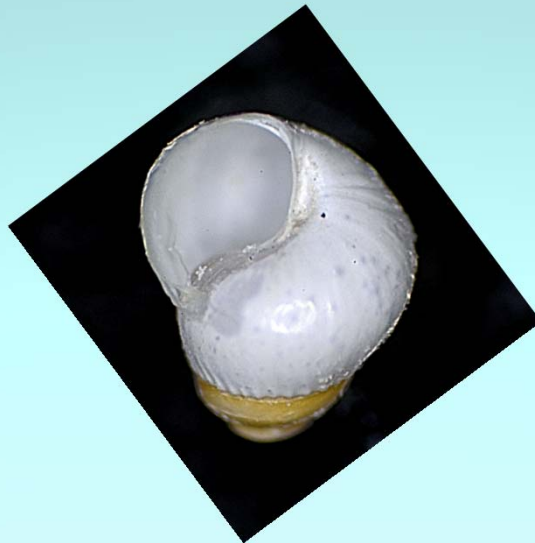


**WORKSHOP ON
TAXONOMIC METHODS AND STANDARDIZATION OF
MACROFAUNA IN THE CLARION-CLIPPERTON FRACTURE ZONE**

23 - 30 November 2014

Uljin-gun, Gyeongsangbuk-do, South Korea

**Status of Macrofaunal Studies Carried Out by
the Interoceanmetal Joint Organization (IOM)**



Valcana Stoyanova



INTEROCEANMETAL JOINT ORGANIZATION (IOM)



IOM was set up in 1987 on the base of the Intergovernmental Agreement between 6 countries.

OBJECTIVE : To prospect and explore area laid out in the CCZ for polymetallic nodule deposits and to prepare for exploitation their resources .

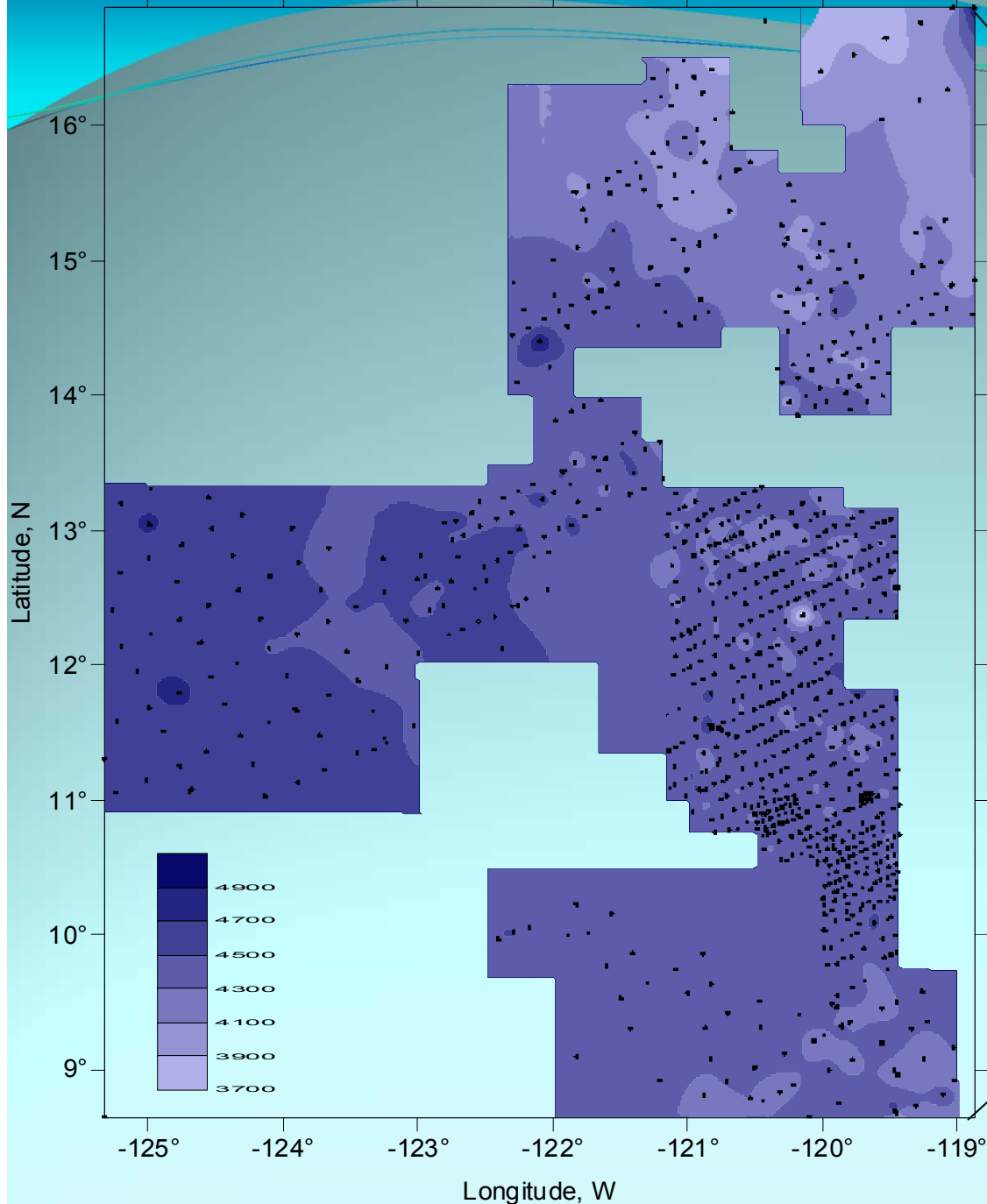


Meeting of the IOM's Council



The headquarters of the IOM at Szczecin, Poland.

IOM PROSPECTING AREA



IOM Sampling Stations

1988 - 2014

Area of survey ~ 800,000 km²

Latitude: 8°38.77' - 16° 52.76' N

Longitude: 118°52.5' - 125° 19.75' W

Depth: 3,470 - 4,940 m

Within 1988 - 2014 IOM carried out a total of 21 research cruises.

Amount of stations: ~ 1036 sampled during 1988 - 2014.



Environmental studies done under different phases of the IOM's activity

Phase I: 1994 – 2000 within the program of the IOM Benthic Impact Experiment (IOM' BIE).

Samples were collected in 1994, 1995, 1997, and 2000.

Phase II: 2001 – to present

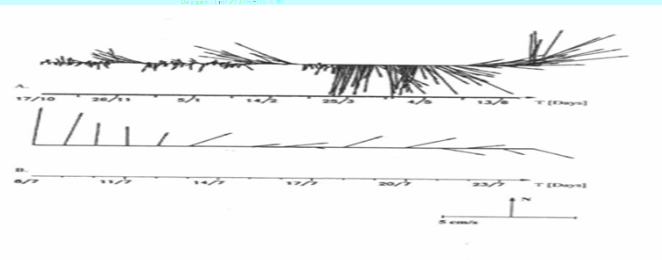
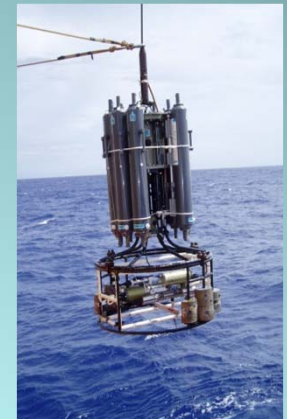
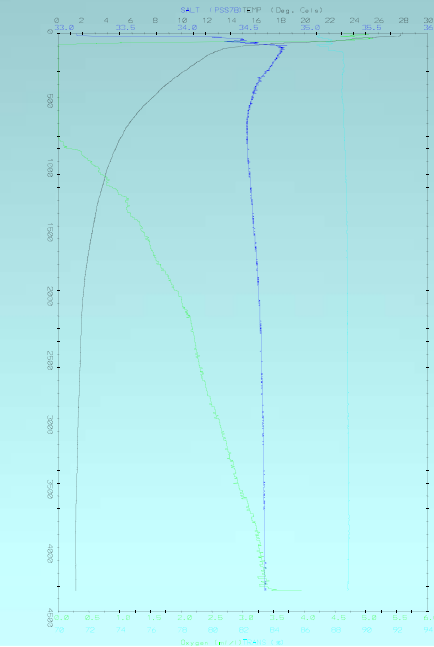
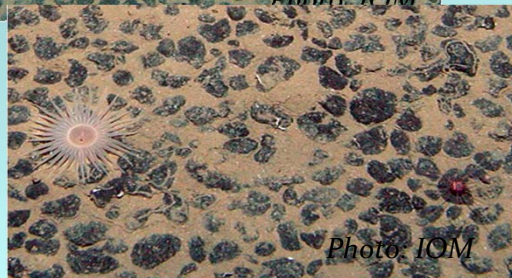
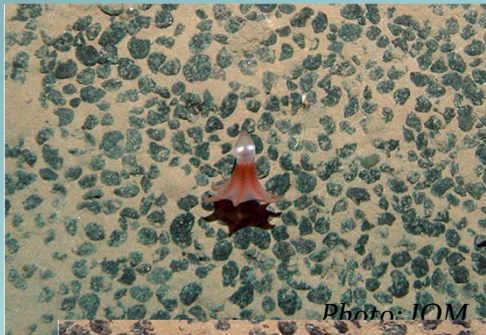
Samples were collected in 2009 and 2014.

MAIN OBJECTIVE: to establish the baseline of natural environment in the IOM claim/exploration area, to assess spatial and temporal variability of their parameters, and to predict/evaluate the potential impacts from nodule mining.



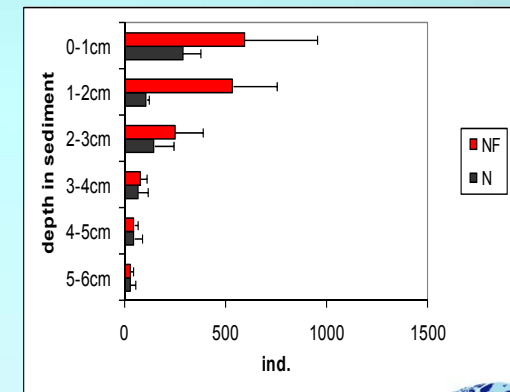
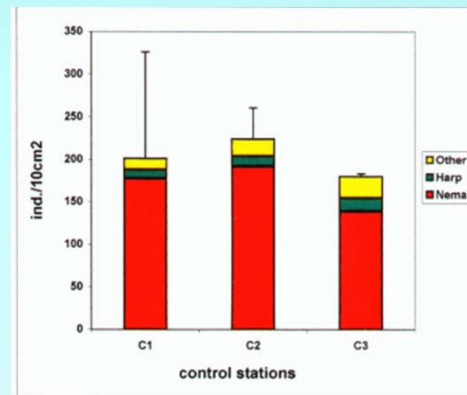
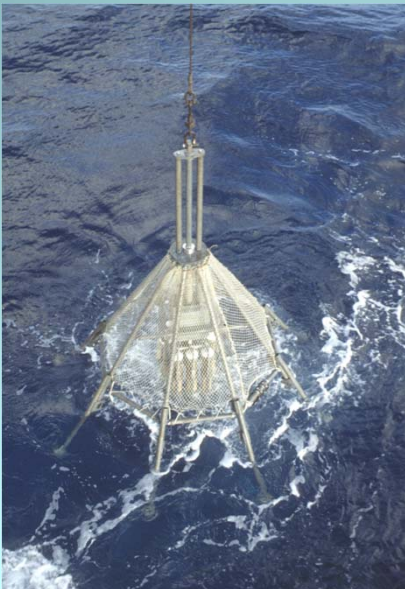
Phase I: Environmental Studies during 1994 - 2000

- ✓ CTD casts, and water sampling by the Rosette sampler (14 stations observed in Oct, 1994 and Mar, 1995);
- ✓ current measurements (4 moorings for 8 months);
- ✓ box core (0.25 m²) and multicore sampling;
- ✓ observation with deep-towed acoustic and TV-photo profiling devices;
- ✓ Meio-, macro-, and megafauna studies.



IOM”BIE Program (1994 – 2000)

- ✓ Deep Sea Sediment Re-suspension System (Disturber; SOSI) was used to create a stress on the benthic environment;
- ✓ Monitoring work included also: sediment sampling by multiple core,
- ✓ mooring system observation using current meters and sediment traps;
- ✓ TV-photo profiling.



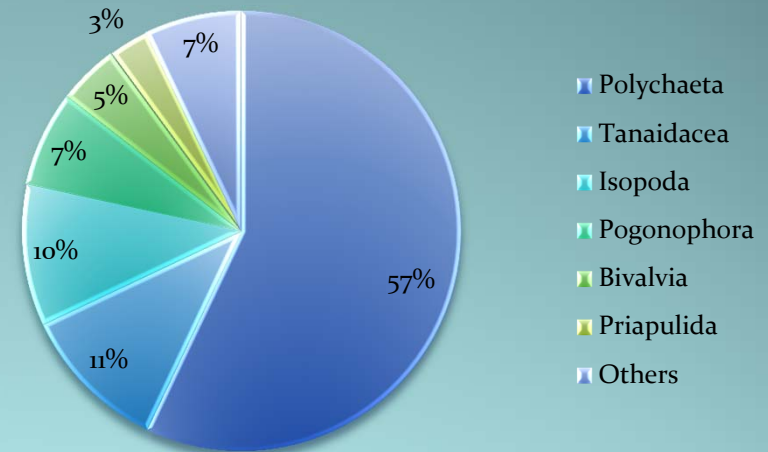
Macrofauna IOM'1997 inds/m²

Taxon	BC1	BC3	BC4	BC10	BC14
Hydrozoa	0	0	0	8	0
Oligochaeta	0	0	0	8	8
Polychaeta	178	386	196	72	224
Cumacea	9	0	0	0	0
Isopoda	17	58	31	12	75
Tanaidacea	83	54	0	24	42
Amphipoda	17	25	0	0	0
Pogonophora	44	25	35	28	0
Priapulida	17	8	0	12	17
Ophiuroidea	9	0	0	0	0
Asteroidea	0	0	8	0	0
Echinoidea	0	0	0	16	0
Bivalvia	22	17	19	8	17
Gastropoda	0	0	8	0	17
total	396	573	296	188	398

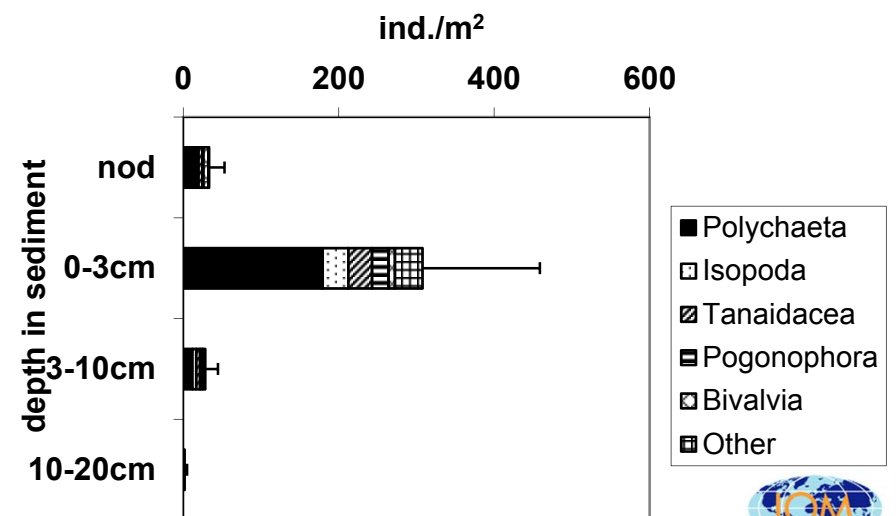
✓ The macrofauna was found to occur at a mean abundance of about 370 inds/m² (ranging from 188 to about 573 inds/m²) and to consist of representatives of 14 taxa.

✓ Macrobenthic abundance peaked at the 0-3 cm layer in the sediment depth.

Macrofauna IOM'1997



Nodulized area macrofauna, 1997 vertical distribution



IOM'1994 macrofaunal taxonomic qualitative data (based on 2 stations)

Analyses were conducted at the Cove Corporation (Lusby, Maryland, USA)

- ✓ **Polychaetes** (Polychaeta) represented by members of the families Paraonidae, Amphinomidae, Sabellidae, Cirratulidae, Spionidae, Oweniidae, Phyllodocidae, and Hesionidae,
 - ✓ as well as specimens identified as belonging to the genera *Lumbrinereides*, *Progoniada*, *Kesun*, *Prionospio*, *Poecilochaetus*, *Sclerobregma*, *Tharyx*, *Aphelochaeta*, and *Pherusa*, and individuals of *Paralacydomia paradoxa*.

- ✓ **Scaphopods** (Scaphopoda) were represented by *Dentalium* sp.

- ✓ **Crustaceans** (Crustacea) included isopods (Isopoda) and tanaidaceans (Tanaidacea).
 - ✓ Isopods were identified as belonging to *Paraleptognathia* sp., *Armatognathia birsteini*, *Colletea* sp., and unidentified members of the family Macrostylidae, Desmosomatidae, and Thambematidae.
 - ✓ Tanaidaceans were assigned to the genera and species *Paranarthura* cf. *tridens*, *Parafilitanais* sp., *Stenotanais* sp., and *Filitanais* cf. *rebainsi*.

- ✓ **Bivalves** (Bivalvia) were represented by *Neilonella* sp. and *Bathyarca corpulenta*.



IOM's Exploration Area

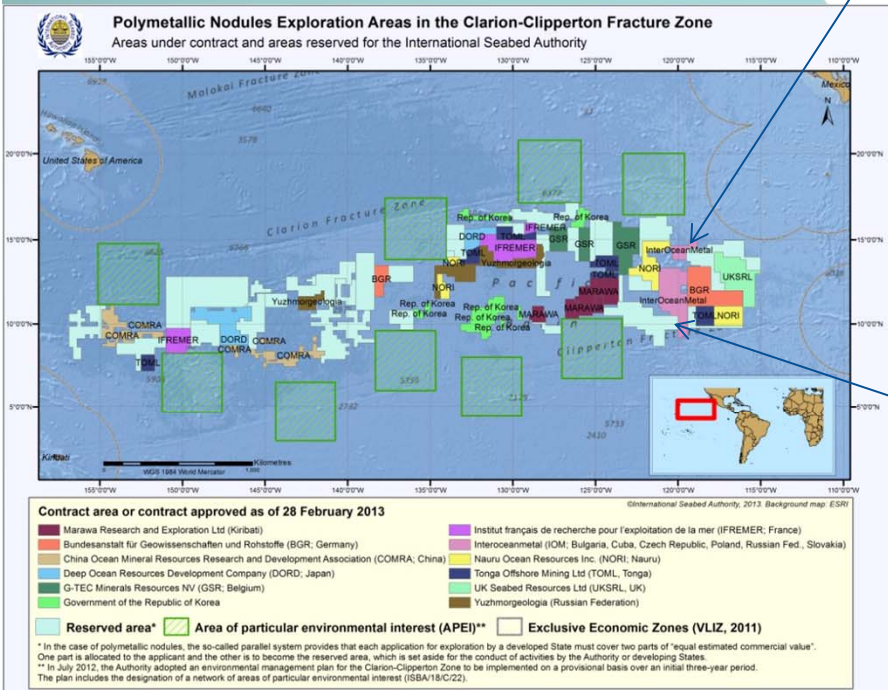
In 2001, IOM signed a 15-ys contract with the ISA for exploration of polymetallic nodules.

IOM exploration area (B1 & B2 Sectors) covers 75 000 km² in the eastern part of the CCZ.

Sector B1

Sector B2

IOM'2014
IOM'2009
BIE



<http://isa.org.jm/files/images/maps/CCZ-Sep2012-Official.jpg>

Macrofaunal studies: IOM'2009 and IOM'2014 areas of prime interest for future nodule exploitation.



Phase II: Environmental work (2001 – to present)

In accordance with the standard contract requirements and Recommendations for the guidance of contractors for the assessment of the possible environmental impacts arising from exploration for marine minerals in the Area (ISBA/19/LTC/8),

the IOM environmental research were focused on:

“gathering environmental baseline data necessary for establishment of baselines against which to assess the likely effects of its activities on marine environment and development of marine environment monitoring program”.

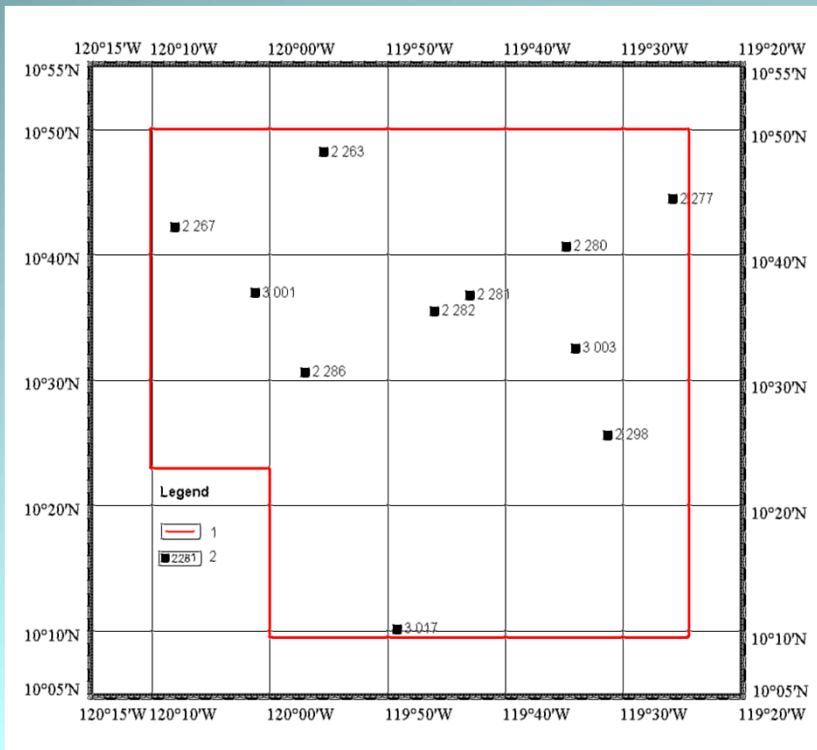
In the subject of this Workshop, and in accordance of the Recommendations:

“Macrofauna. Data on macrofauna (>250 μm) abundance, species structure, biomass and diversity should be obtained through a quantitative analysis of samples. In soft sediments, vertical profiles with a suitable depth distribution (suggested depths: 0-1, 1-5, 5-10 cm) should be obtained from box cores (0.25 m²) or multiple corers, as appropriate.”



IOM'2009 Study Area

Location of stations sampled for macrofauna



✓ In 2009, **11 stations** randomly selected in the exploration block H11 (~ 4 000 km²) of the IOM licence area were studied for macrofaunal composition and abundance.

✓ Sampling density for macrofaunal study was about 1 station per 360 km².

✓ A total of **44** macrofauna samples were processed from sediment intervals: 0-3, 3-5, and 5-10 cm.

✓ Raw data on macrofaunal research were prepared and were submitted to the Secretary General of ISA in accordance with the Recommendations laid out in the documents ISBA/19/LTC/8.

IOM'2014 Study Area

✓ In April-May 2014, **16 stations** randomly selected in the exploration block H22 (~4000 km²) were studied for macrofauna.

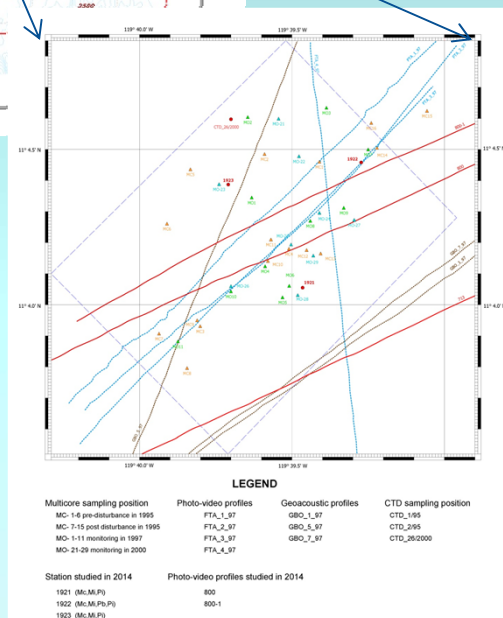
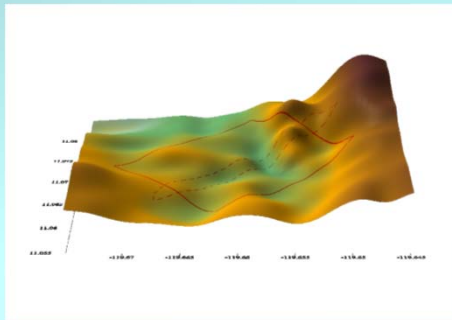
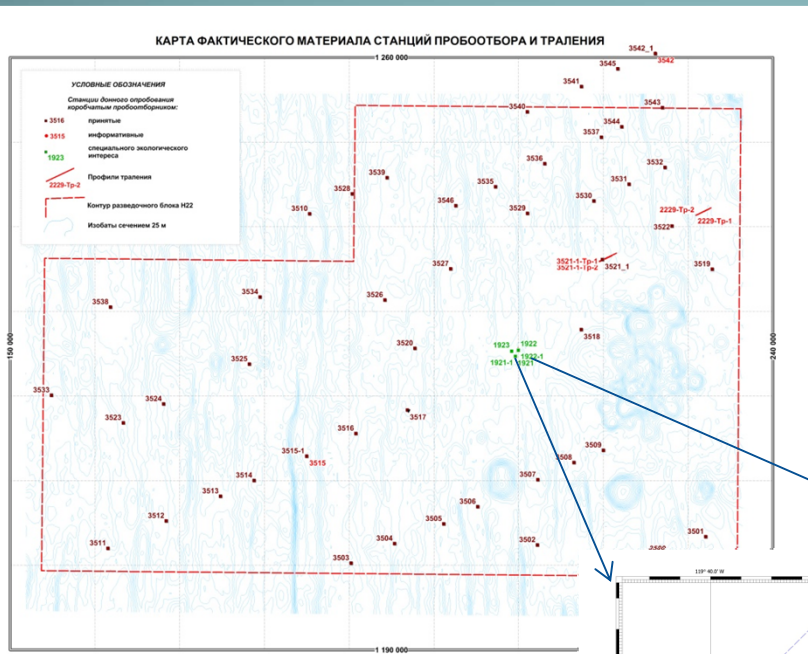
✓ A total of **48** macrofauna samples were processed from sediment intervals: 0-3, 3-5, and 5-10 cm.

✓ Sampling density for macrofaunal study was about 1 station per 250 km²

✓ In addition, **46** macrofauna samples were collected at 46 stations by rinsing the sediment from nodules.

✓ **71** individuals of nodule fauna were collected for DNA studies.

✓ Laboratory analyses of samples is planning to start in



✓ Within the IOM'BIE site 3 stations were sampled for macro- and meiofauna.



Collecting Techniques and Procedures



✓ Sediment samples for quantitative analyses of macrofauna were taken with a 0.25-m² box corer.

✓ The location of the box corer was determined with a POSIDONIA 6000 ultra-short baseline underwater acoustic system (iXSEA Oceano, France). Average accuracy of box core position was estimated about 50 m.

✓ As the box corer was lifted and deposited on the deck, its content was photographed and processed as required by the sampling protocol.



Top water in the box was siphoned off onto the sieve a 0.25-mm mesh-size and the residue was later added to the 0-3 cm layer sample.

Collecting Techniques and Procedures (cont.)



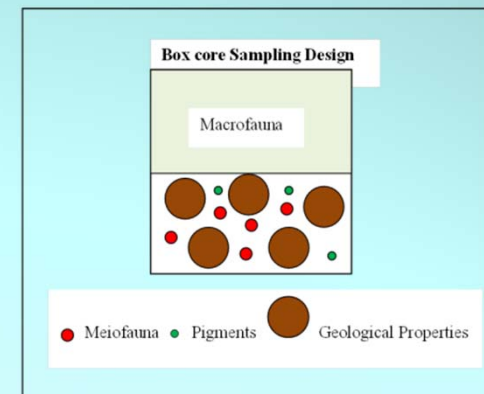
✓The nodules contained in the sediment collected by the box corer were carefully removed from the sediment and placed on a plastic tray for further processing.



✓Initially, all the nodules were rinsed on board with filtered seawater onto 0.25-mm sieve to collect the sediment remains on their surface.



✓The box core was divided by plastic plate into two equal parts, each with a 0.125-m² surface area for further geological and biological analyses in depends of the IOM research strategy.



Processing of Samples



- ✓ The macrofauna was extracted from 3 depth-layers: 0-3; 3-5; and 5-10 cm.
- ✓ The sediment from each layer was placed in a washing trough, washed with filtered seawater, and passed onto a 0.25-mm sieve-size.
- ✓ The sieving residue from each sediment layer was transferred to separate plastic containers and fixed with 4% buffered formaldehyde in seawater.
- ✓ About 5 days later, the samples were rinsed and preserved in 80% alcohol with Rose Bengal.
- ✓ All the animals were placed in plastic jars, and were subsequently delivered to the IOM headquarters laboratory for further studies and storage.
- ✓ Specimens for DNA extraction were put in EtOH 96% and were stored in the fridge (4°C).

Onshore Processing of Samples



✓ In the onshore laboratory, prior to examination, each sample was rinsed with small portions of running water onto a 0.25 mm mesh-size sieve to remove the excess of the fixative and dye.

✓ Analyses were carried out using a Nikon SMZ 745T stereomicroscope.

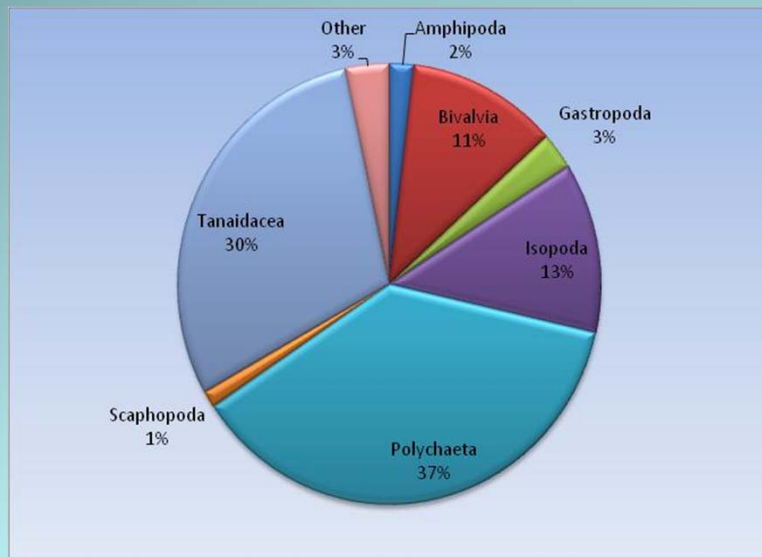
✓ All the animals from each station were sorted into major taxonomic groups, and the specimens of one taxon from each sample were transferred into 10 ml and 7 ml glass vials with 80 % ethanol and stored for further, more detailed, species identification.



Macrofauna abundance (inds/m²) within 0-10 cm sediment layer at IOM'2009 studied area

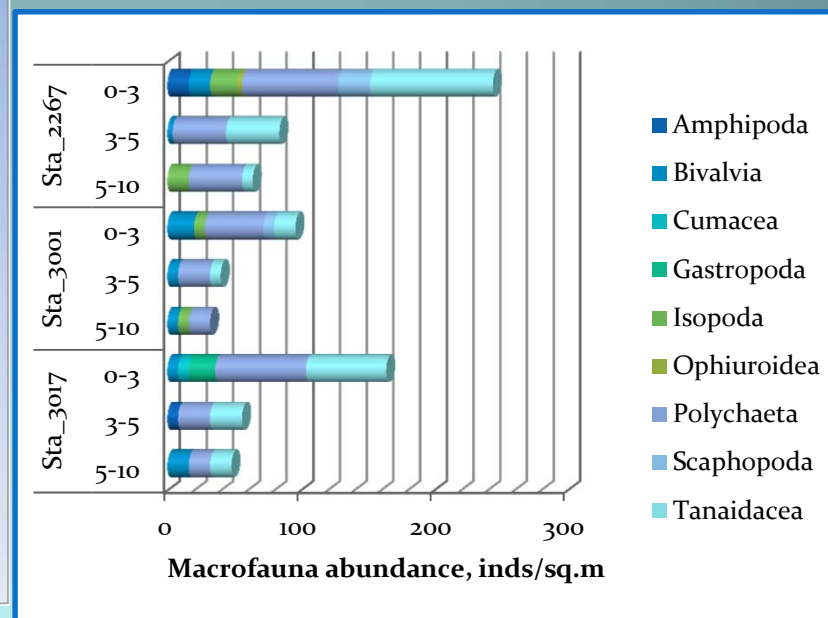
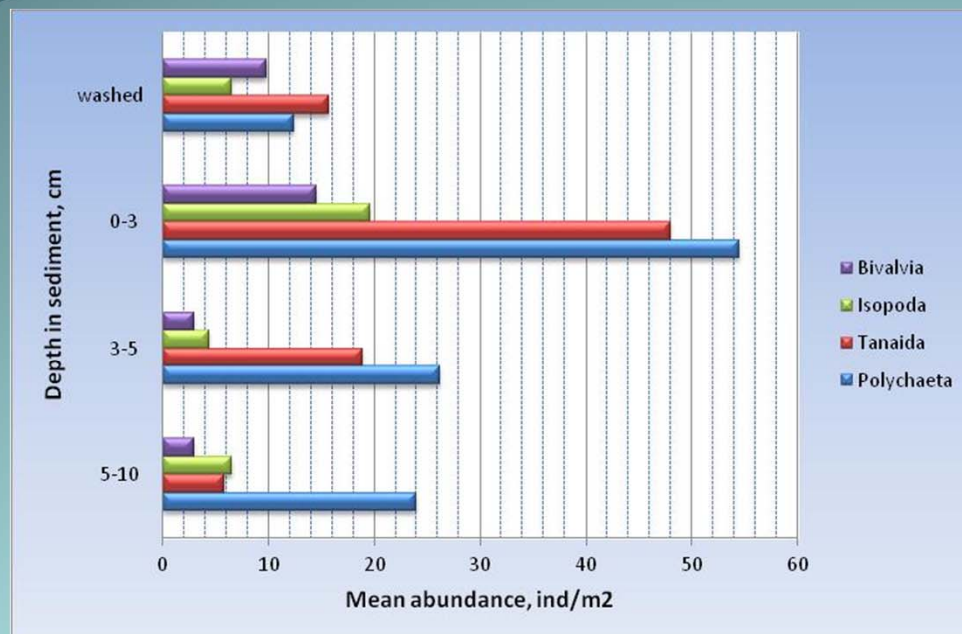
Taxon	Stations											Mean abundance
	2263	2267	2277	2280	2281	2282	2286	2298	3001	3003	3017	
Amphipoda		16	4	20			8			8	8	6
Aplacophora										4		0
Bivalvia	28	20	12	56	8	24	24	68	36	32	24	30
Brachiopoda							8					1
Cumacea	8									8		1
Decapoda				8				8				1
Echinoidea				16		8						2
Gastropoda	4		8	8	20	8		8		12	20	8
Isopoda	56	36	100	92	32	24	16	12	16	24		37
Ophiuroidea		4	4					8		8		2
Polychaeta	148	152	60	104	144	232	80	80	84	96	108	117
Polyplacophora						12						1
Priapulidae				8								1
Scaphopoda		24	4						8	8		4
Tanaidacea	76	140	116	148	84	92	52	52	24	88	100	88
Total	320	392	308	460	288	400	188	236	168	280	268	301

Macrofauna community structure



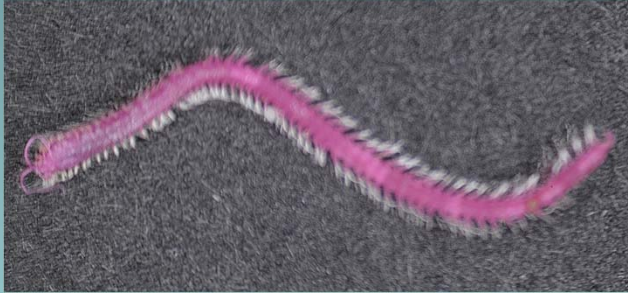
- ✓ A total of 480 macrofauna individuals representing 15 taxa were found.
- ✓ Mean abundance of macrofauna was found to occur about 301 inds/m², ranging from 168 to 460 inds/m².
- ✓ Polychaetes were represented in the samples most abundantly and accounted for 37% of all the macrofauna.
- ✓ Tanaid crustaceans contributed 30%.
- ✓ The contributions of isopods and bivalves were very similar (13 and 11%, respectively).
- ✓ The other taxa combined contributed about 9% .

Depth distribution of the main macrofauna taxa in the IOM'2009 area



- ✓ The macrofauna abundance peaked in the 0-3 cm depth-layer in the sediment at all the stations sampled.
- ✓ The mean polychaete abundance was 55 inds/m² at 0-3 cm, and decreased slightly down from 26 inds/m² at 3-5 cm depth interval to 24 inds/m² at 5-10 cm.
- ✓ The tanaid abundance reduction with depth was more pronounced than that of polychaetes.
- ✓ The vertical distribution of isopods in the sediment differed from that shown by both polychaetes and tanaids.

**Polychaete worms comprised the greatest proportion of individuals
(37% of the all taxa recognized)**



Polychaeta_2267_3_1_1



Polychaeta_2267



Calcareous polychaets
tube_2267



Polychaeta_sediment
tube_3001_0165_R

**A mean abundance of polychaete worms was counted about
117 inds/m², ranging from 60 to 232 inds/m².**

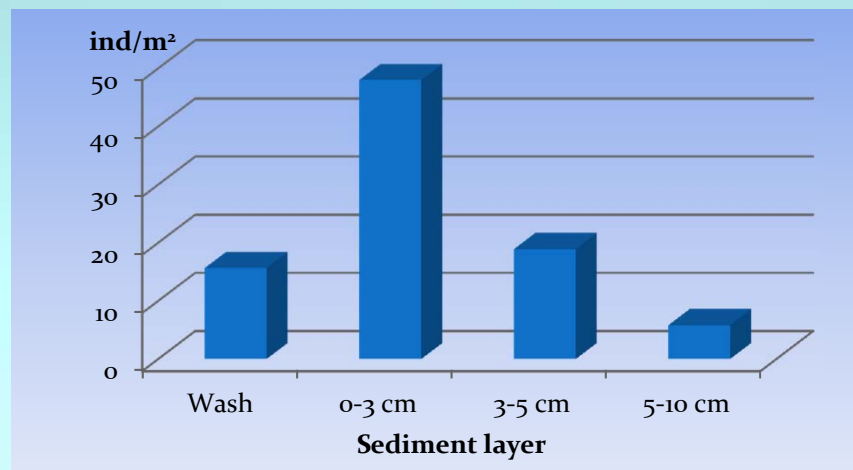
Tanaid crustaceans were the second most abundant taxon in the IOM'2009 studied area



Tanaida_2267_Apseudes galatheaen. sp.

Tanaid crustaceans were found at all the stations studied.

Tanaid abundance varied from 24 to 148 inds/m², averaging 88 inds/m².

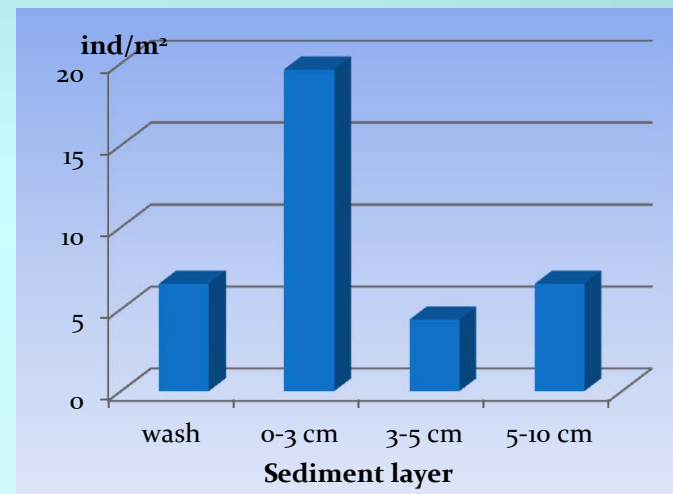


Average tanaid numbers per layers in sediment



Isopoda_3001_0158_R

- ✓ Isopod abundance varied from 0 to 100 inds/m², averaging 37 inds/m².
- ✓ In the deepest (5-10 cm) sediment layer, isopods were more abundant than in the 3-5 cm layer, their abundances amounting to 7 and 4 inds/m², respectively.





Amphipoda_2267



Amphipoda_3017



Scaphopoda_2267_1

Amphipods and scaphopods were found at 6 and 4 stations, with a mean abundance of 6 and 4 inds/m², respectively.



Bivalvia_3001_R

✓ Bivalve molluscs were recorded at all the stations with abundance varying from 8 to 68 inds/m², averaging 30 inds/m² across the area studied.

✓ The largest among the bivalves found in the area was a representative of the genus *Kelliella* (family Kelliellidae, order Veneroidea).



Bivalve_2267_1_1



Bivalvia_3017



Gastropoda_n_3017



Gastropoda_3003_1_R

Gastropods were recognized at 8 stations with abundance varying from 4 to 20 inds/m², averaging 8 inds/m² across the area studied.

Nodule Fauna

✓ The polymetallic nodules provide a unique hard substratum supporting abundant and diverse epibenthic biota that include all animal size classes, from micro- to megafauna.

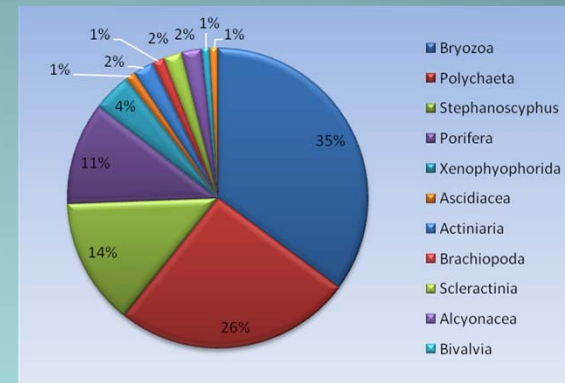
✓ A total of 461 (without foraminifera) nodule faunal individuals were collected during the IOM'2009 study, belonging to different taxonomic groups.

✓ Analysis of the nodule faunal composition indicated that more than 80% of all indentified specimens belong to four taxa: Bryozoan (bryozoans' colony-forms), Polychaeta, Stephanoscyphus, and Porifera.



Taxonomic structure and number of the nodule fauna

Taxon	Affiliation	Quantity, inds.
Phylum <i>Bryozoa</i>	Phylum <i>Bryozoa</i>	162
Class <i>Polychaeta</i>	Phylum <i>Annelida</i>	118
Genus <i>Stephanoscyphus</i>	Phylum <i>Cnidaria</i> Class <i>Scyphozoa</i> Order <i>Coronatae</i> Family <i>Atorellidae</i>	63
Class <i>Demospongiae</i>	Phylum <i>Porifera</i>	51
Class <i>Xenophyophorida</i>	Phylum <i>Protozoa</i>	19
Order <i>Actinaria</i>	Phylum <i>Cnidaria</i> Class <i>Anthozoa</i>	10
Order <i>Alcyonacea</i>	Phylum <i>Cnidaria</i> Class <i>Anthozoa</i>	10
Order <i>Scleractinia</i>	Phylum <i>Cnidaria</i> Class <i>Anthozoa</i>	9
Phylum <i>Brachiopoda</i>	Phylum <i>Brachiopoda</i>	6
Class <i>Ascidiacea</i>	Phylum <i>Tunicata</i>	5
Class <i>Bivalvia</i> (bivalves)	Phylum <i>Mollusca</i>	4
Class <i>Hydrozoa</i>	Phylum <i>Cnidaria</i>	1
Class <i>Crinoidea</i>	Phylum <i>Echinodermata</i>	1
Family <i>Scalpellidae</i>	Phylum <i>Arthropoda</i> Class <i>Maxillopoda</i> Order <i>Pedunculata</i>	1
<i>Antipatharia</i> (black corals)	Phylum <i>Cnidaria</i> Class <i>Anthozoa</i>	1
Total		461



Conclusion Remarks:

- Macrofauna has been subjected to qualitative and limited taxonomic analysis at the early stage of the IOM's activity.
- During the IOM'2009 and IOM'2014 cruises, macrofauna was collected from a half of the contents of 27 box corers deployed in the most promising for the future mining blocks of the IOM exploration area.
- The data collected in 2009 were quantitative and represent information on macrofaunal composition, densities, and vertical distribution in the sediment.
- Assessed and interpreted results of macrofauna studies together with the raw data were reported to the ISA in accordance with the IOM obligations as a contractor.
- IOM is open for cooperative research of macrofaunal samples collected during the IOM'2014 cruise with purpose to unify the taxonomic methods and standards used by others contractors and scientists as well as to contribute the knowledge on the deep-sea macrofauna in the CCZ.





THANK YOU VERY MUCH

ACKNOWLEDGEMENTS

**This study was supported by the IOM'
sponsoring states.**

