PICES 2015 Annual Meeting

Using 454 pyrosequencing to analyze the *in situ* diet of the marine copepod *Calanus sinicus*

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What copepods eat?

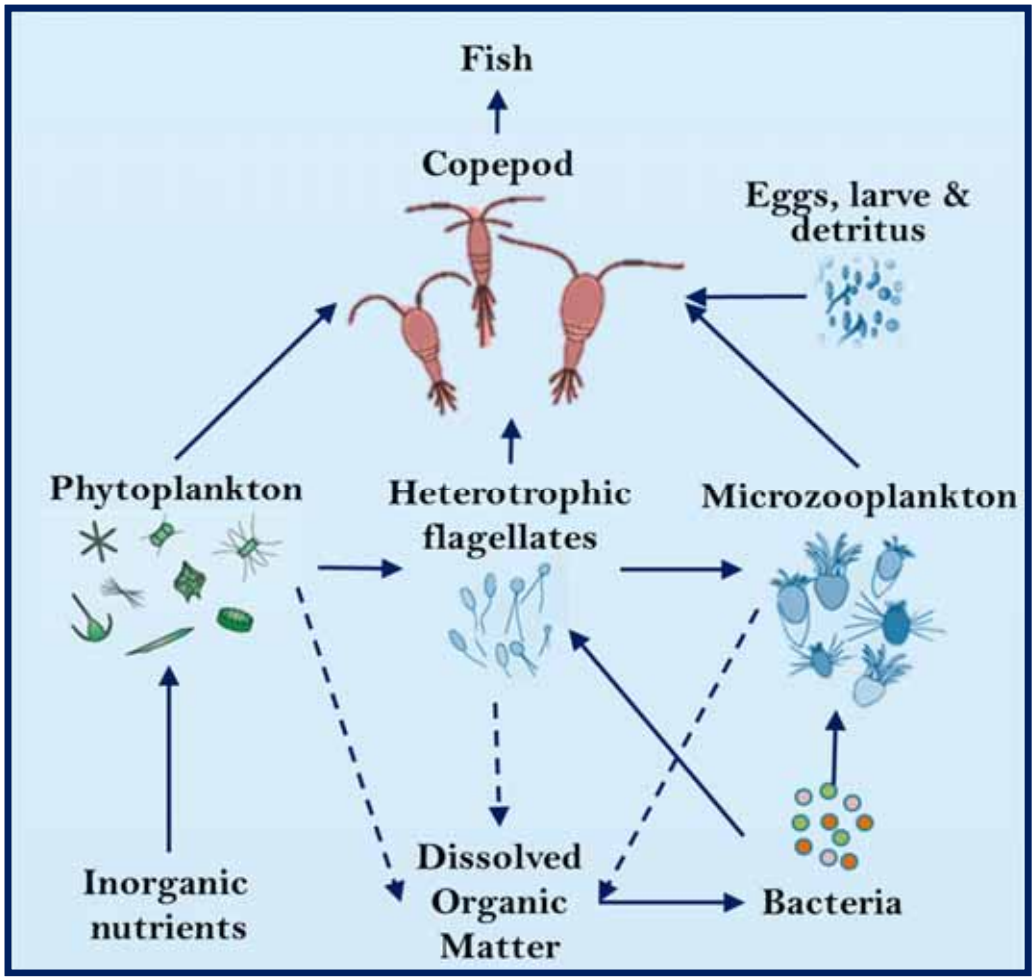
Copepod diet

- Diverse
- Flexible
- Specific preference

Calanus sinicus

- Calanoid copepod
- Large size (2-3mm)
- Common in the shelf waters of China, Japan & Korea
- Ecological important

http://mits10.aori.u-tokyo.ac.jp/lirc



***in situ* diet composition?**

How to assess copepod diet composition?

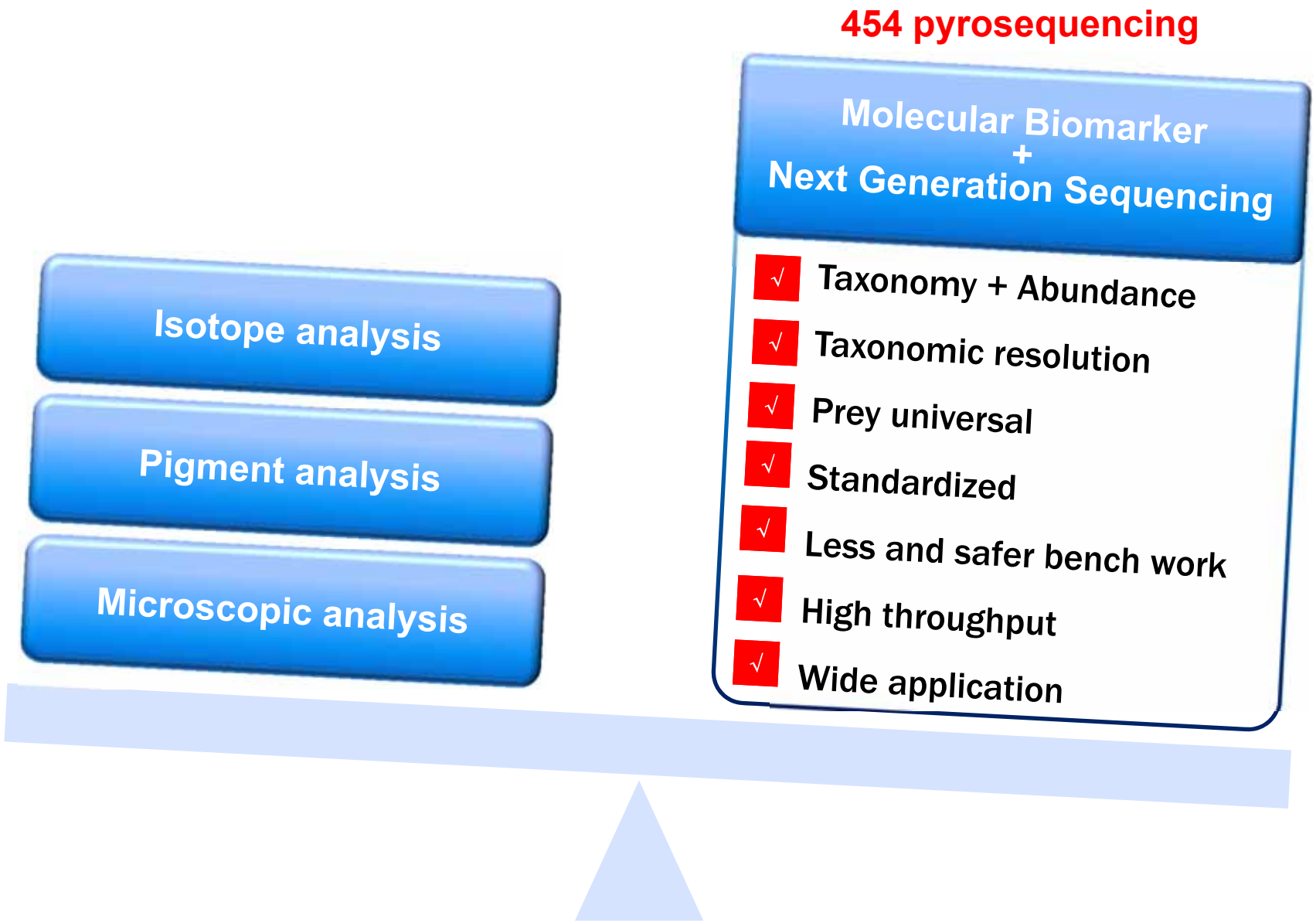
- Isotope analysis
- Pigment analysis
- Microscopic analysis

X

Molecular Biomarker of prey (rRNA, COI)

- √ Taxonomy + Abundance
- √ Taxonomic resolution
- √ Prey universal
- √ Standardized

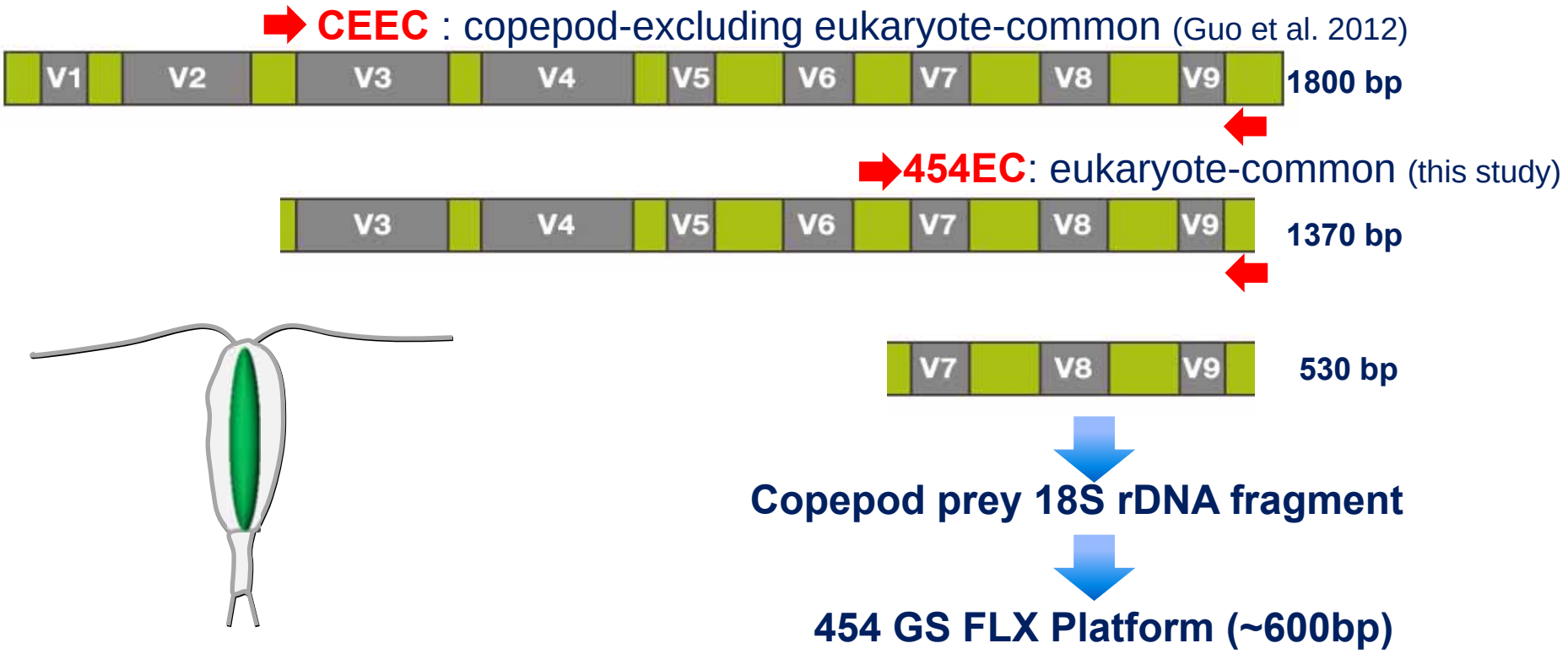
How to assess copepod diet composition?



Copepod prey biomarker in this study

Eukaryotic small subunit ribosomal RNA gene: 18S rDNA

- Prevalent in all eukaryotes
- Informative: large size (~1.8kb) with both conserved and variable region
- Different taxonomic resolution
- Largest eukaryotic database



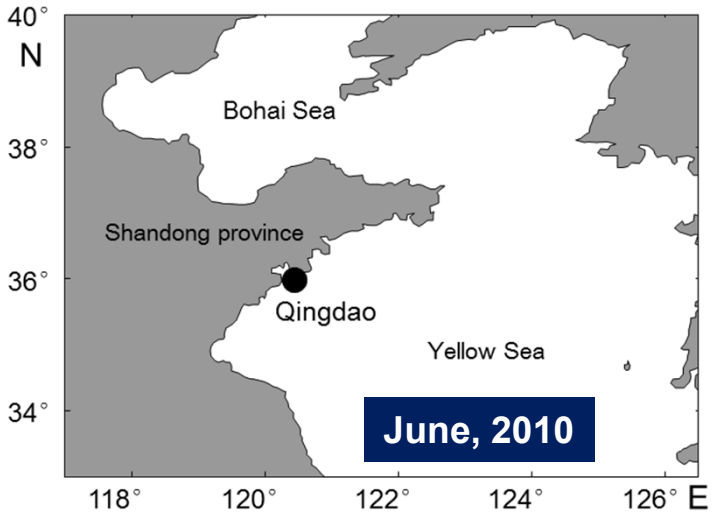
Objectives

Method Validation

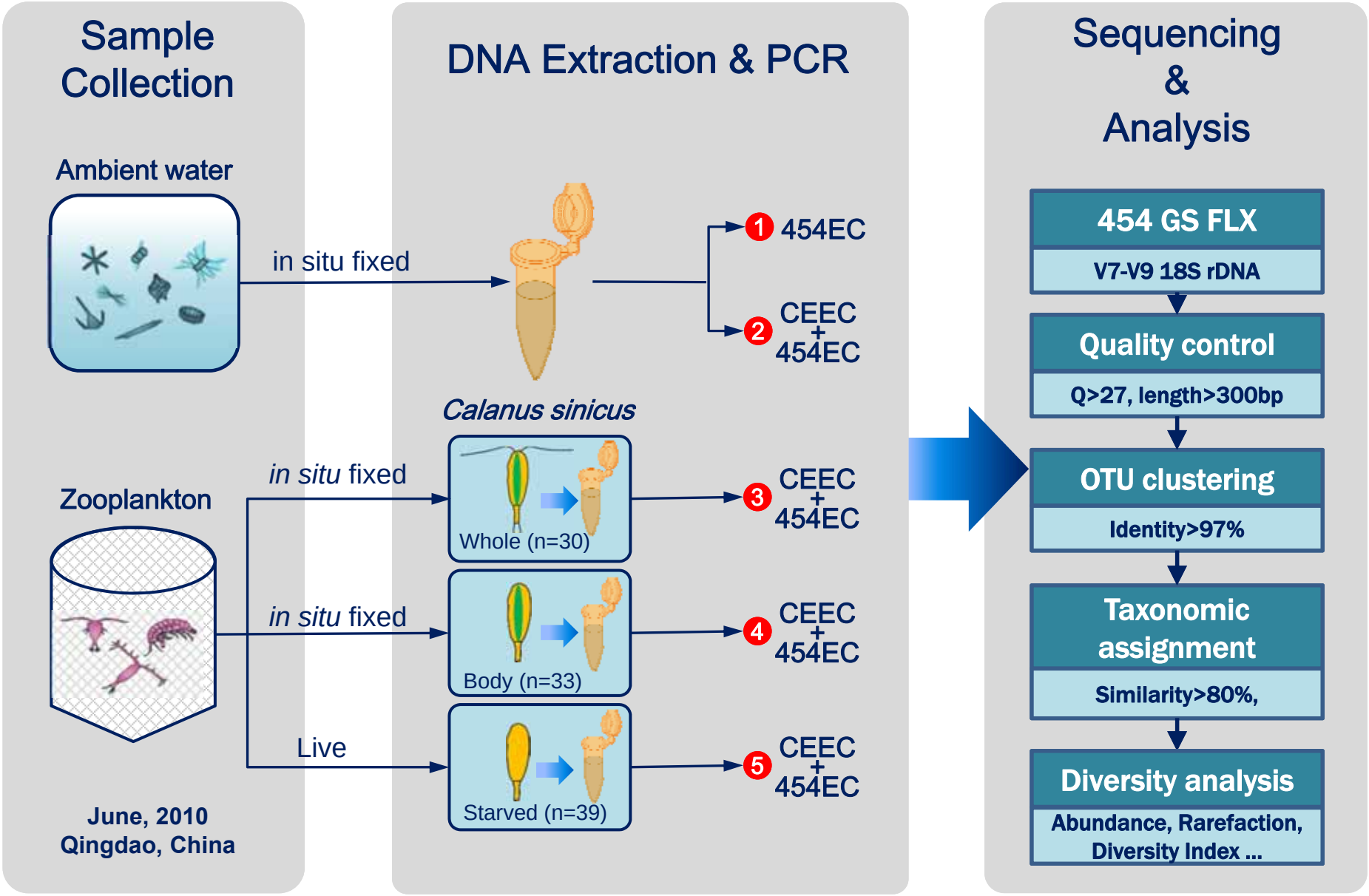
- Is primer CEEC + 454EC copepod-excluding?
- Is primer CEEC + 454EC eukaryote-common?
- How is the taxonomic resolution?

Case Study: *Calanus sinicus*

- What do they eat?
- Any feeding preference?



Work flow


Sample Collection
Ambient water
in situ fixed
Zooplankton
in situ fixed
in situ fixed
Live
June, 2010
Qingdao, China
DNA Extraction & PCR
Calanus sinicus
Whole (n=30)
Body (n=33)
Starved (n=39)
1 454EC
2 CEEC + 454EC
3 CEEC + 454EC
4 CEEC + 454EC
5 CEEC + 454EC
Sequencing & Analysis
454 GS FLX
V7-V9 18S rDNA
Quality control
Q>27, length>300bp
OTU clustering
Identity>97%
Taxonomic assignment
Similarity>80%,
Diversity analysis
Abundance, Rarefaction, Diversity Index ...

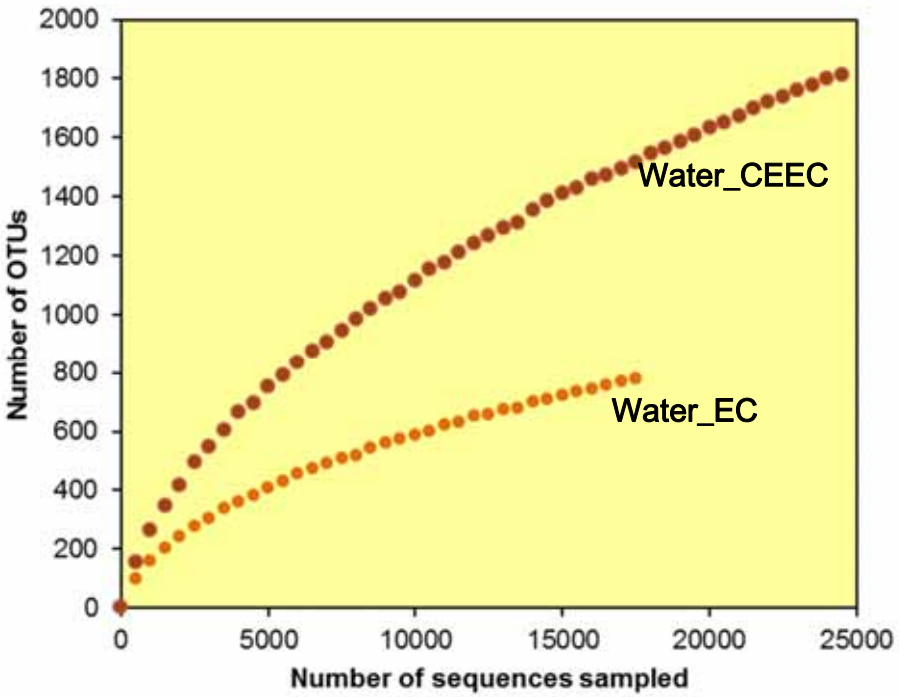
Results: sequencing data overview

Library ID	Water_CEEC	Water_EC	Calsi_Whole	Calsi_Body	Calsi_Starved	Total
Samples						
DNA source	Ambient Water		Copepod			
			Whole	Body	Starved	
Primers	CEEC+454EC	454EC	CEEC+454EC			
Raw data						
No. of raw reads	19,196	29,306	3,393	7,306	7,236	66,437
Average length (bp)	457	432	475	460	450	455
Total no. of bases (Mbp)	8.77	12.65	1.61	3.36	3.26	29.66
Quality filtering						
No. of high-qual reads	19,172	29,289	3,376	7,287	7,230	66,354
Average ength (bp)	394	385	415	402	405	400
Effective (%)	99.87	99.94	99.5	99.74	99.92	99.79
OTU clustering						
No. of non-singleton OTUs	973	559	70	139	25	1278
No. of singleton	868	218	39	127	10	1262
% of singleton	47.15	28.06	35.78	47.74	28.57	49.69
Total no. of OTUs	1,841	777	109	266	35	2,540
Taxonomic assignment						
Undetermined OTU (%)	4.73	6.83	2.75	6.39	2.86	5.63
Assigned at class (%)	94.35	92.14	96.33	90.98	97.14	93.19

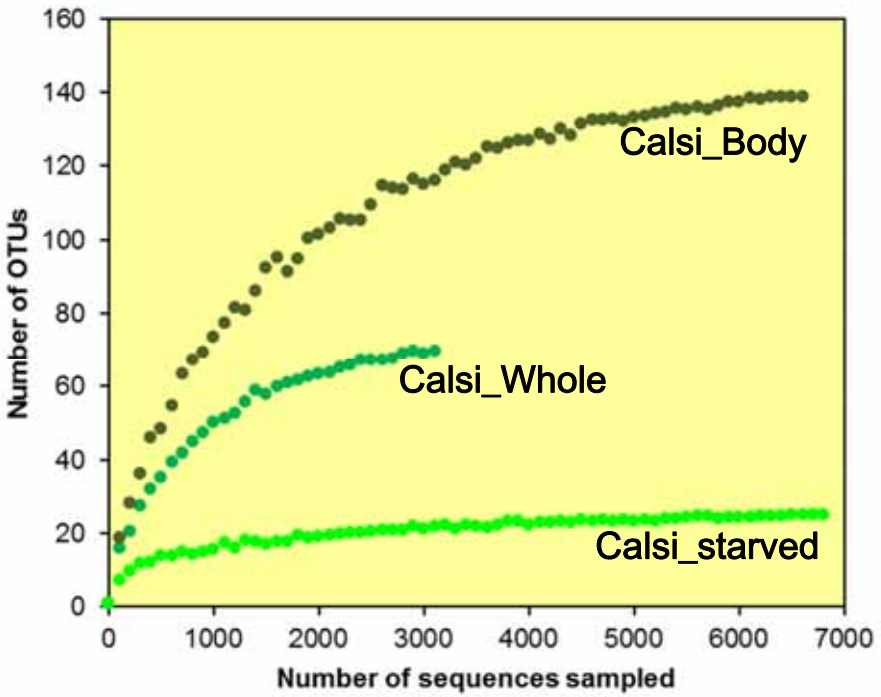
Results: sequencing depth enough?

Rarefaction curve

Water



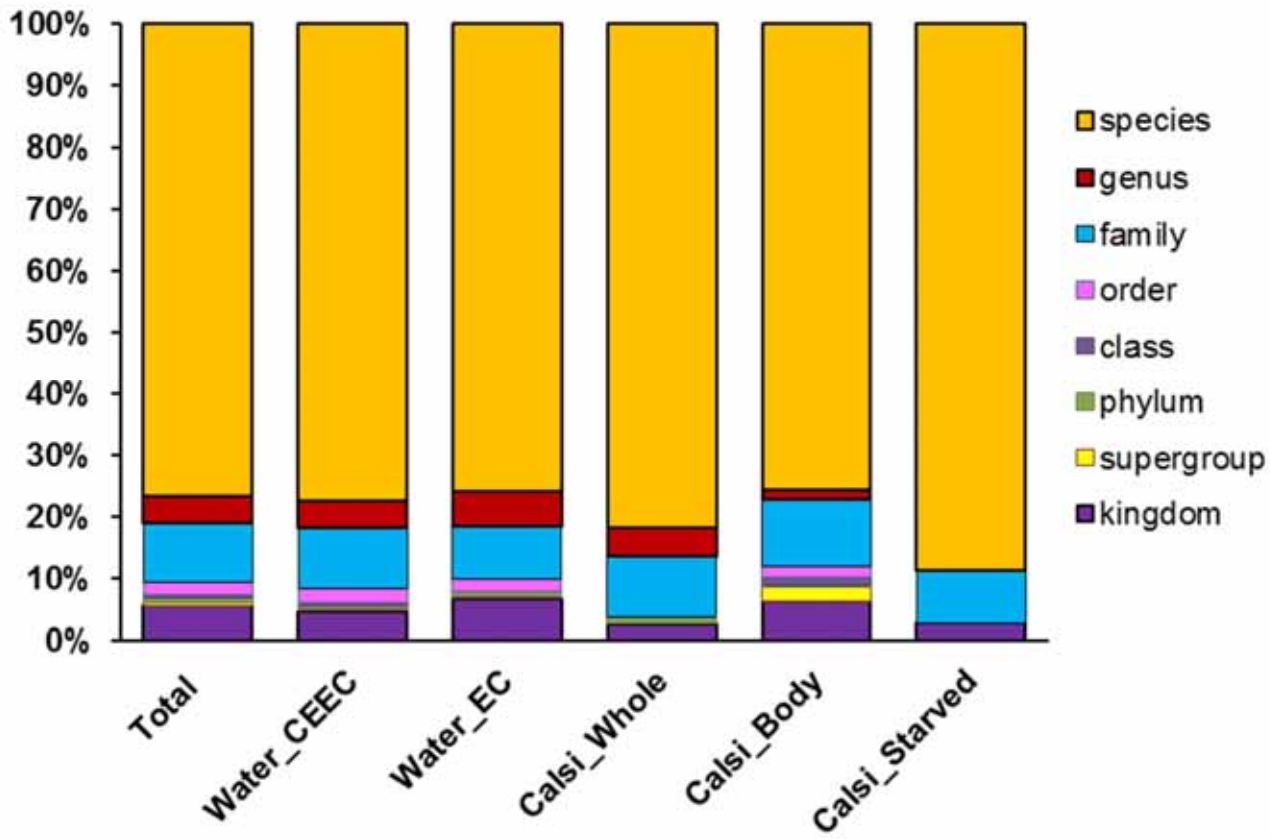
Calanus sinicus



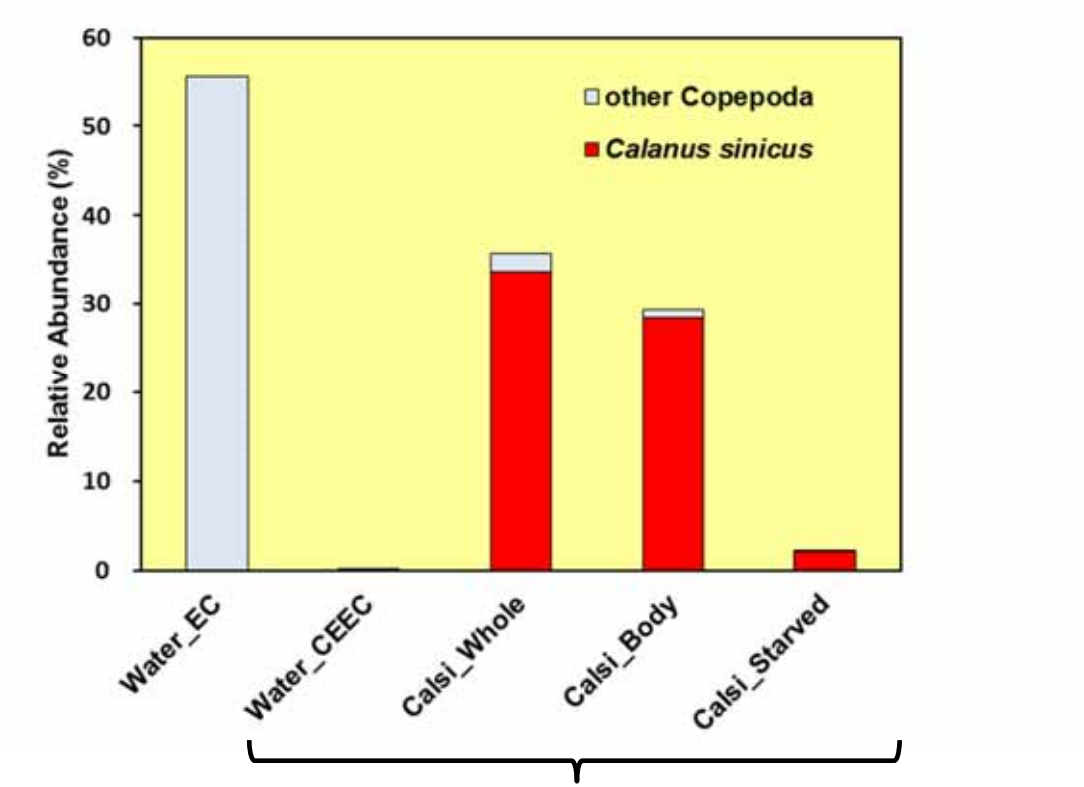
Results: taxonomic resolution

Total 66,354 sequences, 2,540 OTUs

>93% assigned taxonomy at genus/species



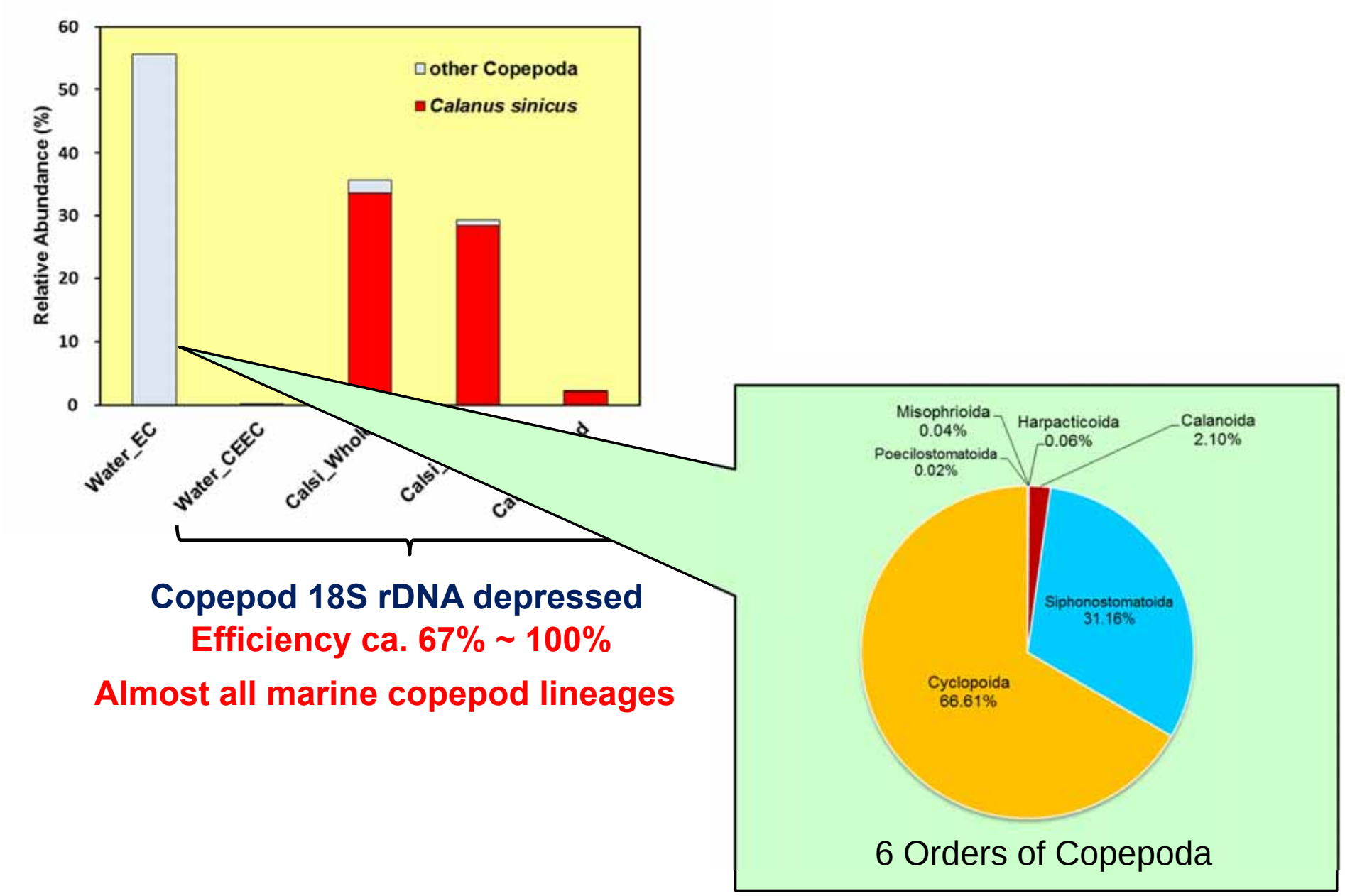
Results: CEEC+454EC copepod excluding?



Copepod 18S rDNA depressed

Efficiency ca. 67% ~ 100%

Results: CEEC+454EC copepod excluding?



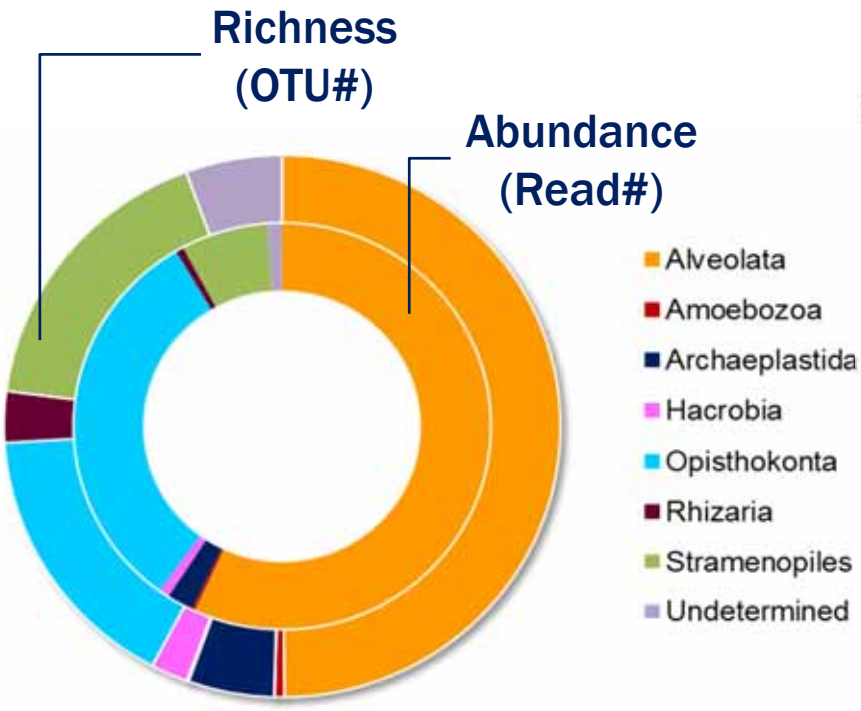
Copepod 18S rDNA depressed

Efficiency ca. 67% ~ 100%

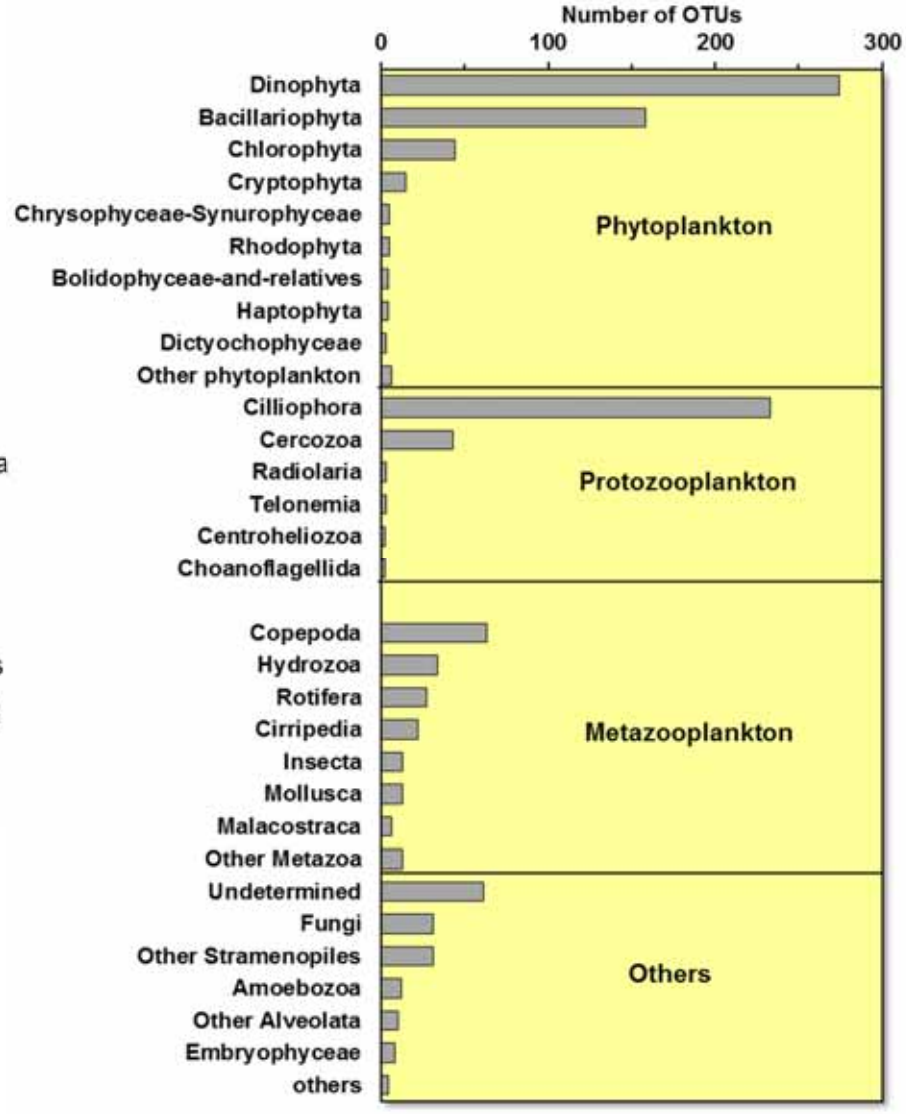
Almost all marine copepod lineages

Results: CEEC+EC eukaryote common?

7 Supergroups

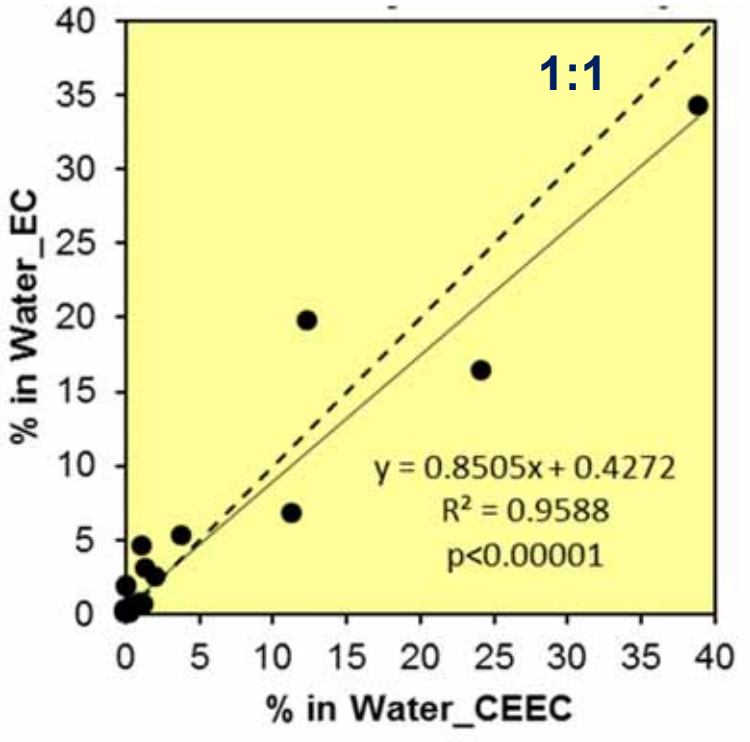


36 Phylum/groups

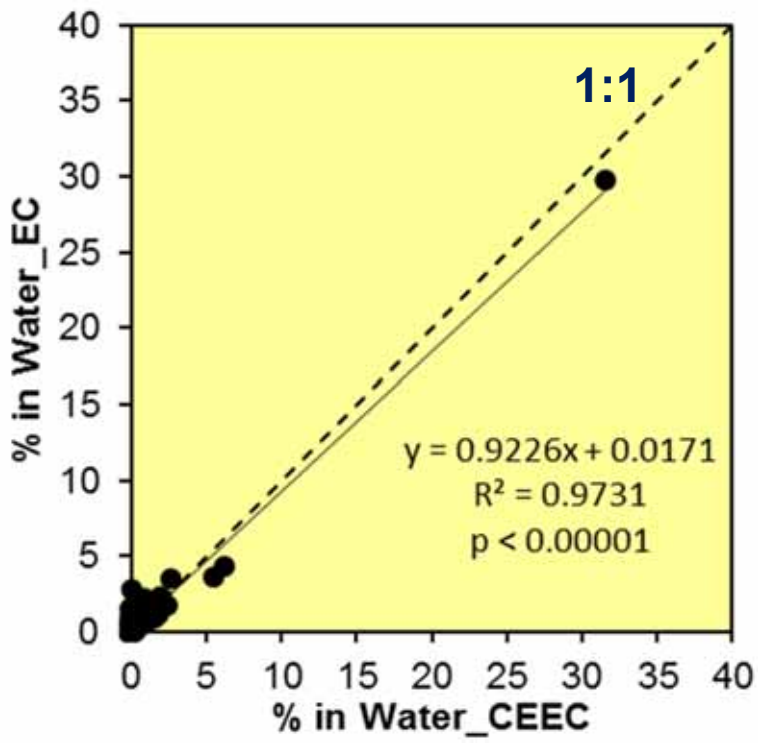


Results: CEEC+454EC vs. 454EC

36 Phylum/groups



411 shared OTUs



No significant difference of community composition!

Objectives

Method Validation

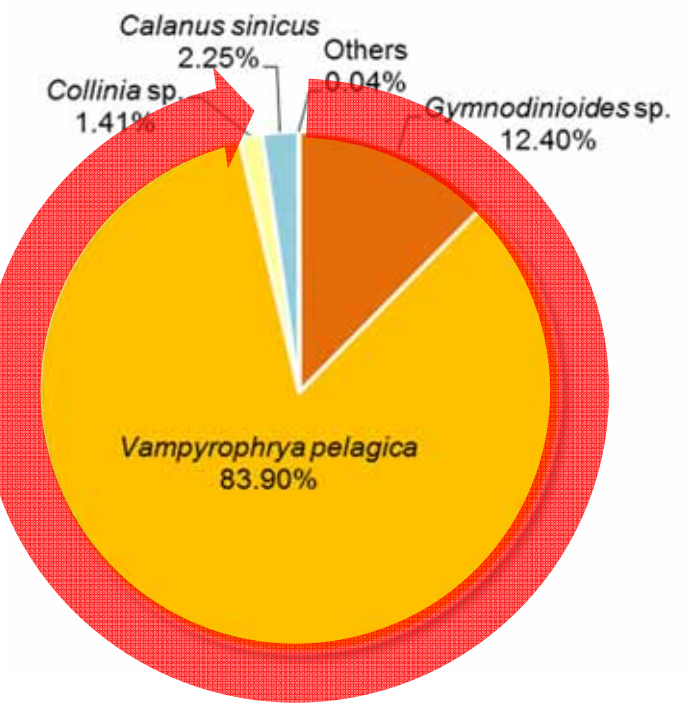
- √ Is CEEC + 454EC copepod excluding?
- √ Is CEEC + 454EC eukaryotes universal?
- √ How is the taxonomic resolution?

Case Study: *Calanus sinicus*

- What do they eat?
- Any feeding preference?

Results: starved copepod as a control

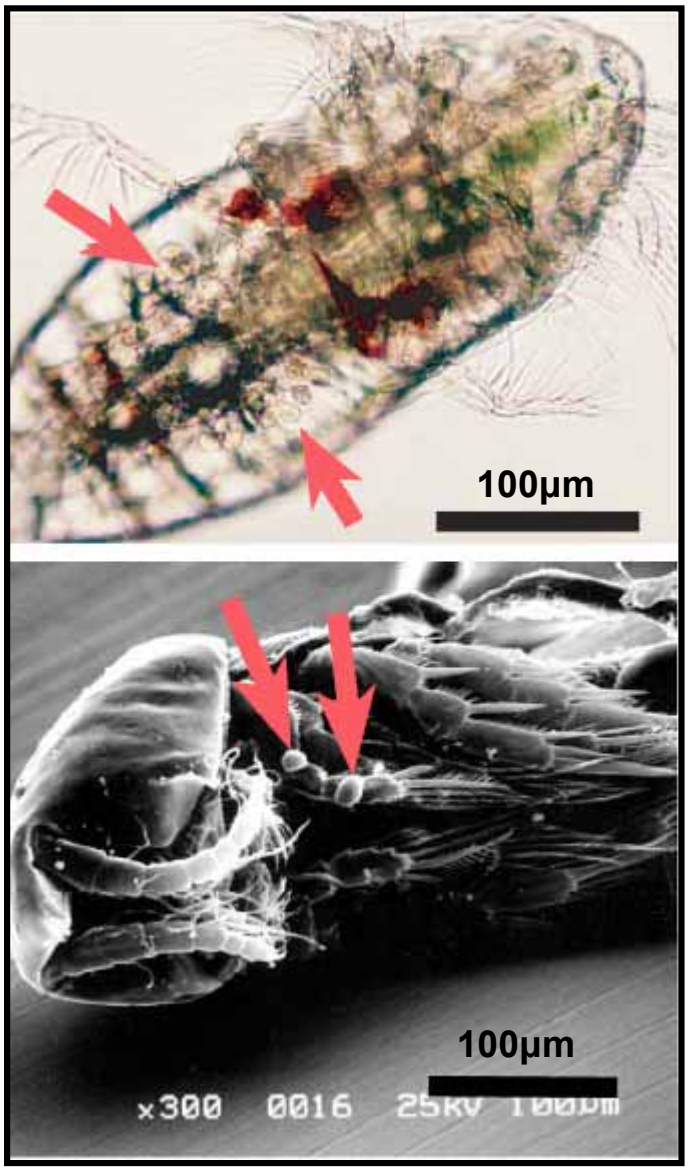
> 97% = symbiotic apostome ciliates



- Prevalent ciliate symbiosis on copepod
- High genetic diversity (Guo et al. 2012)
- On/under skeleton of copepod

Sequences removed from diet analysis!

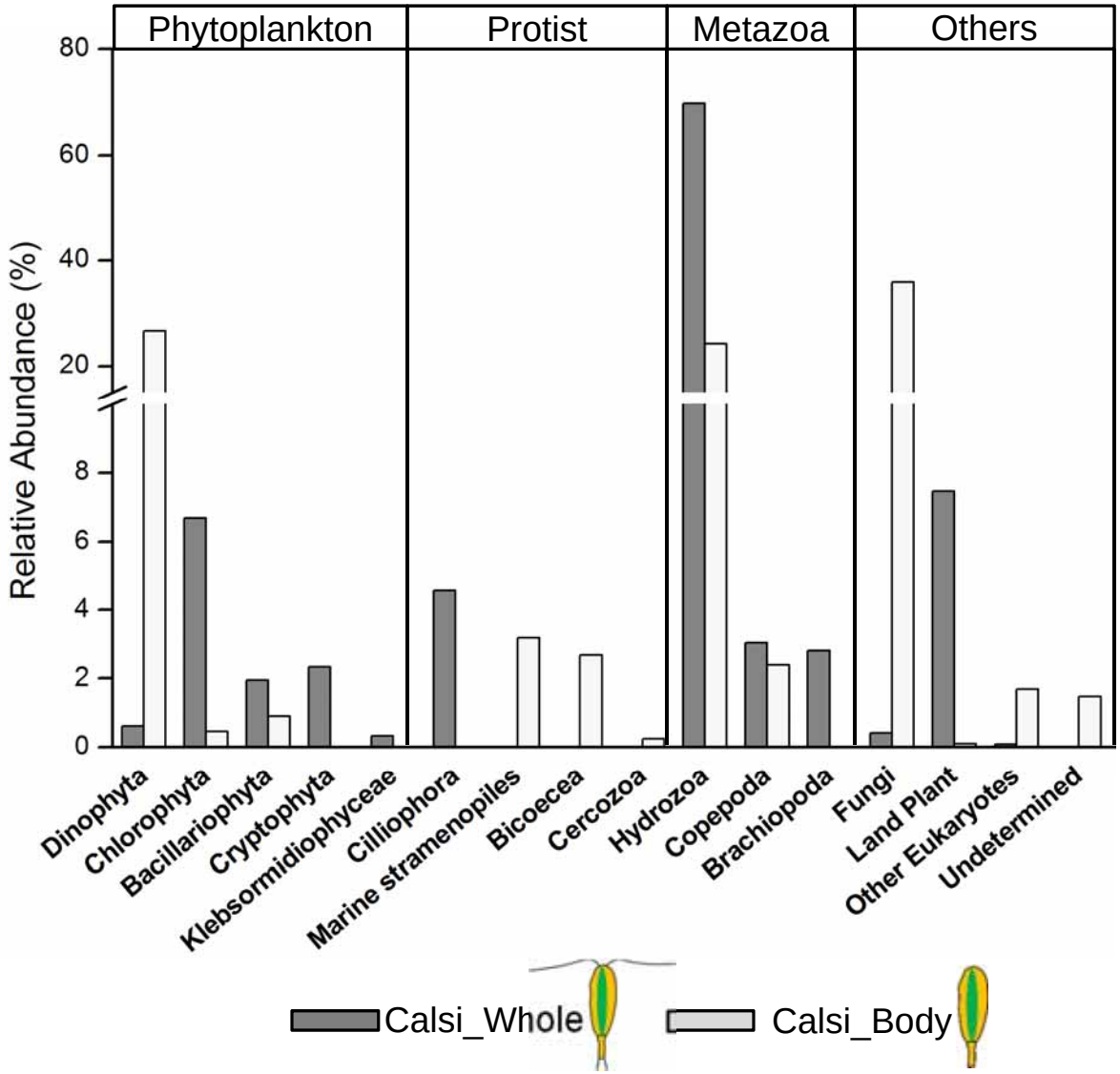
Vampyrophrya pelagica



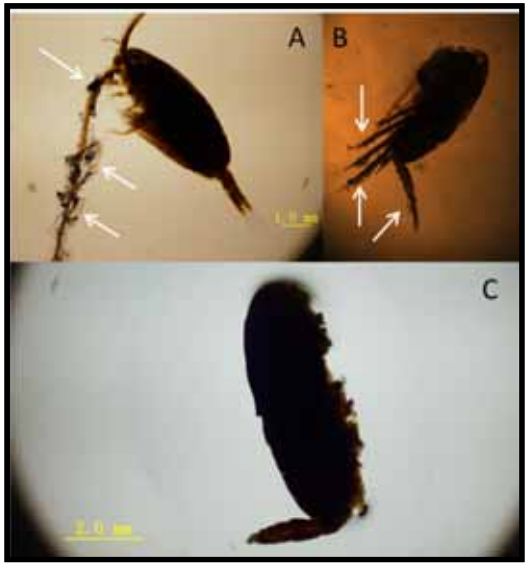
Ostsuka et al. 2004

Results: what do *C. sinicus* eat?

157 OTUs: Omnivorous & Diverse

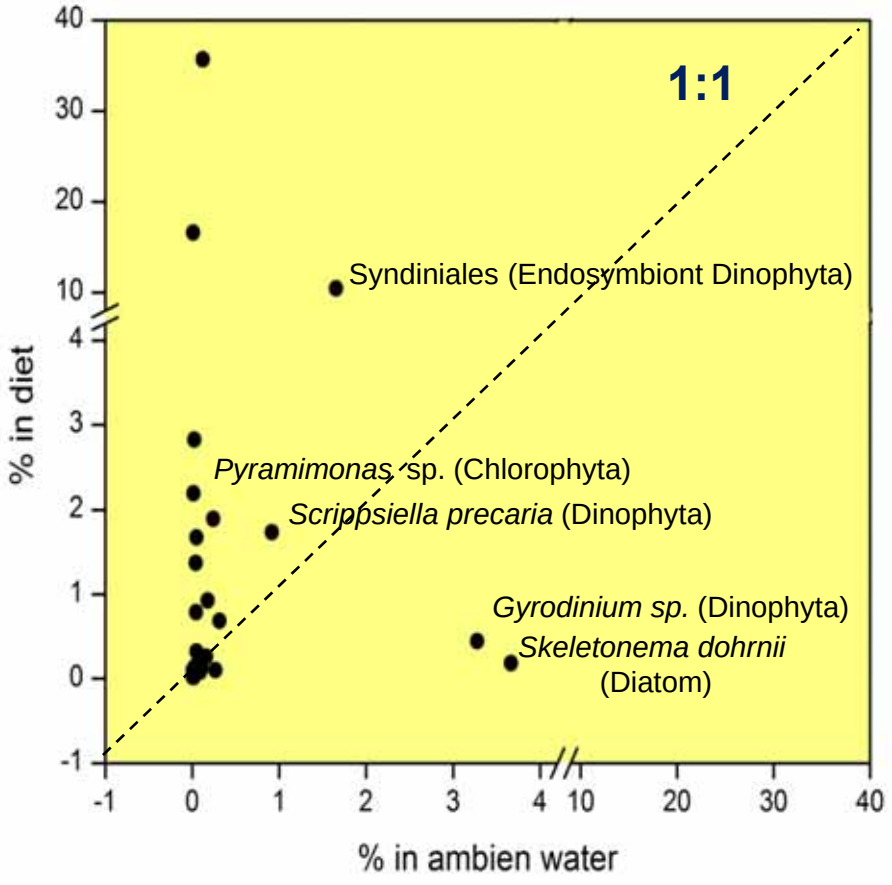


Calanus sinicus

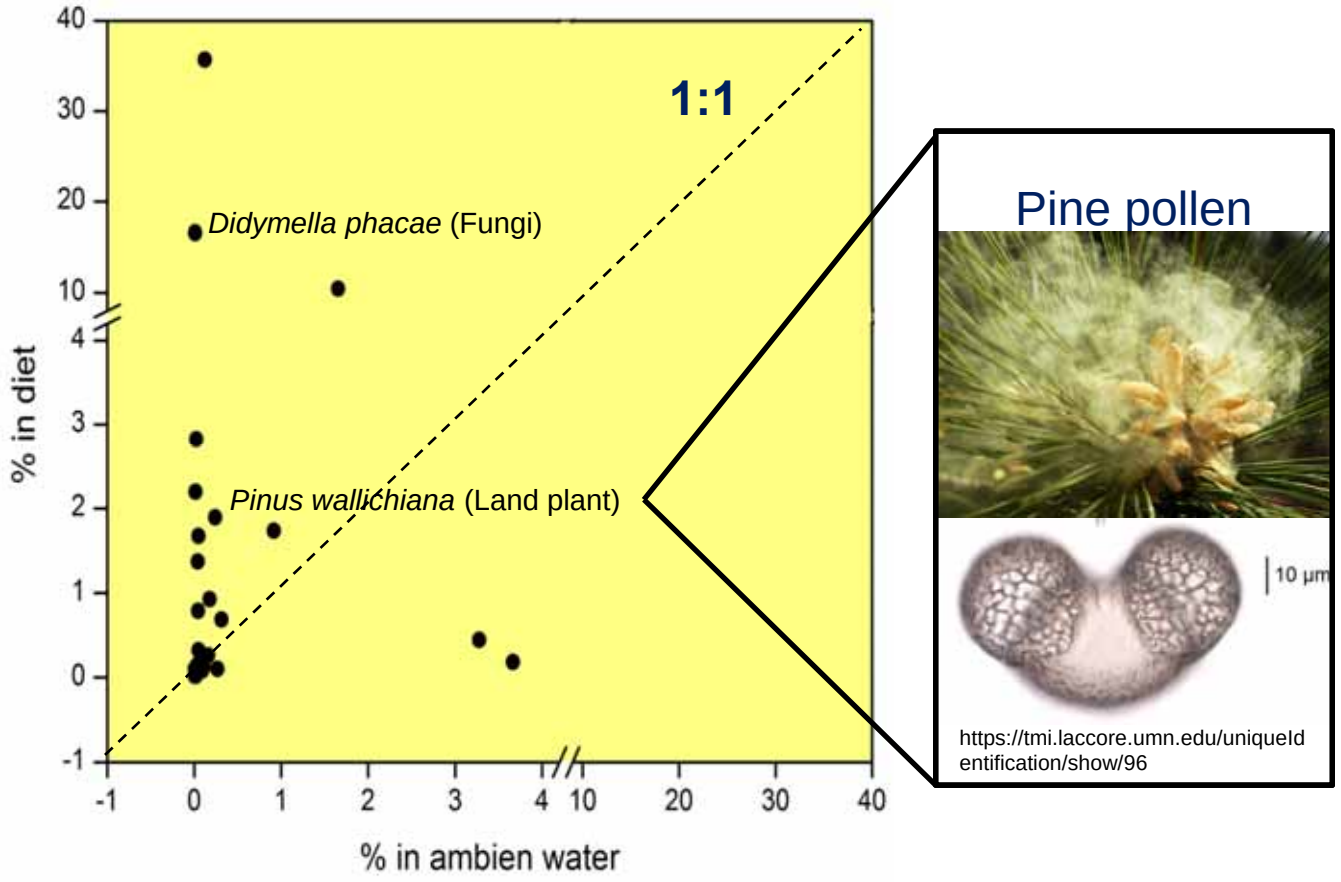


(Photo courtesy: Yousong Huang)

Results: Any feeding preference?



Results: Any feeding preference?

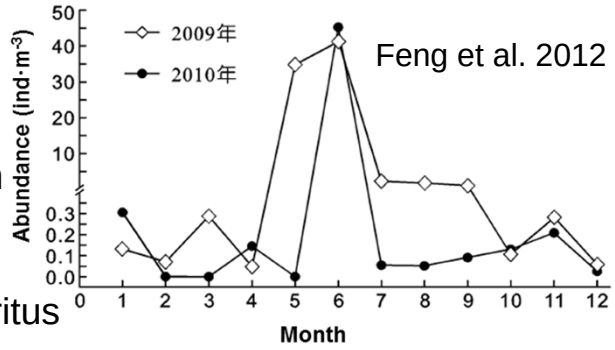


Results: Any feeding preference?

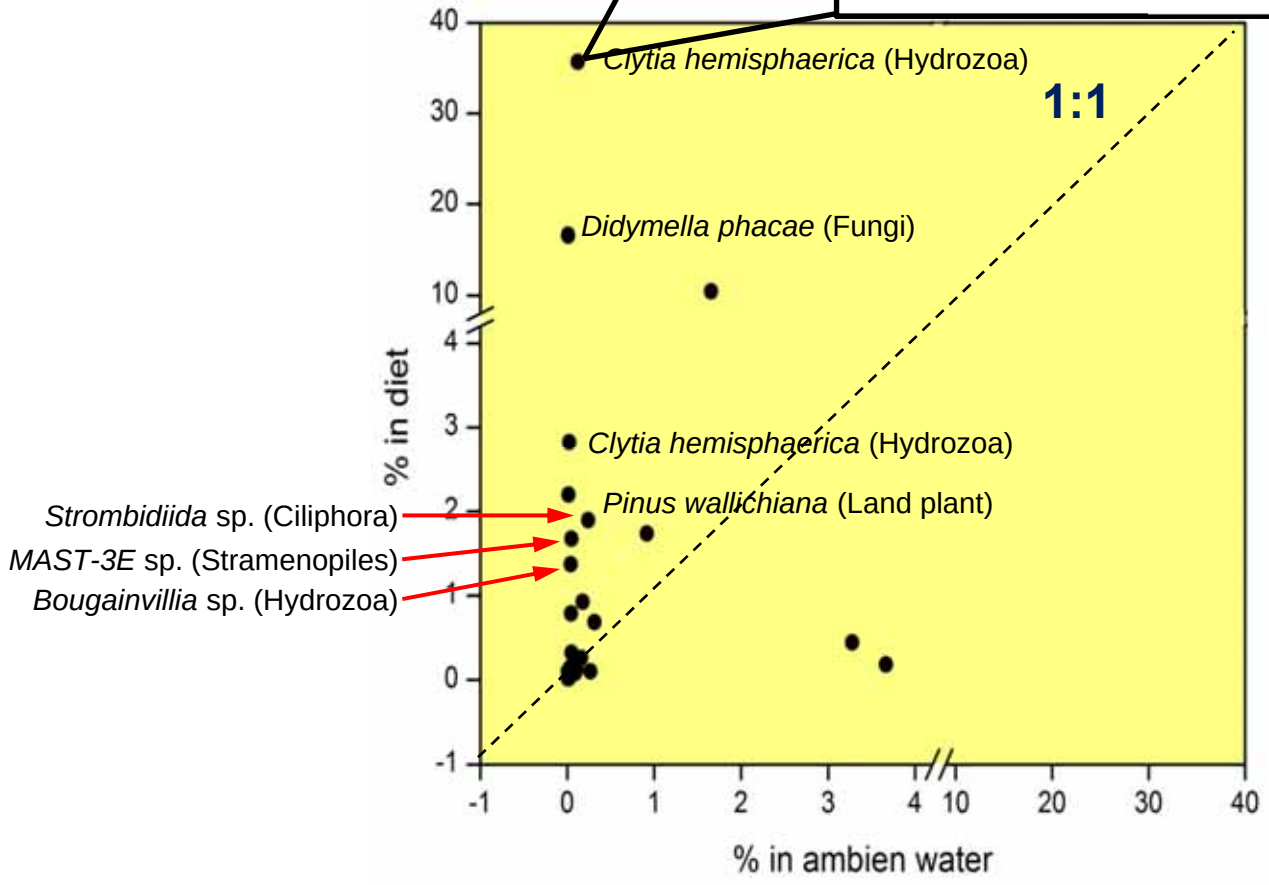
Adult: 2-10mm
Avg. 6mm

Egg, larvae & detritus

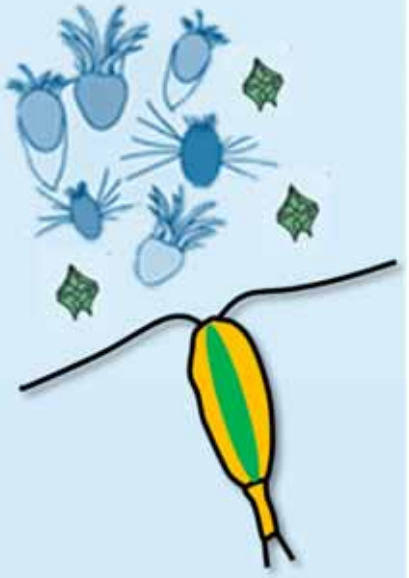
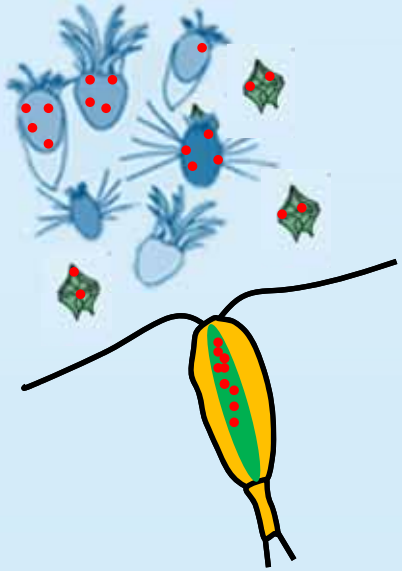
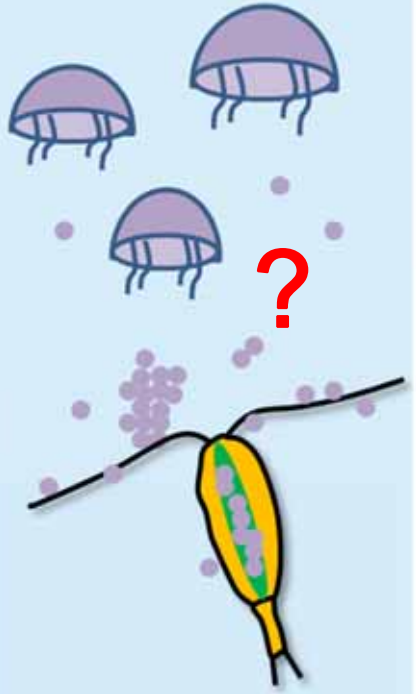
Clytia hemisphaerica bloom



Feng et al. 2012



Discussion: how to get & enrich the food?

Just love it!	Buy 1 get n free	Banquet
Free living	Endosymbionts	Large zooplankton
		

- Zooplankton juvenile, larvae in **marine snow:ambient water >600:1** (Bochdansky and Herndl,1992)
- Copepod use **chemical trail** to find marine snow (Lombard et al. 2013)

Summary

- CEEC + 454EC is eukaryotes universal and copepod excluding.
- Removal of appendages and starved sample are critical controls.
- The diet of *C. sinicus* is omnivorous & diverse with preference.
- This protocol can be applied to other NGS platform and small predators.

However...

- Diet composition = “Snapshot”
- OTU abundance ≠ biomass
- Copepod eating copepod?
- Experimental validation of copepod feeding on hydrozoa.

Acknowledgements

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