

Mucilage events in the Sea of Marmara and the associated processes: Field and laboratory findings

Çolpan Polat-Beken, Vildan Tüfekçi, Başak Sözer,
Emel Yıldız, Fatma Telli-Karakoç, Mustafa Mantıkçı
and Dilek Ediger

***Marmara Research Center (MRC) -
Environment Institute (EI) - Marine and Inland Waters SBU
Chemistry Institute (CI)***



TÜBİTAK

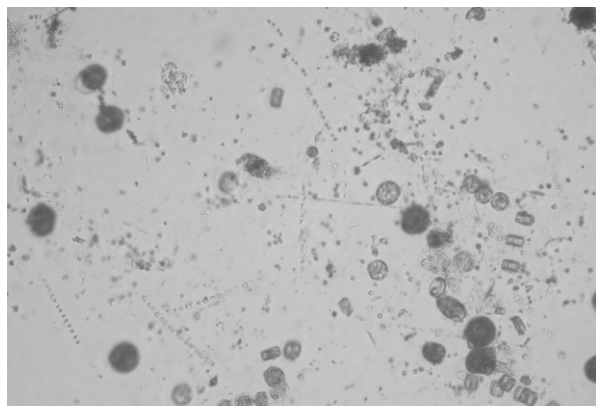
MAM

Mucilage events in the Sea of Marmara

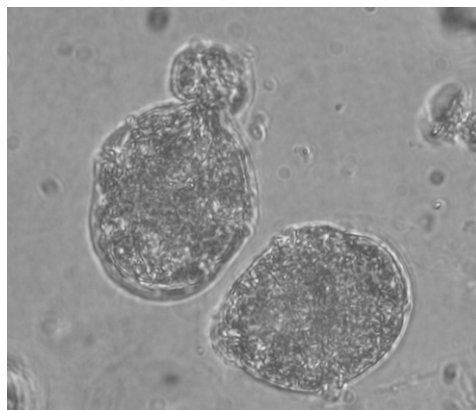
4 major events recorded since 2007:

- October 2007
- January 2008
- Sept-Oct 2008
- December 2009-January 2010

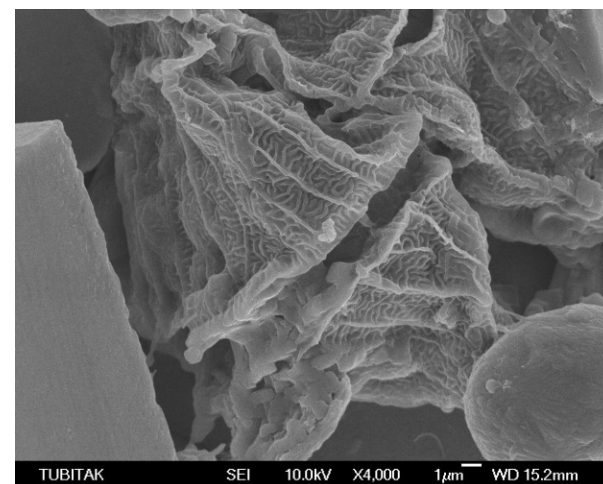
1 massive event in 1992



October 2007



Gonyaulax fragilis



Mucilage events in the Sea of Marmara



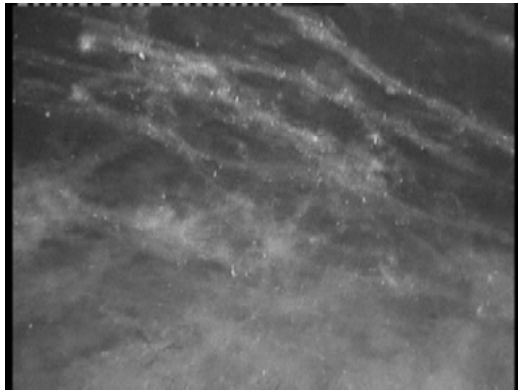
October 2007, İzmit (outer Bay)



January 2008, İzmit (Central basin)



October 2008, İzmit (Inner Bay)

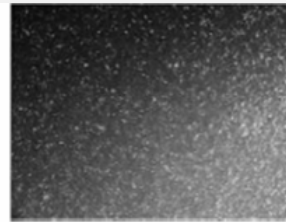


January 2008 , Erdek Bay ,
underwater image



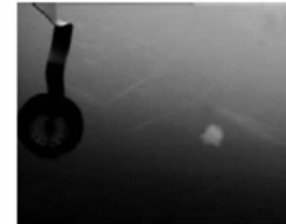
Different mucilage aggregates of phytoplankton origin

Precali et al, 2005



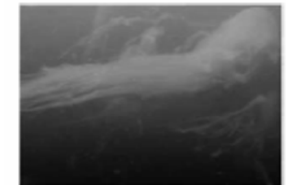
Flocs

Küçük
yumaklar
1-25 mm



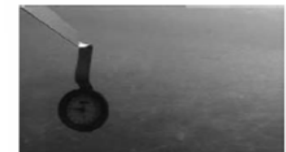
Macroflocs

Yuvarlak/düzensiz
yumaklar, 1-5 cm
Genellikle beyaz



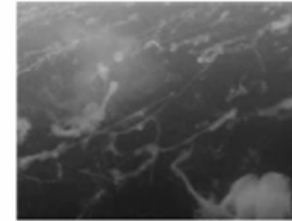
Clouds

Çok büyük yumaklar
0.5m'den 3-4 m
Genellikle sarı



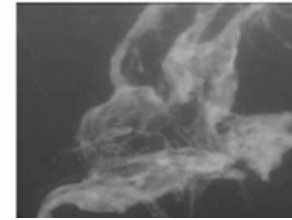
False bottom

Yoğun tabaka halinde
1-2 mm'den 10- ...cm
kalınlığında
Piknokline verleşmiş



Ribbon

Uzamış yumaklar
10-20 cm'den 1 m'ye
Beyaz-sarı



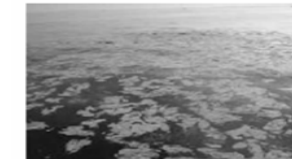
Cobweb

Ağ görüntülü yumaklar
Stringer'lardan oluşmuş
Düşeyde birkaç m.,
yatayda 10'larca m.
Beyazımsı



Creamy surface layer

Su yüzeyinde
veya hemen altında yüzer
Kalınlığı 15 cm'ye kadar
Beyaz



Gelatinous surface layer

Pateklenmiş süngersi yapı
Su yüzeyinde
Sarımsı-kahverengi



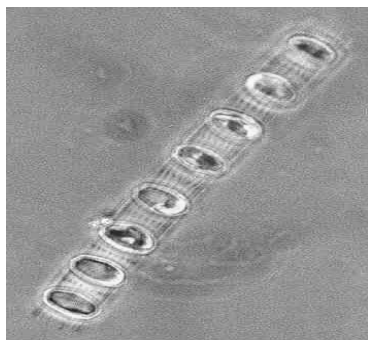
TUBITAK

MAM

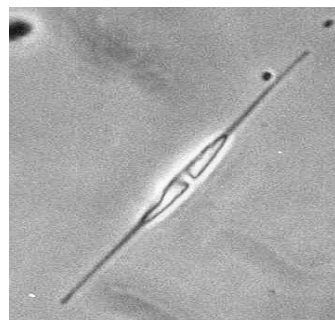
Mucilage events in the Sea of Marmara

A research project (TUBITAK-108Y083), based on laboratory experiments and field studies, was initiated in 2008 to investigate the conditions that may activate these events.

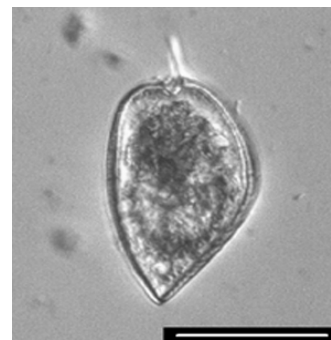
Hypothesis: Phytoplankton extracellular organic material mainly composed of polysaccharides and carbohydrates played a critical role in forming the mucilage aggregates



Skeletonema costatum
(Marchetti, 1990,
M.Mingazzini, 1995, R.
Urbani, 2005)



Cylindrotheca closterium
(Mingazzini vd., 1995,
Alcoverro vd., 2000, Urbani
vd., 2005)



Prorocentrum micans
(Pompei vd., 2003)



Gonyaulax fragilis
(Pompei vd., 2003,
Pistocchi vd., 2005,
Sampedro vd. 2007)

Investigation of factors controlling the marine mucilage/mucus formation with bioassay techniques

Methods:

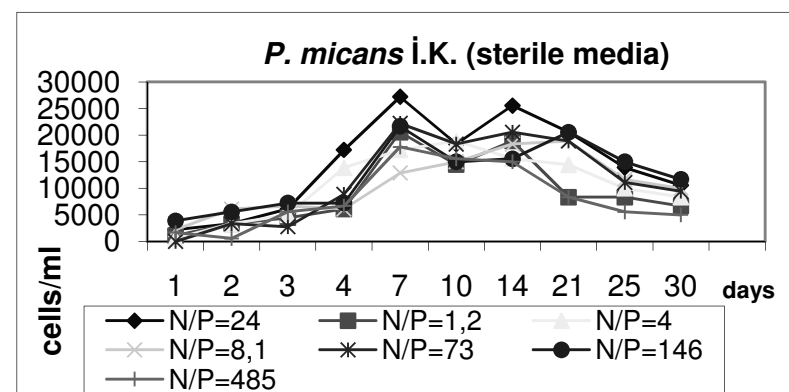
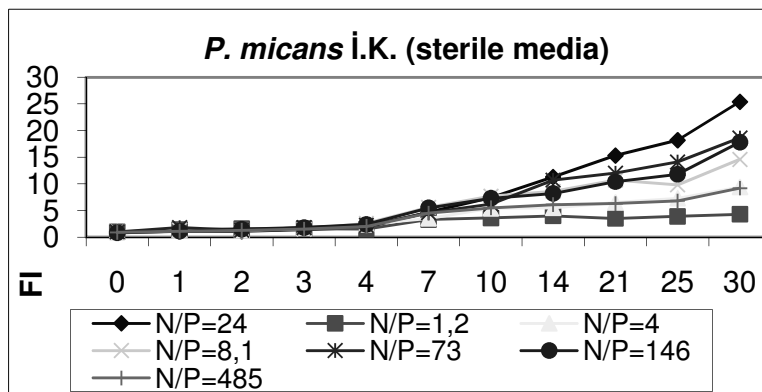
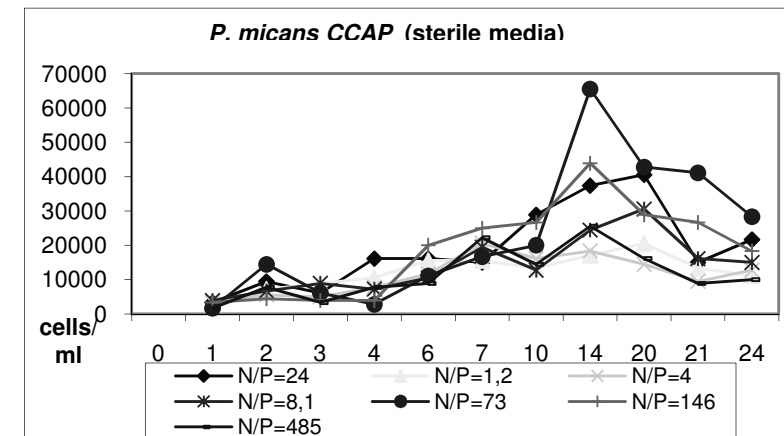
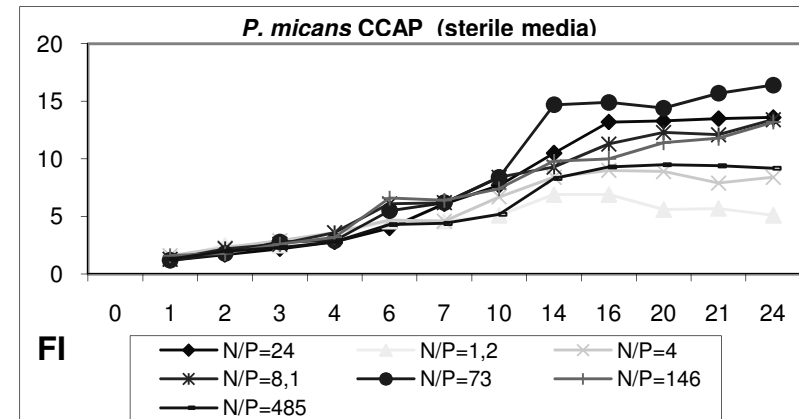
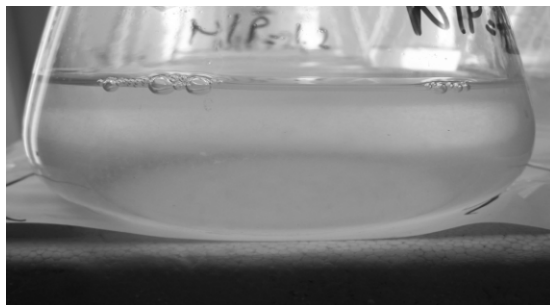
- Temperature, light and nutrient controlled batch culture experiments with phytoplankton cultures; 3-7 weeks
- Chemical and microscopic analysis on dissolved material and aggregates (accumulation of dissolved organic material w/ time and chemical content of aggregates)
- Chemical and microscopic analysis on mucilage aggregates collected from marine environment (chemical and biological content of real material)
- Comparison of results
(Dissolved/particulate organic carbon, carbohydrates, protein, nutrients, FTIR, fluorescence spectra, light/fluorescence microscopy, SEM)
- Visual identification
- Meteorological variability in decades
- Inputs of land-based sources of nutrients
(photography, oceanography and water quality data, meteorological data)



TÜBİTAK

MAM

Experimental design



Experimental design

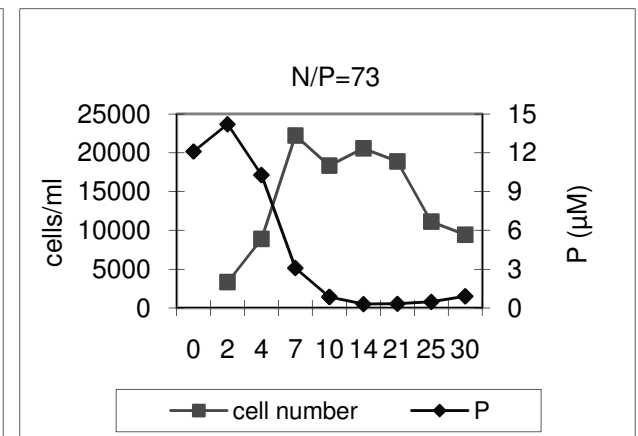
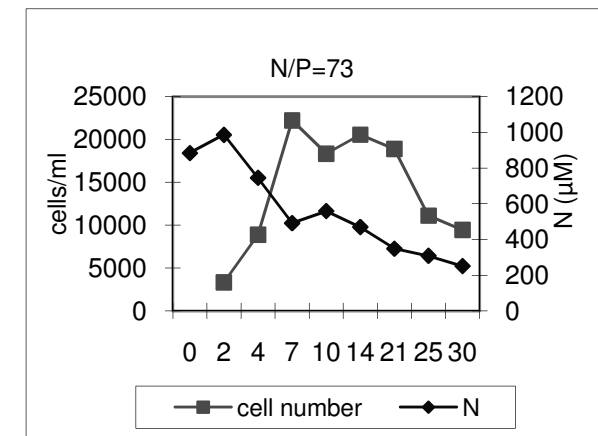
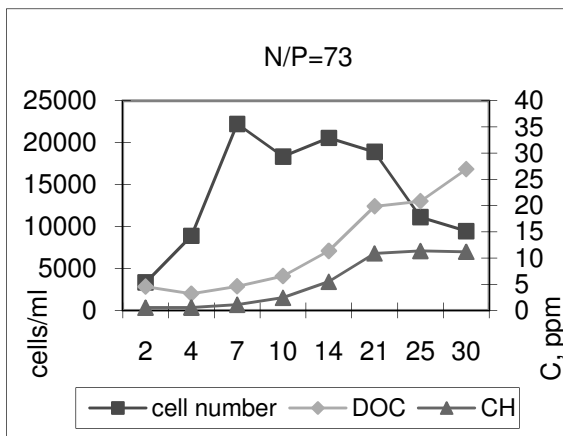
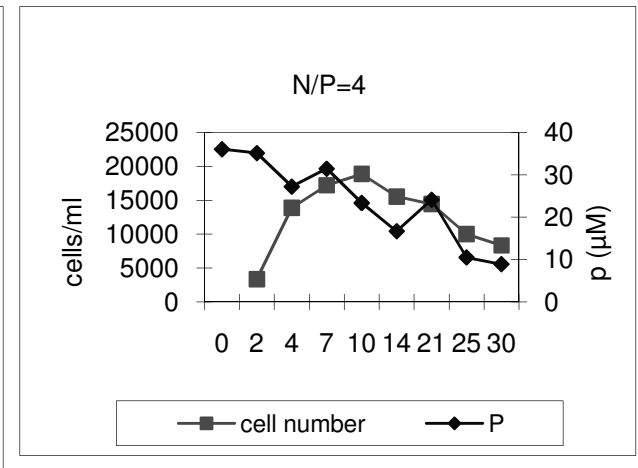
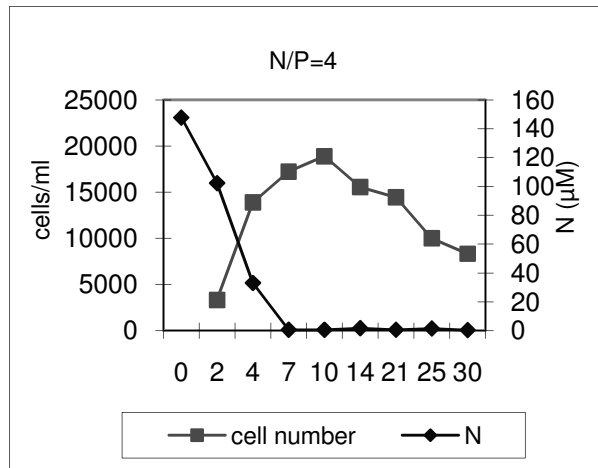
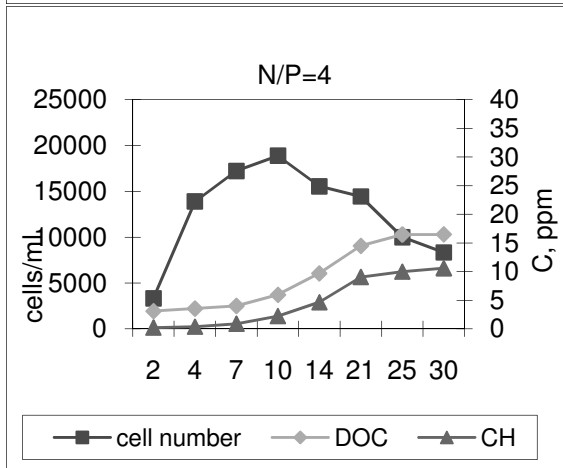
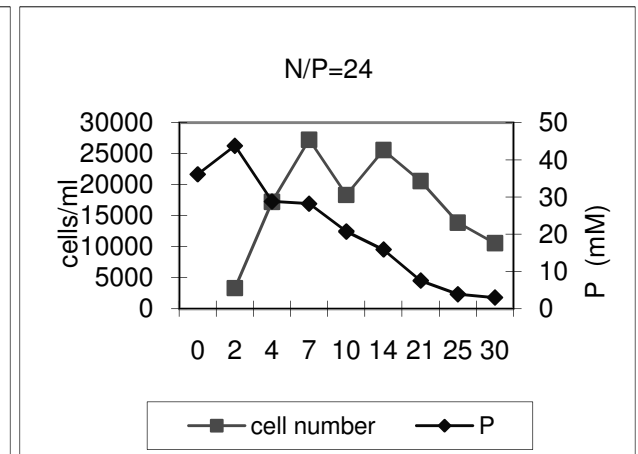
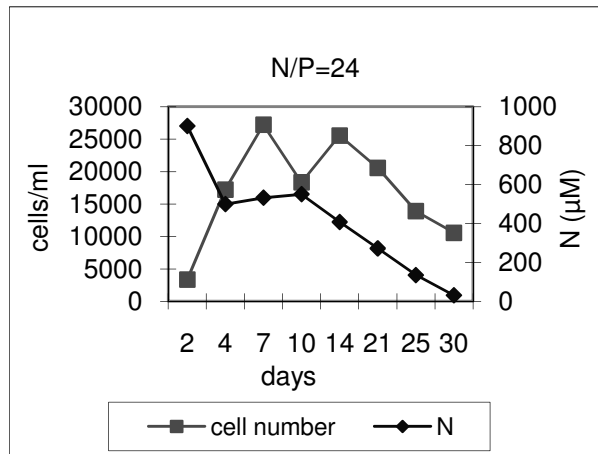
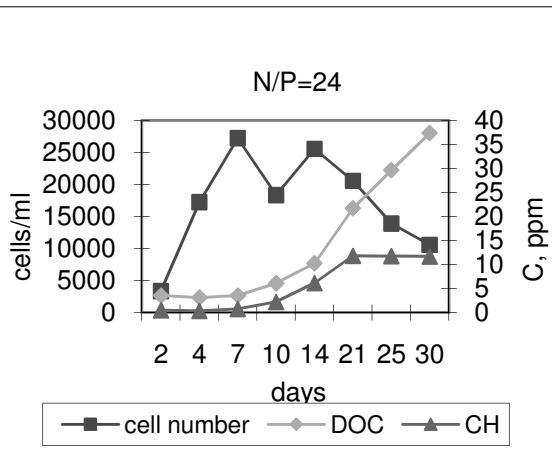
Nr	Species	Time interval	N/P Ratios	Features of the Experiment
1	<i>P. micans</i> CCAP	01.01.2009-26.01.2009	24 – 1,2 - 4 – 8,1 - 73 – 146 - 485	Sterile media
2	<i>S. costatum</i> CCAP	09.02.2009-02.03.2009	24 – 1,2 - 4 – 8,1 - 73 – 146 - 485	Sterile media
3	<i>P. micans</i> İ.K.	30.03.2009-29.04.2009	24 – 1,2 - 4 – 8,1 - 73 – 146 - 485	Sterile media
4	<i>S. costatum</i> İ.K.	13.04.2009-13.05.2009	24 – 1,2 - 4 – 8,1 - 73 – 146 - 485	Sterile media
5	<i>C. closterium</i> CCAP	12.05.2009-08.06.2009	24 – 1,2 - 4 – 8,1 - 73 – 146	Sterile media
6	<i>C. closterium</i> İ.K.	11.08.2009-08.09.2009	24 – 1,2 - 4 – 8,1 - 73 – 146	Sterile media
7	<i>P. micans</i> İ.K.	26.08.2009-18.09.2009	24 – 1,2 - 4 – 15,6 - 73 – 485	NH ₄ addition
8	<i>P. micans</i> İ.K.	19.10.2009-09.11.2009	24 – 4 - 73	Non-sterile media
9	<i>C. closterium</i> İ.K.	09.11.2009-12.01.2009	24 – 4 - 73	Non-sterile media
10	<i>S. costatum</i> İ.K.	09.11.2009-12.01.2009	24 – 4 - 73	Non-sterile media
11	<i>P. micans</i> İ.K.	08.12.2009-04.01.2010	24 + MgCl ₂ +CaCl ₂ 24 + CuCl ₂ +PbCl ₂	Metal addition Non-sterile media
12	<i>S. costatum</i> İ.K.	03.02.2010-24.02.2010	24 – 4	Non-sterile media Low nutrient exposure
13	<i>C. closterium</i> İ.K.	03.02.2010-24.02.2010	24 – 4	Non-sterile media Low nutrient exposure
14	<i>G. fragilis</i> İ.K	10.03.2010-30.03.2010	24 – 4 - 73	Non-sterile media
15	<i>C. closterium</i> İ.K.	15.04.2010-09.06.2010	24 – 4 - 73	Non-sterile media / Longer time period
16	<i>S. costatum</i> İ.K.	31.08.2010-15.10.2010	24 – 4 - 73	Non-sterile media / Longer time period



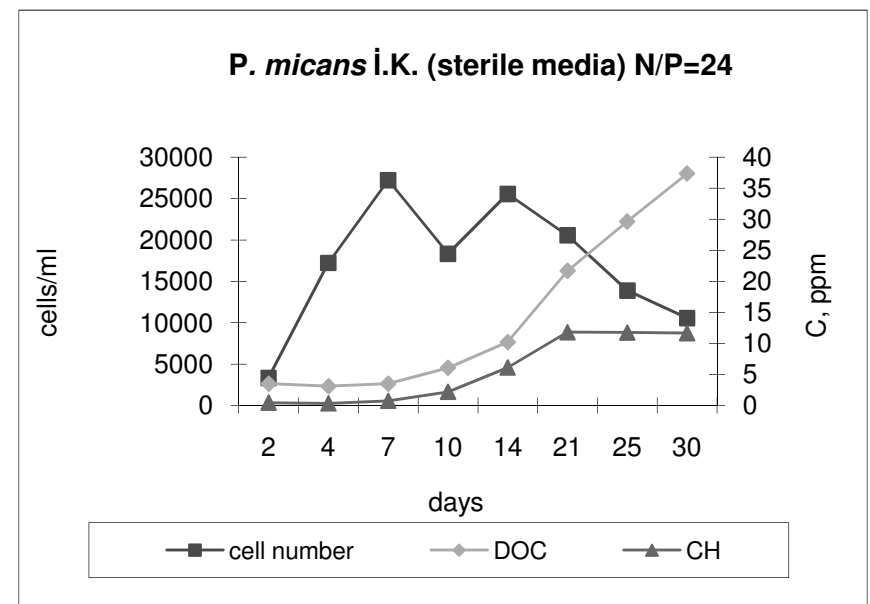
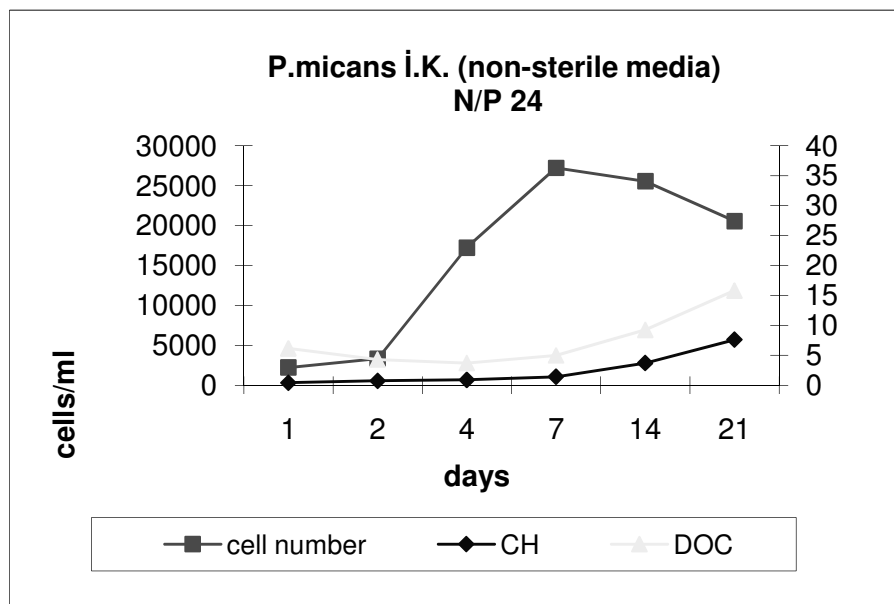
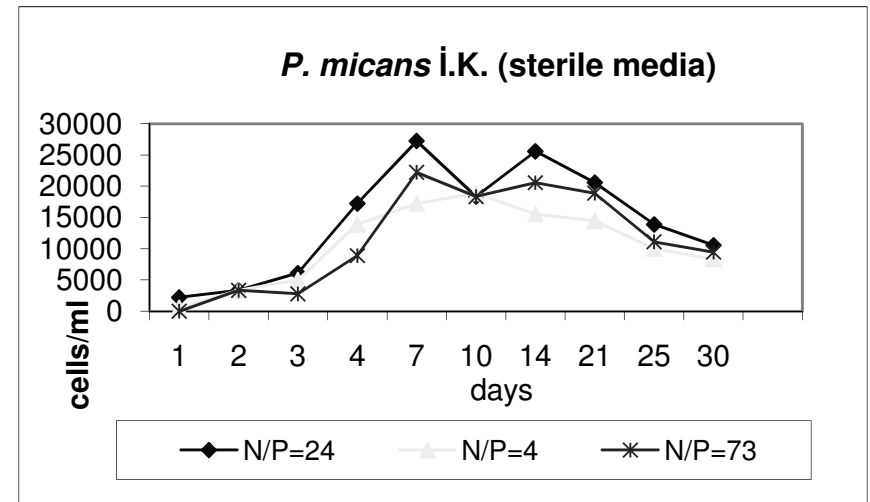
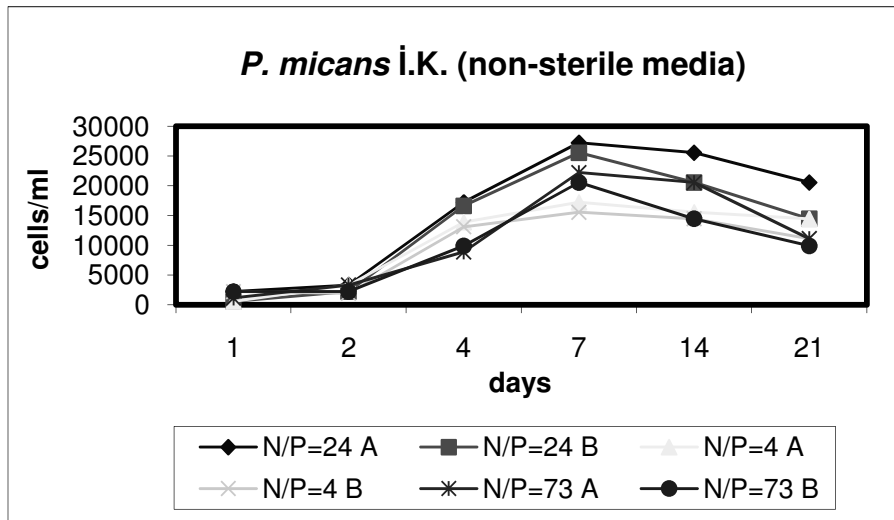
TÜBİTAK

MAM

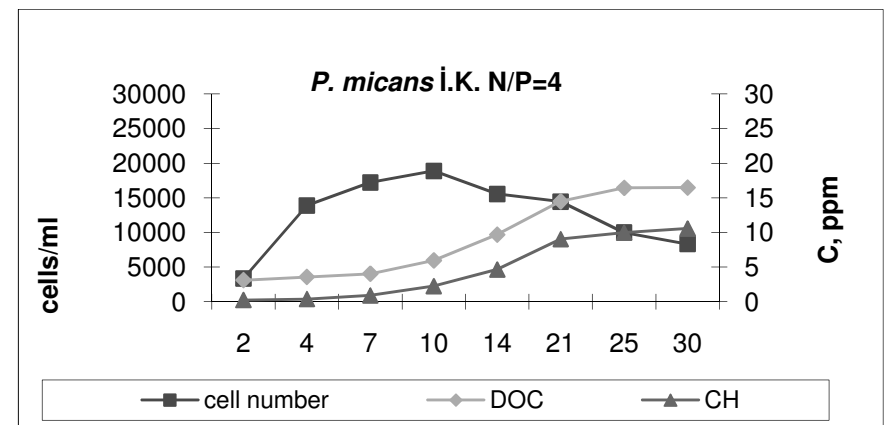
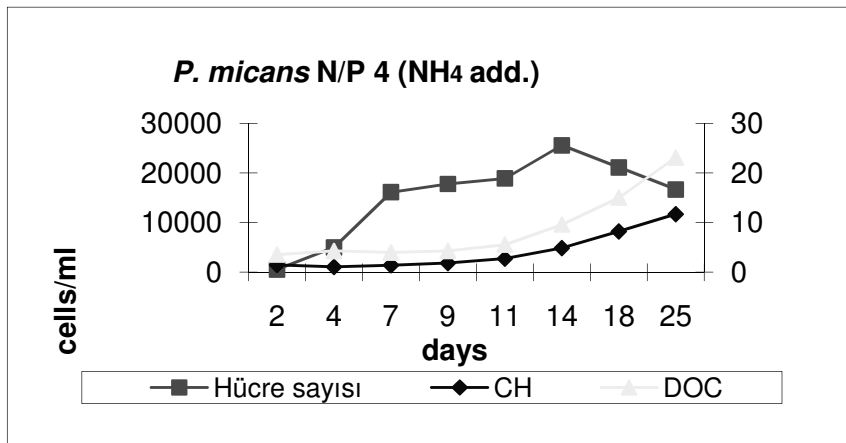
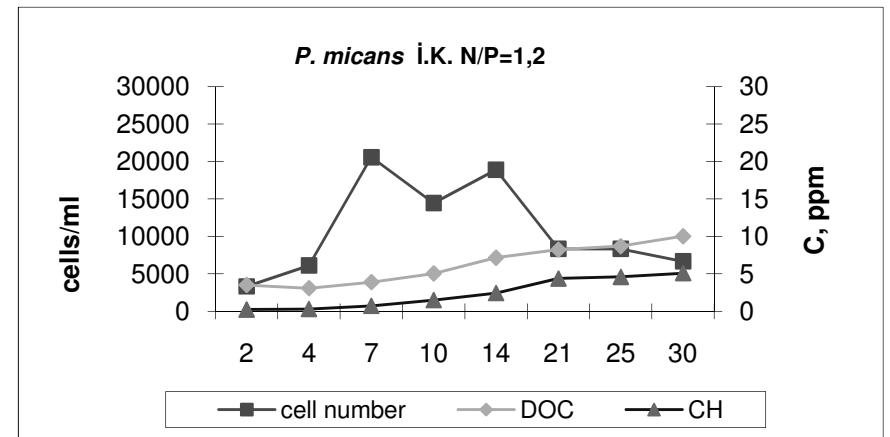
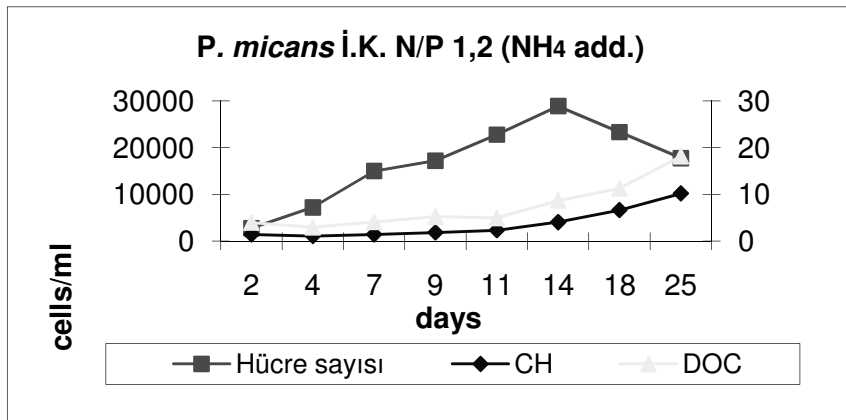
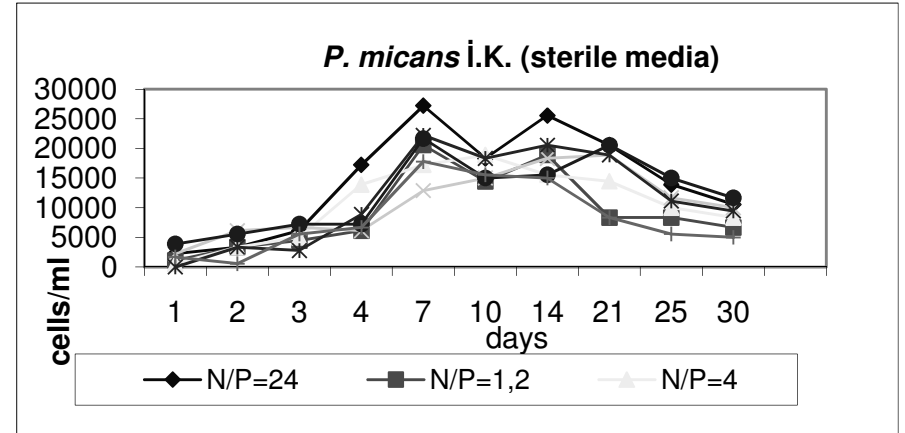
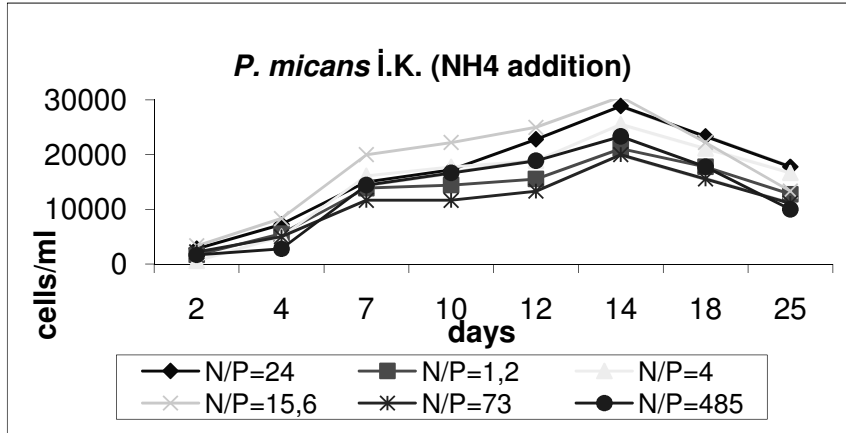
Experiments with *Prorocentrum micans* İ.K.



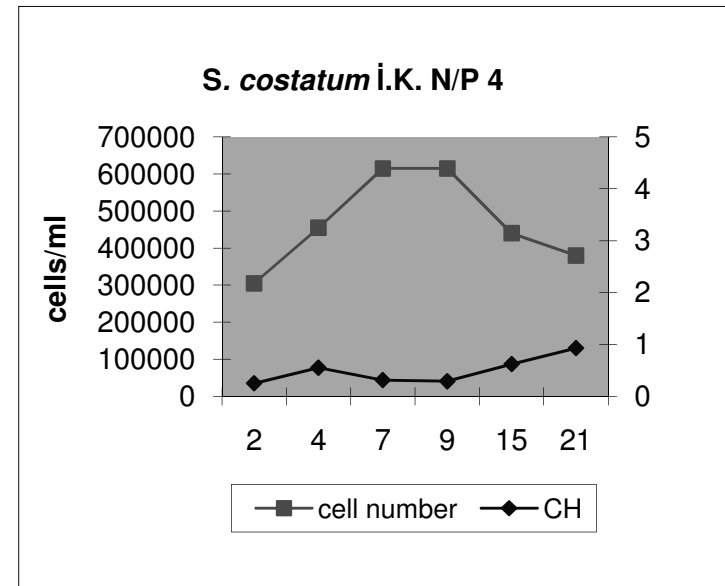
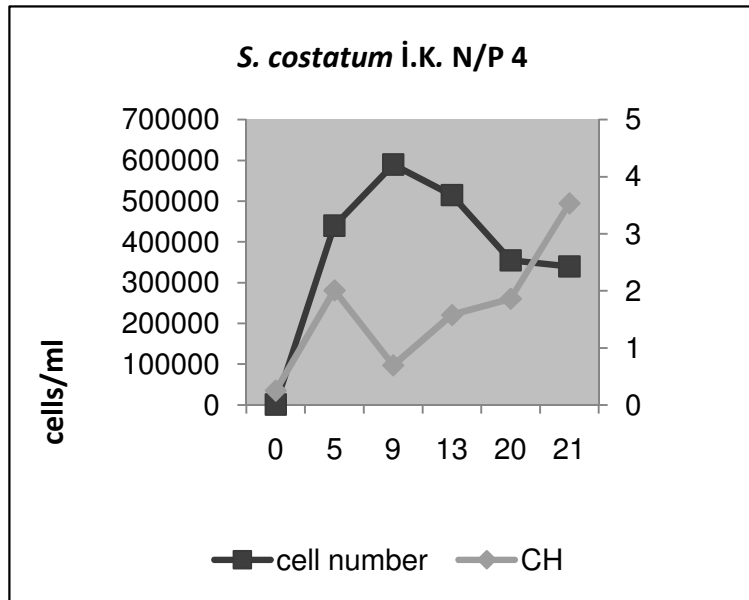
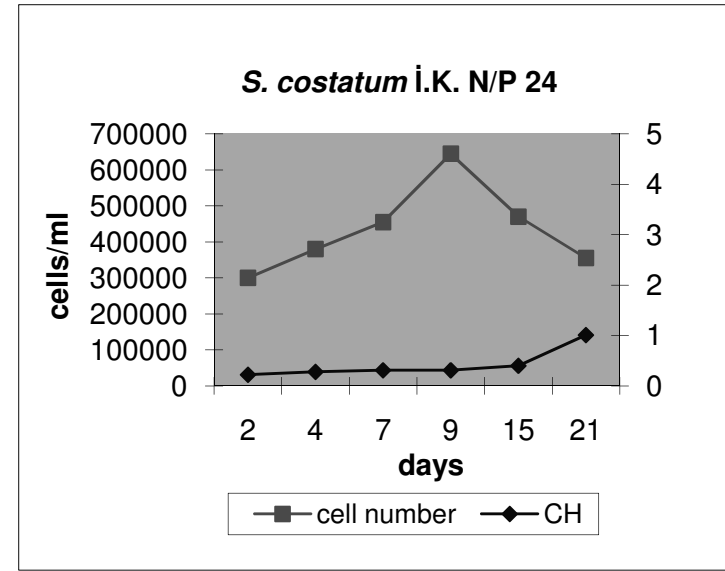
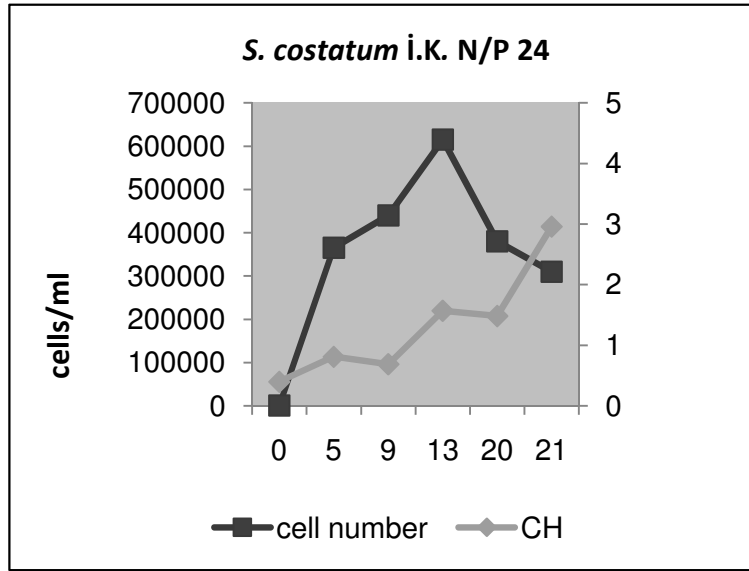
Experiments with *Prorocentrum micans* İ.K.



Experiments with *Prorocentrum micans* İ.K. with external nutrient pulse : N-limited condition



Experiments with *S.costatum* i.K. : CH accumulation in high and low nutrient exposures

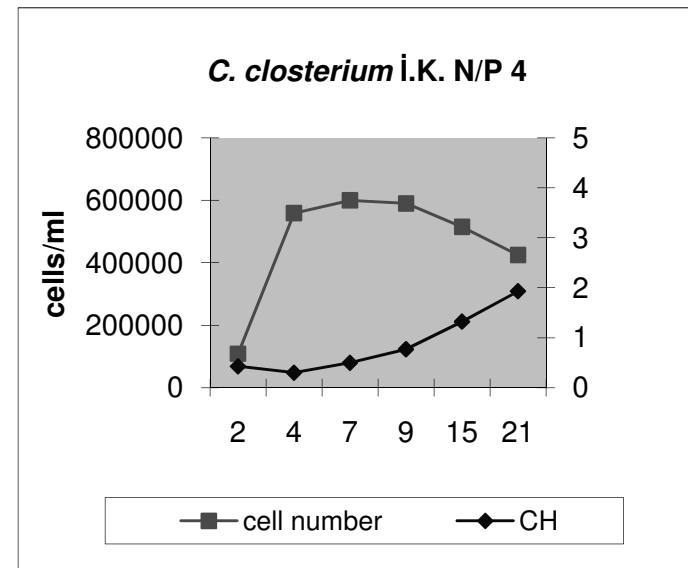
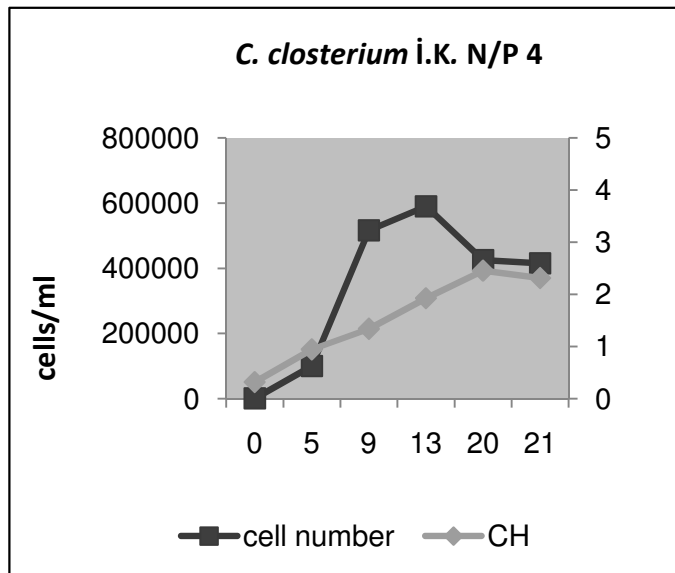
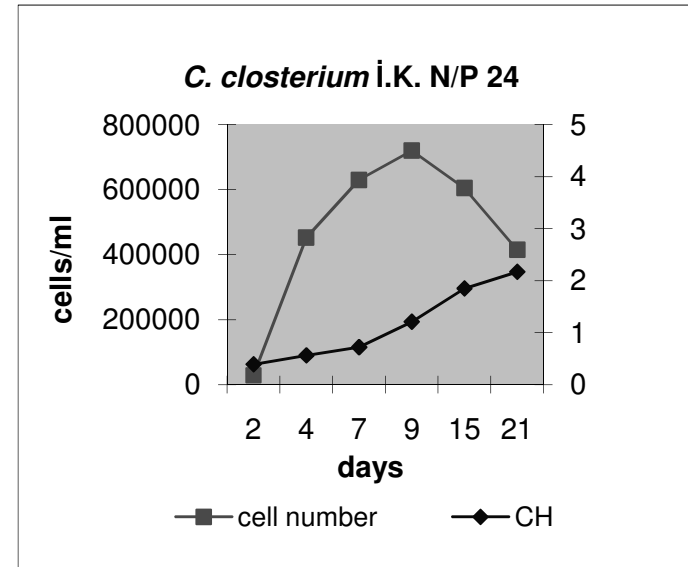
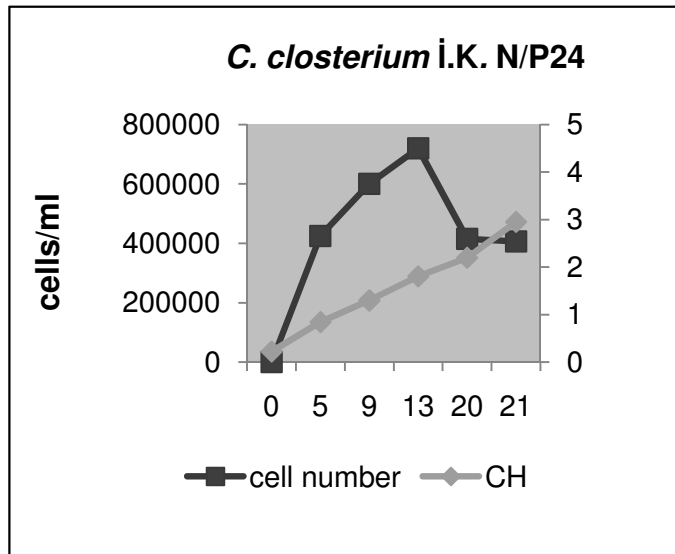


Low

High



Experiments with *C. closterium* i.K. : CH accumulation in high and low nutrient exposures



Low

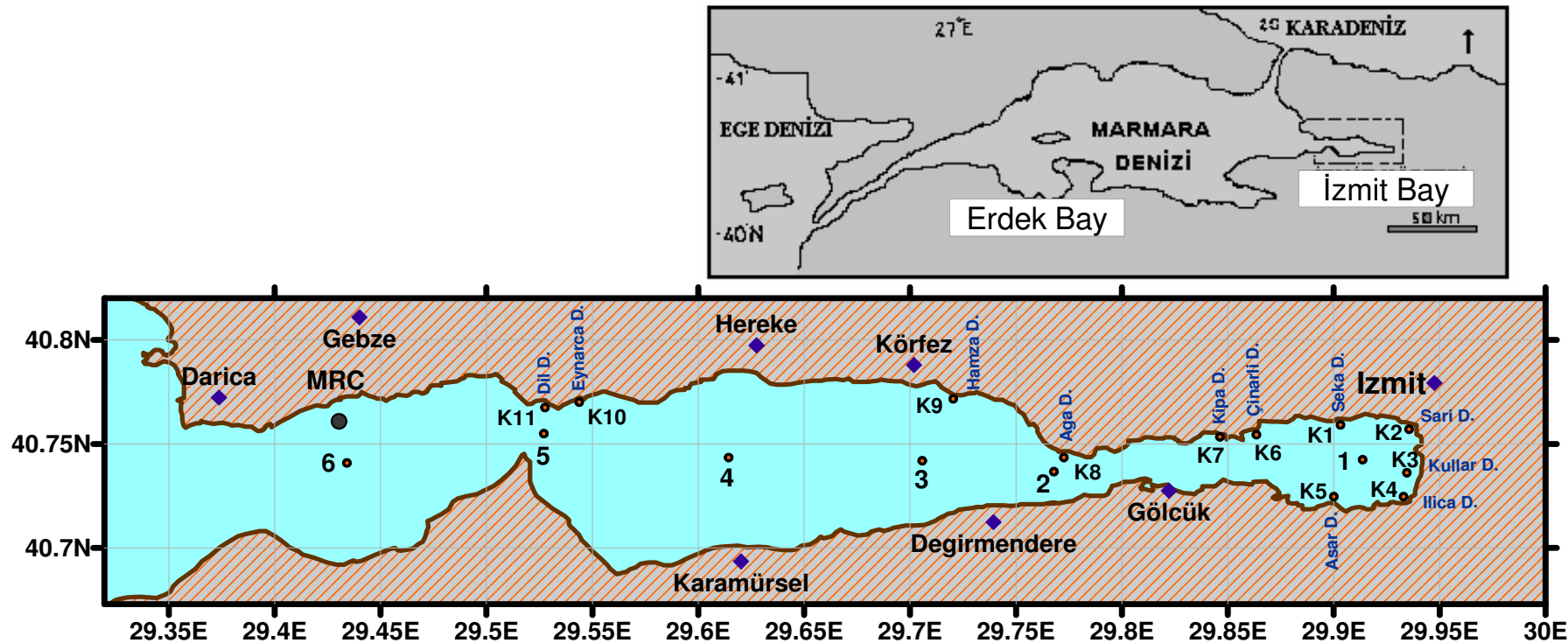
High

Field studies

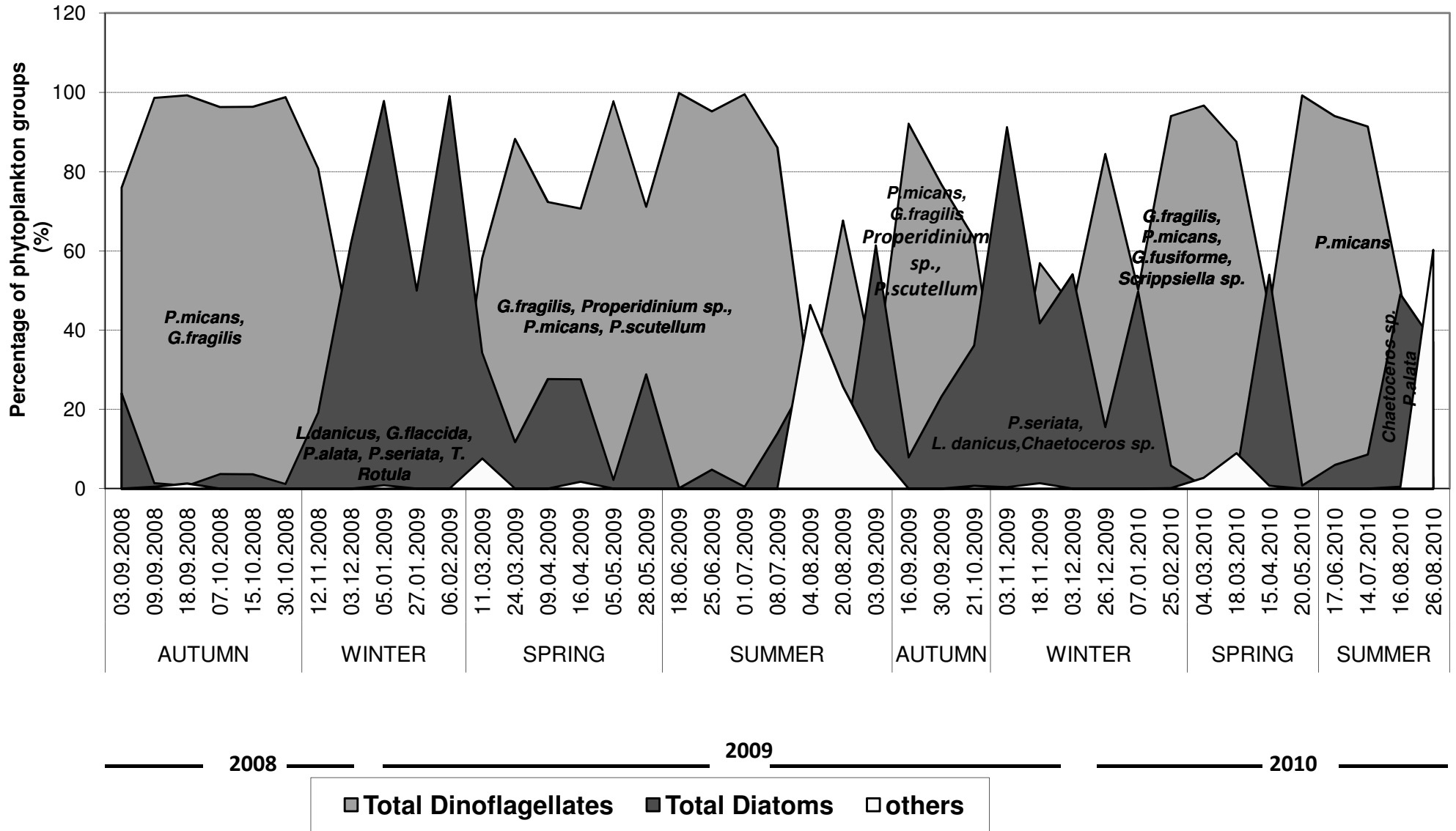
Field studies were basically organized in two ways;

- monitoring at a fixed station in front of the Marmara Research Center (outer İzmit Bay) every 1-3 weeks,
 - monthly monitoring activities in the whole İzmit Bay (supported by Greater Municipality of Kocaeli)
- and also patchily visiting other sites (Erdek Bay) during the events periods.

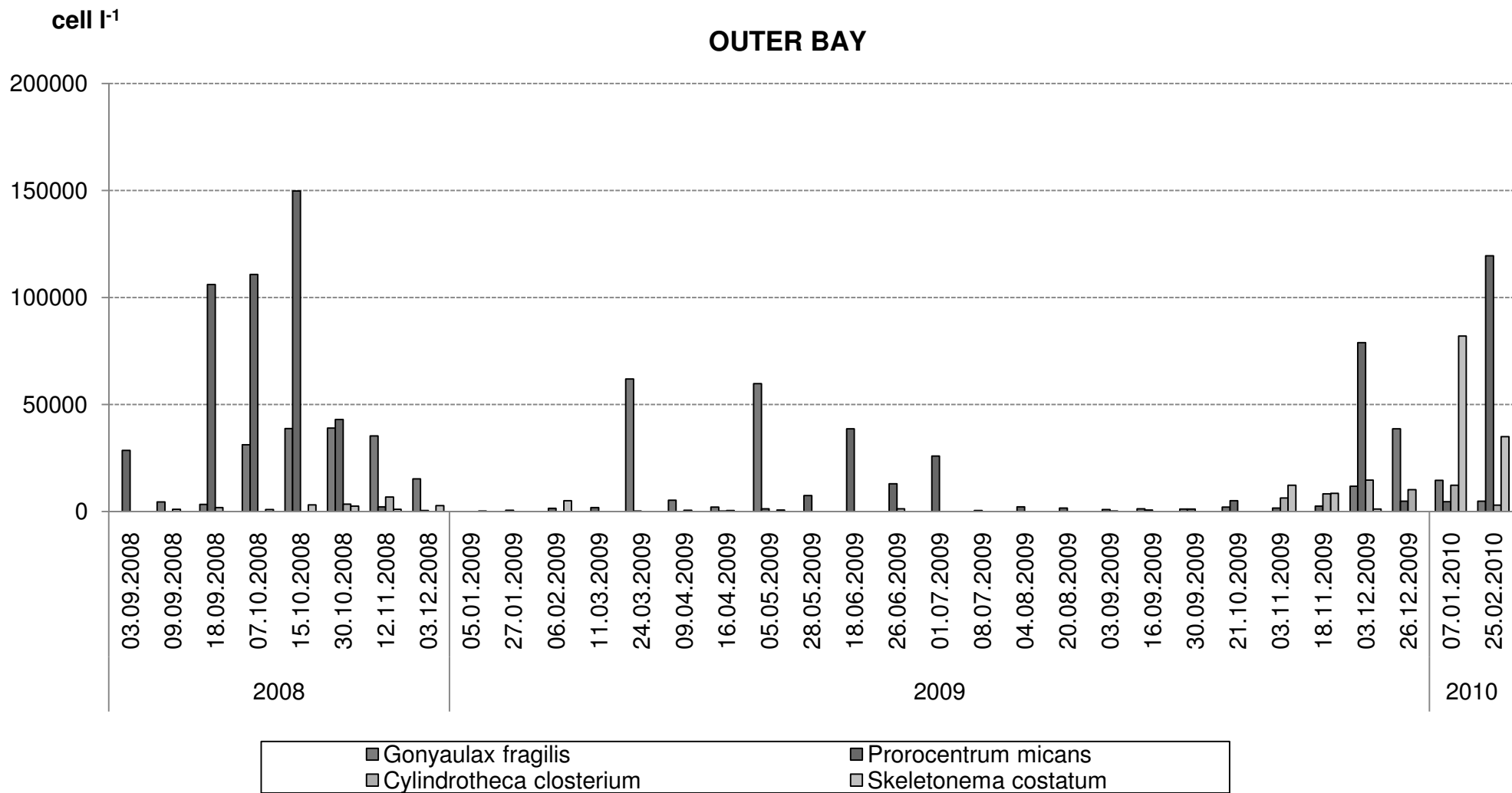
Field investigations were designed basically to monitor the phytoplankton distribution and standard oceanographic and meteorological features to understand better the occurring conditions of the mucilage events.



Field studies at MRC station (Phytoplankton abundance)

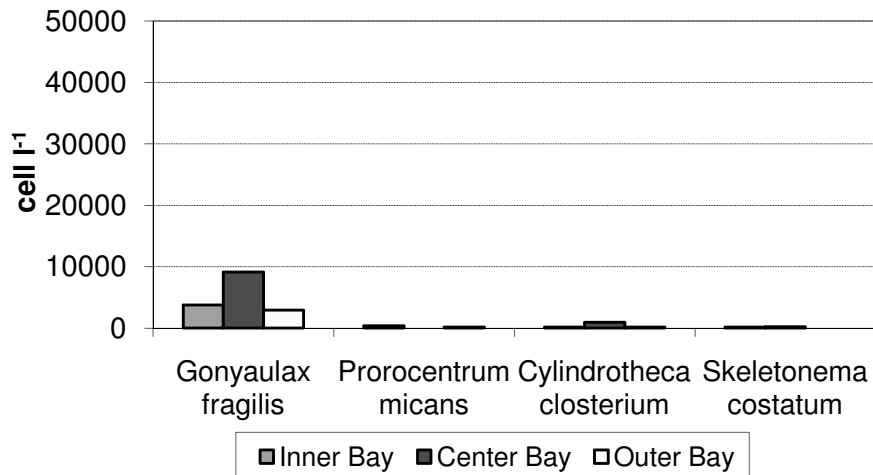


Field studies at MRC station (target species)

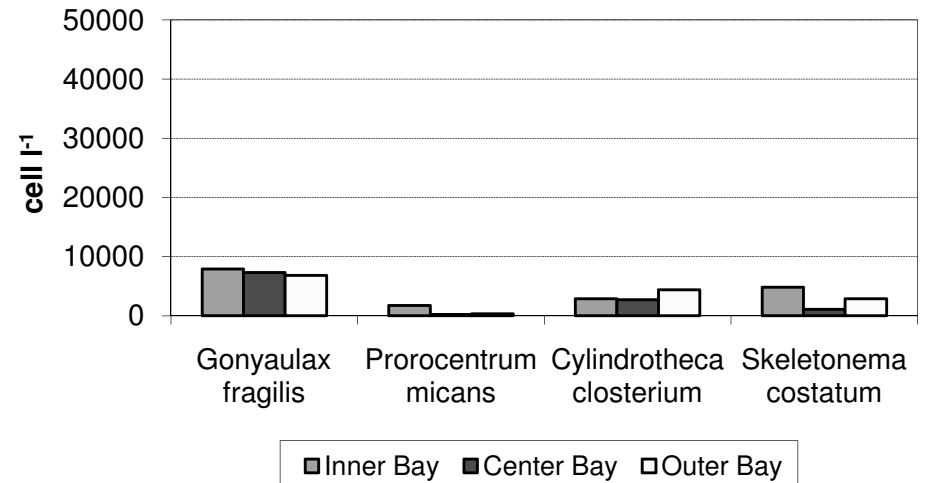


Phytoplankton Distribution in mucilage periods : Jan'08

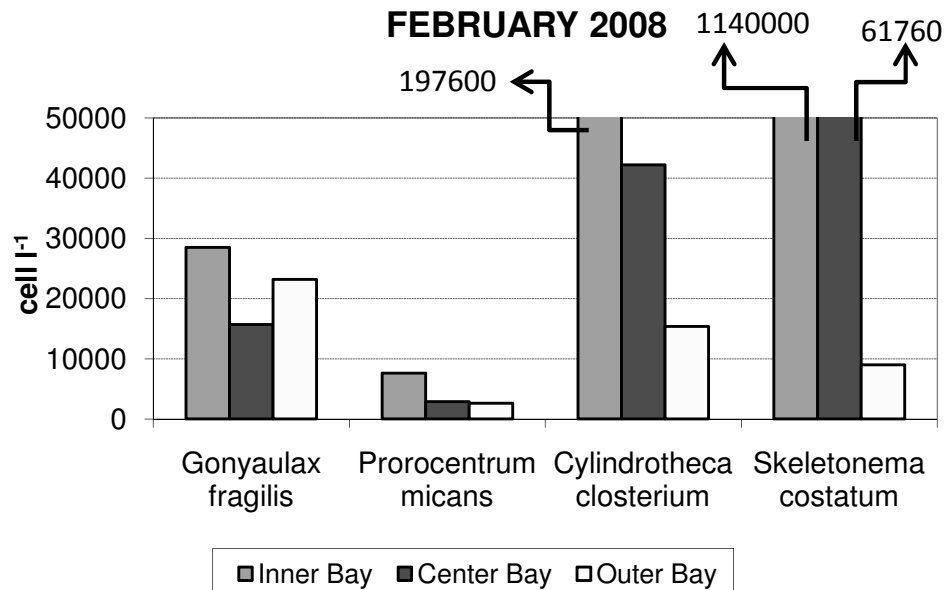
DECEMBER 2007



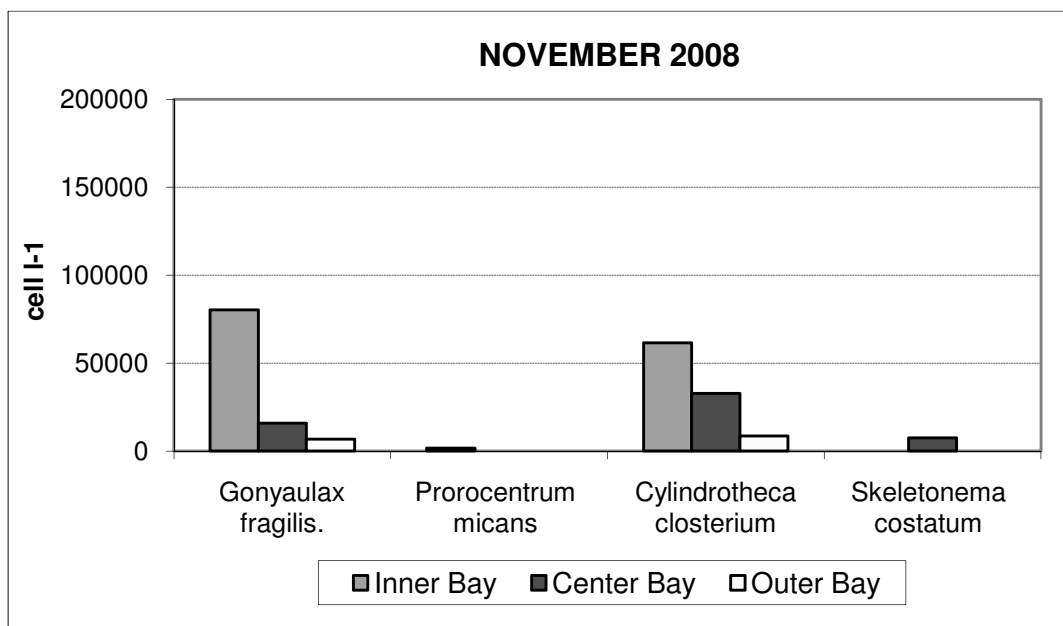
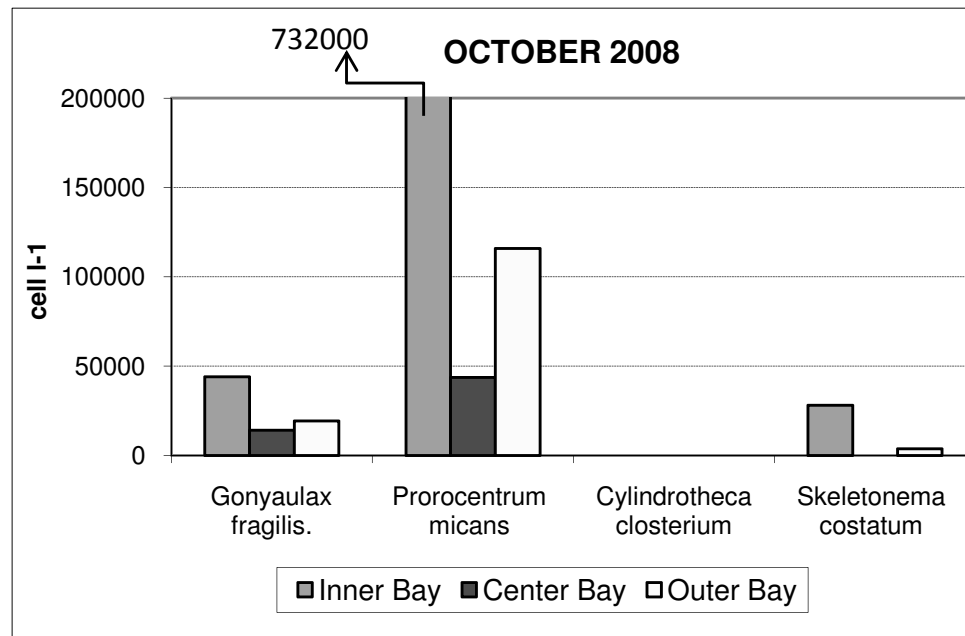
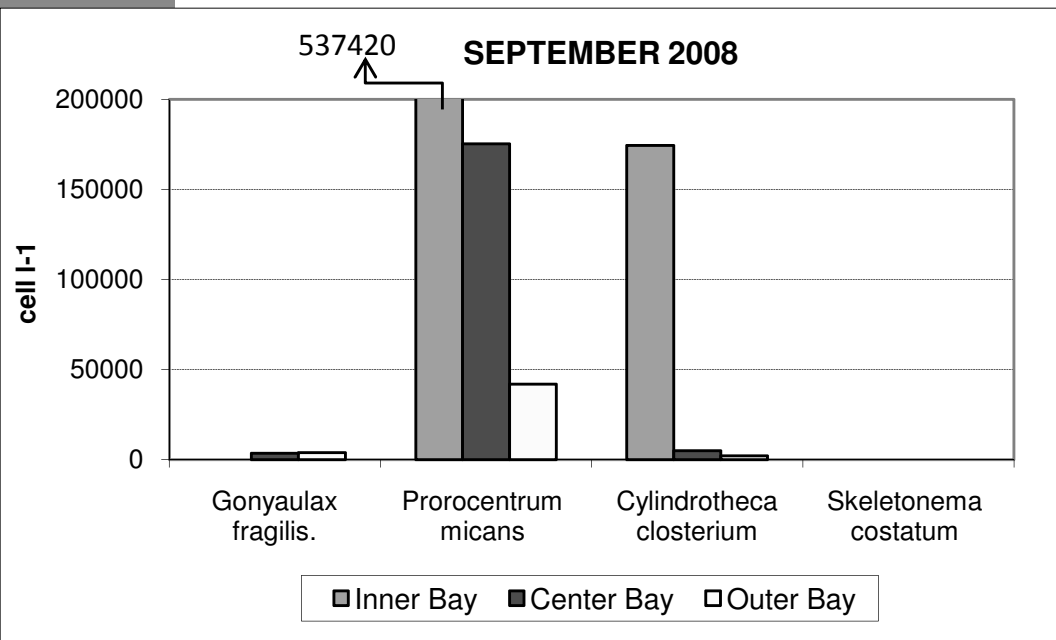
JANUARY 2008



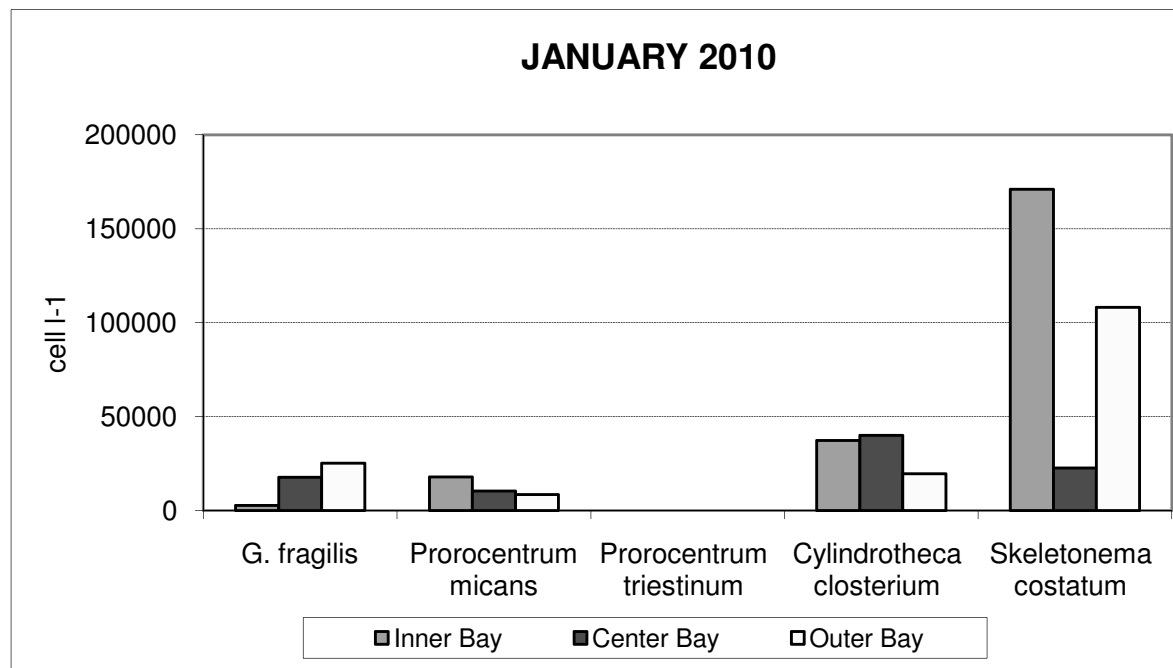
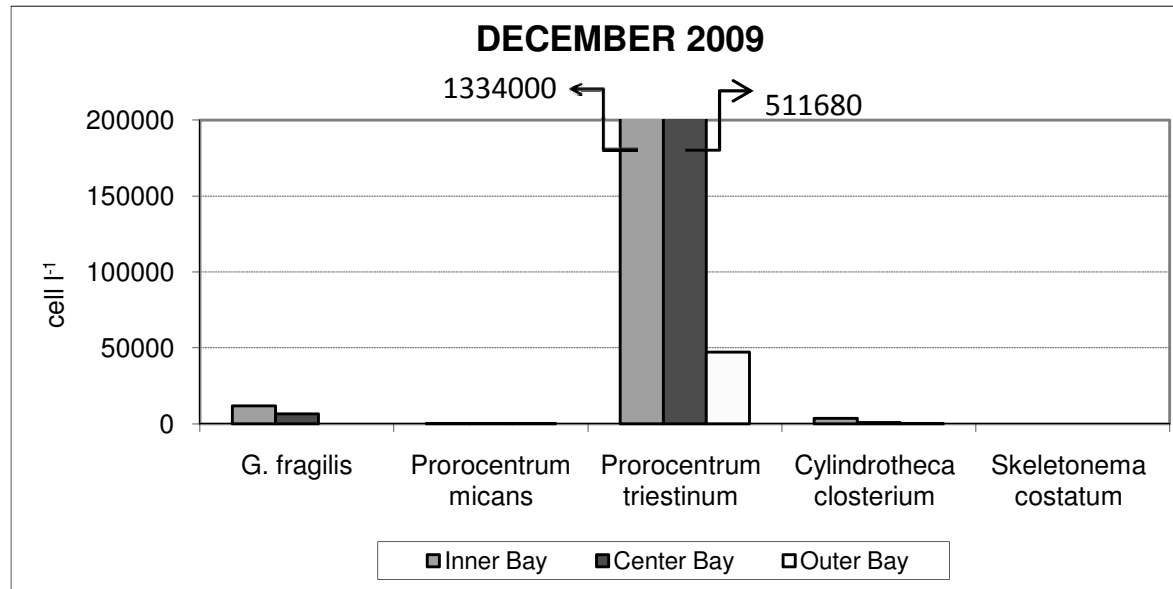
FEBRUARY 2008



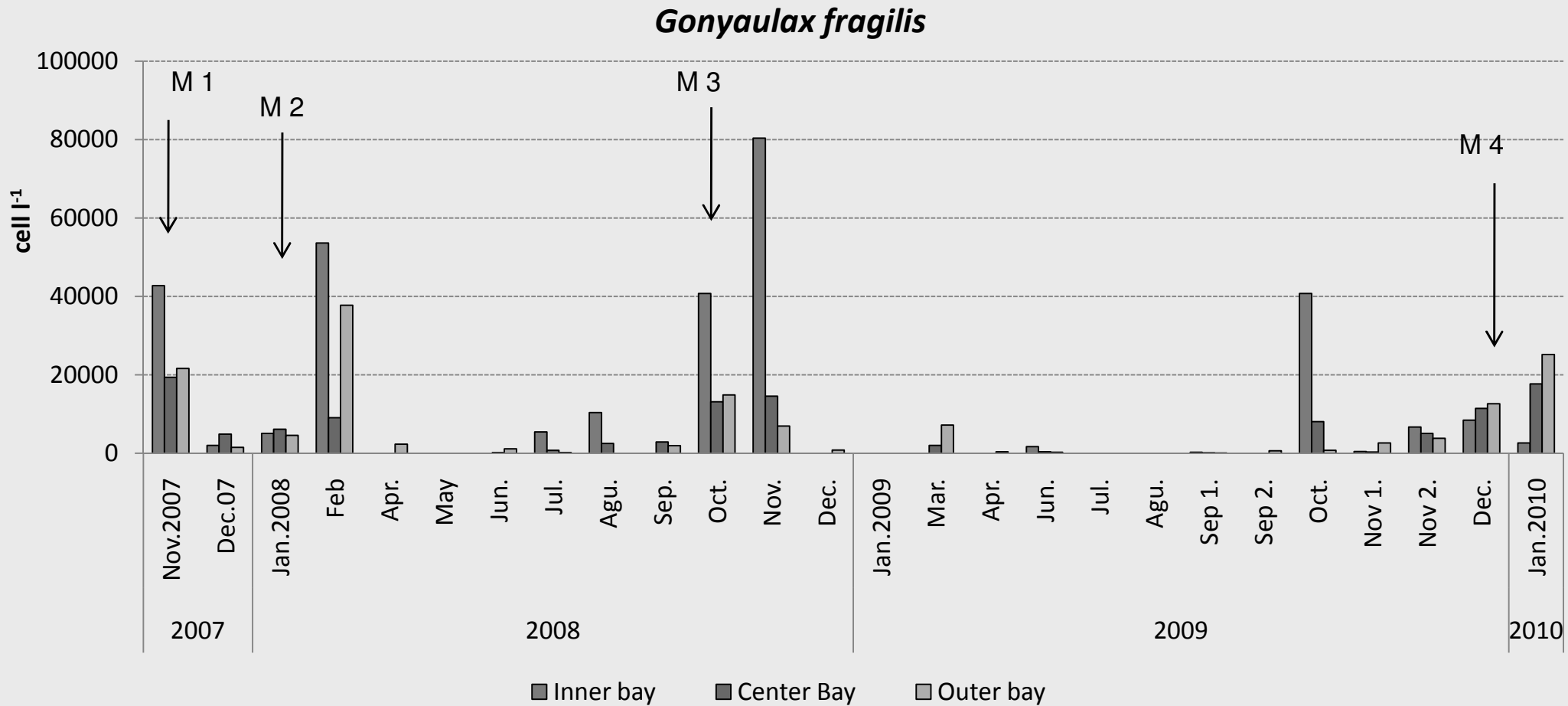
Phytoplankton Distribution in mucilage periods : Oct'08



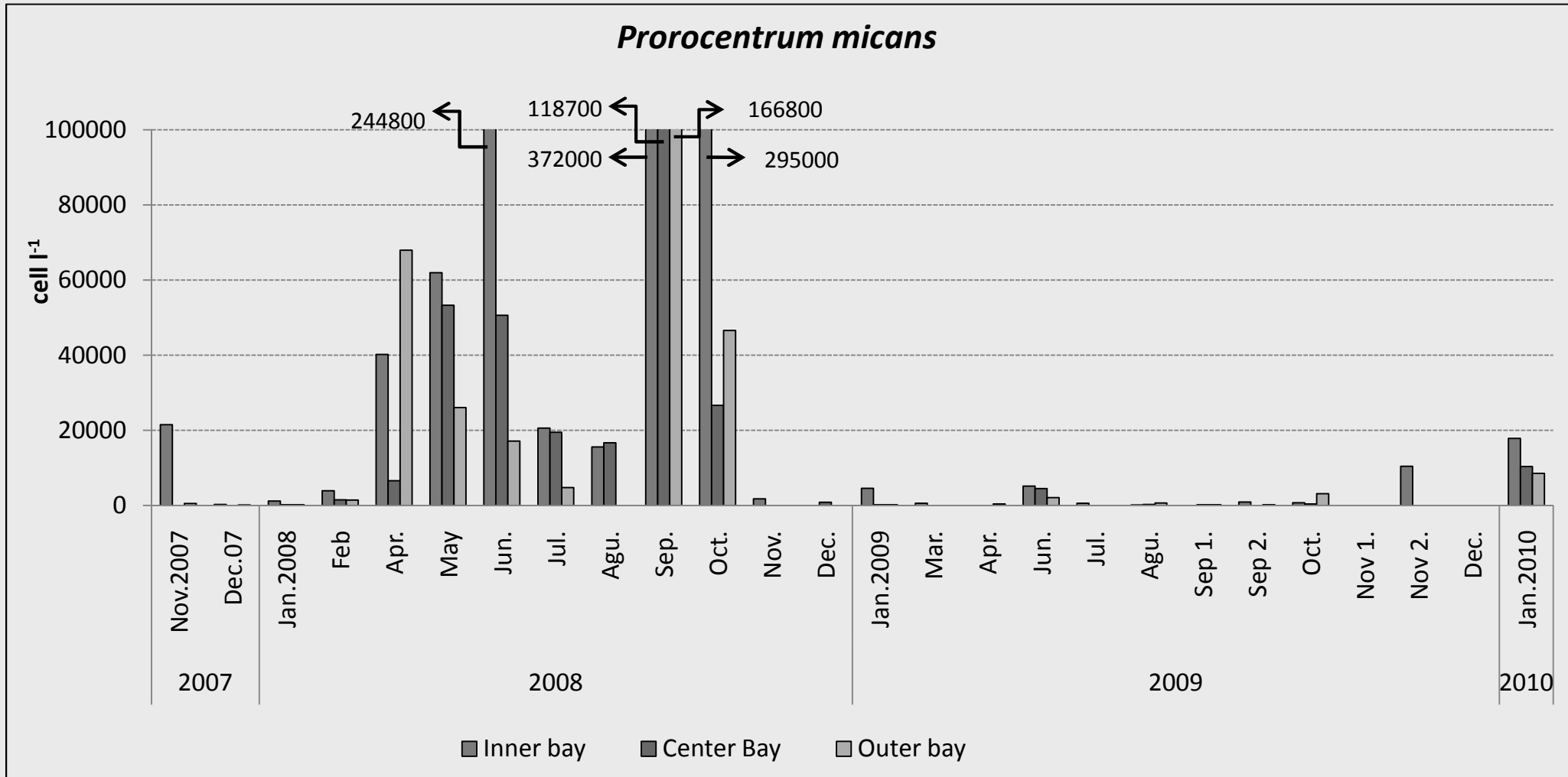
Phytoplankton Distribution in mucilage periods: Dec'09



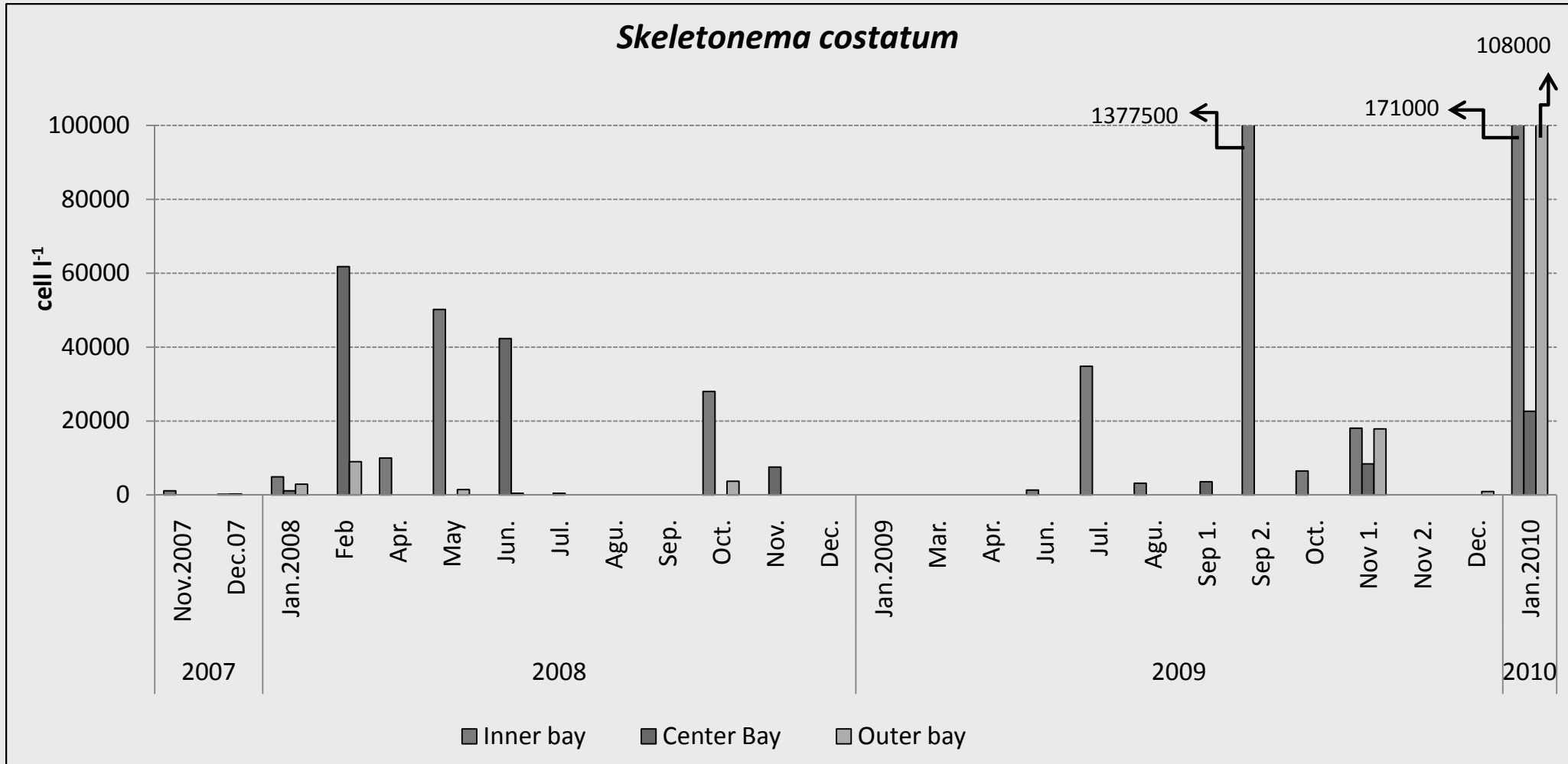
Distribution of target species along the İzmit Bay



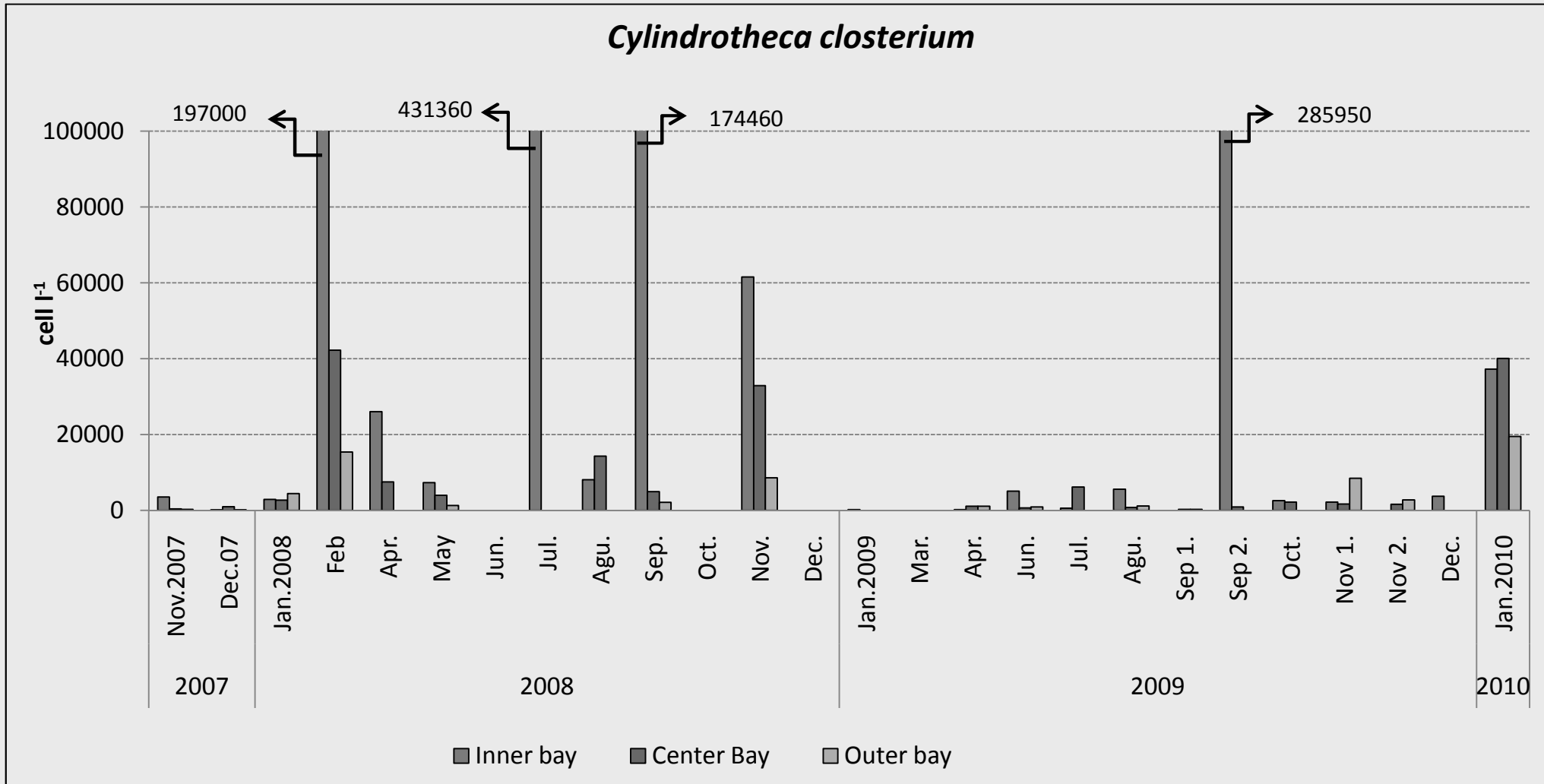
Distribution of target species along the İzmit Bay



Distribution of target species along the İzmit Bay

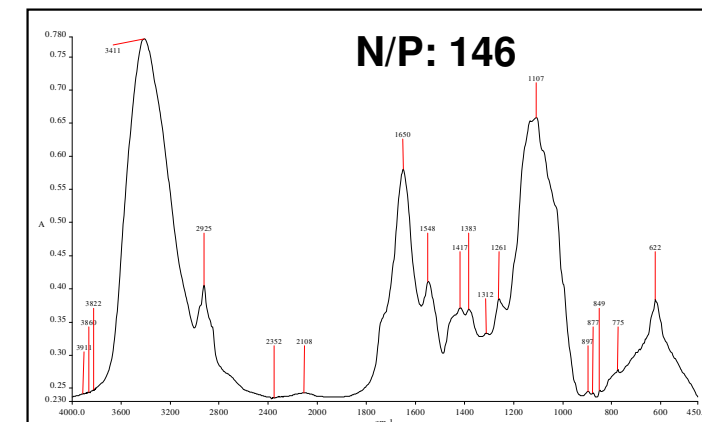
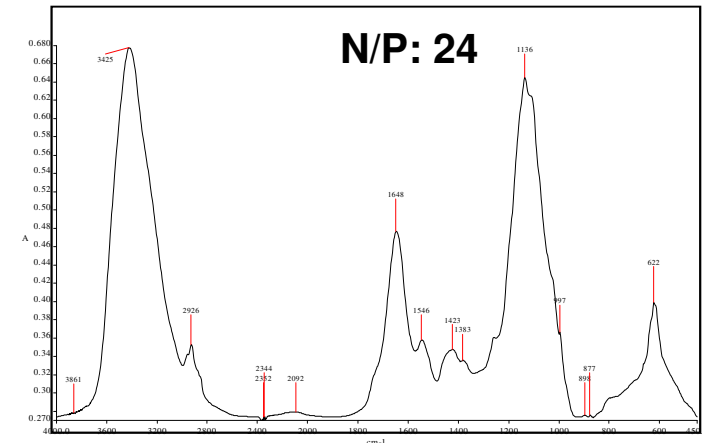
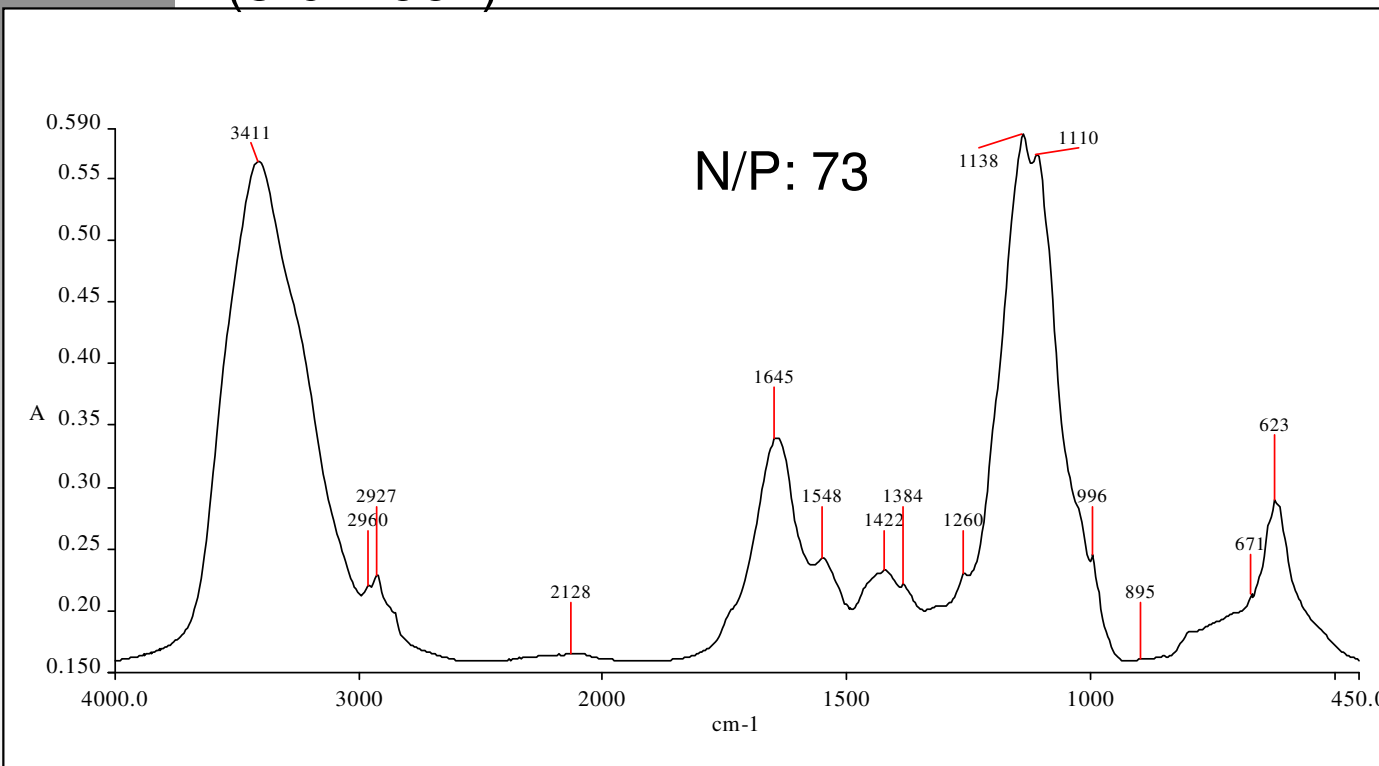


Distribution of target species along the İzmit Bay



Chemical composition of mucilage aggregates : FTIR

Laboratory aggregates : Obtained during single specie batch cultures (3rd week)



Wavenumber (cm-1)

Functional groups

73	24	146
3411	3401	3423
2927	2925	2926
1645	1650	1648
1541	1548	1547
1258	1259	1423
1138-1108	1107	1136
623	622	622

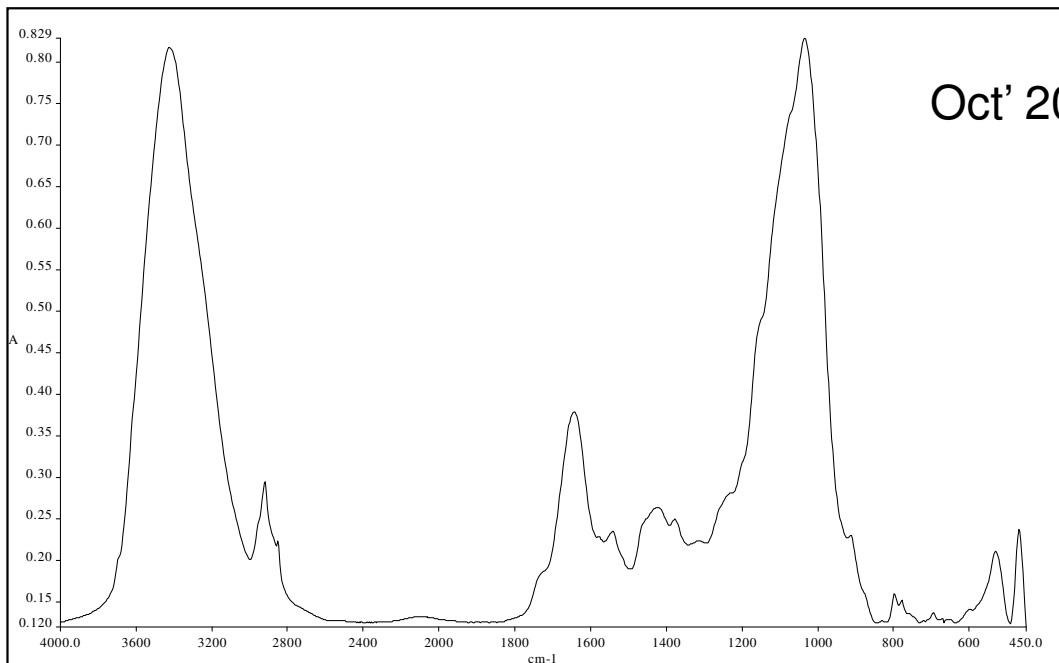
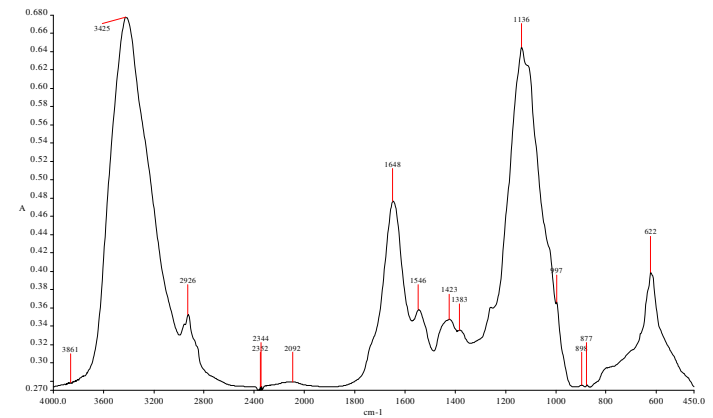
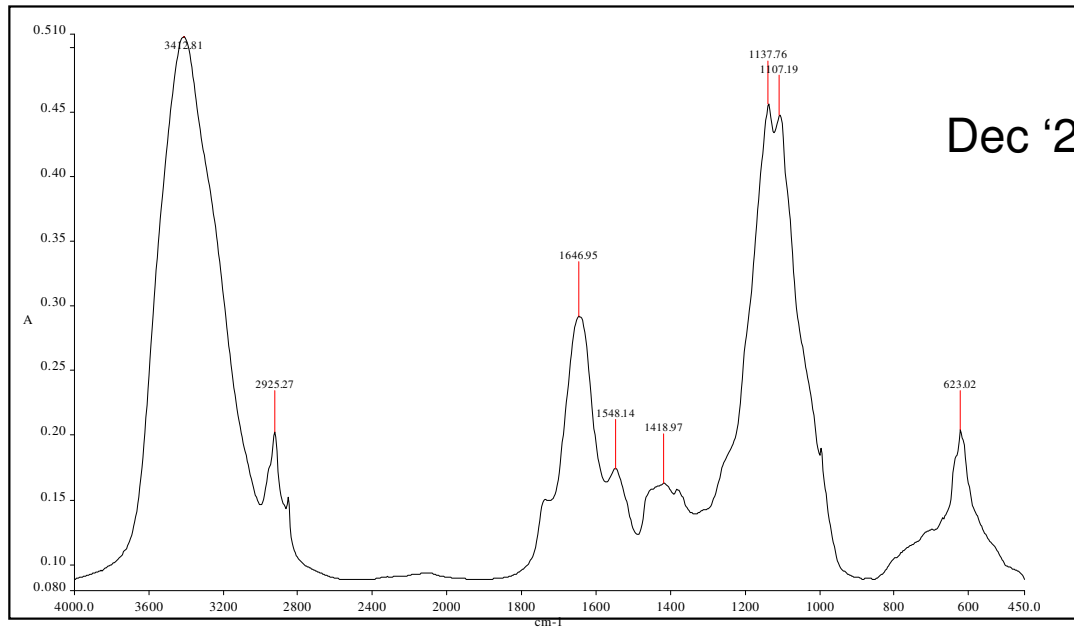
-OH, Karbonhidrat
 CH ve CH2 alifatik
 C=O Amid I band
 C-N Amid II band
 C-N Amid III band
 C-O-C polisakkarit
 Streching – aromatic ring

Major difference in N- and P-limited media:

C-O-C not exist in **N/P:8.1**
 instead C-O carbohydrate exist
 at 1075 cm⁻¹

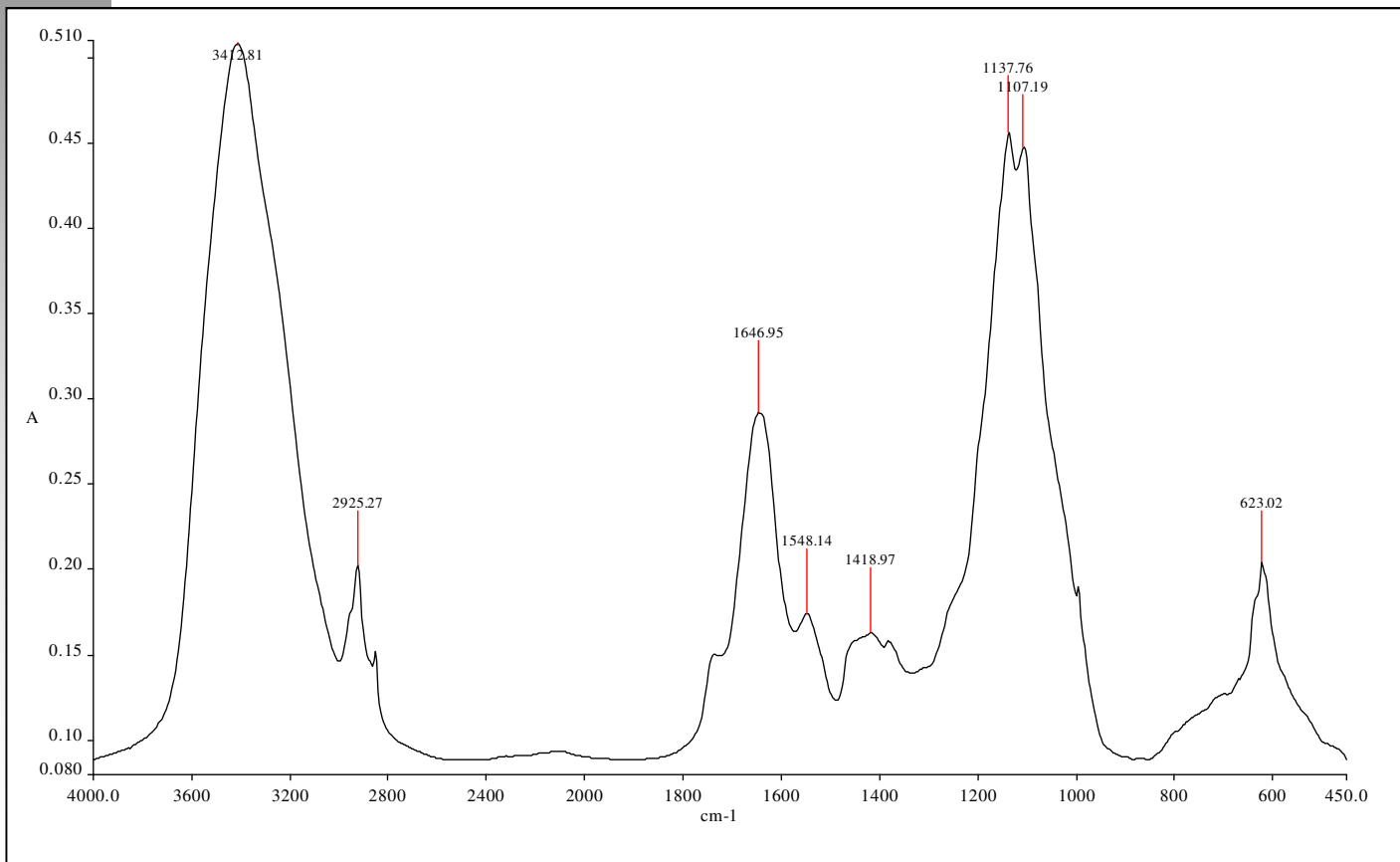
Chemical composition of mucilage aggregates : FTIR

Field mucilage aggregates : Sampled from surface in İzmit Bay



Chemical composition of mucilage aggregates : FTIR

Laboratory aggregates : Obtained in natural media -mixed species- at laboratory incubation (6-11 days: 07-13-18.01.2010)

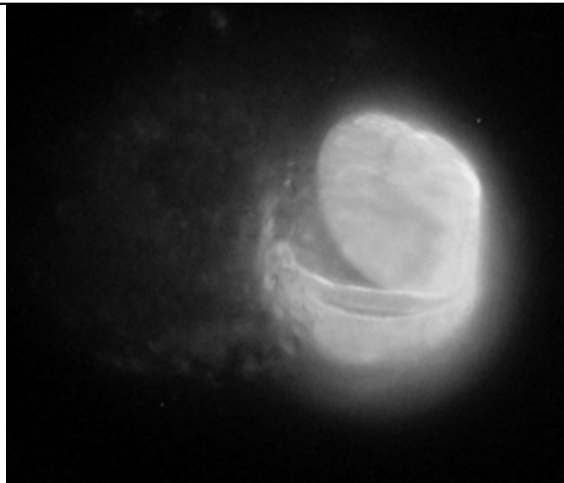


Total abundance: 45540 cell/L
50 % dinoflagellate
49 % diatom

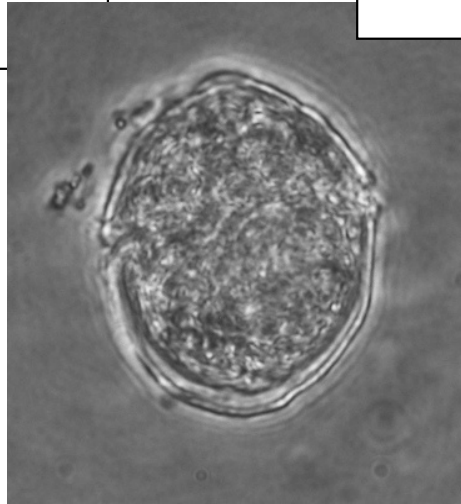
G.fragilis: 64 % of dinoflagellates
P.micans: 20% of dinoflagellates
S. costatum: 23 % of diatoms
C.closterium: 19 % of diatoms

Investigation of factors controlling the marine mucilage/mucus formation with bioassay techniques

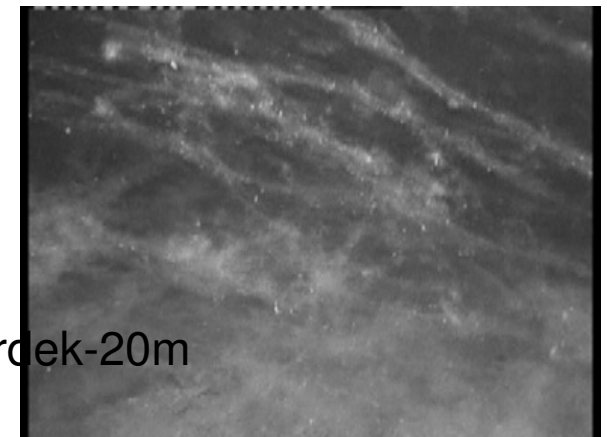
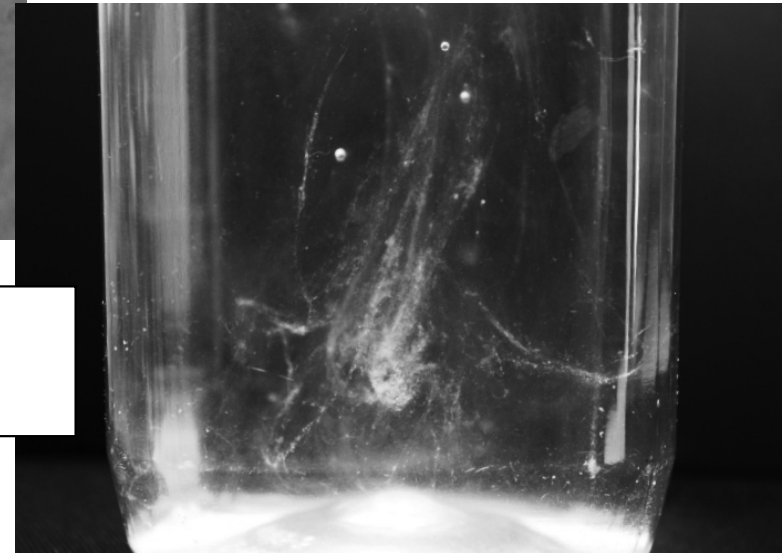
Gonyaulax fragilis and intercellular material– Epiflouresans microscope, 25 /12/2009 sampling



Gonyaulax fragilis – Light microscope 25 / 12 / 2009 sampling



Clear water sampled on 7 January 2010 and incubated at laboratory conditions for 6-11 days. Aggregates were formed in jars.



Jan'08 Erdlek-20m



TUBITAK

MAM

Inspiration from recent literature

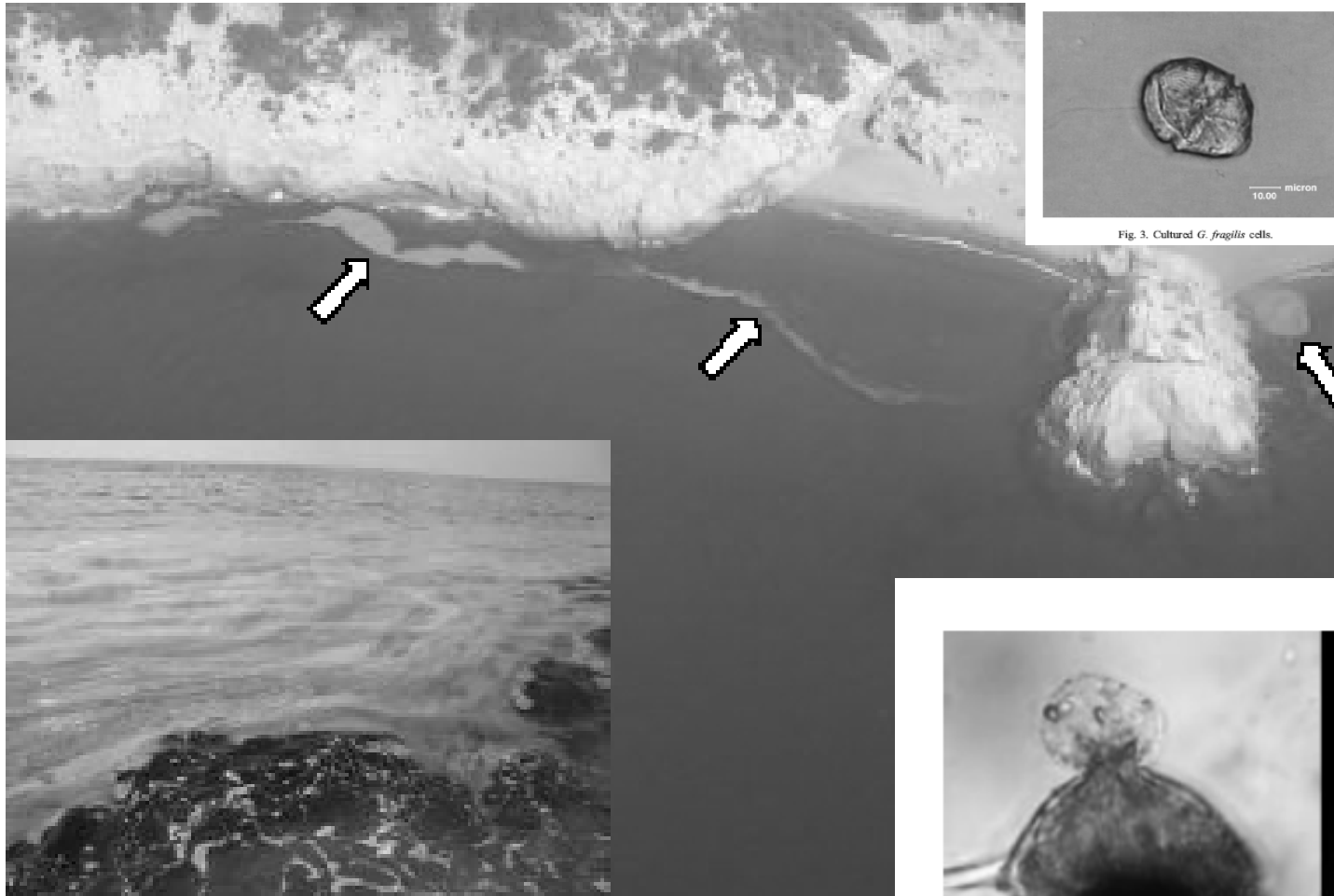


Fig. 2. Aerial and coastal photographs of mucilage (from ACA)

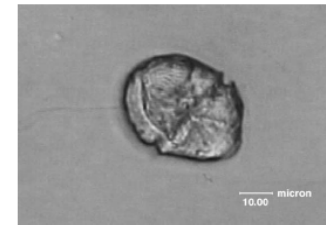


Fig. 3. Cultured *G. fragilis* cells.

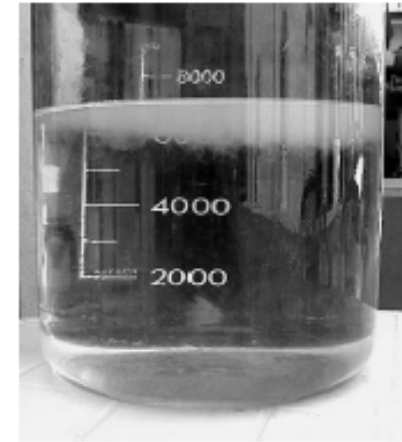


Fig. 6. Mucilage formations by cultured *G. fragilis*.

Pistocchi et al.2005

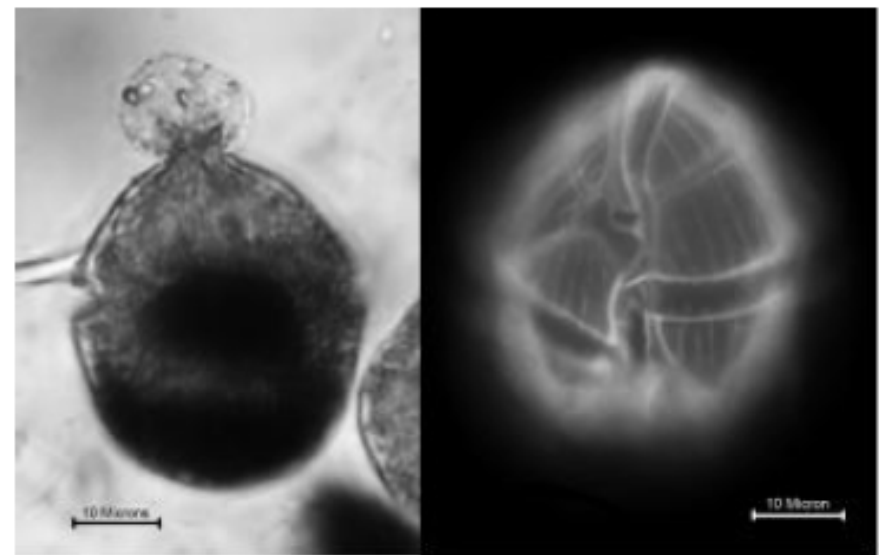


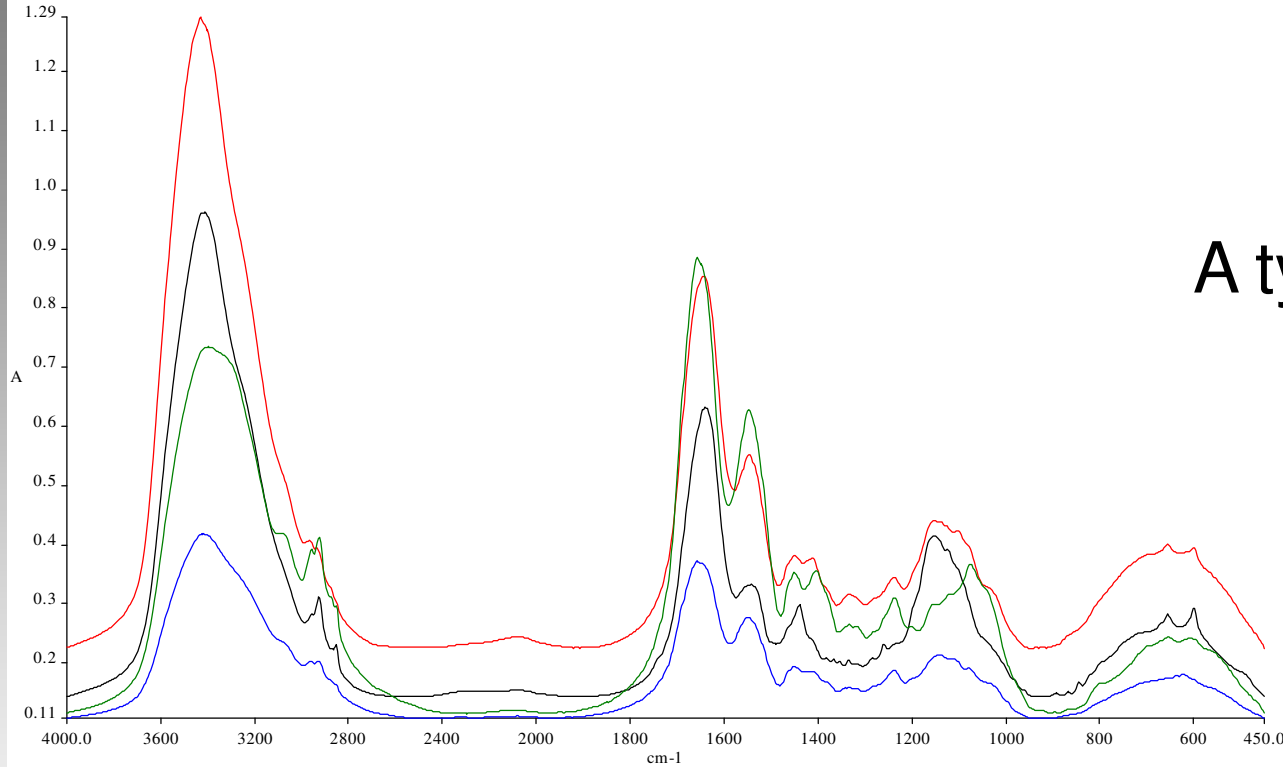
Fig. 3. Light and epifluorescence microscopy photographs of *G. fragilis*. Note the sticky material leaving from their apical part.

Sampedro et al.2007



TÜBİTAK
MAM

Chemical composition of *Aurelia aurita* : FTIR



A typical protein
structure

- _____ : Denizanası 1 25.08.10/03.09.10 F-Dr
_____ : 16.09.10 suzmeli/21.09.10 Deniz A.4
_____ : 07.09.10 suzmeli/21.09.10 Deniz A.3
_____ : Denizanası 2 27.08.10 suzme/03.09.10 F-Dr

Wavelength(cm-1)

3700-3000

2923

1654

1547

1400-1450

1237

1075

Functional group

-OH ve -NH gruplarından kaynaklanan hidrojen bağı

-CH₂ ve -CH₃ alkil gruplarına ait Stretching vibration bandı

C=O Amid I bandı

C-N Amid II bandı

İnorganik karbonatlar

C-N Amid III bandı

C-O karbonhidrat



TUBITAK

MAM

Summary of presented (1)

Field studies indicate that

- *Gonyaulax fragilis* is newly recorded starting from the first mucilage event in 2007.
- There are other inhabitants as mucilage producers
- Blooming of any of these species before and during the events is not the case

Light, temperature and nutrient controlled experiments indicate that

- DOC and dissolved carbohydrates considerably increase after 2 weeks
- Under P-limitation DOC accumulation is more pronounced whereas carbohydrate accumulation is at the same level in N and P limitation
- NH₄ additions at severe N-limited condition increase DOC and CH accumulation
- At low nutrient exposures (same N/P) CH accumulation is 3-4 times more in diatoms



Summary of presented (2)

- FTIR results prove that chemical composition of field and laboratory aggregates are almost the same and the material is of phytoplankton origin with pronounced polysaccharide and carbohydrate bands
- This spectra is very similar to given in the literature which relate the mucilage phenomena to phytoplankton extracellular material aggregation
- FTIR of jelly fish (*A. aurita*) is different and shows a protein structure

THANK YOU