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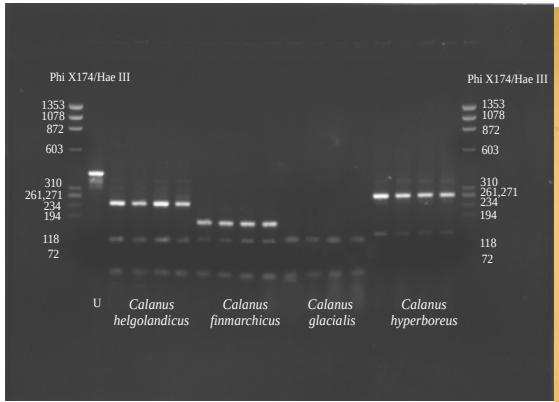
Marine Matters

Molecular Identification of Zooplankton: 10 years on.

Pennie Lindeque



The Problem

- Correct Identification!
  - Zooplankton are systematically diverse
  - Taxonomically challenging
- Why is unambiguous species identification important?
  - Accurate description of zooplankton diversity, distribution and demography
  - Assess biogeographical range or shifts in community composition

Molecular identification of zooplankton: The start

- DNA sequences of homologous gene regions used to design molecular techniques to discriminate closely related spp.

Restriction Fragment Length Polymorphism RFLP

(Lindeque *et al*., 1999; Lindeque *et al*., 2004)

Preserved animal
Egg - Adult

→

Amplification by PCR
of
16S rRNA gene

→

Restriction digest
of the amplified product

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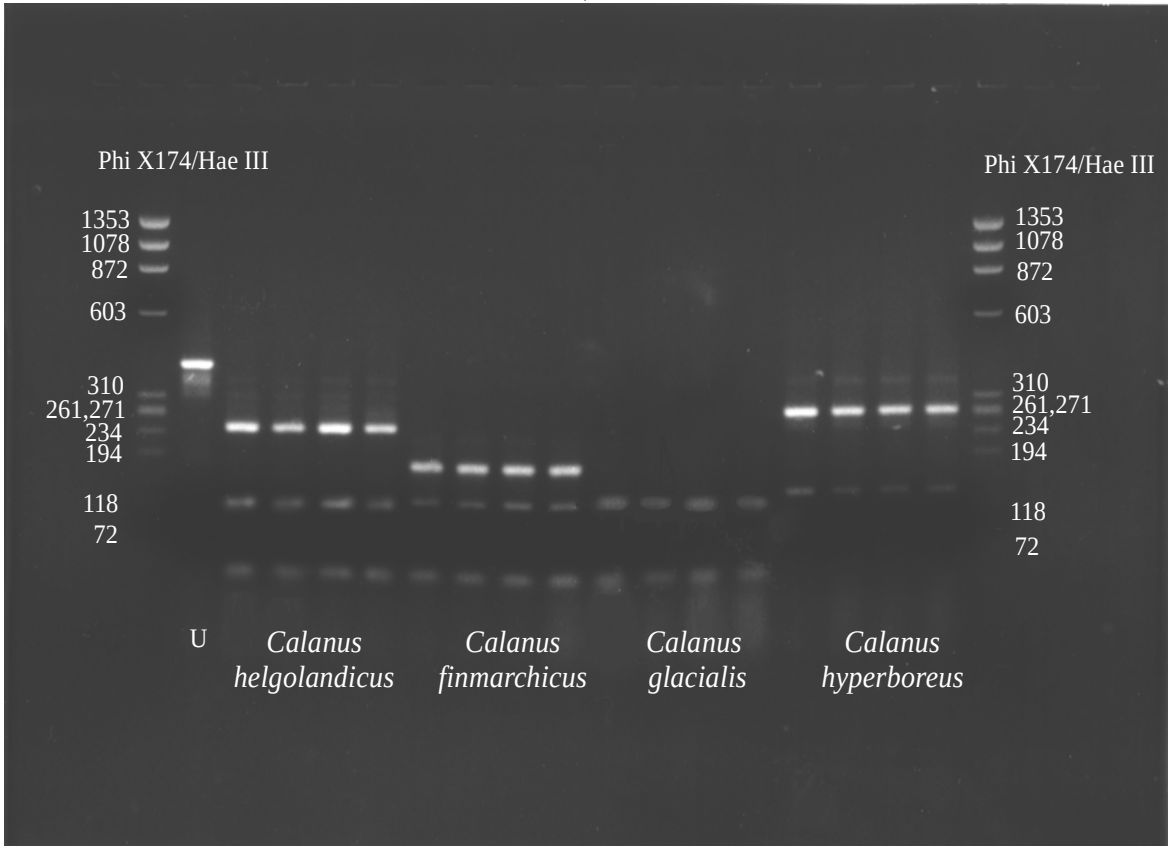
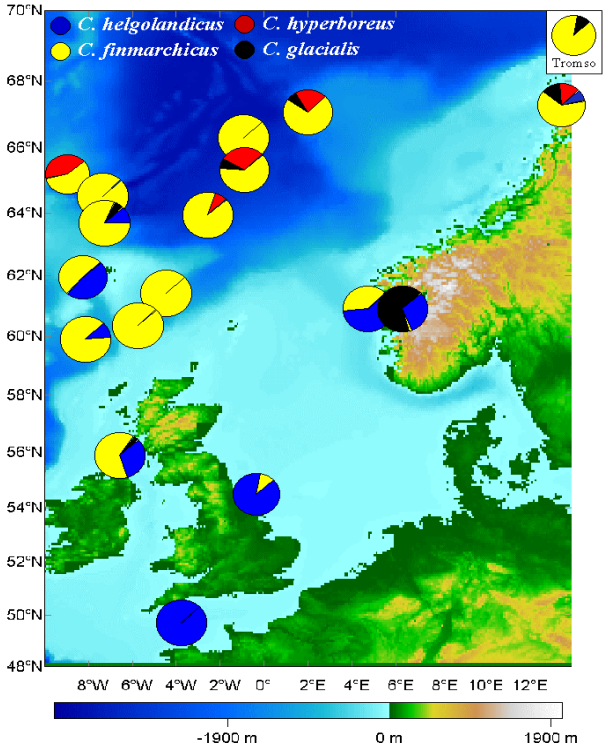


Molecular techniques for zooplankton identification

Date	Author	Organism	Gene	Technique
1998	Bucklin *et al*	*Pseudocalanus moultoni* and *P. newmani*	16S rRNA	Allele-specific PCR amplification
1999	Lindeque *et al*	*Calanus helgolandicus, C.finmarchicus, C. glacials, C. hyperboreus*	16S rRNA	RFLP
1999	Bucklin *et al*	*Calanus helgolandicus, C.finmarchicus, C. glacials* and *Pseudocalanus moultoni*, *P. newmani*	mtCOI	Competitive multiplexed species-specific PCR
2001	Hill *et al*	*Calanus helgolandicus, C.finmarchicus, C. glacials, C. hyperboreus*	mtCOI	Competitive multiplexed species-specific PCR
2007	Blanco-Bercial & Alvarez-Marques	*Clausocalanus jobei, C. lividus, C. arcuicornis, C. pergens*	mtCOI	RFLP
2010	Grabbert *et al*	*Pseudocalanus acuspes & P. elongatus*	mtCOI	Competitive multiplexed species-specific PCR
2010	Sato *et al*	13 species of barnacle larvae	12S rRNA	qPCR

Application of molecular identification technique



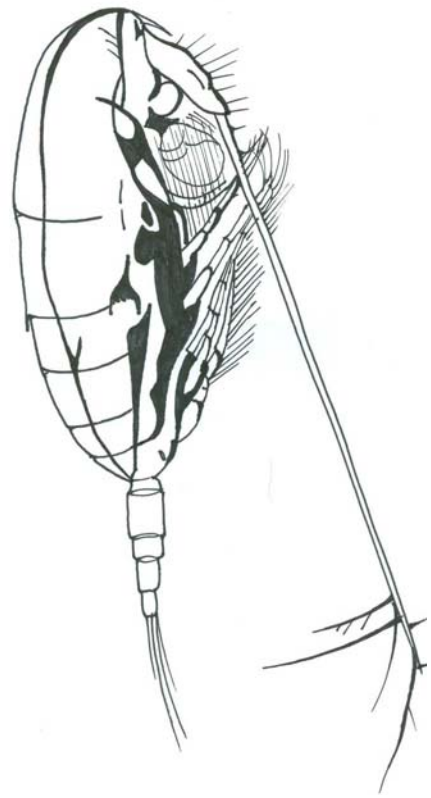
- Distribution of *Calanus spp*. in North east Atlantic
- Mesocosm experiments in Norway
- Onboard nauplii mortality experiments
- Semi-automated for near real-time identification onboard ship
- Merged with conventional microscopy for large-scale field surveys

Implications:

- A better understanding of *Calanus* dynamics, community structure & diversity
- Non-homogenous species composition across developmental stages
- Traditional discriminators unreliable

Barcoding

Short DNA sequences used for species recognition and discrimination

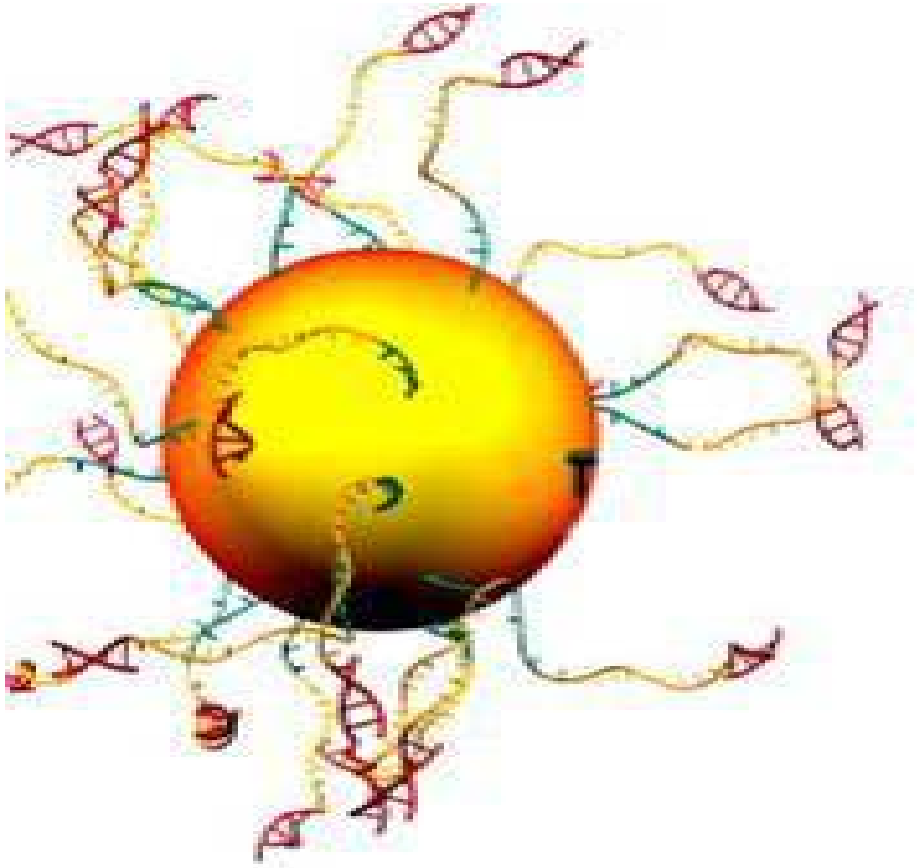


- Common metazoan DNA barcoding gene = mtCOI
- Allows accurate identification of known species
- Assessment of species diversity and distribution
- For Example
  - Webb *et al.*, 2006 'DNA barcoding: A molecular tool to identify Antarctic marine larvae'
  - Bucklin *et al*., 2010 'DNA barcoding of Arctic Ocean holozooplankton for species identification and recognition'

Limitations

- Correct morphological identification ESSENTIAL
- Correct gene usage (NUMTs, pseudogenes)
- High quality molecular data
- Limited to specific genera
- Is DNA Bar-coding and clone sequencing suitable for composition assessment of bulk zooplankton samples?
    - Universal primers
    - Cloning bias
    - Low throughput

Can we use next generation sequencing to assess the composition of zooplankton assemblages?

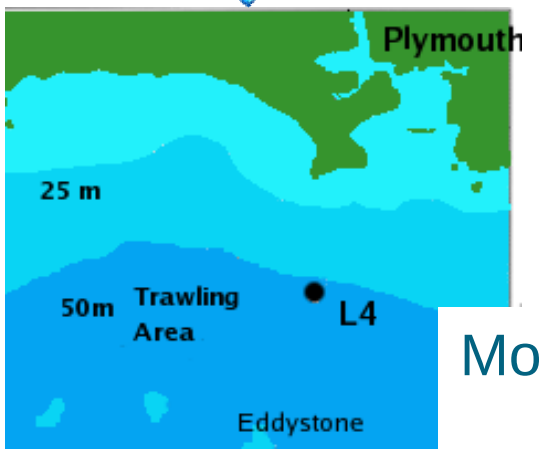


Amplicon application

ROCHE
GS FLX Titanium
454 sequencer

Experimental Design

- Long time series station L4
- Two temporal sampling points
  - September 2010
  - January 2011
- 4 replicate hauls
  - 50 m –surface
  - 200 μM mesh



Bulk Zooplankton Haul

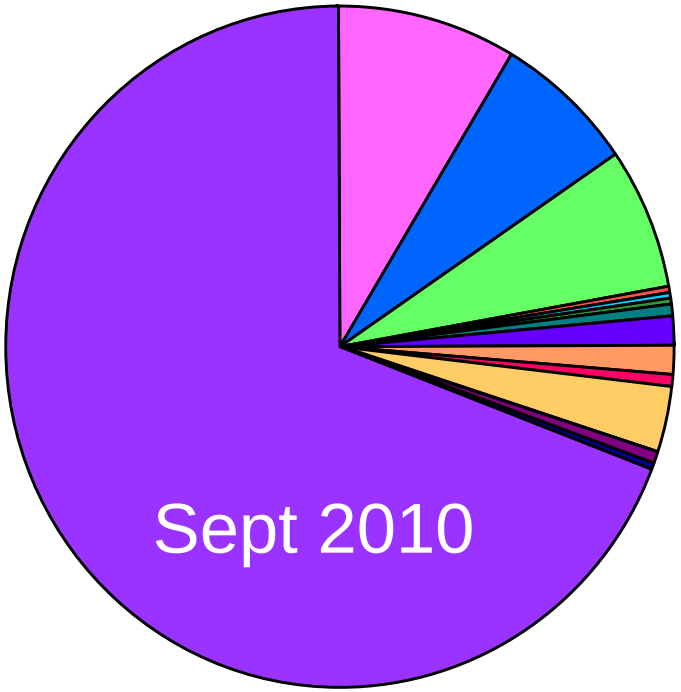
Morphological analysis

Molecular analysis

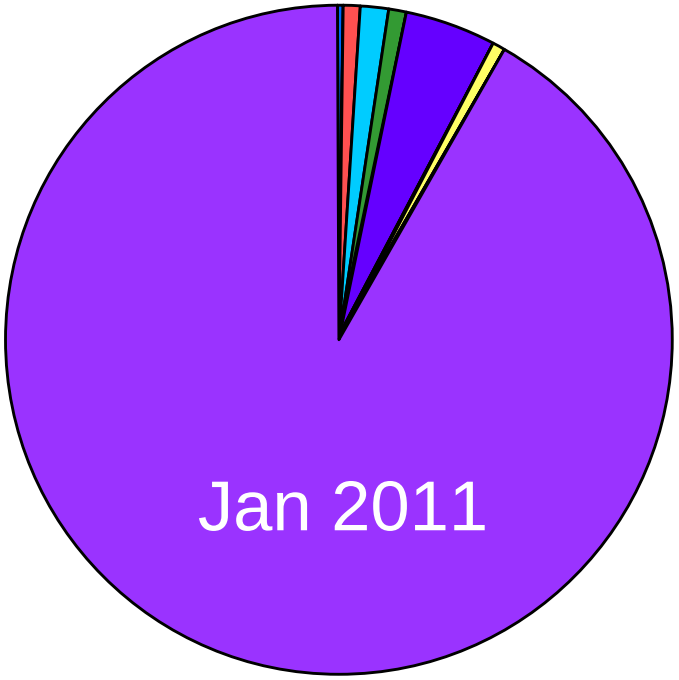
Taxonomic analysis

- 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

Zooplankton Community Structure



Total organisms per m^3 = 3249



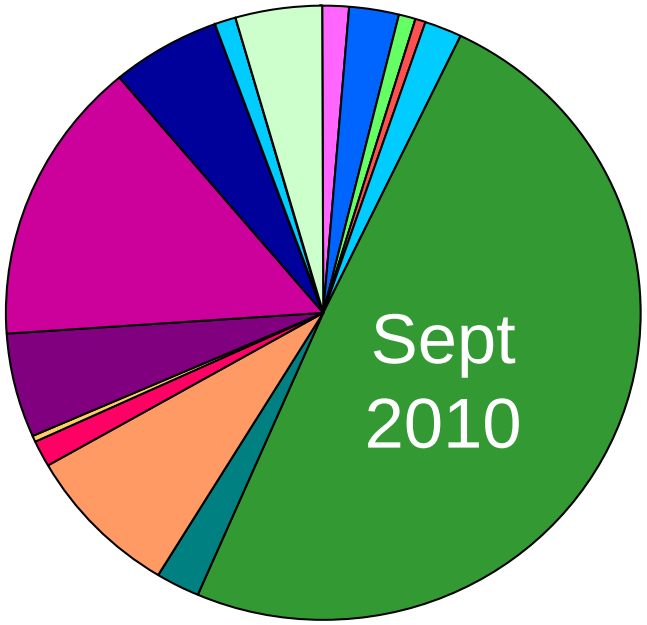
Total organisms per m^3 = 1643

- Noctiluca
- Hydromedusae
- Siphonophores
- Polychaete
- Chaetoganths
- Bryozoan larvae
- Prosobranch Gastropod
- Bivalve
- Other mollusc
- Echinoderm
- Appendicularian
- Fish
- Cladoceran
- Isopoda
- Decapoda
- Copepod

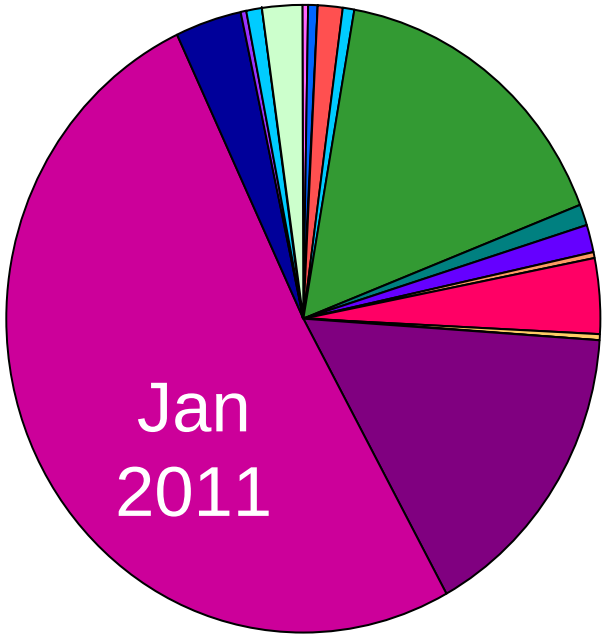
•Samples dominated by copepods in both months (69% September, 92% January)

• High numbers of the dinoflagellate *Noctiluca* as well as gelatinous zooplankton (hydromedusae and siphonophores) contributed to biomass in September

Copepod Community Structure



Total copepods per m^3 = 2251



Total copepods per m^3 = 1506

- Acartia clausi
- Centropages typicus
- Isias clavipes
- Temora longicornis
- Calanus helgolandicus
- Para/Pseudo/Cteno/Clauso Juveniles
- Clausocalanus arcuicornis
- Ctenocalanus vanus
- Paracalanus parvus
- Pseudocalanus elongatus
- Subeucalanus crassus 1-6
- Euchaeta hebes
- Oithona spp. unidentified
- Oncaea spp. unidentified
- Corycaeus spp. unidentified
- Microsetella rosea
- Euterpina acutifrons
- Clytemnestra rostrata
- Copepod nauplii

- Each month a total of 15 copepod species were identified
- September ~ 50% of copepods were copepodites of Calanoid copepods, unidentified to species level due to morphological similarities
- January was dominated by *Oncaea* spp. with high numbers of *Oithona* spp. and juvenile Calanoids as well

Molecular Analysis

DNA Isolation

- Phenol/chloroform extraction of total genomic DNA
- DNA extractions checked by agarose gel electrophoresis and UV absorption on a nanodrop

Fusion primers

18SEUKARY_F CCATCTCATCCCTGCGTGTCTCCGACTCAGgccagtagcatatgcttgtctc
18SEUKARY_R CCTATCCCCTGTGTGCCTTGGCAGTCTCAGagacttgcctccaatggatcc

Adapter sequence Tag/key 18S eukaryotic primers (Holland et al., 1991)

Amplicon PCR

Optimize amplicon PCR with fusion primers

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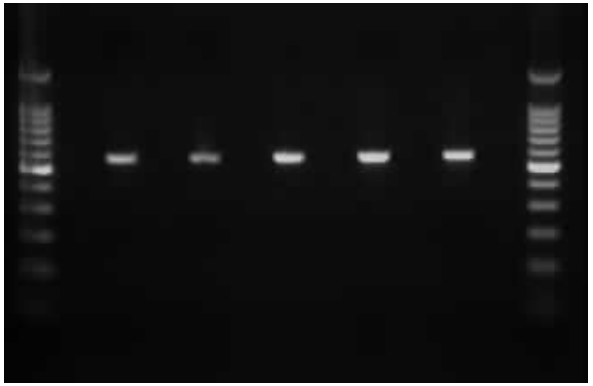
Triplicate PCR on genomic DNA



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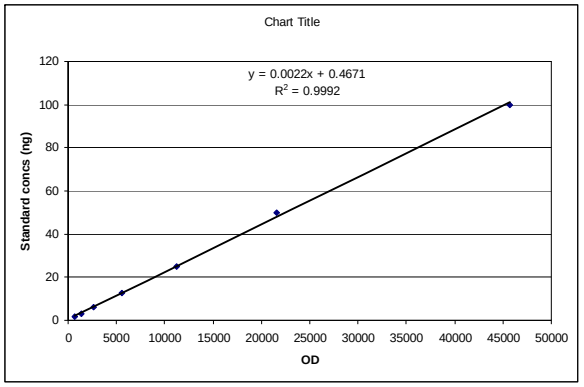
Gel extract amplicons



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Purify amplicon library

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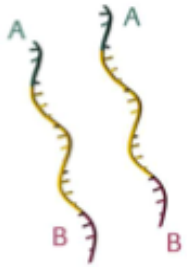
Quantify library of amplicons by fluorometry

454 Sequencing



Library of DNA molecules

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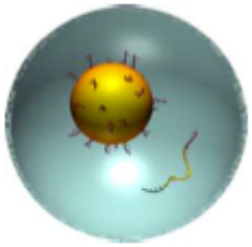

One DNA molecule per bead

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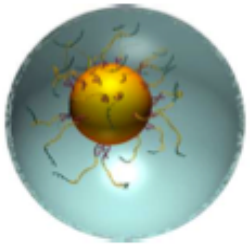

Clonal amplification to ~10 million copies

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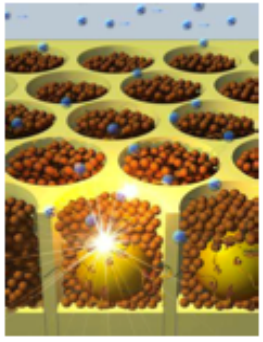

Independent sequencing of each bead

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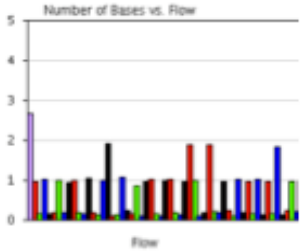


One Bead = One Read = One DNA molecule

Summary

Morphological analysis:

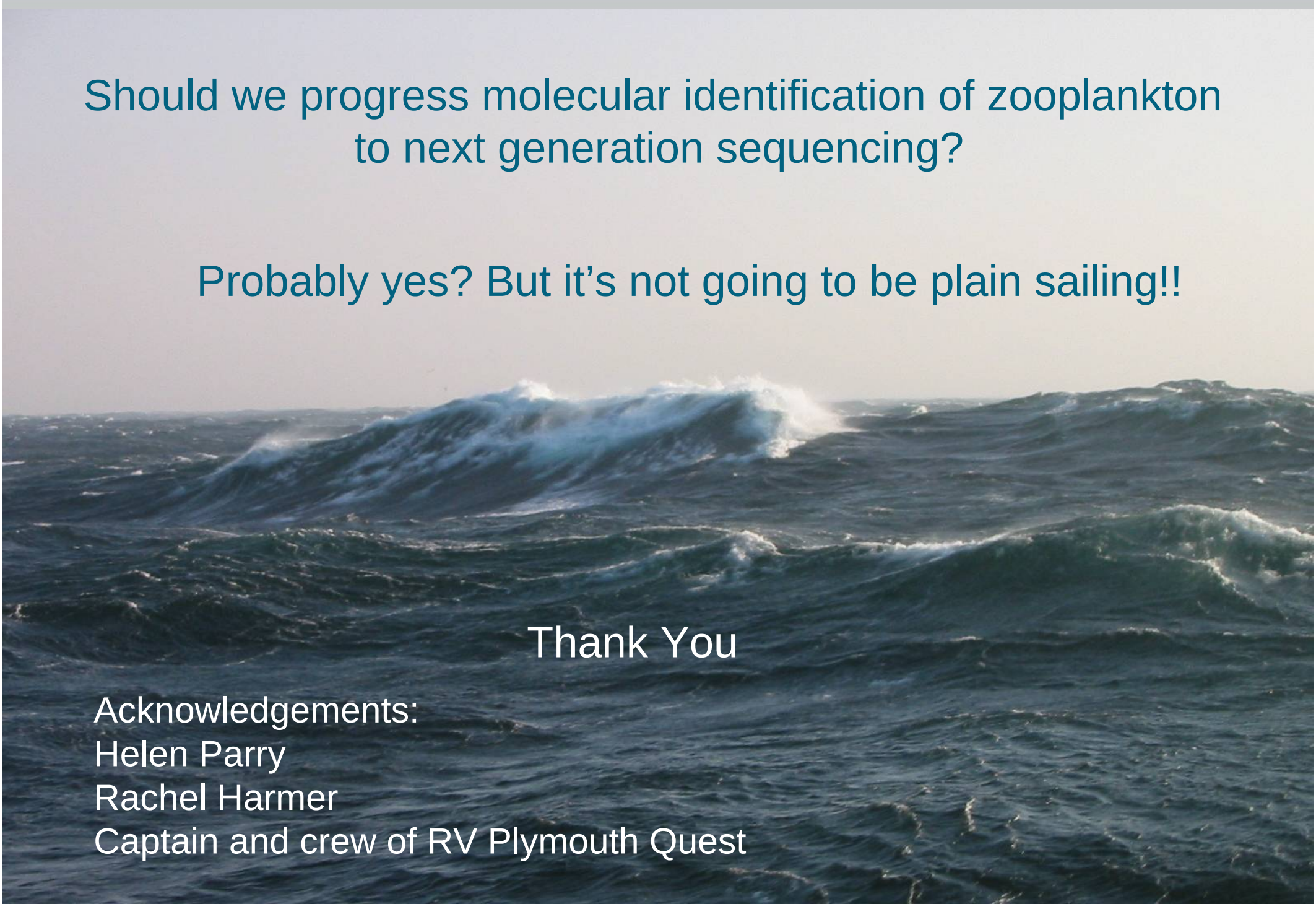
- Taxonomic resolution limited
- Quick, cheap and reliable

Next Generation Sequencing

- Excellent means of estimating species richness
- High throughput, high coverage zooplankton identification, giving improved access to rare genotypes
- Eliminates any cloning bias

However many problems remain:

- Universal primers
- Restricted amplicon length
- Expensive and technically not easy
- Computational resources for data analysis
- Availability of reference sequences in the database



Should we progress molecular identification of zooplankton to next generation sequencing?

Probably yes? But it's not going to be plain sailing!!

Thank You

Acknowledgements:
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