Planktonic microbial food web dynamics in Hong Kong coastal waters

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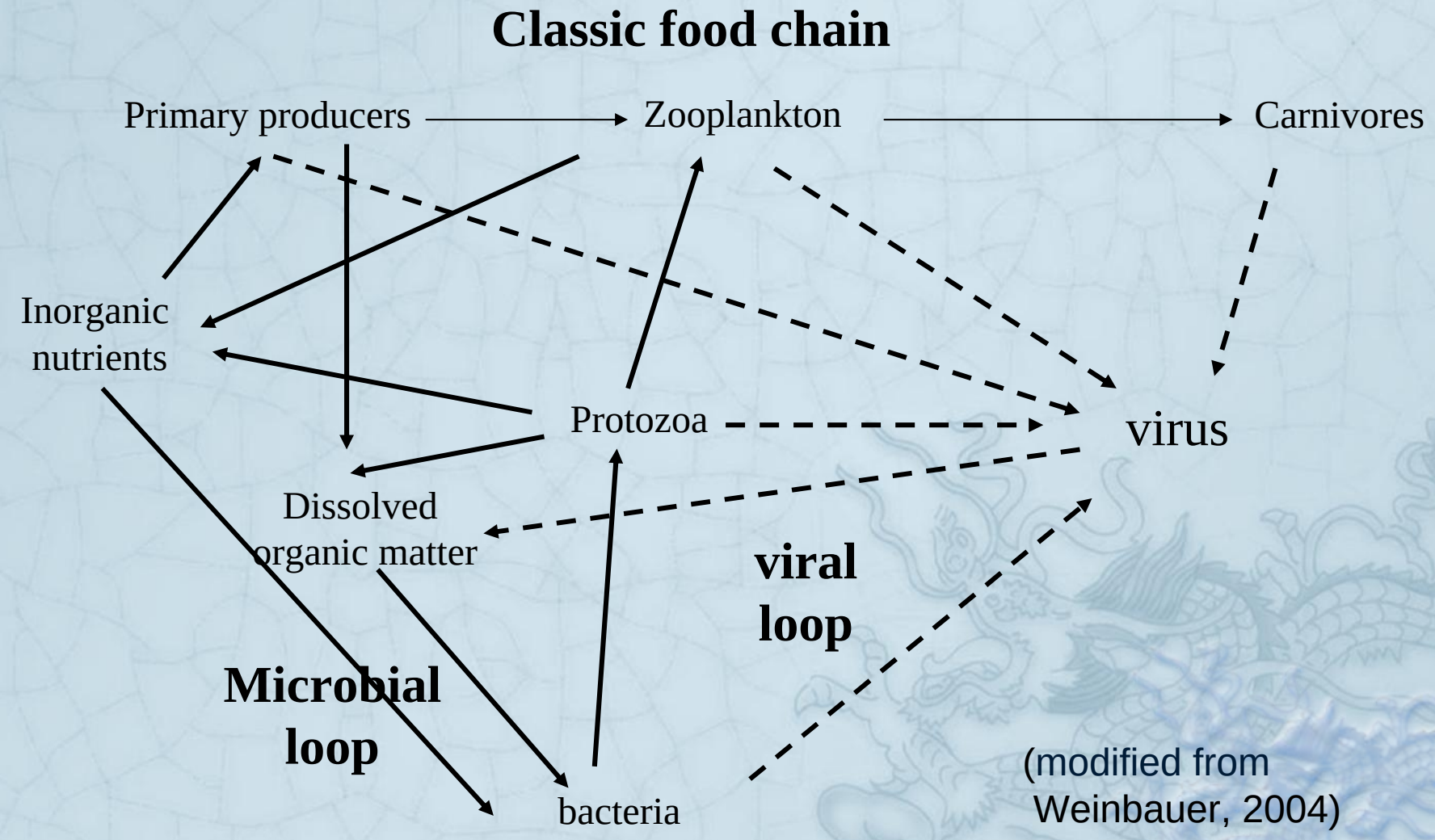
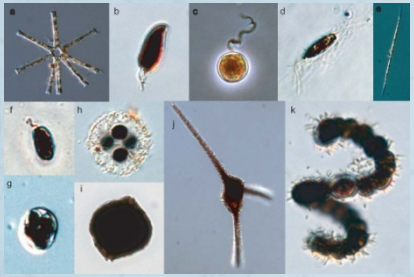
microbial food web



(modified from Weinbauer, 2004)

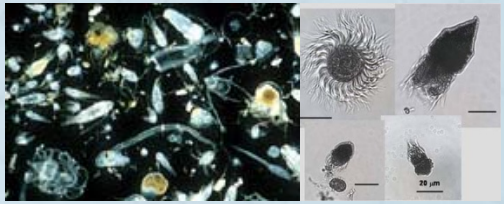
Simplified microbial food web

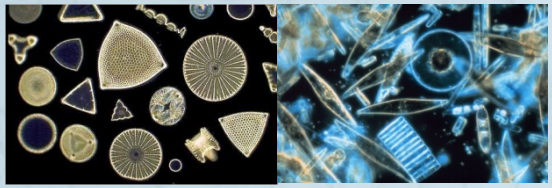
Mesozooplankton



Large-size phytoplankton

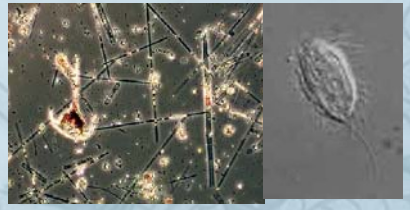
Microzooplankton





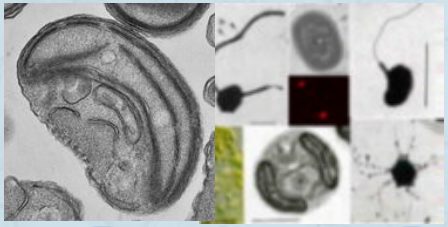
Nano-size phytoplankton

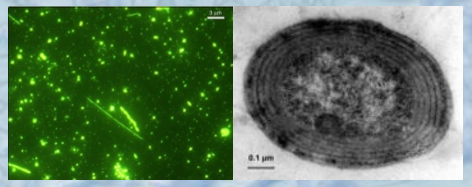
Nanoflagellates



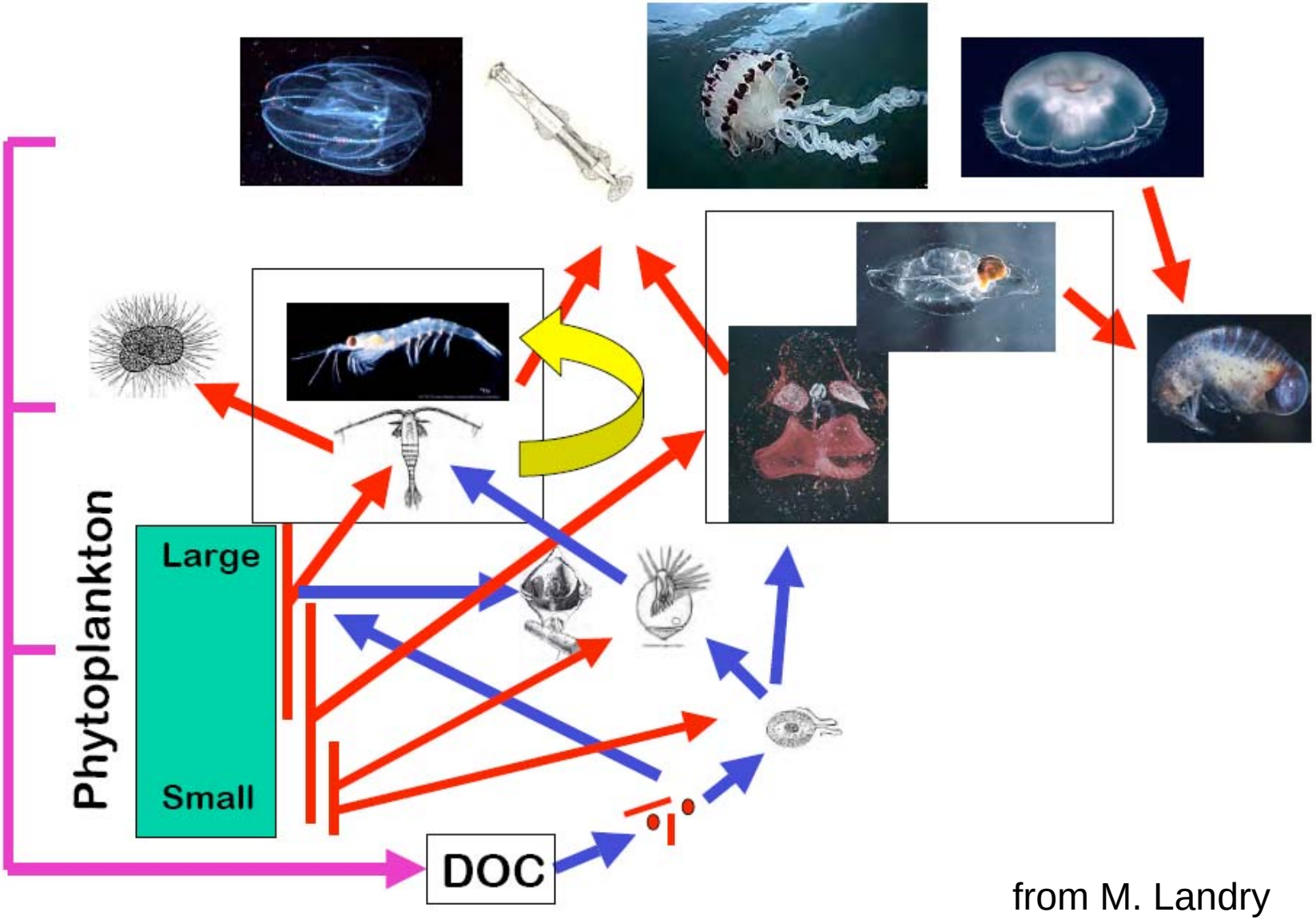
Picoeukaryotes

Bacteria

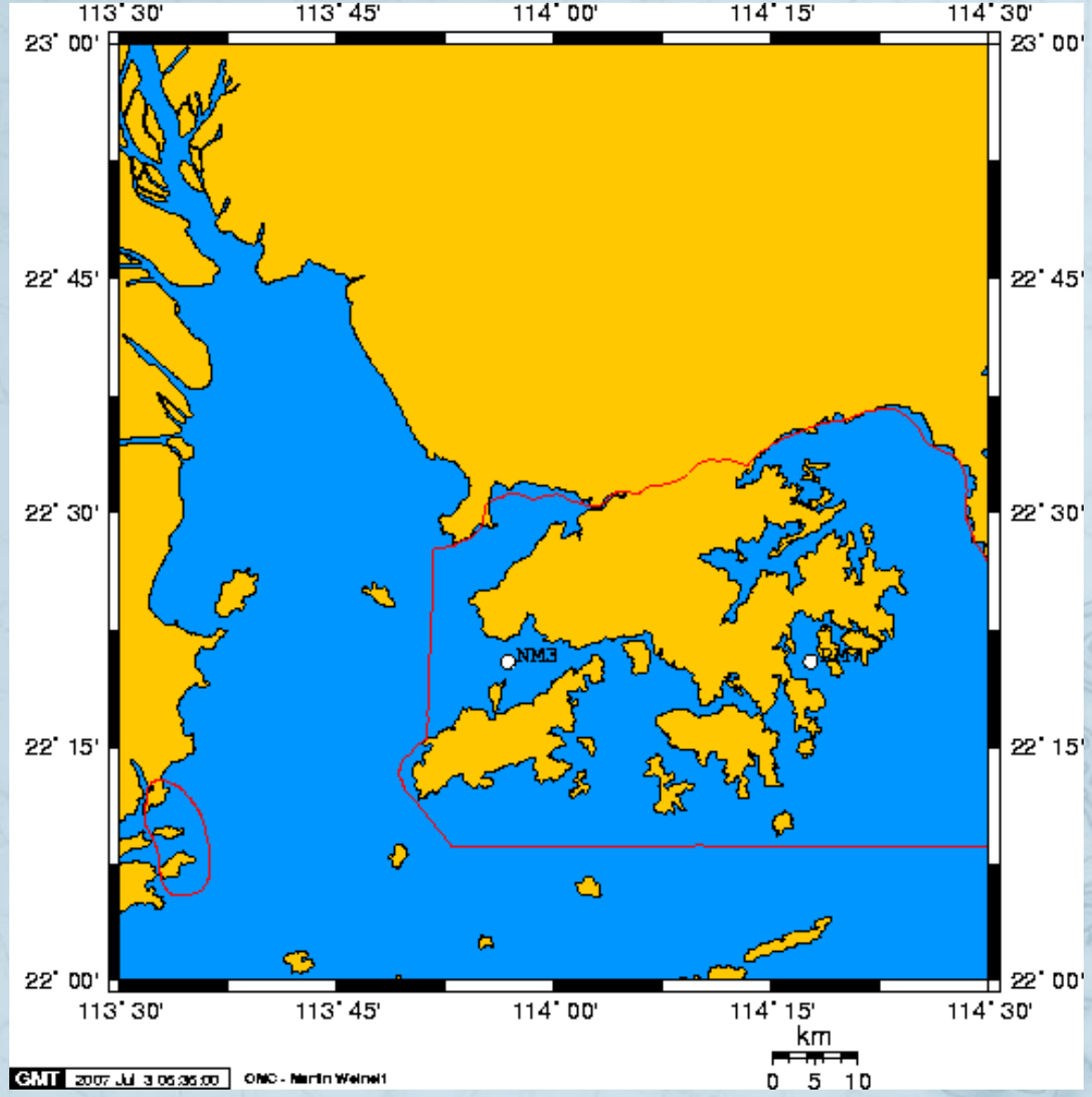




Additional micro- complexities

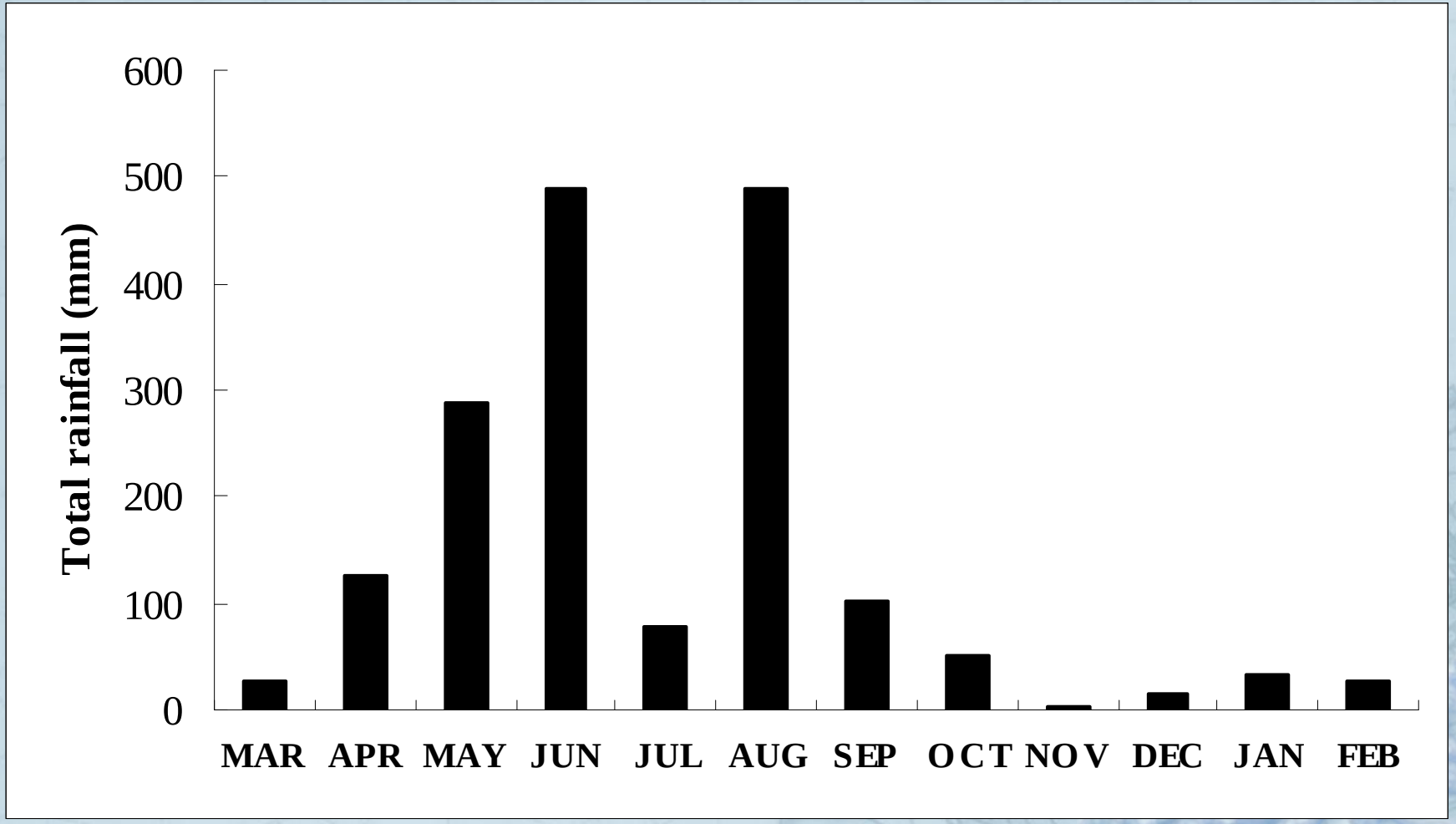


from M. Landry

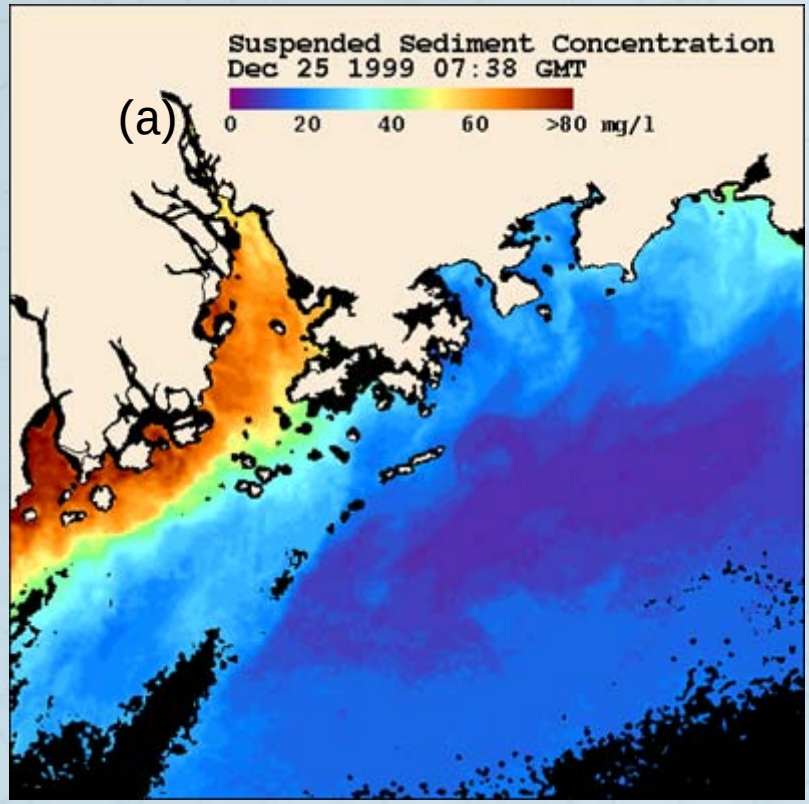


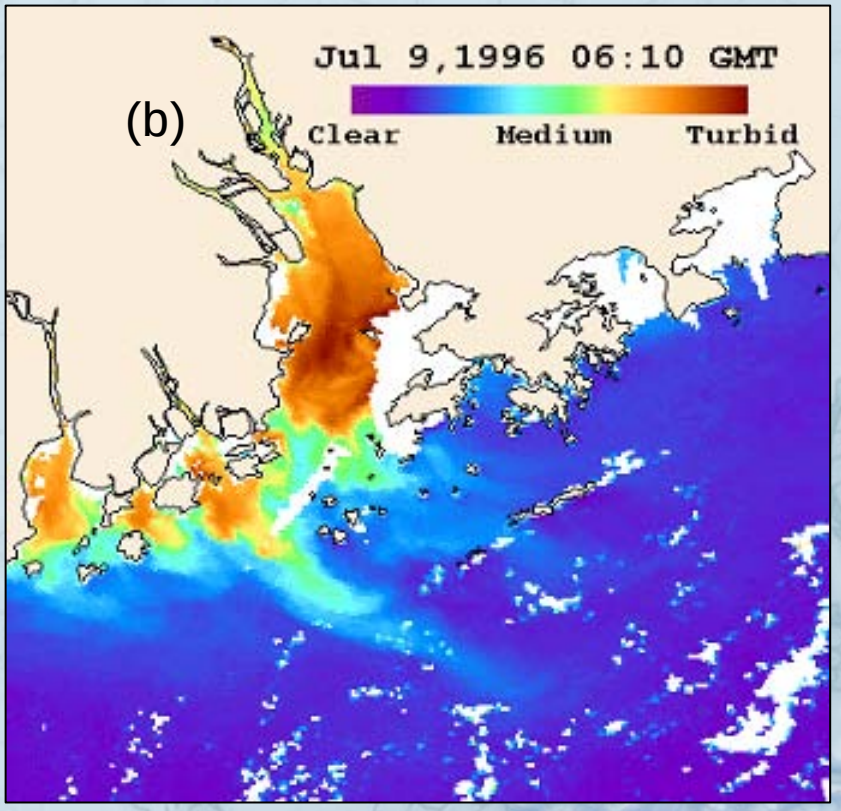
- Contrasting ecosystems in Hong Kong

Monthly total rainfall in Hong Kong from March 2007 to February 2008



Suspended Sediment Concentration (tracer for the plume)





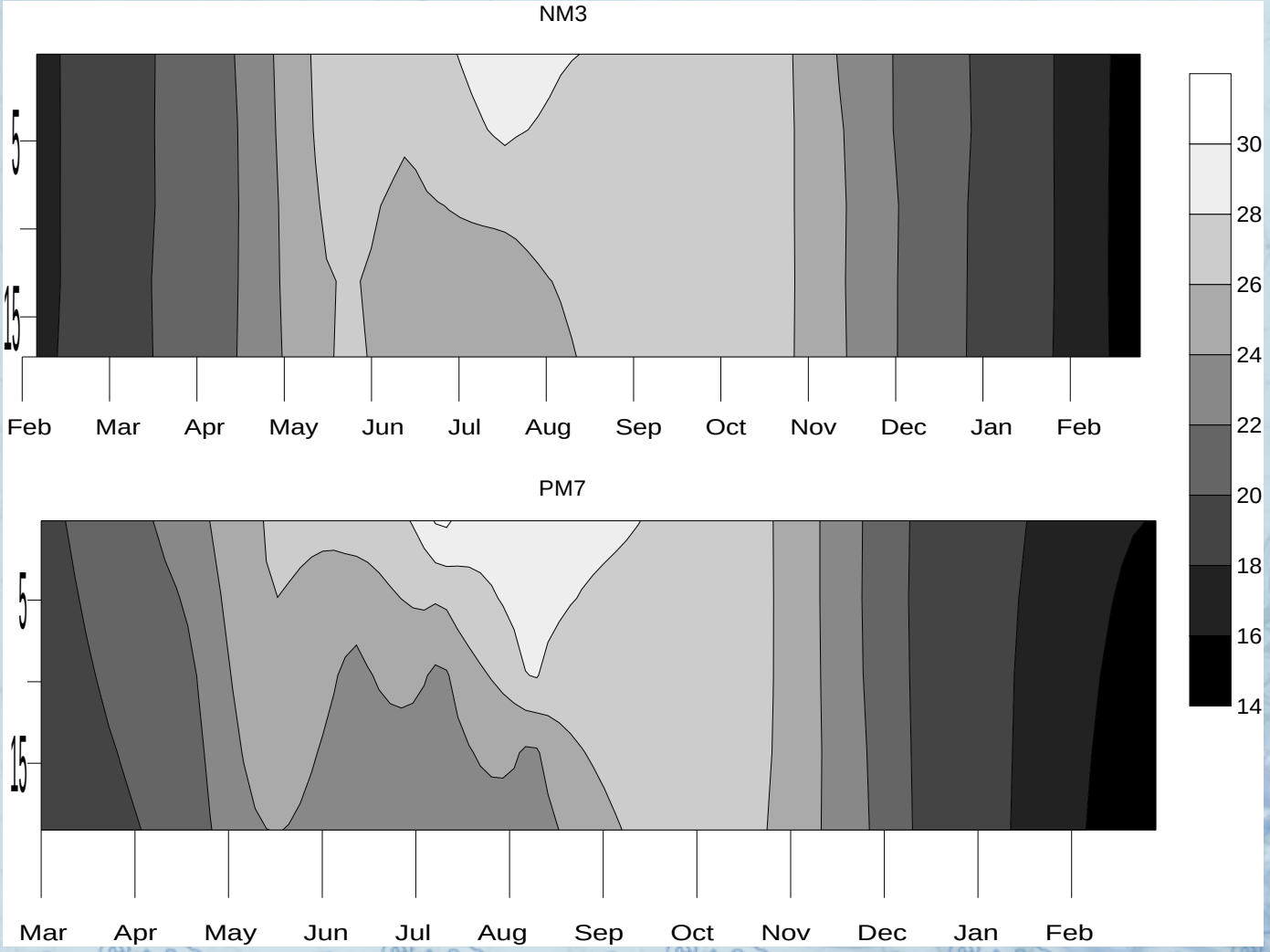
Objectives

- To study microbial community structure and the dynamics of the microbial food web in Hong Kong waters.
- To study micro- and mesozooplankton grazing, as well as viral lysis as possible controlling factors of algal blooms.

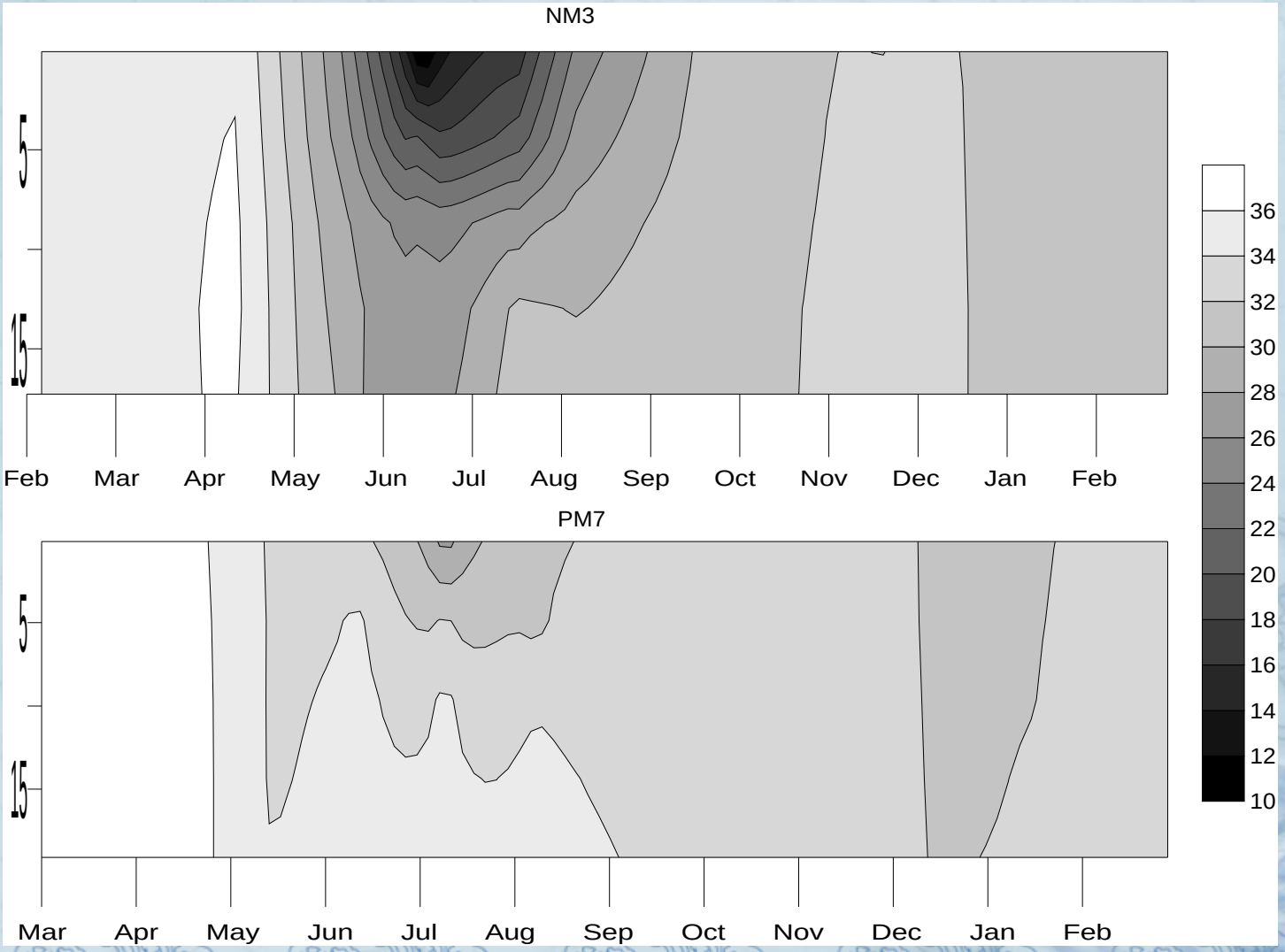
Parameters Measured

- Hydrograph
- Nutrients, POC, PON, DOC, DON
- Meso-, micro-, nano- and picoplankton
- Size-fractionated chlorophyll, pigments
- Phytoplankton growth and mortality (micro- and mesozooplankton grazing)
- Viral dynamics (abundance, production, decay) and viral-induced mortality
- Molecular approaches and stable isotopes

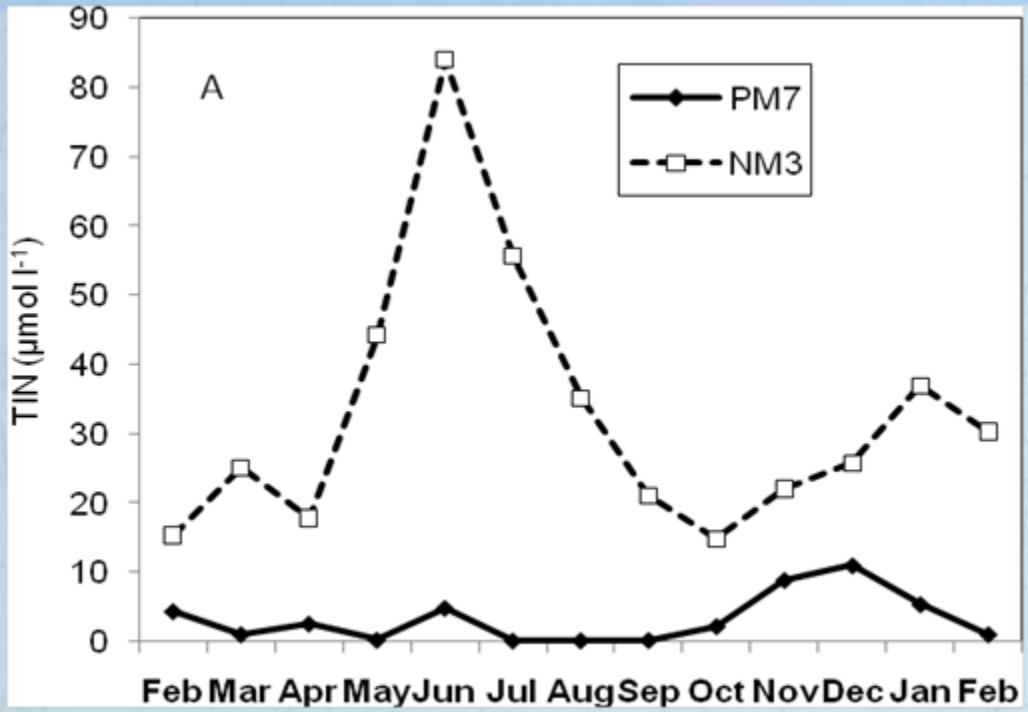
Temperature

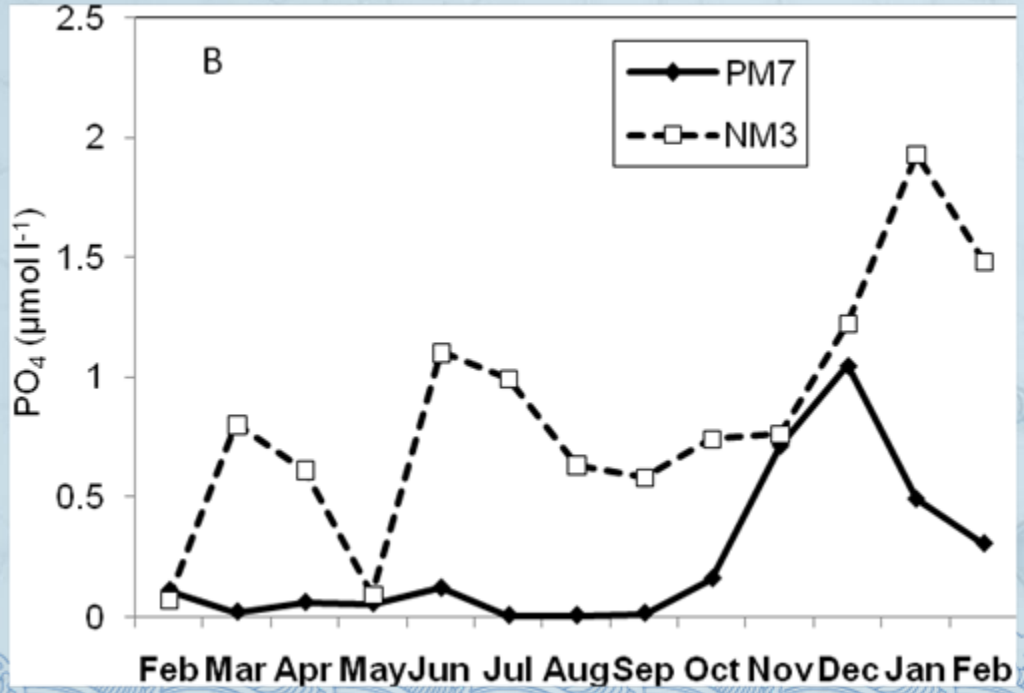


Salinity


NM3
5
15
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
PM7
5
15
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
Feb
36
34
32
30
28
26
24
22
20
18
16
14
12
10

Nutrients


A
PM7
NM3
TIN (µmol l-1)
90
80
70
60
50
40
30
20
10
0
Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb

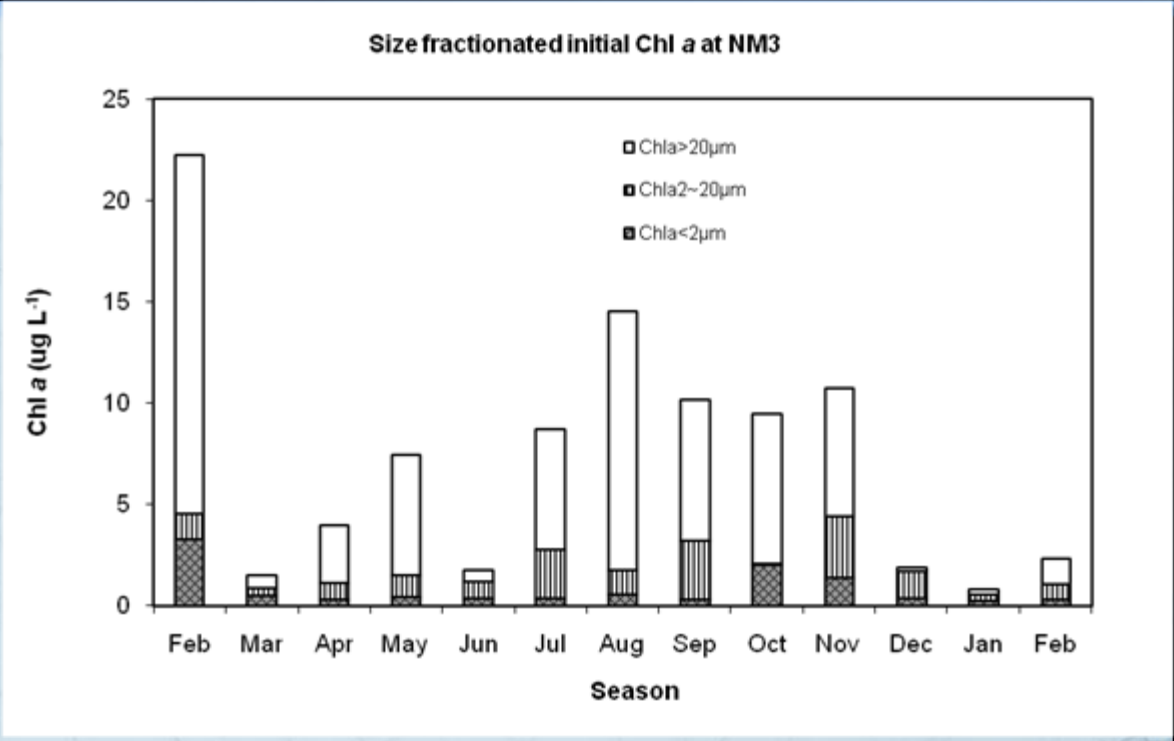

B
PM7
NM3
PO4 (µmol l-1)
2.5
2
1.5
1
0.5
0
Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb

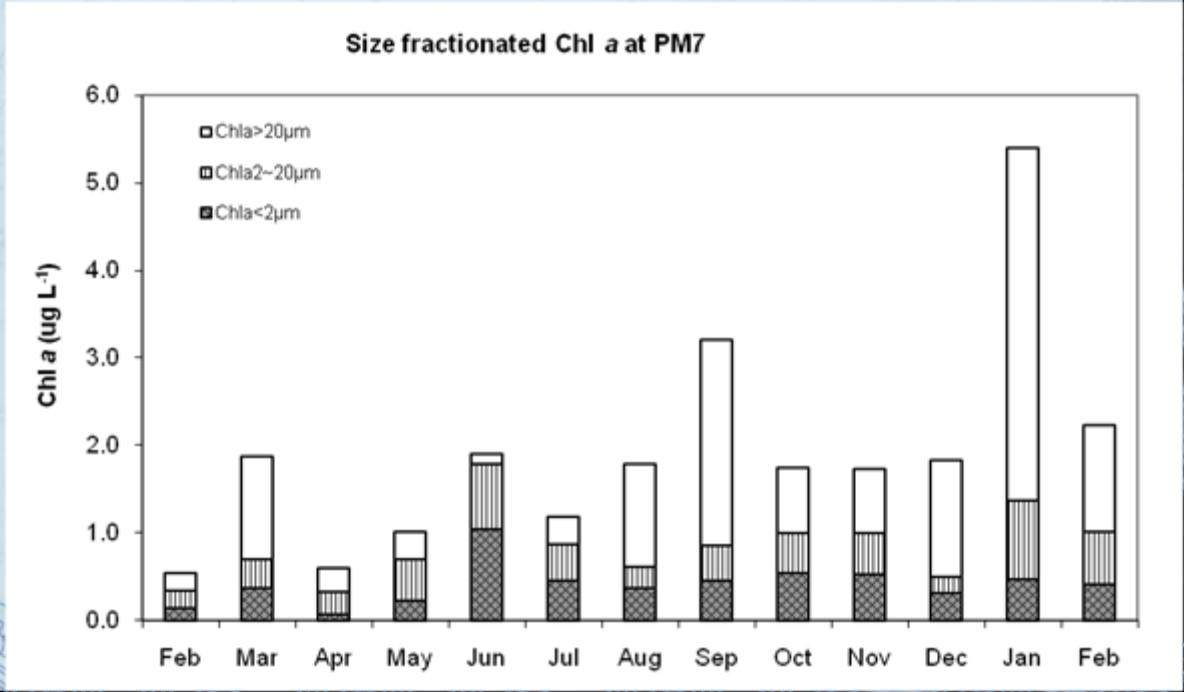
Size-fractionated

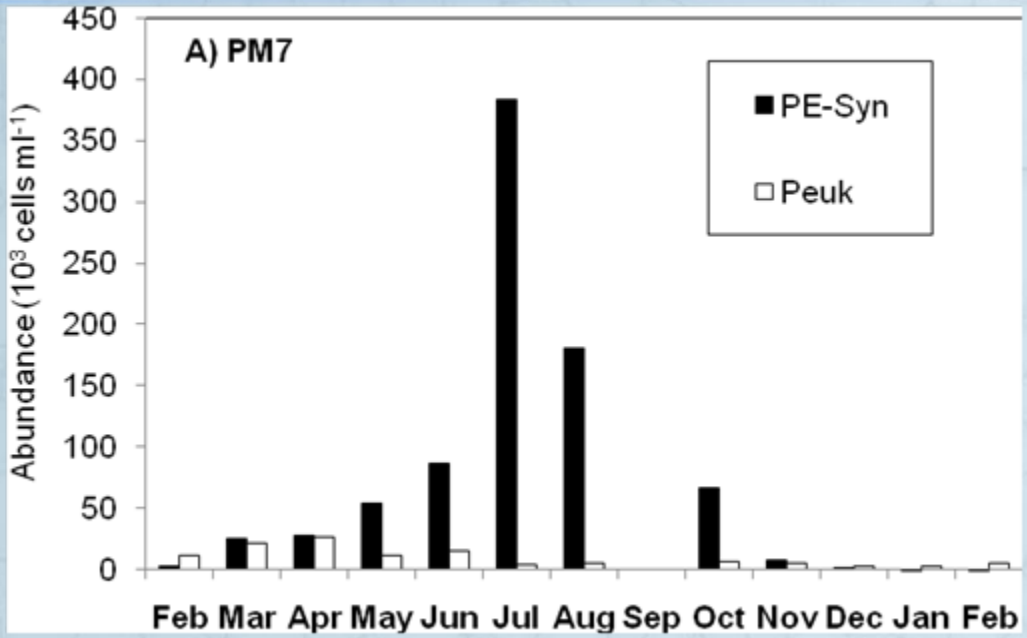
Chl *a*

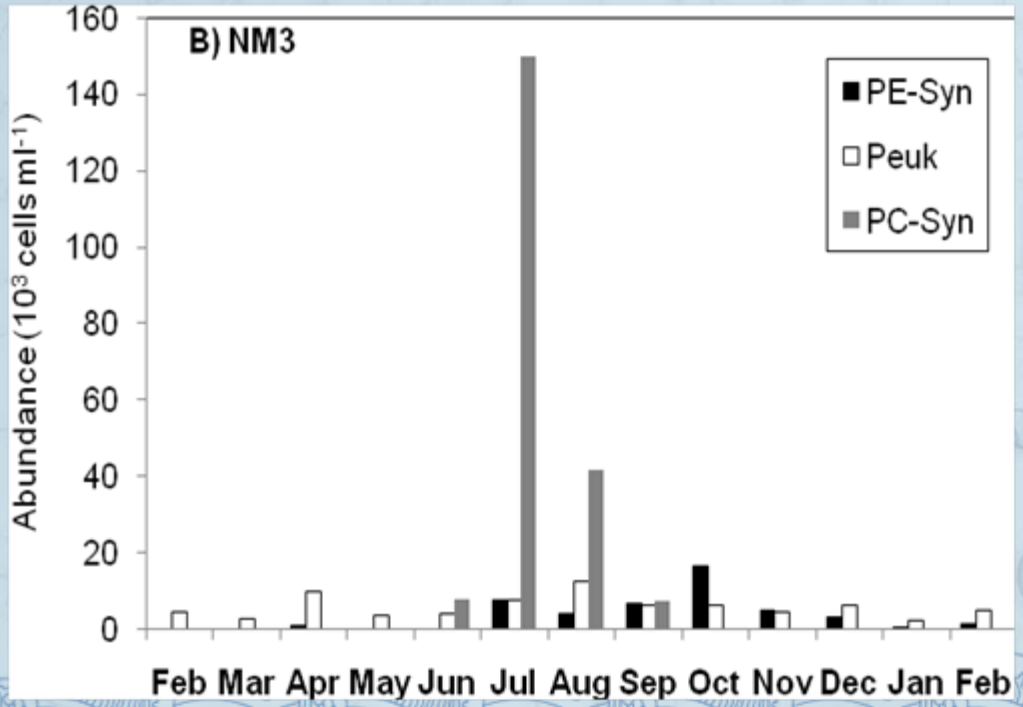
Average percentage of Chl *a* in each size fraction (mean±SD).

	NM3	PM7
Micro	0.59±0.24	0.49±0.21
Nano	0.27±0.19	0.27±0.12
Pico	0.14±0.09	0.25±0.12



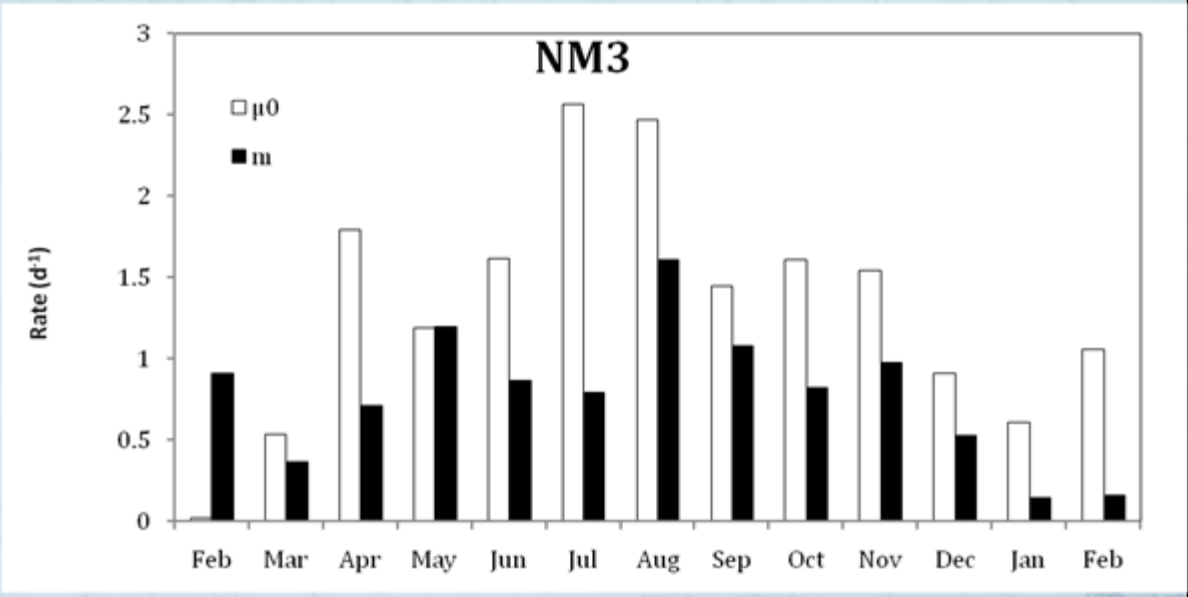


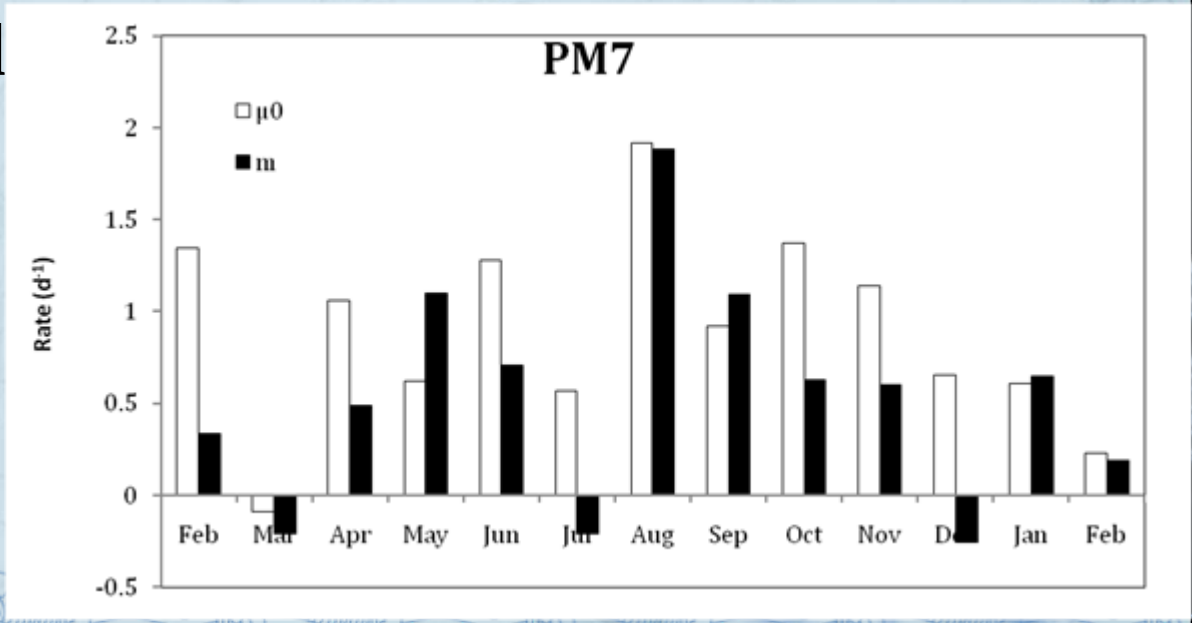


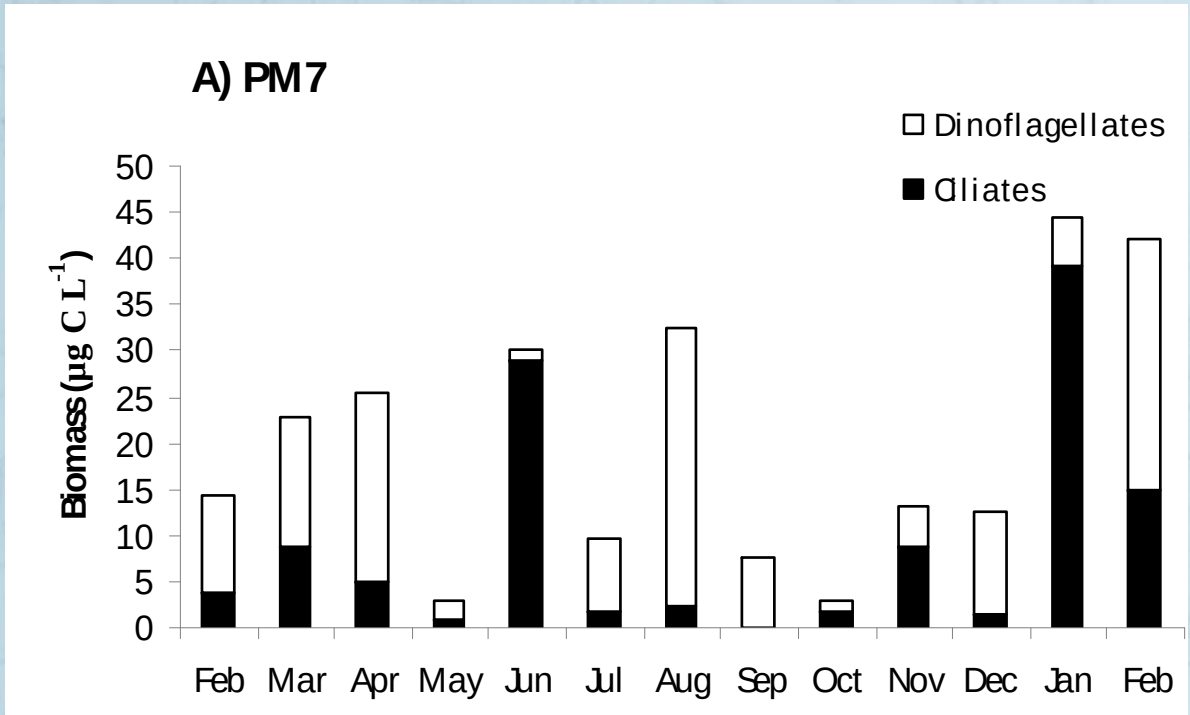


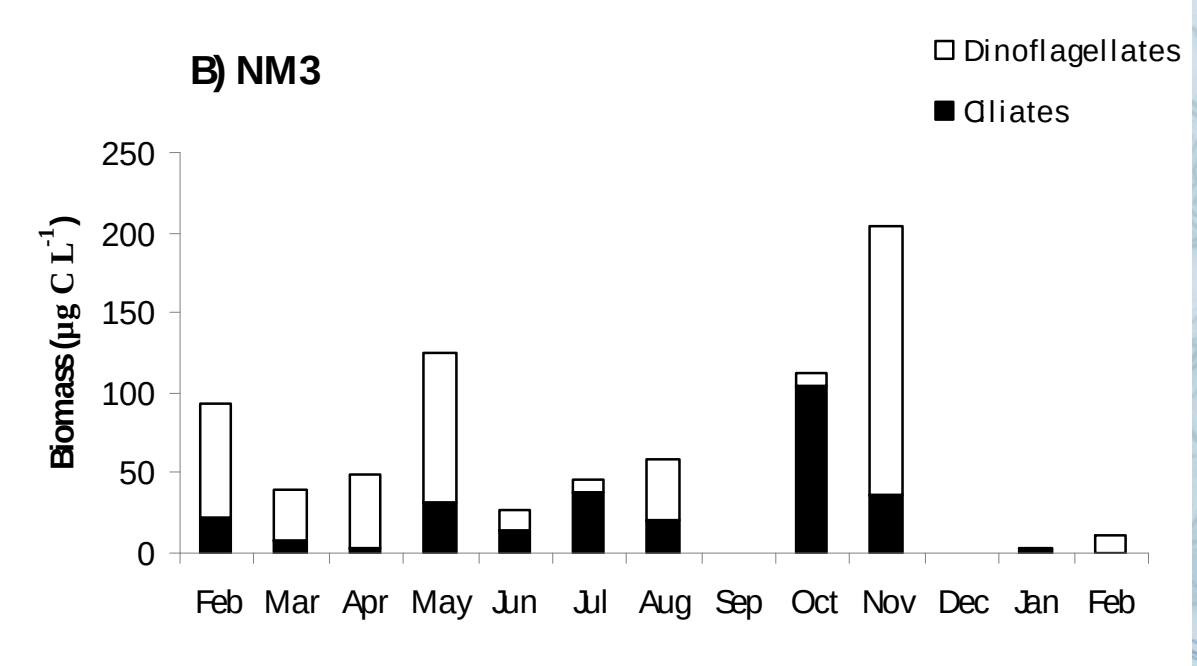
Abundance of *Synechococcus* and picoeukaryotes

- **Phytoplankton growth rates and microzooplankton grazing rates (based on Chl *a*)**



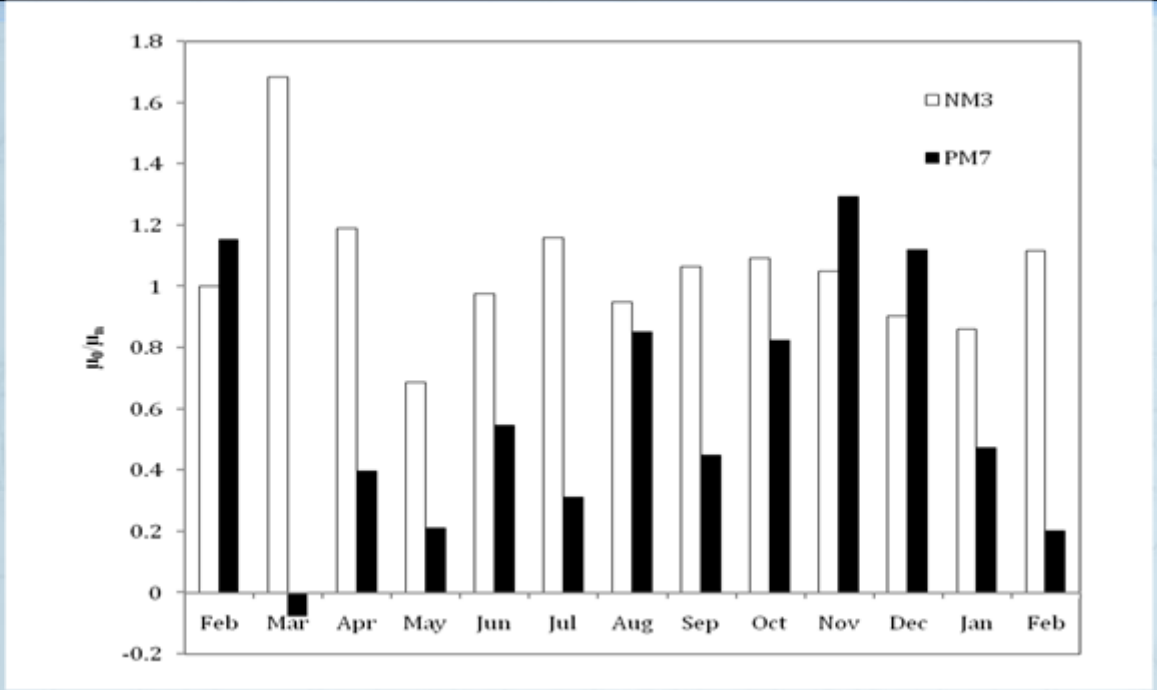


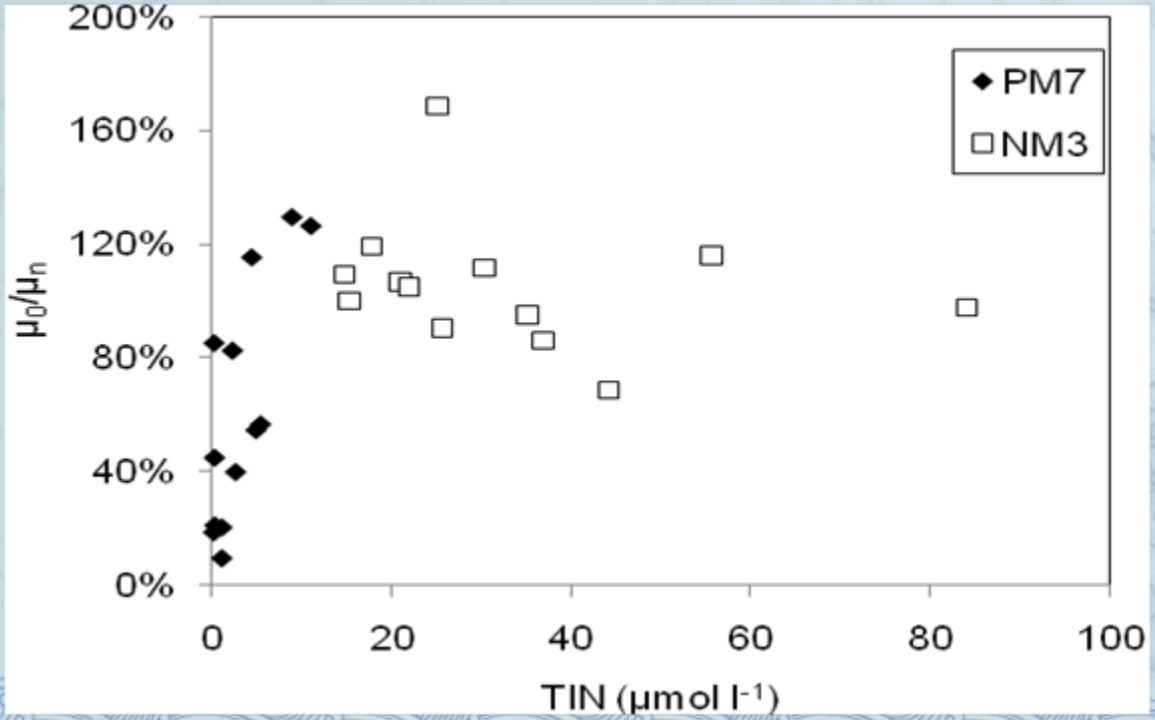


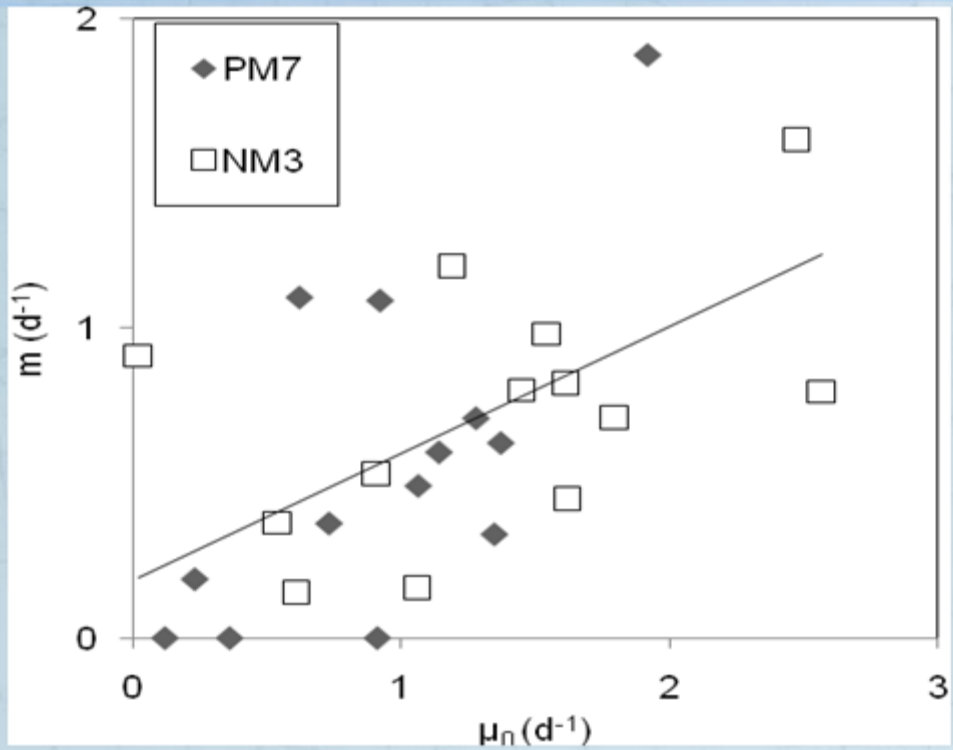


Biomass of ciliates and dinoflagellates

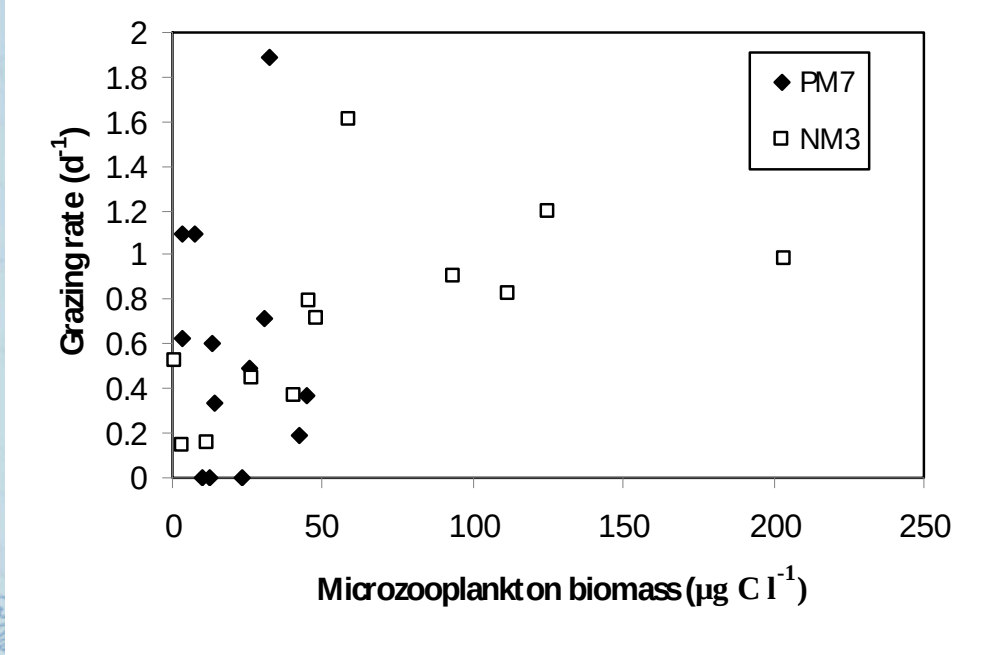
Nutrient limitation index (μ_0/μ_n)





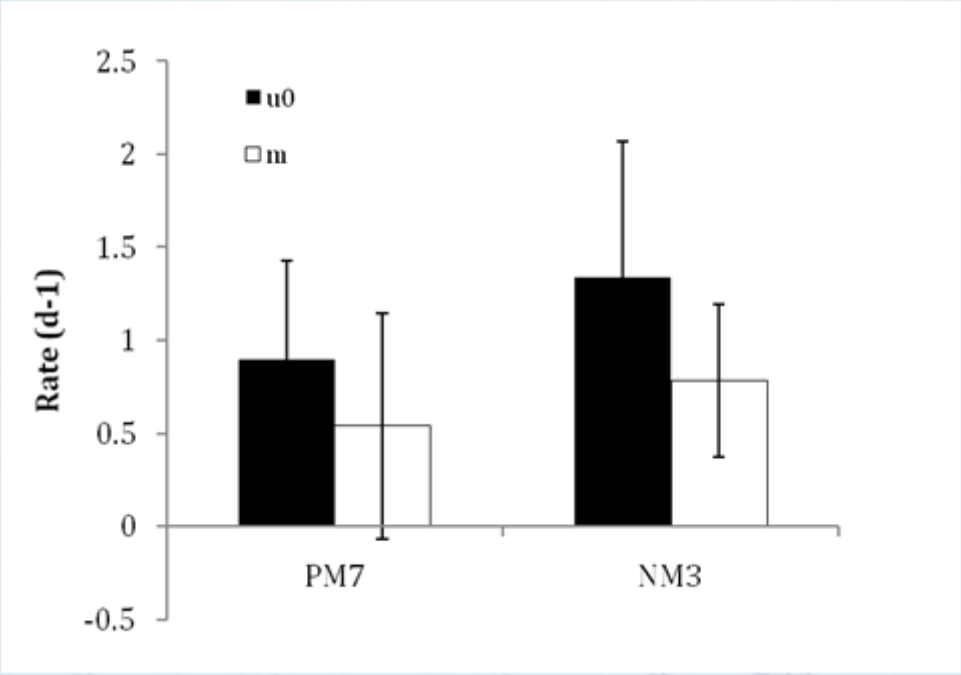


Scatter plots of phytoplankton growth rates (μ_0) and microzooplankton grazing rates (*m*). The Pearson correlation between the two variables is significantly positive ($r = 0.56$, $p < 0.01$, $n = 26$).



Scatter plots of microzooplankton grazing rates (*m*) derived from dilution experiments versus microzooplankton biomass (including both ciliates and dinoflagellates).

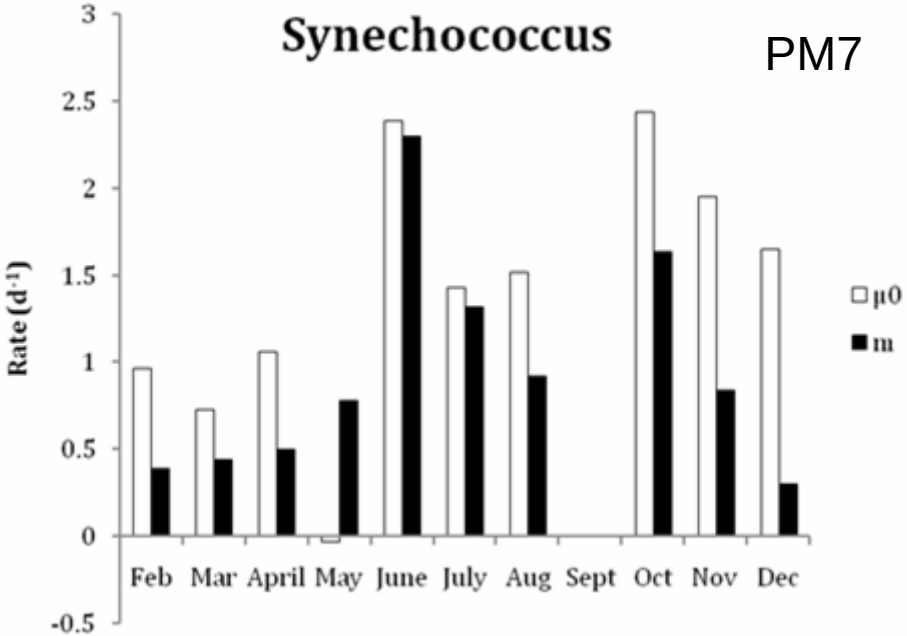
Average growth rates of phytoplankton and grazing rates of microzooplankton

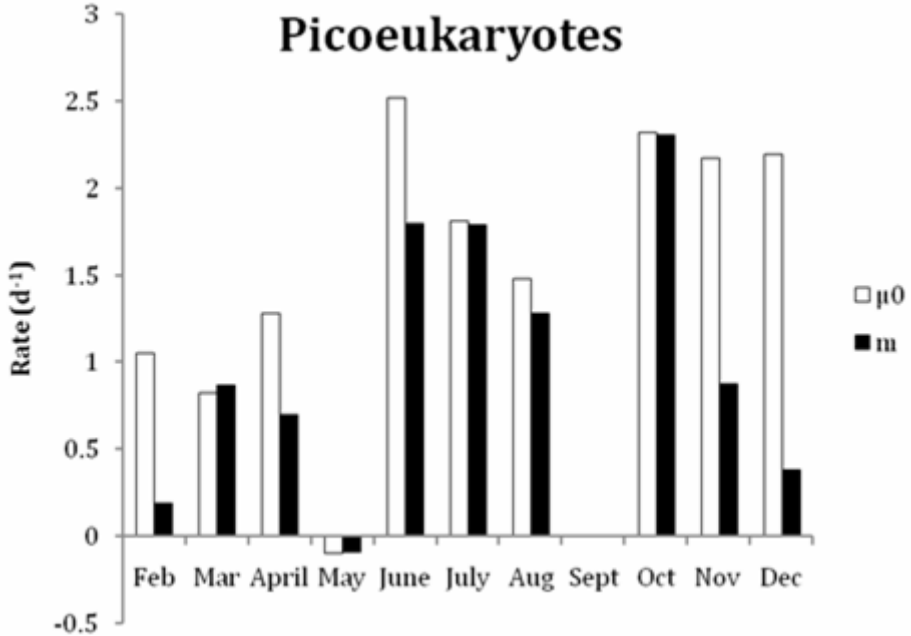


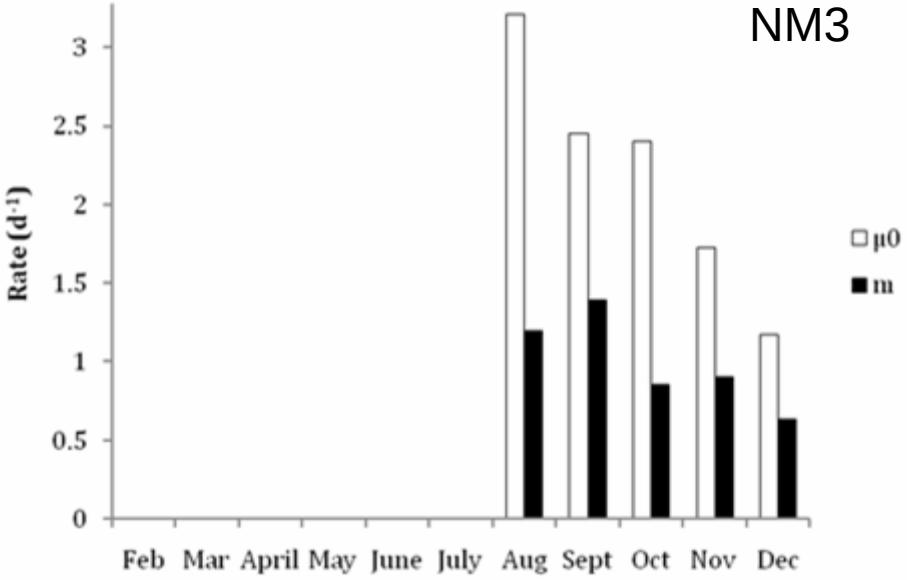
	μ_0		m		m/μ_0	
Stn.	**mean ± sd**	**CV**	**mean ± sd**	**CV**	**Mean***	**Median**
PM7	**0.89±0.54**	**60%**	**0.54±0.60**	**120%**	**0.59**	**0.56**
NM3	**1.34±0.73**	**55%**	**0.78±0.41**	**53%**	**0.54**	**0.58**

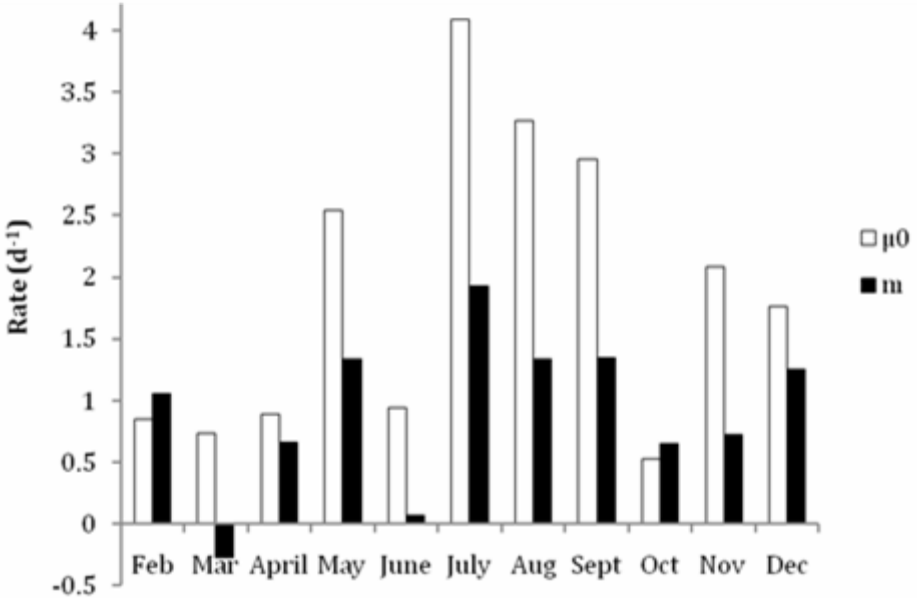
* Excluding data negative and larger than 1.2

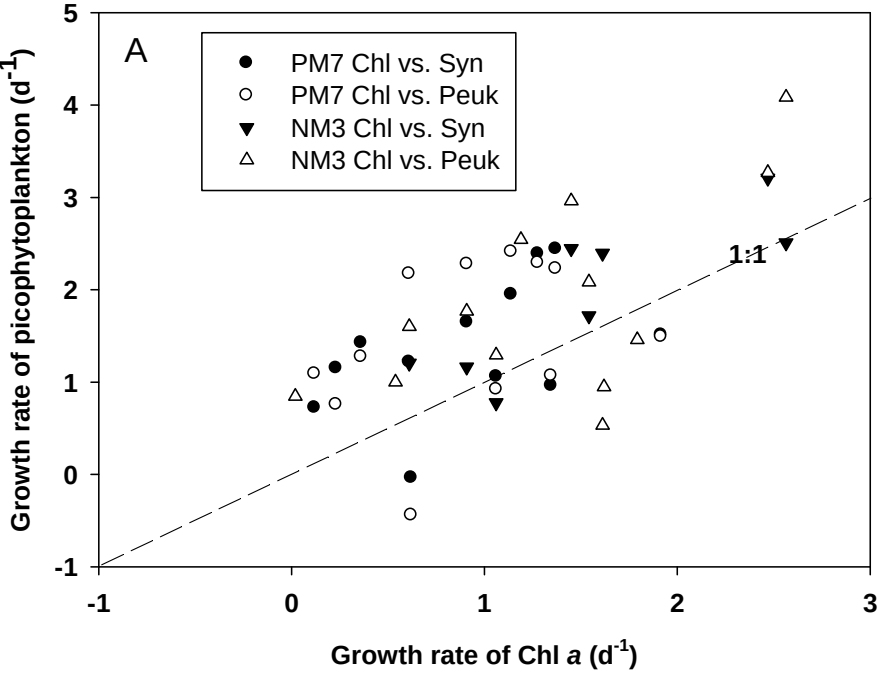
Rate estimates for picophytoplankton

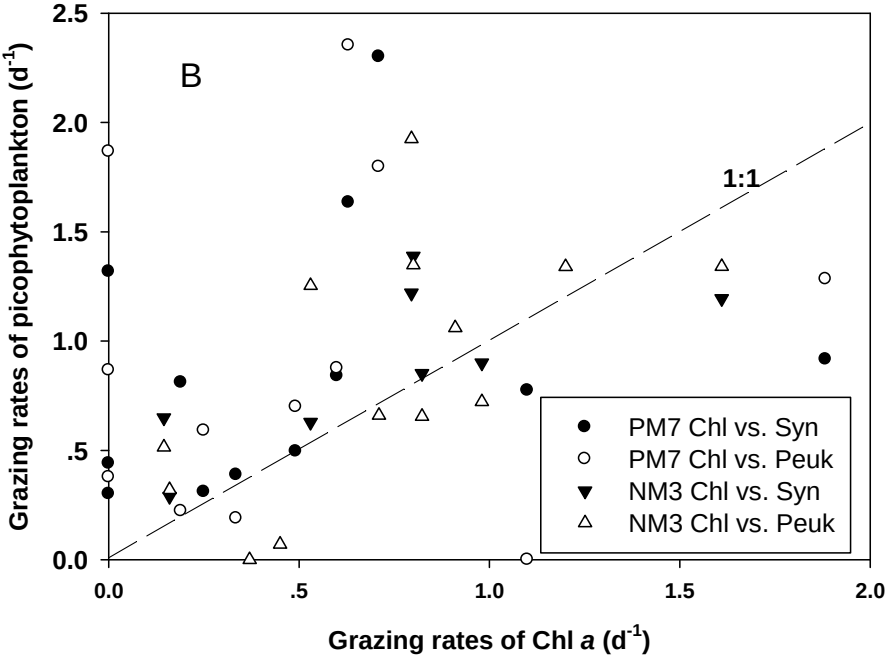






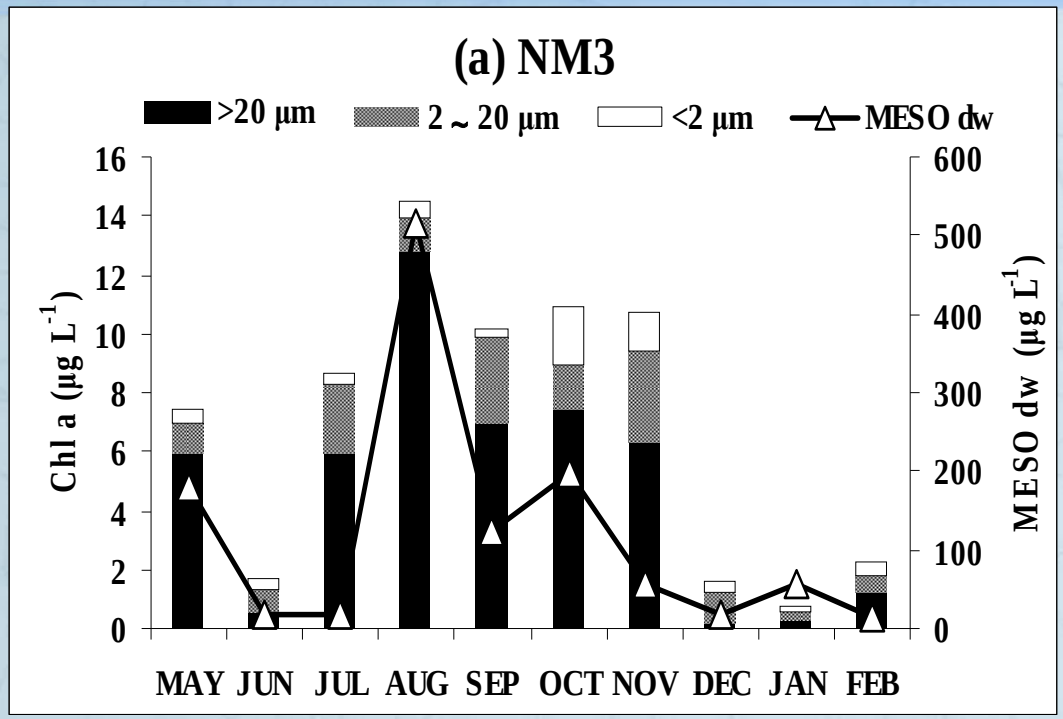


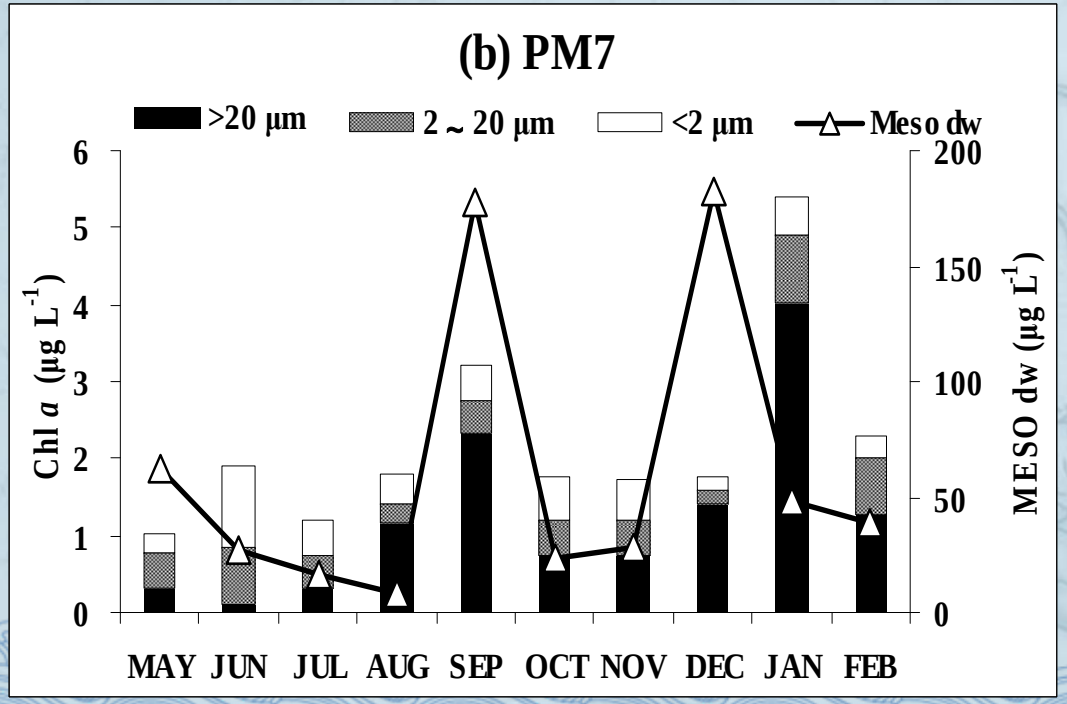


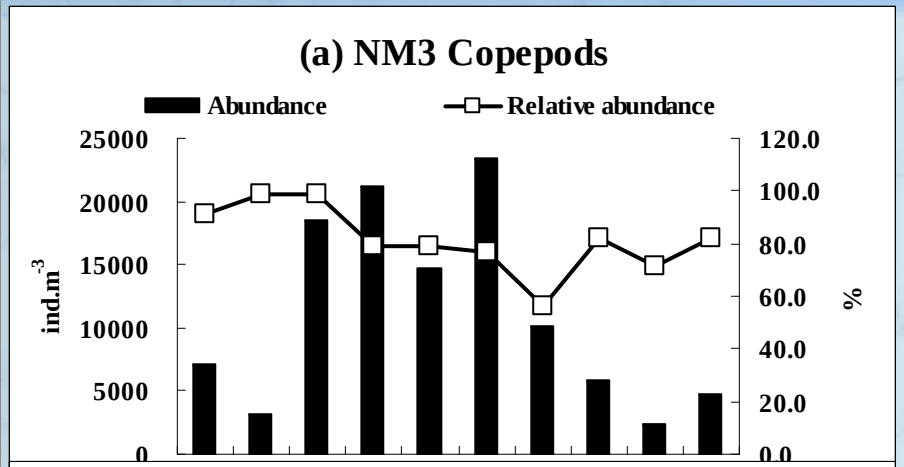


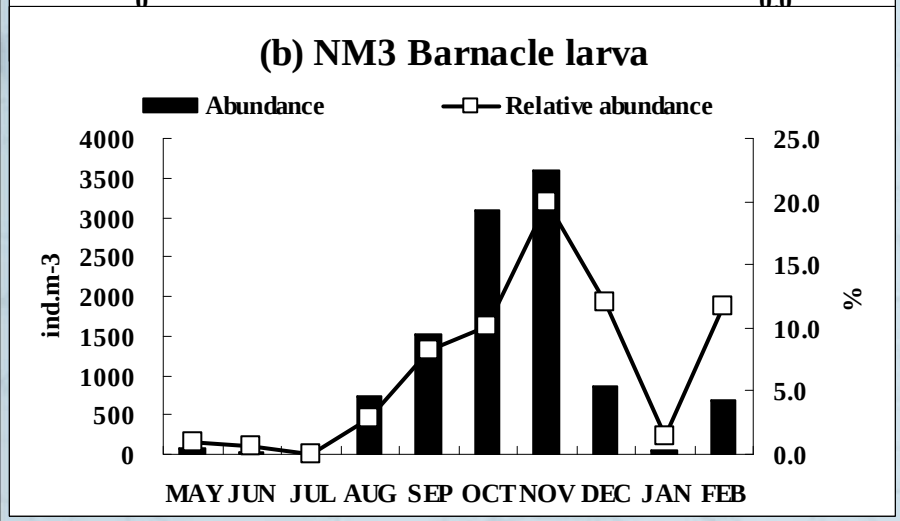
Phytoplankton growth and microzooplankton grazing rates are both faster for smaller than larger cells

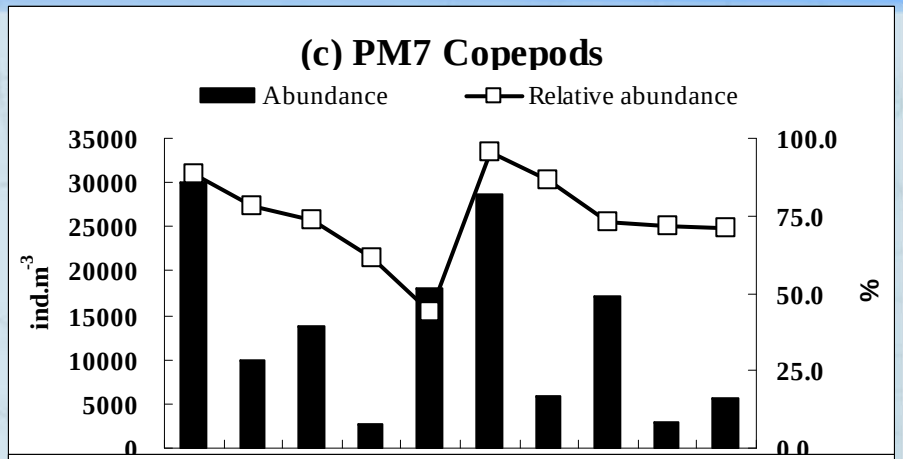
Mesozooplankton dry-weight at two sites

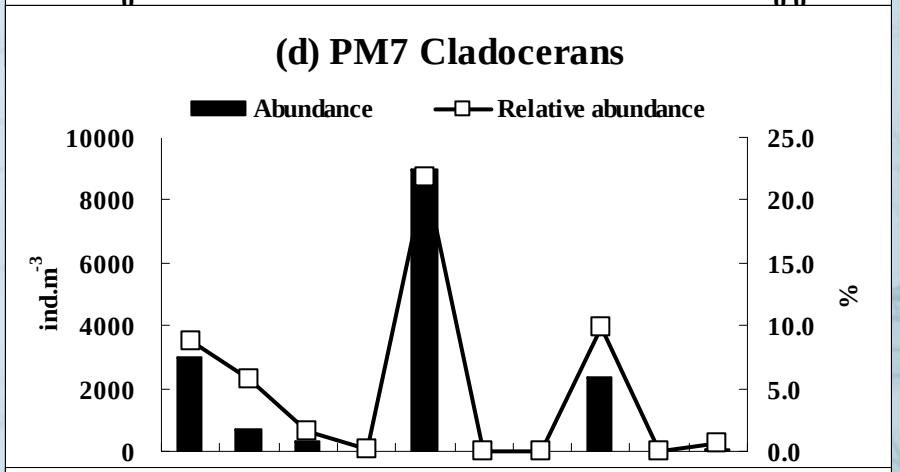


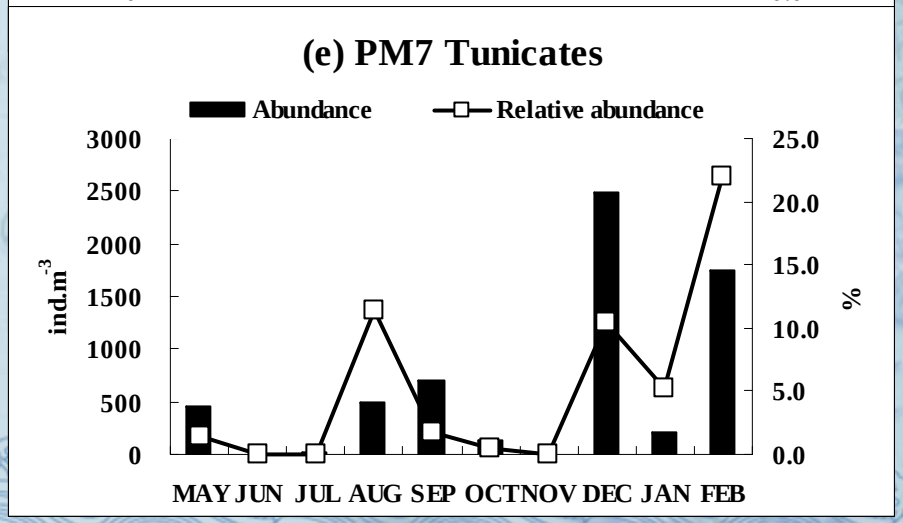






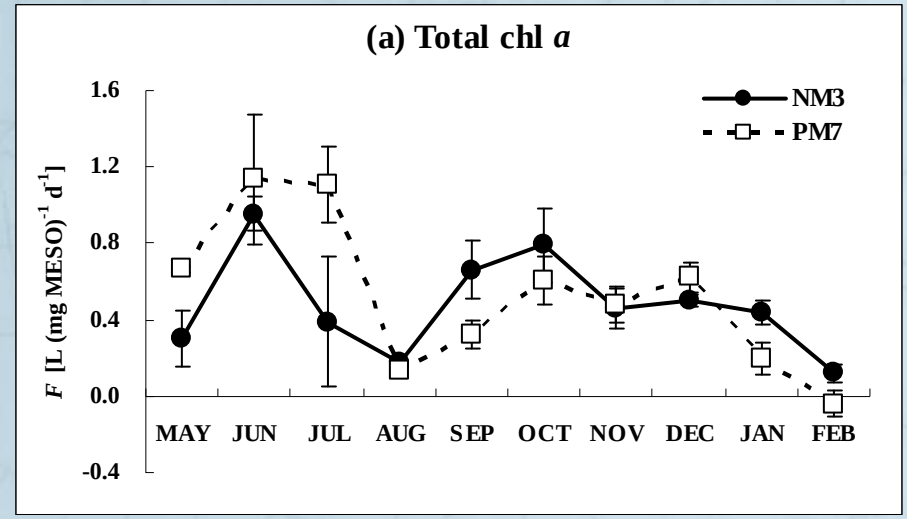


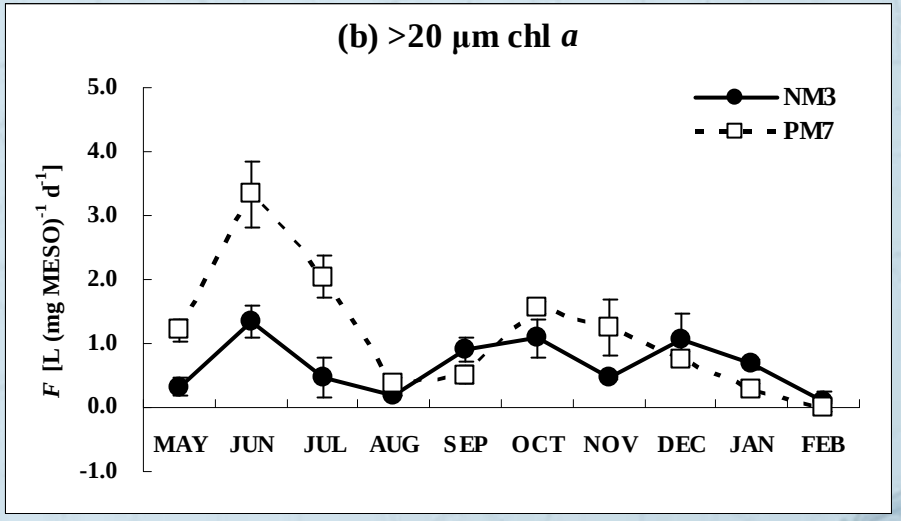


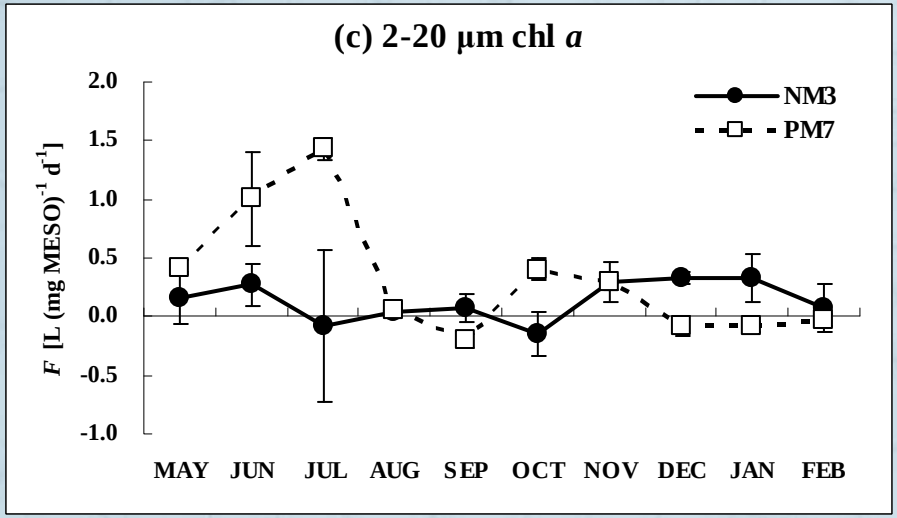


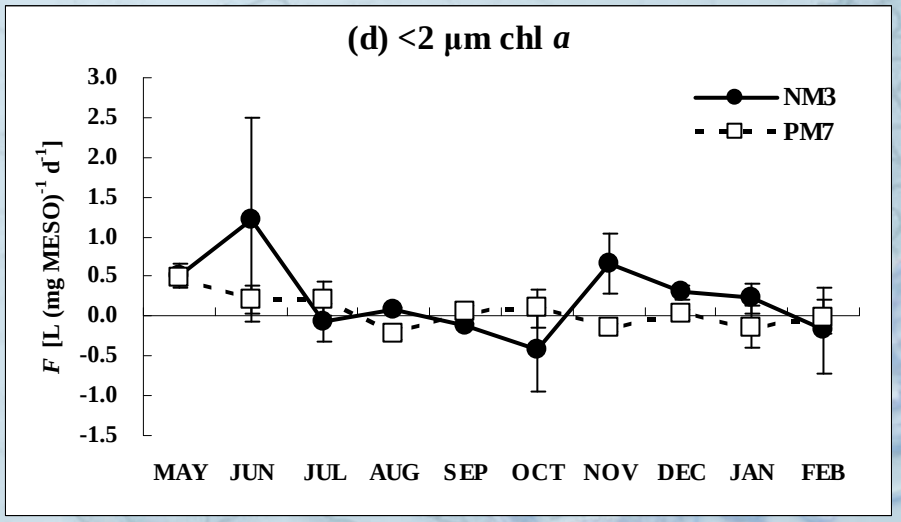
Abundance and relative abundance of major grazers of phytoplankton among metazoan mesozooplankton

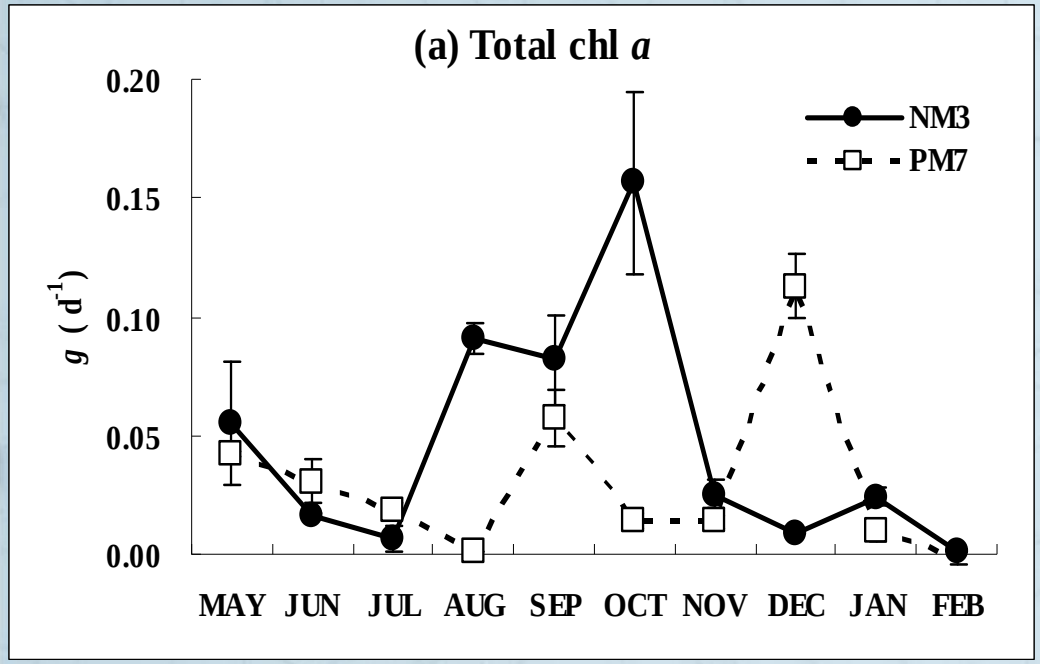
Mesozooplankton clearance rate on different size-fractions



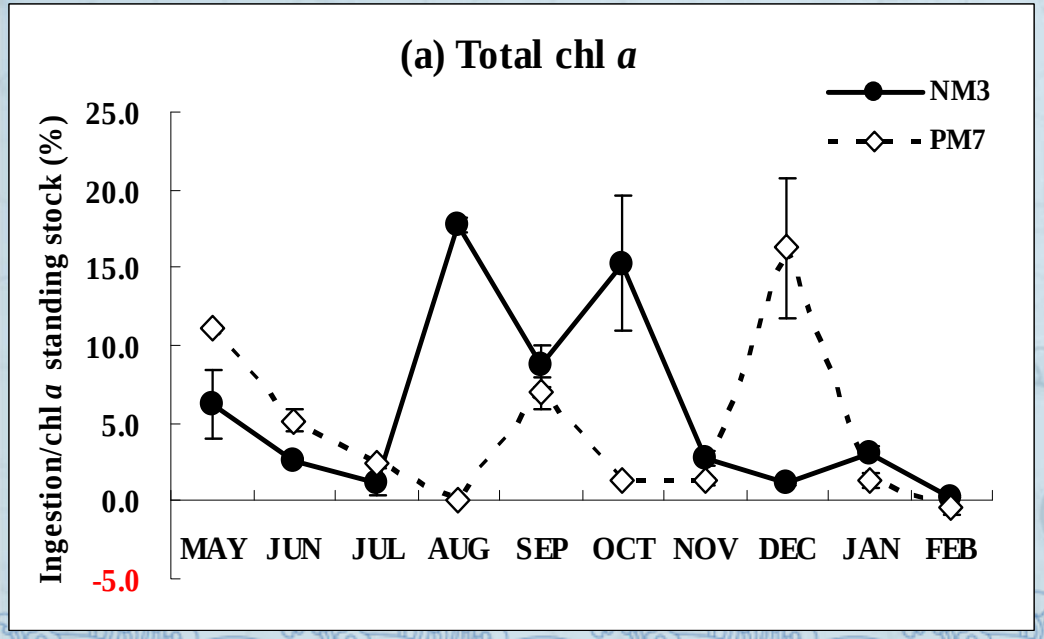






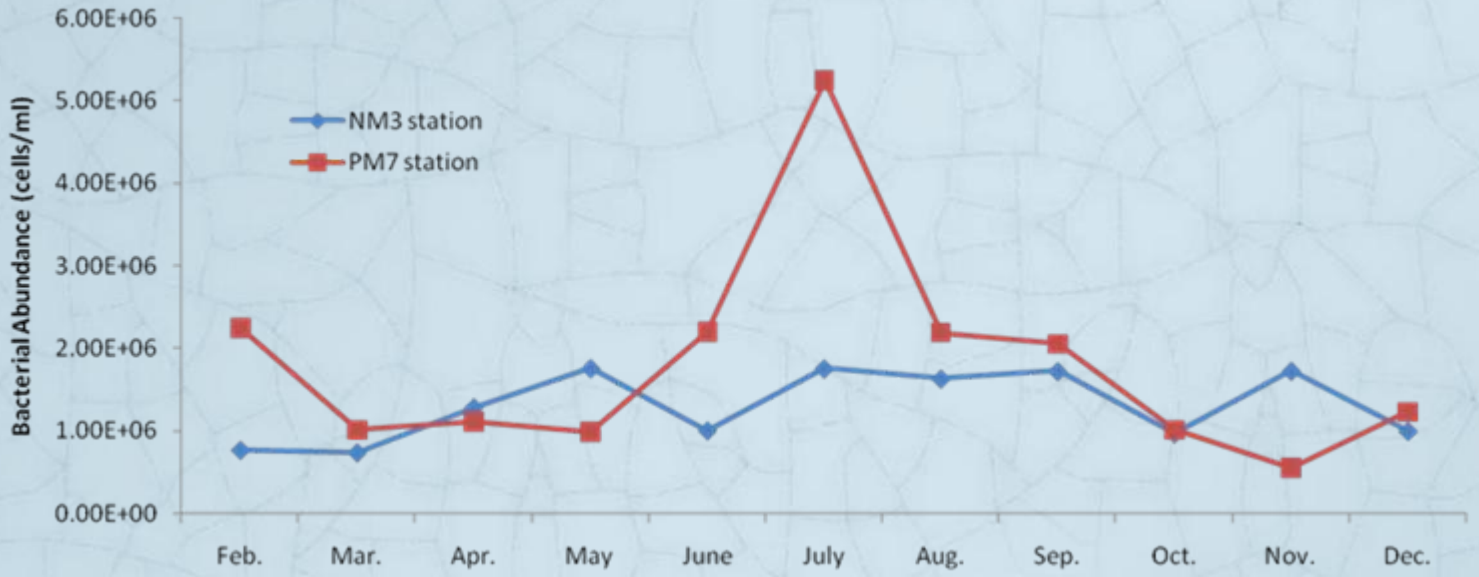


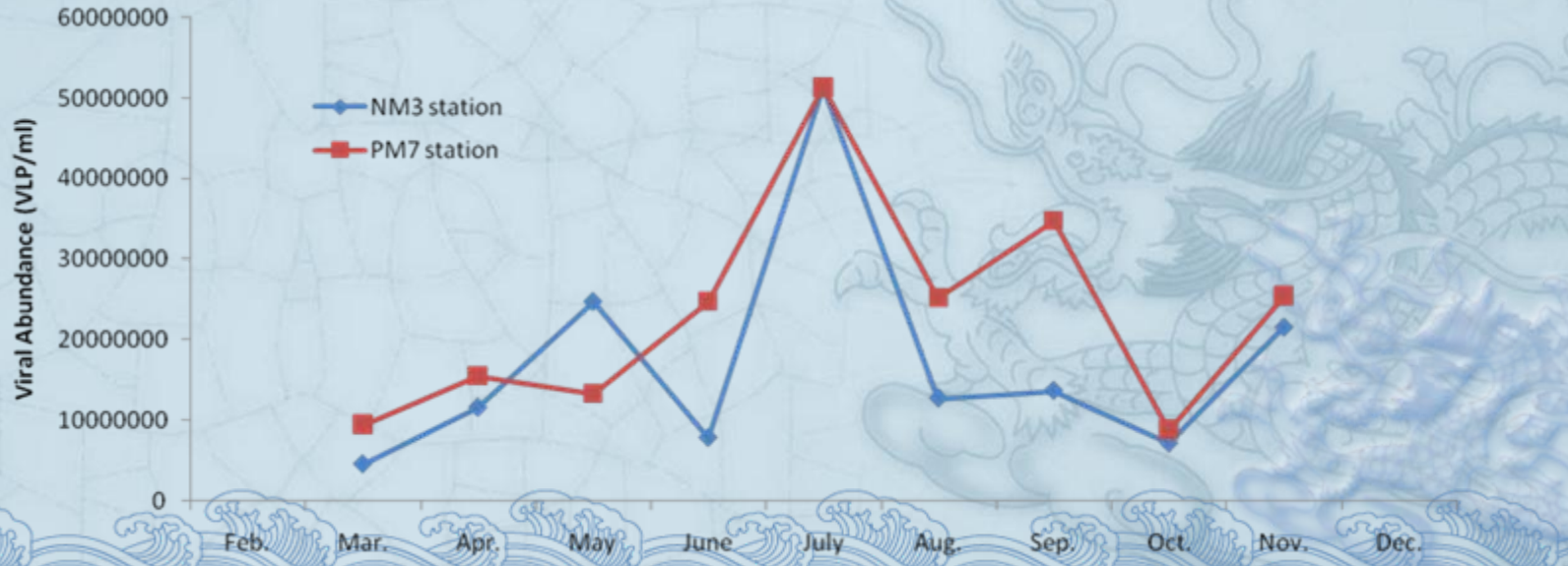
In situ mesozooplankton grazing rates (d^{-1})



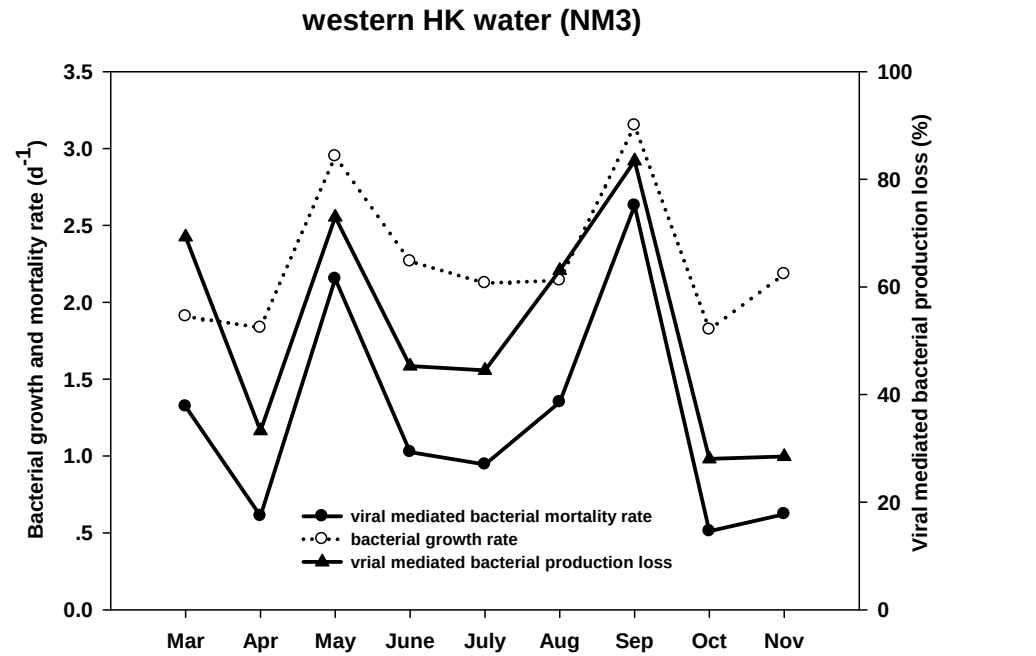
Percentage of phytoplankton standing stock consumed by mesozooplankton

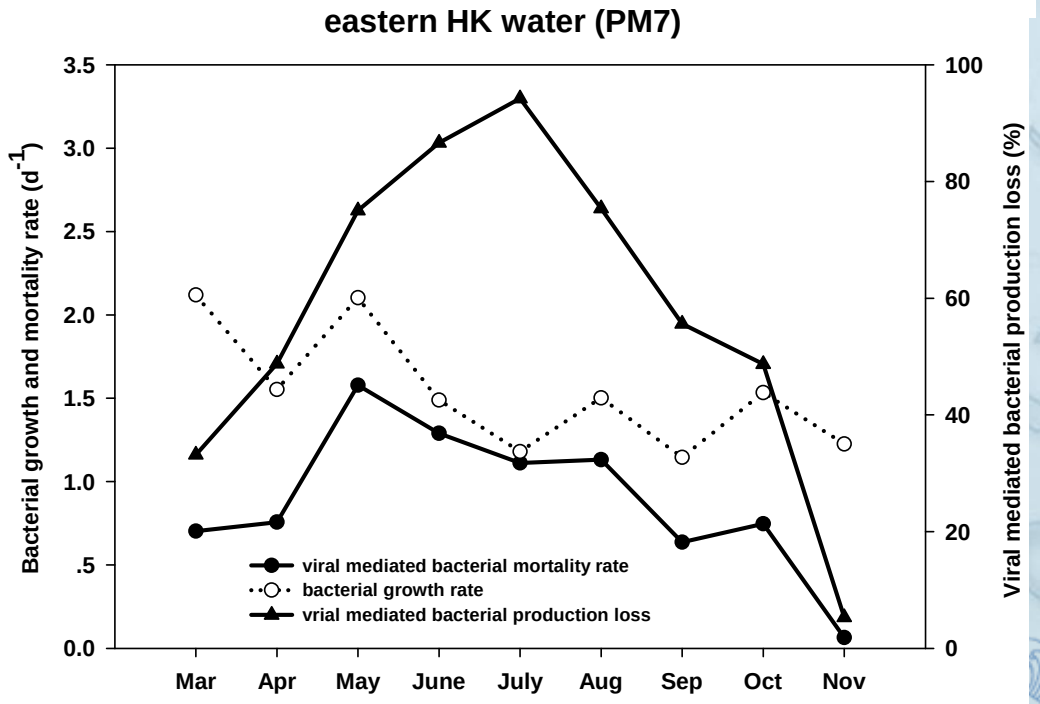
Bacterial and viral abundance





bacterial growth rate and viral induced bacterial mortality rate





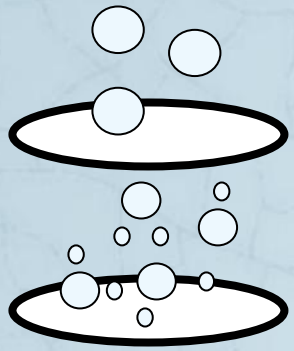
Average estimates for the percentage of different loss factors versus growth rate for phytoplankton

	m/μ	M/μ	h/μ	s/μ	v/μ
PM7	0.59	0.05	<0.05?	?	?
NM3	0.54	0.04	0.12*	?	?

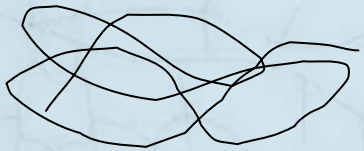
μ: phytoplankton growth rate
m: microzooplankton grazing
M: mesozooplankton grazing
h: horizontal advection
s: sinking
v: virus lysis
*Estimated from Yin et al. (2000)

Water sample

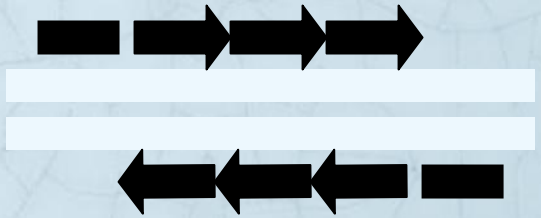
Filtered seawater: 400ml for each 0.22μm / 3μm filter



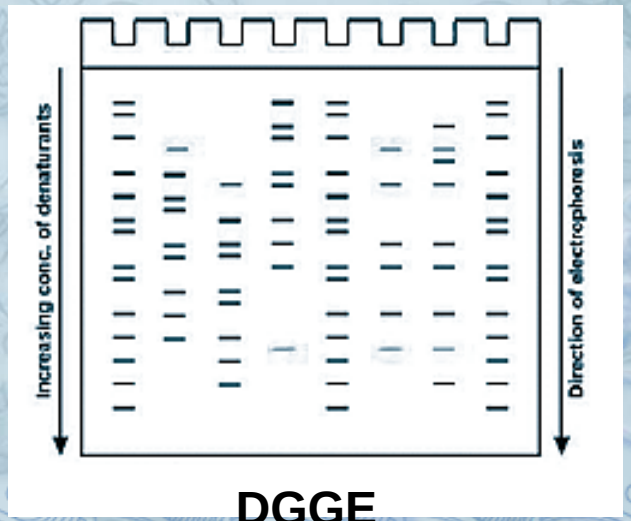
Membrane Filtering



DNA Extraction



PCR Amplification



DGGE

DGGE fingerprint pattern

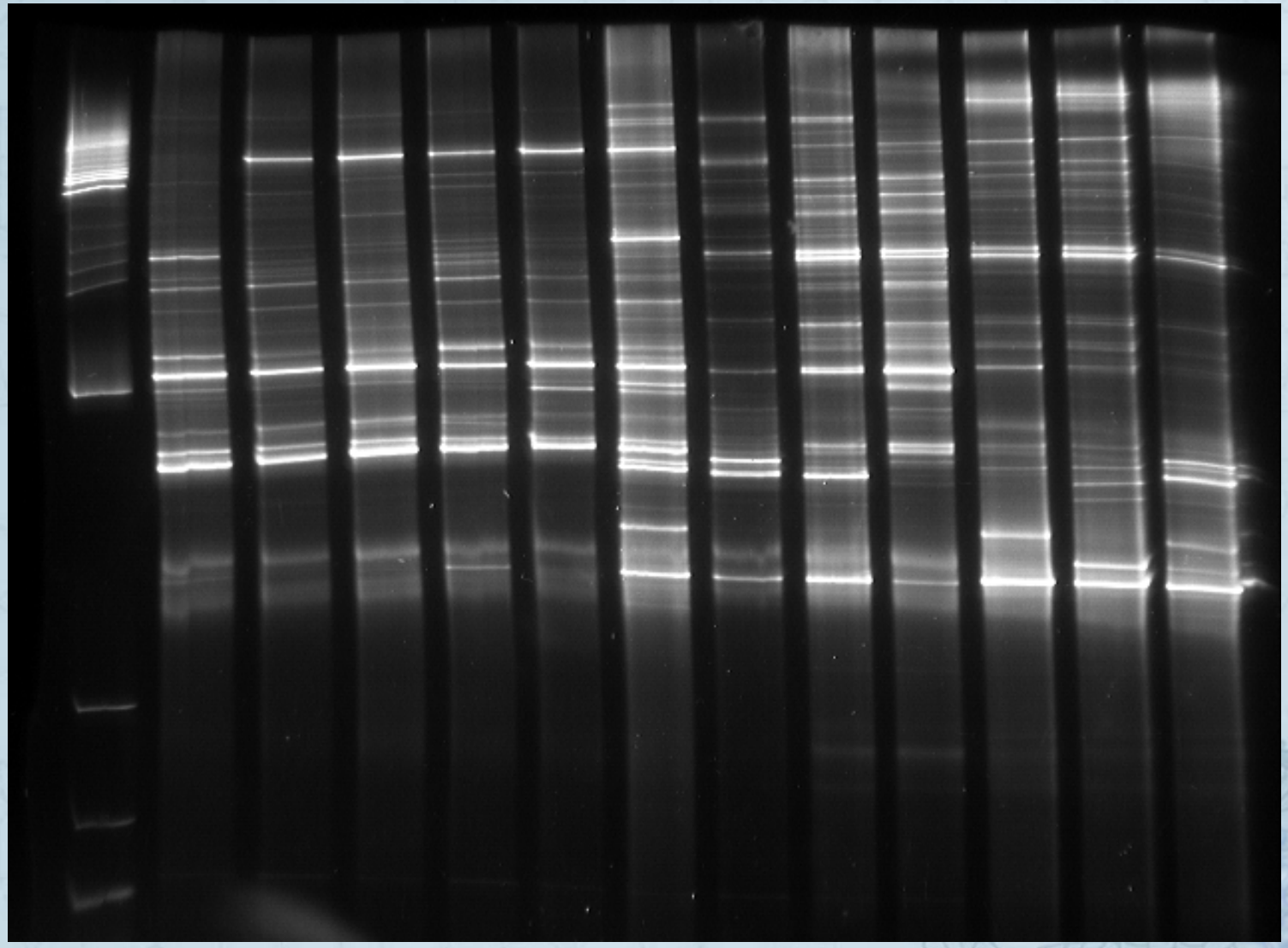
Cluster Analysis &

Gel band quantification

DG band excision, re-amplification, purification

Sequencing &

BLAST search

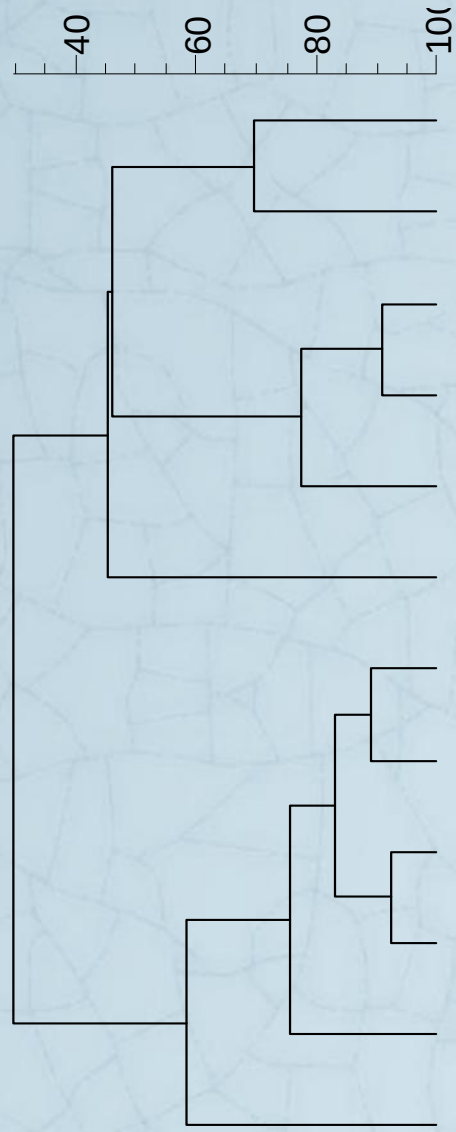


NM3-18S-DGGE
enlarged picture

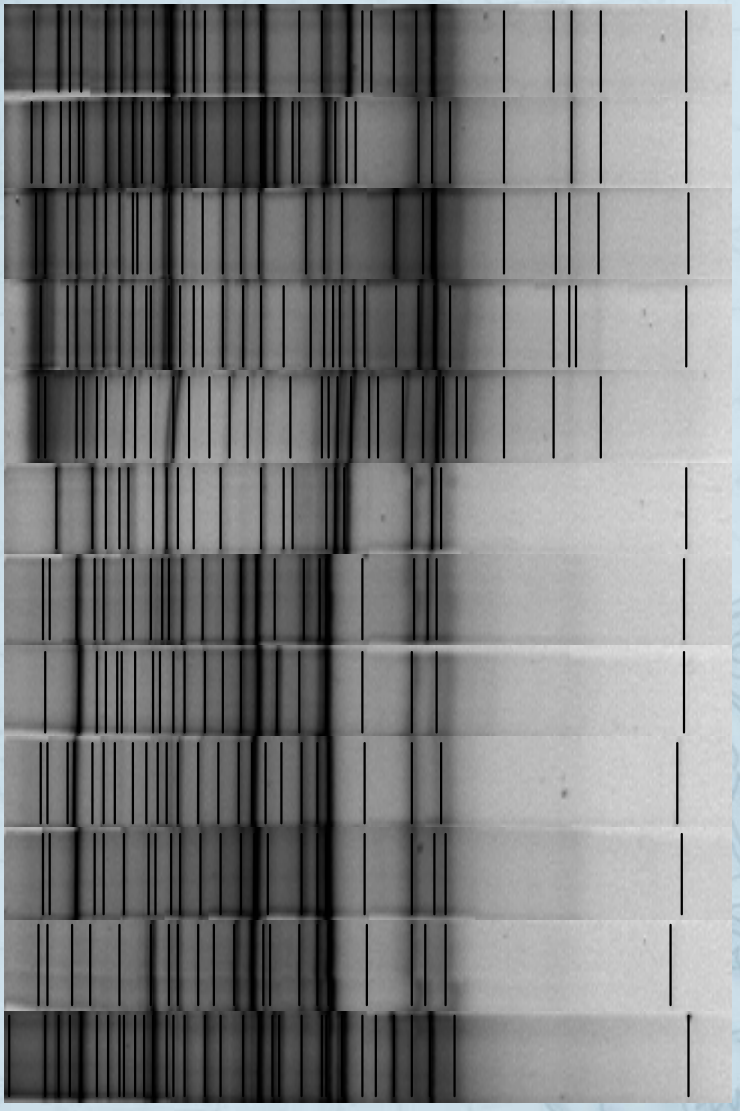
Sample loading order (from **left to right**): ladder, initial (0.22~3um), control A, B, treatment1, 2, 3; initial (>3um), control A, B, treatment 1, 2, 3.

Pearson correlation (Opt:0.01%) [0.0%-100.0%]

DGGE

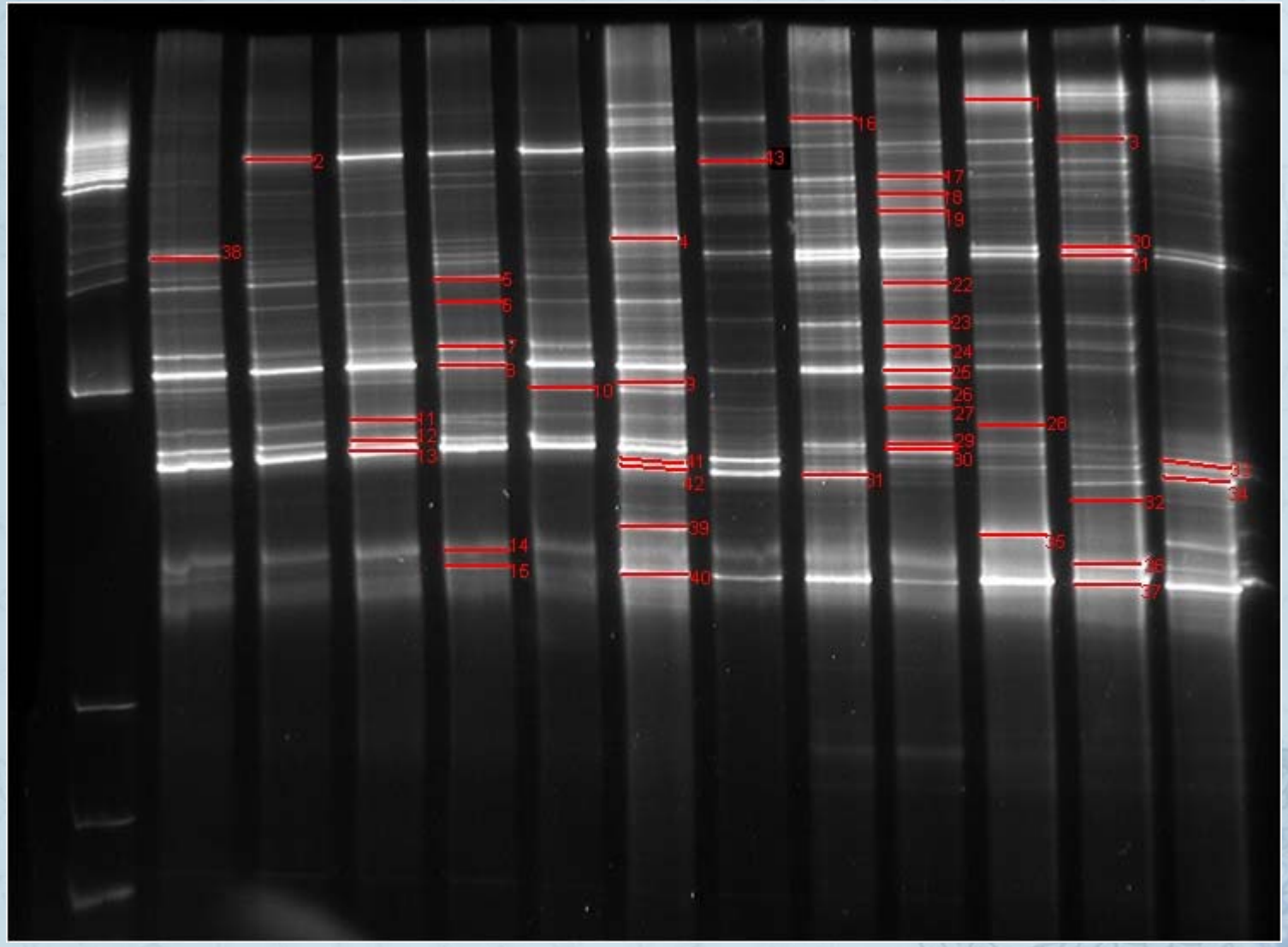


DGGE



Grazing-NM3

grazing-NM3-3-CA
grazing-NM3-3-CB
grazing-NM3-3-T1
grazing-NM3-3-T2
grazing-NM3-3-T3
grazing-NM3-3-initial
grazing-NM3-0.22-t1
grazing-NM3-0.22-T2
grazing-NM3-0.22-CA
grazing-NM3-0.22-CB
grazing-NM3-0.22-Initial
grazing-NM3-0.22-t3



NM3-18S-DGGE enlarged picture

Sample loading order (from **left to right**): ladder, initial (0.22~3um), control A, B, treatment1, 2, 3; initial (>3um), control A, B, treatment 1, 2, 3.

Sequence analysis

- NM3 >3μm
  - Increased after treatment
  - Introduced during zooplankton addition

Code	Closest Identity	Phylogenetic Group
NM3-B01	Uncultured eukaryotic picoplankton isolate DGGE gel band X3	Stramenopiles
NM3-B32	Uncultured copepod clone AT2-18	Copepod
NM3-B33	*Neocalanus cristatus* clone A line	Calanoid copepod
NM3-B34	*Neocalanus cristatus* clone Nihon	Calanoid copepod
NM3-B35	*Asterocheridae sp.* 1 New Caledonia-RJH-2004	Copepod
NM3-B36	*Scambicornus sp.* New Caledonia-RJH-2004	Copepod

- NM3 >3μm
  - Decreased after treatment
  - Grazed by mesozooplankton

Code	Closest Identity	Phylogenetic Group
NM3-B17	Uncultured stramenopile clone GHB34.11	Stramenopiles; Bacillariophyta
NM3-B18	*Woloszynskia pascheri* strain CCAC0075	Alveolata; Dinophyceae
NM3-B19	Uncultured eukaryotic picoplankton clone PG5.8	Stramenopiles; Bacillariophyta
NM3-B22	Uncultured marine eukaryote clone SIF_4H10	Alveolata; Dinophyceae
NM3-B23	Uncultured marine eukaryote clone NA2_2G6	Alveolata; Dinophyceae
NM3-B24	Uncultured marine eukaryote clone js45	Alveolata; Dinophyceae
NM3-B25	*Gyrodinium fusiforme*	Alveolata; Dinophyceae
NM3-B27	Uncultured eukaryote clone SCM28C129	Alveolata; Dinophyceae
NM3-B29	Uncultured marine alveolate clone PROSOPE99.CTD28.50m.DGGE.250803_30	Alveolate
NM3-B30	Uncultured eukaryote clone A01N10	Alveolate

- NM3 0.22-3μm
  - Increased after treatment
  - Enriched when zooplankton were introduced

Code	Closest Identity	Phylogenetic Group
NM3-B10	*Thalassomyces fagei*	Alveolate
NM3-B39	Uncultured eukaryotic picoplankton clone XMCC5	Stramenopiles
NM3-B41	Uncultured eukaryote clone 18BR21	Unknown
NM3-B42	Uncultured eukaryote clone 18BR20	Unknown

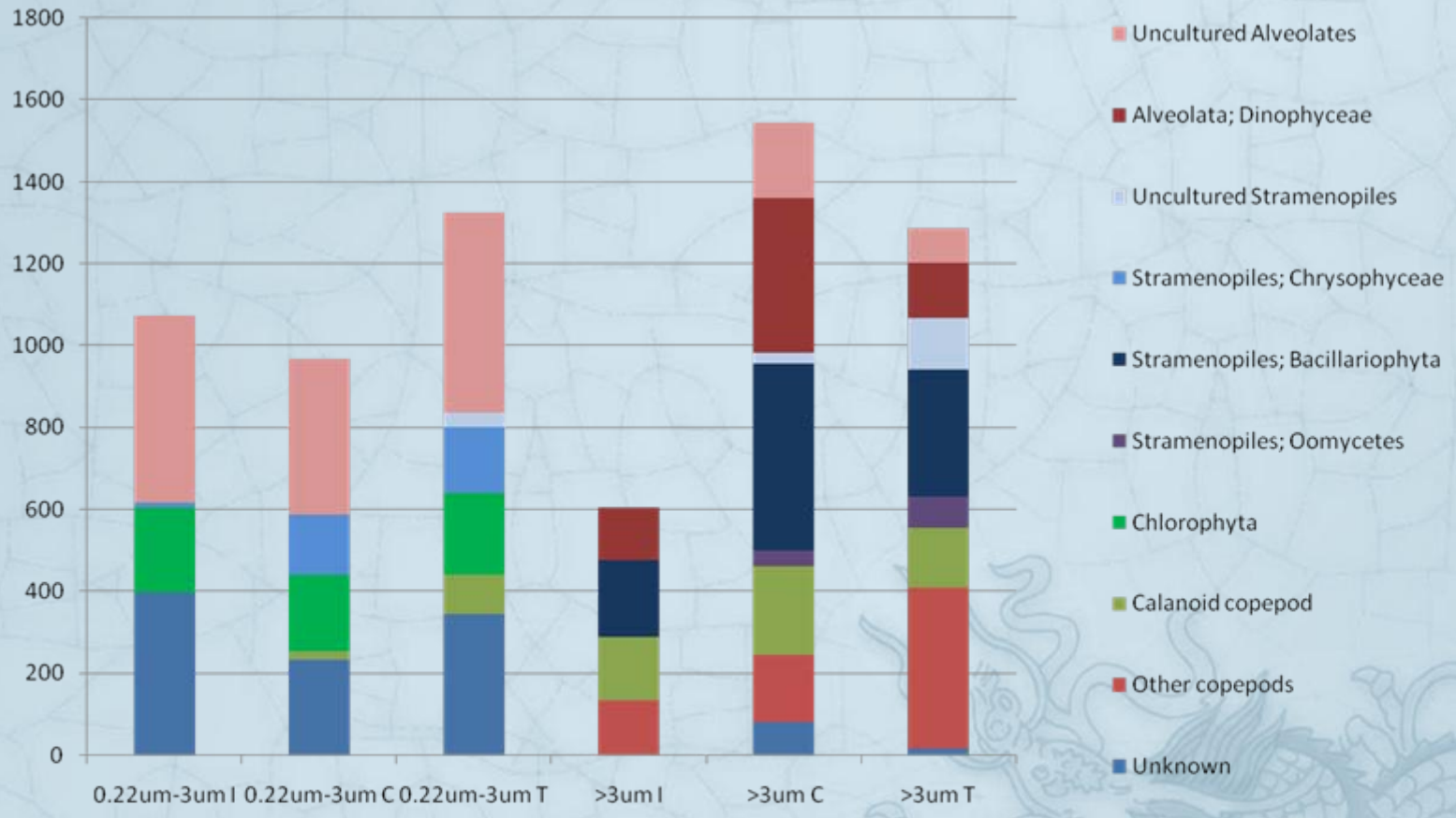


Fig.1 Changes of species group composition in each of the treatments at NM3 station (eutrophic).
I: Initial experiment with no incubation
C: Control experiments with 24 hr incubation
T: Treatments with copepod addition and 24 hr incubation

Summaries

- Large phytoplankton dominates the biomass at the western site, while pico-phytoplankton are more important at the eastern site.
- Nutrient limitation of phytoplankton growth is common at the eastern site, but not at the western site.
- Microzooplankton consumes a great proportion of primary production at both sites, while the impact of mesozooplankton grazing is very small.
- Viral mediated bacterial mortality is significant, but viral lysis of phytoplankton is likely small.


THANK YOU

Regional & Habitat Comparisons

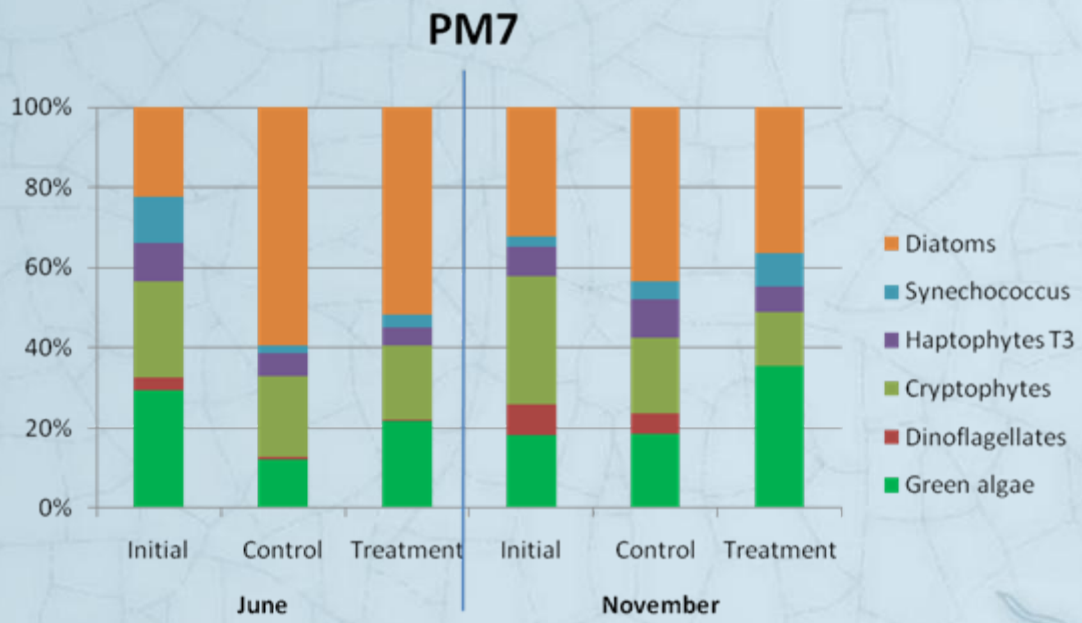
Means ± standard errors

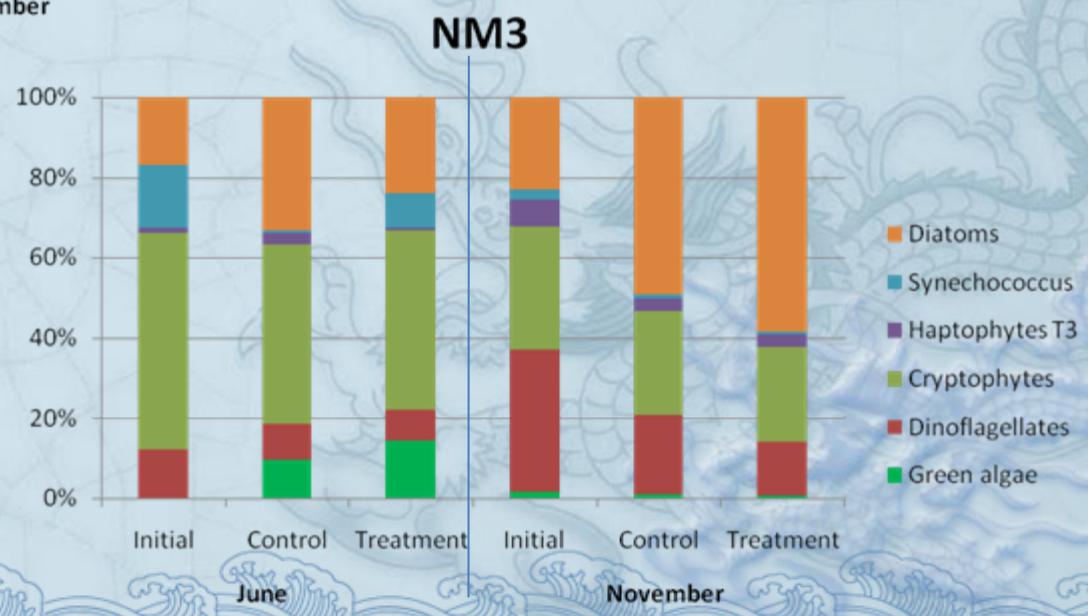
	Chl*a* (µg/L)	μ (d^{-1})	%PP grazed
Oceanic	0.6 ± 0.03	0.59 ± 0.02	70 ± 1.5
Coastal	3.1 ± 0.5	0.67 ± 0.05	60 ± 3.3
Estuarine	13.0 ± 1.8	0.97 ± 0.07	60 ± 2.7
Tropical	1.0 ± 0.2	0.72 ± 0.02	75 ± 2.0
Temperate	5.2 ± 0.7	0.69 ± 0.03	61 ± 1.8
Polar	0.6 ± 0.1	0.44 ± 0.05	59 ± 3.3

Calbet & Landry (2004) L&O

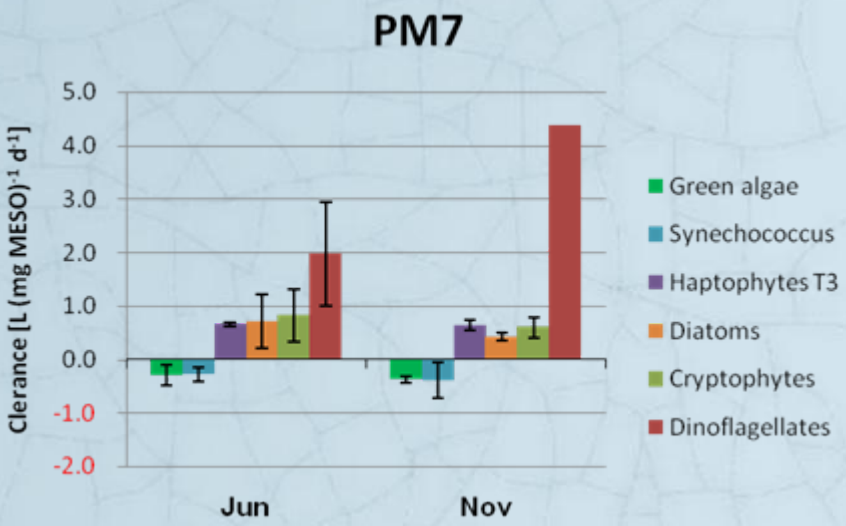
Composition changes by mesozooplankton grazing

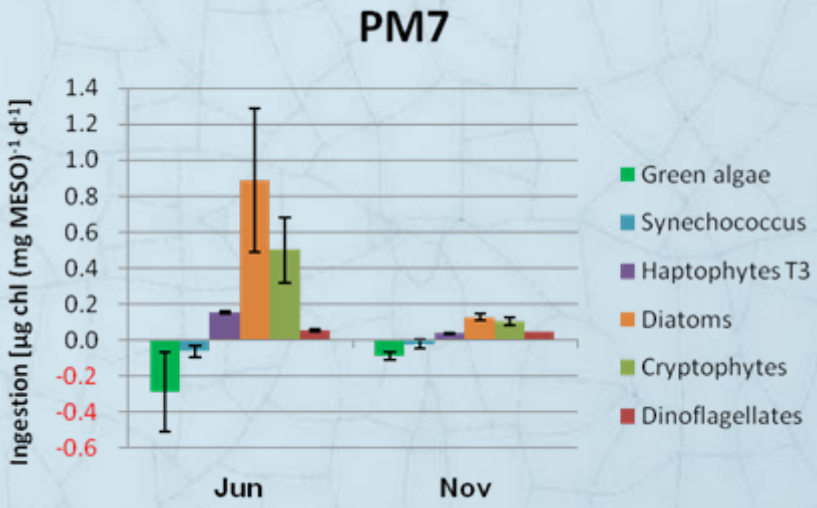
HPLC pigment analysis

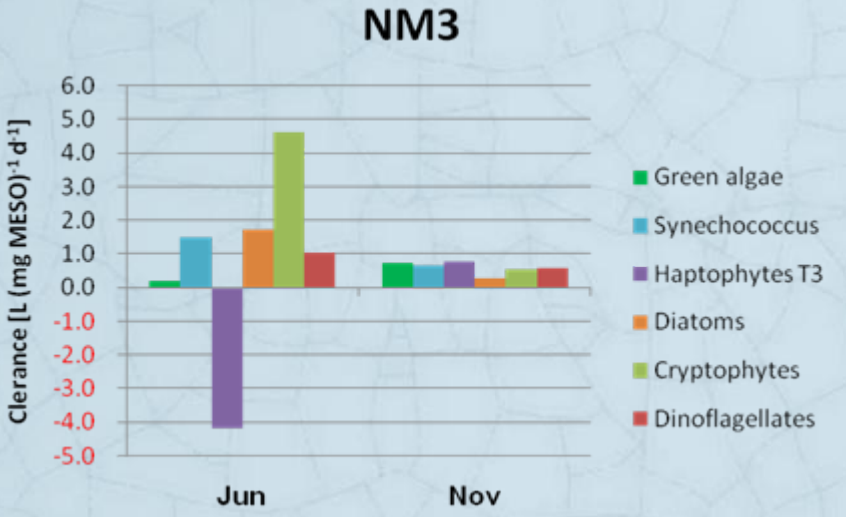


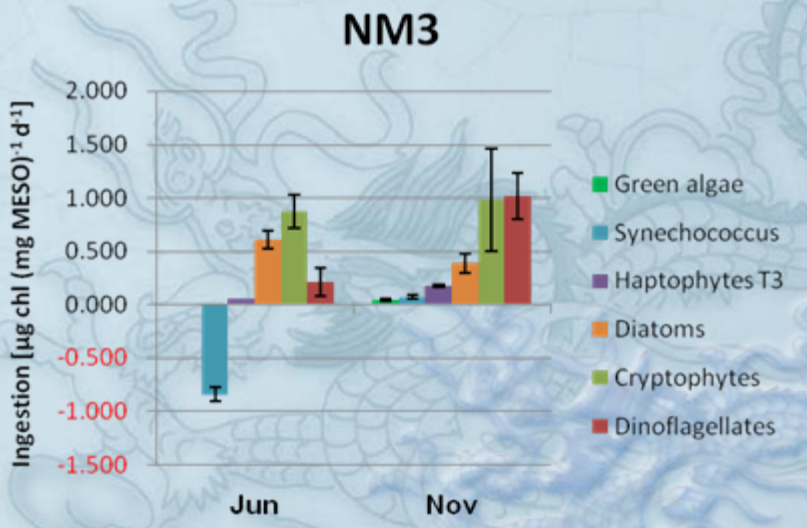


Mesozooplankton clearance rates and ingestion rates

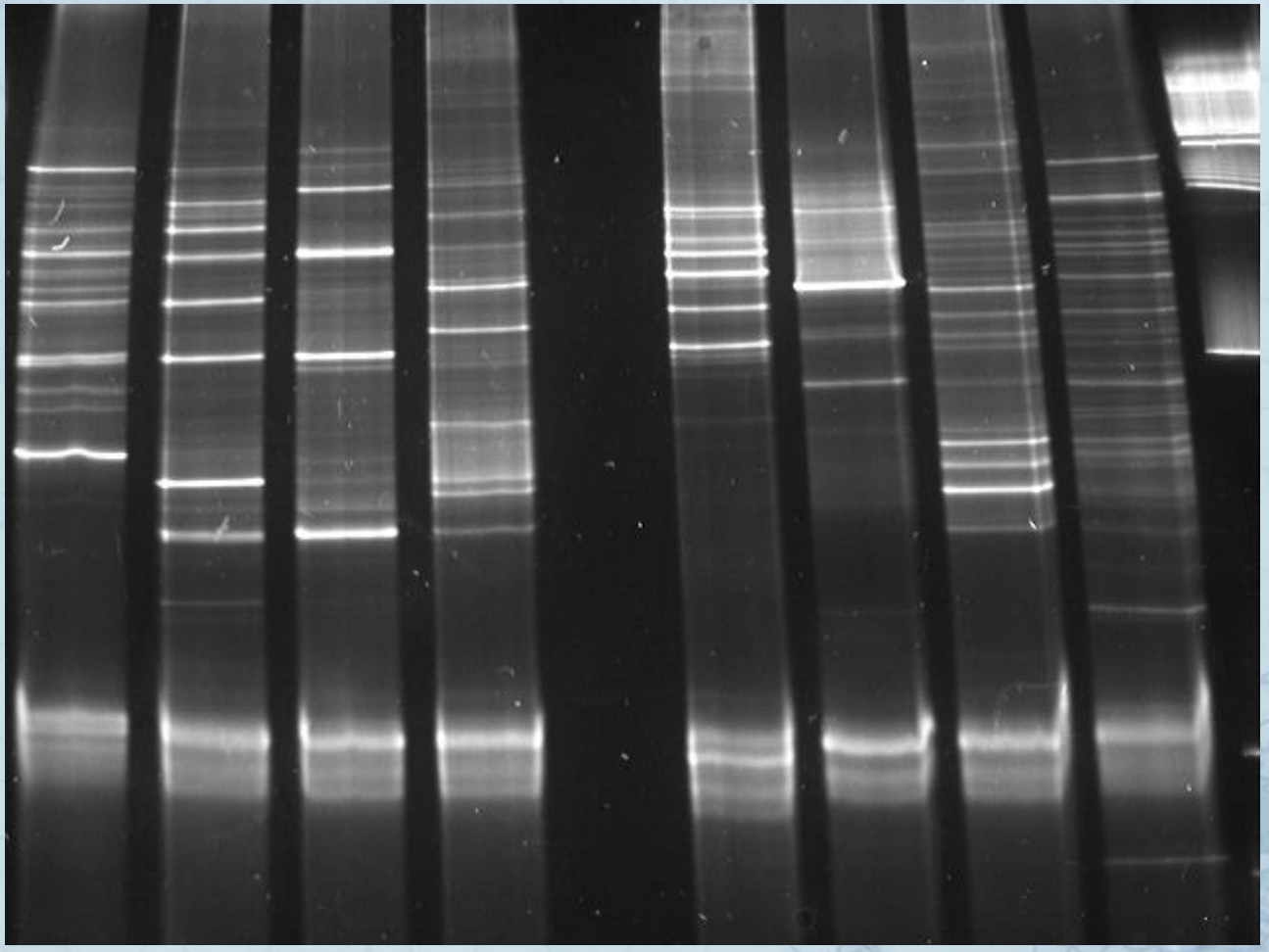








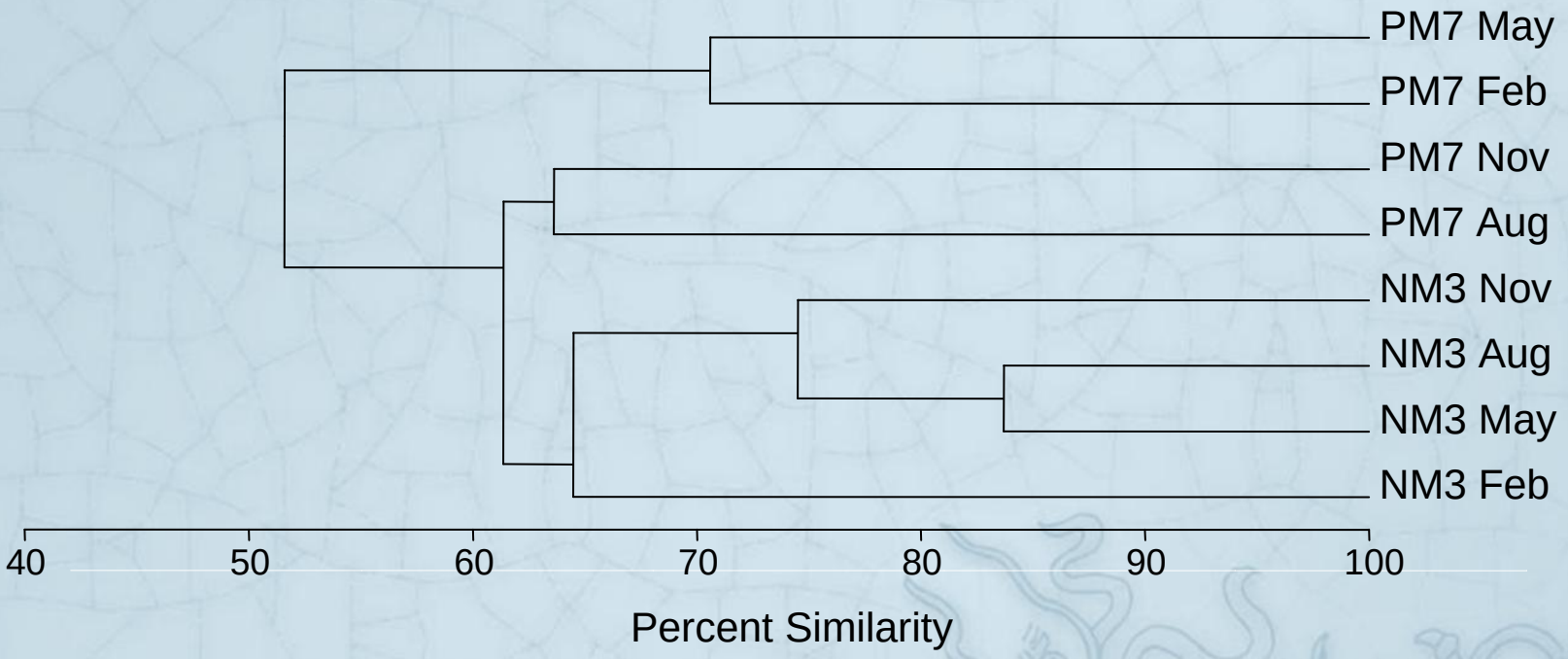
Picoeukaryotes diversity as shown in denaturing gradient gel electrophoresis (DGGE) gel patterns of PCR amplified 18S rRNA genes



2 5 8 11 2 5 8 11

NM3 PM7

DGGE cluster analysis
MVSPW: UPGMA method



Species richness (no. of bands)

NM3 Feb: 15

NM3 May: 20

NM3 Aug: 23

NM3 Nov: 24

Species richness (no. of bands)

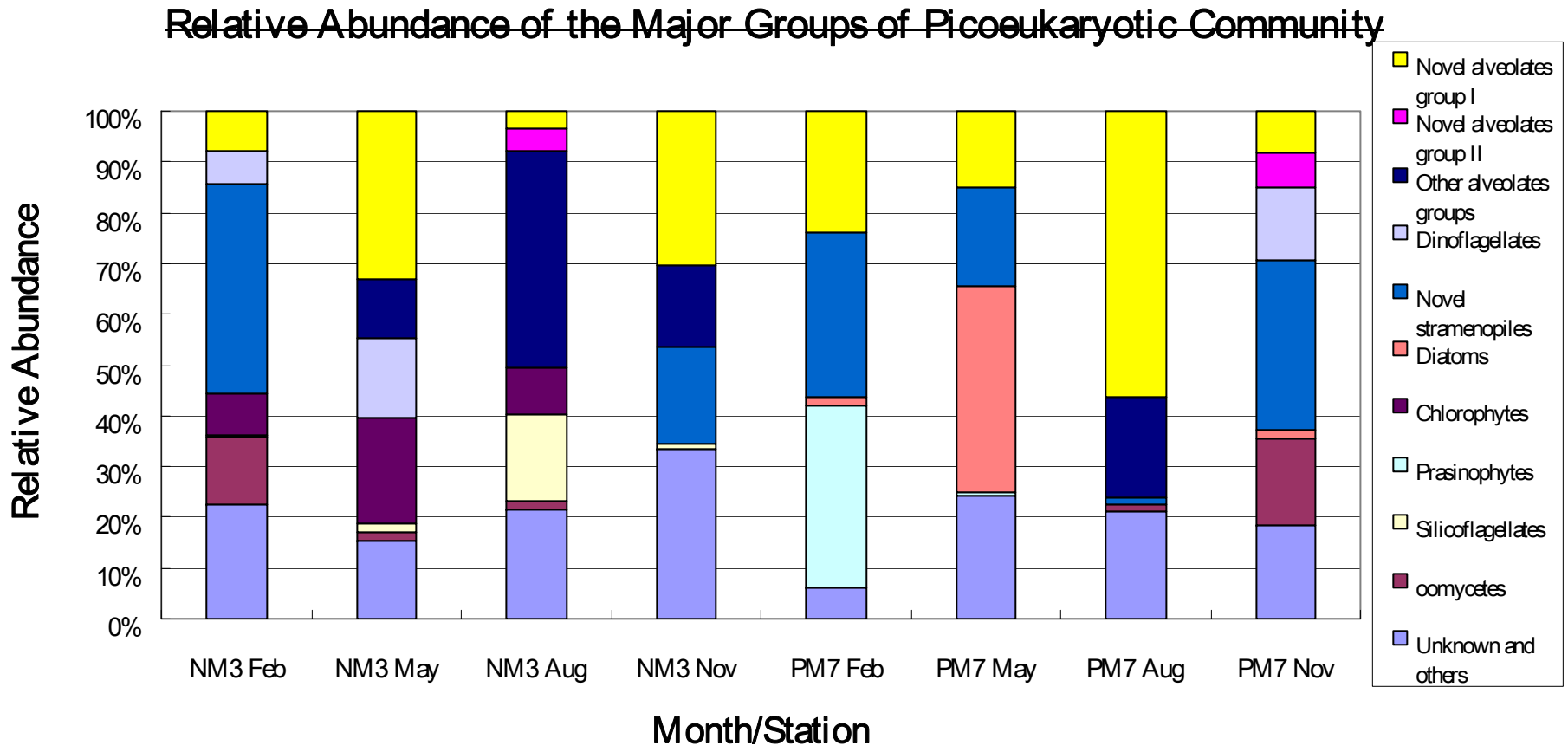
PM7 Feb: 18

PM7 May: 16

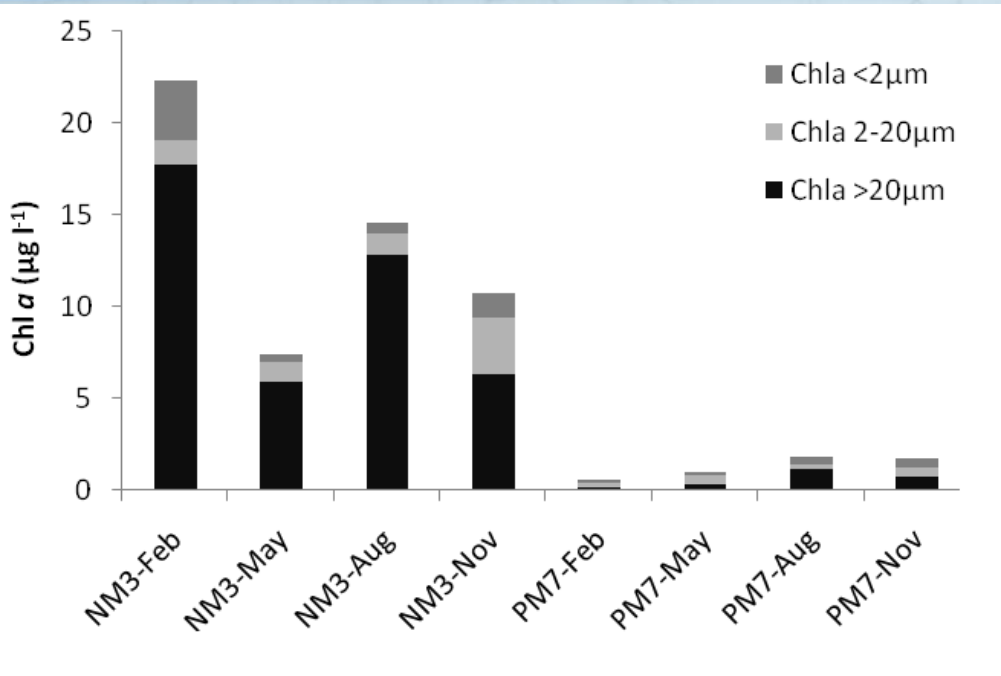
PM7 Aug: 21

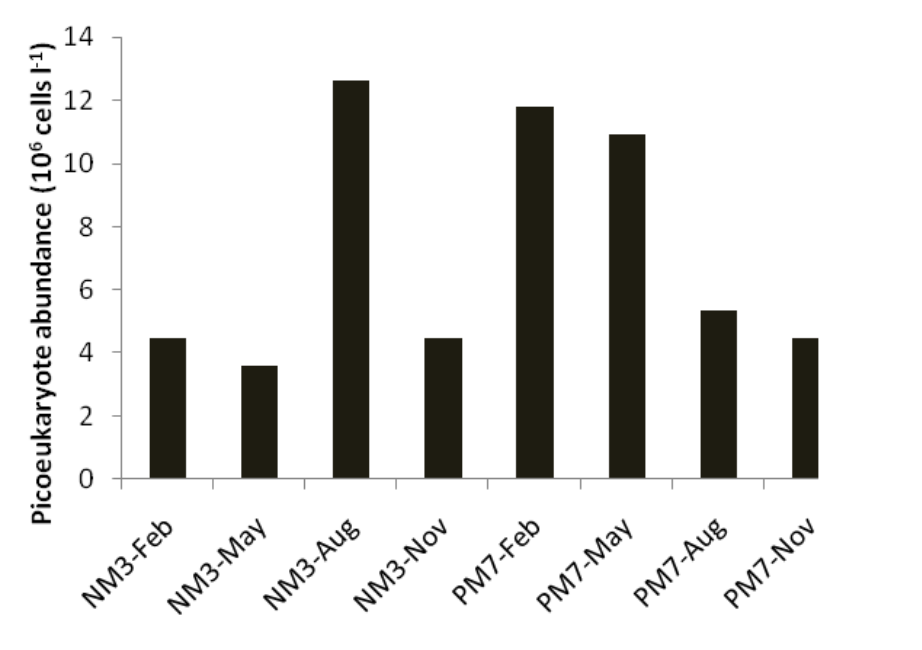
PM7 Nov: 23

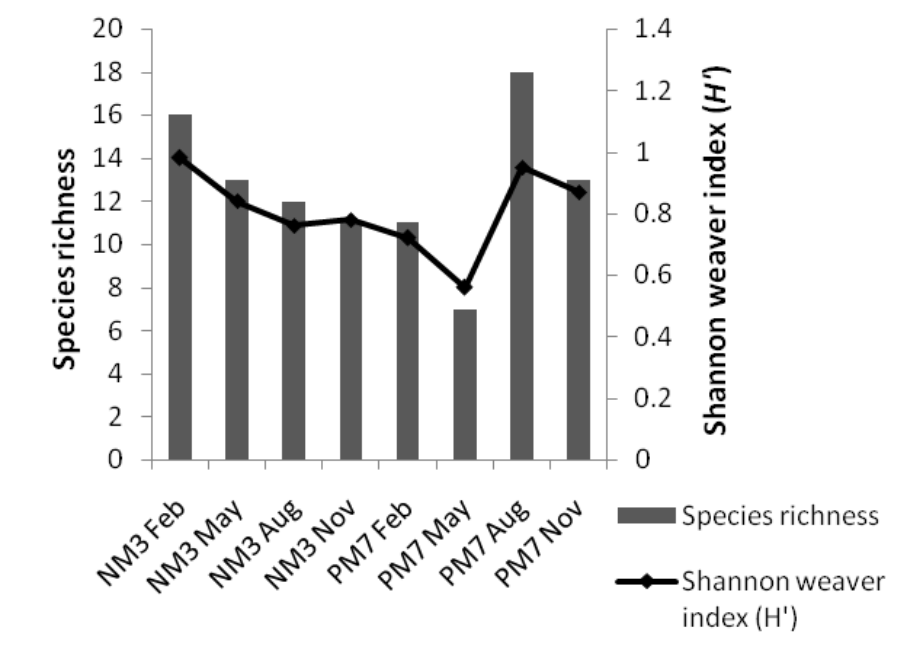
Temporal variation

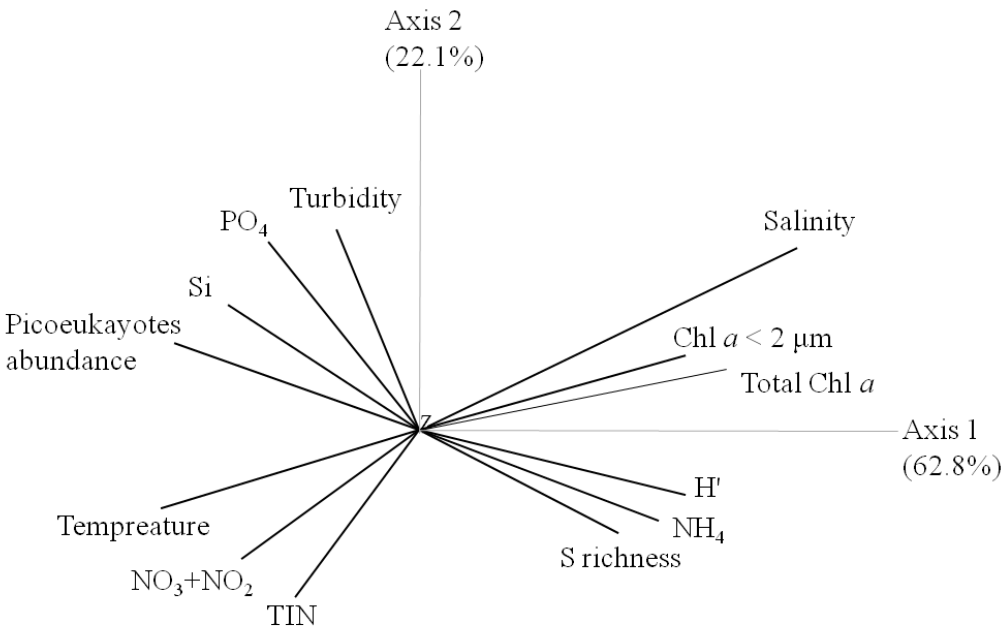


- Generally higher diversity in NM3 summer and higher diversity in PM7 winter
- **Novel alveolate groups** presisted throughout the year in both station
- **Dinoflagellates** were found primarily during late winter and early summer in NM3
- **Novel stramenopiles** were found primarily during winter time in both station
- **Chlorophytes and Silicoflagellates / Diatoms** were present during summer time in NM3 / PM7 station
- **Chlorophytes / Prasinophytes** (tiny chlorophytes) were found in NM3 / PM7 respectively


Chl a (µg l-1)
25
20
15
10
5
0
Chla <2µm
Chla 2-20µm
Chla >20µm
NM3-Feb
NM3-May
NM3-Aug
NM3-Nov
PM7-Feb
PM7-May
PM7-Aug
PM7-Nov

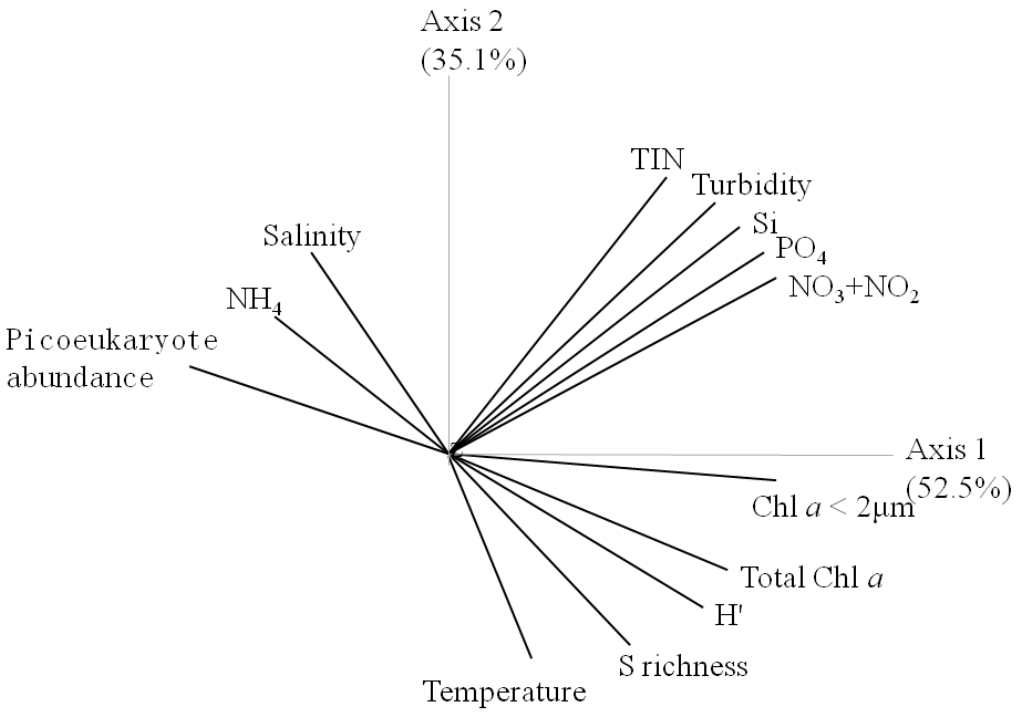

Picoeukaryote abundance (10^6 cells l-1)
14
12
10
8
6
4
2
0
NM3-Feb
NM3-May
NM3-Aug
NM3-Nov
PM7-Feb
PM7-May
PM7-Aug
PM7-Nov

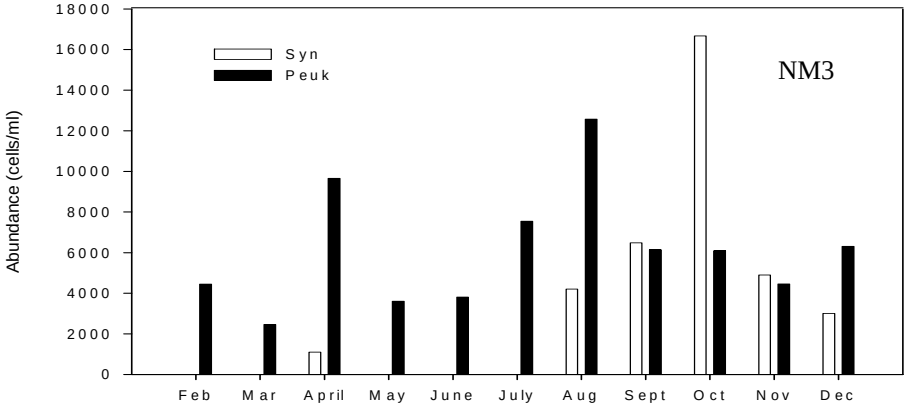

Species richness
20
18
16
14
12
10
8
6
4
2
0
Shannon weaver index (H')
1.4
1.2
1
0.8
0.6
0.4
0.2
0
NM3 Feb
NM3 May
NM3 Aug
NM3 Nov
PM7 Feb
PM7 May
PM7 Aug
PM7 Nov
Species richness
Shannon weaver index (H')

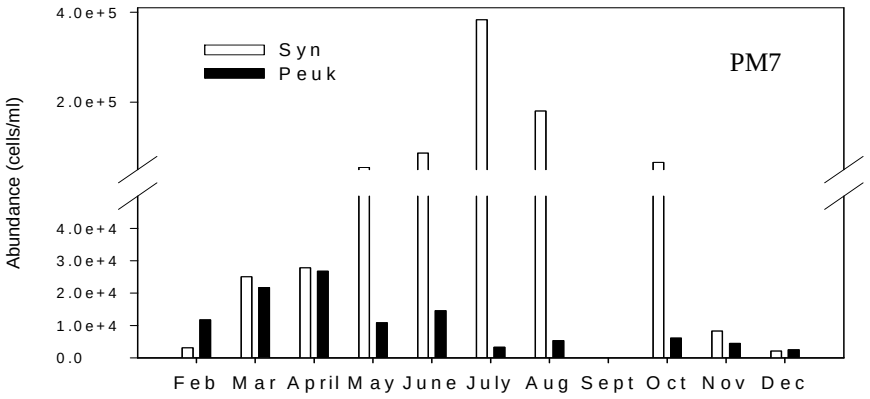

Axis 2
(22.1%)
Turbidity
PO4
Si
Picoeukayotes
abundance
Salinity
Chl a < 2 µm
Total Chl a
Axis 1
(62.8%)
H'
NH4
S richness
Tempreature
NO3+NO2
TIN

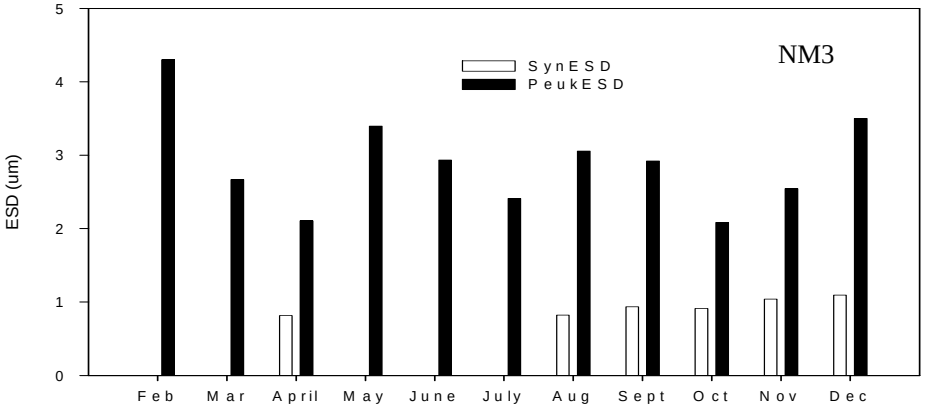
NM3

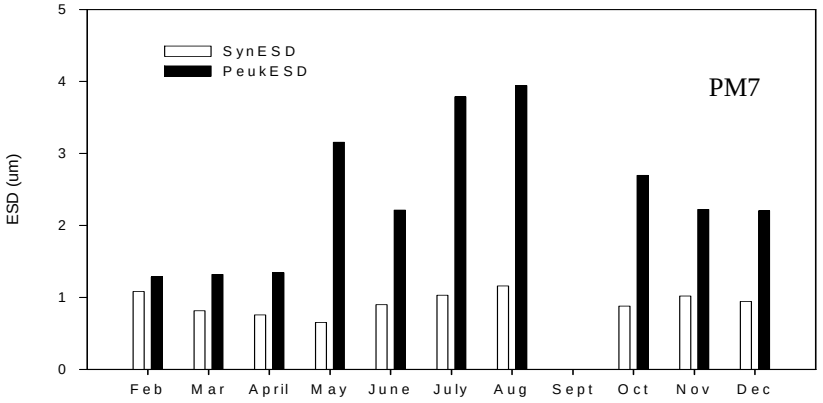
PM7

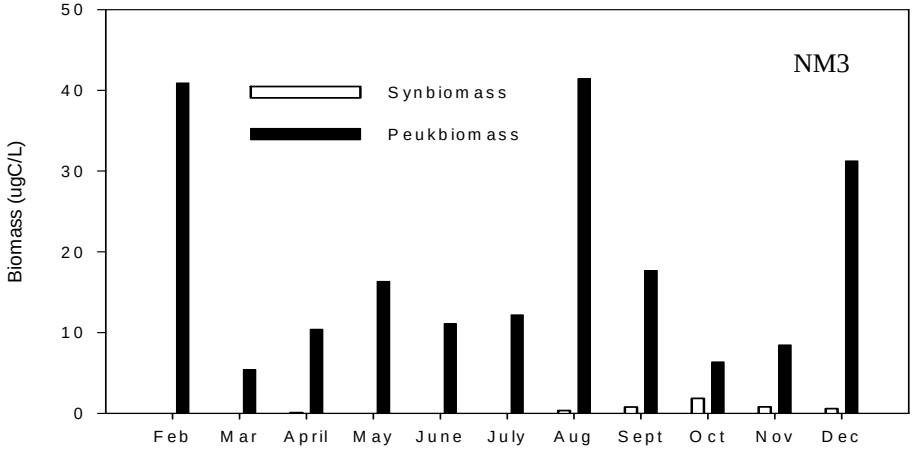

Axis 2
(35.1%)
TIN
Turbidity
Si
PO4
NO3+NO2
Salinity
NH4
Picoeukaryote
abundance
Axis 1
(52.5%)
Chl a < 2µm
Total Chl a
H'
S richness
Temperature

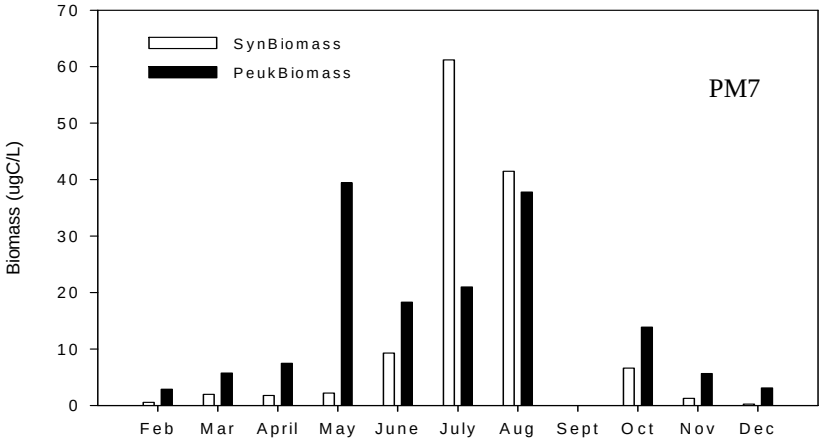

NM3
Syn
Peuk
Abundance (cells/ml)
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Feb
Mar
April
May
June
July
Aug
Sept
Oct
Nov
Dec


PM7
Syn
Peuk
Abundance (cells/ml)
4.0e+5
2.0e+5
4.0e+4
3.0e+4
2.0e+4
1.0e+4
0.0
Feb
Mar
April
May
June
July
Aug
Sept
Oct
Nov
Dec


NM3
SynESD
PeukESD
ESD (um)
5
4
3
2
1
0
Feb
Mar
April
May
June
July
Aug
Sept
Oct
Nov
Dec


PM7
SynESD
PeukESD
ESD (um)
5
4
3
2
1
0
Feb
Mar
April
May
June
July
Aug
Sept
Oct
Nov
Dec


NM3
Synbiomass
Peukbiomass
Biomass (ugC/L)
50
40
30
20
10
0
Feb
Mar
April
May
June
July
Aug
Sept
Oct
Nov
Dec


PM7
SynBiomass
PeukBiomass
Biomass (ugC/L)
70
60
50
40
30
20
10
0
Feb
Mar
April
May
June
July
Aug
Sept
Oct
Nov
Dec