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Signatures of pigments and processes in the south Adriatic Pit - project MEDUZA

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During two cruises of the MEDUZA experiment conducted in July 2003 and May 2005 various data were obtained over the deepest part of the southern Adriatic Pit. Temperature, salinity, light transmission and fluorescence were measured in July 2003. During May 2005, except CTD measurements the samples were taken for laboratory determination of phytoplankton pigments with HPLC technique. During both cruises irradiance and radiance were measured at 14 wavelengths in the range 340-715 nm with optical probe to about 100 m depth. Bad weather conditions during both cruises caused interruption of measurements that continued after 24 to 48 hours. Stormy conditions have resulted in a slightly different structure of the water column. In the periods of measurements recorded were diurnal rhythms in a series of parameters, caused by solar radiation cycles in the surface layers as well as fluctuations in the deeper layers under the influence of the internal tide. Meteorological conditions and changes in sea level are also described. The analysis included the principal component and correlation analysis between the measured and derived parameters in order to establish a link between the in-situ pigments and multispectral data. During both cruises diurnal vertical oscillations were observed in biotic and abiotic parameters. Chlorophyll pigments prevailed over other pigments preventing other pigments to be distinguished spectrally. Regression models for chlorophyll **a** *were established from radiance and reflectance ratios.*

Key words: South Adriatic Sea, thermohaline properties, diurnal rhythm, internal tide,

INTRODUCTION

The Adriatic Sea (Fig. 1.) is a landlocked sea of the Mediterranean. It is very shallow in its northern part and deepens southward reaching 1200 m. The Otranto Strait connects the Adriatic Sea to the Ionian Sea and the rest of the Mediterranean.

Buoyancy driven cyclonic flow is a permanent feature of the Adriatic Sea (ZORE, 1956), delineated by the East Adriatic Current (EAC) and West Adriatic Current (WAC) (ARTEGIANI *et al*., 1997; POULAIN & CUSHMAN-ROISIN, 2001; VILIBIĆ *et al*., 2009). The WAC brings fresh water south-eastward along the west coast while EAC brings saltier and warmer waters from the Ionian Sea and Levantine basin to the Adriatic Sea. The intrusion of Mediterranean waters in the intermediate layer was proved to influence higher salinity as well as higher productivity along the

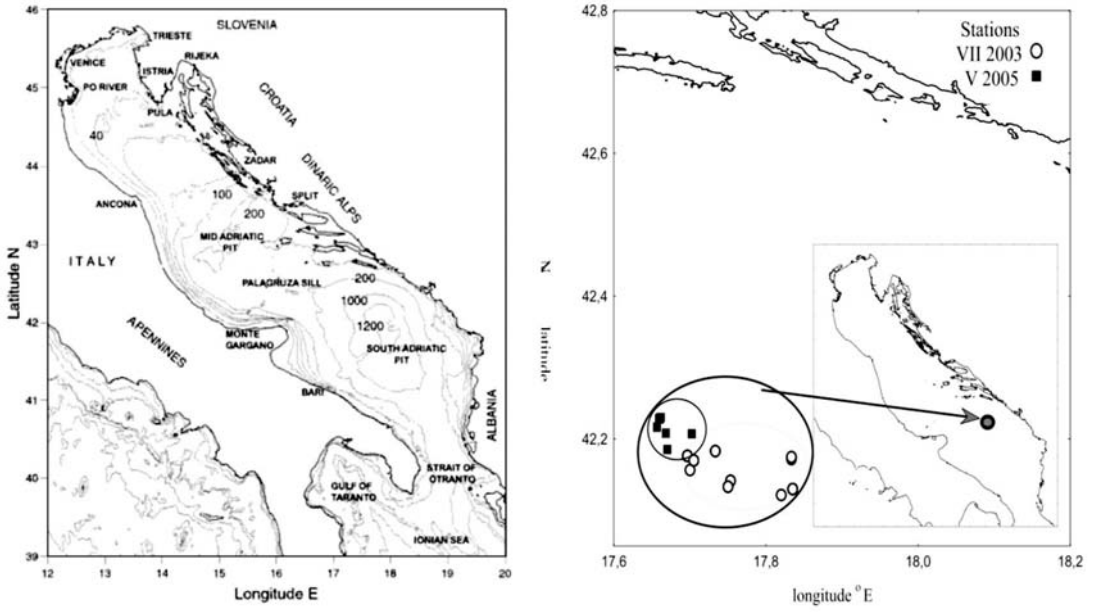

Fig. 1. Bathymetry of the Adriatic and locations during the cruises in July 2003 and May 2005 in the south Adriatic Pit

east Adriatic coast (BULJAN & ZORE-ARMANDA, 1976; MARASOVIĆ *et al*., 1995). Such currents and topography enable formation of cyclonic gyres, like the South Adriatic Gyre. Cross-basin flows are also present mainly in transitional seasons. The Adriatic Sea tides are mainly caused by the tides in the Mediterranean. There are two types of tidal waves, diurnal and semidiurnal. The main components are in phase for east and west Adriatic sides, and phase depends on the distance from the Otranto strait.

In addition to astronomic tide, there may be fluctuations in the water column due to internal tides of typically diurnal period, which may cause significant sea level variations after a wake of a storm (CUSHMAN-ROISIN *et al*., 2001).

The waters from the Mediterranean through the Otranto Strait are mainly transported in the intermediate layers. The intruding waters are saltier and warmer (VILIBIĆ & ORLIĆ, 2002) being influenced by Levantine Intermediate Water (LIW) (BULJAN & ZORE-ARMANDA, 1976). This water is also richer in nutrients and enriches the Adriatic see with some critical elements, so interannual fluctuations of water intrusions from the Mediterranean correspond to the Adriatic increased productivity (MARASOVIĆ *et al*., 1995, 2005; GRBEC *et al*., 2009).

The northern Adriatic, because of its hydromorphology and various coastal (largest Adriatic river Po) and anthropogenic inputs (populated north Italian cities) that cause nutrient over enrichment is among the most eutrophic regions of the Mediterranean (REVELANTE & GILMARTIN, 1995). In the optical sense, the north Adriatic waters and the waters within the coastal color front of the west coast belong to the case 2 waters (BARALE, 1984; MOROVIĆ & PRECALI, 2004). With the exception of some enclosed bays of the east coast in some seasons, the rest of the Adriatic is oligotrophic, especially the waters of the south Adriatic. In the last decades several times in the late winter-early spring season the phytoplankton blooms have occurred offshore, having higher than usual chlorophyll concentrations (VILIČIĆ, 1989), these conditions were also recorded recently by satellite images (MOROVIĆ *et al*., 2004; MOROVIĆ *et al*., 2006).

In this paper we have presented some results of measurements throughout the MEDUZA cruises in late July 2003 and late May 2005, particularly the pigments and optical properties as well as some other supporting data collected simultaneously that could help explaining the variability of optical properties. Our analyses were focused on the several aspects. For both

cruises we have described the meteorological and thermohaline conditions and aimed to understand the dynamical processes.

For the cruise in 2003, optical properties were analyzed via attenuation of light. For the cruise in 2005, optical properties were analyzed also in relation to different pigments.

Many papers that treated remote sensing of ocean color attempted to retrieve concentrations of pigments via different combinations of normalized water leaving radiances and reflectances (SATHYENDRANATH *et al.*, 2004; BARNARD *et al.*, 1999) with high success. Under certain conditions even some phytoplankton species were recognized. In May 2005 we have measured high number of pigments and on the basis of this and the data from *in situ* profiling radiometer we tried to establish algorithms for chlorophyll pigment.

MATERIAL AND METHODS

In the following chapter, brief descriptions of the utilized measurement methodologies are given. In the Tables 1 and 2, the times of acquisition for different parameters are presented in order to compare the number of simultaneous data.

Table 1. Measurement times in July 2003 for CTD casts and for light measurements; correspondence of both measurements are shaded.

July 2003	CTD: SBE25-X; Idronaut-O				PRR800 - X		
Date/ hours	6	12	18	24	6	12	18
22			O	X			
23	X	X	X	X	X	X	X
24	X		O	X	X	X	X
25	X				X	X	X
26							
27	X			X	X	X	X
28			O	X	X	X	X
29							

Pigments

The water samples for determination of pigments were regularly taken with Niskin bottles at depths of 0.5 m, 5m, 15 m, 30 m, 54 m and 100 m, while samplings at 200 m, 300 m, 400 m, 500 m, 700 m, 900 m, 1000 m and 1100 m were taken only twice during measurements. The qualitative and quantitative analyses of pigments in the water samples were performed using a reverse-phase HPLC (High Performance Liquid Chromatography) method (MANTOURA & LLEWELLYN, 1983; BARLOW *et al.*, 1993). Water samples were filtered through Whatman GF/F filters and immediately frozen until analyzed (at -80° C). Frozen samples were extracted in 4 ml of 90% acetone, using sonication, and centrifuged 10 min at 4.000 rpm to remove particles. An aliquot (300 μl) of clarified extract was mixed with 300 μl 1 M ammonium acetate and 500 μl of mixture injected in a gradient HPLC system with 200 μl loop. The HPLC system was equipped with a reverse phase 3 μm C_{18} column (Pecosphere, 35x4.5 mm, Perkin Elmer). Solvent A consisted of 80 % of methanol and 20 % of M ammonium acetate and solvent B contained 60 % of methanol and 40 % of acetone. A linear gradient from 0 % B to 100 % B for 10 min was followed by an isocratic hold at 100 % B for 6 min. The rinse flow was 1 ml min^{-1}. Chlorophylls and carotenoids were detected by absorbance at 440 nm using an UV/Vis spectrophotometric detector (Spectra Physics, Model UV2000). The degradation products of chlorophyll *a* were detected by measuring fluorescence (420/672 nm) with a spectrofluorimetric detector (Spectra Physics, Model FL2000). The data collection and integration was performed utilizing Agilent ChemStation software. Abbreviations for measured pigments are listed in the Table 4.

CTD measurements

During the cruise in July 2003 the thermohaline measurements were performed with the two CTD probes (provided by IOF, Split). The Sea-Bird (SBE) instrument measured temperature, salinity, oxygen, transmission and fluorescence to approximately 250 m depth. This probe was used few times per day. The Idronaut probe was

Table 2. Measurement times in May 2005 for CTD casts, light measurements and sampling of pigments; correspondence of CTD measurements and pigments are shaded light gray; correspondence of light measurements and pigments are shaded medium gray; correspondence of CTD and light measurements are dark gray.

May 2005	CTD					PRR800			PIGMENTS				
Date/ hours	6	12-15	16-17	19-20	24	6	12	18	7-9	12-14	15-18	20	24x
23		X	X	X	X	X	X	X			X	X	X
24		X				X	X	X	X	X			
25													
26			X	X							X	X	
27	X	X		X		X	X	X	X	X		X	X
28						X	X	X					

Table 3. Biomarker pigments as markers of different phytoplankton groups

BIOMARKER PIGMENT	PHYTOPLANKTON GROUP
fucoxanthin	diatoms
19'-hexanoyloxyfucoxanthin	prymnesiophytes
peridinin	dinoflagellates
zeaxanthin	cyanophytes
19'-butanoyloxyfucoxanthin	silicoflagellates
alloxanthin	cryptophytes
chlorophyll b	green algae (chlorophytes, euglenophytes)

used down to 1200 m (temperature and salinity sensors) only few times during the cruise to control the conditions in the deep layers.

During the cruise in 2005 thermohaline parameters and oxygen were measured with the SBE probe (provided by MBS) with available depths until 45 m depth.

Sea level conditions

The data from the station Bari were provided from Istituto Superiore per la Protezzione e la Ricercha Ambientale (Via Vitaliano Brancati, 48, 00144 Roma, Italy). The data for Dubrovnik are acquired from Hydrographic Institute of Republic of Croatia via European Sea Level Database (ESEAS[1]).

Optical measurements

Wetlabs C-star measured beam attenuation along the 25 cm path length, installed on SBE. The measurement results are expressed in terms of beam attenuation coefficient c (m^{-1}). Chlorophyll-like pigments were obtained from fluorescence measured with Wetlabs Fluo sensor, also installed on SBE. These measurements were available only in July 2003.

Spectral irradiance and radiance were measured with the profiling radiometer PRR-800 (Biospherical inc.; http://www.biospherical.com) at 14 wavelengths (340; 380; 412; 443; 465; 490; 510; 532; 555; 589; 625; 665; 683 and 710nm). In both periods these measurements were performed three times a day. The profiler was slowly lowered manually from sunny side of the ship. Calibrated data were processed to 1m depth and reflectance and attenuation coefficients for irradiance were calculated for each meter depth (JERLOV, 1976; KIRK, 1994; PREISENDORFER, 1961). From surface normalized irradiances and radiances the reflectance values were calculated as: $R_\lambda = E_{d\lambda}/L_{u\lambda}$ where $E_{d\lambda}$ denotes downward irradiance for wavelength λ and $L_{u\lambda}$ is upward radiance and $K_{d\lambda}$ is diffuse attenuation coefficient $K_{d\lambda}=d\ln(E_{d\lambda}(z))/dz$.

[1] EUROPEAN SEA LEVEL SERVICE, ESEAS-RI at http://www.eseas.org

The optical data served for calculation of different relations among radiances and among reflectances. These data were put in correlation to concentrations of pigments measured with HPLC. The pigments data were also subject to PCA and correlation analyses.

RESULTS AND DISCUSSION

Pigments

HPLC (High Performance Liquid Chromatography) pigment analysis may be used to determine phytoplankton community structure. Some photosynthetic pigments, above all carotenoids, are specific for particular phytoplankton taxonomic groups (Table 3).

The concentration of carotenoid pigments can be defined only with use of HPLC, no other techniques like spectrophotometric or spectrofluorimetric are appropriate. The photosynthetic pigments have proved to be useful biomarkers of the abundance, composition and physiological status of the phytoplankton biomass in the marine environment although they cannot be considered as fully specific diagnostic markers of individual phylogenetic groups of phytoplankton, and their use should therefore be exercised with caution (JEFFREY *et al.*, 1997). Not all species of particular phytoplankton group contain the same accessory pigment. Pigments concentrations in the cells normally vary with different light quality and intensity, nutrients availability and physiological status of the cell. Because all photosynthetic microalgae (except prochlorophytes) contain chlorophyll *a* the concentration of this photosynthetic pigment can be used to estimate the spatial and temporal changes in phytoplankton biomass as well as the abundance of phytoplankton. The chlorophyll *a* concentrations below the 500 ng l^{-1} indicate oligotrophic conditions while concentrations higher than the 10.000 ng l^{-1} indicate eutrophic conditions (MILLER, 2002). The chlorophyll *a* degradation products are indicators of physiological status of the phytoplankton community. Chlorophyllide *a* and pheophorbide *a* indicate

Table 4. Measured pigments, abbreviations and maximum absorption peaks

Abreviations – Pigments	Color	Wavelengths of absorption peaks (nm) according to Jeffreys et al. (Eds.) 1997		
Chl_a - chlorophyll a	blue-green	430	662	411
Chl_b - chlorophyll b	olive-green	457	646	350
Chl_c2 - chlorophyll c2	light-green	450	396	630
Per - peridinin	brick-red	456	485	430
But - butanoyloxyfucoxanthin	yellow-orange	445	470	418
Fuc - fucoxanthin	orange	446	468	
Neox - neoxanthin	yellow	439	467	415
Hex - hexanoyloxyfucoxanthin	orange	444	470	420
Prasino - prasinoxanthin	deep-pink	454	482	432
Viola - violaxanthin	yellow	470	440	472
Diadino - diadinoxanthin	yellow	448	478	425
Anthera - antheraxanthin	yellow	444	471	
Allo - alloxanthin	yellow-orange	454	484	428
Diato - diatoxanthin	yellow	448	478	425
Zea+Lut - lutein+zeaxanthin	yellow-orange	450	454	481
B_car - beta carotene	yellow-orange	445	448	474
Chlid_a - chlorophyllide a	blue-green	450	478	454
Phbid - pheophorbide a	grey	430	664	412
Pheo - pheophytin a	grey	410	666	505

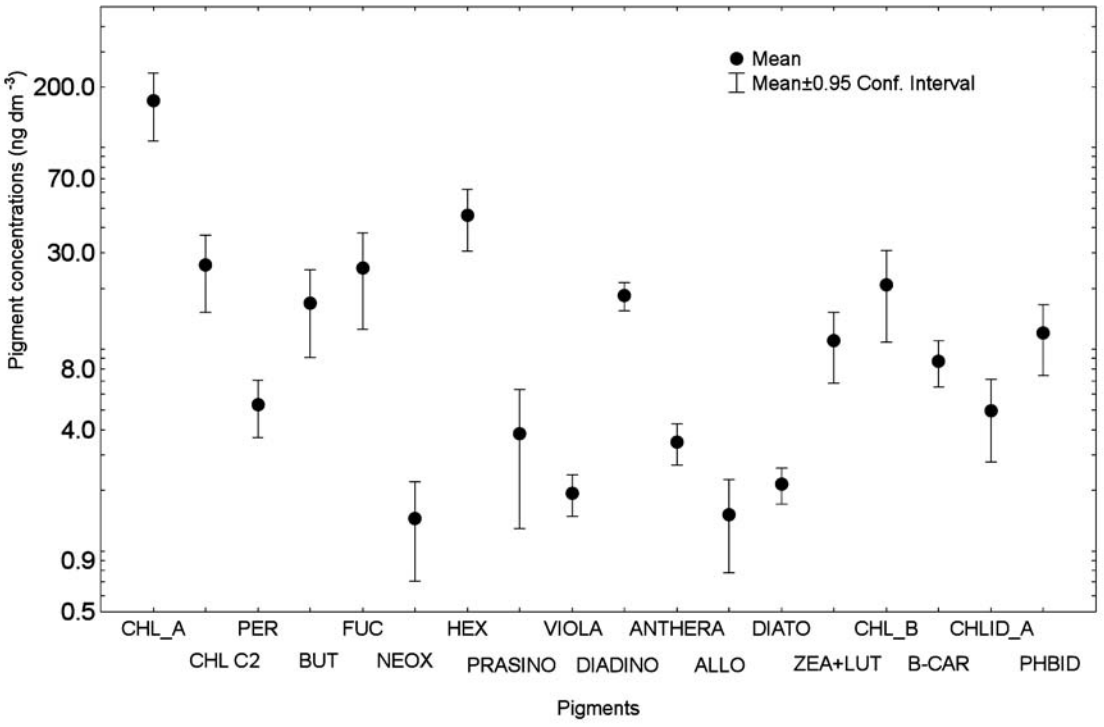

Fig. 2. Average pigments concentrations within 95% confidence limit for pigments measured in May 2005

the dying algae (HEAD *et al*., 1994). Pheopigments, above all pheophorbide *a*, are also indicators of zooplankton grazing and senescent algae (JEFFREY *et al*., 1997).

REVELANTE & GILMARTIN (1995) hypothesized that in the Adriatic Sea the DCM (deep chlorophyll maximum), layer is located at the lowest depth where available light makes it possible for phototrophic populations to exploit nutrients advection from sub-euphotic depths, well below the depth maximum density gradient and associated with the nutricline. The depth and the amount of the pigments in DCM differ during the year, being more evident during the stratification regime (TOTTI *et al*., 2000). When the peak of certain biomarker pigment is represented in higher concentration, it concurs with chlorophyll *a* peak, meaning that this pigment (i.e. phytoplankton group) contributes also to the total phytoplankton biomass. From concentrations of accessory pigments in southern Adriatic samples we can gather prymnesiophytes, diatoms and green algae make the prevailing part in the phytoplankton biomass. Diatom maxima is normally observed in spring and autumn. TOTTI *et al*. (2000) reports about the significant negative correlation between diatoms and salinity values in southern sub-basin in all the periods. Diatom maxima are normally observed in spring and autumn.

In the western areas of the middle Adriatic basin the phytoplankton community with the dominance of large cells (i.e. diatoms) probably represents a response to higher nutrient availability derived from northern Adriatic waters, while in the more oligotrophic waters of the central and eastern areas the phytoplankton assemblages at the DCM layer were dominated by phytoflagellates and nanoplanktonic dinoflagellates (TOTTI *et al*., 2000).

Fig. 2 shows average concentrations of all measured pigments in May 2005 in the Southern Adriatic for each pigment within 95% confidence limits. Contribution of chlorophyll *a* to all chlorophyll-like pigments was about 44%, while all chlorophyll-like pigments consisted 81% of the total concentrations of all pigments.

Generally chlorophyll *a* concentrations are the lowest at the surface and the concentrations increase with depth until deep chlorophyll maxi-

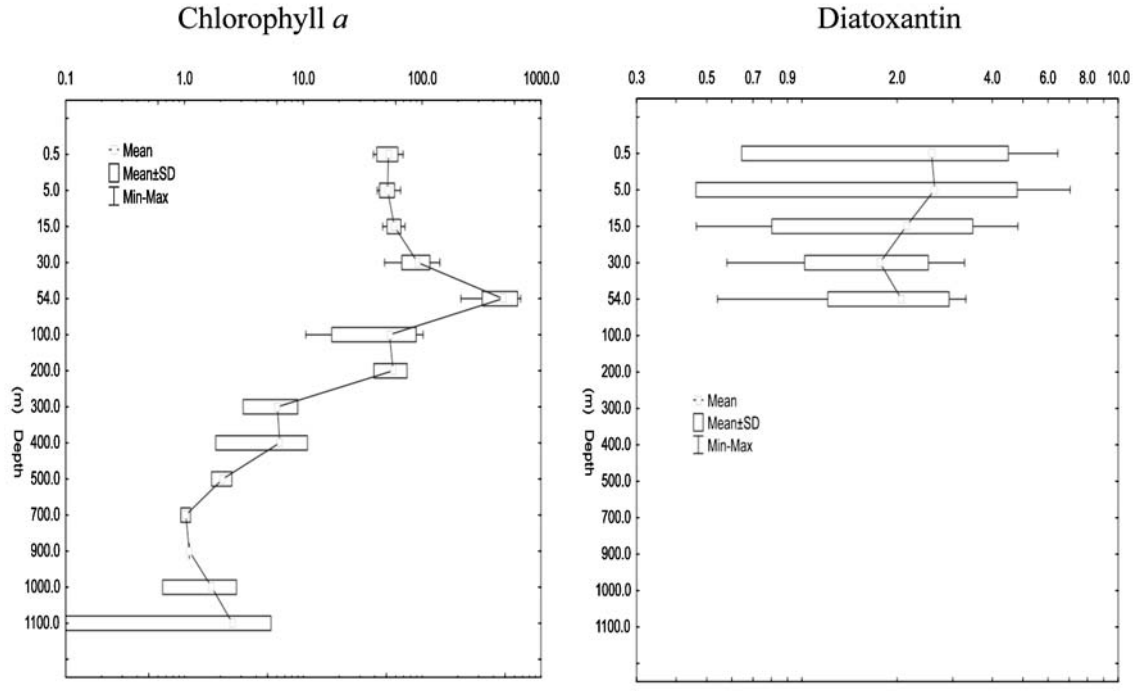

Fig. 3. Mean vertical distribution of chlorophyll a and diatoxantin pigments in the surface layer within 95% confidence limits measured in May 2005

mum, and in May 2005 this layer was around 54 m (Fig. 3). Below this depth the concentrations decreased with depth. Vertical distributions of most pigments are similar to chlorophyll a vertical distribution. Some of the pigments of non-chlorophyll type in some measurements terms show different vertical distribution, like diatoxantin which had minimum concentration at 30 m and maximum usualy at the surface. However, the concentration of this pigment are two orders of magnitude lower than the concentrations of chlorophyll *a*.

Oceanographic and meteorological conditions

Summer season 2003 (GRBEC *et. al.*, 2007) was extremely warm and dry. Exceptionally high air temperatures occurred while precipitation and river runoff were extremely low (ČAČIĆ & KATUŠIN, 2004). Salinity was also higher than the average in the intermediate layers of the Middle Adriatic due to enhanced Levantine Intermediate Water (LIW) inflow, and surface salinity was also higher because of strong evaporation (GRBEC *et al.*, 2006). July 22nd cruise started in late afternoon with the calm and clear weather, which turned to partially cloudy with a slight southern wind. Second day was cloudy with slight wind and on the 24th July strong southeast wind was blowing with high waves that didn't allow further measurements. During 36 hours no data were acquired. After returning to the position, the sea was rather calm and sky was clear until the end of the cruise. The ship corrected the position every 6 hours and changes occurred due to drift caused by currents or wind.

Tidal fluctuations at both sides of the Adriatic, in Bari and in Dubrovnik (Fig. 4) in July 2003 were as usually in phase (CUSHMAN-ROISIN *et al.*, 2001), showing transition between diurnal tidal type, in the beginning of measurements, to semi diurnal tidal type fluctuation. Sea level vertical fluctuations were about 15 cm at the beginning of measurements and increased to 40 cm at the end of measurements period at both stations.

Vertical thermal distribution shows developed stratified conditions (Fig. 5). Throughout the measurement period the depth of the thermocline was fluctuating between 8 and 13 m, with a tendency of slight deepening in

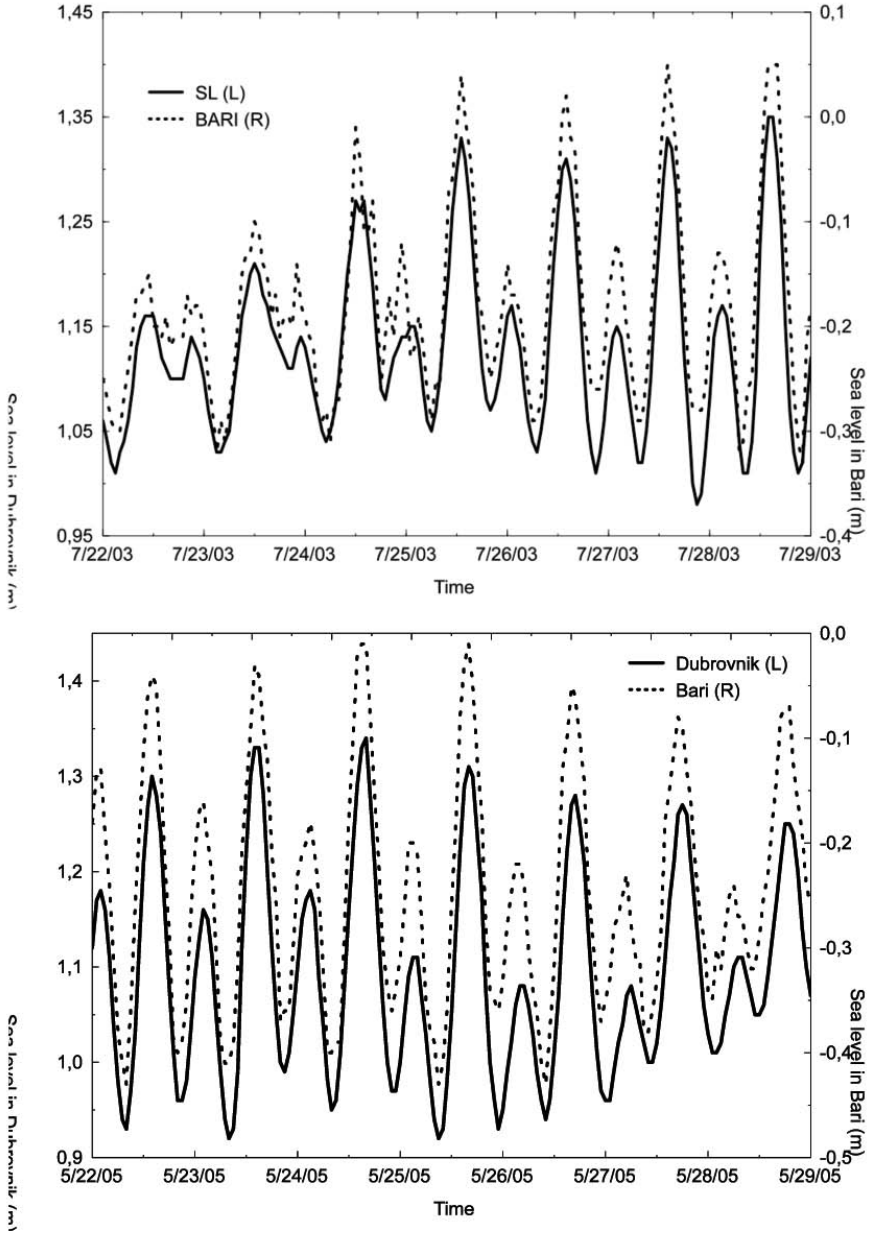

Fig. 4. Sea level data from the two opposite sides of the southern Adriatic Sea from stations Dubrovnik (ESEAS IR) and Bari (I.S.P.R.A. Italy) in July 2003 (up) and May 2005 (down)

the course of time. Surface temperatures were exceptionally high, reaching 27° C. Salinity was measured within a range 38.69 - 38.96 in the first 45 m. Salinity was also high in the deep layers where it was above 38.72. Overall salinitiy range around the thermocline was 0.65 units, where minimum and maximum salinities were recorded. Above the thermocline, a pool of relatively lower salinity water was residing. The change of temperature was expressed in the surface layer due to the diurnal warming (Fig. 6) with diurnal amplitudes decreased with depth. In the beginning of measurements diurnal oscillations were observed in the thermal structure in the layers down to 200 m (Fig. 7), with the same diurnal rhythm. After returning to the position, 24 hours fluctuation cannot be recognized from CTD data since measurements 27th July midday were missing. Climatologically, spring 2005 was generally a normal season, with a warm

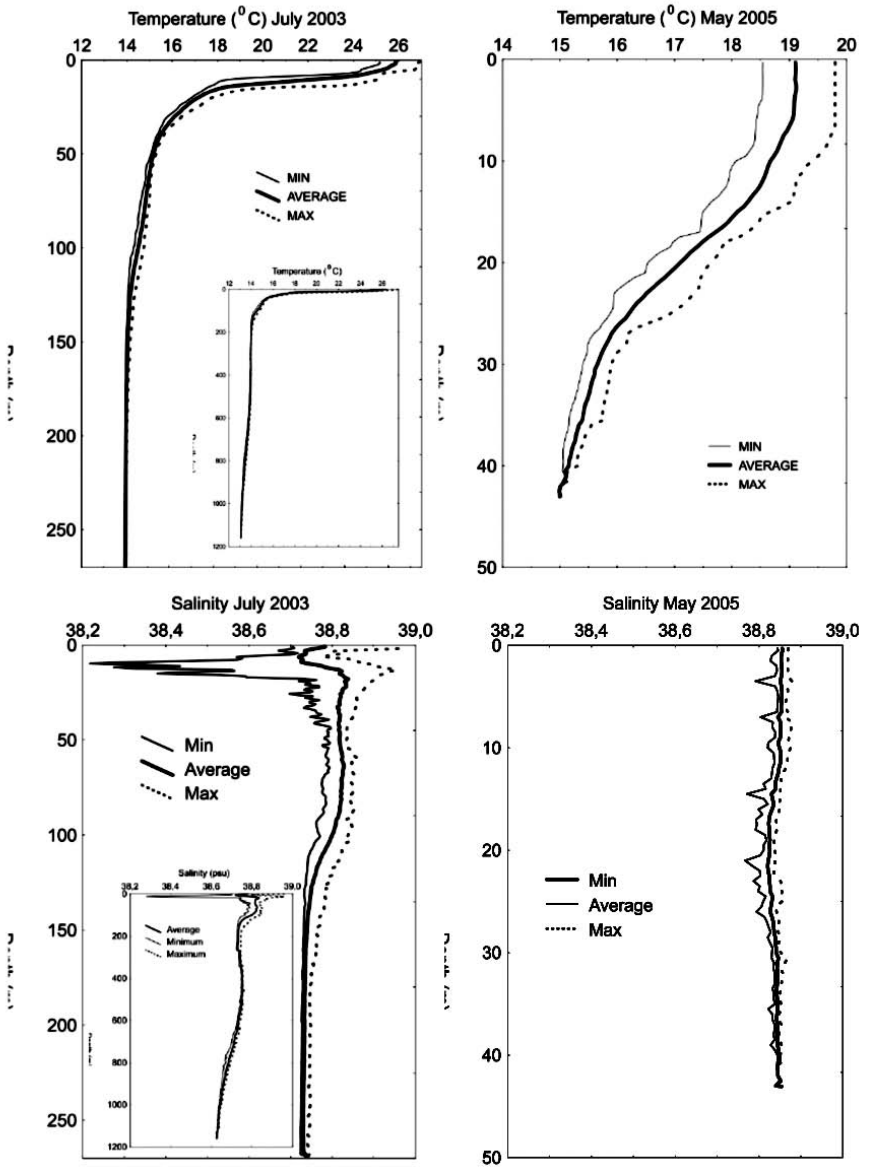

Fig. 5. Temperature and salinity vertical structure during MEDUZA cruises in the south Adriatic Pit. Small figures show veritacal structure until 1200 m.

and dry May. Meteorological conditions of this cruise were rather bad. The sky was overcast on 23rd and partially cloudy on 24th. The weather was worsening, with strong winds and waves, and measurements were interrupted until the 26th in the afternoon hours. Tidal fluctuations in May 2005 were again closely in phase in Bari and in Dubrovnik (Fig. 4). The semi-diurnal tidal oscillations were present in the period of measurements, tending towards diurnal tidal type fluctuation at the end of measurements. Sea level fluctuations in the beginning of measurements had amplitudes of about 40 cm and were decreasing towards the 15 cm amplitude on 28th May. Thermal conditions in 2005 indicate that warming of surface layers has just started (Fig. 5) after a relatively colder April month in the atmosphere (ČAČIĆ & KATUŠIN, 2006). During the first part of measurements the surface temperatures were below 19°C. The difference in the first 20 m was just two degrees, and the thermocline was not yet formed. At the depth of 30 m observed were diurnal temperature fluctuations (Fig. 6), caused by daily warming of the

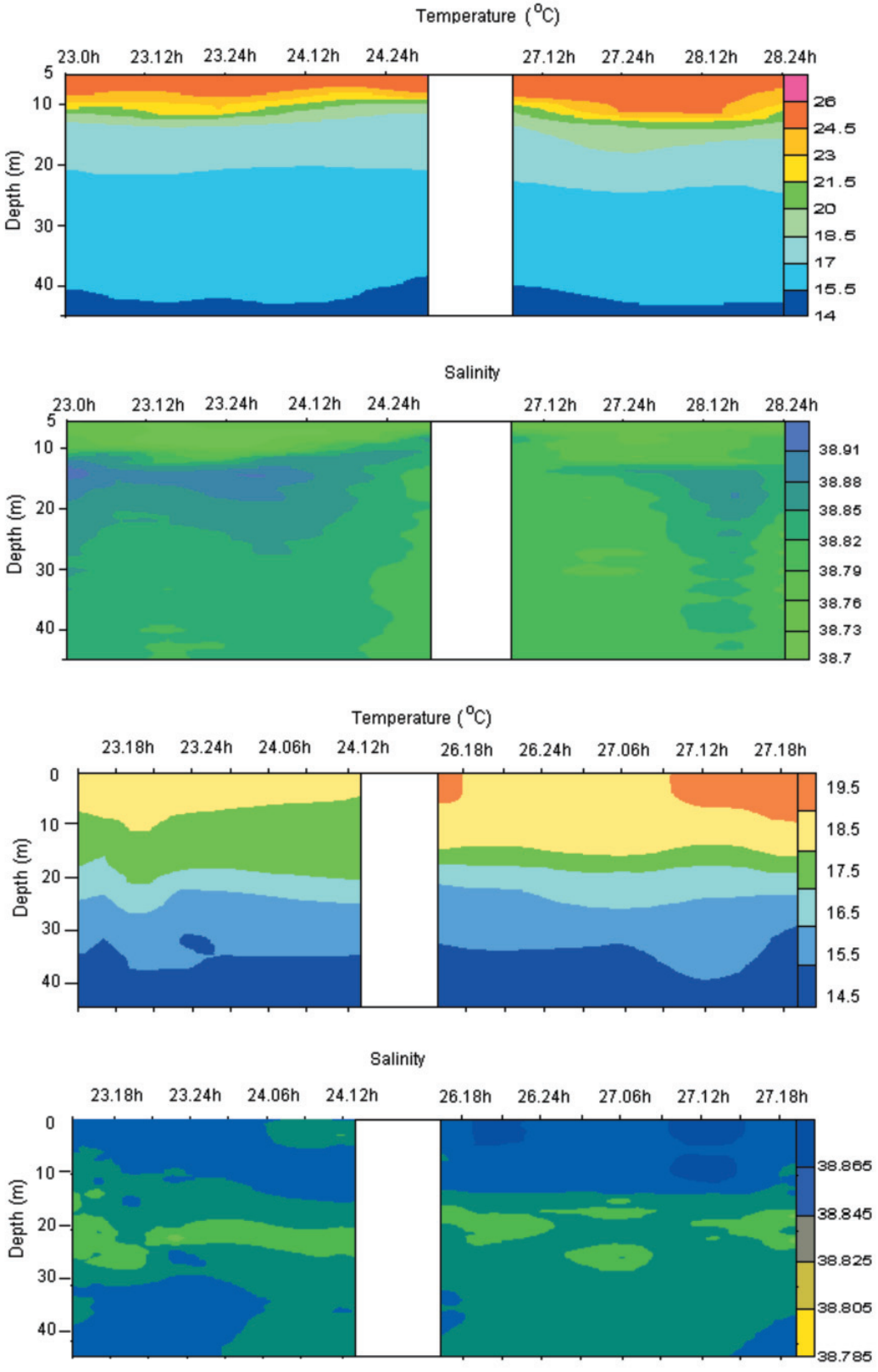

Fig. 6. Temperature and salinity course between 23rd and 28th July 2003 (left) and between 23rd and 28th May 2005 (right) in the South Adriatic Pit. Measurement day and time are indicated as dd. hh.

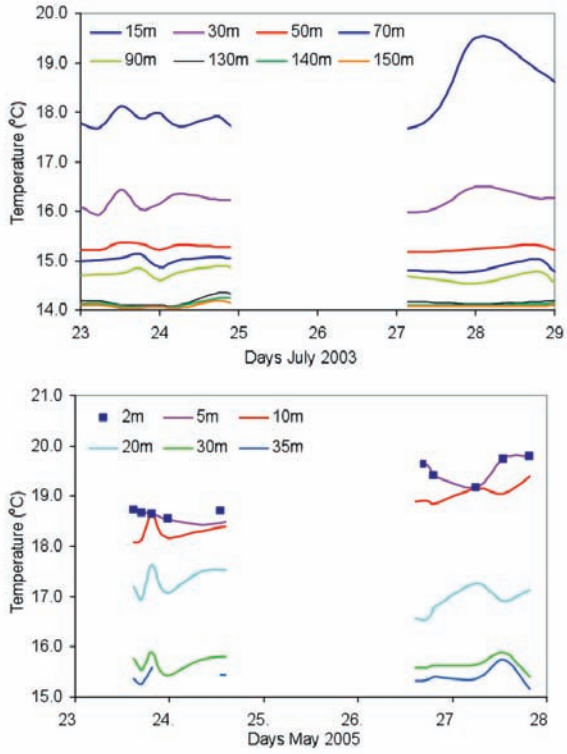

Fig. 7. Temperature course in different layers in July 2003 (up) and in May 2005 (down) in the South Adriatic Pit

surface. After returning to the position 26th May temperature was somewhat above 19°C. Salinity was generally high and varied as little as 0.06 units around 38.84. Compared to July 2003, salinity had lower variations in May 2005 in the first 40 m, but had somewhat higher mean value (38.84 compared to 38.81 in 2003).

The Fig. 7 shows variations of temperature in different depth layers at diurnal scale period in the begining of measurements in both cruises. Strong oscillations of isothermal surfaces were often observed in the Adriatic Sea especially in summer. These were confirmed through the modelling experiment (MIHANOVIĆ *et al.*, 2006) and were explained in terms of internal tides, having typically diurnal oscillations. Our measurement area was recognized from a modeling experiment as a location where high internal tide amplitudes can occur (ORLIĆ *et al.* 2006).

Optical properties and relations to pigments

Transmission of light and fluorescence were measured in July 2003 few times per day down to variable depths of 180-270 m (Fig. 8), depending on the drift of the CTD probe by currents. The range of measured beam attenuation coefficients were between 0.431 and 0.112 m^{-1}. In the first few measurements there was a rather homogenous depth distribution. However, beam attenuation coefficients increased in the course of time, especially in the layers bellow 80 m. After the storm these coefficients increased even more especially in deeper layers but have somewhat decreased at the surface layer. It can be observed how the coefficients changed at different depths throughout the measurements (Fig. 9). In July 2003 at different depths coefficients show fluctuations with a period of about 24 hours in the first part of measurement. Through these fluctuations in the first 24 hours in the deep layers beam attenuation coefficients varied between 0.261-0.117 m^{-1}. In the second part of measurement they increased considerably, than dropped and at the end considerably increased. In the first 100 m fluctuations of beam attenuation were rather small, except that strong increase of beam attenuation coefficients was observed in the last measurement for the whole water column. However, highly pronounced fluctuations were observed more in the layers below 120 m. These changes may have the cause in internal tide (MOROVIĆ *et al.*, 2010) and/or in mixing of the water column that occurred during and after the storm, either bringing to the surface the stuff from the deeper layers that could enhance beam attenuation coefficients or/and bringing the nutrients from the deeper nutricline that via biota indirectly influenced optical properties.

Chlorophyll-like pigments measured with Wetlabs fluo sensor in 2003 show an increase from the surface to the deep chlorophyll maximum layer (Fig. 8). The depth of DCM was changing between 60 and 90 m during the measurements in July 2003.

In May 2005 the chlorophyll pigments show pronounced DCM layer at about 54 m depth (see Fig.3) (we have to keep in mind that pigments were measured at discrete depths). The deep chlorophyll maximum layer was deeper in July than in May as a consequence of higher light

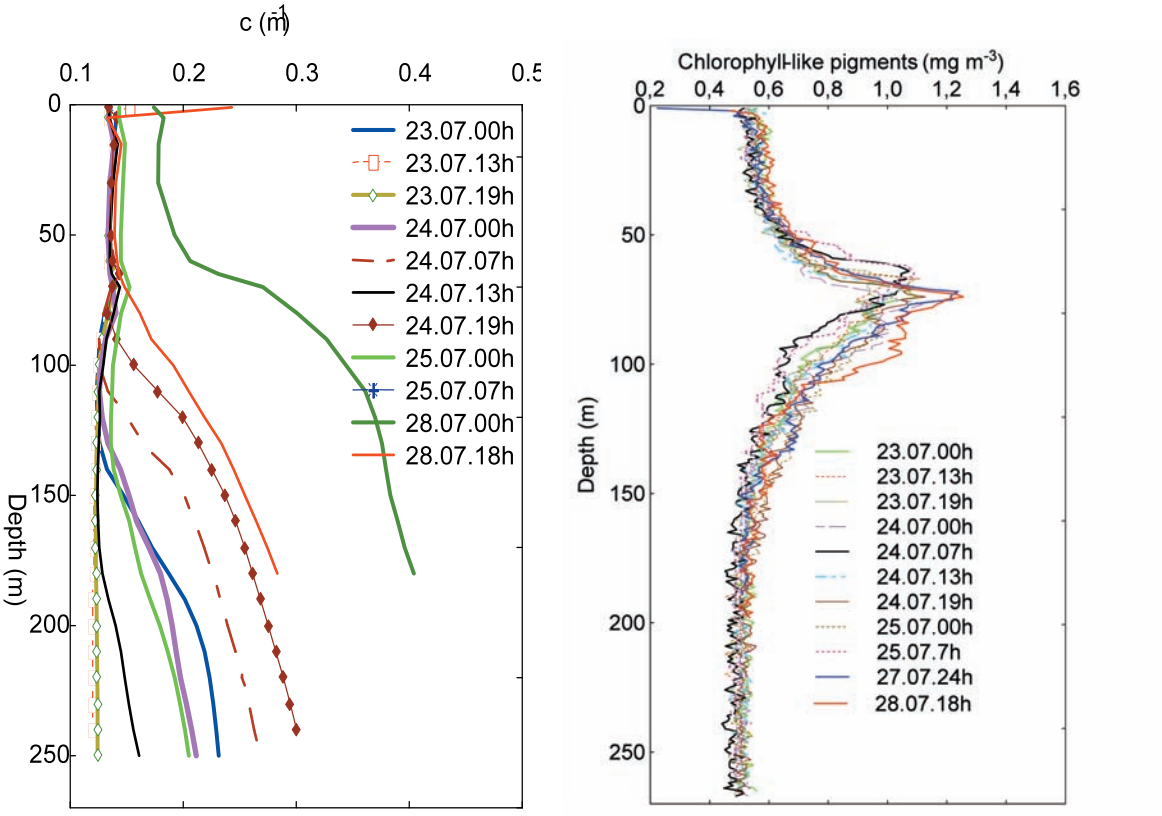

Fig. 8. Vertical distribution of individual measurements of attenuation coefficients and chlorophyll-like pigments in July 2003 in the south Adriatic Pit (measured with Wetlabs c-star and Fluo sensors)

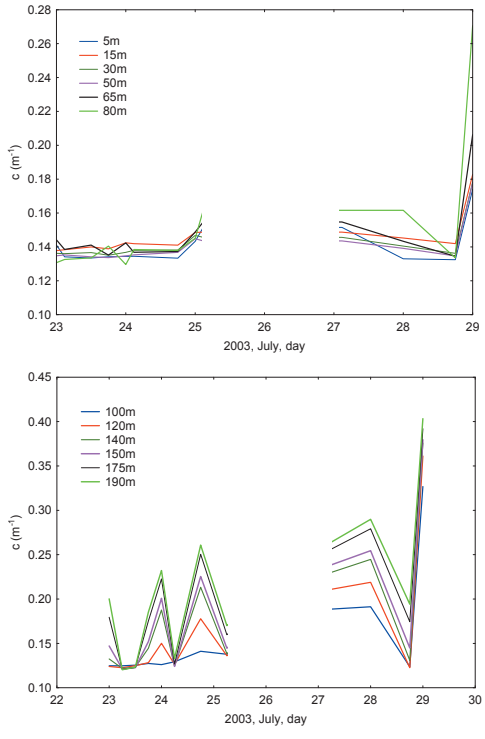

Fig. 9. Course of attenuation coefficient at various depths in July 2003

intensity in July, which the phytoplankton tends to avoid.

Although pigments were determined by HPLC method only in May 2005, the same pigments/species, were probably present in July 2003, with similar vertical distribution but more likely in lower quantities, since species like Diatoms (containing among other the Diatoxantin) would be less abundant in summer than in spring. The abundances of phytoplankton species behave differently in relation to light availability. The Diatoms have higher tolerance to light intensity and can place themselves closer to the surface layer. The species with lower tolerance to light would tend to stay in deeper layers.

Fluctuation of chlorophyll like pigments concentrations in the course time at selected depths during both cruises were high (Fig. 10 and Fig. 11). They resemble to fluctuations of beam attenuation since the phytoplankton (and its pigments) besides absorbing, influences the transmission of light, acting like the particles of the same size. Looking at the data for exam-

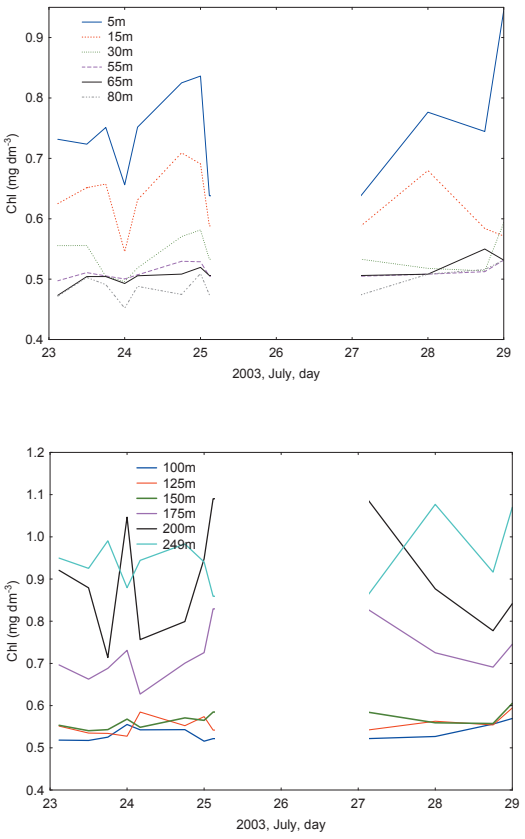

Fig. 10. Course of cshlorophyll-like pigments concentrations (Wetlabs sensor) at various depths in July 2003

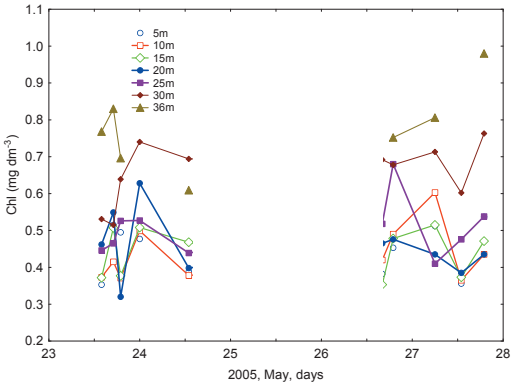

Fig. 11. Course of chlorophyll-like pigments concentrations (Wetlabs sensor) at various depths in May 2005

ple from the DCM layer, we can see that beam attenuation and pigments are in phase.

Changes of chlorophyll concentrations may have different causes. Partly these may be due to normal diurnal migration of phytoplankton. However, since the vertical displacements due to internal tide are observed in temperature, salinity and beam attenuation coefficients, the phytoplankton should also be influenced. Here it is necessary to keep in mind that chlorophyll *a* in the marine environment is accompanied with other pigments with similar absorption peaks as well as fluorescent properties. Although the *in-situ* fluorometers declare to measure chlorophyll a, they cannot distinguish other chlorophyll-like pigments from chlorophyll a, which is the reason why we prefer to call the pigments measured by Wetlab sensor the chlorophyll-like pigments

Table 5. The first three significant factors from the principal components analysis, their eigen values and explained variability with cumulative values

	Eigenvalue	% Total var.	Cumul. Eigenvalue	Cumul. %
f1	13.76	76.45	13.76	76.45
f2	1.79	9.94	15.55	86.39
f3	1.01	5.59	16.56	91.98

PCA and correlation analyses

The concentrations of pigments from May 2005 are subject to principal component analyses (PREISENDORFER, 1982). The matrix of pigments (18*60) consisted of 10 series of measurements at 6 depth levels (depth below 100 m were excluded, since were sampled only twice). The analysis resulted with the three significant factors (Table 5) with eigen values >1. The first factor carried above 76% of the overall variance of pigment concentrations and with the other two factors they explain about 92% of variability. Varimax normalized rotation was applied to the extracted factors. The first two factors have significant loadings with many pigments (Table 6). The first factor has the highest correlation coefficients (>0.9) with prasinoxanthin, neoxanthin, chlorophyll *b* and butanoyloxyfucoxanthin. The second factor has the highest correlation coefficients with Pheophorbide a and chlorophyllide a. The third factor is well correlated only with diatoxantin. The two representations of the three factor loadings (Fig. 12) separated on one side only diatoxantin, the pigment whose vertical distribution is rather different from all other pigments.

The concentration of pigments and the scores of the three factors were put in correlation to the values of temperature, salinity, oxygen and depth (Table 6). The second factor has high correspondence to temperature and oxygen and third to depth. Most pigments corresponded to temperature and oxygen while only diatoxantin corresponded to salinity.

The analysis of correlation coefficients between pigments and reflectance is presented in the Fig.13. Positive correlation coefficients of pigments with reflectance are found only for wavelengths higher than 555 nm (highest at 598 nm). Correlation coefficients have opposite signs for shorter than for longer wavelengths. There are no significant correlation coefficients in the range of wavelengths from 490 nm to 555 nm, except for diatoxantin.

Positive correlation coefficients exist between concentrations of all pigments and K_d for wavelengths shorter than 555 nm, except for diatoxantin (Fig. 14). Higher concentrations of pigments lead to higher attenuation of light in concordance to their spectral absorption properties (JEFFREY *et al*., 1997). There are no significant correlation coefficients of pigments and attenuation coefficients for irradiance K_d at 555 nm. The fact is that none of these pigments absorbs at 555 nm, neither Diatoxantin. What makes the difference with Diatoxantin in many of these cases probably is due to combination of conditions such as different characteristics of the phytoplankton that carry Diatoxantin, combination of accompanying pigments and maybe its vertical distribution.

Table 6. Correlation coefficients between concentrations of pigments with PC factors, depth-D, temperature-T, salinity-S and oxygen-O_2, p - significance level

p<0.01	N=60 depth ≤ 100m				N=36 depth ≤ 30m		
Pigments	f1	f2	f3	D	T	S	O_2
chl a	0.76	0.64			-0.77		0.69
chl c2	0.70	0.70			-0.76		0.73
per	0.59	0.77			-0.80		0.78
but	0.91			0.45	-0.66		0.61
fuc	0.45	0.85			-0.77		0.73
neox	0.94				-0.67	-0.43	0.65
hex	0.79	0.59			-0.76		0.72
prasino	0.96				-0.51		0.53
viola	0.73	0.48			-0.47		0.48
diadino	0.45	0.74	0.36				
anthera	0.83	0.34					
allo	0.87	0.42					
diato			0.96	-0.52			
zea+lut	0.61	0.59			-0.68		0.64
chl b	0.92	0.36			-0.83		0.76
B-car	0.60	0.74			-0.65		0.62
chlid a		0.90			-0.69		0.75
phbid a		0.94			-0.70		0.62
f1					0.43		
f2					-0.76		0.70
f3				-0.65			

Correlation coefficients of pigments and K_d have maximum at 412 nm, which is shorter wavelength than the absorption peaks of all these pigments (Table 7). However, the absorption characteristics are determined in the laboratory conditions, and besides not being in a living phytoplankton cells the pigments are diluted in

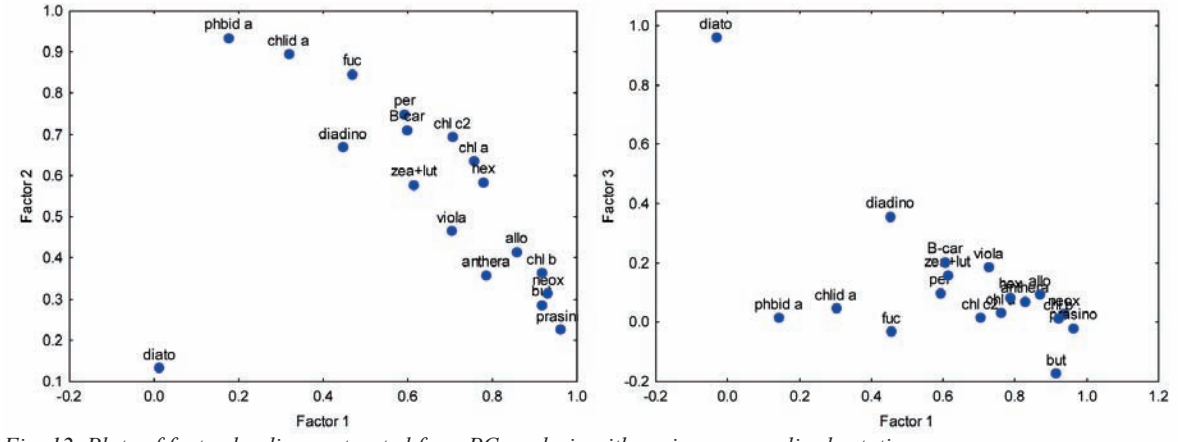

Fig. 12. Plots of factor loadings extracted from PC analysis with varimax normalized rotation

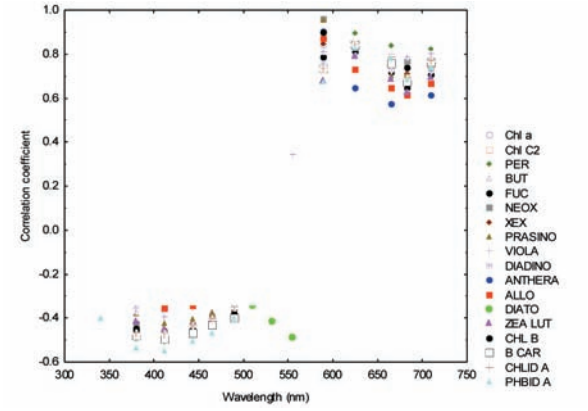

Fig. 13. Distribution of correlation coefficients between pigments concentrations and reflectances R according to wavelengths at p<0.05 level of significance

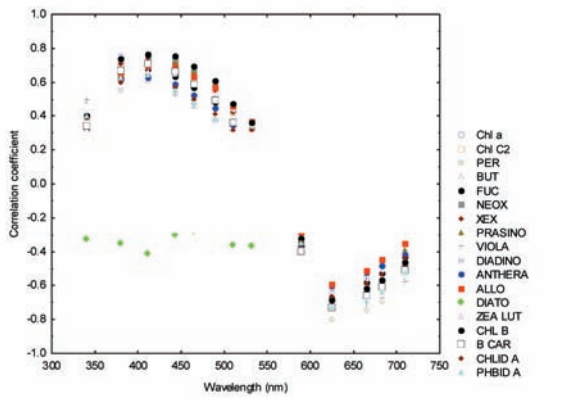

Fig. 14. Distribution of correlation coefficients between pigments concentrations and attenuation coefficients $K_{d\lambda}$ (m^{-1}) according to wavelengths at p<0.05 level of significance

solvents, the shape of pigment is different and the response of living pigment cell is missing in the laboratory compared to conditions of *in-situ* light attenuation measurements. All these can result in different spectral properties of K_d relative to absorption measurements in the laboratory.

It is characteristic that most of the chlorophyll-like pigments have high correlation coefficients with many reflectance ratios and radiance ratios (Table 8). This may be very useful for obtaining regression models for concentration of pigments. Interesting are ratios that relate radiances at the wavelengths characteristic for absorption of pigments and the middle of the upward spectrum, where there is no absorption at the wavelength of clear water transmission maximum (JERLOV, 1976). We have tried analysis

Table 7. Correlation coefficients between ratios of radiances ($L_{\lambda 1}/L_{\lambda 2}$) and concentration of pigments, statistically significant at p<0.05 level of significance

Pigment	L_{412}/L_{532}	L_{412}/L_{589}	L_{412}/L_{683}	L_{443}/L_{555}	L_{443}/L_{532}	L_{443}/L_{589}	L_{443}/L_{465}	L_{443}/L_{683}	L_{465}/L_{490}	L_{465}/L_{532}	L_{465}/L_{589}	L_{10}/L_{532}	L_{683}/L_{555}
CHL_A	-0.84		-0.56		-0.56		-0.89	-0.56	-0.89	0.48			0.92
CHL_C2	-0.85		-0.58		-0.56		-0.90	-0.57	-0.90	0.50			0.93
PER	-0.90		-0.57		-0.54		-0.95	-0.55	-0.95	0.55		-0.43	0.93
BUT	-0.82		-0.55		-0.55		-0.86	-0.55	-0.86	0.46			0.89
FUC	-0.82	-0.44	-0.57		-0.54		-0.87	-0.57	-0.87	0.49			0.92
NEOX	-0.84		-0.56		-0.46		-0.90	-0.54	-0.90	0.59			0.86
HEX	-0.83		-0.55		-0.54		-0.88	-0.54	-0.88	0.48			0.89
PRASINO	-0.78		-0.55		-0.50		-0.82	-0.54	-0.82	0.47			0.85
VIOLA	-0.80						-0.87		-0.88	0.63	0.55	-0.56	0.80
DIADINO	-0.77				-0.43		-0.84		-0.84	0.55			0.82
ANTHERA	-0.60						-0.67		-0.67	0.50			0.52
ALLO	-0.71		-0.47		-0.45		-0.74		-0.74	0.44			0.70
DIATO				-0.48		-0.46	0.33			-0.49	-0.48	0.44	
ZEA_LUT	-0.75		-0.55		-0.59		-0.78	-0.55	-0.79				0.90
CHL_B	-0.86		-0.58		-0.58		-0.90	-0.57	-0.90	0.46			0.94
B_CAR	-0.85		-0.57		-0.58		-0.89	-0.57	-0.90	0.46			0.93
CHLID_A	-0.80		-0.58		-0.50		-0.85	-0.56	-0.85	0.50			0.90
PHBID_A	-0.88		-0.62		-0.52		-0.91	-0.60	-0.92	0.54			0.88
SUMA	-0.86		-0.57		-0.56		-0.90	-0.57	-0.90	0.49			0.93

Table 8. Correlation coefficients between ratios of reflectances ($R_{\lambda 1}/R_{\lambda 2}$) and concentration of pigments, statistically significant at $p<0.05$ level

Pigment	R_{412}/R_{532}	R_{412}/R_{589}	R_{412}/R_{683}	R_{443}/R_{555}	R_{443}/R_{532}	R_{443}/R_{589}	R_{443}/R_{465}	R_{443}/R_{683}	R_{465}/R_{490}	R_{465}/R_{532}	R_{465}/R_{589}	R_{589}/R_{532}	R_{683}/R_{555}
CHL_A	-0.59	-0.59		-0.73	-0.69	-0.60			-0.57	-0.73	-0.61	0.98	0.70
CHL_C2	-0.60	-0.60		-0.74	-0.70	-0.62			-0.57	-0.74	-0.63	0.99	0.72
PER	-0.71	-0.71		-0.83	-0.80	-0.72	-0.52		-0.67	-0.83	-0.73	0.96	0.79
BUT	-0.58	-0.57		-0.71	-0.67	-0.58			-0.56	-0.71	-0.59	0.96	0.68
FUC	-0.56	-0.56		-0.70	-0.65	-0.58			-0.52	-0.69	-0.59	0.99	0.68
NEOX	-0.67	-0.66		-0.79	-0.75	-0.67	-0.47		-0.64	-0.79	-0.68	0.97	0.73
HEX	-0.62	-0.61		-0.75	-0.71	-0.63	-0.42		-0.60	-0.75	-0.64	0.94	0.71
PRASINO	-0.54	-0.55		-0.68	-0.63	-0.57			-0.52	-0.68	-0.58	0.96	0.65
VIOLA	-0.79	-0.78	-0.52	-0.87	-0.85	-0.78	-0.64	-0.52	-0.78	-0.87	-0.78	0.85	0.78
DIADINO	-0.65	-0.62		-0.75	-0.72	-0.62	-0.46		-0.64	-0.75	-0.62	0.95	0.70
ANTHERA	-0.55	-0.57		-0.62	-0.60	-0.56	-0.41		-0.48	-0.62	-0.56	0.76	0.51
ALLO	-0.47	-0.44		-0.60	-0.56	-0.46			-0.43	-0.60	-0.47	0.90	0.51
DIATO	0.52	0.45		0.46	0.48		0.60		0.57	0.45			
ZEA_LUT	-0.49	-0.52		-0.63	-0.59	-0.54			-0.49	-0.63	-0.55	0.84	0.67
CHL_B	-0.61	-0.61		-0.75	-0.70	-0.63	-0.41		-0.58	-0.74	-0.64	0.95	0.73
B_CAR	-0.61	-0.63		-0.75	-0.71	-0.64			-0.59	-0.75	-0.65	0.96	0.74
CHLID_A	-0.58	-0.61		-0.69	-0.65	-0.63			-0.52	-0.69	-0.64	0.94	0.75
PHBID_A	-0.67	-0.66		-0.79	-0.75	-0.68	-0.49		-0.61	-0.79	-0.69	0.92	0.80
SUMA	-0.61	-0.61		-0.75	-0.70	-0.62	-0.41		-0.58	-0.74	-0.63	0.98	0.72

Table 9. Elements of multiple regression models for chlorophyll a pigment based on ratios of radiances and reflectances. (The values in the table are R multiple regression coefficient; p significance level; β - beta coefficient (standardized regression coefficient); B regression coefficient.)

R= 0.965 ; R^2=0.932						
F(2,25)=171 p<,00000 Std.Error of estimate: 0.24						
Log Chl a	b	St. Err. of b	B	St. Err.of B	t(25)	p-level
Intercept	8.63			1.00	8.61	6.04E-09
L_{u683}/L_{u555}	0.44	0.11	4.67	1.18	3.94	0.000573
L_{u465}/L_{u490}	-0.55	0.11	-4.09	0.83	-4.93	4.44E-05

R= 0.943 ; R^2=0.89						
F(2,25)=104 p<0,00000 Std.Error of estimate: 0.302						
log Chl a	b	St. Err. of b	B	St.Err.of B	t(25)	p-level
Intercept	3.11			0.26	11.85	5.54E-12
R_{683}/R_{555}	1.26	0.14	0.08	0.01	8.86	2.5E-09
R_{465}/R_{490}	0.38	0.14	0.09	0.03	2.66	1.3E-2

with several ratios that are highly correlated to concentration of pigments.

Combining the two ratios in a multiple regression relation with chlorophyll *a*, high multiple correlation coefficients can be obtained. We have used these parameters to produce chlorophyll-like pigments concentrations from the radiance and reflectance ratios. Very high multiple correlation coefficient is obtained for log chlorophyll *a* and both the reflectance and radiance ratios (Table 9). Somewhat lower multiple correlation coefficient is obtained for non-transformed chlorophyll *a* values (original values and radiance ratios did not give significant result for beta coefficients).

For these same experiments in July 2003 and May 2005 it was found that distribution of some zooplankton species show specific diurnal vertical behavior in relation to light intensities (LUČIĆ *et al*., 2009, 2011; GANGAI *et al*., 2012). Since zooplanktons are rather large and not completely transparent, apart from response of zooplankton to light, zooplankton to some extent may also influence the light attenuation, reflectance, etc.

Most of the marine pigments overlap in some of their spectral absorption properties and it is very difficult to distinguish them in the presence of high chlorophyll concentrations. We could eventually better discern other pigments in conditions of their exceptional blooms. New hyper-spectral *in-situ* instruments eventually would allow better discrimination of pigments.

CONCLUSIONS

The investigated area in the South Adriatic Sea is oligotrophic most of the time but still offers a variety of phytoplankton pigments that are major optically active constituents in the open sea.

Among the pigments in the southern Adriatic the chlorophyll *a* and other similar pigments strongly prevailed over other groups. Pigments with somewhat different absorption characteristics were present in too low concentrations to have any effect on *in-situ* measured spectral characteristics of the ensamble of pigments. More experiments with hyperspectral instruments are needed to suceed to distinguish individual pigments from chlorophyll-like pigments. The property of the pigment diatoxantin is the opposite sign of correlation coefficients relative to other pigments, lack of correlation or significant correlation when other pigment did not show correlations.

Vertical distributions of most pigments were similar, with the maximum concentration in DCM layer, except the diatoxin, which often shows surface maximum. The deep chlorophyll maximum in July 2003 was present at about 70 m depth and in May 2005 at about 54 m because of different seasons of measurements.

During both cruises vertical oscillations were observed in the water column from the surface to deep layers probably caused by internal tide of about diurnal period. Such oscillations can be observed in temperature, salinity, oxygen, attenuation of light and concentrations of pigments. Additional mixing of the water column could have come from the storm that occured in the middle of both cruises.

It was possible to establish regression model between radiance ratios L_{u683}/L_{u555} and L_{u465}/L_{u490} and reflectance ratios with concentrations of Chlorphyll from data in May 2005 which worked particularly well for log transformed chlorophyll values.

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Spektralni odraz pigmenata i procesi u južnom Jadranu - projekt MEDUZA

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SAŽETAK

Tijekom nekoliko krstarenja u okviru Meduza eksperimenta obavljenih u srpnju 2003 i svibnju 2005 (http://jadran.izor.hr/meduza/meduza_g.htm), pribavljeni su raznovrsni podaci iznad najdubljeg dijela južnog Jadrana. Temperatura, salinitet, propusnost za svjetlost i fluorescencija mjereni su u srpnju 2003. Tijekom svibnja 2005., osim CTD mjerenja uzeti su uzorci za laboratorijsko određivanje pigmenata fitoplanktona. Tijekom oba krstarenja mjereno je zračenje na 14 valnih duljina u rasponu 340-715nm optičkom sondom do oko 100m dubine. Loši vremenski uvjeti tijekom oba krstarenja uzrokovali su prekid mjerenja koja se nastavljaju nakon 24 odnosno 48 sati. Olujni uvjeti rezultirali su nešto drugačijom strukturom vodenog stupca. U razdobljima mjerenja zabilježeni su dnevni ritmovi u nizu parametara, uzrokovani ciklusima sunčeva zračenja u površinskim slojevima kao i oscilacije u dubljim slojevima zbog unutarnje plime. Meteorološki uvjeti i promjene razine mora su također diskutirani. Analiza je obuhvatila i korelacije između izvedenih i mjerenih parametara s ciljem da uspostavi veza između koncentracija mjerenog klorofila i multispektralnih podataka.

Tijekom oba krstarenja opažene su dnevne vertikalne oscilacije biotskih i abiotskih pametera. Klorofilni su pigmenti prevladavali onemogućujući spektralno prepoznavanje ostalih pigmenata. Utvrđeni su regresivni modeli za klorofil **a** na osnovi mjerenja radijanca i reflektancija.

Ključne riječi*:* Južni Jadran, termohalina svojstva, dnevni ciklusi, pigmenti fitoplanktona, optička svojstva, unutrašnja plima