

# PML

Plymouth Marine  
Laboratory

Listen to the ocean

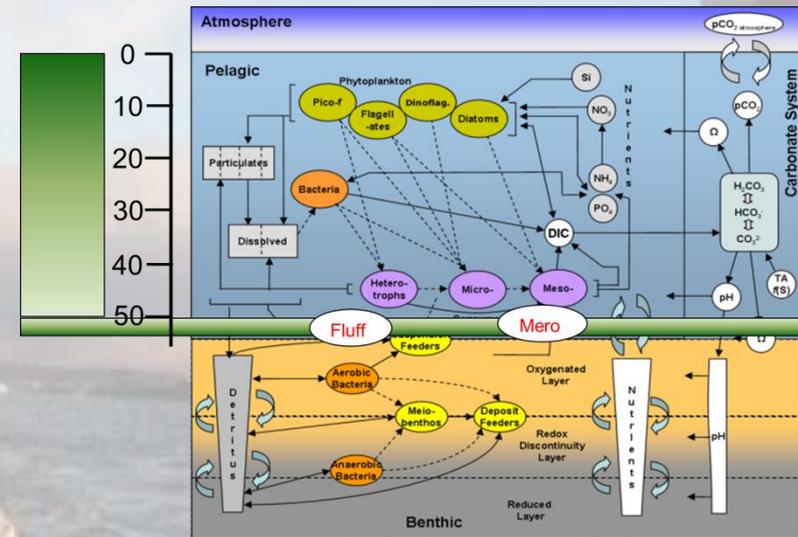
## What does standard plankton monitoring miss? Using meta-barcoding and an epibenthic sledge to reveal the hidden diversity of the shelf sea zooplankton

Pennie Lindeque, Helen Parry and  
Angus Atkinson



## Traditional monitoring of shelf sea zooplankton

- ❖ Vertical hauled plankton nets
- ❖ Microscope-based identification of the catch

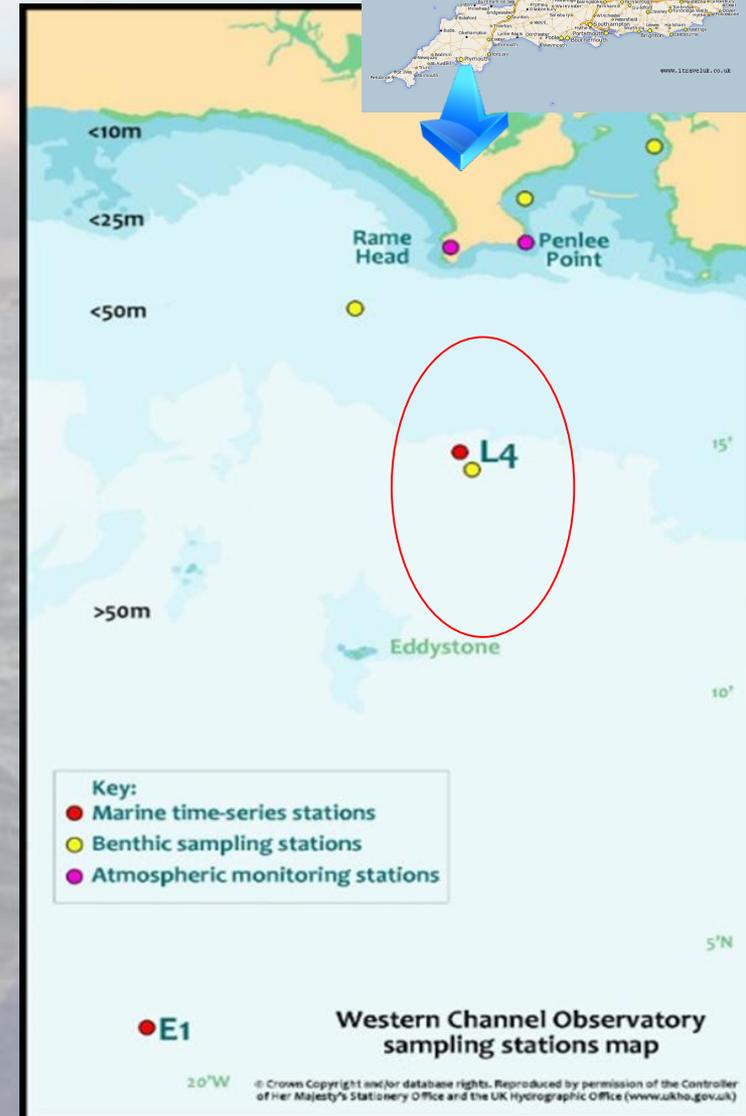


## But what are we missing.....?

- ❖ Does microscopy give a true diversity of the assemblage?
- ❖ What about small/rare/cryptic taxa?
- ❖ What about 'difficult to identify' organisms (larvae, meroplankton)?
- ❖ What about taxa close to seabed?

# Studied zooplankton at the L4 time series site in the Western English Channel

- 1) Microscopy vs Metabarcoding  
Compare morphologically- and metagenetically-derived Operational Taxonomic Units (OTUs) assigned to major taxa
- 2) Vertical nets vs Epibenthic sled  
Use metabarcoding to analyse the catch from both vertical nets and epibenthic sledge over a seasonal cycle



## Experimental Design

- Long time series station L4, WCO
- Two temporal sampling points
  - September 2010
  - January 2011
- 4 replicate hauls
  - Vertical 50 m –surface
  - 200  $\mu$ M mesh

Bulk Zooplankton Haul

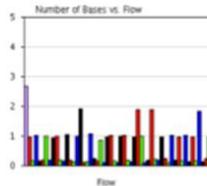
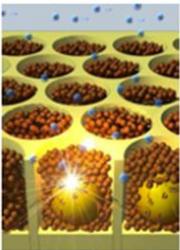
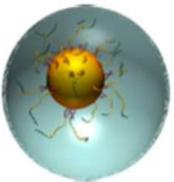
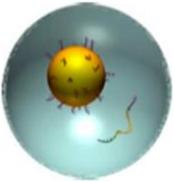
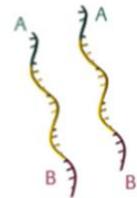
Morphological  
analysis

Molecular  
analysis



## Metabarcoding

- DNA Extraction from whole community samples
- Triplicate PCR of 18S nuclear small subunit rRNA gene (SSU\_FO4 + SSU\_R22; Fonseca et al., 2010)
- Amplicons purified
- Sequenced on a Roche 454 FLX platform
- Reads passed through Qiime pipeline.
- OTUs assigned @ 97% homology
- Assigned taxonomy by BLASTN search of NCBI dataset (homology > 97%)



## Microscopy

- Samples were analysed using light microscopy
- Organisms identified to genus or species level where possible
- A small subsample was analysed first, and then a larger subsample, to ensure rare/large organisms were represented in the analysis



## Results

### Metabarcoding

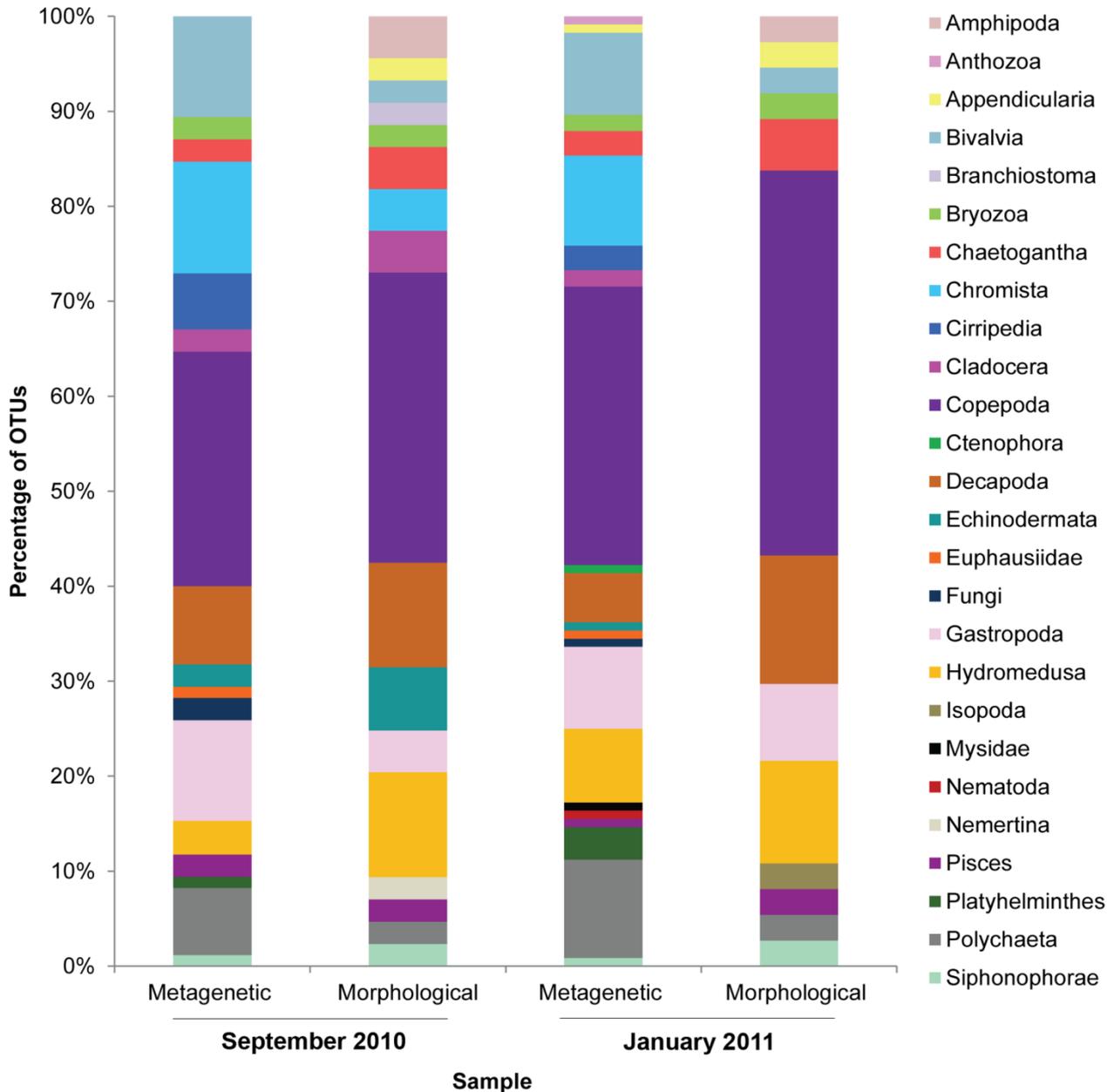
- 419,041 sequences (QC = loss of 7%-30%)
- 205 OTUs (@ 97% similarity cut-off)
- 135 OTUS – Species, 11 OTUS – Genus, 1 OTU – Order
- Unknowns – 58 OTUS <2.5 % of sequences

### Microscopy

- By skilled analyst
- Total of 2058 organisms counted
- 58 taxonomic groups (OTUs) recorded
- 4 – phyla, 9 – class, 5- order, 2- family, 8 – genus, 30 – species
- For many copepod OTUs, sex and developmental stage also were recorded

	SEPTEMBER 2010		JANUARY 2011		TOTAL OTUs	
	Metagenetic # OTUs	Morphological # OTUs	Metagenetic# OTUs	Morphological # OTUs	Metagenetic	Morphological
Amphipoda	0	2	0	1	0	2
Anthozoa	0	0	1	0	1	0
Appendicularia	0	1	1	1	1	1
Bivalvia	9	1	10	1	13	1
Branchiostoma	0	1	0	0	0	1
Bryozoa	2	1	2	1	3	1
Chaetognatha	2	2	3	2	3	3
Chromista	10	2	11	0	18	2
Cirripedia	5	0	3	0	6	0
Cladocera	2	2	2	0	2	2
Copepoda	21	14	34	15	40	17
Ctenophora	0	0	1	0	1	0
Decapoda	7	5	6	5	8	8
Echinodermata	2	3	1	0	2	3
Euphausiidae	1	0	1	0	1	0
Fungi	2	0	1	0	2	0
Gastropoda	9	2	10	3	13	4
Hydromedusae	3	5	9	4	9	8
Isopoda	0	0	0	1	0	1
Mysidae	0	0	1	0	1	0
Nematoda	0	0	1	0	1	0
Nermertina	0	1	0	0	0	1
Pisces	2	1	1	1	2	1
Platyhelminthes	1	0	4	0	5	0
Polychaeta	6	1	12	1	14	1
Siphonophorae	1	1	1	1	1	1
Unknowns	26	0	45	0	58	0
<b>Total OTUs</b>	<b>111</b>	<b>45</b>	<b>161</b>	<b>37</b>	<b>205</b>	<b>58</b>

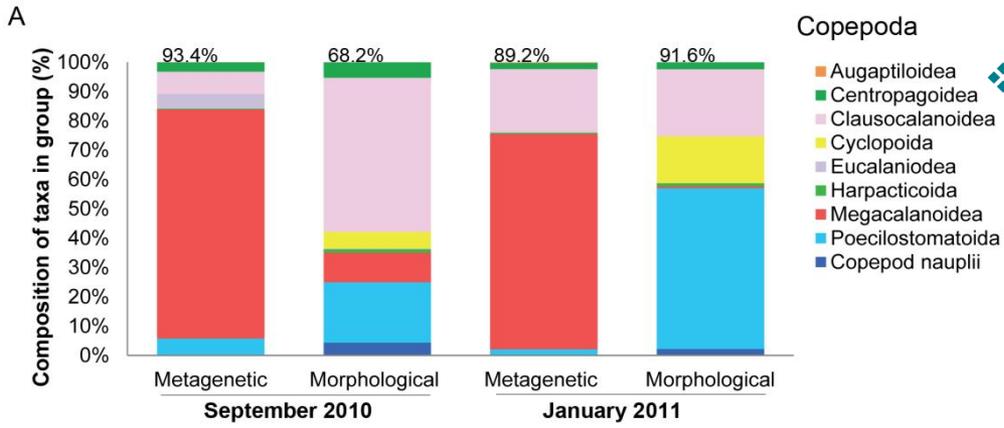
# Microscopy vs Metabarcoding



- OTUs - broad taxonomic groups to allow a comparison between microscopy and metabarcoding
- Groups constrained by level of identification possible by morphological analysis
- At this resolution metabarcoding broadly aligns with morphological analysis
- Diverse range of taxa dominated by copepoda

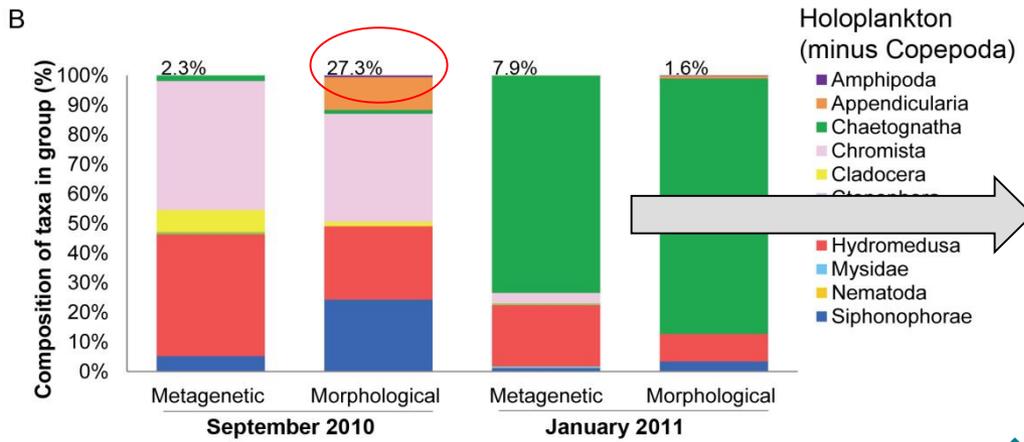
# Composition of taxa in the zooplankton derived from morphological and metagenetic analysis

❖ Copepods strongly dominated in terms of number of reads/abundance of organism



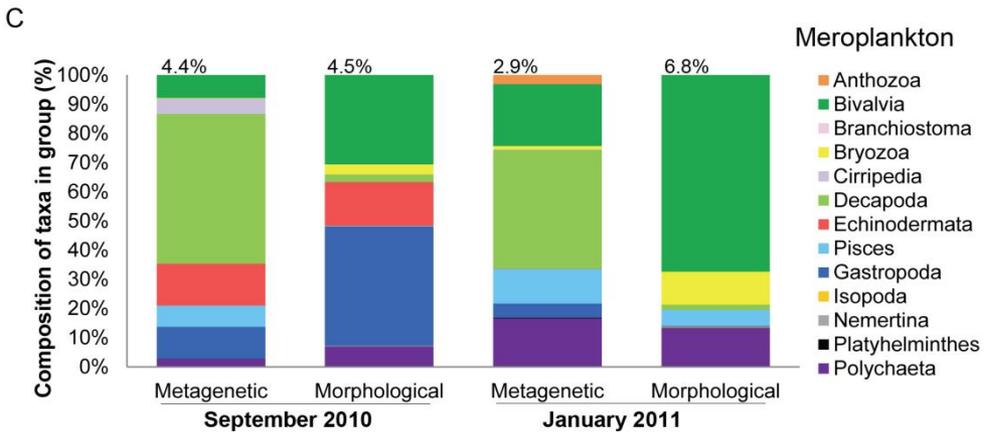
❖ Relative magnitude and composition of copepod subgroup varied between method and timepoint

❖ High proportion of holoplankton due to large numbers of Noctiluca and Hydromedusa



❖ Chaetognatha (Sagitta) dominated both datasets

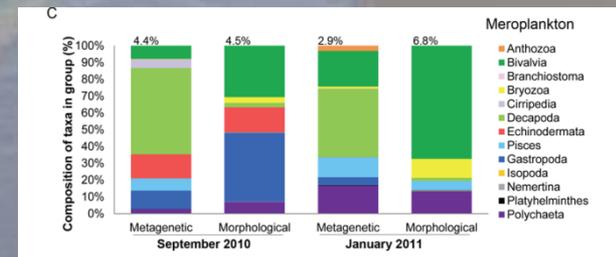
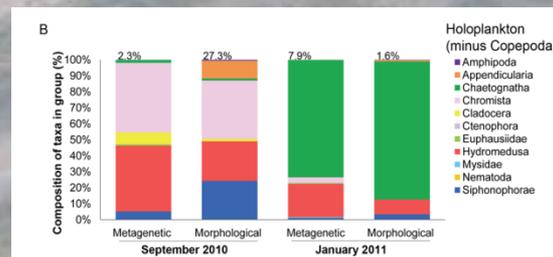
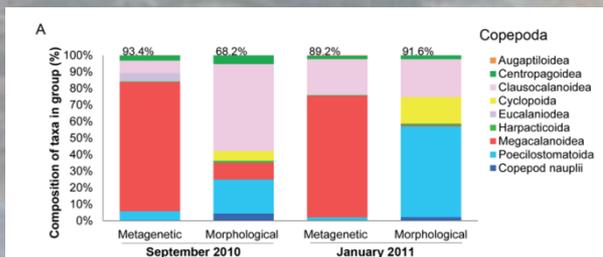
❖ Methods reveal domination of meroplankton by different taxa



❖ Metagenetics – dominated by Decapoda (Liocarcinus spp.)  
 ❖ Morphological – dominated by Gastropoda & Bivalvia in Sept and Bivalvia in Jan

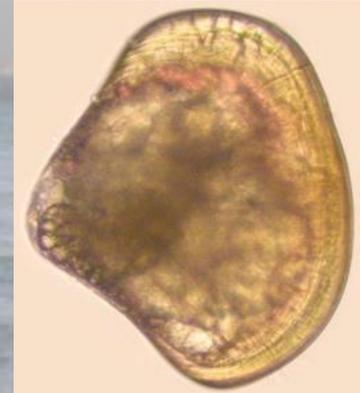
## Why the variation between datasets?

- 1) Morphological analysis measures abundance whereas metagenetic analysis more closely relates to biomass.
  - Eg. Copepoda sequences dominated by *Calanus helgolandicus* (relatively large biomass); morphological analysis dominated by juvenile stages of *Pseudo-/Cteno-/Clausocalanus* (high abundance)
  
- 2) Lack of annotation of the metagenetically derived unknowns.
  - Eg. Dominance in January morphological dataset of *Oncaea*, however, NCBI database poorly populated with substantial length reference sequences.
  
- 3) Primer mismatch; zero tolerance in Qiime pipeline quality control
  - Reduced amplification of Cnidarian DNA due to bp mismatch at 3' end of reverse primer.



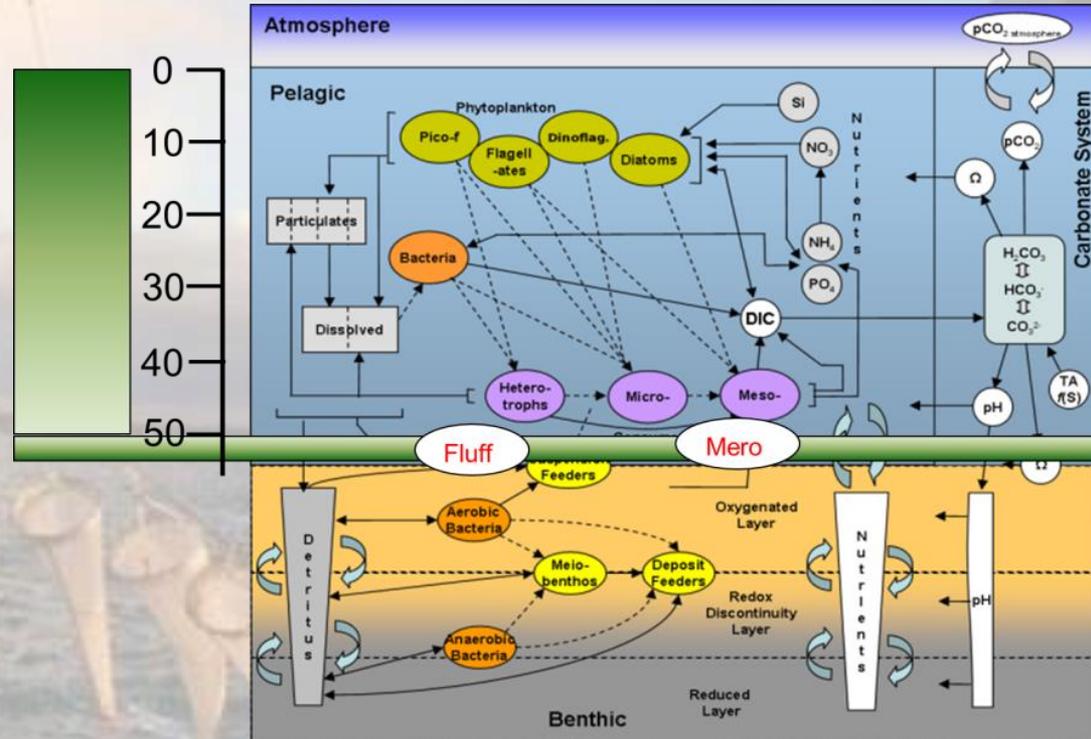
## Taxonomic resolution of the metagenetic and morphological datasets.

- ❖ Metagenetic analysis revealed greater species richness than morphological identification.
- ❖ Meroplanktonic larvae.
  - ❖ Morphological limitations mean microscopy revealed 1 OTU for each polychaete, bivalve and gastropod group.
  - ❖ Metagenetic analysis revealed 14 polychaete spp., 13 gastropod spp. and 13 bivalve spp.
- ❖ Copepoda.
  - ❖ Metagenetics revealed more copepod OTUs with all but 1 identified to species
  - ❖ Microscopy revealed less OTUs but gave quantification of life stage and sex of adults.
- ❖ Parasitic spp.
  - ❖ Metagenetic analysis uniquely revealed a number of parasitic spp. (9OTUs).



## Traditional monitoring of shelf sea zooplankton

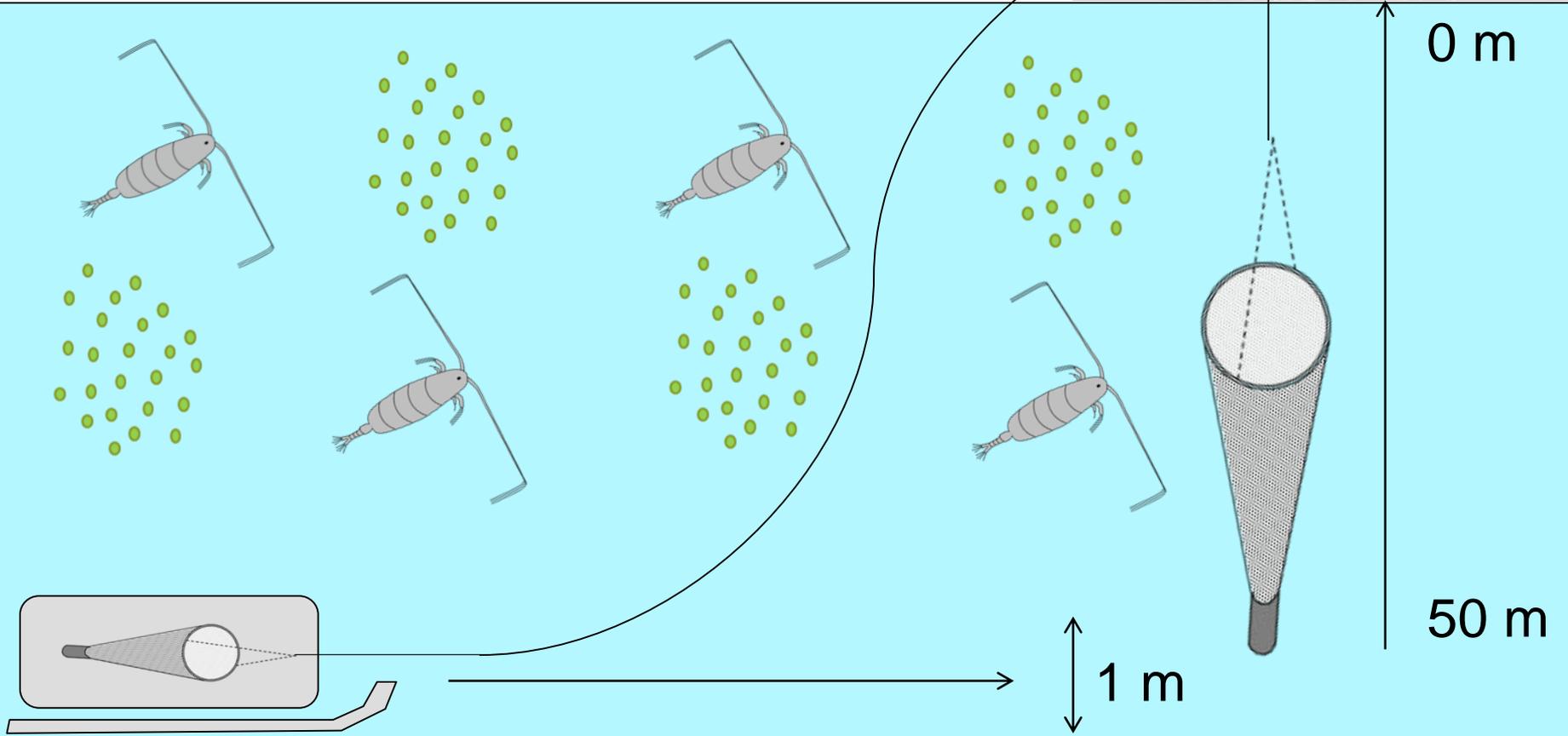
- ❖ Vertical hauled plankton nets
- ❖ Microscope-based identification of the catch



## But what are we missing.....?

- ❖ Does microscopy give a true diversity of the assemblage?
- ❖ What about small/rare/cryptic taxa?
- ❖ What about 'difficult to identify' organisms (larvae, meroplankton)?
- ❖ **What about taxa close to seabed?**

1. Sample epi-benthic boundary layer with sled.
2. Determine how this differs from the upper 50 m using metabarcoding.



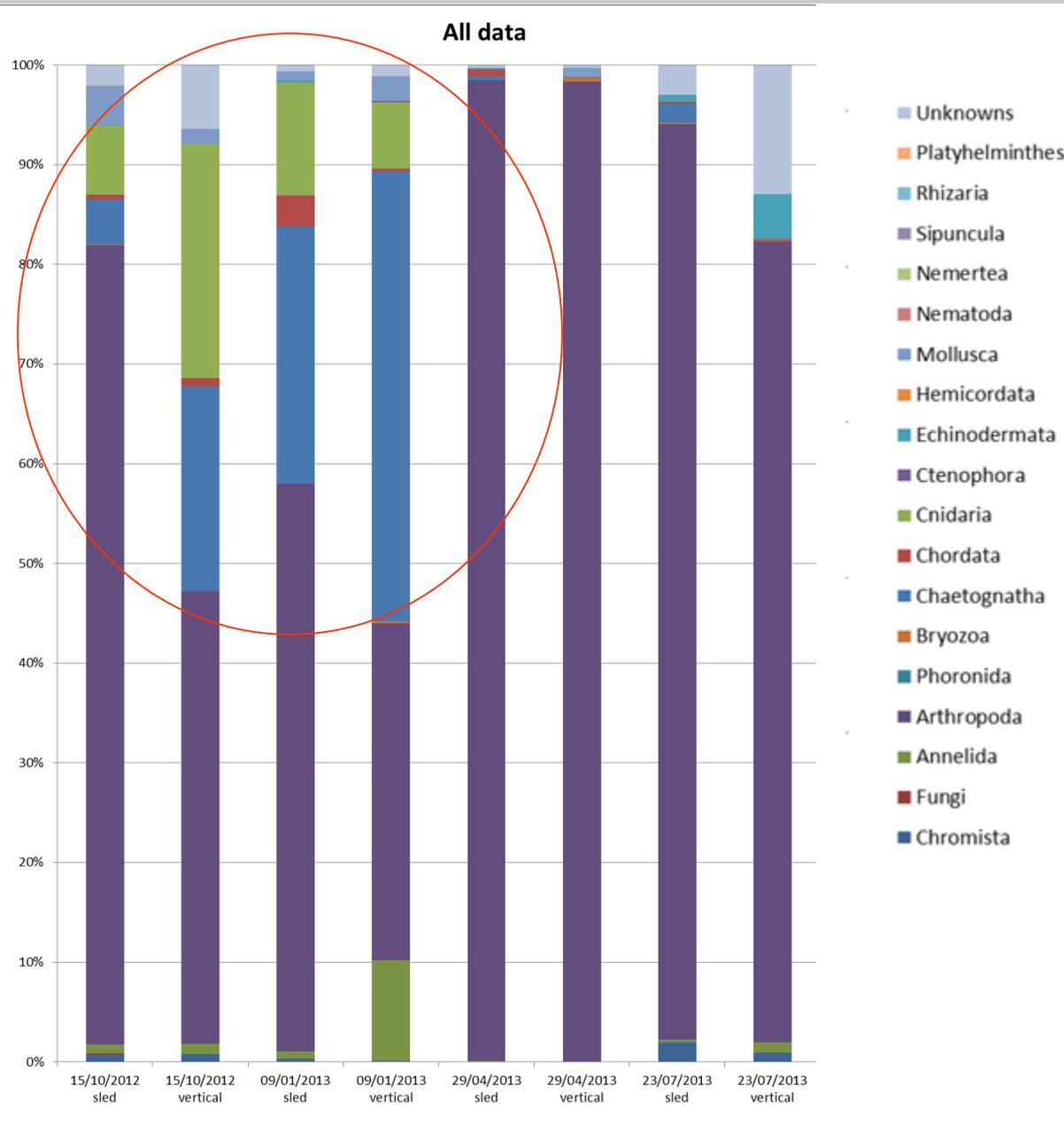
- Long time series station L4, WCO
- Four temporal sampling points
  - October 2012
  - January 2013
  - April 2013
  - July 2013
- Vertical 63  $\mu$ M net haul ~ 50 m to 0 m
- Horizontal 63  $\mu$ M net tow (700 m)
- Samples were preserved in 95% ethanol and stored at 4° C.

## Metabarcoding

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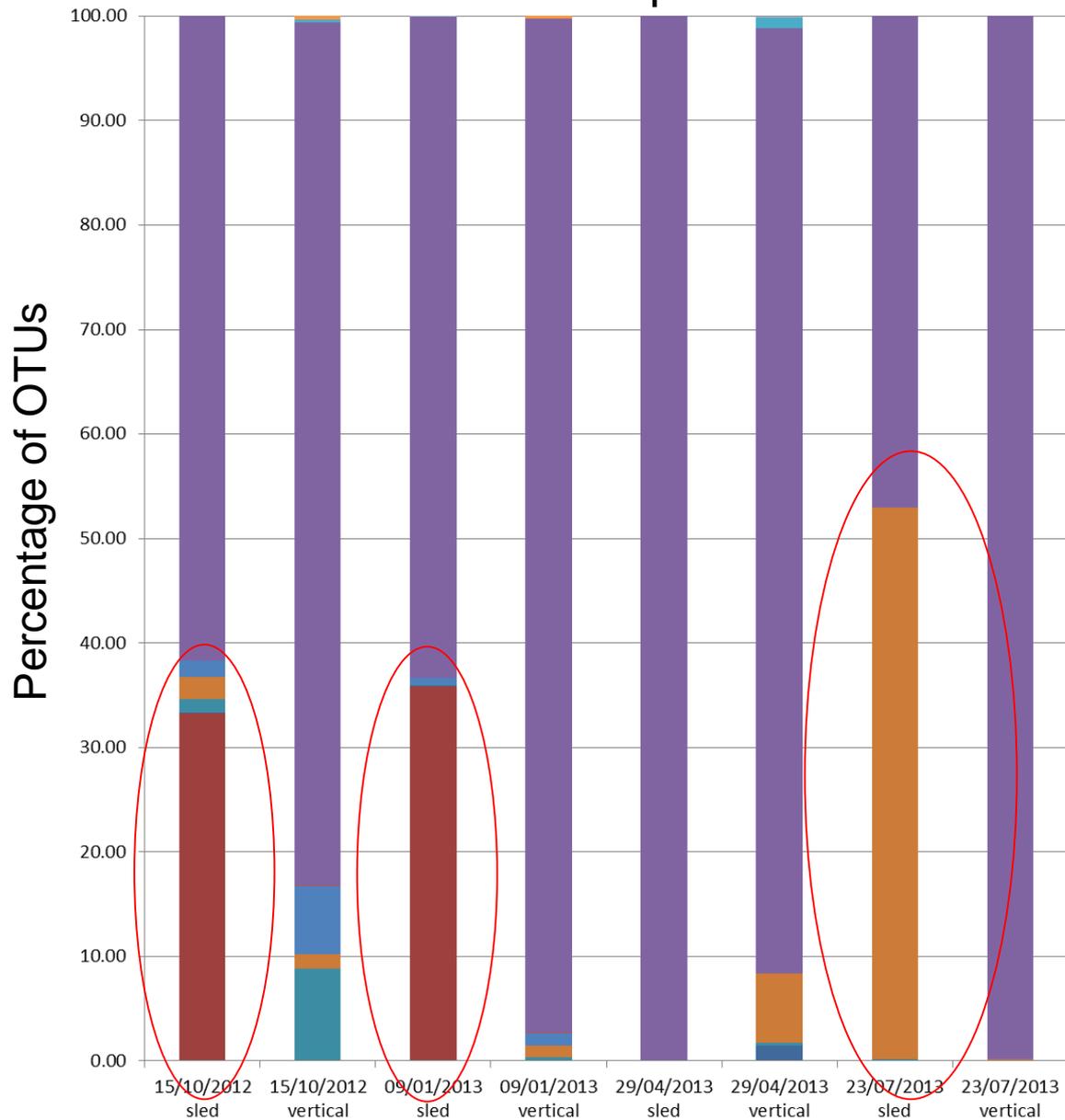


Percentage of OTUs

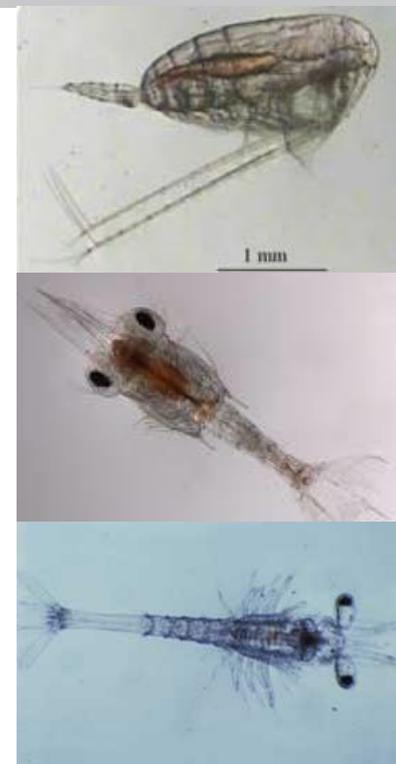


- Most samples are dominated by Arthropoda
- Relatively high proportion of Chaetognaths, and to a lesser extent Cnidarians in Oct and Jan.

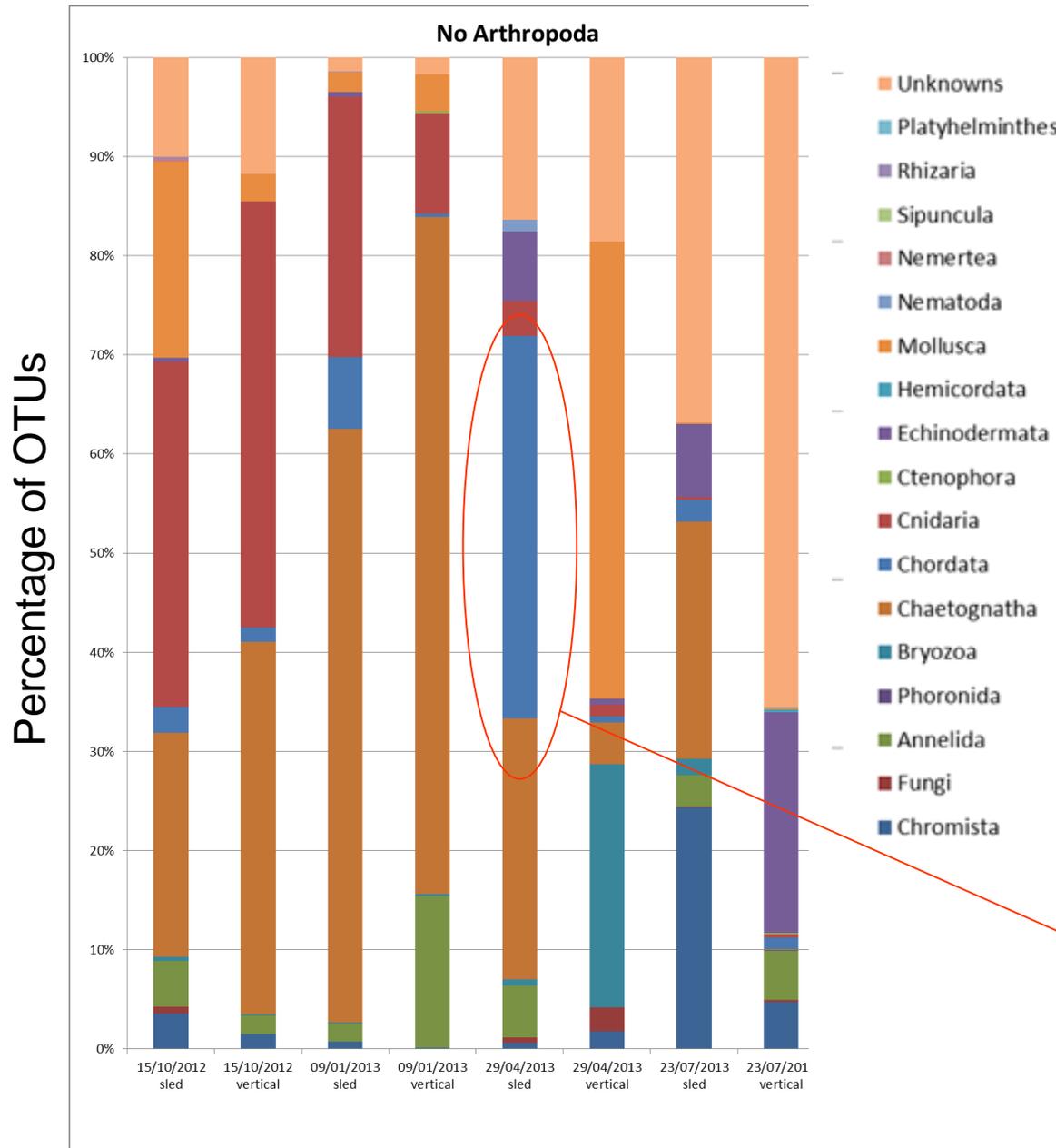
## Arthropoda



- Poecilostomatoida
- Siphonostomatoida
- Cyclopoida
- Euphausiacea
- Calanoida
- Diplostraca
- Amphipoda
- Poecilostomatoida
- Decapoda
- Harpacticoida
- Podocopida
- Trombidiformes
- Mysida
- Sessilia

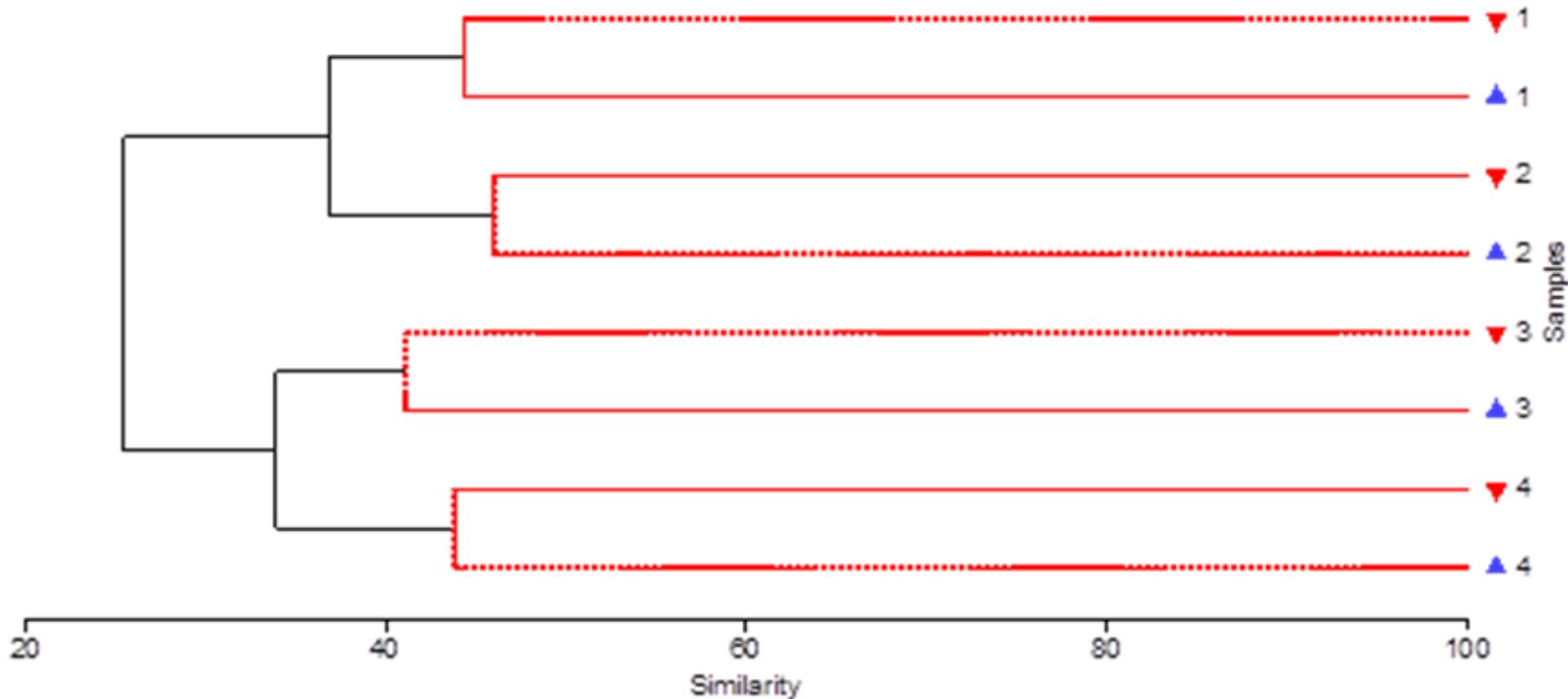


- Arthropoda dominated by Copepoda
- Oct and Jan sled samples have significant contribution of Mysida
- July sled has significant contribution of Decapoda



- Both sled and vertical haul dominated by Chaetognaths and Cnidaria in Oct and Jan
- April: greatest variation between sled and vertical
- ❖ Vertical – Molluscs (Bivalves and Gastropods)
- ❖ Sled – Chordata (Oikopleura)
- Abandoned mucus houses of Oikopleura known to make an important contribution to marine snow

Differences in community structure between groups of samples were explored using Bray-Curtis similarities calculated from square-root transformed abundances (PRIMER 6)



▲ sled ▼ vertical

No significant differences seen between the community structure sampled by horizontal sled tow at the epibenthic layer and a vertical haul from 50m to surface.

- Metabarcoding of 18S amplicons is a powerful tool for elucidating the true diversity and species richness of zooplankton communities
- Reveals a previously hidden taxonomic richness
  - Copepoda
  - Meroplankton (Bivalvia, Gastropoda and Polychaeta)
- Reveals rare species and parasites.
- ❖ Critical need for reference libraries of accurately identified individuals
- Traditional monitoring of shelf sea zooplankton with vertical hauled plankton nets does not critically misrepresent zooplankton in the water column by under-sampling those close to the sea floor
- But epibenthic sled does provide more information.





Thank You  
Captain and Crew of RV Plymouth Quest, Paul Somerfield,  
Rachel Harmer