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SEASONAL VARIATIONS OF PHOTO-ACCLIMATION IN THE PHYTOPLANKTON ASSEMBLAGES OF THE TUSCAN TYRRHENIAN SEA

VARIAZIONI STAGIONALI DELLA RISPOSTA DI FOTOACCLIMATAZIONE
NEI POPOLAMENTI FITOPLANCTONICI DEL MAR TIRRENO TOSCANO

Abstract - The photo-acclimation responses of natural phytoplankton from the Tuscan Tyrrhenian Sea were assessed in different seasons through the measurements of Photosynthesis-Irradiance curves (PEc) by pulse amplitude modulated (PAM, Walz) variable fluorescence. The analysis of photosynthetic parameter variations allowed distinguishing between low-light (shade-acclimation) and high-light (sun-acclimation) responses. The relationship between phytoplankton response to light and the structure of the water column (mixed or stratified) is discussed, together with the taxonomic composition. Variable fluorescence PEc measurement turned out to be particularly suitable in these low chlorophyll *a* concentration conditions, finally allowing the assessment of photosynthetic parameters as a valid alternative to more traditional gas exchange techniques.

Key-words: adaptation, irradiance, phytoplankton, photosynthesis.

Introduction - Photosynthesis-Irradiance curves (PEc) are a widely used tool for assessing phytoplankton response to light variations, i.e. photo-acclimation. A microalgal sample is exposed for a chosen time lag to a series of increasing irradiances E and the photosynthetic rate P is measured for each E value. Any PEc is associated to its photosynthetic parameters α (the light-limited slope), P_{\max} (the light-saturated rate), E_k ($=P_{\max}/\alpha$, the light-saturation index) and β (the light-inhibiting slope, usually detectable in aquatic autotrophs; Saggiomo *et al.*, 2010). Furthermore, the calculation of PE parameters is essential for primary productivity modelling (in particular P_{\max}^B , i.e. P_{\max} on a chlorophyll *a* basis as an index of phytoplankton productivity; Behrenfeld and Falkowski, 1997). The analysis of E_k allows distinguishing between a low-light acclimation (low E_k =shade-acclimation) and conversely a high-light acclimation (high E_k =sun-acclimation). As an alternative to PEc by gas exchange, the measurement of photosynthetic rate by pulse amplitude modulated (PAM, Walz) variable fluorescence is quick and non-invasive (Lazzara *et al.*, 2010b). In particular, the WaterPAM (Walz) has a high sensitivity, which makes it suitable for low chlorophyll-*a* suspensions ($>0.1 \mu\text{g L}^{-1}$). Furthermore, the chance to measure automatic Rapid Light Curves (RLC; White and Critchley, 1999), choosing a very short acclimation time lag (<1 min), allows assessing phytoplankton short-term scale responses just after sampling. Within the framework of the cross-border cooperation project MOMAR (Sistema integrato per il MOnitoraggio e il controllo dell'ambiente MARino, 2010-2011), PEc were measured on natural phytoplankton samples collected on surface and at depth during three different seasons in the Tuscan Tyrrhenian Sea, and photosynthetic parameter variations were analysed in relation with the water column structure (mixed/stratified) as an indirect index of light-field variations. The aim of this study was the investigation of phytoplankton short-term scale response to light, so as to assess a preliminary seasonal characterization of photo-acclimation of the microalgal assemblages of the Tuscan Tyrrhenian Sea.

Materials and methods - Samples were collected at three sites of the Tuscany coast opposite San Rossore (SR), Piombino (PB) and Talamone (T), along cross transects with sampling stations at 3 km, 10 km and 25-30 km offshore, in spring 2010 (March-April), summer 2010 (July) and winter 2011 (January-February). At each station, temperature and salinity along the water column were measured by an Idronaut Ocean Seven CTD probe. Water samples for biological analysis were collected in 10-L Niskin bottles at discrete depths between the surface and 50 m. Pigments were detected by HPLC (Class VP HPLC system, Shimadzu) according to Barlow *et al.* (1997). PE curves were measured on both surface coastal (3 km) and offshore (25-30 km) samples, and deep offshore (30 km) samples (23 total samples) by variable fluorescence using the WaterPAM fluorometer (Walz). PEc were measured immediately after their collection following the RLC protocol: 4 subsamples of each sample were kept in the dark for 3 min, then exposed for 20 s at each PAR of a series of 9 (maximum PAR value: 1300 $\mu\text{E m}^{-2} \text{s}^{-1}$). For each sample, a single curve was calculated after interpolation of the 4 replicates, according to Platt *et al.* (1980)'s equation using the SPSS-TC2D software (Lazzara *et al.*, 2010b). Finally, 500 mL water samples were collected and fixed in dark PVC flasks (neutralised formaldehyde at a final concentration of 1%) and analysed by counting after sedimentation of 50-100 mL on an inverted optical microscope (40 \times , ZEISS IM, Zingone *et al.*, 2010).

Results - During winter, the mean photosynthetic parameters of all samples were similar ($\alpha=0.17 \mu\text{mol e}^{-} \mu\text{E}^{-1}$, $P_{\max}=\text{ETR}_{\max}$ (maximum Electron Transport Rate)=12.8 $\mu\text{mol e}^{-} \text{m}^{-2} \text{s}^{-1}$, $E_k=76 \mu\text{E m}^{-2} \text{s}^{-1}$, $\beta=0.095 \mu\text{mol e}^{-} \mu\text{E}^{-1}$, Fig. 1 left), thus indicating that both surface and deep, coastal and offshore phytoplankton were shade-acclimated under winter low-light conditions. Shade-acclimation was particularly stressed in the deep offshore sample of Talamone (T30m50: $\text{ETR}_{\max}=7.2 \mu\text{mol e}^{-} \text{m}^{-2} \text{s}^{-1}$, $E_k=47 \mu\text{E m}^{-2} \text{s}^{-1}$), whose phytoplankton assemblage was dominated by Coccolithophores (>60%, with *Emiliania huxleyi* the most abundant species). A similar composition was detected in the corresponding surface sample T30, nevertheless PEc enabled us to differentiate two distinct photo-acclimation responses. In winter, the water column was homogeneously mixed (temperature around 14-15 °C and salinity ~37.5 psu, respectively) both in the coastal and offshore stations, with the exception of SR, which showed lower surface salinity values, due to freshwater inputs from the Arno river outlet. Due to vertical mixing, all phytoplankton assemblages were subjected to a similar light field, thus photo-acclimation responses were similar. Further, high Fv/Fm (mean: 0.598) indicate that PSII performance was efficient. High surface chlorophyll *a* was detected at the coastal stations SR3 (2.7 $\mu\text{g L}^{-1}$, around 10⁶ cell/L, 37% diatoms, with *Pseudo-nitzschia* sp. *delicatissima* complex dominating the assemblage, and 25% Cryptophyceae) and T3 (1.3 $\mu\text{g L}^{-1}$, 45% Cryptophyceae, 26% Coccolithophores - *Emiliania huxleyi* - and 17% diatoms - *Chaetoceros* spp.), while generally lower values were present at depth and offshore (mean: 0.55 $\mu\text{g L}^{-1}$). In spring 2010, the surface coastal assemblage in SR3 was clearly sun-acclimated ($E_k=133 \mu\text{E m}^{-2} \text{s}^{-1}$, $\text{ETR}_{\max}=26.4 \mu\text{mol e}^{-} \text{m}^{-2} \text{s}^{-1}$, $\beta=0.027 \mu\text{mol e}^{-} \mu\text{E}^{-1}$, Fv/Fm=0.479): here phytoplankton was confined to surface higher light-conditions due to the evident halocline (between surface and 10 m, minimum coastal salinity value: 36.2 psu). *Chaetoceros socialis* and *Thalassionema nitzschioides* were the dominant species (60% diatoms, 20% Cryptophyceae). Conversely, E_k was only 54 $\mu\text{E m}^{-2} \text{s}^{-1}$ in the deep (50 m) offshore sample SR28, which was confined to dim light at depth ($\beta=0.177 \mu\text{mol e}^{-} \mu\text{E}^{-1}$, Fv/Fm=0.527). This sample showed a different composition as well: 25% Cryptophyceae, unidentified nanoflagellates were abundant too, while diatoms were absent. Elsewhere, all surface samples were still generally shade-acclimated ($E_k=82 \mu\text{E m}^{-2} \text{s}^{-1}$ at T3, 77 $\mu\text{E m}^{-2} \text{s}^{-1}$ at P3, 75 $\mu\text{E m}^{-2} \text{s}^{-1}$ at SR28; mean $\beta=0.131$

$\mu\text{mol e}^{-}\mu\text{E}^{-1}$, $\text{Fv/Fm}=0.474$). A shallow thermocline was detected between surface and 5 m at stations P, while the water column was still homogeneously mixed at stations T. The maximum chlorophyll *a* concentration was measured at surface at T3: $1.1 \mu\text{g L}^{-1}$ ($>50\%$ Cryptophyceae, nanoflagellates were abundant, in particular *Pyramimonas* spp.), elsewhere it was homogeneous along the water column (mean: $0.59 \mu\text{g L}^{-1}$). Finally, in summer 2010 surface assemblages were all sun-acclimated (mean $E_k=114 \mu\text{Em}^{-2}\text{s}^{-1}$), conversely deep samples were shade-acclimated (mean $E_k=62 \mu\text{Em}^{-2}\text{s}^{-1}$, Fig. 1 right). A clear mixed layer was present between surface and 12 m, with the thermocline reaching 50 m, thus surface assemblages were confined into high-light conditions, while the deep ones experienced low-light. The comparison between surface and deep phytoplankton composition revealed that the dominant species were the same, but the relative ratios between classes changed: for example, in the offshore P25 station, nanoflagellates and Coccolithophores were abundant in surface waters, while dinoflagellates dominated at depth. The stratification appeared to play the main role in determining the different photo-acclimation responses and we can hypothesize that the variations in the relative abundances of the phytoplankton groups can reflect the different responses to light. Summer mean PSII efficiencies were similar to spring ($\text{Fv/Fm}=0.482$), with the exception of the offshore assemblages in SR28, whose values were markedly lower (surface: 0.319, 50 m: 0.253). Both the two assemblages showed similar composition; in fact, they were dominated by the same classes (Dinoflagellates, Coccolithophores, Prasinophyceae) though in a slightly different relative ratio. Chlorophyll *a* was generally low both in surface and deep, coastal and offshore samples (mean: $0.12 \mu\text{g L}^{-1}$). When studying the seasonal variability of mean ETR_{\max}^B , an index of phytoplankton productivity, the summer values were around seven-fold higher ($143 \mu\text{mol e}^{-}\text{m}^{-2} \text{s}^{-1} (\mu\text{g chla L}^{-1})^{-1}$) than in winter and spring (19 and $23 \mu\text{mol e}^{-}\text{m}^{-2} \text{s}^{-1} (\mu\text{g chla L}^{-1})^{-1}$, respectively), thus indicating that in summer phytoplankton productivity was clearly higher though biomass was lower. An analogous observation has been made on P^B through the use of bio-optical models of pelagic primary production (Lazzara *et al.*, 2010a) in the Ligurian and North Tyrrhenian Seas.

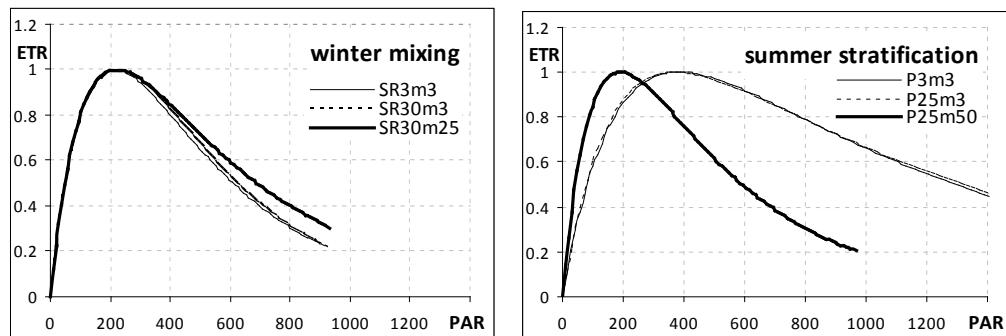


Fig. 1 - ETR-normalized PEc: in winter, the water column is homogeneously mixed and curves are similar (San Rossore, left), while curves are clearly different under conditions of summer stratification (Piombino, right).

Curve PE normalizzate per il valore di ETR: curve simili in condizione di rimescolamento verticale (San Rossore, sinistra), curve ben diversificate durante la stratificazione estiva (Piombino, destra).

Conclusions - A general seasonal variability in the photo-acclimation of phytoplankton from the Tuscan Tyrrhenian Sea was detected. The assemblages were

generally shade-adapted to winter low-light. This feature was more evident in the offshore deep samples at Talamone and the analysis of PE parameters revealed a different short-term acclimation, when compared to the corresponding surface sample, though a similar taxonomic composition. Shade-acclimation was still generally present in spring, with the exception of the San Rossore samples, where distinct responses were detected in a diatom dominated assemblage. In this case, the different taxonomic composition could partially explain the different responses to light. Finally, summer stratification could determine distinct whether shade or light acclimation in assemblages with similar composition but different relative abundances. Our preliminary study supports the choice of modulated variable fluorescence measurements as an effective tool in assessing *in situ* photo-acclimation of natural low-chlorophyll assemblages. Future investigations along our coasts should comprise further PE measurements and their interannual comparisons, with the aim to better characterize photo-acclimation ability in the water column and to eventually detect its link with the taxonomic composition of the assemblages.

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