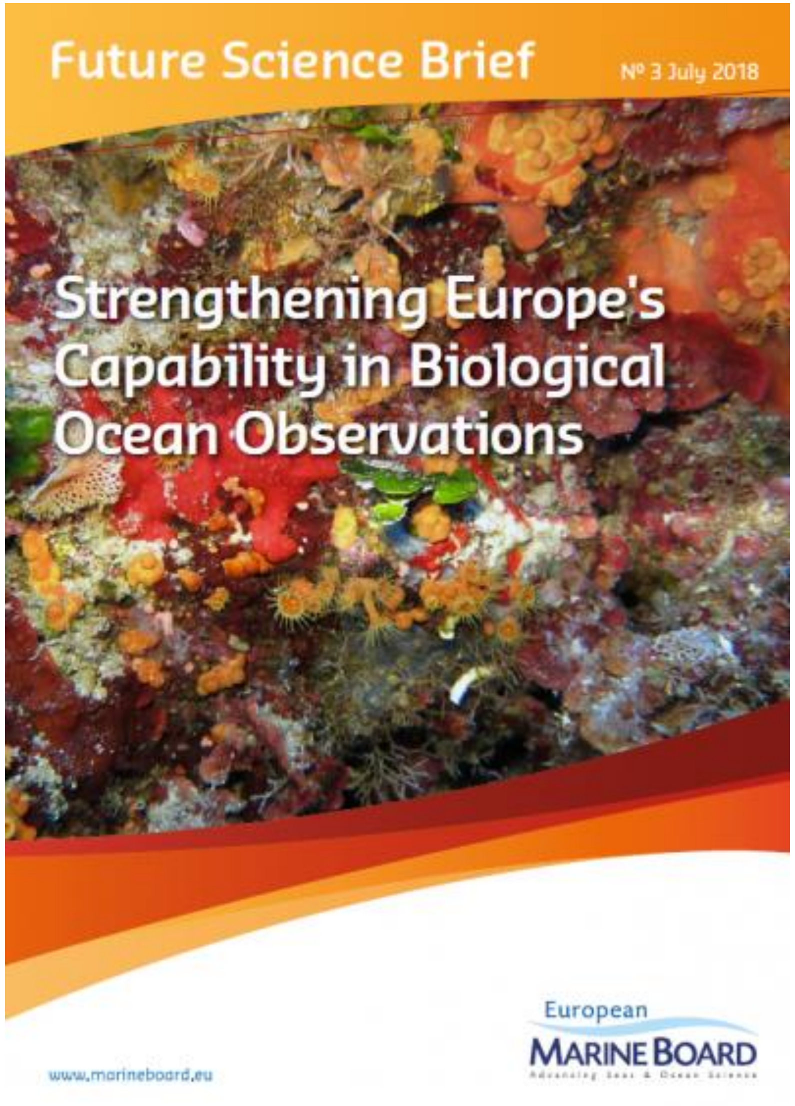
European
MARINE BOARD
Advancing Seas & Oceans Science

Biological Time Series for Science and Marine Status Assessment

Adriana Zingone

STAZIONE ZOOLOGICA ANTON DOHRN
• Napoli •



Biological time series: do we need them?

There is a great concern about how ecosystems will change under the increasing impacts of climate change and human population growth

Ecosystems trajectories are driven by poorly known laws and contingencies: low predictability

Time series offera unique opportunity to assess marine status and track ecosystem changes

Boero et al. 2015
Karl et al. 2010
Edwards et al. 2010
Hughes et al. 2017
Benwey et al. 2019
Muelbert et al. 2019

Review

Special Issue: Long-term ecological research

Multi-decadal oceanic ecological datasets and their application in marine policy and management

Martin Edwards[1,2], Gregory Beaugrand[3], Graeme C. Hays[4], J. Anthony Koslow[5] and Anthony J. Richardson[6,7]

An instrument of the California Cooperative Oceanic Fisheries Investigations survey profiles ocean conditions off the US west coast.

Follow the fish

Koslow and **Couture**

10 OCTOBER 2013 | VOL 502 | NATURE | 163

Overview Articles

Long-Term Studies Contribute Disproportionately to Ecology and Policy

http://bioscience.oxfordjournals.org

BRENT B. HUGHES, RODRIGO BEAS-LUNA, ALLISON K. BARNER, KIMBERLY BREWITT, DANIEL R. BRUMBAUGH, ELIZABETH B. CERNY-CHIPMAN, SARAH L. CLOSE, KYLE E. COBLENTZ, KRISTIN L. DE NESNERA, SARAH T. DROBNITCH, JARED D. FIGURSKI, BECKY FOCHT, MAYA FRIEDMAN, JAN FREIWALD, KRISTEN K. HEADY, WALTER N. HEADY, ANNALIESE HETTINGER, ANGELA JOHNSON, KENDRA A. KARR, BRENNA MAHONEY, MONICA M. MORITSCH, ANN-MARIE K. OSTERBACK, JESSICA REIMER, JONATHAN ROBINSON, TULLY ROHRER, JEREMY M. ROSE, MEGAN SABAL, LEAH M. SEGUI, CHENCHEN SHEN, JENNA SULLIVAN, RACHEL ZUERCHER, PETER T. RAIMONDI, BRUCE A. MENGE, KIRSTEN GRORUD-COLVERT, MARK NOVAK, AND MARK H. CARR

Journal of Sea Research 101 (2015) 12–18

Contents lists available at ScienceDirect

Journal of Sea Research

journal homepage: www.elsevier.com/locate/seares

ELSEVIER

ime is an affliction: Why ecology cannot be as predictive as physics and why it needs time series

Boero[a,b], A.C. Kraberg[c,*], G. Krause[d], K.H. Wiltshire[c]

Review

Special Issue: Long

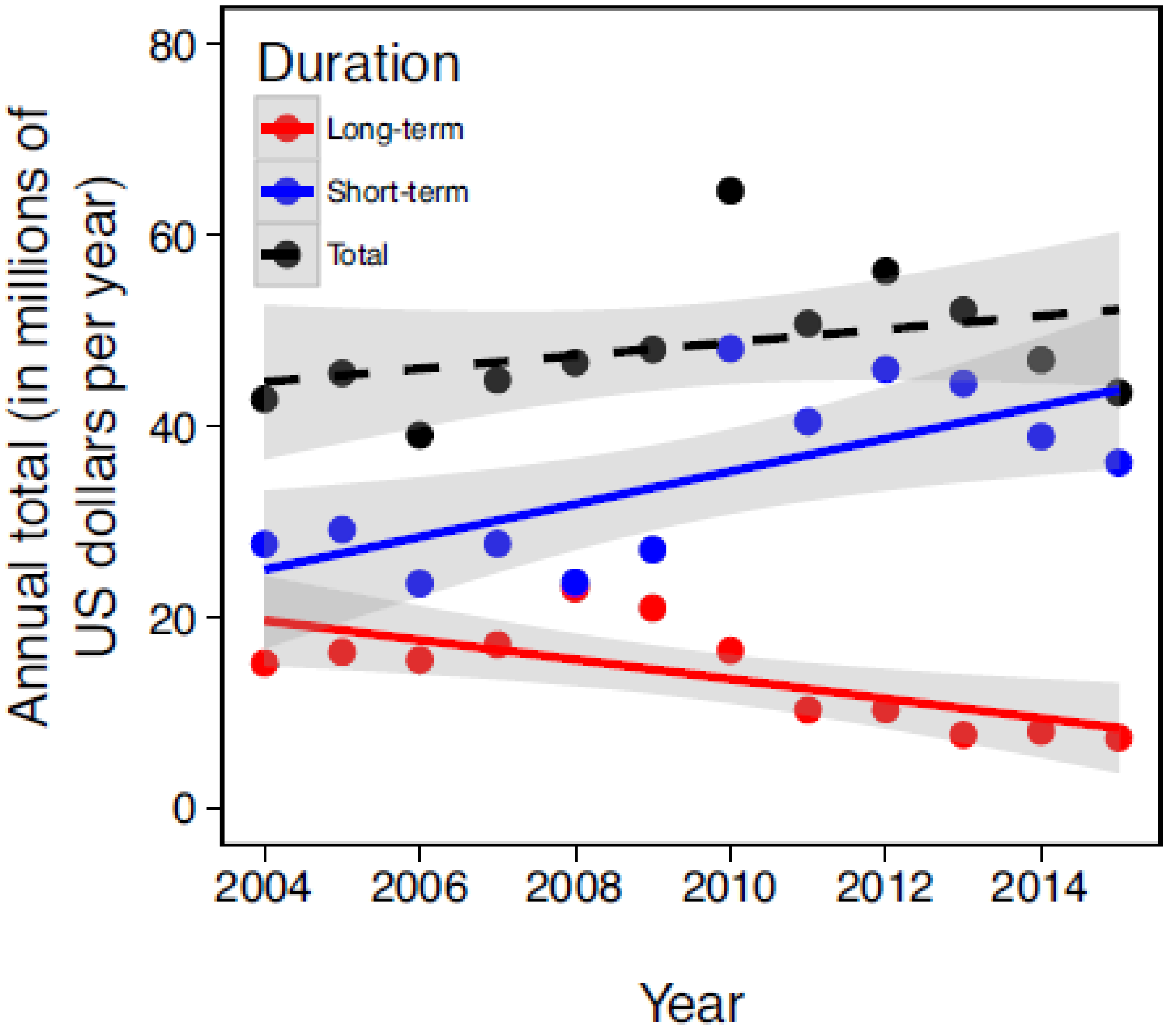
**Multi-de
datasets
marine**

Martin Edwards[1,
Anthony J. Richa

**Long-Term
Disproport
Ecology ar**

http://bios

BRENT B. HUGHES, RODRIGO B
ELIZABETH B. CERNY-CHIPMAN,
SARAH T. DROBNITCH, JARED D
KRISTEN K. HEADY, WALTER N.
BRENNA MAHONEY, MONICA M.
JONATHAN ROBINSON, TULLY R
JENNA SULLIVAN, RACHEL ZUEF
MARK NOVAK, AND MARK H. CA



ne fish

ure

L 502 | NATURE | 163

Sea Research 101 (2015) 12–18

sts available at ScienceDirect

l of Sea Research

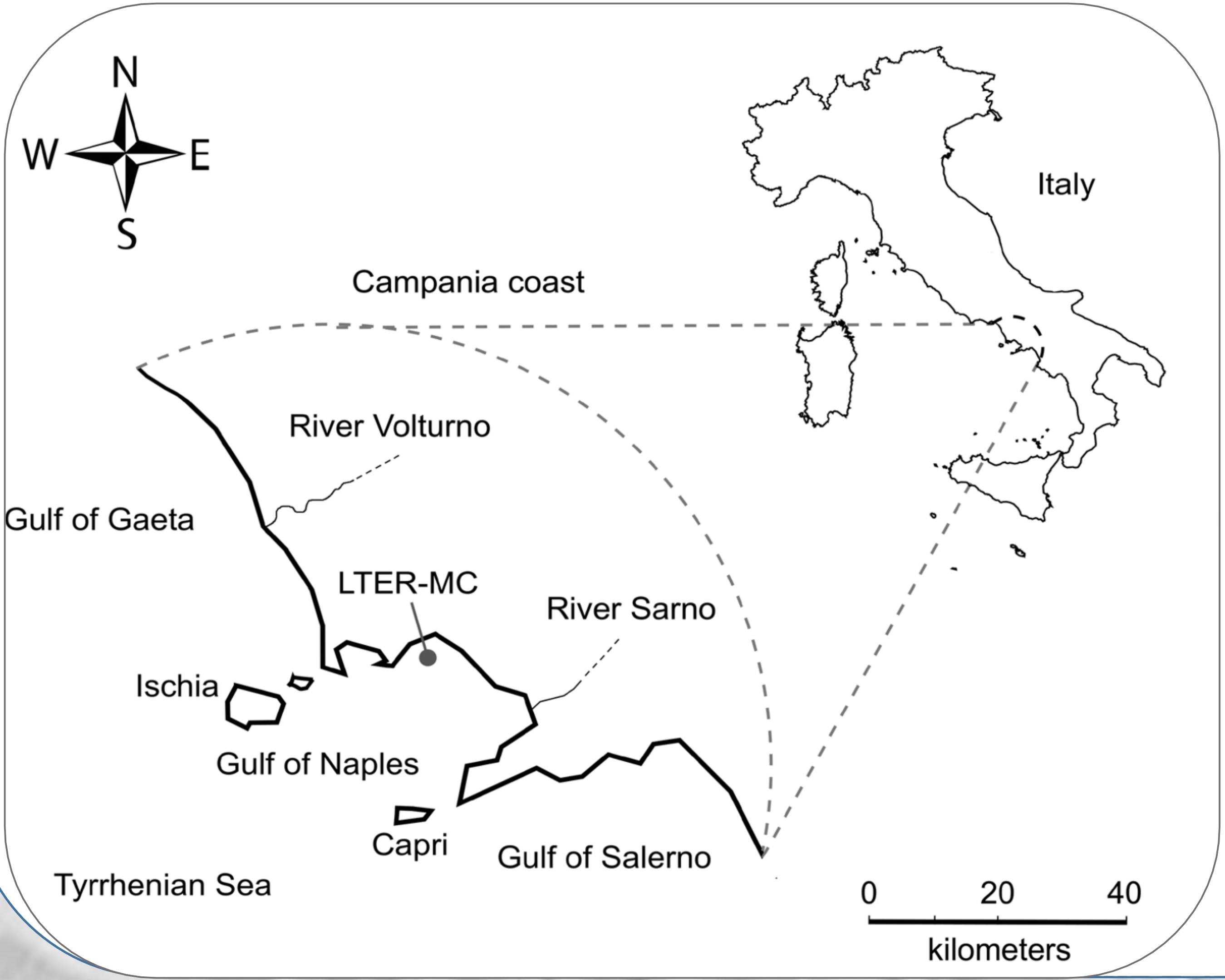
www.elsevier.com/locate/seares

not be as predictive as physics and

tshire [c]

The plankton time-series LTER-MC in the Gulf of Naples

WHERE




inputs from the coast
High human density and industrial activities
LTER-MC
Influence of offshore water



HOW

- GPS
- Radar
- Echo sounder
- VHF
- Multiparametric Probe SBE 911Plus
- Hydraulic portal for oceanographic instruments and sampling of planktonic and benthic communities
- Hydraulic winch with double drum
- Cable (1500 m) for multiparameter probes and samplers
- Automatic sampler Carousell SBE with 12 bottles of 10 liters
- Steel cord (600 m, 5 mm diameter)
- Wet laboratory for filtration and first processing of samples for chemical and biological analysis

M/N Vettoria

What

ON BOARD

Temperature
Salinity,
Oxygen (CTD)
Photosynthetic active radiation (PAR), surface and underwater CTD continuous
Transparency (Secchi disk)
Fluorescence by CTD, continuous, as a proxy of Chlorophyll a

IN THE LAB

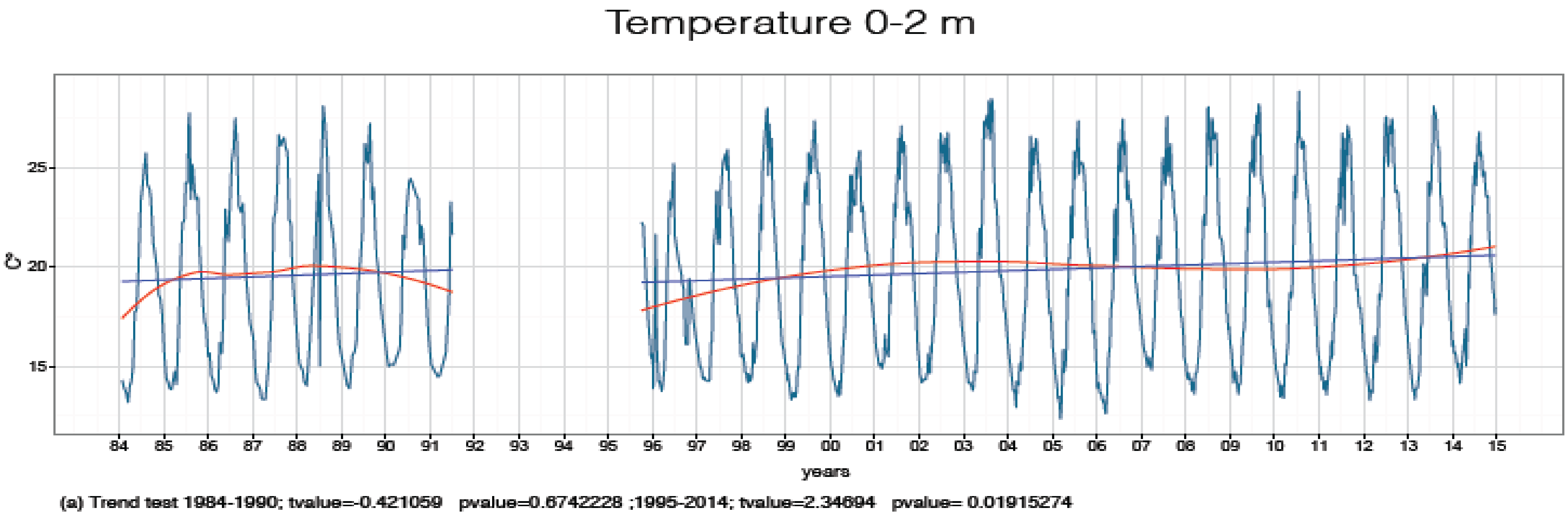
Inorganic Nutrients (Nitrates, nitrites, ammonia, phosphates, silicates
(10 depths, AutoAnalyser)
Total nitrogen, Total phosphorus, Particulate Organic Carbon, Particulate organic nitrogen
Chlorophyll a (7 depths, fluorometer)
Chl a and accessory pigments (4 depths, HPLC, since 1996)
Zooplankton biomass
Bacteria and picoplankton (Flow-cytometry, since 2007)
e-DNA on filters for metabarcoding (since 2010)

AT THE MICROSCOPE

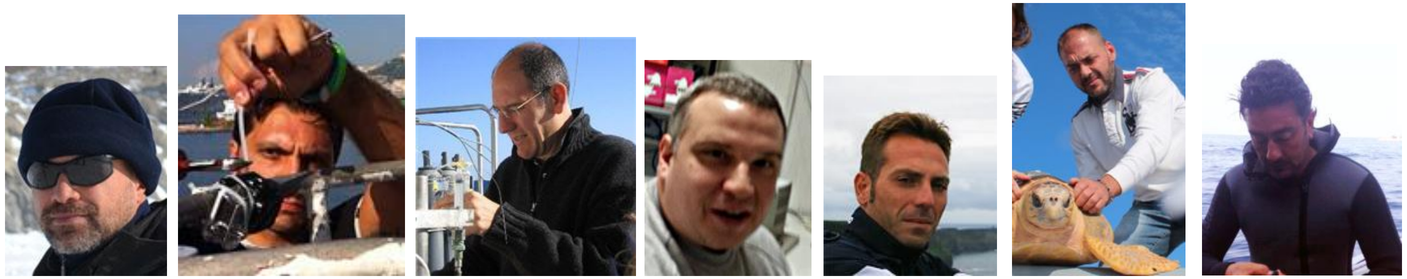
Phytoplankton diversity and abundance (surface)
Microzooplankton (surface or two depths, 1997-2008)
Zooplankton diversity and abundance (50-0 m)

When

- January 1984: start, with **fortnightly sampling**
- July 1991: break
- February 1995, restart with **weekly sampling**

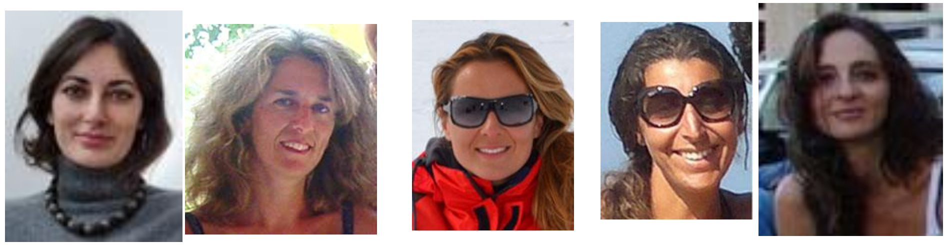


Stn LTER-MC: 1,396 sampling cruises since 1984



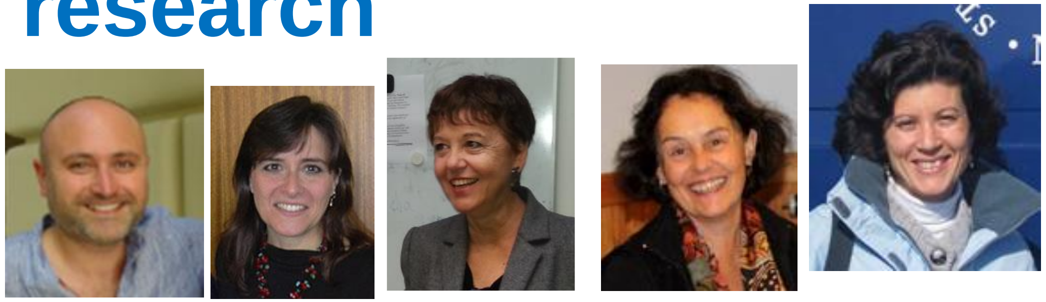
Who

at sea and in the lab



Integrative Marine Ecology Lab
Taxonomy Unit MOTAX
Microscopy UNIT AMOBIO
Molecular Biology UNIT MB&BI
Crew of R/V Vettoria

research



PhD's

postdoc's

and several others in the past

2006: The Gulf of Naples becomes part of the Long Term Ecological Research Network: LTER-MC and LTER-LA

2012: LTER MC is a founding site of the Genom Observatory Network

2012: MIUR Flagship Project RITMARE SP5- Marine Observatories

2013: PON EMSO MEDIT: structural enhancement and networking

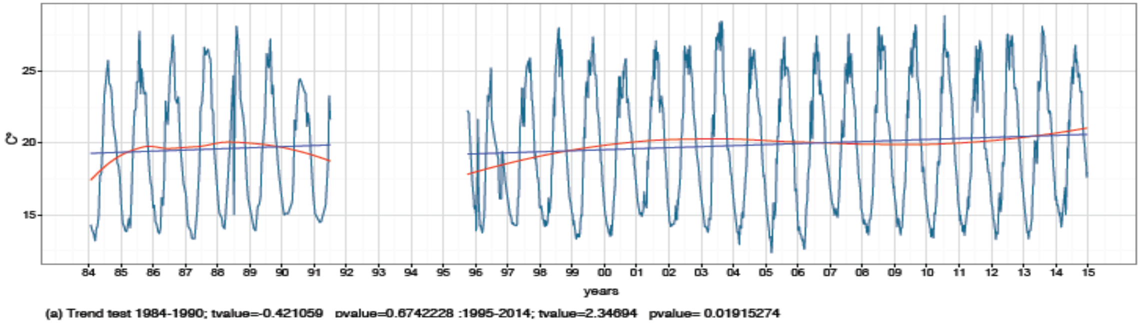
Towards a marine observatory: meeting societal needs

- **Interoperability** *QA/QC, data management* & data sharing
- **Interpretation** research and models
- **Innovation** new technologies and approaches
- **Integration** of different observational systems
- **Internationalization** through networks, working groups, projects
- **Information** publications, website, dissemination
- **Interest to Society:**, fishery, tourism, human health, GES, ocean literacy

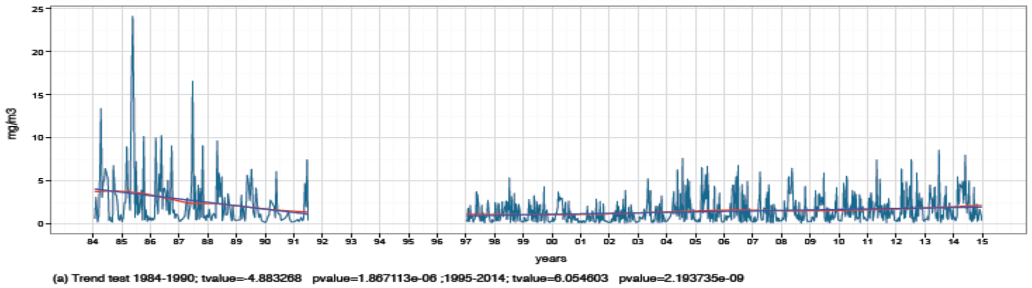
trends

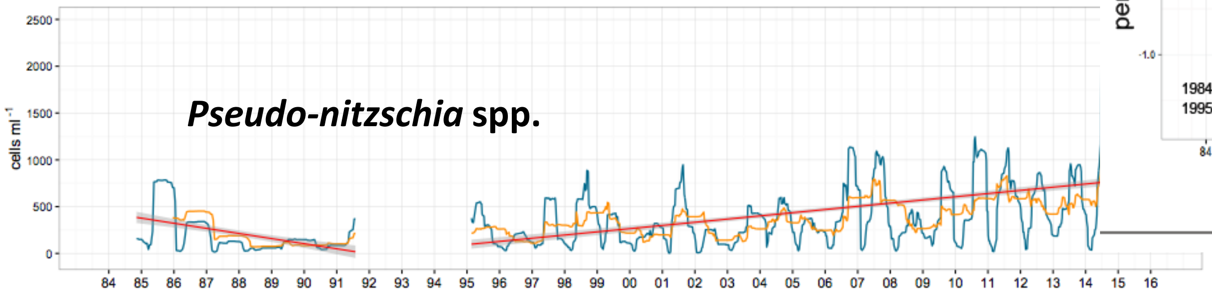
tracking environmental variability

temperature

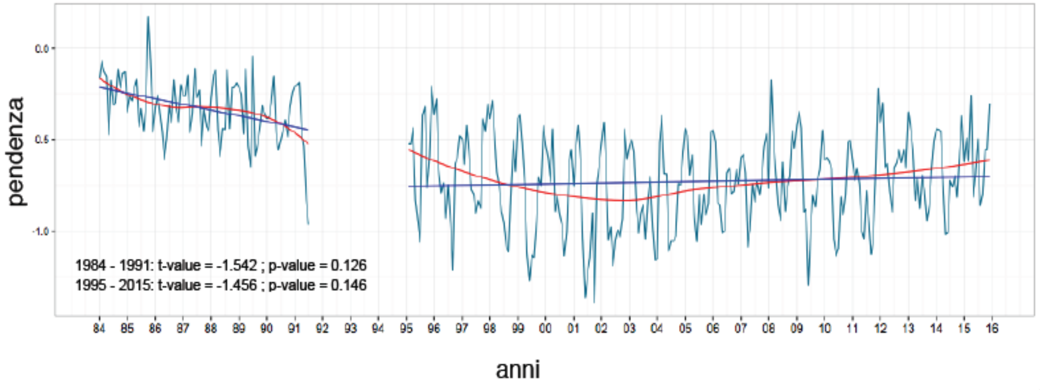


chlorophyll a





phytplankton species size



Nature Conservation 34: 273–310 (2019)
doi: 10.3897/natureconservation.34.30789
http://natureconservation.pensoft.net

REVIEW ARTICLE

A peer-reviewed open-access journal
Nature Conservation
Launched to accelerate biodiversity conservation

Time series and beyond: multifaceted plankton research at a marine Mediterranean LTER site

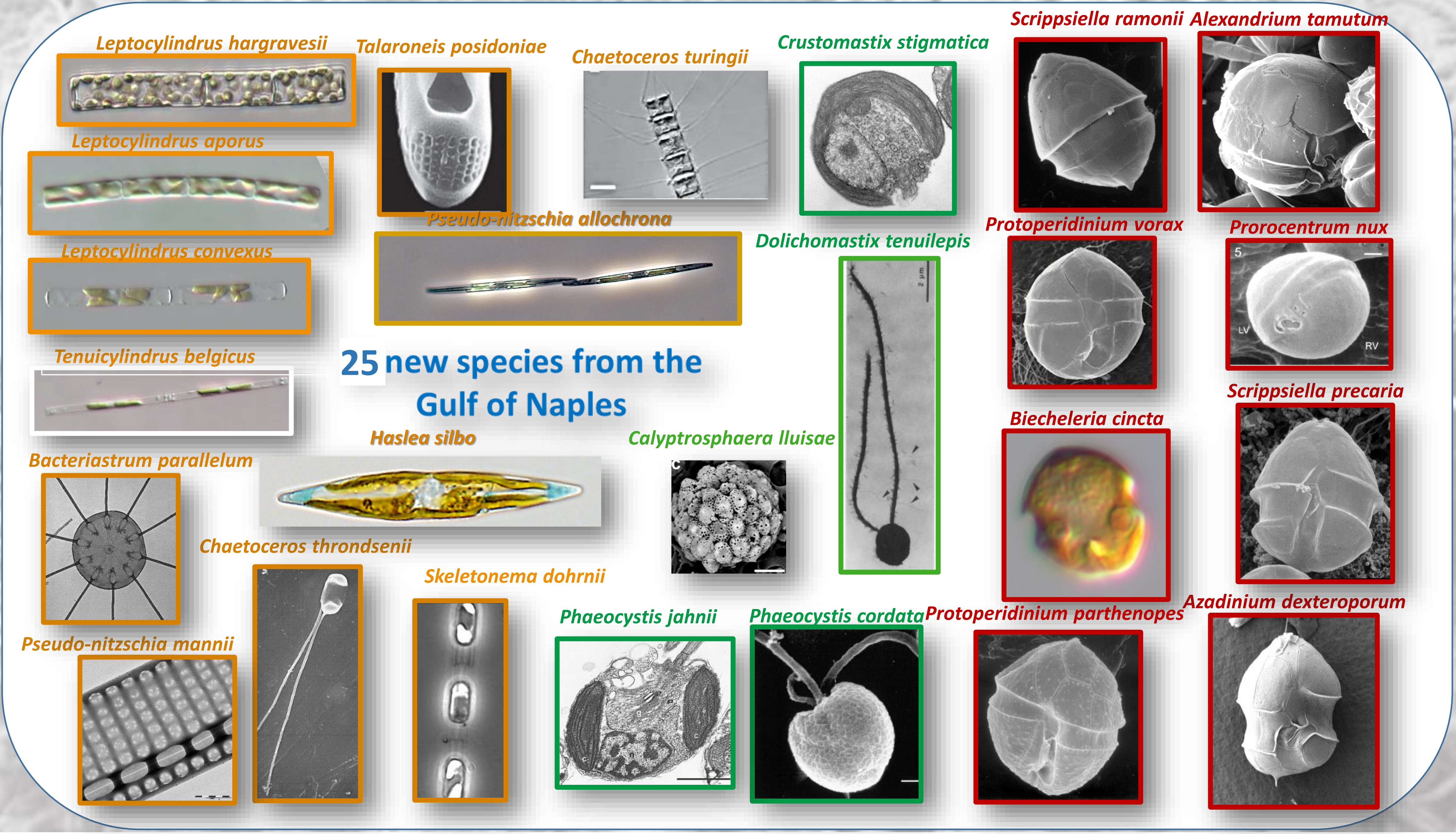
Adriana Zingone[1], Domenico D'Alelio[1], Maria Grazia Mazzocchi[1], Marina Montresor[1], Diana Sarno[1], LTER-MC team[1]

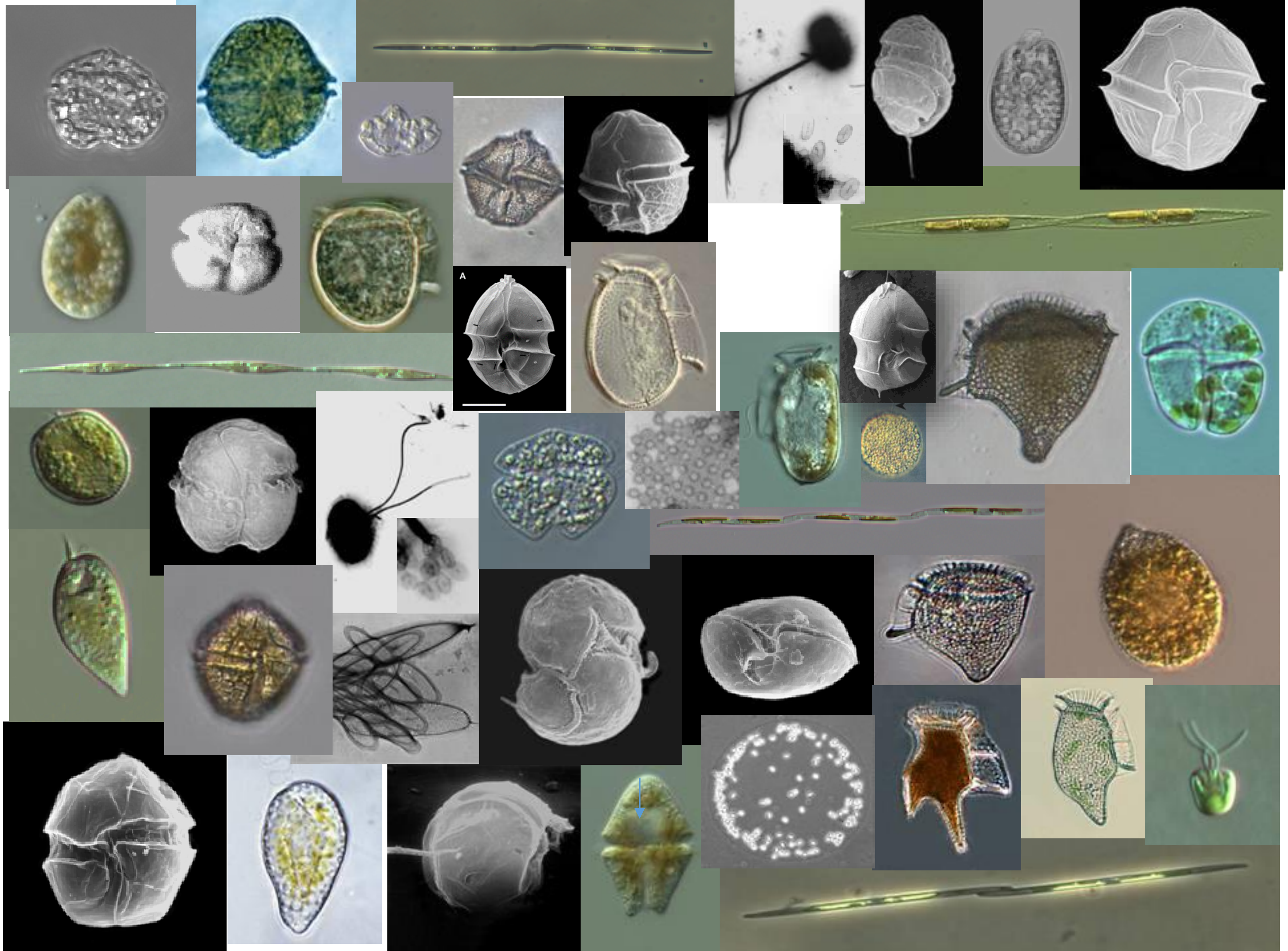
...time-series programs act as intellectual flywheels that create and sustain ever larger, complementary programs where the scientific outcome of the integrated effort is much larger than the sum of its parts

David Karl (2010)

More than 140 studies published around LTER-MC

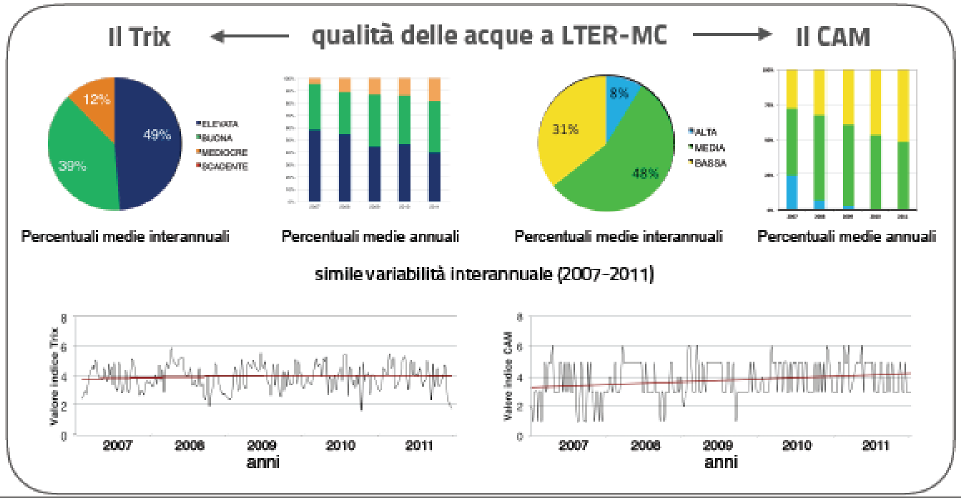
- seasonal patterns and trends;
- taxonomic diversity with a focus on key or harmful algal species;
- molecular diversity of selected species, groups of species or the whole planktonic community;
- life cycles of phyto- and zooplankton species: cysts, spores, resting stages
- interactions: trophic relationships, parasites and viruses.


25 new species from the Gulf of Naples
Leptocylindrus hargravesii
Talaroneis posidoniae
Chaetoceros turingii
Crustomastix stigmatica
Scrippsiella ramonii
Alexandrium tamutum
Leptocylindrus aporus
Pseudo-nitzschia allochrona
Protoperidinium vorax
Prorocentrum nux
Dolichomastix tenuilepis
Leptocylindrus convexus
Tenuicylindrus belgicus
Scrippsiella precaria
Biecheleria cincta
Haslea silbo
Calyptrosphaera lluisae
Bacteriastrum parallelum
Chaetoceros throndsenii
Skeletonema dohrnii
Phaeocystis jahnii
Phaeocystis cordata
Protoperidinium parthenopes
Azadinium dexteroporum
Pseudo-nitzschia mannii

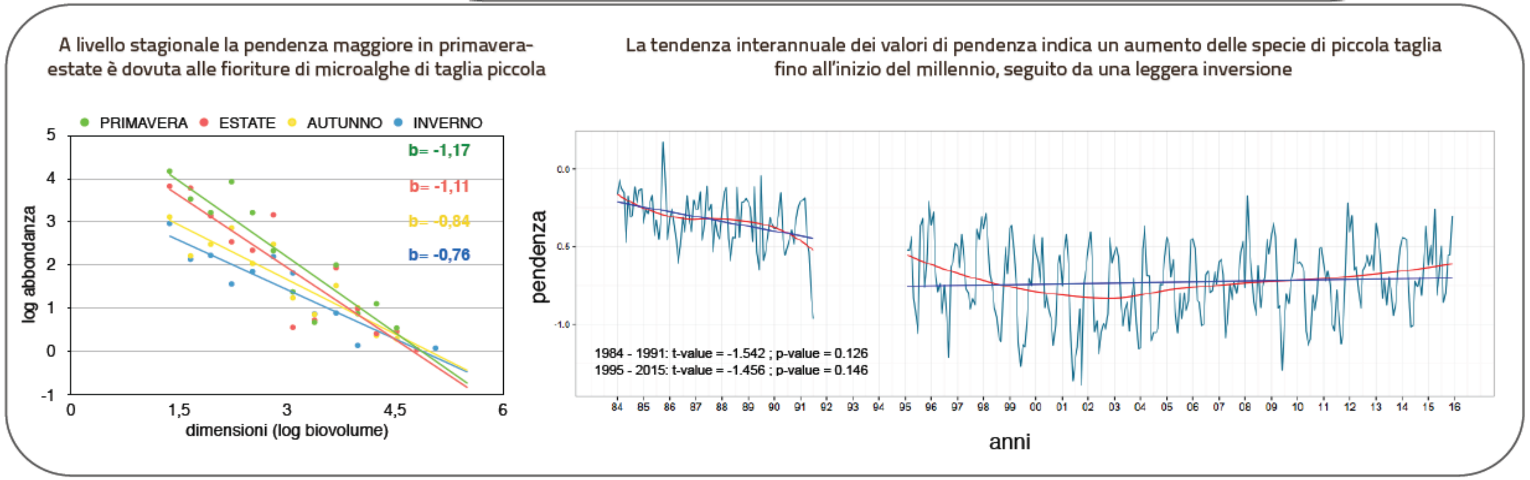


>50 potentially toxic species in Campania waters

➢ Testing classical and new synthetic **indicators** of environmental status



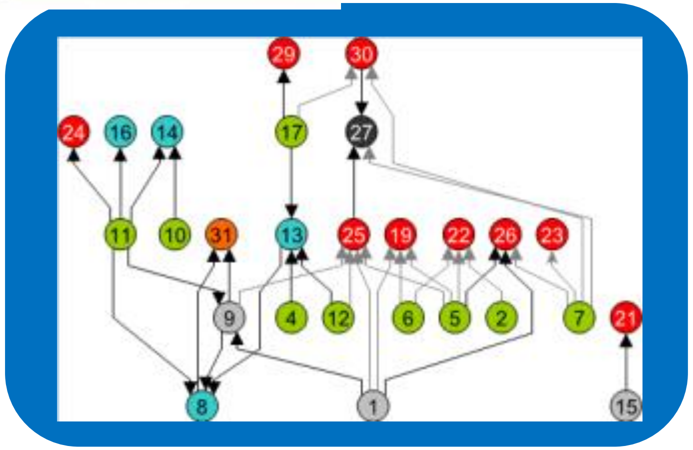
Based on a combination of variables, i.e. nutrients, oxygen, biomass, water transparency, etc.

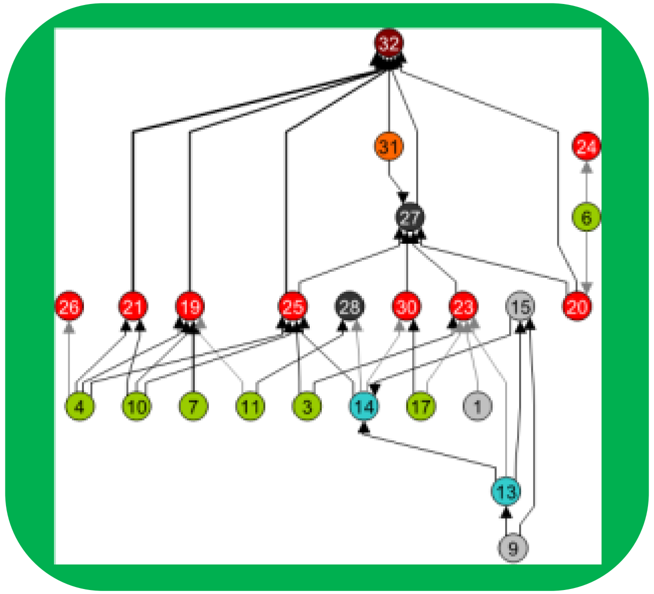


Based on organisms' size

LTER MC Marechiara

Food-web analysis





Protists

- Autotrophic
- Mixotrophic
- Heterotrophic

Mesozooplankon

- Feeding on protists
- Detritivores
- Carnivores
- Feeding on both protists and mesozooplankton

n Taxon code

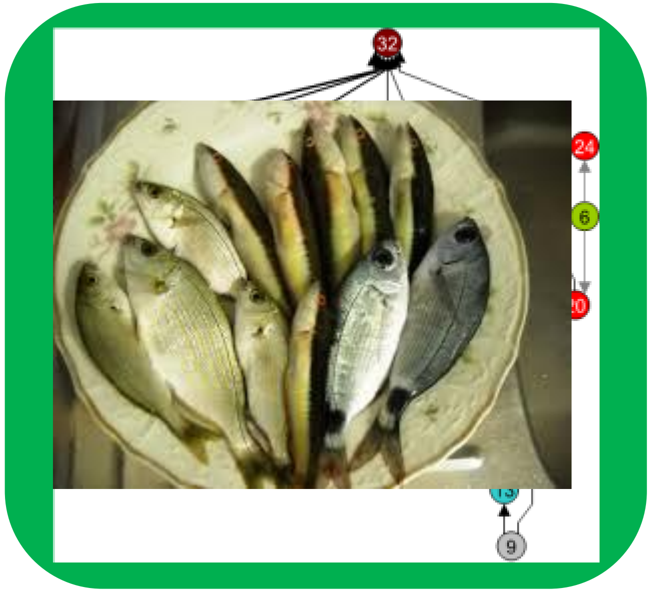
The same planktonic community shows two different organizations based on the state of the system

In <u>Blue Waters</u>, the flux of organic matter is more scattered and integrated into a microbial loop

<u>In Green Waters</u>, the flux of organic matter is mainly oriented from protists to animals at higher trophic levels

D'Alelio et al. 2016

Food-web analysis



Protists

- Autotrophic
- Mixotrophic
- Heterotrophic

Mesozooplankon

- Feeding on protists
- Detritivores
- Carnivores
- Feeding on both protists and mesozooplankton

n Taxon code

The same planktonic community shows two different organizations based on the state of the system

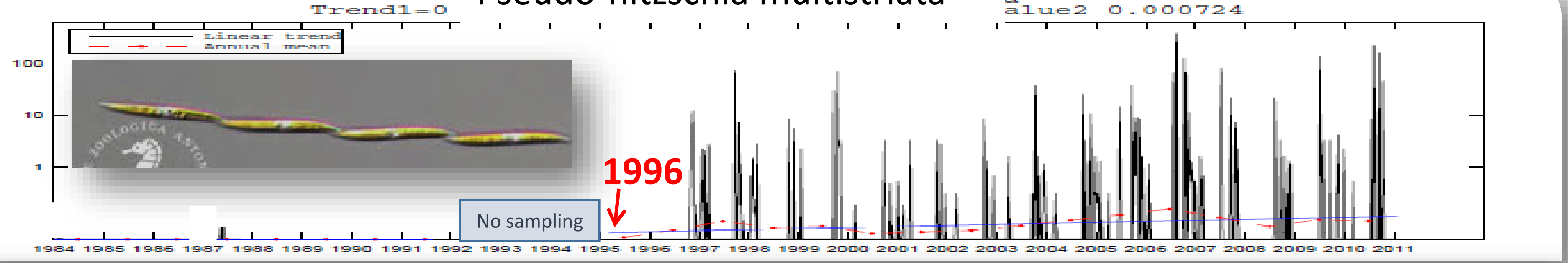
In Blue Waters, the flux of organic matter is more scattered and integrated into a microbial loop

- In Green Waters, the flux of organic matter is mainly oriented from protists to animals at higher trophic levels

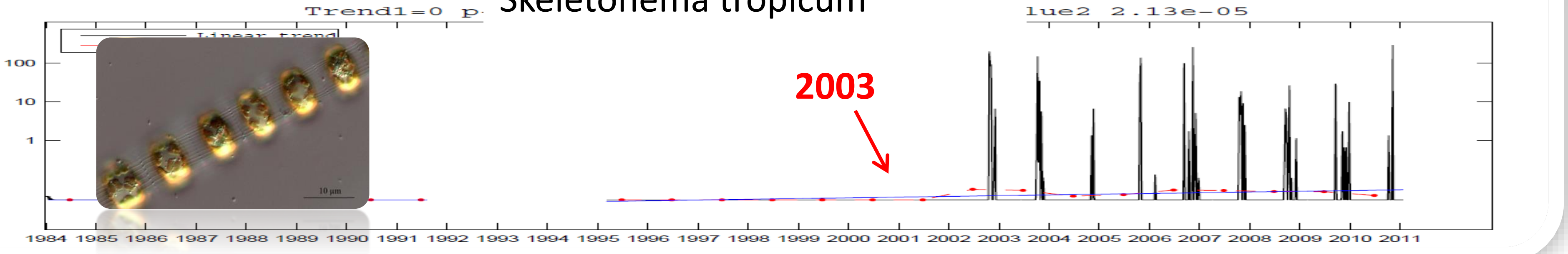
D'Alelio et al. 2016

non-indigenous species

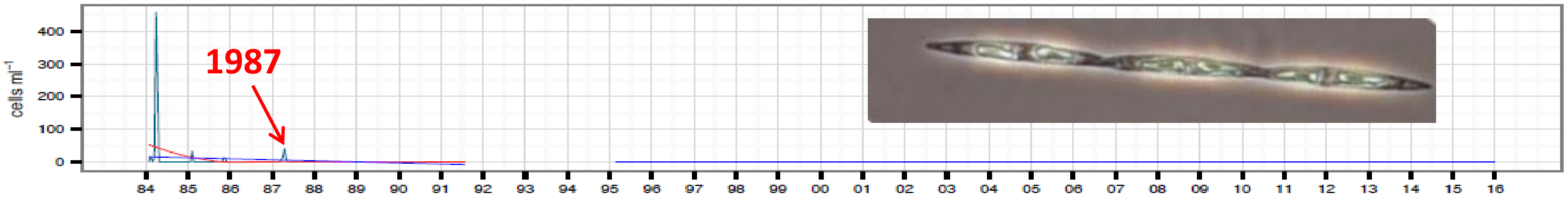
Pseudo-nitzschia multistriata



Skeletonema tropicum



Pseudo–nitzschia subpacifica



Phytoplankton variability and periodicity at LTER-MC

Lorenzo Longobardi, PhD thesis

Diana Sarno, Motax, SZN

Laurent Dubroca, IFREMER

Lorenzo Longobardi, PhD thesis

Environmental variability at LTER-MC

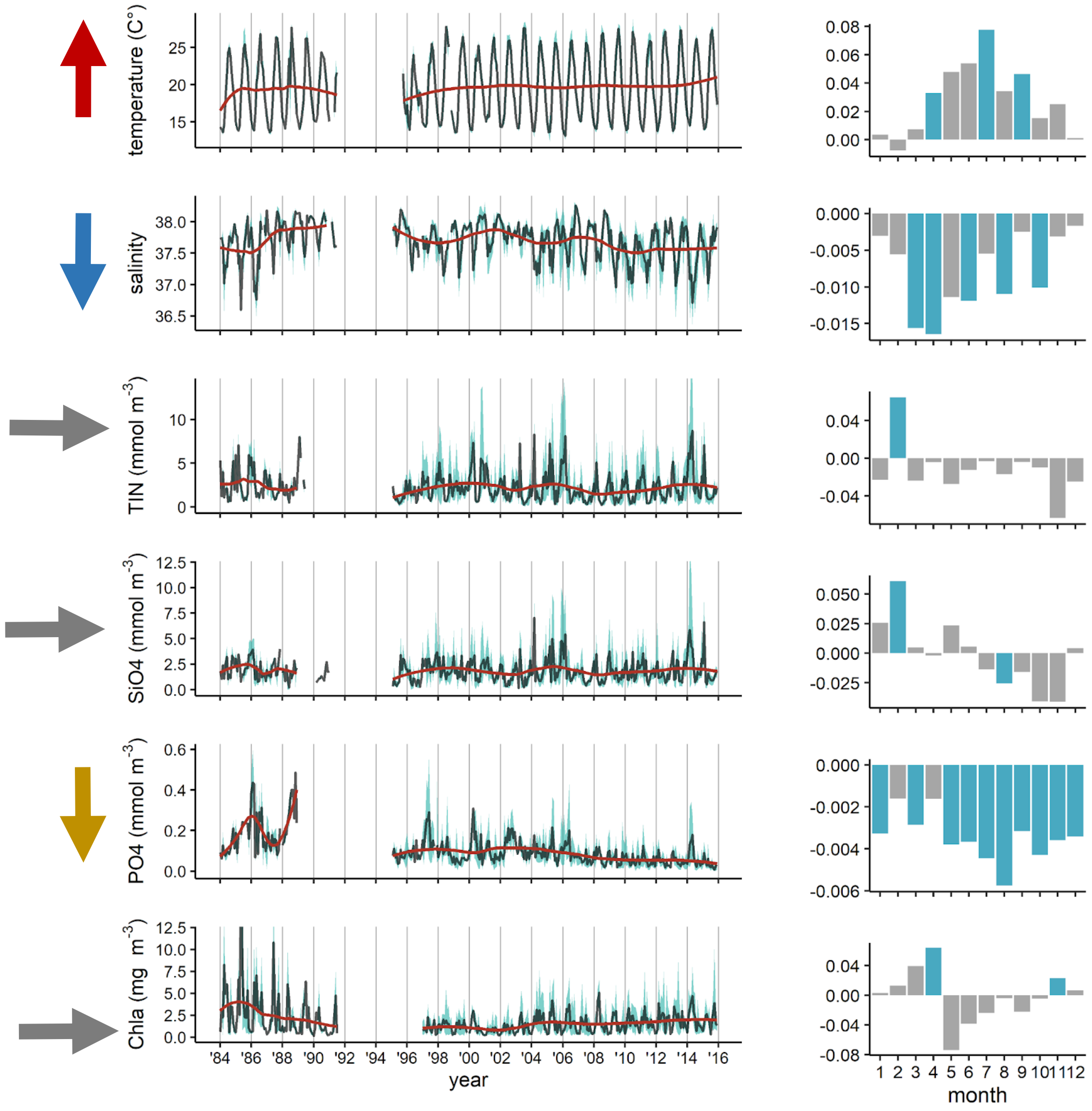
temperature ↑

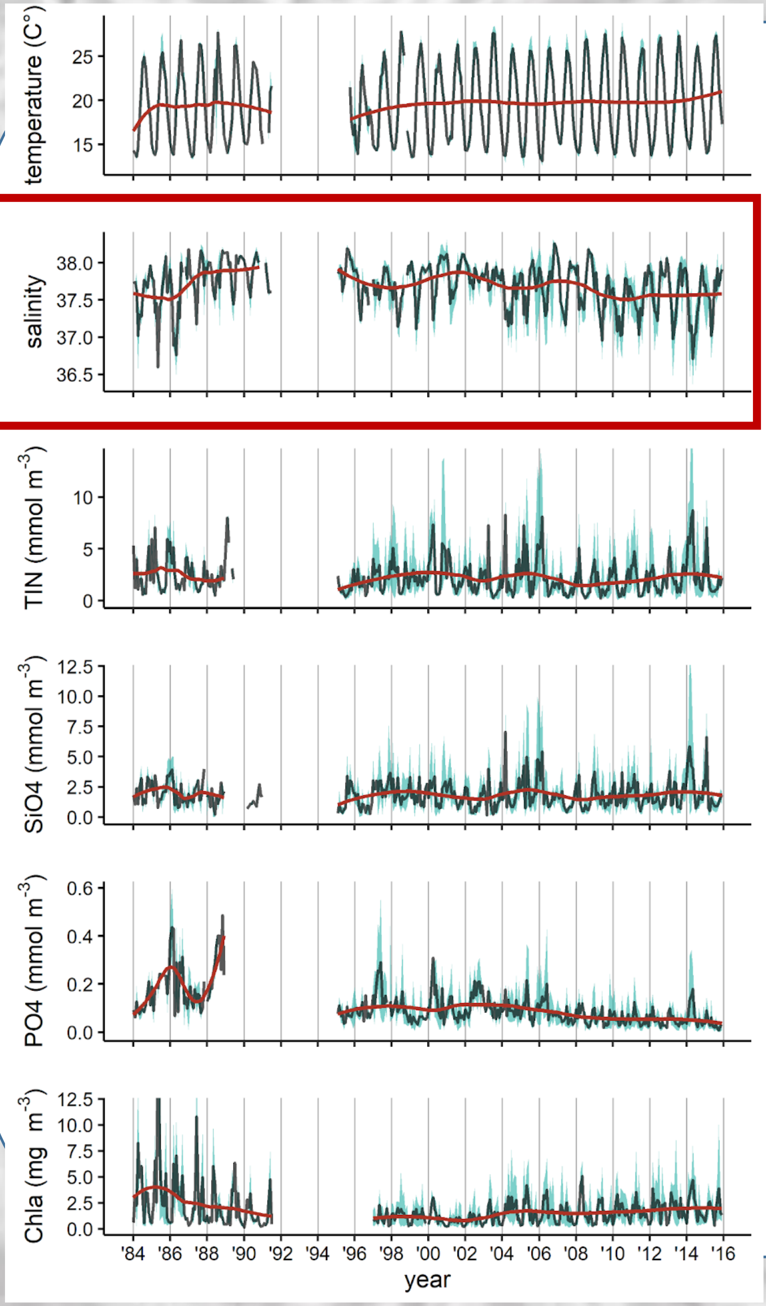
salinity ↓

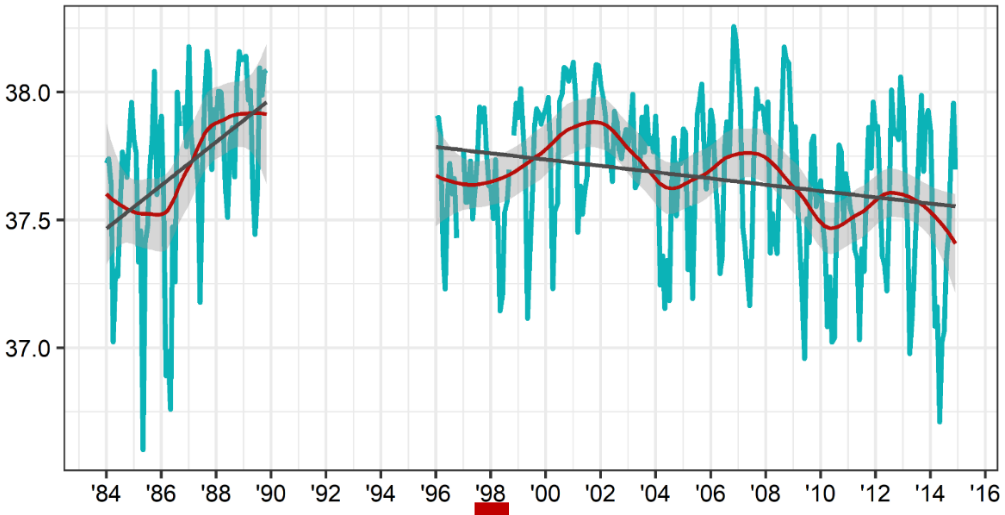
phosphates ↓

TIN, SO_4, Chla → no clear trend, irregular fluctuations

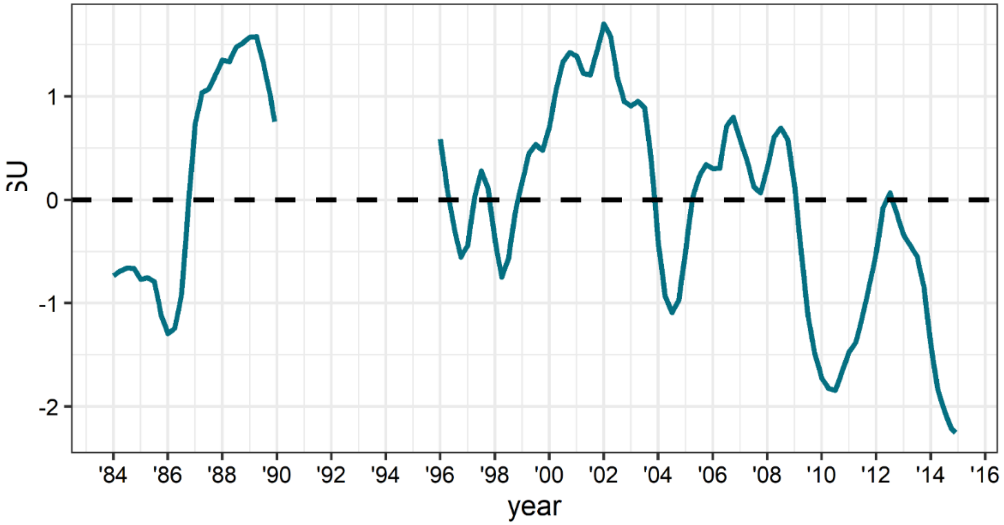
LTER-MC is characterized high environmental variability, with strong seasonal patterns, isolated events, fluctuations and trends

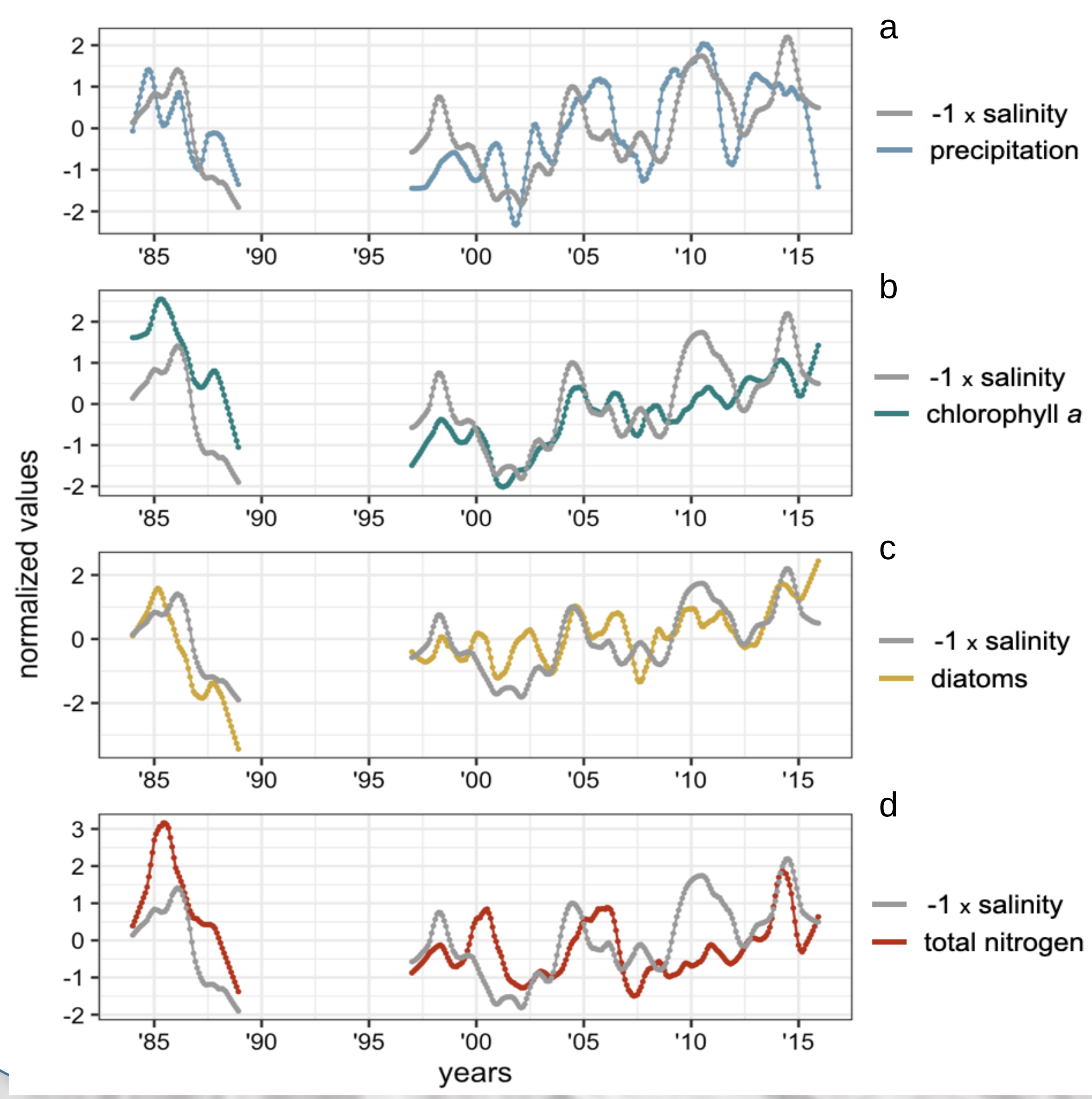






Trend extraction (LOESS)





+ precipitations

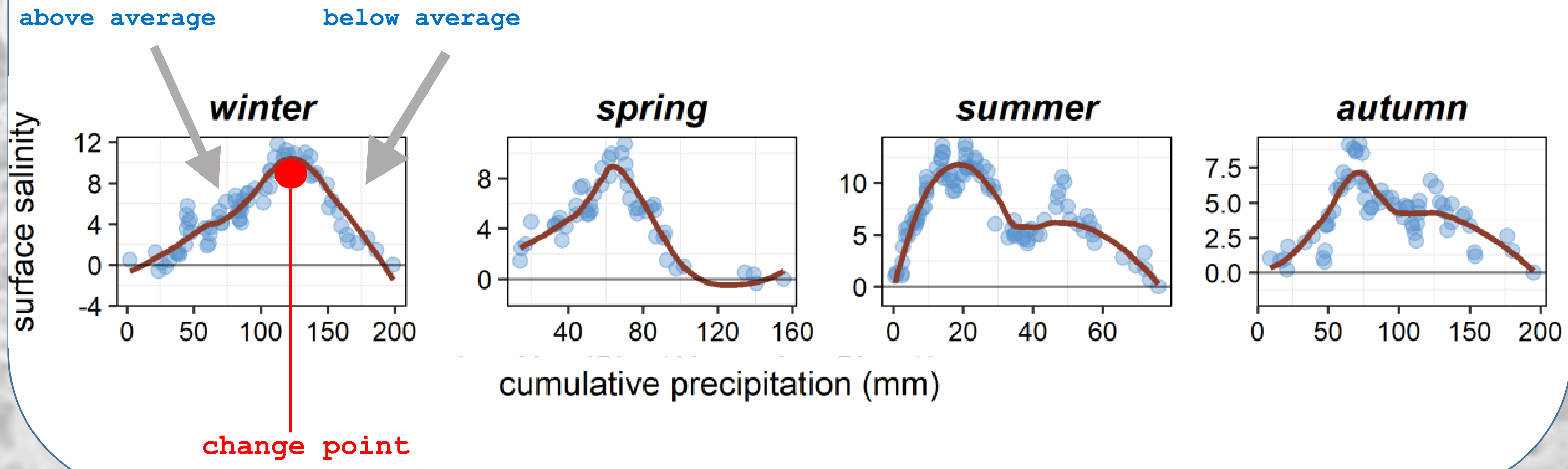
runoff

- salinity

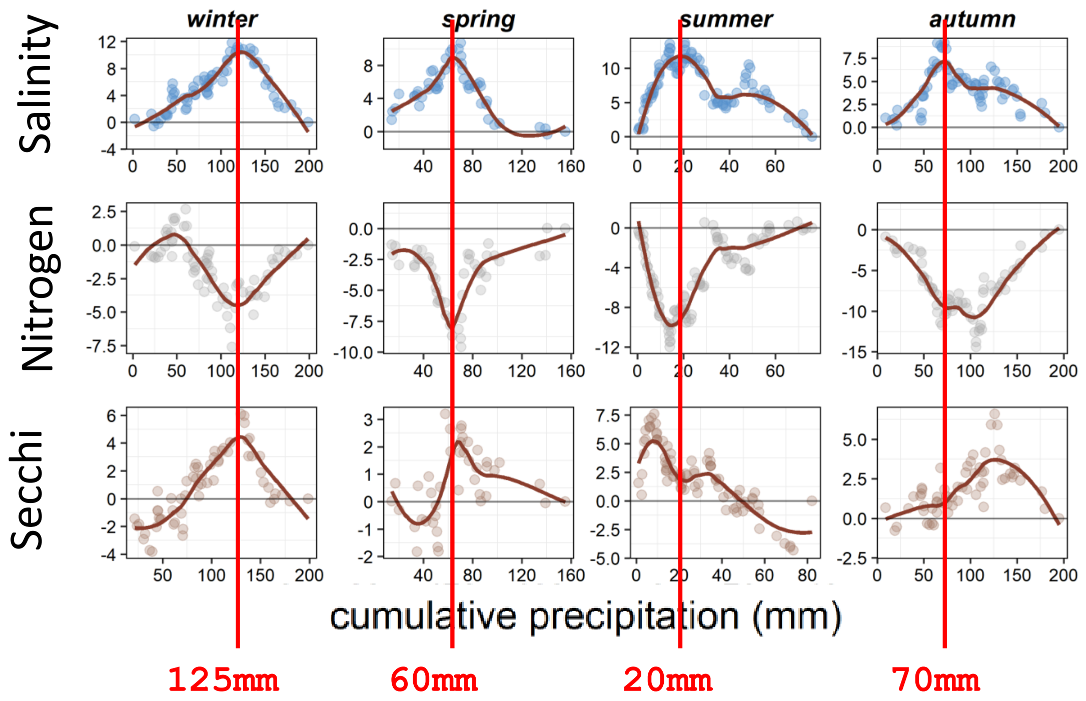
+ diatoms biomass

Testing the seasonal impact of precipitations on the LTER-MC system

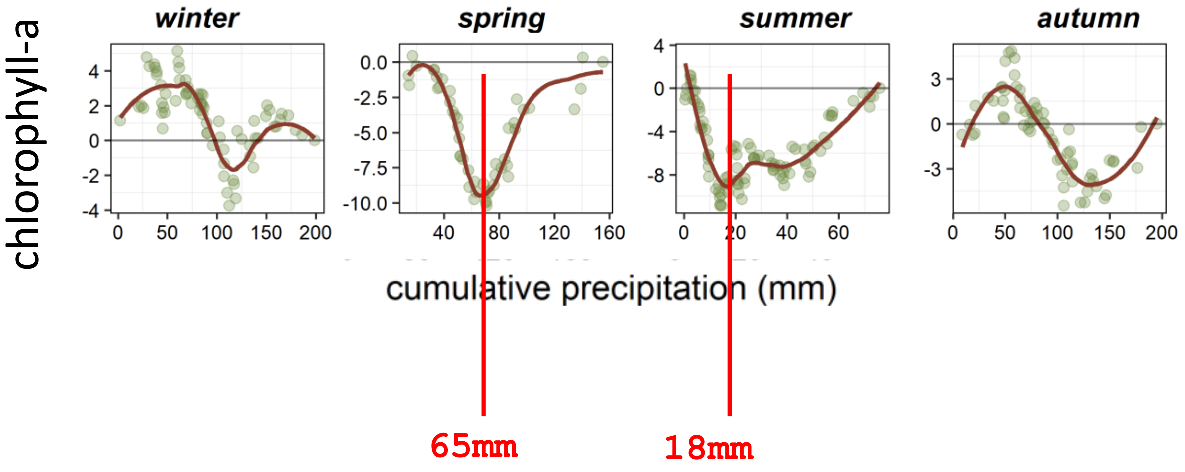
Driver-response curves: Cumulative distribution of environmental and biological anomalies ranked on the precipitation gradient (Regier et al. 2019)



Environmental parameters' response



Biological response



- **Change point at different precipitation values over the seasons**
- **Complex biological responses in winter and autumn: more complex meteorological patterns? weaker biological signal?**

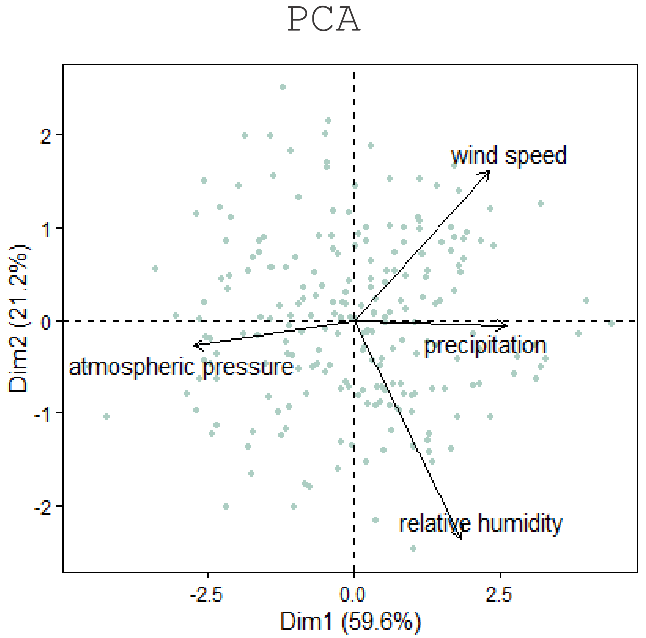
LTER-MC climatic index

Precipitation

Wind speed

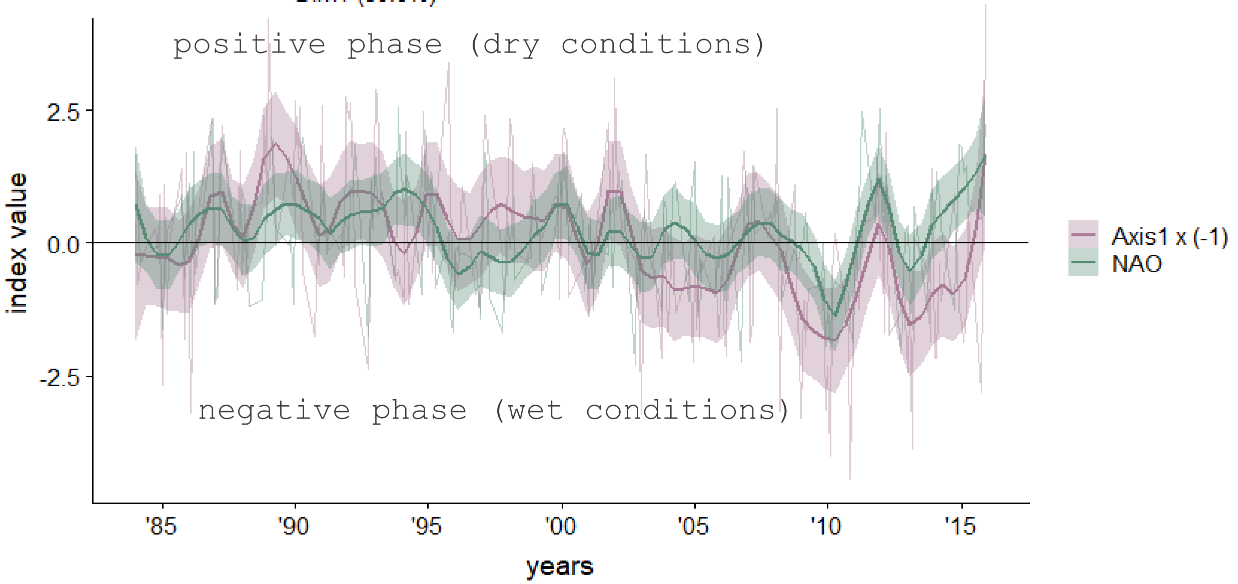
Atmospheric pressure

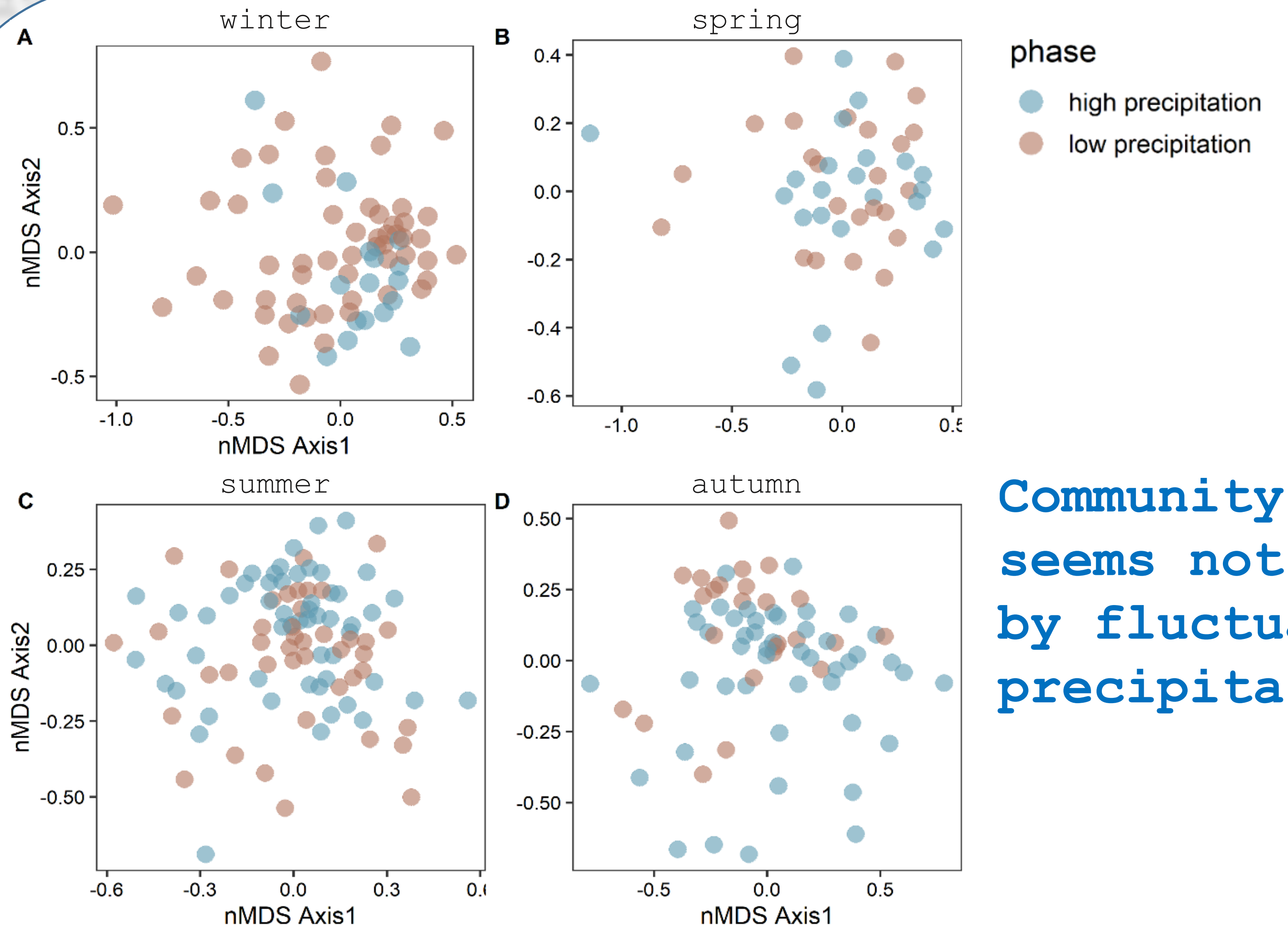
Relative humidity



First axis explains 60% of climate variability at LTER-MC and can be used as a climate index

LTER-MC climate index is correlated with the North Atlantic Oscillation (NAO) (Pearson corr. = -0.39).

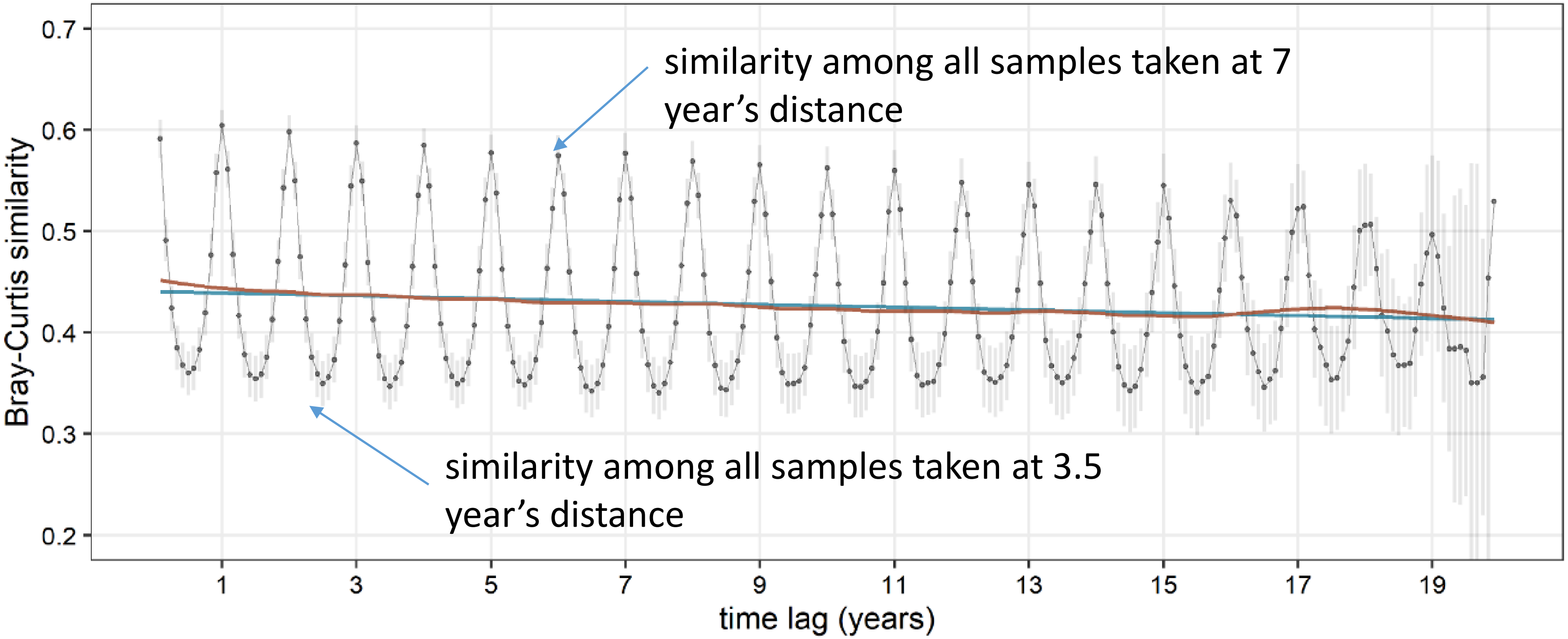




Community composition seems not to be affected by fluctuations in precipitation

Phytoplankton periodicity

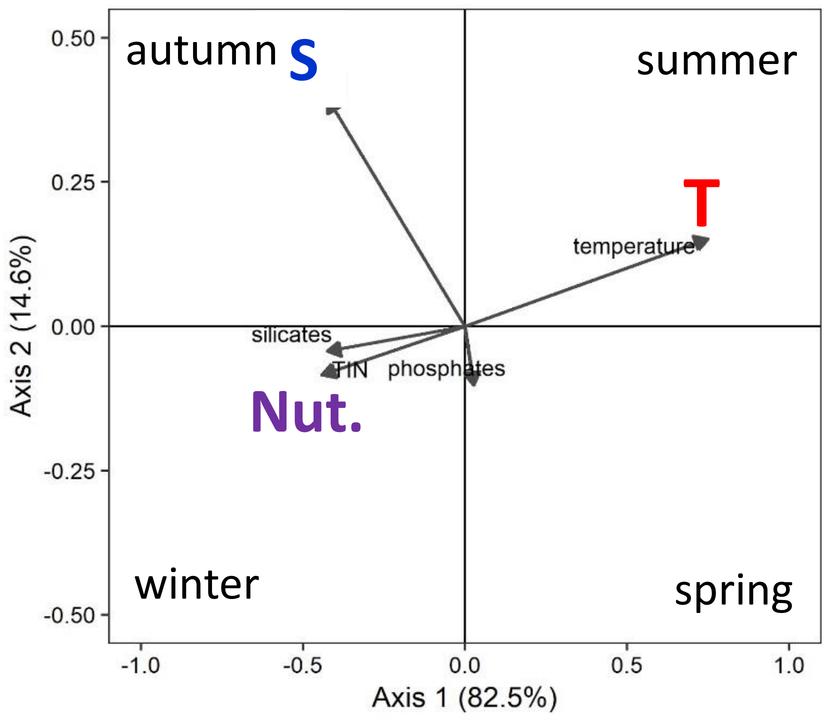
community similarity at different time lags

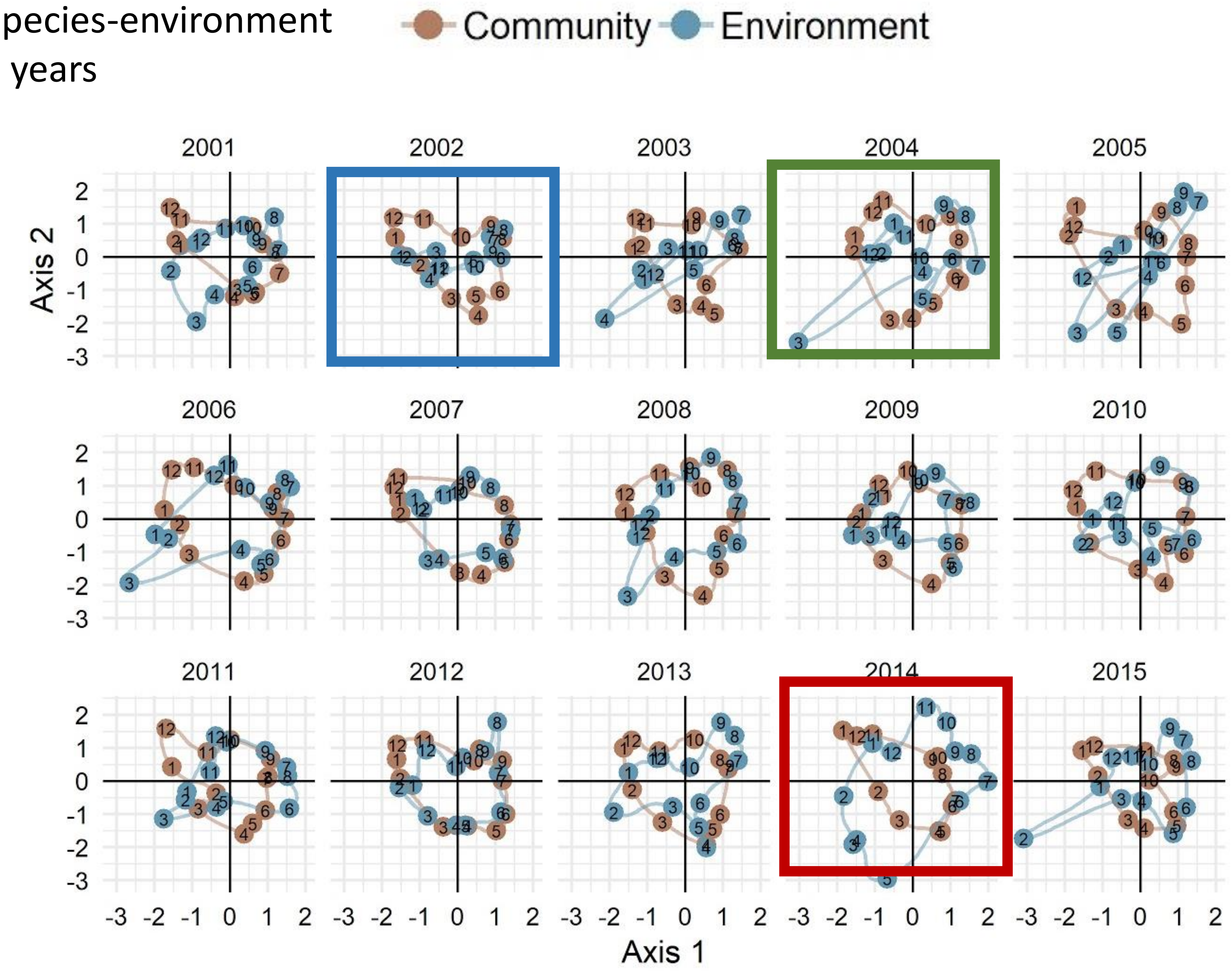


Discriminant Function Analysis (DFA) predicts the sampling month in 89% of the cases**. Day length** and **temperature** explain 44% and 24 % of the variance, respectively

STATICO

compromise: common structure of the species-environment relationship over the different years

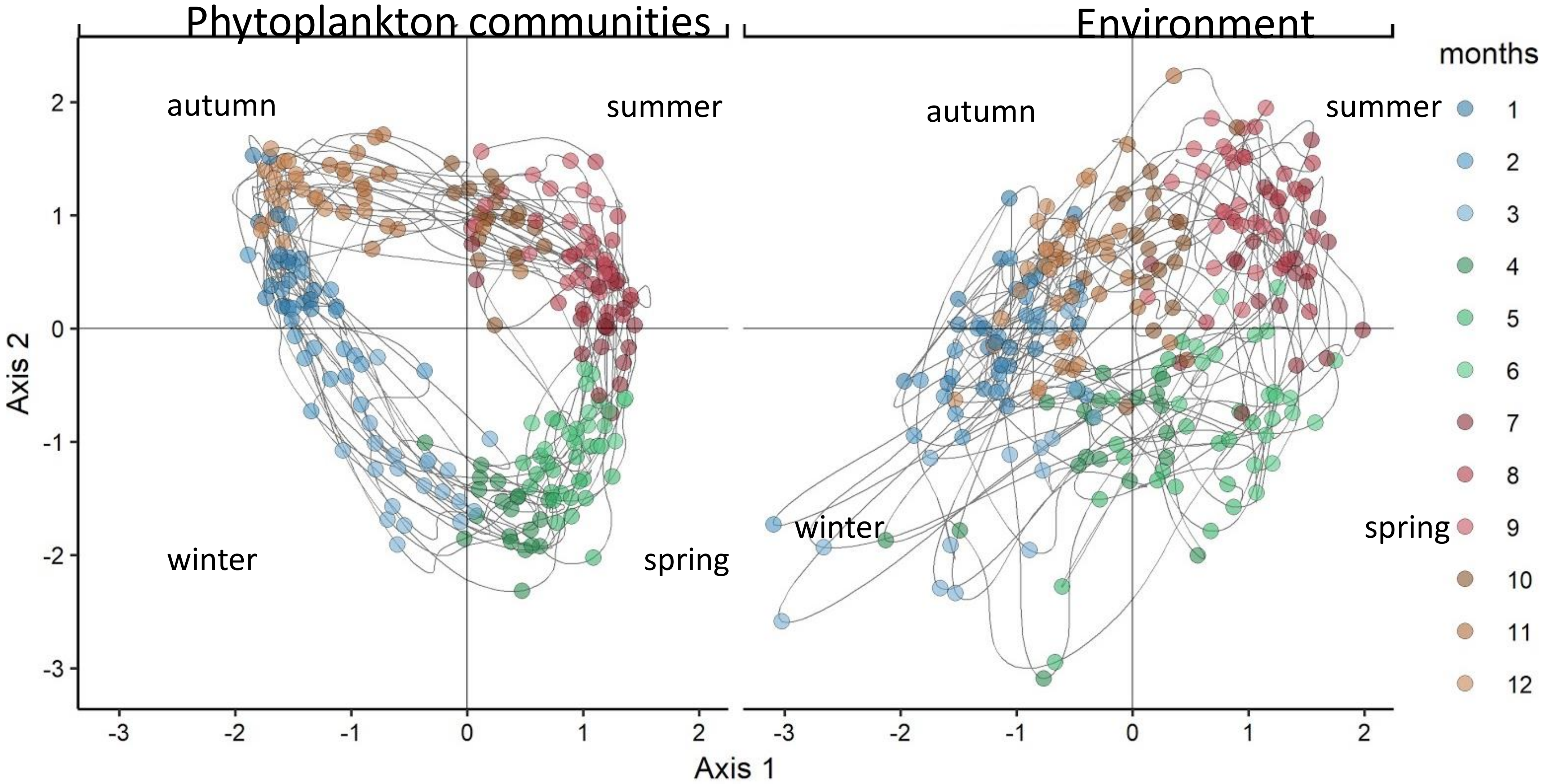




salinity fluctuations

nutrients fluctuations

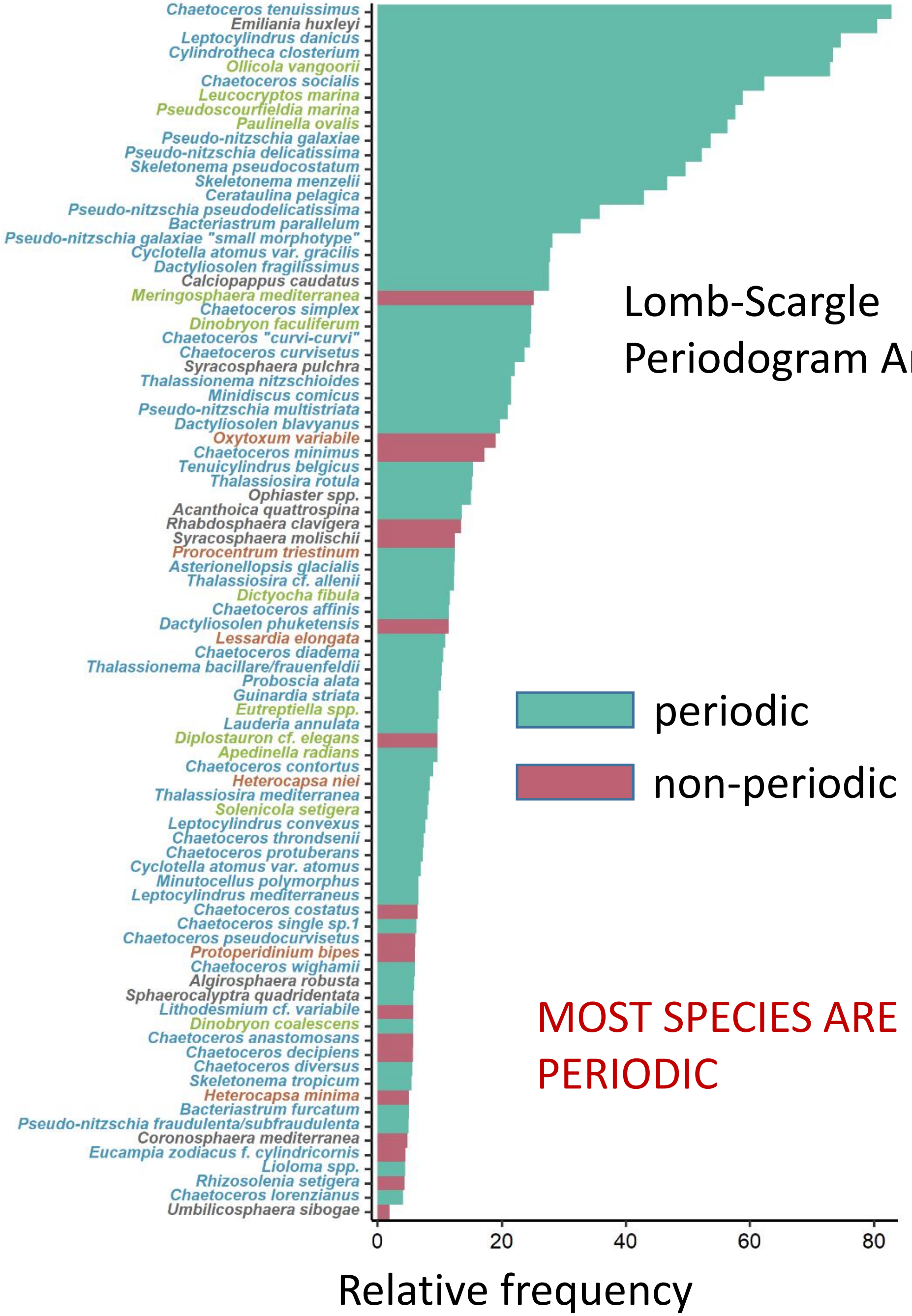
temperature fluctuations



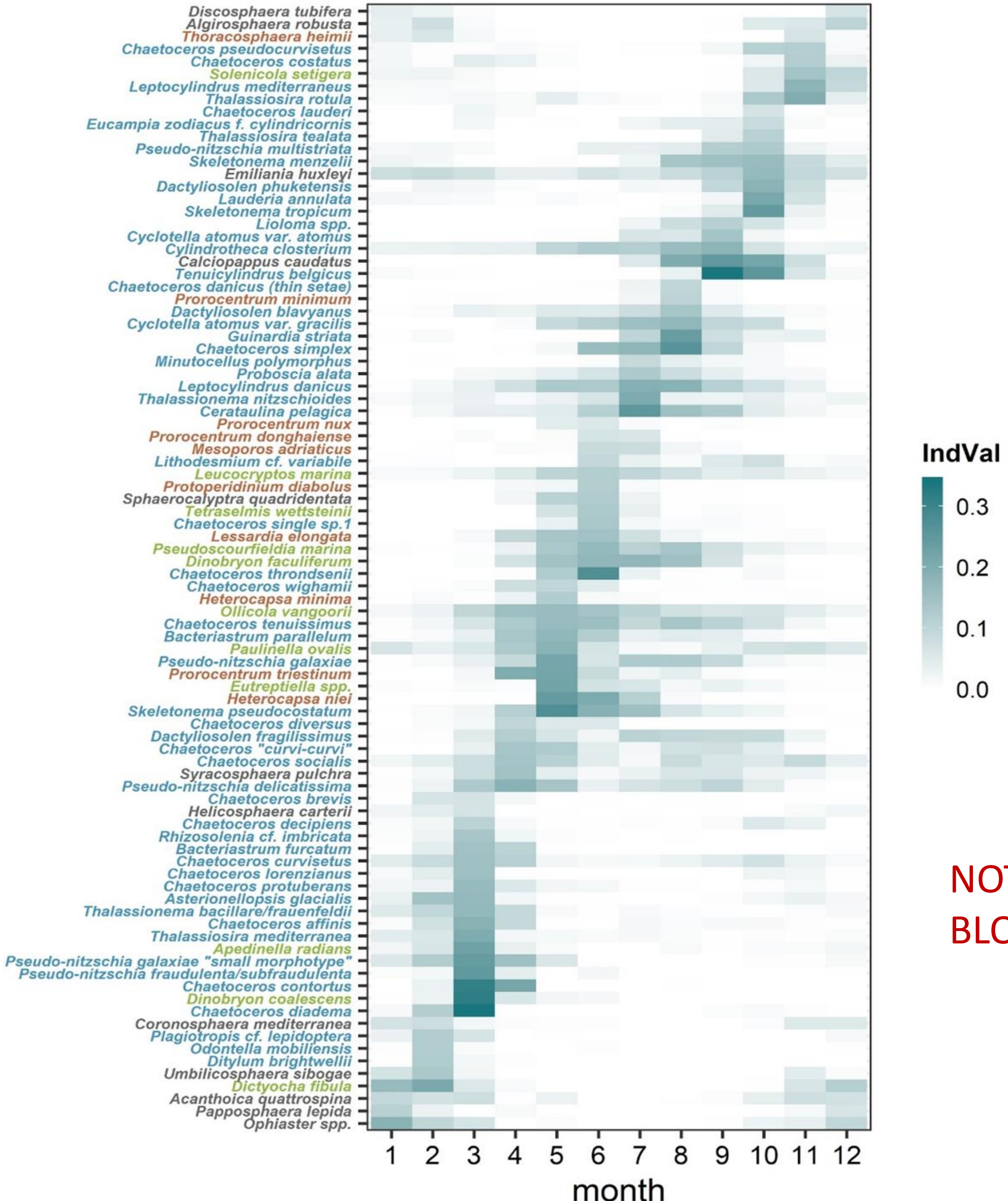
Phytoplankton community are resistant/resilient to trends, fluctuations and environmental perturbations:
Not a good indicator of environemntal changes?
Signals may be hidden in more subtle changes
Systems may shift abruptly – tipping points

Longobardi, Dubroca et al., in preparation

Lomb-Scargle Periodogram Analysis



MOST SPECIES ARE PERIODIC

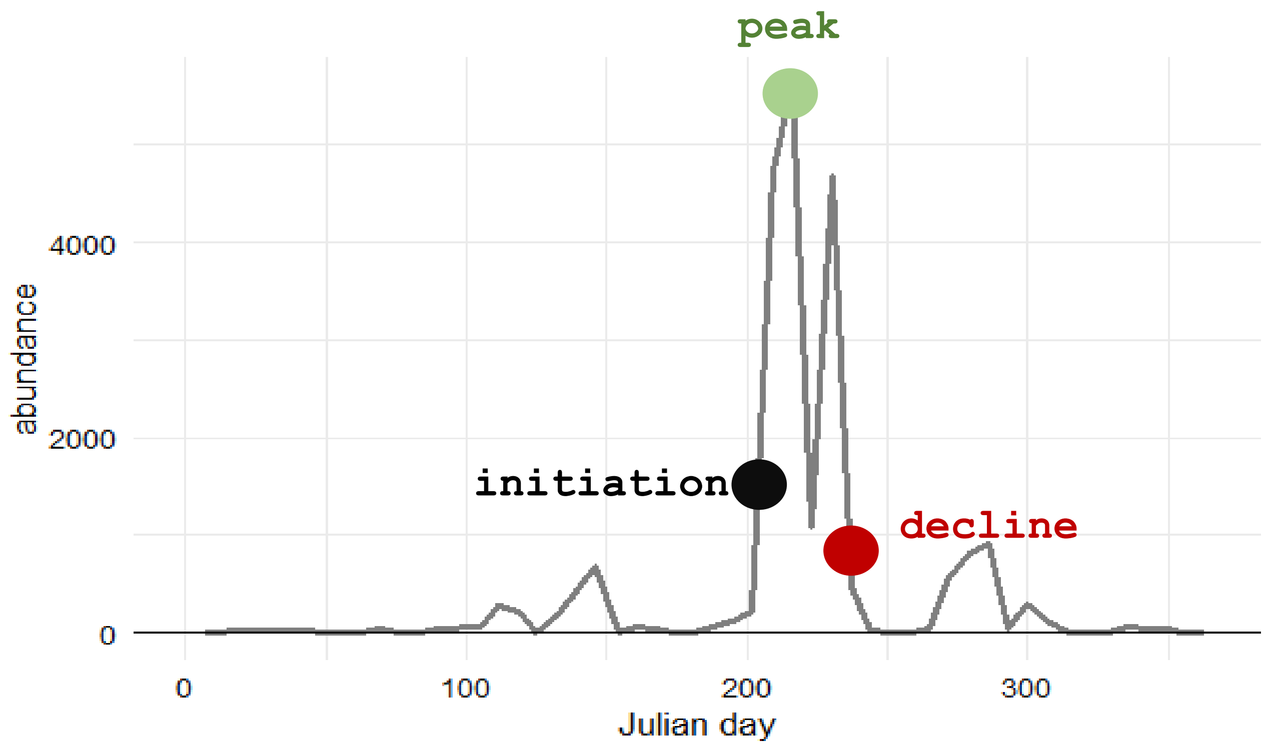


= fidelity and relative abundance

NOT ALL SPECIES BLOOM IN SPRING

The drivers of species' phenological patterns

Extraction of ecologically relevant temporal points

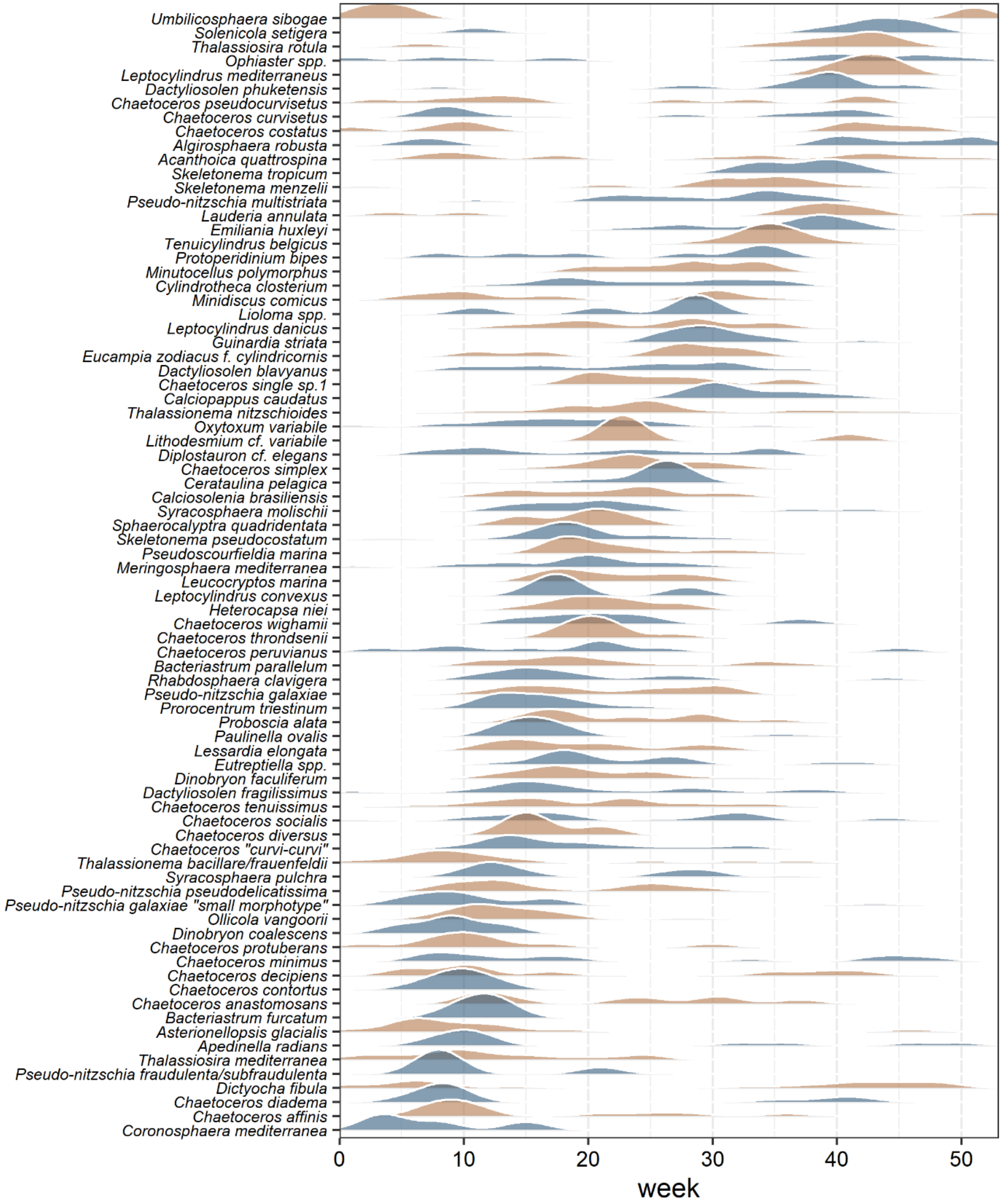


R package "Rplanktonanalytic"

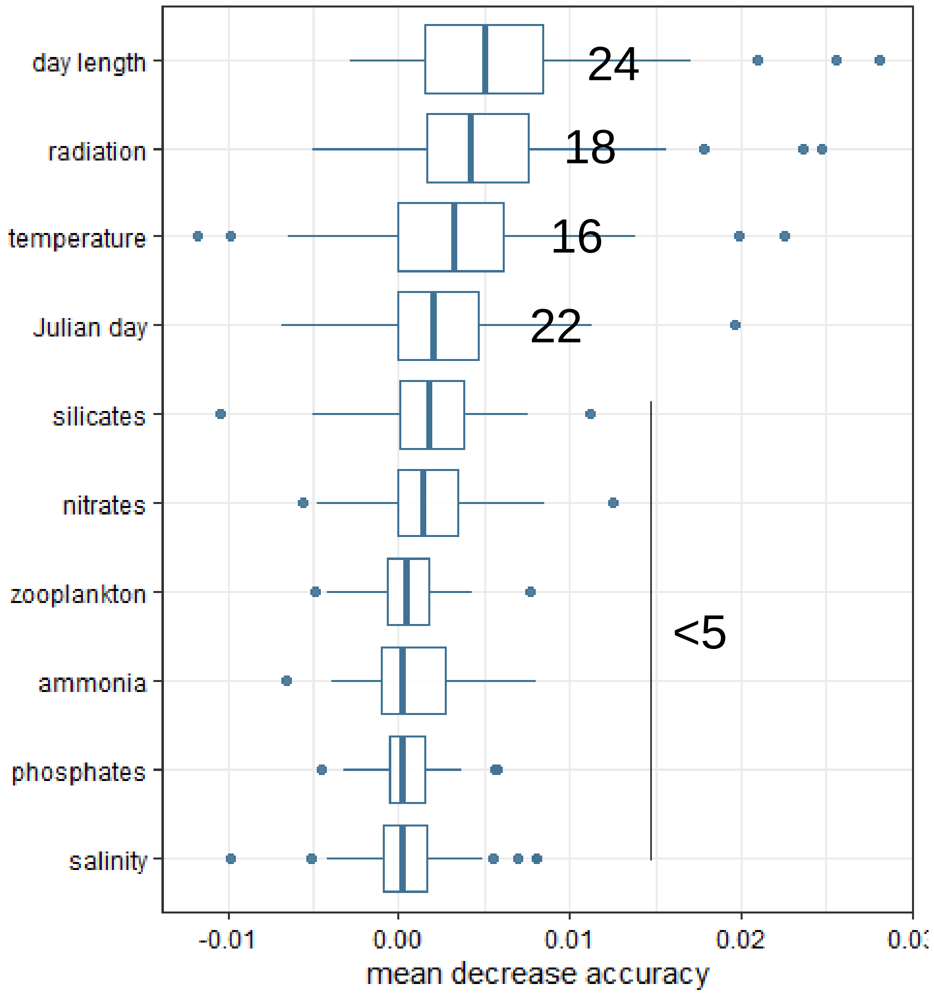
by Lorenzo Longobardi and Laurent Dubroca

GitHub https://github.com/ldbk/Rplanktonanalytic

- Bloom phases
- Bloom duration
- Temporal variability of bloom timing
- Trends related to phenological patterns



Species bloom initiation



random forest model

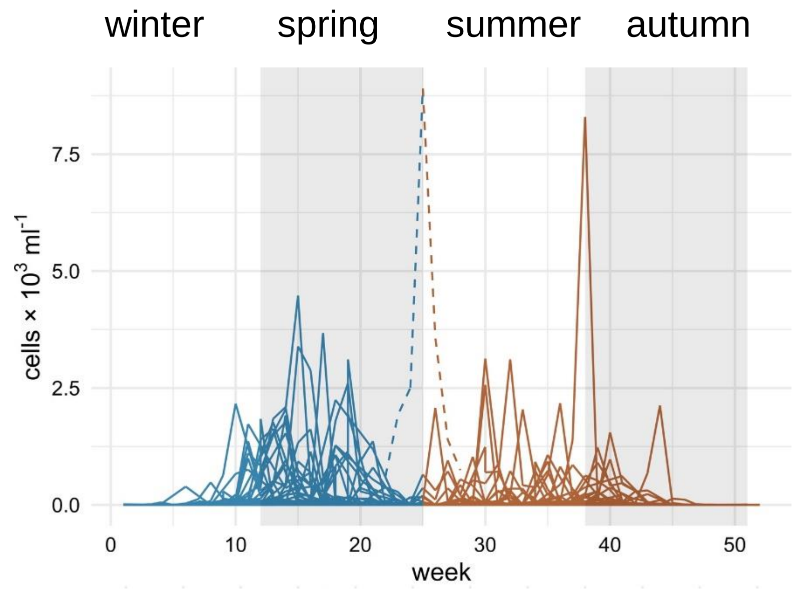
Photoperiod is the predominant factor regulating species turnover and replacement:

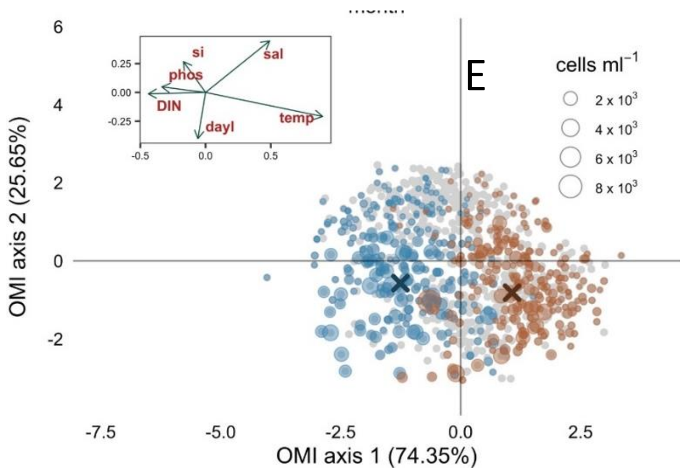
Photoperiod is a reliable factor to synchronise growth and allow sexual reproduction

Like in terrestrial plants, a strong biological component drives species phenology

Phenological differences among cryptic species

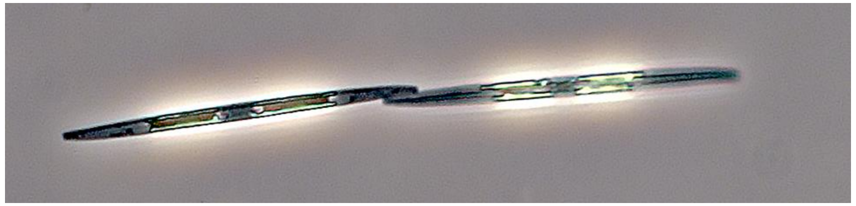
D



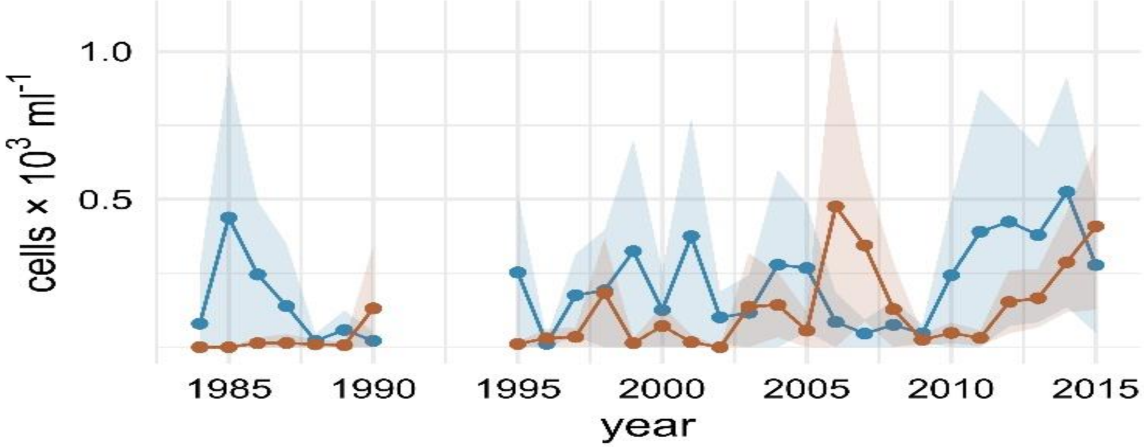


P. delicatissima/ P. arenysensis/ P. dolorosa

P. allochrona



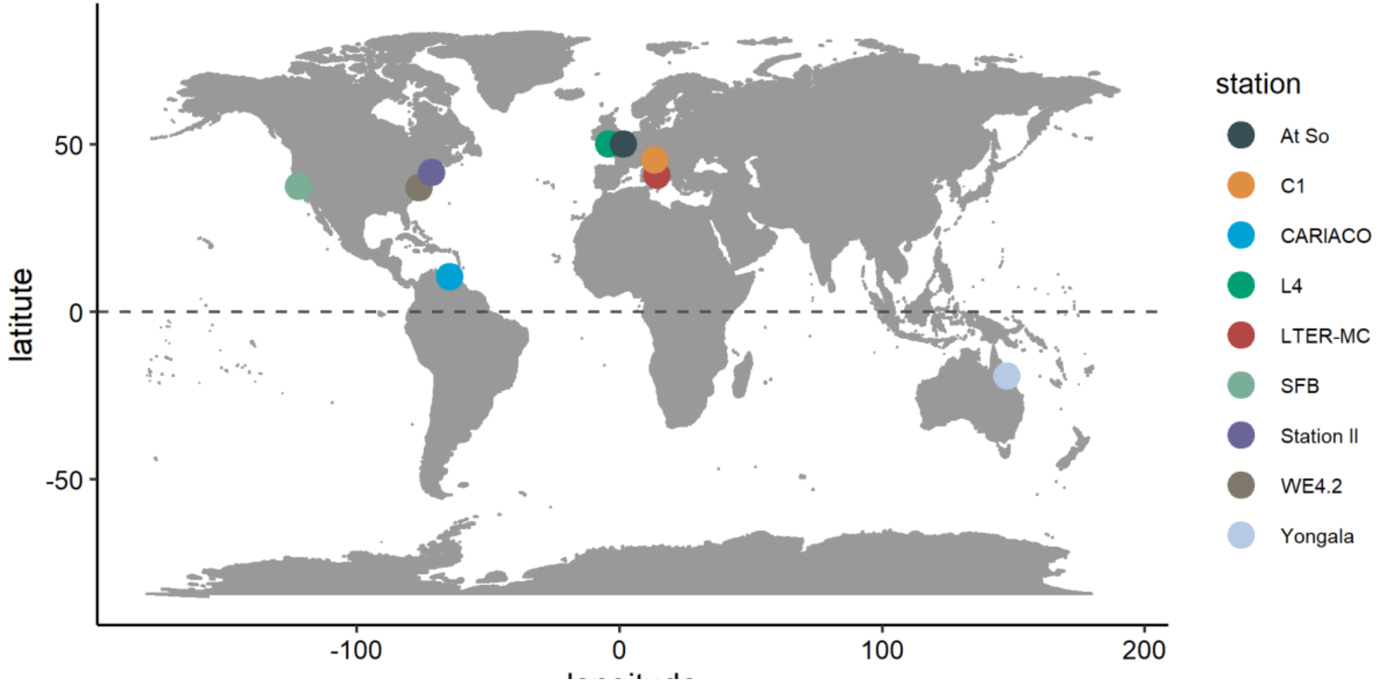
Phenological segregation suggests speciation by time in Pseudo-nitzschia allochrona sp. nov.



Percopo et al., BioRxiv 2021

Beyond LTER -MC

Do phytoplankton species show the same ecological niche over the global coastal seas?



Environmental parameters

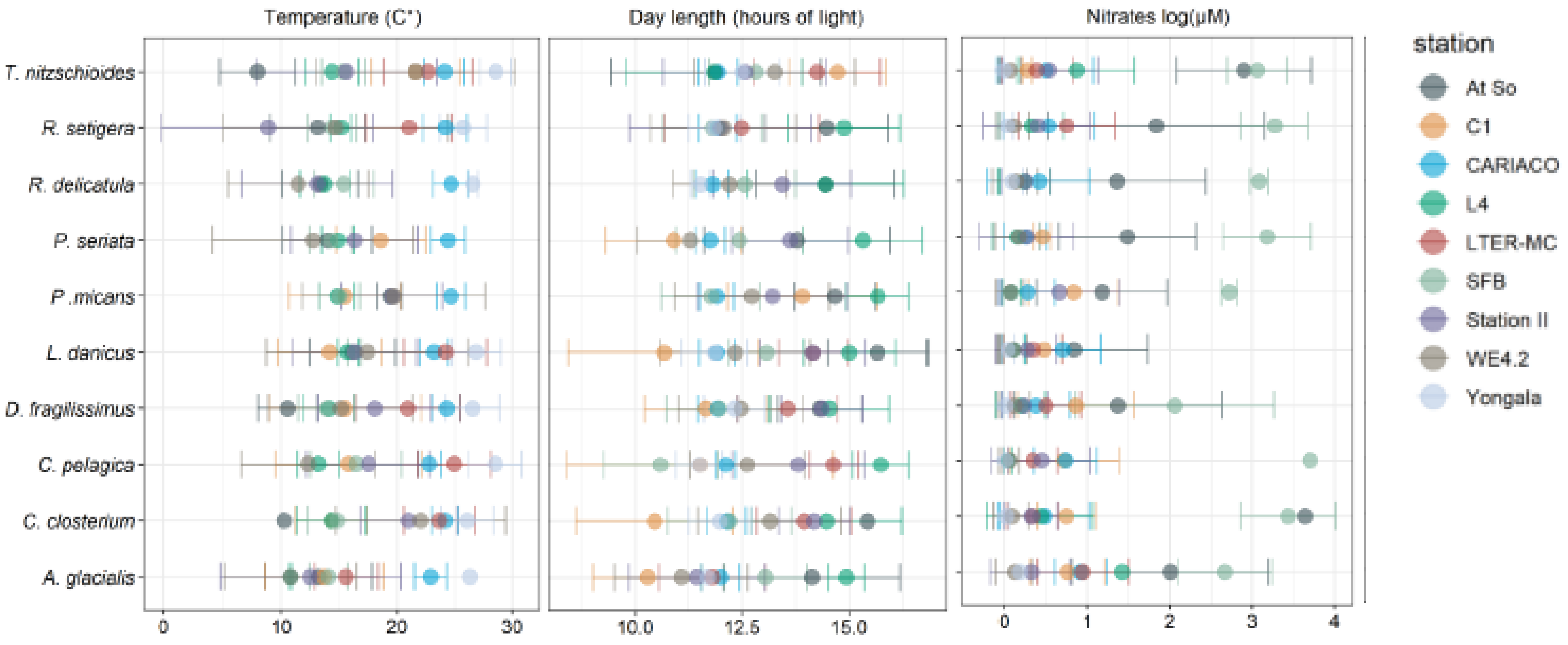
- Daylength
- Temperature
- Salinity
- Nitrates
- Silicates
- Phosphates

Region	Station	Latitude	Longitude	Frequency	Monitored period	Length (years)
Chesapeake Bay (USA)	*WE4.2*	37.11	-76.29	monthly	1985-2018	33
Cariaco basin (Venezuela)	*CARIACO*	10.50	-64.67	monthly	1995-2017	22
San Francisco Bay (USA)	*SFB*	37.50	-122.10	monthly	1992-2011	19
Gulf of Naples (Italy)	*LTER-MC*	40.82	14.25	weekly	1984-2015	31
Narragansett Bay (USA)	*Station II*	41.57	-71.39	weekly	1959-2018	59
Western English Channel (England)	*L4*	50.15	-4.13	weekly	1992-2015	23
Gulf of Trieste (Italy)	*C1*	45.42	13.42	monthly	1986-2010	24
Eastern English Channel (France)	*At so*	50.20	1.47	fortnightly	1990-2018	28
NE Australia (Australia)	*Yongala*	-19.19	147.37	monthly	2009-2019	10

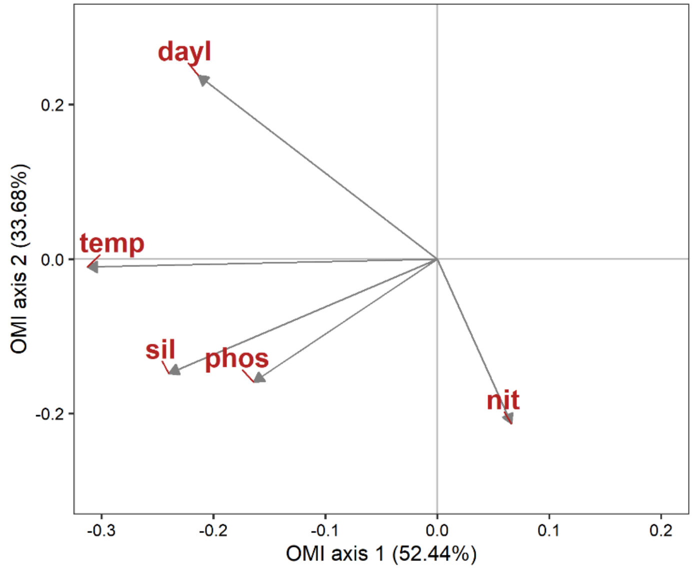
Physical-chemical ranges

species do not seem to share same/similar ecophysiological characteristics

Species annual maxima distribution



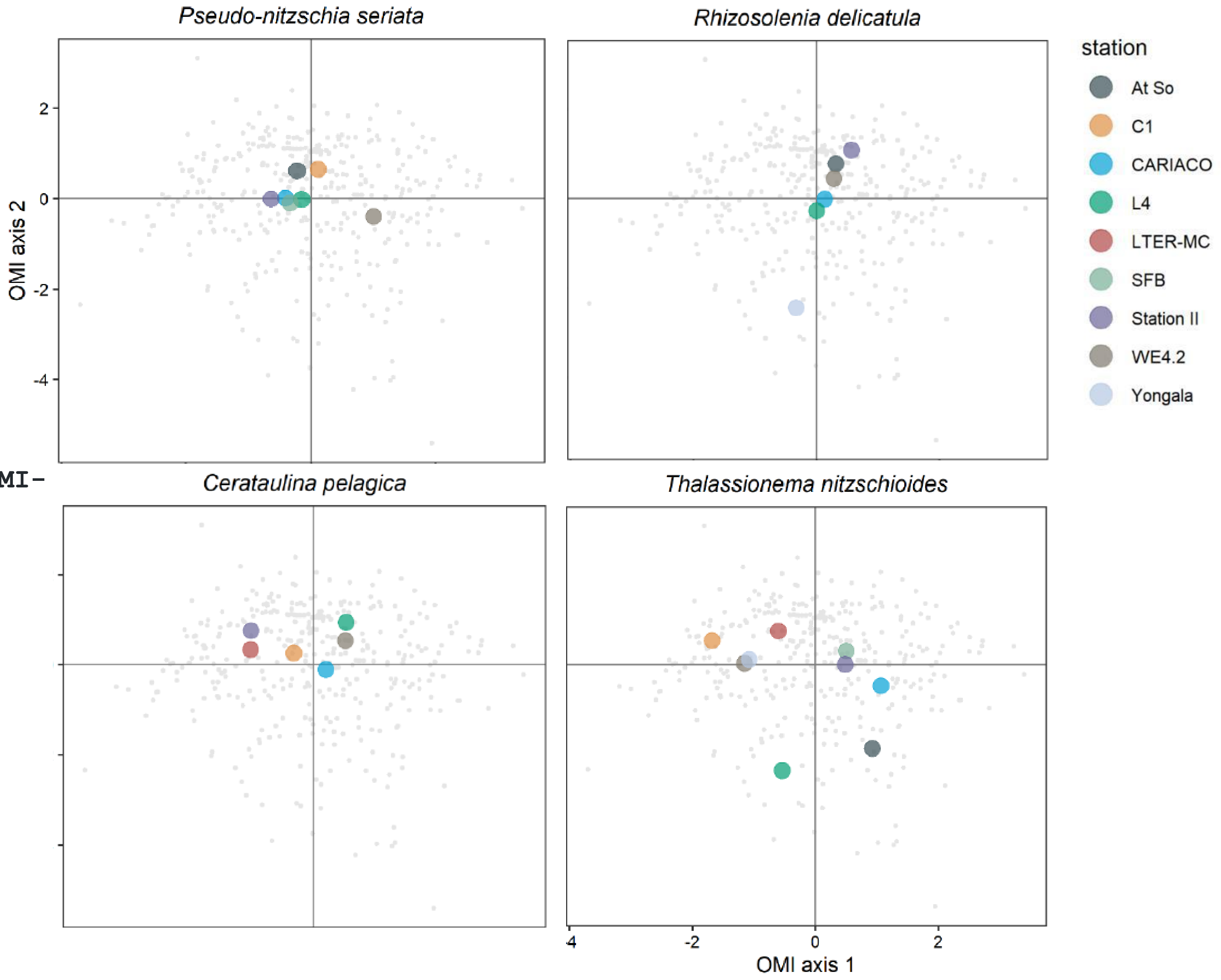
common environmental space



Within Outlying Mean Index – WitOMI-

Doledec (2000)

Karasiewicz et al. (2017)

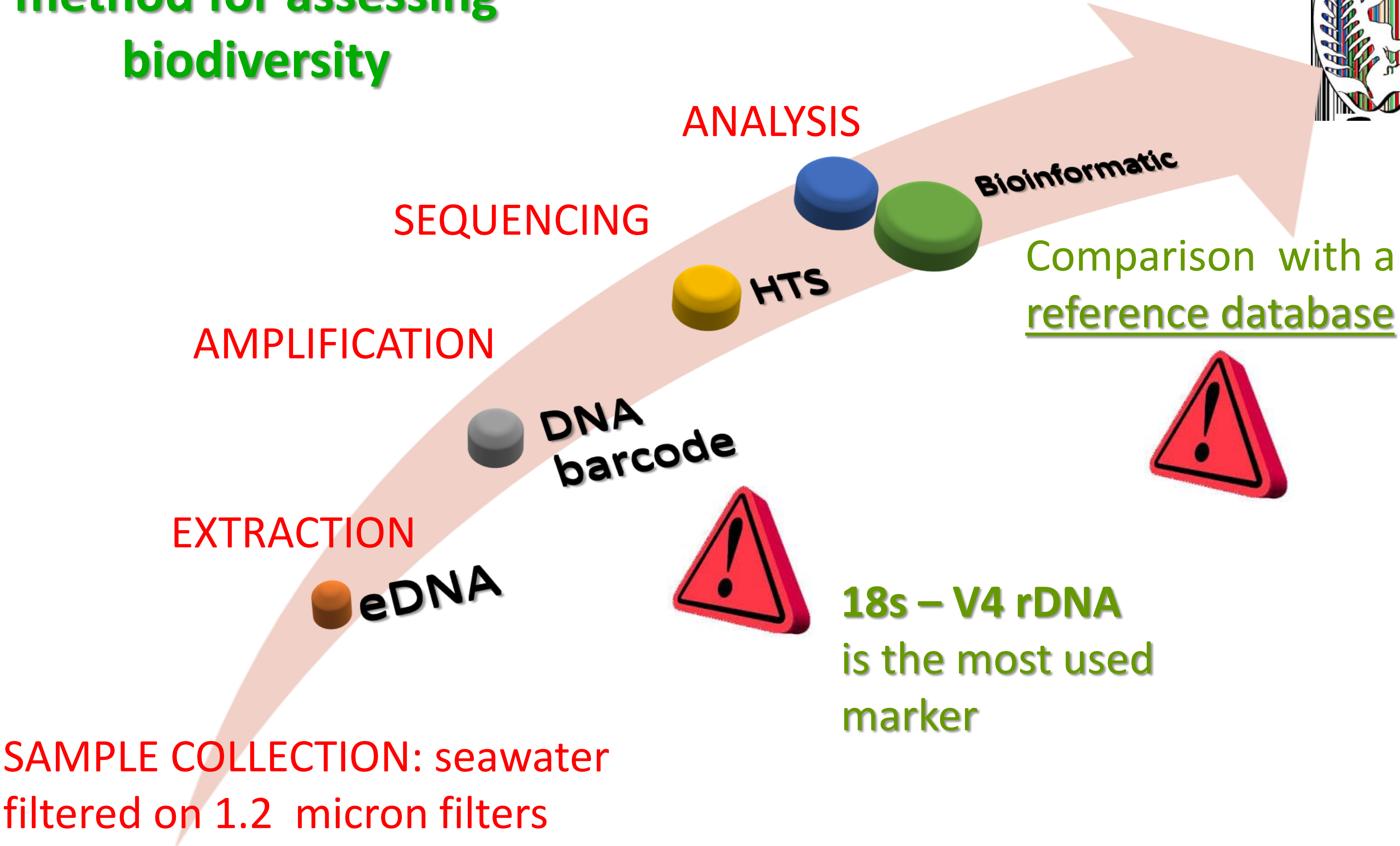


Niche overlap within a species is significantly higher than that among different species in 4 out of 12 cases

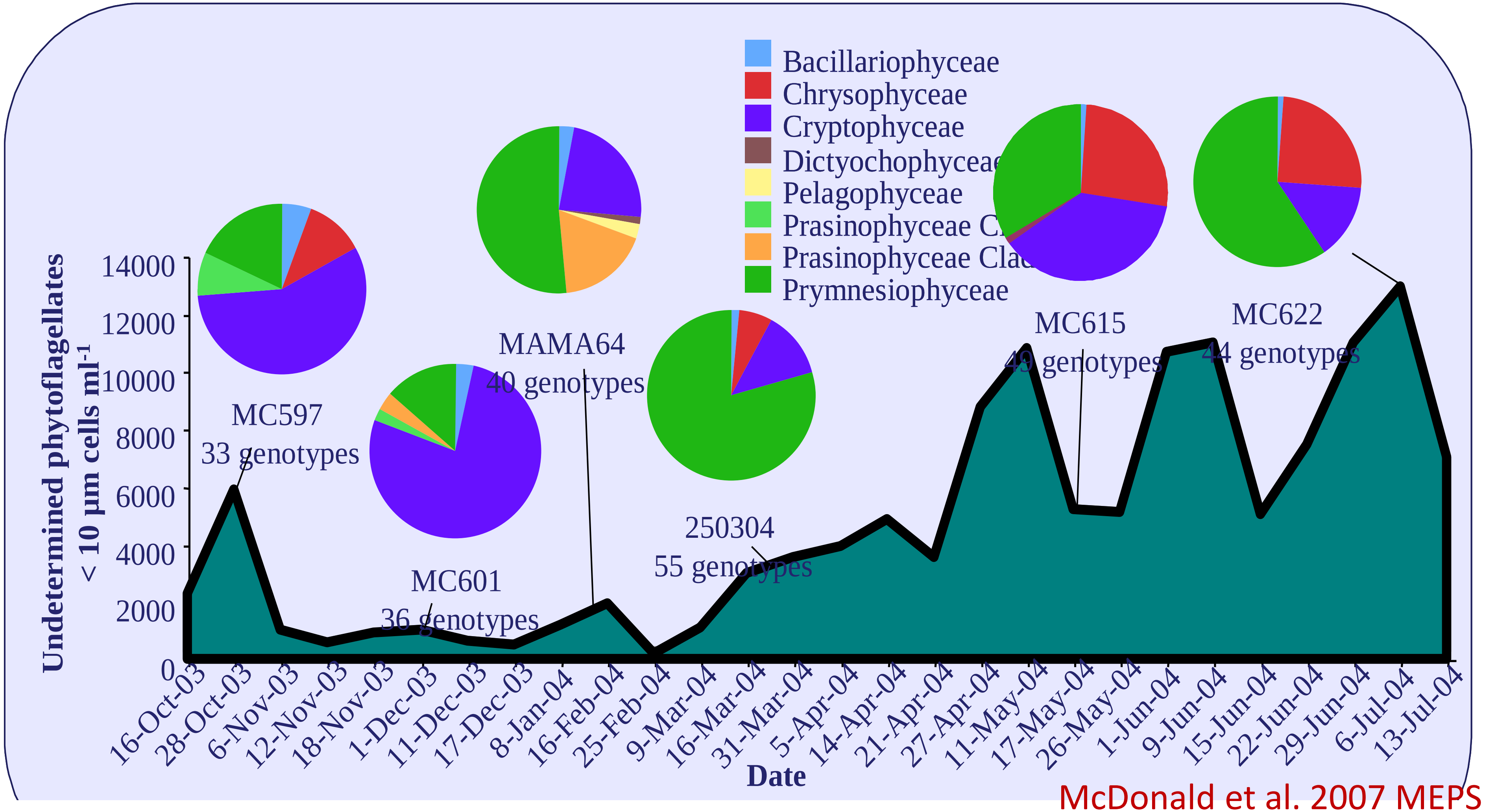
Wide plasticity?
Different populations?
Cryptic species?
Identification problems?

HTS DNA-metabarcoding is a rapidly evolving method for assessing biodiversity

Assign a species name to an e-DNA sequence

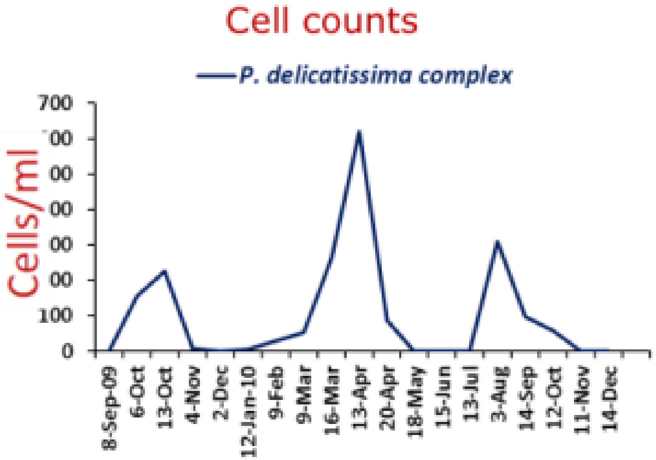


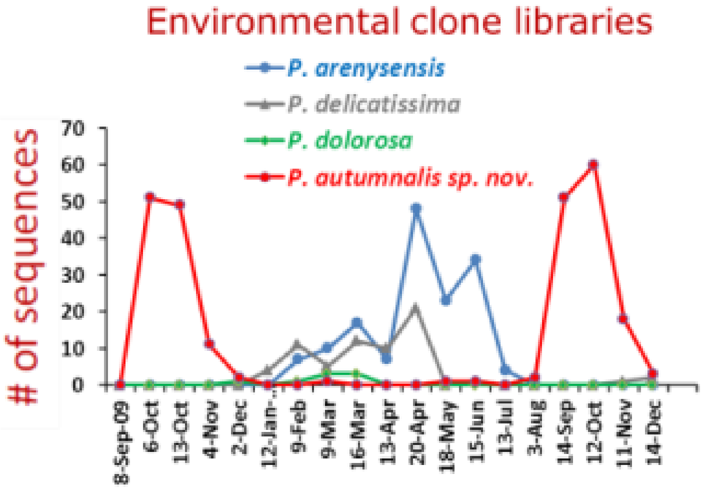
Biodiversity through the molecular lenses
Metabarcoding - Clone Libraries

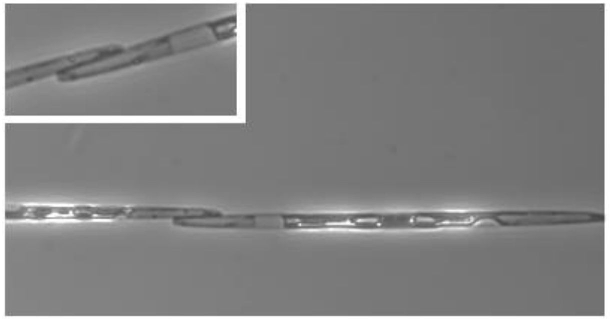


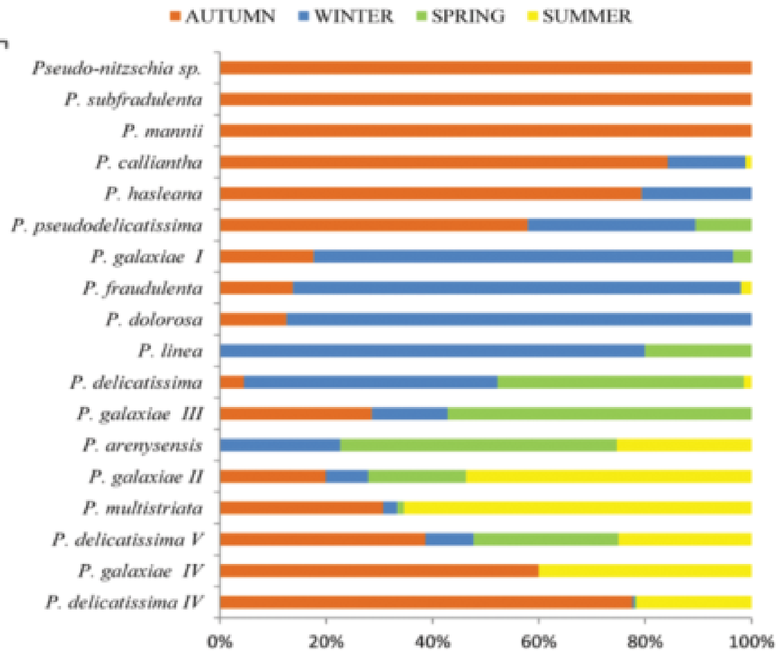
McDonald et al. 2007 MEPS

Biodiversity through the molecular lenses
Seasonality of cryptic species







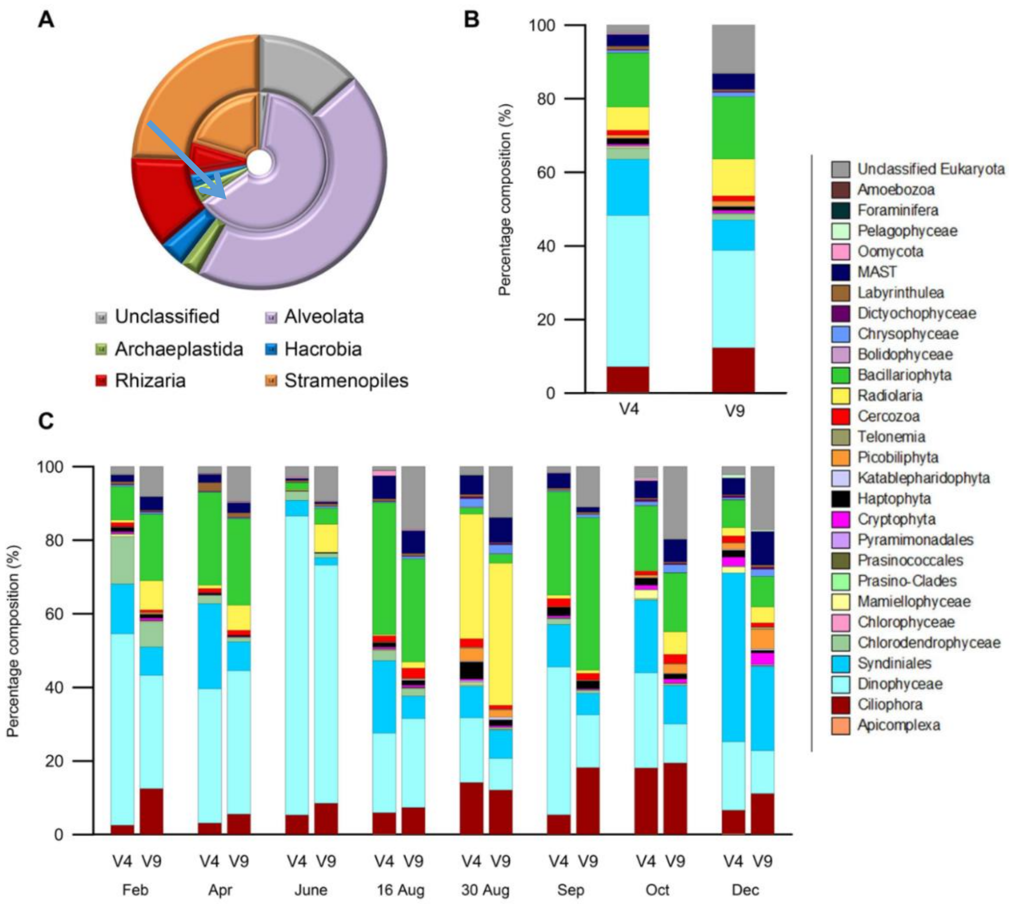


19 clone libraries (16 mo)
1653 sequences
84 ribotypes (97%)
16 species + 3 new ones

Cryptic species have distinct seasonal patterns

Ruggiero et al., Harmful Algae 2016

Biodiversity through the molecular lenses
Metabarcoding with High Throughput Sequencing (HTS)

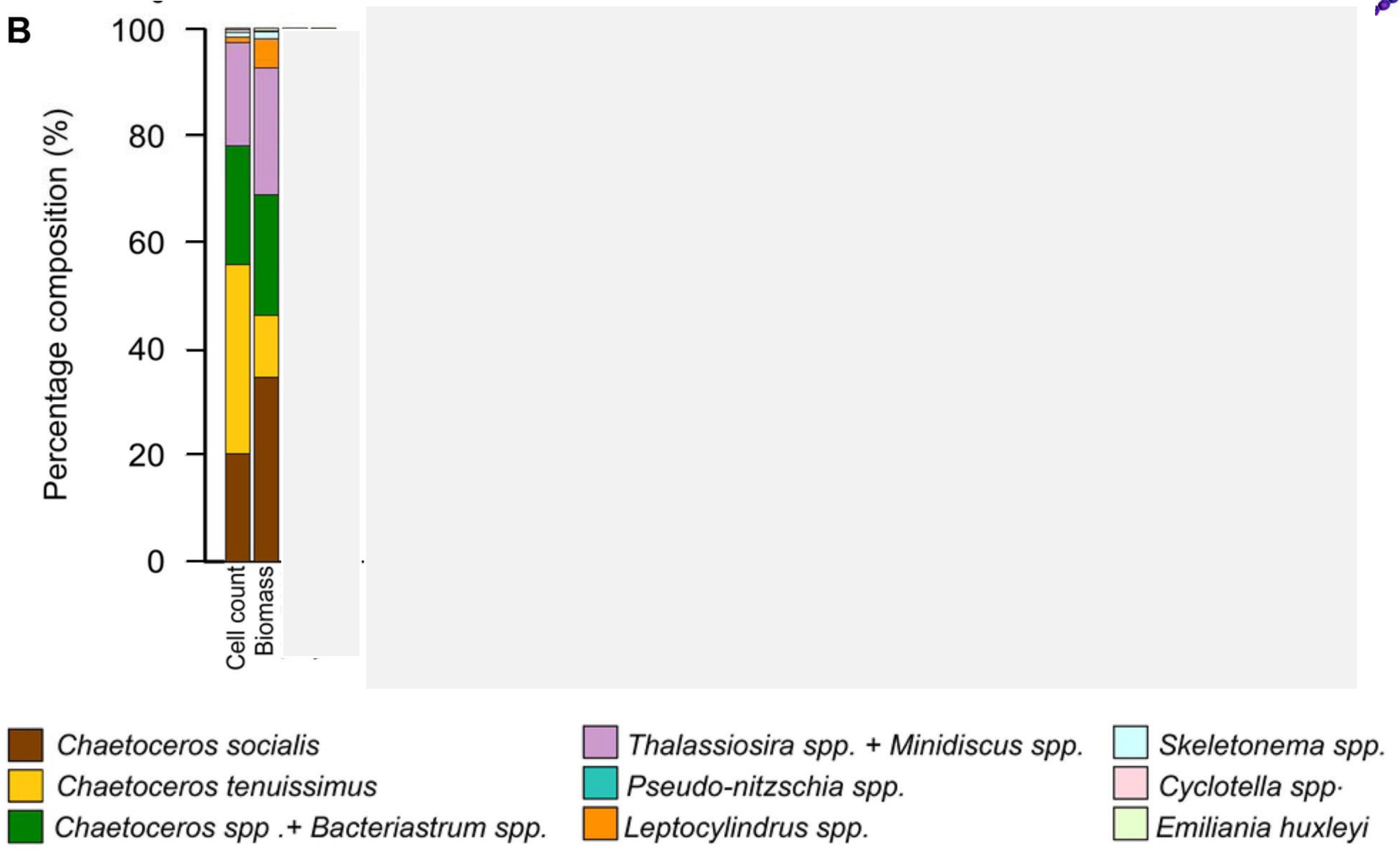


6,000 OTU$_{95}$= >6,000 species

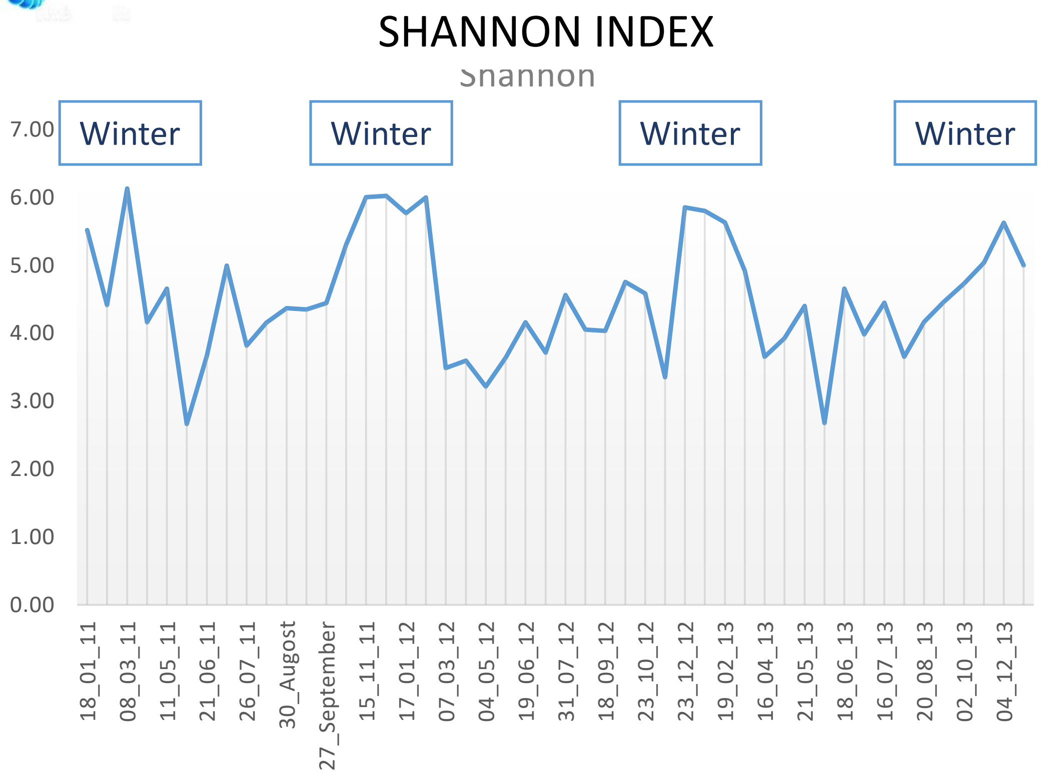
- **6 times the species diversity detected in more than 30 ys studies**
- **V4 and V9 provide similar results**
- **Dominance of dinoflagellates**

Piredda et al., FEMS 2017a

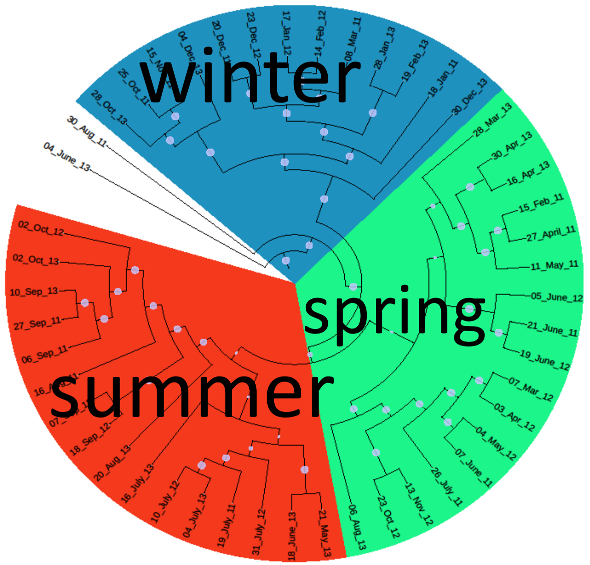
Molecular data match light microscopy



Seasonal cycle, 48 dates over three years



Diversity peaks in winter

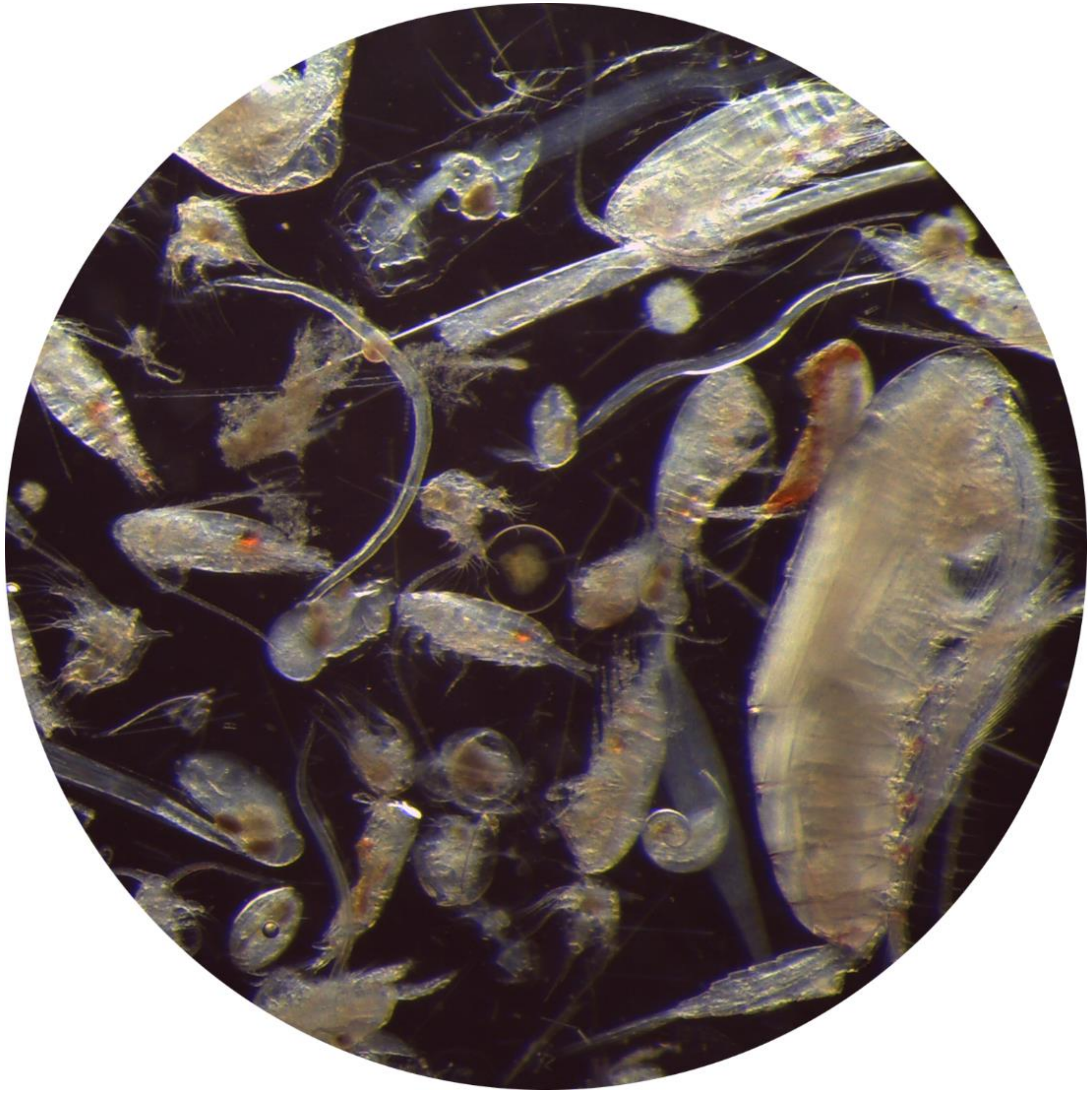


Metabarcoding data from ca 120 samples over 10 years now available

DNA-METABARCODING METAZOAN

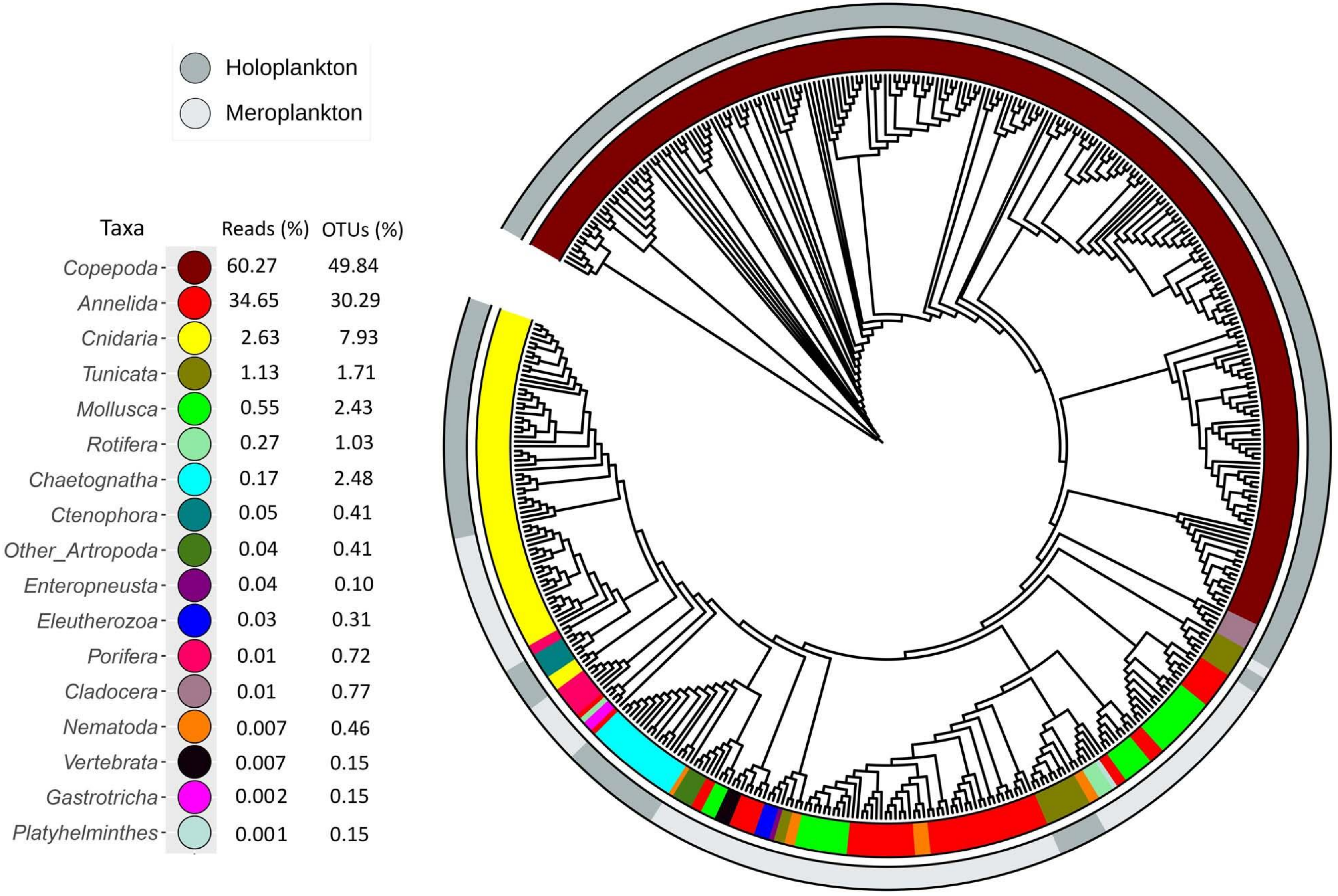
Holoplankton
Meroplankton

Taxa	Reads (%)	OTUs (%)
Copepoda	60.27	49.84
Annelida	34.65	30.29
Cnidaria	2.63	7.93
Tunicata	1.13	1.71
Mollusca	0.55	2.43
Rotifera	0.27	1.03
Chaetognatha	0.17	2.48
Ctenophora	0.05	0.41
Other_Artropoda	0.04	0.41
Enteropneusta	0.04	0.10
Eleutherozoa	0.03	0.31
Porifera	0.01	0.72
Cladocera	0.01	0.77
Nematoda	0.007	0.46
Vertebrata	0.007	0.15
Gastrotricha	0.002	0.15
Platyhelminthes	0.001	0.15



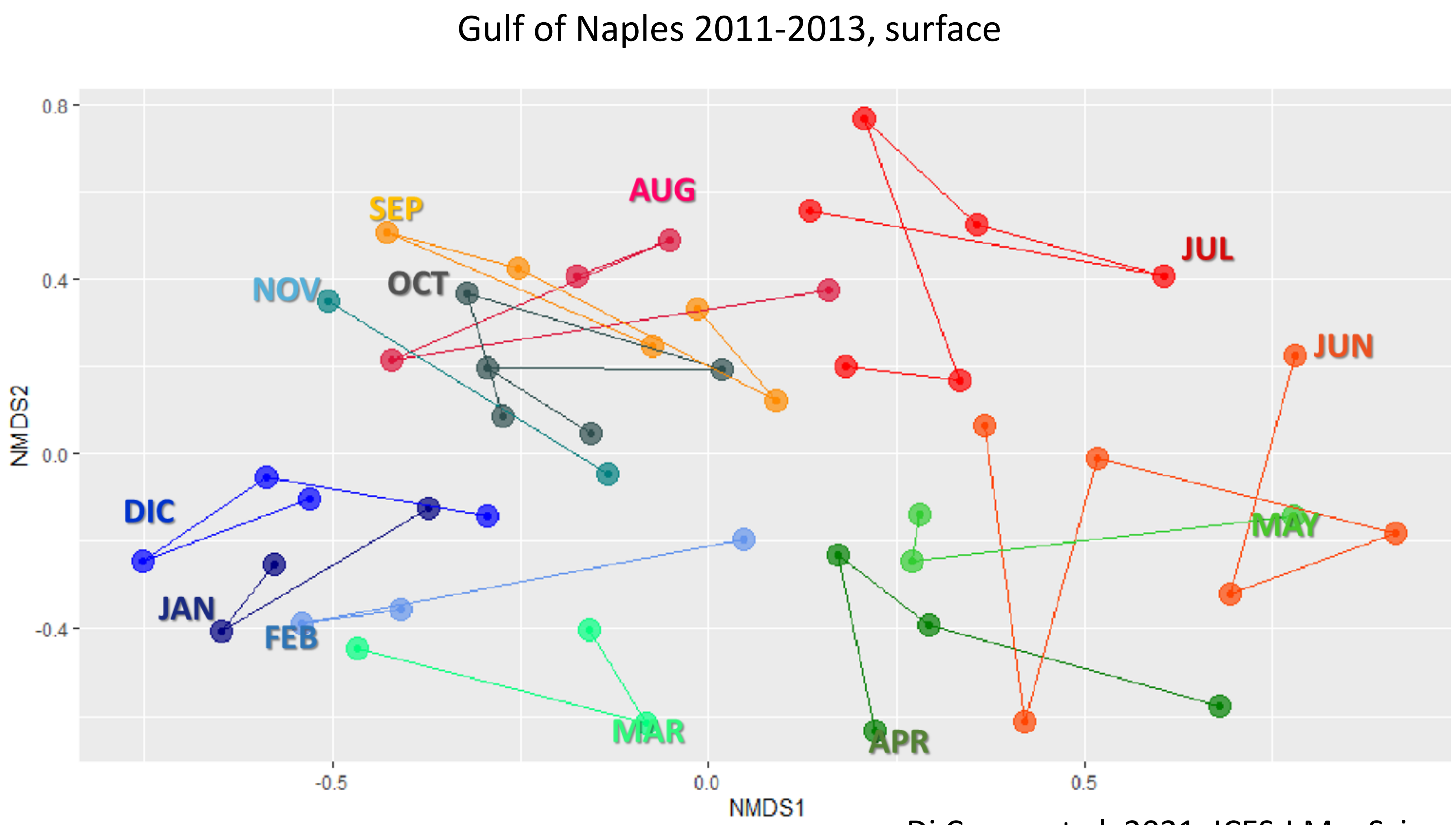
Di Capua et al. 2021, ICES J.Mar.Sci.

DNA-METABARCODING METAZOAN



Di Capua et al. 2021, ICES J.Mar.Sci.

DNA-METABARCODING METAZOAN

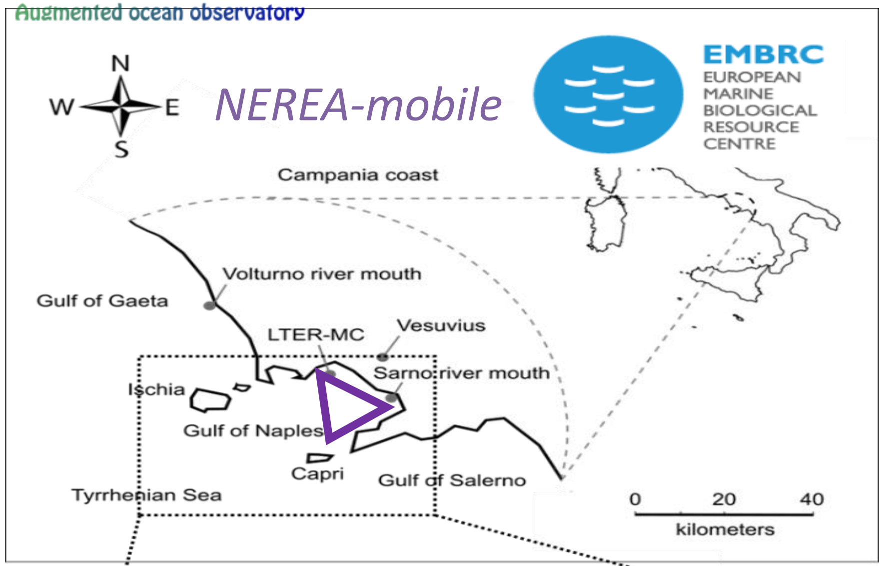


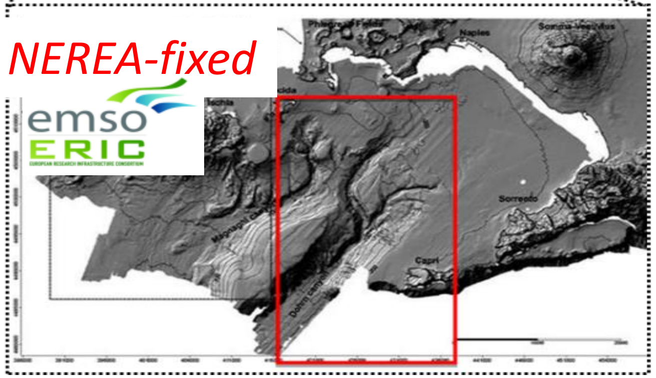
Di Capua et al. 2021, ICES J.Mar.Sci.

www.nerea-observatory.org

NEREA, the Naples Ecological REsearch for Augmented observatories:

Towards an end-to-end transdisciplinary approach for the study of marine ecosystems





Monthly sampling at LTER-MC station, plus process studies at LTER-MC, River Sarno and Canyon Dohrn (max depth = - 750 m)

- Meta-omics, trace metals, turbulence profiles, high frequency ongoing flow cytometry, environmental DNA, etc.

[1] *EuroSea - Improving and Integrating European Ocean Observing and Forecasting Systems for Sustainable use of the Oceans* (EU H2020 Blue Growth 07). Coord. GEOMAR Helmholtz-Zentrum für Ozeanforschung Kiel

Ocean Sampling Day

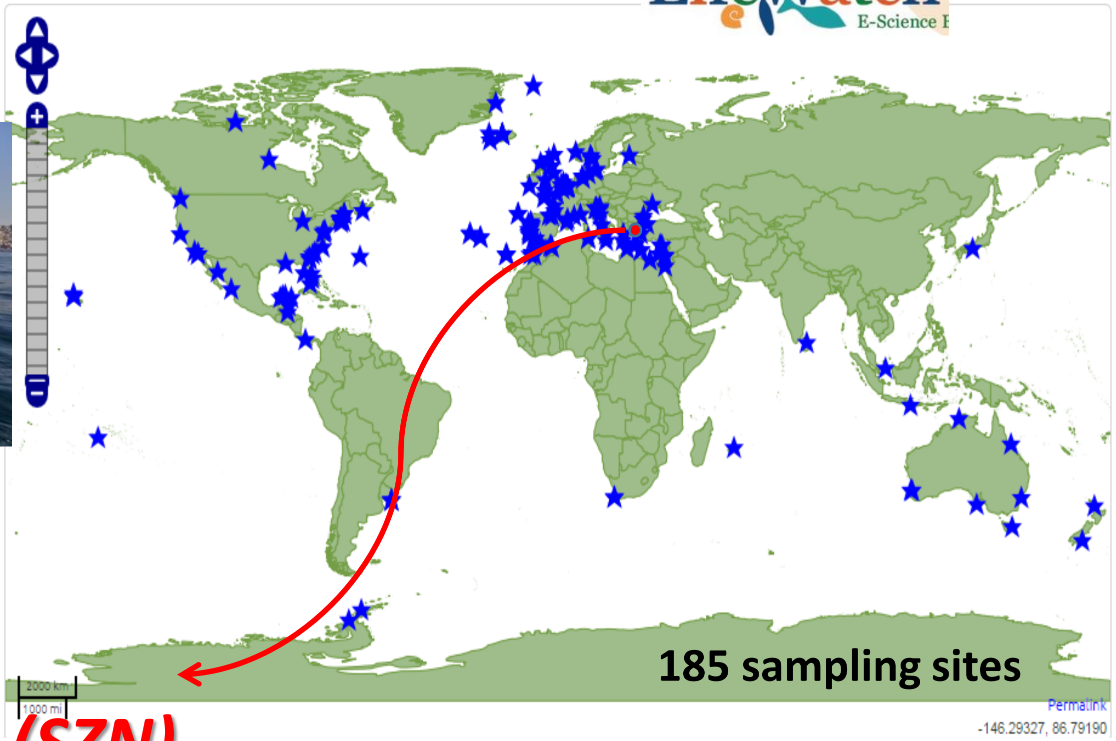
Internationalization
Information

OSD
Ocean Sampling Day

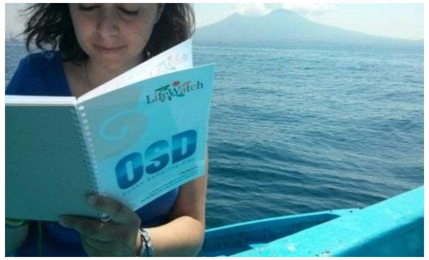
June 21st 2014 -Summer solstice

Map of OSD Participant sites

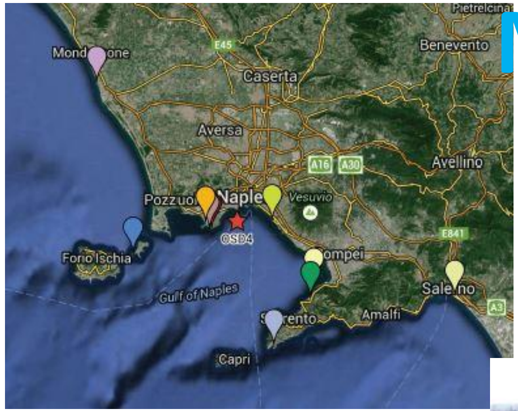
LifeWatch
E-Science



Naples (SZN)
LTER- Mare Chiara
Site OSD 4
MC 1008



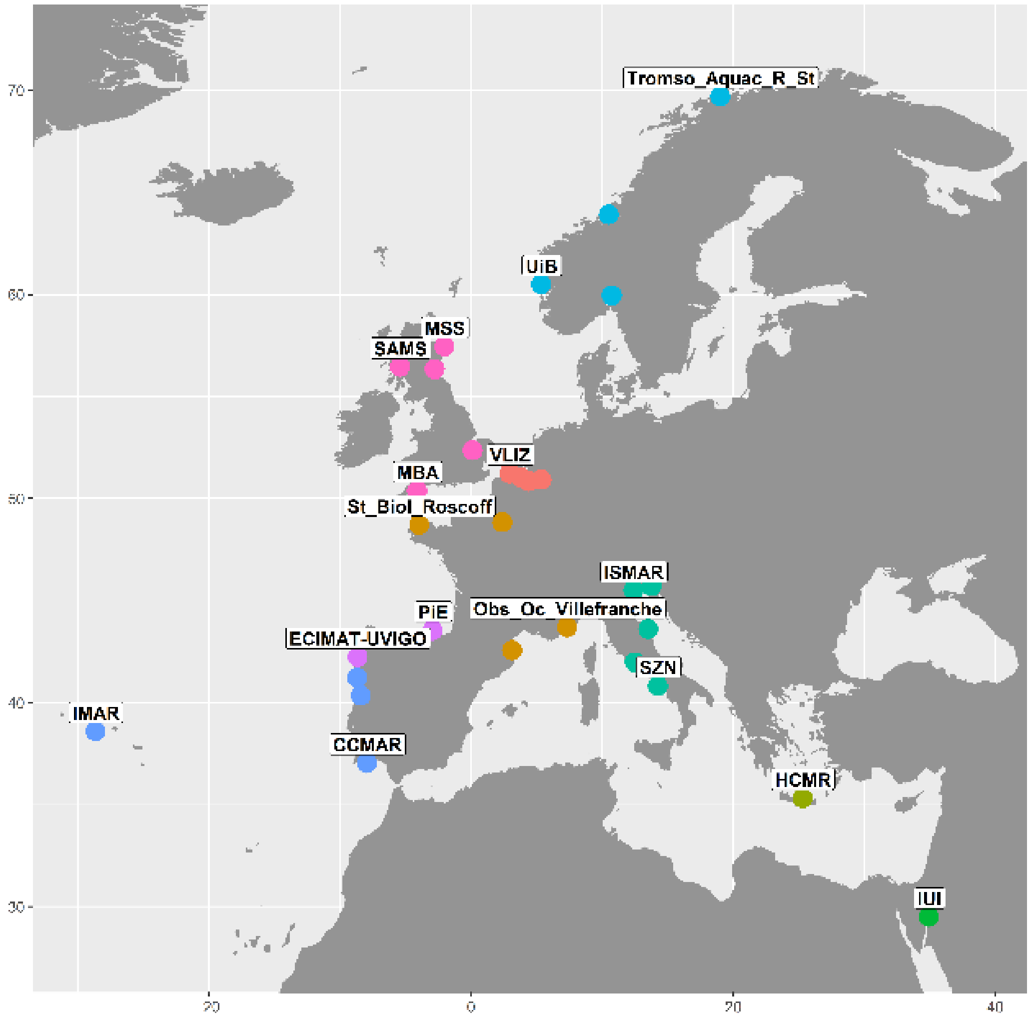
MY OSD 2015 Naples



European Marine Omics Biodiversity Observation Network – EMO BON

EMBRC
EUROPEAN MARINE BIOLOGICAL RESOURCE CENTRE

- Pilot study (2 years – bimonthly)
- 15 sites across Europe
- Mostly long-term observation sites
- Three protocols:
  - Water column (mandatory)
  - Soft Sediment
  - Hard substrates

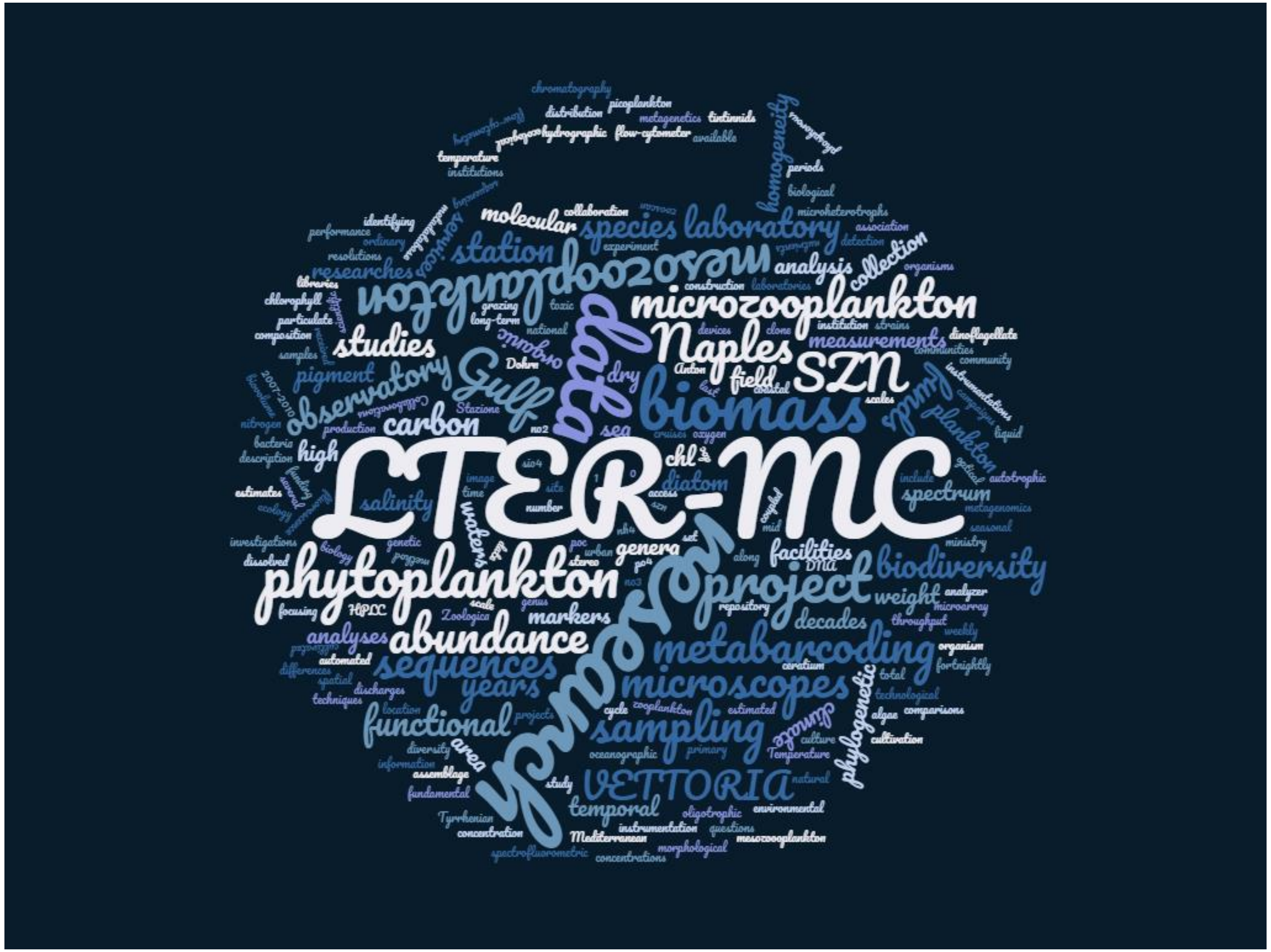


By Nicholas Pade

LTER MareChiara:
a multipurpose research infrastructure in the Gulf of Naples

- **A long term ecological project, to study decadal variations of the ecosystem**
- **A natural laboratory** to test hypotheses and address basic questions on plankton biology
- **A source of material, information and inspiration** for research
- **A resource allowing** participation in international comparison exercises **and increase SZN visibility**
- **An attractions** for researchers from other institutions
- A contribution to the **EU Marine Strategy Framework Directive**
- A chance for **dissemination and social engagement**
- **...**

http://szn.macisteweb.com/


LTER-MC
research
data
biomass
phytoplankton
project
mesozooplankton
microzooplankton
Naples
SZN
Gulf
observatory
metabarcoding
microscopes
sampling
VETTORIA
abundance
sequences
functional
biodiversity
species
laboratory
station
studies
carbon
salinity
pigment
analyses
years
temporal
markers
genera
facilities
diatom
spectrum
plankton
weight
decades
measurements
collection
analysis
homogeneity
molecular
researches
high
chl
waters
climate
phylogenetic
area
field
dry
organic
sea