate diversity at the Solwara inactive site (Es_{41}=8.11), and lowest diversity at South Su active sites (Es_{41}=5.15) (Fig. 5). Tube cores revealed similar diversity patterns (not shown); South Su active sediments had lowest diversity, with Es_{41} =2.85.

Fig. 5. Rarefaction curves illustrating diversity for macrofauna collected in scoop samples from Solwara and South Su active and inactive hydothermal sediments.



In scoop samples the majority of the most common species were deposit feeders (Table 5). These include *Heteromastus* sp., *Prionospio* sp., *Cossura* sp., the nuculanoid bivalve, and probably the tanaids. It is not unrealistic to think that the most numerous taxa are taking advantage of enhanced chemosynthetic bacterial biomass within sediments, but there are no data to support this idea at present.

Hydrothermal Sediment Observations.

ROV still images were examined to provide information about the sedimentary habitats present in macrofaunal sampling areas. Photographs revealed a mosaic of habitat patches comprising the seafloor sediments in the vicinity of Solwara and South Su (Appendix 3). In addition selected cores from each location and activity stage were observed under a dissecting microscope for detailed sediment observations and photography (Table. 6). Observations are summarize below.

Solwara active.

Surface sediments often appeared orange or tan, with subsurface sediments being grey or lighter. Within tubecore samples we observed sediment of hydrothermal origin including white grey sediment particles. Light brown fecal pellets of macrofaunal origin (capitellid or bivalve) were moderately abundant. Tube fragments of a chaetopterid polychaete were present.

Solwara inactive. Sediments were gray and orange with plate-size orange iron oxide crusts visible. Fine-scale ripples, pits, burrows tubes and dimples were evident on sediment surfaces. Extensive bacterial mats (presumably sulfide oxidizers) were present at the sediment surface in some areas, and at the base of rocks. Some sediments appeared very coarse grained. Xenophyophores (large agglutinating foraminifera) were visible, and aggregations of tubes, likely agglutinated rhizopods, were also present on the sediment surface. Small piles of dead snails were visible atop sediments. Predators visible in photographs included octopus, pycnogonids, and blob fish. Wood fragments (branches) were visible on the sediment, covered with crabs.

Microscopic examination revealed crusty orange sediments and a thinner white porous crust also present. Grey sediment of hydrothermal origin and black crystals (glass) of volcanic origin were observed. Particles < 0.3 mm were mainly orange (iron) oxidized material.

South Su active. Both massive and small patches of thick, white bacterial mat were observed in photos of the sediment surface. Lamellibrach tube worms were present as solitary individuals in sediments. Synaphobranchid eels, blobfish, ratails and even gastropods were present on the sediment surface. Tube aggregations (most likely polychaetes) were evident.

Sediments from two cores from this site were observed; each was very different. One core's sediments were comprised mainly of biogenic fecal pellets, probably made by nuculanoid bivalves. The finer fraction presented a thick conglomerate of organic material; this could be the result of microbial decomposition of fecal pellets. The second core contained pyrite in a grey/white sediment matrix of hydrothermal origin. Coarser black volcanic fragments were also present. The fine fraction included organic matter aggregates and soft white material, possibly amorphous iron.

South Su inactive. Distinctive features of this site were small white patches of bacteria, syringamminid xenophyophore tests, *Bathysiphon* tubes, an egg case (maldanid polychaete?), an encrusting tunicate or sponge, a lamellibrach in sediments, worm casts, and a vent fish. Large wood debris and vertebrate bones were also observed on the sediment surface; both are thought to support chemosynthetic communities.

The sediment from this core resembles that of the Solwara active core. No fecal pellets were observed. Meiofauna werea abundant and included nematodes, *Bathysiphon* and

agglutinated foraminifera. The sediment is of hydrothermal origin and includes mainly grey particles, black grains of volcanic origin and white material as well. An organically rich fine fraction (<0.3 mm) is populated by small *Bathysiphon* and nematodes.

Discussion

Scales of habitat heterogeneity

A cursory review of the still photographs of Solwara and South Su sediments taken by ROV in March and April 2007 reveals a significant amount of sediment heterogeneity over scales from cm to 10's of m. Sediment surfaces exhibited heterogeneous features that provide evidence of physical influence (ripples and dimples) and biogeochemical influence (bacterial mats, orange, grey and white color variations). Biotic structures also provide significant sources of heterogeneity in the Manus Basin sediments. Pits, mounds, tube aggregations, clam (lucinid?) aggregations, burrow openings, and xenophyophore tests were all visible on the sediment surface (Appendix III). It appears that none of these features were explicitly sampled by coring, so it is difficult to determine which taxa are associated with specific features, or whether the full spectrum of biota was sampled via coring and scoop sampling. The limited number of cores taken at each of the 4 study areas is inadequate to fully characterize the macrofauna, given the heterogeneity evident in sediments. Past research provides strong evidence that features such as rippled sediments, microbial mats (Bernhard et al. 2000, Levin et al. 2003), xenophyophore tests (Levin and Thomas 1989), tube beds (Gooday et al. 1992), pits and mounds (Schaff and Levin 1994) are likely to support distinct species and contribute to diversity maintenance in deep-sea sediments (Snelgrove and Smith 2002). Mining has the potential to reduce this heterogeneity through physical disruption or sediment deposition.

We note that much of the macrofaunal material examined was in poor shape, with the majority of organisms fragmented. It is not known whether this is a result of poor preservation, damage during processing on the ship, or damage from exposure to changing chemical conditions during recovery.

Relationship to other sedimented vents

Densities. The macrofauna of sediment-hosted hydrothermal vents have been studied in Guaymas Basin in the Gulf of California (1800-2000 m), in Escanaba Trough on Gorda Ridge (3250 m), and in Middle Valley in northern Juan de Fuca Ridge (2400 m) (Table 7); all of these locations are in the eastern Pacific Ocean. All of the eastern Pacific hydrothermal sediment sites are significantly deeper than those sampled at Solwara and South Su in the Manus Basin, yet they exhibited higher macrofaunal densities than found in 3 of the 4 sampled Manus Basin (Table 7). Only in hot muds (up to 94^{o}C) from Middle Valley were macrofaunal densities as low as those at Solwara and South Su inactive sites (<1000 ind./m^2). The South Su active site (3700 ind./m^2) resembled the Middle Valley, Escanaba and Guaymas vent sediments in having somewhat higher densities (Table 7), and dominance by a few species, with rare species absent. The lower

Manus Basin densities may be due to (a) more oligotrophic conditions in overlying waters off Papua New Guinea than in the eastern Pacific regions which are upwelling areas and (b) lesser hydrothermal activity and associated microbial food availability than in in the sampled sediments of Guaymas, Escanaba Trough and Middle Valley

Composition. Despite density differences between the Manus Basin and the eastern Pacific hydrothermal sediments, there were some similarities observed in taxonomic composition, most notably the abundance of the genus *Prionospio* (*Minuspio*) (South Su active only) and nuculanoid bivalves (all sites). The genus *Prionospio* (*Minuspio*) is reported from Escanaba Trough and Middle Valley hydrothermal sediments and wood falls (Grassle and Petrecca 1994), and in low abundance from hydrothermal vents in the Indian Ocean and E. Pacific (21^{o}N). This widespread and speciose genus is very abundant in shallow hypoxic sediments on the Oman margin (Levin et al. 2000), and in polluted harbors, but even occurs in N. Atlantic abyssal plains (Levin and Gooday 2003). Some specimens of *Laonice* sp., a genus in the "*Prionospio* complex" (Sigvaldadottir 1998) and small specimens of a spionid that appears to belong to *Prionospio* have been found in white microbial mat and inactive sediments at Middle Valley (Levin et al., unpublished). *Prionospio* sp. was a dominant colonizer in organically enriched sediments at 2160 m in the Bay of Biscaye (Debruyeres et al. 1980). The limpet *Lepetodrilus* sp., typically attached to hard substrates or vetimentiferan tubes, is a genus broadly distributed in eastern Pacific hydrothermal vents. This taxon and the gastropod *Olgasolaris* sp. are likely to be associated with sulfide precipitates.

Nuculanoid bivalves, which comprised 17-43% of the fauna at the 4 Manus Basin sites (Table 3) are abundant in Guaymas Basin hydrothermal clam bed sediments (*Nuculana* sp.) as well (Petrecca and Grassle 1990). The maldanid polychaete *Nicomache* sp., common in South Su active scoop sediments, is characteristic of vent environments and some methane seeps (FL Escarpment) globally (Desbruyeres et al. 2006).

Peracarid crustaceans (mainly tanaids and isopods) appeared more abundant in the Manus Basin than in other hydrothermal sediments, but related taxa (*Pseudotanais* species, and *Leptognathia* spp.) are reported from Escanaba Trough. In Middle Valley a small unidentified species of tanaid was the only crustacean collected (Levin et al., unpublished). Orbiniid, ampharetid, dorvilleid and hesionid polychaetes, which are abundant in eastern Pacific hydrothermal sediments are less common (ampharetids), rare or absent. We note that the spatial coverage, habitat coverage and number of individuals sampled in the Manus Basin are insufficient to assess taxon absence. Even with similarities at the generic level or higher, there appears to be no overlap in species composition. To our knowledge, all of the Manus Basin macrofauna sampled appear to be new to science.

Diversity. Reduced diversity (relative to non-hydrothermal background sediments) and high dominance is characteristic of all active hydrothermal sediments sampled thus far and applies most strongly to the South Su Active site. The number of species expected from a collection of 100 individuals (Es_{100}) ranges from 6 (South Su Active) to 20 (South Su Inactive and Solwara Active) (Figure 5). This is comparable to or higher than

diversity recorded at Guaymas Basin and Escanaba Trough hydrothermal sediments (Grassle and Petrecca 1990; Petrecca and Grassle 1990). Wood falls in the vicinity of vents appear to support slightly higher diversity.

Relation to Non-Vent Fauna

Most of the western South Pacific non-vent infaunal literature addresses deeper sites (> 2000 m) (Alongi 1992) or focuses on metazoan and protozoan meiofauna (Shirayama 1984 a,b; Alongi 1987; Alongi and Pichon 1988; 1990). Use of a 0.5 mm mesh yielded 736 macrofaunal ind./m^2 and biomass 2.44 g/m^2 at 695 m on the Papuan Barrier Reef Slope (roughly 11° S 151°E), and 120 individuals/m^2 and 0.33 g/m^2 at 1454 m on the Coral Sea Plateau (roughly 14°S 146 °E), south of Papua New Guinea (Alongi 1992). The Coral Sea density (from depths comparable to those sampled in the present study) was 4-30 times lower than the South Su and Solwara densities, but the biomass was of the same order of magnitude as 3 out of 4 sites (Table 3). Notably, Shirayama (1983) reported ~ 1000 ind/m^2 and biomass of 1-2 g/m^2 at 2100-2200 m on the Solomon Rise (roughly 0-3° S 159° E). In general, density is more sensitive to sieve size than is biomass (Gage et al. 2002). Our use of a 0.3 mm mesh may contribute to (but probably does not fully account for) the observation of higher densities in the Manus Basin hydrothermal sediments. Greater food supply associated with hydrothermal activity is also likely to elevate densities.

Additional sampling of macrofauna is taking place in the PNG region (by British investigators) at the time this is being written.

Many of the genera identified in this study are commonly found in non-vent, deep-sea sediments. For example, *Heteromastus (filliformis)*, is a dominant taxon from 1100-1437 m, and the nuculanoid *Deminucula cancellata* is dominant from 1707-1815 m on the New England margin (Rowe et al. 1982). *Cossura* sp., present only in inactive hydrothermal sediments, is reported broadly from continental margins around the world, including many oxygen minimum zones (Levin 2003).

Endemicity

All of the species we collected that were examined by systematists appear to be new to science. This was true for the tanaids (2 spp.), isopods (3 spp.) and bivalves (2 spp.). Most of the polychaetes also appear to be new, and several of these taxa may also belong to new genera. Because deep-water sediments in the PNG region, and the western Pacific in general are poorly sampled it is not possible to determine whether the taxa identified are (a) endemic to the PNG region, (b) endemic to hydrothermal sediment settings, or (c) opportunistic fauna taking advantage of a high-food or stressed ecosystem.

Mining disturbance and resilience

Sources of mining disturbance include removal of substrate and associated organisms, physical disruption of sediment, plume presence and deposition, return water, and waste disposal. It is impossible to know how individual species will respond to these disturbances without conducting pilot studies. However, based on lifestyle information, and behaviors of similar taxa elsewhere, we have tried to identify taxa likely to be more and less sensitive to disturbance.

Substrate removal should have the greatest negative effect on those species that a) are endemic to the local area and have small populations sizes, or b) species without significant dispersal stages. No information is available on the endemicity or broader distribution of the species recovered in this study. All peracarids (isopods, tanaids, amphipods, mysids and cumaceans) have direct development and lack larval stages. While some adults can swim it is anticipated that dispersal is especially restricted in these taxa. Spionids, including *Prionospio*, typically have mixotrophic development with initial brooded stages and later development by feeding larval stages that may remain planktonic for 1-3 weeks. This strategy typically allows rapid recolonization when source populations are nearby (Levin 1984). Nuculanoidea typically have planktonic larvae, though brooding has been documented. Two *Nuculana* species colonized defaunated sediment trays placed at 1800 m in the NW Atlantic and one of these, *N. cancellata* grew to maturity in 2 years (Grassle and Morse Porteous 1987).

Reproductive studies of the remaining fauna (e.g., larval trophic mode and planktonic stages assessed from egg size) or colonization studies involving defaunated sediments would permit a more accurate assessment of rates and potential for recovery for individual species.

Even in the absence of physical disruption, massive plumes of turbid water are likely to negatively affect suspension/surface feeder by clogging feeding apparatus (Rhoads and Boyer 1982). Among the Manus Basin macrofauna, only *Prionospio (Minuspio)* sp., the ampharetids, and the gastropods are distinctly surface feeders. The majority of the other abundant species present in both active and inactive hydrothermal sediments (e.g., bivalves, capitellids, maldanids), are likely to be subsurface deposit feeders. These taxa may be more tolerant of limited burial by resuspended sediments. Carnivory and a scavenging lifestyle are best represented among the less abundant species; sediment deposition may interfere with various sensory cues involved in prey location and capture. For all of these extensive deposition (many cm) will smother and bury resident taxa.

Return water and waste disposal may act to concentrate toxic substances. The effects of these cannot be predicted without knowing mining protocols and carrying out pilot studies.

Describing Species

Because the Manus Basin hydrothermal sediments are remote, and have been studied mainly by geologists and geochemists rather than biologists, most of the infaunal species sampled appear to be previously unseen and new to science. To facilitate further study of the communities, monitoring of their response to mining disturbance and recovery potential, as well as to extend the value of the study beyond the scope of this mining location, it is desirable to have the species formally described by trained systematists. Such descriptions require the placement of a preserved type specimen (holotype), as well as several additional specimens (paratypes) in one or more museums. Often the placement is best made in a Museum at or near where the describing systematists work. Thus, it is optimal for the government of Papua New Guinea to agree to either (a) turn over at least paratype material to the systematist's museum or (b) agree to a permanent loan [though this appears to be unacceptable to many systematists]. There should not be concern about use of material for commercial purposes, as the type specimens will never be destroyed, damaged or used for this purpose. We should also encourage retention of shells and other hard parts (and placement in museums) when soft tissues are removed for isotope or other chemical analyses.

Involve the scientific community

The vent-savy scientific community recognizes the potential economic value of polymetalic sulfide deposits. Several international programs (InterRidge and Chess (Census of Marine Life) have engaged in discussions about deep-sea mining and the role of the scientific community. These discussions have highlighted the patchy nature of active hydrothermal sites, the existence of significant genetic isolation creating distinct biogeographic provinces (Van Dover et al. 2002), and the limits to knowledge of global distributions. They point to a dearth of knowledge about inactive hydrothermal sites, their trophic pathways and other functional aspects. The scientists acknowledge a need to develop novel techniques to sample and quantify inactive sites (which are targeted for mining) and evaluate their ecosystem functioning. Interaction between active and inactive sites also requires study. At present, major international programs - e.g. InterRidge, ChEss (Census of Marine Life), are actively discussing the need for, and how best to implement, a larger-scale pilot study to evaluate mining effects.

Recommendations

A. Expand baseline infaunal studies. Because of low faunal densities initial sampling was insufficient to fully characterize faunal assemblages in the targeted sediments. Conduct further sampling of inactive and active areas, focusing on small-scale heterogeneity (clam patches, tube aggregations, xenophyophore tests, microbial mats) as depicted in Appendix III. Other sources of small-scale heterogeneity may include wood and animal bones – both provide sources of organic matter and can support chemosynthetic communities.

B. Characterize (assess) densities and taxonomic composition of (1) sediment dwelling megabenthos, (2) metazoan meiofauna and (3) protozoan macro- and meiofauna – Foraminifera are clearly abundant on the sediment surface. They form 29 to 99% of the nearby Coral Sea meiofauna. These smaller organisms may be more easily damaged and slower to recover than the larger macrofauna.

C. Characterize sediment structure and composition, surface features, biogenic structures and generate x- radiographs (x-rays of sediments slabs) to document levels of disturbance and bioturbation. These can yield information about presence or absence of organism activity.

D. Provide abiotic indicators to better characterize 'active' vs 'inactive' hydrothermal sediments. In the present study Solwara active and South Su inactive communities were most alike. Continuous temperature records, data on sediment composition and texture, oxygen penetration data, and quantification of bacterial biomass, could all contribute to interpretation of faunal assemblages and aid assessment of faunal resilience.

E. Conduct pilot disturbance studies that mimic physical sediment disruption, plume formation, and chemical modifications. Identify undisturbed reference sites to document natural variability. Evaluate a) the nature of faunal response to disturbance in terms of density, biomass, composition, diversity and behavior, b) rates and trajectories of faunal recovery, c) identify indicator taxa that are either highly sensitive to disturbance or when present, reflect active disturbance, and d) characterize disturbed sediment and plume physical and chemical properties.

F. Promote global exchange of scientific information across ecosystems among scientists and industry. Develop a model protocol for responsible pre-mining investigations in new areas being considered for mining, that can be adopted as a global standard.

G. Engage systematists and forge an arrangement with the PNG government that allows description of new species. This must include permission for paratypes to reside in PNG museums and type specimens to reside in (and be owned by) museums outside PNG that will make the material available for comparative work worldwide. This is fundamental to assessment of species endemicity and vulnerability to mining disturbance.

H. Develop a process-oriented understanding of inactive hydrothermal sediments, including drivers of composition, diversity, and trophic structure.

Elements of a Pilot Study for Hydrothermal (Inactive) Sediments

Knowledge of near bottom current directions and velocities as well as particle concentrations in the water (determined via nephelometer) are required to design an appropriate pilot study (Thiel et al. 2001). Ideally these should be measured for many months (up to a year). Sites should be selected along the direction of mean current flow and pre disturbance sampling should take place to select representative settings. Experimental disturbance of a nature similar to that incurred by mining should be

generated, ideally with replication. If toxic substances are associated with minerals processing, these should be included in the experiment. Appropriate physical, chemical and biological sampling should be carried out before, immediately after and for multiple years (up to 10) after disturbance. A combination of suspended sediment load/particle plume measurements, quantification of sediment deposition patterns, x - radiography, video imaging to quantify lebenspurren and megafauna, and sediment coring for meio- and macrofauna should be incorporated. Mining disturbance studies in the DISCOL and ATESEPP programs conducted in manganese nodule areas of the abyssal Peru Basin revealed that disturbance effects on sediment structure, faunal diversity and abundance can be detected even 7 years after initial disturbance (Thiel et al. 2001, Borowski 2001). Although recovery at the shallower Solwara sites could be more rapid than at the 4150 m Peru Basin, a plan should be in place to document the full time scales, short- and long-term community responses and trajectory of faunal recovery (in terms of animal density, biomass, diversity, composition, spatial heterogeneity, and functional attributes [e.g., trophic structure, bioturbation]).

Acknowledgements. Assistance at sea was provided by Canyon Offshore (ROV driving/collecting, photos and tube core development). Elitsa Hrischeva (Univ. of Toronto), Samantha Smith (Nautilus), Alison Swaddling (Nautilus), Patrick Collins (Duke), and Carol Logan (Duke) helped with sample collection and onboard processing of samples. Assistance in the laboratory with sample sorting was provided by Shira Maezumi and Sarah Wheeler. Jennifer Gonzalez and Sarah Wheeler provided help with data compilation. Species/genus identifications were provided by Buz Wilson (isopods), Graham Oliver (bivalves) and Isabelle Williams (tanaids).

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Table 1. List of tube cores collected from active and inactive sediments at the Solwara and South Su sites of Manus Basin.

Site:	Feature:	Col. Date: (day/mo/y)	Dive:	Depth: (m)	T:	Core:	Vert. Fraction:	Easting:	Northing:	Lat	Long
Solwara	Inactive	24/3/07	7	1513m	T:5	Core:4	(0-10cm)	399534	9581109	-3.78926	152.095
Solwara	Inactive	24/3/07	7	1504m	T:5	Core:6	(0-10cm)	399534	9581109	-3.78926	152.095
Solwara	Active	25/3/07	8	1530m	T:6	Core:7	(0-10cm)	399457	9581267	-3.78787	152.095
Solwara	Active	25/3/07	9	1514m	T:7	Core:27	(0-10cm)	399488	9581251	-3.78801	152.095
Solwara	Inactive	25/3/07	8	1532m	T:8	Core:10	(0-10cm)	399442	9581278	-3.78777	152.094
Solwara	Inactive	25/3/07	9	1523m	T:9	Core:6	(0-10cm)	399455	9581225	-3.78446	152.091
Solwara	Active	26/3/07	10	1511m	T:10	Core:27	(0-10cm)	399402	9581178	-3.78867	152.094
Solwara	Inactive	27/3/07	11	1625m	T:12	Core:17	(0-10cm)	398905	9580986	-3.79034	152.09
Solwara	Inactive	28/3/07	12	1634m	T:13	Core:16	(0-10cm)	398888	9581045	-3.78985	152.089
Solwara	Inactive	28/3/07	12	1601m	T:14	Core:12	(0-10cm)	398990	9580952	-3.79072	152.09
Solwara	Active	3/4/07	14	1575m	T:15	Core:30	(0-?cm) Whole Core	399069	9580967	-3.79062	152.091
Solwara	Active	3/4/07	14	1575m	T:15	Core:15	(0-?cm) Whole Core	399069	9580967	-3.79062	152.091
Solwara	Active	3/4/07	14	1575m	T:15	Core:13	(0-10cm)	399069	9580967	-3.79062	152.091
Solwara	Active	4/4/07	15	1516m	T:16	Core:30	(0-?cm) Whole Core	399469	9581250	-3.79061	152.091
Solwara	Active	4/4/07	15	1516m	T:16	Core:8	(0-?cm) Whole Core	399469	9581250	-3.79061	152.091
Solwara	Active	4/4/07	15	1516m	T:16	Core:11	(0-?cm) Whole Core	399469	9581250	-3.79061	152.091
Solwara	Active	4/4/07	15	1516m	T:16	Core:9	(0-10cm)	399469	9581250	-3.79061	152.091
Southsu	Active	8/4/07	24	1325m	T:17	Core:4	(0-2cm)	400622	9578929	-3.80901	152.105
Southsu	Active	8/4/07	24	1325m	T:17	Core:8	(0-10cm)	400622	9578929	-3.80901	152.105
Southsu	Active	8/4/07	24	1325m	T:17	Core:13	(0-5cm)	400622	9578929	-3.80901	152.105
Southsu	Inactive	8/4/07	24	1347m	T:18	Core:24	(0-10cm)	400527	9578955	-3.80877	152.104
Southsu	Active	9/4/07	25	1325m	T:19	Core:7	(0-?cm) Whole Core	400678	9578870	-3.80959	152.105
Southsu	Active	9/4/07	26	1312m	T:20	Core:30	(0-10cm)	400632	9578895	-3.80934	152.105
Southsu	Inactive	10/4/07	27	1374m	T:21	Core:21	(0-10cm)	400667	9578812	-3.8101	152.105
Southsu	Active	4/14/07	34	1313m	T:22	Core:8	(0-10cm)	400625	9578911	-3.80919	152.105
Southsu	Active	10/4/07	28	1312m	T:22	Core:11	(0-10cm)	400625	9578911	-3.80919	152.105
Southsu	Inactive	11/4/07	29	1406m	T:23	Core:21	(0-10cm)	400601	9578669	-3.81132	152.105
Southsu	Active	11/4/07	30	1340m	T:24	Core:14	(0-?cm) Whole Core	400700	9578907	-3.80922	152.106
Southsu	Inactive	11/4/07	30	1356m	T:25	Core:28	(0-10cm)	400733	9578947	-3.80888	152.106
Southsu	Inactive	12/4/07	31	1430m	T:26	Core:21	(0-10cm)	400745	9579128	-3.80723	152.106
Southsu	Inactive	13/4/07	33	1423m	T:27	Core:21	(0-10cm)	400386	9579192	-3.80667	152.103
Southsu	Inactive	13/4/07	33	1423m	T:27	Core:30	(0-3cm)	400386	9579192	-3.80667	152.103
Southsu	Inactive	15/4/07	35	1452m	T:28	Core:17	(0-10cm)	400345	9578703	-3.81106	152.103
Southsu	Active	16/4/07	36	1352m	T:29	Core:8	(0-?cm) Whole Core	400663	9578871	-3.80958	152.105
Southsu	Active	16/4/07	36	1362m	T:30	Core:28	(0-10cm)	400677	9578852	-3.80974	152.105

Table. 2. List of scoop samples collected from active and inactive sediments at the Solwara and South Su sites of Manus Basin.

Site:	Feature:	Col. Date:	Dive:	Depth:	T:	Core:	Type	Easting	Northing	Lat	Long
Solwara	Active	25/3/07	9	1515m	T:6	Core:Unknown	Suction	399457	9581267	-3.78787	152.095
Solwara	Active	25/3/07	9	1515m	T:6	Core:Unknown	Scoop	399457	9581267	-3.78787	152.095
Solwara	Inactive	22/4/07	48	1532m	T:8	Core:Yellow Scoop	Scoop	399442	9581278	-3.78777	152.094
Solwara	Inactive	26/3/07	10	1512m	T:11	Core:BB1	Scoop	399469	9581250	-3.78998	152.096
Solwara	Inactive	26/3/07	10	1512m	T:11	Core:BB2	Scoop	399469	9581250	-3.78998	152.096
Solwara	Inactive	26/3/07	10	1512m	T:11	Core:BB3	Scoop	399469	9581250	-3.78998	152.096
Solwara	Inactive	26/3/07	10	1510m	T:11	Core:BB4	Scoop	399469	9581250	-3.78998	152.096
Solwara	Inactive	28/3/07	12	1601m	T:14	Core:Scoop	Scoop	398990	9580952	-3.79072	152.09
Southsu	Active	15/4/07	25	1369m	T:21.3	Core: Scoop	Scoop	400667	9578810	-3.8101	152.105
Southsu	Active	10/3/03	28	1312m	T:22	Core:Scoop #1	Scoop #1	400625	9578911	-3.80919	152.105
Southsu	Inactive	14/4/07	34	1335m	T:25	Core:Scoop #6	Scoop #6	400733	9578947	-3.80888	152.106
Southsu	Inactive	14/4/07	34	1334m	T:25	Core:Scoop #7	Scoop #7	400733	9578947	-3.80888	152.106
Southsu	Inactive	13/4/07	33	1423m	T:27	Core:Scoop #2	Scoop #2	400386	9579192	-3.80667	152.103
Southsu	Inactive	14/4/07	33	1367m	T:27	Core:Scoop #4	Scoop #4	400386	9579192	-3.80667	152.103
Southsu	Inactive	13/4/07	33	1423m	T:27	Core:Scoop #5	Scoop #5	400386	9579192	-3.80667	152.103
Southsu	Inactive	13/4/07	33	1423m	T:27	Core:Scoop #8	Scoop #8	400386	9579192	-3.80667	152.103
Southsu	Inactive	15/4/07	35	1452m	T:28	Core:Scoop #4	Scoop #4	400345	9578703	-3.81106	152.103

Table 3. Number of individuals and biomass (average, S.E. and percentage) of macrofauna >0.3 mm in sediment cores (7 cm diameter) from Solwara and South Su active and inactive hydrothermal sediments..

	Solwara_Active			Solwara_Inactive			Southsu_Active			Southsu_Inactive	
n=	10			7			10			8	
	Average	S.E.	Percentage	Average	S.E.	Percentage	Average	S.E.	Percentage	Average	S.E
Counts = no. individuals /38 cm2 core											
Heteromastus sp.	0.5	0.31	13.89%	0.14	0.14	8.33%	0.00	0.00	0.00%	0.00	0.00
Glyceridae	0.1	0.10	2.78%	0.00	0.00	0.00%	0.00	0.00	0.00%	0.00	0.00
Cossura sp.	0	0.00	0.00%	0.14	0.14	8.33%	0.00	0.00	0.00%	0.25	0.25
Sigalionidae	0	0.00	0.00%	0.14	0.14	8.33%	0.00	0.00	0.00%	0.00	0.00
Prionospio (Minuspio) sp.	0	0.00	0.00%	0.00	0.00	0.00%	11.10	4.66	77.08%	0.38	0.18
Nereis sp.	0	0.00	0.00%	0.00	0.00	0.00%	0.10	0.10	0.69%	0.00	0.00
Spiochaetopterus sp.	0.1	0.10	2.78%	0.00	0.00	0.00%	0.00	0.00	0.00%	0.00	0.00
Lumbrineridae	0	0.00	0.00%	0.00	0.00	0.00%	0.00	0.00	0.00%	0.13	0.13
Cumacea	0	0.00	0.00%	0.00	0.00	0.00%	0.00	0.00	0.00%	0.13	0.13
Paraleptognathia sp.	1.3	0.26	36.11%	1.00	0.85	58.33%	0.00	0.00	0.00%	1.00	0.38
Pseudotanais sp.	0.2	0.13	5.56%	0.00	0.00	0.00%	0.00	0.00	0.00%	0.00	0.00
Isopoda	0.7	0.40	19.44%	0.00	0.00	0.00%	0.00	0.00	0.00%	0.13	0.13
Gammaridea	0	0.00	0.00%	0.00	0.00	0.00%	0.10	0.10	0.69%	0.00	0.00
Nuculanoid bivalve	0.7	0.26	19.44%	0.29	0.18	16.67%	3.00	0.60	20.83%	1.50	0.50
Lepetodrilus?	0	0	0.00%	0.00	0	0.00%	0.10	0.1	0.69%	0.00	0
TOTAL	3.60	1.56	1.00	1.71	1.46	1.00	14.40	5.56	1.00	3.50	1.69
No./m2	934.92	404.39		445.20	378.68		3739.68	1443.74		908.95	437.84

	Solwara_Active			Solwara_Inactive			Southsu_Active			Southsu_Inactive	
n=	n10			n7			n10			n8	
	Average	S.E.	Percentage	Average	S.E.	Percentage	Average	S.E.	Percentage	Average	S.E
BIOMASS = mg/38 cm2 core											
Heteromastus sp.	0.060	0.041	6.10%	0.014	0.041	0.35%	0.000	0.000	0.00%	0.000	0.000
Glyceridae	0.110	0.110	11.19%	0.000	0.011	0.00%	0.000	0.000	0.00%	0.000	0.000
Cossura sp.	0.000	0.000	0.00%	0.013	0.009	0.31%	0.000	0.000	0.00%	0.020	0.016
Sigalionidae	0.000	0.000	0.00%	3.341	2.339	80.93%	0.000	0.000	0.00%	0.000	0.000
Prionospio (Minuspio) sp.	0.000	0.000	0.00%	0.000	0.000	0.00%	34.643	11.948	68.56%	0.115	1.186
Nereis sp.	0.000	0.000	0.00%	0.000	0.000	0.00%	3.104	3.104	6.14%	0.000	0.310
Spiochaetopterus sp.	0.106	0.106	10.78%	0.000	0.011	0.00%	0.000	0.000	0.00%	0.000	0.000
Lumbrineridae	0.000	0.000	0.00%	0.000	0.000	0.00%	0.000	0.000	0.00%	0.018	0.014
Cumacea	0.000	0.000	0.00%	0.000	0.000	0.00%	0.000	0.000	0.00%	0.020	0.016
Paraleptognathia sp.	0.403	0.282	41.00%	0.134	0.291	3.25%	0.000	0.000	0.00%	0.074	0.025
Pseudotanais sp.	0.008	0.008	0.81%	0.000	0.001	0.00%	0.000	0.000	0.00%	0.000	0.000
Isopoda	0.155	0.086	15.77%	0.000	0.069	0.00%	0.000	0.000	0.00%	0.014	0.011
Gammaridea	0.000	0.000	0.00%	0.000	0.000	0.00%	0.008	0.008	0.02%	0.000	0.001
Nuculanoid bivalve	0.141	0.078	14.34%	0.626	0.405	15.16%	12.723	7.328	25.18%	0.263	0.731
Lepetodrilus?	0.000	0.000	0.00%	0.000	0.000	0.00%	0.053	0.053	0.10%	0.000	0.005
TOTAL	0.983	0.711	100.00%	4.129	3.176	100.00%	50.531	22.441	100.00%	0.523	2.315
mg/m2	255.285	184.638		1072.190	824.878		13122.901	5828.027		135.693	601.282
g/m2	0.255	0.185		1.072	0.825		13.123	5.828		0.136	0.601

S.E. = standard error.

Table 4. Macrofaunal counts from scoop samples collected from active and inactive hydrothermal sediments from Solwara and South Su in Manus Basin.

Location	Solwara	Solwara	Solwara	Solwara	South Su	South Su	South Su	South Su			
Activity	Active	Active	Inactive	Inactive	Active	Active	Inactive	Inactive			
Depth Range	1515 m		1510-1601 m		1312-1369		1334-1452 m			TOTAL	Percent
n	2		6		2		6				
Vert. Fraction:	SUM	Percentage	SUM	Percent	SUM	Percent	SUM	Percent			
Heteromastus sp.	0	0.000%	42	12.389%	0	0.000%	1	1.010%		43	6.48%
cf. *Scyphoproctus* sp.	0	0.000%	0	0.000%	1	0.541%	0	0.000%		1	0.15%
Nicomache sp	0	0.000%	0	0.000%	15	8.108%	0	0.000%		15	2.26%
Glyceridae	0	0.000%	1	0.295%	0	0.000%	3	3.030%		4	0.60%
Cossura sp.	0	0.000%	17	5.015%	0	0.000%	14	14.141%		31	4.67%
Polynoidae	0	0.000%	0	0.000%	0	0.000%	1	1.010%		1	0.15%
Sigalionidae	1	2.439%	3	0.885%	0	0.000%	3	3.030%		7	1.05%
Prionospio(?) sp.	0	0.000%	31	9.145%	36	19.459%	0	0.000%		67	10.09%
Prionospio (Minuspio) sp.	0	0.000%	0	0.000%	0	0.000%	0	0.000%		0	0.00%
Nereis sp.	0	0.000%	0	0.000%	0	0.000%	1	1.010%		1	0.15%
Spiochaetopterus sp.	3	7.317%	0	0.000%	0	0.000%	0	0.000%		3	0.45%
Lumbrineridae	0	0.000%	0	0.000%	0	0.000%	2	2.020%		2	0.30%
Hesionidae	0	0.000%	1	0.295%	0	0.000%	0	0.000%		1	0.15%
Opheliidae	0	0.000%	0	0.000%	0	0.000%	0	0.000%		0	0.00%
Phyllodocidae	0	0.000%	0	0.000%	0	0.000%	2	2.020%		2	0.30%
Ampharetidae	2	4.878%	3	0.885%	6	3.243%	2	2.020%		13	1.96%
Nemertinea	0	0.000%	0	0.000%	0	0.000%	1	1.010%		1	0.15%
Cumacea	0	0.000%	1	0.295%	0	0.000%	1	1.010%		2	0.30%
Paraleptognathia sp.	11	26.829%	175	51.622%	1	0.541%	23	23.232%		210	31.63%
Pseudotanais sp.	3	7.317%	24	7.080%	1	0.541%	8	8.081%		36	5.42%
Isopoda	3	7.317%	23	6.785%	0	0.000%	1	1.010%		27	4.07%
Gammaridea	1	2.439%	1	0.295%	0	0.000%	1	1.010%		3	0.45%
Lyssianasid gammarid	0	0.000%	0	0.000%	0	0.000%	1	1.010%		1	0.15%
Phoxocephalid gammarid	0	0.000%	0	0.000%	0	0.000%	1	1.010%		1	0.15%
Alvinocaridid decapod	1	2.439%	0	0.000%	0	0.000%	0	0.000%		1	0.15%
Nuculanoid bivalve	2	4.878%	14	4.130%	120	64.865%	26	26.263%		162	24.40%
Cuspidaria sp.	1	2.439%	2	0.590%	0	0.000%	0	0.000%		3	0.45%
unid. bivalve	0	0.000%	0	0.000%	0	0.000%	0	0.000%		0	0.00%
Lepetodrilus	8	19.512%	0	0.000%	5	2.703%	7	7.071%		20	3.01%
Olgasolaris sp.	5	12.195%	0	0.000%	0	0.000%	0	0.000%		5	0.75%
Turritelid gastropod	0	0.000%	1	0.295%	0	0.000%		0.000%		1	0.15%
TOTAL	41		339		185		99			664	

Table 5. Feeding mode and dwelling modes of macrofaunal invertebrates collected from active and inactive hydrothermal sediments at South Su and Solwara sites in the Manus Basin.

Species	Feeding mode	Dwelling Habit
Heteromastus sp.	Deposit Feeder (Subsurface)	Burrower
cf. *Scyphoproctus* sp.	Deposit Feeder	Burrower
Nicomache sp	Deposit Feeder/Bacterivore	Tube Builder
Glyceridae	Carnivore/Omnivore	Motile
Cossura sp.	Deposit Feeder (Surface)	Burrower
Polynoidae	Carnivore/Omnivore	Motile
Sigalionidae	Carnivore/Omnivore	Motile
Spionidae (Prionospio?)	Deposit Feeder (Surface)	Tube Builder
Prionospio (Minuspio) sp.	Deposit Feeder (Surface)	Tube Builder
Nereis sp.	Carnivore/Omnivore	Tube Builder
Spiochaetopterus sp.	Suspension Feeder	Tube Builder
Lumbrineridae	Deposit Feeder	Burrower
Hesionidae	Carnivore/Omnivore	Motile
Opheliidae	Deposit Feeder (Subsurface)	Burrower
Phyllodocidae	Carnivore/Omnivore	Motile
Ampharetidae	Deposit Feeder (Surface)	Tube Builder
Nemertinea	Carnivore/Omnivore	Motile
Cumacea	Scavenger/Omnivore	Motile
Paraleptognathia sp. (sp.1)	Scavenger/Omnivore	Tube Builder
Pseudotanais sp. (sp. 2)	Scavenger/Omnivore	Tube Builder
Isopoda (3n. sp.)	Scavenger/Omnivore	Motile
Gammaridea	Suspension Feeder?	Motile
Lyssianasid gammarid	Scavenger	Motile
Phoxocephalid gammarid	Carnivore/Omnivore	Motile
Alvinocaridid decapod	Carnivore/Omnivore	Motile
Nuculanoid bivalve n. sp. (nr	Deposit-Feeder	Burrower
Cuspidaria sp.	Carnivore/Omnivore	Burrower
unid. bivalve	Deposit-Feeder	Burrower
Lepetodrilus	Bacterial Grazer/Suspension feeder?	Epifaunal
Olgasolaris sp.	Bacterial Grazer	Epifaunal
Turritelid gastropod	Carnivore/Omnivore	Epifaunal

Table 6. Microscope images and observations of hydrothermal sediments from Solwara and South Su sites.

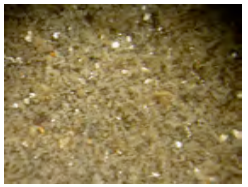
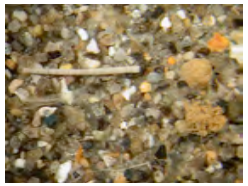
Site	Feature	Sediment Observations	Non-microscope close up	12x Image (>300 micron)	25x Image (<300 micron)
Solwara	Active	Grey sediment of hydrothermal vent origin including white particles Light brown capitellid/bivalve fecal pellets moderately abundant low visible organic matter content Chaetopterid tubes present			
Solwara	Inactive	Thick plate-like iron oxidized orange crust Thin white porous crust fragments present Fine grey sandy sediment of hydrothermal vent origin black volcanic origin finer material Finer fraction mostly orange color			
SouthSu	Active	Mostly dark grey fecal pellets, probably from bivalve molluscs finer fraction with dense organic material conglomerate			
SouthSu	Active	Golden shiny sulfate deposits (pyrite?) Grey sediment of hydrothermal vent origin Tube linings and organic aggregates Some pellets present Finer material with conglomerated organic matrix			
SouthSu	Inactive	Similar to Solwara Active Grey sediment of hydrothermal origin No pellets Abundant Nematodes, Bathysiphon and a few xenophyophores Abundant small Bathysiphon and nematodes in fine fraction			

Table 7. Summary of macrofaunal community structure in hydrothermal sediments

SITE	Habitat	Region	Depth (m)	No. Macrofauna/m 2	Rank 1 Dominance	Dominant Taxa	Other Taxa	REFERENCE
Middle Valley		NE Pacific	2410					
	bacterial mat			5589 ± 2022	22%	Ampharetidae, Orbinidae, Dorvilleidae		Levin et al. unpubl.
	hot mud			910 ± 263	50%	Syllidae, Spionidae		Levin et al. unpubl.
	clam bed			16769 ± 1106	34%	Syllidae, Orbinidae		Levin et al. unpubl.
Escanaba Trough		NE Pacific	3254					
	hydrothermal sediment + near background			18,709		Ampharetidae, Orbiniidae, Spionidae	nuculanoid, *Provanna, Neolepetosis*	Grassle & Petrecca 1994
	near wood			16,932		Dorvilleidae, Hesionidae, *Provanna*		Grassle & Petrecca 1994
	mounds		3274		<8%	Cirratulidae, Spionidae, Paraonidae	similar to ambient	Grassle & Petrecca 1994
	combined hydrothermal	Gulf of CA		3722-5443				
Guaymas Basi	petroleum sed					Hesionidae, Dorvileidae		Petrecca & Grassle 1990
	clam bed					Nuculanid bivalves		Petrecca & Grassle 1990
	microbial mat					*Amphisamytha* (Ampharetidae)		Petrecca & Grassle 1990
	background		3270	1200-1900				
Solwara		Papua New Guinea						
	active		1511-1575	934	36%	*Paraleptognathia* (Tanaidacea)	Isopoda, Nuculanoid bivalve, Heteromastus	This study
	inactive		1504-1634	445	58%	*Paraleptognathia* (Tanaidacea)	Nuculanoid bivalve	This study
South Su	active	Papua New Guinea	1312-1369	3740	77%	*Prionospio (Minuspio)*	Nuculanoid bivalve	This study
	inactive		1356-1452	908	43%	Nuculanoid bivalve	*Paraleptognathia* sp., *Prionospio* sp.	This study

Appendix IA. Macrofaunal counts for tube core samples (7 cm diameter) taken from Solwara and South Su active and inactive hydrothermal sediments.

Site:	Solwara	Solwara	Solwara	Solwara	Solwara	Solwara	Solwara	Solwara	Solwara	Solwara	Solwara	Solwara	Solwara	Solwara	Solwara	Solwara	Solwara
T:	T:16	T:16	T15	T15	T:16	T:6	T:7	T:10	T:15	T:16	T:5	T:5	T:8	T:9	T:14	T:13	T:12
Dive:	Dive:15	Dive:15	Dive:14	Dive:14	Dive:15	Dive:8	Dive:9	Dive:10	Dive: 14	Dive:15	Dive:7	Dive:7	Dive:8	Dive:9	Dive:12	Dive:12	Dive:11
Core:	Core:30	Core:8	Core:30	Core:15	Core:11	Core:7	Core:27	Core:27	Core:13	Core:9	Core:4	Core:6	Core:10	Core:6	Core:12	Core:16	Core:17
Depth:	1576m	1576m	1575m	1575m	1576m	1530m	1514m	1511m	1575m	1576m	1513m	1504m	1532m	1523m	1601m	1634m	1625m
Activity:	Active	Active	Active	Active	Active	Active	Active	Active	Active	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Col. Date:	Col. Date: 4/	Col. Date:4/	Col.Date:3/4	Col.Date:3/4	Col. Date:4/	Col. Date:25	Col.Date:25/	Col.Date:26/	Col. Date: 3/	Col.Data:4/4	Col.Date: 24	Col.Date:24/	Col.Date:25/	Col.Date:25/	Col.Date: 28	Col.Date:28/	Col. Date: 27/3/07
Vert. Fraction:	(0-?cm) Who	(0-?cm) Wh	(0-?cm) Wh	(0-?cm) Wh	(0-?) Whole	(0-10 cm)	(0-10 cm)	(0-10 cm)	(0-10 cm)	(0-10 cm)	(0-10 cm)	(0-10 cm)	(0-10 cm)	(0-10 cm)	(0-10 cm)	(0-10 cm)	(0-10 cm)
Heteromastus sp.	0	0	0	1	1	0	0	0	3	0	0	0	0	1	0	0	0
Glyceridae	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Cossura sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Sigalionidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Prionospio (Minuspio) sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nereis sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spiochaetopterus sp.	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lumbrineridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cumacea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paraleptognathia sp.	1	2	1	1	1	2	0	1	1	3	6	0	0	1	0	0	0
Pseudotanais sp.	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Isopoda	1	0	0	1	0	0	0	0	1	4	0	0	0	0	0	0	0
Gammaridea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nuculanoid bivalve	2	0	1	0	1	0	0	2	1	0	0	0	1	0	0	0	1
Lepetodrilus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Site:	Southsu	Southsu	Southsu	Southsu	Southsu	Southsu	Southsu	Southsu	Southsu	Southsu	Southsu	Southsu	Southsu	Southsu	Southsu	Southsu	Southsu	Southsu
T:	T:29	T:30	T:20	T:17	T:17	T:17	T:19	T:22	T:22	T:24	T:18	T:21	T:23	T:25	T:26	T:27	T:28	T:27
Dive:	Dive:36	Dive:36	Dive:26	Dive:24	Dive:24	Dive:24	Dive:25	Dive:34	Dive:28	Dive:30	Dive:24	Dive:27	Dive:29	Dive:30	Dive:31	Dive:33	Dive:35	Dive:33
Core:	Core:8	Core:28	Core:30	Core:4	Core:8	Core:13	Core:7	Core:8	Core:11	Core:14	Core:24	Core:21	Core:21	Core:28	Core:21	Core:21	Core:17	Core:30
Depth:	1352m	1362m	1312m	1325m	1325m	1325m	1325m	1313m	1312m	1340m	1347m	1374m	1406m	1356m	1430m	1423m	1452m	1423m
Activity:	Active	Active	Active	Active	Active	Active	Active	Active	Active	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Col. Date:	Col.Date:16/	Col.date:16/	Col.Date:9/4	Col.Date:8/4	Col.Date:8/4	Col.Date:8/4	Col.Date:9/4	Col.Date:4/1	Col. Date: 1(	Col. Date: 1	Col. Date:8/	Col.Date:10/	Col. Date:11	Col. Date:11	Col. Date: 1	Col. Date:13	Col. Date:15	Col. Date:13
Vert. Fraction:	(0-?cm) Wh	(0-10 cm)	(0-10 cm)	(0-2cm)	(0-10 cm)	(0-5cm)	Whole core	(0-10 cm)	(0-10 cm)	Whole Core	(0-10 cm)	(0-10 cm)	(0-10 cm)	(0-10 cm)	(0-10 cm)	(0-10 cm)	(0-10 cm)	(0-3cm)
Heteromastus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glyceridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cossura sp.	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
Sigalionidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prionospio (Minuspio) sp.	0	0	14	19	47	6	5	1	19	0	0	1	1	0	0	0	0	1
Nereis sp.	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Spiochaetopterus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lumbrineridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Cumacea	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Paraleptognathia sp.	0	0	0	0	0	0	0	0	0	0	2	2	2	0	2	0	0	0
Pseudotanais sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Isopoda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Gammaridea	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nuculanoid bivalve	3	3	3	3	7	1	2	0	4	4	1	1	3	0	2	0	4	1
Lepetodrilus	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix IB: Macrofaunal counts in scoop samples from Solwara and South Su active and inactive hydrothermal sediments.

Site:	Solwara	Solwara	Solwara	Solwara	Solwara	Solwara	Solwara	Solwara	Southsu	Southsu	Southsu	Southsu	Southsu	Southsu	Southsu	Southsu
T:	T:6	T:6	T:8	T:11	T:11	T:11	T:11	T:14	T:21.3	T:22	T:25	T:27	T:27	T:27	T:27	T:28
Dive:	Dive:9	Dive:9	Dive:48	Dive:10	Dive:10	Dive:10	Dive:10	Dive:12	Dive:25	Dive:28	Dive:34	Dive:33	Dive:33	Dive:33	Dive:33	Dive:35
Core:	Core:Unknown	Core:Unknown	Core:Yellow Scoop	Core:Scoop	Core:Scoop	Core:Scoop	Core:Scoop	Core:Scoop	Core: Scoop	Core:Scoop #1	Core:Scoop #6	Core:Scoop #2	Core:Scoop #4	Core:Scoop #5	Core:Scoop #8	Core:Scoop #4
Depth (m):	1515m	1515m	1532m	1512m	1512m	1512m	1510m	1601m	1369m	1312m	1335m	1423m	1367m	1423m	1423m	1452m
Activity:	Active	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Collection Date:	25/3/07	25/3/07	22/4/07	26/3/07	26/3/07	26/3/07	26/3/07	28/3/07	15/4/07	10/4/07	14/4/07	13/4/07	14/4/07	13/4/07	13/4/07	15/4/07
Vert. Fraction:	Suction Sample	Scoop	Scoop	Scoop	Scoop	Scoop	Scoop	Scoop	Scoop	Scoop	Scoop	Scoop	Scoop	Scoop	Scoop	Scoop
Heteromastus sp.	0	0	5	8	0	13	0	16	0	0	0	0	0	0	0	1
cf. *Scyphoproctus* sp.	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Nicomache sp	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0
Glyceridae	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	3
Cossura sp.	0	0	0	0	0	0	0	17	0	0	0	0	0	0	0	14
Polynoidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Sigalionidae	1	0	0	0	0	0	2	1	0	0	0	0	0	0	0	3
Prionospio (?) sp.	0	0	0	0	0	0	0	31	32	4	0	0	0	0	0	0
Prionospio (Minuspio) sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nereis sp.	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Spiochaetopterus sp.	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lumbrineridae	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
Hesionidae	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Opheliidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phyllodocidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Ampharetidae	0	2	0	1	0	0	1	1	3	3	0	0	2	0	0	0
Nemertinea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Cumacea	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
Paraleptognathia sp.	11	0	124	38	0	0	12	1	1	0	7	0	9	6	0	1
Pseudotanais sp.	3	0	4	11	2	1	6	0	1	0	5	0	2	0	0	1
Isopoda	3	0	6	2	0	4	1	10	0	0	0	0	0	1	0	0
Gammaridea	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
Lyssianasid gammarid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Phoxocephalid gammarid	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Alvinocaridid decapod	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nuculanoid bivalve	2	0	5	0	0	0	0	9	104	16	2	0	2	0	11	11
Cuspidaria sp.	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
unid. bivalve	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lepetodrilus	0	8	0	0	0	0	0	0	2	3	6	1	0	0	0	0
Olgasolaris sp.	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Turritelid gastropod	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0

Appendix II. Community comparisons of Solwara and South Su Active and Inactive sites: ANOSIM and SIMPER results

Global Test
Sample statistic (Global R): 0.315
Significance level of sample statistic: 0.1%
Number of permutations: 999 (Random sample from a large number)
Number of permuted statistics greater than or equal to Global R: 0

Pairwise Tests

Groups	R Statistic	Significance Level %	Possible Permutations	Actual Permutations	Number >= Observed
Solwara_Active, Solwara_Inactive	0.211	3	19448	999	29
Solwara_Active, Southsu_Active	0.658	0.1	92378	999	0
Solwara_Active, Southsu_Inactive	0.138	2.9	43758	999	28
Solwara_Inactive, Southsu_Active	0.501	0.1	19448	999	0
Solwara_Inactive, Southsu_Inactive	0.016	30.4	6435	999	303
Southsu_Active, Southsu_Inactive	0.249	0.4	43758	999	3

Groups Solwara_Active & Solwara_Inactive
Average dissimilarity = 81.21

Species	Group Solwara_Active Av.Abund	Group Solwara_Inactive Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
tanaid.sp1	1.06	0.49	25.91	1.57	31.91	31.91
bivalve.sp.1	0.58	0.29	15.53	0.91	19.13	51.03
isopod	0.5	0	9.93	0.75	12.23	63.26
heteromastus	0.37	0.14	9.49	0.72	11.68	74.95
glycerid	0.1	0	5.65	0.29	6.96	81.91
tanaid.sp2	0.2	0	4.69	0.46	5.77	87.68
cossura	0	0.14	3.98	0.38	4.9	92.58

Groups Southsu_Active & Southsu_Inactive
Average dissimilarity = 70.71

Species	Group Southsu_Active Av.Abund	Group Southsu_Inactive Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
prionospio.(minusp)	2.5	0.38	31.33	1.25	44.31	44.31
bivalve.sp.1	1.6	1.02	17.67	0.78	24.98	69.3
tanaid.sp1	0	0.71	9.54	0.9	13.5	82.8
cossura	0	0.18	2.05	0.36	2.9	85.7
lumbrinerid	0	0.13	1.89	0.35	2.67	88.37
isopod	0	0.13	1.89	0.35	2.67	91.04

Groups Solwara_Inactive & Southsu_Active
Average dissimilarity = 90.49

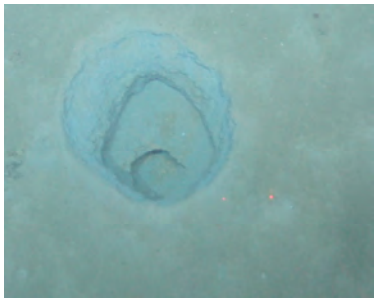
Species	Group Solwara_Inactive Av.Abund	Group Southsu_Active Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
prionospio.(minusp)	0	2.5	39.12	1.36	43.24	43.24
bivalve.sp.1	0.29	1.6	28.43	1.25	31.42	74.65
tanaid.sp1	0.49	0	8.44	0.52	9.32	83.98
cossura	0.14	0	3.34	0.35	3.69	87.67
heteromastus	0.14	0	2.61	0.37	2.88	90.55

Groups Solwara_Inactive & Southsu_Inactive
Average dissimilarity = 83.67

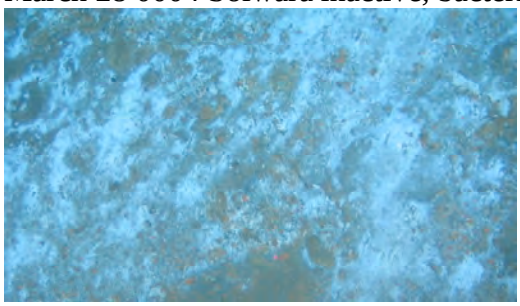
Species	Group Solwara_Inactive Av.Abund	Group Southsu_Inactive Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
bivalve.sp.1	0.29	1.02	25.94	1.11	31	31
tanaid.sp1	0.49	0.71	22.62	0.98	27.04	58.04
cossura	0.14	0.18	9.02	0.43	10.78	68.82
prionospio.(minusp)	0	0.38	8.99	0.78	10.75	79.57
heteromastus	0.14	0	4.04	0.37	4.83	84.4
sigalionid	0.14	0	4.04	0.37	4.83	89.23
lumbrinerid	0	0.13	3.37	0.38	4.02	93.25

Appendix III. Images of sediments, surface features, and sediment-fauna from Solwara and South Su sediments.

March 27 0026 Solwara inactive

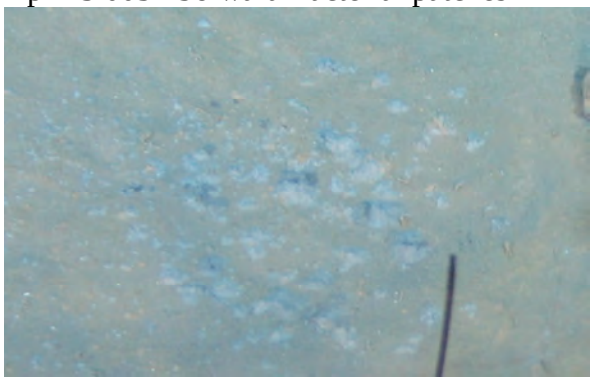


March 28 0004 Solwara inactive, bacterial mat



March 29 0052 Xenophyophores

April 5 0034 Solwara Bacterial patches



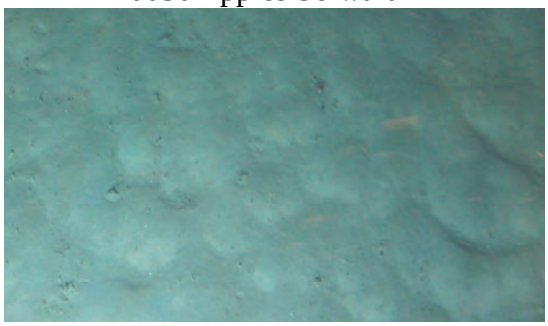
April 5 0037 Solwara

April 6 0088 Solwara 0102

0106 Solwara

APRIL 7 0068 Solwara

APRIL 7 0050 ripples Solwara



APRIL 7 005 Site?

APRIL 7 0077 ? Lucinid Bed?

APRIL 8 0065 South Su

APRIL 8 0150 South Su

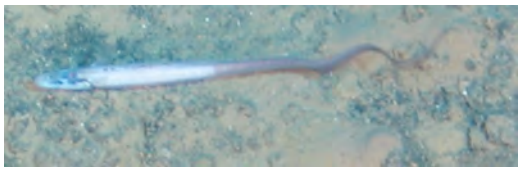
APRIL 8 0165 South Su

APRIL 8 0169 South Su active?

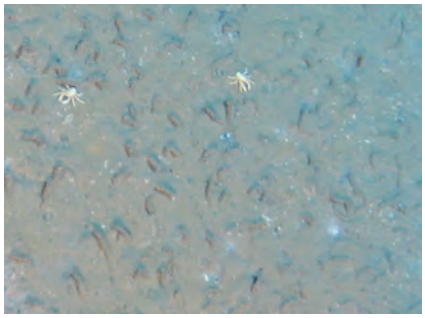
APRIL 8 0170 South Su

APRIL 8 0220 South Su

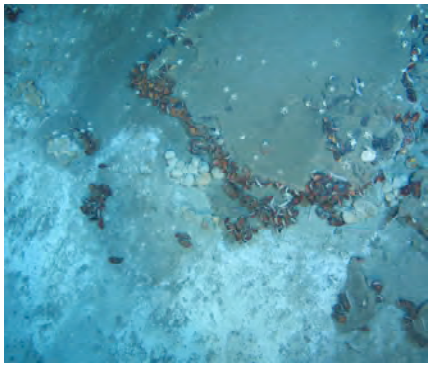
APRIL 8 0019 South Su



APRIL 9 0024 South Su active



APRIL 9 0032 South Su active



APRIL 15 0165 South Su inactive?

APRIL 15 0218 South Su Inactive

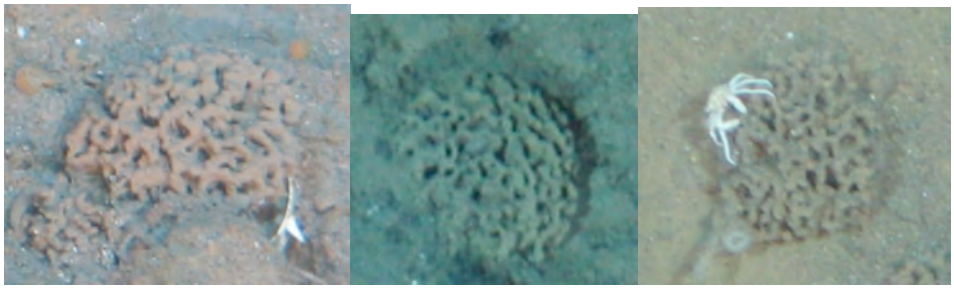
APRIL 15 0279 South Su Inactive

APRIL 15 0275 South Su Inactive

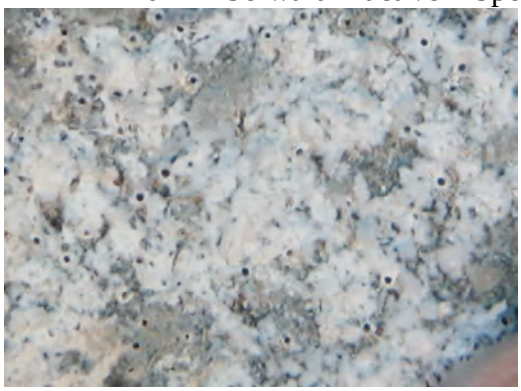
APRIL 15 0296 South Su inactive

APRIL 15 0343

APRIL 15 0126 SS Inactive/ APRIL 22 0004/ 0016 – Solwara inactive



APRIL 22 0222 Solwara Inactive – Sponge or Tunicate?



APRIL 25 0027 – Site Unknown

Appendix 6

Quality Including Trace Elements of Sediments from the SuSu Knolls, Manus Basin, Bismarck Sea, Papua New Guinea

Report to Nautilus Minerals Inc.

February 15, 2008

Quality Including Trace Elements of Sediments from the SuSu Knolls, Manus Basin, Bismarck Sea, Papua New Guinea

A contribution to environmental assessment

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Table of contents

Figures

Tables

1. Executive Summary

The research project comprises studies of mineralogy and geochemistry of sediments within the tenements held by Nautilus Minerals in the vicinity of SuSu Knolls, eastern Manus basin, PNG, that aim to establish environmental baselines as well as guidelines for exploration for seafloor massive sulfide deposits. Sediment cores for the study were collected during cruises of Nautilus Minerals Inc. in 2006 and 2007 (Luk Luk 07) using short and 1 m long push corers operated by ROV. Studied were 33 cores from the Solwara 1 sulfide mound and 13 cores from South Su. The analytical work included examination of thin and polished sections of sediments by light microscope and scanning electron microscope equipped with EDS, grain-size analysis, X-ray diffraction analysis, X-ray fluorescence analysis and instrumental neutron activation analysis.

Solwara 1 and South Su are covered by an apron of dark gray volcanic sandy silts and silty sands composed of various amounts of volcanic rock fragments, volcanic glass, Ca plagioclase, pyroxene, cristobalite, Si-rich amorphous material, alunite, pyrite, barite and magnetite. In many cases, the gray volcaniclastic sediments of Solwara 1 exhibit patches and layers having a black or greenish-brown color that contain fecal pellets. On the western slope of Solwara 1, gray volcaniclastic sediments overlie greenish, greenish-brown and greenish-black sediments that are primarily composed of fine-grained material containing volcanic glass and products of it alteration such as smectite, chlorite and X-ray amorphous material as well as sand-size fragments of volcanic glass and volcanic rocks. The sediments also contain minor plagioclase and traces of sulfides and barite. In most cases the greenish and greenish-black colored sediments have fecal pellets at different stages of preservation.

The distributions of Au (19 ppb to 2 ppm), Cu (159 ppm to 1 wt %), Zn (35 ppm to 1333 ppm), Pb (not detected to 977 ppm), Ba (0.05 wt % to 2.8 wt %) and As (12 ppm to 654 ppm) outline patchy anomalies throughout the sediments. The volcaniclastic sediments of Solwara 1 and South Su have similar background concentrations of Cu (up to 500 ppm), Zn (up to 100 ppm), Pb (up to 50 ppm), Ba (up to 0.1 wt %) and As (up to 100 ppm). The concentrations of metals in much of the sediment of South Su are within background levels whereas the sediments of Solwara 1 exhibit pronounced metal anomalies at depths greater than 25 cm below the

sediment-water interface. We suggest that there are two major sources of metal anomalies: dispersion of hydrothermal particles from eroded chimneys and deposition of hydrothermal particles within fecal pellets. A strong metal anomaly created by dispersion of sulfides and barite from chimneys was found only locally in sediments from the southern rim of Solwara 1. More widespread but weaker metal anomalies in sediments from the western slope of Solwara 1 appear to have been produced by dispersal of sulfide and barite particles from chimneys and biogenic deposition of hydrothermal particles within fecal pellets. With the exception of local weak anomalies in surface sediments around active chimneys, the current particulate plume emanating from black smokers at Solwara 1 and South Su does not leave a widespread clear signal in the surface sediments.

2. Objectives

The objectives of the proposed study are to establish environmental baselines as well as guidelines for exploration for seafloor massive sulfide (SMS) deposits based on sedimentological (mineralogy and grain-size) and geochemical analyses of sediments within the tenements held by Nautilus Minerals in the vicinity of SuSu Knolls, eastern Manus basin, PNG.

Mineralogical and grain-size analyses were performed in order to determine the physical properties and origin of sediments. Geochemical analysis determined the baseline/pre-mining concentrations of Au, Cu, Zn, Pb, Ba, Co, Cr, Ni, Mo, Fe, Mn and As as well as the spatial distributions of metals in sediments. Concentrations of metals in sediments associated with SMS are typically elevated with respect to the background levels. The sediment can be contaminated by hydrothermal particles derived from erosion of chimneys and other hydrothermal precipitates and from fallout from a hydrothermal plume (Mills, 1995).

3. Sediments in back arcs

Most studies of sediments associated with SMS have been near vents and on ridge flanks of the Mid-Atlantic Ridge and the East Pacific Rise where hydrothermally derived material is associated with pelagic carbonate ooze having

only a minor detrital component (e.g., Ruhlin and Owen, 1986; Barrett et al., 1987; Owen and Olivarez, 1988; Metz et al., 1988; Kyte et al., 1993; Mills et al., 1993; German et al., 1993, 1997, 1999; Cave et al., 2002). In contrast to mid-ocean ridges, the sedimentation in back-arc environments is more complex, producing sedimentary sequences that comprise volcaniclastic sediments interbedded with hemipelagic and pelagic sediments. Models of sedimentation in back-arc basins have shown that the bordering volcanic arc supplies large volumes of volcaniclastic material (Carey and Sigurdsson, 1984; Clift, 1995; Marsaglia et al., 1995). Some of the volcaniclastic material may be derived locally from the spreading centers (e.g., hyaloclastite) or from eruptions of intra-basinal seamount volcanoes, as is inferred to be the case in the Lau basin (Clift et al., 1994; Marsaglia et al., 1995). Volcaniclastic material derived from hydrothermally altered and disintegrated volcanic rocks also has been found in a hydrothermal field on the Valu Fa Ridge, an active back-arc spreading center of the southern Lau basin (Fouquet et al., 1993).

Metalliferous sediments deposited in modern back-arc environments have been studied from the North Fiji basin (e.g., McMurtry et al., 1991), the Lau basin (e.g., Cronan et al., 1984; Hodkinson and Cronan, 1991) and from and peripheral to the PACMANUS area, eastern Manus basin (Ortega-Osorio, 1996; Sebastian et al., 1999, 2000; Binns, 2004). These studies have shown that material deposited from hydrothermal plumes is superimposed on hemipelagic and pelagic sediments containing variable amounts of biogenic and volcaniclastic material.

4. Geologic setting and previous work

The Manus basin is located off the eastern coast of Papua New Guinea in the Bismarck Sea (Fig. 1, inset). It is bounded along its northeast side by a series of Eocene to Oligocene island arcs generated by subduction of the Pacific plate along the now inactive Manus trench (Martinez and Taylor, 1996). On its western border lies the island of New Guinea and, to the south, the New Britain island arc. On the south side of this volcanically active island arc is the New Britain trench, which is the current locus of subduction of the northward moving Indo-Australian Plate. Subduction along this trench is believed to have begun at approximately 15 Ma, after

subduction of the westward moving Pacific plate came to a halt upon collision of the Ontong Java plateau with the Manus trench (Martinez and Taylor, 1996).

The Manus basin shows typical back-arc characteristics with basalt dominated volcanism occurring along the more mature western and central seafloor spreading segments, whereas mafic to felsic magmas characterize the rifting of the eastern Manus basin. These three zones of active volcanism are separated by NW-WNW trending transform faults (Martinez and Taylor, 1996). The eastern Manus basin, which is the least mature of the three active extensional zones, is an 80-100 km wide pull-apart structure enclosed by a set of sinistral faults; on its western edge, the Djaul fault, and on its eastern edge, the Weitin fault (Fig. 1). It is between these transform faults that the gold- rich PACMANUS and SuSu Knolls hydrothermal fields were discovered (Binns and Scott, 1993; Scott and Binns, 1995; Binns et al., 1997a). Rifting began in the Pliocene at approximately 3.5 Ma (Binns et al., 1995). Located in this immature back-arc basin is a series of NE trending en-echelon volcanic ridges with a wide range of rock compositions from basaltic andesite to highly differentiated dacite and rhyolite. Exposures of arc crust, Eocene to Oligocene in age, on the islands of New Britain and New Ireland are thought to be continuous with the basement lithologies of the eastern Manus basin (Binns et al., 1995).

Solwara 1 is a sulfide mound that is part of the SuSu Knolls. The SuSu Knolls are in the eastern part of eastern Manus basin, at a water depth of 1160-1550 m and extending for approximately 4 km in a NNW-SSE direction. They consist of two prominent porphyritic dacite domes, North Su and South Su, and a deeper and lower andesite edifice Solwara 1 (Binns et al., 1997b). The hydrothermal field at SuSu Knolls consists of four main sites: Solwara 1, South Su, North Su and a valley between North Su and Solwara 1.

The PACMANUS and SuSu Knolls hydrothermal fields were discovered during the 1991 PACMANUS I and 1996 PACMANUS III marine expeditions, respectively (Binns and Scott, 1993; Scott and Binns, 1995; Binns et al., 1997b; Parr and Binns, 1997) by means of multiple real-time CTD (conductivity, temperature, depth) and transmissometry plume-detection profiles across what was believed, from their bathymetry, to be active volcanoes (Parr and Binns, 1997; McDonald et al., 1998). During the 1996 PACMANUS III expedition, there were two recognizable particulate plumes, the fallout from which could have contributed hydrothermal

constituents to the seafloor sediments, a lower plume at 1260-1460 m water depth and an upper plume at 1060-1140 m water depth. Eleven months later during the 1997 PACMANUS IV cruise, the lower plume had all but disappeared but the upper plume had intensified. The upper plume clearly emanated from the top of North Su. The lower plume appears to have had two sources, a strong source from South Su and a weaker one from Solwara 1.

The South Su and North Su sites are characterized by extensive natroalunite-bearing alteration of the porphyritic dacite as well as scattered sulfide chimneys and mounds dominated by pyrite-enargite-fukuchilite mineralization (Yeats et al., 2000; Binns, 2004). In contrast, the deeper and lower andesitic edifice at Solwara 1 displays a north-south aligned field of pyrite-poor , chalcopyrite- and gold-rich chimneys with barite gangue, similar to those of the PACMANUS site, located 50 km to the west (Binns and Scott, 1993; Scott and Binns, 1995; Moss and Scott, 2001). The SuSu Knolls are covered by a thick (up to several meters) and widespread “tuffite” apron of layered, dark, locally sulfidic sandy sediment (Binns, 2004). Binns (2004) proposed derivation of components of the sediment from violent hydrothermal eruptions at North Su and South Su.

5. Sampling and methods

5.1. Sampling

Sediment cores for the study were collected during cruises of Nautilus Minerals Inc. in 2006 and 2007 (Luk Luk 07 cruise). A list of the collected cores including core labels and corresponding cores’ names as entered in the sample log as well as core lengths, sampled intervals, coordinates of the sampling sites and water depths is in Tables 1 and 2. Some of the collected cores were not analyzed because they were disturbed or recovered insufficient sample; this is indicated in Table 2. Locations of the sites of sediment cores taken from Solwara 1 and South Su are shown in Figures 2 and 3, respectively. Sixteen short sediment cores taken during the 2006 Nautilus cruise were provided by Samantha Smith (cores were labeled SC-2 to SC-78). The cores recovered sediments from Solwara 1 at a maximum depth of 33 cm below the seafloor. The majority of the cores (cores SC-16 to SC-44) were taken

along a profile in a chimney field in the western limb of Solwara 1. Transects during the Luk Luk 07 cruise showed occurrences of both active and inactive chimneys in the eastern part of the field. The western extension of the field consists of thick sediment containing weathered chimney fragments. The chimney fragments are covered by sediment and probably a large part of them is buried below the sediment cover. The surface of the sediment is burrowed and covered by long tracks. Core 46 was taken from a heavily sedimented slope approximately 200 m west of the rim of Solwara 1. Cores SC-2 and SC-3 were from sediments in proximity to chimney fields in the western limb of Solwara 1. Core SC-4 was taken from thick sediment on the SE rim and cores SC-73 and SC-78 from sediment on the eastern rim of Solwara 1.

During the Luk Luk 07 cruise, sediment cores from Solwara 1 and South Su were collected using both short and 1 m long push cores. Sampling with 1 m long push cores was proposed in the sampling strategy for Luk Luk 07 cruise in order to attempt recovery of deep sediments from the rim and slope of Solwara 1. Long push cores were deployed on the seafloor in a cage and the sampling was manually operated by an ROV arm. In many cases, the attempts to retrieve deep sediments by long push cores were unsuccessful. One of the reasons for the difficulties could be the physical properties of the sediments. Most of the sediments covering Solwara 1 are gritty, silty and sandy volcaniclastics. Although long push cores penetrated fully in the sediments, in many cases upon retrieval, the cores were only ¼ full, probably because of settling of the upper sediment that was saturated with water and/or the loss of sediment from the bottom of the corer during its removal from the seabed. The longest cores obtained were two 50 cm long and one 80 cm long from sediments on the western slope of Solwara 1. A reason for the better recovery here could have been presence of a minor clay component in the silty and sandy sediments from this area, which increases the cohesiveness of the sediments.

A total of 30 sediment cores were collected during the two cruises from Solwara 1, of which 17 were analyzed. In addition, 16 cores were collected from South Su, of which 13 were analyzed. At Solwara 1, cores were taken from different parts of the rim and slope of the mound. Core SC-83 was taken approximately 10 m from an active sulfide chimney in a chimney field in the western limb of Solwara 1. Cores SC-81 and SC-113 were from thick sediment on the steep northern slope of Solwara 1. Cores that were taken from the eastern limb of Solwara 1 are SC-87 from

thick sediment in proximity to chimney fragments, SC-85 from thick sediment with worms near a weathered chimney and SC-110 from the eastern slope of Solwara 1. The eastern slope of Solwara 1 is steep and along the rim there are blocks of volcanic rocks. Core SC-108 was taken from a heavily sedimented area on the southern rim of Solwara 1. The western rim and slope of Solwara 1 were sampled using both short and long push cores at increasing distance from the mound. Short cores SC-117, SC-118 and SC-121 were taken along the western rim from a heavily sedimented area with scattered chimney fragments. Cores SC-114, SC-116, SC-122 and SC-123 were taken from the western slope of Solwara 1 and retrieved sediment at a maximum depth of 50 cm. Core SC-124 was taken approximately 350 m away from the western rim of Solwara 1 and recovered sediment as deep as 80 cm. The western slope of Solwara 1 deepens gradually and the surface sediments exhibit ripples indicative of bottom currents.

Sediments from the slope and surrounding South Su were sampled by short push cores. The sediment cover at South Su is thinner then that at Solwara 1. Cliffs and outcrops of volcanic rock occur close to the rim of the dome. Large parts of the slopes are covered by a coarse talus of volcanic blocks and pockets of fine sediment. In some areas, the fine sediment was thick enough for full penetration of short push cores. In most cases, although the cores penetrated fully into the sediment, they recovered only small amount of sample, a large portion of which was in the core catcher. We were not able to obtain good sediment cores here and, for this reason, only the surface sediment (0-2 cm and 0-5 cm) was analyzed. Cores taken from the slope of South Su were at different distances from active and inactive chimneys. Core SC-89 was taken from sediment in proximity to an inactive chimney, core SC-90 from sediment at the base of a chimney venting black smoke, cores SC-91 and SC-92 from sediment in an area with small active chimneys and core SC-95 from sediment away from the chimney field. Also collected were short sediment cores near sediment traps surrounding South Su (cores SC-96 to SC-104).

5.2. Methods

Upon retrieval, sediment cores were split into half, photographed and logged. One half of the cores was archived and the other half was sectioned into sub-samples that were air-dried. The majority of the analyses were conducted in the laboratories of

the University of Toronto. Grain-size analysis was performed for selected samples. The sand (> 2 mm) and silt fractions (2-0.063 mm) were separated by sieving and the clay fraction (< 4 μm) by settling in water. Samples for geochemical and X-ray diffraction analyses were washed with distilled water to remove salts, dried at 70^0C and ground.

X-ray diffraction analysis (Philips diffractometer, Cu radiation, 40 kV and 40 mA, scan rate 1^0 per minute) of bulk samples and clay fractions was used to determine the mineralogy of the sediments. Clay fractions were settled on glass slides and analyzed air-dry and after saturation with ethylene glycol. Characterization of the mineral composition, morphology and elemental composition of particles was carried out using a Leitz light microscope and a Jeol JSM-840 scanning electron microscope (SEM) equipped with an IXRF energy dispersive analytical X-ray system operated at 15-20 kV. Polished sections were prepared from various depth intervals and layers in cores from different parts of Solwara 1. Polished thin sections for SEM analysis were carbon coated and analyzed in both secondary and backscattered electron mode. The backscattered images were further processed with Image Pro Plus software in order to determine the percentages of the mineral phases.

Concentrations of SiO_2, TiO_2, Al_2O_3, Fe_2O_3, MnO, MgO, CaO, K_2O, Na_2O, P_2O_5, S as well as trace elements Cu, Zn, Pb, Ba and As were determined by X-ray fluorescence spectroscopy (XRF) applying the pressed pellet method. The concentrations were determined quantitatively using a standard and the difference from 100 % was calculated as H_2O. The detection limit for Cu, Zn and Pb is 3 ppm. The accuracy of the XRF analysis was less then 5 % for all elements except for As and Pb for which the accuracy was about 20 %.

Concentrations of Au, Ag, As, Ba, Co, Cr, Hf, Ir, Mo, Ni, Rb, Sb, Sc, Se, Sr, Ta, U, Th, W, Zn and REE's were determined for selected samples by instrumental neutron activation analysis (INAA). The INAA was contracted out to ACTLABS because the University of Toronto equipment is currently inoperative. The detection limits for elements are in Table 5. The precision and accuracy of the INAA for most of the analyzed elements were less than 10 %. Exception is Ba and Zn, for which the precision and accuracy were about 30 % and U - 20 %. We have used data on concentrations of Ba and Zn determined by XRF analysis.

Maps of element distributions in surface sediments and in depth profiles were drawn using Surfer software. Because of the paucity of sample points, maps of element distributions in surface sediments of South Su are based on a very coarse grid.

6. Results

6.1. Mineralogical composition of sediments

Descriptions of sediments from sampled core intervals are in Table 3. Photographs of cores taken from Solwara 1 are shown in Figures 4, 5 and 6 and cores taken from South Su in Figure 7. Solwara 1 is covered by a thick apron of volcaniclastic sediments. The sediments are volcanic sandy silts and silty sands, the majority of which contain from 30 to 70 wt % sand fraction (> 63 μm), from 20 to 60 wt % silt fraction (4-63 μm) and from 2 to 10 wt % clay fraction (< 4 μm). Over much of Solwara 1, the volcaniclastic sediments are dark gray with patches and layers of orange, greenish-brown and black color (Fig. 4 and 6). Backscattered electron images of gray volcaniclastic sediments of Solwara 1 are shown in Figures 8 and 9. The mineral abundances of sediments from Solwara 1 and South Su are in Table 6. The gray volcaniclastic sediments are composed of about 50-60 vol. % fine-grained material that comprises glass shards, X-ray amorphous Si- and Si-Al-rich phases, cristobalite and alunite, 15-25 vol. % fragments of volcanic rocks, 15-20 vol. % Ca plagioclase, about 3 vol. % pyroxene and 2 vol. % pyrite, barite and magnetite. Volcanic rock fragments are of dacitic composition and contain microlites of plagioclase, pyroxene and magnetite. They are angular to subrounded and show different degrees of alteration. Plagioclase and pyroxene occur mainly as angular broken crystal fragments ranging in size from about 10 μm up to 200 μm. Alunite occurs as euhedral crystals, some of platy habit, or aggregated with amorphous silica (Fig. 8D). The compositional heterogeneity of alunite marked by the presence of variable amounts of K, Na, Ca and P, as well as XRD reflections at 2.95 and 2.93 Å, suggest the presence of different aluminum sulfate phases, such as natroalunite (Na-K alunite) and woodhouseite (Ca-P alunite). Barite forms disseminated platy crystals and rosettes (Fig. 8C and 9A). Pyrite occurs as disseminated euhedral crystals or

aggregates, in some cases intergrown with Si-rich amorphous material, and as mineralization in volcanic rock fragments (Fig. 8C and D). This morphology is similar to that of disseminated pyrite found in altered and mineralized volcanic rocks. Large aggregates of pyrite and Fe-Cu sulfide that account for increased sulfide concentrations (up to 5 wt %) were found in grayish-black volcanic sands from the 23-33 cm interval of core SC-4 from the southeastern rim of Solwara 1 (Fig. 9C). Some of the aggregates consist of globules and framboids. (Fig. 9D).

Both black and greenish-brown patches of the gray volcaniclastic sediments contain aggregates of fine-grain particles that are similar to fecal pellets (e.g. Cuomo and Bartholomew, 1991), which suggests that the coloration is a result of the presence of organic matter (Fig. 10). The size of the fecal pellets varies between about 0.2 mm to 0.5 mm. Most of the fecal pellets are of ellipsoidal shape, while ovoid pellets are rarer. The fecal pellets are composed of fine-grained particles of Si-Al composition, some of which could be altered glass, and of fine-grained plagioclase and pyroxene particles. In addition, many of the fecal pellets contain hydrothermal particles such as barite and Fe- and Fe-Cu sulfides (Fig. 10 E and F).

On the western slope of Solwara 1, laminated gray volcaniclastic sediments overlie greenish, greenish-brown and greenish-black sediments (Fig. 5 and 6). The greenish, greenish-brown and greenish-black sediments contain larger amounts of fine-grained Si-Al- rich material (around 80 vol. %) and volcanic glass fragments but less mineral fragments than the overlying gray sediment. The clay fraction (< 4 μm) of the sediments is up to 10 wt % and is composed of smectite, chlorite and X-ray amorphous material that could be alteration products of volcanic glass. Vesicular glass fragments of andesitic composition are abundant (about 15 vol. %) in sandy intervals of greenish and greenish-black sediments (Fig. 11E and F). Some sediment also contains pumice fragments, some altered to clayey material. Fecal pellets at different stages of preservation were identified in association with the fine-grained material. The fecal pellets are better preserved in sediments from cores located closer to the mound (e.g. cores SC118 and SC116) (Fig. 10), while poorly preserved aggregates resembling fecal pellets were found in sediments away from the mound (e.g. cores SC114 and SC124) (Fig. 11A and C). Sulfides and barite constitute up to 2 vol. % of the sediments. They occur as particles and small aggregates in both fine-grained material and preserved fecal pellets, and as larger aggregates of about 200 μm

(Fig. 11). Greenish and greenish-black sediments on the western slope of Solwara 1 as deep as 80 cm contain calcareous tests of foraminifers and siliceous tests of other organisms (Fig. 11D and F).

Sediments of South Su are gray volcaniclastic sandy silts and silty sands similar to the gray volcaniclastic sediments of Solwara 1. Photographs of cores from South Su are shown in Figure 7 and BSE images in Figure 12. The sediments are composed of 45-50 vol. % fine-grained material that comprises X-ray amorphous Si- and Si-Al-rich phases, cristobalite and alunite, 25 to 35 vol. % volcanic rock fragments and glass shards, about 15 vol. % Ca plagioclase, 2 vol. % pyroxene, and 1-2 vol. % pyrite and magnetite. A large aggregate of pyrite was found in sediments of core SC-90 collected near an active chimney (Fig. 12D). Most of the cores from South Su recovered insufficient sample and did not exhibit clear layering of the sediments as was seen at Solwara 1. However, in some cases, there were recognizable greenish-brown patches that, as in Solwara 1, could be due to the presence of fecal pellets. Figure 12F, a BSE image of sediment with greenish-brown patches, shows rare aggregates of fine-grained particles that resemble fecal pellets. Greenish, greenish-brown and greenish-black sediments recovered from depth on the slopes of Solwara 1 were not found in South Su.

6.2. Geochemical composition of sediments

Concentrations of major and trace elements determined by XRF are in Table 4 and those determined by INAA are in Table 5. The concentrations of SiO_2 range from 44.9 wt % to 64.6 wt % (average 58.3 wt %), Al_2O_3 from 12.9 wt % to 15.9 wt % (average 14.9 wt %), TiO_2 from 0.5 wt % to 0.7 wt % (average 0.6 wt %), Fe_2O_3 from 5.0 wt % to 11.4 wt % (average 7.0 wt %), MnO from 0.05 wt % to 0.13 wt % (average 0.08 wt %), MgO from 1.1 wt % to 2.7 wt % (average 1.5 wt %), CaO from 3.2 wt % to 7.6 wt % (average 4.6 wt %), Na_2O from 1.8 wt % to 3.8 wt % (average 2.9 wt %)and K_2O from 0.6 wt % to 2.2 wt % (average 0.8 wt %). Lower concentrations of SiO_2 and Al_2O_3 accompanied by high amounts of H_2O are in greenish and greenish-black sediments from the western slope of Solwara 1. These concentrations are similar to those in hemipelagic sediments of the eastern Manus basin (Hrischeva et al., 2007) and is probably a result of their increased clay content.

Concentrations of SiO_2 and Al_2O_3 are high in the grey volcaniclastic sediments and are similar to their concentrations in SuSu Knolls feldspar-phyric dacite (Moss et al., 2001) and in typical silicic volcaniclastic sediments (Kyte et al., 1993). The highest CaO concentrations are in intervals of greenish and greenish-black sediments from the slope of Solwara 1 that contain calcareous tests of foraminifers. The highest concentrations of Fe_2O_3 and S are in sediment of core SC-4 that has an increased amount of sulfides.

The sediments of Solwara 1 have highly variable concentrations of Au (from 19 ppb to 2 ppm, average 350 ppb), Cu (from 177 ppm to 1 wt %, average 1015 ppm), Zn (from 35 ppm to 1333 ppm, average 176 ppm), Pb (from 7 ppm to 977 ppm, average 100), BaO (from 0.04 wt % to 2.8 wt %, average 0.194 wt %) and As (from 12 ppm to 654 ppm, average 128 ppm). The concentrations of Co (from 19 to 80 ppm, average 37 ppm), Cr (from 13 to 43 ppm, average 22 ppm), Ni (<50 ppm), Mo (<2 ppm to 15 ppm, average 4), Th (from <0.1 ppm to 1.3 ppm, average 0.8 ppm) and U (from <0.1 ppm to 8.1 ppm, average 2 ppm) are relatively low and less variable. Distributions of the concentrations of Cu, Zn, Pb, Ba and As in sediments down to a depth of 50 cm in three profiles along the southern, central and northern part of Solwara 1 (profiles are shown in Fig. 2) are illustrated in Figures 13A, 14A, 15A, 16A and 17A. Much of the gray volcaniclastic sediments of Solwara 1 have background concentrations of Cu between 177 and 500 ppm, which are higher than average Cu in hemipelagic sediments of eastern Manus basin (81 to 124 ppm; Hrischeva et al., 2007), in hemipelagic Pacific sediments from other areas (20 to 75 ppm; Goodfellow and Peter, 1991) and in typical volcaniclastic sediments (107 to 140 ppm; Cronan et al., 1984). Background concentrations of both Cu and Au are higher than in the SuSu Knolls dacite (104 to 151 ppm Cu and 7 to 9 ppb Au; Moss et al., 2001).

The background concentrations of Zn (up to 100 ppm), Pb (up to 50 ppm) and Ba (up to 0.1 wt %) in a large part of the gray volcaniclastic sediments of Solwara 1 are comparable to the average concentrations of these elements in hemipelagic sediments. Concentrations of metals that exceed the background values for Solwara 1 outline patchy anomalies within the sediments of the mound. A local strong anomaly in metal concentrations is found in sediment containing fragments of chimney sulfides and barite from the 23 to 33 cm depth interval of core SC-4 (Fig. 9). The sediment shows strong co-enrichments of Au (up to 2 ppm), Cu (up to 1 wt %), Ba (up to 2.8

wt %) and As (up to 654 ppm) and also has high concentrations of Zn (589 ppm) and Pb (422 ppm). More widespread anomalies of Au (up to 2.3 ppm), Cu (up to 4960 ppm), Zn (up to 1333 ppm), Pb (up to 977 ppm), Ba (up to 1.1 wt %) and As (up to 268 ppm) occur in greenish and greenish-black sediments from the western rim and slope of Solwara 1 (Fig. 5 and Fig. 6). The anomalies can be traced down to a depth of 80 cm and approximately 350 m away from the rim of the mound. Anomalous concentrations of metals are also detected in greenish-brown and black layers in gray volcaniclastic sediments from the western limb and western rim of the mound (Fig. 4 and Fig. 6). A local strong anomaly of Ba is in sediments of core SC-33.

The surface (0-2 cm) sediment covering Solwara 1 was studied in order to assess the contribution of fallout of hydrothermal particles from the present-day particulate plume. The distributions of Cu, Zn, Pb, Ba and As in the surface sediment outline weak patchy anomalies (Fig. 13B, 14B, 15B, 16B and 17B). In much of the surface sediment, concentrations of Cu, Zn, Pb, Ba and As are around the background values for the sediments of the mound. Co-enrichments of metals and As are found in two sites (cores SC-28 and SC-83) that are near chimneys venting black smoke.

At South Su, we were able to study only the surface sediment from 0-2 cm and 0-5 cm because, in most cases, the cores retrieved insufficient sample. In other cases, where sediment was collected from deeper intervals, it was a monotonous gray volcaniclastic without clear layering (Fig. 7). The goal of studying the topmost sediment was to assess the contribution of plume fallout in the metal content of the sediments. The major element composition of the volcaniclastic sediments of South Su is similar to that of gray volcaniclastic sediments of Solwara 1, which reflects their common mineral composition (Table 4). Distributions of concentrations of Cu, Zn, Ba, Pb and As in surface sediments of South Su are shown in Figures 18 and 19. In the majority of the surface sediment of South Su, the concentrations of Cu are from 156 to 308 ppm, Zn from 32 to 262 ppm, Pb from not detected to 47 ppm, Ba from 0.04 to 0.1 wt % and As from 21 ppm to 94 ppm (Table 4). These values are comparable to the background values of metals and As in gray volcaniclastic sediments of Solwara 1. A local anomaly in concentrations of Cu (1274 ppm), Zn (235 ppm), Pb (89 ppm) and Ba (0.4 wt %) was found for one sample taken at the foot of an active chimney. This enrichment could be a result of hydrothermal particles that were settled from the black smoke and/or eroded from the chimney.

7. Discussion

Both Solwara 1 and South Su are covered by an apron of gray volcanic sandy silts and silty sands. The sediment cover over Solwara 1 is thick and, in parts of the mound, blocks of chimneys and volcanic rocks are buried under sediment. The sediment cover over South Su is thinner than that over Solwara 1 and, in a large part of the steep slopes of South Su, fine volcaniclastic sediments are associated with coarse volcanic blocks. The grey volcaniclastic sediments of Solwara 1 and South Su have similar mineral compositions that suggest a common source. They are composed of angular fragments of volcanic rock, Ca plagioclase and pyroxene that are equivalent to the plagioclase-pyroxene porphyritic dacite lavas building North Su and South Su (Binns, 2004). The sediments also contain cristobalite, alunite, pyrite and barite that are typical products of subsea-floor hydrothermal alteration and mineralization of volcanic rocks and were found in altered volcanics in the eastern Manus basin (Yeats et al., 2000). Binns (2004) proposed derivation of components of the volcaniclastic sediment at SuSu Knolls from violent hydrothermal eruptions at North Su and South Su. Our results support this interpretation. The gray volcaniclastic sediments at Solwara 1 and South Su exhibit characteristics similar to those of volcaniclastic deposits interpreted to be a product of phreatic eruptions (Heiken and Wohletz, 1985), including those occurring at or below 1350 m water depth (Clague et al., 2003). The ash produced from these eruptions consists of fragments from vent walls and crater fields, including hydrothermal alteration products. The volcaniclastic sediment could have been emplaced as submarine ash falls or turbidity currents.

At Solwara 1, much of the gray volcaniclastic sediments contain greenish-brown and black patches and layers that have fecal pellets. Unlike Solwara 1, the volcaniclastic sediments at South Su do not exhibit clear layering. The reason for this could be that the sediment cover at South Su is thinner and we were not able to retrieve sufficient amount for study. A characteristic feature of Solwara 1 is the occurrence of greenish, greenish-brown and greenish-black sediments on the western rim and slope of the mound that are covered by gray volcaniclastic sediments. The greenish, greenish-brown and greenish-black sediments contain up to 10 % clay component and fecal pellets at different stages of preservation. It is suggested that part

of these sediments is of biogenic origin. Probably the sediments were originally deposited near chimney fields where there was an abundance of organisms producing fecal pellets. The organisms were ingesting sediment particles including hydrothermal particles. The fact that the fecal pellets contain dominantly lithic particles indicates that they were produced by benthic deposit-feeding organisms. The fecal pellets in the sediments of Solwara 1 are similar to those produced by benthic worms (Cuomo and Bartholomew, 1991). The sediments were later transported and re-deposited on the slopes of the mound together with glass fragments. The absence of well preserved fecal pellets in sediments away from the mound could be a result of their destruction during the down-slope transportation.

Our previous study of an 80 cm long gravity core (MS-36) close to the western rim of Solwara 1 showed the occurrence of Cu- and Au-rich metalliferous sediment that contains glass fragments, amorphous Si-rich material, pyrite, chalcopyrite, barite, gypsum and atacamite (Hrischeva et al., 2007). The highly metalliferous sediment was recovered below 39 cm depth and was overlaid by gray volcaniclastic sediment. We proposed that the metalliferous sediment originated from erosion of old oxidized sulfide chimneys and is an indicator for proximity to a chimney field. Similar metalliferous sediments may have broader distribution in depth along the western rim of Solwara 1. During the Luk Luk 07 cruise, we attempted to collect 1m long push cores from the western rim of the mound in the hope of recovering such metalliferous sediment. However, the attempts were not successful as the maximum recovered depth was 28 cm. A more robust gravity coring operation is required.

The gray volcaniclastic sediments of Solwara 1 and South Su have similar major element compositions that are analogous to that of the feldspar-phyric dacite at SuSu Knolls (Moss et al., 2001). The gray volcaniclastic sediments also have comparable background values of trace metals and As. The background concentration of Cu in the sediments of both Solwara 1 and South Su is higher than average Cu in hemipelagic sediments of eastern Manus basin (Hrischeva et al., 2007), in hemipelagic Pacific sediments from other areas (Goodfellow and Peter, 1991) and in typical volcaniclastic sediments (Cronan et al., 1984). Copper and Au have background concentrations that are significantly higher than that in the SuSu Knolls dacite (Moss et al., 2001). A source for this anomalous Cu and Au is probably

products of volcanic rock alteration and mineralization in the volcaniclastic sediments. The background concentrations of Zn, Pb, Ba, Co, Cr, Ni and Mo in the gray volcaniclastic sediments of Solwara 1 and South Su are comparable to the average concentrations of these elements in hemipelagic sediments. Much of the studied volcaniclastic sediments of South Su are not enriched in trace metal above the background levels. In contrast, the volcaniclastic sediments of Solwara 1 show patchy anomalies in the concentrations of trace metals and As. We suggest that there are two major sources of metal anomalies: dispersal of hydrothermal particles from eroded chimneys and deposition of hydrothermal particles within fecal pellets. Fallout from particulate plumes appears to be minor. A strong anomaly created by dispersal of sulfides and barite that may have been derived from erosion of proximal chimneys was found at a depth below 25 cm in core SC-4 from the southeastern rim of Solwara 1. In most cases, though, volcaniclastic sediments as deep as 25 cm that are proximal to chimneys and chimney fragments do not exhibit metal anomalies created by dispersal of chimney fragments. Probably, anomalies created by mass wasting of chimneys are more extensive in deeper intervals of sediments from the western rim of the mound, as suggested by our previous study (Hrischeva et al., 2007). More widespread but weaker metal anomalies in greenish, greenish-brown and greenish-black sediments from the western slope of Solwara 1 appear to have been produced by dispersal of sulfide and barite particles from chimneys and biogenic deposition of hydrothermal particles within fecal pellets. Organisms inhabiting areas close to venting are another factor contributing to the metal enrichment of the sediments of Solwara 1. The presence of hydrothermal particles in fecal pellets has created small scale metal anomalies in black and brownish-green patches of grey volcaniclastic sediments.

The study showed that the current plume from local venting at Solwara 1 and South Su is not producing obvious, extensive anomalies in metal concentrations over much of the surface sediments. Only local metal enrichments in sediments of two sites (cores SC-28 and SC-83) near active vents at Solwara 1 could be a result of settling of hydrothermal particles from distinct black smoker plumes. The lack of a clear signal from plume fallout could be attributed to recent deposition of volcaniclastic material. Hemipelagic sediments, indicative of long-time exposure to the water column, were not recovered during the sampling in 2006 and 2007. Sediments as far as

approximately 350 m to the west of Solwara 1 contain volcaniclastic and biogenic components that were re-deposited from the mound. Therefore, we do not have data on concentrations of trace metals in hemipelagic sediments from the far-field area surrounding Solwara 1 for making a pre- vs post-mining comparison. However, the background concentrations of metals, except for Cu, in the volcaniclastic sediments of Solwara 1 and South Su are comparable to metal concentrations in hemipelagic sediment approximately 10 km east of the SuSu Knolls (Hrischeva et al., 2007) and could be referred to as pre-mining concentrations of metals in sediments from a broader area surrounding the SuSu Knolls.

8. Conclusions

Solwara 1 and South Su are covered by an apron of volcaniclastic sediments. The dark grey volcaniclastic sediments of Solwara 1 and South Su are composed of volcanic rock fragments, Ca plagioclase, pyroxene, glass shards, amorphous Si-rich phases, cristobalite, alunite, pyrite, barite and magnetite and have a common origin. Our results support the interpretation of Binns (2004) that the source of the volcaniclastic material was likely hydrothermal eruptions at North Su and South Su. On the western rim and slope of Solwara 1, grey volcaniclastic sediments overly greenish, greenish-brown and greenish-black sediments that contain volcaniclastic material and fecal pellets at different stages of preservation. We suggest that some of the components of these sediments have a biogenic origin being originally deposited near hydrothermal vents as fecal pellets by organisms that ingested sediment and hydrothermal particles. In a later event, the biogenic sediment was transported and re-deposited down-slope together with glass fragments and rare barite and sulfide aggregates possibly derived from chimneys.

The grey volcaniclastic sediments of Solwara 1 and South Su have a similar major element composition and background levels of trace metals. The background concentrations of Zn, Pb, Ba, Co, Cr and Ni are comparable to the average concentrations of these elements in hemipelagic sediments. The background concentrations of Cu are higher than the average Cu in hemipelagic and volcaniclastic sediments likely as a result of the presence of products of volcanic rock alteration and mineralization in the gray volcaniclastic sediments.

Distributions of metal concentrations that are above background create patchy anomalies in the volcaniclastic sediments. Pronounced metal anomalies were found in Solwara 1 in sediment intervals deeper than 25 cm. We suggest that there are two major sources of metal anomalies: dispersal of hydrothermal particles from eroded chimneys and deposition of hydrothermal particles within fecal pellets. A strong metal anomaly created by dispersion of chimney sulfides and barite was found only locally in sediments from the southern rim of Solwara 1. More widespread metal anomalies in sediments from the western slope of Solwara 1 likely resulted from deposition of sulfides and barite within fecal pellets and derived from chimneys. With the exception of local weak anomalies in surface sediments around active chimneys, the current particulate plume emanating from black smokers at Solwara 1 and South Su does not leave a clear signal in the sediments.

Acknowledgement

This research was funded by a grant from Nautilus Minerals Inc. and the Natural Sciences and Engineering Research Council of Canada (NSERC).

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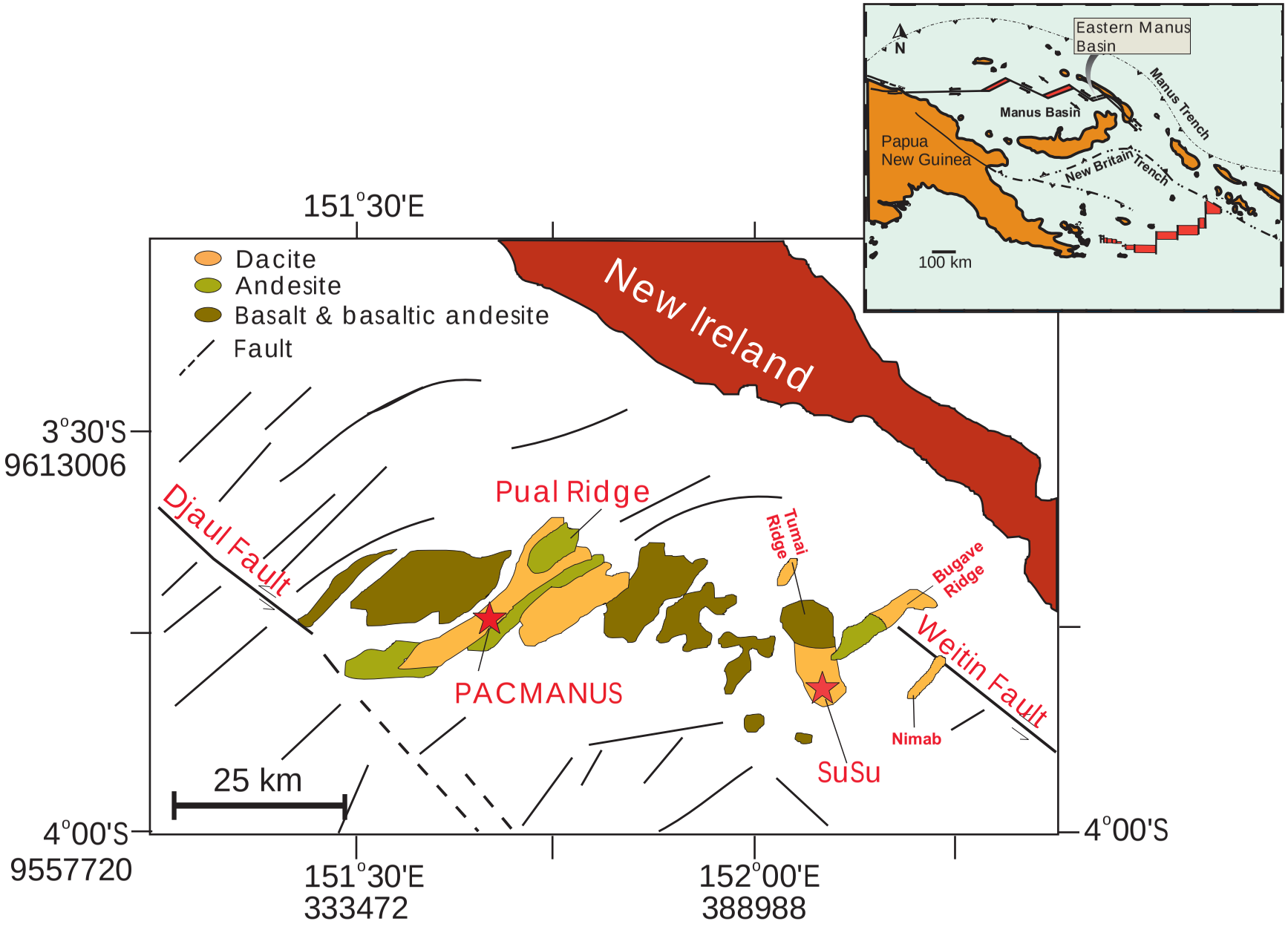

Figure 1. Regional setting (inset) after Benes et al. (1994) and geology of the eastern Manus back-arc basin after Binns et al. (1995).

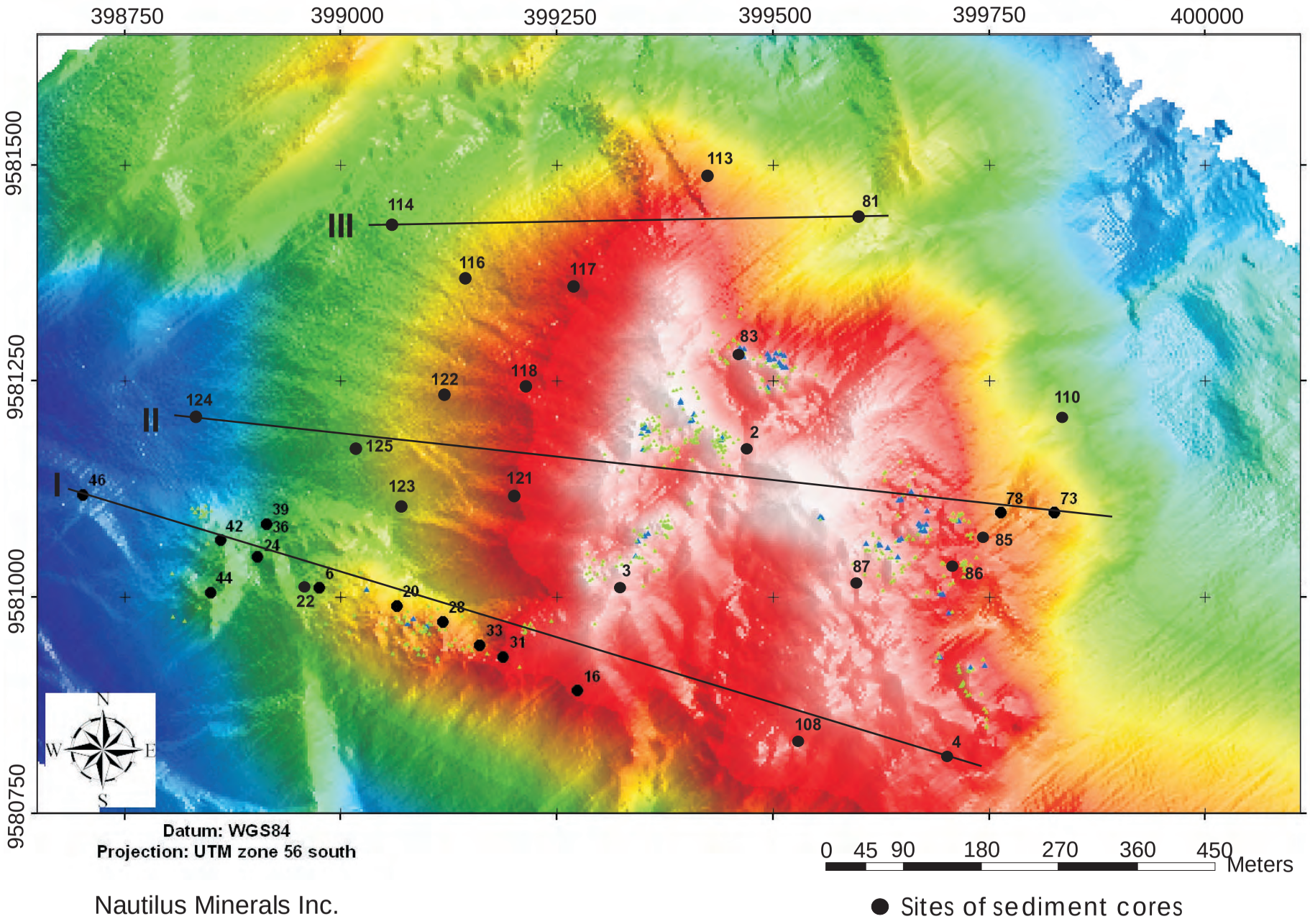

Figure 2. Map of Solwara 1 and locations of sediment cores. Lines are profiles I, II and III. Core numbers have been abbreviated from SC-## to ## for better presentation.

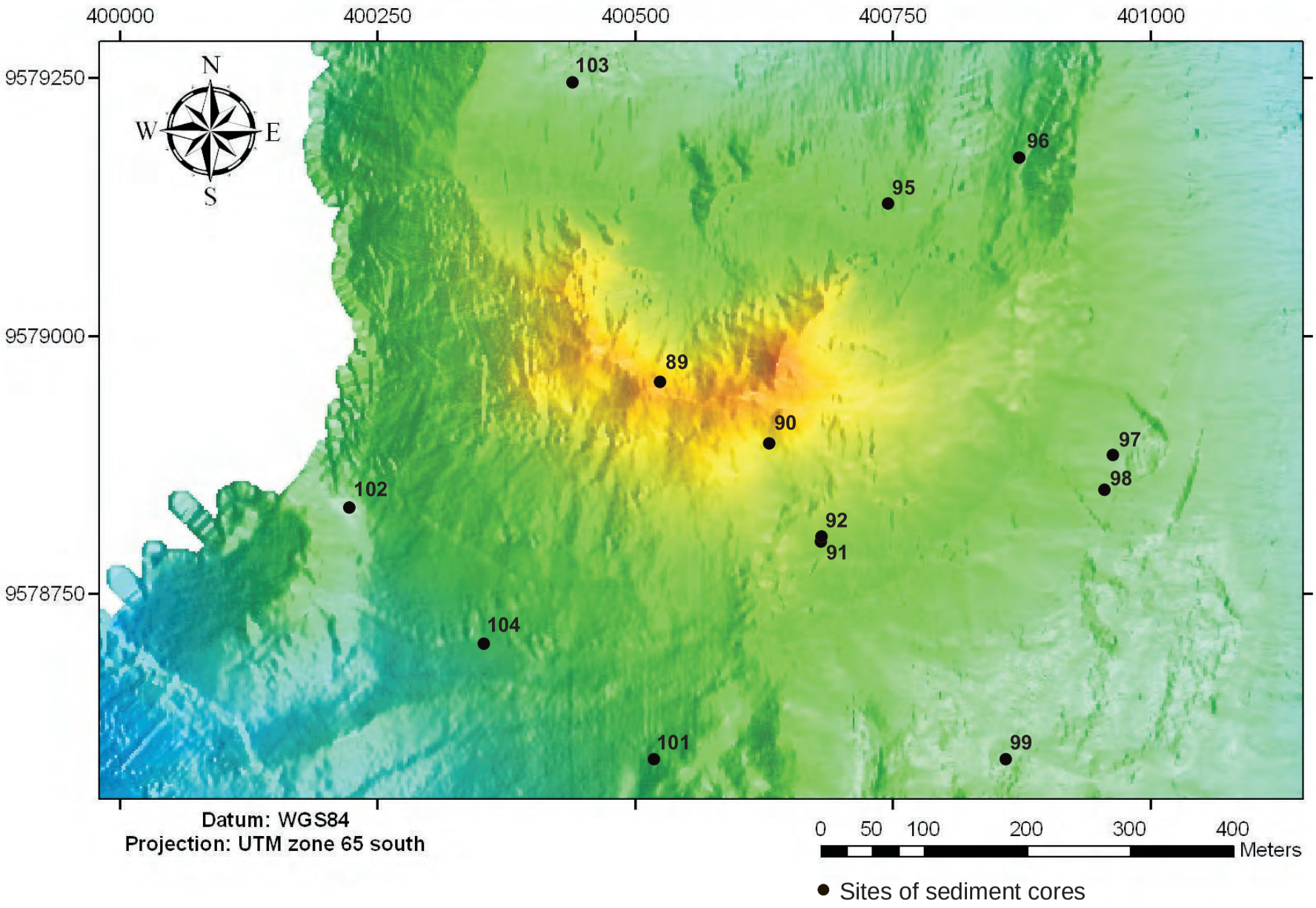

Figure 3. Map of South Su and locations of sediment cores. Core numbers have been abbreviated from SC-## to ##.

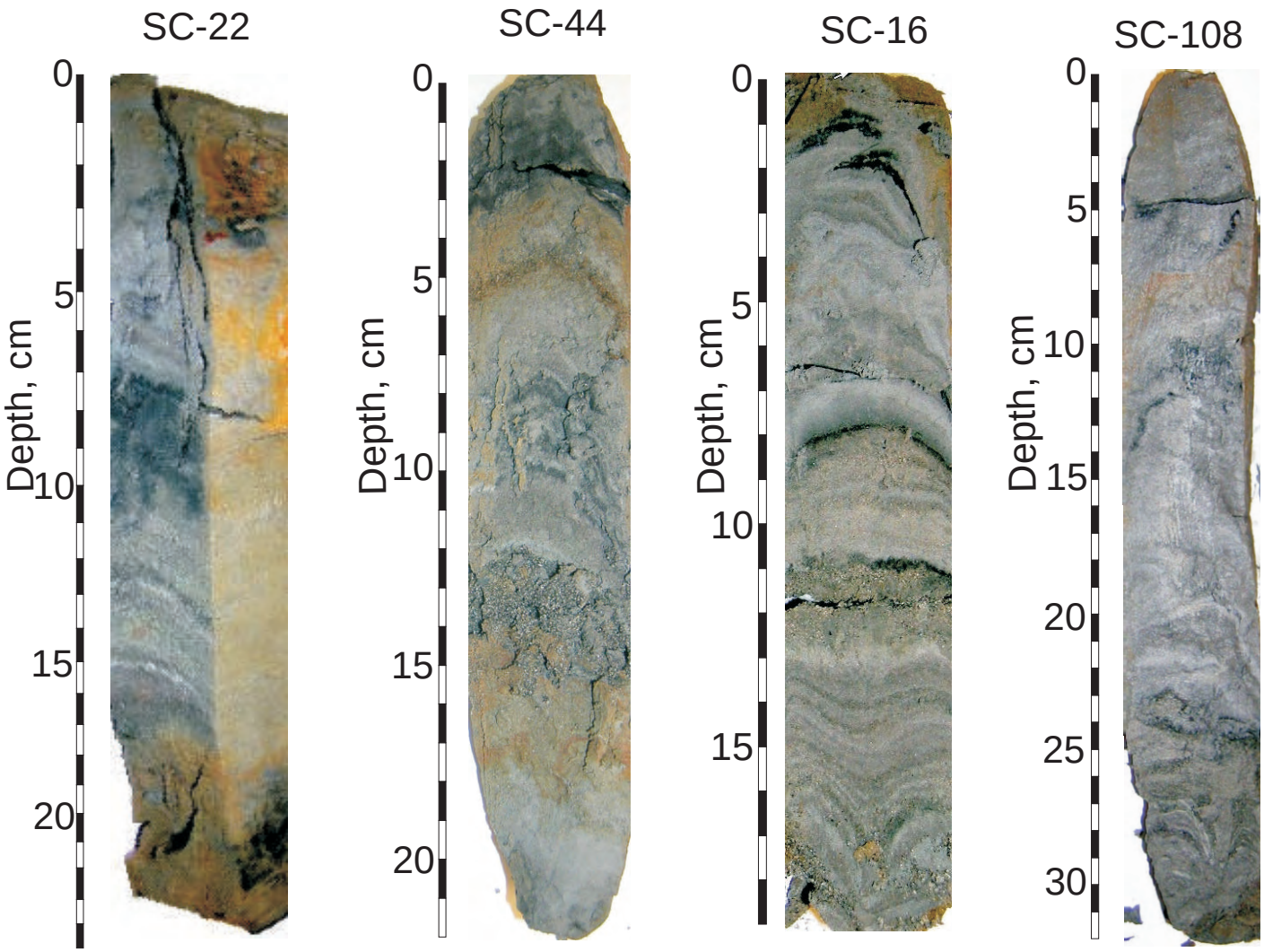

Figure 4. Photographs of cores SC-22, SC-44, SC-16 and SC-108 located along profile I in Fig. 2.

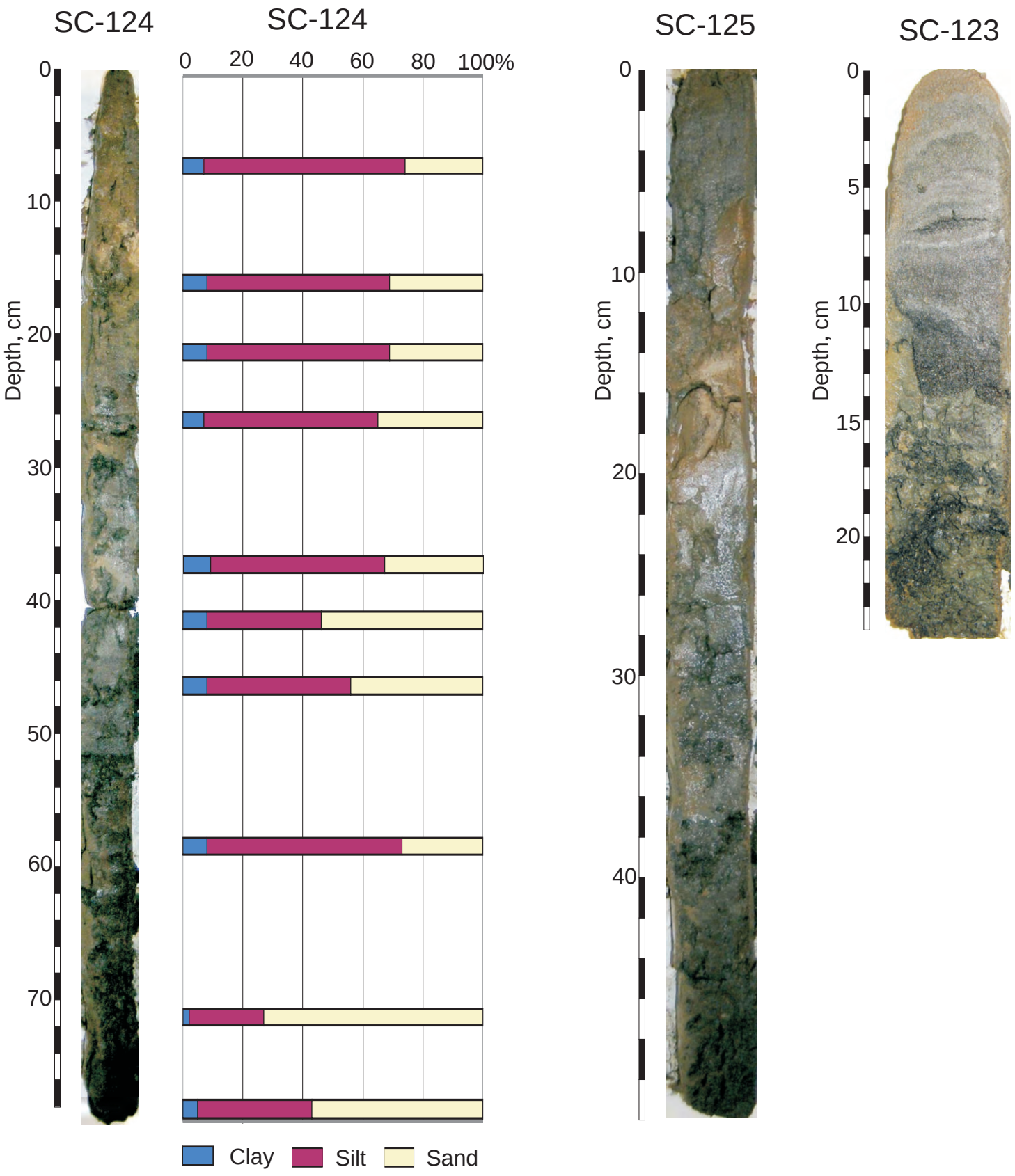

Figure 5. Photographs of cores SC-124, SC-125 and SC-123 located along profile II in Fig. 2 and grain-size composition of sediments of core SC-124.

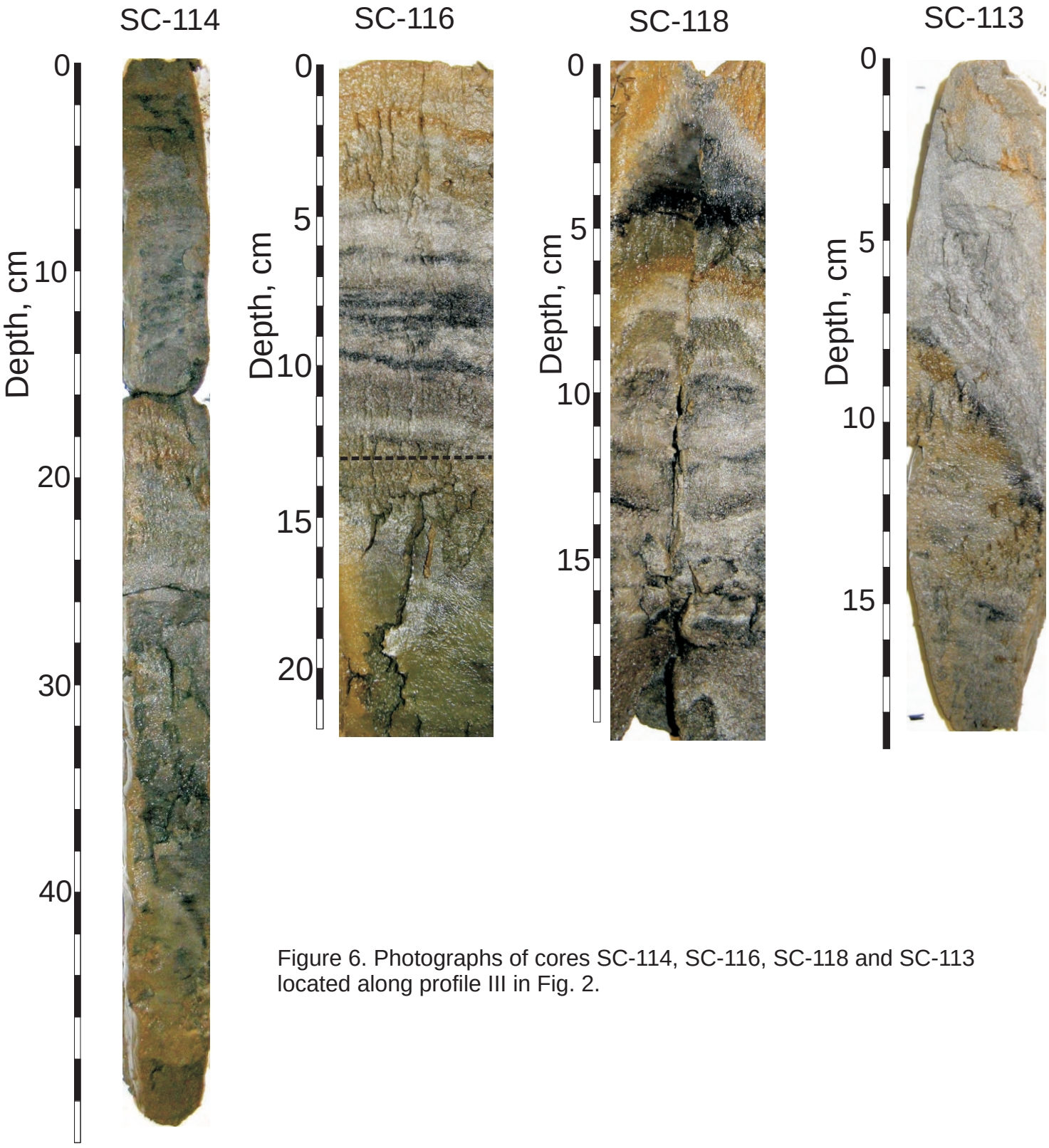

Figure 6. Photographs of cores SC-114, SC-116, SC-118 and SC-113 located along profile III in Fig. 2.

SC-89

SC-98

SC-99

Figure 7. Photographs of sediment cores taken from South Su. Scale is in inches and centimeters.

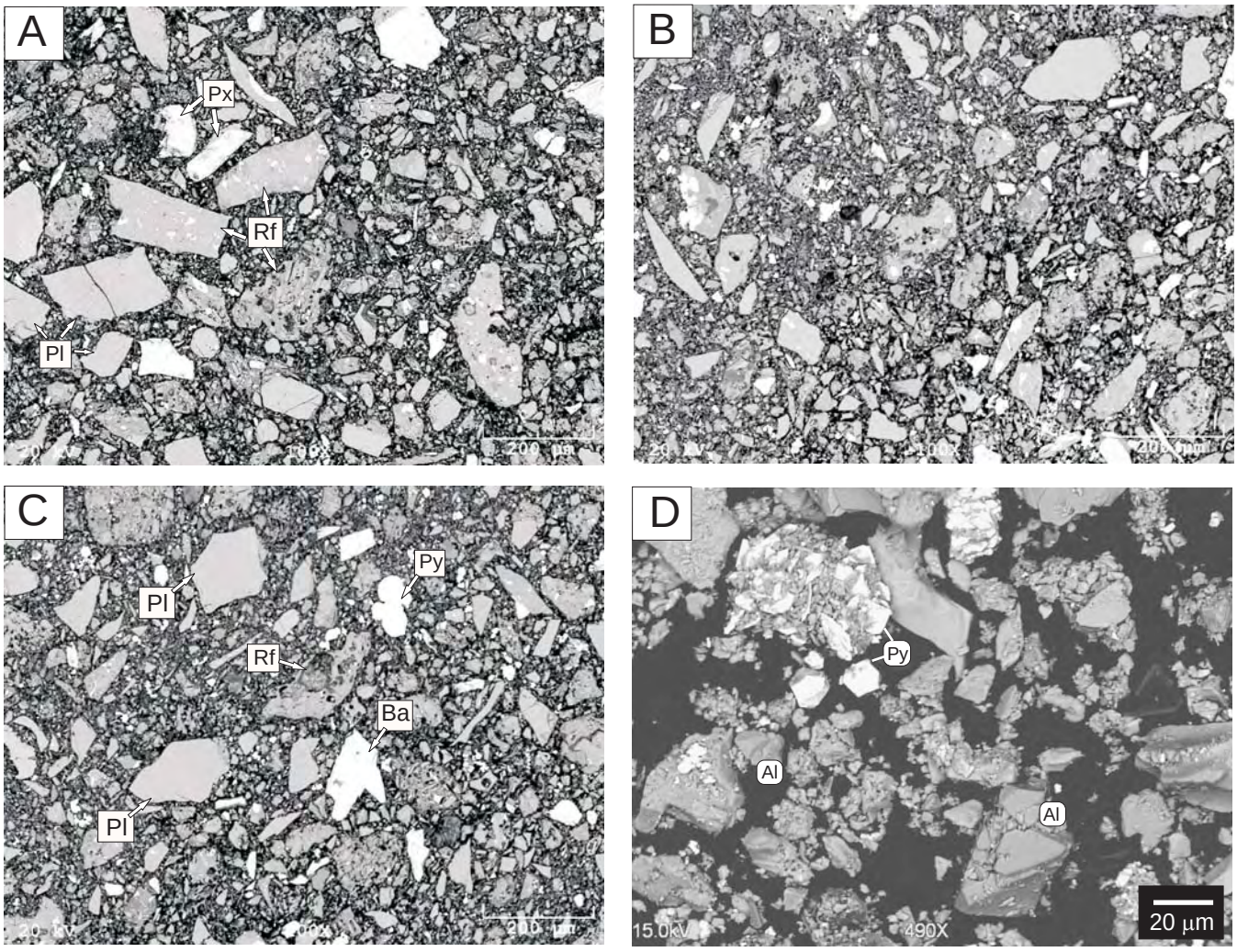

Figure 8. BSE images of sediments of Solwara 1. A, B and C. Images of gray volcaniclastic sediment of core SC-118 showing volcanic rock fragments (Rf), angular fragments of plagioclase (Pl) and pyroxene (Px), pyrite (Py), barite (Ba) and fine-grained material. D. An image of gray volcaniclastic sediment showing pyrite (Py) as disseminated euhedral crystals, aggregated with Si-rich material and as a replacement in volcanic rock fragments. Darker grains are euhedral alunite crystals (Al) and volcanic glass.

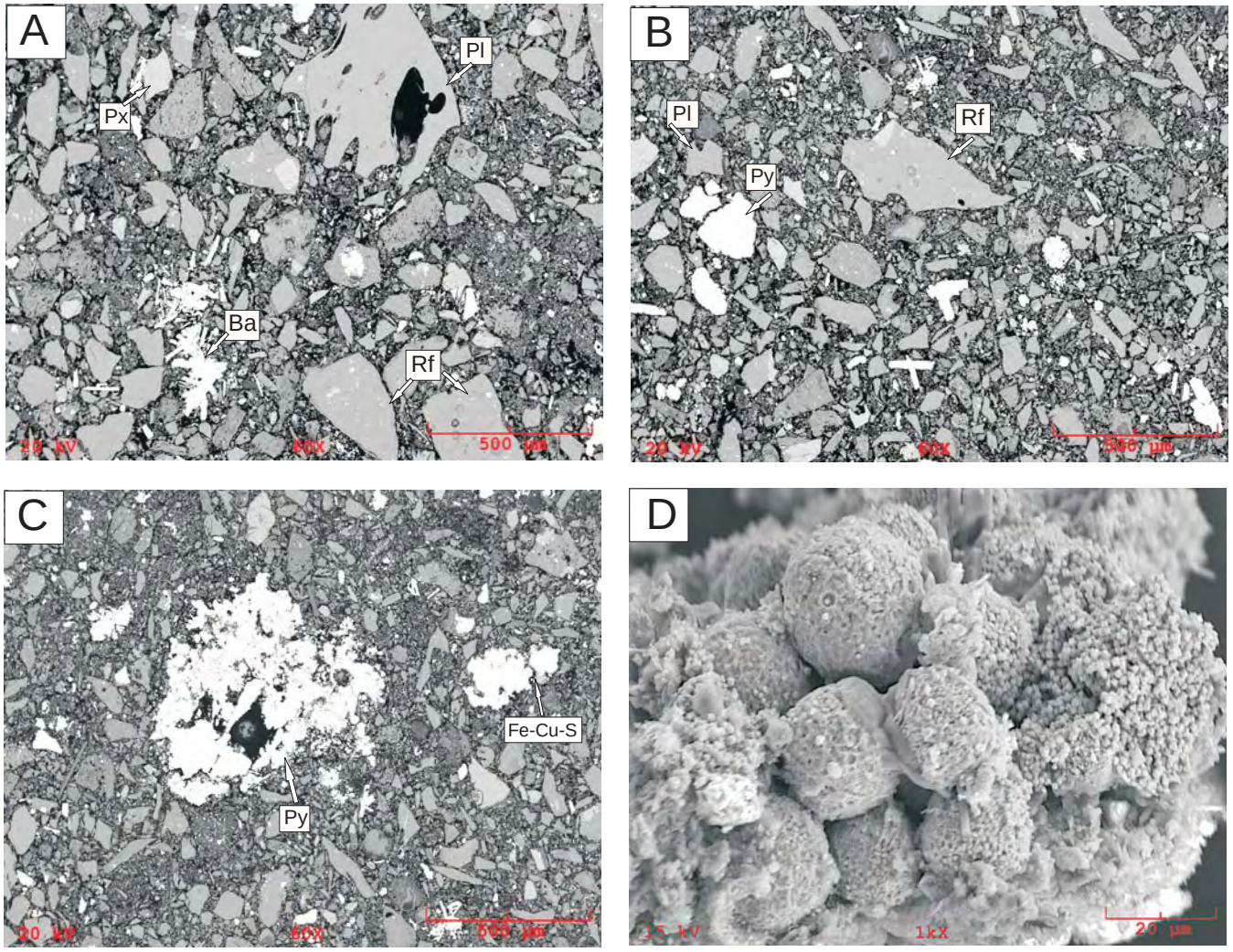

Figure 9. BSE and SE images of grayish-black volcaniclastic sediment of core SC-4 from Solwara 1. A and B. Images showing fragments of volcanic rock (Rf), plagioclase (Pl) and pyroxene (Px), aggregates of barite (Ba) and pyrite (Py) and fine-grained material. C. Aggregates of pyrite (Py) and Fe-Cu-sulfide (Fe-Cu-S). D. SE image of pyrite aggregate composed of globules and framboids.

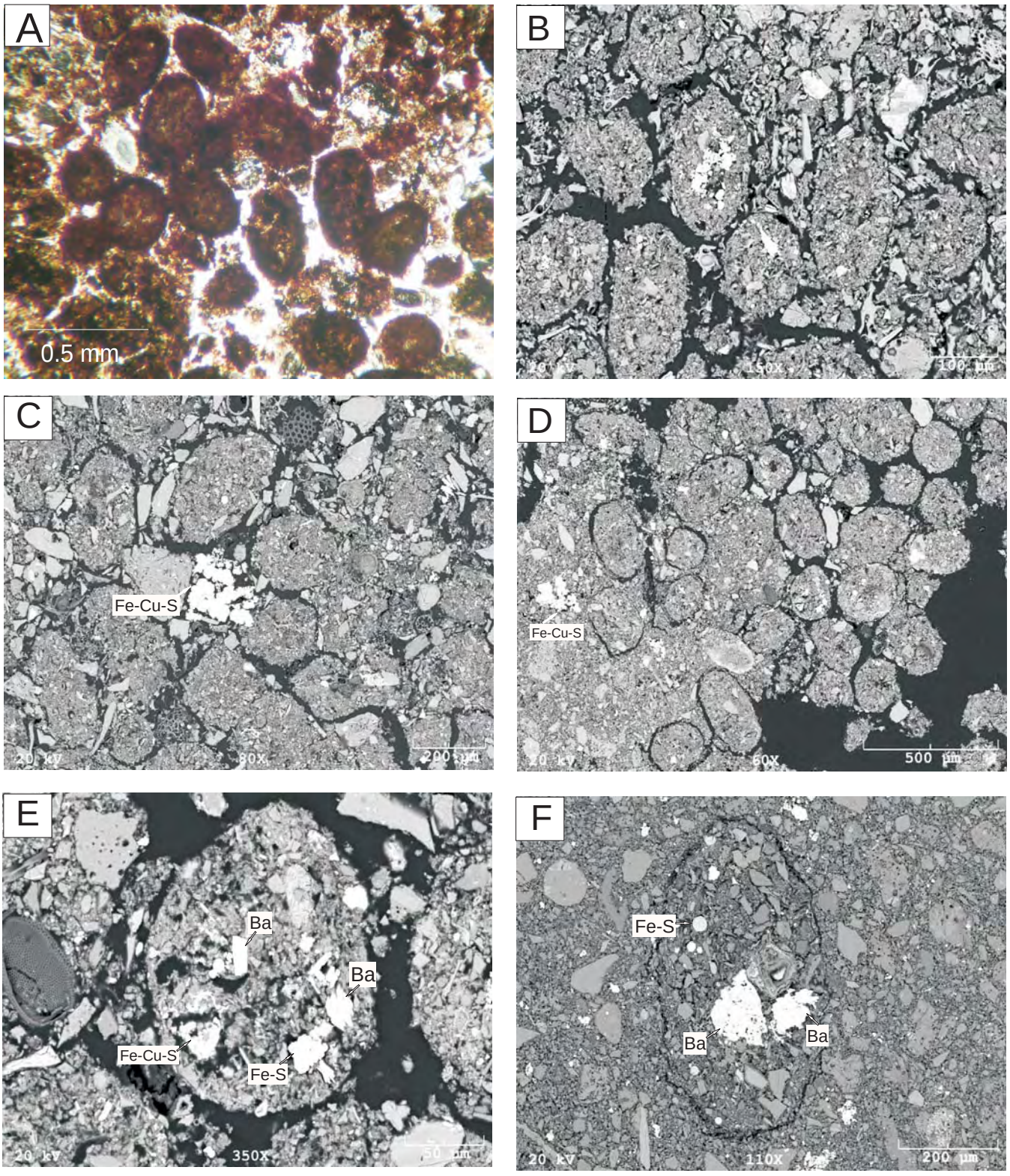

Figure 10. Images of fecal pellets in sediments of Solwara 1. A. Light microscopy image of fecal pellets. B. BSE image of fecal pellets containing hydrothermal particles (bright particles) in greenish sediment of core SC-116. C and D. BSE images of fecal pellets and aggregates of Fe-Cu-sulfides (Fe-Cu-S) in a black patch of gray volcaniclastic sediment of core SC-118. E. An enlarged image of D showing fecal pellet containing barite (Ba), Fe sulfide (Fe-S) and Fe-Cu sulfide (Fe-Cu-S) particles. F. BSE image of fecal pellet containing barite and Fe-sulfide from a black layer in gray volcaniclastic sediment of core SC-33.

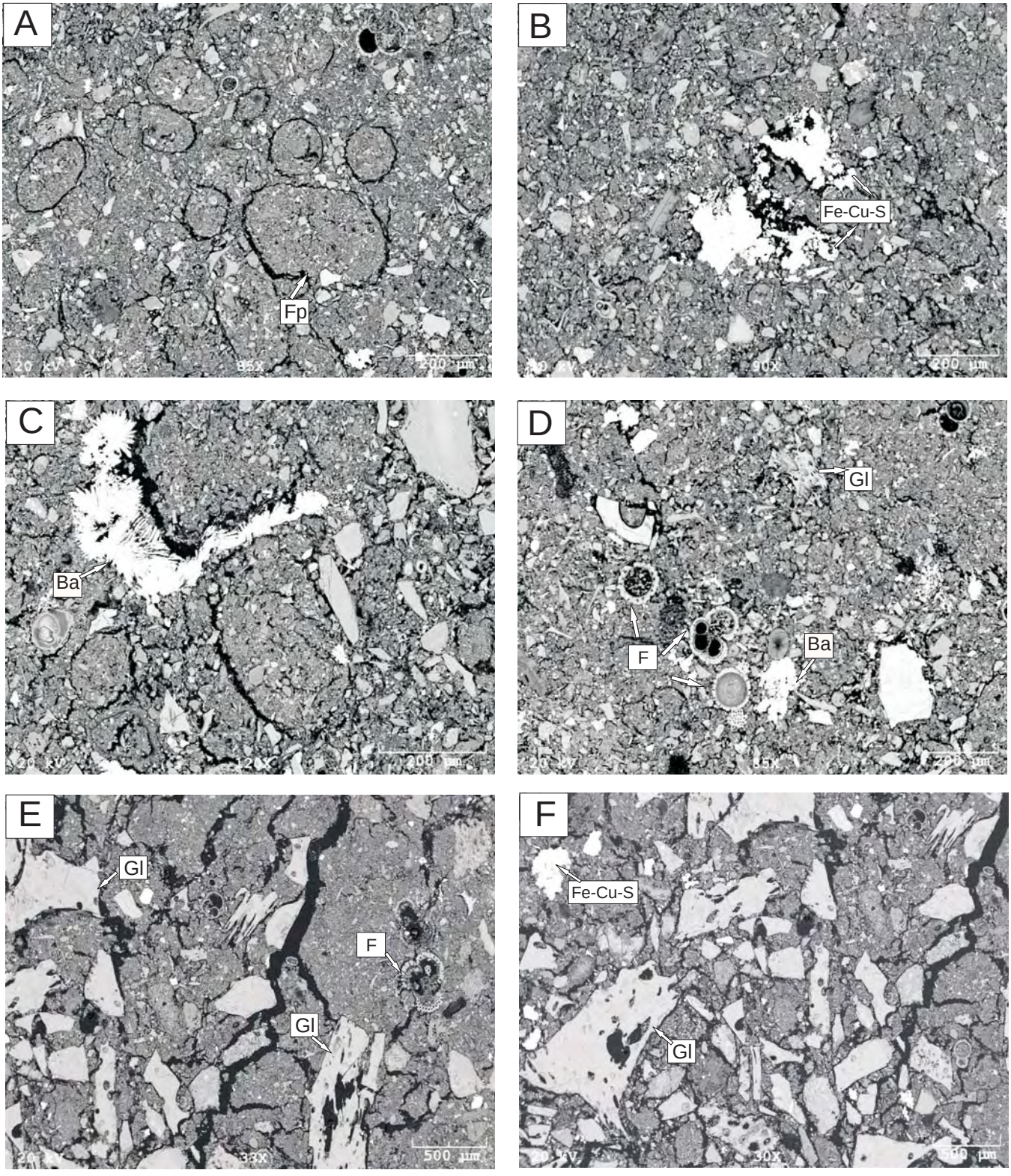

Figure 11. BSE images of greenish-black sediment of core SC-124 from Solwara 1. A. An image showing fecal pellets at different stages of preservation in fine-grained sediment. The bright particles are barite and sulfides. B. An aggregate of Fe-Cu sulfide (Fe-Cu-S) in fine-grained sediment. C. An aggregate of barite (Ba) associated with aggregates of fine-grained material that could be poorly preserved fecal pellets. D. Foraminifera's tests (F), barite aggregates (Ba) and glass shards (Gl) in fine-grained sediment. E and F. Images of sandy volcaniclastic sediment from the lower intervals of core SC-124 composed of vesicular glass fragments (Gl) and fine-grained matrix. The sediment also contains aggregates of Fe-Cu sulfide and tests of foraminifera (F).

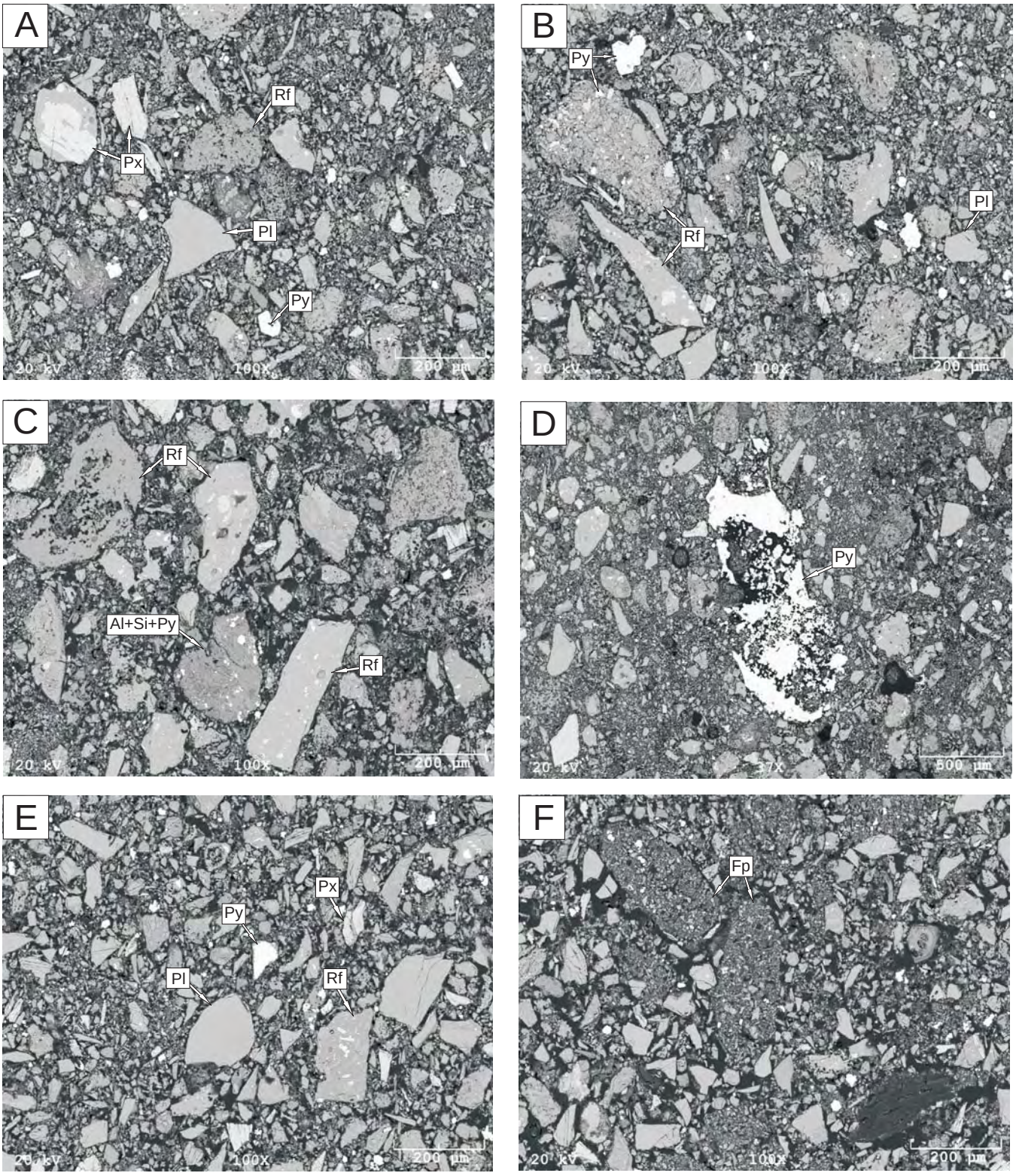

Figure 12 . BSE of grey volcaniclastic sediments of South Su. A and B. Images of volcaniclastic sediment of core SC-89 showing fragments of volcanic rocks (Rf), plagioclase (Pl) and pyroxene (Px), pyrite (Py) and fine-grained material. C and D. Images of volcaniclastic sediment of core SC-90 showing large volcanic rock fragments (Rf) and an aggregate of alunite-amorphous Si-pyrite (Al+Si+Py) (C) and a large pyrite aggregate (D). E and F. Images of volcaniclastic sediment of core SC-98 showing fragments of volcanic rocks(Rf), plagioclase (Pl), pyroxene (Px) and pyrite (Py) and fine-grained material (E) and aggregates of fine-grained material that may be fecal pellets (Fp) (F).

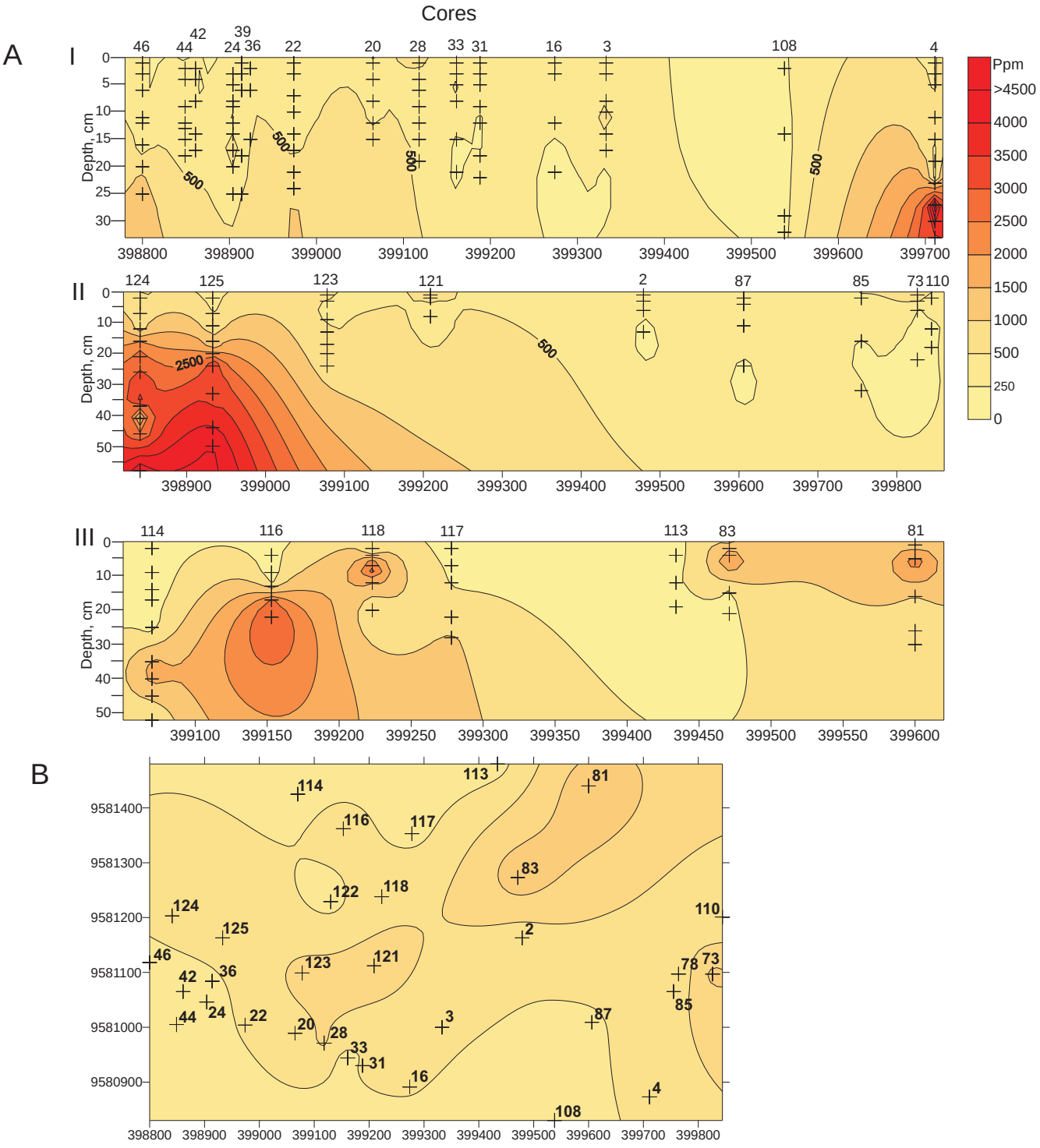

Figure 13. Distribution of Cu in sediments in profiles I, II and III in Fig. 2 (A) and in surface sediments (B) of Solwara 1. Sample locations are shown as crosses.

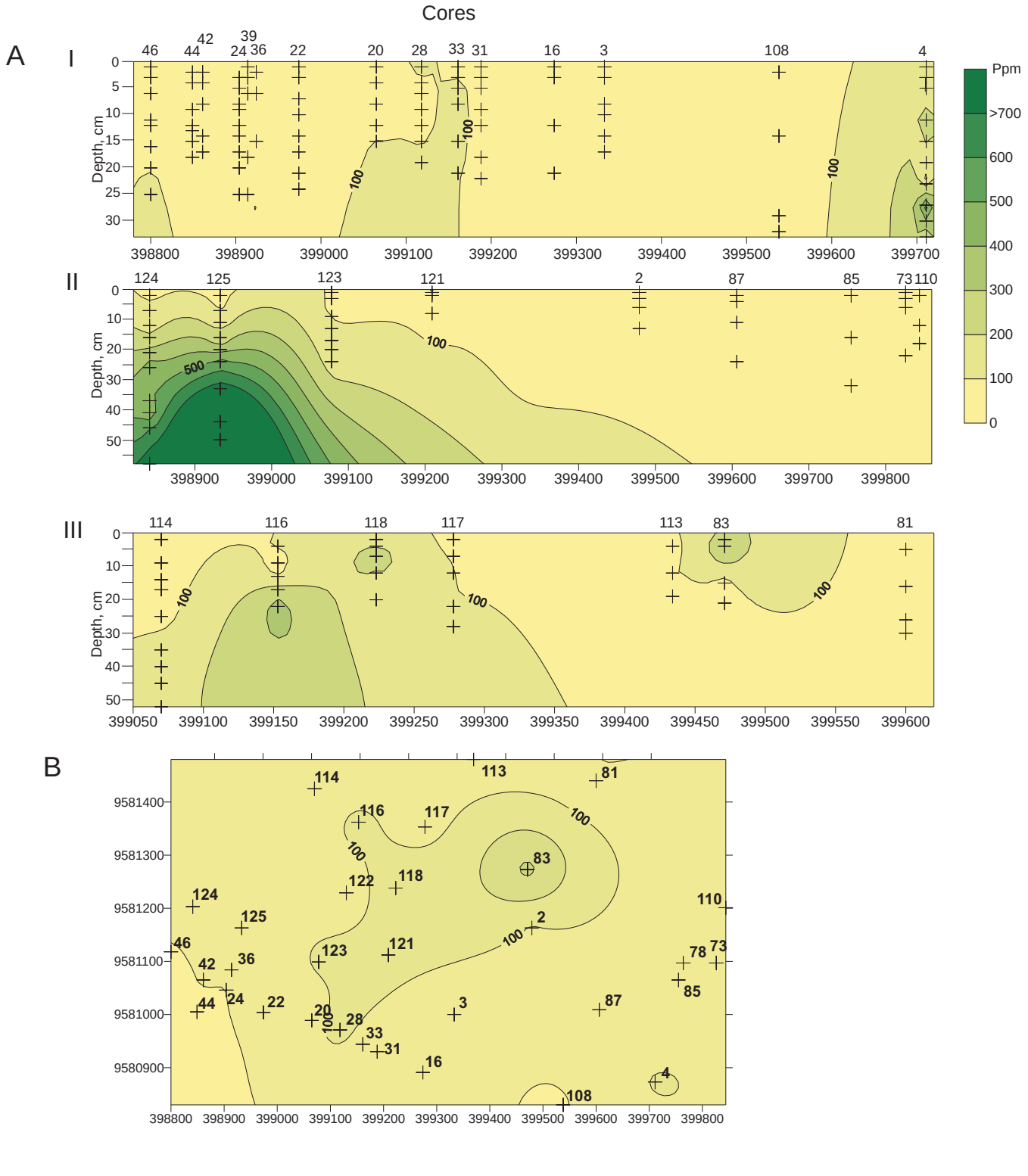

Figure 14. Distribution of Zn in sediments in profiles I, II and III in Fig. 2 (A) and in surface sediments (B) of Solwara 1.

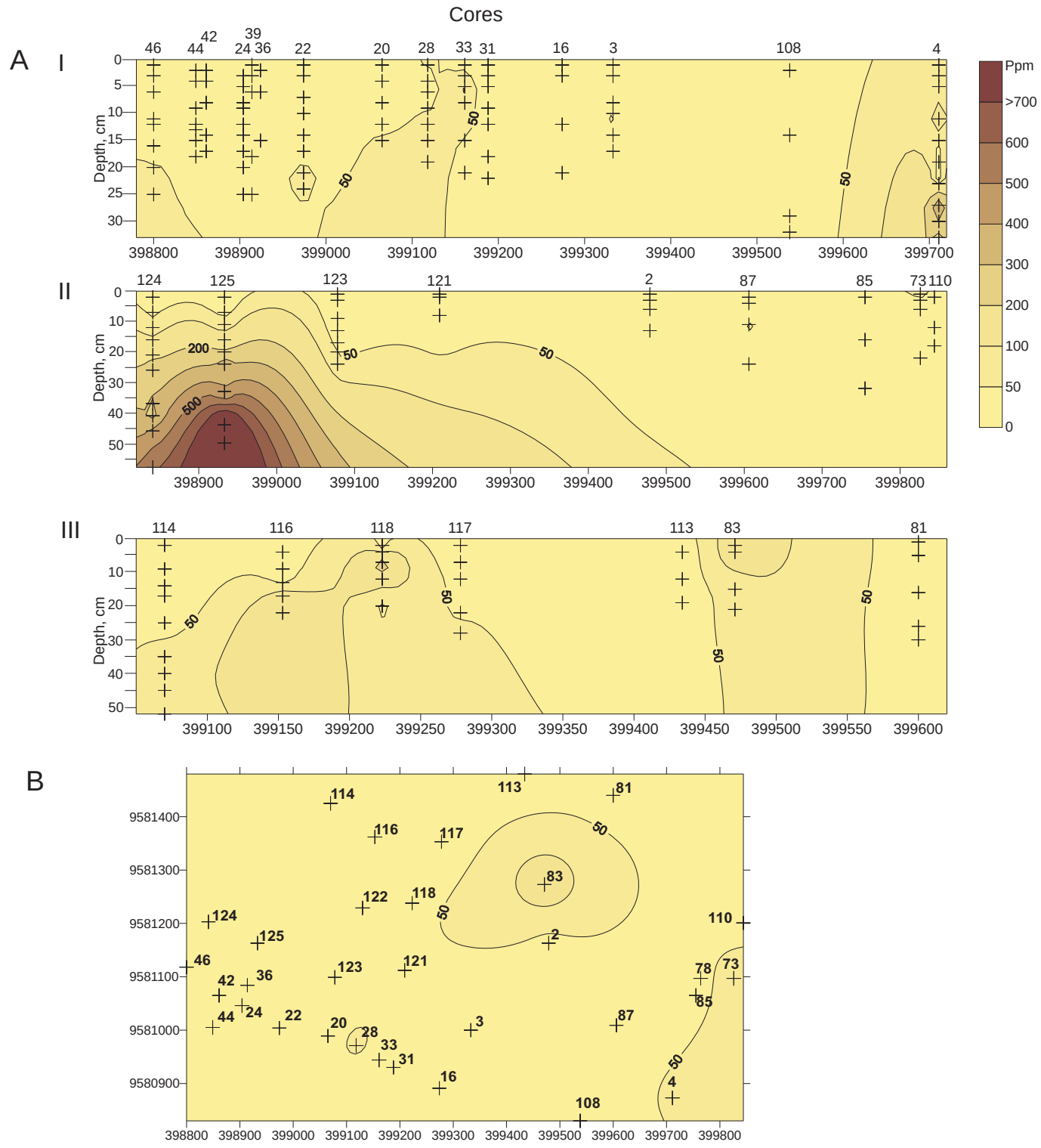

Figure 15. Distribution of Pb in sediments in profiles I, II and III in Fig. 2 (A) and in surface sediments (B) of Solwara 1.

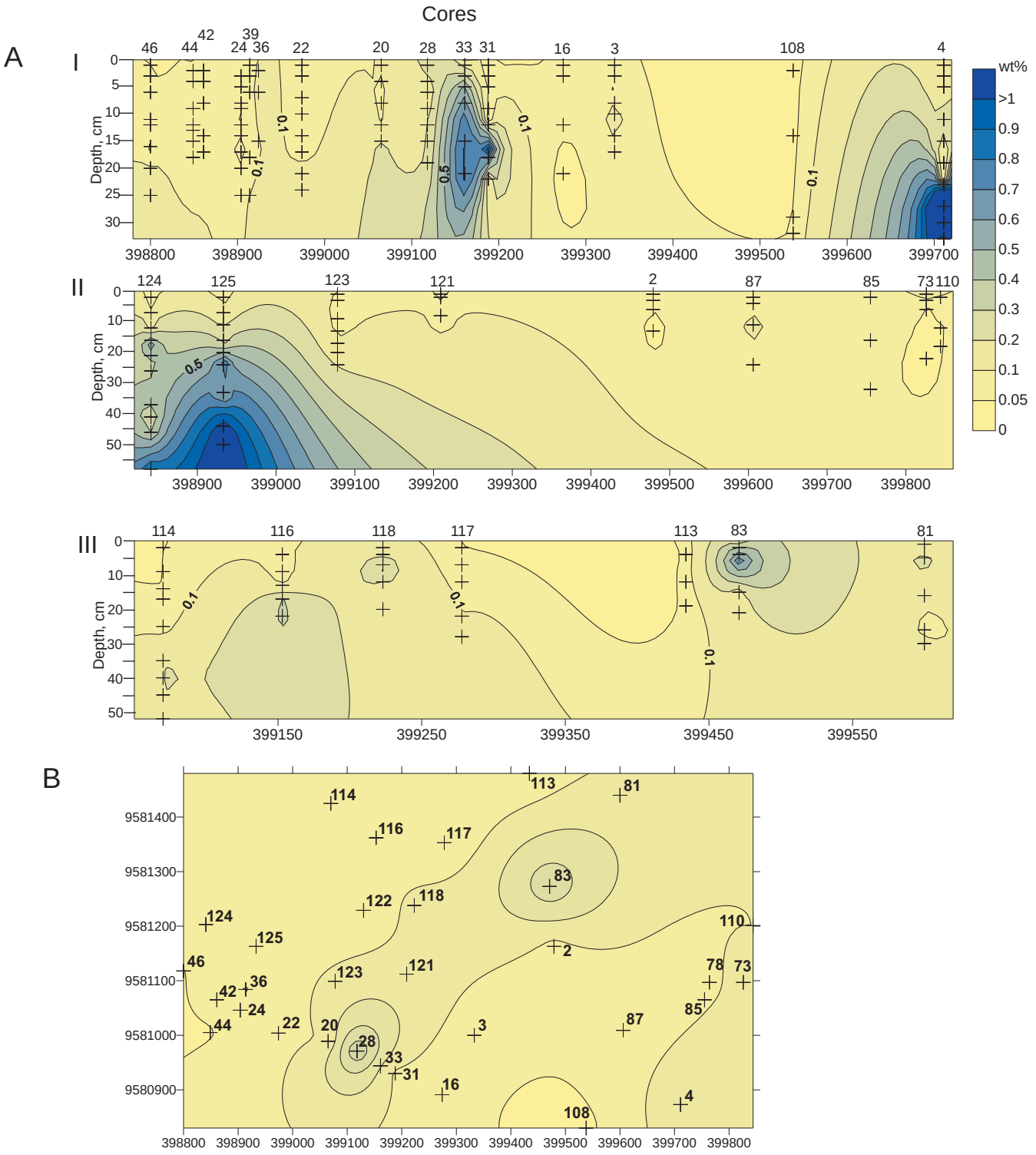

Figure 16. Distribution of Ba in sediments in profiles I, II and II in Fig. 2 (A) and in surface sediments (B) of Solwara 1.

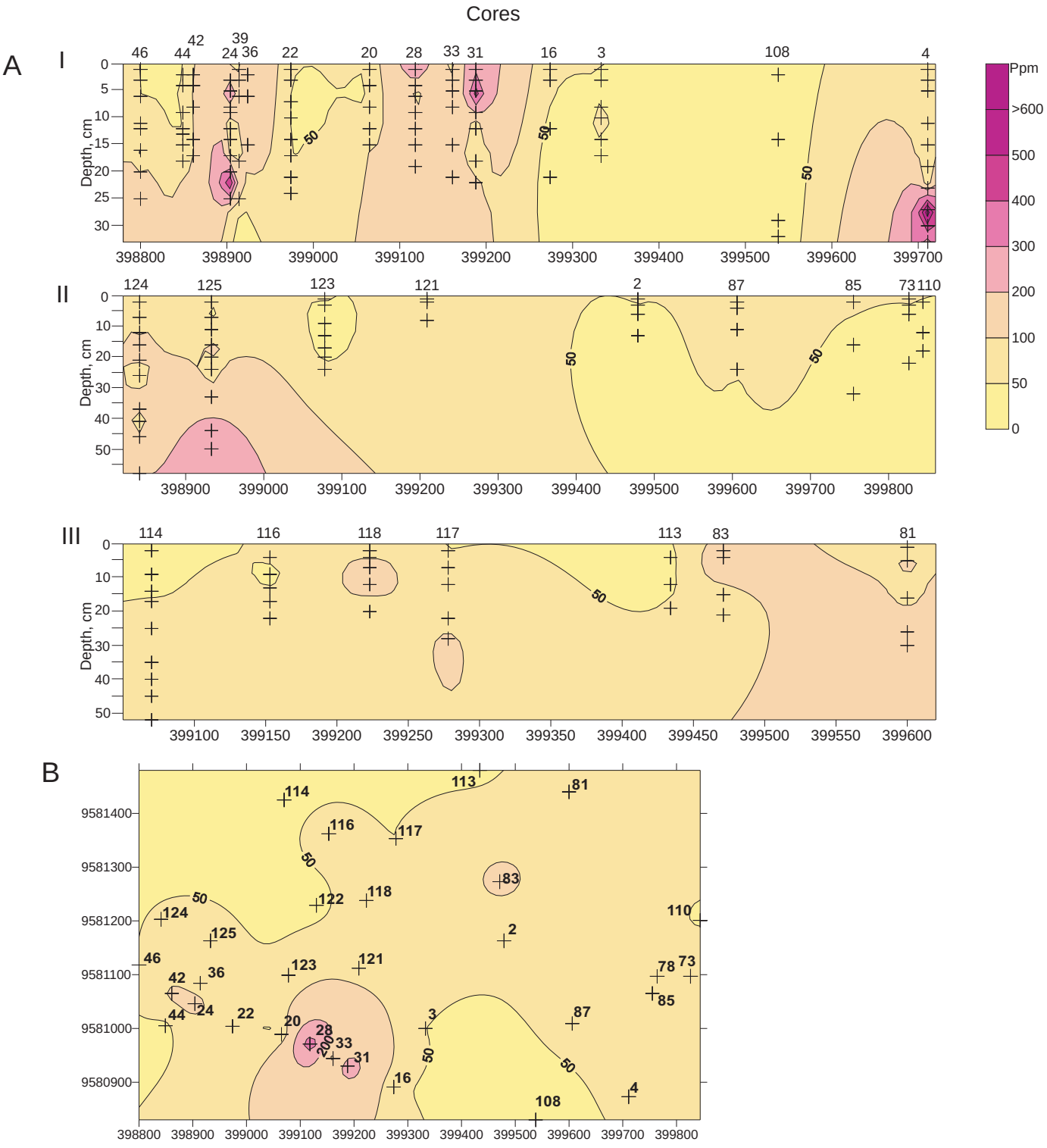

Figure 17. Distribution of As in sediments in profiles I, II and III in Fig. 2 (A) and in surface sediments (B) of Solwara 1.

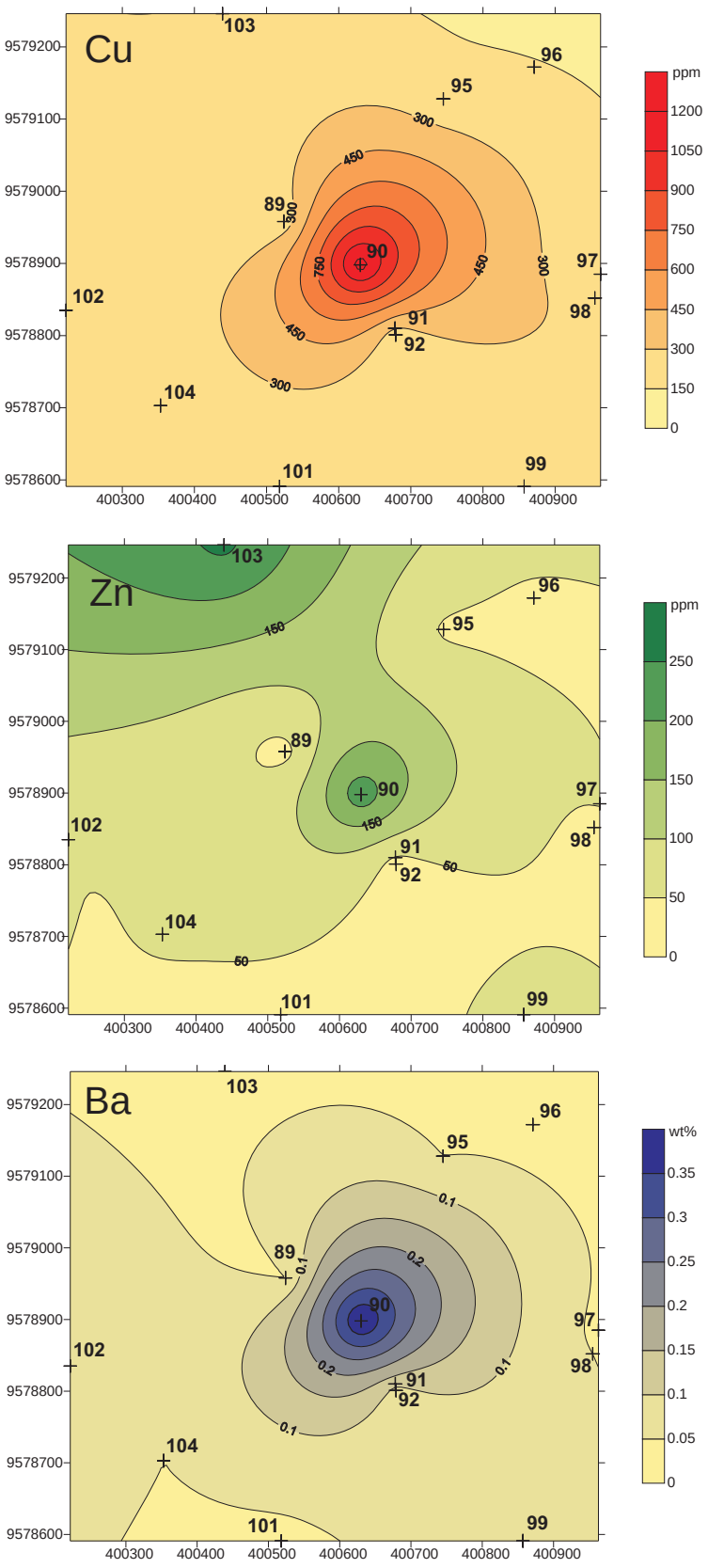

Figure 18. Distributions of Cu, Zn and Ba in surface sediments of South Su. Sample locations are shown as crosses and core numbers - #.

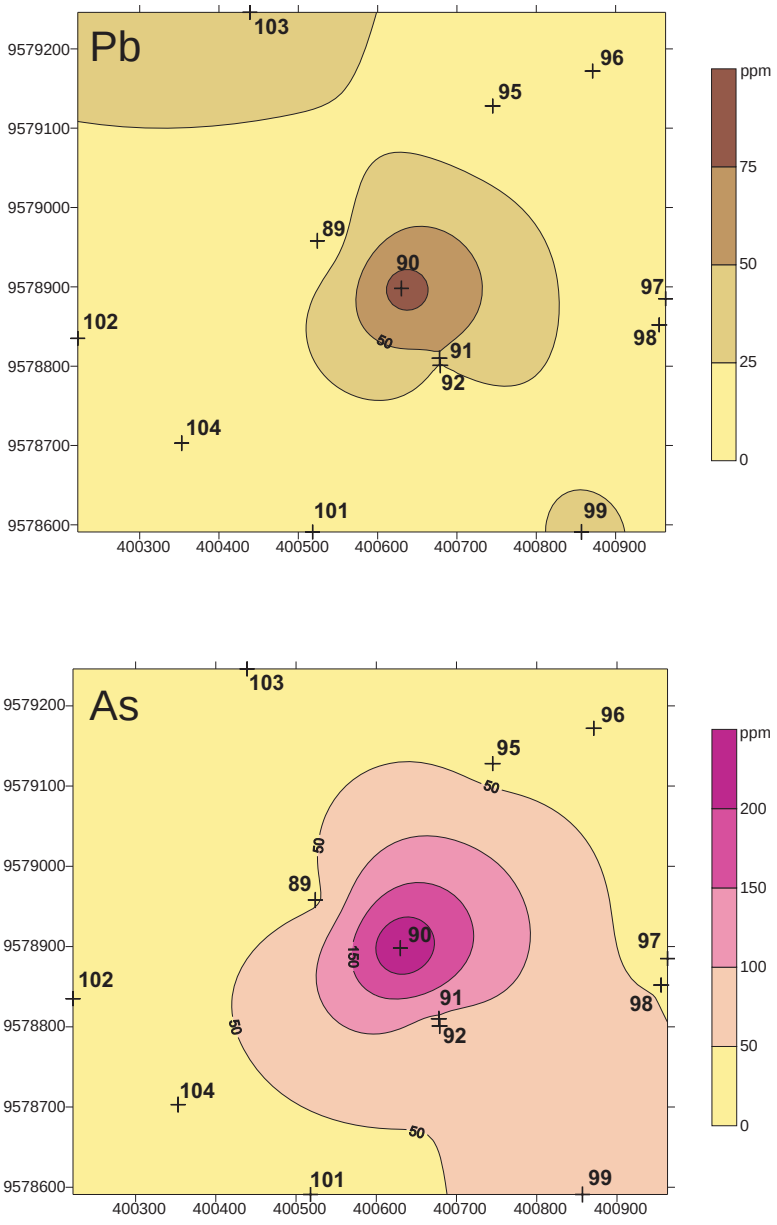

Figure 19. Distributions of Pb and As in surface sediments of South Su. Sample locations are shown as crosses and core #.

Table 1. List of sediment cores collected during Nautilus cruise in 2006

Core label	Corer	Dive	Easting	Northing	Depth (m)	Site	Core length	Intervals analysed
SC-2	short		399479	9581163		Solwara 1	13 cm	0-1, 1-3, 3-6, 9-13 cm
SC-3	short		399333	9581000		Solwara 1	17 cm	0-1, 1-3, 5-8, 8-10, 10-14, 14-17 cm
SC-4	short		399711	9580873	1530	Solwara 1	33 cm	0-1, 1-3, 3-5, 7-11, 11-15, 15-19, 19-23, 23-27, 27-30, 30-33 cm
SC-16	short		399274	9580891	1558	Solwara 1	21 cm	0-1, 1-3, 7-12, 16-21 cm
SC-20	short		399065	9580989	1569	Solwara 1	15 cm	0-1, 1-4, 4-8, 8-12, 12-15 cm
SC-22	short		398974	9581004	1593	Solwara 1	24 cm	0-1, 1-3, 3-7, 7-10, 10-14, 14-17, 17-21, 21-24 cm
SC-24	short		398904	9581046	1620	Solwara 1	25 cm	0-1, 1-3, 3-5, 5-8, 8-10, 10-12, 12-14, 14-17, 17-20, 20-25 cm
SC-28	short		399118	9580971	1580	Solwara 1	19 cm	0-1, 1-4, 4-6, 6-9, 9-12, 12-15, 15-19 cm
SC-31	short		399188	9580930	1558	Solwara 1	22 cm	0-1, 1-3, 3-5, 5-

								10, 10-12, 15-18, 18-22 cm
SC-33	short		399161	9580944	1555	Solwara 1	21 cm	0-1, 1-3, 3-5, 5-8, 12-15, 18-21 cm
SC-36	short		398914	9581084	1595	Solwara 1	15 cm	0-2, 2-6, 10-15 cm
SC-39	short		398914	9581084	1625	Solwara 1	25 cm	0-1, 1-3, 3-6, 14-18, 22-25 cm
SC-42	short		398861	9581065	1645	Solwara 1	17 cm	0-2, 2-4, 6-8, 11-14, 14-17 cm
SC-44	short		398849	9581005	1679	Solwara 1	22 cm	0-2, 2-4, 4-9, 9-13, 13-15, 15-18, 18-22 cm
SC-46	short			9581118	1669	Solwara 1	25 cm	0-1, 1-3, 3-6, 6-11, 11-12, 12-16, 16-20, 20-25 cm
SC-73	short		399826	9581097	1559	Solwara 1	22 cm	0-1, 1-3, 3-6, 18-22 cm
SC-78	short		399764	9581097	1573	Solwara 1	15 cm	0-2, 2-4, 4-6, 10-15 cm

Table 2. List of sediments cores collected during Luk Luk 07 cruise

Ops log name	Core label	Corer	Dive	Easting	Northing	Depth (m)	Site	Core length	Intervals analysed	Comments
PC-1	SC-80	Short		399578	9581436	1579	Solwara 1	20 cm	Not analysed	Disturbed
PC-2	SC-81	Short		399600	9581440	1570	Solwara 1	30 cm	0-1, 1-5, 5-11, 11-16, 16-20, 20-26, 26-30 cm	
T-15	SC-82	Short	14	399069	9580967	1575	Solwara 1	18 cm	Not analysed	Duplicate Talina's cores
T-16	SC-83	Short	15	399471	9581273	1516	Solwara 1	21 cm	0-2, 2-4, 4-8, 8-11, 11-15, 15-21 cm	
E-5	SC-84	Short	15	399552	9581229	1522	Solwara 1		Not analysed	Insufficient sample
E-8	SC-85	Short	16	399755	9581065	1542	Solwara 1	32 cm	0-2, 2-5, 5-8, 8-12, 12-16, 16-23, 23-32 cm	
E-9	SC-86	Short	16	399718	9581030	1535	Solwara 1		Not analysed	Insufficient sample
E-11	SC-87	Short	20	399606	9581009	1512	Solwara 1	24 cm	0-2, 2-4, 4-6, 6-9, 9-11, 11-15, 15-20, 20-24 cm	
E-12	SC-88	Short	21				Solwara 1			Disturbed
T-18	SC-89	Short	24	400524	9578958	1347	South Su	17 cm	0-2, 2-4 cm	Duplicate Talina's cores
T-20	SC-90	Short	26	400630	9578898	1312	South Su	15 cm	0-2, 2-4 cm	Duplicate Talina's

										cores
E-13	SC-91	Short	26	400679	9578801	1374	South Su	5 cm	0-2 cm	
E-14	SC-92	Short	26	400678	9578810	1372	South Su	5 cm	0-2 cm	
T-23	SC-93	Short		400597	9578677	1406	South Su			Duplicate Talina's cores
T-25	SC-94	Short	30	400736	9578953	1354	South Su			Duplicate Talina's cores
T-26	SC-95	Short	31	400745	9579128	1430	South Su	20 cm	0-2 cm	Duplicate Talina's cores
E-15/SS_STSED_1	SC-96	Short	32	400871	9579172	1443	South Su		0-2 cm	Insufficient sample
E-16/SS_STSED_2	SC-97	Short	32	400963	9578885	1429	South Su		0-5 cm	Insufficient sample
E-17/SS_STSED_2A	SC-98	Short	32	400955	9578852	1426	South Su	15 cm	0-3, 3-7 cm	Insufficient sample
E-18/SS_STSED_3	SC-99	Short	32	400857	9578591	1440	South Su	15 cm	0-2 cm	Insufficient sample
E-19/SS_STSED_4	SC-100	Short	32	400518	9578591	1441	South Su		Not analysed	Insufficient sample
E-20/SS_STSED_4	SC-101	Short	32	400518	9578591	1441	South Su		0-5 cm	Insufficient sample
E-21/SS_STSED_5	SC-102	Short	32	400222	9578835	1447	South Su		0-5 cm	Insufficient sample
E-22/SS_STSED_6	SC-103	Short	32	400439	9579246	1441	South Su		0-5 cm	Insufficient sample
T-28	SC-104	Short	35	400353	9578703	1425	South Su		0-5 cm	Insufficient sample
E-23	SC-105	Short	37	399205	9580935	1536	Solwara 1		Not analysed	Insufficient sample
E-24	SC-106	long	37	399177	9580974	1437	Solwara 1		Not analysed	Disturbed
E-25	SC-107	Short	37	399538	9580830	1530	Solwara 1		Not analysed	Insufficient sample
E-26	SC-108	long	37	399538	9580830	1530	Solwara 1	32 cm	0-2, 2-4, 4-9, 9-14, 14-19, 19-24, 24-29, 29-32 cm	
E-27	SC-109	Short	37	399799	9581226	1581	Solwara 1		Not analysed	Insufficient sample
E-28	SC-110	Short	37	399844	9581201		Solwara 1	18 cm	0-2, 2-4, 4-8, 8-	

									12, 12-16, 16-18 cm	
E-29	SC-111	Short	37	399820	9581156	1571	Solwara 1		Not analysed	Insufficient sample
E-30	SC-112	Short	37	399916	9581180	1618	Solwara 1		Not analysed	Insufficient sample
E-31/Sed_Sample Ea	SC-113	Short	38	399434	9581480	1549	Solwara 1	19 cm	0-2, 2-4, 4-7, 7-12, 12-15, 15-19 cm	
E-32/Sed_Sample Ec	SC-114	long	38	399070	9581425	1596	Solwara 1	52 cm	0-2, 2-4, 4-9, 9-14, 14-17, 17-20, 20-25, 25-30, 30-35, 35-40, 40-45, 45-52 cm	
E-33/Sed_Sample Eb	SC-115	Short	38	399413	9581433	1536	Solwara 1	8 cm	Not analysed	Insufficient sample
E-34/Sed_Sample Ec	SC-116	Short	38	399153	9581362	1543	Solwara 1	22 cm	0-2, 2-4, 4-9, 9-13, 13-17, 17-22 cm	
E-35/Sed_Sample Ed	SC-117	Short	38	399278	9581353	1541	Solwara 1	28 cm	0-2, 2-7, 7-12, 12-17, 17-22, 22-28 cm	
E-36/Sed_Sample Ee	SC-118	Short	38	399223	9581238	1540	Solwara 1	20 cm	0-2, 2-4, 4-7, 7-12, 12-16, 16-20 cm	
E-37/Sed_Sample Ef	SC-119	Short	38	399133	9581227	1560	Solwara 1		Not analysed	Insufficient sample
E-38/Sed_Sample Eg	SC-120	Short	38	399085	9581101	1582	Solwara 1		Not analysed	Insufficient sample
E-39/Sed_Sample Eh	SC-121	Short	38	399209	9581112	1539	Solwara 1	8 cm	0-1, 1-2, 2-5, 5-8cm	
E-40/Sed_Sample Ef	SC-122	long	38	399130	9581229	1558	Solwara 1	34 cm	0-2, 2-4, 4-9, 9-	

									14, 14-19, 19-23, 23-26, 26-30, 30-34 cm	
E-41/Sed_Sample Eg	SC-123	long	38	399078	9581099	1582	Solwara 1	24 cm	0-1, 1-3, 3-6, 6-9, 9-13, 13-17, 17-20, 20-24 cm	
E-42	SC-124	long	43	398841	9581203	1652	Solwara 1	80 cm	0-2, 2-7, 7-12, 12-16, 16-21, 21-26, 26-31, 31-37, 37-41, 41-46, 46-52, 52-58, 58-65, 65-73, 73-80 cm	
E-43	SC-125	long	43	398933	9581163	1625	Solwara 1	50 cm	0-2, 2-4, 4-7, 7-11, 11-16, 16-20, 20-24, 24-28, 28-33, 33-38, 38-43, 43-50 cm	

Table 3. Sampled core intervals and description of sediments.

Sediment core label	Samples	Depth intervals	Description
SC-2	SC-2-1	0-1cm	volcanic sandy silt, dark gray
	SC-2-2	1-3 cm	volcanic sandy silt, dark gray
	SC-2-3	3-6 cm	volcanic fine sand, olive-gray
	SC-2-4	6-9 cm	volcanic fine sand, olive-gray
	SC-2-5	9-13 cm	volcanic fine sand, olive-gray
SC-3	SC-3-1	0-1 cm	volcanic sandy silt, dark gray with greenish-brown patches
	SC-3-2	1-3 cm	volcanc sandy silt, dark gray
	SC-3-3	3-5 cm	volcanc sandy silt, dark gray
	SC-3-4	5-8 cm	volcanc sandy silt, dark gray
	SC-3-5	8-10 cm	volcanic fine sand, olive-gray with black patch
	SC-3-6	10-14 cm	volcanic fine sand with coarse sand layer, olive-gray
	SC-3-7	14-17 cm	volcanic fine sand, olive-gray
SC-4	SC-4-1	0-1 cm	volcanic sandy silt, dark gray
	SC-4-2	1-3 cm	volcanic sandy silt, dark gray
	SC-4-3	3-5 cm	volcanic sandy silt, dark gray
	SC-4-4	5-7 cm	volcanic sandy silt, dark gray
	SC-4-5	7-11 cm	olive-gray volcanic fine sand, olive-gray with black layer at 7 cm
	SC-4-6	11-15 cm	volcanic fine sand, olive-gray
	SC-4-7	15-19 cm	volcanic fine sand, olive-gray
	SC-4-8	19-23 cm	volcanic fine sand, olive-gray
	SC-4-9	23-27 cm	volcanic fine sand, contain fine-grained black material and chimney fragments
	SC-4-10	27-30 cm	olive-gray volcanic fine sand, olive-gray with black patches
	SC-4-11	30-33 cm	volcanic fine sand, olive-gray with rare black patches
SC-16	SC-16-1	0-1 cm	volcanic sandy silt, dark gray
	SC-16-2	1-3 cm	volcanic sandy silt, dark gray with black banded layers at ~1.5 and 2.5 cm
	SC-16-3	3-7 cm	volcanic sandy silt, dark gray
	SC-16-4	7-12 cm	transition between dark gray volcanic silt and olive-gray fine sand with a black layer at 8cm
	SC-16-5	12-16 cm	volcanic fine sand with coarser sand layer at ~ 12 cm
	SC-16-6	16-21 cm	volcanic sand, olive gray
SC-20	SC-20-1	0-1 cm	volcanic sandy silt, dark gray with greenish-brown patches
	SC-20-2	1-4 cm	volcanic sandy silt, dark gray with greenish-brown patches
	SC-20-3	4-8 cm	volcanic fine sand with coarser sand layer at ~8 cm and black layer at 4 cm
	SC-20-4	8-12 cm	volcanic sand, olive-gray
	SC-20-5	12-15 cm	volcanic sand, olive-gray
SC-22	SC-22-1	0-1 cm	volcanic sandy silt with black and greenish-brown patches
	SC-22-2	1-3 cm	volcanic sandy silt with black and greenish-brown patches
	SC-22-3	3-7 cm	volcanic sandy silt, dark gray with black and greenish-brown patches
	SC-22-4	7-10 cm	volcanic fine sand, olive-gray with black material
	SC-22-5	10-14 cm	volcanic find sand with coarser sandy lamina at 12.5 cm, olive-gray
	SC-22-6	14-17 cm	volcanic find sand, olive-gray
	SC-22-7	17-21 cm	patchy dark gray and greenish-brown fine sand
	SC-22-8	21-24 cm	patchy dark gray and greenish-brown fine sand
SC-24	SC-24-1	0-1 cm	volcanic sandy silt, dark gray with black and greenish-brown patches
	SC-24-2	1-3 cm	volcanic sandy silt, dark gray with black and greenish-brown patches
	SC-24-3	3-5 cm	volcanic sandy silt, dark gray with black and greenish-brown patches
	SC-24-4	5-8 cm	volcanic sandy silt, dark gray with black and greenish-brown patches
	SC-24-5	8-9.5 cm	coarse sand layer
	SC-24-6	9.5-12.5 cm	volcanic fine sand, dark gray to olive-gray with black layers
	SC-24-7	12.5-14.5 cm	volcanic fine sand, dark gray to olive-gray
	SC-24-8	14.5-17 cm	coarse sand layer
	SC-24-9	17-20 cm	greenish-brown fine sand
	SC-24-10	20-25 cm	dark gray fine sand with greenish-brown patches
SC-28	SC-28-1	0-1 cm	volcanic sandy silt, dark gray with greenish-brown patches
	SC-28-2	1-4 cm	volcanic sandy silt, dark gray with greenish-brown patches and clasts of Fe-oxyhydroxides
	SC-28-3	4-6 cm	volcanic sandy silt, dark gray with bended black layer at ~6 cm
	SC-28-4	6-9 cm	volcanic fine sand, olive gray
	SC-28-5	9-12 cm	volcanic fine sand, olive gray
	SC-28-6	12-15 cm	volcanic fine sand, olive gray
	SC-28-7	15-19 cm	volcanic fine sand, olive gray
SC-31	SC-31-1	0-1 cm	volcanic sandy silt, dark gray with bended black layers and greenish-brown patches
	SC-31-2	1-3 cm	volcanic sandy silt, dark gray with bended black layers and greenish-brown patches
	SC-31-3	3-5 cm	volcanic sandy silt, dark gray with bended black layers and greenish-brown patches
	SC-31-4	5-9.5 cm	volcanic sandy silt, dark gray with bended black layers and greenish-brown patches
	SC-31-5	9.5-12 cm	volcanic fine sand, dark gray with bended black layers and cosrser sand layers
	SC-31-6	12-15 cm	volcanic sand, dark gray
	SC-31-7	15-18 cm	volcanic fine sand, olive-gray
	SC-31-8	18-22.5 cm	volcanic fine sand, olive-gray with rusty coarser sand patches
SC-33	SC-33-1	0-1 cm	volcanic sandy silt, dark gray
	SC-33-2	1-3 cm	volcanic sandy silt, dark gray
	SC-33-3	3-5 cm	volcanic sandy silt, dark gray with bended black layer at 5 cm
	SC-33-4	5-8 cm	volcanic fine sand, olive-gray
	SC-33-5	8-10 cm	volcanic fine sand, olive-gray

	SC-33-6	10-12 cm	volcanic fine sand, olive-gray
	SC-33-7	12-15 cm	volcanic fine sand, olive-gray
	SC-33-8	15-18 cm	volcanic sand, olive-gray
	SC-33-9	18-21 cm	volcanic sand, olive-gray
SC-36	SC-36-1	0-2 cm	volcanic sandy silt, dark gray with greenish-brown patches and pieces of orange oxyhydroxides
	SC-36-2	2-6 cm	volcanic sandy silt, dark gray with greenish-brown patches and pieces of orange oxyhydroxides
	SC-36-3	6-10 cm	volcanic sandy silt, dark gray with greenish-brown patches and pieces of orange oxyhydroxides
	SC-36-4	10-15 cm	volcanic sandy silt, dark gray with greenish-brown patches and pieces of orange oxyhydroxides
SC-39	SC-39-1	0-1 cm	volcanic sandy silt, dark gray with greenish-brown patches
	SC-39-2	1-3 cm	volcanic sandy silt, dark gray with greenish-brown patches
	SC-39-3	3-6 cm	volcanic sandy silt, dark gray with greenish-brown patches
	SC-39-4	6-10 cm	volcanic sandy silt, dark gray with greenish-brown patches
	SC-39-5	10-14 cm	volcanic sandy silt, dark gray with greenish-brown patches
	SC-39-6	14-18 cm	volcanic fine sand, dark gray with bended black layers
	SC-39-7	18-22 cm	volcanic fine sand, dark gray
	SC-39-8	22-25.5 cm	volcanic fine sand, dark gray
SC-42	SC-42-1	0-2 cm	dark gray volcanic sandy silt, dark gray with greenish-brown patches
	SC-42-2	2-4 cm	volcanic sandy silt, dark gray with greenish-brown and black patches
	SC-42-3	4-6.5 cm	volcanic sandy silt, dark gray with greenish-brown and black patches
	SC-42-4	6.5-8 cm	rusty sand layer
	SC-42-5	8-11 cm	volcanic fine sand, dark gray
	SC-42-6	11-14 cm	volcanic fine sand, dark gray with greenish-brown patches
	SC-42-7	14-17 cm	volcanic fine sand, dark gray with greenish-brown patches
SC-44	SC-44-1	0-2 cm	volcanic sandy silt, dark gray with greenish-brown patches
	SC-44-2	2-4 cm	volcanic sandy silt, dark gray with greenish-brown and black patches
	SC-44-3	4-9 cm	volcanic fine sand, dark gray with greenish-brown patches and rusty sand layer at 6-6.5 cm
	SC-44-4	9-13 cm	volcanic fine sand, dark gray
	SC-44-5	13-15 cm	coarse sand layer
	SC-44-6	15-18 cm	volcanic fine sand, dark gray and greenish-brown patches
	SC-44-7	18-22 cm	volcanic fine sand, dark gray
SC-46	SC-46-1	0-1 cm	volcanic sandy silt, dark gray with greenish-brown patches
	SC-46-2	1-3 cm	volcanic sandy silt, dark gray with greenish-brown patches and black laminae
	SC-46-3	3-6 cm	volcanic sandy silt, dark gray with greenish-brown patches and black laminae
	SC-46-4	6-11 cm	volcanic sandy silt, dark gray with greenish-brown patches and black laminae
	SC-46-5	11-12 cm	brownish silt
	SC-46-6	12-16 cm	dark gray silt
	SC-46-7	16-20 cm	patchy dark gray and greenish-brown silt
	SC-46-8	20-25 cm	patchy dark gray and greenish-brown silt
SC-73	SC-73-1	0-1 cm	volcanic sandy silt, dark gray
	SC-73-2	1-3 cm	volcanic sandy silt, dark gray
	SC-73-3	3-6 cm	volcanic sandy silt, dark gray
	SC-73-4	6-8 cm	volcanic fine sand, dark gray
	SC-73-5	8-10 cm	volcanic fine sand, dark gray
	SC-73-6	10-14 cm	volcanic fine sand, dark gray
	SC-73-7	14-18 cm	volcanic fine sand, dark gray
	SC-73-8	18-22 cm	volcanic fine sand, dark gray
SC-78	SC-78-1	0-2 cm	volcanic sandy silt, dark gray
	SC-78-2	2-4 cm	volcanic sandy silt, dark gray
	SC-78-3	4-6 cm	volcanic sandy silt, dark gray with black lamina at ~5 cm
	SC-78-4	6-10 cm	volcanic fine sand, dark gray
	SC-78-5	10-15 cm	volcanic fine sand, dark gray
SC-83	SC-83-1	0-2 cm	volcanic sandy silt, dark gray with orange patches
	SC-83-2	2-4 cm	volcanic sandy silt, dark gray
	SC-83-3	4-8 cm	volcanic sandy silt, dark gray
	SC-83-4	8-11 cm	volcanic sandy silt, dark gray
	SC-83-5	11-15 cm	volcanic sandy silt, dark gray
	SC-83-6	15-21 cm	volcanic sandy silt, dark gray
SC-85	SC-85-1	0-2 cm	volcanic sandy silt, dark gray with greenish-brown patches
	SC-85-2	2-5 cm	volcanic sandy silt, dark gray
	SC-85-3	5-8 cm	volcanic sandy silt, dark gray with black layers
	SC-85-4	8-12 cm	volcanic sandy silt, dark gray
	SC-85-5	12-16 cm	volcanic sandy silt, dark gray
	SC-85-6	16-23 cm	volcanic sandy silt, dark gray
	SC-85-7	23-32 cm	volcanic sand
SC-87	SC-87-1	0-2 cm	volcanic sandy silt, dark gray with orange layer
	SC-87-2	2-4 cm	volcanic sandy silt, dark gray with greenish-brown patches
	SC-87-3	4-6 cm	volcanic sandy silt, dark gray
	SC-87-4	6-9 cm	volcanic sandy silt, dark gray
	SC-87-5	9-11 cm	volcanic sandy silt and sand, dark gray with orange and black layers
	SC-87-6	11-15 cm	volcanic sandy silt, dark gray
	SC-87-7	15-20 cm	volcanic sandy silt, dark gray
	SC-87-8	20-24 cm	volcanic sandy silt, dark gray

SC-108	SC-108-1	0-2 cm	volcanic sandy silt, dark gray with greenish-brown patches
	SC-108-2	2-4 cm	volcanic sandy silt, dark gray
	SC-108-3	4-9 cm	volcanic sandy silt, dark gray
	SC-108-4	9-14 cm	volcanic sandy silt, dark gray with some black layers
	SC-108-5	14-19 cm	volcanic sandy silt, dark gray
	SC-108-6	19-24 cm	volcanic sandy silt, dark gray with some black layers
	SC-108-7	24-29 cm	volcanic sandy silt and sand, dark gray
	SC-108-8	29-32 cm	volcanic sand, dark gray
SC-110	SC-110-1	0-2 cm	volcanic sandy silt, dark gray
	SC-110-2	2-4 cm	volcanic fine sand, dark gray and olive-gray
	SC-110-3	4-8 cm	volcanic fine sand, dark gray and olive-gray
	SC-110-4	8-12 cm	volcanic sand, dark gray
	SC-110-5	12-16 cm	volcanic sand, dark gray
	SC-110-6	16-18 cm	volcanic sand, dark gray
SC-113	SC-113-1	0-2 cm	volcanic sandy silt, dark gray with orange patches
	SC-113-2	2-4 cm	volcanic sandy silt, dark gray with orange patches
	SC-113-3	4-7 cm	volcanic sandy silt, dark gray
	SC-113-4	7-12 cm	volcanic sandy silt, dark gray and greenish-brown with black layer between
	SC-113-5	12-15 cm	volcanic sandy silt, dark gray and greenish-brown with some black patches
	SC-113-6	15-19 cm	volcanic sandy silt,dark gray and greenish-brown
SC-114	SC-114-1	0-2 cm	volcanic sandy silt, dark gray with greenish-brown and black patches
	SC-114-2	2-4 cm	volcanic sandy silt, dark gray with greenish-brown and black patches
	SC-114-3	4-9 cm	volcanic sandy silt, dark gray with greenish-brown and black patches
	SC-114-4	9-14 cm	volcanic sandy silt, dark gray with greenish-brown and black patches
	SC-114-5	14-17 cm	volcanic silt and sand, dark gray and greenish-brown
	SC-114-6	17-20 cm	volcanic silt, dark gray and greenish-brown
	SC-114-7	20-25 cm	volcanic silt, dark gray and greenish-brown
	SC-114-8	25-30 cm	dark gray volcanic silt and greenish mud
	SC-114-9	30-35 cm	dark gray volcanic silt and greenish mud with black patches
	SC-114-10	35-40 cm	greenish mud with black patches
	SC-114-11	40-45 cm	greenish-brown mud with black patches
	SC-114-12	45-52 cm	greenish-brown mud ; pumice clast and white clay that could be altered pumice
SC-116	SC-116-1	0-2 cm	volcanic sandy silt, dark gray with orange patches
	SC-116-2	2-4 cm	volcanic sandy silt, dark gray with orange patches
	SC-116-3	4-9 cm	volcanic sandy silt,dark gray with black layers, tubeworm
	SC-116-4	9-13 cm	volcanic sandy silt,dark gray with black layers, tubeworm
	SC-116-5	13-17 cm	volcanic silt, dark gray and brownish-green, foraminiferas
	SC-116-6	17-22 cm	brownish-green mud, foraminiferas
SC-117	SC-117-1	0-2 cm	volcanic sandy silt, dark gray with greenish-brown patches
	SC-117-2	2-7 cm	volcanic sandy silt, dark gray to black with greenish-brown patches
	SC-117-3	7-12 cm	volcanic sandy silt, dark gray with greenish-brown patches
	SC-117-4	12-17 cm	volcanic sandy silt, dark gray
	SC-117-5	17-22 cm	volcanic sandy silt, dark gray with greenish-brown patches
	SC-117-6	22-28 cm	volcanic sandy silt, dark gray with greenish-brown patches
SC-118	SC-118-1	0-2 cm	volcanic sandy silt, dark gray with orange patches
	SC-118-2	2-4 cm	volcanic sandy silt, dark gray with orange and black patches
	SC-118-3	4-7 cm	volcanic sandy silt, dark gray to black with some greenish-brown patches, foraminifers
	SC-118-4	7-12 cm	volcanic sandy silt, dark gray
	SC-118-5	12-16 cm	volcanic sandy silt and sand, dark gray
	SC-118-6	16-20 cm	volcanic sandy silt, dark gray
SC-121	SC-121-1	0-1 cm	volcanic sandy silt, dark gray with greenish-brown and black patches
	SC-121-2	1-5 cm	volcanic sandy silt, dark gray
	SC-121-3	5-8 cm	volcanic sandy silt, dark gray
SC-122	SC-122-1	0-2 cm	volcanic sandy silt, dark gray with black and greenish-brown patches
	SC-122-2	2-4 cm	volcanic sandy silt, dark gray with black and greenish-brown patches
	SC-122-3	4-9 cm	volcanic sandy silt, dark gray with black and greenish-brown patches
	SC-122-4	9-14 cm	volcanic sandy silt, dark gray with black layers
	SC-122-5	14-19 cm	volcanic sandy silt, dark gray
	SC-122-6	19-23 cm	volcanic sandy silt, dark gray
	SC-122-7	23-26 cm	volcanic sandy silt, dark gray and brownish-green, clasts of volcanic glass
	SC-122-8	26-30 cm	volcanic silt and sand, dark gray and brownish-green
	SC-122-9	30-34 cm	greenish mud
SC-123	SC-123-1	0-1 cm	volcanic sandy silt, dark gray with orange patches
	SC-123-2	1-3 cm	volcanic sandy silt, dark gray
	SC-123-3	3-6 cm	volcanic sandy silt, dark gray
	SC-123-4	6-9 cm	volcanic sandy silt, dark gray
	SC-123-5	9-13 cm	volcanic sand, dark greenish-gray
	SC-123-6	13-17 cm	volcanic sand with mud matrix, greenish, sand graines are mostly volcanic glass
	SC-123-7	17-20 cm	volcanic sand with mud matrix, greenish, sand graines are mostly volcanic glass
	SC-123-8	20-24 cm	volcanic sand with mud matrix, greenish, sand graines are mostly volcanic glass
SC-124	SC-124-1	0-2 cm	volcanic sandy silt, dark gray and greenish-brown
	SC-124-2	2-7 cm	volcanic sandy silt, dark gray and greenish-brown
	SC-124-3	7-12 cm	volcanic sandy silt, dark gray and greenish-brown, foraminifers

	SC-124-4	12-16 cm	greenish-brown silty sand; clast of pumice and patch of white clay that probably is altered pumice
	SC-124-5	16-21 cm	greenish sandy silt, foraminifers
	SC-124-6	21-26 cm	dark green sandy silt with black patches, foraminifers
	SC-124-7	26-31 cm	volcanic sandy silt,dark green with black patches, foraminifers
	SC-124-8	31-37 cm	sandy silt,dark green with black patches, foraminifers
	SC-124-9	37-41 cm	dark green sand, sand grains are mostly volcanic glass
	SC-124-10	41-46 cm	volcanic sand with mud matrix, dark greenish-black, foraminifers
	SC-124-11	46-52 cm	sandy silt, dark greenish-black, foraminifers
	SC-124-12	52-58 cm	sandy silt, dark greenish-black, foraminifers
	SC-124-13	58-65 cm	sandy silt, dark greenish-black, foraminifers
	SC-124-14	65-73 cm	volcanic sand with mud matrix, dark greenish-black, sand grains are mostly volcanic glass
	SC-124-15	73-80 cm	volcanic sand with mud matrix, dark greenish-black, sulfides
SC-125	SC-125-1	0-2 cm	volcanic sandy silt, dark gray with greenish-brown patches
	SC-125-2	2-4 cm	volcanic sandy silt, dark gray with greenish-brown patches
	SC-125-3	4-7 cm	volcanic sandy silt, dark gray with greenish-brown patches
	SC-125-4	7-11 cm	volcanic sandy silt, dark gray with greenish-brown patches
	SC-125-5	11-16 cm	greenish-brown silt; pumice clast partially altered to white clay, foraminifers
	SC-125-6	16-20 cm	greenish-brown silt, foraminifers
	SC-125-7	20-24 cm	volcanic sand with mud matrix, dark green, foraminifers
	SC-125-8	24-28 cm	volcanic sand with mud matrix, dark green, foraminifers
	SC-125-9	28-33 cm	volcanic sand with mud matrix, dark greenish-black, foraminifers
	SC-125-10	33-38 cm	volcanic sand, dark greenish-black, sand grains are mostly volcanic glass, foraminifers
	SC-125-11	38-43 cm	volcanic sand with mud matrix, dark greenish-black, foraminifers
	SC-125-12	43-50 cm	volcanic sand with mud matrix, dark greenish-black, foraminifers

Table 4. Geochemical composition of sediments of Solwara 1 and South Su determined by XRF analysis.

Sample name	Depth, cm	SiO2, wt %	TiO2, wt %	Al2O3, wt %	Fe2O3, wt %	MnO, wt %	MgO, wt %	CaO, wt %	Na2O, wt %	K2O, wt %	P2O5, wt %	Cu, ppm	Zn, ppm	As, wt %	Pb, ppm	S, wt %	BaO, wt %	H2O, wt %
SC-81-1	0-1cm	62.6	0.6	15.2	6.6	0.059	1.1	4.1	3.4	0.7	0.2	915	53	0.0069	21	2.5	0.118	2
SC-81-2	1-5 cm	62	0.5	14.9	6.9	0.062	1.1	4.3	3.4	0.7	0.2	2380	84	0.0112	41	2.7	0.238	2
SC-81-4	11-16 cm	61.3	0.5	15.4	6.5	0.068	1.1	4.4	3.1	0.7	0.1	927	48	0.0074	20	2.6	0.115	3
SC-81-6	20-26 cm	60.4	0.5	15.9	6.9	0.071	1.1	4.4	3.2	0.8	0.3	554	55	0.0184	20	2.6	0.094	3
SC-81-7	26-30 cm	59.7	0.5	15.5	6.6	0.07	1.1	4.2	3.1	0.8	0.3	671	58	0.019	36	2.4	0.104	5
SC-83-1	0-2 cm	62.4	0.6	14.7	7.2	0.079	1.2	4.2	3.4	0.7	0.3	908	209	0.0113	139	1.4	0.236	3
SC-83-2	2-4 cm	59.6	0.6	13.8	8	0.059	1.1	3.8	3.2	0.6	0.5	2210	306	0.0189	143	2.1	0.745	5
SC-83-5	11-15 cm	61.8	0.5	15.6	6.4	0.069	1.1	4.5	3.1	0.7	0.2	388	67	0.0065	57	2.8	0.151	2
SC-83-6	15-21 cm	62	0.6	15.9	6.1	0.069	1.1	4.5	3.2	0.7	0.2	427	75	0.0061	75	2.6	0.174	2
SC-85-1	0-2 cm	57.2	0.6	14.1	5.6	0.055	1.1	3.7	3	0.6	0.2	453	71	0.0054	34	1.7	0.083	11
SC-85-5	12-16 cm	57.6	0.5	14.5	6	0.064	1	4.1	2.7	0.7	0.1	216	46	0.0042	20	2.6	0.058	9
SC-85-7	23-32 cm	63.1	0.5	15.1	6.5	0.089	1.2	4.6	3.1	0.8	0.1	268	50	0.004	8	2.1	0.065	2
SC-87-1	0-2 cm	62.1	0.6	14.8	7.2	0.07	1.1	4.1	3.3	0.7	0.5	374	54	0.0063	26	1.9	0.073	3
SC-87-2	2-4 cm	57.6	0.5	13.6	6.2	0.058	1	3.6	3	0.7	0.6	292	41	0.0051	20	1.8	0.063	10
SC-87-5	9-11 cm	60.3	0.5	14.7	7.3	0.072	1.2	4.4	3	0.7	0.3	428	51	0.0088	58	2.7	0.13	4
SC-87-8	20-24 cm	63.1	0.5	15.6	6.1	0.06	0.9	4.1	3	0.8	0.1	216	37	0.005	5	2.9	0.054	2
SC-108-1	0-2 cm	64.6	0.6	15.3	6.1	0.058	1.1	4.1	3.6	0.7	0.2	246	47	0.0027	7	1.8	0.044	1
SC-108-4	9-14 cm	58.9	0.6	14	5.2	0.053	1.1	3.6	3.2	0.6	0.2	177	40	0.0029	4	1.9	0.041	10
SC-108-7	24-29 cm	55	0.5	14.2	5.1	0.067	1	3.9	2.7	0.6	0.2	180	51	0.0025	8	2	0.044	14
SC-108-8	29-32 cm	60.7	0.5	15.1	5.8	0.074	1	4.3	3	0.7	0.2	169	42	0.002	< 3	2.1	0.045	6
SC-110-1	0-2 cm	60.9	0.5	15.8	6.5	0.078	1.3	4.5	2.9	0.7	0.2	406	72	0.0048	30	2.2	0.105	3
SC-110-4	8-12 cm	63.1	0.5	15.5	6.4	0.079	1.3	4.8	3.3	0.7	0.2	187	44	0.0025	< 3	2.1	0.04	1
SC-110-6	16-18 cm	62.7	0.5	15.8	6.2	0.086	1.4	5.2	3.6	0.8	0.2	258	56	0.0039	< 3	1.6	0.049	1
SC-113-1	0-2 cm	63	0.6	15.5	6.2	0.076	1.2	4.4	3.3	0.7	0.2	303	72	0.0041	12	2	0.061	2
SC-113-2	2-4 cm	63.3	0.6	15.5	6.2	0.075	1	4.2	3.4	0.6	0.2	311	67	0.0044	11	2	0.061	2
SC-113-4	7-12 cm	61.1	0.6	15.5	6.9	0.069	1.2	4.3	3	0.7	0.3	449	96	0.0055	27	2.7	0.075	3
SC-113-6	15-19 cm	61.3	0.6	15.7	6.3	0.066	1.1	4.4	3.1	0.7	0.3	309	67	0.0038	11	2.4	0.057	3
SC-114-1	0-2 cm	63.1	0.6	15.5	6.1	0.071	1.2	4.4	3.4	0.6	0.2	335	69	0.0042	12	1.7	0.061	2.5
SC-114-3	4-9 cm	62.2	0.6	15.7	6.4	0.066	1.2	4.4	3.2	0.7	0.2	279	59	0.0034	9	2.2	0.054	2.5
SC-114-4	9-14 cm	59.8	0.5	15.7	5.9	0.064	1.1	4.2	3.1	0.7	0.2	240	55	0.0032	5	2.5	0.05	5
SC-114-5	14-17 cm	59	0.6	15.3	7.2	0.089	1.4	4.7	3.2	0.7	0.3	417	77	0.0064	13	1.9	0.071	5
SC-114-7	20-25 cm	62.9	0.6	15.7	6.2	0.072	1.2	4.5	3.4	0.8	0.2	327	65	0.0034	8	1.8	0.055	2
SC-114-9	30-35 cm	55.3	0.6	15.4	7.4	0.088	1.8	4.8	2.5	0.9	0.2	1587	146	0.0069	96	1.3	0.175	8
SC-114-10	35-40 cm	50.6	0.6	14.7	6.9	0.081	1.9	4.7	2.1	0.9	0.2	1644	148	0.0072	93	1.2	0.135	15
SC-114-11	40-45 cm	52.9	0.7	15	8.1	0.098	2	4.8	2.2	1	0.2	1116	169	0.0095	77	1.1	0.169	11
SC-114-12	45-52 cm	51	0.6	13.1	8.3	0.112	1.9	5.9	2.3	1	0.3	490	160	0.0094	54	0.4	0.095	14
SC-116-2	2-4 cm	60.1	0.6	15.5	6.7	0.067	1.2	4.5	2.8	0.7	0.3	477	109	0.0059	29	2	0.087	4.5
SC-116-3	4-9 cm	61.2	0.6	16	6.6	0.064	1.1	4.4	3.2	0.7	0.1	318	68	0.0036	9	2.8	0.057	2.5
SC-116-4	9-13 cm	58.8	0.5	15.9	6.1	0.076	1.3	4.5	3.2	0.8	0.2	488	88	0.005	28	2.2	0.095	5.5
SC-116-5	13-17 cm	53.7	0.6	14.9	8.3	0.086	1.8	4.3	2.4	0.9	0.2	2644	253	0.0086	149	1.4	0.312	10
SC-116-6	17-22 cm	50.9	0.6	13.8	9	0.093	2	5.2	2	1	0.2	3107	334	0.009	166	1.2	0.31	12
SC-117-1	0-2 cm	63.7	0.6	15.3	6.1	0.059	1.1	4.1	3.4	0.7	0.2	334	81	0.005	14	2	0.056	2
SC-117-2	2-7 cm	63	0.6	15.3	6.5	0.062	1.2	4.3	3.3	0.7	0.2	445	97	0.006	25	2.2	0.076	2
SC-117-3	7-12 cm	55.2	0.6	14.2	6	0.06	1.1	3.8	2.6	0.7	0.5	473	100	0.008	41	2	0.088	12
SC-117-5	17-22 cm	59.3	0.6	16.1	6.8	0.071	1.2	4.3	3.2	0.8	0.2	716	101	0.0087	39	2.5	0.107	4
SC-117-6	22-28 cm	58.7	0.6	16	6.6	0.072	1.3	4.4	3	0.8	0.2	1070	139	0.0106	71	2.1	0.159	5
SC-118-1	0-2 cm	62.7	0.6	15.3	6.5	0.086	1.3	4.5	3.3	0.6	0.2	552	119	0.0064	43	1.7	0.115	2
SC-118-2	2-4 cm	62	0.6	15.1	6.8	0.077	1.4	4.5	3.2	0.6	0.2	653	159	0.0069	52	1.6	0.115	3
SC-118-3	4-7 cm	55.1	0.6	14.1	8.5	0.072	1.5	5.5	2.6	0.7	0.4	3246	336	0.0176	292	2.1	0.362	7
SC-118-4	7-12 cm	58.9	0.6	15	7.3	0.069	1.2	4.7	2.8	0.7	0.4	1435	182	0.0128	126	2	0.196	5
SC-118-6	16-20 cm	59.3	0.5	16.1	5.9	0.069	1.1	4.3	3.2	0.8	0.2	558	119	0.0061	42	2.6	0.104	5
SC-121-1	0-1 cm	62.1	0.6	15.4	6.6	0.065	1.2	4.3	3.2	0.7	0.3	669	105	0.0078	46	2	0.116	3
SC-121-2	1-2 cm	62.4	0.5	15.4	6.3	0.067	1.2	4.5	3.2	0.7	0.2	426	57	0.0057	26	2.1	0.09	2
SC-121-4	5-8 cm	61.6	0.5	15.9	6.4	0.071	1.2	4.5	3.2	0.7	0.2	365	69	0.005	15	2.5	0.082	2
SC-122-1	0-2 cm	58.5	0.6	14.1	5.5	0.051	1.1	3.6	3.1	0.6	0.3	326	79	0.0043	17	1.7	0.056	10
SC-122-3	4-9 cm	60	0.5	15.2	6.4	0.066	1.2	4.3	3	0.7	0.3	563	105	0.0063	39	2.2	0.1	5
SC-122-5	14-19 cm	56.8	0.5	14.6	5.6	0.064	1.1	3.9	3.1	0.7	0.2	562	94	0.0049	24	2	0.08	10
SC-122-6	19-23 cm	60	0.6	15.7	5.9	0.068	1.2	4.1	3.1	0.8	0.2	1075	112	0.0055	44	2	0.107	5

SC-122-7	23-26 cm	53.1	0.6	13.7	7.7	0.074	1.5	3.2	2.5	0.9	0.3	1223	209	0.0103	92	1.3	0.169	14
SC-122-8	26-30 cm	60.8	0.6	14.9	7	0.086	1.4	3.3	2.5	1	0.3	1053	185	0.0113	60	1.2	0.174	6
SC-122-9	30-34 cm	62.5	0.7	14.2	5.8	0.126	1.4	2.9	3.5	2.2	0.2	480	159	0.0032	28	0.4	0.116	5
SC-123-1	0-1 cm	62.5	0.6	15.3	6.5	0.066	1.3	4.3	3.3	0.7	0.2	660	106	0.0062	39	1.5	0.111	3
SC-123-2	1-3 cm	56.6	0.5	14.3	5	0.057	1.1	3.7	2.8	0.6	0.2	399	89	0.0031	22	1.9	0.07	12
SC-123-4	6-9 cm	62.4	0.5	16	5.9	0.074	1.2	4.7	3.4	0.7	0.2	321	78	0.0034	10	2	0.063	2
SC-123-5	9-13 cm	58.3	0.6	14.7	7.9	0.137	2.4	5.8	3	0.7	0.2	692	130	0.0045	33	1.3	0.103	4
SC-123-6	13-17 cm	57.7	0.6	14.4	7.7	0.119	2.2	6.1	3	1	0.2	490	133	0.0035	38	0.5	0.111	5
SC-123-7	17-20 cm	57.2	0.6	14.4	7.9	0.13	2	5.5	2.9	1.1	0.2	681	155	0.004	52	0.7	0.146	6
SC-123-8	20-24 cm	56.5	0.7	14.2	8.5	0.122	2.1	5.9	3	1.1	0.3	745	143	0.0062	56	0.5	0.197	6
SC-124-1	0-2 cm	60.1	0.6	15.4	7.3	0.076	1.4	4.4	3	0.8	0.3	541	86	0.0054	26	1.7	0.099	4
SC-124-2	2-7 cm	52.2	0.6	14.4	7.9	0.088	1.7	4.2	2.4	0.9	0.4	480	122	0.0082	48	1.4	0.113	13
SC-124-3	7-12 cm	54.9	0.7	15.2	8.9	0.104	1.9	4.3	2.4	0.9	0.3	627	137	0.0097	55	1.3	0.165	8
SC-124-4	12-16 cm	52.1	0.6	13.1	9.6	0.101	1.9	4.7	2.3	1.1	0.4	2214	216	0.0165	111	0.9	0.612	12
SC-124-5	16-21 cm	49.8	0.6	13.2	9.3	0.086	2.1	4.9	2.1	1	0.3	2750	337	0.0102	188	0.8	0.401	14
SC-124-6	21-26 cm	48.7	0.7	14	8.8	0.088	2.2	5.2	2	0.9	0.2	3013	465	0.0063	235	0.9	0.361	15
SC-124-8	31-37 cm	45.1	0.6	12.9	9.9	0.097	2.4	7.6	1.8	1	0.2	3761	489	0.0158	319	0.8	0.515	16
SC-124-9	37-41 cm	51.7	0.7	13.8	8.8	0.11	2.4	6.5	2.4	0.9	0.2	1138	397	0.0057	255	0.5	0.275	11
SC-124-10	41-46 cm	44.9	0.7	13.7	8.7	0.093	2.4	7.4	1.8	0.9	0.2	2585	590	0.011	426	0.9	0.391	17
SC-124-12	52-58 cm	47.3	0.7	13.7	9.3	0.09	2.4	5.9	1.9	1	0.2	4960	771	0.0198	578	1.3	0.911	14
SC-124-13	58-65 cm	50.4	0.7	14.1	8.7	0.1	2.2	5	2.1	1.1	0.2	4526	703	0.0209	396	1.2	0.71	12
SC-124-14	65-73 cm	55	0.7	13.8	8.6	0.115	2	5.4	2.8	0.9	0.2	2093	462	0.0142	246	0.7	0.572	8
SC-124-15	73-80 cm	51.9	0.8	14.2	9.9	0.101	2.4	4.3	2.3	0.9	0.2	3794	664	0.0305	323	1.9	0.888	9
SC-125-1	0-2 cm	61.1	0.6	15.3	7	0.072	1.4	4.5	3.2	0.7	0.3	481	89	0.0059	24	1.7	0.098	3.5
SC-125-3	4-7 cm	61.5	0.6	15.8	6.8	0.074	1.4	4.4	3.1	0.7	0.2	424	83	0.004	19	2.3	0.087	2.5
SC-125-4	7-11 cm	55.1	0.6	15.3	8.5	0.109	1.8	4.1	2.4	0.9	0.3	794	171	0.0114	81	1.4	0.189	8
SC-125-5	11-16 cm	52.6	0.6	13.8	8.8	0.127	1.9	6	2.3	1	0.3	952	218	0.0129	111	0.7	0.306	11
SC-125-6	16-20 cm	48.6	0.6	13.2	8.8	0.109	2	6	2.1	1.1	0.3	1654	331	0.0097	192	0.6	0.304	15
SC-125-7	20-24 cm	48.4	0.7	13.8	9.5	0.089	2.5	4.6	1.8	1	0.2	3893	547	0.0067	369	1	0.919	14
SC-125-9	28-33 cm	48.2	0.7	13.6	9.1	0.104	2.4	6.5	2	0.9	0.2	3183	746	0.013	356	0.9	0.517	14
SC-125-11	38-44 cm	47.8	0.7	13.6	9.6	0.096	2.4	5.7	2.1	0.9	0.2	4038	978	0.0244	969	1.3	1.13	13
SC-125-12	44-50 cm	48	0.7	13.7	9.1	0.096	2.3	5.8	2.1	0.9	0.2	4521	1333	0.03	977	1.6	1.122	13
SC-89-1	0-2 cm	64.4	0.6	15.3	5.9	0.061	1.1	4.3	3.6	0.7	0.2	213	35	0.004	4	1.7	0.048	1
SC-90-1	0-2 cm	63.5	0.5	15.1	5.6	0.068	1.2	4.6	3.4	0.6	0.2	1274	235	0.0258	89	2.4	0.416	1
SC-91-1	0-2 cm	63.4	0.6	15	6.1	0.06	1	4.2	3.3	0.7	0.2	212	32	0.0056	21	2.4	0.084	2
SC-92-1	0-2 cm	62.3	0.6	15	6.1	0.067	1.2	4.3	3.2	0.7	0.2	308	48	0.0094	47	2.7	0.12	3
SC-95-1	0-2 cm	61.6	0.5	16	6.2	0.079	1.3	4.8	3.1	0.7	0.2	201	45	0.0034	< 3	1.7	0.048	3
SC-96-1	0-2 cm	62.2	0.5	14.6	6.1	0.088	1.5	5.1	3.1	0.5	0.2	156	44	0.0023	< 3	1.3	0.034	4
SC-97	0-5 cm	62.8	0.5	15.8	5.5	0.088	1.3	5.1	3.4	0.7	0.2	159	51	0.0021	< 3	1.1	0.045	3
SC-98-1	0-3 cm	62.6	0.6	15.2	7	0.062	1.1	3.9	3.2	0.7	0.2	275	42	0.0047	7	1.9	0.052	3
SC-99-1	0-2 cm	59.7	0.5	15	7.3	0.091	1.6	5	3	0.7	0.2	259	61	0.0075	29	2.1	0.078	4
SC-101-1	0-5 cm	62.7	0.5	15.8	6	0.081	1.4	5	3.5	0.7	0.2	177	45	0.003	< 3	1.3	0.044	2
SC-102-1	0-5 cm	61.5	0.5	14.3	5.9	0.088	1.1	4.3	2.9	0.7	0.2	273	52	0.0038	9	1.6	0.096	6
SC-103	0-5 cm	66.5	0.5	15	5.2	0.085	1.3	4.7	3.4	0.7	0.2	156	262	0.0021	47	0.9	0.041	1
SC-104-1	0-5 cm	62.5	0.6	16	5.6	0.07	1.2	4.7	3.2	0.7	0.2	196	52	0.0028	< 3	1.4	0.047	3

Table 5. Geochemical composition of sediments determined by INAA. Concentrations of As, Ba and Zn in all of the studied sediment samples were also determined by XRF.

Analyte Symbol	Au	Ag	As	Ba	Br	Ca	Co	Cr	Cs	Fe	Hf	Ir	Mo	Na	Ni	Rb	Sb	Sc	Se	Sr	Ta	Th	U	W	Zn	La	Ce	Nd	Sm	Eu	Tb	Yb	Lu
Unit Symbol	ppb	ppm	ppm	ppm	ppm	%	ppm	ppm	ppm	%	ppm	ppb	ppm	%	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Detection Limit	2	2	1	20	0.5	1	0.1	0.5	0.2	0.05	0.2	2	2	0.001	50	10	0.1	0.01	0.5	100	0.3	0.1	0.1	1	10	0.05	1	1	0.01	0.05	0.1	0.05	0.01
Analysis Method	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA	INAA
SC-2-1	66	< 2	40	700	13.4	3	33.4	33.8	< 0.2	3.87	0.9	< 2	5	2.4	< 50	< 10	1.3	15.1	< 0.5	< 100	< 0.3	0.6	0.6	140	120	4.86	10	6	1.91	0.69	0.4	1.54	0.24
SC-2-5	20	< 2	23	380	7.3	3	41.8	19.6	0.8	4.62	1	< 2	5	2.79	< 50	< 10	0.5	18.5	< 0.5	< 100	< 0.3	0.7	1.6	161	40	5.58	12	7	1.99	0.81	0.4	1.61	0.26
SC-3-2	43	< 2	31	380	7.6	3	28	18	0.8	3.92	2.2	< 2	5	2.61	< 50	10	0.9	16.7	< 0.5	< 100	< 0.3	0.7	0.9	95	90	5.22	11	7	1.93	0.72	0.4	1.58	0.25
SC-3-5	152	< 2	53	1560	11.4	4	27.5	16.7	0.3	3.6	0.9	< 2	7	2.38	< 50	20	1.7	17.5	< 0.5	< 100	< 0.3	0.6	3.3	137	110	5.52	11	7	2.07	0.81	0.4	1.7	0.28
SC-4-5	192	< 2	64	2620	13.2	3	27	18	< 0.2	3.94	0.9	< 2	4	2.53	< 50	< 10	3.6	18.6	6.6	< 100	< 0.3	0.8	2.3	92	360	5.59	11	7	2.01	0.82	0.5	1.75	0.28
SC-4-9	1970	< 2	595	> 10000	15.7	3	75.6	20.3	< 0.2	7.89	1	< 2	15	2.07	< 50	< 10	15.9	15.6	18.9	< 100	< 0.3	0.2	8.1	100	590	8.38	12	6	1.82	0.84	0.4	1.54	0.24
SC-4-10	2000	< 2	654	> 10000	17.4	2	80.4	21.9	0.6	7.95	1.1	< 2	11	2.11	< 50	< 10	15.6	15.7	19.7	< 100	< 0.3	0.6	7.8	103	690	8.34	13	7	1.89	0.81	0.4	1.44	0.23
SC-22-4	66	< 2	31	740	15.6	3	42	22.1	0.8	4.28	1.2	< 2	5	2.54	< 50	< 10	1.2	18.4	< 0.5	< 100	< 0.3	0.8	2.3	189	100	6.35	12	7	2.06	0.79	0.5	1.82	0.28
SC-22-7	165	< 2	87	1590	24.5	3	34.1	20.9	0.6	4.78	1	< 2	3	2.51	< 50	< 10	1.7	20	< 0.5	< 100	< 0.3	0.9	1.1	209	170	6.82	13	8	2.28	0.8	0.5	1.84	0.28
SC-22-8	81	< 2	66	770	18.6	3	39	15.3	0.6	4.66	0.9	< 2	4	2.64	< 50	< 10	1.1	18.7	10.5	< 100	< 0.3	0.9	1.5	180	120	6.47	13	7	2.07	0.8	0.5	1.8	0.28
SC-24-5	20	< 2	58	460	8.4	3	35.3	15.9	0.6	5.31	2.3	< 2	6	2.65	< 50	20	0.6	18.3	< 0.5	< 100	< 0.3	0.7	0.8	144	90	5.55	12	7	2.07	0.81	0.5	1.74	0.27
SC-24-9	59	< 2	639	560	19.1	3	22.7	18.4	0.5	5.15	1.8	< 2	3	2.36	< 50	< 10	< 0.1	18	< 0.5	< 100	< 0.3	0.8	< 0.1	61	130	6.11	12	7	2.05	0.77	0.5	1.7	0.24
SC-28-1	142	< 2	300	2520	12.4	3	27.3	18.7	1	4.8	1.8	< 2	5	2.38	< 50	10	1.8	17.5	< 0.5	< 100	< 0.3	0.5	2.3	106	200	5.56	11	7	2.08	0.91	0.5	1.61	0.26
SC-28-7	79	< 2	186	2040	8.9	3	30.3	14.6	1.4	4.43	1.9	< 2	6	2.52	< 50	< 10	1.2	18.9	< 0.5	< 100	< 0.3	0.6	3.3	98	230	5.88	12	7	1.95	0.83	0.4	1.56	0.25
SC-31-5	20	< 2	40	360	6.2	3	34.8	14.4	0.6	4.85	1.9	< 2	4	2.4	< 50	< 10	0.5	18.6	< 0.5	< 100	< 0.3	0.6	1	123	100	5.25	11	7	2	0.75	0.5	1.68	0.27
SC-31-2	90	< 2	383	780	15.3	3	32.8	19.2	0.8	6.1	1.5	< 2	< 2	2.17	< 50	< 10	1.8	16.8	3.7	< 100	< 0.3	0.4	1	128	130	5.18	10	7	1.87	0.69	0.3	1.54	0.26
SC-33-2	30	< 2	46	490	7.9	3	29.4	19.1	0.4	4.57	1.9	< 2	4	2.64	< 50	20	0.7	18.8	< 0.5	< 100	0.5	0.7	0.8	65	110	5.9	12	7	2.12	0.9	0.4	1.65	0.26
SC-33-7	44	< 2	100	6800	6.3	4	30.9	11.4	0.9	4.07	1.6	< 2	3	2.45	< 50	10	1.1	18.4	< 0.5	< 100	< 0.3	0.5	1.5	129	170	5.26	9	7	1.79	0.82	0.4	1.53	0.24
SC-36-1	63	< 2	204	930	13	3	24	20.3	0.9	6.14	1.8	< 2	4	2.12	< 50	< 10	1.6	16.6	7.8	< 100	< 0.3	0.6	1.5	78	120	5.23	10	7	1.83	0.75	0.4	1.51	0.23
SC-46-7	152	< 2	105	1300	31.6	3	28.1	29.2	0.7	5.86	2.2	< 2	< 2	2.05	< 50	20	1.9	20.6	< 0.5	< 100	< 0.3	0.7	0.9	76	170	7.95	15	9	2.6	0.94	0.5	2.16	0.34
SC-46-8	176	< 2	111	1310	30.8	2	32.8	43.2	0.8	5.88	2.3	< 2	2	1.9	< 50	20	1.8	22	< 0.5	< 100	< 0.3	1.3	0.6	72	170	8.61	17	10	2.72	1.01	0.6	2.22	0.36
SC-73-1	174	< 2	50	1300	16	4	31.1	18.4	0.4	4.24	1.5	< 2	3	2.44	< 50	< 10	1.4	18.4	< 0.5	< 100	0.5	0.6	< 0.1	149	120	5.51	10	7	2.14	0.82	0.5	1.68	0.26
SC-73-8	19	< 2	17	460	6	3	40.7	13.9	< 0.2	4.59	1.9	< 2	5	2.57	< 50	10	0.6	18.6	< 0.5	< 100	< 0.3	0.7	1.4	208	80	5.58	12	7	2.07	0.78	0.5	1.7	0.24
SC-83-2	510	< 2	171	7030	14.4	3	39.2	17.4	< 0.2	5.51	1.6	< 2	3	2.32	< 50	< 10	5.4	17.1	< 0.5	< 100	< 0.3	1	0.8	157	270	6.8	13	8	2.36	0.89	0.5	1.61	0.25
SC-83-6	120	< 2	52	1440	9.8	3	49.3	15.6	< 0.2	4.19	< 0.2	< 2	6	2.21	< 50	< 10	3.6	17.6	< 0.5	< 100	0.3	0.9	1.3	298	80	5.75	12	7	1.93	0.72	0.4	1.49	0.23
SC-85-2	66	< 2	45	810	11.7	3	19.8	19	< 0.2	4.42	2.1	< 2	4	2.39	< 50	< 10	1.5	17.7	7.7	< 100	< 0.3	1	0.9	< 1	80	5.72	12	8	2.13	0.72	0.5	1.62	0.25
SC-85-8	30	< 2	31	670	6.2	3	60.6	13.1	< 0.2	4.33	< 0.2	< 2	4	2.14	110	< 10	1.1	17.5	< 0.5	< 100	< 0.3	1.2	0.7	421	70	6.05	12	8	2.17	0.74	0.5	1.61	0.25
SC-108-2	10	< 2	27	380	8.1	2	36.1	18	0.9	3.74	2.3	< 2	6	2.41	< 50	< 10	1.1	17.4	< 0.5	< 100	< 0.3	0.8	0.5	155	30	5.93	12	7	1.95	0.83	0.4	1.44	0.21
SC-108-8	10	< 2	12	320	6.9	3	38.4	16.1	0.5	4.17	2	< 2	3	2.17	< 50	< 10	0.4	17.4	< 0.5	< 100	< 0.3	0.6	1.1	202	60	6.03	11	7	2.16	0.81	0.5	1.56	0.25
SC-114-3	23	< 2	23	500	12.5	3	34.8	14.9	0.7	4.24	1.9	< 2	2	2.09	< 50	< 10	0.7	16.4	< 0.5	< 100	< 0.3	< 0.1	0.4	172	70	5.58	11	7	1.99	0.5	0.4	1.47	0.24
SC-114-7	28	< 2	24	500	11	3	45.2	13.2	< 0.2	4.16	1.3	< 2	3	2.36	< 50	40	0.8	16.5	< 0.5	< 100	< 0.3	1	1.2	241	70	6.37	12	7	2.17	0.87	0.5	1.95	0.3
SC-114-9	194	< 2	57	1700	31.7	3	32	39	< 0.2	4.91	2	< 2	< 2	1.84	< 50	< 10	2.4	18.8	< 0.5	< 100	< 0.3	0.6	1.8	128	140	8.02	14	10	2.48	0.85	0.5	1.92	0.29
SC-114-11	146	< 2	76	1400	24.9	3	25	37.8	0.6	4.99	1.7	< 2	3	1.61	< 50	< 10	2.3	18.5	< 0.5	< 100	0.5	1.1	0.6	86	170	8.27	16	9	2.75	0.91	0.6	2.23	0.35
SC-116-3	24	< 2	27	540	9.3	3	40.4	15.8	0.8	4.68	1.8	< 2	3	2.13	< 50	< 10	0.8	17.3	< 0.5	< 100	< 0.3	0.7	1.2	213	90	5.69	11	7	1.94	0.81	0.4	1.62	0.26
SC-116-6	499	< 2	68	2320	36.7	3	38.1	33.8	< 0.2	5.61	1.9	< 2	2	1.63	< 50	< 10	4.7	17.6	< 0.5	< 100	< 0.3	0.9	1.9	89	190	8.53	16	10	2.67	0.92	0.6	2.12	0.33
SC-124-2	173	< 2	70	1120	20.9	3	22.3	26.4	< 0.2	5.69	2.2	< 2	< 2	2.01	< 50	< 10	1.5	18.9	4	< 100	< 0.3	1.1	1.3	< 1	80	7.51	12	8	2.41	0.83	0.5	1.98	0.3
SC-124-6	826	< 2	58	4400	28.7	3	29	31	< 0.2	5.9	2.9	< 2	3	1.77	< 50	< 10	4.7	19.9	3.9	< 100	< 0.3	0.4	3.6	< 1	500	9.39	15	10	2.63	0.89	0.5	1.98	0.3
SC-124-12	1620	< 2	177	6400	39.6	4	39.2	35	< 0.2	5.66	1.8	< 2	3	1.47	< 50	< 10	10.8	18.8	3.8	< 100	< 0.3	1.1	4.3	106	500	8.09	14	9	2.51	0.58	0.5	1.9	0.3
SC-124-15	1100	< 2	265	6320	38.9	3	47.8	31	1.5	6.47	1.7	< 2	9	1.83	< 50	< 10	8.9	21.6	11.2	< 100	< 0.3	0.9	5.8	211	470	7.88	15	9	2.4	1.01	0.5	1.91	0.3
SC-125-3	70	< 2	29	670	13.2	3	48.3	23.8	< 0.2	4.75	1.9	< 2	< 2	2.29	< 50	< 10	1.1	18.6	4.6	< 100	< 0.3	0.9	1.4	247	< 10	6.66	11	10	2.14	0.67	0.4	1.68	0.27
SC-125-7	1180	< 2	53	6800	35.6	3	36.4	33.8	1.3	5.97	2	< 2	< 2	1.55	< 50	< 10	3.2	19.2	1.7	< 100	0.9	1	2.3	58	360	8.69	15	10	2.56	0.81	0.5	1.9	0.3
SC-125-12	2270	< 2	268	7920	33.5	4	38.8	24.3	< 0.2	5.74	1.3	< 2	5	1.62	280	30	20.9	18.7	3.1	< 100	< 0.3	1.1	4.8	136	790	8.54	15	10	2.41	0.87	0.4	1.78	0.3

Table 6. Mineral abundances of representative samples from Solwara 1 and South Su based on XRD and SEM analyses

Core	Depth interval, cm	Site	Sediment	Minerals													
				Rf	Gl	Pl	Px	Si-Al-rich	Cr	Al	Qz	Cl	Ca	Py	Ba	Mt	Fp
SC-4	23-27	Solwara 1	Gray sand	xxx	xx	xxx	xx	xx	xx	xx	x	x		xx	x	x	
SC-22	7-10	Solwara 1	Black patch	xxx	xx	xxx	xx	xxx	xx	xx	x	x		x	x	x	*
SC-22	17-21	Solwara 1	Greenish-brown silt	xxx	xx	xxx	xx	xxx	xx	x	x	x		x	x	x	*
SC-28	0-1	Solwara 1	Gray silt	xxx	xx	xxx	xx	xx	xx	xx		x		x	x	x	
SC-28	12-15	Solwara 1	Gray silt	xxx	xx	xxx	xx	xx	xx	x		x		x	x	x	
SC-114	40-45	Solwara 1	Greenish silt	x	xx	xx	x	xxxx	x		x	xx		x	x		*
SC-116	0-2	Solwara 1	Orange-gray silt	xxx	xx	xxx	xx	xx	xx	x		x		x	x	x	
SC-116	17-22	Solwara 1	Greenish silt	x	xx	xx	x	xxxx	xx		xx	xx	x	x	x		*
SC-118	2-4	Solwara 1	Gray silt	xxx	xx	xxx	xx	xx	xx	x		x		x	x	x	
SC-118	4-7	Solwara 1	Black patch	xxx	xx	xx	x	xxx	x	x		x		x	x	x	*
SC-124	7-12	Solwara 1	Brownish silt	xxx	xx	xx	x	xxxx	x		x	xx	x	x	x		

Table . Continue

Core	Depth interval, cm	Site	Sediment	Minerals													
				Rf	Gl	Pl	Px	Si-Al-rich	Cr	Al	Qz	Cl	Ca	Py	Ba	Mt	Fp
SC-124	52-58	Solwara 1	Greenish-black silt		xx	xx	x	xxxx	x		xx	xx	x	x	x		*
SC-124	73-80	Solwara 1	Greenish-black sand		xx	xx	x	xxxx	x	x	x	xx		x	x		
SC-89	2-4	South Su	Gray sand	xxx	xx	xxx	x	xx	xx	x	x			x	x	x	
SC-90	2-4	South Su	Gray sand	xxx	xx	xxx	x	xx	xx	x	x			x	x	x	
SC-98	3-7	South Su	Gray silt	xxx	xx	xxx	x	xx	xx	x	x			x	x	x	

Abbreviations: Al = alunite, Ba = barite, Ca = calcite, Cl = clay minerals, Cr = cristobalite, Fp = fecal pellets, Gl = volcanic glass, Mt = magnetite, Pl = plagioclase, Px = pyroxene, Py = pyrite, Qz = quartz, Rf = rock fragments, Si-Al-rich = Si- and Si-Al-rich fine-grained material; * = present, x = trace (<3 %), xx = minor (4-10 %), xxx = major (11-50 %), xxxx = dominant (>50 %)

Appendix 7

Water and Sediment Characterisation and Toxicity Assessment for the Solwara 1 Project

Water and Sediment Characterisation and Toxicity Assessment for the Solwara 1 Project

Stuart Simpson, Brad Angel, Ian Hamilton, David Spadaro, and Monique Binet.

CSIRO Land and Water Science Report 68/07
Saturday 5, January
Prepared for Coffey Natural Systems Pty Ltd

Commercial-in-confidence

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EXECUTIVE SUMMARY

Nautilus Minerals Niugini (Nautilus) is proposing to mine the copper- and gold-rich seafloor sulfide (SMS) deposits, associated with active and inactive hydrothermal vent systems, in the eastern Manus Basin, Bismarck Sea, Papua New Guinea. The mined material will be pumped to the surface vessel where the ore will be dewatered and the process water will be discharged back to the sea at a considerable depth.

Coffey Natural Systems Pty Ltd requested (on behalf of Nautilus) that the Centre for Environmental Contaminants Research, CSIRO Land and Water (CLW) conduct a field sampling of waters using ultra trace-metal techniques, analyses of waters for trace-metals, characterisation of minerals, and laboratory-based tests to assess the release of metals that may occur during the dewatering of crushed minerals.

Total (unfiltered) concentrations of arsenic (As), selenium (Se) and mercury (Hg) in the waters collected in the vicinity of the proposed mining operations were at concentrations typical of ocean water. Total concentrations of Cu, Pb and Zn exceeded water quality guideline levels in a number of water samples. Dissolved metal concentrations in the waters near the proposed ore body, and in the surrounding ocean waters, were generally very low and below water quality guideline concentrations (99% protection levels) (ANZECC/ARMCANZ, 2000).

The maximum strong acid-extractable metal concentrations measured in the mineral samples (in mg/kg) were 182 Ag, 8420 As, 580 Cd, 670 Co, 4.9 Cr, 147000 Cu, 108000 Mn, 20 Ni, 9570 Pb, and 76700 Zn. Weak-acid extractable (1-M HCl) metal concentrations in the mineral samples were also high, with maximum metal concentrations (in mg/kg) of 9 Ag, 490 As, 44 Cd, 20 Co,1.4 Cr, 2400 Cu, 1700 Mn, 11 Ni, 1400 Pb, and 1000 Zn. These high particulate metal concentrations were expected due to the high grade ore in the proposed mining area. The behaviour of the fine particulates in the seawater discharge produced from dewatering the crushed ore remains a significant environmental concern.

Measurements of acid-volatile sulfide (AVS) and simultaneous extractable metals (SEM) were undertaken to investigate sulfide-binding of metals. The AVS concentrations of the mineral samples ranged from 0.1 to 18 µmol/g, however, all the chimney samples (active, inactive and weathered) had a molar excess of SEM, indicating that there was insufficient AVS to bind all of the SEM fraction should it dissolve.

Elutriate tests, involving the shaking of crushed mineral samples in oxygenated seawater, were undertaken to investigate metal release processes. In general, metal release was high for As, Cu, Mn and Zn and relatively low for Ag, Cd, Ni and Pb. The maximum concentrations of the metals measured (including results from elutriate tests performed for toxicity testing) were 8900 µg/L Mn, 6200 µg/L Zn, 3300 µg/L Cu, 2800 µg/L As, 120 µg/L Pb, 80 µg/L Cd, 40 µg/L Ni, 30 µg/L Ag. Based on the metals concentrations measured in the elutriate waters (Cu and Zn from the chimney materials M and Ch-I, respectively), dilutions of greater than 4000 times may be necessary before the concentrations would be below the 99% protection levels of the ANZECC/ARMCANZ (2000) water quality guidelines.

The mechanism controlling metal release was not determined. The total suspended solids concentration and the resuspension time had the greatest effect on metal concentrations in the elutriate waters. It remains unclear from these tests whether the mineral sample type or metal concentrations most greatly influence the metal release. The role of the reactive sulfide phases, AVS, in modifying the rate and magnitude of metal release was not fully quantified.

The toxicity of the elutriate waters was investigated using tests that determine inhibition of growth of the alga, *Nitzschia closterium*., and inhibition of mobility of the marine copepod, *Acartia sinjiensis*. The undiluted elutriate waters prepared from several mineral samples were toxic to both the algae and copepod. Dilutions of up to 700 times would be required to result in no toxicity from the elutriate waters to these two species.

The procedures used for collection, transportation, crushing and testing of the samples in the present study were quite likely to cause an over-estimation of the metals release that is likely to occur under the 'real' conditions. For example, the water temperature during the elutriate tests was higher than what is expected to be reached during the dewatering operations and greater oxidative-dissolution of sulfide mineral phases may have occurred due to exposure of samples to air during crushing.

The following ongoing studies are proposed:

1. Develop relationships between sediment/chimney mineral material properties and metal concentrations released in elutriates that are suitable for predicting (with reasonable confidence) maximum concentrations of metals in the elutriate waters. This might be achieved by characterisation of sediment/chimneys with a greater range of properties and further investigation of the release parameters such as processing method, time, suspended solids concentration, water temperature, water pressure and dissolved oxygen concentration.

2. Quantify the effects of water temperature and dissolved oxygen concentration on metal release rates from mineral-sediment samples.

3. Determine whether AVS and SEM measurements of mineral-sediment samples provide information that is suitable for modifying predictions of metal release rates. Quantify the rate of oxidation of AVS occurring during mineral processing.

4. Determine the metal concentrations of the fine particulates that will be discharged with the bulk elutriate.

5. Determine the toxicity of the fine particulates that will be discharged with the bulk elutriate.

6. Determine the whole-sediment toxicity discharged material that may deposit on the sea floor.

7. Investigate process changes that can be used to reduce concentrations of total and dissolved metals in the discharge elutriate.

CONTENTS

List of Tables

List of Figures

List of Plates

INTRODUCTION

Nautilus Minerals Niugini (Nautilus) is proposing to develop the Solwara 1 Project. The project proposes to commercialise the copper- and gold-rich seafloor sulfide (SMS) deposits, associated with active and inactive hydrothermal vent systems, in the eastern Manus Basin, Bismarck Sea, Papua New Guinea (PNG).

The offshore component of the project will involve mining SMS deposits at the Solwara 1 Prospect at approximately 1,700 m water depth. The SMS deposits are associated with both active and inactive hydrothermal vent systems. The mining will be conducted from a surface vessel. The mined material (maximum rock size of 30 mm) will be pumped to the surface vessel where the ore will be dewatered. The dewatering system will remove a high percentage of the seawater, retaining an ore slurry.. The process water will be discharged back to the sea at a depth to be determined (but well below the productive surface mixed layers and euphotic zones). As the process water is expected to be approximately 2°C and denser than surface waters, it will not rise above the depth of discharge.

The Centre for Environmental Contaminants Research, CSIRO Land and Water (CLW) was engaged by Coffey Natural Systems Pty Ltd (known as ENESAR Consulting Ptd Ltd when this project commenced) to provide scientific advice and experimentation to assess the water toxicity for the preparation of an Environmental Impact Statement (EIS).

Scope of Work

The scope of work that Coffey Natural Systems requested that CSIRO carry out included determining:

1. Background concentrations of metals in the water column in the vicinity of the SMS deposits.

2. Metals concentrations in plumes created during sampling of SMS deposits.

3. Potential mobilisation of metals from solid to dissolved phases from ore dewatering processes.

4. The toxicity of the ore dewatering water on aquatic organisms.

The specific services requested were:

- Undertake measurements of sediment samples to determine physico-chemical properties (e.g. particle size fractions, acid-volatile sulfide, total organic carbon).

- Analyse total and dilute acid-extractable (1 M HCl) metals concentrations in sediment samples.

- Conduct elutriate tests (shaking sediment in seawater and measuring dissolved contaminants) to determine the potential for release of contaminants from sediment in ore dewatering plumes.

- Conduct bioassays to assess the toxicity to organisms in the water column due to ore dewatering plumes, e.g., by conducting elutriate toxicity tests.

- Prepare a scientific report on the results, discusses the significance of the findings, and provide recommendations for future work.

Proposed Approach

Coffey Natural Systems originally proposed to collect the seawater and sediment samples and supply these to CSIRO for the physico-chemical and toxicity tests. Following discussion, between Coffey Natural Systems and CSIRO, of the logistics of collecting seawater samples for trace-metal analyses, it was agreed that CSIRO should send a staff member to collect the seawater samples. The sediment samples would be collected by Coffey Natural Systems and supplied to CSIRO.

Waters

Ultra-trace sampling and analysis techniques were used for collection of water samples (USEPA 1996; Apte et al. 1998; Apte et al. 2002). Coffey Natural Systems specified that seawater samples be collected from 1, 5, 10 and 20 m from the seabed in the vicinity of a black smoker at Solwara 1 at four different sites. Seawater samples were also requested and taken at 1000 m depth, to target a 'plume' (of suspended material) that had previously been observed at approximately this depth. Background seawater samples were collected (in duplicate) at 500 m water depth. Seawater samples for possible analyses of total and dissolved metals (only totals for Hg), were to be collected (simultaneously) for each location (water depth and site). Total particulate solid (TSS) concentrations were to be measured at each location. Seawater blanks comprised background water samples and field blanks for bottles. In total, water samples from twenty one locations, plus two duplicates and two blanks, were to be collected for dissolved and total metals and total mercury analyses.

Considerable discussion was held between Coffey Natural Systems and CSIRO regarding the determination of trace metals in seawater and the requirements for specialist sample collection and analysis protocols to be adhered to in order to achieve the required detection limits and suitable quality assurance (QA). Typically trace metal concentrations in ocean waters are at low parts per trillion concentrations (e.g. 10-100 ng/L). It was discussed that for dissolved metals analyses in seawater, water samples would, ideally, require filtration and preservation within 24-h of collection, so clean-laboratory facilities needed to be available locally. For ultra-trace metal analyses, several litres of Analytical Reagent (AR) grade HNO_3 is required for washing of Niskin bottles used for water collection, and one litre of high-purity HNO_3 is also needed for washing of filtration apparatus and acidification of filtered seawaters. As the air-freighting of concentrated acids is not permitted by most countries, the AR-grade HNO_3 was obtained within PNG, but the high-purity HNO_3 could not be obtained. Consequently, water filtration and sample preservation techniques were modified by CSIRO to achieve the best possible outcome. The modified approach involved use of filtration equipment with acid-washed filters prepared at CSIRO (high-purity HNO_3, clean room environment) and acidification of samples following return of samples to CSIRO.[2]

For water samples, analyses of total metals (unfiltered samples) were made initially by inductively coupled plasma atomic emission spectrometry (ICP-AES, 3-20 µg/L detection limits). Total analyses of As, Hg, and Se would be by atomic florescence spectroscopy (AFS). Following these analyses, decisions were made whether to progress to ultra-trace methods for selected metals in total and dissolved (<0.45 µm filtered) samples to achieve the low detection limits (e.g. ng/L concentrations).

Sediments (mineral/ores samples)

Approximately four kilograms (4 kg, wet weight) of mineral sample was collected from each location. The sample was homogenised and split into two bags; 1 kg for physico-chemical tests (characterisation, elutriate tests); and 2-3 kg of sediment for undertaking toxicity testing.

The choice of analyses of the mineral samples depended, in part, on the physical state of samples collected. For all sediments, analyses of total metals (aqua regia digestion) and dilute acid-extractable

metals (1 M HCl) were made on crushed samples. For bottom sediments with particle size less than 2 mm, analyses of moisture, particle size <63 µm and total organic carbon (TOC) would be made. For mineral samples (from active and inactive hydrothermal vent systems – black smokers), the materials would require crushing before analyses could be undertaken and analyses such as TOC may not be applicable. Analyses of acid-volatile sulfide (AVS) were also undertaken. All samples were stored chilled from time of collection until delivery to CSIRO.

Elutriate Tests

Experiments were undertaken to determine the rate of metal release from crushed rock and sediment materials. The effect on metal release of resuspension time, total suspended solids concentration and particles size was investigated. The experiments were conducted at room temperature in fully oxygenated seawater.

Toxicity Tests

The toxicity of selected elutriate waters prepared was assessed by determining chronic (population growth rate) effects on marine algae and acute (inhibition of mobility) effects on pelagic copepods.

The toxicity assessment used surrogate species as no tests using local species were available. The philosophy of using surrogate species is that it is expected that species with similar exposure pathways and similar sensitivity will exist at the local sites. It is likely, however, that species exist at the field sites that have both differing exposure pathways (e.g. water versus food) and sensitivity to metals. In the metal-enriched sediments and waters surrounding the black smokers it is likely that endemic species have specialised behaviour (e.g. feeding) adapted for the local conditions.

Notes: The background information for this Introduction was taken from a project brief to CSIRO prepared by Coffey Natural Systems Pty Ltd (ENESAR, 2007)

[2] While preservation of samples with high-purity HNO_3 within 24-h of collection is preferred, past experience and QA tests by CSIRO have found that delayed acidification, post-filtration provides accurate results and is much preferred than risking sample contamination by using HNO_3 of lower quality.

METHODS

Boat Operations

All water and sediment sampling operations were conducted from the stern of the boat, Mercury Wave (Plate 1). A remote operating vehicle (ROV) was used to obtain water samples from the desired depth and location (Plate 1). The ROV operated from a winch that lowered it to the water adjacent stern of the boat.

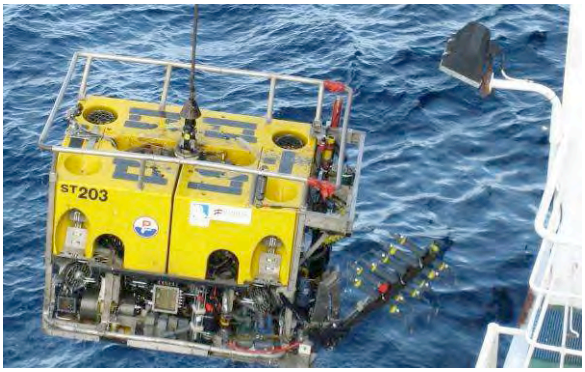

Plate 1. The Mercury Wave, the remote operating vehicle (ROV) and lowering of ROV to water using the crane at rear of boat

Laboratory preparation

To minimise the risk of metal contamination of samples, extensive cleaning of the laboratory was carried out before any equipment was set up (Plate 2). Cleaning involved sweeping and mopping floors, and repeated wiping walls and ceiling with moist Kim wipes®. Most of the laboratory was cleaned in this way, with areas adjacent to where sample processing was to be carried out and air conditioning vents receiving particular attention. These surfaces were cleaned with a cloth moistened with tap water, then repeatedly with tissues moistened with mineral water until no dirt was visible, and finally wiping with tissues (Kim wipes) moistened with mineral water.

To further minimise the risk of sample contamination from laboratory surfaces, all benches were coated with plastic (white Bench-kote®) and white plastic bags were stuck to walls. This was also

performed to minimise air from air conditioning vents from blowing air onto the sample processing area and potentially causing contamination. A Laminar Flow Cabinet was provided in the laboratory, however, as it was not operating at the time, all sample filtrations were undertaken inside the cabinet, but without air flows.

Plate 2. The cleaned laboratory work-space on the boat

Water Sample Collection and Handling

All seawater samples were collected using rigorous 'clean hands/dirty hands' sampling protocols to avoid sample contamination (USEPA 1996; Apte et al. 1998; Apte et al. 2002). This included the

wearing of clean powder-free vinyl gloves for the handling of all sample bottles and sampling equipment.

Trace metal sample bottles

One-litre low density polyethylene (Nalgene) bottles were cleaned using a three stage sequence. First, the bottles and caps were submerged for at least 2 hours in 2% Extran® detergent solution, followed by rinsing with copious amounts of deionised water. The bottles were then soaked for a minimum of 24 hours in 10% nitric acid (analytical reagent grade) contained in a covered plastic tank. They were then rinsed with Milli-Q (MQ) high purity water and then filled with 1% high purity nitric acid (Merck Suprapur), capped and left to stand for at least 48 hours. The bottles were then rinsed three times with MQ water and 'double-bagged' in two polyethylene bags.

Mercury sample bottles

One-litre fluorinated ethylene propylene (FEP) (Nalgene) bottles were used for collection and storage for samples for mercury analysis. Bottles were cleaned by soaking in 10% analytical grade (AR) hydrochloric acid for greater than 2 days and were rinsed with copious quantities of MQ water. The bottles were then filled with 20% ultra-pure grade hydrochloric acid (Merck Suprapur) and left for a minimum of 5 days. The bottles were thoroughly rinsed with MQ water, filled with MQ water, capped and left for at least 2 days. The bottles were then emptied and 'double-bagged' in polyethylene bags (double bagged) prior to shipping.

Sample collection using Niskin bottles operated from ROV

The sample collection was undertaken by a CSIRO staff member assisted by a crew member on board the vessel Mercury Wave. The ROV was used to obtain samples from the desired water depth and location. Water samples were collected using six 2.5 Litre Niskin water sampling bottles mounted on a mechanical arm extending from the ROV (Plate 3). Niskin bottles are non-metallic free-flushing sampling bottles able to be remotely triggered to close and allow the collection of water samples at chosen water depths and locations. The Niskin bottles were cleaned before use by soaking in acidified seawater collected from 500 m water depth (control sample site). The procedure involved lowering Niskin bottles to 500 m depth and standing for 30 min. The ROV was then moved up and down by 10 m twice to flush the seawater through the Niskin bottles, and then closing the Niskin bottles at this depth and returning to the surface. The Niskin bottles were then removed from the arm on the ROV and transported into the laboratory. A small volume of water (~40 mL) was removed and 35 mL of high-purity HNO_3 (Aristar Grade) added to produce a concentration of approximately 1.5 % [3]. The Niskin bottles were then inverted ten times and left to stand for 30 min, with inversions every ten minutes. Following the 30 min acid-soaking period, the acidified water was removed from the Niskin bottles, and they were attached to the arm of the ROV. The ROV then lowered the Niskin bottles to 500 m depth for water sampling at this site, with the residual acid being rinsed from the bottles during the descent.

[3] The AR-grade HNO_3 requested by CSIRO was not available and high-purity HNO_3 (Aristar Grade) was used to acidify the seawater used for washing of Niskin bottles.

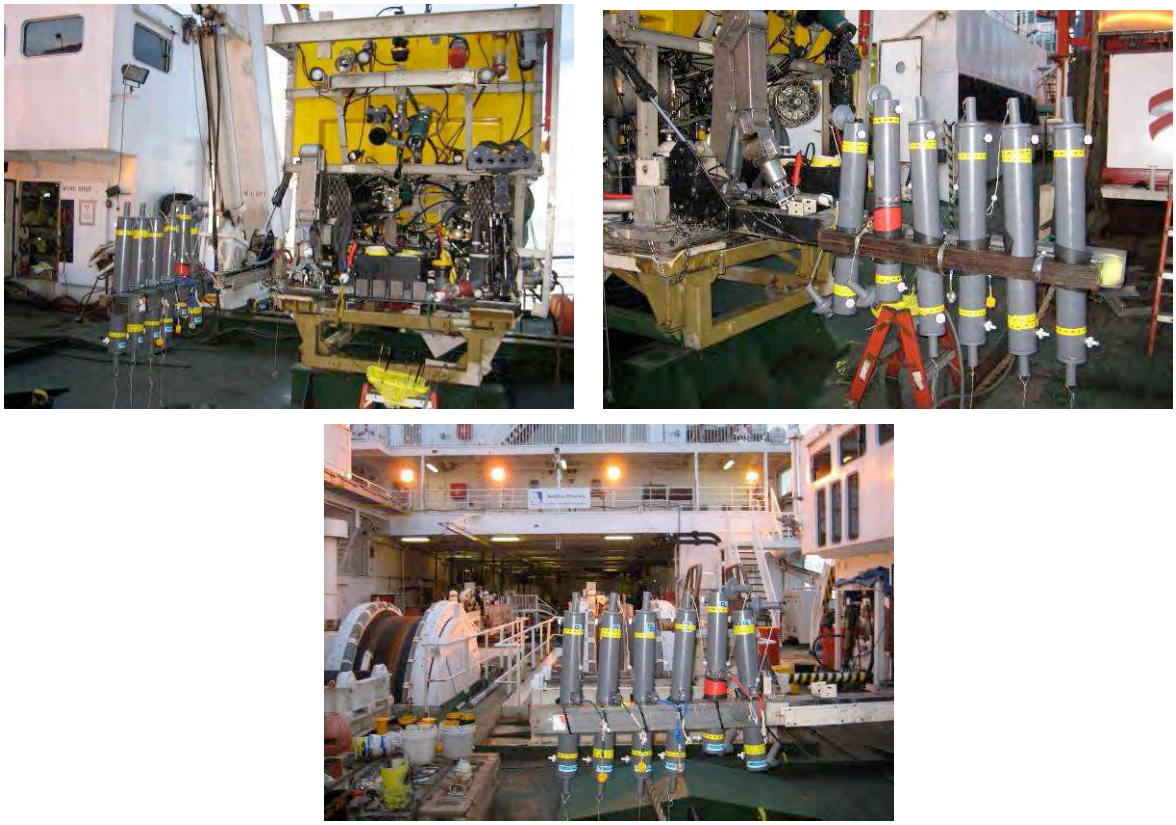

Plate 3. Niskin bottles attached to mechanical arm of ROV for water sampling

The water sampling occurred continuously over a 4-day period and the Niskin bottles remained attached to the ROV during this entire period. Sampling depths were chosen by Coffey Natural Systems as 1, 5, 10, 20 m from the ocean floor, a plume previously detected at 1000 m depth. The locations of the sampling sites were determined in consultation between CSIRO and Nautilus staff on the boat. A 500- m depth location was chosen by CSIRO staff for collection of the field blank. The positioning of the ROV and attached Niskin bottles was made by other staff aboard the boat. The Niskin bottles were deployed in an open position and conditioned with seawater as the ROV travelled to the collection sites 500 m to 1700 m below the sea surface. At the collection sites, the Niskin bottles were similarly conditioned for at least 2 minutes at each depth of water collection before closing to collect samples. The Niskin bottles were triggered to close using a mechanical arm on the ROV. As the ROV was being used for multiple tasks during each dive (e.g. benthos and sediment collections) the time between closure (sample collection) of the Niskin bottles and return to the surface varied from one to five hours.

Upon surfacing, the arm of the ROV containing the Niskin bottles was manoeuvred to ensure minimal disturbance and then the ROV was winched to the boat deck (approximately 15 m above the sea surface). The positioning of the ROV on the deck allowed water samples to be subsequently transferred to acid-washed polyethylene bottles for trace-metal samples and to acid-washed FEP bottles for mercury samples. Polyethylene bottles were partially removed from the sealable bags and the clean hands/dirty hands technique (two persons with specific tasks) was used to acquire the water samples from the Niskin bottles. A staff member from CSIRO acted as the clean hands person and a staff member on board acted as the dirty hands person (sampling timing determined which staff member acted as the dirty hands person).

The samples collected during the water sampling program are shown in Table 1. Water samples were collected from a total of 21 locations (including control site), with replicate samples collected at the control site and at one field site (A3.20, Table 1). From these samples, a total of 22 water sub-samples for total metals (TM) analyses and 22 water sub-samples for dissolved metals (DM) analyses were prepared. In addition, 16 waters were collected for total mercury (THg) analyses and 21 water samples were filtered for total suspended solids (TSS) analyses. Two field-blanks (FB) were prepared using water collected from the Control site (C500). Two operation-blanks (OB) were prepared by opening sample bottles in the sample location for the same period of time required to fill sample bottles with water from the Niskin bottles. The OB samples were filled with high-purity deionised water (Mill-Q, 18 MW) upon return of samples to CSIRO.

Water sample filtration and preservation for dissolved metals analyses

All samples were otherwise treated in an identical manner. Following water collection, all sample bottles were transferred to the on-board laboratory and filtered within 2 h of collection (Plate 2). Water samples for dissolved metals analysis were filtered using polycarbonate filter rigs (Sartorius) fitted with 0.45 µm Millipore membrane filters. All filtration assemblies had been rigorously cleaned at CSIRO in Sydney by first filtering 100 mL volumes of 10% nitric acid solution followed by a ca. 150 mL of MQ water. The filter rigs had then been 'double-bagged' in two polyethylene bags prior to shipping. On board the boat, the filters and rigs were conditioned with a 50 mL volume of sample. The filtrate was used to condition the bottles and then discarded to waste. Most of the remaining sample (generally 750 mL) was then filtered and the filtrates were transferred to acid-washed polyethylene bottles, which had been rinsed with the same 50 mL aliquot used to rinse filtration rigs. The unfiltered water remaining in the sample bottles (generally 200-250 mL) was retained for analysis of total metal concentrations, following acidification.

Following filtration, all sample bottles were double-bagged in polythene zip-lock bags and stored in a refrigerator at 4ºC. All water samples were transferred to CSIRO in cooler-boxes (Eskies) containing ice to ensure the samples remained cold. Upon receipt at CSIRO the water samples for trace-metals analyses were preserved by addition of 2 mL/L concentrated nitric acid (Merck Tracepur). Total mercury samples were preserved by addition of 2 mL/L concentrated high purity nitric acid to the FEP sample bottles.

Total suspended solids (TSS) collection and analysis

Filter papers/membranes were pre-weighed on an analytical balance and the weight recorded accurately to ±0.1 mg. The filter membrane was placed on a clean filtration rig and the vacuum applied. The water to be filtered was thoroughly mixed by shaking and then passed through the filter. The volume of sample passed through the filter was recorded. Any solid material stuck to the side of the upper part of the filtration rig was removed with clean water (e.g. Milli-Q). Once the filtration was complete, the filtrate was discarded (or stored if required for analysis of dissolved analytes) and the filter transferred into a clean holding tray and capped. Upon return to CSIRO, the filters were dried to constant weight on a watch glass in a clean oven set at 60ºC, placed in a desiccator to cool, and then weighing was repeated three times to ensure precise weight readings. TSS concentrations are reported in mg/L.

Table 1. Water samples collected for Solwara 1 project

Dive, Date, time	Location	Name	Depth, m	Proposed analyses			
41, 20/4, 8 am	South Su	Field Blank, FB1	500 (from surface)	TM	DM	THg	-
41, 20/4, 8 am	South Su	Field Blank, FB2	500 (from surface)	TM	DM	-	-
22/4, 4 pm	Milli-Q	Method Blank, OB1	CSIRO water	TM	DM	THg	-
22/4, 6 pm	Milli-Q	Method Blank, OB2	CSIRO water	TM	DM	THg	-
41, 20/4, 8 am	South Su	Control, C500	500 (from surface)	TM	DM	THg	TSS
41, 20/4, 8 am	Duplicate	Control, C500r	500 (from surface)	TM	DM	THg	-
43, 20/4, 6 pm	Williamson mound	Active 1, A1.01	1 m (from bottom)	TM	DM	THg	TSS
42, 20/4, 10 am	Williamson mound	A1.05	5 (from bottom)	TM	DM	THg	TSS
42, 20/4, 10 am	Williamson mound	A1.10	10 (from bottom)	TM	DM	THg	TSS
42, 20/4, 10 am	Williamson mound	A1.20	20 (from bottom)	TM	DM	THg	TSS
43, 20/4, 6 pm	Williamson mound	A1.1000	1000 (from surface)	TM	DM	-	TSS
44, 21/4, 2 am	Binns mound	Active 2, A2.01	1 m (from bottom)	TM	DM	THg	TSS
45, 21/4, 9 pm	Binns mound	A2.05	5 (from bottom)	TM	DM	THg	TSS
44, 21/4, 2 am	Binns mound	A2.10	10 (from bottom)	TM	DM	THg	TSS
44, 21/4, 2 am	Binns mound	A2.20	20 (from bottom)	TM	DM	THg	TSS
45, 21/4, 9 pm	Binns mound	A2.1000	1000 (from surface)	TM	DM	-	TSS
46, 22/4, 8 am	South of Kawalczyk	Cold 1, C1.01	1 m (from bottom)	TM	DM	THg	TSS
46, 22/4, 8 am	South of Kawalczyk	C1.05	5 (from bottom)	TM	DM	THg	TSS
46, 22/4, 8 am	South of Kawalczyk	C1.10	10 (from bottom)	TM	DM	THg	TSS
47, 22/4, 1 pm	South of Kawalczyk	C1.20	20 (from bottom)	TM	DM	THg	TSS
47, 22/4, 1 pm	South of Kawalczyk	A1.1000	1000 (from surface)	TM	DM	-	TSS
48, 22/4, 9 pm	Kawalczyk (active)	Active 3, A3.01	1 m (from bottom)	TM	DM	THg	TSS
48, 22/4, 9 pm	Kawalczyk (active)	A3.05	5 (from bottom)	TM	DM	-	TSS
48, 22/4, 9 pm	Kawalczyk (active)	A3.10	10 (from bottom)	TM	DM	THg	TSS
49, 23/4, 10 am	Kawalczyk (active)	A3.20	20 (from bottom)	TM	DM	THg	TSS
49, 23/4, 10 am	Kawalczyk (active)	A3.1000	1000 (from surface)	TM	DM	-	TSS

'Dive' number was determined by the ROV operators. Date 20/4 = 20/04/2007 etc

TM = total metals, DM = dissolved metals, THg = total mercury (Hg), TSS = total suspended solids, - = sample not collected. Australian Quarantine and Inspection Service (AQIS) protocols applied to handling of all samples

Sediment-Mineral Sample Collection and Handling

Nine sediment (mineral ore) samples were collected from the locations specified in Table 2. The samples were obtained using a specialised robotic-arm of the ROV to collect sediments from the bottom or break off parts of chimnies and place the samples in an adjacent container (Plate 4). The containers used were a jug/scoop for bottom sediments and labelled bins for chimney samples. Upon surfacing of the sample containers, sediment samples were removed and triple bagged then stored in a refrigerator at 4ºC on board the boat. All samples were transported to CSIRO in Eskies chilled with ice. At CSIRO, the sediment samples were re-homogenised using a plastic spoon and sub-samples stored for the various physico-chemical analyses. The chimney samples were stored as received. The bulk samples were stored at CSIRO in a refrigerator at 4ºC. Although analyses of acid-volatile sulfide (AVS) were not part of the initial suite of tests, sub-samples of each sediment-mineral were taken when received at CSIRO for later AVS analyses.

Table 2. Sediment-mineral samples collected for Solwara 1 project

Dive, Date, time	Location	Material Type	Name	Depth, m
42, Jug container, 20/4/7, 10 am	Williamson mound	Sediment: Active silt	A-c	Sea floor
42, Jug container, 20/4/7, 10 am	Williamson mound	Sediment: Active silt	A-t,	Sea floor
43, Jug container, 20/4/7, 6 pm	Williamson mound	Sediment: Cold silt	C-c	Sea floor
43, Jug container, 20/4/7, 6 pm	Williamson mound	Sediment: Cold silt	C-t	Sea floor
43, Bin D container, 20/4/7, 6 pm	Williamson mound	Chimney (active)	Ch-A-c	Chimney
43, Bin D container, 20/4/7, 6 pm	Williamson mound	Chimney (active)	Ch-A-t	Chimney
43, Bin F container, 20/4/7, 6 pm	Williamson mound	Chimney (inactive)	Ch-I-c	Chimney
43, Bin F container, 20/4/7, 6 pm	Williamson mound	Chimney (inactive)	Ch-I-t	Chimney
43, Bin B container, 20/4/7, 6 pm	Williamson mound	Weatherd Chimney	M-t	Sea floor

'Dive' number was determined by the ROV operators. Date 20/4 = 20/04/2007, Time is when ROV decent commenced (24 h). Material Type was a description provided by Nautilus staff

Plate 4. Containers from which the sediment (mineral ore) samples were collected

Quality Control: General Procedures

To check on analytical accuracy, aliquots of a National Research Council Canada (NRC) and National Institute of Standards (NIST) Certified Reference Materials were analysed with each batch of samples whenever a suitable reference material was available. Reference standards have certified concentrations of elements for a range of sample matrices such as seawater and sediment, allowing the performance of the analytical procedures to be assessed by a comparison of the results obtained with the certified concentrations. The following reference materials were used: CASS-4 for metals in saline waters, NASS-5 for As in waters; and PACS-2 for total metals in sediments. In addition, laboratory blanks, analytical duplicates and spiked samples (where appropriate) were included in every sample batch. Method detection limits (3 times the standard deviation of the blank measurements) were calculated from the laboratory blank data.

Delayed water-sample acidification

As a result of the insufficient quantifies of high-purity HNO_3 (Aristar Grade) available when the samples were collected, all the water samples used for trace-metal analyses were acidified upon return to CSIRO.

To demonstrate that the delayed acidification of the samples would not affect the results, experiments were undertaken at CSIRO that mimicked the procedures used for the Solwara water samples before analyses. These experiments used seawater collected from Cronulla, NSW, Australia. Filtered Cronulla seawater was transferred to 15 bottles cleaned using the same procedures as used for the Solwara water samples. Six bottles were unmodified, six bottles spiked with 0.100 µg/L (100 ng/L) metals (Ag, Cd, Co, Cr, Cu, Ni, Pb and Zn), and six bottles spiked with 50 µg/L metals (Ag, Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn). Three of each set of these samples were immediately acidified to 2 mL/L concentrated nitric acid (Merck Tracepur), while the remaining three bottles were acidified with the same quantify and quality of acid seven (7) days later. The concentrations of metals in the un-spiked samples and samples spiked with 0.10 µg/L metals was analysed using trace-metal techniques, and the samples spiked with 50 µg/L metals were analysed by ICP-AES.

Analytical Methods for Waters

General dissolved metals analysis

Water samples were subjected to metal chelation and solvent extraction prior to the analysis of cadmium, copper, lead and silver in order to separate the metals of interest from major ions in the salt matrix that interfere with the final spectrometric measurements. The extraction procedures also preconcentrate the metals by typically 25 to 30-fold, thus making them easier to quantify. Dissolved copper and zinc were analysed in the saline water samples using a dithiocarbamate complexation/solvent extraction graphite furnace atomic absorption spectrometry (GFAAS) method based on the procedure described by Magnusson and Westerlund (1981). The major differences were the use of a combined sodium bicarbonate buffer/ammonium pyrrolidine dithiocarbamate reagent (Apte and Gunn 1987) and 1,1,1-trichloroethane as the extraction solvent in place of Freon. Sample aliquots (250 mL) were buffered to pH 5 by addition of the combined reagent and extracted with two 10 mL portions of double-distilled trichloroethane. The extracts were combined and the metals back-extracted into 1 mL of concentrated nitric acid (Merck Suprapur). The back extracts were diluted to a final volume of 10 mL by addition of deionised water and analysed by GFAAS (Perkin Elmer

4100ZL) using Zeeman effect background correction and operating conditions recommended by the manufacturer.

Dissolved chromium was measured directly by GFAAS (Perkin Elmer 4100ZL) using Zeeman effect background correction and standard additions calibration. Dissolved iron and manganese were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) (Spectroflame EOP, SPECTRO Analytical Instruments, Kleve, Germany) using matrix matched standards. The instruments were operated under the standard conditions recommended by the manufacturer.

Arsenic

Arsenic concentrations were determined by hydride-generation atomic spectrometry, using procedures based on the standard methods described by APHA (1998). Samples were first digested by addition of potassium persulfate (1% m/V final concentration) and heating at 120ºC for 30 minutes. Hydrochloric acid, (3 M final concentration) was then added to the samples. Pentavalent arsenic was then pre-reduced to As(III) by addition of potassium iodide (1% (m/v final concentration) and ascorbic acid (0.2% (m/v final concentration) and standing for at least 20 min at room temperature prior to analysis. Arsenic concentrations were then determined by hydride-generation atomic absorption spectrometry (AAS) using a Varian SpectrAA with a VGA77 hydride generator and heated quartz cell (925°C) using conditions recommended by the manufacturer. Arsenic absorbance was measured at 189 nm using an ultra lamp and background correction.

Selenium

Selenium concentrations were determined by hydride-generation atomic spectrometry, using procedures based on the standard methods described by APHA (1998). Samples were first digested by addition of potassium persulfate (1% m/V final concentration) and heating at 120ºC for 30 minutes, converting all forms of selenium to Se(VI). Hydrochloric acid, (4 M final concentration) was then added to the samples and they were heated (95°C for 45 minutes) forming Se(IV). After cooling sulfanilamide (0.02% final concentration) was added. Selenium concentrations were then determined by hydride-generation atomic absorption spectrometry (AAS) using a Varian SpectrAA with a VGA77 hydride generator and heated quartz cell (850°C) using conditions recommended by the manufacturer. Selenium absorbance was measured at 196 nm using an ultra lamp and background correction.

Total mercury

Total mercury in both filtered and unfiltered water samples was determined by cold vapour AFS (Liang and Bloom, 1993). Eighty mL of sample was dispensed into a Pyrex-glass purging vessel and a 0.4 mL aliquot of bromine monochloride (0.2 M) in hydrochloric acid (BrCl) added to allow oxidation of any organic mercury present to inorganic mercury. The mixture was allowed to stand for a minimum of 90 min. Fifty µL of hydroxylamine solution (3 M) was added to destroy any residual BrCl. The vessel was connected to a custom-built purge trap system and 0.5 mL stannous chloride solution (20% m/V) was then added to reduce the inorganic mercury to elemental mercury. The elemental mercury was purged from solution in a nitrogen stream (20 min purge time) and trapped on a gold-coated sand trap. The trap was transferred to a thermal desorption unit interfaced to a Brooks Rand Atomic Fluorescence Spectrometer. The trap was connected to a mercury-free helium gas stream and rapidly heated to 320ºC. The released mercury was quantified by the AFS. The same analytical procedure was also applied to samples for filterable mercury.

Silver

Dissolved silver concentrations were determined by microsolvent extraction GFAAS (Apte and Gunn 1987) using dithizone as the metal complexing ligand. A stock dithizone solution (0.1% w/v) was prepared in double-distilled trichloroethane and was further diluted with double-distilled trichloroethane to give a 0.01% (v/v) working solution (prepared on a daily basis). A 30 mL sample aliquot was accurately transferred to an acid-washed fluorinated ethylene propylene (FEP) Oak Ridge centrifuge tube to which 1.3 mL of 3M sodium acetate buffer (final pH 5.0-5.5) and 1 mL of the dithizone solution was added. The centrifuge tube was tightly capped and shaken for 5 minutes. Following standing for 10 minutes the tubes were uncapped and 1.5 mL of the lower portion of solution (comprising the organic extract plus some of the aqueous layer) was pipetted into a dry acid washed PTFE furnace cup. The silver content of the solvent layer was determined using a Perkin Elmer 4100ZL graphite furnace atomic absorption spectrometer equipped with Zeeman effect background correction and a silver hollow cathode lamp. The furnace operating conditions recommended by the manufacturer were used. The autosampler arm was adjusted so that the sampling probe penetrated the upper aqueous layer and sampled from the lower organic layer only. Standards of concentration 0, 40, 80 120, and 160 ng/L were prepared by spiking amounts of stock silver standard into 2 mL/L nitric acid solutions, which were extracted alongside samples in every batch.

Analytical Methods for Sediment Samples

Physico-chemical measurements on sediments

Sediment moisture contents were determined according to standard methods (Mudroch et al., 1997). Sediment grain size fractionation was determined by sieving a known weight of wet sediment sequentially through stainless steel sieves of decreasing aperture (4, 2 , 1, 0.5, 0.25 and 0.063 mm) using a minimal volume of Milli-Q water. The remaining sediment on each sieve was then dried in beakers (110ºC, >24 h). The dry weight and wet weight were then used to determine grain size fractionation of the sediments.

Total organic carbon (TOC) analyses in sediments were undertaken by the National Measurement Institute (NMI, Pymble, Australia) in accordance with NMI Method No: NWS 15 “Determination of total organic carbon in soil and total (non-volatile) organic carbon in water.” The sample was weighed into a small platinum boat and acid added to remove inorganic carbon (carbonates and bicarbonates). The residue was heated in an oven at 75ºC to dryness and the boat with the dried residue was placed in the boat accessory of a Dohrmann DC-190 high temperature TOC analyser. The boat was heated in a furnace to convert all organic carbon to carbon dioxide and the gas so produced swept into the main body of the TOC analyser where its concentration was determined by a non-dispersive infrared detector. The instrument was fitted with software enabling it to give a read out of per cent organic carbon in the sample. The method had a limit of determination of 100 mg/kg. For QA, per batch or every 20 samples, two reagent blanks, one sample duplicate, one blank spike, one post-digestion matrix spike or sample spike and a reference material (where available) were analysed. The sediment and chimney samples were dried, homogenised and equal portions of the ‘-c’ and ‘-t ‘ replicate samples (Table 2) were combined before analyses.

Determination of total sediment metals (TPM)

Frozen sediments were left to thaw overnight in a nitrogen atmosphere. Each sample was then thoroughly homogenised by vigorous stirring with a plastic rod. A sub-sample was then taken and dried at 110 ºC (16 h). The dried samples were ground to a fine powder using a mortar and pestle.

Two hundred and fifty milligrams of the sediment powder was weighed into a polycarbonate vial and 0.5 mL of concentrated nitric acid (Merck Suprapur) and 1 mL of concentrated hydrochloric acid (Merck Suprapur) was added. All sample digests were prepared in triplicate. The samples were allowed to stand for between 12 to 24 hours and were then twice heated in a domestic microwave oven for 20 min at 10% power. After cooling to room temperature, the solutions were made up to a final volume of 25.0 mL with deionised water. To provide a check on analytical quality, a digestion blank and a certified reference sediment (PACS-2, National Research Council, Canada) was analysed with every batch of microwave samples. The concentrations of Ag, As, Cd, Cr, Cu, Fe, Hg, Mn, Pb and Zn in the final digest solutions were analysed using a range of analytical techniques. Cu, Fe, Mn and Zn were measured by ICPAES (Spectroflame EOP, SPECTRO Analytical Instruments, Kleve, Germany).

Determination of 1-M HCl (dilute acid) extractable metals (AEM) and simultaneously extractable metals (SEM)

The methods for determining acid extractable metals (AEM) and simultaneously extractable metals (SEM) were identical; both involving extraction of wet sediment with cold 1-M HCl (dilute acid). The AEM and SEM analyses were conducted on frozen sediments/minerals samples (sub-samples of bulk material) that were thawed overnight in a nitrogen-filled glove box before analysis. All subsequent sample handling and manipulation was carried out in the glove box. A known wet weight of sediment was extracted with 1 M HCl for 30 min at room temperature (~10 g sediment/L). Acid-extracts were filtered through 0.45 µm membrane filters and analysed by ICP-AES against matrix-matched standards. This extraction is analogous to the acid-extraction used for simultaneously extractable metals (SEM) analyses (Allen et al., 1993). The detection limits for the metals of interest were approximately 0.4 mg/kg for Al, Cd, Co, Cr, Cu, Mn, Ni and Zn, and 1 mg/kg for Ag, As, Fe and Pb (by ICP-AES).

Determination of acid-volatile sulfide (AVS)

The AVS analyses were conducted on frozen sediments/minerals samples (sub-samples of bulk material) that were thawed overnight in a nitrogen-filled glove box before analysis. All subsequent sample handling and manipulation was carried out in the glove box. The method for determining sediment AVS was based on the procedure described by Simpson (2001). Deoxygenation of waters for these tests was achieved by sparging deionised water with N_2(g) in glass containers. The method is applicable to sediments having AVS concentrations in the range 0.5 to 300 µmol/g (sediment, dry weight). The limit of determination is approximately 0.5 µmol/g. The method utilizes the direct reaction of ‘Clines reagent’ (methylene blue) (Cline, 1969) with small amounts of sediment followed by colorimetric determination of sulfide release by 1-M acid.

As noted earlier, analyses of acid-volatile sulfide (AVS) were not included in the analyte list at the time of sample collection. For this reason, samples of the collected sediment/minerals were not preserved by freezing immediately after collection. The samples were stored cold, and sub-samples frozen upon arrival at CSIRO and all further handling and manipulation of the samples was undertaken in a nitrogen-gas filled glove box.

Elutriate Tests

Elutriate tests were undertaken on sediment/rock samples on-board the boat (in the field) two days after collection and also at CSIRO (3-6 months after collection). During this period the samples were stored refrigerated at 4ºC. The basic methodology for the field-based elutriate tests was prescribed by Samantha Smith (Environmental Manager, Nautilus Minerals) and the technique used developed by both Nautilus and CSIRO. All tests were conducted by CSIRO.

Field-based elutriate tests

The field-based elutriate tests investigated the effect of elutriate time (5 min, 6 h, 12 h, 24 h) and 'chip' (TSS) concentration (1, 4, 10 chips; equivalent to ~10±2, 40±8, 90±8 g/L) on metal release.

These elutriate tests were undertaken using mineral sample M (43, Bin B container, Williamson mound, eroded chimney / sediment). Mineral samples of 2.5 cm diameter were prepared by placing the larger mineral sample M in a plastic bag and hitting with a mallet. Chips of minerals with approximately 2.5 cm diameter were selected for the elutriate tests. The tests were conducted in 1-L plastic bottles (supplied by Nautilus) using 1000 mL of seawater (Field Blank, FB1). The temperature of the boat's laboratory was approximately 20ºC. Immediately following crushing, selection and weighing of chip samples (mineral M), the chips were placed in the bottle, the seawater added and then manually shaken for 30 s and then left to rest/settle for 2 min. The 30 s of shaking, followed by 2 min of resting/settling process, was repeated twice further and allowed sufficient time for all samples to undergo similar treatment using a single person operation. After 5 min from the start of the elutriate tests, two bottles for each of the three chip concentrations were randomly selected and samples were filtered (<0.45 µm) into new 1 L bottles. The remaining elutriate tests were allowed to rest/settle until the required time period, shaken again 5 min before the test completion and then filtered. Filtered water samples were acidified on-site using the high-purity concentrated HNO_3 (sufficient high purity acid was available for acidifiying these samples). The accuracy of the weighing balance used to weigh out chips of rock was ±2 g. All tests were undertaken in duplicate.

Laboratory-based elutriate tests

The laboratory-based elutriate tests characterises the effects of resuspension time, suspended solids concentration, particle size and mineral type (metal concentrations and properties) on metal release from the samples. There were five types of sediment/mineral material (Table 2), and nine samples in total, available for these elutriate tests, all of which were considered to be representative of material that may be mined. From the time the ore is mined, to the time the dewatered seawater will be discharged, the time elapsed is expected to be 10 to 12 min. The particle size of the mined material is expected to be 80% less than 25 mm diameter, with the remaining 20% of smaller, unknown size. No other specifications were provided.

The general methodology provided below was proposed by CSIRO and accepted before proceeding. The test procedures were modified methods commonly used for assessing water quality impacts of disposal of sediments (USEPA/USACE (1991) and (1994) guidelines). The major modifications were (i) the sediment-to-water ratio was 1, 10 or 100 g/L (dry weight) rather than a sediment-to-water ratio of 1:4, and (ii) plastic, rather than glass, bottles were used. The clean seawater used for the elutriate testing was collected from Cronulla, Sydney, filtered (<0.45 µm) and stored in a refrigerator at 4ºC until use. One-litre low density polyethylene (LDPE, Nalgene) bottles were used for the elutriate test bottles because metals, rather than organics, were the contaminants of potential concern. The wide-mouth LDPE bottles were cleaned by acid-washing with 10% AR-grade HNO_3 and rinsing with

copious amounts of deionised water before use. The elutriate was prepared by adding the specified test material to a 250-mL bottle, followed by 300 mL of clean seawater. The mixture was then shaken by end-to-end rolling for the specified time period before collection of a filtered 0.45 µm water sample for chemical analysis. The shaking time period used for many of the tests was 30 min. The seawaters were fully oxygenated, the temperature was 22±3 ºC, and were expected to provide a worst-case scenario for metal sulfide oxidation and metal release. The experiments were performed in the dark.

Measurements of pH (calibrated against pH 4 and 7 buffers, Orion Pacific) used a pH meter (WTW pH 320) equipped with combination pH electrode (Sure-flow 9165BN, Thermo Orion). Dissolved oxygen measurements were made using a WTW Oxi 330 meter and CellOx 325 probe in accordance with the instrument manufacturer's instructions.

Crushing of chimney mineral samples to particle size required for tests

The chimney samples (Table 2) comprised 5-20 cm diameter pieces of rock. For all elutriate tests, including the toxicity tests, mineral samples of the desired diameter were prepared as follows. The larger mineral sample was placed in two heavy duty plastic bags and hit using a mallet with a plastic-coated steel head. The broken mineral was then rapidly sorted by sieving through a series of sieves (all fractions going to separate containers). This process generally took 5-10 min per sample. The material used in the elutriate tests was then prepared by combining suitable amounts of each size fraction (measured by weighing) and then homogenising with a Teflon spatula. This reconstituted material was then used immediately in elutriate tests.

Effect of particle size

Mineral rock/sediment samples of particle size 4-30 mm were used for these experiments. The mineral samples were crushed and sieved (as described above) to provide five size fractions: 4-30 mm, 1-4 mm, 0.25-1 mm, 0.063-0.25 mm, <63 µm. Then elutriate tests were performed immediately. For elutriate tests, the amount of each size fraction equivalent to 10 g/L (dry weight) was added to a bottle containing 300 mL of seawater and shaken for 30 min (continous end-to-end rolling). The pH and dissolved oxygen were measured for all tests. A duplicate test was undertaken with identical conditions for the 0.25-1 mm size fraction. After 30 min, samples were taken in duplicate for dissolved (<0.45 µm filterable) metals and analysed by ICP-AES after acidification.

Effect of total suspended solids (TSS) concentration

Mineral rock/sediment samples of particle size 80% chips (4-30 mm) and 20% finer materials (<0.25 mm) were used for these experiments (prepared as described above). Determination of metal release during elutriate tests was conducted using suspended solids concentrations of 1, 10 and 100 g/L and a time period of 30 min (with continous end-to-end rolling). The pH and dissolved oxygen were measured for all tests. A duplicate test was undertaken with identical conditions for the 10 g/L treatment.

Effect of time

Mineral rock/sediment samples of particle size <0.25 mm were used for these experiments (prepared as described above). The kinetics of metal release during elutriate tests was investigated for time periods of 2, 10, 30, 60 and 240 min (continous end-to-end rolling). For elutriate tests, the amount of each size fraction equivalent to 10 g/L (dry weight) was added to a bottle containing 125 mL of seawater. The pH and dissolved oxygen were measured for all tests. A duplicate test was undertaken with identical conditions for the 30 min treatment.

Elutriate tests on remaining samples

Elutriate tests were performed on the six remaining samples (Table 2) for which elutriate tests had not been performed (including results of toxicity tests): sediment materials A-c / A-t (active silt) and C-t (cold silt), crushed chimney material Ch-A-c (active), Ch-I-c (inactive) and Ch-I-t (inactive). The particle size of the crushed rock materials used to prepare the elutriate waters was 80% 4-25 mm (<25 mm) and 20% <1 mm. The sediment materials did not require crushing and were homogenised then material <25 mm used to prepare the elutriate waters. The particle size range was determined for each material used to prepare elutriates. Elutriate waters of 250 mL volume were prepared by shaking 25 g of each material (100 g/L) in 250 L of clean seawater for 12 min (continous end-to-end rolling), followed by sample filtration. Dissolved metals were determined by ICP-AES on acidified (undiluted) elutriate samples. The test was replicated for sample C-t. Duplicate elutriate samples with identical conditions were prepared and analysed for two tests.

Toxicity Tests

Elutriate water preparation for toxicity tests

Elutriate waters for toxicity tests were prepared for four mineral ore samples (Table 2): sediment materials A-t (active silt) and C-c (cold silt), crushed chimney material Ch-A-t (active), and sediment rock M-t. The particle size of the crushed rock materials used to prepare the elutriate waters was 80% 4-25 mm (<25 mm) and 20% <1 mm. The sediment materials did not require crushing and were homogenised then material <25 mm used to prepare the elutriate waters. The particle size range was determined for each material used to prepare the elutriates.

Elutriate waters of 1 L volume were prepared by shaking 100 g of each material in 1 L of clean seawater for 12 min (continous end-to-end rolling), followed by centrifugation and immediate toxicity testing. The shaking times were chosen to resemble the conditions expected for the mineral processing and dewatering. The solid:water ratio of 100g/L was chosen to provide a high solids load and sufficient elutriate water for the tests. The elutriate water was serially diluted (1:2) with natural seawater (filtered to 0.45 µm) to give test concentrations of 6-100% elutriate water. Dissolved metals were determined by ICP-AES on acidified (undiluted) elutriate samples.

Chronic algal growth toxicity test using the marine alga *Nitzschia closterium*

This test determines the inhibition of growth rate of the marine alga *Nitzschia closterium* over 72 h. The test is based on the OECD Test Guideline 201 (1984) and the protocol of Stauber et al. (1994). The test protocol is summarised in Table 3. This test was carried out at CSIRO.

Algal stock cultures

The unicellular marine diatom *Nitzschia closterium* (Ehrenberg) W. Smith (Strain CS 5) was originally isolated from Port Hacking, NSW. The diatom was cultured in f medium (Guillard and Ryther, 1962) with the iron and trace element concentrations halved. The culture was maintained on a 12 h light:12 h dark cycle (Philips TL 40 W fluorescent daylight, 60 µmol photons s^{-1} m^{-2}) at 21°C without agitation.

Cells in log phase growth were used in the algal bioassays according to the standard protocol (Stauber et al., 1994). The inoculum was washed and centrifuged three times prior to use in the algal bioassays, to remove culture medium.

Table 3. Summary of the Test Protocol for the *Nitzschia closterium* Growth Inhibition Bioassay

Test type	Static, non-renewal
Temperature	21 ± 1°C
Light quality	Daylight fluorescent lighting
Light intensity	150 µmol photons s^{-1} m^{-2}
Photoperiod	12 h light : 12 h dark
Test chamber size	250 mL
Test solution volume	50 mL
Renewal of test solutions	None
Age of test organisms	6 days
Initial cell density in test chambers	2-4 x 10^4 cells/mL
No. of replicates chambers/concentration	3
Shaking rate	Twice daily by hand
Dilution water	Natural 0.45 µm filtered seawater
Seepage water concentrations	Minimum of 5 and a control
Dilution factor	1:2
Test duration	72 h
Endpoint	Growth (cell division rate)
Test acceptability	Cell division rate in controls of 1.4 ± 0.4 doublings per day. Variability in the controls <20%. Reference toxicant IC50 within chart limits.

Algal bioassay

The elutriates and method blanks were filtered through acid washed (10% nitric acid) 0.45µm filters prior to toxicity tests. Physico-chemical conditions of the elutriates and method blanks were measured as received, and again after adjustment and filtration.

Three sets of controls and one reference toxicant (each in triplicate) were tested: (i) seawater controls – filtered (0.45 µm) seawater, (ii) method blanks – filtered (0.45 µm) seawater that had been centrifuged and filtered exactly as the elutriates had been, (iii) reference toxicant (copper – 2.5-40 µg Cu/L).

Up to seven concentrations of elutriate, each in triplicate, were prepared by diluting with filtered seawater. Fifty millilitres of each test solution was dispensed into 250 mL silanised (Coatasil, Ajax) glass Erlenmeyer flasks. To each flask, 0.5 mL of 26 mM sodium nitrate and 0.5 mL of 1.3 mM potassium dihydrogen phosphate were added as nutrients.

Each flask was inoculated with 2-4 x 10^4 cells/mL of a prewashed *Nitzschia* suspension. Flasks were incubated at 21°C on a 12 h light:12 h dark cycle (Philips LTD 36 W fluorescent daylight) at 150 µmol photons s^{-1} m^{-2}. The pH was monitored at the beginning and end of the bioassay.

Cell density in each treatment was determined daily for 3 days using a Becton Dickinson FACSCalibur flow cytometer. A regression line was fitted to a plot of $\log_{10}$ (cell density) versus time (h) for each flask and the cell division rate (µ) determined from the slope. Cell division rates per day (3.32 x µ x 24) were calculated.

Quality assurance

The bioassay was acceptable if the cell division rate in the controls was 1.5 ± 0.4 divisions per day, and if the reference toxicant copper IC50 was within the limits of 18 ± 12 µg Cu/L.

Statistical analyses

The 72-h IC50 value (the inhibitory concentration of elutriate which gave a 50% reduction in cell division rate compared to the controls) was calculated using Linear Interpolation in ToxCalc Version 5.0.23 (Tidepool Software). After testing the data for normality and homogeneity of variance, Dunnett's Multiple Comparison Test was used to determine which elutriate concentrations were significantly different to the controls in order to estimate LOEC and NOEC values.

Copepod immobilisation test

The 48-h acute test was used to determine effects of exposure to elutriates on the marine copepod *Acartia sinjiensis*, with immobilisation as the test endpoint. The test protocol is based on ISO (1999), with modifications by Rose et al. (2006). The test is summarised in Table 4.

Copepods were originally supplied by Gale Semmens from the Queensland Department of Primary Industries, Cairns. Copepods were cultured at CSIRO in 30-33‰ salinity seawater (pH 8.0) at 27°C. Copepods were fed daily with 20,000 cell/mL of the microalga *Cryptomonas* sp. and 25,000 cell/mL of the microalga *Isochrysis galbana*, with water changes weekly. Adult copepods (10-12 days old) were fed for a minimum of 2 h prior to testing.

Elutriates were prepared as described for the algal toxicity tests. The toxicity tests were undertaken on unfiltered elutriate waters. Dissolved metal analysed were made on filtered sub-samples of these waters. Up to seven concentrations of elutriate were prepared by diluting with filtered (0.45 µm) seawater. Twenty millilitres of each concentration and control was pipetted into 20 mL glass scintillation vials. Five copepods were added to each test concentration (four replicates per treatment), to give a total of 20 copepods per treatment. The pH, salinity, dissolved oxygen and temperature was measured on Day 0 and Day 2 of the test. Copepods were examined after 24 and 48 h for immobility.

Several sets of controls were tested within each copepod test: (i) SW control – filtered seawater, (ii) pH controls – filtered seawater adjusted to match the pH of test solutions that were outside the acceptable pH ranges for copepods (i.e. pH was <7.5), and (iii) reference toxicant (copper) tested at 5 concentrations (0-160 µg Cu/L) in 0.45 µm filtered seawater that had been adjusted to pH 7.5.

Tests were considered acceptable if there was ≥80% mobile animals in SW controls and if the EC50 of the reference toxicant (copper) was within the acceptable range of 44 ± 16 µg Cu/L. Toxicity data were arcsine transformed, if necessary, prior to calculation of toxicity endpoints, using ToxCalc Version 5.0.23 (Tidepool Software). Estimates for the EC50 and associated 95% confidence intervals were determined using the Trimmed Spearman-Karber method. After testing the data for normality and homogeneity of variance, Dunnett's Multiple Comparison Test (parametric), Steel's Many-One Rank Test (non-parametric), or Bonferroni's t-Test (parametric with unequal replicates) were used to determine which concentrations were significantly different to the controls (at alpha = 0.05).

Table 4. Summary of Test Conditions for the 48-h Acute Test with the Copepod *Acartia sinjiensis*

Test type	Static non-renewal
Test duration	48 h
Temperature	27±1˚C
Salinity	30 - 35‰
Dissolved oxygen	≥ 80% saturation
Light quality	Cool white
Light intensity	8.1 µmols^{-1} m^{-2}
Photoperiod	12 h light; 12 h dark
Test chamber size	20 mL
Test solution volume	20 mL
Renewal of test solutions	None
Age of test organisms	10-12 days old
No. of organisms per test chamber	5
No. of replicate chambers per concentration	4
No. of organisms per concentration	20
Concentrations	Minimum of 5 and a control
Feeding regime	None (fed 2 h prior to test)
Test chamber aeration	None
Dilution water	0.45 µm filtered seawater or 0.45 µm filtered seawater adjusted to pH 7.5 (reference toxicant test only)
Test endpoint	Immobilisation
Test acceptability	≥80% mobilisation in controls, copper EC50 within chart limits.

RESULTS

Water Analyses

Delayed water-sample acidification

The delay in acidification of the sea water samples by 7 days (from time of collection) was demonstrated to have not affected the dissolved metal concentrations. For sea water spiked with 50 µg/L metals and analysed by ICP-AES or sea water spiked with 100 ng/L metals and analysed using the trace-analysis methods, the recovery of the spiked metals was 90-105% for all metals. This indicated any changes in dissolved metal concentrations that occurred due to storage of samples un-acidified for 7 days were reversible following acidification (Appendix 1).

Analyses of seawater samples by ICP-AES

The concentrations of metals (not including Hg and Se) were initially measured in the total (unfiltered) water samples by ICP-AES to determine if concentrations were high for some metals (Table 5, Appendix 1). The metal concentrations measured by ICP-AES were less than the 3-10 µg/L detection limit for most metals, despite the samples being collected near the black smokers and being unfiltered (i.e. includes acid-soluble metals associated with particles). At some sites (e.g. C1.20 and A3.05 for copper, Table 5), the total concentrations of Cu, Pb or Zn were above the water quality guidelines for dissolved concentrations of these metals (ANZECC/ARMCANZ, 2000). This indicated a need for accurate measurement of dissolved (<0.45 µm filtered) metal concentrations. The ICP-AES analyses indicated that ultra trace-metal analysis procedures would be required for all the metals Ag, As, Cd, Co, Cr, Cu, Hg, Ni, Pb, Se, and Zn. For some water samples, zinc was determined by ICP-AES as well as by ultra trace-metal analysis because of the better accuracy of the trace-analysis methods.

Ultra trace-metal concentration detection limits of quality assurance for seawater

The detection limits for reporting the ultra trace-metal concentrations (calculated as three standard deviations of the blanks, n>10) were in the low ng/L-range: 5 ng Ag/L, 100 ng As/L, 2 ng Cd/L, 24 ng Co/L, 90 ng Cr/L, 30 ng Cu/L, 0.2 ng Hg/L, 31 ng Ni/L, 16 ng Pb/L, 20 ng Se/L, 27 ng Zn/L (Appendix 1). Spike-recoveries were 83-106% (Appendix 1). Analyses of the certified reference water, CASS-4, were within expected ranges (Appendix 1). Duplicate analyses of the same water sample indicated suitable precision (Appendix 1).

To address sample contamination from bottles and sample collection, two operation-blanks (OB) were prepared with bottles handled in an identical manner as samples, but filled with high-purity deionised water (Milli-Q, 18 MW), rather than seawater and two field-blanks (FB) were prepared using water collected from the control site (C500). The metal concentrations in the operation blanks (OB) for both unfiltered and filtered water samples were below the detection limits and indicated that sample bottle contamination due to opening and filling was negligible (Tables 6 and 7). The metal concentrations in the field-blanks (FB) were very similar to the concentrations measured in the seawater samples collected from 500 m depth (Tables 6 and 7).

Table 5. Total (unfiltered) metals in the sea water samples by ICP-AES, µg/L

Location/Name [a]	Ag	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Field Blank 1	<3	<5	<10	<3	<3	<3	<3	<5	<3	<3	<10	9
Field Blank 2	<3	<5	<10	<3	<3	<3	<3	<5	<3	<3	<10	11
Seawater-1 500 m	<3	<5	<10	<3	<3	<3	<3	<5	<3	<3	<10	4
Seawater-2 500 m	<3	<5	<10	<3	<3	<3	<3	<5	<3	<3	<10	5
A1.01	<3	<5	<10	<3	<3	<3	<3	7.0	<3	<3	<10	<3
A1.05	<3	<5	<10	<3	<3	<3	<3	21	9	<3	<10	5
A1.10	<3	<5	<10	<3	<3	<3	<3	7.6	<3	<3	<10	4
A1.20	<3	<5	<10	<3	<3	<3	<3	<5	<3	<3	<10	<3
A1.1000	<3	<5	<10	<3	<3	<3	<3	<5	<3	<3	<10	<3
A2.01	<3	<5	<10	<3	<3	<3	<3	<5	<3	<3	<10	<3
A2.05	<3	<5	<10	<3	<3	<3	<3	<5	<3	<3	<10	<3
A2.10	<3	<5	<10	<3	<3	<3	<3	<5	<3	<3	<10	<3
A2.20	<3	<5	<10	<3	<3	<3	<3	<5	<3	<3	<10	<3
A2.1000	-	-	-	-	-	-	-	-	-	-	-	-
C1.01	<3	<5	<10	<3	<3	<3	<3	19	<3	<3	<10	<3
C1.05	<3	<5	<10	<3	<3	<3	<3	<5	<3	<3	<10	<3
C1.10	<3	<5	<10	<3	<3	<3	<3	<5	<3	<3	<10	<3
C1.20	<3	<5	<10	<3	<3	<3	17	28	<3	<3	<10	5
C1.1000	-	-	-	-	-	-	-	-	-	-	-	-
A3.01	<3	<5	<10	<3	<3	<3	<3	14	7	<3	<10	<3
A3.05	<3	<5	<10	<3	<3	<3	35	76	9	<3	<10	16
A3.10	<3	<5	<10	<3	<3	<3	<3	75	4	<3	12	24
A3.20	<3	<5	<10	<3	<3	<3	<3	45	16	<3	15	74
A3.1000	-	-	-	-	-	-	-	-	-	-	-	-

[a] Location/Name codes in Table 1, [b] TSS = total suspended solids.

Although the Niskin bottles were cleaned by soaking in seawater acidified with high-purity acid before use, the operating area surrounding the Niskin bottles contained many metal sources (Plate 3). The Niskin bottles were loaded in an open position on the ROV (Plate 3). The total metal concentrations in the operation blanks (sample bottles opened in field but filled with Milli-Q water) were below the detection limits.

The trace metal concentrations in the seawater field blanks were similar to those measured for the seawater samples collected from the same site (Seawater 500 m depth), with the exception of zinc. The total and dissolved zinc concentrations in the field blanks were 12-13 µg/L, compared to 5-7 µg/L in the waters collected from the Seawater 500 m site (Tables 5 and 6). The closeness of the filtered and unfiltered (total) zinc concentrations measured for the replicate samples (1 and 2) indicated that the difference was not likely to be due to contamination during filtration and analysis (Tables 5 and 6). The likely reason for the difference was that the samples were taken from different Niskin bottles and the seawater for field blanks was stored in an additional container for approximately 24 h before being used. The Niskin bottles are considered to be the most likely source of the zinc contamination as they were open to the air on board the main deck for some period of time before deployment (Plate 3).

Trace metal concentrations in the seawater samples

The concentrations of trace metals in filtered (dissolved) and unfiltered (total) seawater samples are shown in Tables 6 and 7 (Appendix 1), respectively. All the data are excellent, with the exception of zinc. For zinc, there appeared to be a source of contaminations (either from the Niskin bottles or during sample filtration) and for this reason the dissolved zinc data should be interpreted with some caution.

Table 6. Dissolved (<0.45 µm filtered) metals in the seawater samples, ng/L[b]

Location/Name [a]	Ag	Cd	Co	Cr	Cu	Ni	Pb	Zn
	ng/L		ng/L		ng/L		ng/L	
Operation Blank 1	<5	<2	<24	<90	<30	<31	<15	33
Operation Blank 2	<5	<2	<24	<90	<30	<31	<15	<27
Field Blank 1	<5	44	<24	214	41	361	63	12300
Field Blank 2	<5	43	<24	214	47	389	39	12400
Seawater-1 500 m	<5	45	<24	236	118	293	<15	5020
Seawater-2 500 m	<5	44	<24	200	54	379	34	7390
A1.01	<5	55	<24	220	110	650	27	3300
A1.05	<5	17	<24	170	190	670	72	4900
A1.10	<5	37	<24	220	130	660	29	5600
A1.20	<5	81	<24	210	150	570	16	4200
A1.1000	<5	69	<24	160	100	480	15	2800
A2.01	<5	66	<24	210	99	580	42	4600
A2.05	7.2	85	<24	180	170	620	20	1700
A2.10	<5	72	<24	200	120	640	22	4500
A2.20	<5	84	<24	200	170	660	29	3400
A2.1000	NA	NA	NA	NA	NA	NA	NA	NA
A3.01	<5	35	<24	150	180	550	46	2400
A3.05	<5	28	<24	180	180	570	57	3700
A3.10	<5	70	<24	230	100	520	52	8000
A3.20	<5	15	53	180	140	580	211	6800
A3.1000	NA	NA	NA	NA	NA	NA	NA	NA
C1.01	<5	83	<24	190	140	580	23	1900
C1.05	<5	86	<24	230	160	600	15	1500
C1.10	<5	87	<24	190	160	650	25	1900
C1.20	<5	84	<24	210	210	620	33	4100
C1.1000	NA	NA	NA	NA	NA	NA	NA	NA

[a] Location/Name codes in Table 1, [b] Ag analysed by MSE-GFAAS, Cr analysed by GFAAS, Cd, Cu, Co, Ni, Pb Zn analysed by SE-GFAAS, Hg analysed by AFS, [b] Fe and Mn concentrations analysed by ICP-AES. NA = not analysed.

Table 7. Total (unfiltered) metals in the seawater samples (ng/L or µg/L)[c]

Name [a]	TSS [b]	Ag	Cd	Co	Cr	Cu	Ni	Pb	Zn	Hg	As	Se
Location	mg/L	ng/L		ng/L		ng/L		ng/L		ng/L	µg/L	
Operation Blank 1	NA	<5	<1	<9	<90	<16	<26	26	<26	<0.2	<0.1	<0.02
Operation Blank 2	NA	<5	<1	<9	<90	<16	<26	18	<26	<0.2	<0.1	<0.02
Field Blank 1	NA	<5	42	<24	208	60	349	30	12500	0.4	1.6	0.07
Field Blank 2	NA	<5	43	<24	208	51	363	36	12800	0.3	1.6	0.10
Seawater-1 500 m	3.1	<5	45	<24	230	200	370	28	5020	0.3	1.6	0.08
Seawater-2 500 m	NA	<5	45	<24	220	113	430	42	6910	0.3	1.6	0.09
A1.01	0.2	5	17	<9	200	800	540	100	1200	0.5	1.9	0.15
A1.05	0.5	11	87	<9	230	820	530	320	6000	1.3	2.5	0.12
A1.10	0.2	5	85	<9	330	390	690	140	6900	0.7	1.9	0.14
A1.20	0.2	3	88	<9	180	270	610	70	4400	0.4	1.7	0.08
A1.1000	0.2	<5	72	<9	180	600	410	50	2300	0.2	NA	NA
A2.01	0.1	<5	57	<9	220	150	320	50	3500	0.7	1.7	0.15
A2.05	0.5	<5	83	<9	310	450	450	120	2100	NA	1.7	0.11
A2.10	2.1	5.4	85	<9	170	310	540	70	3400	0.4	1.7	0.14
A2.20	5.1	12	25	12	220	1690	380	160	1300	1.9	1.7	0.14
A2.1000	0.6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
A3.01	2.9	74	96	90	260	2680	620	960	6800	14	2.6	0.14
A3.05 [d]	0.5	<5	135	570	220	31300	590	3940	34600	1.4	31	0.14
A3.10 [d]	0.4	<5	153	77	280	6910	540	11900	42600	NA	12	0.14
A3.20 [d]	6.6	33	551	1300	280	7720	700	37500	27500	7.9	21	0.13
A3.1000	0.6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
C1.01	1.0	<5	73	<9	220	1360	490	140	2500	0.3	1.8	0.14
C1.05	0.4	<5	64	<9	240	210	620	30	1100	0.3	1.8	0.10
C1.10	0.7	<5	85	<9	200	220	550	30	1500	0.4	1.6	0.14
C1.20	0.5	82	121	120	270	53200	530	9230	12400	NA	7.0	0.14
C1.1000	0.8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

[a] Location/Name codes in Table 1, [b] TSS = total suspended solids, [c] Ag analysed by MSE-GFAAS, Cr analysed by GFAAS, Cd, Cu, Co, Ni, Pb Zn analysed by SE-GFAAS, Hg analysed by AFS, [d] Zn concentrations analysed by ICP-AES. NA = not analysed.

For the 500 m seawater site, total and dissolved concentrations of Ag, Co and Pb were 'typical' of ocean waters, the concentrations of Cd and Cu were a 'little higher than typical', and concentrations of Cr, Ni and Zn were higher than is commonly measured for ocean waters. At the sites A1, A2, A3 and C1 (within 20 m of the ocean floor), the dissolved concentrations of Cr and Pb were similar to those measured in the water collected at 500 m depth. The dissolved concentrations of Cd, Cu, and Ni were generally a factor 2-4 greater in the waters collected at the sites A1, A2, A3 and C1 than they were at 500 m depth.

The dissolved zinc concentrations appeared quite variable and indicated a source of contamination. Because of the shortage of high purity acid for acid-washing of equipment and filters *in-situ*, it was decided that filtration equipment included acid-washed filters before departing for the field work. Although this approach has previously been successful in allowing sample filtrations to be achieved with negligible contamination, it is possible that degradation of the acid-washed filters contributed a source of zinc contamination in the present study. As discussed earlier, the laboratory was thoroughly cleaned before use, however, the laminar flow cabinet had to be used without air flows and, dust from within the laboratory may have been another possible source of zinc contamination during sample filtration. Ultra-trace sample filtration undertaken without a laminar flow cabinet has been undertaken by CSIRO (and the personnel used in the current study) without observing any zinc contamination. The reason for the elevated zinc concentrations was not investigated further and the zinc data should be interpreted with some caution.

The concentrations of total suspended solids (TSS) were generally low (mean of 1.2 mg/L and range of 0.1-5.1 mg/L), however, the small concentrations of particles did appear to contribute metals at the A1, A2, A3 and C1 sites, particularly for the A3 locations.

Total concentrations of arsenic (As), selenium (Se) and mercury (Hg) were generally low and dissolved concentrations of these metal(oid)s were not determined (Table 7). For the 500 m depth seawater samples, and for most other samples, total concentrations of As, Se and Hg were at concentrations typical of ocean water. The exception was at site A3, where arsenic concentrations were an order of magnitude higher (up to 31 µg As/L).

Total (unfiltered) concentrations of Cd, Cr, Cu, Ni, and Pb were generally similar or greater than dissolved concentrations. The total concentrations of all metals were greater than dissolved concentrations at all of the A3 locations. At all sites total concentrations of Cu and Pb were greater than dissolved concentrations, particularly for the A3 locations. At approximately half the sites, dissolved zinc concentrations were greater than total concentrations (0.4-2.1 µg/L higher) and as discussed earlier, the zinc data should be interpreted with some caution.

Comparison of metal concentrations to water quality guidelines

The metal concentrations measured in the waters were compared to water quality guidelines in Table 8. The concentrations of all metals were below the 95% protection level (ANZECC/ARMCANZ, 2000) for dissolved metals. At the 99% protection level, there was an exceedance of the guideline for dissolved zinc, but this may have been due to sample contamination. The concentrations of total (unfiltered) Cu, Pb and Zn were greater than the 95% protection level for dissolved metals in three samples for Pb and Zn and six samples for Cu.

Comparison of dissolved metal concentration to other locations

The dissolved metal concentrations measured in the waters were compared to those measured at other locations around the world, including ocean waters of the Pacific and waters of Papua New Guinea (Table 9). Concentrations of dissolved cadmium and zinc were significantly greater than those at comparable locations. As mentioned earlier, the dissolved zinc data may have been subject to sample contamination. Concentrations of dissolved nickel were greater than those measured in Torres Strait and the Gulf of Papua (Papua New Guinea).

Table 8. Comparison of metal concentrations to water quality guidelines

		Dissolved metal, µg/L							
Location		Ag	Cd	Co	Cr	Cu	Ni	Pb	Zn
Seawater at 500 m	Min	<0.005	0.044	<0.024	0.20	0.054	0.29	<0.015	5.0
	Max	<0.005	0.045	<0.024	0.24	0.12	0.38	0.034	7.4
1-20 m off seabed	Min	<0.005	0.015	<0.024	0.15	0.099	0.52	0.015	1.5
	Max	0.0072	0.087	0.053	0.23	0.21	0.67	0.21	8.0
Guidelines ANZECC/ARMCANZ (2000)	95% PL	1.4	5.5	14	4.4 [b]	1.4	120	4.4	15
	99% PL	0.8	0.7	1	0.14 [b]	0.3	14	2.2	7
Number of samples with dissolved metal concentrations exceeding the Guidelines	95% PL	0	0	0	0	0	0	0	0
	99% PL	0	0	0	0 [c]	0	0	0	4 [d]
Number of samples with total metal concentrations exceeding the Guidelines [a]	95% PL	0	0	0	0	6	0	3	3
	99% PL	0	0	1	0 [c]	13	1	4	6

[a] Total metal analysis is used to assess unfiltered waters, however, dissolved metal guideline values were used for comparisons as no guidelines exist for total metals, where PL = protection level.

[b] Guidelines for Cr(VI), with corresponding guidelines for Cr(III) of 10 and 0.8 µg/L, respectively.

[c] If all Cr was present as Cr(VI), then all 21 samples, including field blanks exceeded the Cr guideline, however, as less than half the Cr is expected to be present as Cr(III), the guideline is not considered to be exceeded.

[d] The highest dissolved zinc concentrations were measured in the field blanks and indicate a possible source of sample contamination.

Table 9. Comparison of dissolved metal concentrations in waters with those from other locations

Location	Dissolved metal concentration, µg/L					Reference
	Cadmium	Copper	Nickel	Lead	Zinc	
Solwara, 500 m depth	0.044-0.045	0.054-0.12	0.293-0.379	<0.015-0.034	5.0-7.4	This Study
Solwara, bottom water (1-20 m off seabed)	0.015-0.087	0.099-0.21	0.520-0.670	0.015-0.210	1.4-8.0	This Study
North Pacific Ocean	0.0011	0.04	0.12	-	-	Mackey et al. (2002)
Pacific Ocean	0.01	0.09	0.20	-	0.20	Batley (1996)
Torres Straight & Gulf of Papua (New Guinea)	<0.001-0.029	0.036-0.986	0.94-4.6	-	-	Apte and Day (1998)
North Sulawesi, Indonesia	NM	0.05	NM	NM	0.06	Simpson et al. (2005)
Queensland (QLD) coast, Australia	<0.0015-0.004	<0.019-0.085	0.011-0.19	<0.01-0.12	<0.03-0.14	Angel et al. (2007)
New South Wales (NSW) coast, Australia	0.0025	0.03	0.18	0.01	<0.022	Apte et al. (1998)
Great Barrier Reef, QLD, Australia	<0.010-0.060	0.110-0.240	0.06-0.16	<0.060	0.030-0.35	Denton and Burdon-Jones. (1986)
Nine estuaries, northern Australia	0.0014-0.034	0.15-1.0	0.12-0.50	<0.0020-0.018	<0.010-0.50	Munksguard and Parry (2001)
Port Curtis estuary, QLD, Australia	0.002-0.015	0.41-0.62	0.28-0.47	<0.01-0.44	0.13-0.23	Angel et al. (2007)
Port Jackson estuary, NSW, Australia	0.006-0.10	0.93-2.6	0.18-1.6	-	3.3-9.7	Hatje et al. (2003)
Port Phillip Bay, VIC, Australia	<0.005-0.07	0.40-0.63	0.54-1.1	0.02-0.13	0.25-1.1	Fabris and Monahan (1995)
Bathurst Harbour, Tasmania, Australia	0.0022	0.14	0.14	-	0.39	Mackey et al. (1996)
Four estuaries in the United Kingdom	<0.003-0.45	0.18-10	0.25-12	0.04-0.35	<0.1-21	Leslett and Ball (1995), Comber et al. (1995), Owens and Balls (1997)
Scheldt estuary, Netherlands	0.015-0.10	0.75-1.8	1.0-6.8	0.10-0.68	1.0-10	Baeyens et al. (1998) and (2005)
San Francisco Bay, CA, USA	0.022-0.12	0.32-2.2	0.14-2.4	-	0.16-2.0	Sanudo-Wilhelmy et al. (1996)

Sediment and Chimney Mineral Sample Analyses

The sediment and chimney mineral samples were described by the location from where they were collected (Table 2). Sediment samples were collected from the sea floor and chimney samples were broken off black 'smokers' extending from the sea floor. The sample M-t was collected from the sea floor, but believed to comprise weathered chimney material. Depending on the thermal activity at the collection site, the sediments were described as active or cold and the chimney samples were described as active or inactive.

For the nine sediment/chimney mineral samples collected from the five sites (duplicate samples at four sites), the results of the metal analyses are shown in Tables 10-12 and QA/QC is provided in Appendix 2. The concentrations of metals in the sediment and chimney samples are summarised in Table 10 as total particulate metals (TPM), and in Table 11 as dilute acid-extractable metals (AEM, 1-M HCl, 1 h).

Table 10. Total particulate metals in the mineral samples

Name	Total particulate metals (aqua regia digestion), mg/kg dry weight											
Location	Ag	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
A-c	3.0	1980	248	3.8	20.0	4.9	1680	28400	50.9	8.2	241	1020
A-t,	<0.5	1560	269	1.2	19.9	4.1	1050	29500	44.4	7.9	122	381
C-c	<0.5	1790	112	<0.3	19.8	3.6	256	28600	41.1	7.8	14.3	38.6
C-t	<0.5	1680	103	<0.3	20.8	3.5	278	30300	34.9	8.0	14.1	39.4
Ch-A-c	182	262	8420	580	59.3	1.3	104000	18100	126	3.3	9570	76700
Ch-A-t	142	301	7070	365	77.7	1.2	147000	21300	137	2.8	8710	52100
Ch-I-c	147	150	3930	291	440	0.7	1360	49800	653	4.8	4970	42300
Ch-I-t	98	573	3620	360	670	1.7	22700	58100	2910	20	5770	42900
M-t	83	415	2370	172	61.7	1.3	26700	108000	383	4.2	1480	28000

Key: A = Active silt, C = Cold (inactive), Ch-A = Chimney (active), Ch-I Chimney (inactive), M = Sediment rock, suffix -c = chemistry sample, -t = toxicity sample.

Table 11. Acid-extractable metals (AEM, 1-M HCl, 1 h) in the mineral samples

Name	Acid-extractable metals (AEM, 1-M HCl, 1 h), mg/kg dry weight										
Location	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
A-c	<0.4	62	0.84	1.1	1.0	90	2200	18	<0.4	96	310
A-t,	<0.4	69	0.52	0.79	0.89	120	1200	21	<0.4	63	160
C-c	<0.4	68	<0.4	1.2	1.4	46	2700	9.2	<0.4	8.0	19
C-t	<0.4	97	<0.4	1.6	1.2	47	3800	7.3	<0.4	9.0	20
Ch-A-c	<0.4	430	0.60	0.54	0.22	46	1500	24	<0.4	44	870
Ch-A-t	0.17	440	<0.4	0.32	0.14	110	870	41	<0.4	390	43
Ch-I-c	2.6	212	40	10	0.30	73	1500	560	3.7	720	94
Ch-I-t	9.0	490	44	20	1.0	2400	5600	1700	11	1400	1000
M-t	6.1	110	<0.4	4.1	0.33	1700	2700	340	1.1	150	62

Key: A = Active silt, C = Cold (inactive), Ch-A = Chimney (active), Ch-I Chimney (inactive), M = Sediment rock, suffix -c = chemistry sample, -t = toxicity sample.

For the major metals, the maximum TPM concentrations (in mg/kg) were 180 for Ag, 8400 for As, 580 for Cd, 4.9 for Cr, 670 for Co, 150,000 for Cu, 20 for Ni, 9600 for Pb and 77000 for Zn. The maximum AEM concentrations (in mg/kg) were 9 for Ag, 490 for As, 44 for Cd, 1.4 for Cr, 2400 for Cu, 11 for Ni, 1400 for Pb and 1000 for Zn. The variability in the metal concentratiions measured in samples collected from the same location was sometime very high, e.g. 1360 and 22700 mg/kg Cu measured for samples Ch-I-c and Ch-I-t, respectively (Table 10).

The sample descriptions as sediment/chimney and active/inactive/cold did not provide a useful indication of which samples would have the highest metal concentrations. The active chimney samples (Ch-A-c/Ch-A-t) had the total highest concentrations of Ag, As, Cd, Cu, Pb and Zn (Table 10). The inactive chimney samples (Ch-I-c/Ch-I-t) generally had the highest concentrations of acid-extractable Ag, Cd, Co, Cu, Mn, Pb and Zn (Table 11). The percentage AEM in each sample is shown in Table 12 and indicated that, for most metals, the more reactive AEM fraction comprised (on average) less than 30% of the TPM.

The total organic carbon (TOC) concentrations of the sediment and chimney samples were (in mg/kg) 1300 for A (-c/-t), 1200 for C (-c/-t), 550 for Ch-A (-c/-t), 460 for Ch-I (-c/-t), and 770 for Ch-A(-c/-t).

Table 12. Percent acid-extractable metals in the minerals

Name	AEM/TPM ×100%										
Location	Ag	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
A-c	ND	25	22	5	21	5	8	36	5	40	30
A-t,	ND	26	44	4	22	11	4	48	4	52	42
C-c	ND	61	ND	6	39	18	9	22	4	56	48
C-t	ND	94	ND	8	35	17	13	21	6	63	52
Ch-A-c	ND	5	<1	1	17	<1	8	19	3	<1	1
Ch-A-t	ND	6	ND	0	12	<1	4	30	3	4	<1
Ch-I-c	2	5	14	2	41	5	3	86	76	14	<1
Ch-I-t	9	14	12	3	60	11	10	58	56	24	2
M-t	7	5	0	7	26	6	3	89	27	10	<1
Mean	6	27	15	4	30	8	7	45	21	29	20
SD	4	31	17	3	15	7	4	27	27	24	23

Acid-volatile sulfide

Sulfide is a strong metal binding phase of many metals (e.g. Ag, Cd, Cu, Hg, Ni, Pb and Zn). The acid-volatile sulfide (AVS) concentration is generally considered to be a useful measure of the major reactive sulfide phases that regulate the solubility of these metals (Rickard and Morse, 2005). AVS is operationally-defined as the sulfide liberated from wet sediment/mineral by treatment with 1 M hydrochloric acid, and the concentration of metals (excluding iron) liberated under the same conditions is termed simultaneously extracted (SEM). Where there is a molar excess of AVS over SEM, the metals are predicted, based on thermodynamics, to be bound as sulfide phases that have a very low solubility. The oxidation of metal sulfide phases may result in the release of the metals to solution.

Analyses of AVS and SEM indicated that some samples contained a significant molar excess of AVS over reactive metals (1-M HCl extractable) (Table 13, Appendix 2). However, for the crushed chimney materials (Ch), and sediment rock (M) that had the highest metal concentrations, there was an excess of reactive metals (SEM) compared to AVS. The concentrations of SEM were very high in the inactive chimney samples (Ch-I-c, Ch-I-t), particularly SEM-Cd in both samples and SEM-Cu and SEM-Zn in the sample Ch-I-t. Pure copper sulfide (CuS, Cu_2S) phases do not dissolve in 1-M HCl (the AVS extraction), although some oxidative dissolution does occur due to the dissolution of Fe(III)-phases. NiS phases are also sparingly soluble in 1 M HCl.

As the AVS analyses were not part of the initial analysis plan when sediment sampling was undertaken, the collected samples were stored refrigerated, and subsamples were not frozen, until reaching CSIRO. It is likely that some oxidation of the AVS occurred during cold-storage and transport to CSIRO and it is possible that the oxidative loss of the AVS and changes in SEM in samples shows some effect of sample handling and oxidation during resuspension in oxic seawater (Simpson et al., 1998). However, any oxidation of AVS in the samples is likely to have been restricted to just the surface layers and the excess of SEM over AVS measured for the chimney samples (Table 13) is not likely to have been significantly affected.

Table 13. Acid-volatile sulfide and simultaneous extractable metals in the mineral samples

Name	Concentrations in µmol/g, dry weight												
Location	Fe	Mn	Ag	As	Cd	Cr	Cu	Ni	Pb	Zn	SEM	AVS	Excess
A-c	40	0.33	<0.01	0.83	<0.01	0.02	1.4	<0.01	0.46	4.7	6.6	18	11
A-t,	22	0.39	<0.01	0.92	<0.01	0.02	1.9	<0.01	0.30	2.8	5.1	6.0	0.9
C-c	48	0.17	<0.01	0.91	<0.01	0.03	0.72	<0.01	0.04	0.28	1.0	10	9.1
C-t	67	0.13	<0.01	1.3	<0.01	0.02	0.75	<0.01	0.04	0.31	1.1	8.9	7.8
Ch-A-c	26	0.44	<0.01	5.7	<0.01	<0.01	0.72	<0.01	2.1	13	16	13	-3.1
Ch-A-t	16	0.74	<0.01	5.8	<0.01	<0.01	1.7	<0.01	1.9	6.6	10	2.5	-7.8
Ch-I-c	27	10	0.02	2.8	0.36	<0.01	1.1	0.06	3.5	14	19	0.8	-18
Ch-I-t	99	31	0.08	6.5	0.39	0.02	37	0.19	6.6	15	59	0.1	-59
M-t	49	6.1	0.06	1.4	<0.01	<0.01	27	0.02	0.74	1.0	29	0.2	-29

Key: SEM = Σ(Ag,Cd,Cu,Ni,Pb,Zn), Excess = AVS-SEM

Elutriate Tests

Field-based elutriate tests

The elutriate tests undertaken in the laboratory on board the Mercury Wave used the weathered chimney material, M (Table 2). These tests investigated the effect of mixing time (0, 6, 12, 24 h) and number of rock 'chips' (1, 4, 10 chips; equivalent to ~10±2, 40±8, 90±8 g/L) on metal release. The results of these tests are shown in Table 14.

Table 14. Total dissolved metals (<0.45 µm filtered) in elutriate waters for sample M (field tests)

Number of chips	Mass g	Time h	Metal concentrations in µg/L											
			Ag	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
1	11	0.2	<3	<5	<10	<3	<3	<3	15	<5	17	<3	<10	<3
1	9	0.2	<3	<5	<10	<3	<3	<3	25	<5	37	<3	<10	<3
1	12	6	<3	<5	<10	<3	<3	<3	110	59	28	<3	<10	5
1	14	6	<3	<5	<10	<3	<3	<3	10	9	7	<3	<10	<3
1	12	12	<3	<5	<10	<3	<3	<3	62	38	110	<3	<10	<3
1	13	12	<3	<5	20	<3	<3	<3	57	46	55	<3	10	<3
1	10	24	<3	<5	10	<3	<3	<3	32	22	18	<3	<10	34
1	12	24	<3	<5	<10	<3	<3	<3	41	42	15	<3	<10	11
4	35	0.2	<3	<5	<10	<3	<3	<3	60	<5	47	<3	<10	<3
4	42	0.2	<3	<5	<10	<3	<3	<3	60	<5	65	<3	<10	<3
4	43	6	<3	<5	10	<3	<3	<3	25	4	82	<3	<10	4
4	47	6	<3	33	10	<3	<3	<3	74	110	84	<3	<10	15
4	48	12	<3	<5	<10	<3	10	<3	84	32	240	<3	20	<3
4	42	12	<3	14	10	<3	5	<3	140	90	260	<3	30	10
4	38	24	<3	<5	<10	<3	<3	<3	92	64	69	<3	<10	18
4	43	24	<3	<5	<10	<3	<3	<3	85	54	130	<3	10	8
10	88	0.2	<3	<5	<10	<3	12	<3	83	<5	240	<3	<10	<3
10	96	0.2	<3	<5	<10	<3	5	<3	85	24	220	<3	10	<3
10	85	6	<3	56	50	<3	12	<3	420	310	410	<3	20	76
10	83	6	<3	29	20	<3	8	<3	200	170	310	<3	10	14
10	89	12	<3	9	10	<3	15	<3	210	110	460	<3	20	10
10	96	12	<3	12	20	<3	17	<3	180	81	590	<3	<10	9
10	85	24	<3	<5	20	<3	<3	<3	120	75	150	<3	<10	14
10	88	24	<3	<5	<10	<3	<3	<3	180	110	200	<3	<10	25
Guidelines	(95% level)		1.4	NA	NA	5.5	14	4.4	1.4	NA	NA	120	4.4	15
	(99% level)		0.8	NA	NA	0.7	1	0.14	0.3	NA	NA	14	2.2	7

Key: Chips = pieces of rock approximately 25 mm in size. The ANZECC/ARMCANZ (2000) Guidelines are as described for Table 8.

In the field-based elutriate tests, Cu, Fe, Mn and Zn were the only metals for which measurable concentrations were consistently released. The release of these metals did not increase significantly with time. If the effect of time is ignored, the copper concentration was (mean ± standard deviation) 184±100 µg/L for tests with ten rock chips, which was significantly greater ($p<0.001$) than the copper concentration in tests with one rock chip (44±30 µg/L), or four rock chips (70±30 µg/L).

Laboratory based elutriate tests

The results of elutriate tests (undertaken at CSIRO) to investigate the effect of particle size, particle concentration and time on metal release are shown in Tables 15-17. For chimney material Ch-A-t, moderate to high release of As (100-2800 µg As/L), Pb (10-120 µg Pb/L) and Zn (200-6200 µg Zn/L) was observed. For weathered chimney material M-t, moderate to high release of Mn (50-3300 µg Mn/L) was observed. For the cold silt material (C-c), there was little release of metals other than Fe and Mn.

Effect of particle size

The experiments investigating the effect of particle size provided mixed results (Table 15). Metal release occurred from all size fractions, but release rates from one size fraction were not consistently greater than others. Generally, either the largest or smallest size fractions (<63 µm) resulted in the greatest release of As, Pb or Zn (Figure 1). It was unclear why these results were observed.

Table 15. Effect of particle size on metal release during elutriate tests

Sample	Size	Dissolved metals in µg/L										
	mm	Ag	Al	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Ch-A-t	4-30	<3	<5	140	<3	<3	<3	<5	48	<3	110	720
Ch-A-t	1-4	<3	23	100	<3	<3	<3	<5	38	<3	40	480
Ch-A-t	0.25-1.0	<3	<5	170	<3	<3	<3	<5	24	<3	40	330
Ch-A-t	0.063-0.25	<3	<5	290	6	<3	<3	<5	17	<3	40	340
Ch-A-t	<0.063	<3	<5	840	19	<3	<3	<5	45	<3	110	970
M-t	4-30	<3	<5	<10	<3	<3	140	<5	650	4	<10	46
M-t	1-4	<3	<5	<10	<3	<3	160	<5	900	6	<10	83
M-t	0.25-1.0	<3	44	<10	<3	<3	78	<5	1000	9	<10	110
M-t	0.063-0.25	<3	17	<10	<3	<3	49	<5	370	5	<10	38
M-t	<0.063	<3	83	<10	<3	<3	81	<5	320	5	<10	91
Guidelines	(95% level)	1.4	NA	NA	5.5	4.4	1.4	NA	NA	120	4.4	15
	(99% level)	0.8	NA	NA	0.7	0.14	0.3	NA	NA	14	2.2	7

Key: Chips = pieces of rock approximately 25 mm in size. Time = 30 min.

Guidelines are ANZECC/ARMCANZ (2000), as described for Table 11, with 95% and 99% protection levels.

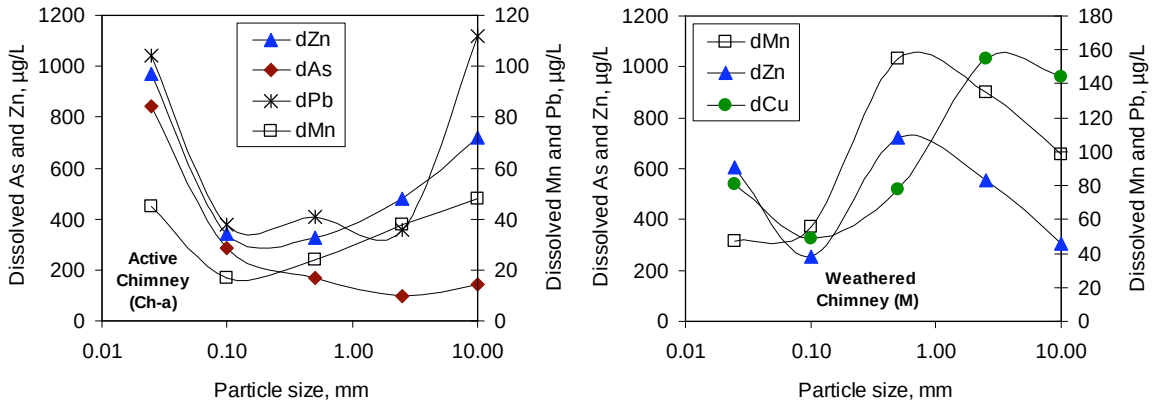

Figure 1. Effect of particle size on metal release from mineral samples: Active Chimney (Ch-a) and Weathered Chimney (M)

Effect of total suspended solids (TSS) concentration

The experiments investigating the effect of particle (TSS) concentration indicated that metal release increased with increasing TSS concentration (load), as evident from As, Mn and Zn (Figure 2). This indicated that the concentrations do not reach equilibrium and the release is affected significantly by mass of solid (Table 16). The pH of waters (Appendix 3) generally decreased from pH ~8 to ~7.4 as TSS concentration increased from 1 g/L to 100 g/L. This indicated that the samples were mildly acidic, and/or oxidation processes involving Fe(II) or sulfide may be causing the water pH to decrease.

Table 16. Effect of total suspended solids (TSS) concentration on metal release during elutriate tests

Sample	TSS	Dissolved metals in µg/L										
	mg/L	Ag	Al	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Ch-A-t	1	<3	<5	110	<3	<3	<3	<5	17	<3	10	200
Ch-A-t	10	<3	<5	520	10	<3	<3	<5	87	<3	20	840
Ch-A-t	100	<3	<5	2700	57	<3	<3	<5	760	26	30	6200
M-t	1	<3	<5	<10	<6	<3	26	<5	48	<3	<10	9
M-t	10	<3	<5	<10	9	<3	38	<5	530	<3	<10	110
M-t	100	<3	<5	10	79	<3	89	15	3020	21	<10	840
C-c	1	<3	<5	<10	<3	<3	<3	39	4	<3	<10	5
C-c	10	<3	91	<10	<3	<3	<3	600	21	<3	<10	45
C-c	100	<3	<5	<10	<3	<3	<3	14	170	<3	<10	18
Guidelines	(95% level)	1.4	NA	NA	5.5	4.4	1.4	NA	NA	120	4.4	15
	(99% level)	0.8	NA	NA	0.7	0.14	0.3	NA	NA	14	2.2	7

TSS concentration = 10 g/L. The guidelines are ANZECC/ARMCANZ (2000), as described for Table 8.

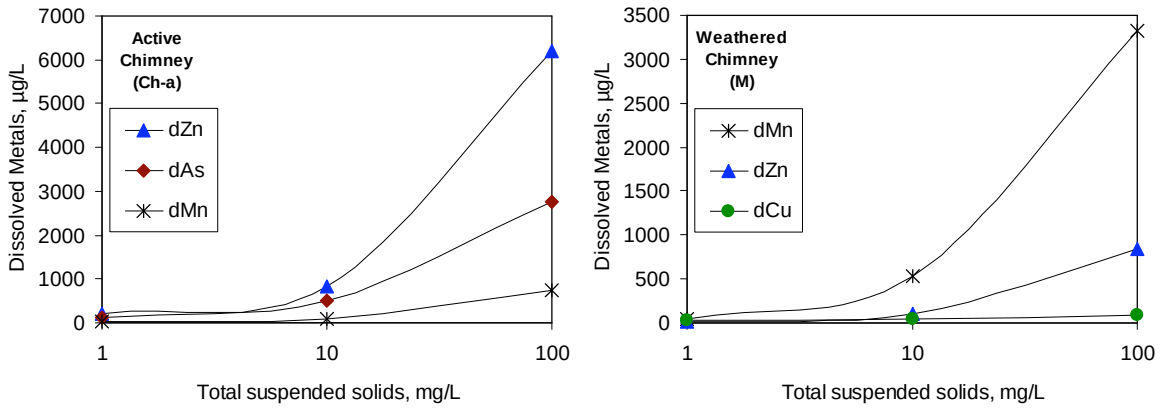

Figure 2. Effect of total suspended solids (TSS) concentration on metal release from mineral samples: Active Chimney (Ch-a) and Weathered Chimney (M)

Effect of time

Metal release during the elutriate tests increased with time, and was most evident for As, Pb, Zn in sample Ch-A-t (Table 17, Figure 3). This indicated that metal release continued beyond the initial displacement of metals from exchange-reactions with the cations in the seawater. The additional release of metals is likely to be from dissolving mineral phases and some of these reactions may involve oxidation of metal sulfide phases. These results are different to those of the field-based elutriate tests, where metal release did not increase significantly with time (Table 14). In the field-based tests the bottles were not shaken continuously and time period was longer (24 h).

Table 17. Effect of time on metal release during elutriate tests

Sample	Time	Dissolved metals in µg/L										
	min	Ag	Al	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Ch-A-t	2	<3	<5	120	<3	<3	<3	<5	23	<3	20	240
Ch-A-t	10	<3	<5	240	5	<3	<3	<5	27	<3	40	350
Ch-A-t	30	<3	<5	360	7	<3	<3	<5	30	<3	60	430
Ch-A-t	60	<3	<5	470	9	<3	<3	<5	34	<3	80	530
Ch-A-t	240	<3	<5	840	18	<3	<3	<5	41	<3	120	880
M-t	2	<3	<5	<10	<3	<3	37	<5	92	<3	<10	16
M-t	10	<3	<5	<10	<3	<3	47	<5	140	<3	<10	19
M-t	30	<3	<5	<10	<3	<3	61	<5	190	<3	<10	24
M-t	60	<3	<5	<10	<3	<3	26	<5	21	<3	<10	9
M-t	240	<3	<5	<10	<3	<3	69	<5	140	<3	<10	37
C-c	2	<3	<5	<10	<3	<3	<3	<5	21	<3	<10	<3
C-c	10	<3	<5	<10	<3	<3	<3	<5	22	<3	<10	<3
C-c	30	<3	<5	<10	<3	<3	<3	<5	22	<3	<10	<3
C-c	60	<3	<5	<10	<3	<3	<3	<5	21	<3	<10	<3
C-c	240	<3	<5	<10	<3	<3	<3	<5	24	<3	<10	<3
Guidelines	(95% level)	1.4	NA	NA	5.5	4.4	1.4	NA	NA	120	4.4	15
	(99% level)	0.8	NA	NA	0.7	0.14	0.3	NA	NA	14	2.2	7

TSS concentration = 10 g/L. The guidelines are ANZECC/ARMCANZ (2000), as described for Table 8.

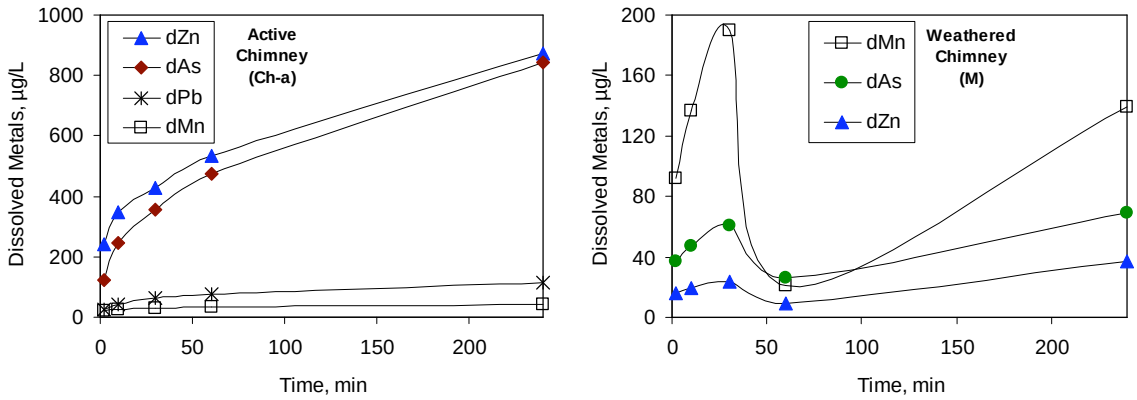

Figure 3. Effect of resuspension time on metal release from mineral samples: Active Chimney (Ch-a) and Weathered Chimney (M)

Elutriate tests on remaining samples

These tests were performed on the six remaining samples for which elutriate tests had not been performed: sediment materials A-c (active silt), A-t (active silt), and C-t (cold silt), crushed chimney material Ch-A-c (active), Ch-I-c (inactive) and Ch-I-t (inactive) (Table 18).

Table 18. Results of elutriate tests on remaining materials

	% particle size, mm				DO	Dissolved (<0.45 µm) metals, µg/L							
Sample	<0.06	<0.25	<1	pH	mg/L	Ag	As	Cd	Cu	Mn	Ni	Pb	Zn
A-c	12.1	19.4	20	7.9	3.7	<3	220	<3	<3	400	<3	6	4
A-t	11.1	18.5	20	7.9	4.1	<3	110	<3	<3	390	<3	<10	290
C-t	11.7	19.4	20	7.9	4.2	<3	53	<3	<3	60	<3	<10	<3
Ch-A-c	3.0	6.4	20	8.1	5.8	<3	795	<3	<3	270	<3	134	5100
Ch-I-c	3.3	5.4	20	7.5	5.7	15	46	735	56	9230	99	330	28000
Ch-I-t	4.6	7.9	20	8.1	5.7	<3	26	345	172	7350	23	116	1550
Guidelines		(95% level)		NA	NA	1.4	NA	5.5	1.4	NA	120	4.4	15
		(99% level)		NA	NA	0.8	NA	0.7	0.3	NA	14	2.2	7

Particle size = 80% 4-25 mm, 20% <1 mm. TSS concentration = 100 g/L. Resuspension time = 12 min. Other metals generally less than detection limit by ICP-AES. The guidelines are ANZECC/ARMCANZ (2000), as described for Table 8.

Summary of metal release from elutriate tests

The TSS concentration and the resuspension time had the greatest effect on metal concentrations in the elutriate waters. In general, metal release was high for As, Cu, Mn and Zn and relatively low for Ag, Cd, Ni and Pb. The maximum concentrations of the metals measured (including results from elutriate tests performed for toxicity testing) were 8900 µg/L Mn, 6200 µg/L Zn, 3300 µg/L Cu, 2800 µg/L As, 120 µg/L Pb, 80 µg/L Cd, 40 µg/L Ni, 30 µg/L Ag.

Based on the metals concentrations measured in the elutriate waters (e.g. 28000 µg Zn/L for Ch-I-c) dilutions of greater than 1000 times may be necessary before the concentrations would be below the 95% protection level of the ANZECC/ARMCANZ (2000) water quality guidelines.

Due to the differences in the experimental methodologies used for the field- and laboratory-based elutriate tests (e.g. rock chips versus mixture of chips and fine materials), it was not possible draw significant conclusions from the differences in metal release from the weathered chimney material (M). In the field-based elutriate tests with 10 g/L, the Cu and Mn release after 30 min was 15-20 and 17-37 µg/L, respectively (Table 14), compared to 38-140 and 190-650 µg/L, respectively, measured in the laboratory-based tests (Tables 15-17). The differences in the metal release are likely to be due to many factors, including differences in sample storage conditions, shaking method and particles size.

Relationships between concentrations of dissolved metals in the elutriate waters (Tables 14-17) and concentrations of (i) total metals (strong acid extractable, Table 10), (ii) dilute acid-extractable (1-M HCl, Table 11) metals, and (iii) the molar (µmol/g) excess of SEM over AVS (Table 13) are shown in Figure 4. These plots indicate that there is unlikely to be a single factor controlling the concentrations of metals released from the minerals, however, both the concentration of dilute acid-extractable metals (AEM = SEM) and the binding of metals to sulfide (AVS) phases are likely to be very important. It remains unclear from these elutriate test results whether the material type (silt, chimney), designated activity (cold, active) or metal concentrations most influence the metal release, or how important AVS is in controlling metal release.

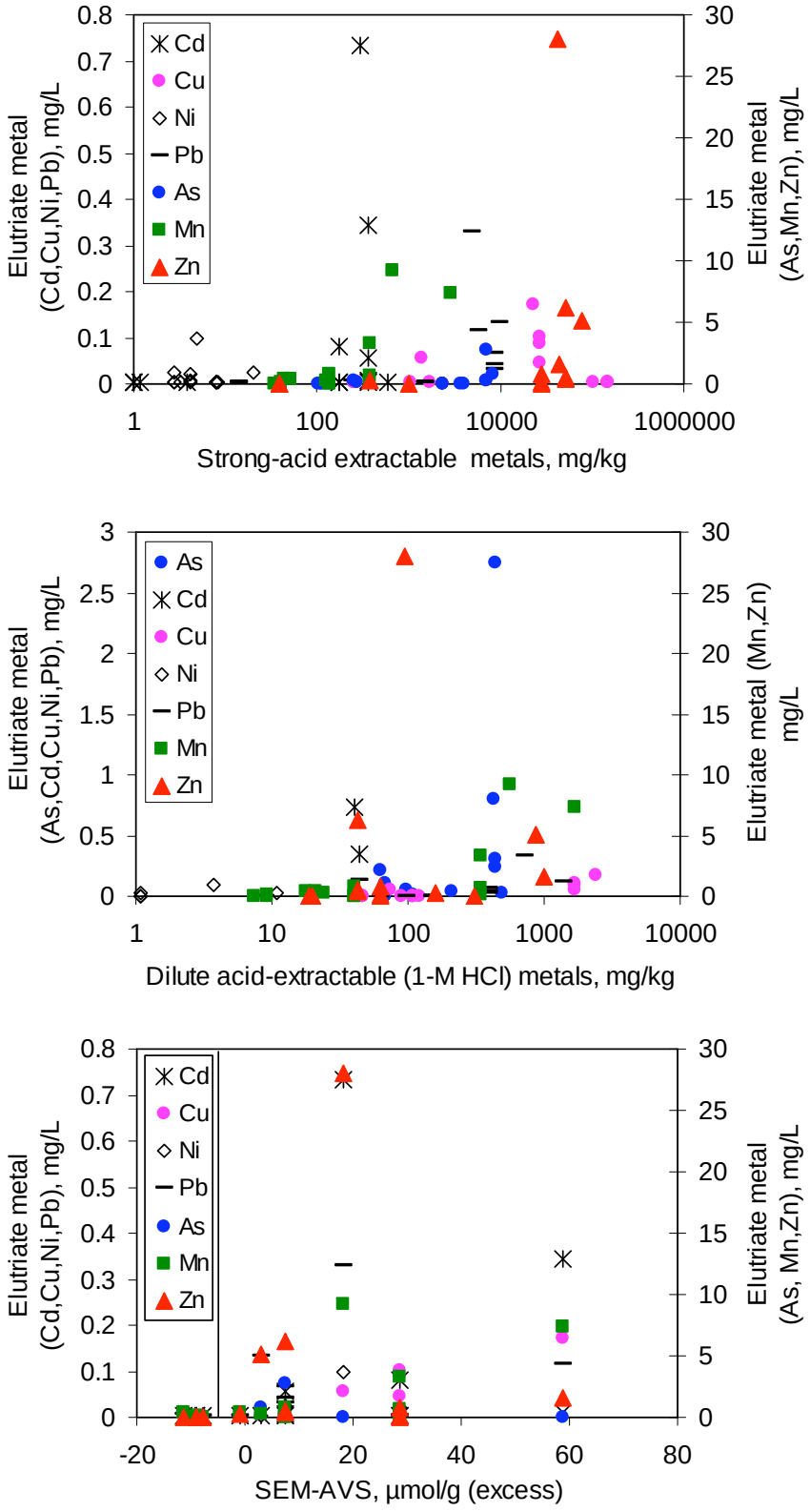

Figure 4. Relationships between concentrations of dissolved metals in the elutriate waters (Tables 16-18) and concentrations of (i) total metals (strong acid-extractable, Table 10), (ii) dilute acid-extractable (1 M HCl, Table 11) metals, and (iii) the molar (µmol/g) excess of SEM over AVS (Table 13).

Toxicity of Elutriate Waters

Toxicity to marine algae

The toxicity to the growth of the alga, *Nitzschia closterium*, of elutriate waters from sediment materials A-t (active silt) and C-c (cold silt), crushed chimney material Ch-A-t (active), and weathered chimney material M-t was tested (Appendix 4). The characteristics of the elutriate waters are shown in Table 19.

As discussed earlier, the time taken for ore mining and dewatering is expected to be approximately 12 min and the particle size of the mined material is expected to be 80% less than 25 mm diameter, with the remaining 20% of smaller, unknown size. These conditions were mimicked for the elutriate waters prepared for the toxicity tests. Although methods for preparing the elutriates differed, a comparison with the metal concentrations in the field-based and laboratory-based elutriate tests of the materials A-t, C-c, Ch-A-t, and M-t (Tables 14-17), can be made. In the toxicity tests the elutriate concentration of As was greater by a factor of 2-4 for Ch-A-t, Cu was greater by more than an order of magnitude for M-t. Zinc was generally greater by a factor of 4-15 for A-t, M-t and Ch-A-t, but similar for C-c, when compared to the elutriate waters prepared previously (Tables 14-17).

Based on the metal concentrations measured in the elutriate waters prepared for the algal toxicity tests, dilutions of ~2400 times for Cu (active chimney material Ch-A-t) and ~400 times for Zn (active silt material A-t) would be necessary to reach the 95% protection level of the ANZECC/ARMCANZ (2000) water quality guidelines (approximately 10,000 times to reach the 99% protection level for Cu).

Algal toxicity test results

The elutriate water from sample C-c was not toxic to *Nitzschia*. The elutriate waters from samples A-t, Ch-a-t and M-t were toxic to *Nitzschia*, causing significant decreases in growth rates over 72-h (Tables 19 and 20, Appendix 4). The no observable effect concentrations (NOECs) for A-t, C-c, Ch-a-t and M-t were 1%, 100%, 0.4% and 0.14% respectively, suggesting that elutriate waters would need to be diluted 100, 0, 250 and 700 times in seawater respectively to eliminate the 72-h toxicity to *Nitzschia*. Note that these dilutions are much lower than "safe" dilutions estimated from the elutriate metal concentrations compared to water quality guidelines for individual metals.

Table 19. Characteristics of elutriate materials and elutriates for *Nitzschia* toxicity tests

	% particle size, mm				DO	Dissolved (<0.45 µm) metals, µg/L							
Sample	<0.06	<0.25	<1	pH	mg/L	Ag	As	Cd	Cu	Mn	Ni	Pb	Zn
A-t	11.6	18.8	20	6.9	4.3	<10	140	<3	<3	640	8	<10	1400
C-c	16.4	19.2	20	7.3	4.6	<10	35	<3	<3	470	<3	<10	5
Ch-A-t	2.8	6.6	20	7.9	8.2	<10	1100	30	<3	410	<3	80	5900
M-t	3.2	8.2	20	7.1	7.8	20	<10	15	3300	8900	40	<10	1600
Guidelines		(95% level)		NA	NA	1.4	NA	5.5	1.4	NA	120	4.4	15
		(99% level)		NA	NA	0.8	NA	0.7	0.3	NA	14	2.2	7

Particle size = 80% 4-25 mm, 20% <1 mm. TSS concentration = 100 g/L. Resuspension time = 12 min.
Other metals generally less than detection limit by ICP-AES.

Table 20. Toxicity to *Nitzschia* of elutriate waters diluted with seawater: A-t, C-c

	Elutriate water dilution in seawater, % elutriate water					
	100	50	25	12.5	6.3	1
Sample	Toxicity (growth inhibition), % control					
A-t	100%	100%	100%	70%	29%	0%
C-c	2%	0%	0%	0%	0%	0%

Table 21. Toxicity to *Nitzschia* of elutriate waters diluted with seawater: Ch-A-t, M-t

	Elutriate water dilution in seawater, % elutriate water						
	100	33	11	3.7	1.2	0.41	0.14
Sample	Toxicity (growth inhibition), % control						
Ch-A-t	100	100	94	62	13	5	0
M-t	100	100	100	100	77	12	3

Toxicity to marine copepods

The toxicity (inhibition of mobility) to the marine copepod, *Acartia sinjiensis,* of elutriate waters from sediment materials A-t (active silt) and C-c (cold silt), crushed chimney material Ch-A-t (active), and weathered chimney material M-t was tested. The characteristics of the elutriate waters are shown in Table 21.

In comparison to the metal concentrations in the field-based and laboratory-based elutriate tests of the materials A-t, C-c, Ch-A-t, and M-t (Tables 14-17), in the toxicity tests the elutriate concentration of As was greater by a factor of 2-4 for Ch-A-t, Cu was greater by more than an order of magnitude for M-t, and Zn was generally greater by a factor of 2-20 for A-t, M-t and Ch-A-t, but similar for C-c

Based on the metals concentrations measured in the elutriate waters prepared for the copepod toxicity tests, dilutions of ~4000 times for Cu (active chimney material Ch-A-t) and ~600 times for Zn (active silt materials A-t) would be necessary to reach the 95% protection level of the ANZECC/ARMCANZ (2000) water quality guidelines (approximately 16,000 times to reach the 99% protection level for Cu).

Copepod toxicity test results

For mineral samples A-t and C-c (Table 22), the toxicity tests were repeated (with freshly prepared elutriate waters) due to less than 80% mobility in controls after 48 h (75% mobile) for the initial tests (Appendix 5). For the repeated tests, there was no significant difference found between the number of mobile copepods in the QA control, A-t pH control, C-c pH control, seawater control or method blank (Appendix 5). However, copepod mobility in both pH controls was lower than the acceptable control rate of 80%, with only 60-75% mobile after a 48-h exposure, indicating that low pH may have contributed to toxicity in undiluted elutriate (i.e. 100% elutriate). In 100% A-t and 100% C-c, mobility decreased by 44%, compared to the seawater control (Table 22). However, due to the variability in copepod mobility among some concentrations of A-t and C-c, this decrease was not statistically significant.

In the initial tests for the mineral samples A-t and C-c (where the Controls failed), the results were quite different to the the repeated tests. For A-t, an EC50 of 11% (95%CLs of 8-15%), was calculated (Appendix 5), compared to the results in Table 22, where the EC50 was >100% and NOEC was 100%. Although the copepods were slightly more sensitive in the first tests (seen from the low mobility in controls), the copper reference toxicity test (EC50 of 33 µg Cu/L) was still within the limit for test acceptability (44±16 µg Cu/L). In the 'toxic' initial tests of mineral sample A-t, the elutriate water

contained ~6000 µg Zn/L, compared to ~600 µg Zn/L in the 'non-toxic' second tests of this mineral elutriate, suggesting that the decrease in toxicity may be due to the lower metal (Zn) concentrations in the second elutriate test.

For mineral samples Ch-A-t and M-t (Table 23), there was no significant difference found between the number of mobile copepods in the QA controls, M-t pH control, seawater control or method blank. However, copepod mobility in the method blank was lower than the acceptable control rate of 80%, with 75% mobile after a 48-h exposure. Therefore, it is possible that some process during elutriate preparation contributed slightly to the toxicity observed in the highest test concentrations (100% elutriates). M-t was more toxic than Ch-A-t, with an EC50 of 1.1% and NOEC value of 0.41%, compared to the Ch-A-t EC50 of 15% and NOEC value of 11%.

The elutriate water from samples A-t and C-c and were not toxic to the copepod (although possible effects from A-t in first tests). The elutriate waters from samples Ch-A-t and M-t were toxic to the copepod, causing significant decreases in mobility (Table 23). NOECs for these two samples, Ch-A-t and M-t were 11% and 0.41% respectively, indicating that dilutions of elutriate in seawater of about 9 times and 250 times respectively would be required to protect the copepods. These dilutions were much lower than "safe" dilutions estimated from the elutriate metal concentrations compared to water quality guidelines for individual metals.

Table 22. Characteristics of elutriate materials and elutriates for copepod toxicity tests

	% particle size, mm			DO		Dissolved (<0.45 µm) metals, µg/L							
Sample	<0.06	<0.25	<1	pH	mg/L	Ag	As	Cd	Cu	Mn	Ni	Pb	Zn
A-t *	11.5	19.0	20	6.8	5.2	<3	74	25	32	615	12	18	6150
C-c *	12.3	19.3	20	7.3	3.8	<3	32	25	32	470	12	18	10
A-t	11.8	18.8	20	6.9	6.8	<3	113	<3	4	471	7	12	609
C-c	11.2	17.6	20	6.4	3.6	<3	30	<3	<3	315	10	<5	76
Ch-A-t	3.4	7.1	20	7.7	6.9	<3	1070	4	4	751	5	214	8970
M-t	3.5	8.0	20	6.8	6.7	<3	10	<3	5520	1010	48	23	2470
Guidelines		(95% level)		NA	NA	1.4	NA	5.5	1.4	NA	120	4.4	15
		(99% level)		NA	NA	0.8	NA	0.7	0.3	NA	14	2.2	7

Particle size = 80% 4-25 mm, 20% <1 mm. TSS concentration = 100 g/L. Resuspension time = 12 min. Other metals generally less than detection limit by ICP-AES. * These tests were later repeated due to less than 80% mobility in controls (75% mobility) for copepod tests (but information still considered useful).

Table 23. Copepod toxicity of elutriate waters diluted with seawater: A-t, C-c

Sample	Elutriate water dilution in seawater, % elutriate water					
A-t	100	33	11	3.7	1.2	0.41
	Toxicity (mobility inhibition), % control					
A-t	44	0	31	6	6	12
	Elutriate water dilution in seawater, % elutriate water					
C-c	100	50	25	12.5	6.5	NA
	Toxicity (mobility inhibition), % control					
C-c	44	31	31	25	6	-

Table 24. Copepod toxicity of elutriate waters diluted with seawater: Ch-A-t, M-t

Sample	Elutriate water dilution in seawater, % elutriate water						
Ch-A-t	100	33	11	3.7	1.2	0.41	0.14
	Toxicity (mobility inhibition), % control						
Ch-A-t	100	100	19	12	6	12	12
	Elutriate water dilution in seawater, % elutriate water						
M-t	100	33	11	3.7	1.2	0.41	0.14
	Toxicity (mobility inhibition), % control						
M-t	100	100	100	100	50	6	0

CONCLUSIONS

Total (unfiltered) concentrations of arsenic (As), selenium (Se) and mercury (Hg) in the waters collected in the vicinity of the proposed mining operations were at concentrations typical of ocean water. The exception was at site A3, where the waters had total arsenic concentrations of up to 31 µg As/L.

Total concentrations of Cd, Cr, Cu, Ni, and Pb were generally similar or greater than dissolved (<0.45 µm filterable) concentrations. Total copper concentrations exceeded the water quality guideline 99% protection level in 13 of the 16 water samples and the 95% protection level in six samples (ANZECC/ARMCANZ, 2000). Total lead concentrations exceeded the water quality guideline 99% protection level in three water samples and the 95% protection level in four samples. Dissolved metal concentrations in the waters near the proposed ore body, and in the surrounding ocean waters, were generally very low and below water quality guideline concentrations (99% protection levels) (ANZECC/ARMCANZ, 2000). Dissolved zinc concentrations exceeded the water quality guideline 95% protection level in four water samples, however two of these samples were field blanks and one a 500-m depth seawater and sample contamination was the possible reason for the exceedances.

The maximum strong acid-extractable (2:1 HCl:HNO_3) metal concentrations measured in the sediment and chimney mineral samples (in mg/kg) were 182 Ag, 8420 As, 580 Cd, 670 Co, 4.9 Cr, 147000 Cu, 108000 Mn, 20 Ni, 9570 Pb, and 76700 Zn. Weak-acid extractable (1-M HCl) metal concentrations in the mineral samples were also high, with maximum metal concentrations (in mg/kg) of 9 Ag, 490 As, 44 Cd, 20 Co,1.4.9 Cr, 2400 Cu, 1700 Mn, 11 Ni, 1400 Pb, and 1000 Zn. These high particulate metal concentrations were expected due to the high grade ore in the proposed mining area. The behaviour of the fine particulates in the seawater discharge produced from dewatering the crushed ore remains a significant environmental concern.

Measurements of acid-volatile sulfide (AVS) and simultaneous extractable metals (SEM) were undertaken to investigate sulfide-binding of metals. Where there is a molar excess of AVS over SEM, Ag, Cd, Cr, Cu, Hg, Ni, Pb, and Zn are predicted to be bound as sulfide phases that have a very low solubility in water. The AVS concentrations of the mineral samples ranged from 0.1 to 18 µmol/g, however, many of the samples had a molar excess of SEM, indicating that there was insufficient AVS to bind all of the SEM fraction should it dissolve. The crushed chimney materials (Ch) and weathered chimney material (M) had the highest metal concentrations and also the greatest excess of reactive metals (SEM) compared to AVS.

Elutriate tests, involving the shaking of crushed sediment/chimney mineral samples in oxygenated seawater, were undertaken to investigate metal release processes. The total suspended solids concentration and the resuspension time had the greatest effect on metal concentrations in the elutriate waters. In general, metal release was high for As, Cu, Mn and Zn and relatively low for Ag, Cd, Ni and Pb. The maximum concentrations of the metals measured (including results from elutriate tests performed for toxicity testing) were 8900 µg/L Mn, 6200 µg/L Zn, 3300 µg/L Cu, 2800 µg/L As, 120 µg/L Pb, 80 µg/L Cd, 40 µg/L Ni, 30 µg/L Ag. Based on the metals concentrations measured in the elutriate waters, dilutions of greater than 1000 times may be necessary before the concentrations would be below the 95% protection levels of the ANZECC/ARMCANZ (2000) water quality guidelines. Dilutions of greater than 4000 times may be necessary before the concentrations were below the 99% protection levels of the ANZECC/ARMCANZ (2000) water quality guidelines. The mechanism controlling metal release was not determined. It remains unclear from these tests whether the sediment/chimney mineral type or metal concentrations most influence the metal release. The role of the reactive sulfide phases, AVS, in modifying the rate and magnitude of metal release was not fully quantified.

The toxicity of the elutriate waters was investigated using tests that determine inhibition of growth rate of the alga, *Nitzschia closterium* and inhibition of mobility of the marine copepod, *Acartia sinjiensis*. Metal concentrations measured in the elutriate waters prepared for the toxicity tests were higher than those measured tests for the same minerals in the earlier elutriate tests. Based on the metals concentrations measured in the elutriate waters prepared for the toxicity tests, dilutions of up to 4000 times would be necessary to reach the 95% protection levels of the ANZECC/ARMCANZ (2000) water quality guidelines (approximately 16,000 times for the 99% protection level for Cu). The undiluted elutriate waters prepared from the cold silt (C), crushed chimney materials (Ch) and weathered chimney (M) mineral samples were toxic to both the algae and copepod. Dilutions of up to 700 times would be required to result in no toxicity from the elutriate waters to these two species.

The procedures used for collection, transportation, crushing and testing of the samples in the present study were quite likely to cause an over-estimation of the metals release that is likely to occur under the 'real' conditions. For example, the water temperature during the elutriate tests was higher than what is expected to be reached during the dewatering operations and greater oxidative-dissolution of sulfide mineral phases may have occurred due to exposure of samples to air during crushing.

PROPOSED ONGOING WORK

1. Develop relationships between sediment/chimney mineral material properties and metal concentrations released in elutriates that are suitable for predicting (with reasonable confidence) maximum concentrations of metals in the elutriate waters. This might be achieved by characterisation of sediment/chimnies with a greater range of the properties and further investigation of the release parameters such as processing method, time, suspended solids concentration, water temperature, water pressure and dissolved oxygen concentration.

2. Quantify the effects of water temperature and dissolved oxygen concentration on metal release rates from mineral-sediment samples.

3. Determine whether AVS and SEM measurements of mineral-sediment samples provide information that is suitable for modifying predictions of metal release rates. Quantify the rate of oxidation of AVS occurring during mineral processing.

4. Determine the metal concentrations of the fine particulates that will be discharged with the bulk elutriate.

5. Determine the toxicity of the fine particulates that will be discharged with the bulk elutriate.

6. Determine the whole-sediment toxicity discharged material that may deposit on the sea floor.

7. Investigate process changes that can be used to reduce concentrations of total and dissolved metals in the discharge elutriate.

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ACKNOWLEDGEMENTS

The following CSIRO Land and Water staff are thanked for contributions to sample analysis and toxicity testing: Leigh Hales, Simon Apte, Rob Jung, James Charlesworth, Merrin Adams and Anthony Platts-Baggs.

Appendix 1: Quality Control for Analyses for Waters

Delayed Water-sample Acidification

Table A2.1: Affect of 7-day delayed acidification on recovery 50 µg/L spiked metals in seawater

Spiked with 50 µg/L	Ag	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
	µg/L											
Immediate acidification 1	49	44	51	55	49	55	56	52	55	48	53	55
Immediate acidification 2	48	44	43	55	49	54	56	53	55	49	60	56
Immediate acidification 3	50	44	54	56	53	56	56	52	56	51	60	56
Delayed acidification 1	46	45	47	56	51	55	56	52	56	51	63	57
Delayed acidification 2	45	43	47	55	49	54	55	52	54	48	55	54
Delayed acidification 3	45	46	41	55	51	55	55	51	53	49	53	55
Spike-recovery, % (relative to mean concentration of metal measured in samples with immediate acidification)												
50 µg/L metals in seawater	92	101	92	99	100	99	99	99	98	101	99	98

[a] Limit of reporting for seawater analyses by ACP-AES = 3 µg/L for Ag, Cd, Co, Cr, Cu, Mn, Ni, and Zn, 5 µg/L for Al and Fe, and 10 µg/L for As and Pb.

Table A2.2: Affect of 7-day delayed acidification on recovery 0.10 µg/L spiked metals in seawater

Spiked with 0.100 µg/L	Cd	Co	Cu	Ni	Pb	Zn
	ng/L					
Cronulla seawater	<10	<10	160	120	110	900
Immediate acidification 1	90	107	256	204	204	1025
Immediate acidification 2	91	116	276	233	233	972
Immediate acidification 3	90	106	264	212	212	1005
Delayed acidification 1	92	99	268	206	206	1005
Delayed acidification 2	91	103	256	208	208	970
Delayed acidification 3	92	111	264	222	222	964
Spike-recovery, % (relative to mean concentration of metal measured in samples with immediate acidification)						
100 ng/L metals in seawater	101	95	99	98	98	98

ICP-AES Analyses

Centre for Environmental Contaminants Research (CECR)
CSIRO Land and Water (CLW)
New Illawarra Road, Lucas Heights, NSW
Private Mail Bag 7, Bangor, NSW, 2234, Australia
Telephone 61 2 9710 6808 Fax 61 2 9710 6837

Total Metals in Waters: Analyses by ICP-AES

Quality Control:
Method blanks prepared using the standard procedure in the absence of sample.

Concentrations in µg/L

Blanks	Ag	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Seawater blank 1	<3	<5	<10	<3	<3	<3	<3	<5	<3	<3	<10	9
Seawater blank 2	<3	<5	<10	<3	<3	<3	<3	<5	<3	<3	<10	11
Milli-Q blank 1	<3	<5	<10	<3	<3	<3	<3	<5	<3	<3	<10	<3
Milli-Q blank 2	<3	<5	<10	<3	<3	<3	<3	<5	<3	<3	<10	<3

Duplicates of field-collected samples

Concentrations in µg/L

Duplicates	Ag	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
A3_20 m, sample 1	<3	<5	<10	<3	<3	<3	<3	69	25	<3	<10	97
A3_20 m, sample 2	<3	<5	<10	<3	<3	<3	<3	21	7	<3	<10	51

Spike-recoveries for ICP-AES analyses
Samples (seawater) were spiked with to 100 µg/L mixed-metals solution and recoveries determined

Percent recovery, %

Sample	Ag	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
A1_5m	105	104	109	102	96	98	99	99	102	99.4	115	102
A2_5m	107	104	105	103	95	101	95	97	104	103	98	108
A3_5m	107	94	58	92	87	90	92	87	96	88.7	110	95
C1_20m	119	108	74	102	97	95	99	96	103	97.1	102	100

Total Suspended Solids (TSS)

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Total Suspended Soilds (TSS) Analyses (filtration, gravimetry)

Detection Limit

	mg/L
Detection Limit	0.1

Quality Control:
Analysis blanks prepared using the standard procedure in the absence of sample.
Duplicate analyses were with subsamples from selected samples.

Method Duplicates

	Replicate 1	Replicate 2	Mean
A3_5m	0.8	0.1	0.5
A3-20m	2.0	11	6.6
A3-20m *	6.6		6.6

** Site duplicates combined 50:50 and filtered*

Note: Site duplicates for Sample A3_20m were taken from the same Niskin bottle and large particles were observed in first sample collected, but not second as these particles sunk to the bottom during the time taken to recover the water from the Niskin bottles. The average TSS concentration is used, but the result highlights the low precision of duplicate samples.

Ultra-trace Metal Analyses

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Ultra-trace analyses of Ag, Cd, Cu, Ni, Pb, Zn in filtered and unfiltered waters

Quality Control:

Method Blank, detection limit and field blanks

	Ag (ng/L)		Cd (ng/L)		Co (ng/L)	
Parameter	**Filtered**	**Totals**	**Filtered**	**Totals**	**Filtered**	**Totals**
Detection limit	5.1	4.8	1.7	0.6	24	9

	Cr (ng/L)		Cu (ng/L)		Ni (ng/L)	
Parameter	**Filtered**	**Totals**	**Filtered**	**Totals**	**Filtered**	**Totals**
Detection limit	90	90	30	16	31	26

	Pb (ng/L)		Zn (ng/L)	
Parameter	**Filtered**	**Totals**	**Filtered**	**Totals**
Detection limit	15	5	27	26

Detection Limit = 3σ of Method Blank

CRM (CASS-4) analysis

Parameter	**Cd (ng/L)**	**Co (ng/L)**	**Cu (ng/L)**	**Ni (ng/L)**	**Zn (ng/L)**
Certified concentration	0.026	0.026	0.592	0.314	0.381
Certified error	±0.003	±0.003	±0.055	±0.030	±0.057
Measured concentration	0.022	0.025	0.593	0.295	0.411
% recovery	85	97	100	94	108

No certified values for Ag, Cd or Cr

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Ultra-trace analyses of Ag, Cd, Cu, Ni, Pb, Zn in filtered and unfiltered waters

Quality Control:

Replicate Determination

Sample	**Ag (ng/L)**		
	Replicate 1	**Replicate 2**	**Average**
Water_C1_1m_filt	<5	<5	<5
Wat_A3_1m_filt	<5	<5	<5
FB1 (filled with site SW & filtered)	<5	<5	<5
Wat_A3_5m_Total	8.5	7.0	7.8
Wat_A2_1m_Total	<5	<5	<5
Wat_A3_10m_Total	<5	<5	<5
Sample	**Cd (ng/L)**		
	Replicate 1	**Replicate 2**	**Average**
Water_C1_10m_filt	87	88	88
Wat_A1_20m_filt	81	83	82
Water_A2_10m_Tot	84	86	85
Water_A1_1000m_Tot	72	73	73
Sample	**Co (ng/L)**		
	Replicate 1	**Replicate 2**	**Average**
Water_C1_10m_filt	<24	<24	<24
Wat_A1_20m_filt	<24	<24	<24
Water_A2_10m_Tot	<9	<9	<9
Water_A1_1000m_Tot	<9	<9	<9
Sample	**Cr (ng/L)**		
	Replicate 1	**Replicate 2**	**Average**
FB3, Filtered	<90	<90	<90
FB4, Filtered	<90	<90	<90
A3_10m_Total	280	270	275
A3_20m_Total, Site dup 1	260	290	275
A3_20m_Total, Site dup 2	250	300	275

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Ultra-trace analyses of Ag, Cd, Cu, Ni, Pb, Zn in filtered and unfiltered waters

Quality Control:

Replicate Determination

Sample	**Cu (ng/L)**		
	Replicate 1	**Replicate 2**	**Average**
Water_C1_10m_filt	157	178	168
Wat_A1_20m_filt	147	144	146
Water_A2_10m_Tot	313	312	313
Water_A1_1000m_Tot	600	407	504
Sample	**Ni (ng/L)**		
	Replicate 1	**Replicate 2**	**Average**
Water_C1_10m_filt	649	638	644
Wat_A1_20m_filt	566	604	585
Water_A2_10m_Tot	541	524	533
Water_A1_1000m_Tot	407	406	407
Sample	**Pb (ng/L)**		
	Replicate 1	**Replicate 2**	**Average**
Water_C1_10m_filt	**25**	25	25
Wat_A1_20m_filt	16	6	11
Water_A2_10m_Tot	72	64	68
Water_A1_1000m_Tot	49	14	32
Sample	**Zn (ng/L)**		
	Replicate 1	**Replicate 2**	**Average**
Water_C1_10m_filt	1980	1800	1890
Wat_A1_20m_filt	4220	4230	4220
Water_A2_10m_Tot	3370	3590	3480
Water_A1_1000m_Tot	2310	2430	2370

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Ultra-trace analyses of Ag, Cd, Cu, Ni, Pb, Zn in filtered and unfiltered waters

Quality Control:

% Recovery of trace metal spikes added to samples prior to solvent extractions.

	% Recovery						
Sample	**Ag**	**Cd**	**Co**	**Cu**	**Ni**	**Pb**	**Zn**
Filtered_A2_5m	NA	91	99	99	97	90	90
Filtered_SS_Control_500m	NA	83	96	90	86	102	nd
Filtered_A1_1000m	NA	89	98	100	103	95	111
Total_A1_10m	NA	88	93	90	89	89	nd
Total_C1_1m	NA	90	97	96	90	87	102
Wat_C1_1m_filt	103	NA	NA	NA	NA	NA	NA
Wat_A3_5m_filt	106	NA	NA	NA	NA	NA	NA

Concentration of spike added: 40 ng/L of Ag, 5000 ng/L of Cd, 25000 ng/L of Cr, Cu, Ni, Pb, and Zn
NA = not analysed

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Total Arsenic in Waters: Analyses by AS

Quality Control:

Method Blank, detection limit and field blanks

Blanks	[As] µg/ L
Method Blanks	<0.1
Detection Limit	0.10
FB1	1.59
FB2	1.62
FB3	<0.1
FB4	<0.1

Detection Limit (3σMethod Blank)

Determinations of replicate field samples

Sample	Replicate 1	Replicate 2	Average
	[As] µg/ L	[As] µg/ L	[As] µg/ L
SS contrl 500m	1.61	1.61	1.61
A3-20m	33.2	8.7	21.0

Replicate AAS determinations of the same solution

Sample	Replicate 1	Replicate 2	Average
	[As] µg/ L	[As] µg/ L	[As] µg/ L
FB1	1.59	1.60	1.59
FB3	<0.1	<0.1	<0.1
FB4	<0.1	<0.1	<0.1
A1-1m	1.90	1.85	1.88
A2 5m	1.74	1.70	1.72
A2-10m	1.73	1.68	1.70
A3 5m	32.8	34.7	33.7
A3-20m dup2	8.23	8.20	8.22
C1-1m	1.84	1.81	1.83
C1 5m	1.83	1.76	1.80
C1-20m	7.07	7.03	7.05

Replicate determinations on different dilutions

Sample	Analysis 1	Analysis 2	Average
	[As] µg/ L	[As] µg/ L	[As] µg/ L
A3-1m	2.54	2.58	2.56
A3-5m	33.7	28.2	31.0
A3-10m	12.9	11.9	12.4
A3-20m dup2	9.22	8.22	8.72

Recovery of As from reference material

Sample	Measured	Certificate value	% Recovery	
	As, µg/L	As, µg/L		
NAAS-5	1.2	1.27 ±0.12	92.1	Coastal seawater
NAAS-5	1.1	1.27 ±0.12	88.8	Coastal seawater
NIST 1643e	60.2	60.45 ±0.7	99.6	Synthetic freshwater

Recovery of As(III) spiked into samples

Sample	Spike concn.	% Recovery
	[As] µg/ L	
A1-10m	8.4	102
A2-20m	8.5	104

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Total Mercury (Hg) Analyses in Waters (unfiltered)

Quality Control:

Detection Limit

	Total mercury
Detection Limit	0.2 ng/L

Spike Recovery

Sample	% Recovery
A1-20m	97.5

A known spike of inorganic Hg was added to sample

Certified Reference Material

Concentrations in ng/L

	Certified Value	Measured
BCR 579 coastal seawater	1.85±0.2	2.0

Method Duplicates

Concentrations in ng/L

Sample	Replicate 1	Replicate 2	Mean
Field Blank	0.3	0.5	0.4
A1-5m	1.3	1.3	1.3
A2-20m	1.7	2.1	1.9
A3-1m	11	17	14
A3-20m	6.8	9.0	7.9

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Total Selenium in Waters: Analyses by AS

Quality Control:

Method Blank, detection limit and field blanks

Blanks	[Se] µg/L
Method Blanks	<0.02
Detection Limit	0.02
FB1	0.07
FB2	0.10
FB3	<0.02
FB4	<0.02

Detection Limit (3σMethod Blank)

Replicate AAS determinations of the same solution

Sample	Replicate 1	Replicate 2	Average
	[Se] µg/L	[Se] µg/L	[Se] µg/L
FB2	0.09	0.10	0.10
FB4	<0.02	<0.02	<0.02
SS control 500m -rep 2	0.10	0.09	0.09
A1 - 1m	0.14	0.15	0.15
A2 - 5m	0.12	0.11	0.11
A2 - 10m	0.14	0.13	0.14
A3 - 20m dup1	0.12	0.13	0.13
A3 - 20m dup2	0.13	0.13	0.13
C1 - 1m	0.14	0.14	0.14
NIST 1643e	12.1	11.6	11.8
NIST 1643e, sample 1	12.0	12.0	12.0
NIST 1643e, sample 2	11.3	11.6	11.4

Replication of different aliquots of the same field sample

Sample	Analysis 1	Analysis 2	Average
	[Se] µg/L	[Se] µg/L	[Se] µg/L
A2-1m	0.15	0.15	0.15
A3-5m	0.14	0.15	0.14
A3-20m dup1	0.13	0.13	0.13
A3-20m dup2	0.13	0.12	0.13
C1-20m	0.14	0.14	0.14

Recovery of Se from reference material

Sample	Measured	Certificate value	% Recovery
	Se, µg/L	Se, µg/L	
NIST 1643e	11.8	11.97±0.14	98
NIST 1643e	12.0		100
NIST 1643e	11.4		96

Recovery of Se(IV) spiked into samples

Sample	Spike concn.	% Recovery
	Se, µg/L	
SS control 500 -rep 1	0.98	94
A1 - 20m	6.8	102
A3 - 5m	6.8	96
A1 - 10m	4.7	94
A3 - 1m	4.7	90
C1 - 1m	4.7	90

Appendix 2: Quality Control for Analyses for Sediments

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Total Particulate Metals (TPM) Analyses (1% HNO3, 2% HCl, microwave digestion)

Quality Control:
Analysis blanks prepared using the standard procedure in the absence of sample.
Duplicate analyses were with subsamples from selected samples.

Repeat Sampling and Analyses of Sediment

Concentrations in µg/g

Sample	Al	Fe	Mn	Ag	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
A-C	1930	28100	50.0	2.9	241	3.7	19.9	4.9	1650	8.3	237	1000
replicate	2020	28600	51.9	3.0	254	3.9	20.0	4.9	1710	8.2	246	1030
Ch-I-T	573	55300	2920	99.6	3450	359	560	1.7	20900	19.4	5430	42600
replicate	574	61000	2900	96.4	3790	361	779	1.7	24500	20.5	6110	43200

Blanks	Al	Fe	Mn	Ag	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
Blank 1	<0.5	<0.5	<0.2	<0.5	<1.0	<0.3	<0.3	<0.3	<0.3	<0.3	<1.0	<0.3
Blank 2	<0.5	<0.5	<0.2	<0.5	<1.0	<0.3	<0.3	<0.3	<0.3	<0.3	<1.0	<0.3
Blank 3	<0.5	<0.5	<0.2	<0.5	<1.0	<0.3	<0.3	<0.3	<0.3	<0.3	<1.0	<0.3

Standard Reference Material Analyses: TPM Metal Concentration (µg/g)

Sample			Mn	Ag	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
PACS2 1			200	1.35	23.9	2.30	17	40	329	25.5	174	351
PACS2 2			204	1.30	27.0	2.26	17	41	334	25.9	176	373
PACS2 3			201	1.28	23.3	2.36	17	40	333	26.0	171	350
Average			202	1.31	24.7	2.31	17	40	332	25.8	174	358
Std. Dev.			2	0.03	1.60	0.04	0	0	2	0.2	2	11
Method: Aqua Regia.			**Mn**	**Ag**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
Certified value (µg/g)			440	1.22	26.2	2.11	11.5	91	310	39.5	183	364
Recovery (%)			46	108	94	109	149	45	107	65	95	98
In-house value (µg/g)			209	1.2	25.0	2.2	18	42	319	27	163	349

Spike-recoveries for ICP-AES analyses
TPM extracts were analysed both undiluted and diluted 1/200 and indicated that there were no significant matrix interferences (results within 10%).
Selected undiluted and diluted extracts were spiked with a 200 ppm mixed-metals solution and recoveries determined.

Spike-recovery, %

Sample	Al	Fe	Mn	Ag	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
Ci-C	83	ND	95	103	100	100	97	97	107	96	97	99
M	85	ND	94	75	75	97	94	94	ND	92	ND	ND
Ch-I-C 1:200	99	97	101	100	99	100	100	100	101	100	99	99
Ch-A-C 1:200	99	99	99	100	99	100	100	99	107	100	99	100

ND = not determined due to concentrations being beyond instrument calibration

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Acid Volatile Sulfide (AVS) and Simultaneous Extractable Metals (SEM)

Quality Control:

Analysis blanks prepared using the standard procedure in the absence of sample.
Duplicate (SEM) and triplicate (AVS) analyses were with subsamples from selected samples.

Repeat Sampling and Analyses of Sediment

	Concentrations in µg/g												AVS
Sample	**Fe**	**Mn**	**Ag**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Hg**	**Ni**	**Pb**	**Zn**	**µmol/g**
A-c	-	-	-	-	-	-	-	-	-	-	-	-	20
A-c	-	-	-	-	-	-	-	-	-	-	-	-	22
A-c	-	-	-	-	-	-	-	-	-	-	-	-	12
A-t	-	-	-	-	-	-	-	-	-	-	-	-	7.8
A-t	-	-	-	-	-	-	-	-	-	-	-	-	4.2
A-t	-	-	-	-	-	-	-	-	-	-	-	-	4.2
Ch-A-T	860	41	<0.4	450	<0.4	<0.4	<0.4	110	NA	<0.4	400	420	3.0
Ch-A-T	880	41	<0.4	430	<0.4	<0.4	<0.4	110	NA	<0.4	390	440	1.9
Ch-A-T	-	-	-	-	-	-	-	-	-	-	-	-	0.9
Ci-T	3700	7.0	<0.4	99	<0.4	1.5	1.2	44	NA	0.47	8.2	19	5.7
Ci-T	3800	7.6	<0.4	96	<0.4	1.7	1.3	50	NA	0.45	9.7	22	12.1
Ci-T	-	-	-	-	-	-	-	-	-	-	-	-	-

Blanks	**Fe**	**Mn**	**Ag**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Hg**	**Ni**	**Pb**	**Zn**	**µmol/g**
Blank 1	<1.5	<0.4	<0.4	<1.5	<0.4	<0.4	<0.4	<0.4	NA	<0.4	<1.5	<0.4	-
Blank 2	<1.6	<0.4	<0.4	<1.6	<0.4	<0.4	<0.4	<0.4	NA	<0.4	<1.6	<0.5	-
Blank 3	<1.6	<0.4	<0.4	<1.6	<0.4	<0.4	<0.4	<0.4	NA	<0.4	<1.6	<0.5	-

CRMs: Concentrations in mg/kg

CRM	**Fe**	**Mn**	**Ag**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Hg**	**Ni**	**Pb**	**Zn**
PACS2	5900	26	<0.4	6.3	<0.4	3.1	5.7	144	NA	3.9	120	210
PACS2	5600	24	<0.4	6.5	<0.4	3.0	5.2	139	NA	3.6	120	190
PACS2	5800	26	<0.4	6.1	<0.4	3.2	5.6	142	NA	3.9	120	200
Average	**5800**	**25**	**<0.4**	**6.3**	**<0.4**	**3.1**	**5.5**	**142**	**NA**	**3.8**	**120**	**200**

Certified value [a]	[a] This value applies to a total-extractable metals analysis of this CRM.											
Total metals	NA	440	1.1	26.2	2.11	11.5	90.7	310	3.04	39.5	183	364

In-house value [b]	[b] This is the mean value obtained for 1-h 1 M HCl extractions of this CRM.											
1 M HCl metal	NA	25	<0.4	6.0	1.4	3.0	6	140	<0.01	4.0	120	200

Spike-recoveries for ICP-AES analyses

SEM extracts were analysed both undiluted and diluted 1/200 and indicated that there were no significant matrix interferences (results within 10%).
Selected undiluted and diluted extracts were spiked with a 200 ppm mixed-metals solution and recoveries determined.

	Spike-recovery, %											
Sample	**Al**	**Fe**	**Mn**	**Ag**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**
A-C	77	78	94	100	97	98	97	96	103	96	95	94
Ci-C	82	86	95	101	100	100	97	97	104	97	99	100
Ch-I-T 1:10	96	91	96	99	97	98	97	98	100	96	95	94
Ch-A-T 1:10	100	98	99	101	97	101	99	100	101	100	101	100

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Total Organic Carbon (TOC) Analyses (by NMI)

Australian Government

National Measurement Institute

QUALITY ASSURANCE REPORT

Client: **CSIRO ENERGY TECHNOLOGY**

NMI Job No: CSIRO07/070522 **Sample Matrix:** Soil

Analyte	Method	LOR	Blank	Duplicates			Recoveries	
				1	2	RPD	Matrix spk	LCS
		mg/kg	mg/kg	mg/kg	mg/kg	%	%	%
Waters Section				N07/020109			N07/020109	
Carbon-Total organic	NWS15	100.0	<100	5050	3910	25.4	84	87

Legend
Acceptable recovery is 80-120%.
Acceptable RPDs on duplicates is 30% at >5 times LOR. Greater RPD may be expected at < 5 LOR.
LOR = Limit Of Reporting ND = Not Determined
RPD = Relative Percent Difference NA = Not Applicable
LCS = Laboratory Control Sample.

Comments
This report shall not be reproduced except in full.
Results greater than ten times LOR have been rounded to two significant figures.

Signed:

Dr Honway Louie
Inorganics
Date: **01/06/2007**

Appendix 3: Quality Control for Elutriate Tests

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Total Dissolved Metals in Elutriate Waters: Analyses by ICP-AES

Quality Control:
Analysis blanks prepared using the standard procedure in the absence of sample.

Spike-recoveries for ICP-AES analyses
dM samples (seawater) were spiked with to 100 µg/L mixed-metals solution and recoveries determined

Percent recovery, %

Sample	Ag	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
A1_5m	105	104	109	102	96	98	99	99	102	99.4	115	102
A2_5m	107	104	105	103	95	101	95	97	104	103	98	108
A3_5m	107	94	58	92	87	90	92	87	96	89	110	95
C1_20m	119	108	74	102	97	95	99	96	103	97	102	100

ND = not determined due to concentrations being beyond instrument calibration

Concentrations in µg/L

Method blanks, µg/L	Ag	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
MQ	<3	<5	<10	<3	<3	<3	<3	<5	<3	<3	<10	<3
MQ	<3	<5	<10	<3	<3	<3	<3	<5	<3	<3	<10	<3

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Total Dissolved Metals in Elutriate Waters: Analyses by ICP-AES

Quality Control: Particle size effect
Analysis blanks prepared using the standard procedure in the absence of sample.

Analyses of duplicate water samples from same elutriate test

Concentrations in µg/L

Sample	Ag	Al	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Ch-a-t A 4-30 mm	<3	<5	150	<3	<3	<3	<5	48	<3	109	711
Ch-a-t B 4-30 mm	<3	<5	138	<3	<3	<3	<5	48	<3	115	729
Ch-a-t A 1-4 mm	<3	25	100	<3	<3	<3	<5	37	<3	39	472
Ch-a-t B 1-4 mm	<3	21	96	<3	<3	<3	<5	38	<3	32	489
Ch-a-t A 0.063-0.25 mm	<3	<5	275	6	<3	<3	<5	17	<3	41	332
Ch-a-t B 0.063-0.25 mm	<3	<5	296	6	<3	<3	<5	17	<3	35	352
Ch-a-t A <63 µm	<3	<5	872	19	<3	<3	<5	46	<3	151	1010
Ch-a-t B <63 µm	<3	<5	810	18	<3	<3	<5	45	<3	58	929
M-t A 4-30 mm	<3	<5	<10	<3	<3	142	<5	648	3	<10	46
M-t B 4-30 mm	<3	<5	<10	<3	<3	145	<5	660	5	<10	47
M-t A 1-4 mm	<3	<5	<10	<3	<3	156	<5	896	6	<10	84
M-t B 1-4 mm	<3	<5	<10	<3	<3	154	<5	901	6	<10	83
M-t A 0.063-0.25 mm	<3	18	<10	<3	<3	49	<5	373	5	<10	39
M-t B 0.063-0.25 mm	<3	15	<10	<3	<3	49	<5	372	5	<10	37
M-t A <0.063 mm	<3	83	<10	<3	<3	83	<5	317	5	<10	92
M-t B <0.063 mm	<3	83	<10	<3	<3	78	<5	316	5	<10	90

Analyses of waters (taken in duplicate) from replicate elutriate tests with same sediment

Concentrations in µg/L

Sample	Ag	Al	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Ch-a-t A 0.25-1 mm	<3	<5	172	<3	<3	<3	<5	23	<3	37	327
Ch-a-t B 0.25-1 mm	<3	<5	175	<3	<3	<3	<5	24	<3	43	329
Ch-a-t A(dup) 0.25-1 mm	<3	<5	162	<3	<3	<3	<5	24	<3	59	320
Ch-a-t B(dup) 0.25-1 mm	<3	<5	161	<3	<3	<3	<5	23	<3	22	322
M-t A 0.25-1 mm	<3	34	<10	<3	<3	77	<5	986	9	<10	110
M-t B 0.25-1 mm	<3	32	<10	<3	<3	74	<5	985	9	<10	104
M-t A(dup) 0.25-1 mm	<3	54	<10	<3	<3	82	<5	1060	9	<10	109
M-t B(dup) 0.25-1 mm	<3	56	<10	<3	<3	80	<5	1080	9	<10	109

ND = not determined due to concentrations being beyond instrument calibration

Spike-recoveries for ICP-AES analyses
dM samples (seawater) were spiked with to 100 µg/L mixed-metals solution and recoveries determined

Percent recovery, %

Sample	Ag	Al	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Ch-a-t 0.25-1 mm 10 g/L	102	84	103	102	100	100	101	101	90.6	105	94
M-t 0.25-1 mm 10 g/L	103	83	105	103	102	99	104	103	89	108	101
Ch-a-t 30 min 10 g/L	102	78	100	102	100	99	101	101	90	102	91
M-t 30 min 10 g/L	103	83	104	102	101	101	101	101	90	102	92
C-c 30 min 10 g/L	101	88	106	101	101	100	98	100	90	103	95

ND = not determined due to concentrations being beyond instrument calibration

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Water pH and Dissolved Oxygen in Elutriate Waters

Quality Control: Particle size effect

Water pH and Dissolved Oxygen in Elutriate Waters

Sample	pH	Dissolved oxygen, mg/L
Blank	8.0	6.8
Ch-a-t A 4-30mm	8.0	6.9
Ch-a-t B 4-30mm		
Ch-a-t A 1-4mm	7.9	
Ch-a-t B 1-4mm		
Ch-a-t A 0.25-1mm	7.9	6.8
Ch-a-t B 0.25-1mm		
Ch-a-t A(dup) 0.25-1mm	7.9	
Ch-a-t B(dup) 0.25-1mm		
Ch-a-t A 0.063-0.25 mm	7.9	
Ch-a-t B 0.063-0.25 mm		
Ch-a-t A <0.063 mm	7.9	
Ch-a-t B <0.063 mm		
M-t A 4-30mm	8.0	7.0
M-t B 4-30mm		
M-t A 1-4mm	7.9	7.0
M-t B 1-4mm		
M-t A 0.25-1mm	7.8	
M-t B 0.25-1mm		
M-t A(dup) 0.25-1mm	7.9	
M-t B(dup) 0.25-1mm		
M-t A 0.063-0.25 mm	7.8	
M-t B 0.063-0.25 mm		
M-t A <0.063 mm	7.8	
M-t B <0.063 mm		

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Total Dissolved Metals in Elutriate Waters: Analyses by ICP-AES

Quality Control: Effect of [TSS]
Analysis blanks prepared using the standard procedure in the absence of sample.

Analyses of duplicate water samples from same elutriate test

Concentrations in µg/L

Sample	Ag	Al	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Ch-a-t A 1 g/L	<3	<5	111	<3	<3	<3	<5	17	<3	24	199
Ch-a-t B 1 g/L	<3	<5	102	<3	<3	<3	<5	17	<3	4	199
Ch-a-t A 100g/L	<3	<5	2690	57	<3	<3	<5	742	25	37	6070
Ch-a-t B 100g/L	<3	<5	2820	57	<3	<3	<5	768	27	22	6350
M-t A 1 g/L	<3	<5	<10	<3	<3	16	<5	43	<3	<10	6
M-t B 1 g/L	<3	<5	<10	<3	<3	14	<5	43	<3	<10	7
M-t A (dup) 10 g/L	<3	<5	<10	9	<3	36	<5	520	<3	<10	108
M-t B (dup) 10 g/L	<3	<5	<10	9	<3	40	<5	542	<3	<10	110
M-t A 100 g/L	<3	<5	14	78	<3	94	29	3300	21	<10	830
M-t B 100 g/L	<3	<5	14	80	<3	84	1	3340	21	<10	848
C-c A 1 g/L	<3	<5	<10	<3	<3	<3	64	4	<3	<10	7
C-c B 1 g/L	<3	<5	<10	<3	<3	<3	15	4	<3	<10	4
C-c A 100 g/L	<3	<5	<10	<3	<3	<3	15	170	<3	<10	18
C-c B 100 g/L	<3	<5	<10	<3	<3	<3	13	169	<3	<10	17

Analyses of waters (taken in duplicate) from replicate elutriate tests with same sediment

Concentrations in µg/L

Sample	Ag	Al	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Ch-a-t A 10 g/L	<3	<5	418	8	<3	<3	<5	77	<3	18	604
Ch-a-t B 10 g/L	<3	<5	501	10	<3	<3	<5	88	<3	10	889
Ch-a-t A (dup) 10 g/L	<3	<5	571	11	<3	<3	<5	90	<3	23	925
Ch-a-t B (dup) 10g/L	<3	<5	583	11	<3	<3	<5	92	<3	21	953
C-c A 10 g/L	<3	<5	<10	<3	<3	<3	331	29	<3	<10	49
C-c B 10 g/L	<3	<5	<10	<3	<3	<3	340	30	<3	<10	51
C-c A (dup) 10 g/L	<3	124	<10	<3	<3	<3	1036	15	<3	<10	47
C-c B (dup) 10 g/L	<3	58	<10	<3	<3	<3	679	11	<3	<10	34

Spike-recoveries for ICP-AES analyses
dM samples (seawater) were spiked with to 100 µg/L mixed-metals solution and recoveries determined

Percent recovery, %

Sample	Ag	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
M-t 30 min 10g/L	103	83	104	102	100	101	101	101	101	90	102	92
C-c 30 min 10g/L	101	88	106	101	101	101	100	98	100	90	103	95

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Water pH and Dissolved Oxygen in Elutriate Waters

Quality Control: Effect of [TSS]

Water pH and Dissolved Oxygen in Elutriate Waters

Sample	TSS, g/L	pH	Dissolved oxygen, mg/L
Blank		8.0	6.2
Ch-A-c	1.0	8.0	
Ch-A-c (dup)	10	7.8	5.6
Ch-A-c	10	7.8	
Ch-A-c	100	7.3	
M-t	1.0	8.1	6.0
M-t	10	7.8	6.0
M-t	100	7.3	
C-c	1.0	7.9	6.0
C-c	10	6.7	
C-c (dup)	10	7.6	
C-c	100	7.5	

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Total Dissolved Metals in Elutriate Waters: Analyses by ICP-AES

Quality Control: Effect of time

Analysis blanks prepared using the standard procedure in the absence of sample.

Analyses of duplicate water samples from same elutriate test

Concentrations in µg/L

Sample	Ag	Al	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Ch-a-t A 2 min	<3	<5	98	<3	<3	<3	<5	20	<3	10	206
Ch-a-t B 2 min	<3	<5	146	<3	<3	<3	<5	27	<3	39	279
Ch-a-t A 60 min	<3	<5	461	9	<3	<3	<5	34	<3	71	533
Ch-a-t B 60 min	<3	<5	488	10	<3	<3	<5	34	<3	81	533
Ch-a-t B 240 min	<3	<5	856	19	<3	<3	<5	42	<3	116	883
Ch-a-t B 240 min	<3	<5	832	18	<3	<3	<5	41	<3	114	867
M-t A 2 min	<3	<5	<10	<3	<3	37	<5	92	<3	<10	16
M-t B 2 min	<3	<5	<10	<3	<3	38	<5	92	<3	<10	15
M-t A 60 min	<3	<5	<10	<3	<3	25	<5	20	<3	<10	9
M-t B 60 min	<3	<5	<10	<3	<3	27	<5	21	<3	<10	9
M-t A 240 min	<3	<5	<10	<3	<3	68	<5	140	<3	<10	37
M-t B 240 min	<3	<5	<10	<3	<3	70	<5	139	<3	<10	36
C-c A 2 min	<3	<5	<10	<3	<3	<3	<5	21	<3	<10	<3
C-c B 2 min	<3	<5	<10	<3	<3	<3	<5	21	<3	<10	<3
C-c A 60 min	<3	<5	<10	<3	<3	<3	<5	21	<3	<10	<3
C-c B 60 min	<3	<5	<10	<3	<3	<3	<5	22	<3	<10	<3
C-c A 240 min	<3	<5	<10	<3	<3	<3	<5	24	<3	<10	<3
C-c B 240 min	<3	<5	<10	<3	<3	<3	<5	25	<3	<10	<3

Analyses of waters (taken in duplicate) from replicate elutriate tests with same sediment

Concentrations in µg/L

Sample	Ag	Al	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Ch-a-t A 10 min	<3	<5	250	5	<3	<3	<5	27	<3	48	351
Ch-a-t B 10 min	<3	<5	257	5	<3	<3	<5	28	<3	48	352
Ch-a-t A (dup) 10 min	<3	<5	235	5	<3	<3	<5	26	<3	42	340
Ch-a-t B (dup) 10 min	<3	<5	234	5	<3	<3	<5	26	<3	34	346
Ch-a-t A 30 min	<3	<5	369	7	<3	<3	<5	31	<3	77	449
Ch-a-t B 30 min	<3	<5	366	7	<3	<3	<5	30	<3	71	455
Ch-a-t A (dup) 30 min	<3	<5	315	7	<3	<3	<5	27	<3	55	351
Ch-a-t B (dup) 30 min	<3	<5	374	7	<3	<3	<5	31	<3	52	449
M-t A 10 min	<3	<5	<10	<3	<3	41	<5	110	<3	<10	18
M-t B 10 min	<3	<5	<10	<3	<3	41	<5	106	<3	<10	17
M-t A (dup) 10 min	<3	<5	<10	<3	<3	52	<5	165	<3	<10	21
M-t B (dup) 10 min	<3	<5	<10	<3	<3	55	<5	166	<3	<10	21
M-t A 30 min	<3	<5	<10	<3	<3	56	<5	142	<3	<10	21
M-t B 30 min	<3	<5	<10	<3	<3	58	<5	149	<3	<10	21
M-t A (dup) 30 min	<3	<5	<10	<3	<3	64	<5	230	<3	<10	27
M-t B (dup) 30 min	<3	<5	<10	<3	<3	67	<5	238	<3	<10	28

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Total Dissolved Metals in Elutriate Waters: Analyses by ICP-AES

Quality Control (continued): Effect of time

Replicate elutriate tests with same sediment (duplicate analyses of waters)

C-c A .10 min	<3	<5	<10	<3	<3	<3	<5	23	<3	<10	<3
C-c B 10 min	<3	<5	<10	<3	<3	<3	<5	23	<3	<10	<3
C-c A (dup) 10 min	<3	<5	<10	<3	<3	<3	<5	21	<3	<10	<3
C-c B (dup) 10 min	<3	<5	<10	<3	<3	<3	<5	21	<3	<10	<3
C-c A 30 min	<3	<5	<10	<3	<3	<3	<5	22	<3	<10	<3
C-c B 30 min	<3	<5	<10	<3	<3	<3	<5	22	<3	<10	<3
C-c A (dup) 30 min	<3	<5	<10	<3	<3	<3	<5	22	<3	<10	<3
C-c B (dup) 30 min	<3	<5	<10	<3	<3	<3	<5	21	<3	<10	<3

Spike-recoveries for ICP-AES analyses
dM samples (seawater) were spiked with to 100 µg/L mixed-metals solution and recoveries determined

Percent recovery, %

Sample	**Ag**	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**
M-t 30 min 10 g/L	103	83	104	102	100	101	101	101	101	90	102	92
C-c 30 min 10 g/L	101	88	106	101	101	101	100	98	100	90	103	95

ND = not determined due to concentrations being beyond instrument calibration

Concentrations in µg/L

Method blanks, µg/L	**Ag**	**Al**	**As**	**Cd**		**Cr**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**
MQ	<3	<5	<10	<3		<3	<3	<5	<3	<3	<10	<3
MQ	<3	<5	<10	<3		<3	<3	<5	<3	<3	<10	<3

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Water pH and Dissolved Oxygen in Elutriate Waters

Quality Control: Effect of time

Water pH and Dissolved Oxygen in Elutriate Waters

Sample	Time, min	pH	Dissolved oxygen, mg/L
Blank	2.0	8.0	6.5
Ch-A-t	2.0	7.9	
M-t	2	7.9	
C-c	2	7.9	7.0
Ch-A-t	10	7.9	
Ch-A-t (dup)	10	7.9	
M-t	10.0	7.9	6.2
M-t (dup)	10	7.9	
C-c	10	8.0	
C-c (dup)	10	8.0	6.3
Ch-A-t	30	7.9	
Ch-A-t (dup)	30.0	7.9	
M-t	30	7.9	
M-t (dup)	30	7.8	6.4
C-c	30	8.0	
C-c (dup)	30	8.0	
Ch-A-t	60	7.9	
M-t	60	7.9	
C-c	60	8.0	7.6
Blank	240	7.9	
Ch-A-t	240	7.8	6.4
M-t	240	7.7	
C-c	240	7.9	

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Total Dissolved Metals in Elutriate Waters: Analyses by ICP-AES

Quality Control: Elutriate - Standarised tests for remaining samples
Analysis blanks prepared using the standard procedure in the absence of sample.

Analyses of duplicate water samples from same elutriate test

Concentrations in µg/L

Sample	Ag	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
A-t	<3	<3	220	<3	<3	<3	<3	120	410	<3	6	4
A-t	<3	<3	220	<3	<3	<3	<3	44	390	<3	6	<3
Ch-a-c	<3	<3	710	<3	<3	<3	<3	6	267	<3	68	4700
Ch-a-c	<3	<3	880	<3	<3	<3	<3	15	272	<3	200	5500
Ch-i-t	<3	<3	32	350	120	<3	270	18	7400	23	200	1900
Ch-i-t	<3	<3	19	340	120	<3	74	9	7300	23	32	1200
Ch-i-c	15	<3	46	730	1500	<3	68	10	9119	98	420	28000
Ch-i-c	15	<3	45	740	1500	<3	45	<5	9334	99	240	28000

Analyses of waters (taken in duplicate) from replicate elutriate tests with same sediment

Concentrations in µg/L

Sample	Ag	Al	As	Cd		Cr	Cu	Fe	Mn	Ni	Pb	Zn
C-t	<3	21	57	<3	<3	<3	<3	260	57	<3	<3	<3
C-t	<3	32	57	<3	<3	<3	<3	420	56	<3	<3	<3
C-t duplicate	<3	11	49	<3	<3	<3	<3	170	62	<3	5	<3
C-t duplicate	<3	30	50	<3	<3	<3	<3	320	63	<3	<3	<3

Water pH and Dissolved Oxygen in Elutriate Waters

Quality Control: Elutriate - Standarised tests for remaining samples

Water pH and Dissolved Oxygen in Elutriate Waters

Sample	pH	Dissolved oxygen, mg/L
Blank	8.0	6.5
A-c	7.9	3.7
C-t	7.9	4.2
Ch-A-c	8.1	5.8
Ch-I-c	7.5	5.7
Ch-I-t	8.1	5.7

Appendix 4: Toxicity of Elutriate Waters to Algae

Algal (Nitzchia) Growth Bioassay

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Chronic Algal Growth Test Report NcG 180907 **Page 1 of 2**

Client: ENESAR (Coffey Natural Systems)
Project: Solwara
Test Performed: 72-h chronic algal growth toxicity test with the marine alga *Nitzschia closterium*

72-hour chronic growth bioassay, *Nitzschia Closterium*

Test Method

This test measures the decrease (inhibition) in cell growth of the temperate marine alga *Nitzschia closterium* after exposure to the sample for 72 h (initial cell density 2-4 × 10^4 cells/mL). The test protocol is based on the OECD Test Guideline 201(1984) and the protocol of Stauber et. al. (1994). The 72-h IC50, LOEC and NOEC values were calculated using ToxCalc Version 5.0.23 (Tidepool Software).

References

OECD (1984) Guideline for testing of chemicals. Alga growth inhibition test. Test Guideline No. 201. Organisation for Economic Cooperation and Development, Paris, France.

Stauber, J.L., Tsai, J., Vaughan, G.T., Peterson, S.M. and Brockbank, C.I. (1994) Algae as indicators of toxicity of the effluent from bleached eucalypt kraft paper mills. National Pulp Mills Research Program Technical Report No. 3 Canberra: CSIRO, 146 pp.

Samples and Testing

Samples Prepared:	11/09/2007	(Mineral samples collected on 20/04/2007)
Samples Received:	11/09/2007	Test Initiated: 11/09/2007
CSIRO Sample No.	**Sample Name**	**Sample Description**
NA	Ch-a-t	Elutriate sample
NA	M-t	Elutriate sample

Sample Physico-Chemistry and Preparation:
The elutriates were prepared by rolling 100 g of the rock sample for 12 minutes in 1 L of seawater. The samples was centrifuged and filtered through a 0.45 µm filter. The physio-chemistry of the elutriate samples was measure prior to use in the test

Sample	Physio-chemistry				Comments
	pH	‰	mS/cm	DO (%)	
Ch-a-t	7.85	34.8	52.7	100	-
M-t	8.24	35.2	53.2	100	-

‰ = Salinity; µS/cm = Conductivity; [a] Dissolved Oxygen (%); [b] mg N/L ± 2 SD;

Results

Survival

Sample	Growth Rate (Doublings/day)	% of QA Control	CV (%)
QA Control	1.67	-	1
Method Blank	1.67	100	9
Ch-a-t			
0.14%	1.69	101	4
0.4%	1.58	95	5
1.2%	1.46	87[a]	8
3.7%	0.63	38[a]	2
11%	0.10	6[a]	87
33%	-0.15	-9[a]	0
100%	-0.34	-20[a]	0
M-t			
0.14%	1.62	97	6
0.4%	1.46	88[a]	4
1.2%	0.39	23[a]	20
3.7%	-0.13	-8[a]	0
11%	-0.76	-46[a]	0
33%	-1.78	-107[a]	0
100%	NA	NA[a]	NA

Sample	IC50 (%)	LOEC (%)	NOEC (%)
Ch-a-t	3.06	1.2	0.4
M-t	0.87	0.4	0.14

[a] Significantly less than QA control; NA – Growth rate could not be establish as there was a zero cell count in all replicates on day 1, 2 and 3

Quality Assurance/Quality Control

Parameter	Criterion	This Test	Criterion Met?
Control growth rate (doublings/day)	1.5 ± 0.4	1.67	Yes
Control growth rate CV	<20%	1	Yes
Reference toxicant IC50 (measured copper, µg Cu/L)	18 ± 12	18 (16-22)	Yes

Testing and Reporting

Test carried out and report prepared by: Anthony Platt-Baggs and David Spadaro
Experimental Scientist (Ph: 02 9710 6801)

Test report authorised by: Stuart Simpson
Principal Research Scientist (Ph: 02 9710 6807)

Date: 19/09/2007

CSIRO Land and Water
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ABN 41 687 119 230

Client: ENESAR (Coffey Natural Systems)
Project: Solwara
Test Performed: 72-h chronic algal growth toxicity test with the marine alga *Nitzschia closterium*

72-hour chronic growth bioassay, *Nitzschia Closterium*

Test Method

This test measures the decrease (inhibition) in cell growth of the temperate marine alga *Nitzschia closterium* after exposure to the sample for 72 h (initial cell density 2-4 × 10^4 cells/mL). The test protocol is based on the OECD Test Guideline 201(1984) and the protocol of Stauber et. al. (1994). The 72-h IC50, LOEC and NOEC values were calculated using ToxCalc Version 5.0.23 (Tidepool Software).

References

OECD (1984) Guideline for testing of chemicals. Alga growth inhibition test. Test Guideline No. 201. Organisation for Economic Cooperation and Development, Paris, France.

Stauber, J.L., Tsai, J., Vaughan, G.T., Peterson, S.M. and Brockbank, C.I. (1994) Algae as indicators of toxicity of the effluent from bleached eucalypt kraft paper mills. National Pulp Mills Research Program Technical Report No. 3 Canberra: CSIRO, 146 pp.

Samples and Testing

Samples Prepared:	28/08/2007	(Mineral samples collected on 20/04/2007)
Samples Received:	28/08/2007	Test Initiated: 28/08/2007
CSIRO Sample No.	**Sample Name**	**Sample Description**
NA	C-c	Elutriate sample
NA	A-t	Elutriate sample

Sample Physico-Chemistry and Preparation:
The elutriates were prepared by rolling 100 g of the rock sample for 12 minutes in 1 L of seawater. The samples was centrifuged and filtered through a 0.45 µm filter. The physio-chemistry of the elutriate samples was measure prior to use in the test

Sample	Physio-chemistry				Comments
	pH	‰	mS/cm	DO (%)	
C-c	7.89	35	48	89	-
A-t	8.12	36	49	93	-

‰ = Salinity; µS/cm = Conductivity; [a] Dissolved Oxygen (%); [b] mg N/L ± 2 SD;

Results

Survival

Sample	Growth Rate (Doublings/day)	% of QA Control	CV (%)
QA Control	1.94	-	3
Method Blank	2.05	105	5
C-c			
100%	1.90	98	4
50%	2.03	104	4
25%	2.08	107	10
12.5%	2.10	108	6
6.25%	1.95	101	1
1%	2.02	104	5
A-t			
100%	-0.37 [a]	-19	0
50%	-0.26 [a]	-13	0
25%	-0.05 [a]	-2	173
12.5%	0.59 [a]	30	19
6.25%	1.37 [a]	71	6
1%	1.95	100	5
			3
Sample	**IC50 (%)**	**LOEC (%)**	**NOEC (%)**
C-c	>100	>100	>100
A-t	9.43	1	6.25

[a] Significantly less than QA control

Quality Assurance/Quality Control

Parameter	Criterion	This Test	Criterion Met?
Control growth rate (doublings/day)	1.5 ± 0.4	1.9	Yes
Control growth rate CV	<20%	2.6	Yes
Reference toxicant IC50 (measured copper, µg Cu/L)	18 ± 12	7 (5-9)	Yes

Testing and Reporting

Test carried out and report prepared by: David Spadaro
Experimental Scientist (Ph: 02 9710 6801)

Test report authorised by: Stuart Simpson
Principal Research Scientist (Ph: 02 9710 6807)

Date: 15/09/2007

Appendix 5: Toxicity of Elutriate Waters to Copepods

Copepod Mobility Bioassays for Samples A-t and C-c

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New Illawarra Road, Lucas Heights, NSW
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Acute Copepod Toxicity Test Report 07082C

Client: Solwara
Project: Solwara
Test Performed: 48-h acute copepod immobilisation with the marine copepod *Acartia sinjiensis*

Elutriate Prepared:	30/10/07		
Elutriate Received:	30/10/07	**Test Initiated:**	30/10/07
CSIRO Sample No.	**Sample Name**	**Sample Description**	
WQE07082	Method Blank	Seawater blank sample	
WQE07083	A-t	Elutriate	
WQE07084	C-c	Elutriate	

Sample Physico-Chemistry and Preparation: The elutriates had pH values of 6.8-7.1 and a salinity of 35‰ while the Method Blank had a pH of 8.2 and a salinity of 35‰. All QA test solutions were prepared in filtered (0.45µm) seawater, which had been adjusted to a pH of 7.5 ± 0.1. Additional pH controls were prepared to match the pH of both elutriates.

The pH, salinity, temperature and dissolved oxygen were measured in sub-samples of test concentrations at the beginning and end of each test. The measurements below represent the physico-chemical properties of the samples as received. However, by the time of test commencement, the pH of both the samples and the pH controls had drifted upwards, probably due to the buffering capacity of natural seawater. Therefore, the values below are slightly different to that at the time of test commencement, reported in the toxicity test spreadsheets (attached at end of test report).

Sample	Physico-chemistry				Comments
	pH	‰	mS/cm	DO (%)	
WQE07082 (Method Blank)	8.17	35	52	102	
WQE07083 (A-t)	7.12	35	53	98	
WQE07084 (C-c)	6.80	35	53	97	
A-t pH control	7.05	35	53	98	
C-c pH control	6.76	35	53	99	
pH 7.5 Control	7.40	35	54	102	
Seawater Control	8.21	35	54	104	

‰ = Salinity; µS/cm = Conductivity; DO = Dissolved Oxygen (%);

Test Method: This test measures the mobility of the marine copepod *Acartia sinjiensis* during a 48-h exposure period. The test protocol is based on the protocol described in Rose et al. (2006). Copepods were fed a minimum of two hours prior to test commencement with a mixture of two algal species *Isochrysis* sp. and *Proteomonas sulcata* (previously known as *Cryptomonas* sp.). The 48-h EC50, LOEC and NOEC values were calculated using ToxCalc Version 5.0.23 (Tidepool Software).

Results: There was no significant difference found between the number of mobile copepods in the QA control, A-t pH Control, C-c pH Control, Seawater Control or Method Blank. However, copepod mobility in both pH Controls were lower than the acceptable control rate of 80%, with only 60-75% mobile after a 48 h exposure, indicating that low pH may have contributed to toxicity in undiluted elutriate (i.e. 100% elutriate). In 100% A-t and 100% C-c, mobility decreased by 44%, compared to the Seawater Control. However, due to the variability in copepod mobility among some concentrations of A-t and C-c, this decrease was not statistically significant.

Sample	Mean Mobile (%)	% of Seawater Control	% Inhibition
QA Control (pH 7.5)	80	100	0
Seawater Control	80	100	0
A-t pH Control	60	75	25
C-c pH Control	75	94	6
Method Blank (WQE07082)	80	100	0
A-t (WQE07083)			
0.41%	70	88	12
1.2%	75	94	6
3.7%	75	94	6
11%	55	69	31
33%	80	100	0
100%	45	56	44
C-c (WQE07084)			
6.25%	75	94	6
12.5%	60	75	25
25%	55	69	31
50%	55	69	31
100%	45	56	44
Sample	**IC50 (%)**	**LOEC (%)**	**NOEC (%)**
A-t Elutriate	>100	>100	100
C-c Elutriate	>100	>100	100

[a] Significantly less than Seawater Control

Quality Assurance/Quality Control	Criterion	This Test	Criterion Met?
Control copepod mobility (%)	>80	80	Yes
Reference toxicant EC50 (measured copper, µg Cu/L)	44 ± 16	42 (27-65)	Yes

Comments:

References:

Rose, A., Carruthers, A-M, Stauber, J.L., Lim, R. and Blockwell, S.J. (2006). Development of an acute toxicity test with the marine copepod *Acartia sinjiensis*. *Australasian Journal of Ecotoxicology*. 12: 73-81.

Test carried out by: Monique Binet
Test supervised by: Monique Binet

Test report prepared by: Monique Binet
Experimental Scientist (ph: 02 9710 6812)

Test report authorised by: Jenny Stauber
Senior Principal Research Scientist (ph: 02 9710 6808)

Date: 26/11/07

Statistics –

30/10/2007 **Acute Toxicity of A-t (WQE07083, AQIS ID: 2007-184) to *Acartia sinjiensis***

								Number of mobile organisms										
	Sample (%)	pH		Salinity		D.O.		Time			Mean		Prop'n mob		Mean % Control		Mean Mobile (%)	
Vial		0 hr	48 hr	0 hr	48 hr	0 hr	48 hr	0 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	***48 hr***	24 hr	***48 hr***
1								5	5	4			1.00	0.80				
2	Seawater Control	8.27	8.29	35.6	36.1	104%	94%	5	5	5	4.25	4.00	1.00	1.00	100%	***100%***	85%	***80%***
3								5	4	4			0.80	0.80				
4								5	3	3			0.60	0.60				
								Mean Mobility in Seawater Control (%) =					***85%***	***80%***				
1								5	5	5			1.00	1.00				
2	Method Blank	8.24	8.23	35.7	36.1	99%	96%	5	5	3	5.00	4.00	1.00	0.60	118%	***100%***	100%	***80%***
3								5	5	4			1.00	0.80				
4								5	5	4			1.00	0.80				
1								5	2	2			0.40	0.40				
2	A-t pH Control	7.23	8.07	35.7	36.1	99%	93%	5	5	3	3.75	3.00	1.00	0.60	88%	***75%***	75%	***60%***
3								5	4	4			0.80	0.80				
4								5	4	3			0.80	0.60				
A-t Concentrations (diluted in seawater)																		
1								5	3	3			0.60	0.60				
2	0.41%	8.27	8.25	35.7	35.8	104%	95%	5	4	3	4.00	3.50	0.80	0.60	94%	***88%***	80%	***70%***
3								5	5	4			1.00	0.80				
4								5	4	4			0.80	0.80				
1								5	3	3			0.60	0.60				
2	1.2%	8.32	8.29	35.7	35.9	108%	93%	5	5	5	4.00	3.75	1.00	1.00	94%	***94%***	80%	***75%***
3								5	5	4			1.00	0.80				
4								5	3	3			0.60	0.60				
1								5	5	4			1.00	0.80				
2	3.7%	8.31	8.3	35.6	36.1	105%	94%	5	2	2	4.00	3.75	0.40	0.40	94%	***94%***	80%	***75%***
3								5	4	4			0.80	0.80				
4								5	5	5			1.00	1.00				
1								5	4	4			0.80	0.80				
2	11%	8.27	8.28	35.6	36.0	104%	94%	5	4	3	3.50	2.75	0.80	0.60	82%	***69%***	70%	***55%***
3								5	3	2			0.60	0.40				
4								5	3	2			0.60	0.40				
1								5	4	4			0.80	0.80				
2	33%	8.12	8.25	35.7	36.2	104%	93%	5	5	5	4.00	4.00	1.00	1.00	94%	***100%***	80%	***80%***
3								5	2	2			0.40	0.40				
4								5	5	5			1.00	1.00				
1								5	3	2			0.60	0.40				
2	100%	7.36	8.16	35.7	36.0	101%	93%	5	1	1	2.50	2.25	0.20	0.20	59%	***56%***	50%	***45%***
3								5	3	3			0.60	0.60				
4								5	3	3			0.60	0.60				

30/10/2007 **Acute Toxicity of C-c (WQE07084, AQIS ID: 2007-185) to *Acartia sinjiensis***

								Number of mobile organisms										
	Sample (%)	pH		Salinity		D.O.		Time			Mean		Prop'n mob		Mean % Control		Mean Mobile (%)	
Vial		0 hr	48 hr	0 hr	48 hr	0 hr	48 hr	0 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	*48 hr*	24 hr	*48 hr*
1								5	5	4			1.00	0.80				
2	Seawater Control	8.27	8.29	35.6	36.1	104%	94%	5	5	5	4.25	4.00	1.00	1.00	100%	*100%*	85%	*80%*
3								5	4	4			0.80	0.80				
4								5	3	3			0.60	0.60				
								Mean Mobility in Seawater Control (%) =					*85%*	*80%*				
1								5	5	5			1.00	1.00				
2	Method Blank	8.24	8.23	35.7	36.1	99%	96%	5	5	3	5.00	4.00	1.00	0.60	118%	*100%*	100%	*80%*
3								5	5	4			1.00	0.80				
4								5	5	4			1.00	0.80				
1								5	4	4			0.80	0.80				
2	C-c pH Control	7.05	8.11	35.6	36.0	99%	95%	5	5	5	3.75	3.75	1.00	1.00	88%	*94%*	75%	*75%*
3								5	4	4			0.80	0.80				
4								5	2	2			0.40	0.40				
C-c Concentrations (diluted in seawater)																		
1								5	3	3			0.60	0.60				
2	6.3%	8.26	8.29	35.7	36.1	102%	93%	5	4	4	4.00	3.75	0.80	0.80	94%	*94%*	80%	*75%*
3								5	5	5			1.00	1.00				
4								5	4	3			0.80	0.60				
1								5	2	2			0.40	0.40				
2	12.5%	8.21	8.28	35.6	36.3	101%	93%	5	4	3	3.50	3.00	0.80	0.60	82%	*75%*	70%	*60%*
3								5	4	4			0.80	0.80				
4								5	4	3			0.80	0.60				
1								5	4	4			0.80	0.80				
2	25%	8.12	8.25	35.7	36.3	98%	94%	5	2	0	3.50	2.75	0.40	0.00	82%	*69%*	70%	*55%*
3								5	5	5			1.00	1.00				
4								5	3	2			0.60	0.40				
1								5	3	3			0.60	0.60				
2	50%	7.76	8.20	35.7	36.1	98%	94%	5	5	5	2.75	2.75	1.00	1.00	65%	*69%*	55%	*55%*
3								5	1	1			0.20	0.20				
4								5	2	2			0.40	0.40				
1								5	3	3			0.60	0.60				
2	100%	7.00	8.06	35.7	36.2	94%	93%	5	3	2	3.00	2.25	0.60	0.40	71%	*56%*	60%	*45%*
3								5	3	2			0.60	0.40				
4								5	3	2			0.60	0.40				

Copepod Survival Test-48h Survival

Start Date:	30/10/2007	Test ID:	Sol	Sample ID:	WQE07084
End Date:	1/11/2007	Lab ID:	CSIRO-CECR	Sample Type:	C-c
Sample Date:		Protocol:	ROSE 06	Test Species:	AS-Acartia sinjiensis
Comments:	Test 2				

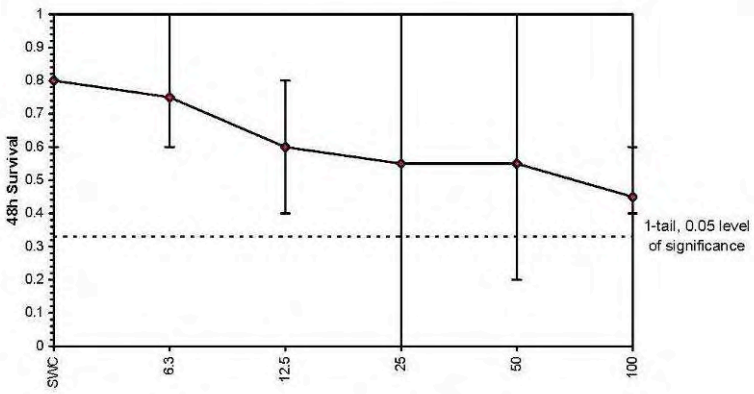
Conc-%	1	2	3	4
SWC	0.8000	1.0000	0.8000	0.6000
6.3	0.6000	0.8000	1.0000	0.6000
12.5	0.4000	0.6000	0.8000	0.6000
25	0.8000	0.0000	1.0000	0.4000
50	0.6000	1.0000	0.2000	0.4000
100	0.6000	0.4000	0.4000	0.4000

			Transform: Arcsin Square Root					1-Tailed		
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
SWC	0.8000	1.0000	1.1114	0.8861	1.3453	16.874	4			
6.3	0.7500	0.9375	1.0561	0.8861	1.3453	20.748	4	0.269	2.410	0.4953
12.5	0.6000	0.7500	0.8910	0.6847	1.1071	19.366	4	1.072	2.410	0.4953
25	0.5500	0.6875	0.8407	0.2255	1.3453	58.613	4	1.317	2.410	0.4953
50	0.5500	0.6875	0.8449	0.4636	1.3453	44.446	4	1.297	2.410	0.4953
100	0.4500	0.5625	0.7351	0.6847	0.8861	13.697	4	1.831	2.410	0.4953

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.963966	0.884	-0.00884	0.658097
Bartlett's Test indicates equal variances (p = 0.15)	8.065272	15.08627		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	100	>100		1	0.469499	0.58439	0.081497	0.084487	0.465092	5, 18

Dose-Response Plot



Statistics – QA

30/10/2007 **Acute Toxicity of the Reference Toxicant Copper to *Acartia sinjiensis***

									Number of mobile organisms									
	Copper (µg/L)		pH		Salinity		D.O.		Time			Mean		Prop'n mob		Mean % Control		Mean Mobile (%)	
Vessel	Nominal	Measured	0 hr	48 hr	0 hr	48 hr	0 hr	48 hr	0 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	***48 hr***	24 hr	***48 hr***
1									5	4	3			0.80	0.60				
2	pH 7.5	<2	7.60	8.14	35.3	35.9	99%	94%	5	3	3	4.25	4.00	0.60	0.60	100%	***100%***	85%	***80%***
3	Control								5	5	5			1.00	1.00				
4									5	5	5			1.00	1.00				
									Mean Mobility in Control (%) =					***85%***	***80%***				
1									5	5	5			1.00	1.00				
2	Method	-	8.24	8.23	35.7	36.1	99%	96%	5	5	3	5.00	4.00	1.00	0.60	118%	***100%***	100%	***80%***
3	Blank								5	5	4			1.00	0.80				
4									5	5	4			1.00	0.80				
1									5	4	4			0.80	0.80				
2	C-c pH	-	7.05	8.11	35.6	36.0	99%	95%	5	5	5	3.75	3.75	1.00	1.00	88%	***94%***	75%	***75%***
3	Control								5	4	4			0.80	0.80				
4									5	2	2			0.40	0.40				
1									5	2	2			0.40	0.40				
2	A-t pH	-	7.23	8.07	35.7	36.1	99%	93%	5	5	3	3.75	3.00	1.00	0.60	88%	***75%***	75%	***60%***
3	Control								5	4	4			0.80	0.80				
4									5	4	3			0.80	0.60				
Copper RefTox concentrations diluted in pH 7.5 seawater																			
1									5	3	3			0.60	0.60				
2	15	14	7.62	8.17	35.4	35.9	101%	94%	5	4	4	3.50	3.00	0.80	0.80	82%	***75%***	70%	***60%***
3									5	2	2			0.40	0.40				
4									5	5	3			1.00	0.60				
1									5	4	3			0.80	0.60				
2	30	27	7.63	8.18	35.6	36.4	98%	94%	5	4	2	3.50	2.75	0.80	0.40	82%	***69%***	70%	***55%***
3									5	4	4			0.80	0.80				
4									5	2	2			0.40	0.40				
1									5	3	1			0.60	0.20				
2	60	58	7.69	8.20	35.6	36.5	99%	93%	5	2	2	2.75	1.50	0.40	0.40	65%	***38%***	55%	***30%***
3									5	3	2			0.60	0.40				
4									5	3	1			0.60	0.20				
1									5	1	0			0.20	0.00				
2	120	110	7.70	8.21	35.7	36.5	98%	94%	5	4	1	2.50	0.75	0.80	0.20	59%	***19%***	50%	***15%***
3									5	2	0			0.40	0.00				
4									5	3	2			0.60	0.40				

Copepod Survival Test-48h Survival

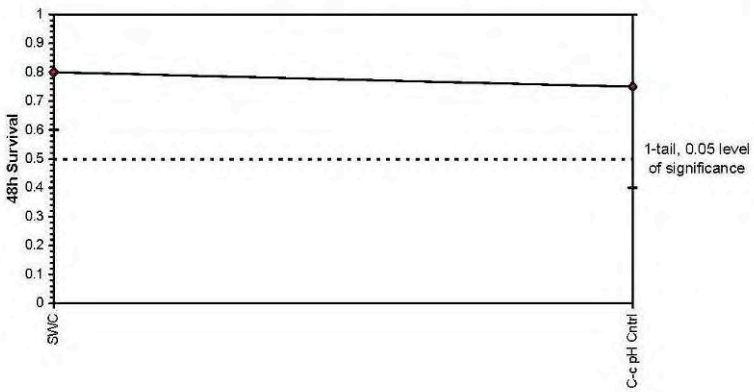
Start Date:	30/10/2007	Test ID:	Solwarra	Sample ID:	pH Controls
End Date:	1/11/2007	Lab ID:	CSIRO-CECR	Sample Type:	C-c pH Control
Sample Date:		Protocol:	ROSE 06-Modified	Test Species:	AS-Acartia sinjiensis
Comments:					

Conc-%	1	2	3	4
SWC	0.8000	1.0000	0.8000	0.6000
C-c pH Cntrl	0.8000	1.0000	0.8000	0.4000

			Transform: Arcsin Square Root						1-Tailed	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
SWC	0.8000	1.0000	1.1114	0.8861	1.3453	16.874	4			
C-c pH Cntrl	0.7500	0.9375	1.0611	0.6847	1.3453	25.905	4	0.303	1.943	0.3233

Auxiliary Tests	Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.932877		0.749		-0.53081	-0.00238
F-Test indicates equal variances (p = 0.55)	2.14831		47.46723			
Hypothesis Test (1-tail, 0.05)	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Homoscedastic t Test indicates no significant differences	0.300684	0.374263	0.005068	0.055362	0.772436	1, 6

Dose-Response Plot



Copepod Survival Test-48h Survival

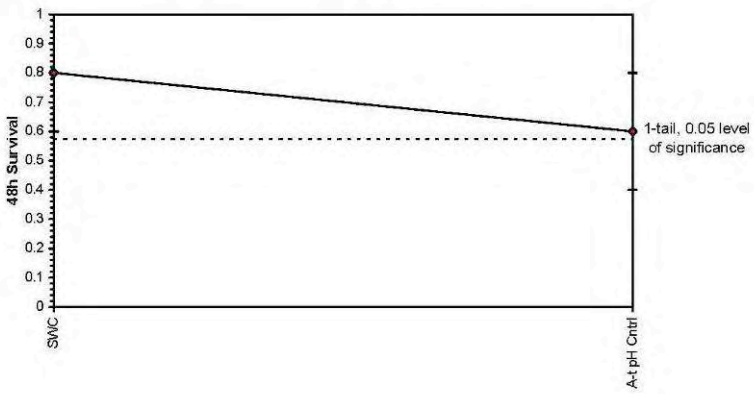
Start Date:	30/10/2007	Test ID:	Solwarra	Sample ID:	pH Controls
End Date:	1/11/2007	Lab ID:	CSIRO-CECR	Sample Type:	A-t pH Control
Sample Date:		Protocol:	ROSE 06-Modified	Test Species:	AS-Acartia sinjiensis
Comments:					

Conc-%	1	2	3	4
SWC	0.8000	1.0000	0.8000	0.6000
A-t pH Cntrl	0.4000	0.6000	0.8000	0.6000

			Transform: Arcsin Square Root						1-Tailed	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
SWC	0.8000	1.0000	1.1114	0.8861	1.3453	16.874	4			
A-t pH Cntrl	0.6000	0.7500	0.8910	0.6847	1.1071	19.366	4	1.730	1.943	0.2476

Auxiliary Tests	Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.870069		0.749		0.109463	-0.66739
F-Test indicates equal variances (p = 0.89)	1.181227		47.46723			
Hypothesis Test (1-tail, 0.05)	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Homoscedastic t Test indicates no significant differences	0.225305	0.280439	0.09716	0.032471	0.134394	1, 6

Dose-Response Plot



Copepod Survival Test-48h Survival

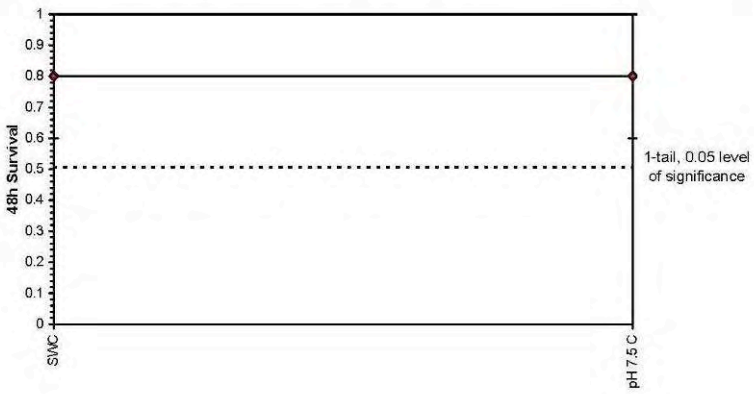
Start Date:	30/10/2007	Test ID:	Solwarra	Sample ID:	Controls
End Date:	1/11/2007	Lab ID:	CSIRO-CECR	Sample Type:	SWC v pH7C
Sample Date:		Protocol:	ROSE 06-Modified	Test Species:	AS-Acartia sinjiensis
Comments:					

Conc-%	1	2	3	4
SWC	0.8000	1.0000	0.8000	0.6000
pH 7.5 C	0.6000	0.6000	1.0000	1.0000

			Transform: Arcsin Square Root					1-Tailed		
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
SWC	0.8000	1.0000	1.1114	0.8861	1.3453	16.874	4			
pH 7.5 C	0.8000	1.0000	1.1157	0.8861	1.3453	23.763	4	-0.026	1.943	0.3155

Auxiliary Tests	Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.806338		0.749		0.026747	-2.09936
F-Test indicates equal variances (p = 0.58)	1.99862		47.46723			
Hypothesis Test (1-tail, 0.05)	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Homoscedastic t Test indicates no significant differences	0.292905	0.364581	3.64E-05	0.05273	0.979893	1, 6

Dose-Response Plot



Copepod Survival Test-48h Survival

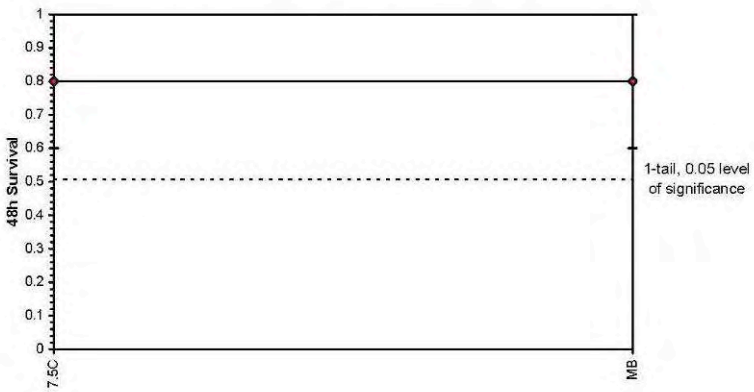
Start Date:	30/10/2007	Test ID:	Sol	Sample ID:	Controls
End Date:	1/11/2007	Lab ID:	CSIRO-CECR	Sample Type:	Method Blank
Sample Date:		Protocol:	ROSE 06	Test Species:	AS-Acartia sinjiensis
Comments:	Controls for 30.10.07 copepod Solwarra Test				

Conc-%	1	2	3	4
7.5C	0.6000	0.6000	1.0000	1.0000
MB	1.0000	0.6000	0.8000	0.8000

			Transform: Arcsin Square Root						1-Tailed	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
7.5C	0.8000	1.0000	1.1157	0.8861	1.3453	23.763	4			
MB	0.8000	1.0000	1.1114	0.8861	1.3453	16.874	4	0.026	1.943	0.3155

Auxiliary Tests	Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.806338		0.749		0.026747	-2.09936
F-Test indicates equal variances (p = 0.58)	1.99862		47.46723			
Hypothesis Test (1-tail, 0.05)	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Homoscedastic t Test indicates no significant differences	0.29202	0.361957	3.64E-05	0.05273	0.979893	1, 6

Dose-Response Plot



Copepod Survival Test-48h Survival

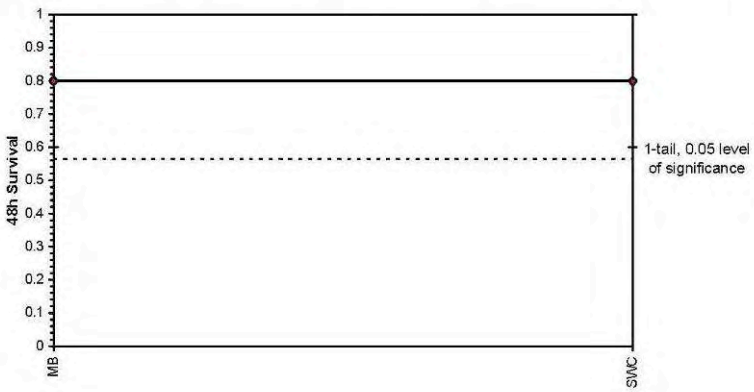
Start Date:	30/10/2007	Test ID:	Sol	Sample ID:	Controls
End Date:	1/11/2007	Lab ID:	CSIRO-CECR	Sample Type:	SWC
Sample Date:		Protocol:	ROSE 06	Test Species:	AS-Acartia sinjiensis
Comments:	Controls for 30.10.07 copepod Solwarra Test				

Conc-%	1	2	3	4
MB	1.0000	0.6000	0.8000	0.8000
SWC	0.8000	1.0000	0.8000	0.6000

			Transform: Arcsin Square Root					1-Tailed		
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
MB	0.8000	1.0000	1.1114	0.8861	1.3453	16.874	4			
SWC	0.8000	1.0000	1.1114	0.8861	1.3453	16.874	4	0.000	1.943	0.2577

Auxiliary Tests	Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.848761		0.749		0.098206	-0.69711
F-Test indicates equal variances (p = 1.00)	1		47.46723			
Hypothesis Test (1-tail, 0.05)	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Homoscedastic t Test indicates no significant differences	0.235277	0.292851	0	0.035169	1	1, 6

Dose-Response Plot



Copepod Survival Test-48h Survival

Start Date:	30/10/2007	Test ID:	Sol	Sample ID:	REF-Ref Toxicant
End Date:	1/11/2007	Lab ID:	CSIRO-CECR	Sample Type:	CUSO-Copper sulfate
Sample Date:		Protocol:	ROSE 06	Test Species:	AS-Acartia sinjiensis
Comments:	RefTox for 30.10.07 copepod Solwarra Test				

Conc-ug/L	1	2	3	4
7.5C	0.6000	0.6000	1.0000	1.0000
14	0.6000	0.8000	0.4000	0.6000
27	0.6000	0.4000	0.8000	0.4000
58	0.2000	0.4000	0.4000	0.2000
110	0.0000	0.2000	0.0000	0.4000

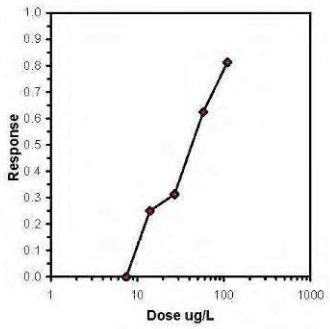
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
7.5C	0.8000	1.0000	1.1157	0.8861	1.3453	23.763	4				4	20
14	0.6000	0.7500	0.8910	0.6847	1.1071	19.366	4	1.567	2.360	0.3384	8	20
27	0.5500	0.6875	0.8407	0.6847	1.1071	23.960	4	1.918	2.360	0.3384	9	20
*58	0.3000	0.3750	0.5742	0.4636	0.6847	22.229	4	3.777	2.360	0.3384	14	20
*110	0.1500	0.1875	0.3998	0.2255	0.6847	55.174	4	4.992	2.360	0.3384	17	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.902833	0.868	0.25528	-1.45618
Bartlett's Test indicates equal variances (p = 0.83)	1.475435	13.2767		

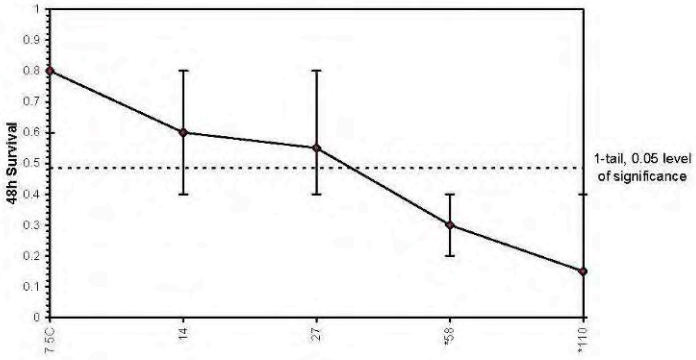
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	27	58	39.57272		0.314889	0.390303	0.314323	0.041119	0.001447	4, 15

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%			
10.0%			
20.0%			
Auto-25.0%	42.023	27.213	64.893



Dose-Response Plot



Copepod Mobility Bioassays for Samples M-t and Ch-a-t

Centre for Environmental Contaminants Research
Lucas Heights Science and Technology Centre
New Illawarra Road, Lucas Heights, NSW
Private Mail Bag 7, Bangor, NSW, 2234, Australia
Telephone 61 2 9710 6812 Fax 61 2 9710 6837

Acute Copepod Toxicity Test Report 07086C

Client: Solwara
Project: Solwara
Test Performed: 48-h acute copepod immobilisation with the marine copepod *Acartia sinjiensis*

Elutriate Prepared: 13/11/07
Elutriate Received: 13/11/07 **Test Initiated:** 13/11/07

CSIRO Sample No.	Sample Name	Sample Description
WQE07086	Method Blank	Seawater blank sample
WQE07087	M-t	Elutriate
WQE07088	Ch-a-t	Elutriate

Sample Physico-Chemistry and Preparation: The elutriates had pH values of 7.0-7.7 and a salinity of 35‰ while the Method Blank had a pH of 8.3 and a salinity of 35‰. All QA test solutions were prepared in filtered (0.45µm) seawater, which had been adjusted to a pH of 7.5 ± 0.1. An additional pH control was prepared to match the pH of the M-t elutriate.

The pH, salinity, temperature and dissolved oxygen were measured in sub-samples of test concentrations at the beginning and end of each test. The measurements below represent the physico-chemical properties of the samples as received. However, by the time of test commencement, the pH of both the samples and the pH controls had drifted upwards, probably due to the buffering capacity of natural seawater. Therefore, the values below are slightly different to that at the time of test commencement, reported in the toxicity test spreadsheets (attached at end of test report).

Sample	Physico-chemistry				Comments
	pH	‰	mS/cm	DO (%)	
WQE07086 (Method Blank)	8.25	35	53	99	
WQE07087 (M-t)	7.00	35	53	96	
WQE07088 (Ch-a-t)	7.74	35	53	96	
M-t pH control	7.03	35	54	106	
pH 7.5 Control	7.47	35	54	108	
Filtered Natural Seawater Control	8.25	36	54	107	

‰ = Salinity; µS/cm = Conductivity; DO = Dissolved Oxygen (%);

Test Method: This test measures the mobility of the marine copepod *Acartia sinjiensis* during a 48-h exposure period. The test protocol is based on the protocol described in Rose et al. (2006). Copepods were fed a minimum of two hours prior to test commencement with a mixture of two algal species *Isochrysis* sp. and *Proteomonas sulcata* (previously known as *Cryptomonas* sp.). The 48-h EC50, LOEC and NOEC values were calculated using ToxCalc Version 5.0.23 (Tidepool Software).

Results: There was no significant difference found between the number of mobile copepods in the QA controls, M-t pH Control, Seawater Control or Method Blank. However, copepod mobility in the Method Blank was lower than the acceptable control rate of 80%, with 75% mobile after a 48 h exposure. Therefore, it is possible that some process during elutriate preparation contributed slightly to the toxicity observed in the highest test concentrations (100% elutriates). M-t was more toxic than Ch-a-t, with an EC50 of 1.1% and NOEC value of 0.41%, compared to the Ch-a-t EC50 of 15% and NOEC value of 11%.

Sample	Mean Mobile (%)	% of Seawater Control	% Inhibition
QA Control (pH 7.5)	80	100	0
M-t pH Control	80	100	0
Seawater Control	80	100	0
Method Blank (WQE07086)	75	94	6
M-t (WQE07087)			
0.14%	85	106	0
0.41%	75	94	6
1.2%	40	50[a]	50
3.7%	0	0[a]	100
11%	0	0[a]	100
33%	0	0[a]	100
100%	0	0[a]	100
Ch-a-t (WQE07088)			
0.14%	70	88	12
0.41%	70	88	12
1.2%	75	94	6
3.7%	70	88	12
11%	65	81	19
33%	0	0[a]	100
100%	0	0[a]	100
Sample	**IC50 (%)**	**LOEC (%)**	**NOEC (%)**
M-t Elutriate	1.1 (0.8-1.4)	-	0.41
Ch-a-t Elutriate	15 (11-22)	-	11

[a] Significantly less than Seawater Control

Quality Assurance/Quality Control	Criterion	This Test	Criterion Met?
Control copepod mobility (%)	>80	80	Yes
Reference toxicant EC50 (measured copper, µg Cu/L)	44 ± 16	58 (45-75)	Yes

Comments:

References:

Rose, A., Carruthers, A-M, Stauber, J.L., Lim, R. and Blockwell, S.J. (2006). Development of an acute toxicity test with the marine copepod *Acartia sinjiensis*. *Australasian Journal of Ecotoxicology*. 12: 73-81.

Test carried out by: Monique Binet
Test supervised by: Monique Binet

Test report prepared by: Monique Binet
Experimental Scientist (ph: 02 9710 6812)

Test report authorised by: Jenny Stauber
Senior Principal Research Scientist (ph: 02 9710 6808)

Date: 26/11/07

Statistics –

13/11/2007 **Acute Toxicity of M-t (WQE07087, AQIS ID: 2007-187) to *Acartia sinjiensis***

								Number of mobile organisms									
	Sample (%)	pH		Salinity		D.O.		Time			Mean		Prop'n mob		Mean % Control		Mean Mobile (%)	
Vial		0 hr	48 hr	0 hr	48 hr	0 hr	48 hr	0 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	***48 hr***	24 hr	***48 hr***
1								5	5	4			1.00	0.80				
2	Seawater Control	8.3	8.27	36	36	98%	95%	5	3	2	4.50	4.00	0.60	0.40	100%	***100%***	90%	***80%***
3								5	5	5			1.00	1.00				
4								5	5	5			1.00	1.00				
								Mean Mobility in Seawater Control (%) =					***90%***	***80%***				
1								5	4	4			0.80	0.80				
2	Method Blank	8.30	8.30	36	36	96%	94%	5	5	4	4.50	3.75	1.00	0.80	100%	***94%***	90%	***75%***
3	*(WQE07086)*							5	4	3			0.80	0.60				
4								5	5	4			1.00	0.80				
1								5	5	4			1.00	0.80				
2	M-t pH Control	7.37	8.11	36	35	98%	97%	5	4	3	4.75	4.00	0.80	0.60	119%	***100%***	95%	***80%***
3								5	5	5			1.00	1.00				
4								5	5	4			1.00	0.80				
M-t Concentrations (diluted in seawater)																		
1								5	4	4			0.80	0.80				
2	0.14%	8.31	8.3	36	36	97%	95%	5	5	5	4.25	4.25	1.00	1.00	94%	***106%***	85%	***85%***
3								5	5	5			1.00	1.00				
4								5	3	3			0.60	0.60				
1								5	4	3			0.80	0.60				
2	0.41%	8.31	8.31	36	36	97%	97%	5	5	3	4.75	3.75	1.00	0.60	106%	***94%***	95%	***75%***
3								5	5	5			1.00	1.00				
4								5	5	4			1.00	0.80				
1								5	4	2			0.80	0.40				
2	1.2%	8.31	8.31	36	36	96%	97%	5	4	1	4.00	2.00	0.80	0.20	89%	***50%***	80%	***40%***
3								5	3	2			0.60	0.40				
4								5	5	3			1.00	0.60				
1								5	5	0			1.00	0.00				
2	3.7%	8.3	8.3	36	36	96%	96%	5	3	0	3.25	0.00	0.60	0.00	72%	***0%***	65%	***0%***
3								5	2	0			0.40	0.00				
4								5	3	0			0.60	0.00				
1								5	1	0			0.20	0.00				
2	11%	8.26	8.28	36	36	97%	96%	5	1	0	0.50	0.00	0.20	0.00	11%	***0%***	10%	***0%***
3								5	0	0			0.00	0.00				
4								5	0	0			0.00	0.00				
1								5	0	0			0.00	0.00				
2	33%	8.09	8.21	36	36	96%	96%	5	0	0	0.00	0.00	0.00	0.00	0%	***0%***	0%	***0%***
3								5	0	0			0.00	0.00				
4								5	0	0			0.00	0.00				
1								5	0	0			0.00	0.00				
2	100%	7.64	8	36	36	95%	96%	5	0	0	0.00	0.00	0.00	0.00	0%	***0%***	0%	***0%***
3								5	0	0			0.00	0.00				
4								5	0	0			0.00	0.00				

Copepod Survival Test-48h Survival

Start Date:	13/11/2007	Test ID:	Solwarra	Sample ID:	WQE07087
End Date:	15/11/2007	Lab ID:	CSIRO-CECR	Sample Type:	M-t Elutriate
Sample Date:		Protocol:	ROSE 06-Modified	Test Species:	AS-Acartia sinjiensis
Comments:	Elutriate prepared 13.11.07				

Conc-%	1	2	3	4
SWC	0.8000	0.4000	1.0000	1.0000
0.14	0.8000	1.0000	1.0000	0.6000
0.41	0.6000	0.6000	1.0000	0.8000
1.2	0.4000	0.2000	0.4000	0.6000
3.7	0.0000	0.0000	0.0000	0.0000
11	0.0000	0.0000	0.0000	0.0000
33	0.0000	0.0000	0.0000	0.0000
100	0.0000	0.0000	0.0000	0.0000

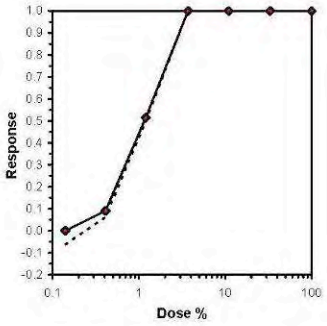
			Transform: Arcsin Square Root					1-Tailed			Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
SWC	0.8000	1.0000	1.1206	0.6847	1.3453	27.799	4				4	20
0.14	0.8500	1.0625	1.1709	0.8861	1.3453	18.840	4	-0.301	2.290	0.3828	3	20
0.41	0.7500	0.9375	1.0561	0.8861	1.3453	20.748	4	0.386	2.290	0.3828	5	20
*1.2	0.4000	0.5000	0.6798	0.4636	0.8861	25.383	4	2.637	2.290	0.3828	12	20
3.7	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4				20	20
11	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4				20	20
33	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4				20	20
100	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4				20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.944756	0.844	-0.48186	-0.60197
Bartlett's Test indicates equal variances (p = 0.81)	0.967173	11.34487		

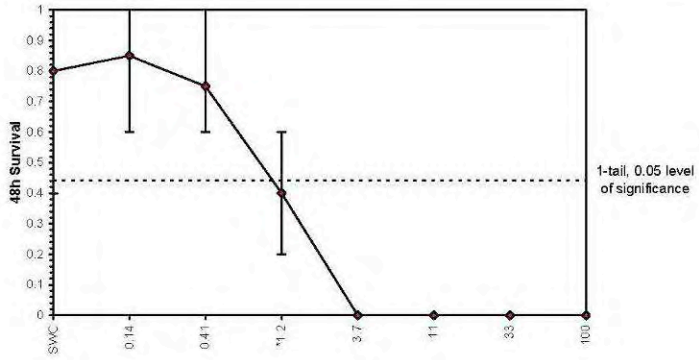
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	0.41	1.2	0.701427	243.9024	0.358142	0.441792	0.199023	0.055876	0.047418	3, 12

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%	1.0844	0.8179	1.4376
5.0%	1.1201	0.8287	1.5139
10.0%	1.1327	0.8293	1.5472
20.0%	1.1386	0.7690	1.6859
Auto-0.0%	1.0844	0.8179	1.4376



Dose-Response Plot



13/11/2007 **Acute Toxicity of Ch-a-t (WQE07088, AQIS ID: 2007-189) to *Acartia sinjiensis***

								Number of mobile organisms											
	Sample (%)	pH		Salinity		D.O.		Time			Mean		Prop'n mob		Mean % Control		Mean Mobile (%)	
Vial		0 hr	48 hr	0 hr	48 hr	0 hr	48 hr	0 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	***48 hr***	24 hr	***48 hr***
1								5	5	4			1.00	0.80				
2	Seawater Control	8.30	8.27	36	36	98%	95%	5	3	2	4.50	4.00	0.60	0.40	100%	***100%***	90%	***80%***
3								5	5	5			1.00	1.00				
4								5	5	5			1.00	1.00				
								Mean Mobility in Seawater Control (%) =					***90%***	***80%***				
1								5	4	4			0.80	0.80				
2	Method Blank	8.30	8.30	36	36	96%	94%	5	5	4	4.50	3.75	1.00	0.80	100%	***94%***	90%	***75%***
3	*(WQE07086)*							5	4	3			0.80	0.60				
4								5	5	4			1.00	0.80				
Ch-a-t Concentrations (diluted in seawater)																		
1								5	3	3			0.60	0.60				
2	0.14%	8.3	8.3	36	36	96%	96%	5	3	3	3.75	3.50	0.60	0.60	83%	***88%***	75%	***70%***
3								5	5	5			1.00	1.00				
4								5	4	3			0.80	0.60				
1								5	5	4			1.00	0.80				
2	0.41%	8.31	8.31	36	36	95%	96%	5	3	2	4.00	3.50	0.60	0.40	89%	***88%***	80%	***70%***
3								5	5	5			1.00	1.00				
4								5	3	3			0.60	0.60				
1								5	4	4			0.80	0.80				
2	1.2%	8.32	8.29	36	36	98%	97%	5	5	4	4.00	3.75	1.00	0.80	89%	***94%***	80%	***75%***
3								5	3	3			0.60	0.60				
4								5	4	4			0.80	0.80				
1								5	3	2			0.60	0.40				
2	3.7%	8.29	8.3	36	36	98%	96%	5	4	4	4.00	3.50	0.80	0.80	89%	***88%***	80%	***70%***
3								5	5	4			1.00	0.80				
4								5	4	4			0.80	0.80				
1								5	4	4			0.80	0.80				
2	11%	8.26	8.29	36	36.0	98%	95%	5	4	2	4.25	3.25	0.80	0.40	94%	***81%***	85%	***65%***
3								5	4	3			0.80	0.60				
4								5	5	4			1.00	0.80				
1								5	0	0			0.00	0.00				
2	33%	8.13	8.23	36	36	95%	95%	5	0	0	0.00	0.00	0.00	0.00	0%	***0%***	0%	***0%***
3								5	0	0			0.00	0.00				
4								5	0	0			0.00	0.00				
1								5	0	0			0.00	0.00				
2	100%	7.88	8	36	36.0	95%	97%	5	0	0	0.00	0.00	0.00	0.00	0%	***0%***	0%	***0%***
3								5	0	0			0.00	0.00				
4								5	0	0			0.00	0.00				

Copepod Survival Test-48h Survival

Start Date:	13/11/2007	Test ID:	Solwarra	Sample ID:	WQE07088
End Date:	15/11/2007	Lab ID:	CSIRO-CECR	Sample Type:	Ch-a-t Elutriate
Sample Date:		Protocol:	ROSE 06-Modified	Test Species:	AS-Acartia sinjiensis
Comments:	Elutriate prepared 13.11.07				

Conc-%	1	2	3	4
SWC	0.8000	0.4000	1.0000	1.0000
0.14	0.6000	0.6000	1.0000	0.6000
0.41	0.8000	0.4000	1.0000	0.6000
1.2	0.8000	0.8000	0.6000	0.8000
3.7	0.4000	0.8000	0.8000	0.8000
11	0.8000	0.4000	0.6000	0.8000
33	0.0000	0.0000	0.0000	0.0000
100	0.0000	0.0000	0.0000	0.0000

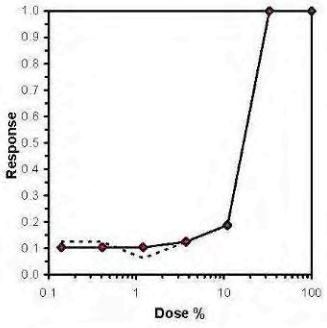
			Transform: Arcsin Square Root					1-Tailed			Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
SWC	0.8000	1.0000	1.1206	0.6847	1.3453	27.799	4				4	20
0.14	0.7000	0.8750	1.0009	0.8861	1.3453	22.940	4	0.723	2.410	0.3990	6	20
0.41	0.7000	0.8750	1.0058	0.6847	1.3453	28.293	4	0.693	2.410	0.3990	6	20
1.2	0.7500	0.9375	1.0519	0.8861	1.1071	10.508	4	0.415	2.410	0.3990	5	20
3.7	0.7000	0.8750	1.0015	0.6847	1.1071	21.089	4	0.719	2.410	0.3990	6	20
11	0.6500	0.8125	0.9463	0.6847	1.1071	21.467	4	1.053	2.410	0.3990	7	20
33	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4				20	20
100	0.0000	0.0000	0.2255	0.2255	0.2255	0.000	4				20	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.966479	0.884	-0.32349	-0.44958
Bartlett's Test indicates equal variances (p = 0.73)	2.83522	15.08627		

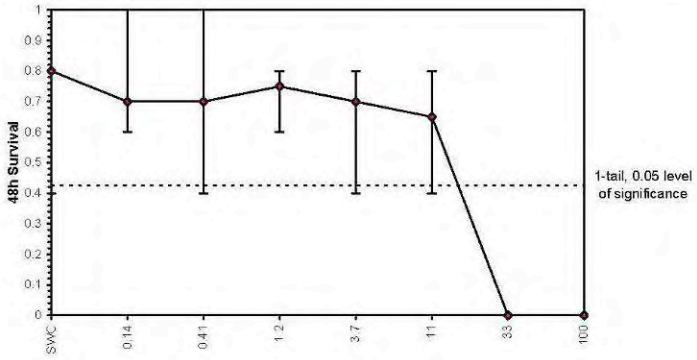
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	11	33	19.05256	9.090909	0.374226	0.461632	0.013979	0.054807	0.931693	5, 18

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%			
10.0%			
20.0%	16.784	14.515	19.408
Auto-10.4%	15.485	11.122	21.559



Dose-Response Plot



Statistics – QA

13/11/2007

Acute Toxicity of the Reference Toxicant Copper to Acartia sinjiensis

									Number of mobile organisms									
	Copper (µg/L)		pH		Salinity		D.O.		Time			Mean		Prop'n mob		Mean % Control		Mean Mobile (%)	
Vessel	Nominal	Measured	0 hr	48 hr	0 hr	48 hr	0 hr	48 hr	0 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	***48 hr***	24 hr	***48 hr***
1									5	4	3			0.80	0.60				
2	pH 7.5	<2	7.77	8.17	36	36	96%	97%	5	5	4	4.75	4.00	1.00	0.80	100%	***100%***	95%	***80%***
3	Control								5	5	5			1.00	1.00				
4									5	5	4			1.00	0.80				
									Mean Mobility in Control (%) =					***95%***	***80%***				
1									5	4	4			0.80	0.80				
2	Method	<2	8.3	8.3	36	36	96%	94%	5	5	4	4.50	3.75	1.00	0.80	95%	***94%***	90%	***75%***
3	Blank								5	4	3			0.80	0.60				
4	*(WQE07086)*								5	5	4			1.00	0.80				
1									5	5	4			1.00	0.80				
2	M-t pH	-	7.37	8.11	36	35	98%	97%	5	4	3	4.75	4.00	0.80	0.60	100%	***100%***	95%	***80%***
3	Control								5	5	5			1.00	1.00				
4									5	5	4			1.00	0.80				
Copper RefTox concentrations diluted in pH 7.5 seawater																			
1									5	4	3			0.80	0.60				
2	15	13	7.8	8.19	36	36	95%	96%	5	5	3	4.75	4.00	1.00	0.60	100%	***100%***	95%	***80%***
3									5	5	5			1.00	1.00				
4									5	5	5			1.00	1.00				
1									5	3	3			0.60	0.60				
2	30	25	7.88	8.2	36	36	96%	95%	5	4	3	3.75	3.50	0.80	0.60	79%	***88%***	75%	***70%***
3									5	3	3			0.60	0.60				
4									5	5	5			1.00	1.00				
1									5	3	2			0.60	0.40				
2	60	50	7.88	8.20	36	36	96%	94%	5	5	3	3.75	2.50	1.00	0.60	79%	***63%***	75%	***50%***
3									5	5	3			1.00	0.60				
4									5	2	2			0.40	0.40				
1									5	4	0			0.80	0.00				
2	120	94	7.90	8.2	36	36	95%	95%	5	4	1	4.00	0.75	0.80	0.20	84%	***19%***	80%	***15%***
3									5	4	0			0.80	0.00				
4									5	4	2			0.80	0.40				

Copepod Survival Test-48h Survival

Start Date:	13/11/2007	Test ID:	Solwara	Sample ID:	REF-Ref Toxicant
End Date:	15/11/2007	Lab ID:	CSIRO-CECR	Sample Type:	CUSO-Copper sulfate
Sample Date:		Protocol:	ROSE 06-Modified	Test Species:	AS-Acartia sinjiensis
Comments:	Measured concentrations				

Conc-ug/L	1	2	3	4
Cntrl (pH7.5)	0.6000	0.8000	1.0000	0.8000
13	0.6000	0.6000	1.0000	1.0000
25	0.6000	0.6000	0.6000	1.0000
50	0.4000	0.6000	0.6000	0.4000
94	0.0000	0.2000	0.0000	0.4000

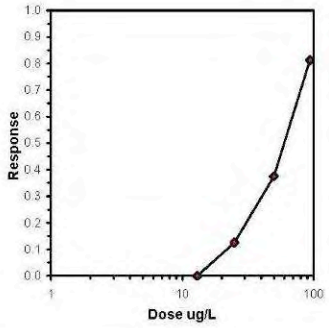
			Transform: Arcsin Square Root						1-Tailed		Number	Total
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
Cntrl (pH7.5)	0.8000	1.0000	1.1114	0.8861	1.3453	16.874	4				4	20
13	0.8000	1.0000	1.1157	0.8861	1.3453	23.763	4	-0.029	2.360	0.3503	4	20
25	0.7000	0.8750	1.0009	0.8861	1.3453	22.940	4	0.745	2.360	0.3503	6	20
50	0.5000	0.6250	0.7854	0.6847	0.8861	14.802	4	2.196	2.360	0.3503	10	20
94	0.1500	0.1875	0.3998	0.2255	0.6847	55.174	4	4.793	2.360	0.3503	17	20

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.91027	0.868	0.454508	-1.14414
Bartlett's Test indicates equal variances (p = 0.78)	1.753805	13.2767		

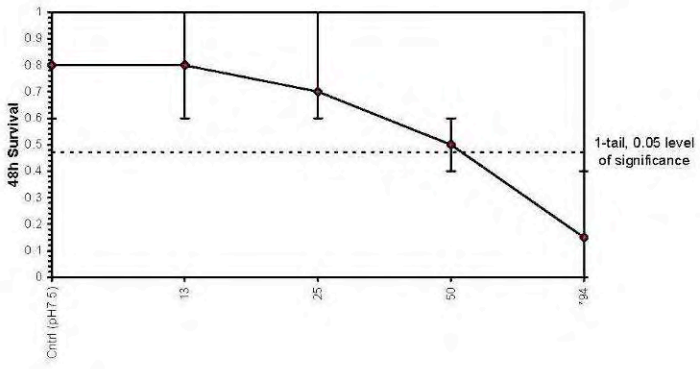
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	50	94	68.55655		0.327707	0.4079	0.363169	0.044072	0.001007	4, 15

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%			
10.0%			
20.0%	57.885	44.277	75.675
Auto-18.8%	57.685	44.614	74.585



Dose-Response Plot



Copepod Survival Test-48h Survival

Start Date:	13/11/2007		Test ID:	Solwarra	Sample ID:	Controls
End Date:	15/11/2007		Lab ID:	CSIRO-CECR	Sample Type:	SWC V M-t pH Cntrl
Sample Date:			Protocol:	ROSE 06-Modified	Test Species:	AS-Acartia sinjiensis
Comments:						

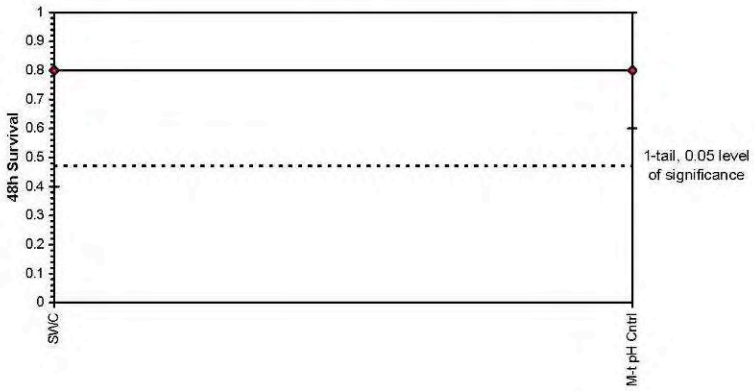
Conc-%	1	2	3	4
SWC	0.8000	0.4000	1.0000	1.0000
M-t pH Cntrl	0.8000	0.6000	1.0000	0.8000

			Transform: Arcsin Square Root					1-Tailed		
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
SWC	0.8000	1.0000	1.1206	0.6847	1.3453	27.799	4			
M-t pH Cntrl	0.8000	1.0000	1.1114	0.8861	1.3453	16.874	4	0.051	1.943	0.3533

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.875314	0.749	-0.8302	0.093812
F-Test indicates equal variances (p = 0.43)	2.759397	47.46723		

Hypothesis Test (1-tail, 0.05)	MSDu	MSDp	MSB	MSE	F-Prob	df
Homoscedastic t Test indicates no significant differences	0.328727	0.405507	0.000169	0.066108	0.961309	1, 6

Dose-Response Plot



Copepod Survival Test-48h Survival

Start Date:	13/11/2007	Test ID:	Solwarra	Sample ID:	Controls
End Date:	15/11/2007	Lab ID:	CSIRO-CECR	Sample Type:	SWC v MB
Sample Date:		Protocol:	ROSE 06-Modified	Test Species:	AS-Acartia sinjiensis
Comments:					

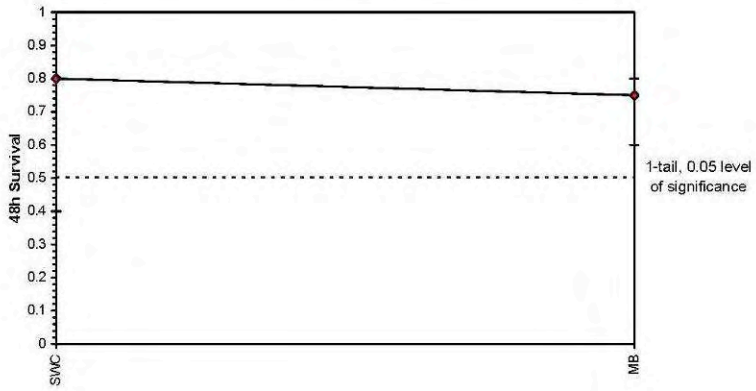
Conc-%	1	2	3	4
SWC	0.8000	0.4000	1.0000	1.0000
MB	0.8000	0.8000	0.6000	0.8000

			Transform: Arcsin Square Root					1-Tailed		
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
SWC	0.8000	1.0000	1.1206	0.6847	1.3453	27.799	4			
MB	0.7500	0.9375	1.0519	0.8861	1.1071	10.508	4	0.416	1.943	0.3212

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.874169	0.749	-1.20659	1.663741
F-Test indicates equal variances (p = 0.12)	7.942761	47.46723		

Hypothesis Test (1-tail, 0.05)	MSDu	MSDp	MSB	MSE	F-Prob	df
Homoscedastic t Test indicates no significant differences	0.29661	0.365888	0.009447	0.054632	0.691993	1, 6

Dose-Response Plot



Copepod Survival Test-48h Survival

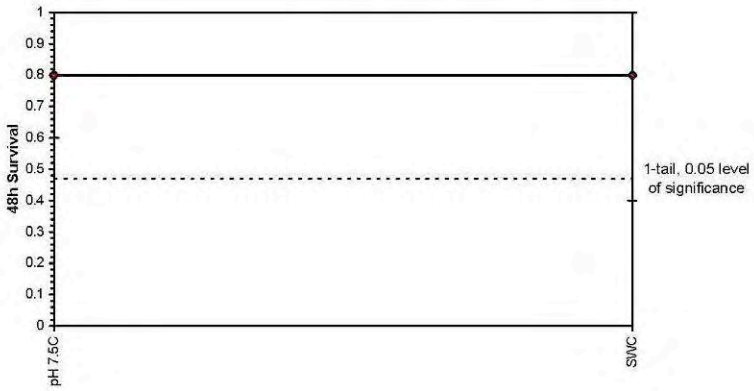
Start Date:	13/11/2007	Test ID:	Solwarra	Sample ID:	Controls
End Date:	15/11/2007	Lab ID:	CSIRO-CECR	Sample Type:	pH 7.5C v SWC
Sample Date:		Protocol:	ROSE 06-Modified	Test Species:	AS-Acartia sinjiensis
Comments:					

Conc-%	1	2	3	4
pH 7.5C	0.6000	0.8000	1.0000	0.8000
SWC	0.8000	0.4000	1.0000	1.0000

			Transform: Arcsin Square Root					1-Tailed		
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
pH 7.5C	0.8000	1.0000	1.1114	0.8861	1.3453	16.874	4			
SWC	0.8000	1.0000	1.1206	0.6847	1.3453	27.799	4	-0.051	1.943	0.3533

Auxiliary Tests	Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.875314		0.749		-0.8302	0.093812
F-Test indicates equal variances (p = 0.43)	2.759397		47.46723			
Hypothesis Test (1-tail, 0.05)	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Homoscedastic t Test indicates no significant differences	0.330656	0.41157	0.000169	0.066108	0.961309	1, 6

Dose-Response Plot



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