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Non-Photochemical Quenching of Chlorophyl Fluorescence and Operation of the Xanthophyll Cycle in Estuarine Microphytobenthos

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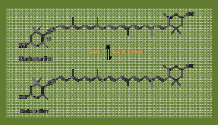
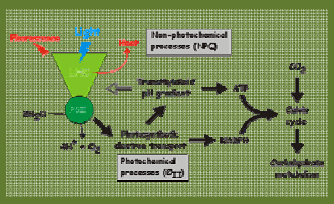
Photoprotective response of microphytobenthos to excessive light

All photosynthetic organisms have a xanthophyll cycle, in diatoms this cycle comprises only one reversible step: the conversion of an epoxy xanthophyll – **Diadinoxanthin** – into an epoxy-free carotenoid – **Diatoxanthin**



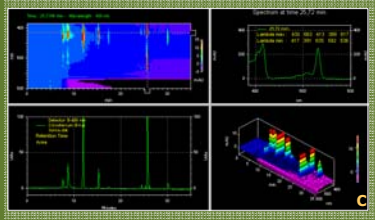


Absorption of sunlight that exceeds the capacity for CO_2 fixation results in a build up of a thylakoid pH gradient which activate the de-epoxidation of **DD** into **DT** leading to a dissipation of excess excitation energy in the antenna of photosystem II (PSII) thereby preventing an over excitation of the PSII reaction center

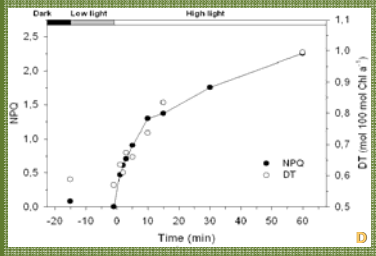
NPQ vs DT

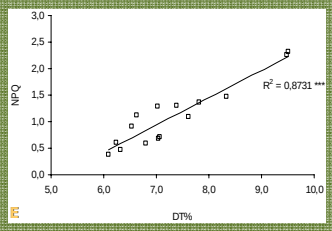
NPQ induction kinetics: Microalgae suspensions were exposure to high actinic irradiance during increasing times (up to 60 min; 1700 µmol quanta m^{-2} s^{-1}). Non-Photochemical quenching (**NPQ**) were measured using the Pulse Amplitude Modulated (**PAM**) fluorometry: $NPQ = (F_{m\,max}' - F_m')/F_m'$

Pigment analysis: High irradiance: 1, 2, 3, 5, 10, 15, 30 and 60 min → liquid nitrogen → **HPLC** → $\%DT = \frac{DT}{(DD + DT)} \times 100$



C. Example of a chromatogram for **Pigment analysis** using **HPLC** to estimate DT content. Pigments were identified from absorption spectra and retention times, and concentrations were calculated from the area of pigment peaks





D. Parallel variation of DT and NPQ leading to an **highly significant correlation** ($P<0,001$) between NPQ and DT content (**E**) showing that NPQ formation was mainly due to photoprotective mechanisms

Conclusion

It was seen that **NPQ has a linear relationship with DT** which means NPQ formation was mainly due to photoprotective mechanisms.

The results of NPQ induction in **microphytobenthos revealed an high capacity for photoprotection**.

The **response to excessive light was significantly higher and faster in November than in July**. NPQ measured after 30 min of high light exposure reached a maximum value of 5.36 – **very high!!!**

Seasonal variability was found: maximum NPQ capacity, NPQ in the dark and NPQ induction Kinetics were higher in November than in July, suggesting important changes in the potential photoprotective response associated to **acclimation to winter conditions**. The enhanced NPQ operation observed in November could result from an **increase of a DD pool** size as a result of acclimation to low temperature. The exposure to the direct sunlight under low temperatures can be particularly damaging to the photosynthetic apparatus. The increase in the **NPQ in the dark** in November would thus represent a side effect of the increase in the DD pool size and the capacity for NPQ operation.

Substantial **changes in taxonomic composition** were found between July and November, considering this, the seasonal variation of the NPQ light responses may also be interpreted as resulting from the appearance of diatom species with the ability of developing an higher photoprotective capacity. Naturally the community composition might have changed due to several causes and the increase in NPQ capacity be the result of the capacity of most species to physiologically acclimate to changing light and temperature conditions on a seasonal scale.

Sampling

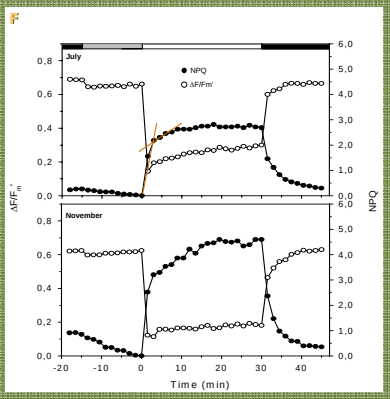
A. Intertidal mudflat located near Vista Alegre (Canal de Ílhavo) on **Ria de Aveiro**, a mesotidal estuary on central west coast of **Portugal**

B. Microphytobenthos – benthic microalgae assemblages dominated by diatoms – were collected from the surface of sediment shortly after the beginning of diurnal low tide. Sampling were carried out during two spring-neap tidal cycles, on **July** and **November 2004**

NPQ induction

F. An **example** of NPQ induction measured in each spring-neap tidal cycles

- Lower values of **NPQ** in **July** than in **November**
- Bi-phasic **NPQ** increase under high light
- Initial large decrease of **$\Delta F/F_m'$** under high light followed by a small and gradual recover
- Microphytobenthos revealed an **high capacity** for **photoprotection** when compared with the published values for diatoms grown in culture. This was particularly true in **November** when NPQ values higher than 5 were measured

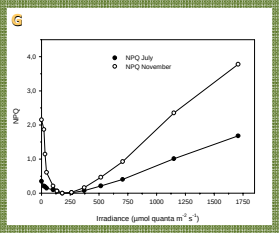


NPQ vs E curves

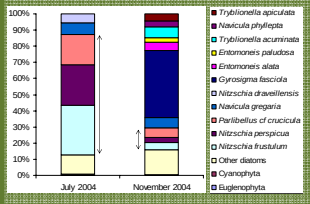
Microalgae suspensions were exposed to increasing irradiance levels (21 to 1707 µmol quanta m^{-2} s^{-1}) and NPQ were measured

G. An **example** of NPQ formation as a response to light for each spring-neap tidal cycles

- **High NPQ** values was formed in the **dark** and decreased gradually with irradiance until a minimum was reached under 100-250 µmol quanta m^{-2} s^{-1}
- Linear increase of NPQ under high light was seen and it was not saturated at 1700 µmol quanta m^{-2} s^{-1}



Taxonomic composition



- Large seasonal variation in the diatom composition
- Higher diversity in November than in July
- **July** was dominated by *Nitzschia perspicua*, *Nitzschia frustulum* and *Parlibellus crucicula*
- In **November** those species occurred in lower abundance relative to the most abundant specie: *Gyrosigma fasciola*

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