Integrating microscopy and multigene metabarcoding to unravel the hidden microzooplankton diversity





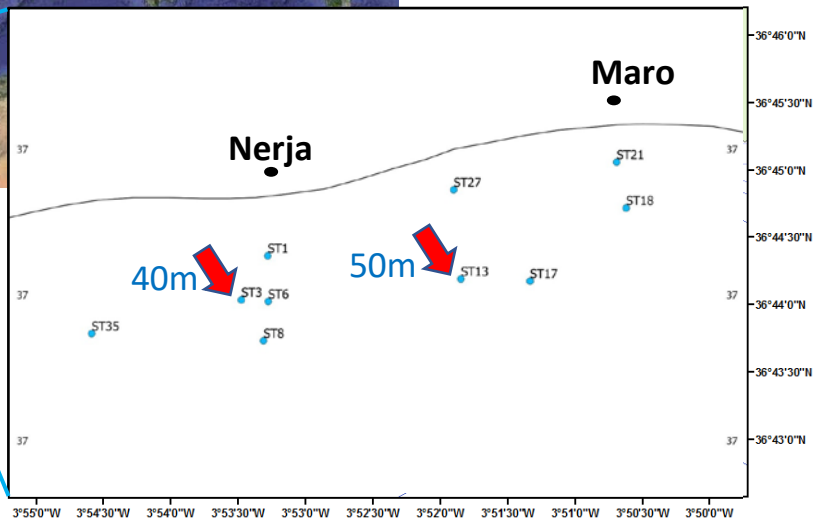
L. Yebra, M. Domínguez, A.M. Cabello, S. Salles, J. Vannini, G. Belmonte, R. Stern, N. Valcárcel-Pérez, I. Ferrera, J.M. Mercado

ECOLOGÍA DEL PLANCTON Y RETOS AMBIENTALES

CSIC

GOBIERNO DE ESPAÑA MINISTERIO DE CIENCIA E INNOVACIÓN

Study area: N Alboran coast in summer



Sampling stations location

9 tonnes of used wet wipes lying on the seabed

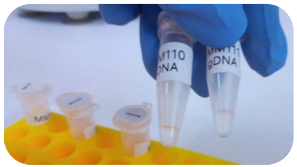
Untreated sewage discharged to the sea at 2 offshore outfalls

Multidisciplinary and integrative approach

- Environmental variables: T, S, nutrients, Chl a
- Microzooplankton: heterotrophs + mixotrophs

CalVET net (50-200 um)
Vertical hauls, bottom to surface

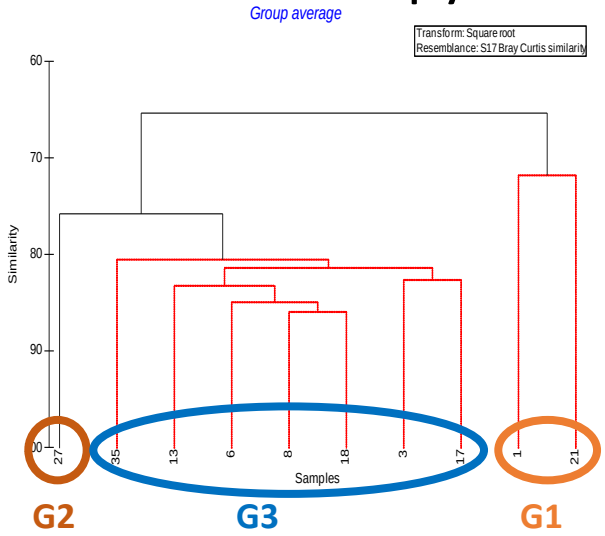
– liquid N_2
Metabarcoding: 18S v4 (PR2)
COI (MIDORI)



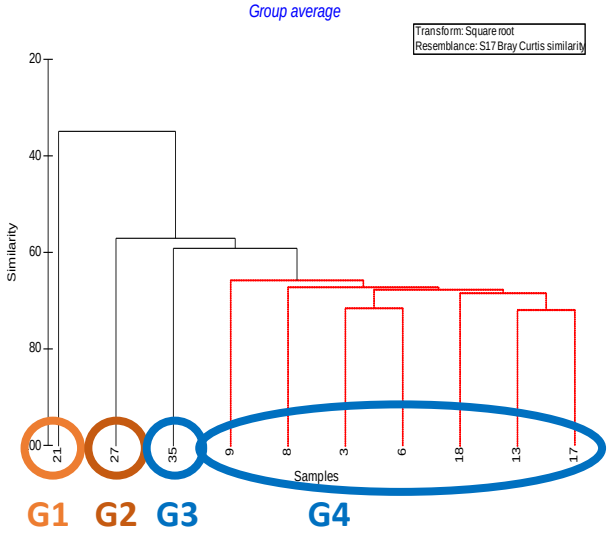
– etOH
Microscopy

Microzooplankton communities structure

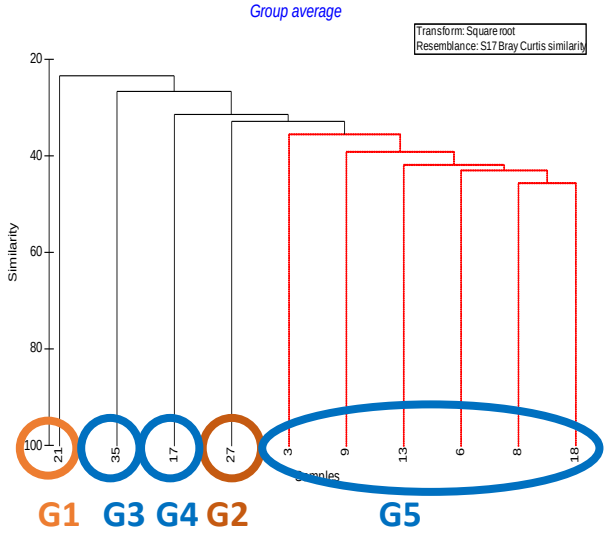
Microscopy



18S v4



COI

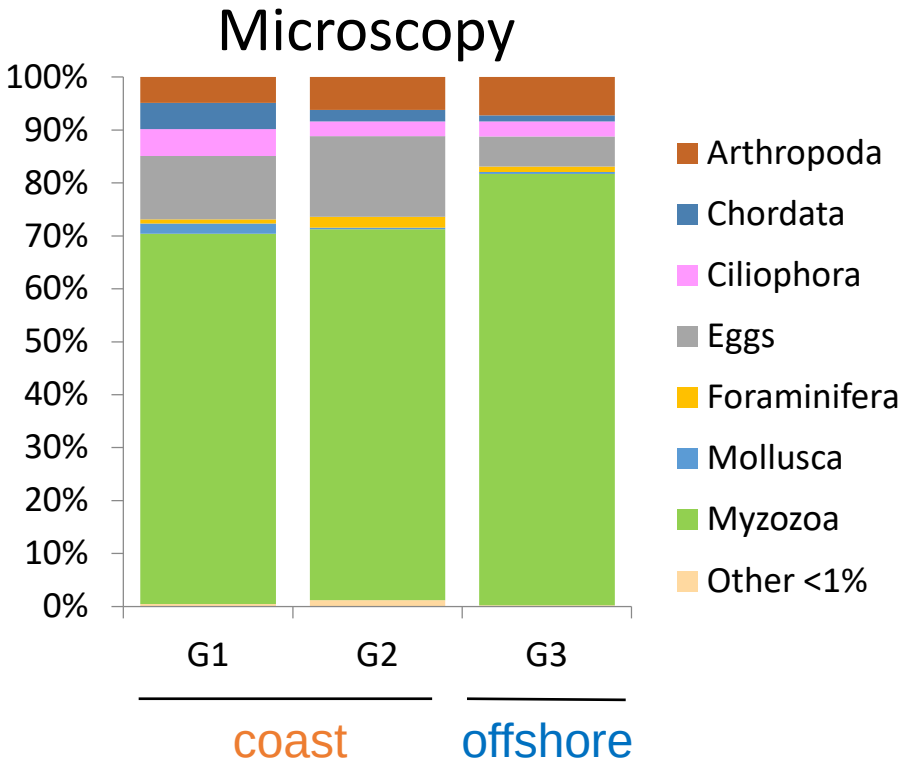


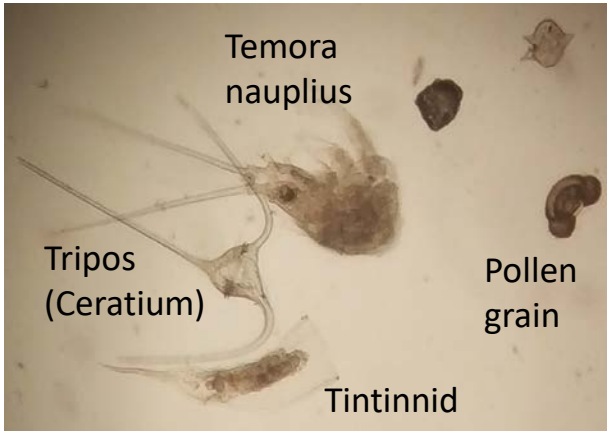
G1-G2 Coast 12 m / 23 m

G3-G5 Offshore 36-65 m

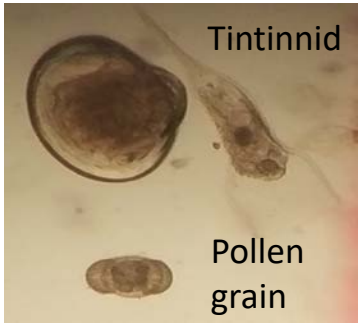
Including unassigned reads

Microzooplankton relative abundances



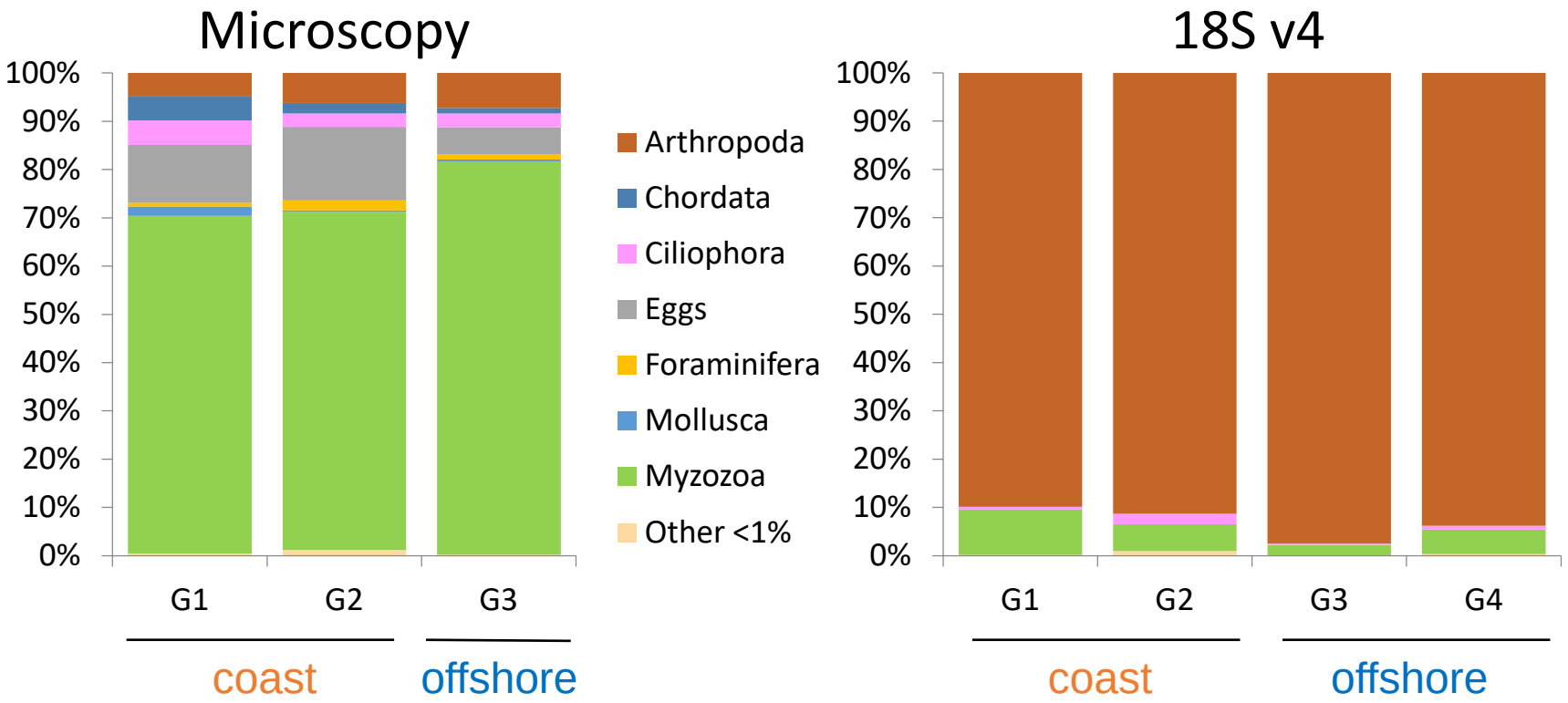


Bivalvia larvae



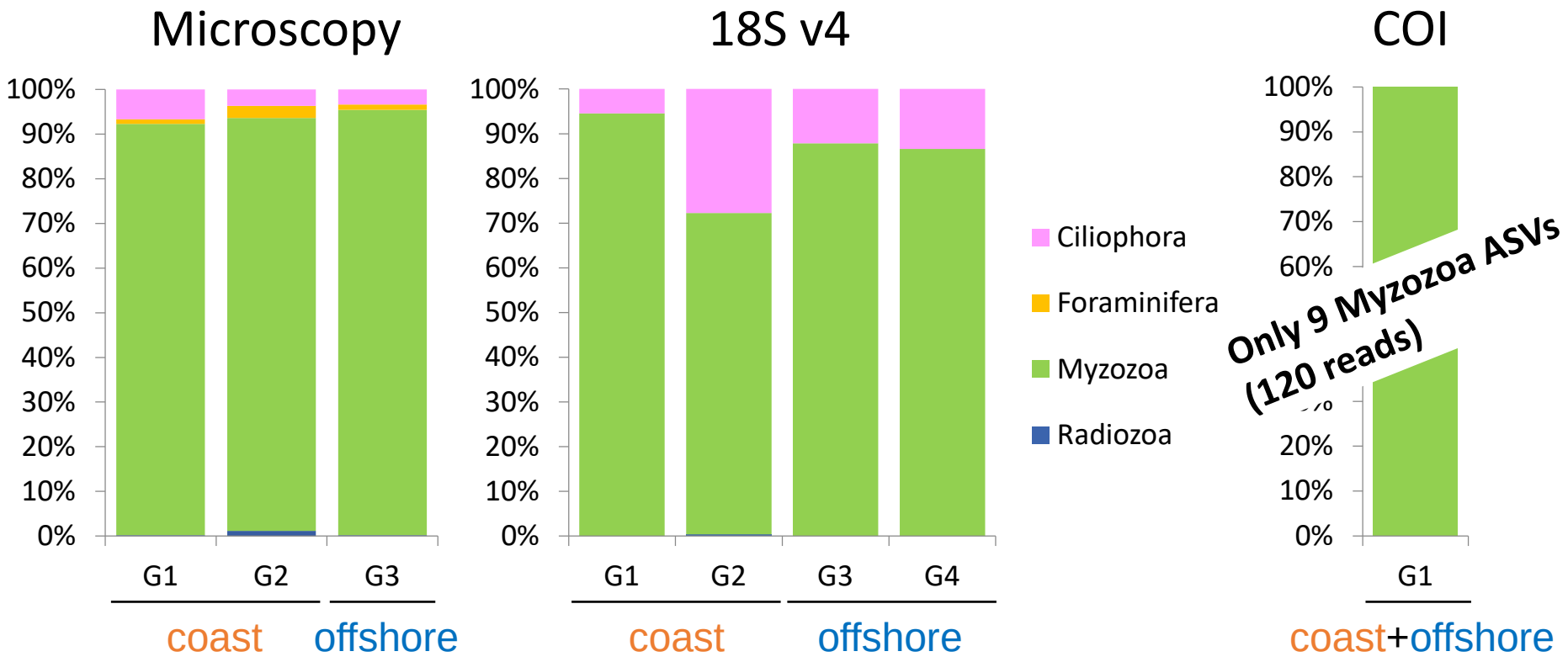
Taxa with abundance <1% were grouped in Other

Microzooplankton relative abundances

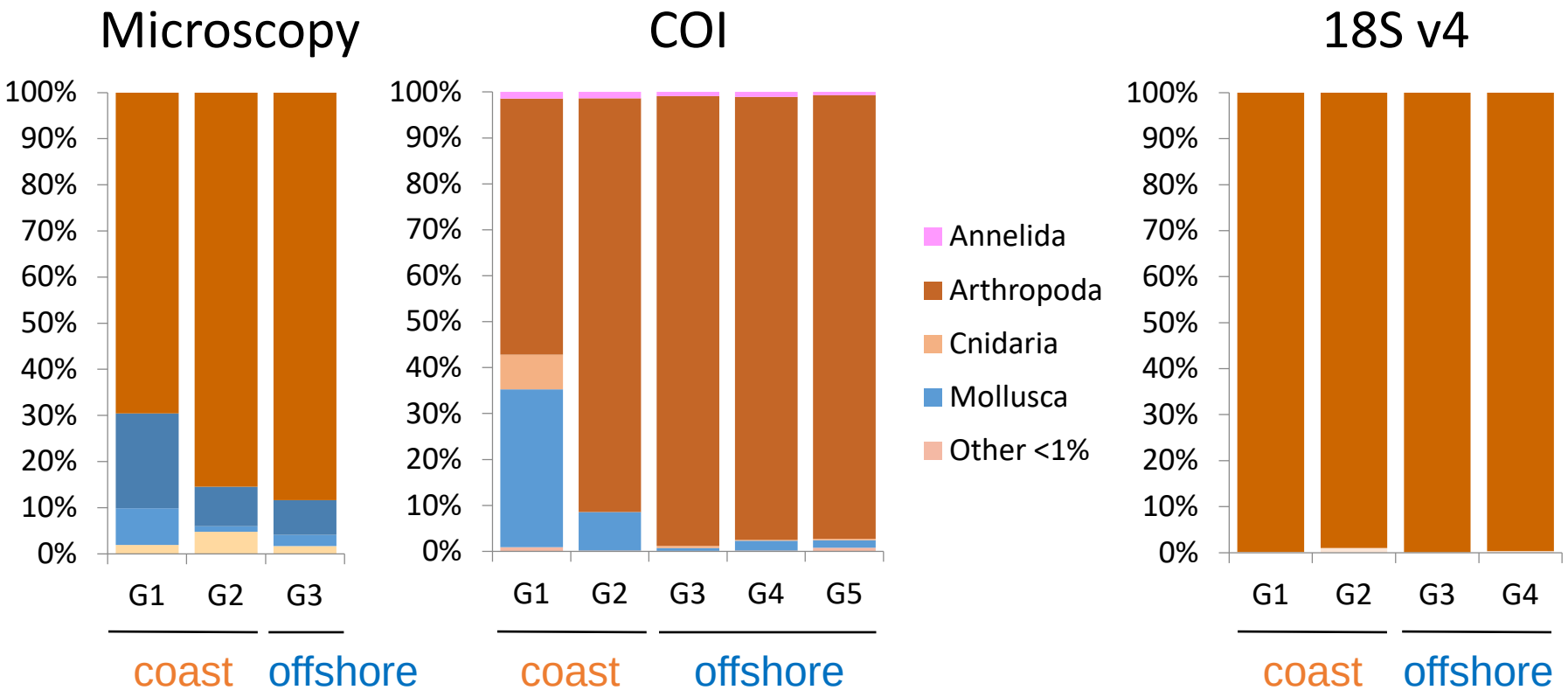


Need to compute protists and metazoans separately

Protista relative abundances



Metazoa relative abundances



Phyla detected

Protista

Microscopy	18Sv4	COI
Ciliophora	Ciliophora	
Foraminifera		
Myzozoa	Myzozoa	Myzozoa
Radiozoa	Radiozoa	

Metazoa

Microscopy	18Sv4	COI
Annelida	Annelida	Annelida
Arthropoda	Arthropoda	Arthropoda
Bryozoa		
Chaetognatha		Chaetognatha
Chordata	Chordata	Chordata
Cnidaria	Cnidaria	Cnidaria
Echinodermata	Echinodermata	Echinodermata
Mollusca	Mollusca	Mollusca
	Nematoda	Nematoda
		Nemertea
Phoronida		Phoronida
	Platyhelminthes	
	Porifera	Porifera
	Xenacoelomorpha	
Rotifera		Rotifera

Identification level

		Microscopy	18Sv4	COI
Protista	Phylum	**4**	3	1
	Class	5	**7**	1
	Order	9	**20**	2
	Family	27	**34**	5
	Genus	28	**41**	6
	Species	23	**24**	5
Metazoa	Phylum	10	10	**12**
	Class	18	15	**22**
	Order	18	18	**55**
	Family	30	32	**118**
	Genus	26	31	**144**
	Species	24	26	**155**

Example coastal station (G2)

Protista

microscopy	%	18S V4	%	COI	%
Protoperidinium sp.	41.1	Ciliophora	25.8	*Ansanella granifera*	100.0
Thecate dinoflagellate	12.7	Dinophyceae	15.5		
Dinophysis caudata	11.8	*Pelagodinium beii*	14.6		
Ceratium sp.	10.6	Fragilidium	11.5		
Tripos candelabrum	4.6	*Triadinium polyedricum*	6.7		
Tripos fusus	2.5	Gymnodinium	3.7		
Dinophysis	2.4	Suessiaceae	2.6		
Stenosemella sp.	2.3	Ichthyosphonida	2.6		
Globigerinidae	2.0	*Gymnodinium catenatum*	2.4		
Athecate dinoflagellate	1.9	Abeoformidae	2.1		
Pyrophacus orologium	1.1	Pelagodinium	1.7		
Tripos furca	1.1	*Protodinium simplex*	1.6		
		Thoracosphaeraceae	1.4		
		Gonyaulax spinifera	1.1		
		Biecheleria	1.0		

Metazoa

microscopy	%	18S V4	%	mtCOI	%
Eggs	63.0	Thecostraca	41.7	*Oithona nana*	50.6
Oncaeidae	7.9	*Temora discaudata*	34.0	*Oncaea waldemari*	22.7
Oithonidae	5.4	Oithona	21.9	*Temora stylifera*	10.9
Doliolidae	5.2	*Paracalanus parvus*	1.0	*Pleurobranchaea meckeli*	2.7
Oikopleura	3.3			*Paracalanus quasimodo*	2.3
Evadne sp.	1.9			Calanoida	1.2
Euterpina acutifrons	1.6			*Haminoea orteai*	1.1
Acartiidae	1.5			*Hancockia uncinata*	1.1
Calanoida	1.4				
Paracalanidae	1.4				
Temora stylifera	1.1				

Summary

- All approaches discriminated coast *vs* offshore
- Dominance of metazoan over protist reads: Separated analyses
- Limited reference databases protists + metazoans
- Identification level differed among methods & taxa
- Integrated approach revealed higher diversity

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