Applicazioni della citometria a flusso allo studio ed al monitoraggio dell'ambiente marino.

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Abstract

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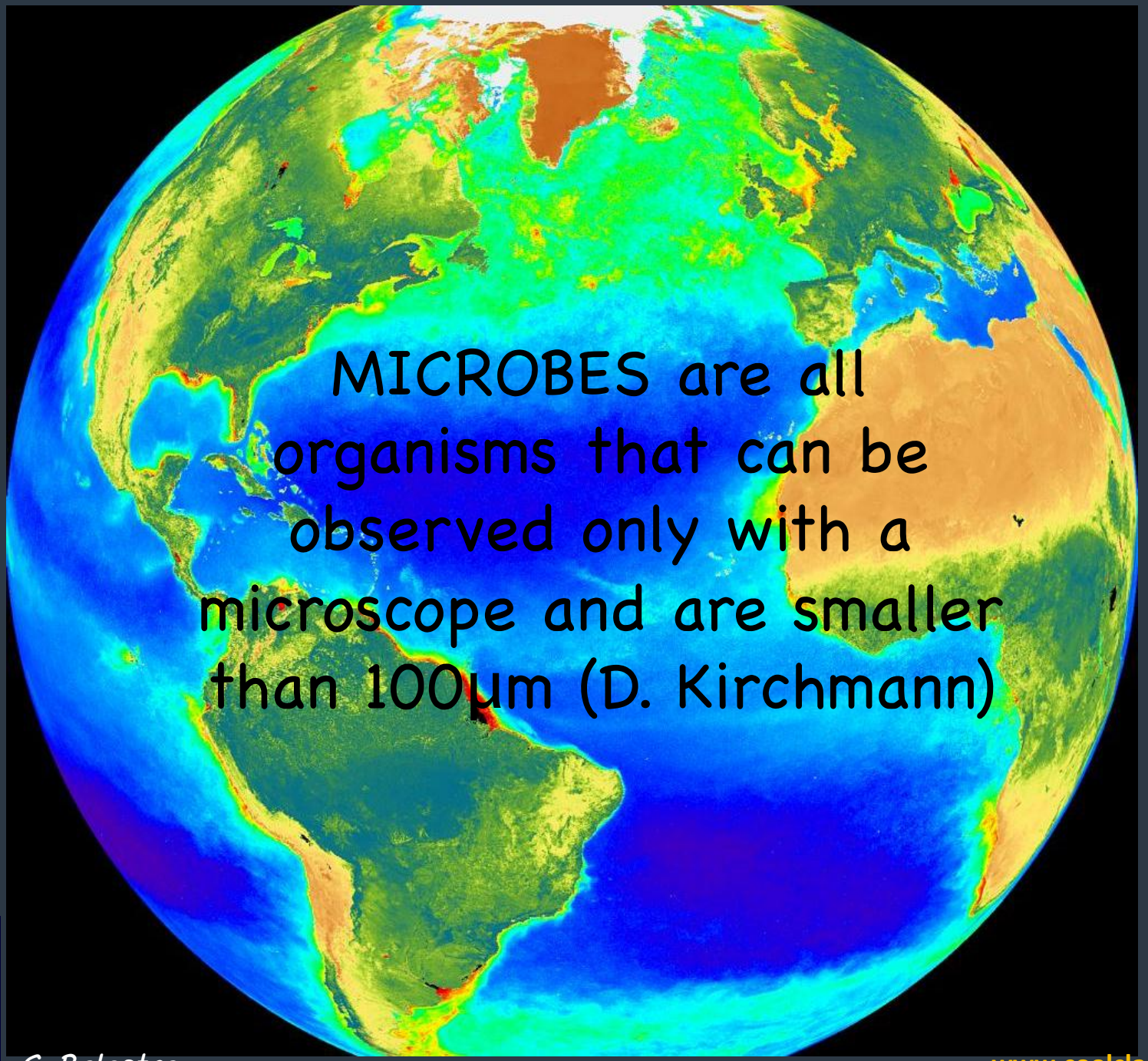
I microorganismi marini cambiano molto rapidamente in conseguenza dei cambiamenti dei fattori ambientali modificando la propria fisiologia, abbondanza numerica e struttura delle popolazioni grazie alla loro alta plasticità fisiologica ed inoltre sono i primi a mutare in risposta alle alterazioni naturali e/o antropiche. La citometria a flusso viene utilizzata in ecologia microbica perché permette di discriminare, in base ai parametri di scatter e di fluorescenza, diversi gruppi cellulari e di stimare l' abbondanza cellulare per ciascun gruppo preso in esame. Con la citometria è, infatti, possibile analizzare in modo diretto gli organismi autotrofi, grazie alla loro autofluorescenza e ai loro pigmenti fotosintetici, ed analizzare, solo dopo colorazione, gli organismi non autotrofi, come ad esempio i batteri eterotrofi e i virus. Abbiamo combinato questi due approcci utilizzando due citometri in linea il CytoSense (Cytobuoy) per l'analisi dei microbi autotrofi e l'onCyt per l'analisi dei microorganismi eterotrofi. Questa tecnologia fornisce in tempo reale le concentrazioni batteriche e del picofitoplancton che sono considerati indicatori della qualità dell'acqua ed è il primo passo per valutare gli effetti dei cambiamenti ambientali.


MICROBES are all organisms that can be observed only with a microscope and are smaller than 100µm (D. Kirchmann)

Copley, Nature 2002 415: 572-574

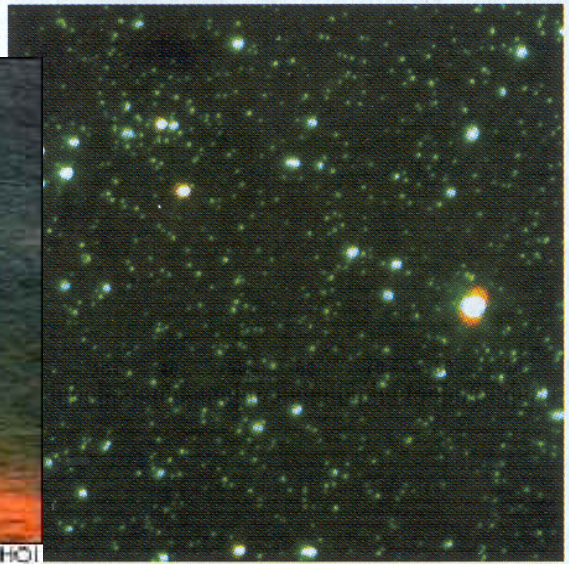
news feature

All at sea

The oceans are full of microorganisms, which are thought to cycle nutrients and mediate climate on a global scale. Despite these environmental consequences, marine microbial biodiversity remains poorly understood. Jon Copley reports.

The role that the ocean plays in structuring the Earth climate is driven by microorganisms (P. Burkill)

Courtesy of WHOI



Send in the marines: researchers such as Ed DeLong (pictured) are finding fresh traits among oceanic microbes (inset). Looking like stars, these organisms outnumber their stellar counterparts.

Falkowski of Rutgers University in New Brunswick, New Jersey, and his colleagues came across one of them by accident.

Falkowski's group was hunting for photosynthetic microbes called α-proteobacteria in the waters around deep-sea vents, reasoning that the incredibly faint glow from the vents might just be sufficient to support photo-

Phytoplankton 3 – 4 gGton carbon (10^{15} g)

Bacteria 2.8 – 13.7 gGton carbon

Viruses 0.027 – 0.27 Gton

Whales 0.0041 – 0.012 Gton

Humans 0.03 Gton carbon

"Life on Earth is microscopic!"

Sean Nee, 2004

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APPLICATIONS TO MARINE ECOLOGY

- Discrimination and counting of different phytoplankton and bacteria at sea.

- Estimates of pigment content and its variations (photoacclimation).

- Growth and grazing losses and selectivity.

- DNA analysis (cell cycle).

- Culture studies (toxicology, physiology: viability, stress, respiration...).

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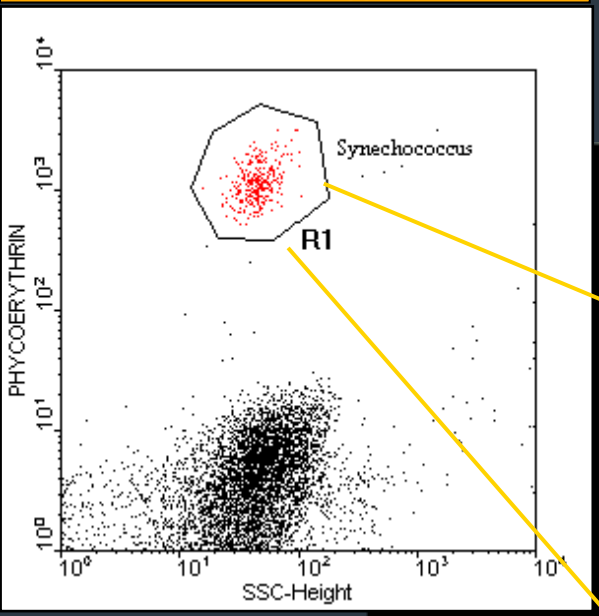
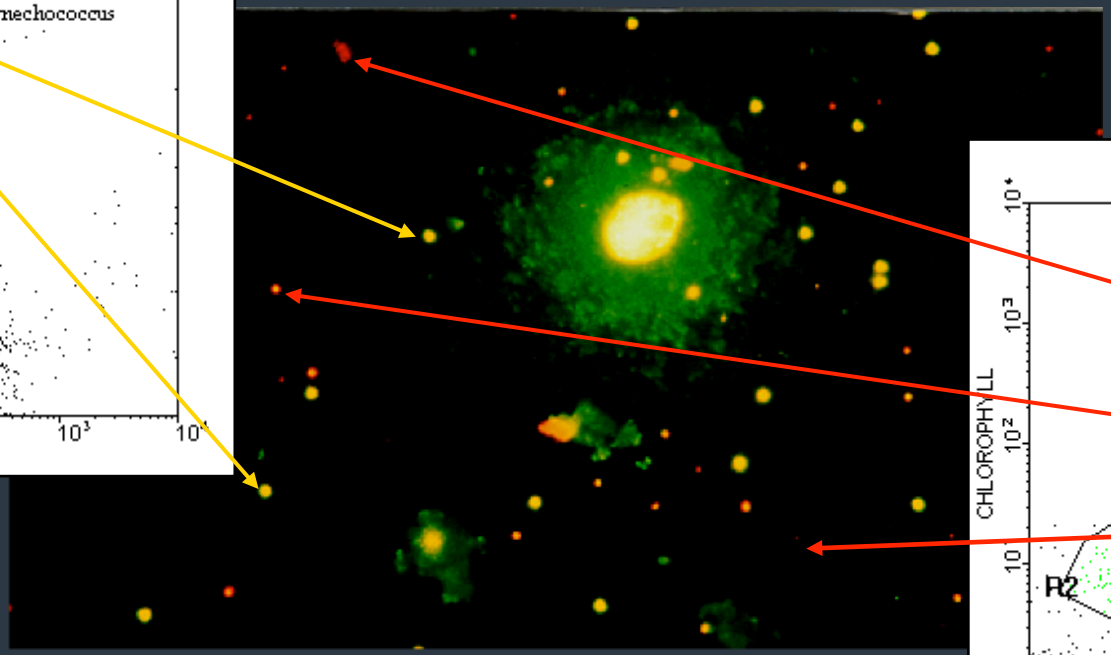
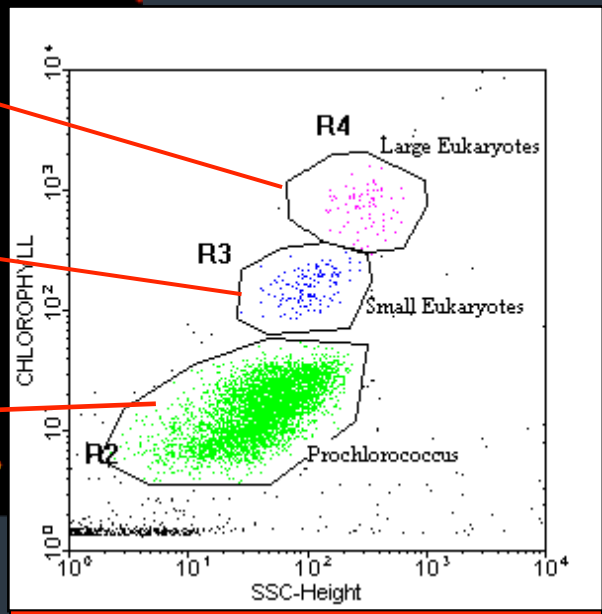
Primary Fluorescence

Phycoerythrine
(Orange Fl. at 488nm Ex.)
vs. Forward Scatter
Cyanobacteria, Cryptophytes

Blue excitation







Chlorophyll
(Red Fl. at 488nm Ex.)
vs. Forward Scatter
All Phytoplankton

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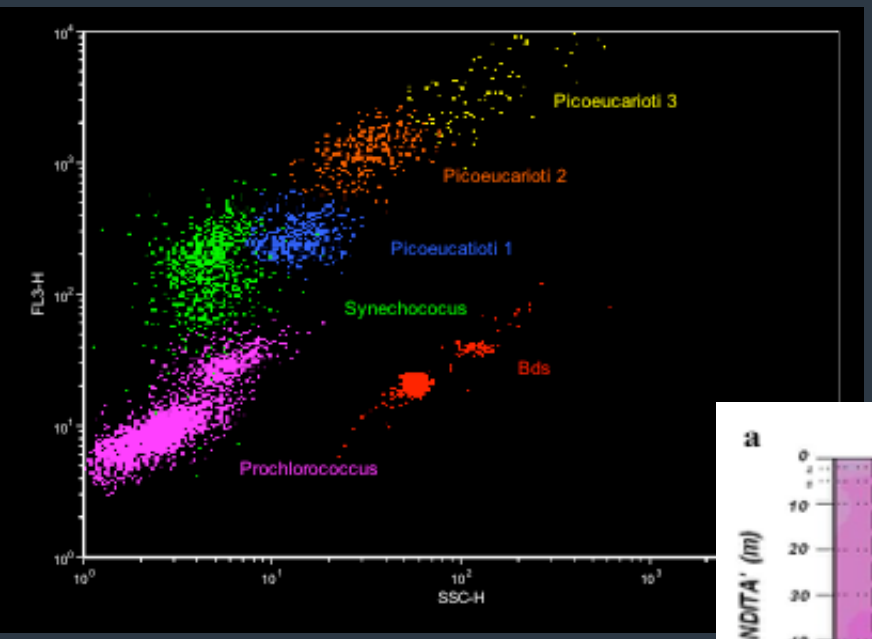
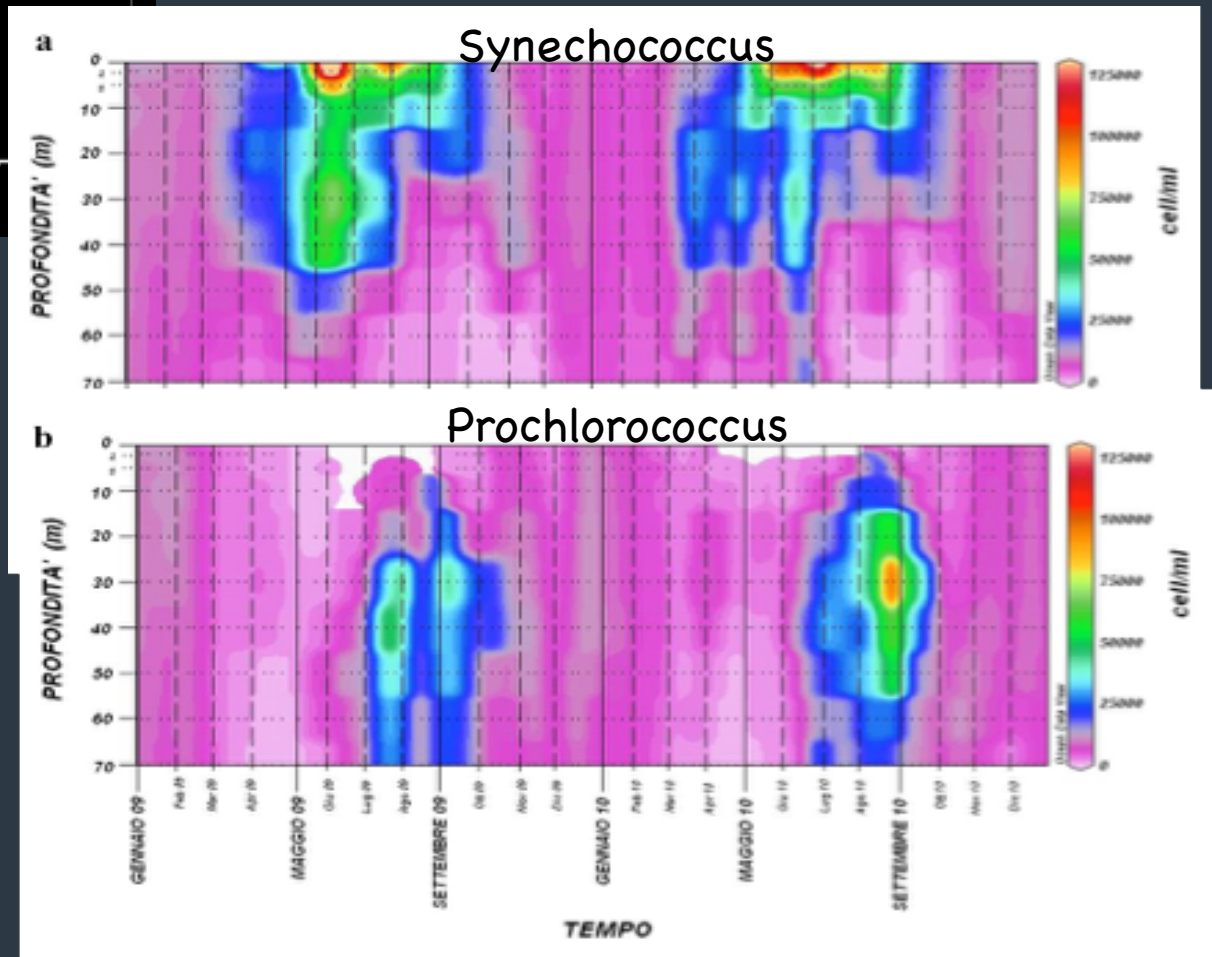
Distribuzione del picoplancton autotrofo nel Golfo di Napoli





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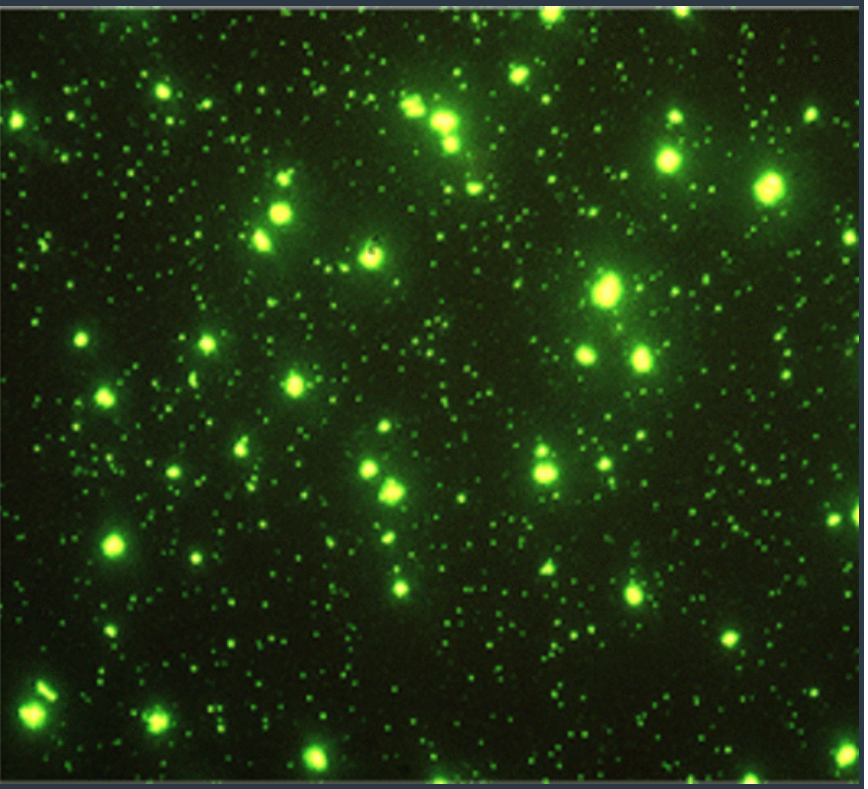
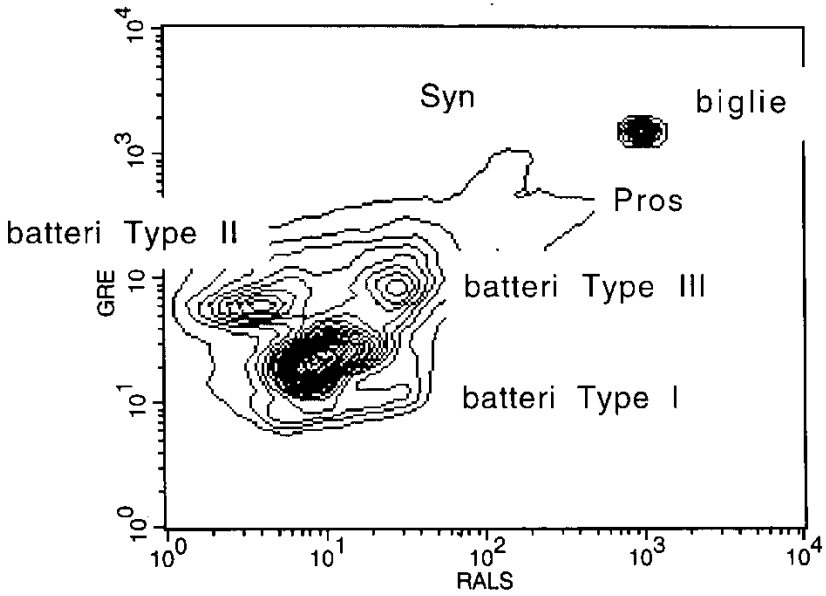
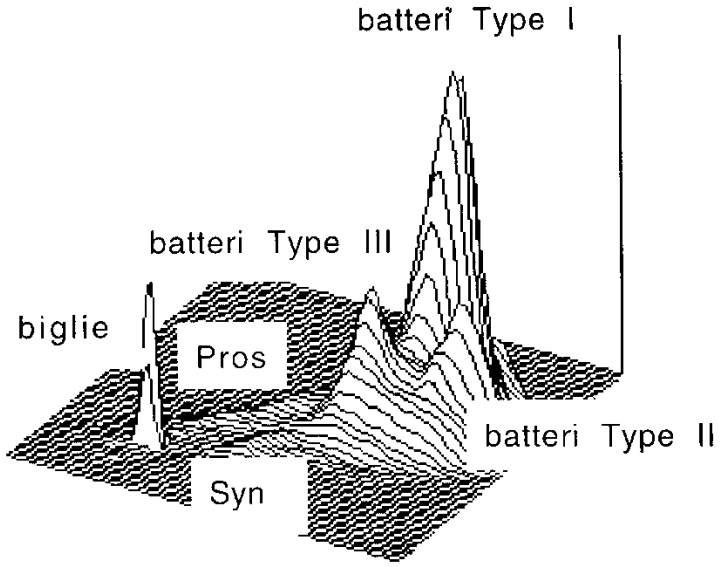
Marine bacteria stained with SYBR Green I







Casotti, 2000

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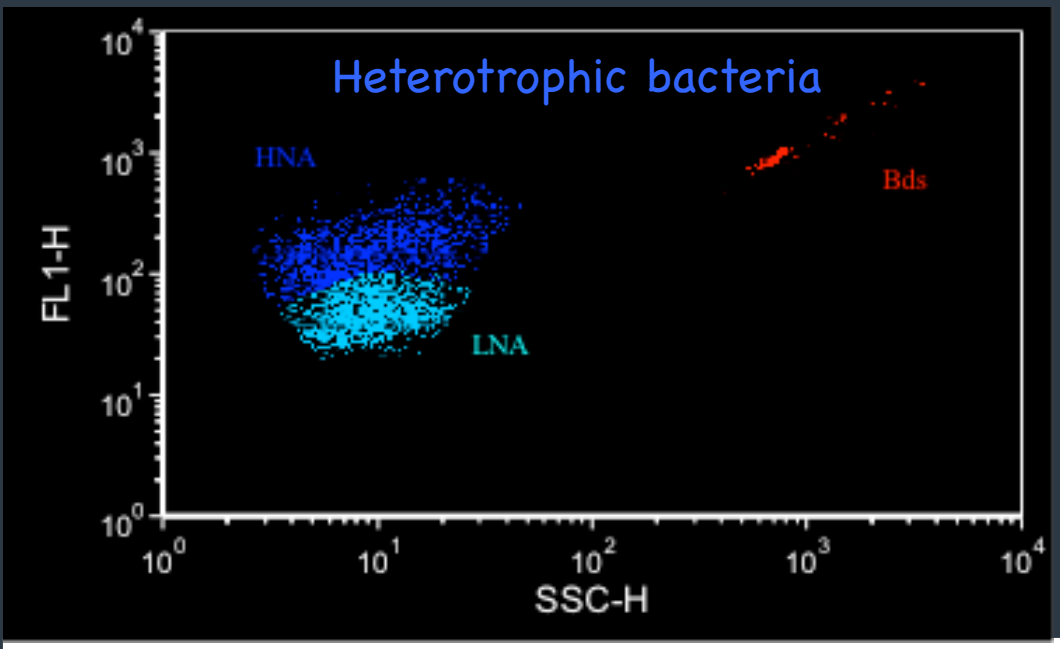
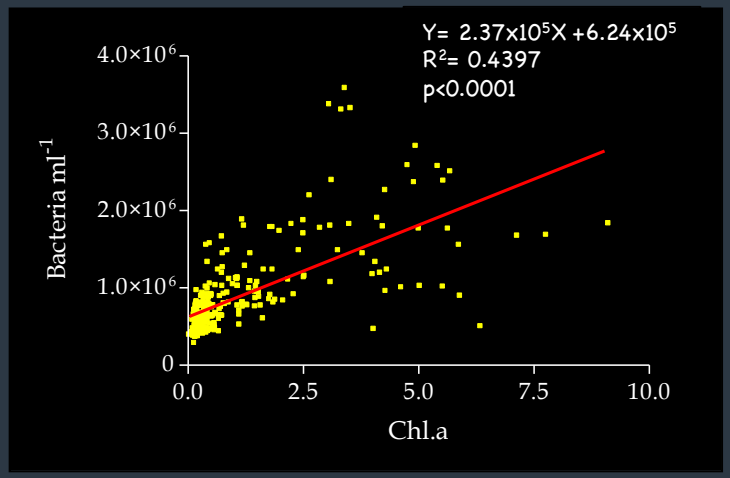
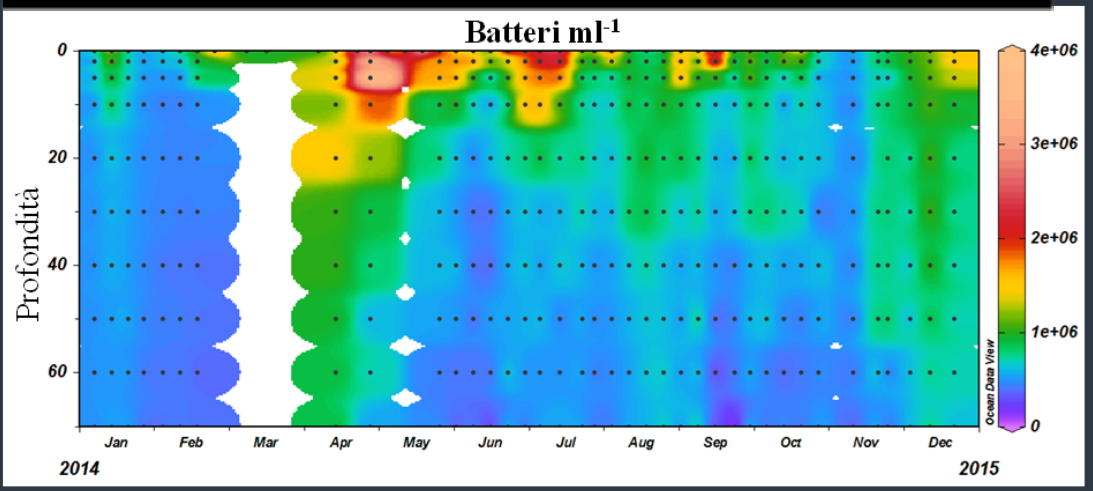
Distribuzione del picoplancton eterotrofo nel Golfo di Napoli







Heterotrophic bacteria used the organic matter released by phytoplankton for their growth.

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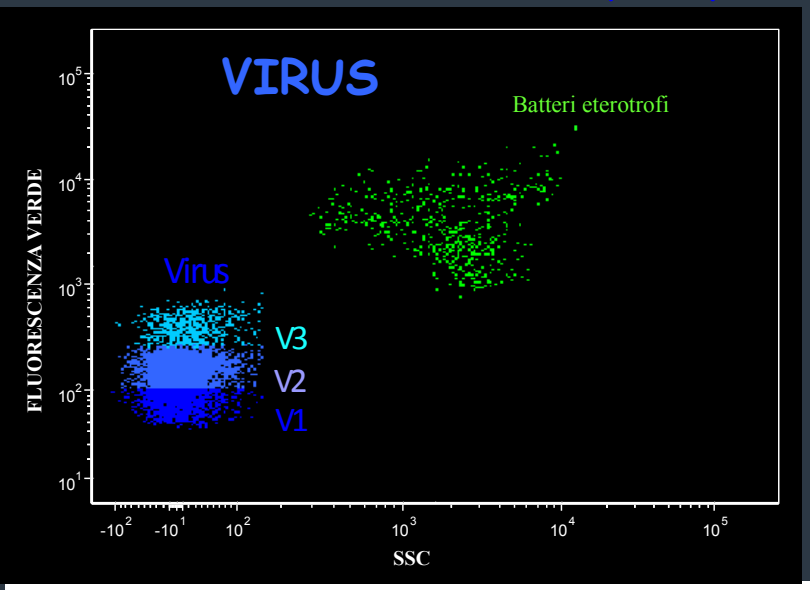
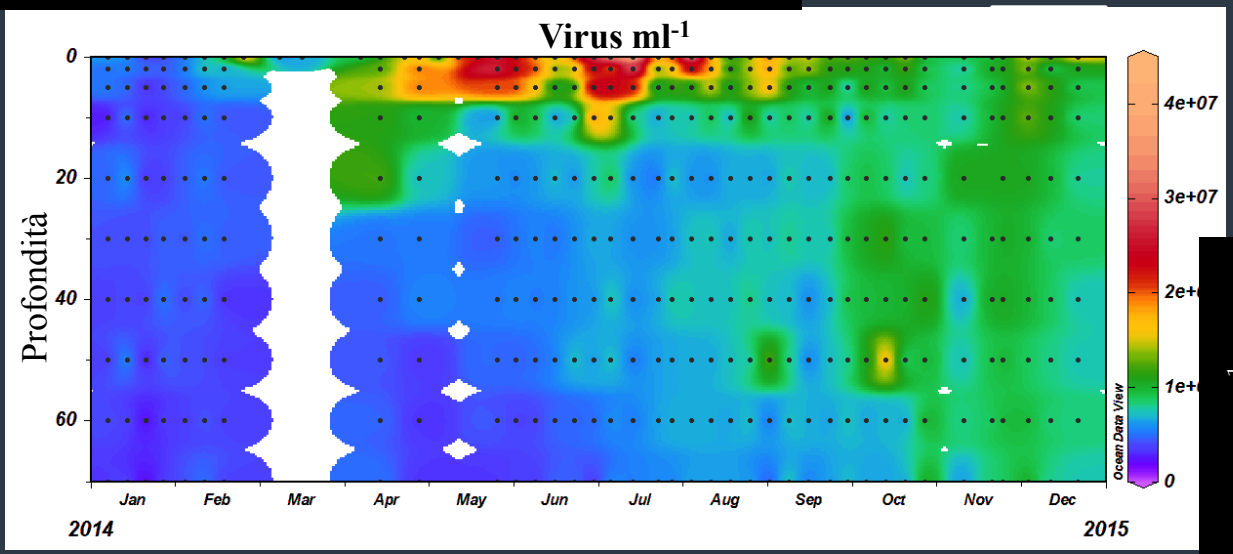
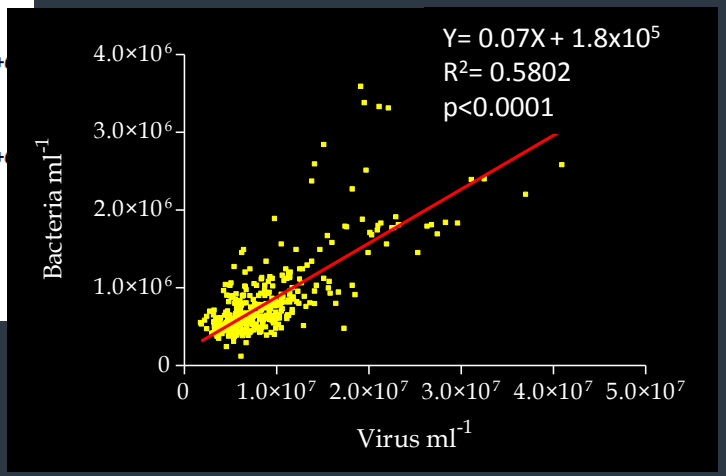
Distribuzione del picoplancton eterotrofo nel Golfo di Napoli



I virus dipendono dalla popolazione ospite batterica





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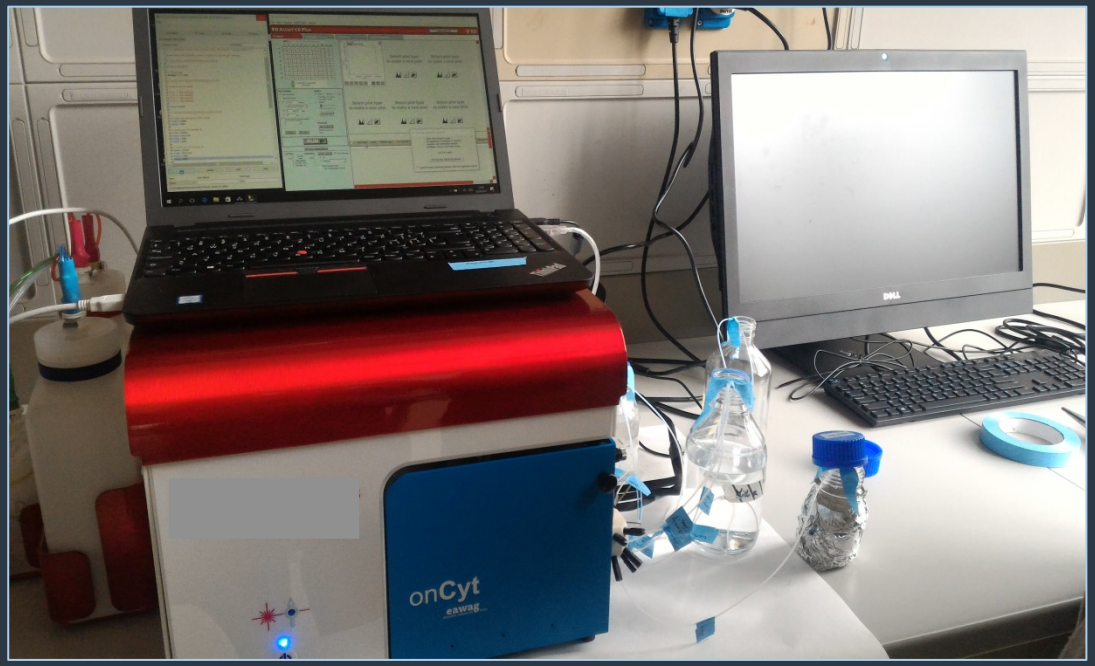
onCyt of eawag

- Incubation chamber connected to the sample inlet.
- Fully automated and programmable.
- Sample acquisition up to every 4 min.

Besmer et al. 2014, Hammes et al. 2012



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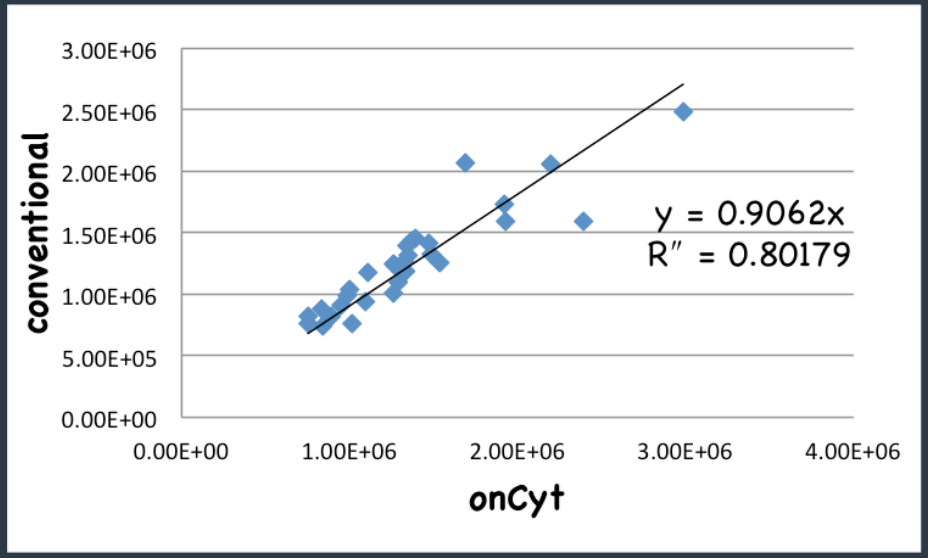
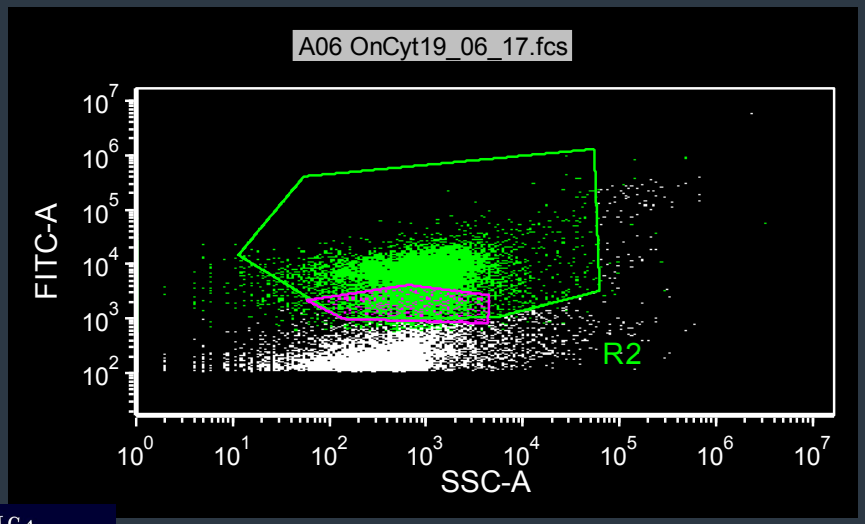
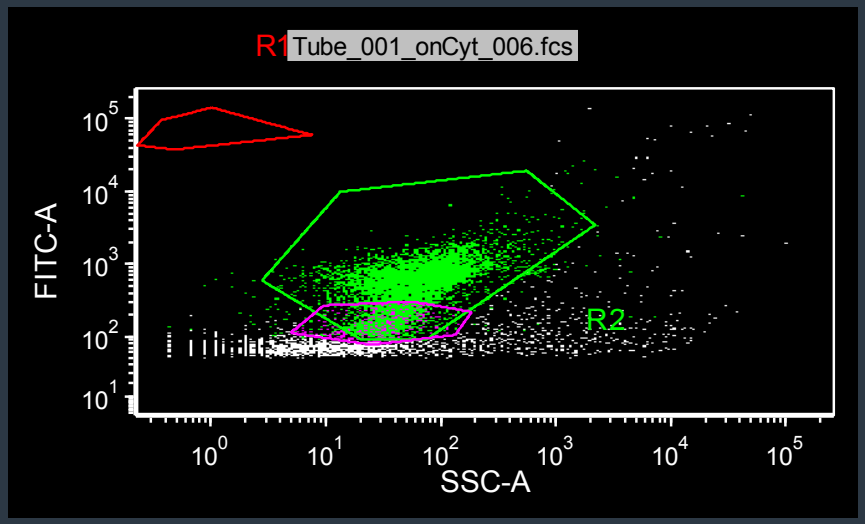
Calibration conventional FCM versus onCyt



onCyt



conventional



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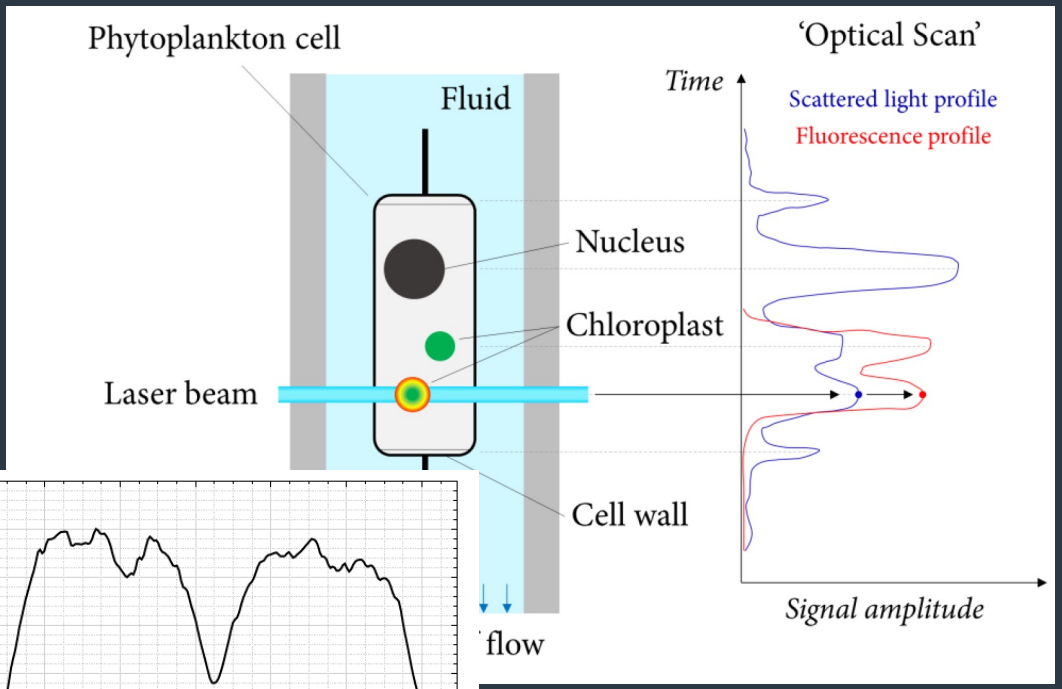
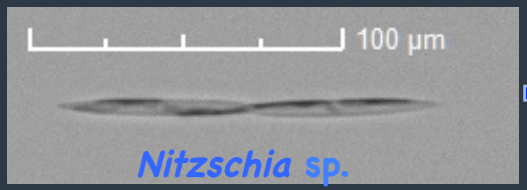
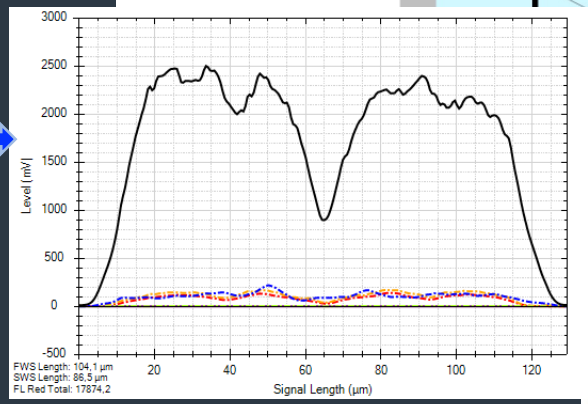
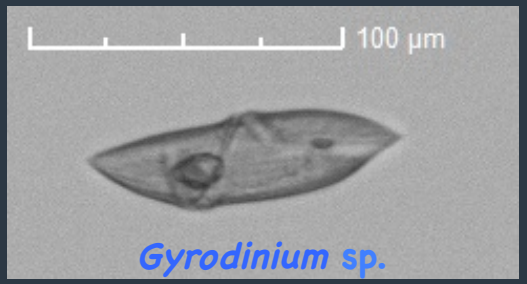
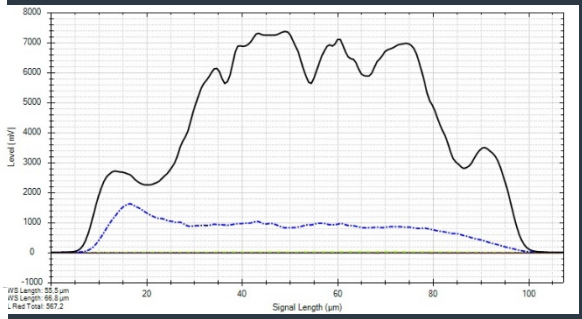
CytoSense scanning flow cytometer

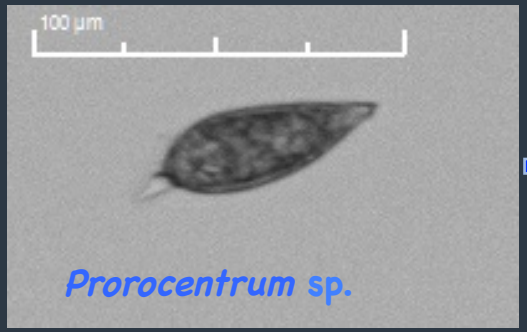


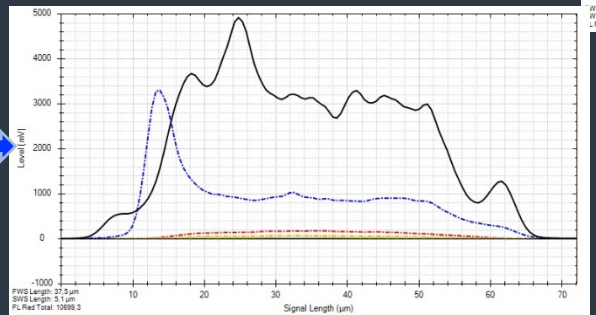












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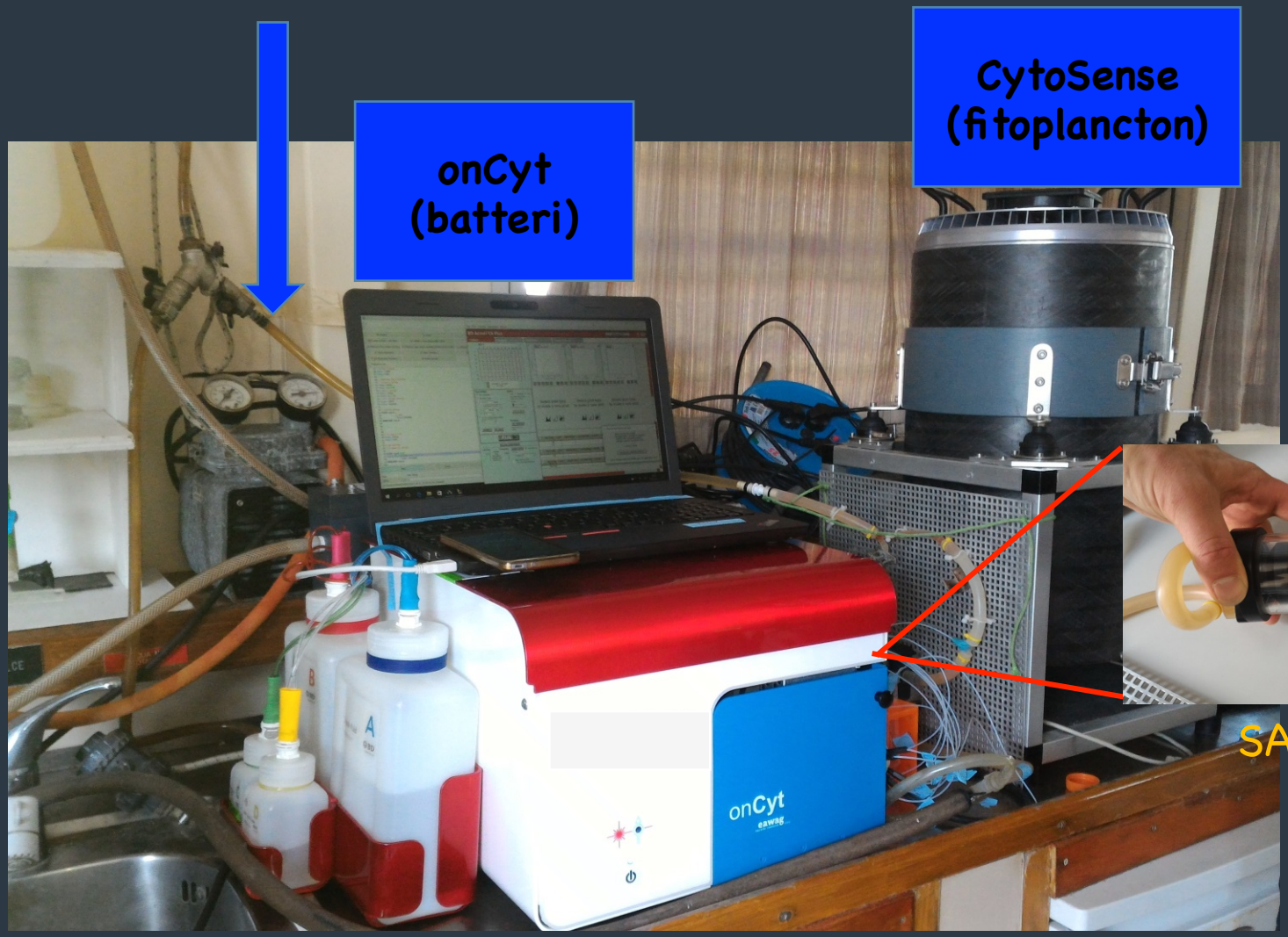
SAMPLE INLET (after thermosalinographer)



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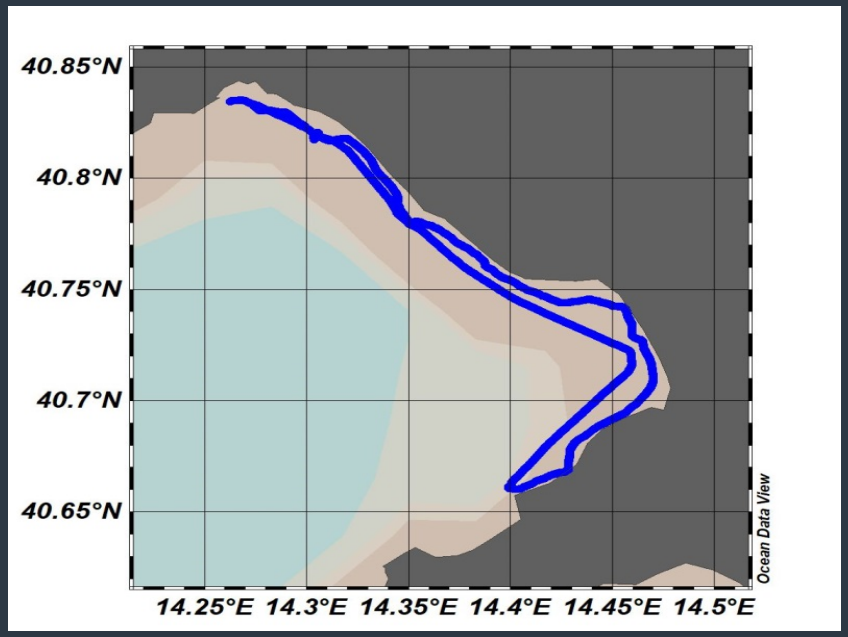
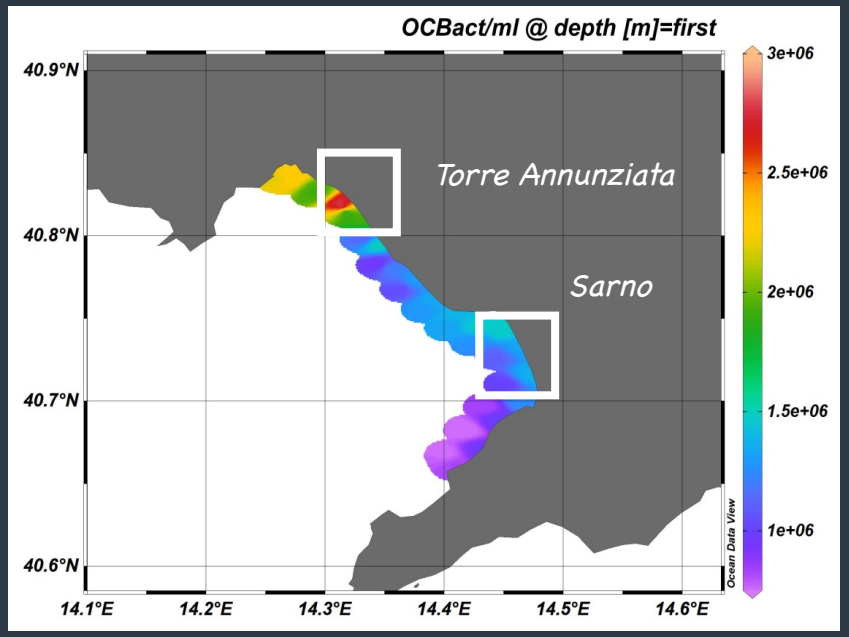
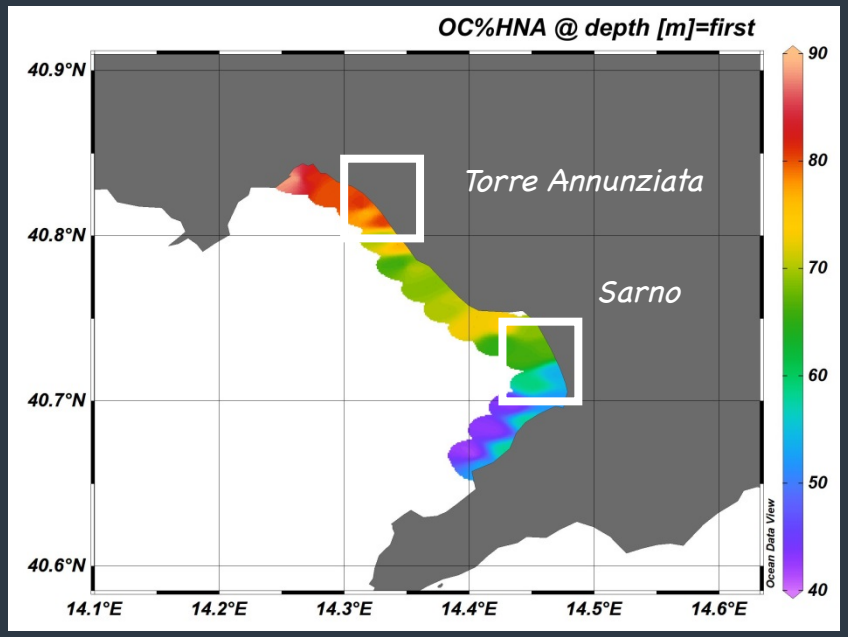
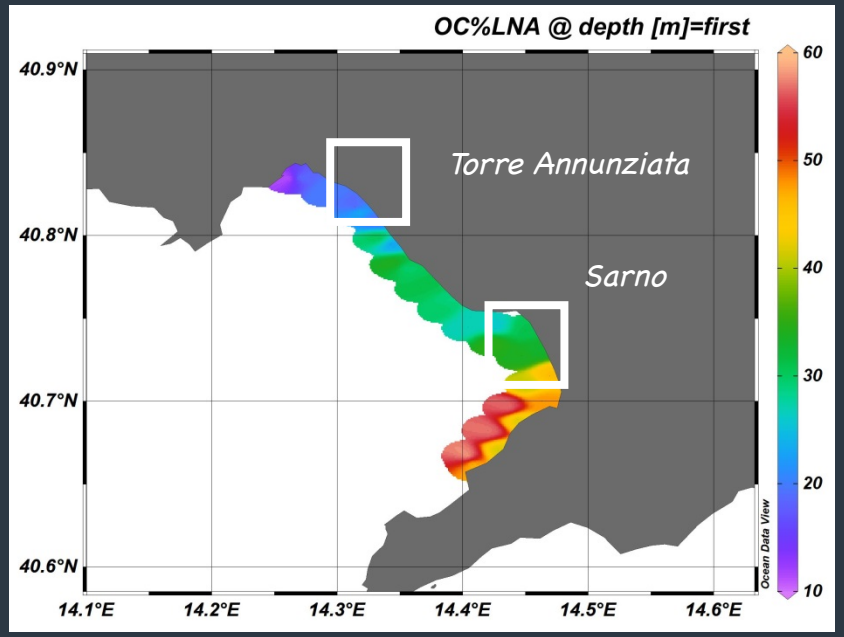

40.85°N
40.8°N
40.75°N
40.7°N
40.65°N
14.25°E 14.3°E 14.35°E 14.4°E 14.45°E 14.5°E
Ocean Data View


OCBact/ml @ depth [m]=first
Torre Annunziata
Sarno
40.9°N
40.8°N
40.7°N
40.6°N
14.1°E 14.2°E 14.3°E 14.4°E 14.5°E 14.6°E
3e+06
2.5e+06
2e+06
1.5e+06
1e+06
Ocean Data View


OC%HNA @ depth [m]=first
Torre Annunziata
Sarno
40.9°N
40.8°N
40.7°N
40.6°N
14.1°E 14.2°E 14.3°E 14.4°E 14.5°E 14.6°E
90
80
70
60
50
40
Ocean Data View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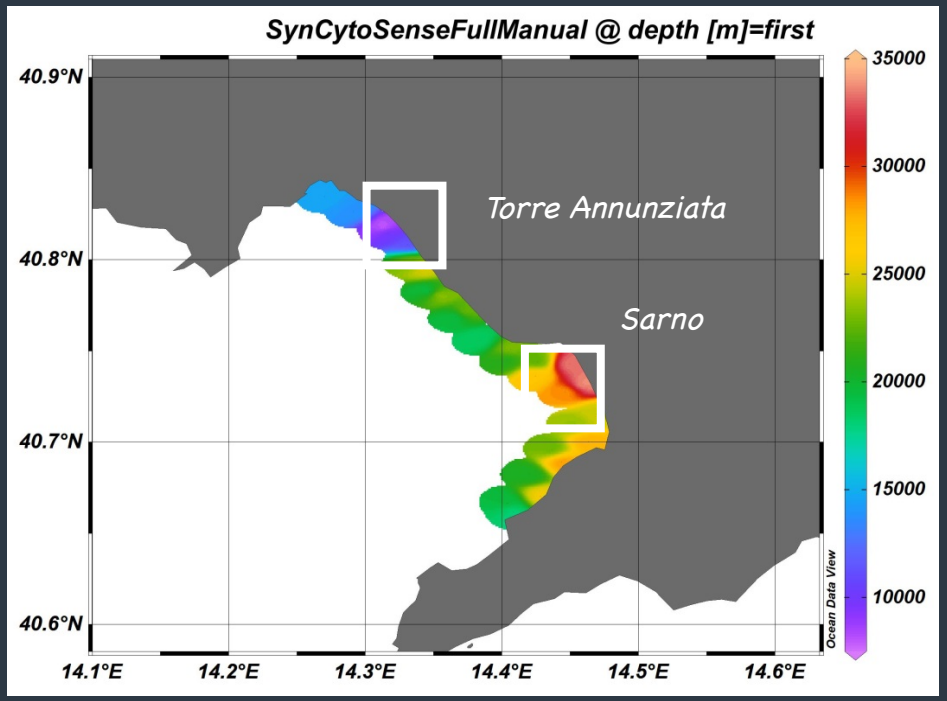
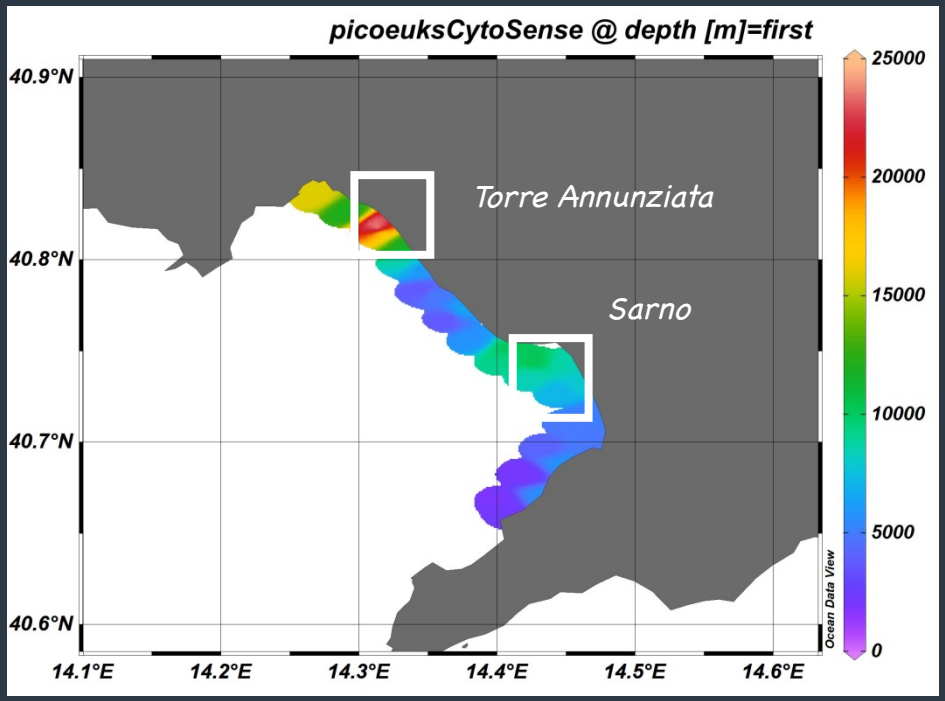
Torre Annunziata
Sarno
40.9°N
40.8°N
40.7°N
40.6°N
14.1°E 14.2°E 14.3°E 14.4°E 14.5°E 14.6°E
60
50
40
30
20
10
Ocean Data View





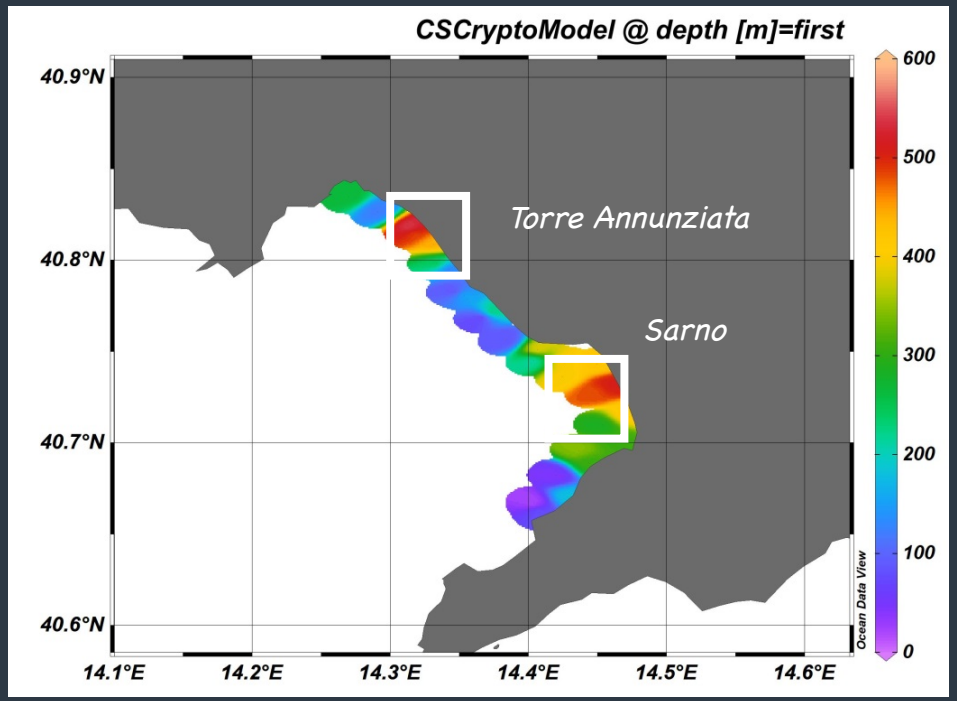


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Tabella 1 – Risultati delle analisi di microbiologia in campioni di acqua di mare prelevati in concomitanza con studi di citometria							
Campione	Coliformi totali MPN ml^{-1}	Escherichia coli MPN ml^{-1}	Enterococchi fecali MPN ml^{-1}	Salmonelle P/A ml^{-1}	Stafilococchi P/A ml^{-1}	Miceti UFC ml^{-1}	Carica microbica totale (Marine Agar) UFC ml^{-1}
T II Torre Annunziata	0	0	0	A	A	0	$2,4 \times 10^4 \pm 7,6 \times 10^3$
# 13 Sarno	0,8	1,3	0	A	A	6,0 x 10	$1,1 \times 10^5 \pm 1,4 \times 10^4$

MPN = Most Probable Number; UFC = Unità Formanti Colonia; P/A = Presenza/Assenza; coag. + = positività prova enzima coagulasi.

Contaminazione fecale recente nella zona del Sarno

Data courtesy Dr. Milva Pepi, Stazione Zoologica Anton Dohrn

CONCLUSIONS

- Single cell methods like flow cytometry provide unique information not obtained with other methods.
- Spatial and vertical studies allow to observe the changes on plankton compartment as reaction to environmental changes.
- High frequency flow cytometry proves to be effective and calibrated against conventional flow cytometry.
- Easy to use also connected to other sensors.
- Use as a first-level alert system.
- Real-time data for sanitary alerts.
- Couple with classical microbiology to support sanitary risk assessment.

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CONCLUSIONS/ FUTURE PERSPECTIVE

- Automated high frequency flow cytometry proves to be a fast method for detection of marine microbes, allowing real-time measurements of cell concentrations to be correlated with environmental factors, in order to be interpreted and potentially used as markers of water quality.

- Microbial communities, in turn, appear to be useful markers of changed environmental conditions, that need to be considered and included in water quality assessment such as those sought by the European Directive 2008/56/EC, which aims at reaching a Good Environmental Status (GES) for all European waters within 2020.


Grazie