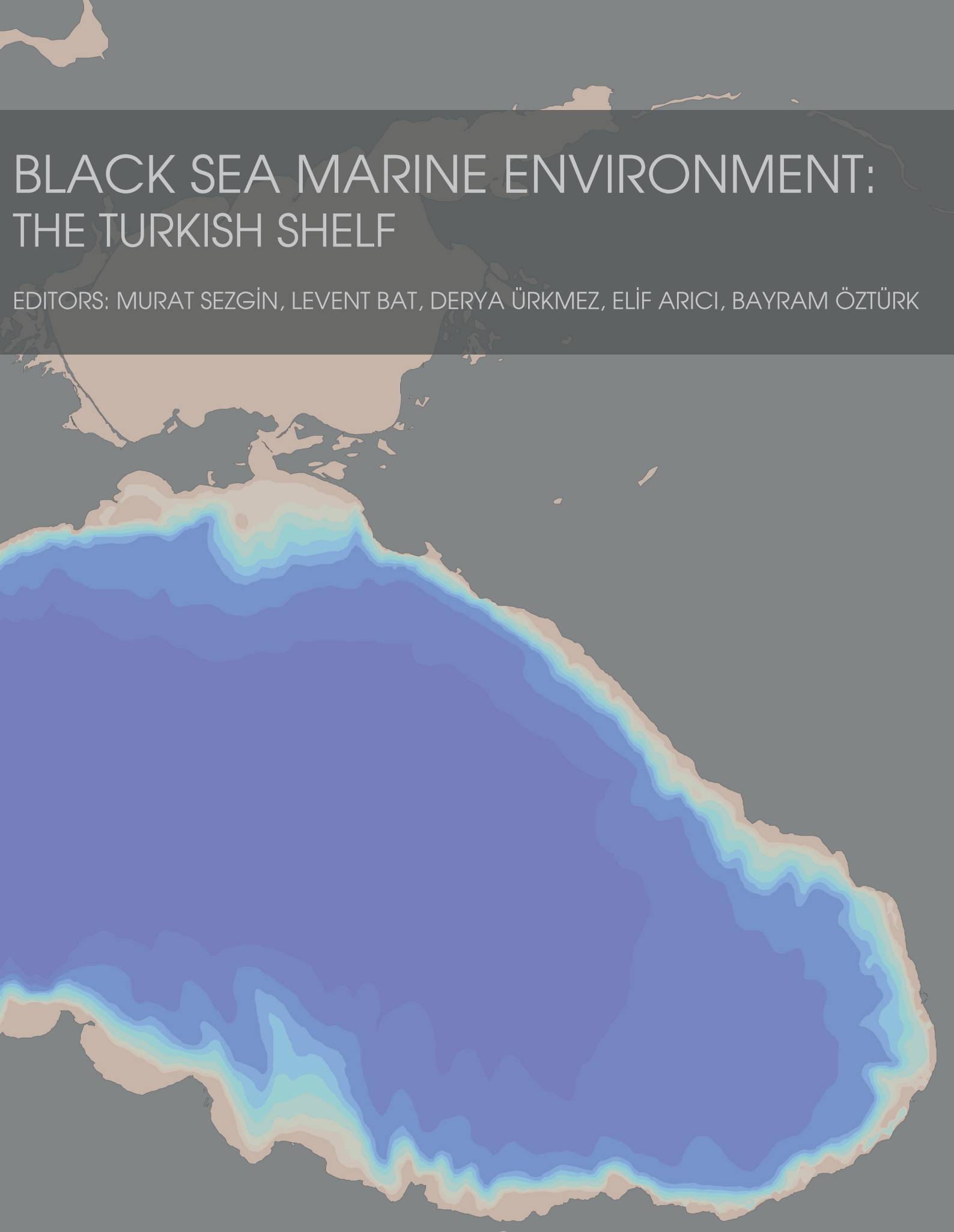
BLACK SEA MARINE ENVIRONMENT:
THE TURKISH SHELF

EDITORS: MURAT SEZGİN, LEVENT BAT, DERYA ÜRKMEZ, ELİF ARICI, BAYRAM ÖZTÜRK



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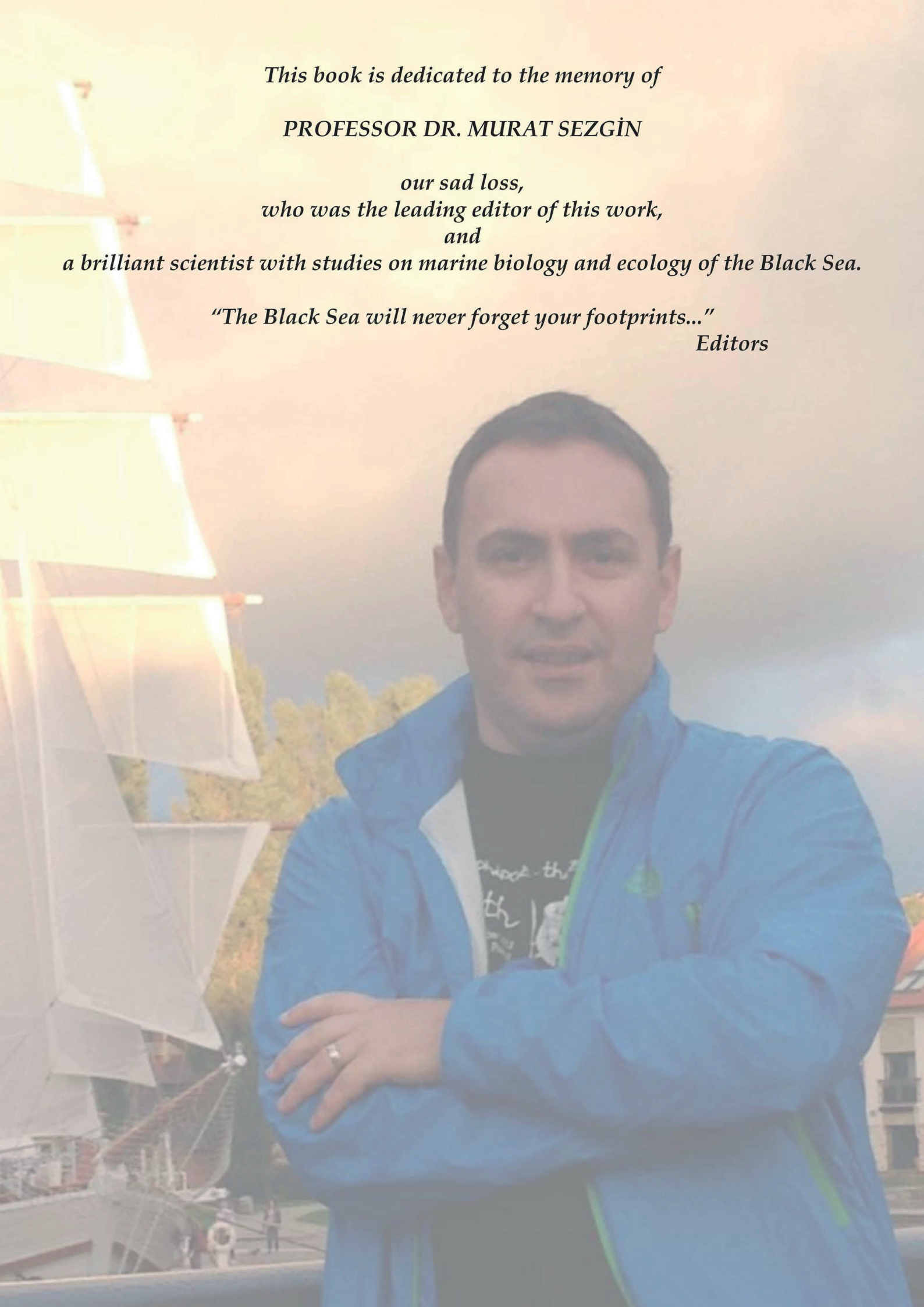
This book is dedicated to the memory of

PROFESSOR DR. MURAT SEZGİN

our sad loss,
who was the leading editor of this work,
and
a brilliant scientist with studies on marine biology and ecology of the Black Sea.

"The Black Sea will never forget your footprints..."

Editors



PREFACE

The Black Sea is one of the major water bodies and a famous inland sea of the world. It is a connecting link between six different countries sharing its coasts with the boundaries of Turkey, Bulgaria, Romania, Ukraine, Russia and Georgia. The Black Sea has extraordinary natural conditions as the largest water body with a meromictic basin. This means that, the interaction between the oxygen rich surface waters and the Black Sea's deeper areas tends to be very limited compared to anywhere else in the world. This leads to a layering structure being created which affects the diversity of the organisms within the Black Sea.

This book aims to be a good compilation bringing together articles focusing on the Black Sea looking at its ecosystem from different aspects. It goes without saying that the Black Sea is like a special kind of living organism which should be known in detail to be protected and saved. And, we deeply recognize that, during the last 50 years, its ecosystem changed extensively making it more vulnerable to anthropogenic effects. Marine resources have declined as a consequence of over-fishing, unplanned development of coastal zones and intense maritime traffic. Therefore, we believe that such kind of a compilation effort with an output can draw a general outline of the picture and help in concentrating attentions on such a special area.

"Black Sea Marine Environment: The Turkish Shelf" is the latest of the series of Turkish Marine Research Foundation (TÜDAV), which covers 29 articles under 4 main chapters (oceanography, biodiversity, pollution and conservation and science and policy) written by 61 authors from various universities and institutions working under different disciplines and who have studies in the Black Sea.

We truly appreciate the contributions of the authors and the editors to this book. We are sure that this publication will serve as an exchange of knowledge with the other bordering countries in the Black Sea and also provide a brief insight to this fragile water body targeting all the readers who care for the better understanding, conservation and sustainable development of the Black Sea.

Prof. Dr. Bayram ÖZTÜRK
Director, Turkish Marine Research Foundation (TÜDAV)
December 2017

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PHYTOPLANKTON PIGMENTS AND USING A MONITORING TOOL FOR THE PHYTOPLANKTON SIZE CLASSES (PSC) IN THE BLACK SEA

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1. Introduction

Phytoplankton account for nearly half of the total primary production on Earth and constitute the base of the marine food web (Jeffrey and Vesk 1997, Falkowski *et al.* 2004). They have important role in modulating the total CO_2 concentration, pH of the ocean and global carbon cycle (Takahashi *et al.* 2002, Brewin *et al.* 2010). Changes in phytoplankton populations also impact the pelagic ecosystem throughout changing trophic transfer, food web and nutrient dynamics (Nagata *et al.* 1996, Pedersen *et al.* 1999). Identifying changes in phytoplankton community composition and diversity is essential to improve our understanding of the responses to climate forcing (Rykaczewski and Dunne 2011). Moreover, phytoplankton quickly response to environmental disturbance, and can be used as indicator for trophic levels, eutrophication and different environmental circumstances (Margalef 1978, Brettum and Andersen 2005).

Studies on phytoplankton are generally conducted by using microscopy (Booth 1993, Eker-Develi *et al.* 2008), which needs taxonomic expertise, time for sample preparation and counting samples. In addition, it is not possible to identify smaller groups (*e.g.* picoplankton) with microscopy due to lack of taxonomically useful external morphological features (Jeffrey *et al.* 1997). However, some photosynthetic pigments (*e.g.* fucoxanthin, peridinin, 19-hexanoyoxifucoxanthin, Chl-*b,* and zeaxanthin etc.) for specific phytoplankton groups derived from high-performance liquid chromatography (HPLC) provide information about the composition of the phytoplankton community (Mantoura and Llewellyn 1983, Wright and Jeffrey 2006). The introduction of HPLC pigment analysis facilitated easy and fast separation, identification and quantification of phytoplankton pigments (Mantoura and Llewellyn 1983). A large number of samples can be processed by HPLC allowing a more thorough examination of the structure and dynamics of phytoplankton populations compared to microscopy (Schlüter *et al.* 2000).

Chlorophyll *a* (Chl-*a*) is accepted as a unique molecular marker of phytoplankton biomass and used as a convenient proxy for phytoplankton biomass (Gibb *et al.* 2000). The major role of Chl-*a* is to absorb light for photosynthesis, however, there are additional accessory pigments such as the chlorophylls *b* (Chl-*b*) and *c* (Chl-*c*) and various carotenoids, which have a significant role in extending the light-harvesting spectrum in different phytoplankton groups (Barlow *et al.* 2004). While Chl-*a* is used as a convenient proxy of phytoplankton biomass, many other phytoplankton pigments (*e.g.*

fucoxanthin, 19-hexanoyloxyfucoxanthin, and 19-butanoyloxyfucoxanthin is accepted as biomarkers for diatoms, prymnesiophytes and chyrsophytes, respectively) exhibit chemotaxonomic associations which may be exploited to map the oceanographic distribution and composition of phytoplankton assemblages (Barlow *et al.* 1993, Gibb *et al.* 2000). Traditionally, spectrophotometry and fluorometry have been used to determine Chl-*a* (*e.g.* Holm-Hansen *et al.* 1965, Lorenzen 1967). However, these methods suffer from inaccuracies associated with spectral interferences from Chl-*b*, carotenoids and from Chl-*a* degradation products (*e.g.* chlorophyllides, phaeophytins and phaeophorbides). Alternatively, using of high performance liquid chromatography (HPLC) facilitated the separation and quantification of other pigments in marine phytoplankton. The exploitation of pigment data generated from HPLC analysis of phytoplankton extracts has greatly advanced our understanding of the distribution, composition and functionality of phytoplankton in the global ocean (Wright *et al.* 1991, Jeffrey, 1997).

A summary of the taxonomic distribution of chlorophyll and carotenoid pigments is presented in Table 1. Photosynthetic pigments also provide the physiological condition of the phytoplankton community, which indicates environmental condition and trophic status for a given area (Roy *et al.* 2006). The majority of phytoplankton carotenoids are photosynthetic. While photosynthetic carotenoids (PSC) absorb light energy and transfer it to chlorophyll, photoprotecting carotenoids (PPC) protect the organism against stressful high light conditions. PPC are capable of quenching excited radicals, and converting their excess energy to heat and dissipating it harmlessly. Moreover, the ratio of PSC to PPC may be used to provide some additional characterisation of transitions in phytoplankton community composition across biogeochemical province boundaries and hence the identification of these boundaries (Gibb *et al.* 2000). Furthermore, PPC and PSC absorb light in different remote sensing spectral bands (*e.g.* SeaWiFS). Thus, variability in their relative abundances has the potential to affect the performance of algorithms used to retrieve Chl *a* values from remotely sensed ocean colour data (Gibb *et al.* 2000).

Table 1. Chemotaxonomic relationships between phytoplankton groups and pigments (Bolds indicate major taxonomic pigments for algal classes. Modified from Aiken *et al.* 2009 and Gibb *et al.* 2001)

Pigments	Occurrence
Chlorophyll a	**Photosynthetic pigment, Total algal biomass**
Divinyl chlorophyll a	**Prochlorophytes**
Chlorophyll b	**Chlorophytes, Prasinophytes,** Prochlorophytes (as DV Chl-*b*)
Chlorophyll c1 c2	**Diatoms,** Prymnesiophytes, Chrysophytes, Dinoflagellates
Chlorophyll c3	Prymnesiophytes, Chrysophytes,
Peridinin	**Dinoflagellates**
19-Butanoyloxyfucoxanthin	**Chrysophytes,** Prymnesiophytes
Fucoxanthin	**Diatoms,** Prymnesiophytes, Chrysophytes,
19-Hexanoyloxyfucoxanthin	**Prymnesiophytes,** Dinoflagellates
Diadinoxanthin	Diatoms, Dinoflagellates, Prymnesiophytes, Chrysophytes,
Alloxanthin	**Cryptophytes**
Lutein	Green algae (Chlorophytes, Prasinophytes)
Diatoxanthin	Diatoms, Dinoflagellates, Prymnesiophytes, Chrysophytes,
Violaxanthin	**Chlorophytes, Prasinophytes**
Zeaxanthin	**Cyanobacteria, Prochlorophytes**
Prasinoxanthin	**Prasinophytes**
β-Carotene	Photoprotective carotenoids

2. Deriving Phytoplankton Size Classes (PSC) from Diagnostic Pigments

Phytoplankton Size Classes (PSC) approach comprises splitting the autotrophic pool into picoplankton (0.2-2 μm), nanoplankton (2–20 μm) and microplankton (>20 μm). These approaches provide relative proportions of classes within the phytoplankton assemblages (Uitz *et al.* 2006). A strong link has been established between PSC and environmental factors (*e.g.* nutrients and light *etc*.) which regulate photosynthesis, phytoplankton dominancy and succession (Chisholm 1992, Bouman *et al.* 2005, Platt *et al.* 2005, Aiken *et al.* 2008). There is also an established connection between the size and the physiology of phytoplankton (Platt and Denman 1976, Chisholm 1992, Raven 1998), the marine food web (Parsons and Lalli 2002), areas of high fish production (Caddy *et al.* 1995) and various metabolic rates (Platt and Denman 1977, 1978).

The pigment index was introduced as an index of trophic status, based on seven diagnostic pigments (DP): Fucoxanthin (Fuco), peridinin (Perid), 19-Hexanoyloxyfucoxanthin (Hex-fuco), 19-Butanoyloxyfucoxanthin (But-fuco), zeaxanthin (Zea), total chlorophyll-b (TChlb =Chl *b* + DVChl *b*), and alloxanthin (Allo).

The relative biomass proportions of phytoplankton size classes [picoplankton (< 2 μm); nanoplankton (2-20 μm) and microplankton (20-200 μm)] are determined depending on the approaches of Uitz *et al.* (2006), Vidussi *et al.* (2001) and Claustre (1994).

$$\Sigma DPw = 1.41[Fuco] + 1.41[Perid] + 1.27[Hex - fuco] + 0.35[But - fuco] + 0.60[Allo] + 1.01[TChlb] + 0.86[Zea]$$

where DP_W represents the chlorophyll a concentration, which can be reconstructed from the knowledge of the concentration of the seven diagnostic pigments. The fractions of the three pigment-based phytoplankton size classes are computed following equations:

$$fmicro = (1.41[Fuco] + 1.41[Perid])/\Sigma DPw$$

$$fnano = (1.27[Hex - fuco] + 0.35[But - fuco] + 0.60[Allo])/\Sigma DPw$$

$$fpico = (1.01[TChlb] + 0.86[Zea])/\Sigma DPw$$

The actual chlorophyll a concentration associated with each class is derived from following equations:

$$Micro - [Chla] = fmicro \times [Chla]$$

$$Nano - [Chla] = fnano \times [Chla]$$

$$Pico - [Chla] = fpico \times [Chla]$$

It should be noted that the pigment grouping here proposed does not strictly reflect the true size of phytoplankton communities (Vidussi *et al.* 2001). Indeed, some taxonomic pigments might be shared by various phytoplankton groups (*e.g.* small amounts of fucoxanthin, the main carotenoid for diatoms, may also be found in some prymnesiophytes and pelagophytes), and also some phytoplankton groups may encompass a wide size range (*e.g.* diatoms are sometimes observed in the nano-size range, even if generally they belong to microplankton; Vidussi *et al.* 2001, Uitz *et al.* 2006).

3. Studies on PSC in the Black Sea

The Black Sea is a unique marine environment which has suffered from severe ecological deteriorations over the last three decades (Oguz 2005). It is a semi-enclosed and largest anoxic marine ecosystem in the world ocean (Tolmazin 1985). Phytoplankton community composition and group ratios have drastically changed due to intense eutrophication occurred in the Black Sea. During that time, phytoplankton responded to qualitative and quantitative changes in community composition, for example, intense blooms and changes in phytoplankton class structure and bloom timing with a pronounced dominance of dinoflagellates compared to diatoms (Kideys 1994). In more recent years, there have been some signs of recovery with a reduction in nutrient loading (McQuatters-

Gollop *et al.* 2008). Phytoplankton in terms of abundance, biomass and species composition, have been measured in the Black Sea over many decades (Eker *et al.* 1999, Agirbas *et al.* 2015). Traditionally, phytoplankton studies in the Black Sea are conducted by microscopic examination (*e.g.* Bologa 1986, Cociasu *et al.* 1997, Moncheva and Krastev 1997 *etc*.). Despite significant roles of phytoplankton communities, information about phytoplankton size classes (PSC) derived from pigment profiles by using HPLC are limited in the Black Sea (Ediger *et al.* 2006, Eker-Develi *et al.* 2012, Agirbas *et al.* 2015).

Pigment data revealed that phytoplankton community in the south-western Black Sea was dominated by dinoflagellates, diatoms and coccolithophores (Ediger *et al.* 2006). Strong correlations (r^2 = 0.56–0.67) between marker pigment concentrations and abundance or biomass in the south-western Black Sea indicated that HPLC pigment analysis can be used to quantify phytoplankton assemblages in the Black Sea (Figure 1).

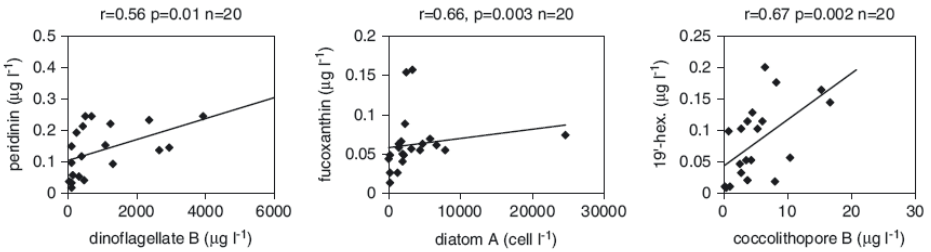

Figure 1. Relationship between phytoplankton abundance (A) or biomass (B) and marker pigments in the south-western Black Sea (Ediger *et al.* 2006)

A strong chemotaxonomic relationship between phytoplankton groups and pigments (*e.g.* between dinoflagellate-C and peridinin; between dinoflagellate-C and diadinoxanthin; between dinoflagellate-C and 19-Hex and between coccolithophore-C and 19'-Hex) was also reported from the south-eastern Black Sea (Figure 2; Agirbas *et al.* 2015). Similarly, high 19-Hex concentrations corresponding to high dinoflagellate-C from the north-western Black Sea was reported (Eker-Develi *et al.* 2012). Besides, fucoxanthin and 19-Hex containing dinoflagellates were reported in the English Channel, previously (Irigoien *et al.* 2004).

There is still certain controversy in interpretation by using diagnostic pigments to infer phytoplankton size classes, and some diagnostic pigments such as fucoxanthin (main indicator of diatoms) may also be found in some flagellates (Vidussi *et al.* 2001). Therefore, the pigment grouping does not strictly reflect true size of phytoplankton communities, and PSC derived from HPLC pigments are indicative but not definitive (Brewin *et al.* 2010).

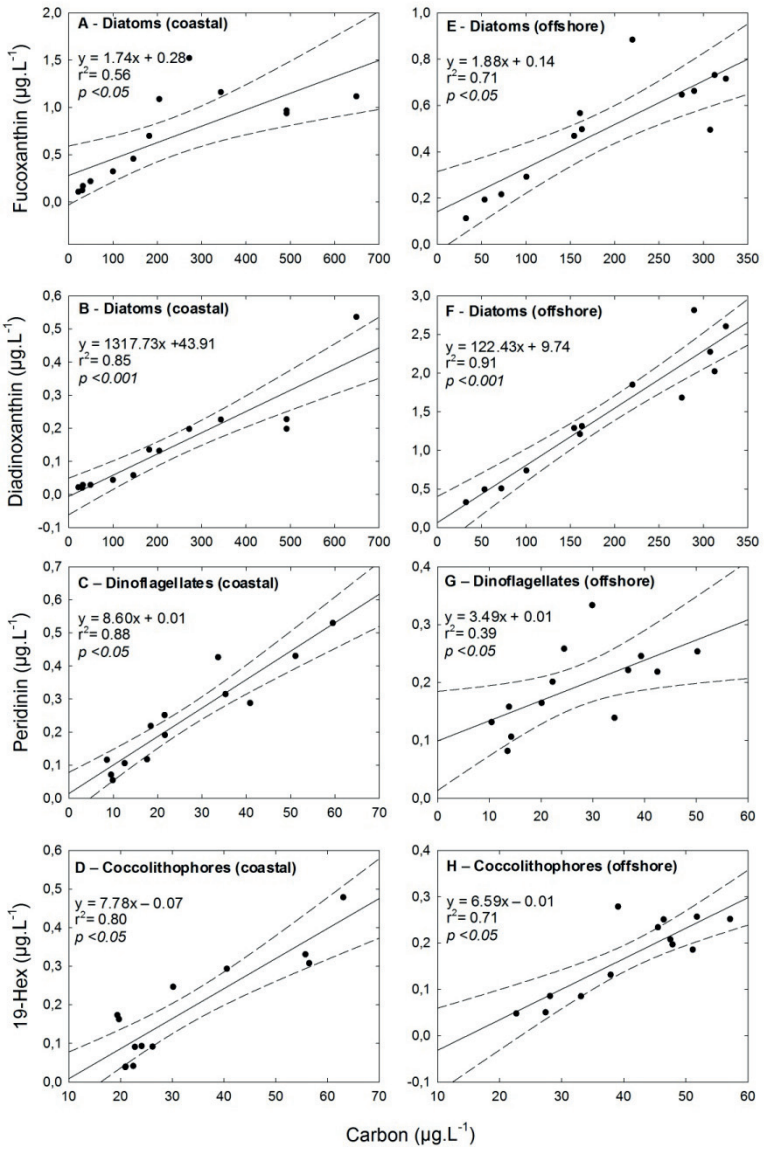

Figure 2. Relationship between phytoplankton groups and marker pigments with 95% confidence intervals (A, C and E indicate coastal station, and B, D and F indicate offshore station in the south-eastern Black Sea, Agirbas *et al.* 2015).

Recently, in another research which was conducted seasonally within the scope of TUBITAK project (113Y189) along the south-eastern coast of the Black Sea (Figure

3), PSC derived from HPLC pigment data revealed a high degree of spatio-temporal variability between November 2014 and August 2015 (Figure 4-6).

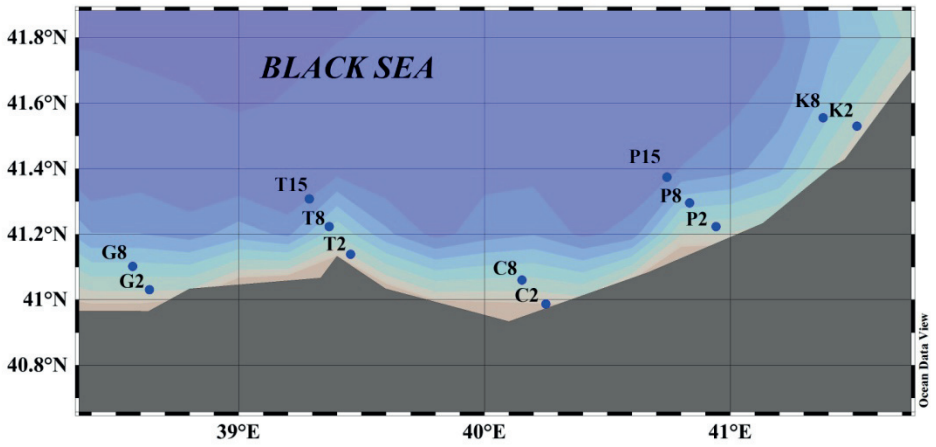

Figure 3. Location map for the project of TUBITAK 113Y189 showing the sampling points in the South-Eastern Black Sea (G2: Giresun 2 nm, G8: Giresun 8 nm, T2: Trabzon 2 nm, T8: Trabzon 8 nm, T15: Trabzon 15 nm, C2: Camburnu 2 nm, C8: Camburnu 8 nm, P2: Pazar 2 nm, P8: Pazar 8 nm, P15: Pazar 15 nm, K2: Kemalpasa 2 nm, K8: Kemalpasa 8 nm)

During this project, the contribution of microplankton ratio (8-93%) was substantial in autumn and spring. On the other hand, the contribution was low in summer (Figure 4). Nanoplankton contribution (1-70%) was generally high in winter and moderately in spring (Figure 5). On the other hand, picoplankton (1- 80%) relatively contributed to PSC in summer at surface waters (Figure 6). Overall, contribution of microplankton to PSC was higher than pico- and nanoplankton contribution along the south-eastern Black Sea. These observations coincide with previous studies reported from the Black Sea (*e.g.* Agirbas *et al.* 2015, Mikaelyan *et al.* 2013). Due to eutrophication, phytoplankton community composition, abundance, biomass, and bloom patterns of phytoplankton changed in the Black Sea. During that period, the biomass of dinoflagellates increased notably in the water column (Mikaelyan *et al.* 2013). However, Black Sea has shown some signs of recovery (*e.g.* increases in diatoms abundance, decrease in the number of monospecific algal blooms etc.) in recent years (McQuatters-Gollop *et al.* 2008). In the present observation, high dominancy of microplankton indicates the recovery reported from pervious observations in the Black Sea.

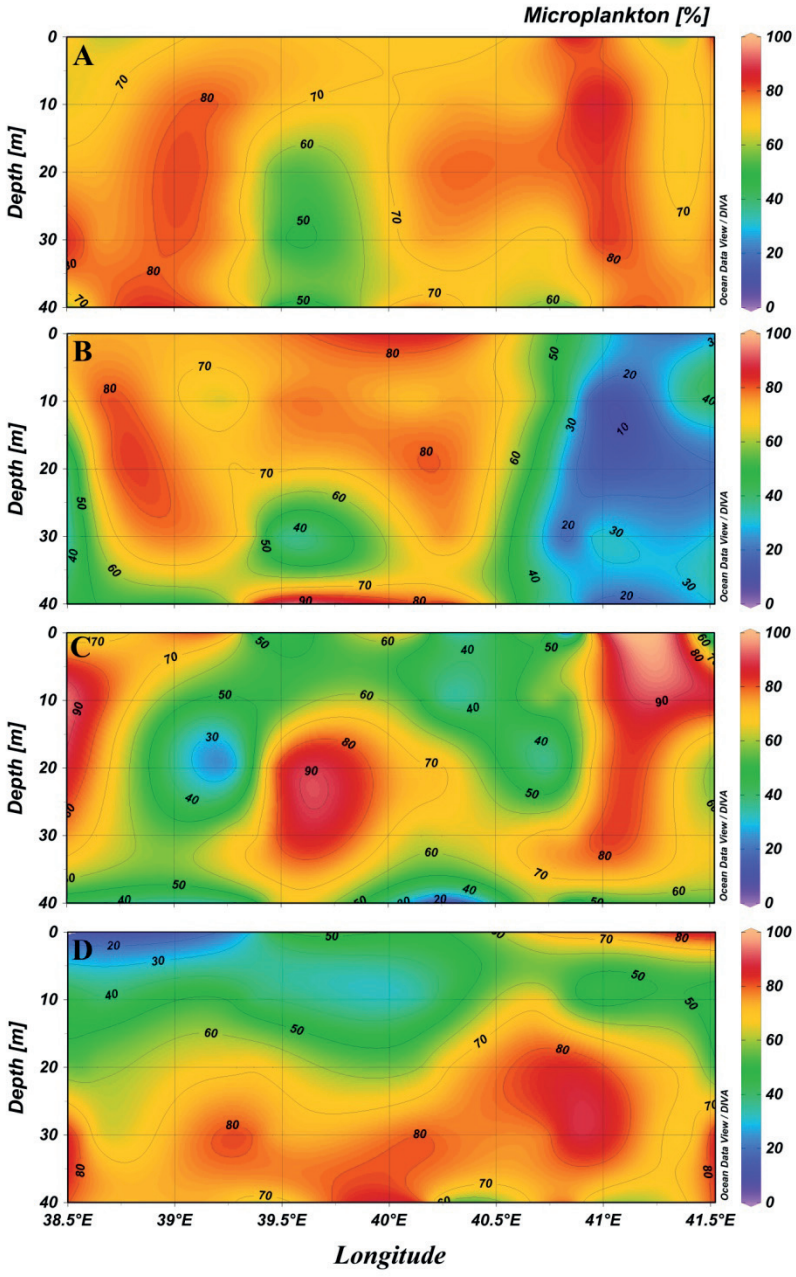

Figure 4. Spatio-temporal variation of microplankton contribution (%) along the study area (A: Autumn 2014, B: Winter 2015, C: Spring 2015 and D: Summer 2015)

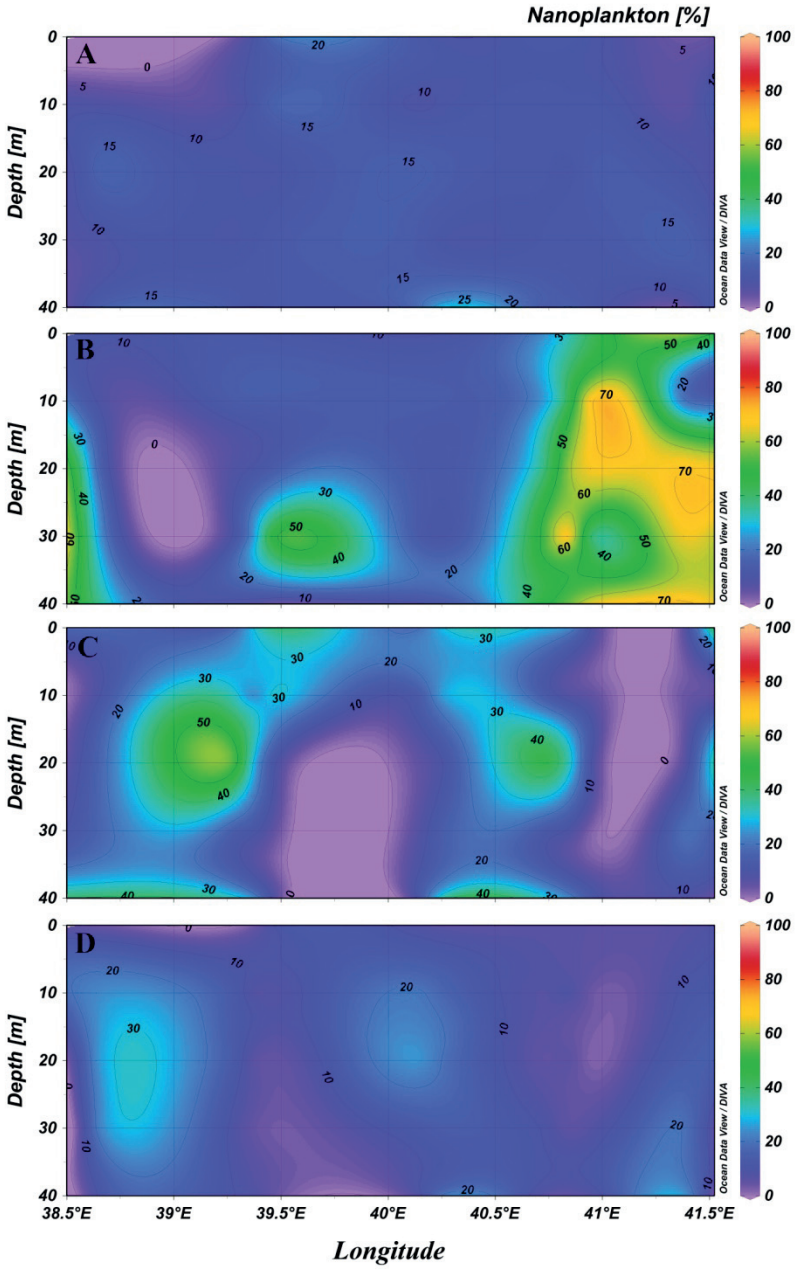

Figure 5. Spatio-temporal variation of nanoplankton contribution (%) along the study area (A: Autumn 2014, B: Winter 2015, C: Spring 2015 and D: Summer 2015)

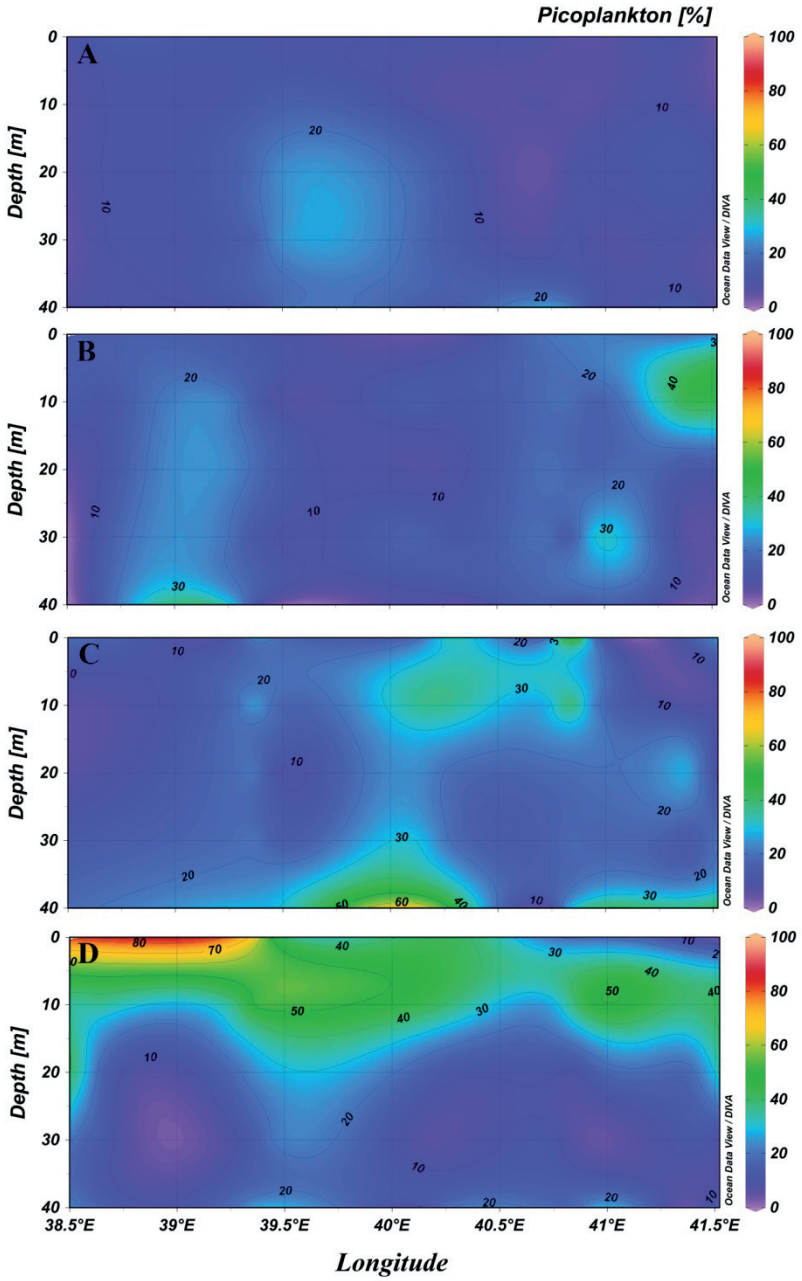

Figure 6. Spatio-temporal variation of picoplankton contribution (%) along the study area (A: Autumn 2014, B: Winter 2015, C: Spring 2015 and D: Summer 2015)

Phytoplankton community composition, and hence pigment concentration are affected by changing environmental factors (Trees *et al.* 2000). Moreover, PSC are closely associated with the trophic status of the environments. Picoplankton generally dominate the surface layers of warm, oligotrophic, low N waters; nanoplankton are more abundant in cooler, mesotrophic, moderate N waters; microplankton are dominant in eutrophic, high N waters (Aiken *et al.* 2009). In this project, the majority of microplankton was observed in autumn and spring, when new nutrient input occurred by vertical mixing and river runoff. Similarly, high picoplankton contribution to total PSC was detected in summer period at the surface waters, where the nutrients were depleted in the water column. On the other hand, nanoplankton contribution was generally substantial in winter and spring below the Chl-*a* maximum, when the extensive vertical mixing occurred throughout the water column. The presence of microplankton in up-welled waters in the Arabian Sea and the dominance of the picoplankton in oligotrophic, low chlorophyll waters along the equator were also reported (Latasa and Bidigare, 1998; Barlow *et al.* 2004). Similarly, high diatom dominancy in nitrate-rich coastal waters and dominancy of picoplankton (*e.g.* Cyanophyta) in nitrate-poor mid-ocean regions from across the north Pacific were also reported (Ondrusek *et al.* 1991). Moreover, surface pigment adaptations are likely to be related to or controlled by the nutrient dynamics (Barlow *et al.* 2004). Diatoms are opportunistic organisms that are able to respond quickly to nitrate enrichment (Fogg, 1991), and Chl-*a* molecules contain nitrogen atoms, while carotenoids do not (Porra *et al.* 1997). Hence, the nitrogen characteristic of diatoms and chlorophylls probably explain high contribution of microplankton in nutrient rich waters during autumn and winter along the study area.

There was great variability in vertical profile of PSC along the south-eastern Black Sea (see also Figures 4-6). Microplankton ratios were high in the whole water column in autumn and spring, relatively in winter. On the other hand, their contribution was low in summer (especially at surface waters), and in winter below the Chl-*a* maximum. Nanoplankton proportions were generally greatest in winter and spring at Chl-*a* maximum or below. In contrast, picoplankton had great proportions at surface waters in summer or at the base of euphotic zone.

Several mechanisms were suggested about vertical structure of phytoplankton size classes (Perez *et al.* 2006). Claustre and Marty (1995) proposed that nanoplankton can develop close to the nutricline and their presence at very low light levels may be governed by nitrate availability as opposed to photoadaptation. They also suggested that reason for nanoplankton below the Chl-*a* maximum may include a decoupling between nitrate assimilation and CO_2 fixation, vertical migration and heterotrophic growth. Similar pattern was also reported from oligotrophic gyres of south pacific with high contribution of nanoplankton below the Chl-*a* maximum (Ras *et al.* 2008). On the other hand, decreasing pattern in picoplankton with depth down to Chl-*a* maximum, and an

increasing pattern of nanoplankton contribution below the Chl-*a* maximum were reported (Brewin 2010).

Chl-*a* is typically used as phytoplankton biomass. It can be routinely estimated *in-situ* (*e.g.* spectrophotometrically, fluorometrically, HPLC) or by using satellite remote-sensing of ocean colour (O'Reilly *et al.* 1998). On the other hand, size-fractionated chlorophyll is used as a metric for phytoplankton size classes and can be estimated through either HPLC diagnostic pigment analysis (*e.g.* Brewin *et al.* 2010, Hirata *et al.* 2011, Uitz *et al.* 2006, Vidussi *et al.* 2001), or through size-fractionated filtration of Chl-*a* (*e.g.* Brewin *et al.* 2014).

Studies revealed a good correlation between Chl-*a*, PSC and physical environment (Behrenfeld *et al.* 2006, Martinez *et al.* 2009, Kostadinov *et al.* 2010, Brewin *et al.* 2012). However, studies on size fractioned Chl-*a* and PSC has remained poorly characterized. Recently, spatio-temporal distribution of *in-situ* Chl-*a*, size fractioned Chl-*a* and their correlation were well established in the south-eastern coast of the Black Sea (Figures 7-11). The data presented here clearly explain general pattern of *in-situ* Chl-*a* and phytoplankton size-classes in relation to seasons and stations. *In-situ* Chl-*a* was ranged from 0.16 µg/L (August 2015) to 4.96 µg/L (February 2015) along the stations, and revealed sub-surface maxima (Figure 7). Sub-surface Chl-*a* maxima was detected between 10 and 20 m depth, except for winter. Seasonally, autumn and winter periods was characterised high Chl-*a* concentrations along the stations.

Size-fractioned Chl-*a* along the station showed high degree of spatio-temporal fluctuation (Figures 8-10). Majority of size-fractioned Chl-*a* was consisted of microplankton, ranged from 0.04-4.79 µg/L (Figure 8). The second group along the stations was nanoplankton and their size-fractioned Chl-*a* concentrations varied between 0.01 and 3.78 µg/L (Figure 9). Picoplankton fractioned Chl-*a* was relatively low along the stations and its concentration ranged from 0.01 to 2.24 µg/L at the base of photic depth (Figure 10). In general stations were dominated by microplankton, however, the contribution of size-fractioned Chl-*a* varied vertically and seasonally. Especially in autumn and occasionally in winter, the water column was dominated by microplankton. However, the majority of microplankton formed at the base of photic depth in spring and summer (see Figure 8). Nanoplankton and picoplankton fractioned Chl-*a* was generally low along the stations except from some periods (*e.g.* winter for nanoplankton and summer for picoplankton).

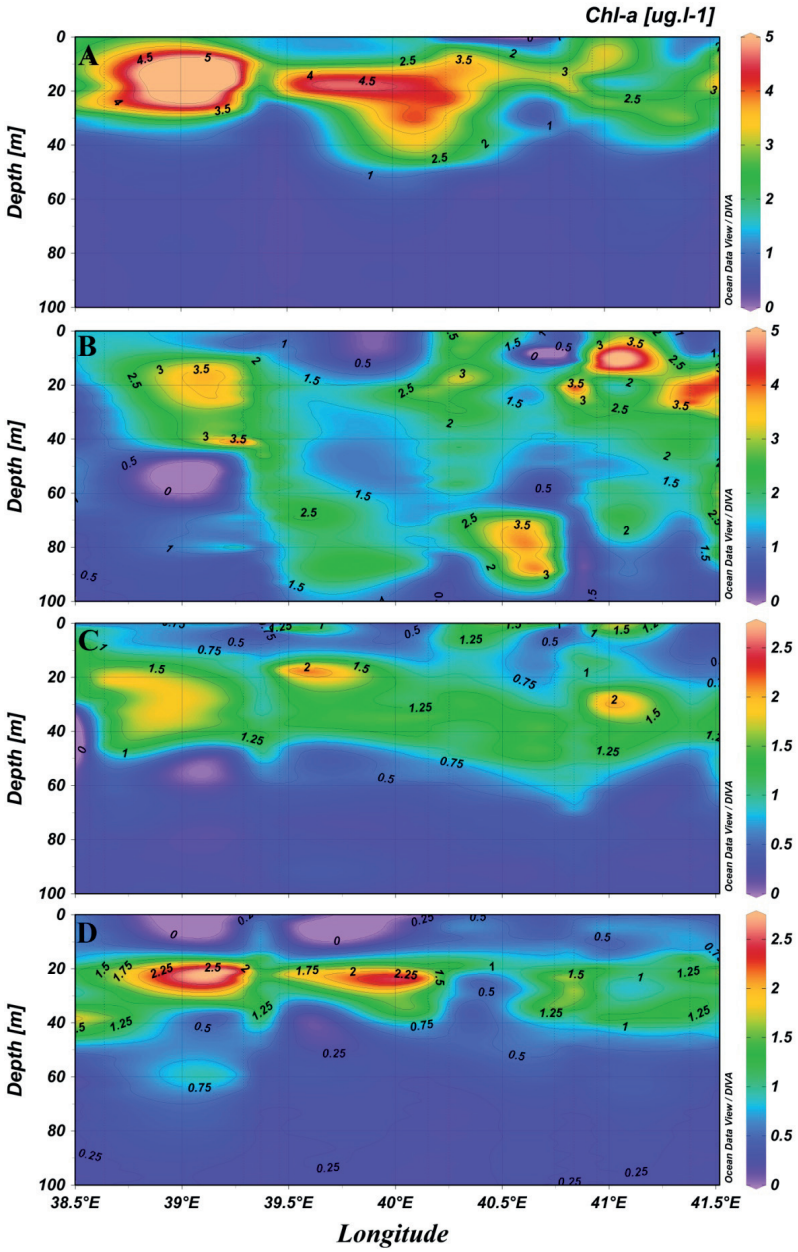

Figure 7. Spatio-temporal variation of Chl-*a* along the study area (A: Autumn 2014, B: Winter 2015, C: Spring 2015 and D: Summer 2015)

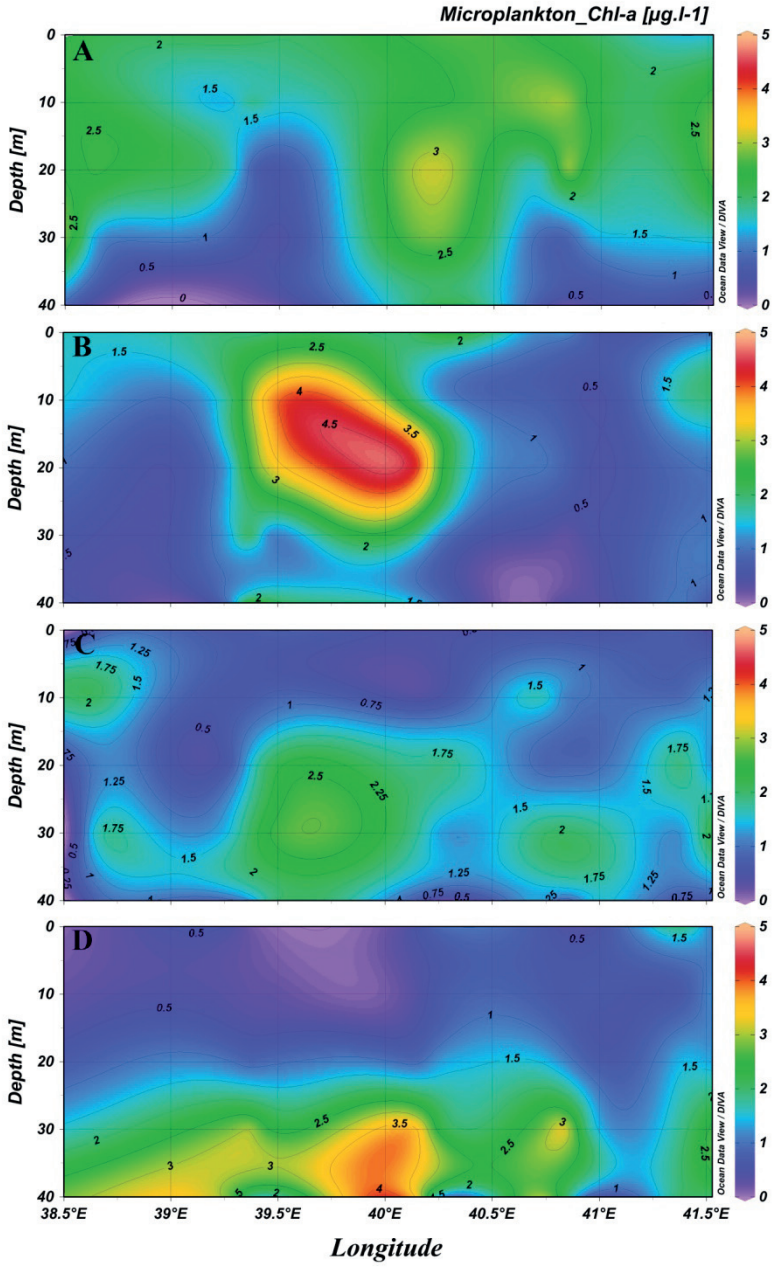

Figure 8. Spatio-temporal variation of microplankton fractioned Chl-*a* along the study area (A: Autumn 2014, B: Winter 2015, C: Spring 2015 and D: Summer 2015)

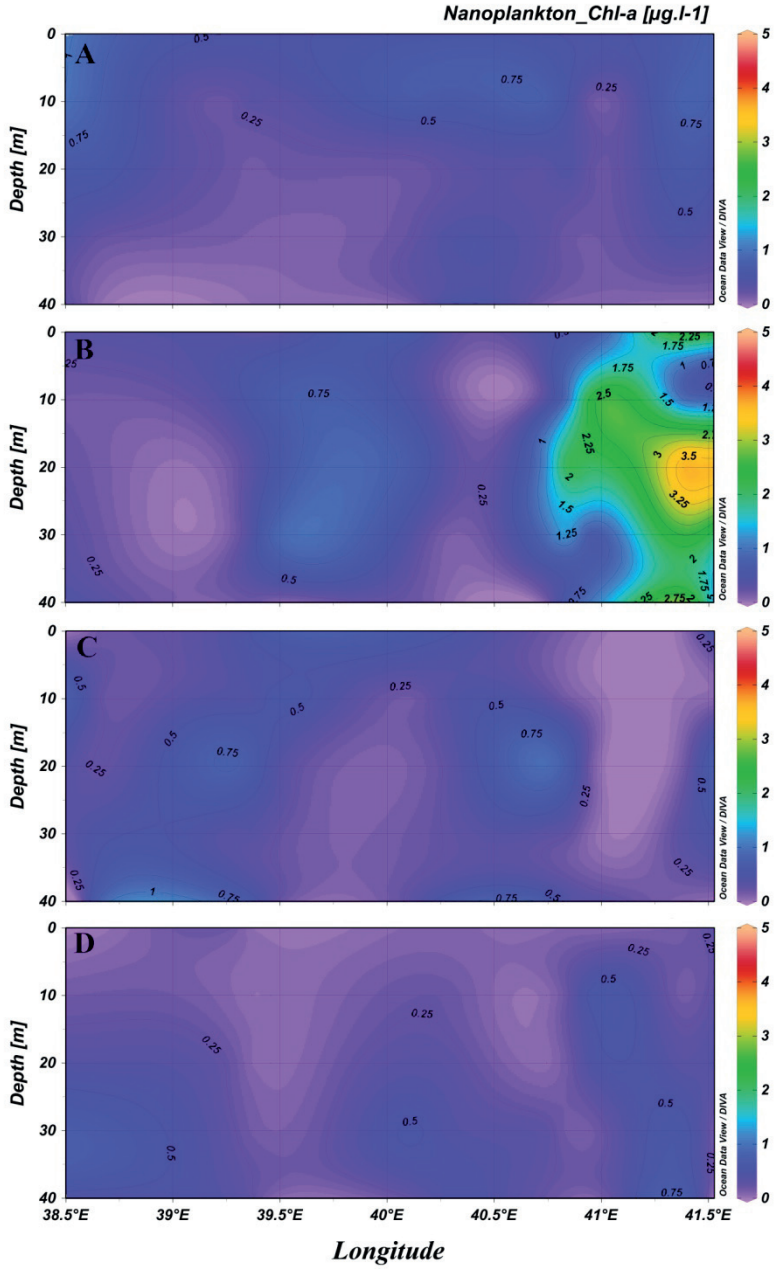

Figure 9. Spatio-temporal variation of nanoplankton fractioned Chl-*a* along the study area (A: Autumn 2014, B: Winter 2015, C: Spring 2015 and D: Summer 2015)

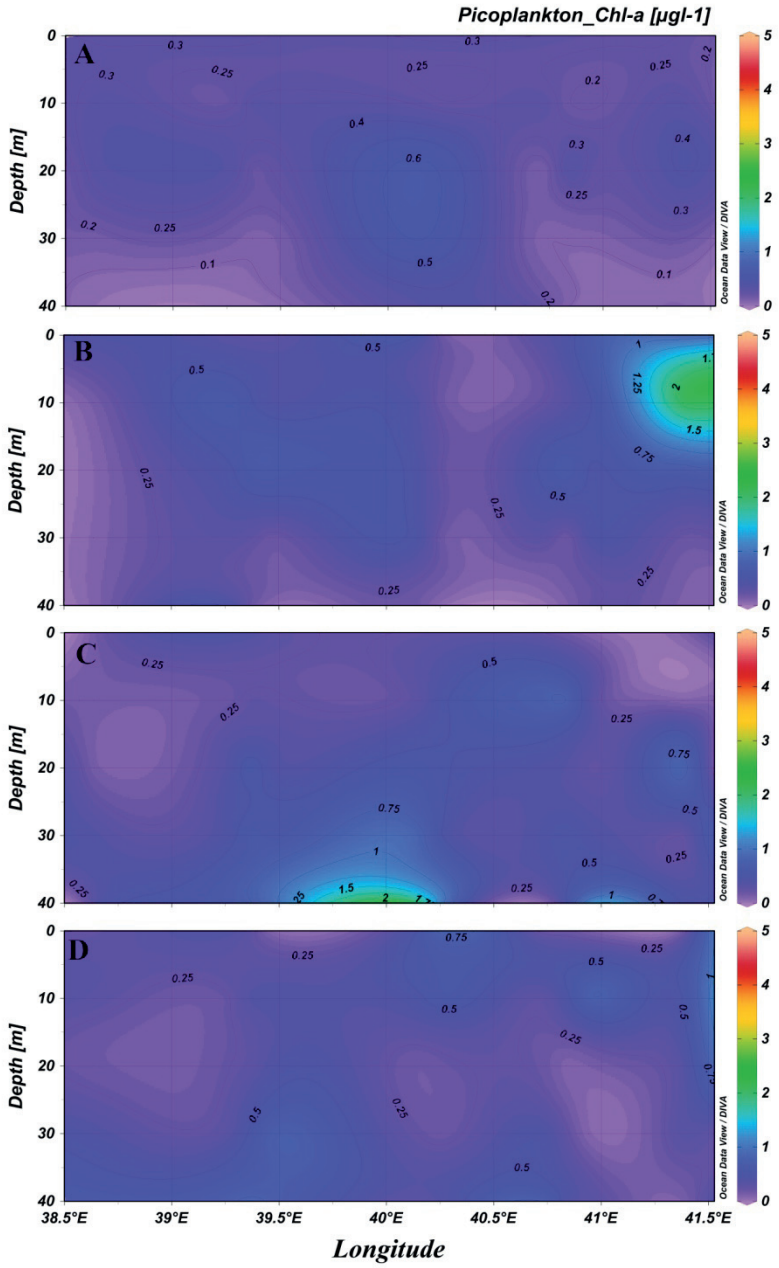

Figure 10. Spatio-temporal variation of picoplankton fractioned Chl-*a* along the study area (A: Autumn 2014, B: Winter 2015, C: Spring 2015 and D: Summer 2015)

The correlation between PSC and size fractioned Chl-*a* was also important along south-eastern Black Sea (Figure 11). However, correlation between nanoplankton fraction and nanoplankton fractioned Chl-*a* was much greater than micro- and picoplankton correlations ($p<0.001$). Overall, statistically significant correlations suggest that pigment derived size fraction give an information about phytoplankton assemblages in the south-eastern Black Sea.

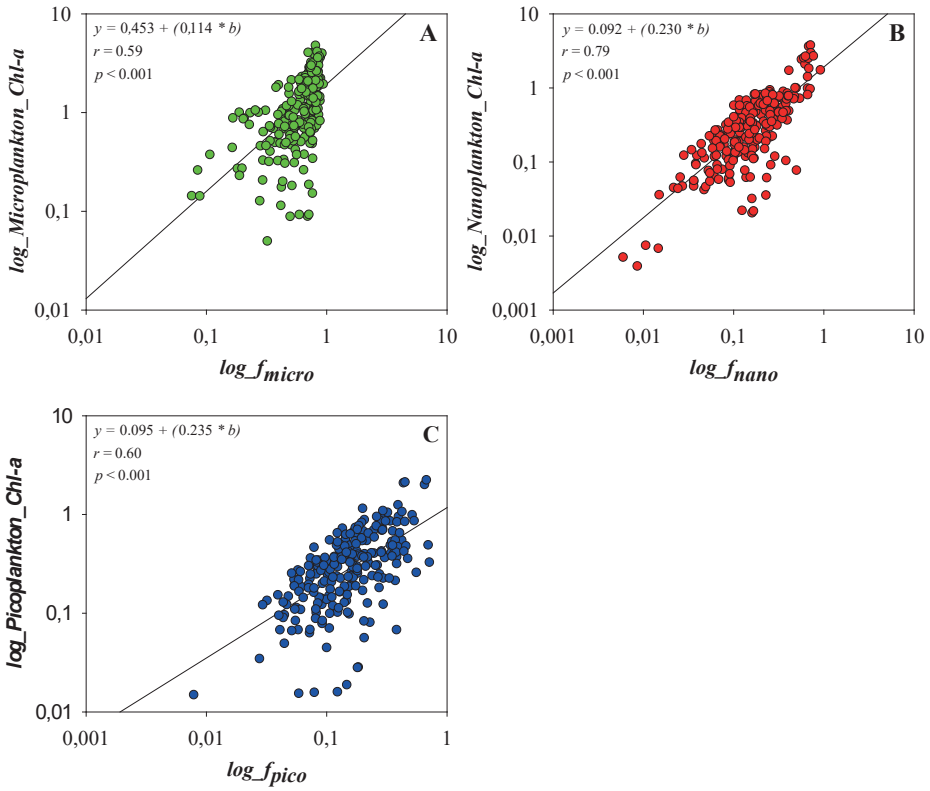

Figure 11. Linear regression between phytoplankton size classes (PSC) and size fractioned Chl-a (A: microplankton, B: nanoplankton, C: picoplankton)

4. Conclusions

In this chapter, phytoplankton pigments and using them as monitoring tool for phytoplankton size classes (PSC) in the Black Sea have been considered and reviewed. Phytoplankton community composition and size structure are admitted as key ecological indicators in the marine environment (Platt and Sathyendranath 2008). Hence, determination of changes in these indicators and monitoring them may help to detect how

the marine ecosystem may respond to natural variability (*e.g.* climate changes) and human induced change (*e.g.* anthropogenic changes).

The data presented here illustrate current state of PSC and mainly dominated by microplankton. In addition, obtained good correlation between PSC and size fractioned Chl-*a* was clearly indicate that pigment derived phytoplankton size classes can be used as a monitoring tool along the south-eastern Black Sea. However, limited researches on PSC deriving from diagnostic pigments have not allowed for comparison present results for the south-eastern Black Sea. Improving the long-term understanding of changes in pelagic ecosystem is only possible by continuous monitoring programmes. Besides, monitoring of such indicators provides comprehensive ecosystem management.

The enclosed seas (*e.g.* Black Sea, Baltic Sea etc) have noticeably undergone far more dramatic changes than the more open seas during the past decades. Even, small changes in the frequency of inflow (as in the Baltic Sea) or in temperature (as in the Eastern Mediterranean and Black Sea) had a strong effect on large parts of the ecosystem, indicating the high sensitivity of these enclosed systems to climate change (Anadon *et al.* 2005). Hence, it is important to determine how the Black Sea ecosystem will respond to changing climate and changes. Finally, the results obtained in the present study provide more comprehensive picture of the phytoplankton size classes for the region, however, it should be validated with other techniques.

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