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**Chlorophyll-*a*
variability in the NW
Adriatic**

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NW Adriatic Sea variability in relation to chlorophyll-*a* dynamics in the last 20 years (1986–2005)

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This paper presents a long-term time series (1986–2005) of hydrological and biogeochemical data, both published and unpublished. Data were collected in the north-western area of the Adriatic Sea, at two stations that are considered hydrodynamically and trophically different. The time series have been statistically and graphically investigated on a monthly scale in order to find not only possible chlorophyll-*a* trends over time, but also links between the concentrations of chlorophyll-*a* and the variability in the environment, as well as trophic differences between the two areas. Basically, in both cases the statistical test results show no significant trends in either the average chlorophyll-*a* values or in dispersion of the data, in contrast with significant trends in temperature and salinity. The two areas have similar hydrological features, yet they present significant differences in the amount of nutrient inputs: these are in fact higher at the coastal site, which is characterized by a prevalence of surface blooms, while they are lower at the offshore station, which is mainly affected by intermediate blooms. Nonetheless, throughout the whole water column, chlorophyll-*a* concentrations are only slightly different. Both areas are affected by riverine discharge, though in the first case considered chlorophyll-*a* concentrations are also driven strongly by the seasonal cycle. Finally, the results show that the two stations are not trophically different, although some controlling factors, such as zooplankton grazing in one case and light attenuation in the other, may regulate the growth of phytoplankton.

1 Introduction

The Northern Adriatic Sea (Fig. 1) is a shallow shelf basin with an average depth of 35 m and a prevalent cyclonic circulation of water masses (Artegiani et al., 1997a). The area is largely affected by riverine inputs that provide the basin with a significant flow of freshwater and land-derived nutrients. The Po river is by far the largest Italian river: in the last 20 years it attained a mean discharge of $1465 \text{ m}^3 \text{ s}^{-1}$ with high intra- and

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inter-annual variability (st. dev.= $1056\text{ m}^3\text{ s}^{-1}$), with peaks in May, mainly due to snow melting, and in October–November, because of high precipitation (Fig. 2). Furthermore, the Northern coast has several smaller rivers that contribute to the overall flow. Consequently, the hydrodynamics of the Northern Adriatic Sea are quite complex and strongly affected by large variations in heat fluxes and the volume of incoming fresh-water (Artegiani et al., 1997a). A temporal succession of two different hydrodynamic patterns has been recognized: between November and March, the westernmost waters are diluted mainly by the Po River outflow and remain separated from the highly saline and vertically-mixed offshore waters thanks to a frontal system located 8–16 km from the coast. The dissolved and particulate matter, coming from the land, therefore remains more or less confined. Between April and October, warmer waters diluted by freshwater inflows are confined to the surface layer and reach almost all of the Northern basin. During that period, one or more pycnoclines separate the water masses of intermediate density, while the high-density waters are confined near the bottom (Artegiani et al., 1997a).

Phytoplankton abundance and distribution is largely dependent on nutrients and light availability, but also on the stability of the water column. The large periodic modification in the structure and dynamics of the Northern Adriatic Sea causes large spatial and temporal distribution of phytoplankton species composition, biomass and production (Fonda Umani, 1996). A general west-to-east decreasing gradient in the phytoplankton standing crop and production has been recognized in winter, while during summer stratification the lateral advection of river run-off in surface layers and the presence of marked pycnoclines result in vertical heterogeneities and local variations in primary productivity (Franco and Michelato, 1992). Primary productivity, representing the immediate result of interactions among physical, chemical and biological variables, gives a dynamic overview of the environment, and is therefore a valuable tool for following the complex effects of freshwater inputs in the Northern Adriatic system (Socal et al., 2002). Although there have been some previous large-scale spatial and temporal studies that focused on the hydrological (e.g., Artégiani et al., 1997a,b; Raicich, 1996) and

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biogeochemical (Zavatarelli et al., 1998) characteristics of the Adriatic Sea, research into the inter-annual variability of the Northern basin productivity related to its hydrology is still scarce.

Recent studies have reported a rise in water temperature (e.g., Corti et al., 1999), in the Mediterranean Sea (Rixen et al., 2005) and in the Northern Adriatic basin (Russo et al., 2002). Not much data analysis, apart from model experiments (Vichi et al., 2003a), has been done, however, on the chlorophyll-*a* response to the changing meteorological conditions in the Northern Adriatic Sea.

We present here the analysis of 20 years (1986–2005) of physical, chemical and biological data, both published and unpublished, collected at two stations, that have operated since the beginning of the twentieth century (Fig. 1). Many authors (e.g., Alberighi et al., 1997; Pugnetti et al., 2003, 2004; Bernardi Aubry et al., 2006) have classified E06 as a station that is influenced by the Po river run-off and characterized by meso-eutrophic waters, while they have represented C10 as a meso-oligotrophic station that is only partially influenced by the Po river discharge; the latter occurs particularly during intense stratification periods, when the Po plume eventually turns north-eastwards. In particular, whereas Alberighi et al. (1997) referred to the two stations as trophically different, Pugnetti et al. (2003) could not typify a significant difference in community composition, because a prevalence of tolerant species is observed in the whole area of the North Adriatic Sea. Bernardi Aubry et al. (2006) concluded that the hydrological and trophic variability seemed mainly to affect phytoplankton abundance and biomass rather than species composition.

The main objectives of this work are:

- (i) to analyse long-term variations in chlorophyll-*a* dynamics at the two stations, either highlighting or excluding possible trends related to climate change and/or to anthropogenic pressures.
- (ii) to compare C10 and E06 chlorophyll-*a* variability on a monthly scale in order to confirm or reject the hypothesis of two very different trophic areas.

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(iii) to relate chlorophyll-*a* to other hydrological and biogeochemical variables so as to identify the principal factors influencing phytoplankton dynamics in both areas.

2 Sampling and methods

The sampling stations C10 (45°15' 00" N, 12°46' 00" E), and E06 (44°57' 50" N, 12°46' 20" E) are located about 20 and 10 nautical miles from the Italian coast and have a maximum depth of 29.5 and 32 m, respectively (Fig. 1). Chlorophyll-*a*, dissolved inorganic nutrients and oxygen samples were taken at fixed depths of 0, 5, 10, 15, 20, 25 m (standard levels, SL). Samples were collected monthly from April 1986 to August 2005, during different cruises and supported by different project funding. Because of the high short-term variability of the Adriatic environment, we decided to analyse the data on a monthly scale, even if, in doing so, the winter months are less represented (Fig. 3). The data frequency shows a different amount of samples for each month, while in the same month the number of samples from both C10 and E06 is comparable. For each station we analysed, at every SL: temperature, salinity and density, obtained using a CTD probe; samples of nutrients (N-NH₃, N-NO₂, N-NO₃, Si-SiO₄, P-PO₄), dissolved oxygen and chlorophyll-*a* using Niskin and Nansen bottles. Dissolved inorganic nutrients were analysed according to the methods described by Strickland and Parsons (1972) and Grasshoff et al. (1999), dissolved oxygen by the Winkler method (Winkler, 1914), while chlorophyll-*a* was assessed according to Holm-Hansen et al. (1965). In the end, 564 samples from C10 and 530 from E06 were fluorometrically analysed.

Statistical analyses (descriptive, non-parametric, principal component analysis and partial regression) were performed using commercial software (Statistica by Statsoft). Test results were considered: significant at p-level <0.05, very significant at p-level <0.01 and highly significant at p-level <0.001. The powerful non-parametric Wilcoxon Matched Pairs test was used to compare the biogeochemical features between the two sampled sites. Both the Cox-Stuart test and the τ -Kendall test were performed to highlight possible trends in time. We only show the Cox-Stuart test results, since it

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better suits our kind of data, which are non contiguous, generic and presenting several anomalous values. A comparison between the vertical distribution of chlorophyll-*a* in the water column at the C10 and E06 stations was carried out by Whisker plots and the significance of the results was tested by the non-parametric Sign test and the Wilcoxon Matched Pairs test. The results of both tests are pretty similar; we present only the results of the Wilcoxon test, since it is considered more powerful (Conover, 1999; Sprent and Smeeton, 2001). The correlation between chlorophyll-*a* and other physical and hydrochemical variables was studied using parametric tests, after the normalization of some of the non-normal distributions. A principal component analysis (PCA) was then applied to identify the weight of each variable, presenting the results of the first two components. To avoid auto-correlation phenomena between the environmental variables considered independent, we finally applied a Ridge Regression analysis to the normalized data for clarifying the statistically-significant, linear correlation between chlorophyll-*a* and the other variables. Ridge regression is used when the independent variables are highly intercorrelated: a constant bias (λ) is added to the diagonal of the correlation matrix, which is then re-standardized so that all diagonal elements are equal to 1 and the off-diagonal elements are divided by the constant. In this way Ridge regression artificially lowers the correlation coefficients so that more stable estimates (β coefficients) can be computed. Graphic linear interpolations were carried out using commercial software (Surfer 8.0 by Golden Software): the median values of all the variables at every SL have been plotted on a monthly scale by the linear Kriging method.

3 Results

3.1 Temperature, salinity and chlorophyll-*a* inter-annual trends

The Cox-Stuart test, which analyses the central trends in the surface water temperature (Table 1), shows a significant increase in May and June and a very significant

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one in July at both sites, in agreement with the rise in air temperature and annual heat fluxes observed in the Northern Adriatic Sea (Russo et al., 2002). Another significant increase is found in December at both sites, indicating a tendency towards milder winters. However, C10 is the only station showing a significant trend in the whole years median values. In fact, between August and November E06 is characterized by a negative temperature trend which, without being a significant value, still agrees with the registered increasing flow of the Po river that causes floods during autumn (Raicich, 2003). The same feature is not found at C10, probably because the freshwater effect is confined to the Northern Adriatic Current region (Artegiani et al., 1997a).

This is further confirmed by the Cox-Stuart test results for surface salinity (Table 1). No significant trend is found at C10, either in spring, or in summertime, when the area is eventually reached by the Po river plume. Significant trends are instead seen at E06: a very significant decrease in February, a significant decrease in October and a very significant increase in November.

Chlorophyll-*a* data for both stations, are plotted in Fig. 4 for the whole period. The Cox-Stuart Sign test (Table 2) reveals no significant long-term trend in either the central (median) value of chlorophyll-*a* concentration, or in its dispersion of data: the slight negative trend in the C10 annual value is considered random, as is the slight increase at E06. On the other hand, C10 shows a significant increase in the central value in April, although this is not really relevant considering the entire annual cycle. The variability is fairly constant, with a small indication of increase for both stations. Any missing result is due to an insufficient number of available comparisons.

3.2 Hydrological and biogeochemical features

In Table 3 we list the number of valid observations, the medians, the ranges and the Wilcoxon Matched Pairs test results of comparison between the two stations for all the available hydrological and biogeochemical variables over the 20-year period. Descriptive statistics reveals that the main differences between the two sites are due to the large dispersion in the E06 variable values, rather than to any differences in the trends

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of the central (median) values. Statistically high significant differences are observed for all the nutrients' concentrations, except N-NO₂ and also, to a less extent ($p < 0.05$), for the chlorophyll-*a* values between the two areas.

The high spatial and temporal variability of the hydrological and biogeochemical features directly and indirectly influencing the chlorophyll-*a* dynamics in the area needs to be analysed on the proper scale, in order to be able to capture the variability. All monthly median values, at every SL, are shown in Hövmöller time-depth plots in Fig. 5 (C10) and in Fig. 6 (E06).

Both sites show similar features in the hydrological variables. The density isolines (panel c) reveal that the water column is mixed between November and March and stratified for the rest of the year. The rise in temperature (panel a) starts to stratify the water in April, with a maximum surface temperature in August (C10 $T = 27.2^{\circ}\text{C}$, E06 $T = 27.1^{\circ}\text{C}$). Freshwater inputs, limited to the first 10 m of the water column, affect the salinity fields in different ways from site to site. The salinity (panel b) at C10 begins to decrease in March, so that the minimum of surface salinity ($S = 34.6$) is found in May–June, reflecting the first annual peak of the Po river discharge (Fig. 2). The salinity starts to increase in July, and in autumn the C10 area is less affected by the second peak of the Po river ($S = 36.3$). The salinity at E06 has a much lower value in March, so it reaches a surface minimum earlier, in April ($S = 31.3$), remaining at constant values around 33–34 until August. In October it detects again the effect of the Po river peak with a second minimum surface value of 34.1.

The nutrients' concentrations show similar distributions at both sites, with lower concentrations at C10. Nutrient profiles are generally characterized by higher surface concentrations down to a depth of 5–10 m. In the last 15 m of the water column, nutrient concentrations are either uniform or increasing with depth, depending on the variable.

The water column is almost completely depleted in ammonia (panel d) throughout the year at C10, and between March and October at E06. Surface peaks are present at C10 in December ($[\text{N-NH}_3] = 1.7 \mu\text{mol dm}^{-3}$), while they are seen at E06 in December ($[\text{N-NH}_3] = 3.1 \mu\text{mol dm}^{-3}$) and January ($[\text{N-NH}_3] = 4.1 \mu\text{mol dm}^{-3}$). In the deep-

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est measured 5 m of the water column, values increase from April onwards, reaching a maximum in September ($[N-NH_3]=1.2 \mu\text{mol dm}^{-3}$) at C10 and in October ($[N-NH_3]=1.8 \mu\text{mol dm}^{-3}$) at E06. Values decrease again to a minimum ($[N-NH_3]=0.3 \mu\text{mol dm}^{-3}$) in January–February at C10 and in March at E06.

5 Nitrite (panel e) remains at low concentrations at both sites: close to zero between April and October and slightly higher for the rest of the year, reaching a maximum in January ($[N-NO_2]=1.0 \mu\text{mol dm}^{-3}$: C10 at 5 m, E06 at the surface).

10 The nitrate time evolution (panel f) indicates that very low concentrations are found between May and October at C10 and between June and September at E06. Higher nitrate values are found at the surface in December, March, April and May at both stations, coinciding with high chlorophyll-*a* concentrations, marking an excess of nitrate. The E06 values are the highest, reaching a maximum of $21.0 \mu\text{mol dm}^{-3}$ in March in correspondence with the Po outflow, while C10 reaches a maximum of $7.0 \mu\text{mol dm}^{-3}$ in January. Because of the second Po river peak and the increase in the vertical mixing processes, which diffuse the bottom-regenerated nutrients, the entire water column shows high values during winter at both sites.

15 Dissolved inorganic nitrogen ($[DIN]=[N-NO_2]+[N-NO_3]+[N-NH_3]$, panel g) reflects mainly the nitrate trend at the surface and the ammonia trend at the bottom.

20 Phosphate concentrations (panel h) are close to zero at both sites, at all depths, during most of the year. Slightly higher concentrations are present in December and January ($[P-PO_4]=0.2 \mu\text{mol dm}^{-3}$), although E06 values are generally higher.

25 Silicate concentrations (panel i) are only high at the surface at E06 (max $[Si-SiO_4]=13.4 \mu\text{mol dm}^{-3}$ in January), reflecting low salinity concentrations and therefore the Po river's influence, while they are high throughout the water column at both sites in December and January, because of mixing processes. Silicate concentrations, increasing with depth at both stations, highlight the importance of benthic regeneration processes to this variable (Giordani et al., 1992).

Dissolved oxygen (panel j) shows comparable concentrations at both sites, as well as a good general oxygenation of the waters and a progressive decrease of concentra-

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tions with depth, well revealing an opposite annual pattern compared to the ammonia, phosphate and silicate concentrations. Minimum values, close to hypoxic phenomena, are found in the deeper SL in September–October (C10: $[O_2]_{\text{sat}}=56.8\%$, E06: $[O_2]_{\text{sat}}=59\%$) at the end of the stratification period. Successively, stratification is broken by surface cooling and wind stirring action, redistributing oxygen concentrations throughout the whole water column during the following months (November, December).

Chlorophyll-*a* (panel k) shows a complex annual cycle at both stations. C10 surface waters are characterized by a January peak and low values for the rest of the year. The lowest concentrations are found between May and October, corresponding to the stratified period (panel k) with low DIN and DIP (dissolved inorganic phosphate) concentrations. Subsurface maxima, below a depth of 15 m in March and in the whole water column in November, coincide with the river nutrient inputs. E06 presents the highest chlorophyll-*a* concentrations at the surface throughout the year, a pattern that is exactly opposite to that of salinity. In fact the highest values are registered in March–April, September and November, months that are characterized by very different hydrological and biogeochemical conditions.

3.3 The vertical variability of chlorophyll-*a*

The Hövmöller plots of Fig. 5 and Fig. 6 give an immediate overview of the median situation characterizing both sites' environment. In this section we focus on an analysis of the variability. Monthly vertical profiles of chlorophyll-*a* concentrations for both sites are shown in Fig. 7, where the Whisker plots represent the median values and the non-outliers ranges (Whisker, coeff.=1) at every SL. Table 4 presents the valid number of observations, the medians, the ranges and the Wilcoxon Matched Pairs test for chlorophyll-*a* on a monthly scale at both stations.

Excluding exceptional values, classified as outliers, median concentrations do not exceed $4 \mu\text{g dm}^{-3}$, and the scale is limited to $8 \mu\text{g dm}^{-3}$. Similar vertical profiles are found between October and February, when mixing processes prevail. During the rest

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of the year, which is mainly characterized by strong temperature and salinity stratification, E06 maintains higher concentrations in the surface layer, while C10 does the same mostly at intermediate depths.

The annual cycle at C10 is characterized by minimum concentrations between May and October, but maximum ones in November, followed by April, March and May, while the other months have intermediate values. Apart from November, E06 is generally characterized by high concentrations limited to the first 5–10 m layer.

Statistically significant differences (Table 4) are found in July, August, September and October. The significant differences detected in July and August and the very significant ones in September are due to sensibly lower concentrations in the first 20 m at C10. October is the only month during which concentrations in the entire water column are very significantly higher at C10 than at E06. For the rest of the year, the chlorophyll-*a* profiles at C10 and E06 have opposite trends and cross each other at different depths between 10 and 20 m.

3.4 Relationships among variables

With the aid of principal component analysis (PCA) we intended to investigate which factors drive the variability of and the interconnections between the physical and biogeochemical variables, particularly in relation to the seasonal cycle and the loads of nutrients.

In order to carry out the PCA and the Ridge Regression tests, it was necessary to test the normality of the data distribution using the Shapiro-Wilk test. Table 5 reports the Shapiro-Wilk test results and the transformation that better suited the normal distribution. A normal distribution was significantly obtained at both sites for all the variables, except for phosphate.

The variables' PCA projection on the factor plane is represented in Fig. 8. Among the variables, we also considered depth as an indicator that explains surface/bottom processes. From the correlation matrix, the two components extracted explained 51.61% of the total variance for C10 and 58.68% for E06. Both stations present all the consid-

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ered variables grouped in three quadrants: the first quadrant is for salinity-depth covariance, the second one is for oxygen-temperature and the third one is for nutrients. At C10 ammonia is situated in the salinity-depth quadrant, opposite to the oxygen concentration. In this graphical representation chlorophyll-*a* is found in different quadrants: in the case of C10 it is located in the nutrients quadrant, while in the case of E06 it has an intermediate position between nutrients and oxygen-temperature.

The Ridge regression results (Table 6) are listed according to the order in which the variable was introduced in the model equation, together with the consequent *p*-level of significance. At C10, the first highly significant positively-related variable is nitrate, followed by the highly significant negatively-related salinity, the very significant positive relation with depth and the significant negative relation with temperature. The negative relation with ammonia and the positive one with nitrite are also introduced in the regression equation, although their relation is not significant. At E06, all the variables introduced in the model are at least significant. The first highly significant negative relation is with salinity, followed by the significant positively-related nitrate, the very significant negative relation with ammonia, the significant negative relation with phosphate, the very significant positive relation with silicate and the significant positive relation with oxygen. The nutrient relations were positive or negative depending on consumption or excess. The strongest chlorophyll-*a* dependence is then positive with nitrate at C10, followed by the hydrological variables, while at E06 it is negative with salinity, followed by the nutrients' concentrations.

4 Discussion

The analysis of relationships among the different physical and biogeochemical variables is hampered by the imposed choice of the sampling frequency with respect to the temporal scale that would better characterize the processes that affected those variables. A monthly frequency analysis is probably sufficient to capture the seasonal cycle in the physical parameters, but not the local, directly-affected biological response. The

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chlorophyll-*a* signature is less constrained by seasonal processes and more affected by short-term disturbances and small-scale spatial distribution.

Indeed, the Cox-Stuart test for temperature (Sect. 3.1) is highly significant, especially in summer, at both stations, although an overall significant trend is detected only at C10. A possible explanation for this behaviour lies in the different relationship that both stations have with the river run-off. This is further confirmed by the test for the salinity trend (Sect. 3.1). The way we explain these results is that both areas detect the effect of the increasing warming (Corti et al., 1999; Rixen et al., 2005), but only E06 is likely to show the effects of the increasing precipitation. In fact, both stations are affected by the rise in summer temperature, due to higher heat fluxes. But, while at E06 the rise in temperature is balanced by an autumn reduction, due to the large quantity of freshwater inflow, at C10 this does not happen, since no effect is detected of the Po water spreading there during autumn. Therefore at C10 the water generally becomes warmer. The analysis of a possible temporal trend for chlorophyll-*a* does not show any significant change in concentrations, either in the central value or in the data dispersion, which is in contrast with the overall positive trend for temperature at C10 and the negative one for salinity at E06 during November. The trends in temperature and salinity are thus not associated with a trend in chlorophyll-*a*, which we may assume to be an indicator of standing biomass (Vichi et al., 2003a).

Despite some indications of altered concentrations in other hydrochemical variables, such as the phosphorous decrease reported by Degobbis et al. (2000) and later by Bernardi Aubry et al. (2004), we are not able to define a possible associated chlorophyll-*a* change on this temporal scale and with this high natural variability. We can then hypothesize that: i) a real change did not happen; ii) the temporal scale of the chlorophyll-*a* processes might have not fitted with the other variable trends; iii) the ecosystem evolution might have favoured new species in the composition of the phytoplankton population without changing the total stock; iv) a possible trend might exist at the extreme concentrations, which are not sufficiently represented in the sample.

The descriptive statistical analysis presented in Sect. 3.2 reveals that, considering

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both sites, the physical variables are not significantly different, while the major nutrients' concentrations are highly different. This indicates that the C10 and E06 areas have significantly comparable hydrological characteristics, while they are differently affected by the river's discharge. However, chlorophyll-*a* only shows a weakly significant difference, indicating that the nutrient standing stocks are not a sufficient criterion to characterize trophic conditions, in contrast with previous analyses of the trophic variability of the area (e.g., Alberighi et al., 1997). High-frequency interconnected physical and biological processes are thus likely to modulate chlorophyll-*a* dynamics, also when the nutrients' availability is dominant.

The hydrological and biogeochemical evolution presented in Sect. 3.2 shows that the C10 station is directly influenced by very high river outflows, which are stronger in May–June when the general circulation and stratification can favour the NE spreading of the Po plume, and weaker in October–November. Conversely, E06 is largely affected by the Po river run-off, irrespective of their magnitude, and also by other smaller rivers, as confirmed by much lower salinity values for all of the non-mixed periods. Finally, at both stations the minimum surface density value is in August, revealing the fact that temperature has a major role in determining the density fields. Another minimum is present in April at E06 when the temperature is still low, indicating that salinity is the major factor responsible for the density value during this phase of the annual cycle.

Generally, the nutrient profiles have higher surface concentrations, reflecting the opposite trend of the vertical distribution of salinity and the direct influence of the river inputs.

The surface peaks of winter ammonia are most likely due to its release by phytoplankton after the November blooming. The lowest 5 m of the water columns show concentrations that well reflect a trend opposite to that of the dissolved oxygen in the same period, indicating benthic recycling of ammonia. The role of benthic nutrient remineralization is then an important factor that can be inferred from the monthly analysis.

Low salinity values during periods of low nitrate concentrations suggest that most of the land-derived nitrate is taken up by phytoplankton. However, while C10 is char-

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acterized by low concentrations of chlorophyll-*a*, E06 maintains higher values. This means that N-NO₃ values are not the only explanation for high surface concentrations of chlorophyll-*a* at E06, but that some other controlling factors may affect phytoplankton growth there. Consequently, either the organisms developed a good adaptation at low nutrient concentrations at E06, or there was zooplankton consumption at C10; it may also have been due to the higher surface concentrations of silicate that characterized only E06 (but not C10) over the year.

High concentrations of chlorophyll-*a*, during those months and at those depths at which the phosphate concentrations are close to zero, suggest that phosphorus is consumed and rapidly remineralized to sustain abundant biomass production.

Despite a good understanding of the behaviour of the hydrological and biogeochemical features, one is faced with a chlorophyll-*a* time evolution that shows a very complex annual cycle in both areas. C10 blooms at different depths during the year, while E06 has mainly surface blooms. The chlorophyll-*a* vertical profiles are similar when mixing processes prevail. For the rest of the year, which is characterized by a strong stratification of temperature and salinity, E06 maintains higher concentrations in the surface layer, where it is mostly affected by riverine loads, while C10 does so mostly at intermediate depths, because a smaller presence of photo-attenuating materials may allow photosynthesis in the entire water column (Vichi et al., 2003b).

At C10, chlorophyll-*a* concentrations seem to pretty well follow the annual cycle of nutrients. At E06, the annual cycle is much more complex, and does not seem to closely follow the temporal succession of nutrients. Except for November, high concentrations are limited to the first 5–10 m layer. However, every period of high concentration is followed by a period of low concentration, indicating that some factor acts successively and regulates phytoplankton abundance. We suppose that, the nutrients being sufficient and sometimes in excess at this site, zooplankton grazing, whose annual cycle is usually out of phase with that of phytoplankton, can be one of the main regulating factors of the chlorophyll-*a* pattern.

This is also confirmed by the PCA and the Ridge regression tests (Sect. 3.4). The

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first principal component of the PCA (Fig. 8) captures the effect of periodical stratification, showing negative correlation between the groups of nutrients, salinity-depth and oxygen-temperature. The second component highlights the effect of river inputs, showing an opposition between nutrients and salinity, with little explanation of temperature-oxygen variability. The chlorophyll-*a* position confirms our previous hypothesis: at C10, chlorophyll-*a* concentrations are mainly correlated to riverine run-off, while at E06, the nutrients being at least sufficient, the correlation is strongly positive with the seasonal cycle and opposite to depth-salinity, because the highest concentrations are found at the surface.

A further confirmation comes from the Ridge Regression test results (Sect. 3.4). At C10, the only nutrient in the equation is nitrate, which is also the closest variable to chlorophyll-*a* in the PCA projection. The following correlations with the physical variables are significant: negative with salinity and temperature, as expected for areas located offshore but affected by land-derived nutrients, and positive with depth because of the mostly intermediate blooms. At E06, the Ridge Regression highlights a strongly opposite trend with salinity, which is responsible for the surface blooms and is well-shown in the PCA diagram (Fig. 8). Finally, the equation includes a positive correlation with nitrate and silicate, indicating an excess of these nutrients for phytoplankton requirements, and a negative correlation with ammonia and phosphate, that are therefore consumed at the site.

5 Conclusions

The long-term set of hydrological and biogeochemical data coming from the C10 and E06 stations, located in the NW Adriatic Sea, allowed us to formulate a reasonable picture of: the chlorophyll-*a* temporal trend, the vertical distributions of chlorophyll-*a* and other biogeochemical properties, their variability on a monthly time scale and the links between them, as well as the trophic differences between the two areas. We recognize that lateral advection might have affected this scenario to some extent:

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at C10, the biomass can indeed be mostly driven by remote production or extended sources (Carniel et al., 2007).

The main difference between the biogeochemical variables was not due to the central (median) values, but to a wider range of data distribution at E06, which showed a generally higher dispersion. Both sites showed similar hydrological features despite their different location, both being clearly influenced by riverine inputs. C10 was mainly affected by river discharge maxima, while E06 was almost permanently stratified because of freshwater inputs. Between June and October both areas have low nutrients and chlorophyll-*a* concentrations, and they have to be considered oligotrophic. For the rest of the year, they display meso-oligotrophic characteristics with temporary eutrophic conditions only at the surface. This leads us to classify the area differently from the previous literature (e.g., Alberighi et al., 1997; Pugnetti et al., 2003, 2004; Bernardi Aubry et al., 2006).

The vertical distribution of properties also showed similar patterns: the hydrological variables, oxygen distribution and phosphate concentrations are comparable in the water column, nitrogen is higher at the surface and silicate is higher at the bottom. Nonetheless, the vertical distribution of chlorophyll-*a* was rather different: E06 had mainly surface blooms in April, September and November, while C10 had blooms in January, March, September and November at different SL depending on the month. The analysis of the relationships among the variables suggested that at C10 chlorophyll-*a* was mainly controlled by river inputs, even though we expected this to be the main regulating factor at E06, which instead was positively correlated with the seasonal cycle and negatively correlated with depth and salinity, because of the mostly surface blooms. We suppose that a high presence of photo-attenuating material limits the photosynthesis at a deeper E06 SL, while nutrient availability is the main regulating factor for phytoplankton growth at C10.

Finally, very low concentrations of phosphate, coupled with exceptional chlorophyll-*a* values, suggest that we should review the thesis of phosphorous being a classical growth-limiting factor of phytoplankton abundance in the North Western Adriatic

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Sea. We suggest that other phosphorous sources besides orthophosphate, such as organic phosphorous and fast regeneration processes, can contribute to maintaining phytoplankton growth. It is therefore necessary to resolve the fast remineralization processes in the pelagic domain on a higher frequency scale in order to clarify the effective co-limiting factors.

A long-term monthly scale analysis was essential to understand typical time scales of the processes that control the concentration and distribution of the properties and the variability of the area. Even if we are not able to assess the climatological change, the Cox-Stuart test for temperature and salinity in the first 5 m of the water column, where the annual variability is higher, agrees pretty well with the Eastern Mediterranean Climatic Transient (EMCT). The EMCT is a global change in the meteo-oceanographic conditions happening over the Mediterranean Sea during recent decades (Rixen et al., 2005), and is defined as a collection of events, such as rising temperature and fewer rainy days with heavier precipitation. However, the absence of a significant chlorophyll-*a* trend in time at either site, in agreement with model results of scenario simulations (Vichi et al., 2003a), is not sufficient to allow us to assume that the communities did not change. Previous studies, based on a shorter time scale (Pugnetti et al., 2003; Bernardi Aubry et al., 2006), could not typify any significant difference in community composition. Our next step will therefore be to couple this study with an analysis of the abundance, biodiversity and distribution of the different species of phytoplankton during the same study period in order to find if an intra- and inter-annual variability is present in the long term instead.

Low salinity values together with low concentrations of nitrate and other nutrients, that result in high chlorophyll-*a* concentrations at E06, but low ones at C10, can probably be explained by zooplankton uptakes at C10 and lower consumptions at E06. We therefore suggest the importance of studying the coupling between the phytoplankton cycle and zooplankton abundance and distribution, since zooplankton is an essential controlling factor in the phytoplankton trend and evolution throughout the trophic chain characterizing an area.

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Table 1. Cox-Stuart test results for surface temperature (left) and salinity (right) at C10 and E06^a.

| Month | Temp | | | | | | | | | | Sal | | | | | | | | | |
|-------|------|----|----|-------|------|-----|----|----|-------|------|-----|----|----|-------|------|-----|----|----|-------|------|
| | C10 | | | | | E06 | | | | | C10 | | | | | E06 | | | | |
| | N | + | - | p | sig. | N | + | - | p | sig. | N | + | - | p | sig. | N | + | - | p | sig. |
| Jan | 6 | 2 | 0 | - | - | 6 | 3 | 0 | - | - | 6 | 0 | 3 | - | - | 6 | 1 | 2 | - | - |
| Feb | 19 | 5 | 4 | 0.500 | ns | 16 | 3 | 5 | 0.344 | ns | 19 | 2 | 7 | 0.090 | ns | 16 | 0 | 8 | 0.004 | ** |
| March | 12 | 3 | 3 | 0.656 | ns | 10 | 2 | 3 | - | - | 12 | 4 | 2 | 0.344 | ns | 10 | 5 | 0 | - | - |
| April | 18 | 5 | 4 | 0.500 | ns | 13 | 4 | 2 | 0.344 | ns | 18 | 6 | 3 | 0.254 | ns | 14 | 4 | 3 | 0.500 | ns |
| May | 26 | 11 | 2 | 0.033 | * | 27 | 11 | 2 | 0.011 | * | 26 | 4 | 9 | 0.133 | ns | 27 | 10 | 2 | 0.055 | ns |
| June | 18 | 8 | 1 | 0.020 | * | 17 | 7 | 1 | 0.035 | * | 18 | 4 | 5 | 0.500 | ns | 17 | 4 | 4 | 0.637 | ns |
| July | 32 | 14 | 2 | 0.002 | ** | 27 | 12 | 1 | 0.002 | ** | 18 | 4 | 5 | 0.500 | ns | 17 | 4 | 4 | 0.637 | ns |
| Aug | 20 | 2 | 6 | 0.055 | ns | 21 | 3 | 7 | 0.172 | ns | 21 | 5 | 5 | 0.623 | ns | 21 | 4 | 5 | 0.500 | ns |
| Sep | 32 | 9 | 6 | 0.227 | ns | 25 | 3 | 9 | 0.073 | ns | 32 | 11 | 5 | 0.105 | ns | 25 | 6 | 6 | 0.613 | ns |
| Oct | 22 | 5 | 6 | 0.500 | ns | 18 | 3 | 6 | 0.254 | ns | 22 | 7 | 4 | 0.274 | ns | 18 | 1 | 8 | 0.035 | * |
| Nov | 19 | 5 | 4 | 0.500 | ns | 15 | 1 | 6 | 0.072 | ns | 19 | 7 | 2 | 0.090 | ns | 15 | 7 | 0 | 0.008 | ** |
| Dec | 12 | 6 | 0 | 0.016 | * | 12 | 6 | 0 | 0.016 | * | 11 | 0 | 5 | - | - | 12 | 0 | 5 | - | - |
| Sum | 236 | 75 | 38 | 0.001 | *** | 207 | 58 | 42 | 0.111 | ns | 236 | 60 | 56 | 0.384 | ns | 208 | 50 | 48 | 0.397 | ns |

^a +: number of increases, -: number of decreases, ns: not significant, * p<0.05: significant, ** p<0.01: very significant, *** p<0.001: highly significant.

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Table 2. Cox-Stuart test results for chlorophyll-*a* at C10 (left) and E06 (right)^b.

| Month | C10 | | | | | | | | | | E06 | | | | | | | | | |
|-------|-------|-----|-----|-------|------|------|----|-------|------|-----|-------|-----|-------|------|----|-------|-------|------|--|--|
| | cent. | | | | | dis. | | | | | cent. | | | | | disp. | | | | |
| | N | + | - | p | sig. | + | - | p | sig. | N | + | - | p | sig. | + | - | p | sig. | | |
| Jan | 10 | 5 | 0 | – | – | 2 | 0 | – | – | 12 | 5 | 1 | 0.109 | ns | 1 | 2 | – | – | | |
| Feb | 48 | 9 | 15 | 0.240 | ns | 4 | 4 | 0.637 | ns | 40 | 7 | 13 | 0.132 | ns | 3 | 3 | 0.656 | ns | | |
| March | 23 | 6 | 5 | 0.500 | ns | 4 | 1 | – | – | 21 | 7 | 3 | 0.172 | ns | 3 | 2 | – | – | | |
| April | 51 | 22 | 3 | 0.006 | ** | 5 | 4 | 0.500 | ns | 38 | 11 | 8 | 0.324 | ns | 4 | 5 | 0.500 | ns | | |
| May | 64 | 17 | 15 | 0.393 | ns | 5 | 3 | 0.363 | ns | 68 | 16 | 18 | 0.393 | ns | 10 | 6 | 0.227 | ns | | |
| June | 46 | 13 | 10 | 0.365 | ns | 8 | 3 | 0.113 | ns | 50 | 14 | 11 | 0.368 | ns | 4 | 3 | 0.500 | ns | | |
| July | 73 | 12 | 24 | 0.075 | ns | 5 | 4 | 0.500 | ns | 62 | 16 | 15 | 0.399 | ns | 8 | 2 | 0.055 | ns | | |
| Aug | 43 | 10 | 13 | 0.365 | ns | 5 | 6 | 0.500 | ns | 54 | 18 | 9 | 0.122 | ns | 5 | 3 | 0.363 | ns | | |
| Sep | 83 | 20 | 21 | 0.399 | ns | 5 | 5 | 0.623 | ns | 74 | 19 | 17 | 0.378 | ns | 4 | 4 | 0.637 | ns | | |
| Oct | 53 | 11 | 15 | 0.340 | ns | 4 | 4 | 0.637 | ns | 50 | 9 | 16 | 0.194 | ns | 3 | 5 | 0.363 | ns | | |
| Nov | 44 | 6 | 15 | 0.058 | ns | 7 | 4 | 0.274 | ns | 28 | 5 | 9 | 0.212 | ns | 3 | 4 | 0.500 | ns | | |
| Dec | 22 | 0 | 11 | – | – | 3 | 2 | – | – | 30 | 9 | 6 | 0.304 | ns | 3 | 4 | 0.500 | ns | | |
| Sum | 278 | 131 | 147 | 0.266 | ns | 48 | 37 | 0.223 | ns | 262 | 136 | 126 | 0.341 | ns | 51 | 43 | 0.261 | ns | | |

^b cent.: central trend, disp.: data dispersion, +: number of increases, -: number of decreases, ns: not significant,

**p<0.01: very significant.

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Table 3. Valid Number of samples, Median, Minimum, Maximum and Wilcoxon Matched Pairs test for all the variables at C10 and E06^c.

| Variable | C10 | | | | E06 | | | | Wilc.test | | | | |
|---------------------|-----|--------|------|-------|-----|--------|------|-------|-----------|-------|------|------|------|
| | N | Median | Min. | Max. | N | Median | Min. | Max. | N | T | Z | p | sig. |
| Temper. | 563 | 15.8 | 7.5 | 28.8 | 528 | 15.2 | 5.8 | 29.4 | 527 | 67738 | 0.45 | 0.65 | ns |
| Salinity | 563 | 37.5 | 31.0 | 38.6 | 528 | 37.6 | 15.7 | 38.5 | 527 | 64877 | 1.34 | 0.18 | ns |
| Density | 563 | 27.6 | 20.5 | 29.9 | 528 | 27.9 | 11.2 | 30.0 | 527 | 65164 | 1.26 | 0.21 | ns |
| OxSatur | 511 | 99.2 | 7.7 | 134.6 | 485 | 99.1 | 13.4 | 158.1 | 451 | 49519 | 0.52 | 0.60 | ns |
| N-NH ₃ | 548 | 0.3 | 0.0 | 21.6 | 507 | 0.6 | 0.00 | 31.7 | 497 | 38806 | 6.98 | 0.00 | *** |
| N-NO ₂ | 549 | 0.1 | 0.0 | 5.0 | 507 | 0.1 | 0.0 | 4.7 | 497 | 52009 | 1.88 | 0.06 | ns |
| N-NO ₃ | 547 | 0.6 | 0.0 | 25.5 | 506 | 0.9 | 0.0 | 93.1 | 494 | 47760 | 4.08 | 0.00 | *** |
| DIN | 555 | 1.3 | 0.0 | 27.8 | 506 | 2.0 | 0.0 | 97.7 | 503 | 43553 | 5.90 | 0.00 | *** |
| Si-SiO ₄ | 548 | 2.1 | 0.0 | 30.1 | 509 | 3.3 | 0.0 | 54.8 | 498 | 47055 | 4.69 | 0.00 | *** |
| P-PO ₄ | 550 | 0.04 | 0.00 | 1.30 | 512 | 0.06 | 0.00 | 1.09 | 503 | 40285 | 5.98 | 0.00 | *** |
| Chl- <i>a</i> | 564 | 0.9 | 0.0 | 11.4 | 530 | 1.1 | 0.0 | 25.3 | 530 | 62886 | 2.05 | 0.04 | * |

^c ns: not significant, * p<0.05: significant, *** p<0.001: highly significant.

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Table 4. Valid Number of samples, Median, Minimum, Maximum and Wilcoxon Matched Pairs test for chlorophyll-*a* at C10 and E06 on a monthly scale^d.

| Month | C10 | | | | E06 | | | | Wilc.test | | | | |
|-------|-----|--------|------|------|-----|--------|------|------|-----------|-------|-------|------|------|
| | N | Median | Min. | Max. | N | Median | Min. | Max. | N | T | Z | p | sig. |
| Jan | 10 | 0.8 | 0.4 | 4.2 | 12 | 1.0 | 0.2 | 2.3 | 10 | 20.0 | 0.764 | 0.45 | ns |
| Feb | 48 | 1.0 | 0.1 | 4.8 | 41 | 1.0 | 0.2 | 5.7 | 41 | 365.5 | 0.598 | 0.55 | ns |
| March | 23 | 1.1 | 0.2 | 5.2 | 21 | 0.8 | 0.2 | 4.4 | 21 | 113.0 | 0.087 | 0.93 | ns |
| April | 51 | 1.2 | 0.3 | 4.8 | 39 | 1.3 | 0.4 | 5.0 | 39 | 277.5 | 1.349 | 0.18 | ns |
| May | 64 | 0.9 | 0.3 | 11.4 | 68 | 1.3 | 0.4 | 25.3 | 63 | 863.0 | 0.993 | 0.32 | ns |
| June | 46 | 0.7 | 0.1 | 2.5 | 50 | 0.8 | 0.2 | 8.1 | 44 | 407.5 | 1.021 | 0.31 | ns |
| July | 73 | 0.6 | 0.1 | 5.3 | 63 | 1.2 | 0.2 | 7.8 | 63 | 645.5 | 2.482 | 0.01 | * |
| Aug | 47 | 0.7 | 0.1 | 10.3 | 54 | 0.9 | 0.2 | 19.4 | 46 | 344.0 | 2.147 | 0.03 | * |
| Sep | 83 | 0.8 | 0.1 | 4.9 | 74 | 0.9 | 0.3 | 8.2 | 72 | 785.5 | 2.966 | 0.00 | ** |
| Oct | 53 | 0.9 | 0.3 | 7.2 | 50 | 0.7 | 0.0 | 5.6 | 47 | 301.5 | 2.778 | 0.00 | ** |
| Nov | 44 | 1.7 | 0.4 | 5.8 | 28 | 1.4 | 0.1 | 3.5 | 28 | 150.0 | 1.207 | 0.23 | ns |
| Dec | 22 | 0.6 | 0.0 | 2.1 | 30 | 1.1 | 0.1 | 2.8 | 22 | 103.0 | 0.763 | 0.45 | ns |

^d ns: not significant, * p<0.05: significant, ** p<0.01: very significant.

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Table 5. Shapiro-Wilk test results and data normalization^e.

| Variable | C10 | | E06 | |
|---------------------|-------|-------|--------|-------|
| | Tran. | Norm. | Tran. | Norm. |
| Temperature | no | y | no | y |
| Salinity | no | y | 3power | y |
| Density | no | y | no | y |
| % Sat. Oxygen | no | y | no | y |
| N-NH ₃ | log | y | log | y |
| N-NO ₂ | 4root | y | 4root | y |
| N-NO ₃ | log | y | log | y |
| DIN | log | y | log | y |
| P-PO ₄ | 4root | n | log | n |
| Si-SiO ₄ | log | y | log | y |
| Chl- <i>a</i> | log | y | log | y |

^e no: normal, log: logarithmic, 3power: cubic power, 4root: quadratic root, y: yes, n: no.

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Table 6. Ridge Regression results for C10 (left) and E06 (right)^f.

| | C10 | | | | | | | E06 | | | | | | | |
|--------------------|--------|----------|--------|-------|--------|-------|------|---------------------|----------|-------|--------|--------|--------|-------|-----|
| | Beta | s.e.Beta | B | s.e.B | t(494) | p | sig. | Beta | s.e.Beta | B | s.e.B | t(455) | p | sig. | |
| Intercept | – | – | 1.667 | 0.411 | 4.057 | 0.000 | – | Intercept | – | – | 0.584 | 0.107 | 5.436 | 0.000 | – |
| NO ₃ | 0.248 | 0.066 | 0.180 | 0.048 | 3.770 | 0.000 | *** | Salinity | –0.361 | 0.055 | –0.000 | 0.000 | –6.578 | 0.000 | *** |
| Salinity | –0.258 | 0.064 | –0.043 | 0.011 | –4.023 | 0.000 | *** | NO ₃ | 0.130 | 0.057 | 0.068 | 0.030 | 2.282 | 0.023 | * |
| Depth | 0.195 | 0.056 | 0.004 | 0.001 | 3.453 | 0.001 | ** | NH ₃ | –0.145 | 0.051 | –0.131 | 0.045 | –2.874 | 0.004 | ** |
| Temper. | –0.113 | 0.055 | –0.004 | 0.002 | –2.055 | 0.040 | * | PO ₄ | –0.083 | 0.041 | –0.205 | 0.101 | –2.033 | 0.043 | * |
| %Ox _{sat} | 0.084 | 0.048 | 0.001 | 0.001 | 1.759 | 0.079 | ns | SiSO ₄ | 0.159 | 0.060 | 0.086 | 0.032 | 2.671 | 0.008 | ** |
| NH ₃ | –0.062 | 0.047 | –0.070 | 0.053 | –1.336 | 0.182 | ns | % Ox _{sat} | 0.138 | 0.057 | 0.001 | 0.001 | 2.437 | 0.015 | ** |
| NO ₂ | 0.062 | 0.055 | 0.126 | 0.112 | 1.119 | 0.264 | ns | | | | | | | | |

^f s.e.: standard error, ns: not significant, * p<0.05: significant, ** p<0.01: very significant, *** p<0.001: highly significant.

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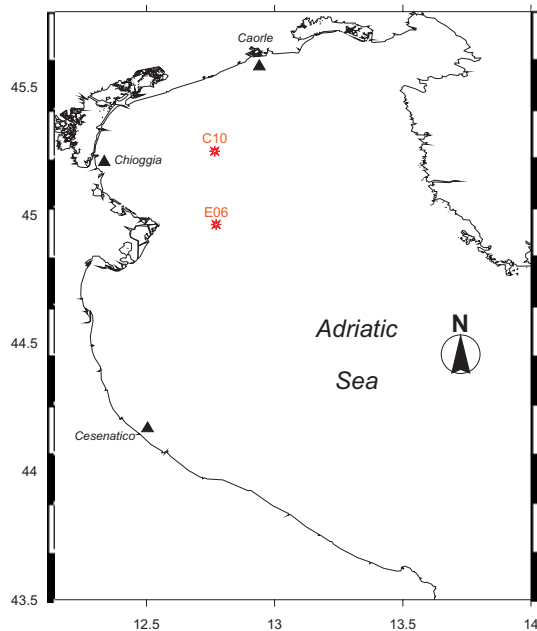


Fig. 1. Study area and location of the sampling stations C10 and E06.

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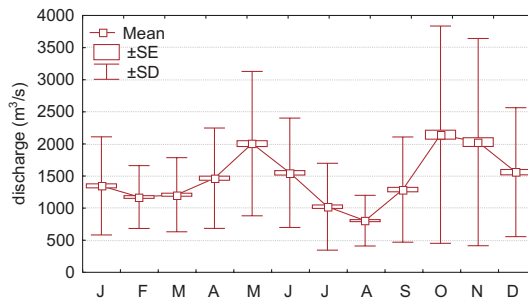


Fig. 2. Monthly mean and variability of the Po river discharge (daily data from “Magistrato del Po”, Parma, Italy).

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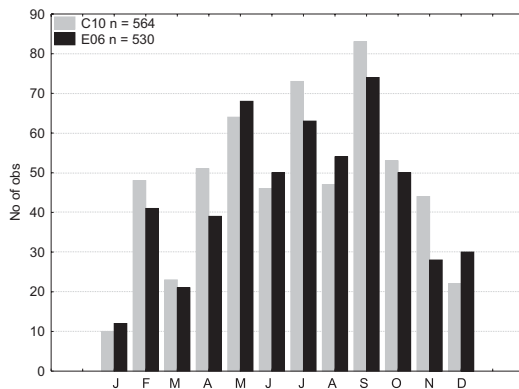


Fig. 3. Number of observations per month.

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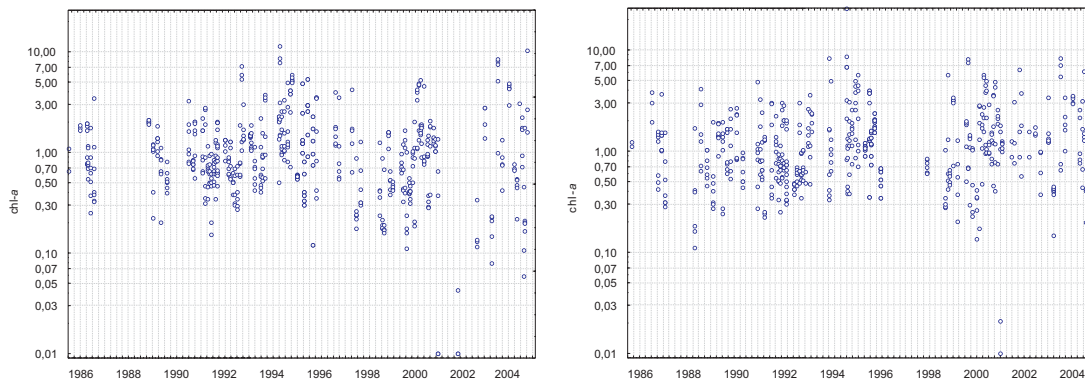


Fig. 4. Chlorophyll-a samples ($\mu\text{g dm}^{-3}$, log scale) collected at C10 (left) and E06 (right).

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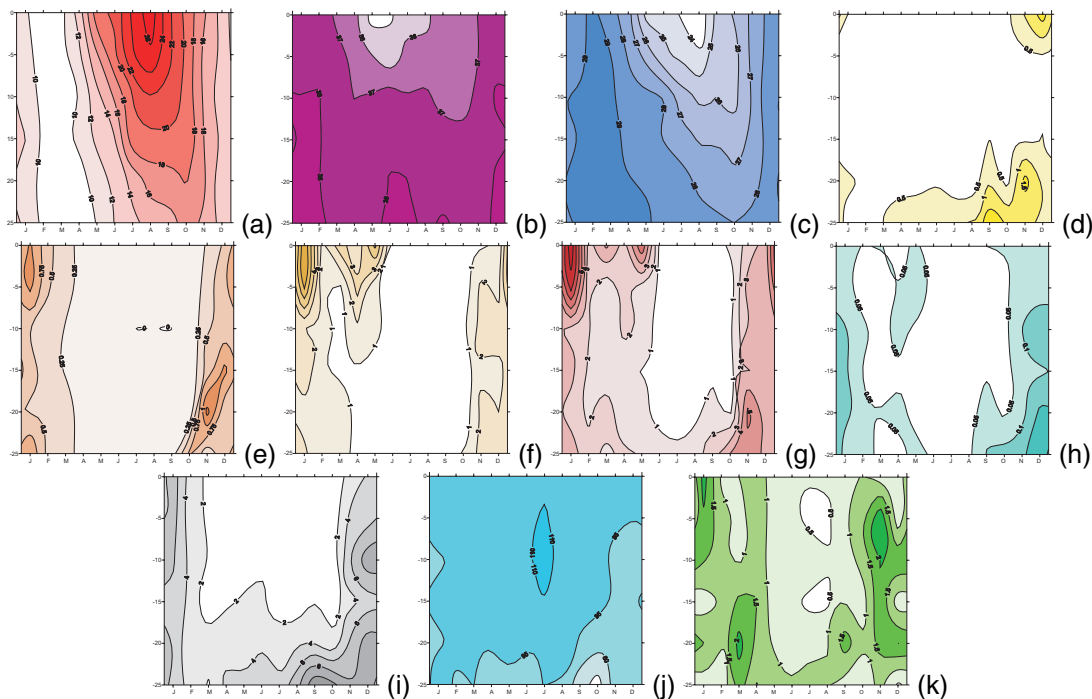
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(a) Temperature ($^{\circ}\text{C}$), **(b)** Salinity, **(c)** Density ($\gamma\text{-t}$), **(d)** Ammonia ($\mu\text{mol dm}^{-3}$), **(e)** Nitrite ($\mu\text{mol dm}^{-3}$), **(f)** Nitrate ($\mu\text{mol dm}^{-3}$), **(g)** Diss. Inor. Nitr. ($\mu\text{mol dm}^{-3}$), **(h)** Phosphate ($\mu\text{mol dm}^{-3}$), **(i)** Silicate ($\mu\text{mol dm}^{-3}$), **(j)** Dissolved oxygen (% sat.), **(k)** Chlorophyll-*a* ($\mu\text{g dm}^{-3}$).

Fig. 5. C10 Hövmöller time-depth plots on a monthly scale.

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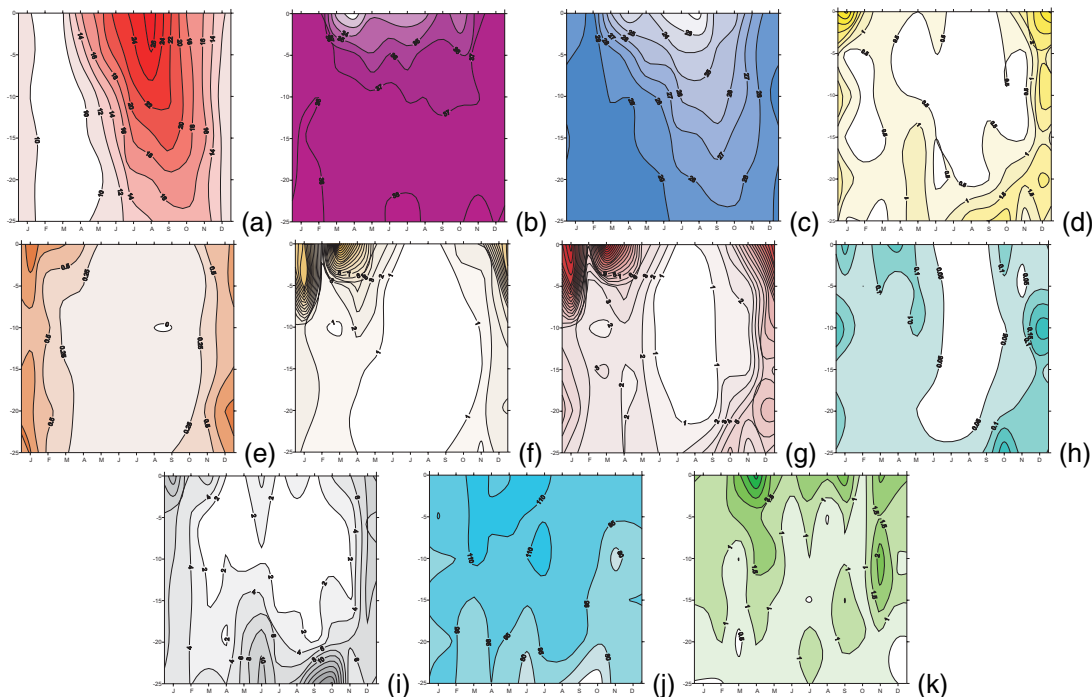
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(a) Temperature ($^{\circ}\text{C}$), **(b)** Salinity, **(c)** Density ($\gamma\text{-t}$), **(d)** Ammonia ($\mu\text{mol dm}^{-3}$), **(e)** Nitrite ($\mu\text{mol dm}^{-3}$), **(f)** Nitrate ($\mu\text{mol dm}^{-3}$), **(g)** Diss. Inor. Nitr. ($\mu\text{mol dm}^{-3}$), **(h)** Phosphate ($\mu\text{mol dm}^{-3}$), **(i)** Silicate ($\mu\text{mol dm}^{-3}$), **(j)** Dissolved oxygen (% sat.), **(k)** Chlorophyll-*a* ($\mu\text{g dm}^{-3}$).

Fig. 6. E06 Hövmöller time-depth plots on a monthly scale.

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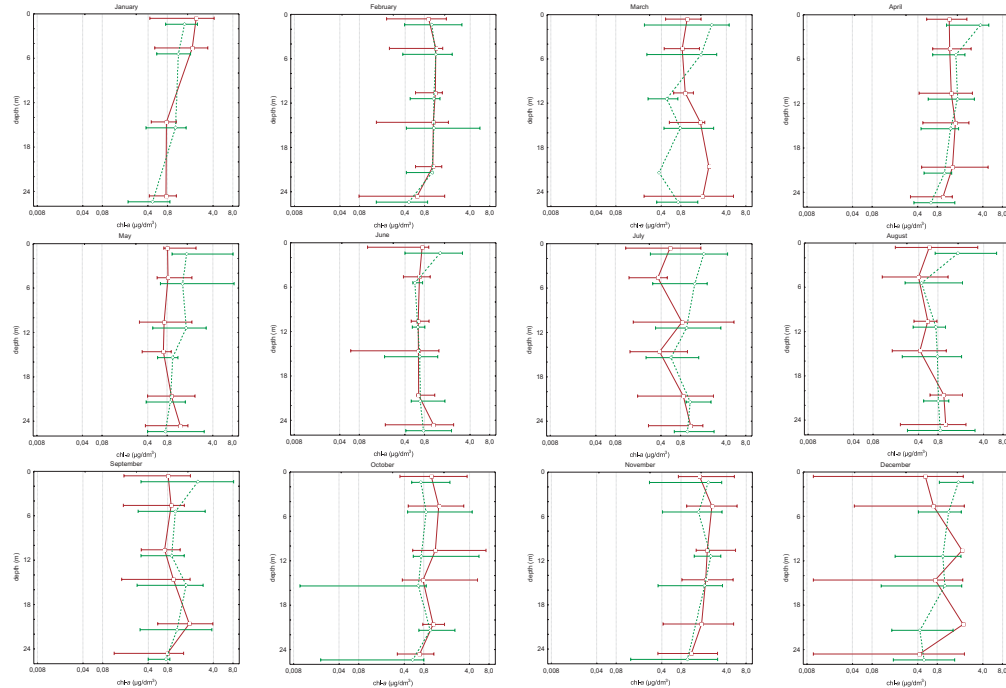


Fig. 7. Monthly vertical profiles of chlorophyll-*a* (log-scale) and range of variability at C10 (red continuous line) and E06 (green dashed line).

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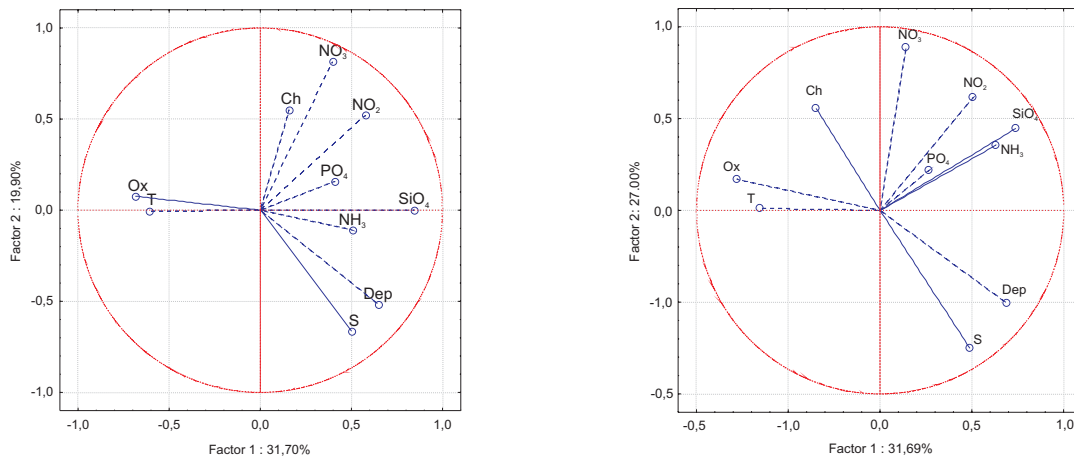


Fig. 8. PCA: projection of the variables (T: temperature, S: salinity, Dep: depth, Ox: oxygen % sat., NH_3 , NO_2 , NO_3 , PO_4 , SiO_4 , Ch: chlorophyll-*a*) on the factor plane (C10, left and E06, right).

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