

# PICODIV meeting

July 2002, Station biologique de Roscoff, Brittany, France

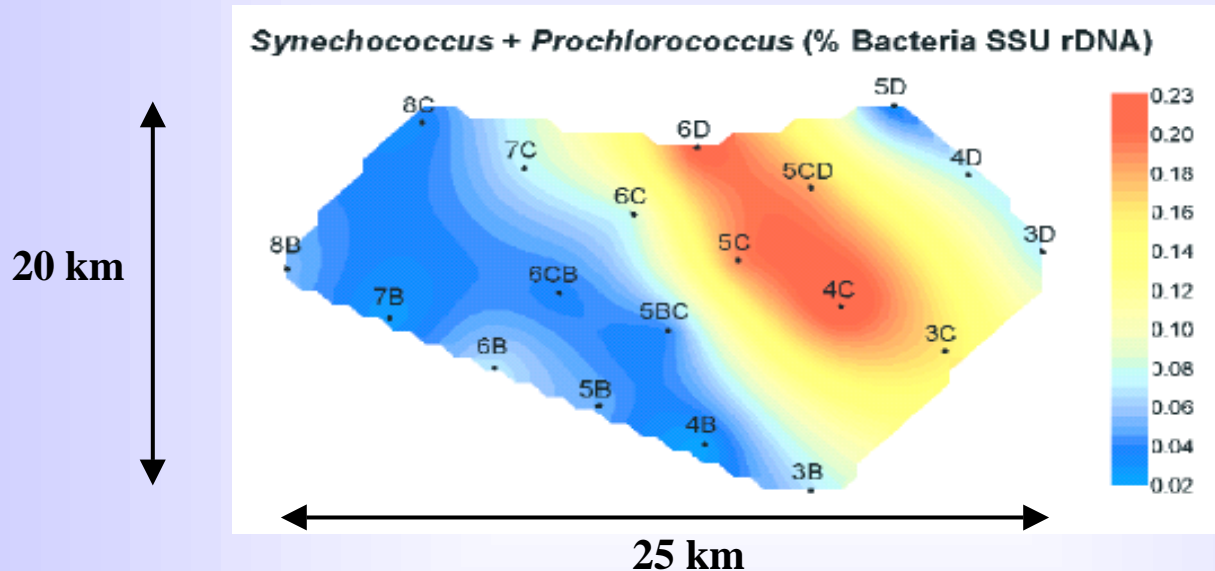
## Quantitative PCR: testing Prasinophyceae primers and preliminary results with natural samples

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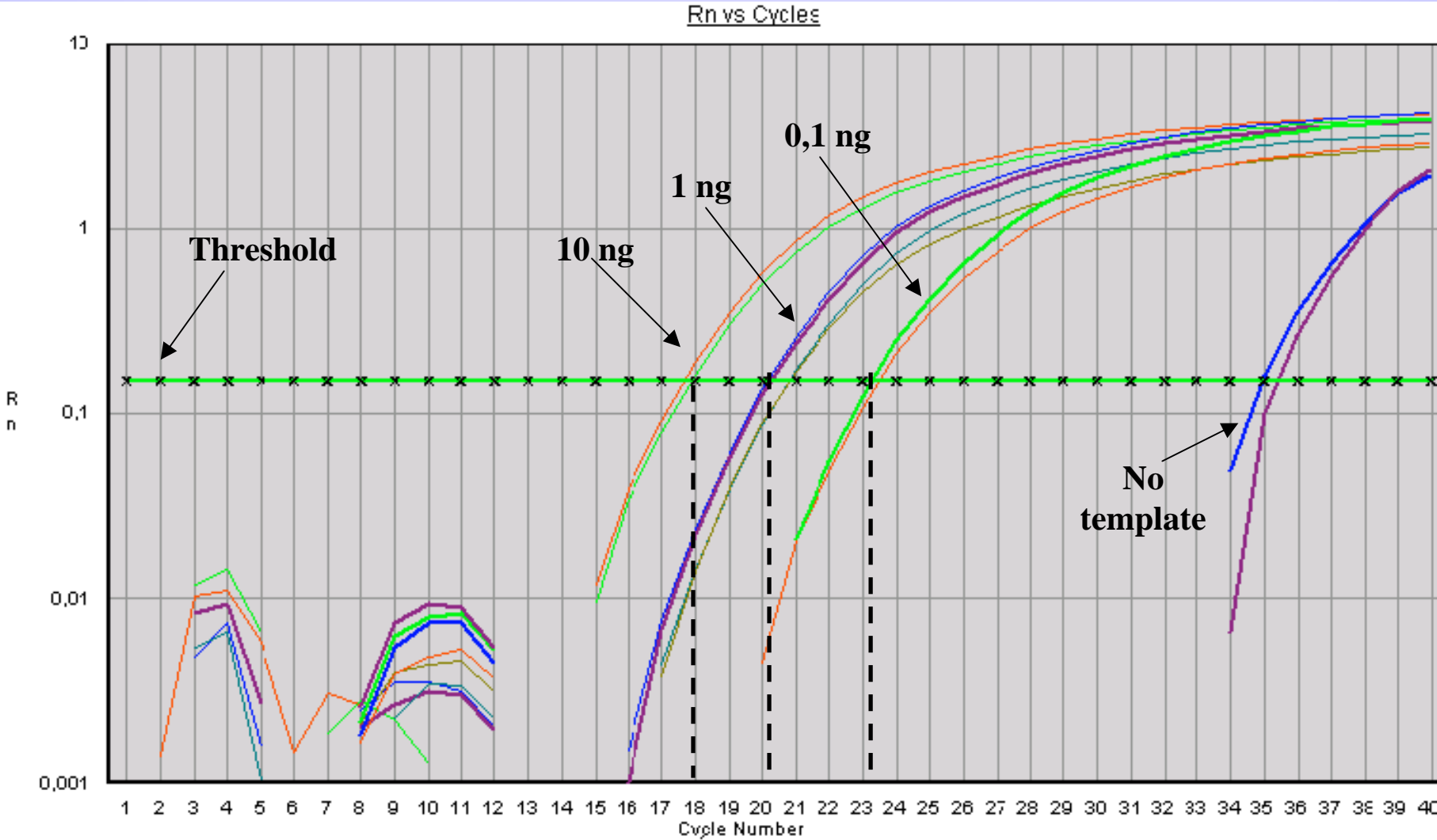
# Quantitative PCR: a powerful tool for large scale quantification

- Applied with success on marine bacteria



- Allow quantification with 30 ml samples
- Never applied on picoeukaryotes

# Quantitative PCR: functioning



# Quantitative PCR

- Primers design (on 18S rDNA gene)
- Specificity and hybridization temperature test
- Primers concentration
- Reproducibility, efficiency
- Detection limits

# Quantitative PCR: primers design

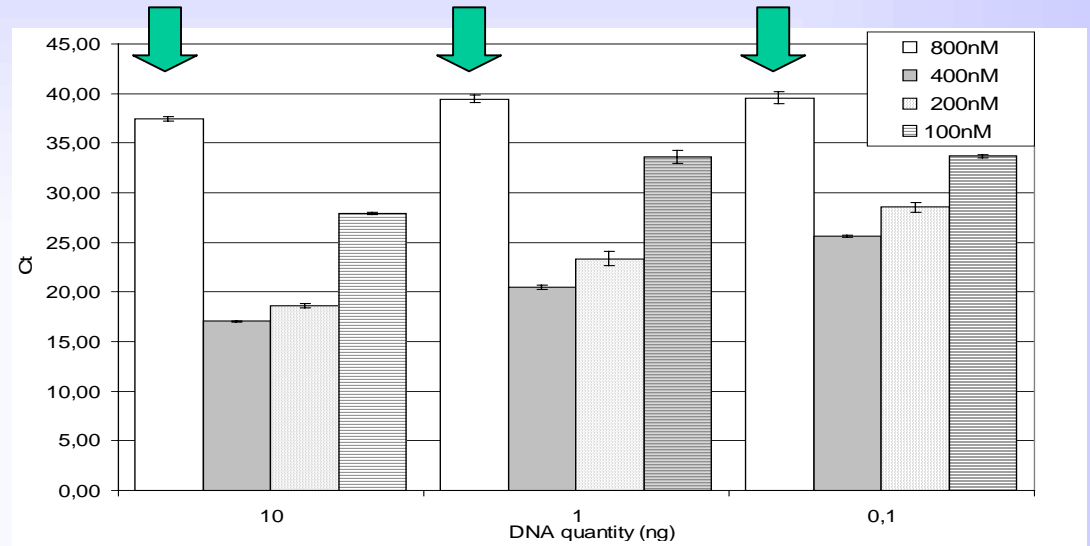
- 22 couples of primers tested: 5 are specific of the targeted strain(s)

ADN	Kingdom	no template (H2O)	Eucaryotes								Procaryote
	Genus		<i>Ostreococcus</i>	<i>Bathycoccus</i>	<i>Micromonas</i>			<i>Rhodomonas</i>	<i>Bolidomonas</i>	<i>Pelagomonas</i>	<i>Escherichia</i>
	Specie		<i>tauri</i>	<i>prasinos</i>	<i>pusilla</i>	<i>pusilla</i>	<i>sp</i>	<i>salina</i>	<i>pacifica</i>	<i>calceolata</i>	<i>coli</i>
	Strain		OTTH 059S		CCMP 490	CCMP 489	BL 122	CCMP 322	OLI 31 SE 3	Prosope_63	
Primers											
Forward	Reverse										
Euk528f	Euk765r	-	+	+	+			+	+	+	-
Euk528f	Pras04r	-	+	+	+				-		-
Euk528f	Ostreo02r	-	+	-		-					-
Euk528f	Bathy03r		-	+	-	-		-	-	-	-
Euk528f	Micro02r	-	-	-	+	+	+				-

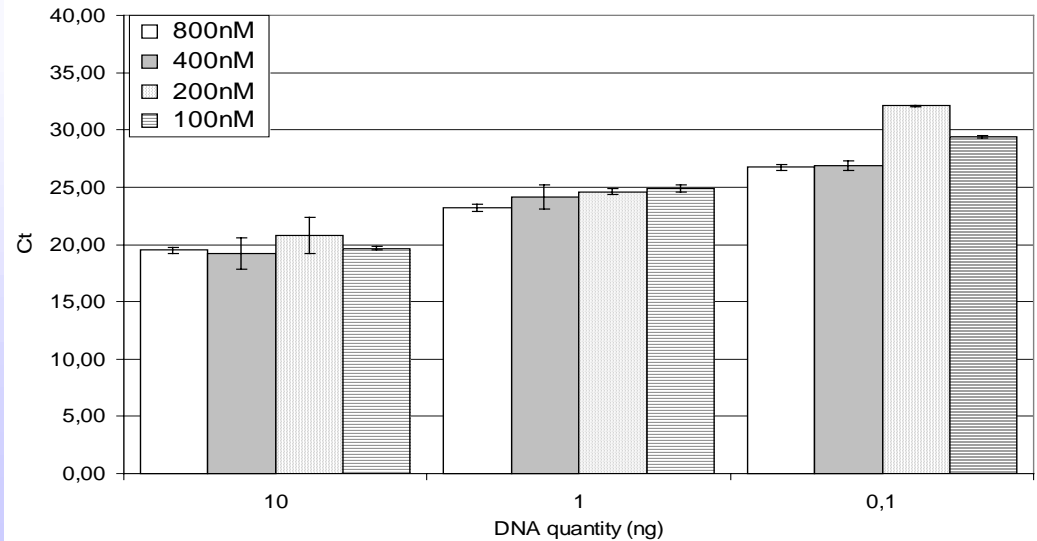
# Quantitative PCR: Optimization of the reaction

- Effect of primer concentration

Primers for  
*Ostreococcus* genus

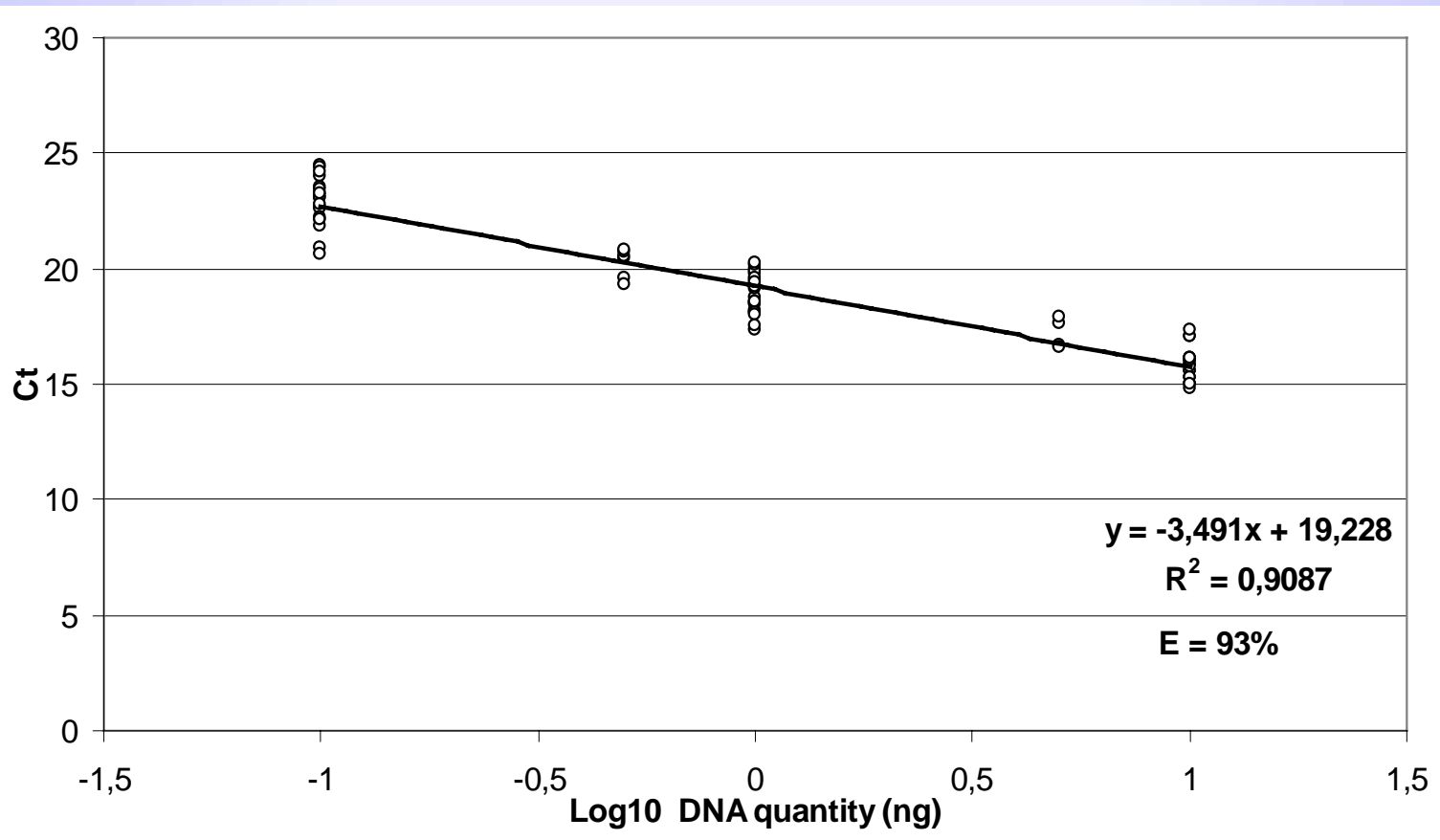


Primers for  
*Bathycoccus* genus



# Quantitative PCR: Reproducibility

- Example of primers for *Ostreococcus* genus: results for 7 QPCR:



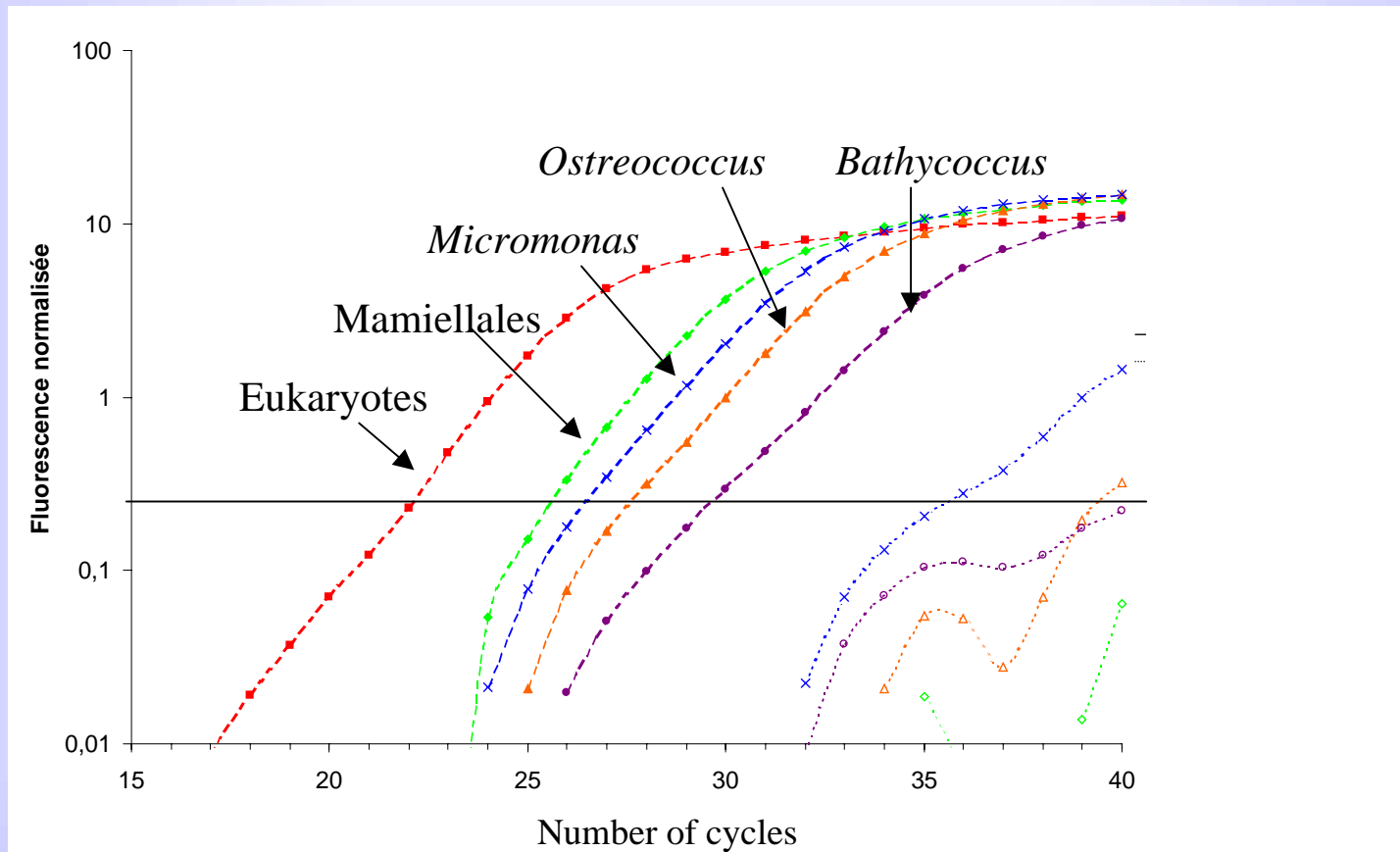
# Quantitative PCR: Detection limits

Target	Theoretical detection limit ( fg DNA)		Theoretical detection limit ( number of cell)	
	maximum	minimum	maximum	minimum
Eukaryots kingdom	1,2E+04	1,5E+01	354	0,4
			376	0,5
			152	0,2
<i>Ostreococcus</i> genus	9,7E+02	5,7E-02	30	0,002
<i>Bathycoccus</i> genus	5,9E+00	2,9E+00	0,1	0,04
<i>Micromonas</i> genus	1,0E+02	9,0E+01	3,0	2,7

- From nanogram to fentogram of DNA
- From hundreds cells to less than one cell

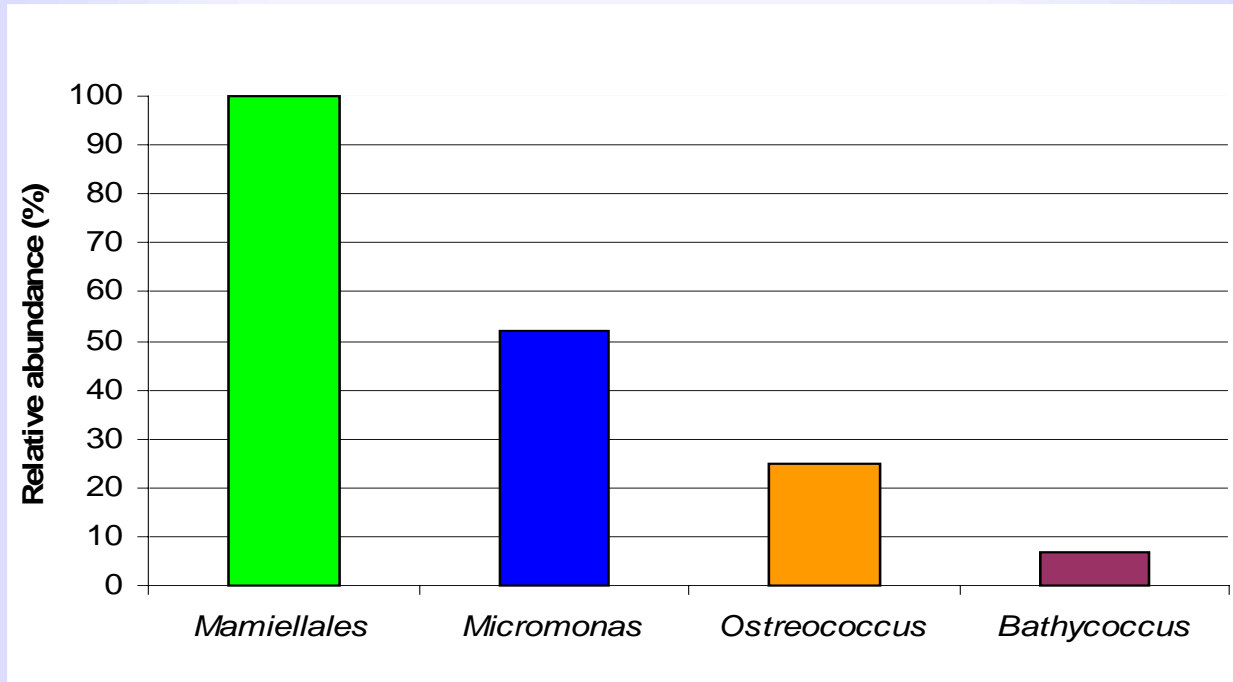
# Quantitative PCR: Application on natural samples

- Sample from Roscoff coast, pre-filtered through 3  $\mu\text{m}$  pore sized filter. Extracted DNA is diluted 100x



# Quantitative PCR: Application on natural samples

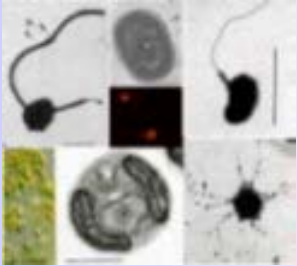
- Relative abundance in Roscoff : preliminary results.



# Conclusion

- Successful application on natural samples
- Primers need some additional development for optimal reaction
- Principal difficulties are :
  - primer design
  - inhibition effect with high quantity of DNA

# Acknowledgements



**PICODIV**

**The Oceanic Phytoplankton team**

**And the Station Biologique de  
Roscoff**

