Flow cytometric retrieval of in situ $\delta^{13}C$ signatures of natural phytoplankton populations using pyrolysis-GC-IRMS: a study of pelagic C-flow

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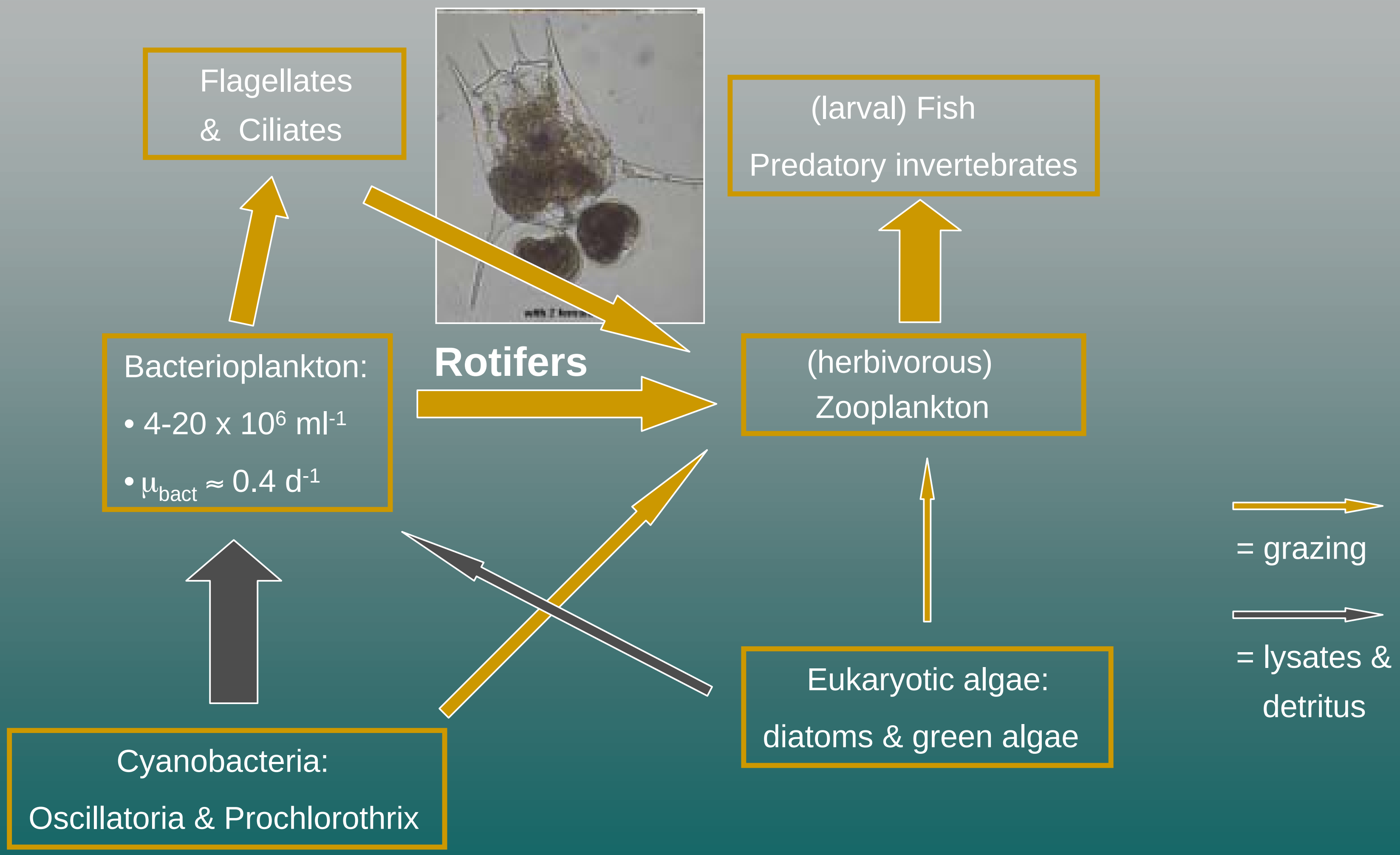
Cyanobacteria-dominated shallow lakes in the Netherlands:
(predominantly filamentous species: - Oscillatoria and Prochlorophytes)

- Use of flow sorting to enhance the resolution of stable isotope analysis in aquatic food web studies: ⇒ retrieval of true *in situ* ^{13}C signals of C-sources
- Relative importance of cyanobacterial and algal-derived carbon in diets of zooplankton: ⇒ assessment of the effects of lake restoration measures

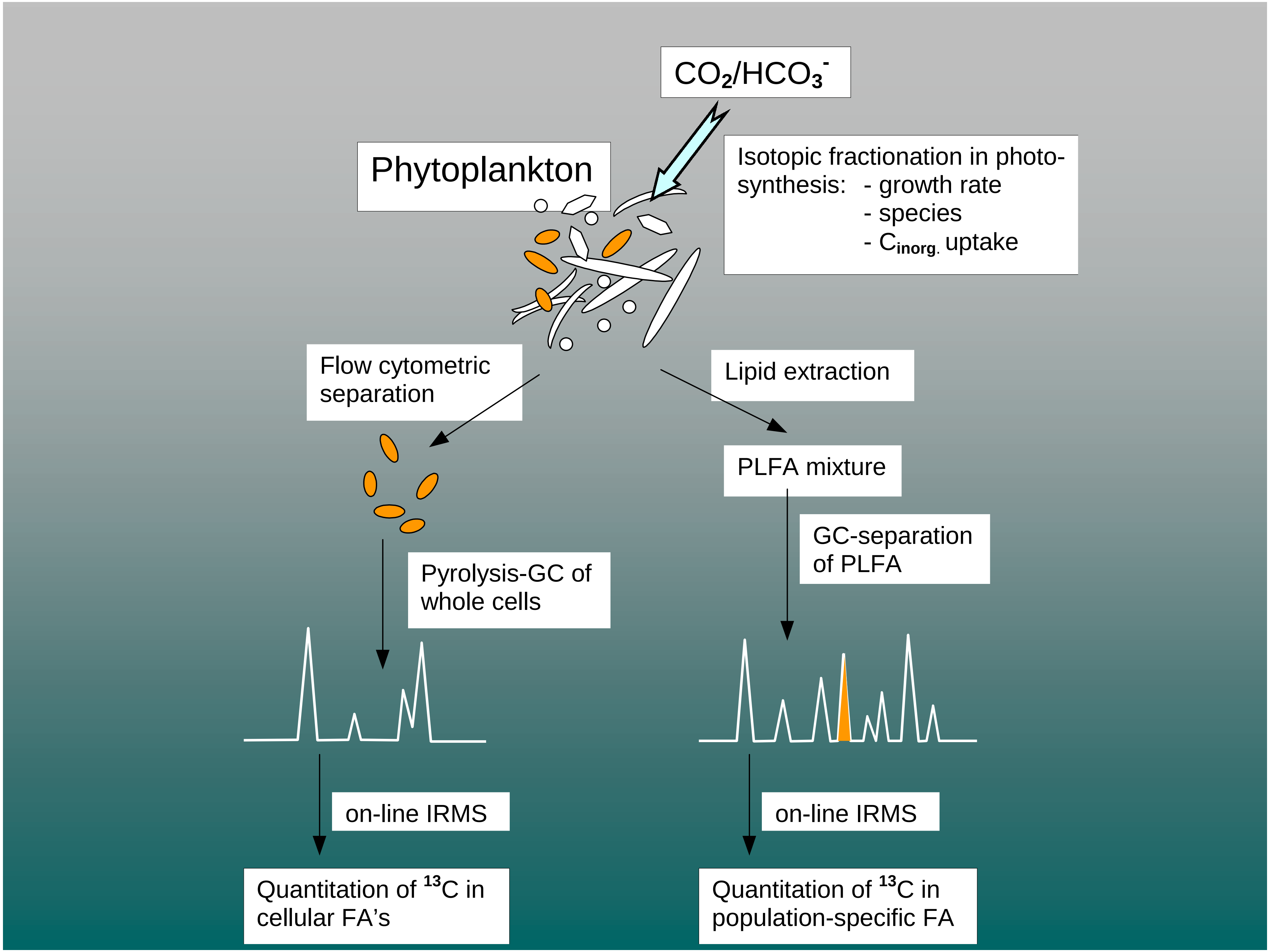
Carbon pathways in cyanobacteria-dominated shallow Lakes



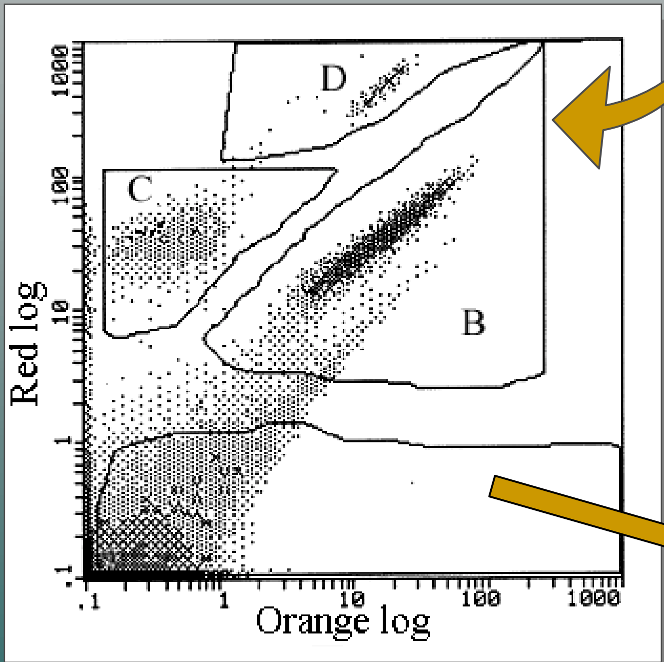
Observations on the pelagic of L. Loosdrecht relating to the reduction of external phosphorus loading

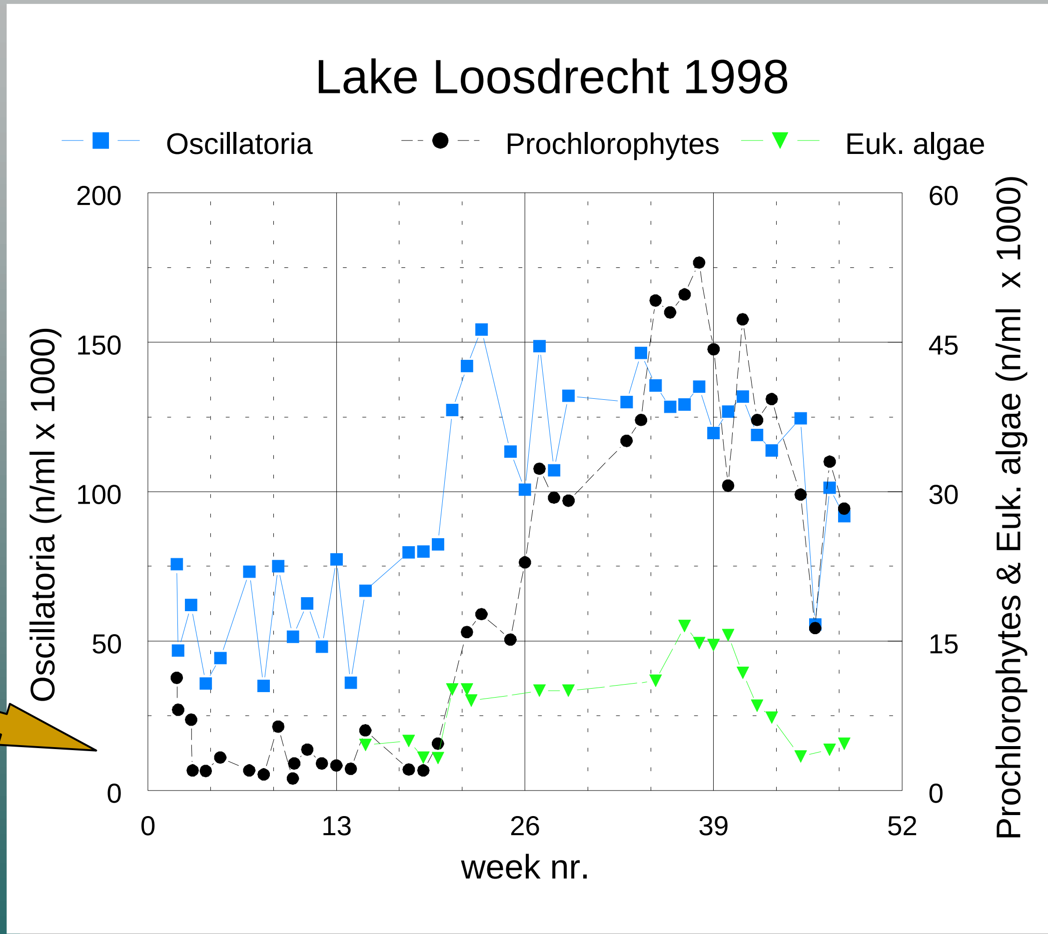
- Effects of reduction in P-loading (start 1984):

- total-P in 1983-1986: 110-140 μg/L; 1987-1990: ± 90 μg/L; from 1992: 50-60 μg/L.
- average spring/summer Chlorophyll: 110-160 μg/L → 50-70 μg/L
- transparency: 0.4 - 0.5 m (waterplants did not return)
- resilience of cyanobacterial community: filament density remained at ~2 x 10^5 per ml


CO_2/HCO_3^-
Phytoplankton
Isotopic fractionation in photo-synthesis: - growth rate
- species
- $C_{inorg.}$ uptake
Flow cytometric separation
Lipid extraction
PLFA mixture
GC-separation of PLFA
Pyrolysis-GC of whole cells
on-line IRMS
on-line IRMS
Quantitation of ^{13}C in cellular FA's
Quantitation of ^{13}C in population-specific FA

Flow cytometric detection and enumeration of phytoplankton taxa

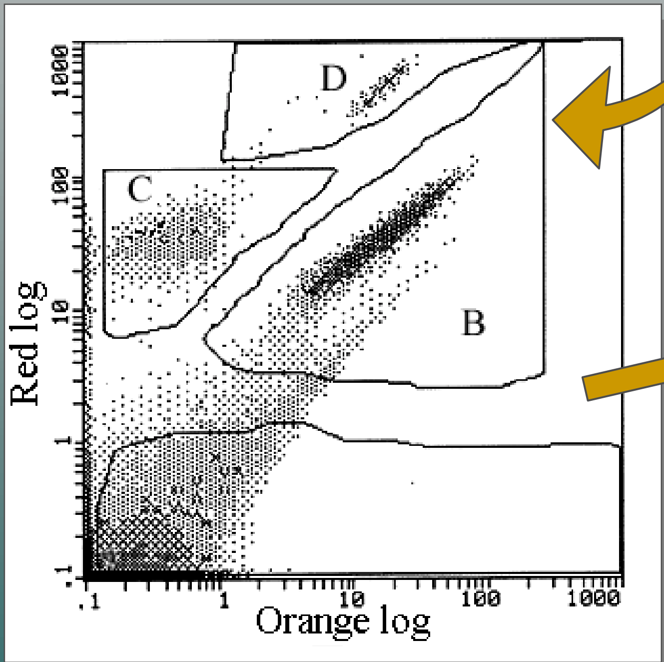




pigment exitation at 488 nm:

- Chlorophyll *a*: red
- Phycoerythrin: orange

Flow cytometric detection and sorting of target cells



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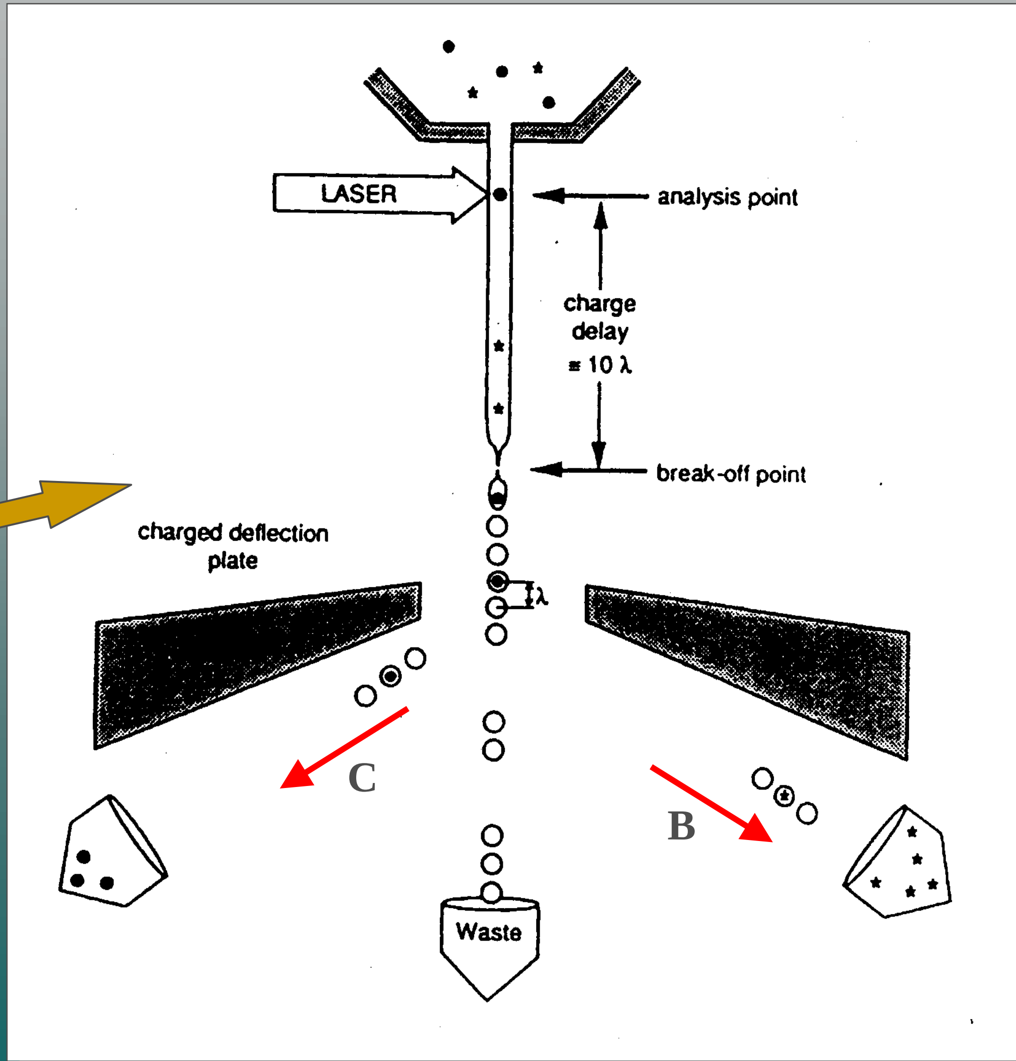
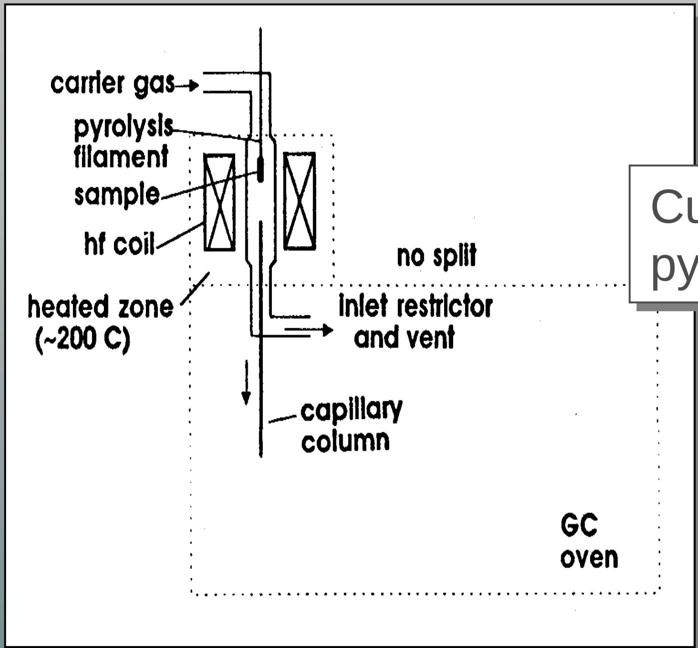
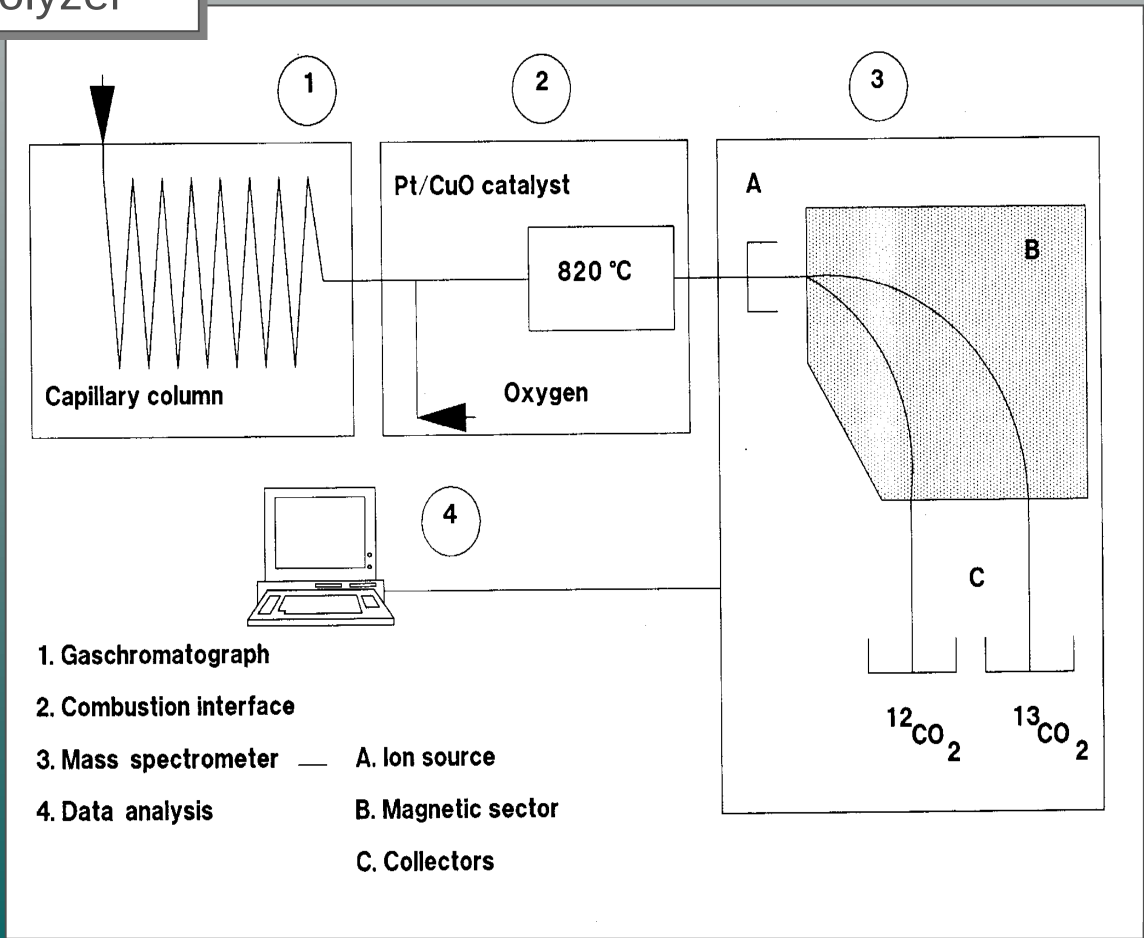


Sample introduction in GC-c-IRMS using Curie-point pyrolytic methylation



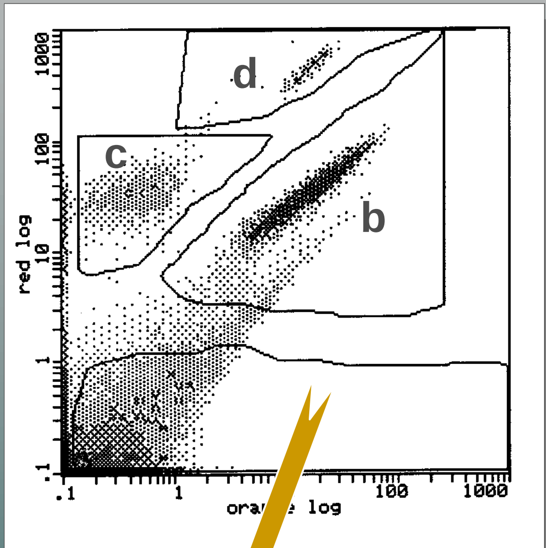
Curie-point pyrolyzer

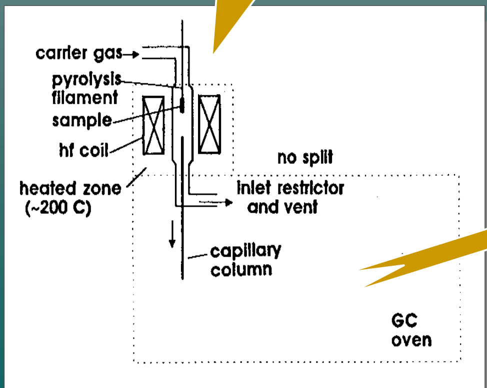


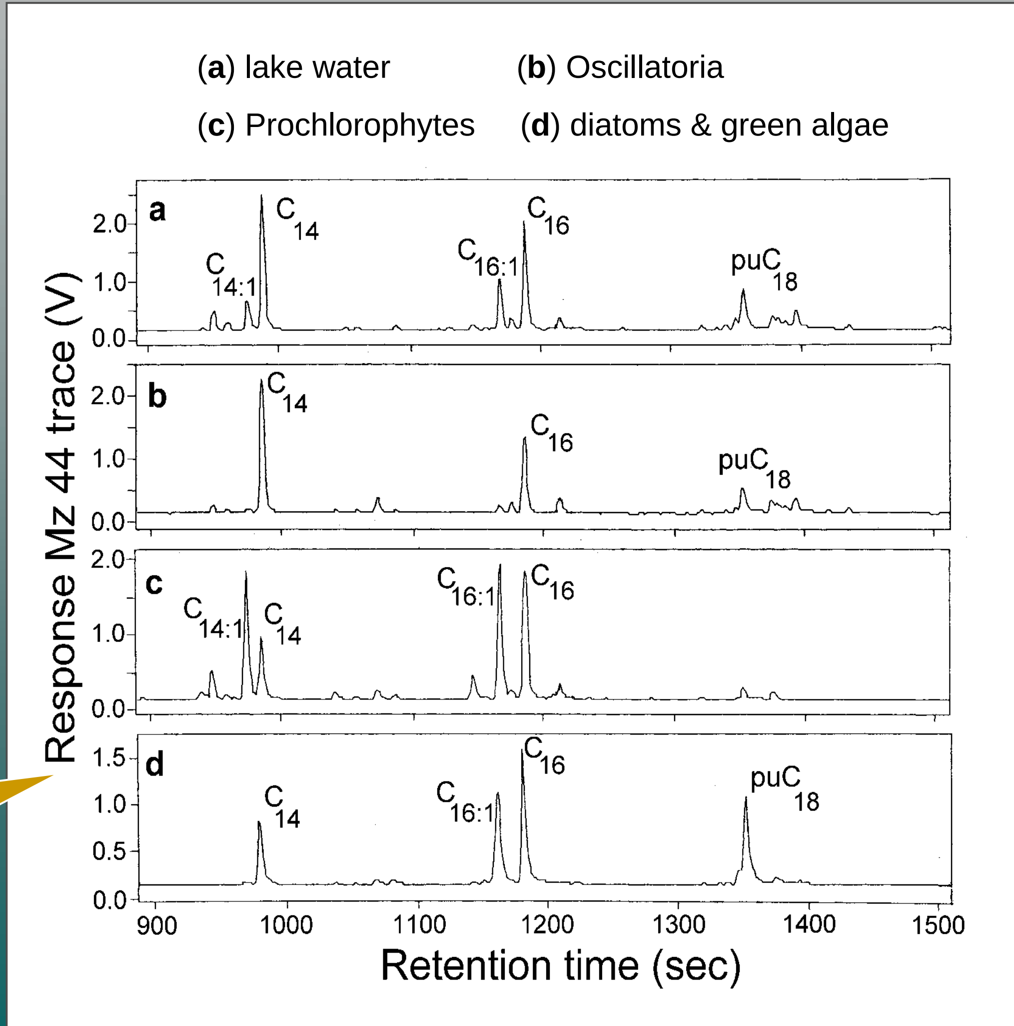
Pyrolytic methylation of cellular FAs:

- application of whole cells and CH_3-donating reagent
- volatilization of FAs at ~500 ^oC

Flow cytometric sorting and reproduction of FA-profiles from target populations by pyrolytic methylation

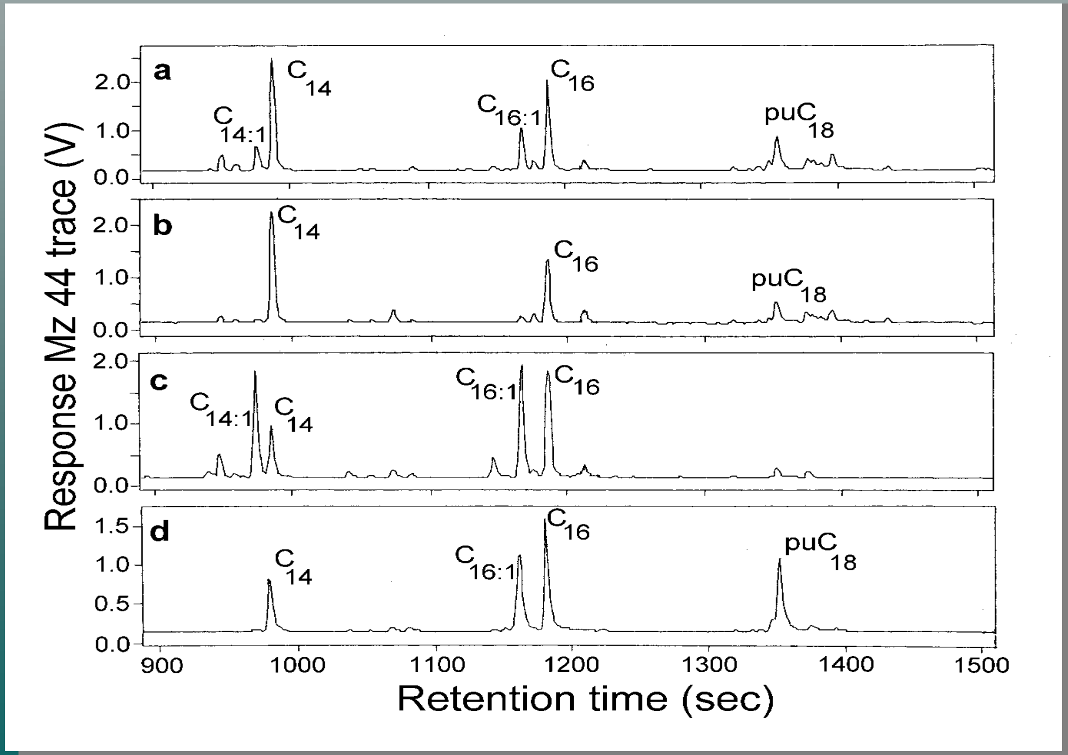




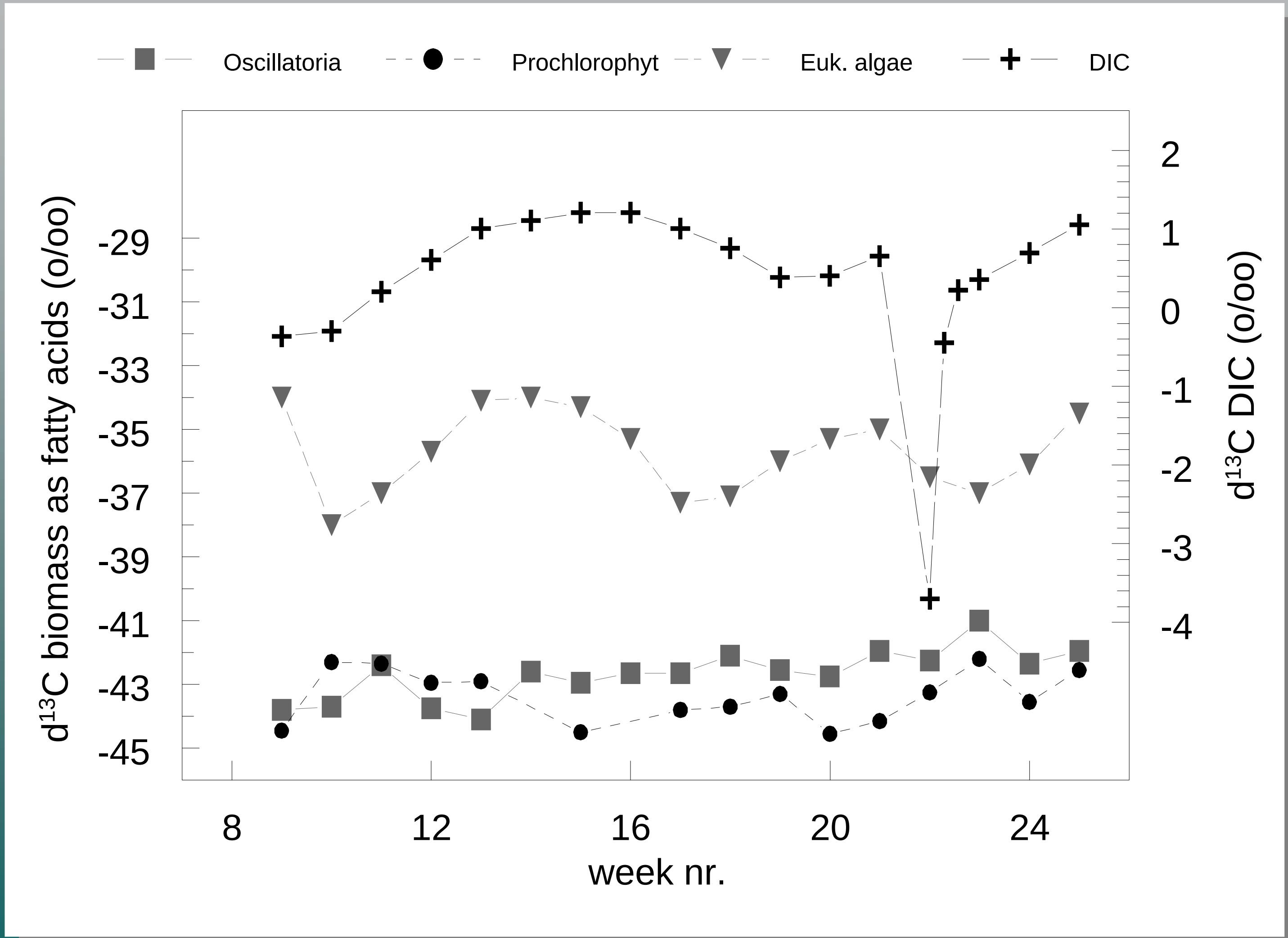


$\delta^{13}C$ values of individual fatty acids present in retrieved lipid profiles

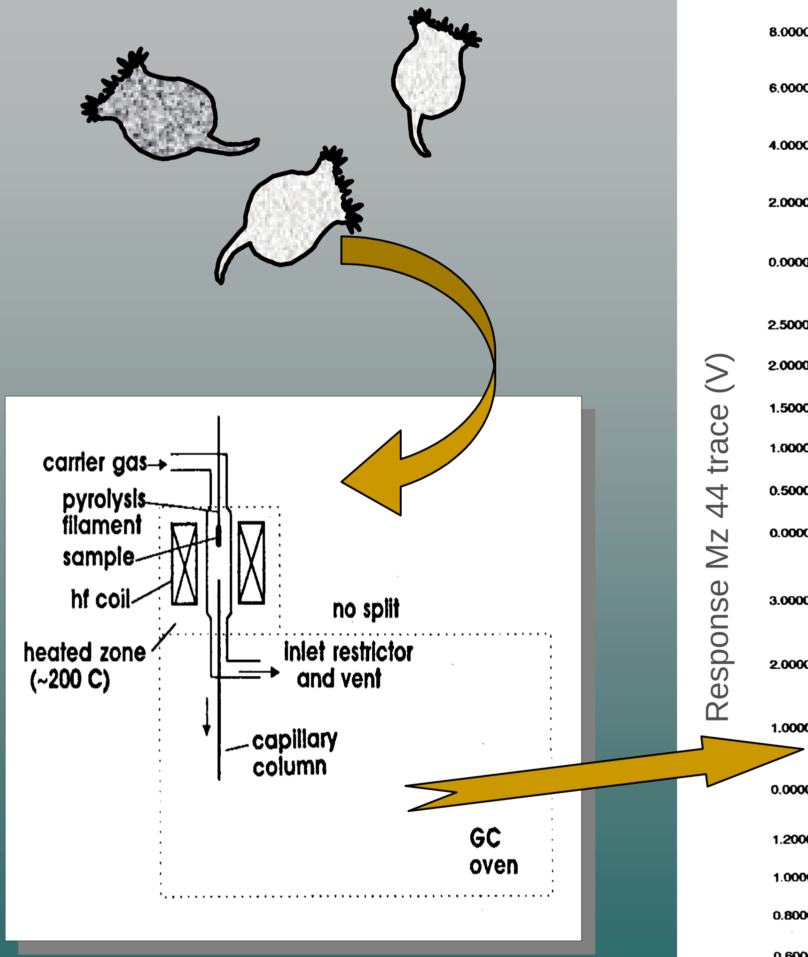
	$\delta^{13}C$ of signature fatty acids (‰)				
	$C_{14:1}$	$C_{14:0}$	$C_{16:1}$	$C_{16:0}$	puC_{18}
(a) lake water	-42.6	-42.2	-40.6	-41.4	-42.1
(b) Oscillatoria	nd	-42.6	tr	-42.1	-42.8
(c) Prochlorophyt	-43.4	-43.3	-45.7	-44.8	tr
(d) Euk. algae	nd	-34.9	-35.3	-34.0	-35.3

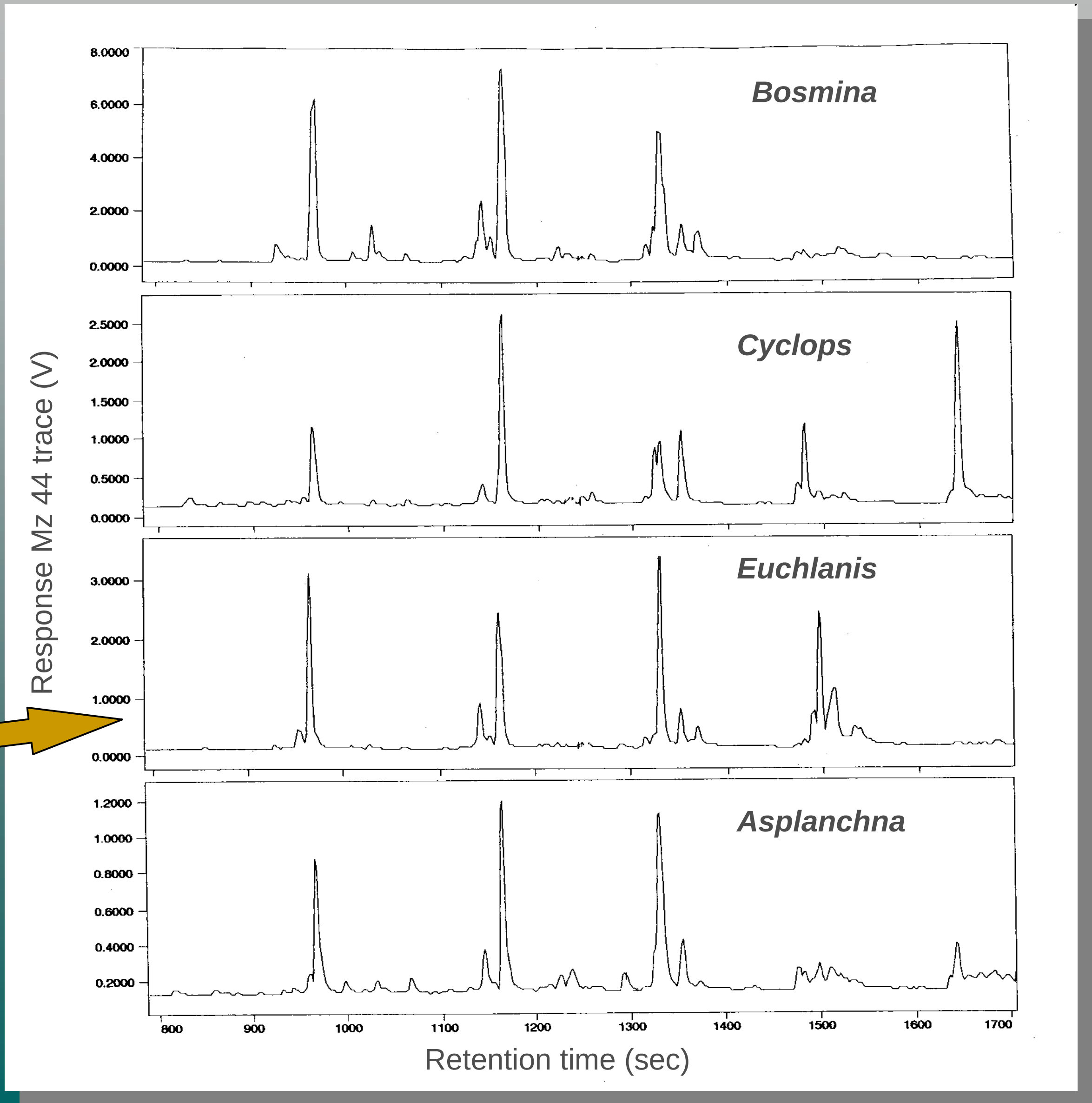


Natural ^{13}C abundance of phytoplankton in Lake Loosdrecht

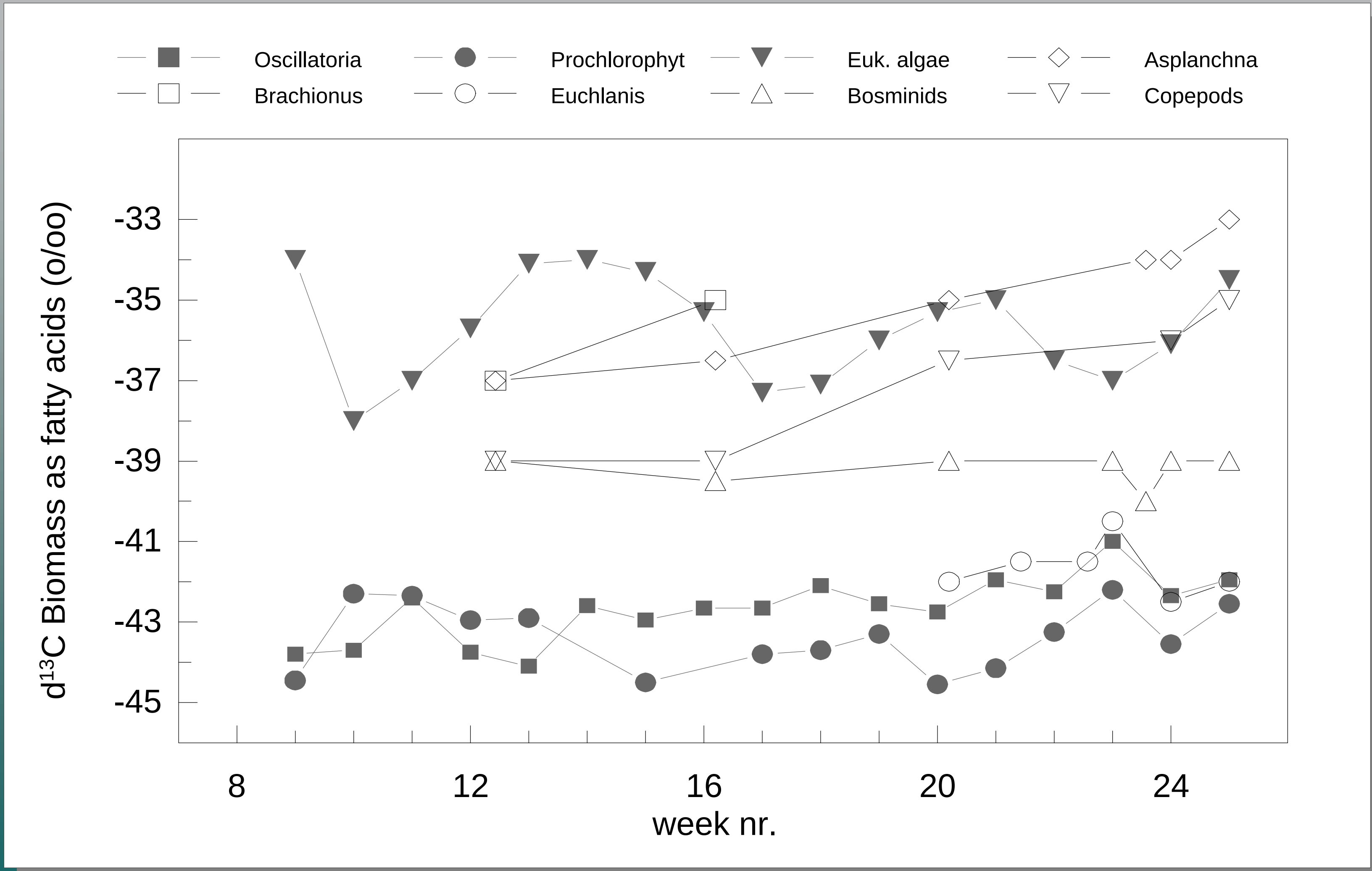


Fatty acid profiles of zooplankton by pyrolytic methylation



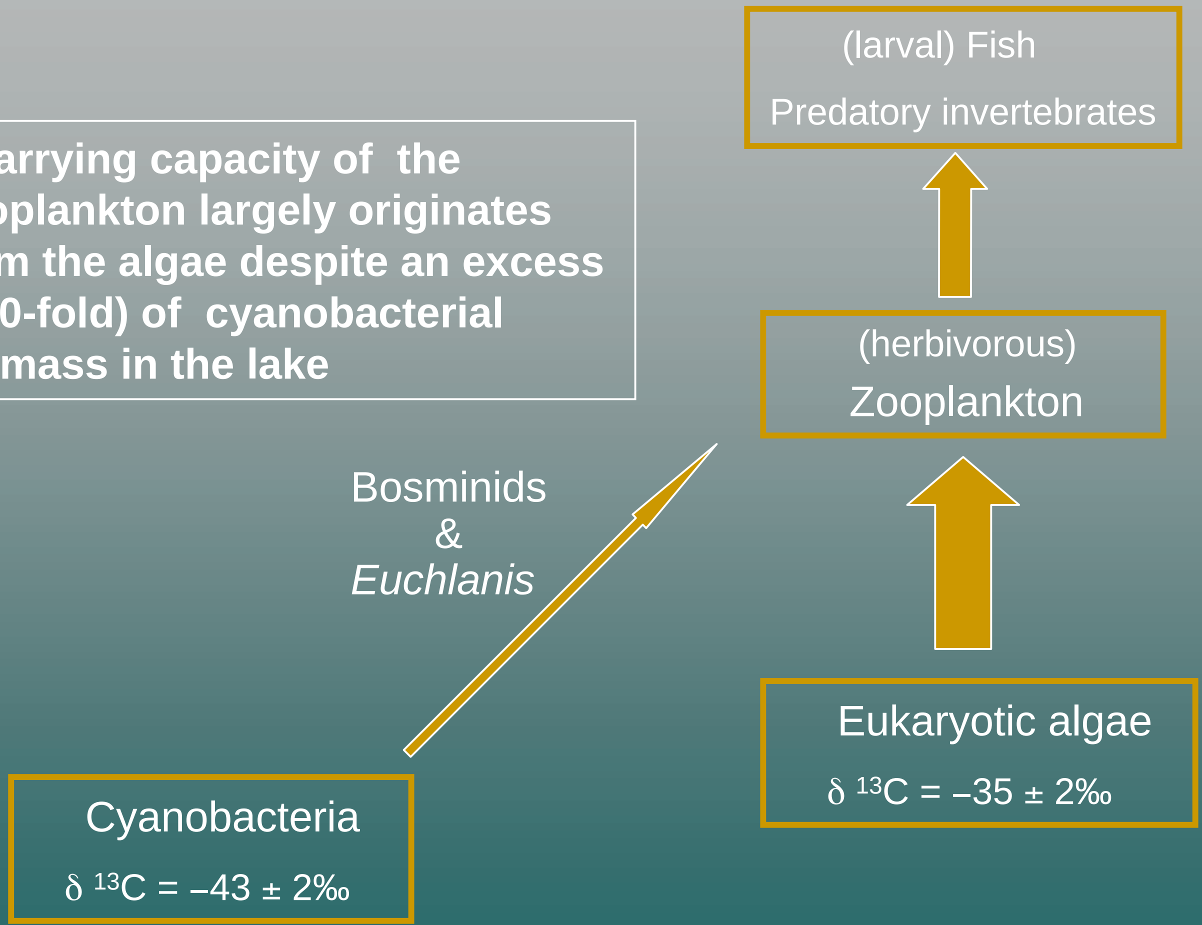


Natural ^{13}C abundance of phyto- and zooplankton in Lake Loosdrecht



Carbon pathways in Lake Loosdrecht

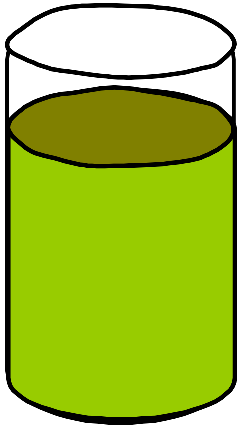
- **Carrying capacity of the zooplankton largely originates from the algae despite an excess (~50-fold) of cyanobacterial biomass in the lake**



Assessment of phytoplankton *in situ* growth rates

light regime:

- L : D ≈ 17h : 7h
- daytime light source 15’ on 15’ off to simulate circulation of water column

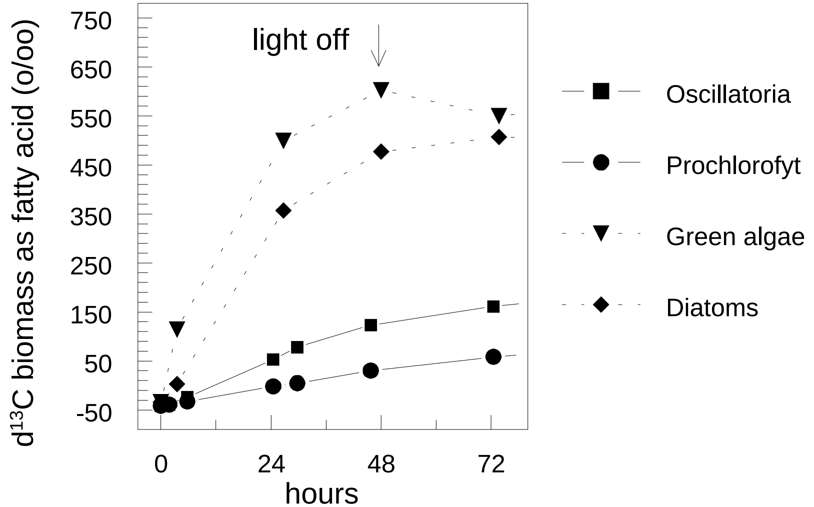


Enclosure:

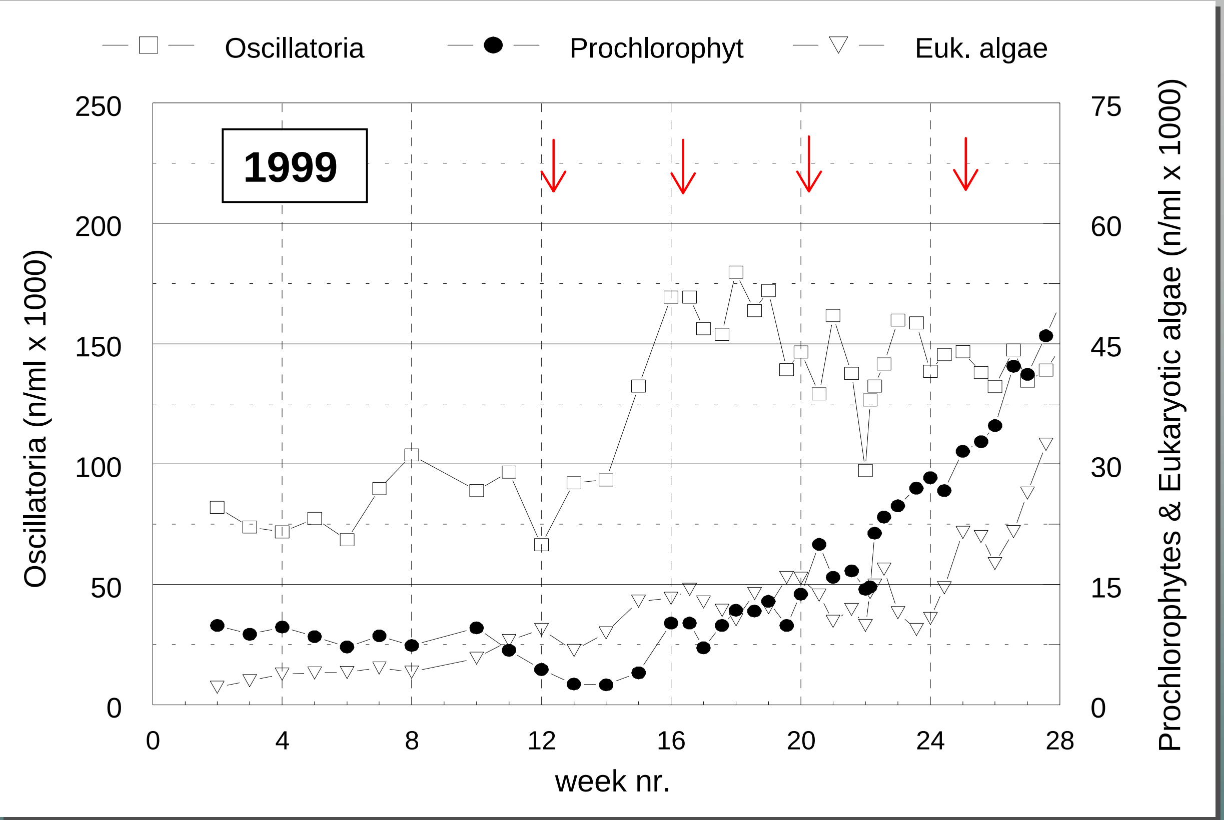
- intact lake water (1.5L)
- *in situ* temperature
- pH ambient (≈ 8.0)
- δ ^{13}C of CO_2/HCO_3^- raised to ca.1300 ‰

Target populations for FCM sorting and pyrolytic methylation:

- Oscillatoria
- Prochlorophytes
- Diatoms
- Green algae



Population dynamics of L. Loosdrecht phytoplankton



Growth rates (d^{-1}) of phytoplankton using ^{13}C-CO_2 lake water enclosure

	March 25	April 20	May 18	June 22
Oscillatoria	0.033	0.031	0.05	0.08
Prochlorophytes	0.014	0.021	0.023	0.046
Diatoms	0.14	0.12	0.15	0.33
Green algae	nd	0.14	0.2	0.43

Conclusions:

• Co-occurrence of phytoplankton populations widely differing in isotopic composition and turnover rates, despite mixed water column:

⇒ **phenomenon of general nature? Yes**

• The zooplankton community in Lake Loosdrecht is largely sustained by algal-derived carbon ($\delta\ ^{13}C \approx -35$ ‰) in spite of the abundance of cyanobacterial biomass ($\delta\ ^{13}C \approx -43$ ‰):

⇒ **'bulk' proxies for phyto- and zooplankton ^{13}C signatures (e.g. chl *a* extracts, filter fractions) easily obscure food-web relations**

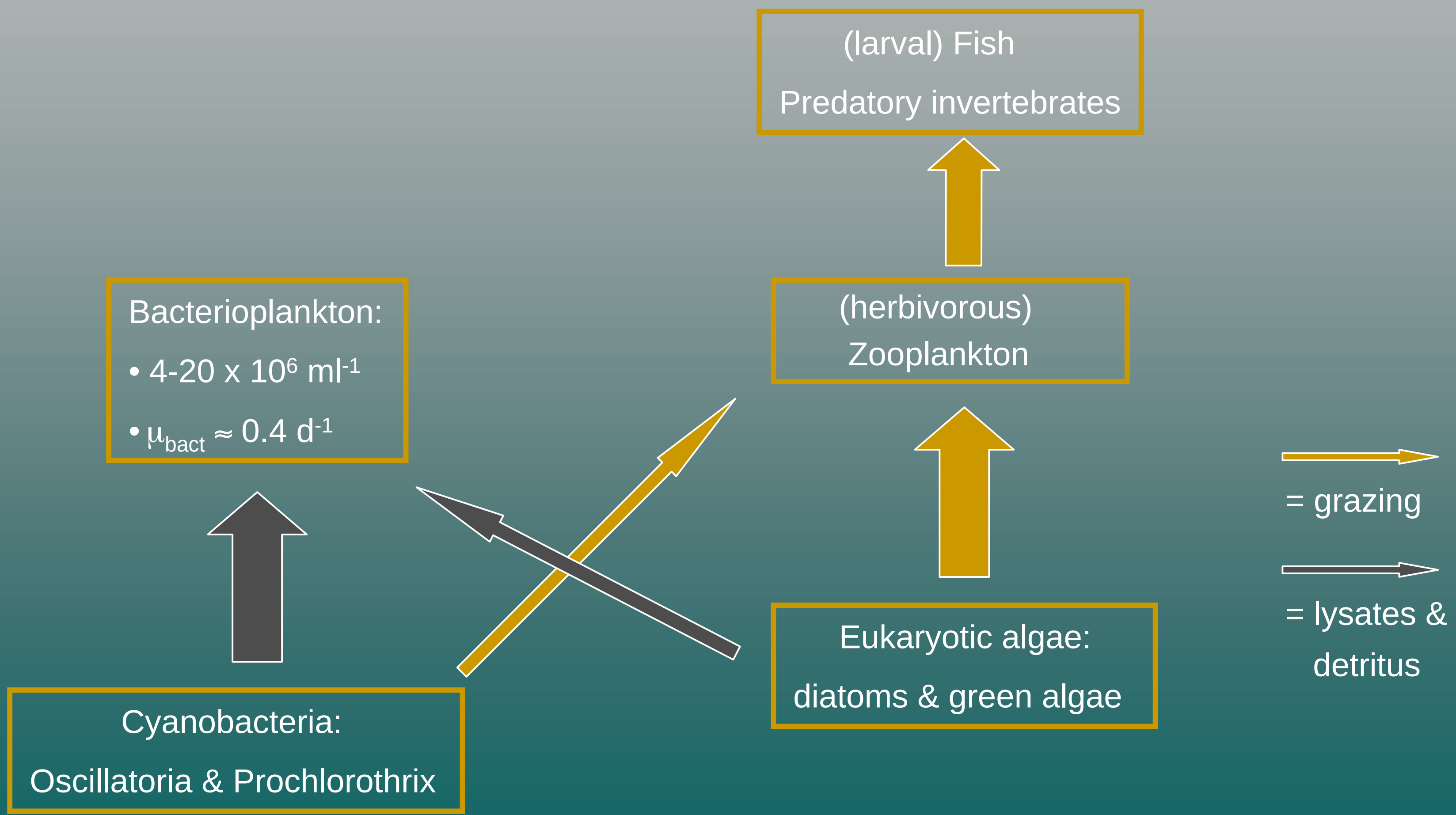
• Bacteria are no important supplemental food source to the zooplankton in lake Loosdrecht:

⇒ **Cyanobacterial-derived carbon is respired in cascading events of mineralization, ultimaltely leading to P-regeneration**

• The flow cytometric retrieval of population-specific ^{13}C signals is a promissing new approach in the analysis of plankton community structure and functioning.

⇒ **monitoring of anthropogenic or climate-mediated stresses on pelagic food webs by a detailed probing of C-transfer**

Carbon pathways in Lake Loosdrecht



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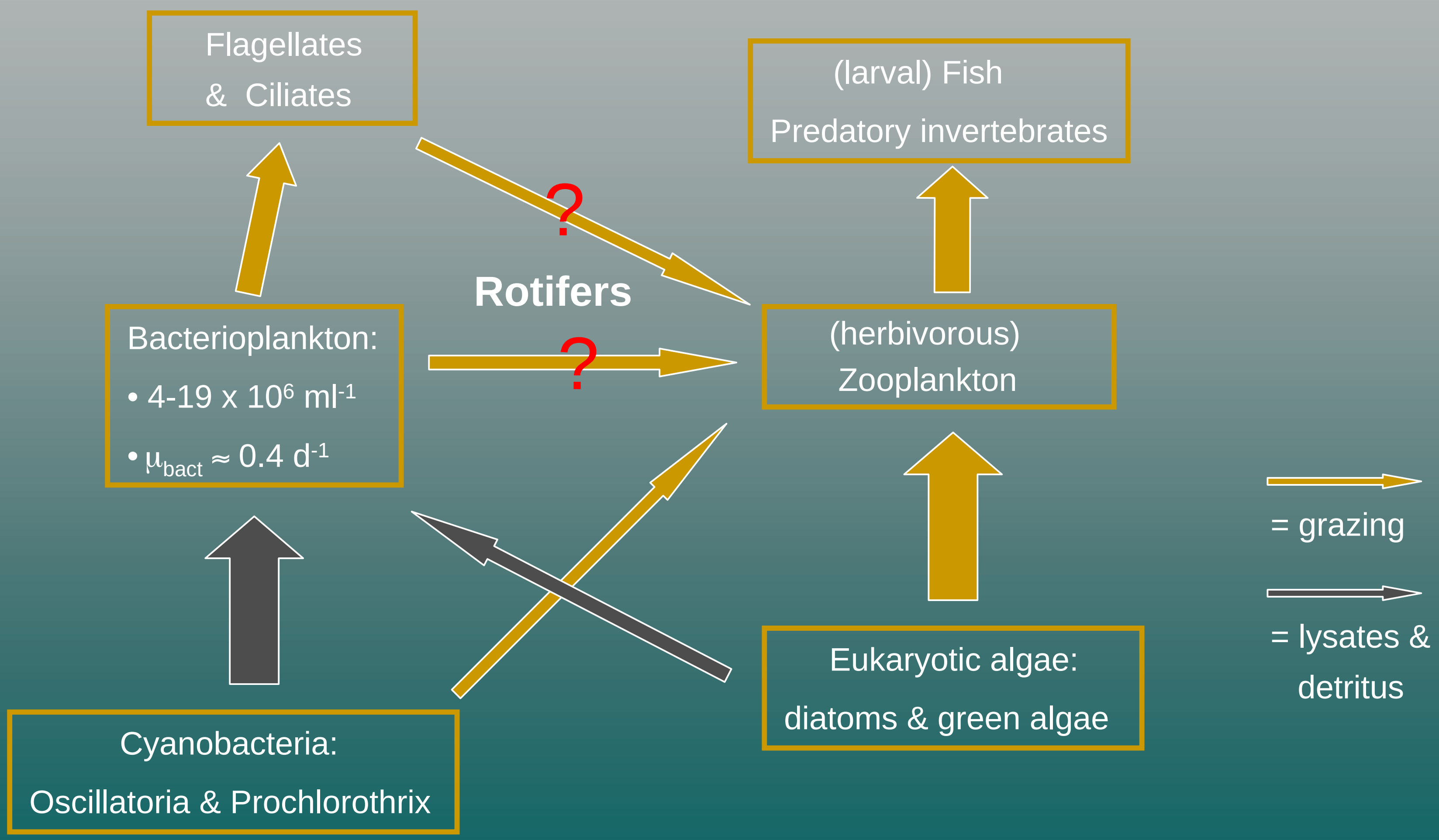

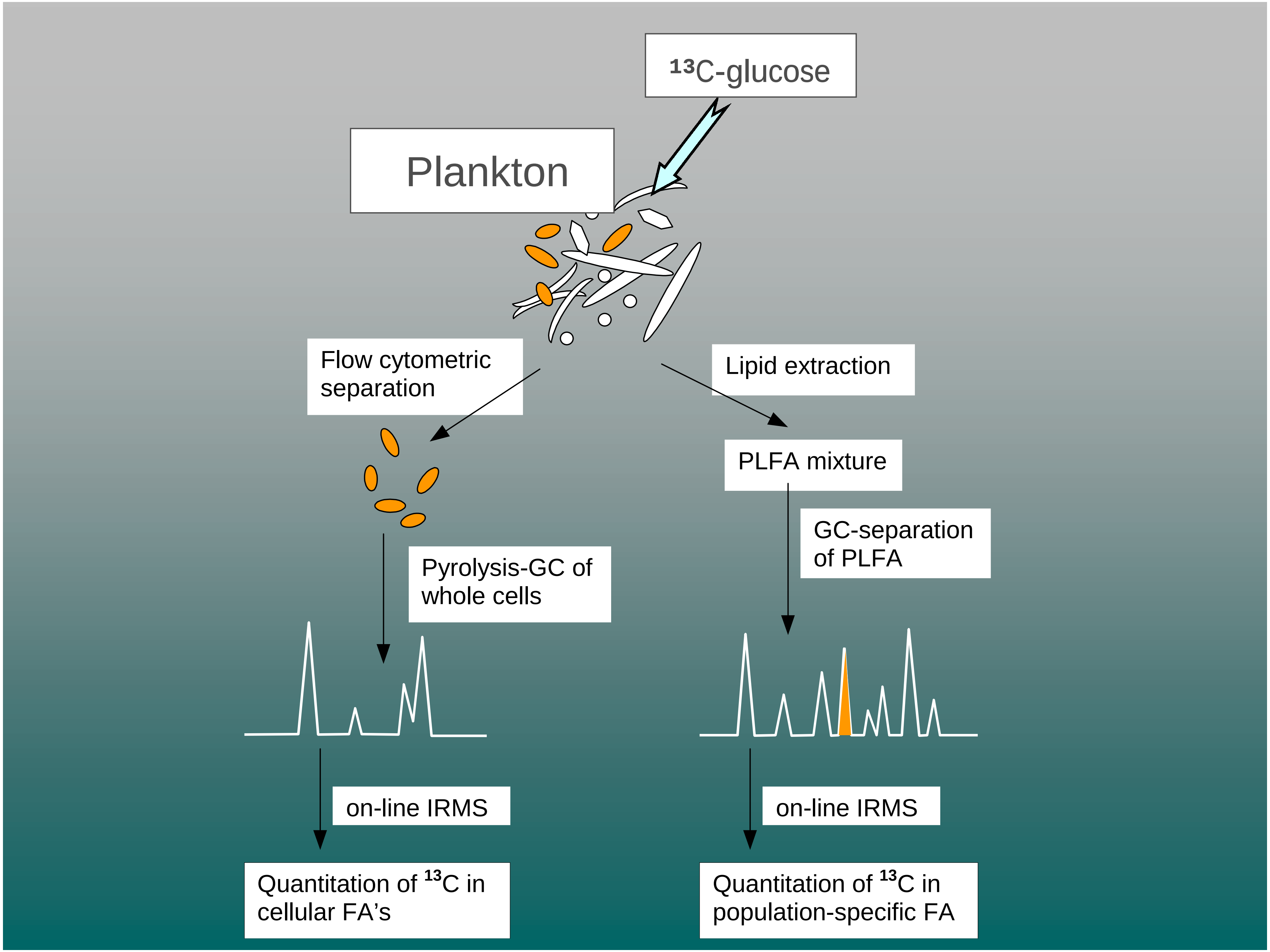

13C-glucose
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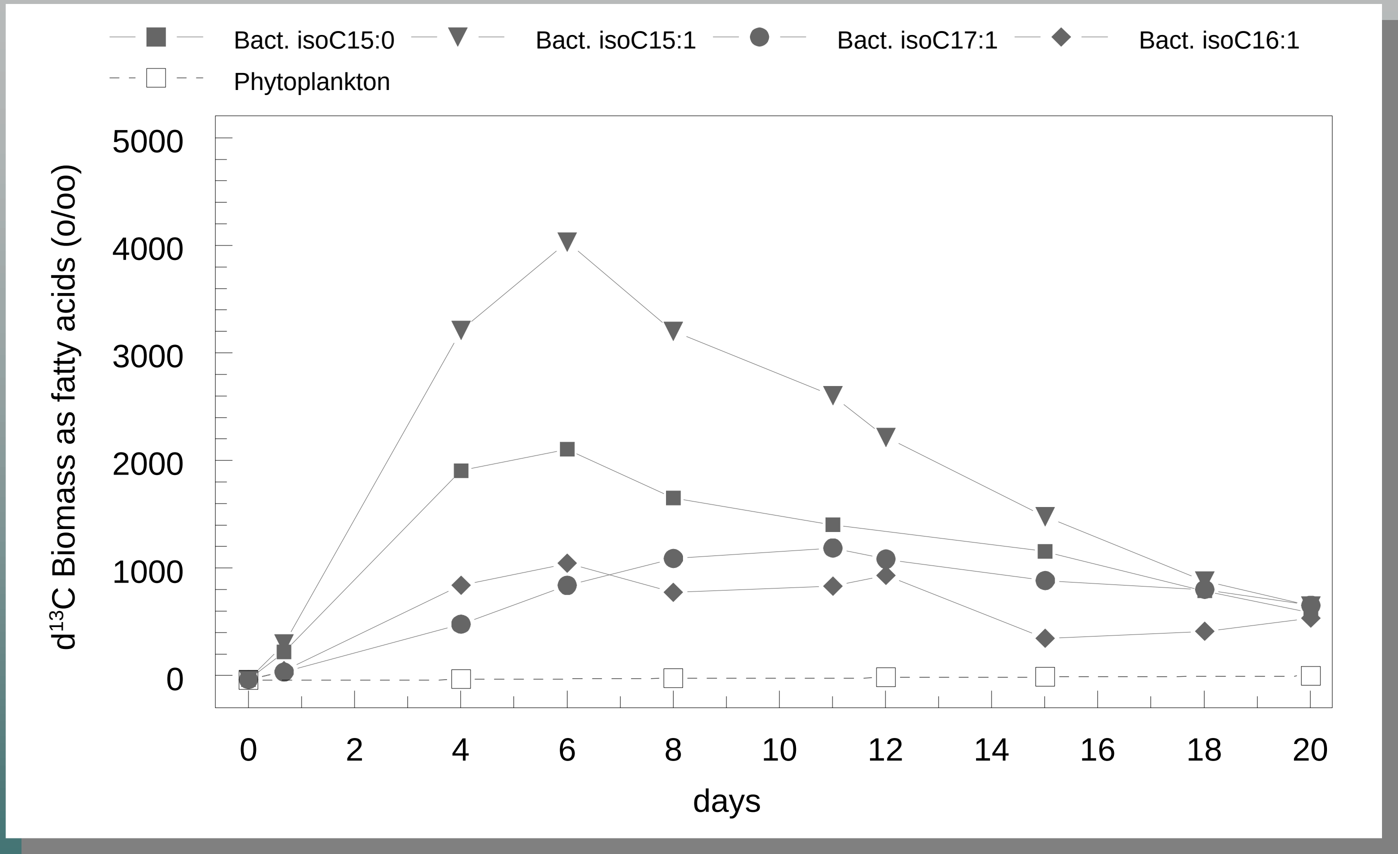
Differential labelling of phyto- and bacterioplankton by ^{13}C-glucose, and label transfer to zooplankton



Enclosure (1.4L):

- ^{13}C-glucose spiked lake water (~4µM)
- incubation at 14 ºC; pH 8.0 - 8.5
- L : D = 10 : 14 h; lamp 15' on/off
- at t=20 d ~70% of ^{13}C-label recovered from DIC

Retrieval of ^{13}C-signatures:

- FCM/py-IRMS: - Cyanobacteria
  - Algae
- IRMS: bacterial lipid markers
- py-IRMS: major zooplankton taxa

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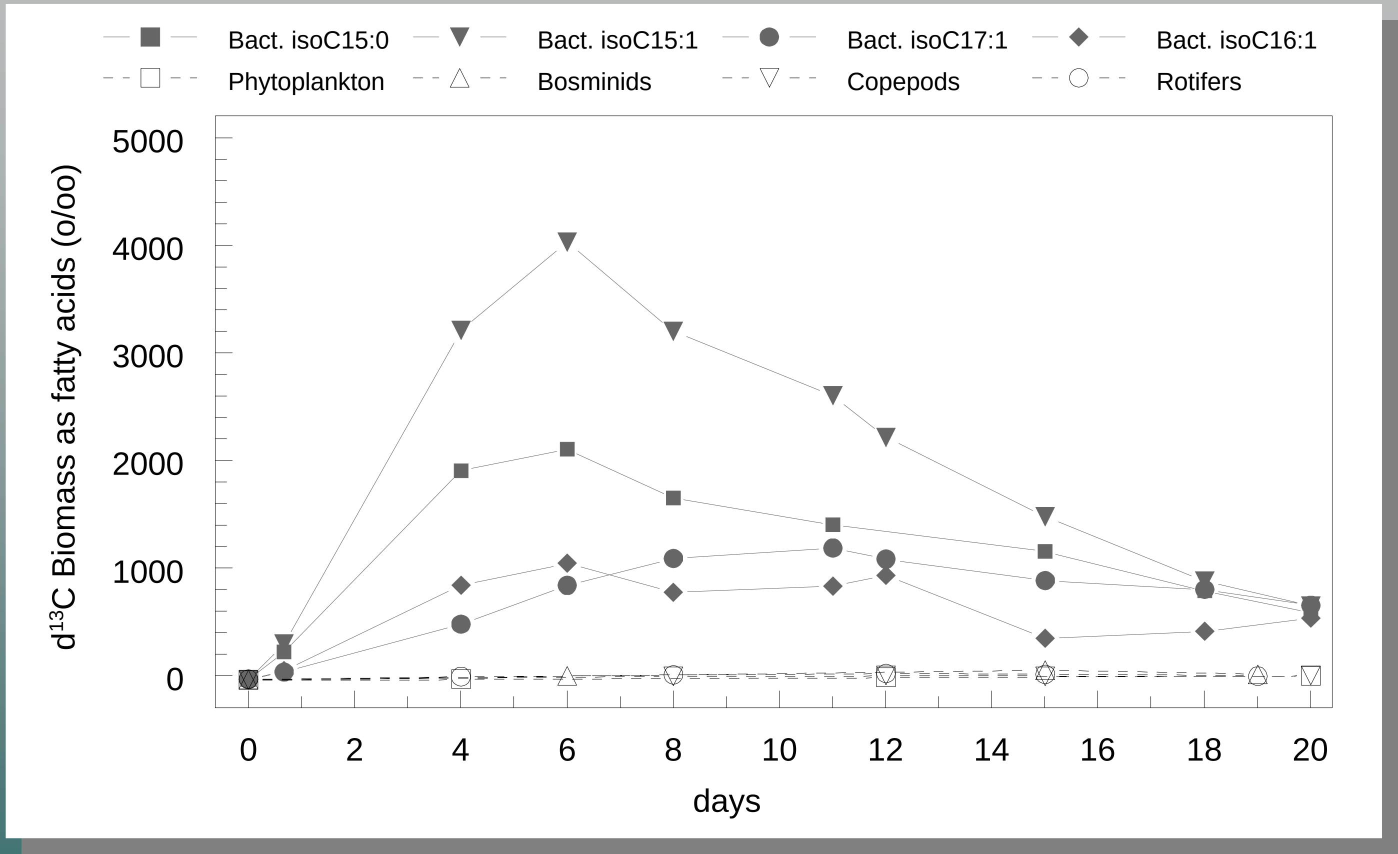

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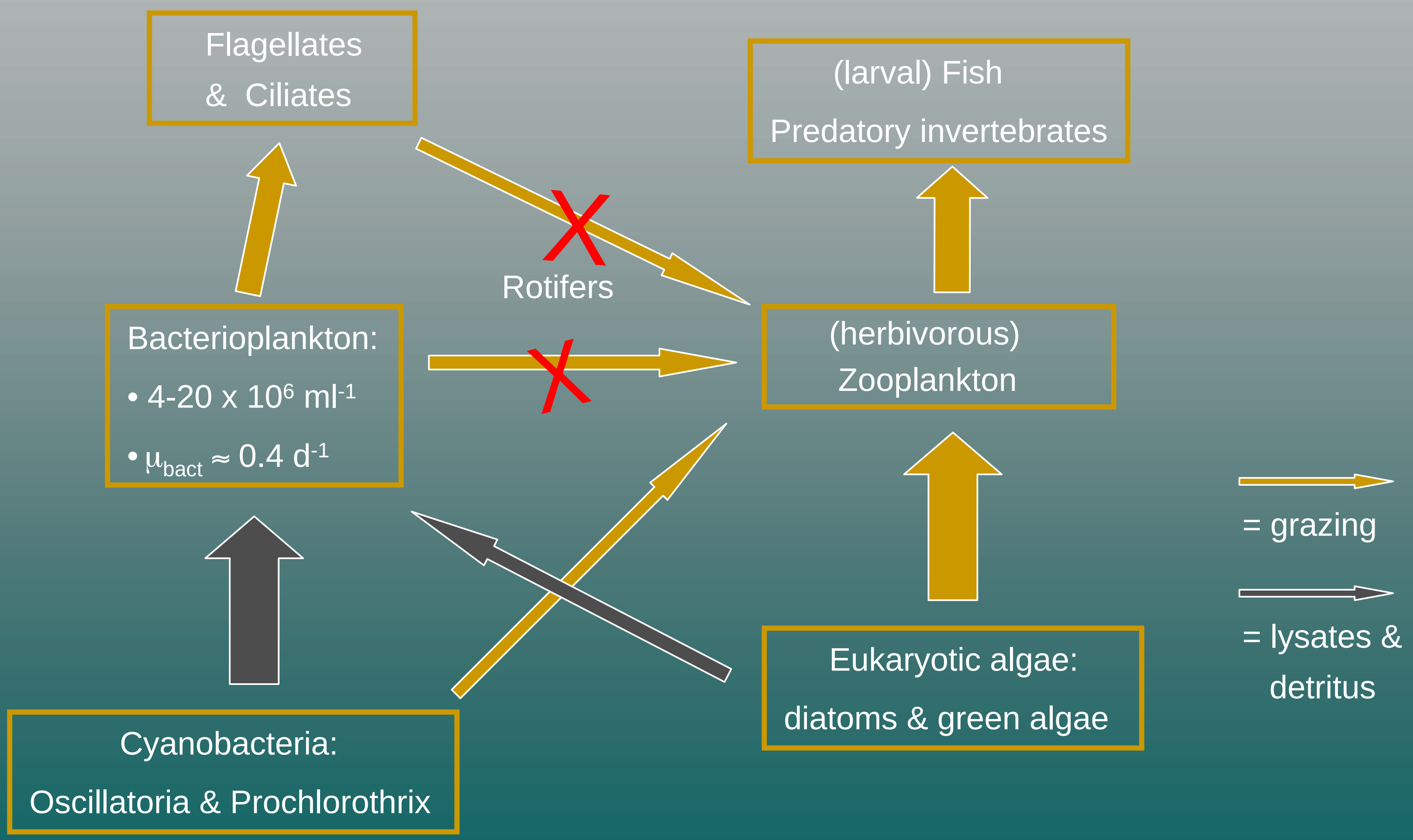

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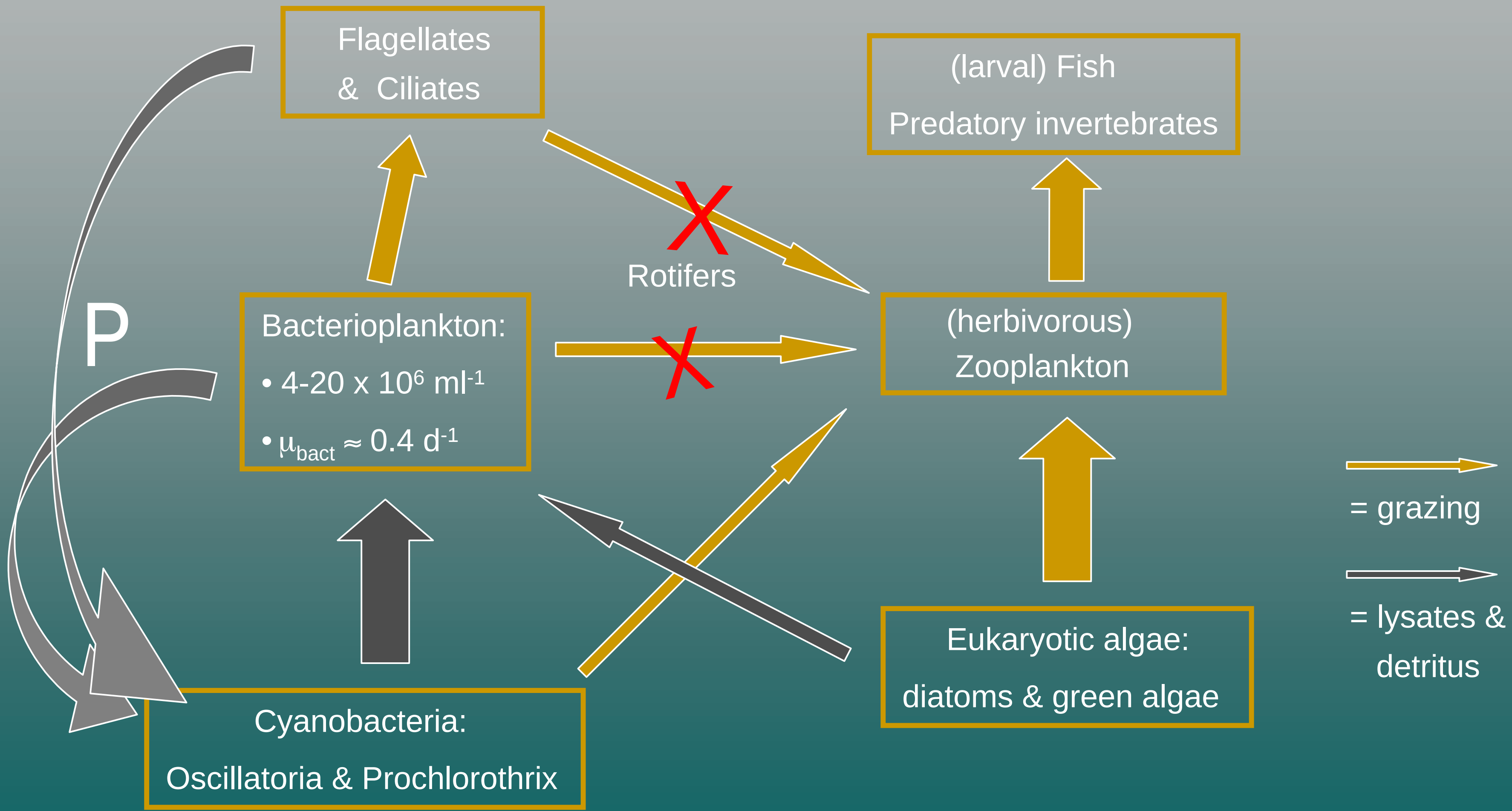
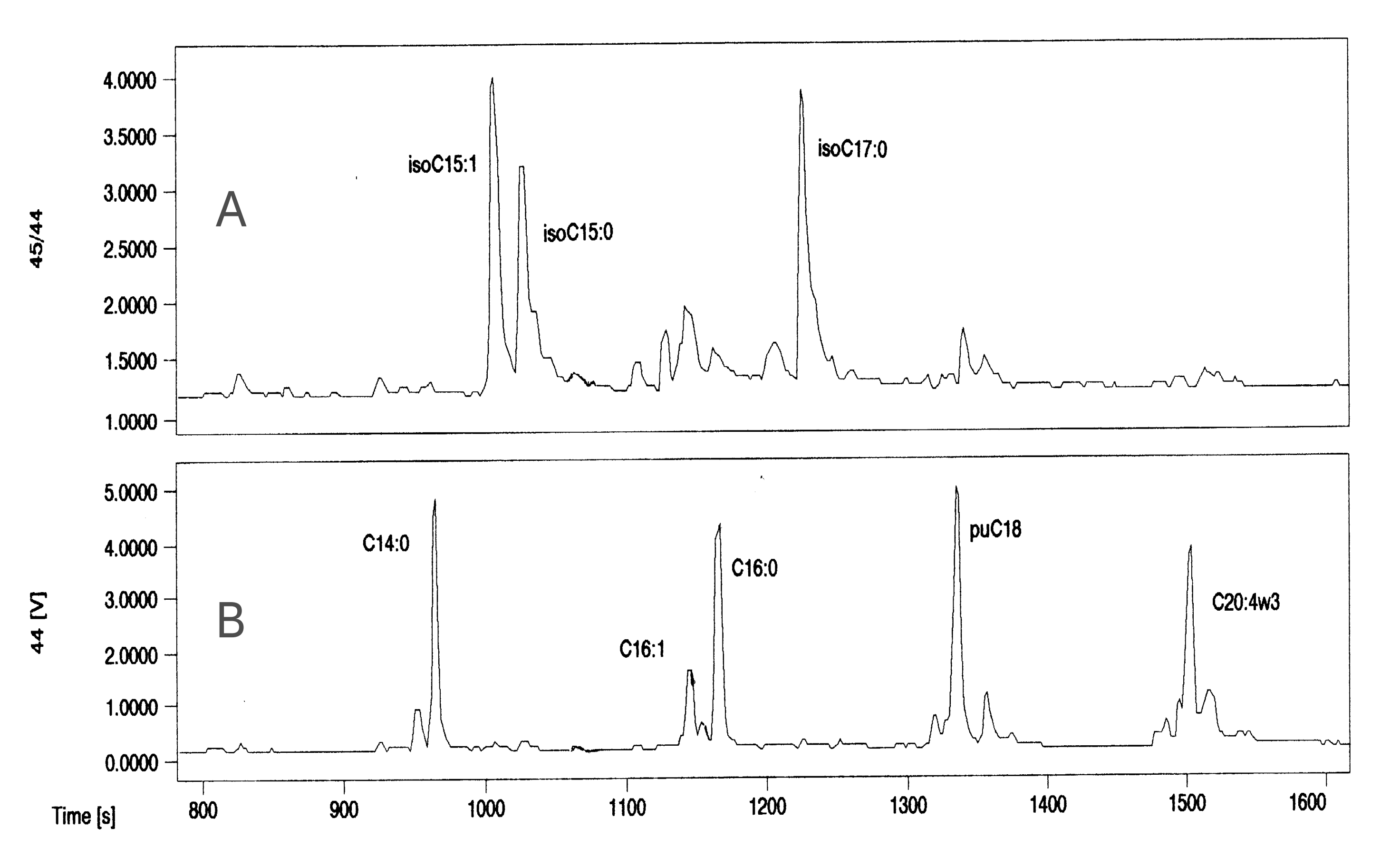


Respons *Euchlanis* FA-profile to ^{13}C-glucose labelling of bacterioplankton



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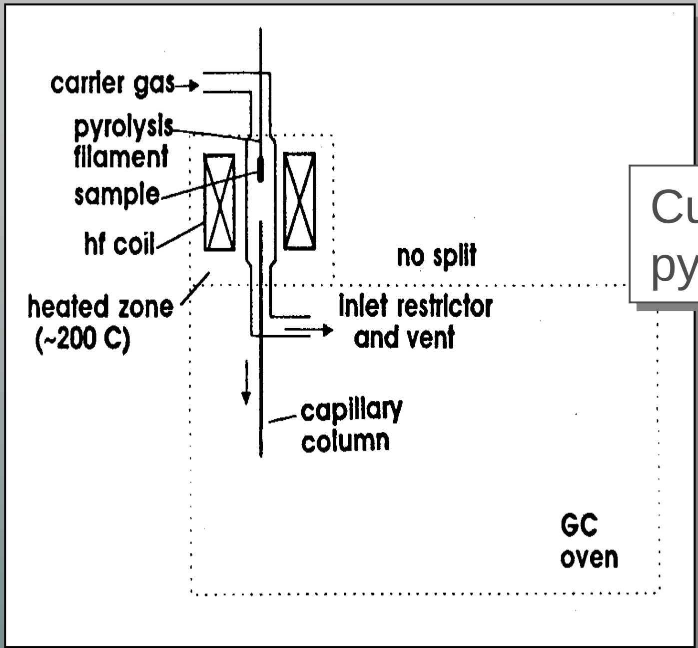

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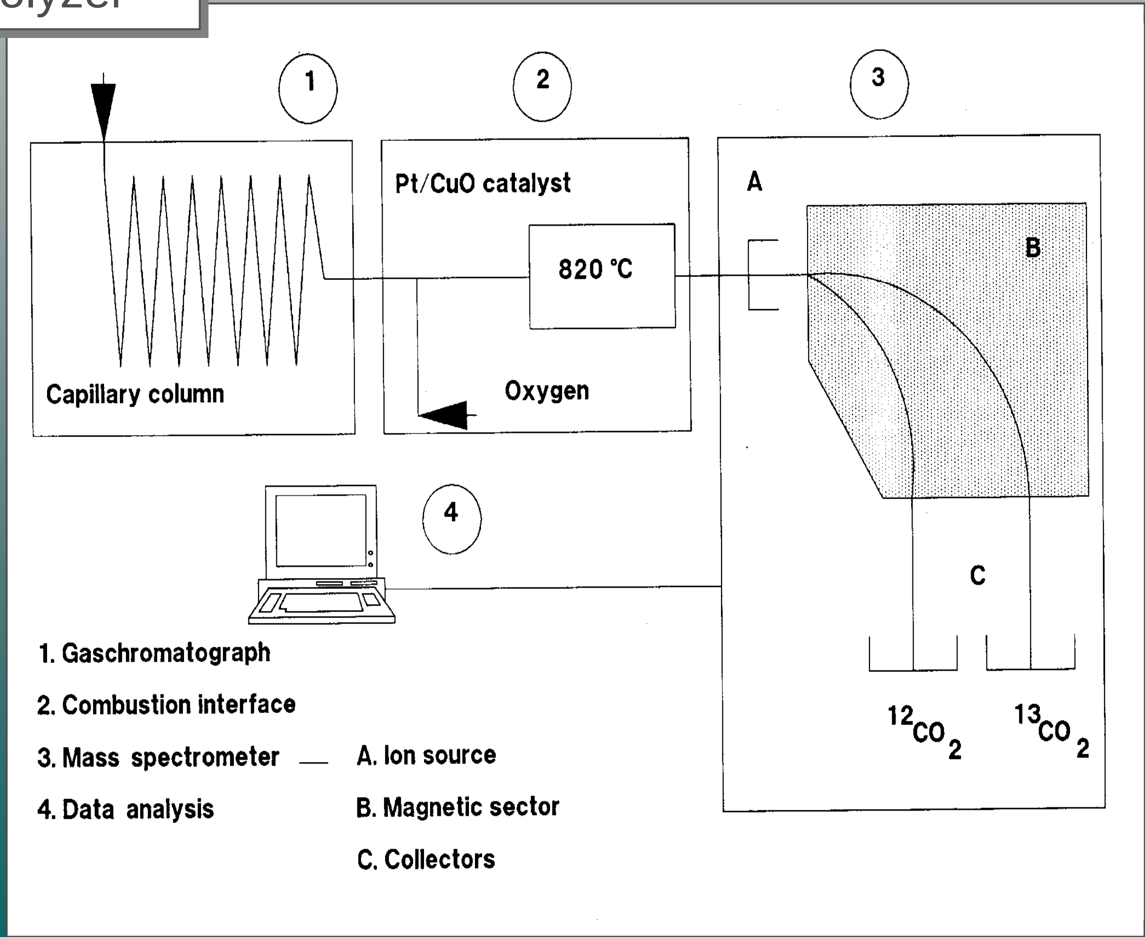

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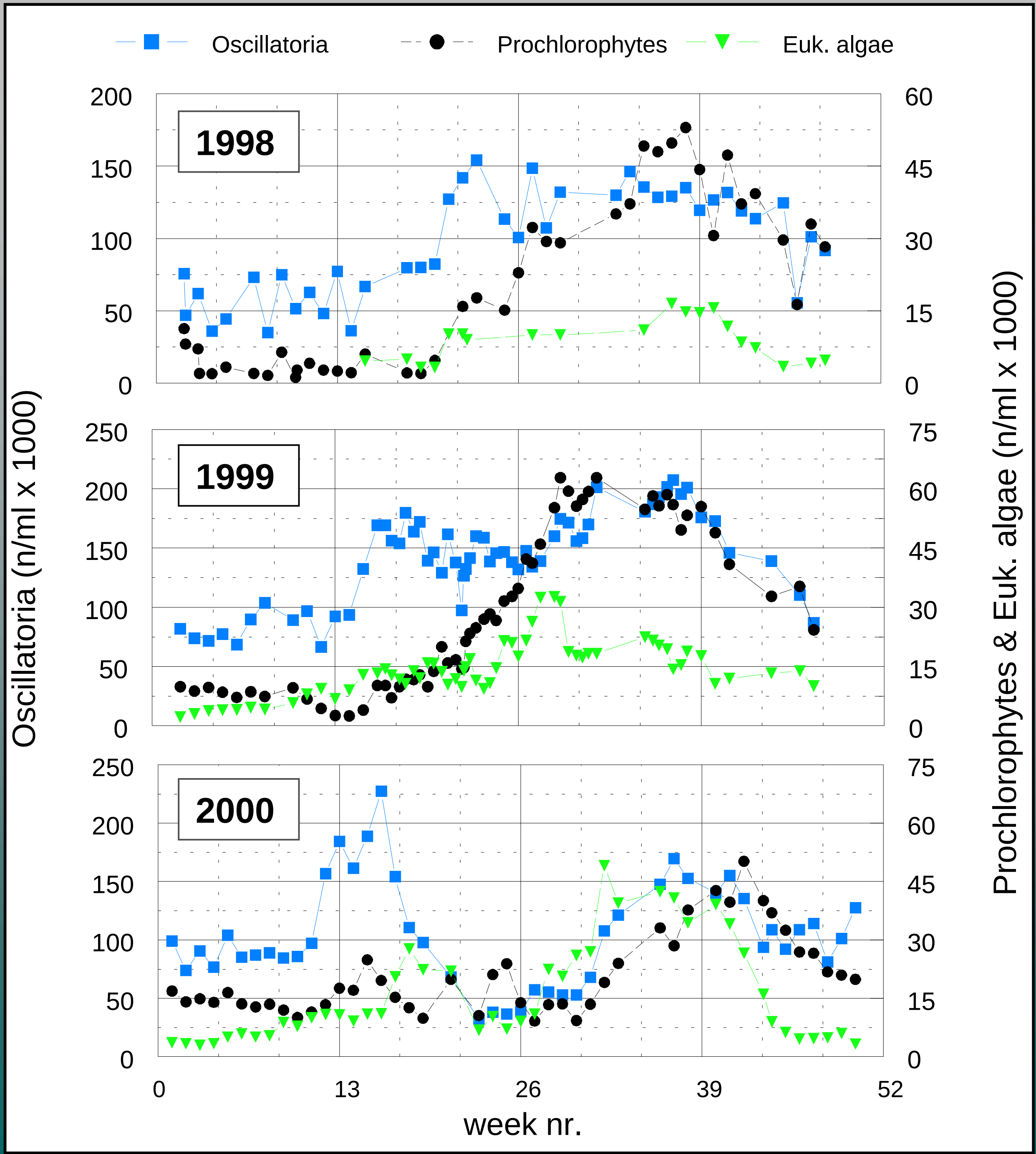


Observations:

• Share of *Prochlorophytes* in total trichome-counts (~20%) has diminished compared to data from 1988-1993 (~50%).

(reduced P-release from upper sediment layers: ⇒
...less variable P-availability)

• Standing stocks of eukaryotic algae in recent years (1998-2001) are 3-5 x higher than the recordings in 1988-1993.

• Shift in start of *Oscillatoria* blooms (lake warming effect?)

Cryptic production fuels lake food webs:

(using the hidden isotopic heterogeneity in phyto- and zooplankton in C-transfer analysis)

• Use of flow sorting to enhance the resolution of stable isotope analysis in aquatic food web studies: ⇒ retrieval of true *in situ* ^{13}C signatures

• Relative importance of cyanobacterial and algal-derived carbon in diets of zooplankton (⇒ assessment of the effects of lake restoration measures)

Problems in isotopic analysis:

• The use of POM as proxy for the phytoplankton ^{13}C signal:

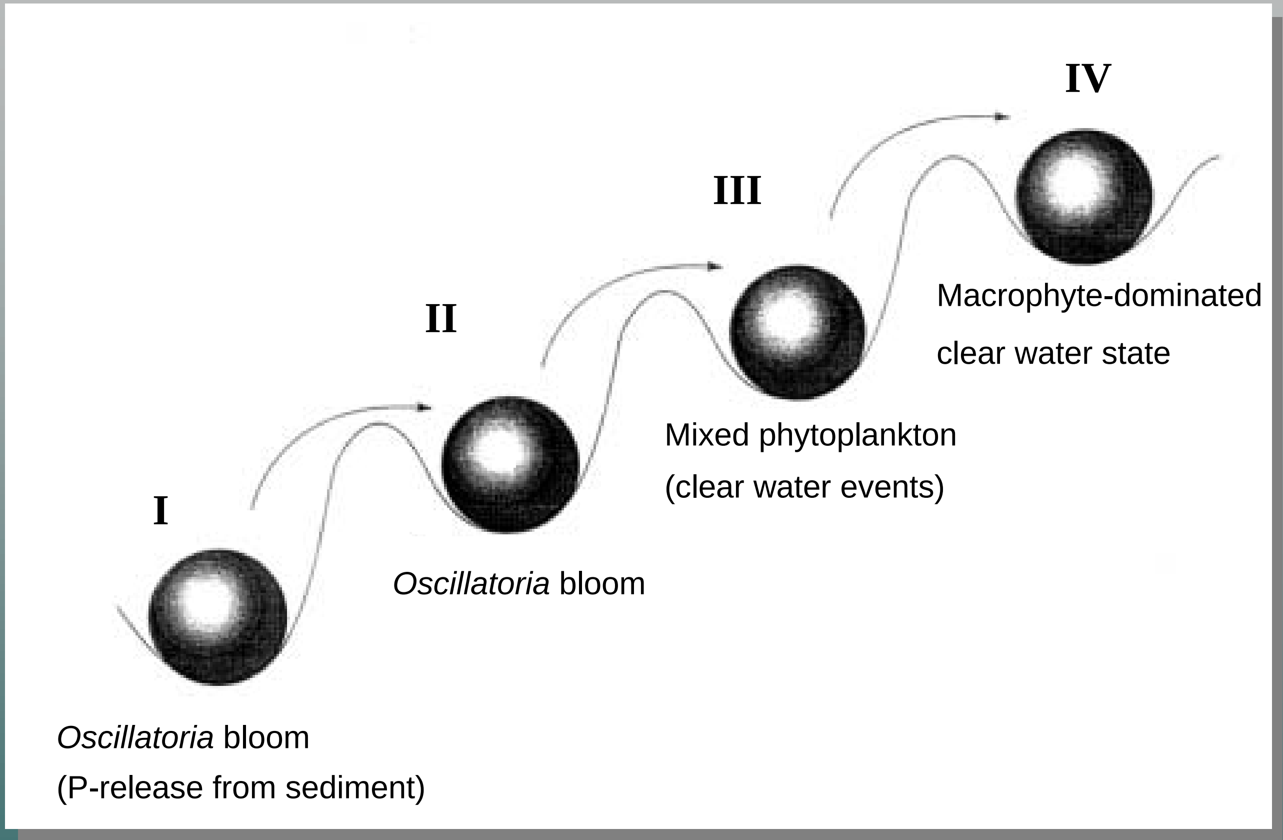
- variable contributions from detritus of diverse origin
- potential heterogeneity in $\delta^{13}C$ of the phytoplankton carbon pool itself because of a multi-species assemblage

Approach:

• Isotopic analysis of *fatty acid* fractions of producers and consumers in stead of measurements on ‘bulk’ biomass C:

⇒ lower limit in ^{13}C-analysis drops from 1-5 μg to 1-5 ng of carbon (using GC-combustion-IRMS)

Alternative stable states in eutrophic shallow lakes



Adapted from Hosper *1997*