Can DNA provide a better representation of zooplankton diversity? A comparison of net versus water sampling

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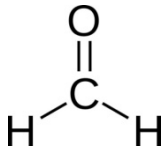
Reasons for genetic studies on preserved samples

- Hindcast genetic surveys are increasingly important to determine long-term biodiversity changes.
- Most samples are preserved immediately yet there are few studies on the effects of preservatives on genetic identification of marine plankton
- Genetics may aid the identification of challenging organisms e.g. jellyfish
- Factors affecting genetic identification
  - Species
  - formulation of preservative
  - sampling method

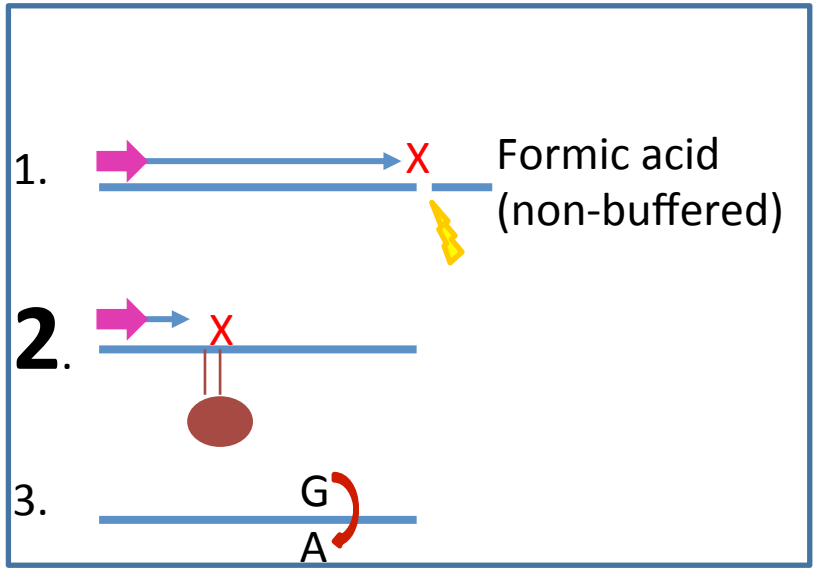
Issues with preservatives

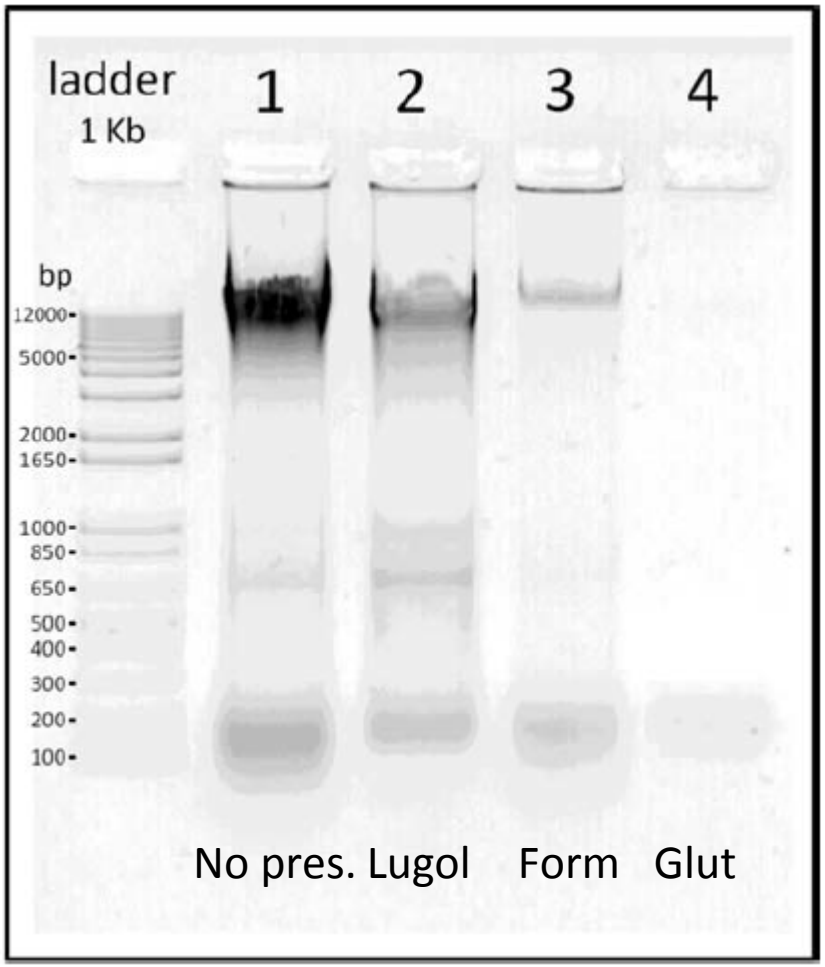
- Not good for all applications
  - Morphological preservatives diminish genetic testing
- Variable preservation effects
  - Water content
- How long do they work for?
- Ultimately end up with biased results: counts and diversity

All preservatives reduce DNA identification but to what degree?



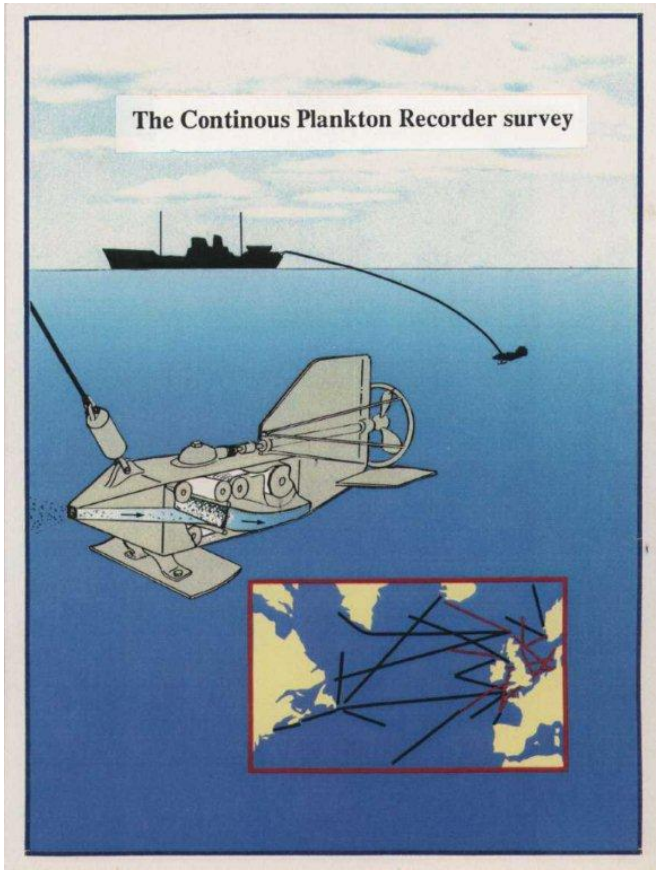
Formalin: Formaldehyde in liquid

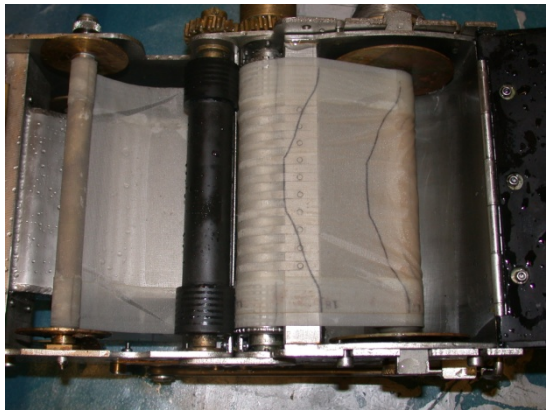


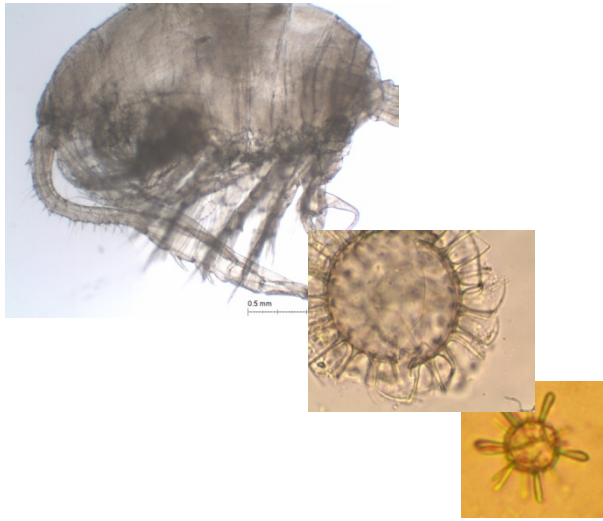


From Churro et al. 2015: Cyanobacterial DNA preservation

Introduction CPR survey

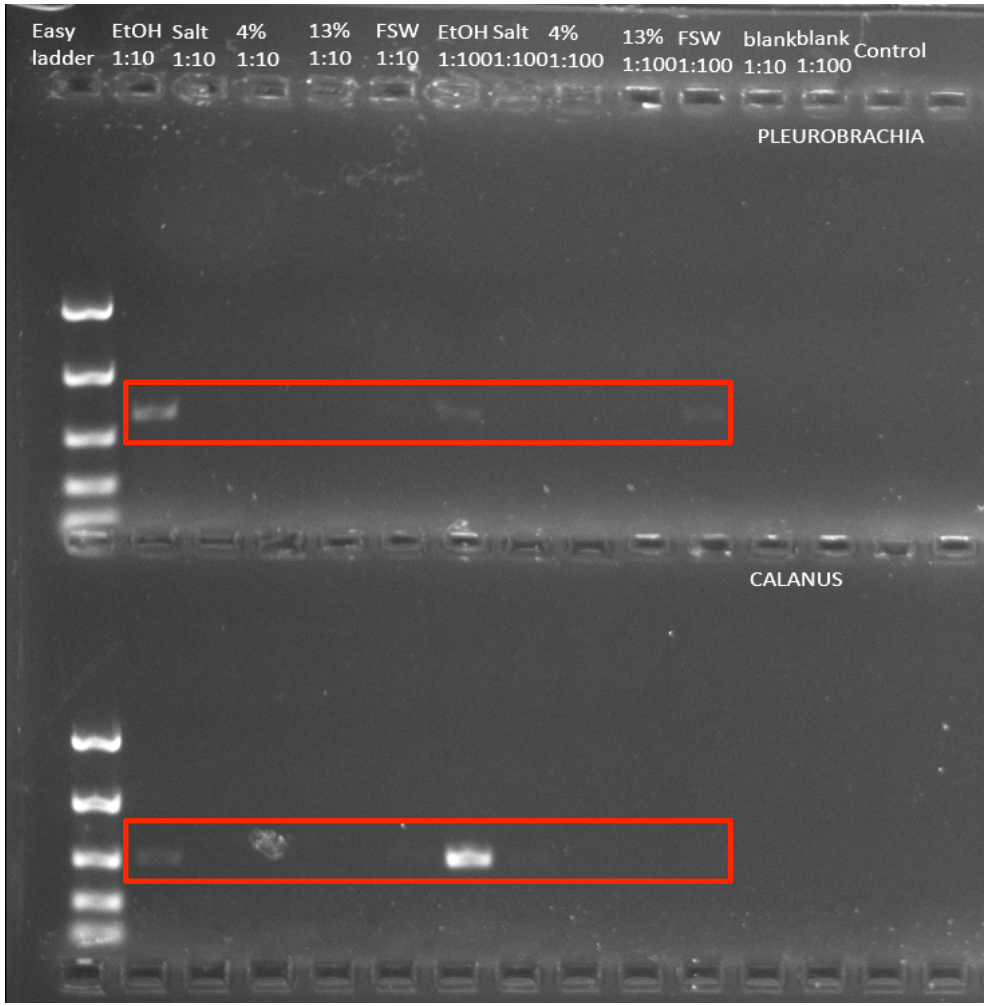






The effects of preservatives on DNA?

PCR from 2 week- preserved zooplankton



Eth: 96% Ethanol
S: RNAlater
Form: Formalin:
4%, 13%

COI Barcode primer
HCO2191/LCO1719
(Folmer et al. 1994)

Gelatinous:
Pleurobrachia sp.

Hard exoskeleton:
Calanus sp.

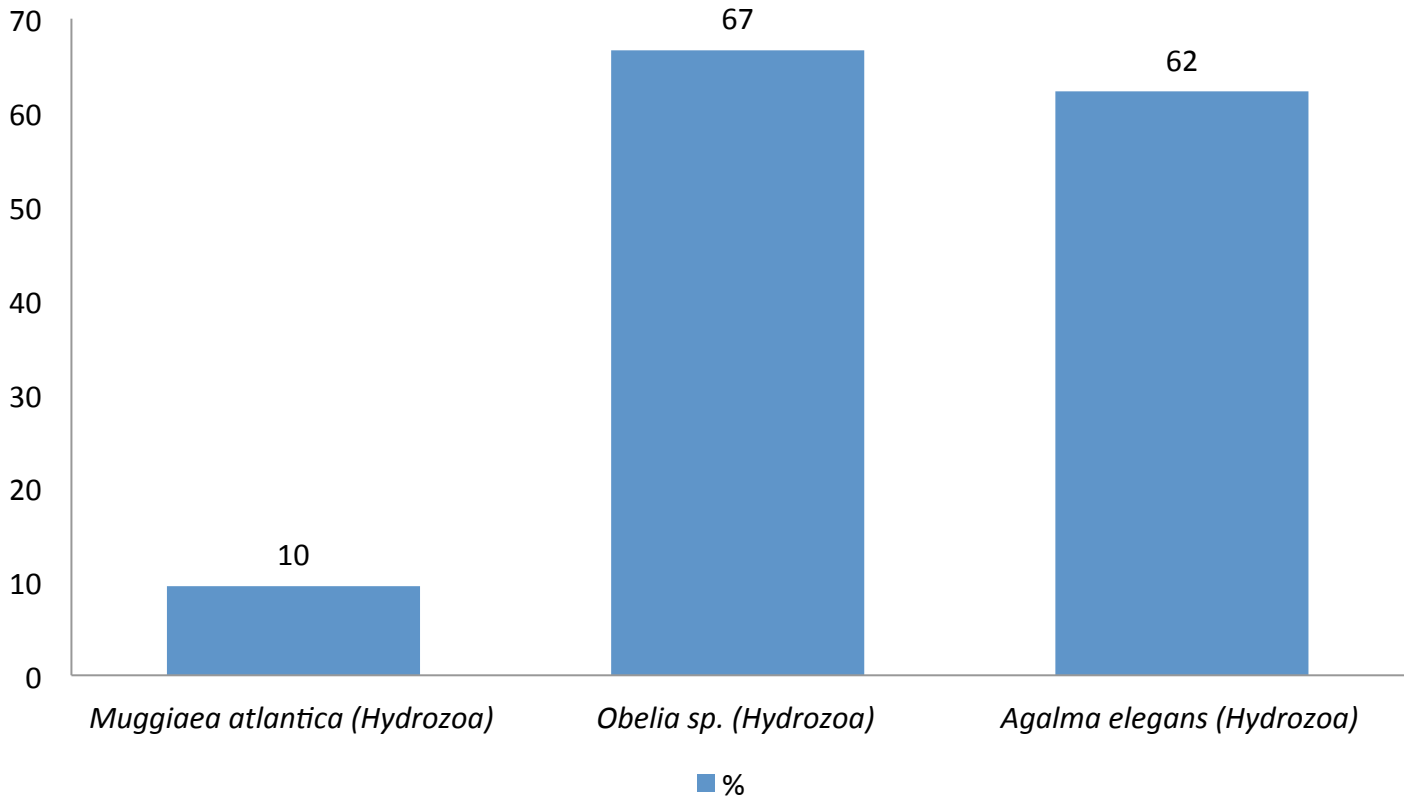
Gel photograph: A. Fischer

Cnidaria- a challenging zooplankton group

16S Primer 1 and 2
(Cunningham & Buss, 1993)

Data: A. Fischer

PCR-based detection varies between species, even within a target group (Cnidaria)

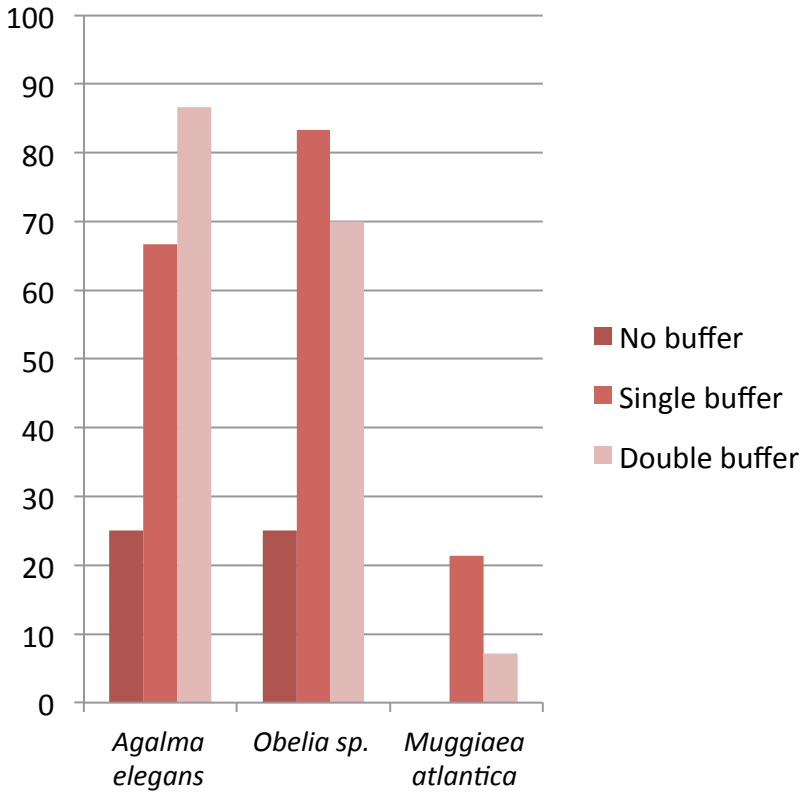


11-12 days in formalin,
Cnidarian 16S mt primer 1 and 2 (Cunningham & Buss, 1993),
DNAzol extraction.

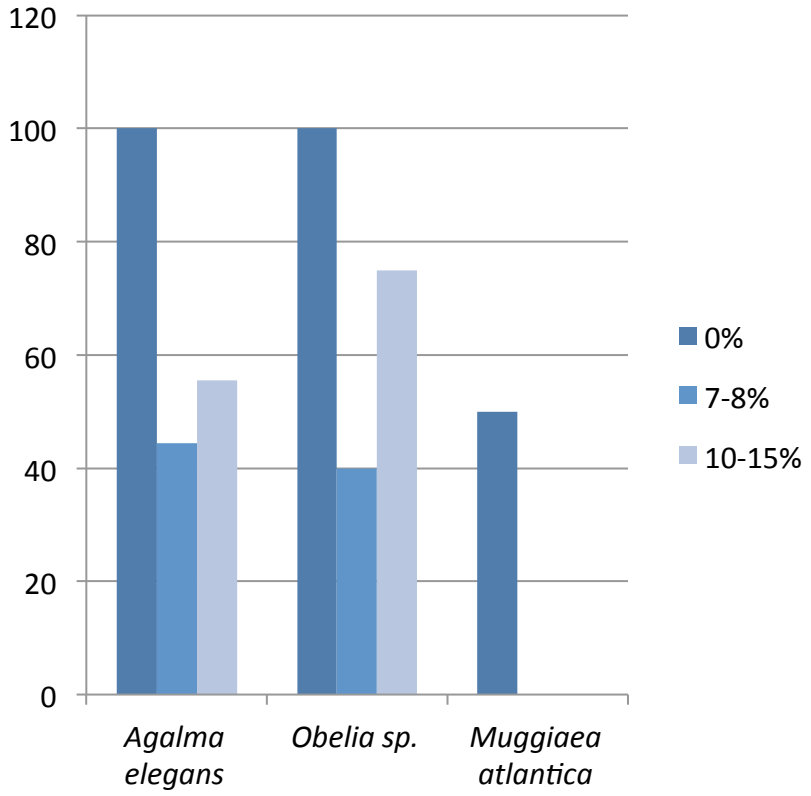
Data: A. Fischer

Formulation of formalin also alters PCR-based detection

Buffer effects



Methanol proportion effects



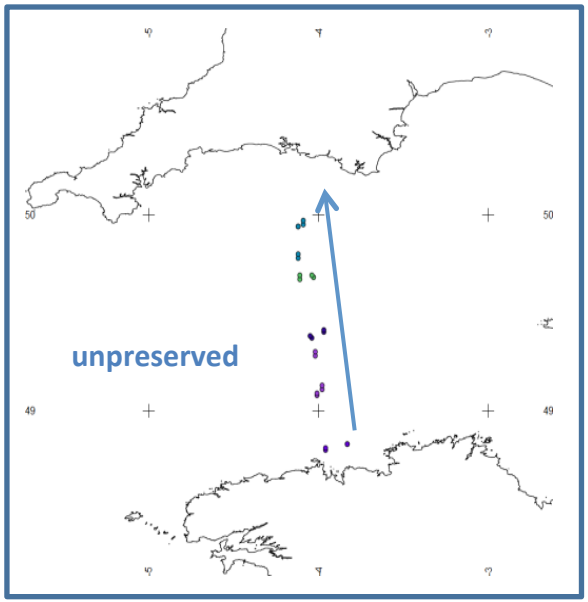
PCR amplification product after 11-12 days in formalin (16S mt primers 1 and 2*). DNAzol extraction.

* Cunningham & Buss, 1993

Data: A. Fischer

Field trial comparing morphological and genetic preservation from CPR and water samples

CPR plankton tow with internal water sampler in the English Channel



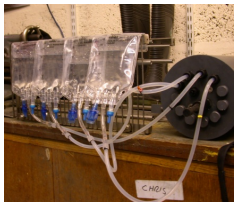
Micro-scopy 0 weeks

Cut ¼.

1: 4% formalin
2: 2% formalin
3: 80% ethanol
4: freezing

Microscopy 4,8 weeks

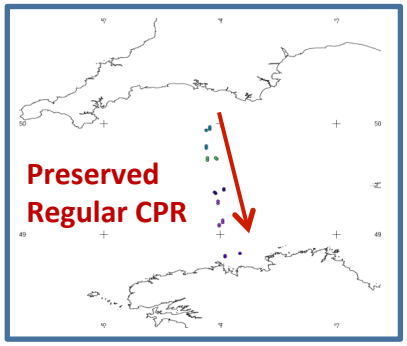
DNA extraction (8 weeks)



0.2l/sample

Post-preserved in ethanol

50ml DNA extraction

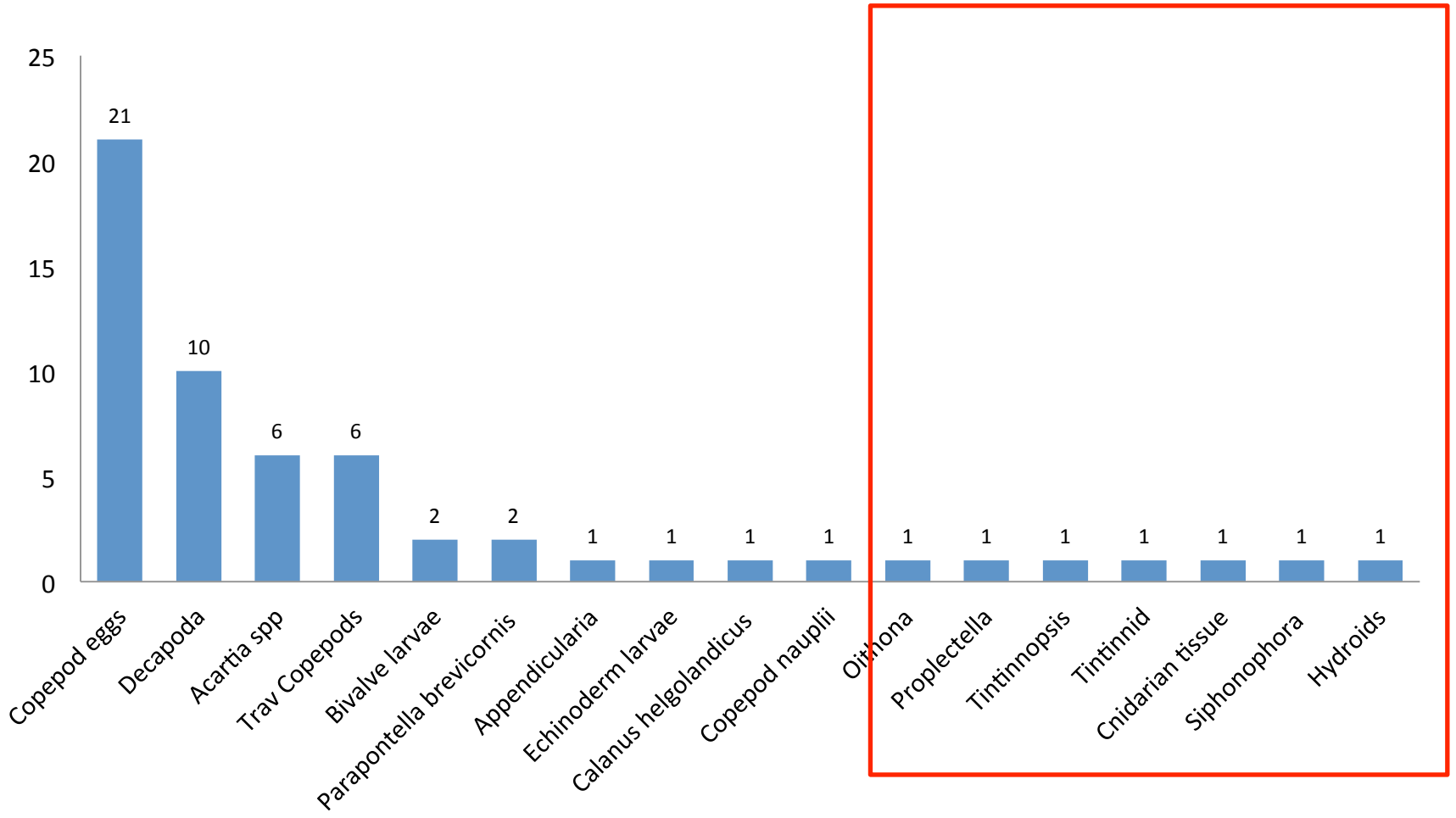


Micro-scopy 0 weeks

3000l/sample

DNA extraction (8 weeks)

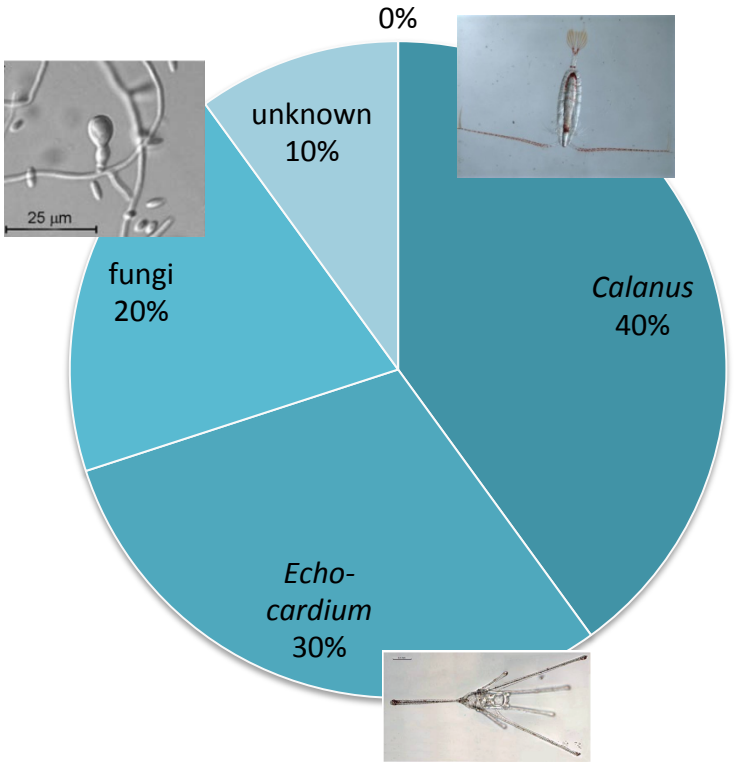
Zooplankton initially observed by microscopy on unpreserved CPR samples



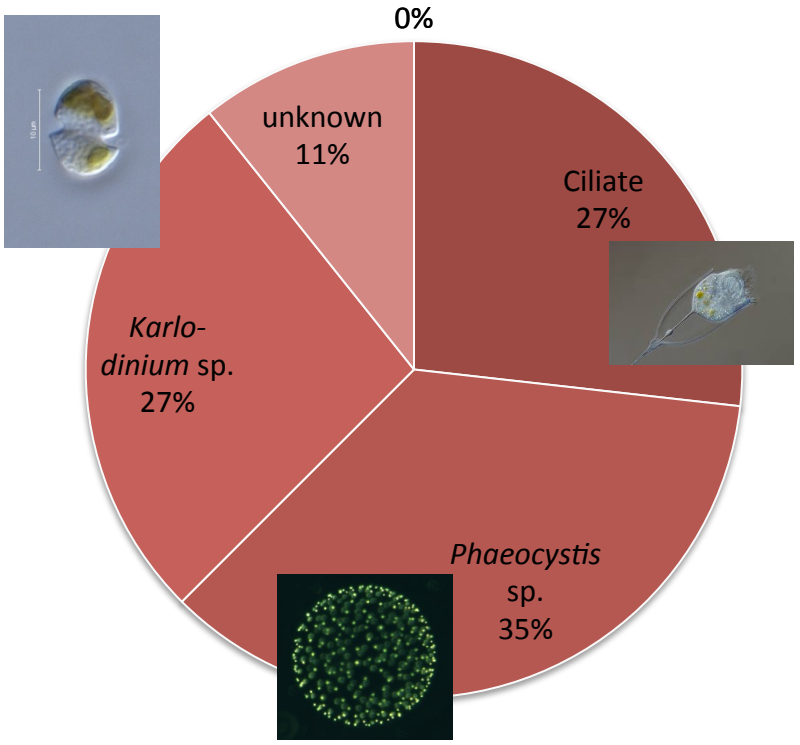
Presence/absence

Molecular identification reveals different taxa to microscopy

Eukaryotes on CPR (28S rDNA) Total preserved samples

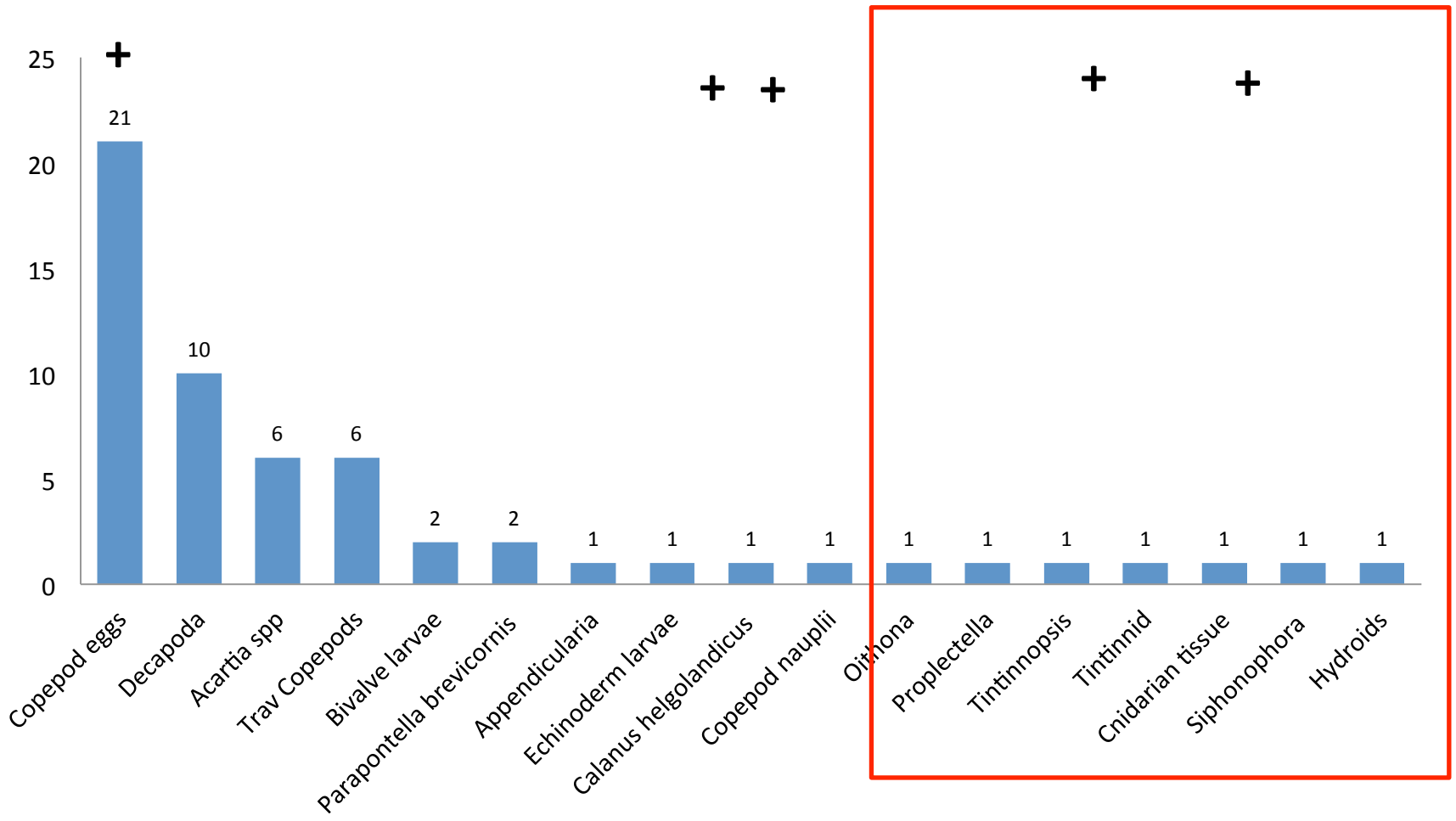


Eukaryotes in water samples (28S rDNA) Post preserved in EtOH



Images from Zhang et al. 2007, by Dr. Peter Countway, SCCOOS, Jean-Marie Cavanihac, microscopy-uk.org.uk

Zooplankton initially observed by microscopy on unpreserved CPR samples



+= also identified by DNA identification

Recorded as Presence/absence

Observations from the trials

- 80% ethanol and 2% formalin worked equally well for some taxa.
- Smaller organisms are observed in low-volume discrete water samples collected with the autonomous water sampler.
- e-DNA from larger organisms are also detected.
- Morphology is relatively intact using 70% ethanol for crustacea, diatoms and hydroids.

Recommendations

- Use a combination of different primer sets
- Investigate preservative formulation (buffers and other additives) and storage
- Lower % Formalin may aid molecular detection later
- De-crosslinking has been shown to much improve nucleic acid detection in formalin preserved samples (Karmakar et al. 2015, Nat. Chem. DOI 10.1038)

Thank you

- SAHFOS operations & analysis team and Ships of Opportunity for enabling this study