Comparison of Metabarcoding and Microscopy for Estuarine Plankton Monitoring: Quantitative Character and Non-Indigenous Species Detectability

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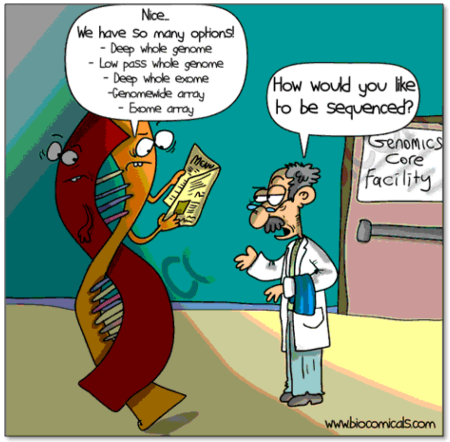
- Plankton is essential for ecosystem functioning
- Used as indicators of ecosystem change
- Limitations:
  - Difficult
  - Time-consuming
  - Expertise
  - Cryptic species

http://slideplayer.com/slide/8127598/



- Metabarcoding as an alternative:
  - Lots of information
  - Sensitivity and resolution
  - Detection of rare taxa, cryptic or NIS

- Limitations: Some groups are poorly represented in databases

- Quantification is affected by:
  - Copy Number Variation (CNV)
  - Technical biases during DNA extraction, PCR or bioinformatics

Main objective: to compare microscopy against metabarcoding to assess the usefulness of metabarcoding for estuarine plankton monitoring

Others:

- Spatio-temporal structure in relation with environmental parameters
- Effects of database completeness in taxon assignment
- Sensitivity for NIS detection

- Macrozooplankton from oceanic samples

- 100% identity for sequences corresponding to the “Para-Und-Euch” group → single OTU for 8 species

Number of individuals per taxa and sample:			A-101	B-101	C-11	D-101
Meganyctiphanes norvegica		Euphausiid	101	33	1	100
Undeuchaeta major	congeneric	Copepod	13	39	1	1
Undeuchaeta plumosa	pair	Copepod	3	9	1	1
Euchirella rostrata	congeneric	Copepod	20	60	1	1
Euchirella curticauda	pair	Copepod	2	6	1	1
Paraeuchaeta gracilis	congeneric	Copepod	22	66	1	1
Paraeuchaeta tonsa	pair	Copepod	12	36	1	1
Euchaeta hebes	congeneric	Copepod	15	45	1	1
Euchaeta acuta	pair	Copepod	3	9	1	1
Pleuromamma robusta		Copepod	23	69	1	1
Candacia armata		Copepod	10	30	1	1
Calanus helgolandicus		Copepod	7	21	1	1
Tomopteris spp.		Polychaeta	25	80	1	1

- 134 OTUs: only 6 from the sorted spp. (89.25% reads)
- Comparison within each particular sample: only mock-D significant ($r = 0.99$ and $P < 0.01$) →

  sample dominated by a taxon (low eveness) → probably due to different CNV between species

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18S rRNA V9 metabarcoding for diet characterization: a critical evaluation with two sympatric zooplanktivorous fish species

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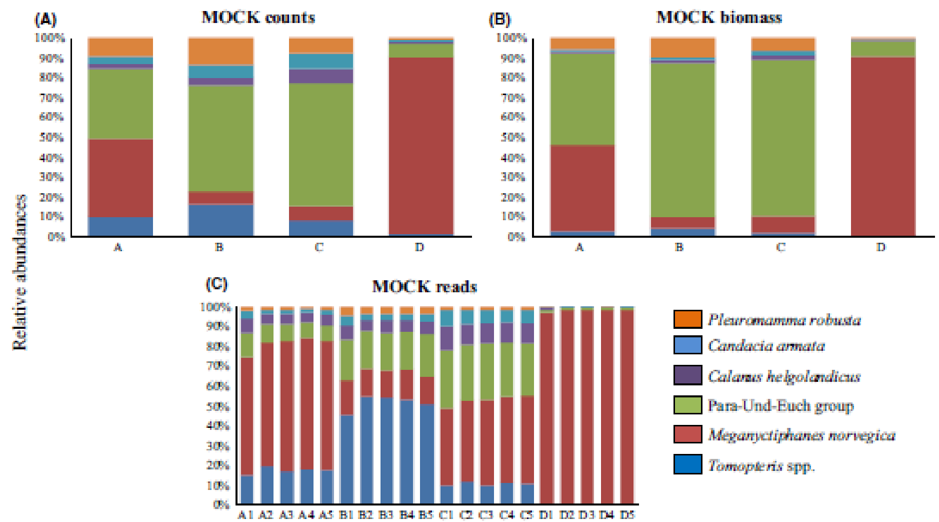


Figure 3. Mock samples. Relative abundance of (A) microscopy counts, (B) estimated biomass (C dry weight) and, (C) 18S V9 reads, for the six OTUs within mock samples. Five technical replicates were sequenced (1–5; bottom graph). No bias in OTUs distribution was reported for the technical replicates (Kruskal–Wallis test). Legend superimposed.

- Estuary of Bilbao
- Huge anthropogenic impact
- Stratified and channeled
- Undergoing a recovery program since the 80s

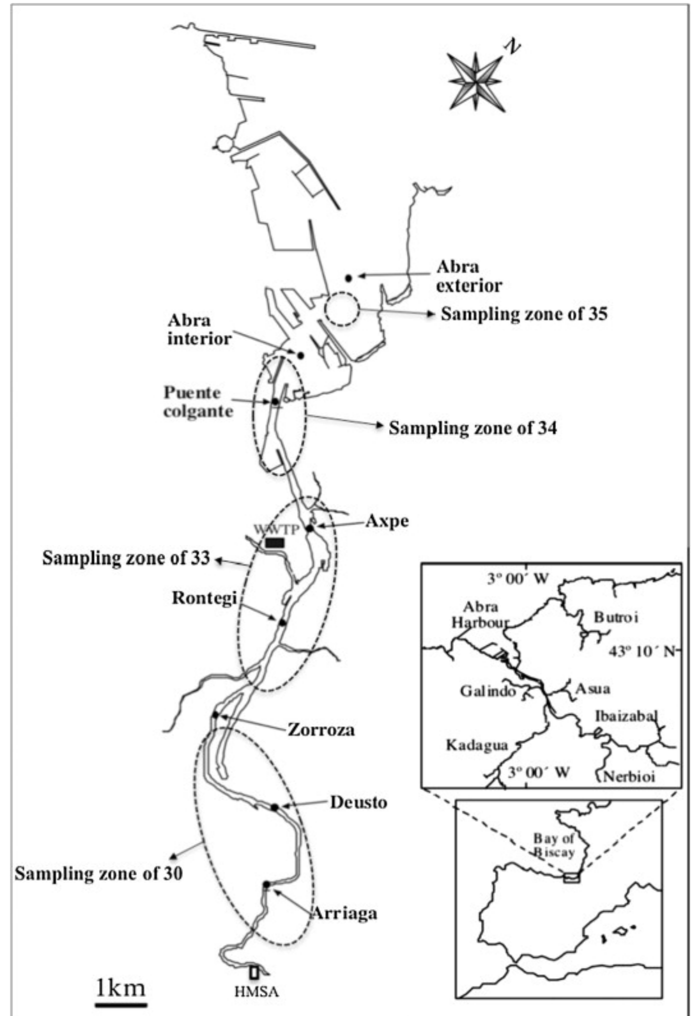


Figure from Villate et al. (2013)

- Three size fractions: 0.22-20, 20-200 and > 200 µm
- Summer (June, July) and Autumn (September, October) in 30 and 35 salinities
- Environmental variables

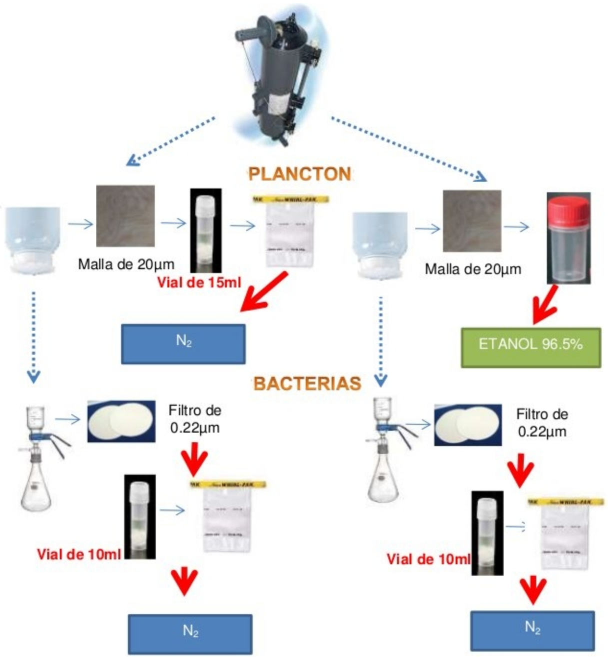
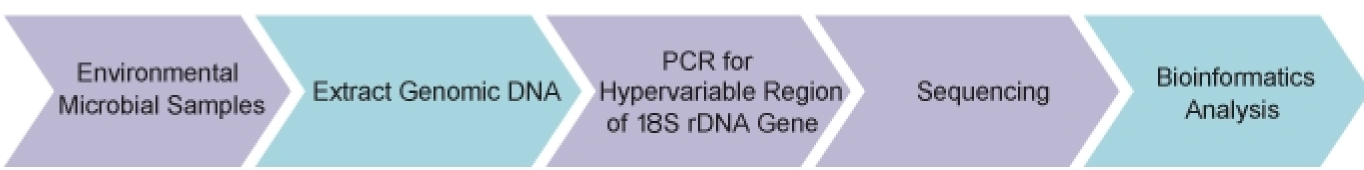


Figure modified from Inma Martín (AZTI; 2013)

- DNA extraction
- 18S V9 amplification (Stoeck *et al*., 2010; EMP)
- Sequencing (Illumina MiSeq 2x150)
- Databases (Silva 111 & 119)
- Bioinformatic analysis (closed-reference, 99% similarity)



- Four “different” databases:
  - Two standard (Silva 111 and 119)
  - Two custom (with addition of 18S sequences)

- Greater number of seqs → higher assignment rate

	Silva 111			Silva 111 Custom			Silva 119			Silva 119 Custom		
	0.20-20	20-200	>200	0.20-20	20-200	>200	0.20-20	20-200	>200	0.20-20	20-200	>200
June 30	28,21	5,25	14,46	40,96	67,99	87,34	55,60	5,63	14,67	55,69	68,12	87,34
June 35	50,71	17,38	24,26	55,62	80,59	86,81	55,26	22,96	48,81	60,09	80,52	86,49
July 30	42,38	1,16	13,69	42,42	10,79	59,68	23,95	0,98	14,85	23,99	10,36	59,47
July 35	46,03	35,28	88,17	46,05	43,39	89,68	53,61	51,20	91,24	53,62	57,81	92,64
Sept 30	22,53	0,75	24,97	22,57	21,67	33,7	22,78	6,55	29,91	22,80	21,68	33,71
Sept 35	38,21	21,30	10,58	38,23	72,84	86,58	54,06	24,55	12,81	54,08	73,71	87,13
Octo 30	30,36	2,31	13,35	30,63	10,16	79,31	35,11	2,44	76,93	35,14	8,85	79,31
Octo 35	25,05	6,63	6,54	25,48	39,69	35,48	42,18	16,38	19,58	42,59	49,41	39,62
Mean	35,44	11,26	24,5	37,75	43,39	69,82	42,82	16,34	38,60	43,50	46,31	70,71
Global	23,73			50,32			32,58			53,51		

Table 2 Percentage of sequences that were assigned to taxonomy using four different databases. Similarity threshold was set at at 99%. Total assignment percentage for each database is shown along with those for each specific size fraction (0.22-20, 20-200 and >200 μm), salinity (30 and 35 ppt) and sampling month (June-October)

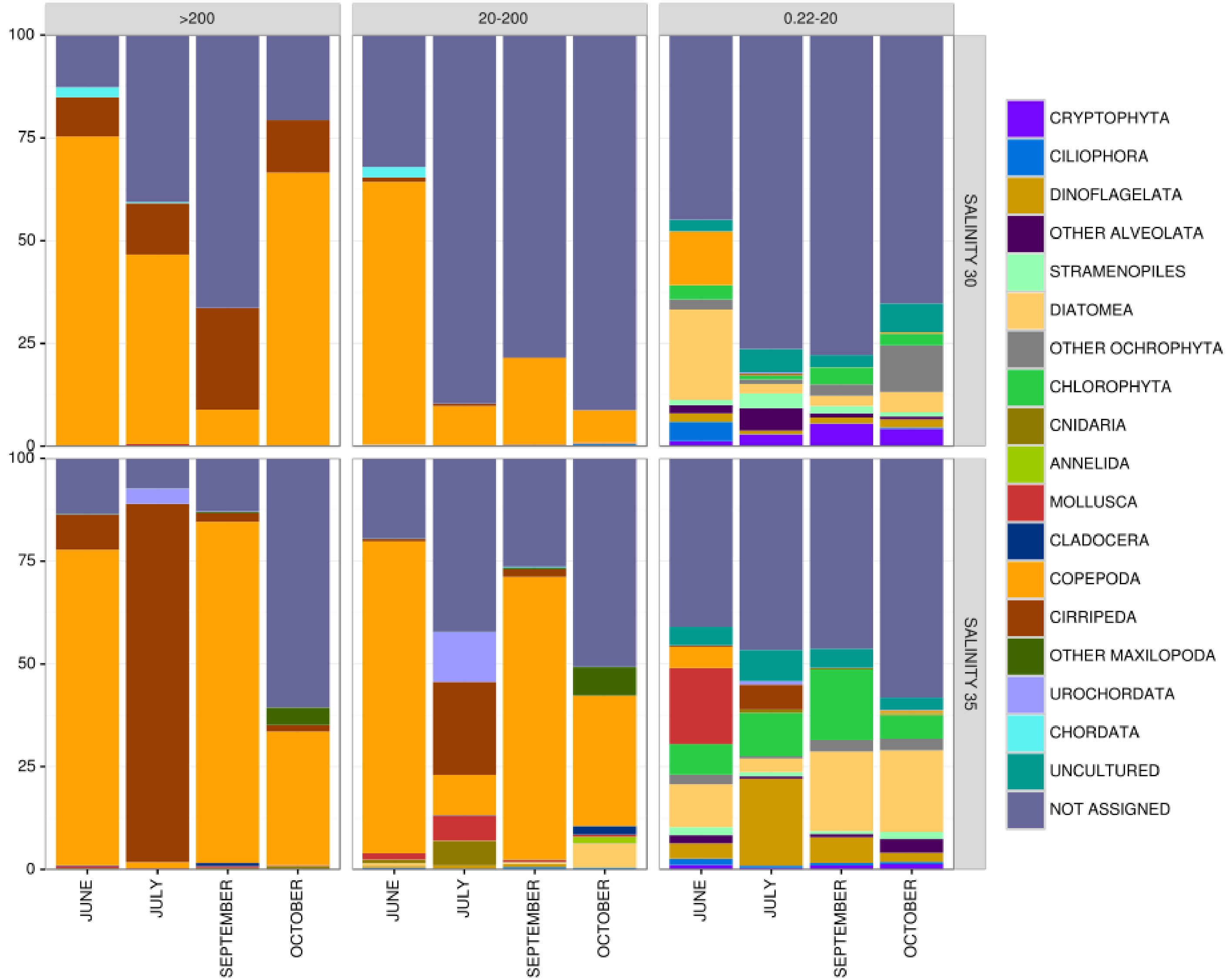


Fig. 1 Proportion of taxonomic ranks in each sample based on the metabarcoding approach. A total of 17 taxonomic ranks (>1% abundance) are shown.

- Higher assignation for 35 (64.8%) than 30 ppt (42.2%) in most of the cases (37 of 48 sequenced samples)

- Unassigned percentage lower as size-fraction increased: 56.5, 53.7 and 29.3%, respectively

- Maxillopoda dominated the 20-200 and >200 µm (mainly copepods and barnacles)

- More diverse assemblage for the 0.22-20 µm (e.g. Dinophyceae, Cryptophyceae, ...)

METABARCODING	MICROSCOPY	ACRONYM	METABARCODING	MICROSCOPY	ACRONYM
	Centric diatoms	CENT		*Scenedesmus* spp.	SCEN*
	Chaetoceros curvisetus	CCUR		*Pyramimonas* spp.	PYRA
	Chaetoceros debilis	CDEB	*Ostreococcus tauri*		OTAU
	Chaetoceros socialis	CSOC	*Ulva intestinalis*		UINT
	Chaetoceros spp.	CHAE	Uncultured phytoplankton		UPHY
	Conticribra weissflogii	CWEI		Choanoflagellates	CHOA
Cyclotella choctawhatcheeana		CCHO		*Leucocryptos* spp.	LEUC
	Cyclotella meneghiniana	CMEN*	*Strombidium basimorphum*		SBAS
Leptocylindrus hargravesii		LHAR	*Chelophyes appendiculata*		CAPP
	L. danicus/hargravesii	LDAN		Unidentified Siphonophora	USIP
	Leptocylindrus aporus	LAPO*		*Evadne nordmanii*	ENOR*
	Leptocylindrus convexus	LCON*		*Evadne spinifera*	ESPI*
	Melosira varians	MVAR	*Podon* spp.		PODO
	Navicula radiosa	NRAD		*Podon intermedius*	PINT
	Pennate diatoms	PENN	*Balanus balanus*		BALA
	Proboscia alata	PALA	*Peltogaster paguri*		PPAG
	Pseudo-nitzschia multistriata	PMUL		Cirripedia nauplius larvae	CNAL
	Pseudo-nitzschia spp.	PSEU		Cirripedia cypris larvae	CCYL
	Skeletonema sp.	SKEL	*Acartia clausi*	*Acartia clausi*	ACLA*
Skeletonema menzelii		SMEN*	*Acartia tonsa*	*Acartia tonsa*	ATON*
Skeletonema pseudocostatum		SPSE		*Acartia* sp. (copepodite)	ASCO
Tenuicylindrus belgicus	*Tenuicylindrus belgicus*	TBEL*	*Calanipeda aquaedulcis*	*Calanipeda aquaedulcis*	CAQU*
	Thalassiosira sp.	THAL	*Calanus helgolandicus*		CHEL
Thalassiosira allenii		TALL	*Centropages hamatus*		CENT*
Thalassiosira delicatula		TDEL		*Cyclops* sp.	CYCL*
Thalassiosira guillardii		TGUI	*Euterpina acutifrons*	*Euterpina acutifrons*	EACU*
Thalassiosira lundiana		TLUN		*Oithona davisae*	OBRE
Heterosigma akashiwo		HAKA	*Oithona nana*	*Oithona nana*	ONAN*
	Apedinella radians	ASPI		*Oithona similis*	OSIM*
	Hemiselmis sp.	HEMI*		*Oncaea media*	OMED*
	Plagioselmis sp.	PLAG	*Paracalanus parvus*	*Paracalanus parvus*	PPAR*
Teleaulax acuta	*Teleaulax acuta*	TACU*		Pcalanus (copepodite)	PCAL
Teleaulax amphioxeia	*Teleaulax amphioxeia*	TAMP*	*Pseudocalanus elongatus*		PELO
	Teleaulax gracilis	TGRA	*Pseudodiaptomus marinus*	*Pseudodiaptomus marinus*	PMAR*
	Teleaulax minuta	TMIN	*Temora longicornis*	*Temora longicornis*	TLON*
	Teleaulax spp.	TELE		Copepod nauplius	CNAU
	Chrysochromulina spp.	CHRY*		Unidentified brachiura larvae	UBRL
	Prymnesiales	PRYM	*Crassostrea gigas*		CGIG
	Gymnodiniales	GYMN	*Mytilus edulis*		MEDU
Gymnodinium aureolum		GAUR	*Littorina littorea*		LITT
	Gyrodinium flagellare	GFLA		Gastropod veliger larvae	GVEL
	Gyrodinium sp.	GYRO		Bivalve veliger larvae	BVEL
	Heterocapsa rotundata	HROT	Uncultured zooplankton		UZOO
	Heterocapsa sp.	HETE		*Oikopleura* sp.	OIKO*
	Katodinium spp.	KATO	*Sabellaria alveolata*		SALV
	Pfiesteria-like	PFIE		*Sagitta* sp.	SAGI
Micromonas pusilla		MPUS	*Scyliorhinus torazame*		STOR

Table 3 List of most abundant taxa from metabarcoding and microscopy. Only taxa with >1% abundance in at least one of the samples are shown. An asterisk marks those taxa identified by both methodologies.

Thalassiosira allenii		TALL
Thalassiosira delicatula		TDEL
Thalassiosira guillardii		TGUI
Thalassiosira lundiana		TLUN
Heterosigma akashiwo		HAKA

	Apedinella radians	ASPI
	Teleaulax gracilis	TGRA

Ostreococcus tauri		OTAU
Micromonas pusilla		MPUS

Littorina littorea		LITT
	Gastropod veliger larvae	GVEL

Balanus balanus		BALA
Peltogaster paguri		PPAG
	Cirripedia nauplius larvae	CNAL
	Cirripedia cypris larvae	CCYL

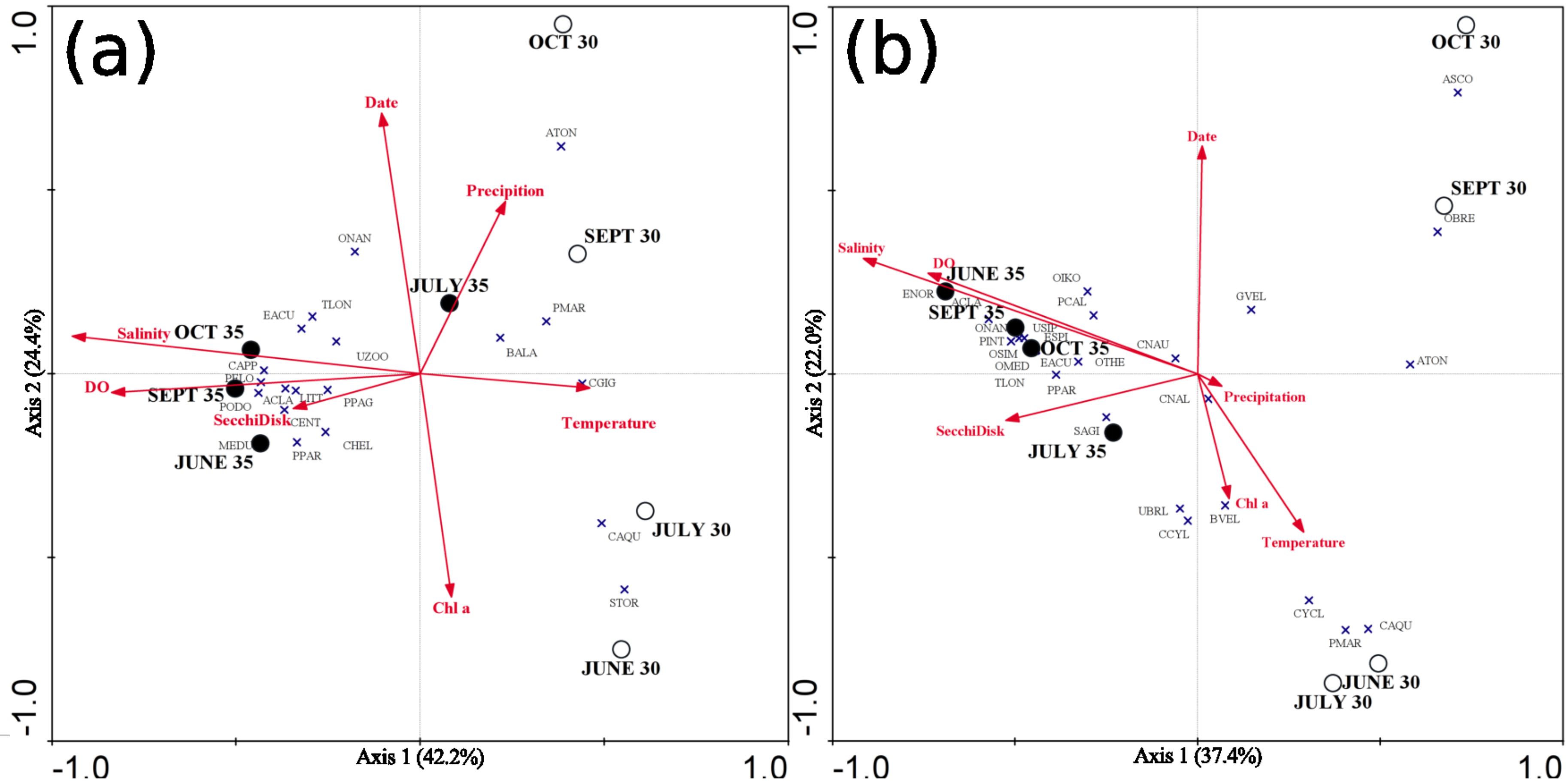
- 44 taxa in common

- Most abundant (>1% abundance):
  - 11 by both
  - 12 only with Microscopy
  - 2 only with Metabarcoding

- Metabarcoding detected congeneric species (e.g genus *Thalassiosira*) but missed others (e.g. *Apedinella radians*, *Teleaulax gracilis*, …)

- Plankton developmental stages

- Comparable spacial and temporal patterns by both methodologies for the >200 µm:
  - DO and water transparency with salinity
  - Precipitation with date



- Neither approach identified a temporal pattern in the 0.22 – 200 µm, but spatial pattern only by microscopy

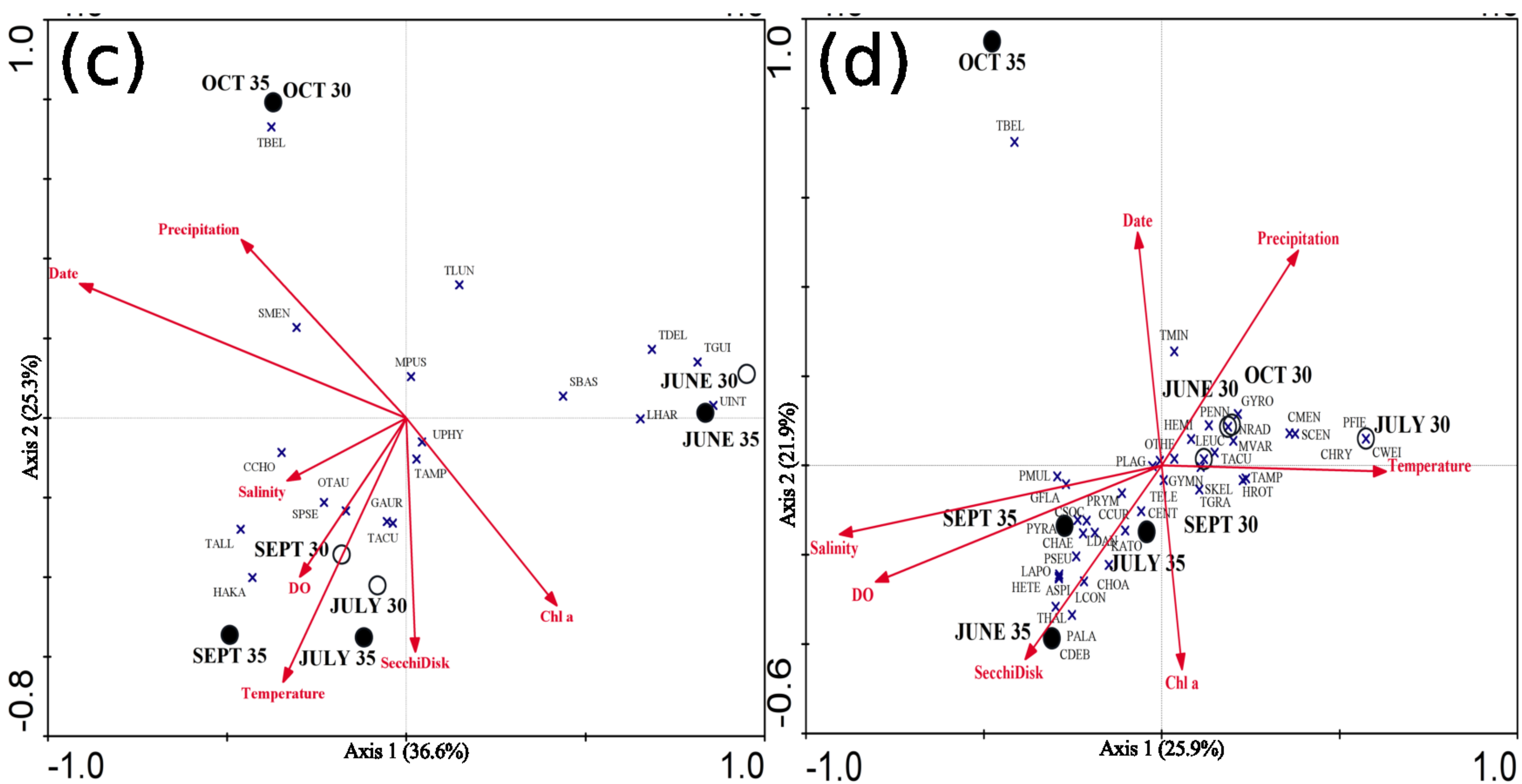


Fig. 2 Metabarcoding and microscopy CCA results.Only taxa with an abundance of 1% or higher in at least one sample were taken into account. (a) >200 µm metabarcoding, (b) >200 µm microscopy, (c) 0.22-200 µm metabarcoding and (d) 0.22-200 µm microscopy.

- Only taxa uncovered by both methods
- Significant correlations when comparing all taxa within each sample in most cases
- Lack of correlation explained by CNV..
- No differences were found for counts or biomass

Fraction	Salinity (n)	Month	ρ (counts)	ρ (biomass)
>200	30 (4)	JUN	0.77*	0.89**
	30 (4)	JUL	0.95***	0.88*
	30 (4)	SEPT	0.65	0.65
	30 (4)	OCT	0.51	0.51
	35 (10)	JUN	0.63**	0.63**
	35 (10)	JUL	-0,27	-0.08
	35 (10)	SEPT	0.51*	0.58**
	35 (10)	OCT	0.52*	0.49*
0.22-200	30 (13)	JUN	0.48**	0.45*
	30 (13)	JUL	0.44*	0.48**
	30 (13)	SEPT	0.67***	0.69***
	30 (13)	OCT	0.75***	0.77***
	35 (22)	JUN	0.72***	0.73***
	35 (22)	JUL	0.55***	0.59***
	35 (22)	SEPT	0.58***	0.74***
	35 (22)	OCT	0.40**	0.44**

Table 4 Correlations between metabarcoding and microscopy-based analysis of community compositions. Spearman's rank correlation coefficient (ρ) and P-values are shown; $P < 0.01$ (***), $P < 0.05$ (**) and $P < 0.1$ (*). Relative abundances from metabarcoding were compared against both microscopy-based relative abundances and biomass.

- Similar relative abundances for *Acartia tonsa* in 30 ppt by both approaches

- Only detected by metabarcoding in 35 ppt

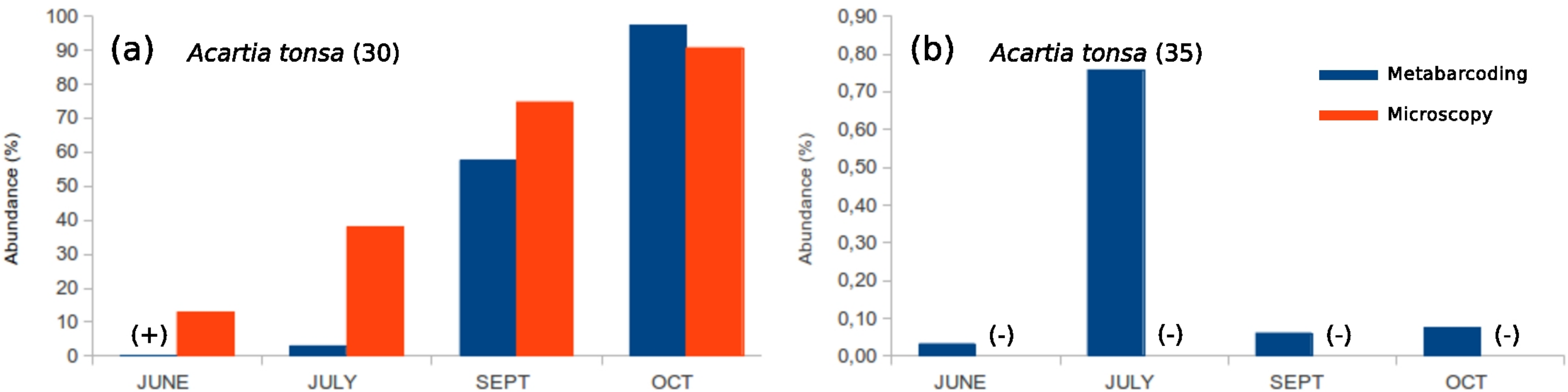
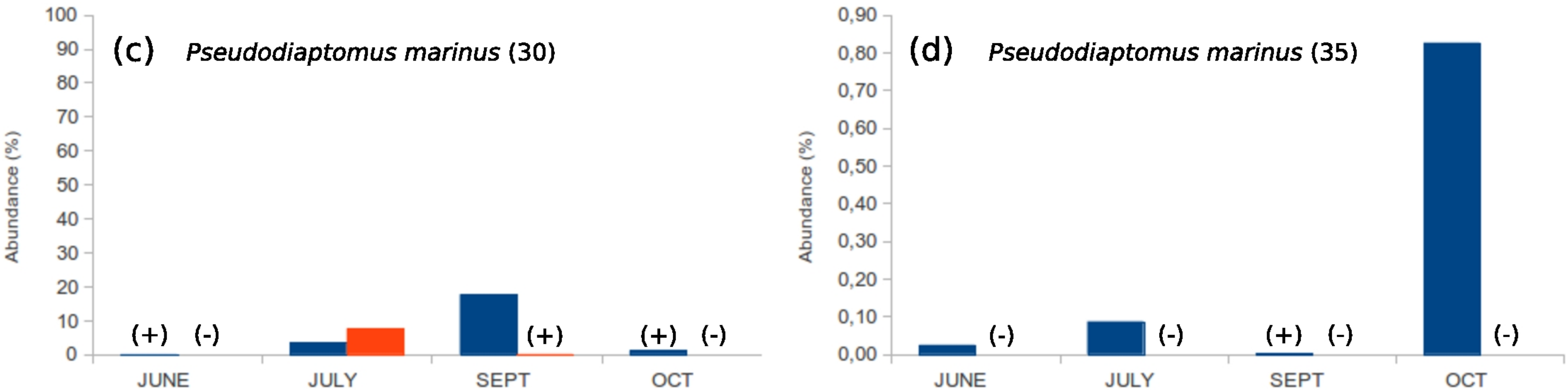


Fig. 3 Comparison of metabarcoding and microscopy when assessing two NIS. *Acartia tonsa* (a, b) and *Pseudodiaptomus marinus* (c, d) relative abundances in the >200 µm size fraction are divided by salinity (30 and 35 ppt). "+" stands for low detection percentages. "-" is showed when the species was not detected.

- *Pseudodiaptomus marinus* was detected in all the samples with metabarcoding
- Microscopy only in two (30 ppt)
- Negative controls/blanks no sequences



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- Similar trends for zooplankton but not for phytoplankton → poor representation of the latter in databases
- Addition of representative sequences from local species → improval in taxonomic assignement
- Correlations between relative abundances → semiquantitative
- Taxonomic resolution issue of 18S V9 → combination with other markers
- Superior sensitivity in the detection of two NIS

- Same set of samples with COI and 18S V1-2
- Similar estimates in most cases, but higher for COI than for the 18S regions
- 46 taxa common to all markers → half of them typically found in the estuary
- Taxonomic composition different in COI for the 0.22-20 size fraction → very few representative sequences for phytoplankton

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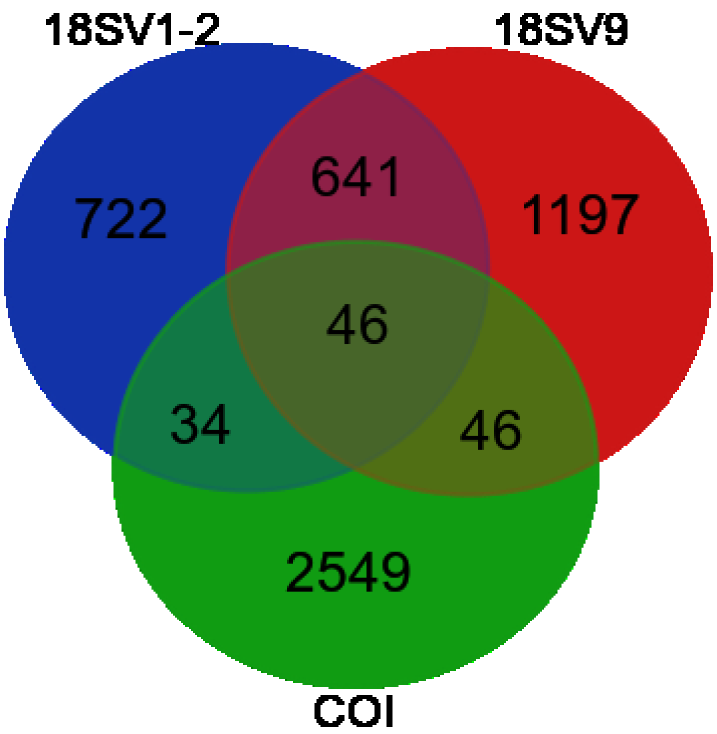
SALINITY	SIZE	MONTH	18SV1-2	18SV9	COI
30	200	JUNE	2,03 (291)	0,80 (438)	2,64 (523)
		JULY	1,74 (204)	1,30 (552)	1,77 (190)
		SEPTEMBER	2,12 (78)	1,75 (423)	2,48 (238)
		OCTOBER	2,75 (170)	1,21 (220)	1,76 (225)
	20 - 200	JUNE	0,94 (893)	1,34 (1241)	3,19 (1782)
		JULY	1,43 (672)	1,22 (908)	2,61 (1812)
		SEPTEMBER	1,96 (178)	1,88 (355)	2,55 (540)
		OCTOBER	2,47 (197)	1,03 (422)	2,70 (592)
	0.22 - 20	JUNE	4,27 (229)	4,39 (239)	4,36 (259)
		JULY	3,86 (274)	3,39 (397)	4,48 (382)
		SEPTEMBER	3,69 (705)	3,68 (893)	4,55 (1764)
		OCTOBER	3,91 (806)	4,24 (755)	4,20 (2129)
35	200	JUNE	2,87 (129)	2,13 (255)	3,40 (239)
		JULY	2,35 (190)	0,64 (378)	1,03 (187)
		SEPTEMBER	2,99 (109)	1,38 (95)	3,54 (182)
		OCTOBER	1,93 (221)	2,13 (291)	3,18 (299)
	20 - 200	JUNE	2,55 (537)	1,66 (477)	3,26 (1724)
		JULY	2,48 (959)	2,60 (1122)	2,35 (1988)
		SEPTEMBER	2,59 (162)	2,10 (359)	3,04 (288)
		OCTOBER	2,77 (132)	2,86 (203)	3,25 (384)
	0.22 - 20	JUNE	4,00 (217)	4,41 (293)	4,40 (260)
		JULY	4,08 (132)	3,78 (386)	4,59 (222)
		SEPTEMBER	4,03 (706)	4,03 (772)	4,77 (1638)
		OCTOBER	4,82 (1233)	4,85 (1460)	4,73 (2528)



Left. Alpha diversities (Shannon index) for each marker. Observed OTUs are included in brackets.

Above. Shared OTUs between markers.

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