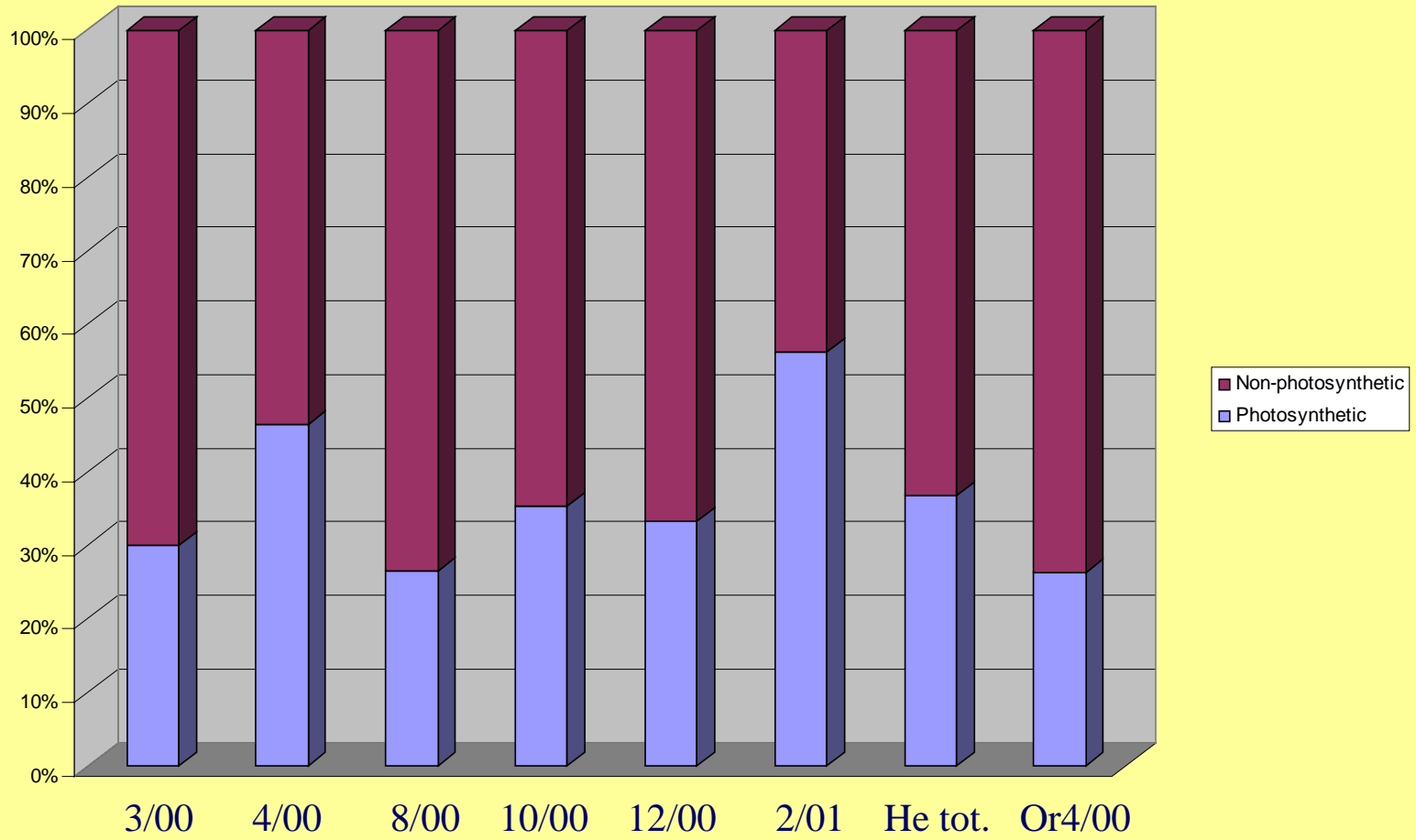
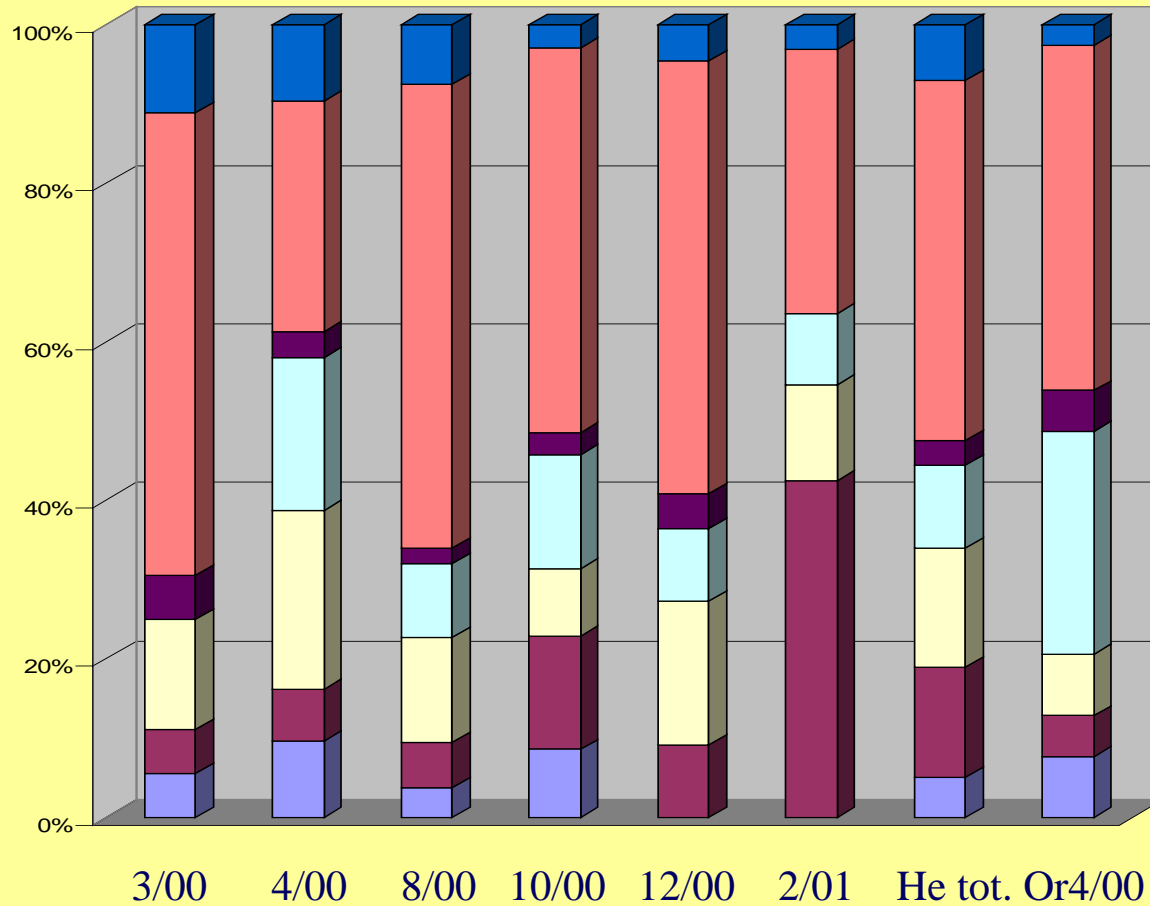


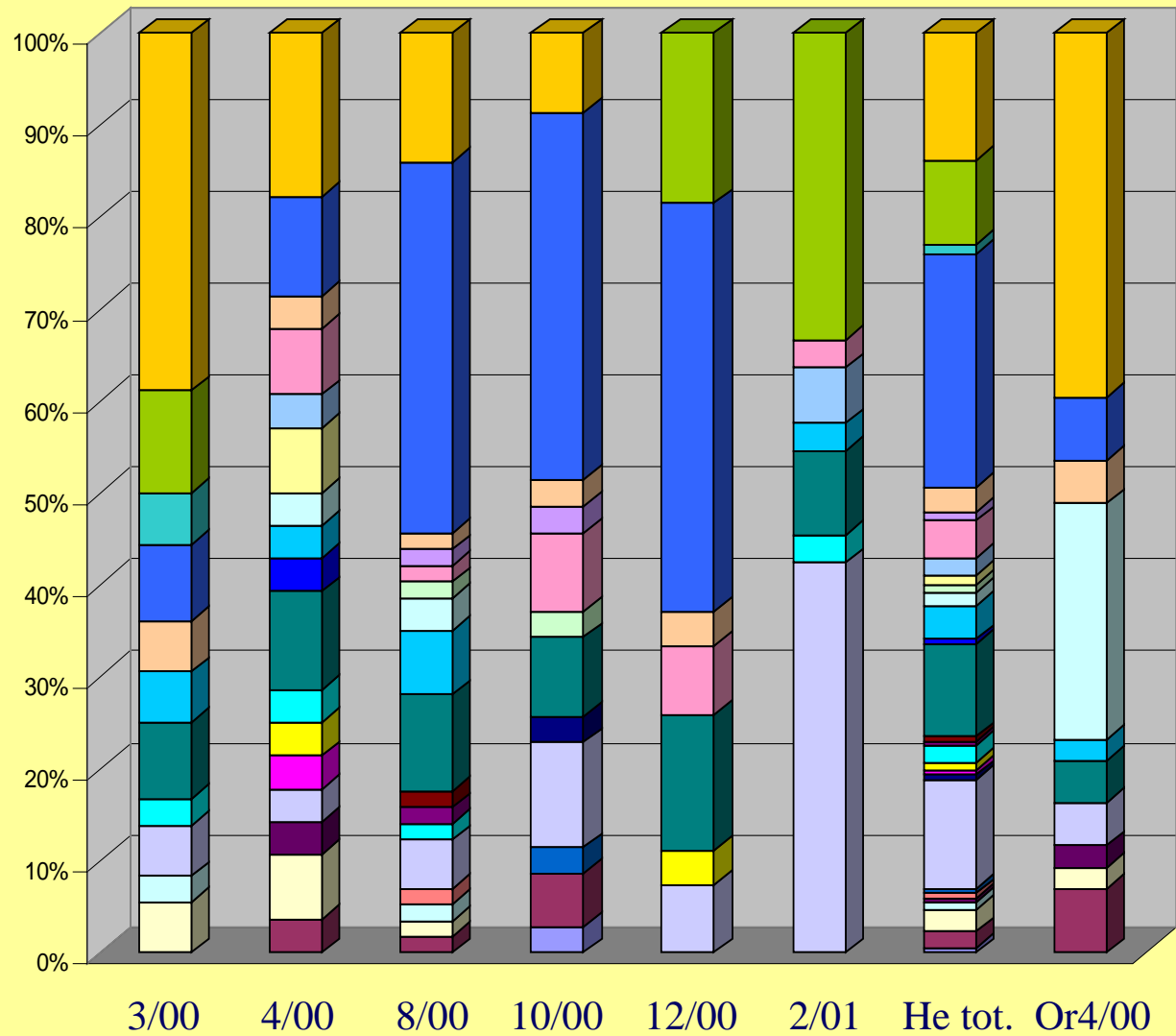
Diversity of the picoplankton community at Helgoland

Klaus Valentin, Helga Mehl and Linda Medlin





- Others
- Alveolates (I, II, and Ciliates), non photosyn.
- Dinoph. (pot. Photosynth.)
- Non-photosynth. Stramenopiles
- Photosynthetic Stramenopiles
- Prasinophytes
- 'Red' and Crypto.



- Alveolates/Ciliates
- Alveolates group I
- He0003 29/72
- Alveolates Group II (Amoebophyra)
- Alveolates/Dinophyta
- Strameno./Traustochytr.-like
- Strameno./Bisecoids (Caecitellus)
- Strameno. III B, non-photosyn.
- Strameno. II, non-photosyn.
- Strameno. IV, non-photosyn.
- Strameno. VII & VIII, non-photosyn.
- Strameno./Chrysophytes
- Strameno./Dictyochiophytes
- Strameno./Bolidophytes
- Strameno./Diatoms
- Chlorarchnioph. Nucleus
- Euglypha
- Haptophyta
- Prasino/Pyramimonadales
- Prasino/Pseudoscourfeldia
- Prasino/Mamiellales
- Chlorophyta/Chlamy-like
- Choanofl.
- "LKM 74" - Perkinsus
- "He0003 26"
- Cryptoph. Nucleus
- Red algal clade ("Porph. 4")
- Cryptoph./Nucleomorph

Conclusions from clone libraries

- There is no pattern visible for photosynthetic versus non photosynthetic picoplankton abundance. Usually the non-photosynthetics dominate.
- Alveolates (I, II and ciliates) are the most dominant group.
- Within the photosynthetic fraction, the Prasinophytes (Mameliales) dominate, followed by stramenopiles (Bolido-, Chrysophytes). Cryptophytes are also present in most libraries.
- The composition of the picoplankton changes drastically throughout the year.

Interesting clades

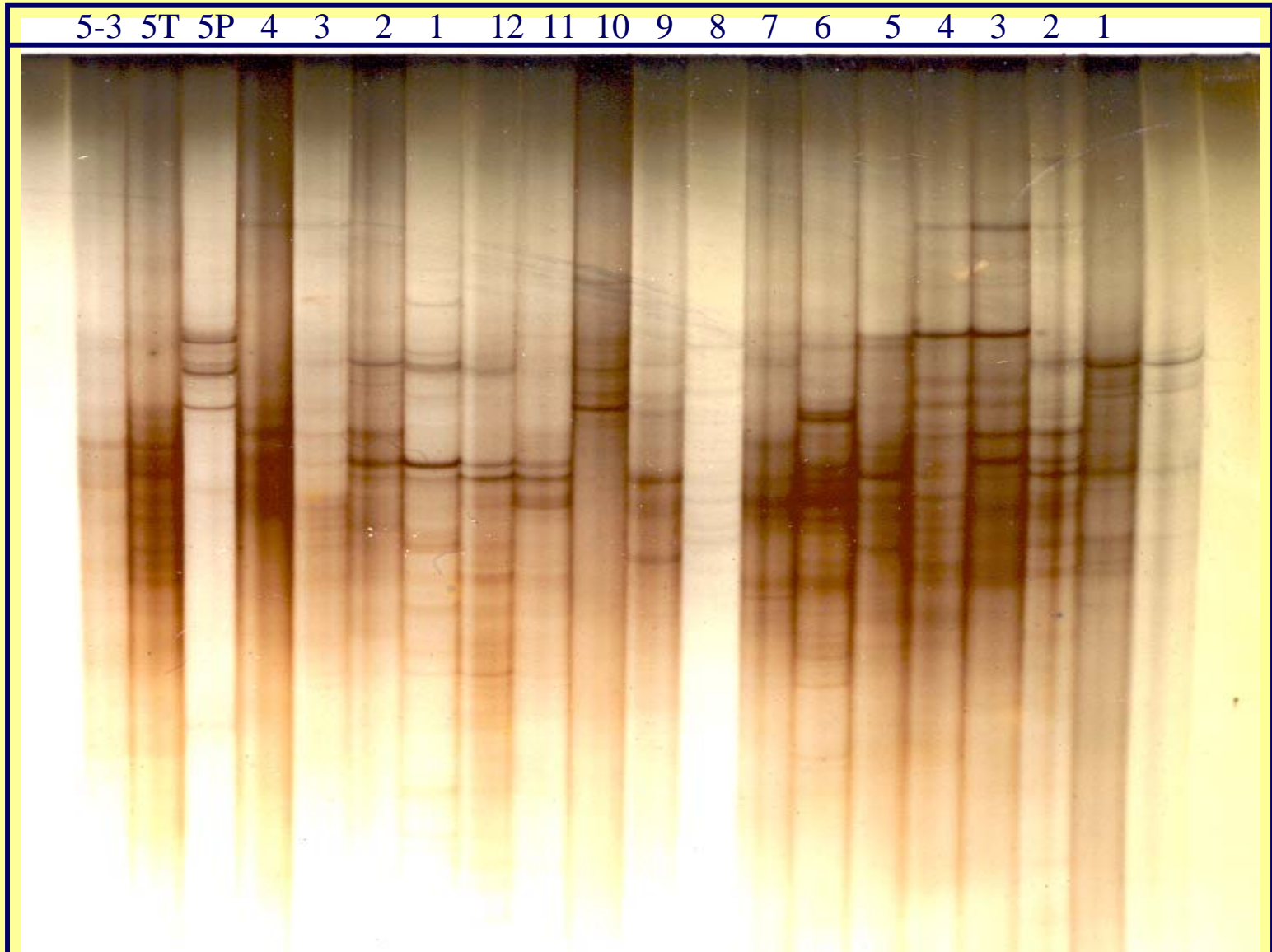
- Novel red algae I: 27 sequences
- Novel red algae II = base of Cryptophyte nucleomorph clade: ca. 10 seq
- Base of Cryptophyte nucleus/Acantamoeba (He0003.26): 4 sequences
- Base of Choanoflagellates: ca. 5 sequences
- Base of Euglypha (3)
- Base of Chlorarachniophytes (4)
- Base of Oomyces (5)
- Base of Amphidinium: (2)

SSCP profiles from environmental DNA - PICODIV

- Isolate environmental DNA from <math><3\mu\text{m}</math> fraction**
- run PCR with 528F and 926R, check on gel**
 - purify product with Qiagen columns**
 - digest with lamda exonuclease**
 - purify again**
 - run PAGE at 20°C, low power (8 W), current (5 mA), and voltage (400 V),**
 - silver stain gel**

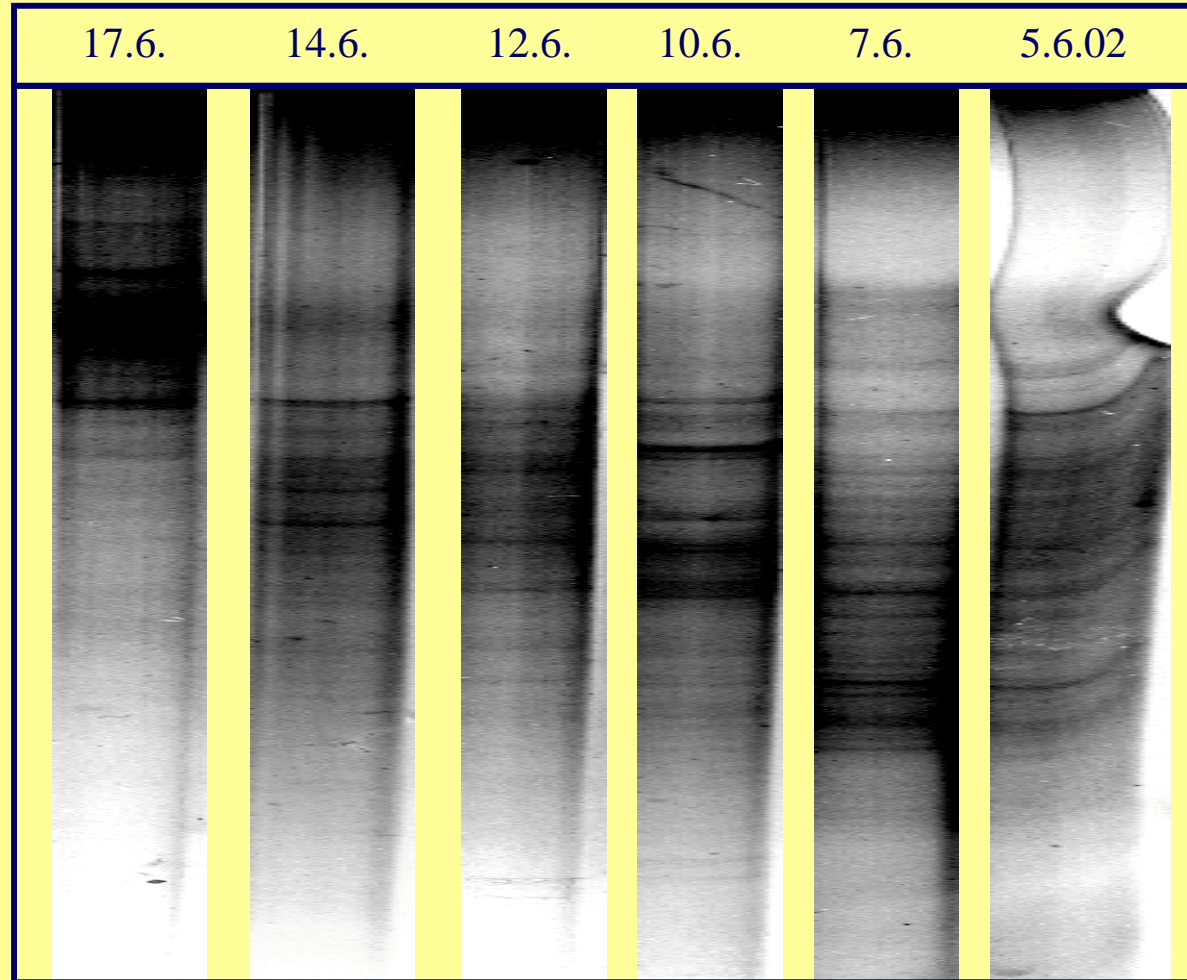
Bands can be cut out, reamplified, checked on an SSCP gel, and sequenced with 528F, allowing direct comparison with the PICODIV data bank.

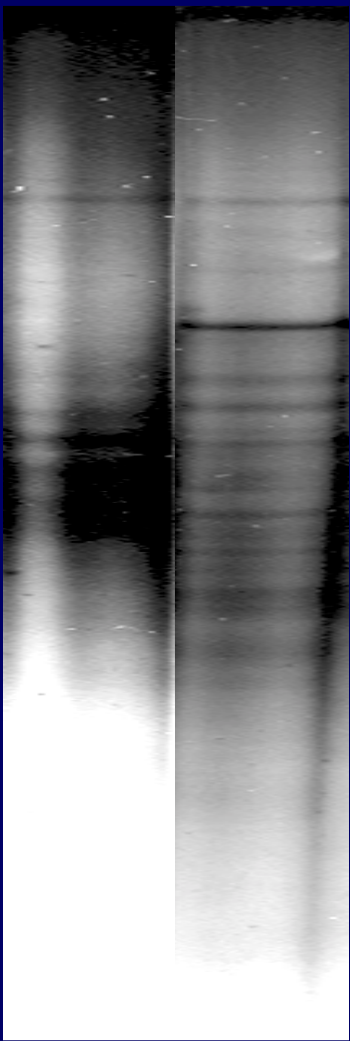
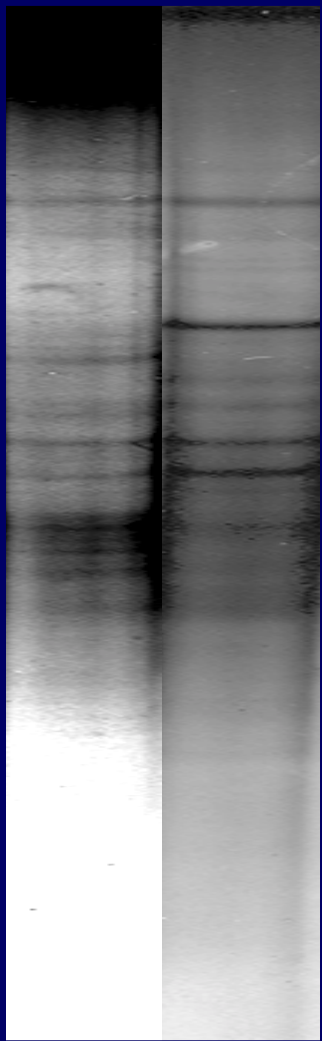
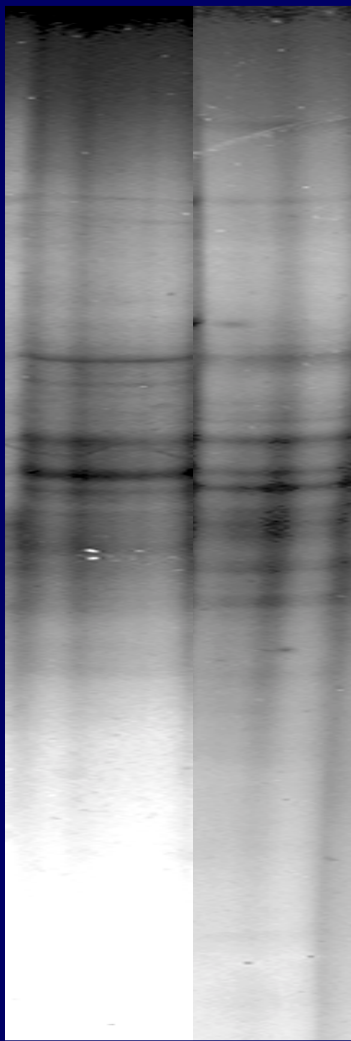
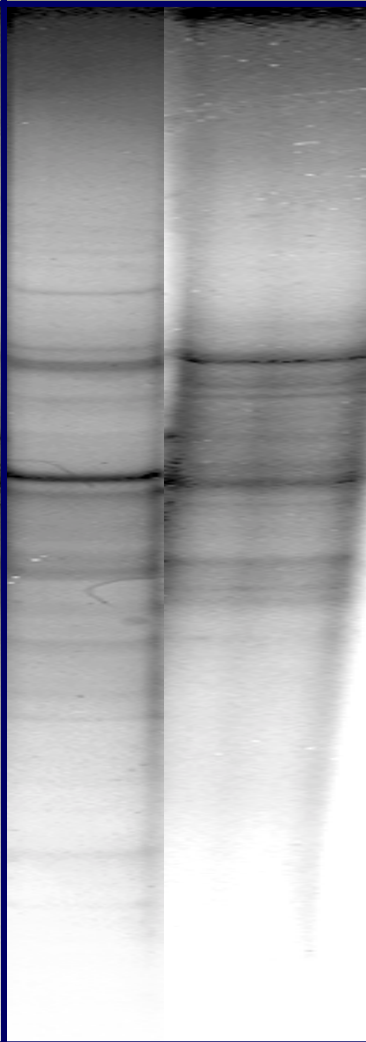
Helgoland picoplankton time series 1/01 - 5/02, SSCP profiles



Short-term time series Helgoland June 2002

SSCP profiles of the picoplankton fraction



April 2002 01	March 02 01	Feb. 02 01	Jan 02 01
			

Conclusions

- 1 – Picoplankton composition changes drastically from month to month as seen by analysis of clone libraries and SSCP profiles.
- 2 – Possibly there is a pattern that is repeated on a yearly basis, as seen by SSCP analysis.
- 3 – Time series should be conducted by SSCP/DGGE over more than one year to identify reoccurring bands which then can be identified.
- 4 – Clone libraries should rather be established for shorter time intervals (i.e. monthly) rather than over prolonged time periods (i.e. not more than a year).

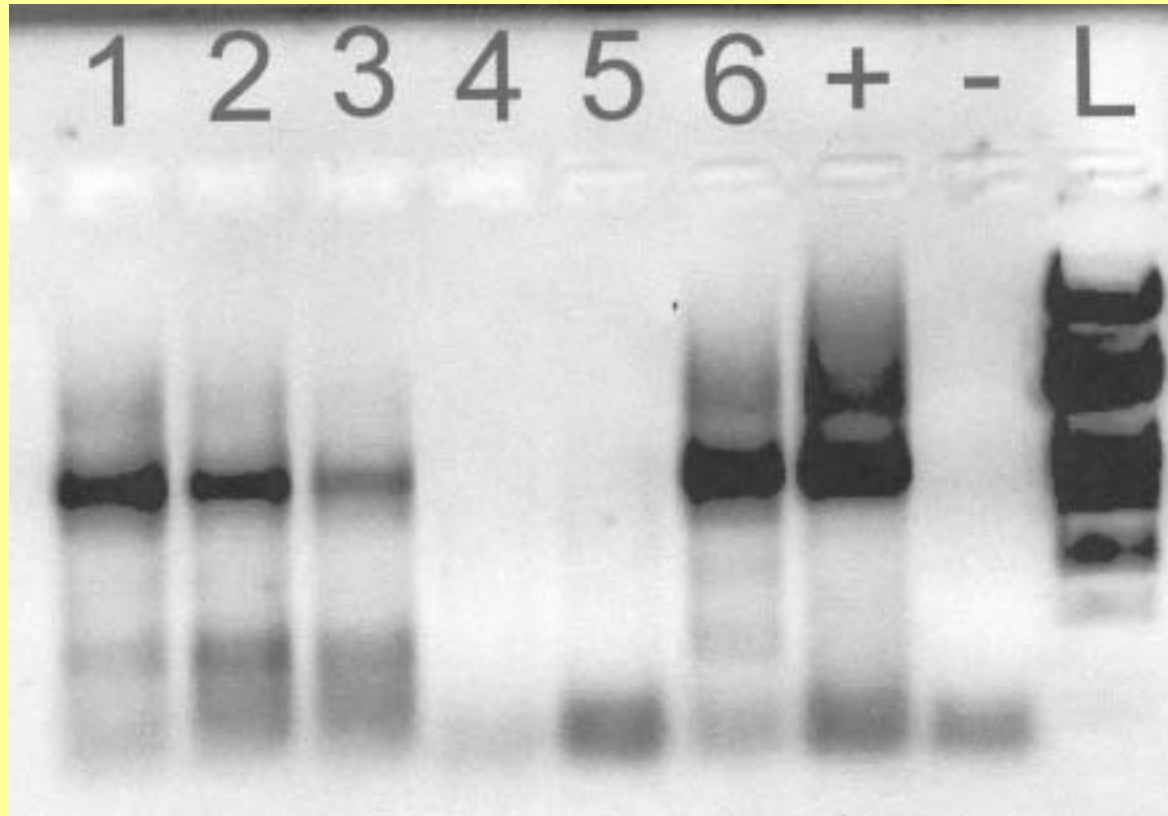
Time series – achievements and desaters

- Monthly sampling was continued until december 2002, analysis of samples was started in January 2003.
- As a result we have samples from two consecutive years, for 1 sample (august) for three years. (Two samples were lost during lab rearrangement.) For three of the samples (8/00, 12/00, 2/01) we also have clone libraries.

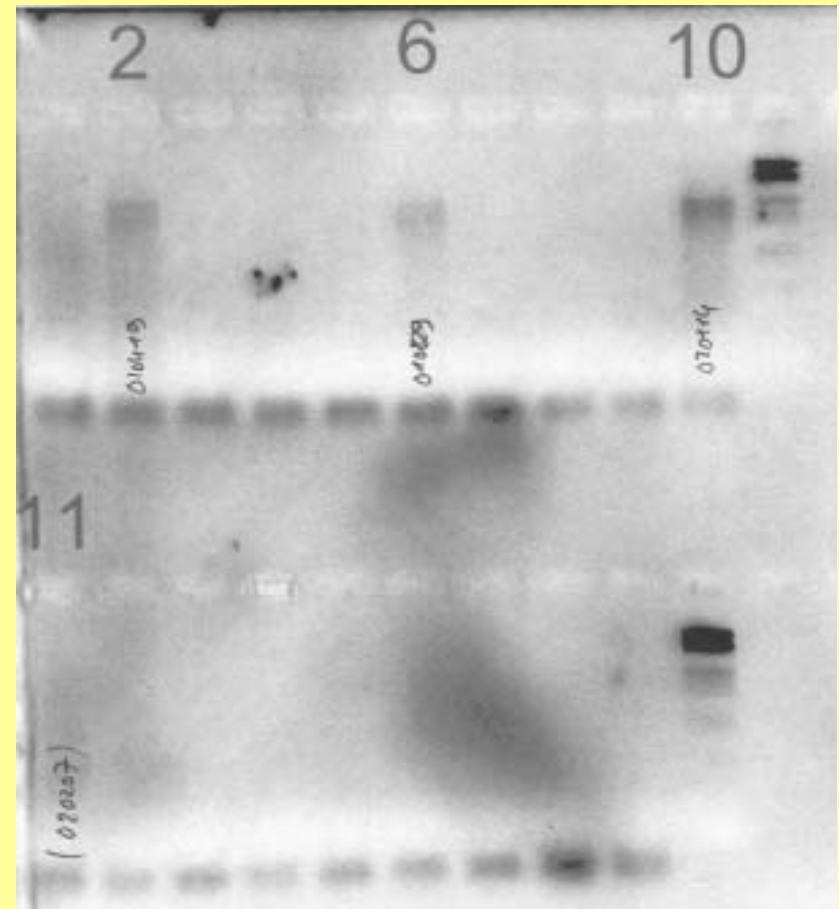
Time series – achievements and desaters

- DNA was prepared twice from all samples using a supposingly identical Kit from another company.
- The yield was very low and the DNA looked poor.
- Numerous attempts to amplify full-length 18S failed completely (-> Katja)
- Several attempts were necessary to generate SSCP products for all samples.
- Four attempts (until exhausting the DNA) failed to produce a good SSCP gel.
- Finally DNA was prepared from a few samples using the remains of the previous kit. This time (i.e. last week) 18S PCR worked.

First successful 18S PCR using He samples in 3 months!



Ramons DNA samples and the corresponding 18S PCR



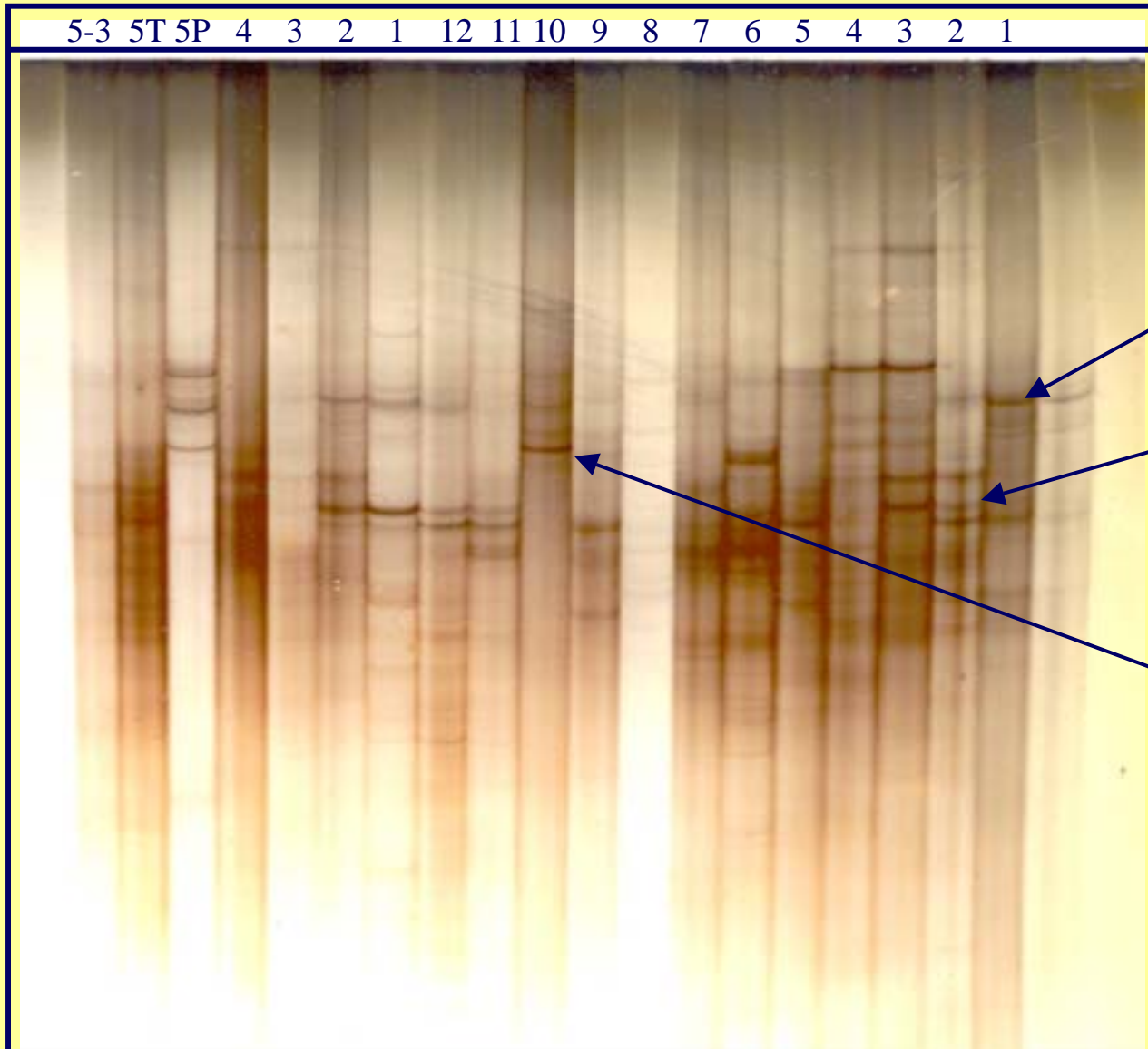
-> Katja

... the corresponding SSCP PCR and the SSCP gel.



**There are still enough filters left to repeat the DNA preps
but it is uncertain whether the complete analysis can be done
until the end of April...**

Helgoland picoplankton time series 1/01 - 5/02, SSCP profiles



Alveolate group I
98% identity

Dinophyte
95% identity

Ciliphora
(Halteria-like)
93% identity