

# Cyanobacteria dot blot probe analysis of time series at coastal stations

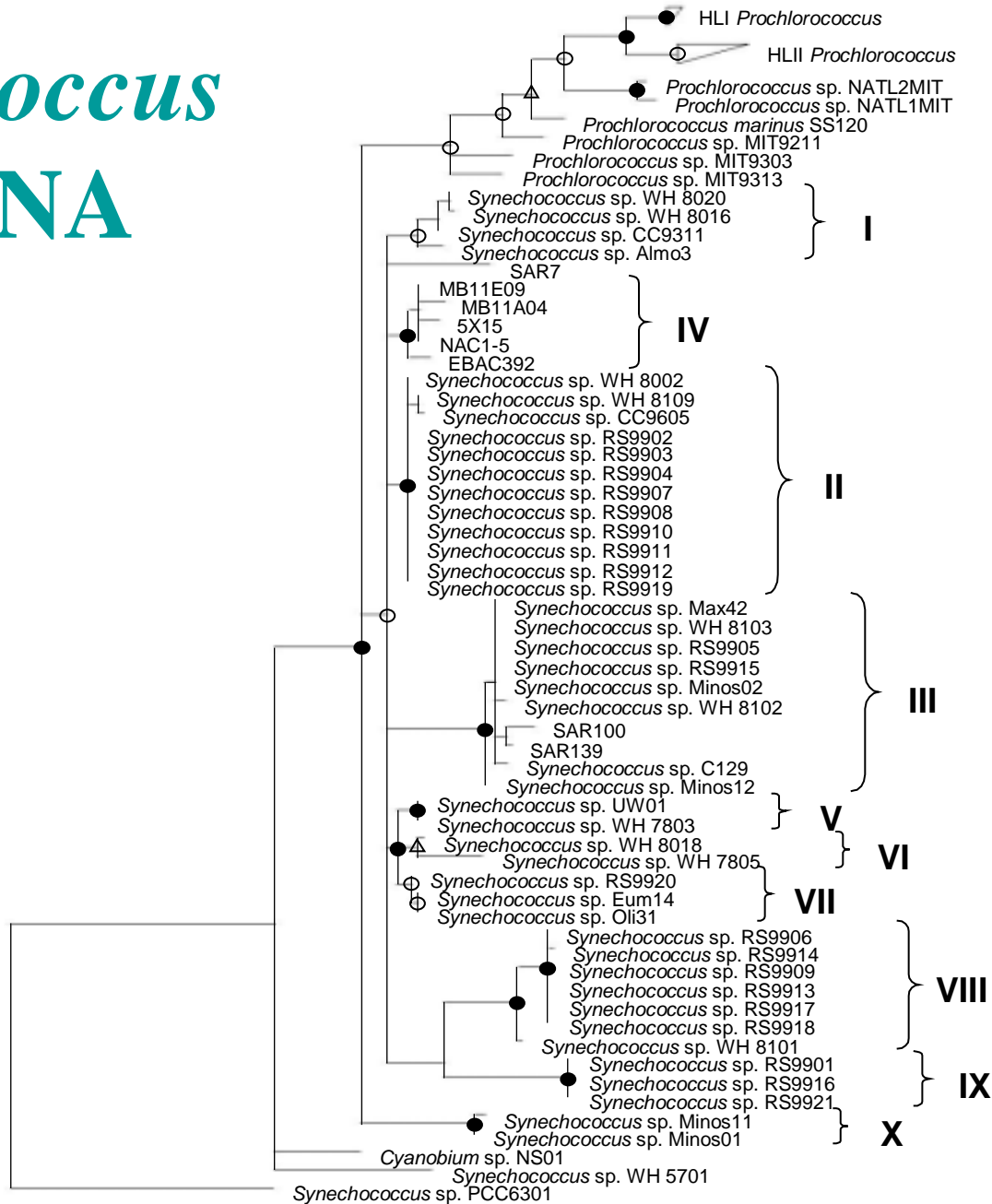
Richard C. Hastings, Nick J. Fuller

Sophie Mazard, Dave J. Scanlan

**University of Warwick UK**

# Synechococcus

## 16S rRNA



- ◆ Boot Strap values >95%
- Boot Strap values 70-95%
- △ Boot Strap values <70%

# Dot blot hybridisation

Environmental sample

↓

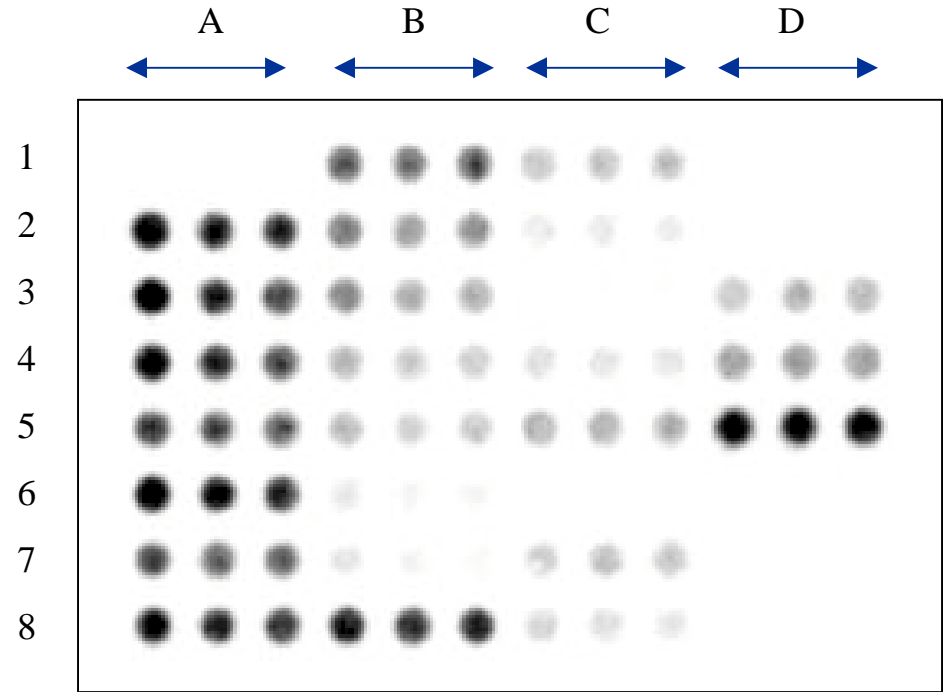
PCR with oxygenic phototroph biased 16S rDNA primers

↓

Immobilise 30ng of product on membrane (in triplicate)

↓

Hybridise <sup>32</sup>P-labelled *Syn*-specific and eubacterial oligonucleotide probe



Determine relative abundance of *Synechococcus* genotypes in environment

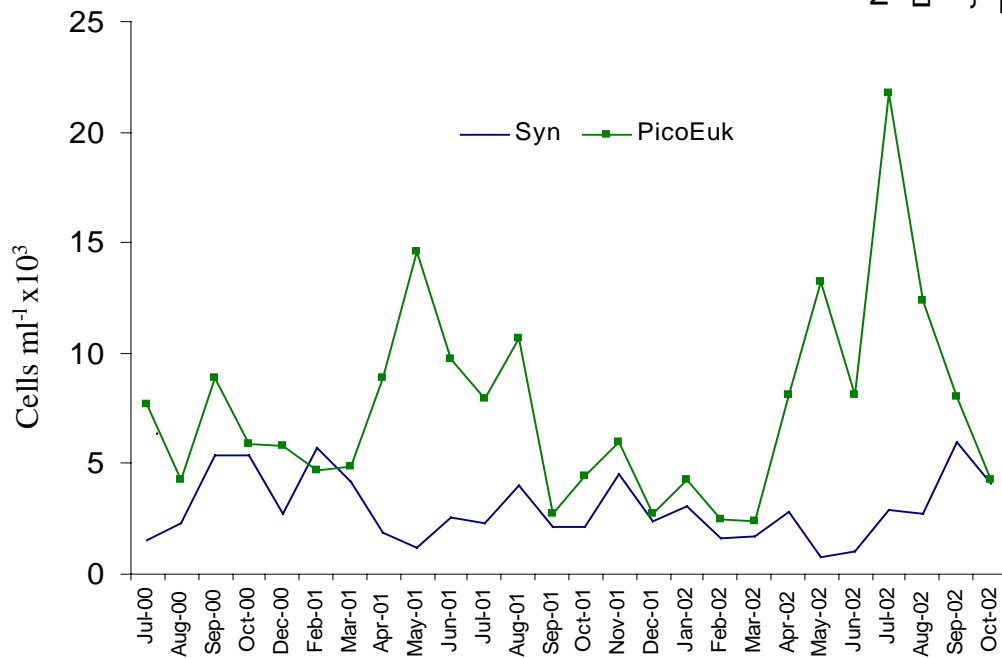
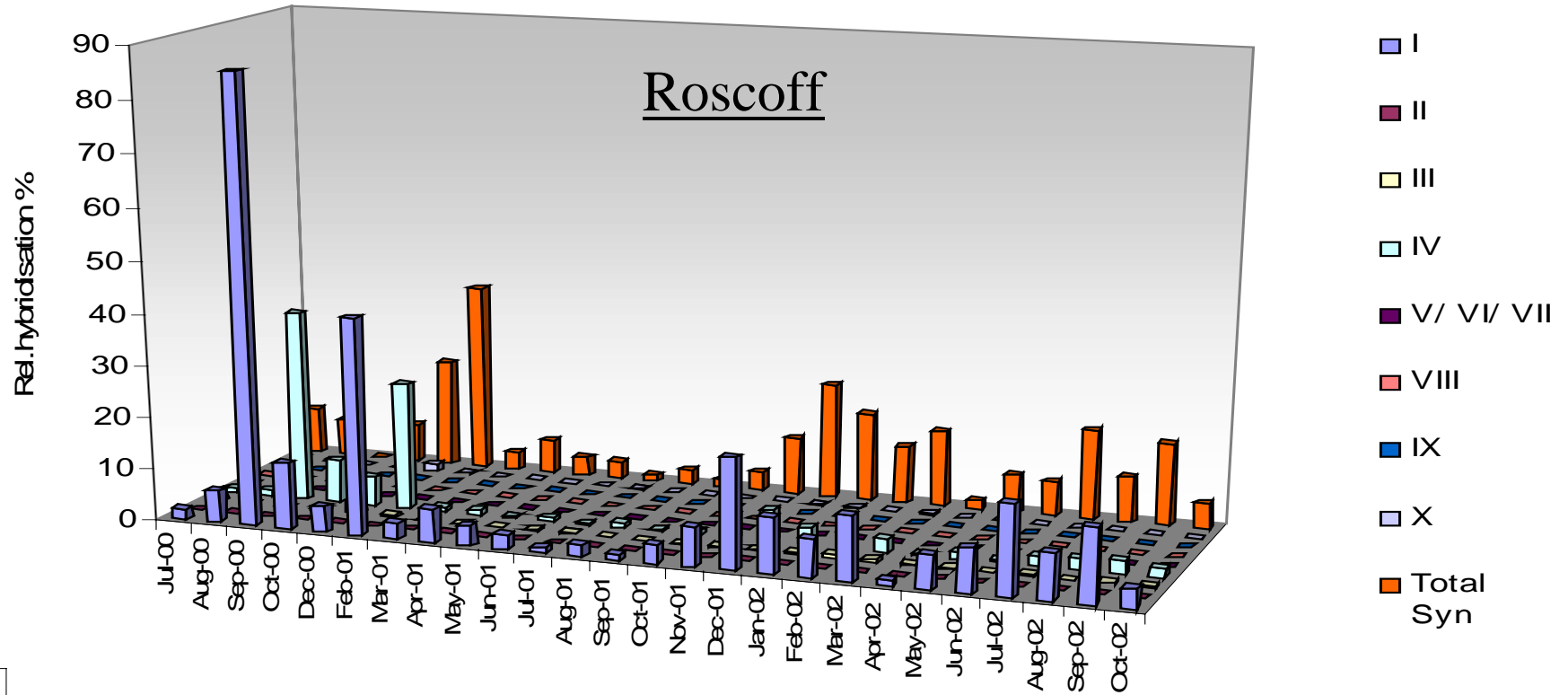
Detect quantity of specifically bound probe (Total Lab. Software)

## Determination of relative hybridisation

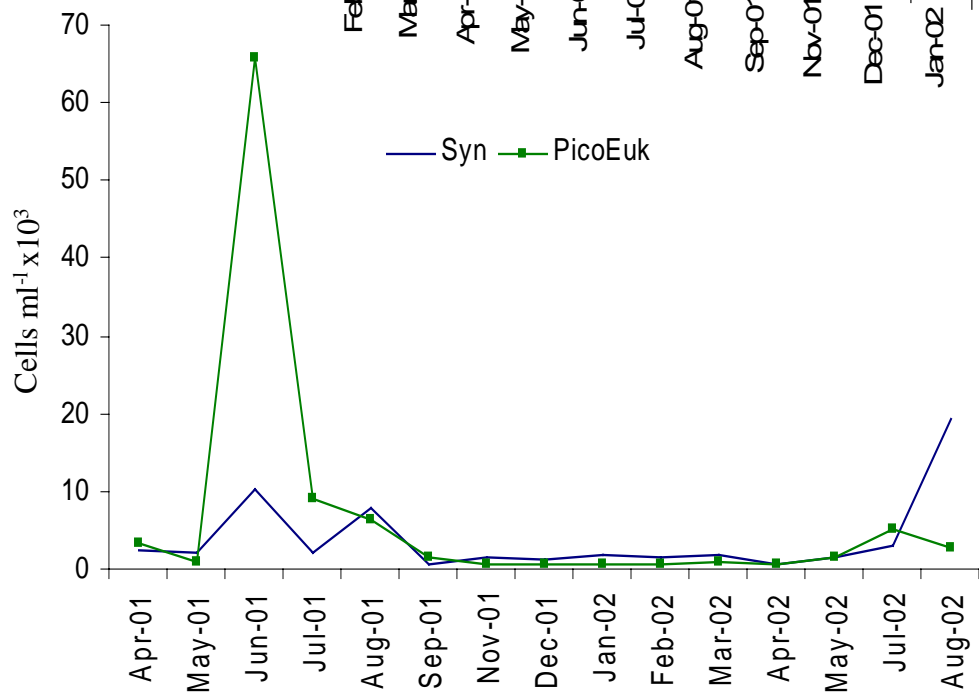
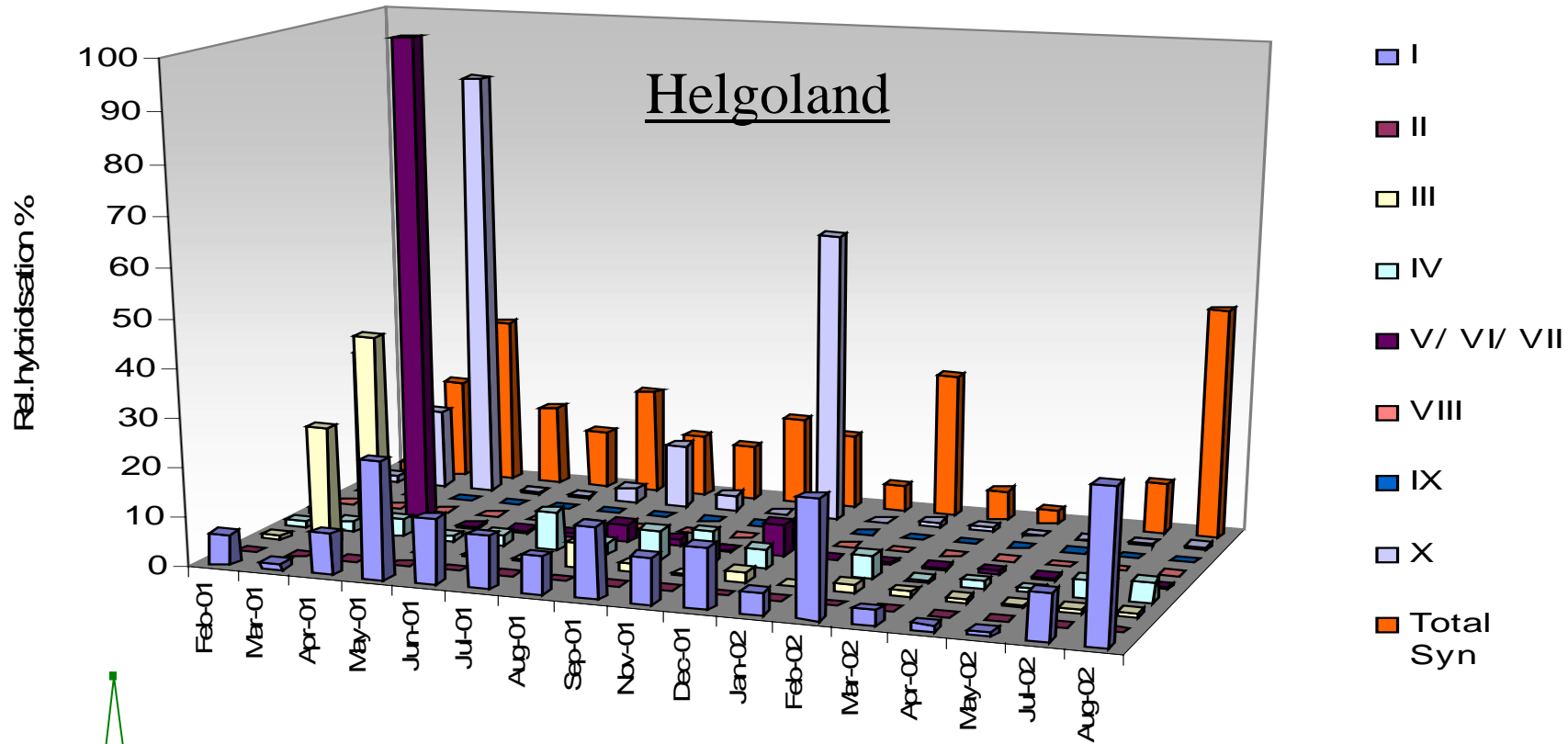
$$\text{Relative Hybridisation (\%)} = \left[ \left[ \frac{S_{env}}{E_{env}} \right] \left[ \frac{S_{con}}{E_{con}} \right]^{-1} \right] \times 100$$

•Where  $S_{env}$  and  $E_{env}$  represent hybridisation to environmental DNA of the specific and eubacterial probes respectively, and  $S_{con}$  and  $E_{con}$  are the slopes of the specific and eubacterial probe-binding curves respectively.

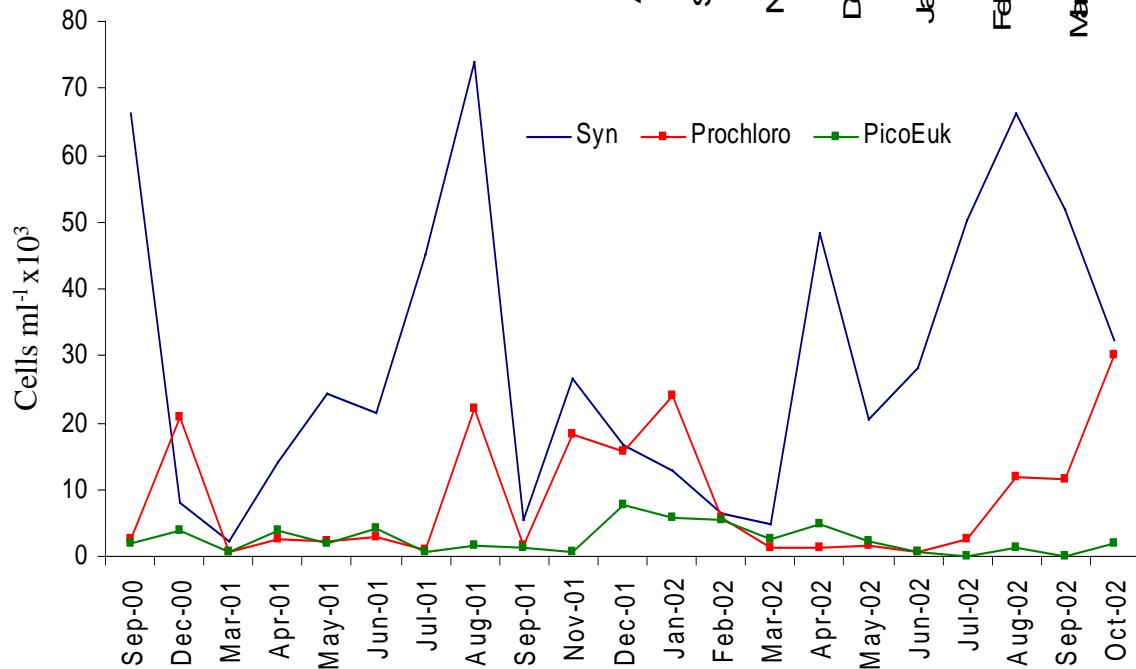
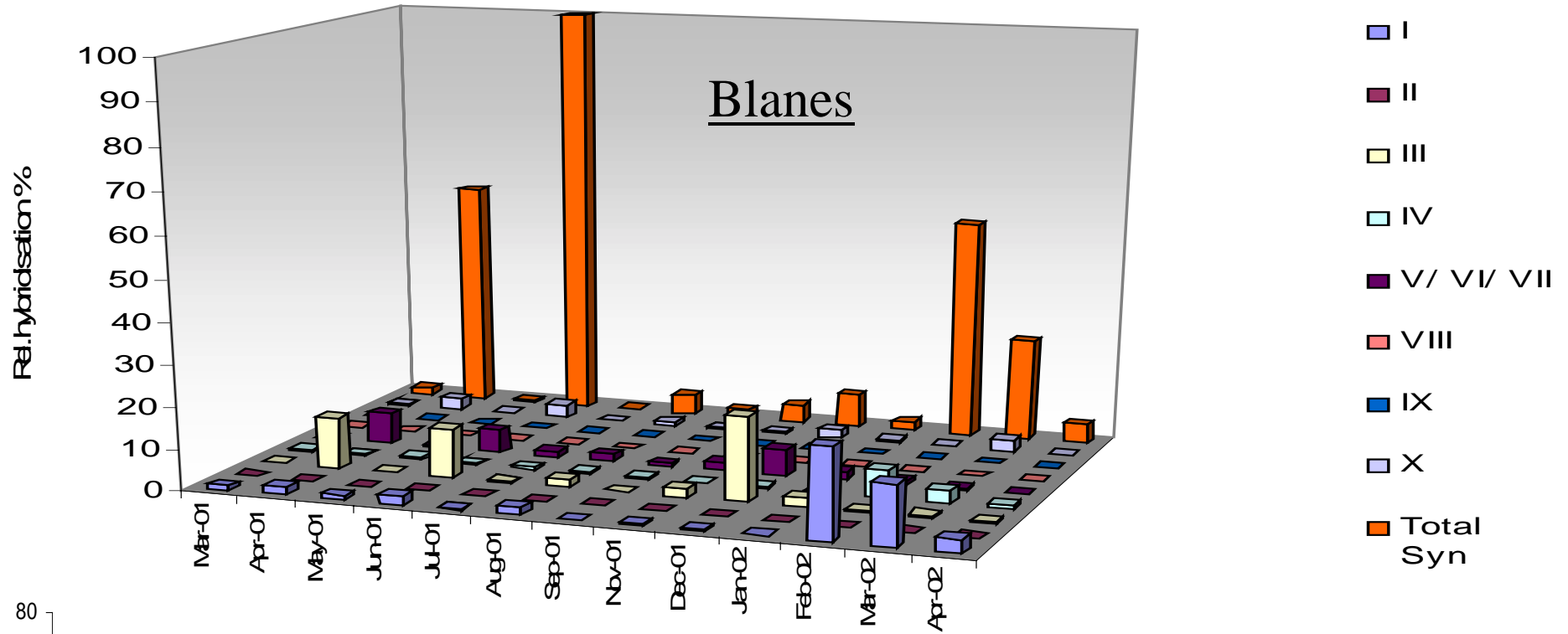
•Calibration curves are used to normalise signals



- Presence of Clades I and IV. Clade IV mostly confined to Sep 00 to Feb 01
- Clades II, V/VI/VII, VIII and IX undetectable



- Presence of Clades I, III, IV, V/ VI/VII and X
- Clades II, VIII and IX undetectable

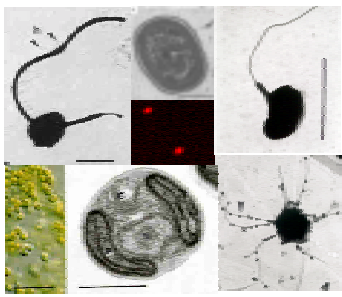


- Presence of Clades I, III, IV, V/VI/VII and X
- Clades II, VIII and IX undetectable
- Shift in community structure from Clades III and V/VI/VII to I and IV (Feb 02)

## Conclusions

- Clades II, VIII and IX undetectable at Roscoff, Helgoland and Blanes coastal sites by dot blot hybridisation. In agreement with clone library data.





## Conclusions

- Clades II, VIII and IX undetectable at Roscoff, Helgoland and Blanes coastal sites by dot blot hybridisation. In agreement with clone library data
- Clade X detected in low abundance
- Clades I and IV present in each site
- Inconsistances between total *Synechococcus* signal and cytometry counts maybe due to:
  - Unknow genotypes not being amplified but detected by FC
  - Differential PCR amplification of heterotrophs and plastids